Histopathological effects and toxicity of atrazine herbicide in Caspian Kutum, *Rutilus frisii kutum*, fry

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Abstract

This study aimed to investigate the toxic effects of atrazine herbicide on the fry of Caspian Kutum (*Rutilus frisii kutum*, Kamensky, 1901). First the 96-h LC50 of the fry were exposed to atrazine at the concentration of 24.95 ppm was determined. Then the toxicity of this herbicide on Caspian kutum fry exposed to the concentration of 12.47ppm (1/2 LC50), for four days was measured and compared with a control group. Comparison of the length, weight and condition factor showed no significant differences between atrazine exposed and control group. The concentration of Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^- in the whole body of fry in control and atrazine exposure groups were as the following order: $Ca^{2+}>K^+> Na^+> Cl^- >Mg^{2+}$ and $Ca^{2+}>Na^+>K^+>Mg^{2+}>Cl^-$, respectively. Results showed that the concentration of all these ions were higher in atrazine exposure group than control group, except for Cl^-, and the only significant differences was found in Na^+ concentration. Major histopathological effects of atrazine on the gills were hyperplasia and thickening of the filaments, separation of the pavement cells of the lamellae epithelium from the pillar cells and swelling of the epithelial cells. Results of the present study showed that atrazine could affect the ion composition of the body, and caused major damages in gill epithelium even at sublethal concentration and acute exposure, but had no effects on the growth parameters.

Keywords: Atrazine, *Rutilus frisii kutum*, Toxicity, Ion, LC50

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Introduction

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) is a pre-emergent herbicide first approved for use in the US in 1958, where it is used primarily on corn, sorghum and sugar cane (Solomon *et al.*, 1996). Atrazine inhibits electron transport in Photosystem II, which results in a disruption of photosynthesis and in turn leads to death from starvation in broad-leaf plants (Giddings *et al.*, 2004).

Herbicides are generally applied in spring or early summer, which often coincide with the breeding season of many fish species. Some of these fishes breed in aquatic habitats receiving the runoff drained from the cultivation fields. Atrazine has low volatility, but its moderate water solubility (33 mg/L at 25 ◦C) makes it relatively mobile in soil and aquatic environments, where it tends to partition into the water column rather than sorbing to sediments (Giddings *et al.*, 2004).

Several recent laboratory studies have shown that environmentally realistic concentrations of atrazine have significant toxic effects on fish. For example, low concentrations of atrazine (1 µg/L) altered olfactory-mediated endocrine function in male Atlantic salmon (*Salmo salar*) (Moore and Lower, 2001). At 100 µg/L, atrazine altered the Na^+, K^+-ATPase activity in common carp (*Cyprinus carpio*) held in fresh water, indicating osmoregulatory disturbances (Hanke *et al.*, 1983). In addition, in vitro studies in fish have shown that atrazine may affect the secretion of cortisol, involved in osmoregulation and stress response (Bisson and Hontela, 2002).

In fishes, gills are vital for respiration and osmoregulatory functions, and respiratory distress is one of the early symptoms of pesticide intoxication (Jayachandran and Pugazhendy, 2009). In recent years considerable histopathological studies have been conducted on fishes exposed to sublethal concentrations of different pesticides and herbicides (Alazemi *et al.*, 1996; Wood, 2001; Cengiz and Unlu, 2006). As a result tissue changes are the functional responses of organisms which provide information on the nature of the toxicant. The present study was an attempt to investigate the histopathological alterations in the gill of *Rutilus frisii kutum* fry exposed to atrazine.

Early developmental stages of the fish life cycle are considered to be the most sensitive stages to the toxic effects of chemical contaminants (Weis and Weis, 1987). Short-term sublethal effects on growth, behavior or osmotic control may affect these critical stages and impact recruitment (Houde, 1987; Sclafani *et al.*, 1997; Alvarez and Fuiman, 2005). For example, loss of osmotic control altering water content may influence larval density and buoyancy. The vertical position of larvae in the water column affects their patterns of drift and their interactions with preys or predators. Thus, a temporary loss of osmotic control in fish larvae may increase their susceptibility to predation or impair their feeding abilities (Sclafani *et al.*, 1997). Disruption of normal cortisol secretion in early life stages may also affect their survival by reducing the ability to cope with acute stressful situations and

by inducing adverse secondary effects on osmoregulation, growth, development and immune function (Benguira *et al*., 2002; Gravel *et al*., 2005; Kennedy and Farrell, 2005).

Caspian kutum is an important commercial fish species in the Caspian Sea in Iran. The sharp decline in its annual catch observed in 1970s and early 1980s (Ghaninejad and Abdulmaleki, 2007) had prompted the Iranian government to launch its restocking project in 1984.

This study is the first attempt to to assess the toxicity of a commercial formulation of the herbicide atrazine on some biochemical indices, of Caspian kutum, a commercially and economically important species of cyprinid fishes in northern Iran. The information obtained may be useful for the management and monitoring of atrazine contamination in the environment.

Material and Methods

Fish and sampling

Caspian kutum, fry, were obtained from the Shahid Ansari Fish Proliferation and Culture Center (Rasht, Iran), in July 2011. Total length (cm) and Body weight (g) were measured, and based on the length and weight, the Condition Factor (CF) was calculated using Williams (2000) method: $K = (100 \times w) \, L^3$.

Determination of LC50 and sub-lethal concentration

Acute toxicity was conducted to determine the 96 h LC50 value of atrazine with definitive test in semi-static system in laboratory as per standard methods (APHA, AWWA, WPCE, 2005). The range finding test was carried out prior to the definitive test to determine the concentration of the test solution. For the test, the atrazine (80% WP, Hangzhuou Ruijiang Chemical Co. Ltd., China) was dissolved in distilled water, and added to the aquarium (20L) following the method of Pluta (1989). In the definitive test, a set of 10 fish specimens were randomly exposed to each of the atrazine concentrations (viz. 20, 22, 24, 28, and 30 mgL^{-1}) and the experiment was set in triplicate to obtain the LC50 value of the herbicide for the species. The LC50 value of test chemical in *R. frisii kutum* was determined by Probit analysis method (Finney, 1971) for 12, 24, 48, 72 and 96 hours Based on the 96h LC50 value, one sublethal test concentration of atrazine was determined and the fish specimens were exposed to this concentration for the assessment of its toxic effects on the osmoregulatory system.

