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ORIGINAL RESEARCH ARTICLE

Behavioural and Respiratory Responses of the Mozambique Tilapia (*Oreochromis mossambicus*) under Sublethal 17α-Ethynylestradiol Exposure

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ABSTRACT

Mozambique tilapia (*Oreochromis mossambicus*; fingerlings and adult) was exposed to the sublethal concentration (30 ng/l) of estrogenic endocrine disrupter (17α-ethynylestradiol) for 14 d and allowed to recover in water free of test substance for 7 days. Behavioral patterns and respiratory responses were observed in sublethal concentration (on 7, 14 and R7 d). Some of the common behavioural changes exhibited by fingerlings in toxic media include disrupted shoaling behaviour, localization of fish to the bottom of the test chamber, independency (spreading out) in swimming, lethargic, restless, secreted slight mucus, and avoidance behaviour. No significant alteration in behaviour of adult fish (except slight mucus secretion on the body) was observed during the test in control and treated fish. The respiratory rate of fingerlings and adult fish in sublethal concentration of 30 ng 17α-ethynylestradiol/l were found in the order of 7 > 14 < R7 and 7 < 14 > R7, respectively. No significant effect of 17α-ethynylestradiol on behaviour and respiratory rate in adult fish might be due to effective detoxification process as compared to the fingerlings. The present study evidenced that 17α-ethynylestradiol had profound impact on the behaviour and respiratory rate in sublethal concentration to fingerlings. This clearly suggests that the 17α-ethynylestradiol undoubtedly affect the sensitive life stages of fish. Deviation in respiratory rate is due to impaired oxidative metabolism and altered respiratory physiological response to 17α-ethynylestradiol stress in the fingerlings and adult fish.

Key words: *Oreochromis mossambicus*, Estrogen, Behaviour, Oxygen consumption.

1. INTRODUCTION

Documentation of pharmaceuticals and personal care products in surface waters has stimulated diverse research examining their environmental impacts [1]. In aquatic environments, endocrine disrupting chemicals (EDCs) that interfere with the endocrine system of males and females form a threat to the maintenance of populations. EDCs are a diverse group of natural and synthetic chemicals that already at very low concentrations (at nanogram levels) can have severe effects on reproduction by individuals, e.g. complete sex reversal, feminisation of males, impaired reproduction even resulting in near extinction of populations.

The potential environmental effects of the synthetic hormone, 17α-ethynylestradiol, used in family planning and hormone replacement therapy, have been the subject of numerous investigations. It has been demonstrated that chronic sublethal exposure to 17α-ethynylestradiol at an environmentally relevant concentration caused the collapse of a fish population [2]. In contrast to the wealth of information about effects of exogenous estrogen exposure on fish reproduction, very fewer studies focused about effects on non-reproductive processes like behaviour and respiratory rate.

EDCs can act as hormone agonists or antagonists. Since sex steroids influence the control of sexual and agonistic behaviour. Moreover, an animal can adjust to changes in the environment with its behaviour, and therefore behaviour (particularly in sexually) of matured fish potentially provides a sensitive early warning signal for the presence of EDCs.

Hormones also affect behaviour indirectly, e.g. through metabolism [3]. Short-term toxicity assays generally ignore the fact that if an animal's

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normal behaviour is altered, it may be unable to function in an ecological context. Thus, behaviour can provide direct information both at the individual and at the population level, and therefore be used as a comprehensive, sensitive and non-invasive measure of exposure to EDCs. Fish with complex reproductive behaviours are particularly at risk to EDCs, as complex behaviours require more coordination [4]. The most intricate reproductive behaviours may be found among fishes with parental care. Among teleost families, 21% of fish population exhibit parental care [5] and most of these fish are small in size and breed in freshwater, estuaries and coastal marine environments, where the risk of EDC contamination is the highest. In theory, any external force that alters the behaviour of individuals away from what is optimal under natural and sexual selection could lead to reduced population sizes [6].

Any change in the behaviour and physiology of fish indicates the deterioration of water quality, as fish are biological indicators. The Mozambique tilapia (*O. mossambicus*), is a very important staple fish generally found in ponds, lakes and sewage fed water bodies. Therapeutically, 17α-ethynylestradiol is extensively used in birth control and hormone replacement treatment. Hence the current study was undertaken to evaluate the effects of 17α-ethynylestradiol with special emphasis on behavioural responses and respiratory performance of the fingerlings and adult tilapia exposed to sublethal concentration (30 ng 17α-ethynylestradiol/l) of xenoestrogen.

2. MATERIALS AND METHODS

2.1 Collection and maintenance of fish

Healthy and active, *O. mossambicus* fish were collected from the Local Fisherman, Tumkur, Karnataka, India. Fish were collected from the pollution free ponds using cast nets (to reduce stress and mechanical damage). Fish were brought to the laboratory in large aerated stainless steel 316 crates. They were held in the laboratory area in large cement tanks (20 ft × 10 ft × 4 ft) for breeding and fed *ad libitum* daily using oil cake and rice bran.

