



DOSE DEPENDENT EFFECT OF SYNTHETIC HERBICIDE (ATRAZINE) ON THE MORPHOLOGICAL PARAMETERS IN *POECILIA SPHENOPS*

S. Vasanth^{1,2*}, T. Siva Vijayakumar^{2,3}, G. Bupesh^{1,2} and P. Subramanian²

¹Research & Development Wing, Sree Balaji Medical College & Hospital (SBMCH), BIHER, Chrompet, Chennai- 600 044.

²Department of Animal Science, School of Life Sciences, Bharathidasan University, Tiruchirappalli-620 024.

³Department of Biotechnology, Srimad Aandavan Arts & Science College, Tiruvanaikovil.

*Corresponding Author: S. Vasanth

Research & Development Wing, Sree Balaji Medical College & Hospital (SBMCH), BIHER, Chrompet, Chennai- 600 044.

Article Received on 12/10/2017

Article Revised on 03/11/2017

Article Accepted on 23/11/2017

ABSTRACT

Atrazine is one of the most efficient and cheapest herbicides in the world and is subsequently used more commonly than any other herbicide. Atrazine is frequently detected in waters, and has been known to affect reproduction of aquatic flora and fauna, which in turn impacts on the community structure as a whole. In the present study was designed to investigate the effect of atrazine on sex ratio, Gonadosomatic index and Hepatosomatic index in *Poecilia sphenops*. Fishes were exposed to atrazine (2.5, 1.25, 0.83 ppm) and control also maintained simultaneously for 100days. In our experiments, atrazine exposures lead to a female-biased sex ratio. A reduction of GSI in *Poecilia sphenops* is observed after exposure to atrazine at all exposure concentrations and all times. Comparative liver weight is increased (dose and duration dependant) in male and female *Poecilia sphenops* treated with atrazine.

KEYWORDS: Atrazine, Sex ratio, Gonadosomatic Index, Hepatosomatic Index, *Poecilia sphenop*.

INTRODUCTION

Morphometric studies are not only help to understand the taxonomy but also the health of a species (including reproduction) in an environment. The shape and structures are unique to the species and the variations in its feature are probably related to the habit and habitat among the variants of a species (Cavalcanti *et al.*, 1999). Morphological characters are phenotypically plastic and are influenced each year by the physical environment during spawning and early juvenile stages (Austin, 1999). Morphometric investigations of an animal species revealed the inter relation between the various bodily parameters like length, weight, etc. Morphometric analysis helps to understand the relation between body parts of the fishes (Carpenter *et al.*, 1996; Manimegalai, 2010).

In numerous fish, reptile, and amphibian produces female-biased or male-biased sex ratios (Navarro-Martin *et al.*, 2009). In atrazine treated *Rana pipiens* Tadpoles produced 20% more female phenotype animals (Valeriem Langlois *et al.*, 2010). In another study sex reversal was demonstrated in *X. laevis* exposed ATZ also showed a dose-dependent increase in female phenotype (Oka *et al.*, 2008). In fish the herbicides atrazine change the sex ratio (Stoker *et al.*, 2000) in addition, atrazine is proved to have definite toxicity on the growth and

reproduction of catfish (Hussein *et al.*, 1996), also can cause impaired embryonic development in zebra fish, caused damage and disorder in the growth stages of embryos (Wiegand *et al.*, 2001). The present study was aimed to investigate the effect of atrazine on sex ratio, Gonadosomatic index and Hepatosomatic index in *Poecilia sphenops*.

MATERIALS AND METHODS

Experimental procedure

The newly hatched juveniles/fries were separated from their respective mother and maintained in 100 L tank. A total of 200 fry (0 day old fry) were separated and used in four equal treatment groups. Fifty individuals' fries in each group were exposed to three different concentrations (1/10th of LC 50, 2.5ppm, 1/20th of LC 50, 1.25ppm and 1/30th of LC 50, 0.83ppm) of atrazine and control also maintained simultaneously. At every 20 days interval Sex ratio, Gonadosomatic indices (GSI) and Hepatosomatic indices (HSI) were analysed and recorded.

