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Influence of cadmium in the testicular recrudescence of fresh water teleost, *Rasbora dandia*

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ABSTRACT

Heavy metals are one of the major pollutants influencing aquatic ecosystems. Toxic heavy metals often interfere with the normal physiological processes of organisms in various levels. Cadmium is a heavy metal with no known biological function, raising severe threat to the environment. In the present study influence of cadmium on the testicular recrudescence of *Rasbora dandia* is evaluated. *R. dandia* were exposed to three sublethal doses of cadmium (0.1 ppm, 0.2 ppm and 0.3 ppm) from February to May during which active gonad development take place before the onset of breeding season associated with south west monsoon in Kerala, India. At the end of the exposure period, control fishes became sexually mature with abundant ripe spermatozoa in the tubular lumen of the testis. Initial stages of spermatogenesis were prominent in cadmium treated fishes indicating that the testes were still in the recrudescence condition. However, reduction in ripe spermatozoa was observed in cadmium exposed fishes. GSI was found to be declined significantly by increasing concentrations of cadmium exposure during gonadal recrudescence.

Key words- Gonadal recrudescence, cadmium, testis, *Rasbora dandia*

INTRODUCTION

Heavy metals are one of the major pollutants influencing aquatic ecosystems. Due to their numerous applications in various human activities, large amount of heavy metals are often entering the environment along with the release from natural phenomenon like volcanic eruptions and weathering (Hong *et al.,* 1996; He *et al.,* 2005; Duruibe *et al.,* 2007; Tchounwou *et al.,* 2012). As they are not degraded, released heavy metals often persist in these eco-systems, making them vicious environmental contaminants. Toxic heavy metals often interfere with the normal physiological processes of organisms in various levels. Cadmium is a heavy metal with no known biological function, raising severe threat to the environment. Despite of the fact that reproduction is a sensitive endpoint, a few comprehensive studies are available regarding the impact of cadmium on fish reproduction (McGeer *et al*., 2012). Ecotoxicology of heavy metals are an area of substantial scientific discovery, while reproductive toxicity deserves significant concern.

Gonadal recrudescence is the revival of regressed gonad into sexually ripe condition ready to undergo breeding. Gonadosomatic index will be elevated considerably in both sexes of fishes after recrudescence (Kime, 1998). Gonadal recrudescence is controlled by environmental factors as well as internal physiological mechanisms like endocrine regulation. Reproduction being a sensitive biological function, various environmental contaminants may disrupt gonadal recrudescence of fishes. Xenobiotics can act directly by toxic effects on gametes as well as indirectly through endocrine disruption or influencing organs like liver or pituitary which involve in reproductive processes (Kime, 1999; Goksøyr, 2006; Scholz and Klüver, 2009).

Rasbora dandia is a cyprinid fish widely distributed in Sri Lanka and southern states of India occurring in rice fields, streams, rivers and reservoirs (Silva *et al.,* 2010). It is a seasonally breeding fish. Gonads start maturing during the summer season. In the natural climatic conditions of Kerala, *R. dandia* complete gonad maturation and become ripe by May and breeding start by the onset of south west monsoon in June. The present study evaluates the influence of cadmium in the testicular recrudescence of *R. dandia.*

METHODOLOGY

Test organism

R. dandia were collected from freshwater bodies of Thrissur, Kerala. These fishes were transported to the laboratory carefully and acclimatized to the laboratory conditions. Fishes were maintained in well water (pH-6.8, dissolved oxygen-6.8 ppm and hardness-36.93 ppm) and fed daily by standard fish feed.

Exposure procedure

Cadmium chloride is used as the source of cadmium exposure. LC_{50} value of cadmium chloride for *R. dandia* was previously reported to be 16.91 ppm (Rajan and Kuzhivelil, 2015). Fishes of sizes used by the earlier experiments of Rajan and Kuzhivelil (2015) were divided into four equal groups, where one of them was maintained as a control group with untreated fishes. Three sublethal doses of cadmium (0.1 ppm, 0.2 ppm and 0.3 ppm) were selected and one experimental group is exposed to each dose. Test solutions were renewed every 24 hours. The exposure period spanned from February to May during which active gonad development take place before the onset of breeding season associated with south west monsoon.

