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Behavioural and Morphological responses in Fishes in Response to Aquatic Toxicants: A Review

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Abstract

Fishes receive stimuli from their surroundings and respond in several ways. Fish behaviour may be considered a sensitive index of environmental stress and may act as a tool for assessing aquatic pollution. Fish is an economically important aquatic animal that responds first when its surrounding environment is contaminated with chemicals. Aquatic toxicants change the physico-chemical characteristics of water and thus impose a drastic negative impact on the health of aquatic inhabitants, particularly in fishes. Therefore, the purpose of this review article is to scan the behavioural and morphological responses in the fishes as a result of exposure to various toxicants. The regular behaviour of fish may be altered due to the toxicant stress and can vary depending on time and dose of exposure. In the present article, behavioural changes reviewed are swimming activity /erratic swimming, opercular movement /surface air gulping, a loss of balance /sluggishness; and morphological changes studied are discoloration of skin/change in body colour /pigmented patches on body and mucus secretion with the sedimentation of chemical on body.

Keywords: *Fish, Behavioural Changes, Morphological Changes, Stress.*

1. Introduction

Monitoring aquatic toxicity is crucial for risk assessment in aquatic ecosystems, especially due to the increasing threats from industrialization, urbanization, and agricultural waste disposal. These activities introduce various toxicants into aquatic environments, overwhelming the natural biological cycles and self-purification capacities. When toxicants exceed threshold levels, they stress aquatic biota, particularly fish, leading to decreased immune responses, increased susceptibility to diseases, and mortality, ultimately reducing fish production. Fish are key indicators of aquatic pollution due to their sensitivity to environmental changes, with hazardous substances like pesticides and heavy metals posing significant threats due to their persistence in aquatic environments (George, 1990). Changes in fish behaviour, influenced by these toxicants, can indicate the health of aquatic ecosystems.

The effects of toxicants can be examined across various levels, from biochemical and cellular changes to population and community impacts. Early biochemical and cellular responses serve as biomarkers for dangerous chemical exposure, although the connections between these levels and broader ecological impacts remain poorly understood. Behaviour, as a response at the organism level, is closely linked to biochemical effects, such as neurotoxicity, and can provide early warnings of environmental stress (Scott and Sloman, 2004). The concept of stress, defined by Hans Selye (1936, 1973) as the body's non-specific response to demands for change, has been applied to understanding the impact of contaminants on both land and aquatic species (Clotfelter et al., 2004; Zala and Penn, 2004), especially fish (Scott and Sloman, 2004; Robinson, 2009).

Fish behaviour is a sensitive indicator of low dissolved concentrations of trace elements, often detectable before other sub-lethal effects. Toxicant-induced stress in fish is reflected in behaviours such as swimming, opercular movement, surfacing, loss of equilibrium, and sluggishness, as well as morphological changes like skin discoloration, pigmented spots, mucus secretion, and descaling (Remyla et al., 2008). The skin, as a biological barrier, not only protects but also signals systemic defence responses, indicating internal disruptions in enzyme activity, neural function, and metabolic pathways. Despite their significance, behavioural and morphological changes in fish are less frequently studied, prompting a need for further research into the impact of environmental contaminants on fish behaviour.

2. Material and Method

This paper is a review of different behavioral and morphological changes recorded against a variety of environmental toxicants to understand toxicological studies in a better way. For this various scientific databases viz., PubMed, Web of Science, Scopus, and Google Scholar were retrieved, reviewed, and compared. The last retrieval time of each database was March 30, 2022.

The keywords for searches are mainly: “environmental toxicant”, “toxicant and fish”, “heavy metal and fish”, “fish behavioral alteration against heavy metal”, “fish behavioral alteration against pesticides”, “fish behavioral alteration against insecticides”, “tannery effluent and fish behavior”, “name of tools for fish behavior study”, “morphology of gill epithelium”, “Histology of fish gill”, “fish morphology and toxicants”, “mechanism of fish morphological alterations”, “discoloration in fish mechanism” and “mechanism of mucous secretion + fish+ toxicants”. The literature searched for environmental toxicants mainly heavy metals, pesticides, and herbicides against their acute and chronic exposure experiments.

Unpublished data and incomplete data like only graphical results were excluded. Attempts have also been made to summarize extracted mechanisms from various searched data and integrate them to outline behavioral and morphological alteration (Fig. 1).

