Eco. Env. & Cons. 30 (January Suppl. Issue) : 2024; pp. (S314-S320)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2024.v30i01s.064

Pigment translocation due to pyrene toxicity in *Cyprinus carpio* and *Ctenopharyngodon idella*

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(Received 17 June, 2023; Accepted 19 August, 2023)

ABSTRACT

Pyrene, a higher molecular weight PAHs compound commonly found in aquatic ecosystem and also used as an indicator of PAHs contamination in the monitoring of the wastes. Aquatic bodies are ultimately the sink of all types of wastes which led to severe deteriorating impacts on aquatic flora and fauna. Fish are highly sensitive to the aquatic pollution and show alteration in their behaviour and morphology, also used as bio indicator to the water pollution. Fish skin is directly expose to the surrounding, displaying many alterations such as lesion, loosening and thinning of scales, pigments mobility, higher mucus secretion etc. Therefore, the present study was carried out to investigate the toxicological impacts of pyrene on chromatophore in fish skin of fresh water fish *Cyprinus carpio* and *Ctenopharyngodon idella*. Fish were exposed to sublethal concentration (mg/l), i.e. 0.125, 0.25, 0.5, 1.0 for 5, 10, 15 days. The number of melanophores were significantly different in *C. carpio* ($P<0.05$) while there were not significantly differences were observed in *C. idella* ($P>0.05$). The calculated mean diameter of melanophores from the treated group of *C. idella* were highly significant different ($F=10.035$, $P<0.01$) on the other hand no significantly differences were seen in calculated mean diameter of melanophores from the treated groups of *C. carpio* ($F=1.161$, $P>0.01$). On the basis of structures the melanophores were seen as punctate, stellate, puntostellate and reticulate.

Key words : *Cyprinus carpio, Ctenopharyngodon idella, Melanophores, PAHs, Pyrene,*

Introduction

Rapid industrialization and urbanization causes water pollution that led to negative impacts on aquatic ecosystem. Fish, known for their nutritional value such as protein, vitamins, poly unsaturated fatty acids and minerals are thrilling due to water pollution. As polycyclic aromatic hydrocarbons (PAHs) were categorized into carcinogenicity and mutagenicity by USEPA are released into water bodies are major concern. They cause many several deleterious issues on fish such as bioaccumulation (Sogbanmu *et al.,* 2019), behavioural changes (Bautista *et al.,* 2019), osteoblastic/osteoclastic on scales (Suzuki *et al.,* 2009), developmental malformation (Heuer *et al.,* 2019), skin lesion (Tauchi *et al.,* 2005), Cancer (Rengrajan *et al.,* 2015), Biochemical changes (Dey and Gosh, 2019). Fish are used as bio indicator to assess the anthropogenic contamination in water bodies (Logan, 2007). Scales that provide the shielding or protection of fish skin, are direct contact to the pollutants present in water bodies (Brraich and Jangu, 2014). They are the components of dermal layer and consequently are covered by epidermal layer including chromatophores and provide complete protection of fish skin (Brraich and Jangu, 2014). Some studies are documented that presenting the scales are used as bioindicator for contaminants such as mercury (Rahman *et al.,* 2018), wastes water (Kaur and Dua, 2012; 2015), Chro-