Experimental design

After the determination of the LC50, one sublethal concentration of atrazine was determined as 1/2LC50 (Ramesh *et al*., 2009). Three aquaria (100L) each containing 50 Caspian kutum fry were exposed to this sublethal concentration for 4 days, and the same number of aquaria and fry in clean water (no atrazine) were held as control group. Sampling begun after 24hrs of exposure and continued every 24 h until the end of the experiment. During the experiment water factors: pH, temperature and dissolved oxygen (DO) were measured using Eutech instruments, pcd650, and fish were not fed during the experiment.

Median Lethal Concentration (LC50)
Median lethal concentration (LC50) is the most widely accepted basis for an acute toxicity test and it is the concentration of a test chemical which kills 50% of the test organisms after a particular length of exposure, usually 96 h. Generally in toxicity tests, death is a decisive criterion because it is easy to determine and has obvious biological and ecological significance. The LC50 values (with 95% confidence limits) of different concentrations of atrazine (Table 2) were found to be 29.22, 28.44, 27.27, 25.78 and 24.95 mg·L^{-1} for 12, 24, 48, 72 and 96 h

LC50, respectively following Finney's (1971) method and using SPSS (version 15). A dose dependent increase and time dependent decrease were observed in mortality rate, such that as the exposure time increases from 12 to 96 h, the median concentration was reduced. It was observed that as the concentration of the herbicide increased, fish mortality also increased, this indicates a direct proportional relationship between mortality and concentration of atrazine herbicide. No mortality was observed in the control during the experimental period.

Table 2: Lethal concentrations (LC) of atrazine depending on exposure time (12-96 h) for *R. frisii kutum*. Different letters show significant differences and similar letters or numbers show no significant differences.

Point	Concentrations (mg·L^{-1}) at various exposure times. (95% confidence intervals)				
	12h	24h	48h	72h	96h
LC$_1$	21.43^a (18.76-22.92)	19.95^b (17.32-21.51)	18.76^c (16.25-20.33)	18.61^c (16.58-19.96)	19.02^d (12.49-21.36)
LC$_{10}$	24.63^a (23.07-25.59)	23.39^b (21.76-24.43)	22.19^c (20.53-23.27)	21.54^d (20.12-22.51)	21.49^d (16.74-23.26)
LC$_{50}$	29.22^a (28.20-30.90)	28.44^b (27.42-29.98)	27.27^c (26.34-28.51)	25.78^d (25.00-26.63)	24.95^e (23.92-25.02)
LC$_{90}$	34.66^a (32.33-39.78)	34.57^a (32.18-39.50)	33.51^b (31.36-37.64)	30.85^c (29.40-33.28)	28.96^d (26.81-36.73)

Length, Weight and Condition Factor
The mean body weight (BW) and mean total length (TL) of fry's were: 0.26±0.01 g and 3.5±0.02 cm, respectively. Measuring the length, weight and condition factor of the control and atrazine exposure groups showed no significant differences ($p>0.05$) between these two experimental groups (Figs. 1 and 2).
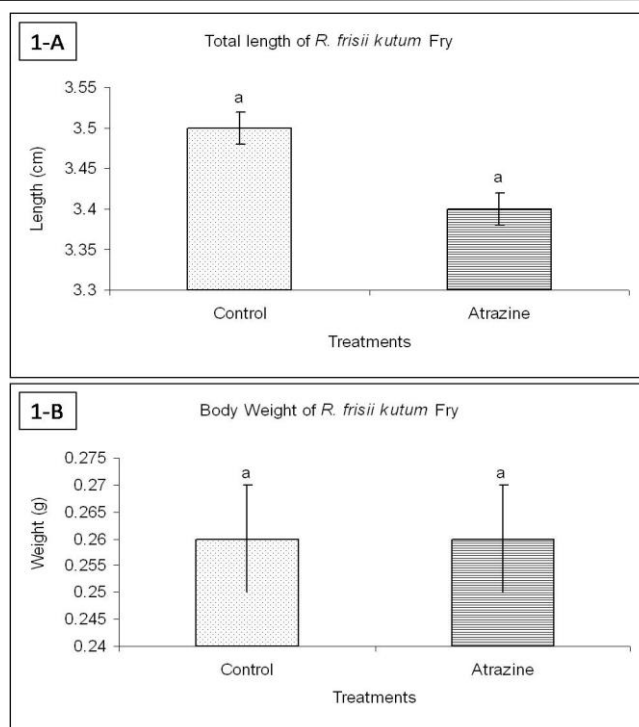
Figure 1: Total Length of *R. frisii kutum* fry (A); Body weight of *R. frisii kutum* fry (B): No significant differences found between two experimental groups ($p>0.05$).
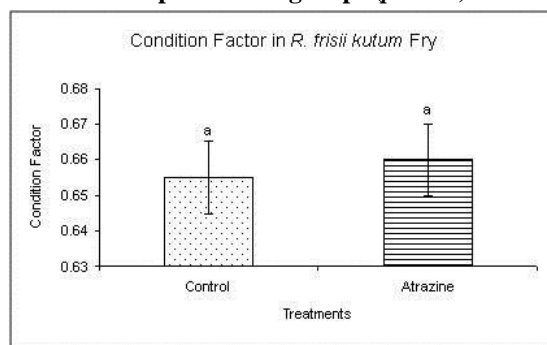
Figure 2: Condition Factor (CF) in *R. frisii kutum* fry. No significant ifferences found between two experimental groups ($p>0.05$).