Histological preparation

Fishes were anaesthetised and sacrificed at the end of exposure period. Testes were dissected out to fix in 10% formalin. They were gradually dehydrated using varying concentrations of ethanol and cleared in xylene. Specimens were paraffin infiltrated and embedded in paraffin blocks. Sections of 1-3 μm thickness were taken in a rotary microtome and double stained using haematoxylin and eosin. Stained sections were observed under light microscope.

Calculation of gonadosomatic index

Gonadosomatic index (GSI) was calculated using the method followed by Robinson *et al*., (2007). Significance of GSI was analysed using one way analysis of variance (ANOVA) followed by Tukey's test using SPSS 16.0 software.

RESULTS

At the end of the exposure period in May, control fishes became sexually mature with abundant ripe spermatozoa in the tubular lumen of the testis. Earlier cell stages of spermatogenesis were not evident in control fishes, other than small patches of spermatids seen infrequently (Figure 1).

0.1 ppm cadmium treated fishes exhibited large and numerous patches of spermatids compared to control fishes. Bundles of ripe spermatozoa appeared less dense in 0.1 ppm cadmium treated fishes (Figure 2). Among 0.2 ppm cadmium treated fishes initial stages of spermatogenesis like spermatocytes were very prominent with further reduction in ripe spermatozoa (Figure 3). 0.3 ppm cadmium treated fishes appeared in very early stages of gonadal recrud-escence with limited presence of sperm bundles (Figure 4). Cadmium treated fishes exhibited lowered gonado-somatic index compared to control fishes (Table 1).

Table 1. Effect of cadmium exposure on GSI of male *R. dandia* (Mean ± SD). Values with different superscript letters vary significantly between exposure groups (($p<0.05$; One way-ANOVA, Tukey's post-hoc test)

Group of fishes	Control	0.1 ppm of cadmium	0.2 ppm of cadmium	0.3 ppm of cadmium
Gonadosomatic index	5.83 ± 0.24^{a}	2.58 ± 0.15^{b}	2.06 ± 0.10^{c}	1.54 ± 0.10^{d}