mium (Coban *et al.* 2013), water quality of River Ganga (Khanna *et al.* 2007), Metal contamination (Jovièiæ *et al.,* 2023). In skin, chromatophores are pigment bearing cells, present in reptiles, amphibians, fishes and mammels. Unlike mammals, fish have two types of chromatophores: light absorbing namely melanophores (black and brown), erythrophores (red), xanthophores (yellow), cyanophores (blue) as well as light reflecting viz. leucophore (whitish), iridophores (metallic or iridescent). They have colour bearing pigment organelle, i.e. xanthosomes, luecosomes, cyanosomes, and erythrosomes are inclusively known as chromatosomes (Fujii, 2000). Among them, melanophores are the abundant chromatophores present in fish skin. Dispersion and aggregation of melanin pigment in melanosomes is regulated by two proteins kinesin and dynein respectively (Skold *et al.,* 2002). During dispersion, the pigment granules spread in cytoplasm giving many dendritic projection in cell led to darkning of skin. On contrary, during aggregation the pigment granules are accumulated at the centre giving lightning of the skin. The change in colour patterns in skin is under the control of endocrine and nervous system (Fujii, 2000). The colour changing information is processed in central nervous system and transmitted to the chromatophores, where both hormonal and neuronal regulations displayed different colour patterns (Shingadia, 2022). Chromatophores also undergo for change in cell numbers, pigment content, cell size and pigment dispersion. Pigment dispersion provide the different structure of melanophores such as punctate, stellate and reticulate. Environmental monitoring factors such as light, temperature, salinity, water quality and chemicals/pollutants also led to colour changes which depends on dispersion or aggregation of chromatophores. Any stimuli/stressed cause dispersion or aggregation of pigments resulting darker or lighting of the skin. (Shingadia, 2022; Logan *et al.,* 2007).

On the basis of dispersion and aggregation of the pigment, melanophores give rise to different shape and size such as punctate, stellate and reticulate. In the present study these types of observations are recorded due to exposure of pyrene in *Cyprinus carpio* and *Ctenopharyngodon idella* to understand the pigment translocation and dynamics of melanin in chromatophores. Pyrene is four ring (Higher molecular weight PAHs) compound commonly found in aquatic ecosystem and also used as an indicator of PAHs contamination in the monitoring of the wastes (Zhang *et al.,* 2004).

Materials and Methods

Animal collection and lab acclimatization

Healthy specimens of *C. carpio* and *C. idella* of either sex (weighing 79.33±5.6 g, 21.6±1.5 cm long) were purchased from local fish farm in plastic bag having complete aeration and transferred to the laboratory. Fish were washed with 0.1% $KMno_4$ solution to cure the dermal infection before introducing into experimental set up. Fish were acclimatized in laboratory condition in natural photoperiodic conditions (12 hr. light: 12 hr. night) for 40 days. Water was replenished at alternate day (every 48 hours). Local commercial food was given to the fish once a day. Faecal matter, unconsumed food and dead fish if any are removed by sieving.

Experimental setup

Fish were introduced in (60 X 30 X 30) cm in glass aquaria and divided into four experimental groups each containing 6 fish to analyse the chromatophore rearrangements in sub lethal dose (as 0.125, 0.25, 0.5, 1.0 mg/l). Semi static bioassay test was performed for 5, 10, 15 days. There was no mortality observed during experimental duration. During exposure, mortality and fish behaviour was also monitored.

Behavioural observation

During the experimental study from 5^{th} to 15^{th} days no fish mortality was observed. Fish were regularly monitored for their morphology and behavioural alteration. Fish were noticed for some behavioural and morphological changes such as roughness of skin, swimming and lethargy activity. After adding 0.125 mg/l, 50% fish start to move in left and right. On contrary, no avoidance behaviour was seen. It is observed that mucus secretion increased significantly as concentration increase. At highest concentration 1 mg/l (1^{st} group), the maximum mucus secretion and lesion/roughness of skin was also observed.

Dermal pigment studies

The scales were removed at different time interval at 5^{th} day, 10^{th} day and 15^{th} day of test exposure concentration as well as control group. Scales were removed with the help of forceps from above and second row of lateral line, behind the operculum (Kaur

and Dua, 2012). The mucus is cleaned by the help of fine brush and dried. The processed scale was placed on glass slides as dorsal side upward with a drop of water, then covered with glass cover slip. The microphotograph was taken under digital microscope (LX model Evos) at 20x magnification.

Microphotograph Analysis

The analysis of microphotograph of the both fish scales were done by Image J (an open access software). Different parameters such as area, length of dendrites, size and numbers of melanophores were measured by different measuring tools in Image J. The numbers of melanophores were counted by watershed selection within binary function tool. The measurement of the size or length of the melanophores were done by line selection tool bar after setting the known scale distance (200 μm) as set scale function within analyse toolbar.