Body Ions

Measurement of the total body ions showed that the highest concentration belongs to Ca^{2+} in the atrazine exposure group and the lowest belongs to the Mg^{2+} in the control group (Fig. 4). Results also showed that all ions in both experimental groups were increased during the experiment (Fig. 3). In atrazine exposed group all the ions showed higher concentrations compared to control group, except for the Cl^-, that had higher concentration in control group (Fig. 4). It seemed that, exposure to atrazine has increased the cations and decreased the anions. On the other side, the concentration of the ions in control group was as the following order: $Ca^{2+}>K^+>Na^+ > Cl^- >Mg^{2+}$, but this order was different in atrazine exposed group: $Ca^{2+}>Na^+>K^+>Mg^{2+}>Cl^-$. The results indicated that besides increasing and

decreasing the concentration of the ions, atrazine has affected the ion composition of the body as well. Statistical analysis showed that, there is no significant difference between the concentrations of the K^+, Ca^{2+}, Mg^{2+}, Cl^- ions in control and atrazine exposure groups, but the concentration of the Na^+ is significantly ($p<0.05$) higher in atrazine exposed group.
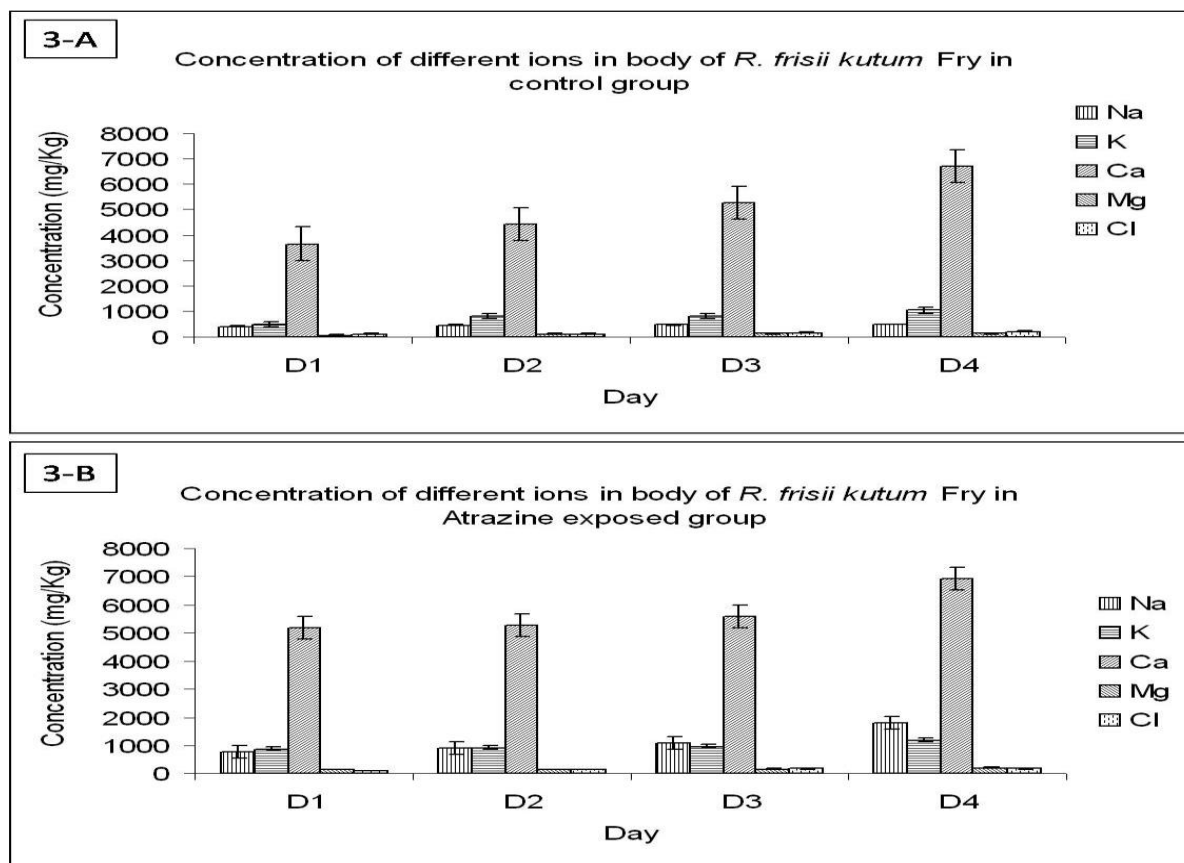
Figure 3: Concentration of different ions in body of control fish during the experiment (A): All concentrations were increased during the experiment; Concentration of different ions in body of atrazine exposed fish during the experiment (B). All concentrations were increased during the experiment.
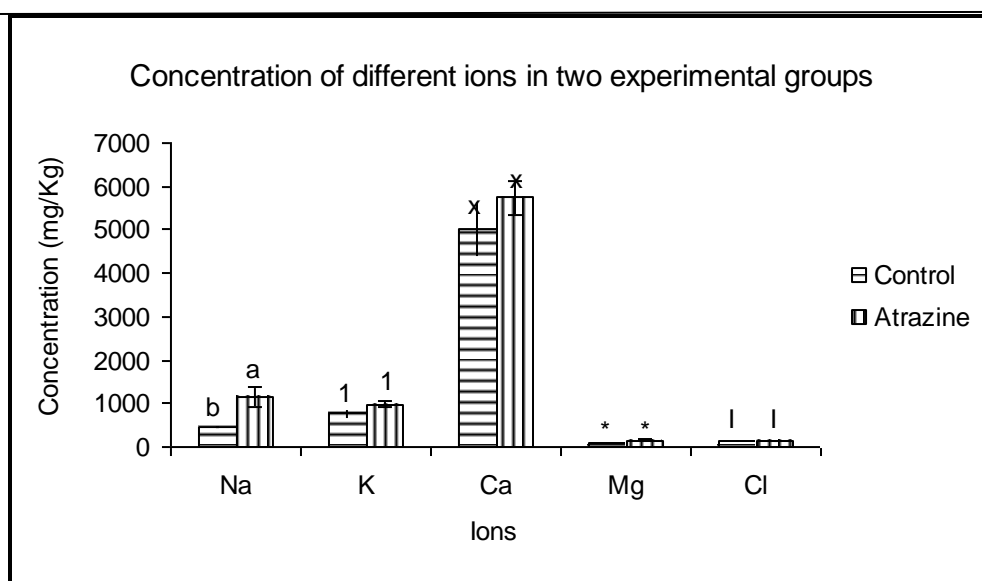
Figure 4: Comparison between mean concentrations of different ions in two experimental groups. The concentration of all ions were higher in atrazine exposed fish compared to control group, but the only significant difference found in Na$^+$ concentration ($p<0.05$). Different letters or numbers show significant differences and similar letters or numbers show no significant differences.