Hepatosomatic index (HSI)

Hepatosomatic index is the relation between liver weights to that of total body weight and has been used to understand any stress in the living condition. The HSI

was determined using the liver weight and total body weight.

$$HSI = \text{Liver weight} / \text{Body weight} \times 100$$

Gonadosomatic index (GSI)

Gonadosomatic index can give an indication of sexual maturity as well as gonadal development. It is a ratio of gonad weight in relation to total body weight.

$$GSI = \text{Gonad weight} / \text{Body weight} \times 100.$$

The sex ratio was calculated after 60 days of exposure based on the development of gonopodium or ovary. Visible sex character variations were noted only after 60 days.

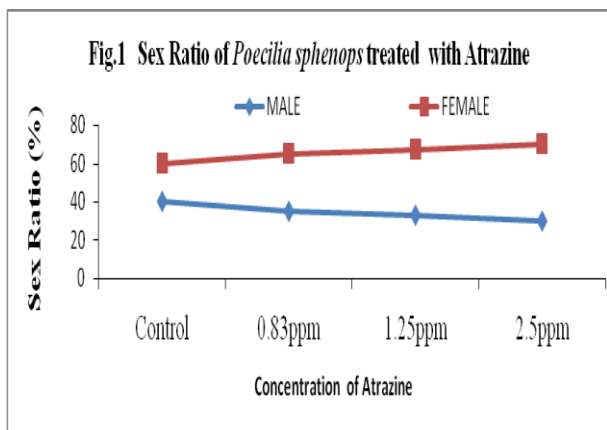
Statistical Analysis

The acquired data were pooled and the above values are expressed as mean ± SE. Differences between groups were assessed by one-way analysis of variance (ANOVA) using the software package for windows (version 16.0). Post -hoc testing was performed for inter group comparisons using the least significant difference (LSD) test (p<0.05) was considered statistically significant.

RESULT

Sex ratio of *Poecilia sphenops* treated with atrazine

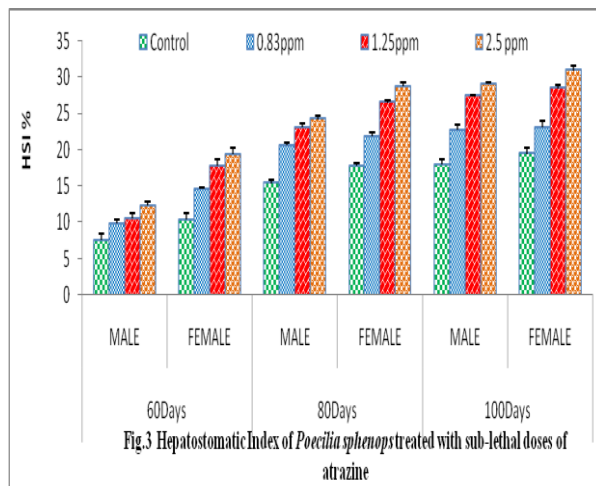
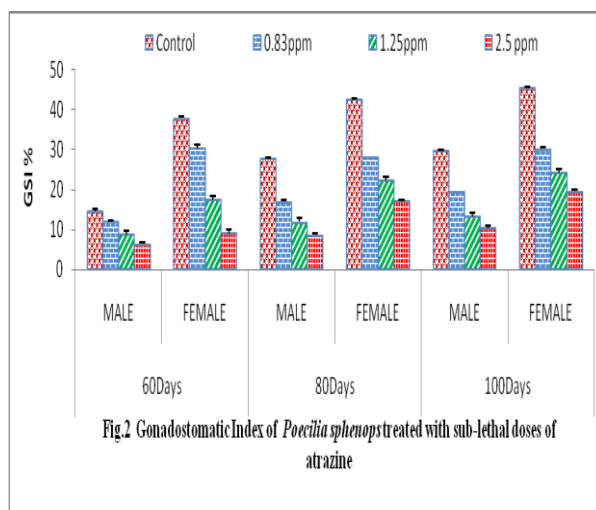
Fig.1 shows that each group of atrazine treated fishes were deviated in the sex ratio when compared to control. Three group of experiment gave increased female population. The sex ratio observed in control 40:60 is (male: female), in 0.83ppm atrazine treatment groups was 35:65, while in 1.25ppm atrazine treatment groups is 33:67 and in 2.5ppm atrazine treatment groups is 30:70. These results are interpreted as an indication that increasing concentrations of atrazine caused a decrease in the number of males produced (Fig.1). In atrazine treated fish, *Poecilia sphenops* shows decreased masculinisation effects during the 60 days exposure period. Dose dependent change in the sex ratio was encountered in 0-day old *Poecilia sphenops* treated with atrazine for 60 days.