Statistical Analysis

The number of chromatophores were counted by Image J was subjected to student t-test ($P<0.05$) for determinining the of effects of pyrene in comparatively three experimental duration (5^{th}, 10^{th} and 15^{th} day) for each test groups to both types of fish scale to assess the impact of pyrene on chromatophores reorganisation. The calculated mean size of melanophores obtained from all treated groups were subjected to one way ANOVA ($P\leq0.01$) to compare significance differences in control and treated groups.

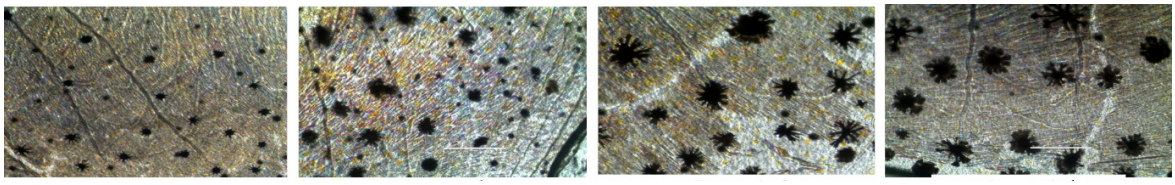
Results and Discussion

Significant changes were observed in morphology of melanophores in *C. carpio* in the experimental groups of the pyrene exposure duration (5^{th} to 10^{th} day, 10^{th} to 15^{th} day). The numbers of melanophores were significantly different in *C. carpio* ($P<0.05$) while there were not significantly difference were observed in *C. idella* ($P>0.05$). The calculated mean diameter of melanophores from the treated group of *C. idella* were highly significant different ($F=10.035$, $P>0.01$) on the other hand no significantly differences were seen in calculated mean diameter of melanophores from the treated groups of *C.carpio* ($F=1.161$, $P<0.01$). As concentration increases, the number of chromatophores were decreases and size of chromatophores were increases. Both types of fish exposed to pyrene presented dispersion of melanin granules into dendritic projections in concentration dependant manner. On 5^{th} day of exposure, at low concentration group (0.125mg/l), the size of chromatophores were start to increase while the numbers of chromatophores were decrease in both types of fish as compare to control Fig. 1. Control group of *C. carpio* showing punctate and puntostellate (small round structure with 5-7 unbranched dendritic process) Fig. 1 (a). At the smallest concentration (0.125 mg/l) 4th group, both types of chromatophores (punctate and puntostallate) were observed but relatively larger in size Fig. 1(b). In group 3^{rd} (0.25 mg/l) punctate and stellate chromatophores were detected and size of dendritic process of melanophores in this group were comparatively larger than group 2^{nd} (0.5 mg/l) Fig. 1 (c) (d) Table 1. In group 2^{nd} stellate chromatophore were measured but minute punctate types of chromatophores were also observed Fig. 1 (d). The size of chromatophores were greater than 3^{rd} group and lesser than 1^{st} group Table 1. At the highest concentration having group (1.0 mg/l), reticulate chromatophores were seen as well as the diameter of chromatophore in this group was greatest than other groups (Table 1). After 10^{th} day of exposure, the size of chromatophores were increasing with concentration Fig. 2. In 4^{th} group having same types of chromatophores (punctate and puntostallate) similarly as 4^{th} group after 5^{th} day of exposure. Surprisingly, in 3^{rd} group, punctate chromatophores are seen larger than 5^{th} day of exposure and seem like start to moving from punctate to puntostate Fig. 2 (b). In 2^{nd} group, the number of chromatophores were comparatively higher than the 1^{st} group and also have stellate types of chro-