Histopathology

Gill in *R. frisii kutum* larvae, as examined are made up of four gill arches in each side of the head. Each arch is carrying two rows of filaments, and the filaments carrying two rows of lamellae. The main epithelial cells of the gill filaments and lamellae are simple squamus cells, called the pavement cells. Each lamella is essentially composed of two epithelial sheets held apart by a series of individual cells termed pillar cells (Fig. 5C & D). The spaces around the pillar cells and between the two epithelial layers are perfused with blood. The most significant alterations in gills caused by atrazine exposure were hyperplasia and thickening of the filaments, separation of the pavement cells of the lamellae epithelium from the pillar cells and swelling of the epithelial cells (Fig. 5A & B).
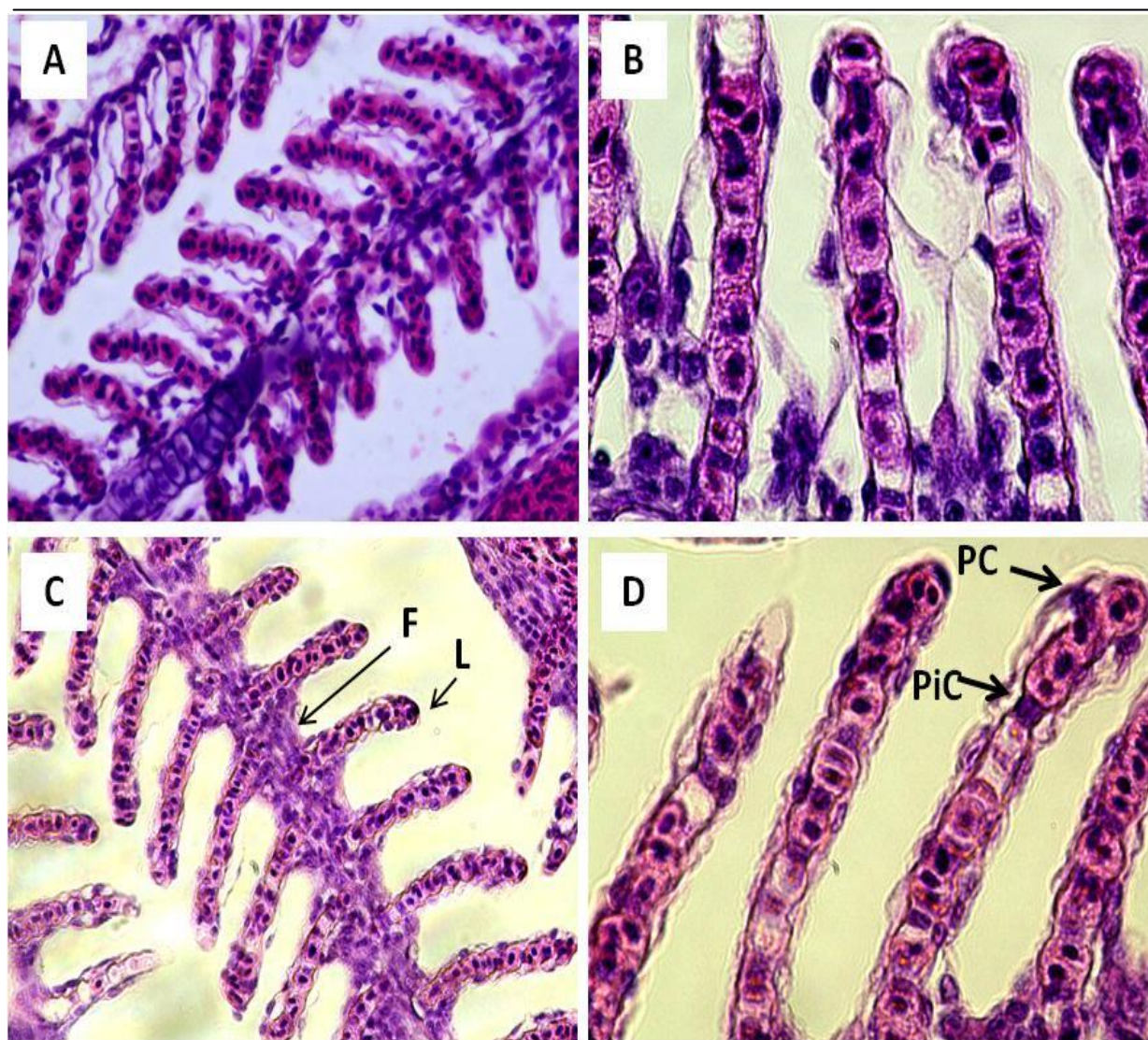
Figure 5: Histological photograph of the gill in *R. frisii kutum* fry. Gill of the atrazine exposed fish (A & B), separation of the pavement cells of the lamellae, thickening of the filament and swelling of the epithelial cells were the major effects of the atrazine. Gill structure in control group (C & D), gill is made up of filaments carrying lamellae. F: Filament; L: Lamellae; PC; Pavement Cell; PiC: Pillar Cell.