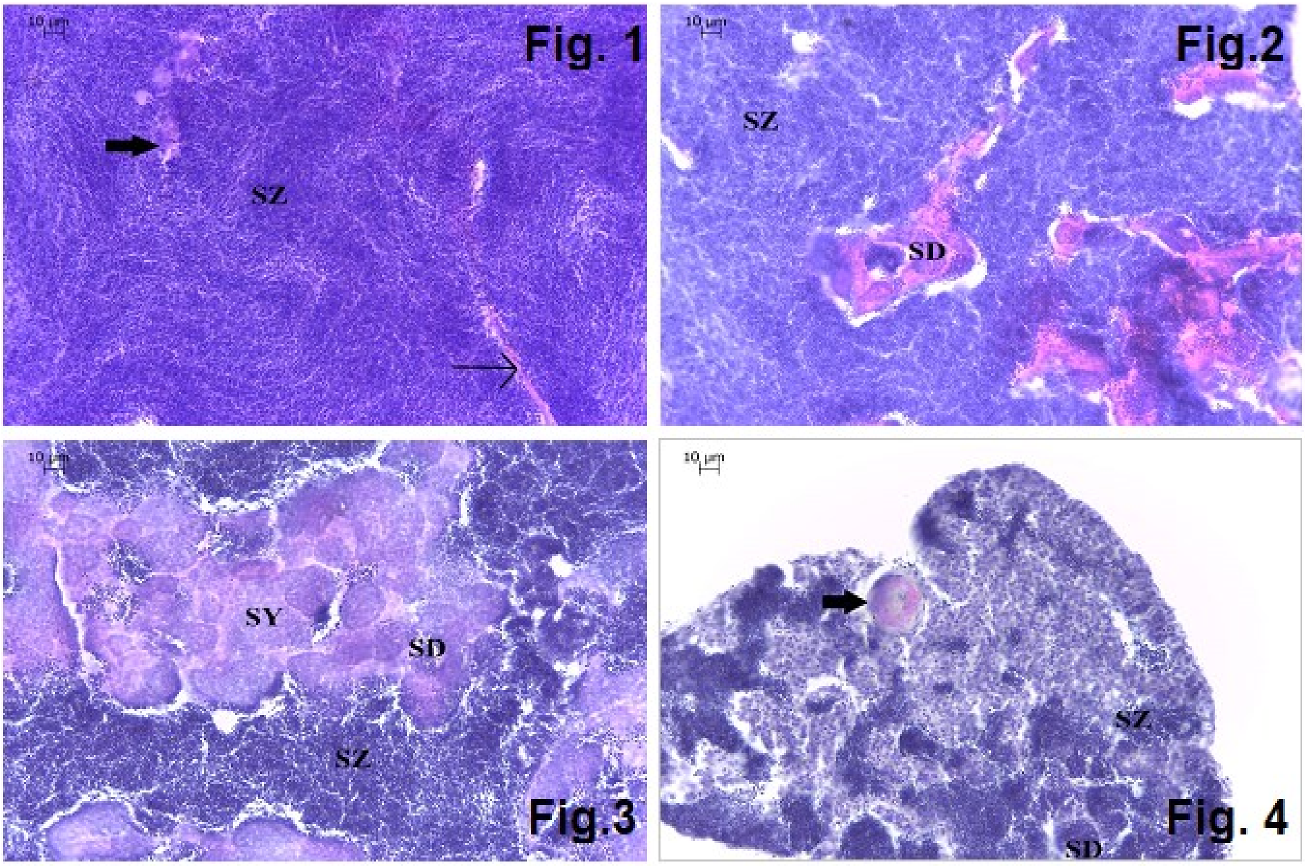

Figure 1: Histological section of testis of *R. dandia* (Control), (H&E; x 400); Spermatozoa (SZ), Spermatids ([→]), interstitial tissue (→); Tubules filled with abundant spermatozoa.

Figure 2: Histological section of testis of *R. dandia* exposed to 0.1 ppm cadmium during gonadal recrudescence, (H&E; x 400); Spermatozoa (SZ), spermatids (SD); Patches spermatids prominent than control testis.

Figure 3: Histological section of testis of *R. dandia* exposed to 0.2 ppm cadmium during gonadal recrudescence, (H&E; x 400); Spermatozoa (SZ), spermatids (SD), spermatocytes (SY); Initial stages of spermatogenesis is prominent.

Figure 4: Histological section of testis of *Rasbora dandia* exposed to 0.3 ppm cadmium during gonadal recrudescence, (H&E; x 400); Spermatozoa (SZ), spermatids (SD); Limited presence of sperm bundles indicating early stage of recrudescence.

DISCUSSION

Gonad maturation is an important phase in the life cycle of fishes as it involves intensive physiological changes leading to spawning with ripe gametes. Environmental toxins like heavy metals have profound influence in altering normal physiological mechanisms. Maintaining homeostasis under toxicant stress is an energetically expensive process demanding high mobilisation of resources (Giesy and Graney, 1989). Survival in polluted ecosystems costs reallocation of energy from other physiological processes resulting in diminished growth rate and reproductive potential (Wendelaar Bonga, 1997; Beyers *et al*., 1999 and Marchand *et al*., 2004).

In the present study, GSI is measured as it is a useful endpoint to evaluate reproductive toxicity of chemicals in fishes (Segner, 2011). Robinson *et al*., (2007) observed that the exposure of 669 ng L^{-1} of 17 β-oestradiol affected testicular development and reduced GSI in the male sand goby, *Pomatoschistus minutus*. Kirubagaran and Joy (1992) observed reduced GSI in male catfish, *Clarias batrachus* exposed to mercuric chloride during preparatory phase of annual reproductive cycle. In the present study GSI was found to be declined significantly in male *Rasbora dandia* by increasing concentrations of cadmium exposure during gonadal recrudescence (Table 1).