Fingerlings and adult fish, *O. mossambicus* which were bred from healthy and active tilapia are considered for this study. Fish were held in glass aquaria containing reverse osmosis water of the quality used in the test for 7 days during acclimatization. Physico-chemical characteristics of reverse osmosis water were analyzed following the methods in APHA [7] and found to be as follows: temperature 25 ± 2 °C, pH 7.0 ± 0.2, dissolved oxygen 9.0 ± 0.3 mg/l, carbon dioxide 1.3 ± 0.1 mg/l, total hardness 123.4 ± 3.4 mg/l as $CaCO_3$, phosphate 0.39 ± 0.002 µg/l, salinity 0.3 ppm, specific gravity 1.001 and conductivity less than 10 µS/cm. Water was renewed every day and a 12 h light and 12 h dark cycle was maintained during acclimatization and test periods. The fish were fed *ad libitum* daily once with commercial food pellets (Kijaro Grow) during acclimatization and test tenures.

17α-ethynylestradiol (≥ 98%) was obtained from the Sigma-Aldrich®, US. The expiry date of the test substance checked prior to initiation of the treatment was found to be suitable for the exposure. Stock solution was made using absolute alcohol and further diluted with distilled water. The secondary stock solution thus prepared was used for preparation of test solution.

The fingerlings (7.0 ± 0.5 g; 5.0 ± 0.3 cm in total length) were exposed to the sublethal concentration of 17α-ethynylestradiol (30 ng/l) in a batch of maximum of 10 fish using glass aquaria of 80 l capacity (150 cm × 30 cm × 50 cm) in 4 replicates (for behaviour and respiratory assessment) in a group along with the control group. Another set of adult fish (40.0 ± 3.5 g; 9.0 ± 1.1 cm) were also exposed to the sublethal concentration of 17α-ethynylestradiol (30 ng/l) in a batch of maximum of 10 fish using glass aquaria of 500 l (350 cm × 150 cm × 250 cm) capacity in 4 replicates in a group along with the control group. Maximum loading (biomass) didn't exceeded 1.0 g wet weight of fish/L during the present study. Replacement of the water medium was followed by the addition of the desired dose of the test compound.

2.2 Exposure and observation periods

Environmentally relevant concentration of 30 ng 17α-ethynylestradiol/l was selected as sublethal concentration. Since, mortality was not observed in either fingerlings or in adult fish at 30 ng 17α-ethynylestradiol/l rendered us to consider this concentration as sublethal concentration.

Behavioral patterns and respiratory responses in fish were studied in sublethal concentration (30 ng 17α-ethynylestradiol/l) at 7 and 14 days. After 14 days of exposure fish were allowed to recover for 7 days in water free of test substance (designated as R7). The control (toxicant free medium) and 17α-ethynylestradiol exposed fish were kept under

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close observation at periodic intervals during the test.

2.3 Assay of respiratory rate

Respiratory rate measurements were made by the method of Welsh and Smith as described by Sameer and David [8]. The dissolved oxygen in test medium (water samples) was estimated adopting Winkler's iodometric method. The respiratory measurements were made in diffused daylight and the time of the experiment was kept constant (12.00 pm to 3.30 pm) to nullify the influence of time on respiratory process of the fish. The oxygen consumed by the fish is expressed as ml of oxygen consumed/g wet weight of fish/h.

At the end of exposure and recovery periods (7, 14 and R7 d), each fish was transferred from the aquaria to a respiratory chamber of 8 L capacity. The fish were allowed to stabilize for 5 min and then the experiment was run for a period of 1 h. After the experiment, the fish were replaced into their respective test chambers. Controls were also run simultaneously to obtain information on the oxygen consumption of the fish in the normal state. Fish were blotted dry and weighed to the nearest mg to calculate the metabolic rate on the day of assessment of respiration.

2.4 Statistical analysis

Data correspond to the average of three replicates. The data obtained were analyzed statistically using Graph Pad Prism Software (Version No. 5).

3. RESULTS AND DISCUSSION

3.1 17α-Ethynylestradiol toxicity

No mortality was observed during the test in control and treated groups (fingerlings or adult *O. mossambicus* in sublethal concentration of 30 ng 17α-ethynylestradiol/l).