Effect of Atrazine on Gonadosomatic and Hepatosomatic Index

Atrazine induces reduction of GSI in both sexes of *Poecilia sphenops*. No significant reduction was observed in fish subjected to low concentration of atrazine when compared to higher. There is significant reduction of GSI is observed in case of male fish subjected atrazine. The GSI of male fish is reduced in all the tested concentration of atrazine (Fig.2).

The HSI values of the fish subjected to atrazine exposure are increased. HSI level is significantly differed in male and female fish subjected to atrazine, whereas significant difference is observed in higher concentration of atrazine treated fish. The atrazine induce significant increase of the HSI of both sexes (Fig.3).



DISCUSSION

Contaminants in the environment can disturb normal physiological processes related to growth, development and behaviour. In this investigation atrazine alter the sex ratio in *Poecilia sphenops*. In our experiments, atrazine exposures lead to a female-biased sex ratio. Chronic exposure to atrazine in zebrafish resulted in a dose-dependent increase in the number of female fish (Miyuki Suzawa and Holly Ingraham, 2008). Similar to our

findings Orn, (2003) also observed increase in the ratio of female fish while exposed to atrazine. Oka *et al.*, (2008) also observed a female-biased sex ratio while atrazine exposed *X. laevis*.

Therefore the usage of high dose of atrazine may produce increased female population in *Poecilia sphenops*. In the present study, 70% female is obtained in *Poecilia sphenops* while treated with atrazine. Further, the present investigations provide evidence that atrazine exposure result in feminization. High doses of atrazine dramatically increase aromatase levels thus increase the percentage of female fish. The present study reveals that atrazine has increased endogenous levels of gonadal CYP19A1 in *Poecilia sphenops* and the target organ is gonads. It is discernible from the study that atrazine changed the male gonadal enzymes levels (in Testis) and induced the functional changes towards femaleness in the reproductive system in *Poecilia sphenops*.

A reduction of GSI in *Poecilia sphenops* is observed after exposure to atrazine at all exposure concentrations and all times. Decreases in GSI are known to occur in reproductive female and male adult goldfish (*Carassius auratus*) exposed to atrazine (Spano *et al.*, 2004). Fidelis Bekeh Ada and Ezekiel Olatunji Ayotunde (2013) also observed the same result in *Oreochromis niloticus* exposed to atrazine herbicide.

Comparative liver weight is increased (dose and duration dependant) in male and female *Poecilia sphenops* treated with atrazine. Increase in HSI is associated with vitellogenesis in both sexes. The atrazine toxicity may impair the liver by making edema or swelling thus the liver weight was increased besides vitellogenin syntheses which are reflected in elevated HIS. Nieves-Puigdoller *et al.*, (2007) also observed increased HSI while exposed to atrazine in female and male Atlantic salmon. In the present study, it concluded that the male sex ratio is decreased in *Poecilia sphenops* due to the atrazine exposure in the hatchlings.