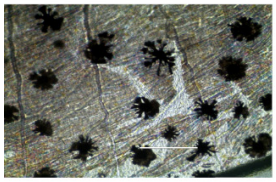
Table 1. Size of melanophores in *Cyprinus carpio* in control as well as exposure groups during 5^{th} to 15^{th} days of experiment

S. No.	Control group	Group 4^{th} (0.125 mg/l)	Group 3^{rd} (0.25 mg/l)	Group 2^{nd} (0.5 mgl)	Group 1^{st} (1.0 mg/l)
5^{th} day	52.178±26.28	171.298±19.191	190.9±29.972	125.017±36.718	202.11±32.719
10^{th} day	33.472±35.30	116.148±1.41	152.45±18.76	158.819±30.343	180.909±22.398
15^{th} day	51.00±17.68	102.223±49.091	117.525±24.96	160.1±78.741	188.908±31.511

matophores. In 1 mg/l exposure group (1st group), reticulostellate types branching were seen Fig. 2 (d). On 15th day of exposure, 4th and 3rd groups showed the same results as 10th day but in 2nd group in addition of reticulate few punctate types of chromatophores were also found Fig. 3 (c). At the last of experiment, in the highest concentration group, only reticulostellate types of chromatophores were seen. On contrary, the dispersion, number and types of chromatophores into control group in *C. idella* Fig. 4 K, are completely different from *C. carpio*, only punctate types of chromatophores were observed and the number of chromatophores were very less as *C. Carpio* (Table 2). After 5th day of exposure, at the smallest concentration group Fig. 4th a group (0.125 mg/l) in *C. idella*, the number of chromatophore were significantly increased while size of chromatophore were decreased Table 2, Fig. 4 (a). One thing is also noticed here that in control group as well as 4th experimental group only punctate type chromatophore were observed but as concentration in increased from 0.125 mg/l to 0.25 mg/l the branching of chromatophores were start and shape will be change from punctate to stellate Fig. 4 a, b. Further, the size of chromatophores were continuously increased and it will converted to punctate, stellate and reticulate in 3rd, 2nd and 1st group respectively Fig. 4 b, c, d. On 10th day of experimental observation, no punctate but puctosttate types of chromatophores were seen in 4th group Fig. 5 a. Similarly, in 3rd group b, the puctosttate types of chromatophores were found. In group (c), very less as well as stellate type chromatophore were observed. At the last group 1st group (d), the breakage of chro-

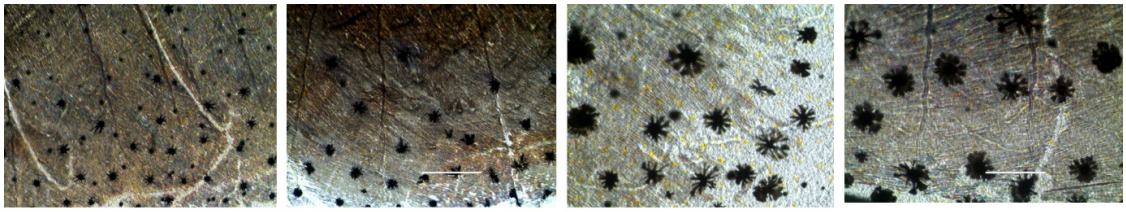


Control K (*Cyprinus carpio*) Group 4th a. (C) Group 3rd b. (C) Group 2nd c. (C)



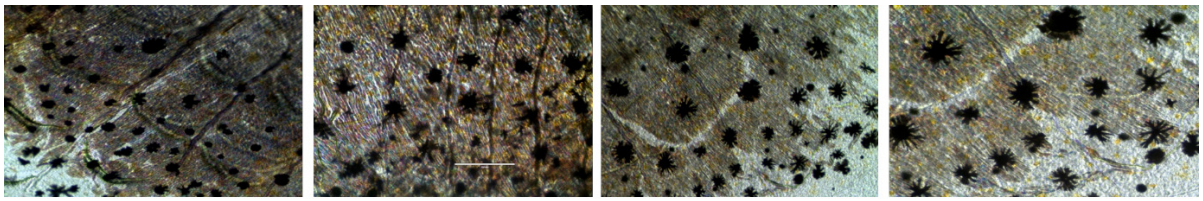
Group 1st d. (C)