According to Kime (1999) it is often unclear whether decreased GSI under xenobiotic influence is attributed to the direct effect on gonads or the result of deficiency in pituitary hormone secretion. Pituitary gland plays critical role in fish reproduction through the secretion of gonadotropins which induce steroid hormones synthesis and stimulate gonadal development (Liley and Stacey, 1983). Gonadotropin secreting cells of pituitary showed inactivation and accumulation of secretary products in catfish, *Clarias batrachus* under cadmium exposure (Jadhao *et al.,* 1994). Apart from the direct influence on gonadal physiology, xenobiotic effects on fish reproduction may be mediated through other components of reproductive system.

Mani and Saxena (1985) studied the effects of carbofuran and fenitrothion on the testicular recrudescence of *Channa punctatus*. Both pesticides delayed the formation of spermatids and sperm as well as caused necrosis in the testes. Ram and Sathyanesan (1983) exposed *Channa punctatus* to mercuric chloride during gonad recrudescence and observed that the testes of exposed fishes were filled with secondary spermatogonial cells, while control fishes exhibited active spermatogenesis with mature sperm. In the present study, spermatogonial cells were prominent in cadmium treated fishes indicating that the testes were still in the recrudescence condition (Figure 2 and 3). Seasonal breeding in fishes is a strategy to synchronize reproductive efforts to periods ideal for juvenile survival and growth (Winemiller, 1993).

Delayed gonad maturation under toxicant stress may prevent utilization of optimal climatic conditions for spawning and larval growth. Reduced chance of larval survival has severe impact on population size. Dietary exposure of cadmium caused reduction in the number of spermatozoa and milt volume in red tilapia (El-Ebiary *et al.,* 2013). Reduction in ripe spermatozoa under the influence of water borne cadmium was evident from the present study (Figure 1, 2, 3 and 4). Most commonly found strategy in teleost fishes is external fertilization in which gametes are released into the external environment and sperm activity decline in a brief period of time (Coward *et al.,* 2002). Ciereszko and Dabrowski, (1994) observed that motility, sperm concentration and fertilization were strongly correlated in Rainbow trout. Reduced number of spermatozoa as observed in the present study impairs chances of fertilization in the external environment resulting in declined number of progeny.

In seasonal breeding fishes, endocrine system cued by environmental factors precisely regulates reproductive cycle to ensure spawning during a specific period of the year (Bhattacharya, 1992). Cadmium was found to cause reproductive endocrine disruption in fishes (Thomas, 1993; Mukherjee *et al.,* 1994; Amutha and Subramanian, 2013; Kim *et al.,* 2016).

Release of gonadal steroids was significantly reduced following exposure to cadmium in Japanese Medaka (Tilton *et al.,* 2003). Ma *et al.,* (1995) observed significant lowering of serum gonadotropin on cadmium exposure in common carp, *Cyprinus carpio*. Sangalang and Freeman, (1974) studied the influence of cadmium in the plasma testosterone and l1-ketotestosterone levels in male brook trout and suggested that utilization and clearance of both steroids are impaired in cadmium treated fish. Variation in steroid production disrupts feedback pathways which orchestrate hypothalamus–pituitary–gonadal axis, ultimately leading to reproductive impairment (Arcand-Hoy and Benson, 1998). Further studies are required in *R. dandia* to prove that how the cadmium affects the reproductive physiology of the fish.

Toxicant stress during gonad maturation results in lowered reproductive potential in fishes leading to the reduction in number of progeny. Thus, reproductive toxicity pose direct threat to the wild fish populations eventually leading to decline in species diversity. Being a sensitive endpoint, fish reproductive dysfunction offers prompt indicators of toxicant stress in ecological monitoring programs.

CONCLUSION

In the present study the effect of cadmium in the gonadal recrudescence of fresh water fish, *R dandia* is studied. It was evident from histological analysis that the experimental fishes were in recrudescent stage while control fishes appear matured. Reduction in GSI was also correlated to delay in gonad maturation of experimental fishes. The present study indicates that the reproductive phases of the fishes are vulnerable to cadmium toxicity.

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