Discussion

Fish are often used as sentinel organisms for ecotoxicological studies because they play a number of roles in the trophic web, accumulate toxic substances and respond to low concentrations of mutagens (Cavas and Ergene-Gözükara, 2005). Therefore, the use of fish biomarkers as indices of the effects of pollution are of increasing importance and can permit early detection of aquatic environmental problems (Lopez-Barea, 1996; Van Der Oost, *et al*., 2003). Acute toxicity data has been used to derive water quality guidelines for regulatory measures (Sunderam, *et al*., 1994).The result of the LC50 (median lethal concentration) for atrazine in the present study at 96 h was 24.95 mg.L^{-1}. The results showed that the toxicity of

atrazine for *R. frisii kutum* is both time and concentration dependent, thus, accounting for differences in LC values obtained at different concentrations and time of exposure. However, some other researchers have shown that exposure time is not significant in LC50 determination for fish (Lakota *et al.*, 1989). The LC50 value obtained for *R. frisii kutum* in this study is higher than that reported by Bathe *et al.* (1973), Neškovic *et al.* (1993), and Hussein *et al.* (1996), who reported LC50 values of 16.0, 18.8 and 9.37 mgl^{-1} for *Lepomis macrochirus* (Bluegill sunfish), *C. carpio* and *Oreochromis niloticus*, respectively, exposed to atrazine. Toxicity of chemicals to aquatic organisms has been shown to be affected by age, size and health of the species (Abdul-Farah *et al.*, 2004). Physiological parameters, and water quality, temperature, pH, dissolved oxygen and turbidity, the, amount and kind of aquatic vegetation, concentration and formulation of the chemical and its exposure also greatly influence such studies (Gupta *et al.*, 1981; Young, 2000). Fish exposed to atrazine were stressed progressively with time before death. The respiratory impairment due to the toxic effect of atrazine on the gills of *R. frisii kutum* is similar to the reports of Abdul-Farah *et al.* (2004); De Mel and Pathiratne (2005); Tilak *et al.* (2007) and Ayoola (2008) that pesticides impair respiratory organs. Death could have, therefore, occurred either by direct poisoning or indirectly by making the medium unconducive for the fish or even by both. The abnormal behavior observed during the exposure period like restlessness and

surface to bottom movement were similar to the observations of Hussein *et al.* (1996); Pandey *et al.* (2005); Chandra (2008) and Naeemi (2013).

The length-weight relationship of fish is an important fishery management tool. Its importance is pronounced in estimating the average weight at a given length group (Beyer, 1987) and in assessing the relative well being of a fish population (Bolger and Connoly, 1989). Consequently, length-weight studies on fish are extensive. Notable among these are the reports Shenouda *et al.* (1994), for *Chrysichthys* spp. from the Southernmost part of the River Nile (Egypt), Alfred-Ockiya and Njoku (1995) for mullet in New Calabar River, Ahmed and Saha (1996) for carps in Lake Kapital, Bangladash, King (1996) for Nigeria fresh water fishes, Hart (1997) for *Mugil cephalus* in Bonny Estuary; Diri (2002) *Tilapia guineensis* in Elechi creek.

Condition factor compares the wellbeing of a fish and is based on the hypothesis that heavier fish of a given length are in better condition (Bagenal and Tesch, 1978). Condition factor has been used as an index of growth and feeding intensity (Fagade, 1979). Condition factor decrease with increase in length (Fagade, 1979); and also influences the reproductive cycle in fish (Welcome, 1979). Condition factors of different species of cichlid fishes have been reported by Siddique (1977), Fagade (1978, 1979, and 1983), Dodzie and Wangila (1980), Arawomo (1982) and Oni *et al.* (1983). Some condition factors reported for other fish species include; Alfred- Ockiya (2000), *Chana chana* in fresh water swamps of Niger Delta and

Hart (1997), *M. cephalus* in Bonny estuary, Abowei and Hart (2007), ten fish species from the lower Nun River, and Abowei and Davies (2009), *Clarotes lateceps* from the fresh water reaches of the lower Nun river. In present study no significant differences were found in atrazine exposed and control group, this result showed that all experimental fish were in the same growth condition and atrazine at sublethal concentration and acute condition have no effects on growth or condition factor of the *R. frisii kutum* fry.

Several studies have tested the effects of atrazine on survival and various measures of iono-regulatory performance in different fishes (Moore *et al*., 2003; Waring and Moore, 2004; Nieves-Puigdoller *et al*., 2007). The present study differs from many in the literature in that the results did not reveal any significant effects of atrazine on survival, body weight, and condition factor or ionoregulatory performance in *R. frisii kutum* fry.

Plasma and whole body electrolyte levels, Na^+/K^+-ATPase activity and muscle water content are commonly measured as indicators of iono-regulatory performance in fishes.

In the present study, atrazine (12.47 mgl^{-1}) elevated whole body Na^+ levels significantly, while not affecting other ions. These results are similar to those of Waring and Moore (2004) and that atrazine elevated plasma Na^+ and had no effects on plasma Cl^- levels. Cassano *et al*. (2006) demonstrated that doses as low as $2\mu g.L^{-1}$ atrazine can stimulate the short-

circuit current of the ventral skin of frog (*Rana esculenta*), resulting in stimulated Na^+ absorption. Increases of plasma Na^+ at intermediate doses of atrazine may be a compensatory response to moderate damage of ion regulatory tissue. In some previous studies, exposure to different levels of atrazine caused elevation in plasma Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^- levels (Nieves-Puigdoller *et al*., 2007).