Fig. 1. 5th day of pyrene exposure on *Cyprinus carpio*in all four groups



4th Group a. (C) 3rd Group b. (C) 2nd Group c. (C) 1st Group d. (C)

Fig. 2. 10th day of pyrene exposure on *Cyprinus carpio*in all four groups



4th Group a. (C) 3rd Group b. (C) 2nd Group c. (C) 3rd Group b. (C)

Fig. 3. 15th day of pyrene exposure on *Cyprinus carpio*in all four groups

Table 2. Size of melanophores in *Ctenopharyngodon idella* in control as well as exposure groups during 5th to 15th days of experiment

S. No.	Control group	Group 4th (0.125 mg/l)	Group 3rd (0.25 mgl)	Group 2nd (0.5 mg/l)	Group 1st (1.0 mg/l)
5th day	101.917±77.578	97.304±6.271	158.605±23.527	181.905±43.168	221.88±34.918
10th day	99.980±8.950	156.37±18.186	215.048±73.997	206.152±58.937	219.325±24.869
15th day	89.450±9.097	171.387±39.176	148.558±35.675	185.494±14.738	231.35±94.499

matophore were seen and size were comparatively greater than other groups Fig 4. At the completion of experiment on 15th day, in 4th group dense with punctate type of chromatophore were seen Fig. 6 (a). Furthermore, in 3rd group (b) the density is more or less same but few small projection of dendrites in chromatophore were observed. In group (c) punctate but few stellate types of chromatophores were seen. At the last of the experiment, in group (b) only reticulate type of chromatophores were noticed Fig 6 (d). Very few reports are available on melanophores related to scales. Although, apart from the scale chromatophores, some authors performed their experiments on transcriptome sequencing on pigmentation of scale and skin in common carp (*C. carpio*) (Zhao *et al.*, 2021), metal contamination on fish scale in Danube River (Jovièiæ *et al*., 2023), Dichromatic color variation in *Pseudochromis diadema* (Goda *et al.* 2011), Cyprinid herpesvirus 3 binding to epidermal cells of *C. carpio* (Raj *et al.*, 2011), com-

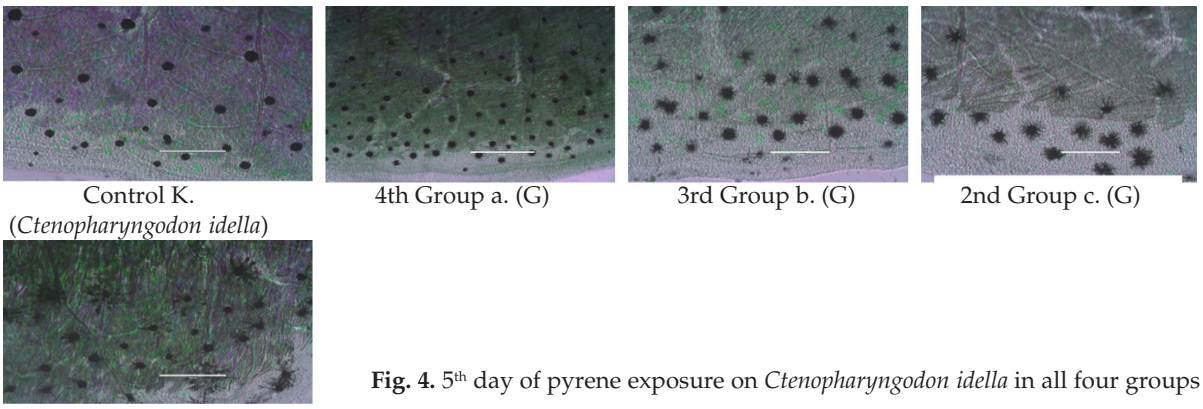


Fig. 4. 5th day of pyrene exposure on *Ctenopharyngodon idella* in all four groups