3. Behavioural and Morphological changes

Fish reactions to stress, according to Barton (2002), may be classified into three stages: primary, secondary, and tertiary. Catecholamines and cortisol are produced during the first phase of the neuroendocrine response (Reid *et al*., 1998). The aforementioned hormones have a secondary effect that affects physiologic and metabolic pathways, leading to hyperglycemia because of increased glycogenolysis and gluconeogenesis, gill filament artery enlargement, and depressed immune function (Gratzek and Reinert, 1984). As these processes support fish survival by maintaining internal homeostasis, the first two steps are regarded as adaptive. On the other hand, tertiary responses involve systemic changes that render animals incapable of responding to stressors, having a detrimental effect on the animals' overall health, including performance, growth, reproduction, disease resistance, and behaviour (Barton, 2002). Depending on its intensity and duration, stress can affect fish at all organisational levels, from the cellular and physiological to the population and community (Adams, 1990) (Fig. 1).

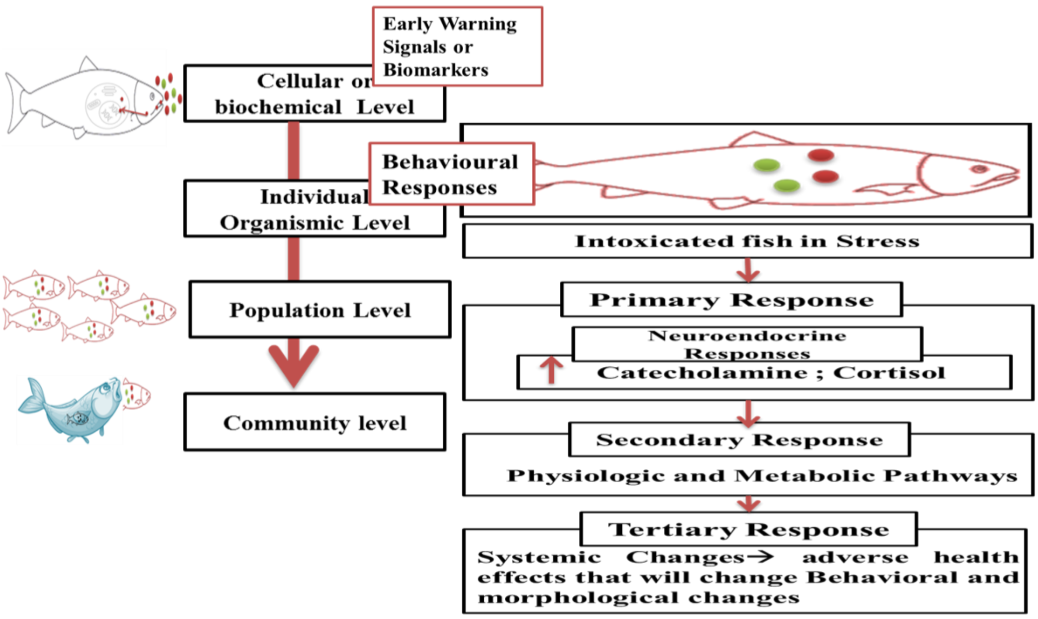

Fig.1. Systemic representation of fish responses against environmental toxicants

Behaviour may be the best way to study the impacts of environmental toxins since it may serve as a link between physiological and ecological systems. Many behavioural acts of fish can be easily observed and quantified in a controlled environmental setting that could be of great relevance for ecological study. Furthermore, behavioural observation in correlation with the well-studied physiology mechanisms of fish will provide an insight for toxic behaviour of toxicant in in-vivo. Indeed, numerous researchers have advocated behavioural indicators in fish to monitor environmental contamination in an ecologically meaningful way (Atchison *et al*., 1987). Now days with advancement of technology, these behavioural changes can be measured and recorded both in lab and field easily. There are a variety of tools available to study the behaviour of fish living in remote areas, such as bio-telemetry and bio-loggers, for example, electromyogram (EMG) telemetry. (Cooke *et al*., 2004)

The available data on the effects of environmental toxicants on fish behaviour and morphological changes are sparse and variable. Table 1 shows some previous studies that explain the behavioural and morphological changes in different fish species against variety of toxicants.

Table 1. Summary of behavioural and morphological alterations in fish induced by exposure of aquatic pollutants.