The high mortality induced by atrazine reported in Waring and Moore (2004) is all the more unexpected given the acute toxicity values for atrazine exposure in freshwater fish. These range from a 96-h LC50 of 4300 μg/L for the guppy (*Poecilia reticulata*) to a 96-h LC50 of N100,000 μg/L for the carp (*Carassius carassius*) (Giddings *et al*., 2004). In the case of salmonids, the 96-h LC50 was 13,000 μg/L for rainbow trout (*Oncorhynchus mykiss*) and 12,000 μg/L for coho salmon (*O. kisutch*). Both short term (4-day; present study) and longer term (21 day, Nieves-Puigdoller *et al*., 2007) exposures to atrazine had no effects on body weight.

Many studies showed the effects of atrazine on gills of the fish species, including increase of epidermal thickness and lamellar width, fusion of secondary lamellae, hyperplasia, club-shaped cartilaginous tissue, aneurysm, and necrosis in epithelium region, (Alazemi *et al*., 1996; Cengiz and Unlu, 2006;Velisek *et al*., 2006 ;Yang *et al*., 2010). Previous studies suggested edematous changes in the gill were most probably due to the increase in capillary permeability. Hyperplasia was considered as a protective

mechanism from environmental irritant by decreasing the respiratory surface and increasing the toxicant–blood diffusion distance (Meissner and Diamandopoulos, 1977), and its intensification could result in the thickness of epithelial layers, which could be supported by the increases of epithermal thickness and lamellar width. Thus, all the lesions found in the present study would probably inhibit the respiratory, secretory and excretory functions in the gill of *R. frisii kutum*.

Results of the present study showed that sublethal concentration of atrazine, even in acute and short term exposure can alter the biochemical composition of the fish body and affect some behavioral responses that could lead to failure of the surviving capabilities of the fish fry.

Acknowledgments

References

Abdul-Farah, M., Ateeq, B., Ali, M.N. and Ahmad, W., 2004. Studies on lethal concentrations and toxicity stress of some xenobiotics on aquatic organisms. *Chemosphere*, 55, 257–265.

Abowei, J.F.N and Hart, A.I. ,2007. Size, Composition, age, growth, mortality and exploitation rate of *Chysichthys nigrodigitatus* from Nun River, Niger Delta, Nigeria. Afr.

Journal of Applied Zoology and Environmental Biology, 9, 44-50.

Abowei, J.F.N. and Davies, A.O., 2009. Some population parameters of *Clarotes laticeps* (Rupell, 1829) from the fresh water reaches of the lower river, Niger Delta, Nigeria. *American Journal of Scientific Research*, 2, 15-19.

Ahmed, K.K. and Saha, S.B., 1996. Length-weight relationship of major carps in Kaptai Lake. Bangladesh. NAGA. *The ICLARM Q.*, 19(**2**), 28.

Alazemi, B.M., Lewis, J.W. and Andrews. E.B., 1996. Gill damage in the freshwater fish *Gnathonemus petersii* (family: Mormyridae) exposed to selected pollutants: An ultrastructural study. *Environmental Technology,* 17, 225-238.

Alfred-Ockiya, J. F. and Njoku, D.C., 1995. A comparative analysis of the length weight relationship and condition factors of four species of grey mullet (pisces/mugildae) from New Calabar River Rivers State, Nigeria. *Journal of Technology and Design Education*, pp. 5-10.

Alfred-Ockiya, J.F., 2000. The length-weight relationship of snake head (*Chana chana*) from the fresh water swamps of Niger Delta. *Journal of Aquatic Science*, 15, 12-14.

Alvarez, M.d.C. and Fuiman, L.A., 2005. Environmental levels of atrazine and its degradation products impair survival skills and growth of red drum larvae. *Aquatic Toxicology*, 74, 229–241.

APHA, AWWA, WPCE, 2005. Standard methods for the examination of water and wastewater, 21st ed. American Publication of Health Association, Washington, DC., 1368P.

Arawomo, G.A.A., 1982. The growth of *Sarotherodon niloticus.* In*:* Proceedings of the 2nd Annual Conference of the Institute. New Bussa, Nigeria. pp. 221-227.

Ayoola, S.O. ,2008. Toxicity of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*) juveline. *African Journal of Agricultural Research*, 3, 825–834.

Bagenal, T.B. and Tesch, A.T. ,1978. Conditions and growth patterns in freshwater habitats. Blackwell Scientific Publications, Oxford. Bakare, O., 1970. Bottom Deposits as Food of Inland Fresh Water Fish. In: Kainji, A Nigerian Manmade Lake. Visser, S.A., (Ed.), Kanyi Lake Studies, Vol.1. Ecology Published for the Nigerian Institute.

Bathe, R., Ullmann, L. and Sachsse, K., 1973. Determination of pesticide toxicity to fish. *Berlin-Dahlem*, 37, 241–246.

Benguira, S., Leblond, V.S., Webert, J.P. and Hontela, A., 2002. Loss of capacity to elevate plasma cortisol in rainbow trout (*Oncorhynchus mykiss*) treated with a single injection of o,p'-dichlorodiphenyldichloroethane. *Environmental Toxicology and Chemistry,* 21, 1753–1756.

Beyer, J.E., 1987. On length-weight relationship computing the mean weight of the fish of a given length class. *Fishbyte*, 5(**1**), 11-13.

Bisson, M. and Hontela, A., 2002. Cytotoxic and endocrine-disrupting potential of atrazine, diazinon, endosulfan, and mancozeb in adrenocortical steroidogenic cells of rainbow trout exposed in vitro. *Toxicology and Applied Pharmacology*, 180, 110–117.

Bolger, T. and Connoly, P.L., 1989. The selection indices for the measurement and analysis of fish condition. *Journal of Fish Biology*, 17(**3**), 1-182.

Cassano, G., Bellantuono,V., Ardizzone, C. and Lippe, C., 2006. Atrazine increases the sodium absorption in frog (*Rana esculenta*) skin. *Environmental Toxicology and Chemistry*, 25, 509–513.