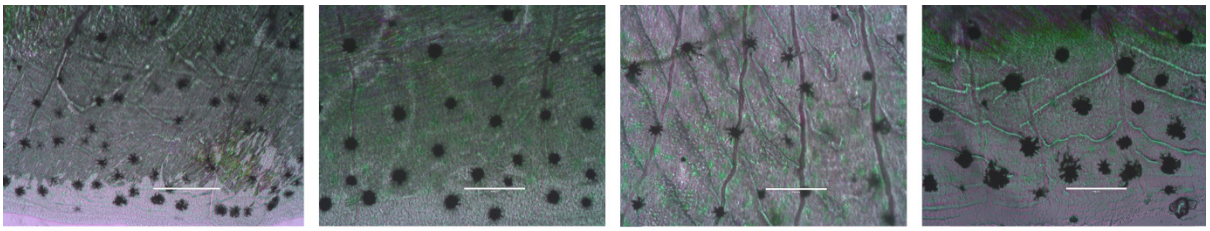


Fig. 5. 10th day of pyrene exposure on *Ctenopharyngodon idella* in all four groups

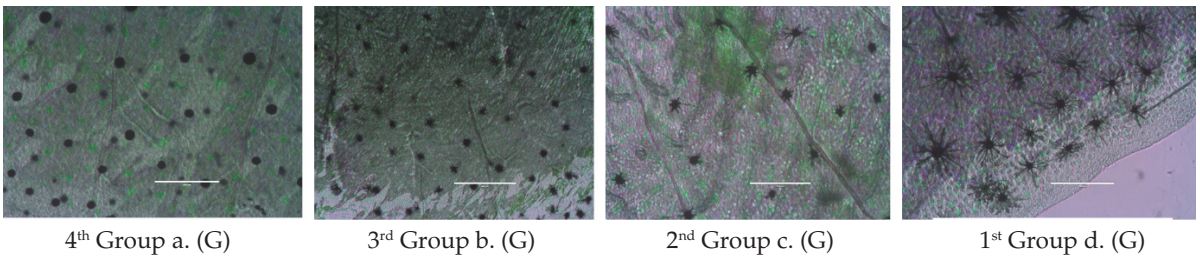


Fig. 6. 15th day of pyrene exposure on *Ctenopharyngodon idella* in all four groups

parative skin transcriptome of two Oujiang color common carp (*C. carpio* var. *color*) (Du *et al.*, 2019), scale morphology in *C. Carpio* (Coban *et al.*, 2016). Acute exposure of pyrene on *C. carpio* and *C. idella* altered the shape, size and structure of melanophores due to melanin dispersion in melanophores. After exposure of pyrene, alteration in melanophores were found similarly studied by Bajpai and Tripathi, 2012. There were no severe differences observed in high sub-lethal concentration of pyrene as studied fenthion exposure on *C. carpio* whereas shape, size and numbers of melanophores were same as Murlidharan and Pillai, 2012. After 10th day of exposure, in 3rd group (0.25 mg/l) of *C. carpio*, breakage of melanophores were seen similarly as on exposure concentration (0.38 mg/l) of fenthion on *C. Carpio* (Murlidharan and Pillai, 2012).

Conclusion

From the observed alterations in chromatophores of both fish (*C. carpio, C. idella*), it is clear that after exposing of pyrene fish scale can be successfully governed as dispersion and aggregation of melanophores is mediated by endocrine system. It is very much evident that findings of present study is much similar as other previous workers. Therefore, present findings indicate the pigment translocation in chromatophores due to pyrene toxicity and to understand the pigment translocation and dynamics of melanin in chromatophores.

Acknowledgement

Authors are very thankful to UGC for provide financial support to perform this experiment. Furthermore, they would like to thanks Department of Zoology, Babasaheb Bhimrao Ambedkar, University to provide instrumentation facilities for present work.

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