Fish Species	**Toxicant**	**Behavioural & Morphological Changes**	**Reference**
Stickleback	Lead nitrate	Resistance at low concentration and migration toward high concentration	Jones, 1947
Bluegill	Cd, Cr, Zn	Hyperactivity	Ellgaard *et al*., 1978
Oreochromis alcalicus grahami	Highly Alkaline Lake (pH~ 10)	Hypoxia	Maina, 1997
Channa punctatus	Cd	sluggishness	Maruthanayagam *et al*. 2002
Ctenopharyngodon idellus	Cd	Loss of balance, excessive mucus secretion, slowness in motion	Yorulmazlar and Gul, 2003.
Channa punctatus	Mercuric Chloride & Malathion	Fast swimming, Opercular movement, gulping of air, loss of equilibrium	Pandey *et al*., 2005
Cyprinus carpio	PCB mixture	Swimming behaviour	Katja *et al*., 2005
Barilius vagra	$CuSO_4$	Mucous Secretion	Bisht and Agarwal, 2007
Channa punctatus	Cr	Increasing in rate of swimming andopercular activity, loss of balance, hyperactivity	Mishra and Mohanty, 2008.
Cyprinus carpio (Linn)	chlorpyrifos	Discoloration of skin, thin chemical deposition on skin, shedding of scales	Halappa and David, 2009
Chanos chanos (Milk fish)	Cd	Mucus secretion, convulsions, loss of balance,	Amrolahi *et al*., 2010
Chanos chanos (Milk fish)	Lead nitrate	Hyper excitability, erratic swimming, jumping and restlessness	Hesni *et al*. (2011)
Channa punctatus	endosulfan	decreased swimming, increase in surfacing frequency	Harit and Srivastava, 2017
Danio rerio, *Oryzias latipes, Gobiocypris rarus*	chlorpyrifos & Iimidacloprid	hyperactivity	Hong and Zha, 2019
Channa punctatus	Cd	restlessness, jumping, erratic swimming and gulping of air; discoloration of skin and pigmented patches on body	Singh and Saxena, 2020
Clarias batrachus	chlopyrifos 50% & cypermethrin 5%EC.	irregular and erratic swimming; body decolouration	Kumar *et al.*, 2020
Hetropneustes-fossilis	Chromium Chloride	restlessens, jumping, erratic swimming, gulping of air, loss of equilibrium, sluggishness, opercular movements	Khan, 2023

3.1. Behavioural changes

Behavioural monitoring can help to investigate environmental conditions by examining the behavioural responses of aquatic animal species and studying their relations to the surrounding environments. Behavioural monitoring is an efficient approach for long-term monitoring of the aquatic ecosystems and water quality assessment.

i. Swimming Activity (erratic swimming)

One of the most frequent and simple to-measure behavioural responses during toxicological investigations is swimming activity which can be utilized as a bioindicator of sub-lethal stress related to exposure to toxicants (Little and Finger, 1990). Swimming can be assessed and monitored in a variety of styles such as burst, critical swimming speed, and endurance. (Beamish, 1978). When exposed to toxicants, fish are likely to get stressed, and they may respond by showing odd behaviours such as swimming swiftly or erratically. Swimming is a sensitive and ecologically significant response in a fish to sublethal exposure to a toxicant (Little and Finger, 1990). The swimming behaviour of young bluegill sunfish (Lepomis macrochirus) was substantially altered after 60 days of exposure to a 6:1 dissolved selenate: selenite ratio (Cleveland et al., 1993). Two to three days of short-term exposure to chlordane, dichlorodiphenyltrichloroethane (DDTs), endosulfan, and polychlorinated biphenyls (PCBs) at concentrations of 1-2 ug/L affected predator avoidance behaviours such as schooling and swimming, and lowered the chance of survival in predator interactions in goldfish, rainbow trout, and medaka (Scott and Sloman, 2004). The impacts of persistent organic pollutants on fish behaviour seem to be caused by two different mechanisms: by altering thyroid function or by altering brain neurotransmitters like serotonin. Serotonin, dopamine, and norepinephrine levels in the brain are affected by DDTs and PCBs, which alter spontaneous activity, learning, and locomotor activity (Khan and Thomas, 2000, 2006; Khan et al., 2001). Hesni et al. (2011) investigated lead-induced alterations in milkfish behaviour. They observed hyperexcitability, erratic swimming, jumping, and restlessness. Walia et al., (2013) noticed erratic swimming in fish Labeo rohita exposed to different concentrations of tannery effluent. Harit and Srivastava (2017) noticed that endosulfan exposure altered the typical behaviour of the fish Channa punctatus. They observed a decrease in swimming activity as well as an increase in surfacing frequency. Singh and Saxena (2020) studied the acute toxicity of cadmium in a freshwater fish, C. punctatus, and the principal behavioural changes they saw during the experiment were restlessness, leaping, irregular swimming, and surface gulping of air. Kumar et al., (2020) also recorded the irregular and erratic swimming pattern in freshwater fish Clarias batrachus exposed to chlorpyrifos 50% & cypermethrin 5% EC.