Cavas, T. and Ergene-Gözükara, S., 2005. Micronucleus test in fish cells, a bioassay for in situ monitoring of genotoxic pollution in the marine environment. *Environmental and Molecular Mutagenesis*, 46, 64–70.

Cengiz, E.I. and Unlu, E., 2006., Histopathology of gills in mosquitofish, *Gambusia affinis* after long-term exposure to sublethal concentrations of malathion. *Journal of Environmental Science and Health*. B38, 581–589.

Chandra, S., 2008. Toxic effect of Malathion on acetylcholinesterase activity of liver, brain and gills of freshwater catfish *Heteropneutes fossilis. Environmental Conservation*, 9, 45–52.

De Mel, G.W.J.L.M.V.T.M. and Pathiratne, A., 2005. Toxicity assessment of insecticides commonly used in rice pest management to the fry of common carp, *Cyprinus carpio*, a food fish culturable in rice fields. *Journal of Applied Ichthyology*, 21, 146–150.

Diri, M.S., 2002. Length-weight relationship of *Sarotheredon melanotheron* and *Tilapia guineensis* in Elechi creek Niger Delta, Nigeria B.Sc. project Rivers State University of Science and Technology Port Harcourt. 33P.

Dodzie, S. and Wangila, B.C.C. ,1980. Reproductive biology, length-weight relationship and relative condition of pond raised tilapia zilli (Gervas). *Journal of Fish Biology*, 17, 243-253.

Fagade, S., 1983. The biology of *chromido Tilapia guntheri* from a small lake. *Archiv fur Hydrobiologie*, 97, 60-72.

Fagade, S.O., 1978. Age determination of *Tilapia melanotheron* (Ruppel) in the Lagos lagoon, Nigeria. International Symposium on Ageing of Fish (In Bagenal), Teseh. pp.71-77.

Fagade, S.O., 1979. Observation of the biology of two species of Tilapia from the Lagos lagoon Nigeria. *Bull. Inst. Fond Afr. Nore (Ser. A),* 41, 627-658.

Finney, D.T. ,1971. Probit Analysis; Cambridge University Press: Cambridge, UK. 272P.

Ghaninejad, D., and S. Abdulmaleki., 2007. Annual stocks assessment of bony fish in the Caspian Sea. Iranian Fisheries research Institute (IFRO), Tehran, Iran. 65P.

Giddings, J.M., Anderson, T.A., Hall Jr., L.W., Kendall, R.J., Richards, R.P., Solomon, K.R. and Williams, W.M., 2004. A probabilistic aquatic ecological risk assessment of atrazine in north American surface waters. SETAC Press, Pensacola, FL, USA.

Gravel, A., Campbell, P.G.C. and Hontela, A., 2005. Disruption of the hypothalamo-pituitary-interrenal axis in 1+ yellow perch (*Perca flavescens*) chronically exposed to metals in the environment. *Canadian Journal of Fisheries and Aquatic Sciences*, 62, 982–990.

Gupta, P.K., Khangant, B.S. and Durve, V.S. ,1981. The temperature dependence of the acute toxicity of copper to freshwater pond snail, *Viviparus bengalensis* L. *Hydrobiologia*, 83, 461–464.

Hanke, W., Gluth, G., Bubel, H. and Muller, R., 1983. Physiological changes in carps induced by pollution. *Ecotoxicology and Environmental Safety*, 7, 229–241.

Hart, S.A., 1997. The Biology of *Mugil cephalus* in Bonny River estuary. M.Sc. Thesis University of Port Harcourt, Nigeria, pp: 42. Hart, A.I. and J.F.N. Abowei, 2007. A study of the length-weight relationship, condition factor and age of ten fish species from the lower Nun river. NigerDelta. *African Journal of Environmental Science and Technology*, 9, 13-19.

Houde, E.D., 1987. Fish early life dynamics and recruitment variability. *American Fisheries Society Symposium*. 2, 17–29.

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 *Chrysichthys auratus* at Assiut Egypt. *Bulletin of Environmental Contamination and Toxicology*, 57, 503–510.

Jayachandran, K. and Pugazhendy, K., 2009. Histopathological changes in the gill of *Labeo rohita* (Hamilton) fingerlings exposed to Atrazine. *American-Eurasian Journal of Scientific Research*, 4(**3**), 219-221.

Kennedy, C.J. and Farrell, A.P., 2005. Ion homeostasis and interregnal stress responses in juvenile Pacific herring, *Clupea pallasi*, exposed to the water-soluble fraction of crude oil. *Journal of Experimental Marine Biology and Ecology*, 323, 43–56.

Khodabandeh, S., Khoshnood, Z. and Mosafer, S. ,2008. Immunolocalization of Na^+, K^+-ATPase-rich cells in the gill and urinary system of Persian sturgeon, *Acipenser persicus*, fry. *Aquaculture Research*, pp.1-8.

King, R.P., 1996. Population dynamics of the mud skipper *Periophthalmus barbarus* (Gobidae) in the estuarine swamps of Cross River Nigeria. *Journal of Aquatic Science*, 11, 31-34.

Lakota, S., Razska, A., Utracki, T. and Chmiel, Z., 1989. Side effect of deltamethrin and cypermthrin in the environment of water biocenoses. *Organika*, 71, 71–77.

Lopez-Barea, J. ,1996. Biomarkers to detect environmental pollution. *Toxicology Letters*, 88, 79.

Meissner, W.A. and Diamandopoulos, G.T., 1977. Neoplasia. In: Anderson, W.A.D., Kissane, J.M. (Eds.), Pathology. The CV Mosby Co., St. Louis. pp. 640–691.

Moore, A. and Lower, N., 2001. The impact of two pesticides on olfactory-mediated endocrine function in mature male Atlantic salmon (*Salmo salar* L.) parr. *Comparative Biochemistry and Physiology*, B129, 269–276.