3.2 Fingerlings behaviour

Experiments were carried to assess the behaviour of tilapia without any toxicant and with one sublethal concentration of 17α-ethynylestradiol. 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 behaviour 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. In toxic media, tilapia fingerlings were normal in their behaviour for the first five days of exposure to sublethal concentration of 30 ng 17α-ethynylestradiol/l. On day 6 fish exhibited disrupted shoaling behaviour, localization to the bottom of the test chamber, and independency (spreading out) in swimming. The above symptoms followed the loss of co-ordination among individuals and occupancy of twice the area of that of the control group were the early symptoms of 17α-ethynylestradiol exposure to 30 ng/l. Subsequently, tilapia fingerlings moved to the corners of the test chambers, which can be viewed as avoidance behaviour of the fish to the sublethal concentration of 30 ng 17α-ethynylestradiol/l. The fish slightly became lethargic, restless, and secreted mucus all over the body on day 6 up to day 14. An excess secretion of mucus in fish is a non-specific response against toxicants, forming a barrier between the body and the toxic medium, so as to minimize its irritating effect, or to scavenge it through epidermal mucus. Similar observations were made by Rao [9] following RPR-V exposure to euryhaline fish, *Oreochromis mossambicus*.

Leaning of tilapia fingerlings indicates reduced feeding behaviour and diversion of fish metabolism towards adaptability to the toxic media. Feeding preferences were affected and consumption of food in fish was impaired and reduced drastically. This was pronounced on day 12 of exposure and recovery in feeding was observed remarkably during expositions period for 7 days. For these animals, it might be profitable to decrease their food uptake under toxic environmental conditions to lower the energetic costs of digestion. Depression in appetite is a common response of fish to stress and intermittence of feeding for longer periods can have a clear impact on growth and reproduction [10]. A substantial growth reduction caused by 17α-ethynylestradiol has important implications for survival of fish as observed in the present study (at sublethal concentration of 30 ng 17α-ethynylestradiol/l).

The control fish were active for feeding and alert to slightest of the disturbance with their well-synchronized movements during the present study. The behaviour did not significantly vary between the control groups; therefore, these results were taken as standards for the entire experimentation.

3.3 Adult fish behaviour

No significant alteration in behaviour of adult fish (except slight mucus secretion on the body) was observed during the test in control and treated groups at the sublethal concentration of 30 ng 17α-ethynylestradiol/l. This might be due to the capacity of adult fish to detoxify the sublethal

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concentration of 30 ng 17α-ethynylestradiol/l by the way of effective detoxification process.

3.4 Respiratory toxicity of 17α-ethynylestradiol

Alteration in the respiratory rate is an indicator of stress, which is frequently used to evaluate the changes in metabolism under environmental deterioration. It is evident from the results that 17α-ethynylestradiol affected respiratory rate (in adults and fingerlings) of *O. mossambius* under sublethal concentration of 30 ng 17α-ethynylestradiol/l (**Table 1**). Studies on respiratory rate in fingerlings depicted time dependency. In fingerlings, the respiratory rate recorded decrease on day 7 and 14, but on seventh day of recovery there is an increase as compared to control (12.1%). Adult fish exposed showed decrease of respiratory rate on day 7. But on subsequent test periods adult fish recorded elevation in the respiratory rate, recording a maximum of 7.5% on day 14 of exposure. The respiratory rate of fingerlings and adult fish in sublethal concentration of 30 ng 17α-ethynylestradiol/l were found in the order of 7 > 14 < R7 and 7 < 14 > R7, respectively. Similar alterations in respiratory rate were made by Sameer and David [8] by exposing common carp to quinalphos.

Table 1: Respiratory Rate (ml of oxygen consumed/g wet wt. of fish/h) of the *O. mossambicus* Fingerlings and Adults Exposed to Sublethal Concentration of 17α-Ethynylestradiol

Experimental Periods	Respiratory Rate		% Change
	Control	Treatment	
Tilapia Fingerlings			
Day-7	0.129 ± 0.009	0.108 ± 0.004	-16.3
Day-14	0.134 ± 0.010	0.099 ± 0.007	-26.1
Post-exposure (R7)	0.132 ± 0.005	0.148 ± 0.005	12.1
Adult Tilapia			
Day-7	0.149 ± 0.005	0.142 ± 0.006	-4.7
Day-14	0.146 ± 0.008	0.157 ± 0.003	7.5
Post-exposure (R7)	0.151 ± 0.010	0.155 ± 0.009	2.6

Data are mean ± SD (n = 3) for a respiratory rate in a column are not statistically significantly different ($p < 0.05$) as compared with their respective controls.

4. CONCLUSION

This study evidenced that 17α-ethynylestradiol had a profound impact on the behaviour and respiratory rate in sublethal concentration to fingerlings. This clearly suggests that the 17α-ethynylestradiol undoubtedly affect the sensitive life stages of fish. Deviation in the respiratory rate is due to impaired oxidative metabolism and altered respiratory physiological response to 17α-ethynylestradiol stress in the fingerlings and adult fish.

CONFLICT OF INTEREST STATEMENT

We declare that we don’t have any sort of conflict of interest.

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