ii. Opercular Movement/ rapid gill movement

When a fish is exposed to a toxicant another behavioural change, we might observe frequently is opercular movement or rapid gill movement. Fish gills are always moving while they breathe, but if the gill movement becomes considerably quicker than usual, this might be an indication of stress. Rapid gill movement might be interpreted as a sign of stress. In fish, the gill epithelium serves a variety of functions including gas exchange, ionic control, acid-base balance, and the elimination of nitrogenous wastes. Since the gills are constantly exposed to the environment, the morphology of the gill epithelium has been discovered to be changed by pollutants such as heavy metals and pesticides from agricultural run-off, and also provide a primary site of action for these environmental contaminants (Macirella, Curcio and Brunelli, 2020; Alesci et al., 2022).

According to Skidmore and Tovell (1972), rainbow trout treated with zinc (40 ppm) for 3 hours showed significant secondary lamellae curling and edoema, as well as epithelial separation from the basement membrane. Partially dislodged and enlarged chloride cells were also present. After exposing rainbow trout to either mercuric chloride or methylmercury (approximately 50 ppb for 1 week or 0.25 ppb for 6-8 weeks), insignificant morphological alterations such as reduced height of lamellar cell ridges, vacuolated epithelial cells, and chloride cell degradation were reported by Olson et al. (1973). The gill epithelium of yearling coho salmon (Oncorhynchus kisutch) exposed to the herbicides dinoseb (100 ppm concentration for 114 hr), paraquat (100 ppm concentration for 120 hr), and atrazine (15 ppm concentration for 140 hr) exhibited necrosis, desquamation, hypertrophy, hyperplasia, and telangiectasia (Meyers and Hendricks, 1985). These changes lead to impaired gaseous exchange resulting in rapid opercular movement. Escape response observed was dose-dependent in larval zebrafish Danio rerio when exposed to mercury (Weber, 2006). Walia et al., (2013) noticed fast opercular movement in fish Labeo rohita just after the exposure of tannery industry effluent.

iii. Sluggishness

Normally, fish stay active at all times; if it gets sluggish, it is most likely a symptom that the fish is stressed. Ammonia toxicity, for example, can make aquarium fish drowsy. Sometimes too cold temperature also affects the fish movement**.** Air-breathing fish, *Channa punctatus* exposed to different test chemicals (Mercuric chloride and Malathion) showed abnormal behaviour. In response to the dramatic shift in the surrounding environment, they stopped swimming, became attentive, and remained still in place. After a while, they attempted to avoid the hazardous water by swimming and leaping quickly. As surfacing and gulping of air occurred, faster opercular activity was seen. Finally, fishes lose their equilibrium, get fatigued, lose consciousness, and become sluggish (Pandey *et al*., 2005). Surfacing or gulping of air may occur as a result of a greater oxygen demand following toxicant exposure (Katja *et al*., 2005). Hesni *et al*. (2011) noticed sluggishness in milk fish exposed to lead nitrate and stated that it might be related to energy loss via irregular swimming, leaping, and restlessness. Walia et al. (2013) made similar observations in fish *Labeo rohita* exposed to various concentrations of tannery effluent. Maruthanayagam *et al*. (2002) and Singh and Saxena (2020) reported sluggishness in fresh water fish, *Channa punctatus* exposed to cadmium.