Moore, A., Scott, A.P., Lower, N., Katsiadaki, I. and Greenwood, L., 2003. The effects of 4- nonylphenol and atrazine on Atlantic salmon (*Salmo salar* L.) smolts. *Aquaculture*, 222, 253–263.

Mortoja, R. and Mortoja – Pierson, M., 1967. Initiation Aux Techniques de l histologe animale. Masson et Cie, Paris, 345P.

Naeemi, A., Jamili, S., Shabanipour, N., Mashinchian, A. and Shariati Feizabadi, S., 2013. Histopathological changes of gill, liver and kidney in Caspian kutum exposed to Linear Alkylbenzene Sulfonate. *Iranian Journal of Fisheries Sciences*, 12(**4**), 887-897.

Nieves-Puigdoller, K., Björnsson, B.T. and McCormick, S.D., 2007. Effects of hexazinone and atrazine on the physiology and endocrinology of smolt development in Atlantic salmon. *Aquatic Toxicology*, 84, 27-37.

Neškovic, N.K., Elezonic, I., Karan, V., Poleksic, V. and Budimir, M., 1993. Acute and sub acute toxicity of atrazine to Carp (*Cyprinus carpio*). Ecotoxicol. *Ecotoxicology and Environmental Safety*, 25, 173–182.

Oni, S.K., Olayemi J.Y. and Adegboye, J.D., 1983. The comparative physiology of three ecologically (Rupel). *Synodonts schall.* Block and Schneider and *Tilapia zilli* (Gervais). *Journal of Fish Biology*, 22, 105-109.

Pandey, S., Kumar, R., Sharma, S., Nagpure, N.S., Sirivastava, S.K. and Verma, M.S., 2005. Acute toxicity bioassays of mercuric chloride and malathion on air breathing fish *Channa punctatus* (Bloch). *Ecotoxicology and Environmental Safety*, 61, 114–120.

Pluta, H.J., 1989. Toxicity of several xenobiotics and waste water effluents measured with a new fish early life stage test. Ger. *Journal of Basic and Applied Zoology,* 76, 195–220.

Ramesh, M., Srinivasan, R., and Saravanan, M. ,2009. Effect of atrazine (Herbicide) on blood parameters of common carp *Cyprinus carpio* (Actinopterygii: Cypriniformes). *African Journal of Environmental Science and Technology*, 3(12), 453-458.

Sclafani, M., Stirling, G. and Leggett, W.C., 1997. Osmoregulation, nutritional effects and buoyancy of marine larval fish: a bioassay for assessing density changes during the earliest life-history stages. *Marine Biology*. 129, 1–9.

Shenouda, T.S., Faten, F.A., Mahmoud M.R. and Ray. M.M. ,1994. A detail study on age and growth for *Chrysichthys auratus* and *Chrysichthys rueppelli* from the southern most part of the River Nile (Egypt). *Egyptian German Society*, 200(1412), 73-101.

Siddique, A.Q., 1977. Reproductive biology, length weight and relative condition of *Tilapia leucostica* (Trewaeva in lake Naivasha, Kenya). *Journal of Fish Biology*, 10, 351-260.

Solomon, K.R., Baker, D.B., Richards, R.P., Dixon, D.R., Klaine, S.J., LaPoint, T.W., Kendall, R.J., Weisskopf, R.J., Giddings, J.M., Giesy, J.P., Hall, L.W. and Williams, W.M., 1996. Ecological risk assessment of atrazine in North American surface waters. *Environmental Toxicology and Chemistry,* 15, 31–74.

Sunderam, R.I.M., Thompson, G.B., Chapman, J.C. and Cheng, D.M.H. ,1994. Acute and chronic toxicity of endosulfan to two Australian Cladocerans and their applicability in deriving water quality criteria. *Archives of Environmental Contamination and Toxicology*, 27, 541–545.

Tilak, K.S., Veeraiah, K., Bhaskara, P. and Butchiram, M.S., 2007. Toxicity studies of Butachlor to the freshwater fish *Channa punctata* (Bloch). *Journal of Environmental Biology,* 28, 285–487.

Van Der Oost, R., Beyer, J. and Vermeulen, N.P.E., 2003. Fish

bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology,* 13, 57–149.

Velisek, J., Wlasow, T., Gomulka, P., Svobodova, Z., Dobsikova, R., Novotny, L. and Dudzik, M., 2006. Effects of cypermethrin on rainbow trout (*Oncorhynchus mykiss*). *Vet. Med.* 51, 469–476.

Waring, C.P. and Moore, A., 2004. The effects of atrazine on Atlantic salmon (*Salmo salar*) smolts in fresh water and after sea water transfer. *Aquatic Toxicology,* 66, 93–104.

Weis, J.S. and Weis, P., 1987. Pollutants as developmental toxicants in aquatic organisms. *Environmental Health Perspectives*, 71, 77–85.

Welcome, R.L., 1979. Fisheries ecology of flood plain rivers. Longman Press, London, 317P.

Williams .J. E., 2000. Manual of fisheries survey methods II: With periodic updates. Chapter 13: The coefficient of condition of fish. Michigan Department of Natural Resources, Fisheries Special Report 25, Ann Arbor.2P.

Wood, C.M., 2001. Toxic responses of the gill. In: Schlenk, D., Benson, W.H. (Eds.), Organs. In: Target organ toxicity in marine and freshwater Teleosts, vol. 1. Taylor & Francis, London, pp. 1–89.

Yang, L., Zha, J., Li, W., Li, Z. and Wang, Z., 2010. Atrazine affects kidney and adrenal hormones (AHs) related genes expressions of rare minnow (*Gobiocypris rarus*). *Aquatic Toxicology*, 97, 204-211.

Young, R.A., 2000. Biomedical discovery with DNA arrays. *Cell*, 102, 9–15.