REFERENCES

1. Cavalcanti, M. J., Monteiro, L. R. and Lopes, P. R. D. (1999). Landmark based morphometric analysis in selected species of Serranid fishes (*Perciformes: Teleostei*), *Zool. Stud.* 38(3): 287-294.
2. Austin, M. (1999). Morphometric separation of annual cohorts within mid Atlantic bluefish, *Pomatomus saltatrix*, using discriminant function analysis. 97: 411-420.
3. Carpenter, K. E., Sommer, H.J. and Marcus, L. (1996). Converting truss interlandmark distances to Cartesian Coordinates. In LF Marcus, M corti, A loy, G. Naylor, DE slice, eds. *Advances in morphometrics. ATO ASI series A; Life Sciences, New York Plenum publ.* 284: 103-111.
4. Manimegalai, M. (2010). Colourmorphism Related Biometry And Biochemistry Influenced By Colour Enhancer Astaxanthine And β -Carotene In Fresh Water Fancy Fish *Etroplus maculatus* (Bloch, 1795). Ph.D Thesis, Bharathidasan University, Tiruchirappalli, Tamilnadu, India.
5. Navarro-Martin, L., Blazquez, M. and Piferrer, F. (2009). Masculinization of the European sea bass (*Dicentrarchus labrax*) by treatment with an androgen or aromatase inhibitor involves different gene expression and has distinct lasting effects on maturation. *Gen Comp Endocrinol.* 160: 3–11.
6. Valeriem, S., Langlois, Amanda C., Carew, Bruce D., Pauli, Michael G., Wade, Gerard M., Cooke. and Vance L. Trudeau. (2010). Low Levels of the Herbicide Atrazine Alter Sex Ratios and Reduce Metamorphic Success in *Rana pipiens* tadpoles raised in Outdoor Mesocosms. *Environmental Health Perspectives.* 118(4): 553-557.
7. Oka, T., Tooi, O., Mitsui, N., Miyahara, M., Ohnishi, Y. and Takase, M. (2008). Effect of atrazine on metamorphosis and sexual differentiation in *Xenopus laevis*. *Aquat Toxicol.* 87: 215–226.
8. Hussein, S. Y., El-Nasser, M. A. and Ahmed, S. M. (1996). Comparative studies on the effects of herbicide atrazine on freshwater fish *Oreochromis niloticus* and *Chrysichteres auratus* at Assiut. *Egypt. Bull. Environ. Contam. Toxicol.* 57: 503–510.
9. Wiegand, C., Krause, E., Steinberg, C. and Pflugmacher, S. (2001). Toxicokinetics of atrazine in embryos of the zebrafish (*Danio rerio*). *Ecotoxicol. Environ. Safe.* 49: 199–205.
10. Miyuki Suzawa. and Holly Ingraham, A. (2008). The Herbicide Atrazine Activates Endocrine Gene Networks via Non-Steroidal NR5A Nuclear Receptors in Fish and Mammalian Cells. *PLoS One.* 3: 5: 2117.
11. Orn, S., Holbeck, H., Madsen, T. H., Norrgren, L. and Petersen, G. I. (2003). Gonad development and vitellogenin production in zebrafish (*Danio rerio*) exposed to ethinylestradiol and methyltestosterone. *Aquat Toxicol.* 65: 397–411.
12. Spano, L., Tyler, C. R., Van Aerle, R., Devos, P., Mandiki, S. N. and Silvestre, F. (2004). Effects of atrazine on sex steroid dynamics, plasma vitellogenin concentration and gonad development in adult goldfish (*Carassius auratus*). *Aquat Toxicol.* 66: 369–79.
13. Fidelis Bekeh Ada. and Ezekiel Olatunji Ayotunde. (2013). Ganado-hepato-somatic index of *Oreochromis niloticus* sub adults exposed to some herbicides. *International Journal of Aquaculture.* 3(11): 49-54.
14. Nieves-Puigdoller Katherine, Bjorn Thrandur Bjornsson, Stephen, D. and Mc Cormicka. (2007). Effects of hexazinone and atrazine on the physiology and endocrinology of smolt development in Atlantic salmon. *Aquatic Toxicology,* 84: 27–37.