3.2 Morphological changes

i. Discoloration of Skin /change in body colour/ dermal ulceration

The skin, with its scales and mucous, serves as a physical barrier for the fish. However, it is vulnerable to damage caused by handling, fighting, viruses, and environmental toxins, and this damage can result in opportunistic infections. Skin ulceration was the most noteworthy finding in a series of research studies in which striped bass (Morone saxatilis) and striped bass hybrids were subjected to acute confinement stress, despite the fact that fish skin has not been widely explored as a stress response target. (Noga *et al*. 1998; Udomkusonsri et al. 2004). Pandey *et al*. (2005) have reported that when fish *Channa punctatus* was exposed to mercuric chloride and malathion, body pigmentation was decreased. Discoloration of skin, thin chemical deposition on skin, shedding of scales have also been reported by Halappa and David (2009). According to Singh and Saxena (2020), *Channa punctatus*, a freshwater fish, was found to have pigmented areas on its body and discoloured skin after being exposed to the heavy metal cadmium. Body decolouration was also noticed by Kumar et al., (2020) in fresh water fish *Clarias batrachus* exposed to chlopyrifos 50% & cypermethrin 5%EC.

ii. Mucus Secretion

Fish mucus serves as a crucial barrier and interface between fish and their environment, playing a significant role in chemical communication (Beklioglu et al., 2006) and immune defense. Composed primarily of mucins, similar to mammalian mucus (Shephard, 1993), it contains immune molecules like mycosporine-like amino acids (Zamzow, 2007). Stress can alter mucus production and composition, leading to increased susceptibility to infections and indicators of poor health, such as increased secretion (Rosseland and Skogheim, 1984; Youson and Neville, 1987), altered chemical composition, and impaired osmotic balance (Rosseland and Skogheim, 1984; Mueller et al., 1991). Mucus production and its chemical changes are adaptive responses to environmental stressors, such as acid exposure

(Zuchelkowski et al., 1985), and may prevent toxins from entering the body (Bisht and Agarwal, 2007). However, excessive mucus, particularly on the gills, can hinder gas exchange, leading to hypoxia and potential mortality (Maina, 1997). Exposure to toxicants, such as mercuric chloride, malathion (Pandey et al., 2005), and tannery effluent, can induce significant mucus secretion, along with other health issues like scale loosening, eye damage, and hemorrhages (Walia et al., 2013).

4. Mechanisms underlying behavioural and Morphological changes

4.1 Mechanisms underlying behavioural changes

Toxicant exposure in fish damages structural and functional components of the nervous system, including nerve cell bodies, axons, and myelin sheaths, leading to altered neurotransmitter synthesis and release, which in turn causes behavioral changes. Neurotransmitters like serotonin, dopamine, acetylcholine, and GABA influence behaviors such as locomotion, aggression, schooling, and feeding (Smith, 1984). Toxicant-induced rapid swimming is an effort to increase oxygen intake to meet the energy demands of elevated muscular activity, as demonstrated by Katja et al. (2005). Mercury exposure in striped mullet (Mugil cephalus) was linked to lower serotonin levels and a progressive loss of motor function. Both organic compounds and metals can influence neurotransmitters and behavior. For instance, DDT treatment increased spontaneous activity and disrupted schooling in goldfish (Carassius auratus) (Weis and Weis, 1974), while parathion decreased brain serotonin and elevated dopamine levels (McDonald, 1979). In killifish (Fundulus grandis), polychlorinated biphenyls (PCBs) altered brain dopamine and norepinephrine levels, affecting locomotor activity (Fingerman and Russell, 1980). Additionally, rainbow trout (Oncorhynchus mykiss) exposed to carbamate insecticides exhibited altered catecholamine levels (Block and Nilsson, 1990).

4.2 Mechanisms underlying Morphological changes

Heavy mucus exudation and depigmentation are thought to be caused by a disturbance in endocrine dysfunction under environmental pollutant stress, resulting in altered number and area of mucus glands and chromatophores (Pandey et al., 1990). Increased mucous secretion is thought to be an adaptive or defense mechanism used to counteract the effects of toxicants for their survival (Santha et al., 2000 and Sivakumar et al., 2006). Bisht and Agarwal (2007) have suggested that mucous neutralizes the effect of toxicants by coagulation and prevents the entry of toxicants into the body.

5. Conclusion

Environmental protection is the major challenge and requirement of the world. Fish is important in an important dietary and nutritional source since it contains high-quality proteins, vitamins, and minerals that are necessary for life and growth. Fish occupy a higher position in the trophic level and hence, accumulate toxicants at high levels and their resultant implications are easily passed on to man. Fish behavioural and morphological impairments can be considered biomarkers to assess the health of toxicant-affected fish and aquatic ecosystems. A combination of multiple behavioral endpoints can provide more comprehensive and valuable information for discerning the impacts of potential aquatic environmental contaminants. The above studies can be useful in studying the effect of aquatic toxicants on fish to find safe ambient concentrations for both humans and fish that do not cause stress or mortality to fishes.

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