Sublethal effects of organophosphate, diazinon on gill tissue and growth performance of Caspian roach (*Rutilus rutilus*) fingerling kept in fresh water and brackish water

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Abstract

The stocks of Caspian roach (*Rutilus rutilus*), an economically important species in the Caspian Sea, is depleting. Annually, millions of fingerlings of these species are produced artificially and restock in the mouth of Qare Soo River in the Southern Caspian Sea, where they exposed to the pesticides coming from rice and orchard farms in the region. The fate of this exposure in hypo-osmoregulatory ability of juvenile fish is important. Therefore, this study initially investigates fingerlings of Caspian roach which was exposed to different concentrations of diazinon (close to estuaries concentration) for 96 h, then they were transferred to the brackish water for 240 h where the growth and survival rate were examined. The major changes in the exposed fish gills to pesticides were collapsed secondary lamellae, oedema, epithelial lifting, and shortening of secondary lamellae. The findings of this study showed decrease of tissue damage in the gill tissue in fingerlings at brackish water, while, a lower growth rate was seen compared to the control group ($P < 0.05$). This study suggests that the exposure of Caspian roach fingerlings to diazinon in fresh water may jeopardize their physiological capabilities and subsequent survive in brackish water conditions which may cause failure in fish stocks rebuild project in the Caspian Sea.

Keywords: Caspian roach, histopathology, gill, growth, diazinon.

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Introduction

Development and optimization of agricultural activities have resulted in an increased use of pesticides in agriculture which has been caused high pollution in aquatic environments. Recently, the occurrence of fish mortality affected by both agriculture pesticides and industrial effluents has been reported in Iran (Bagheri 2007). Among common pesticides, organophosphate pesticides such as diazinon attracted most attention because of their durability in the environment, ability to store in the food web, and the desire to bio accumulate in body of aquatic organisms (Waite, Sommerstad, Grover, Kerr, Westcott & Irvine 1995).

Diazinon is widely used to control the pest population in rice fields in Guilan, Mazandaran, Golestan Provinces (Northern of Iran) as well as other areas (Honarpajouh 2003; Shayeghi, Hosseini & Abtahi 2006). According to the reports of Bulletin of Iran Agriculture Ministry (Banaee, Sureda, Mirvaghefi & Ahmadi 2011), the Iranian annual use of diazinon is about 3775 ton. This pesticide in Qare Soo River estuaries in southeast of Caspian Sea where is site release of juveniles Caspian roach (*Rutilus rutilus*), has been reported (Bagheri 2007). Histopathological studies have revealed that fish organs are adequate indicators for water quality evaluation (Velmurugan, Selvanayagam, Cengiz & Unlu 2007; Velmurugan, Selvanayagam, Cengiz & Unlu 2009). The gills are vital organs in fish that have important roles in the fish such as: respiration, osmoregulation, acid base balance and ammonia excretion (Health 1987). Fish gills are also sensitive to pollutants because have great surface area and located in outside position. For these reasons, fish gills have been considered as the most suitable indicators of water contamination. Although many researchers have reported the effect of organophosphate pesticides on gills (Dutta, Richmonds & Zeno 1993; Fanta, Rios, Romão, Vianna & Freiberger 2003; Velmurugan *et al*. 2009), there is no information as to the effects of toxins on aquatic animals in the Qare Soo River in north Iran.

Caspian roach, an anadromous species, is an economically important species (Coad 1980; Soleimani, Hoseinifar, Merrifield, Barati & Abadi 2011) and is also a main food source for sturgeon fish in the Caspian Sea (Keyvanshokooh & Kalbassi 2006). Similar to other Caspian Sea fishes (e.g. sturgeons) this species is considered at the risk due to overfishing, water pollution, and loss of habitat and spawning sites (Kiabi, Abdoli & Naderi 1999). Therefore Iranian Fisheries Organization attempted to restock this species.

As this fish is an anadromous species, the fingerlings should be released in rivers estuaries. The importance of both marine and freshwater environments in the life cycle of the fishes with reproductive migration has been demonstrated in numerous studies (McCormick, Hansen, Quinn & Saunders 1998; Waring & Moore 2004). Fresh water conditions of the life cycle of anadromous fish during the early stages can have a significant impact on survival and successful return of spawning adults in the marine environment. Thus, there is a significant correlation between the fingerlings which have been exposed to the contaminant and returning their adults (McCormick *et al*. 1998). The fingerlings at this age with adapting to the estuaries preparing themselves for life in the marine environment and this readiness along with many complex behavioral, physiological, and biochemical changes (McCormick *et al*. 1998). Salinity tolerance and increased hypo-osmoregulatory ability are necessary if fingerlings are to success survive in the marine environment. Accordingly, the histological changes of fish which migrate to the sea water have been also considered important. Nevertheless few studies have been done to examine the effects of fresh water pollutants on the subsequent stages of life in marine environment. In this study, we hypothesized that exposure of Caspian roach fingerling to sublethal diazinon in fresh water would lead to subsequent weakened brackish-water tolerance in fingerlings which is due to disruption of gill tissue and reduction in initial growth rate. We examined this hypothesis by exposing Caspian roach fingerling to diazinon for 96 h in fresh water followed by 240 h in brackish water.

Materials and Methods

Fish raring

In July 2012, Caspian roach fingerlings weighing 1.66 ± 0.05 g were obtained from the Caspian Sea Teleost Fish Reproduction Center (Sijowal-Golestan province, Iran). The study was conducted at the time to release fingerlings into river estuaries. Fingerlings were kept in experimental condition for at least one week and were randomly assigned into 12 tanks. Water temperature, dissolved oxygen, pH, salinity and Water were monitored daily and maintaining constant at 25.3°C, 7.01 mg L^{-1}, 7.8, 3.2 ppt and 150 ± 5 mg CO3Ca/l, respectively and were maintained in natural photoperiod (14L:10D). The tanks were continuously aerated and 10% of the water was changed daily. The toxicant was renewed daily in order to maintain constant concentration of the toxicant after exchange of the same volume of water. During the acclimatized period and transfer to brackishwater, the fish were fed twice daily by commercial fingerling food teleost (Sari animal feed and aquatic factories, Iran) and feeding was discontinued for 24 h before the start of the experiment.

Sublethal toxicity experiments

Twelve hundred Caspian roach were randomly divided into twelve 100 L fiberglass tanks (4 treatments in triplicate) to accomplish the 96 h period exposure to sublethal toxicity experiment. One hundred fingerlings were placed in each tank containing diazinon (Basudin 60 EM brand, Partonar Company) at concentrations 1.0, 2.0 and 3.0 mg L^{-1} that are 1/12th, 1/6th and 1/4th of 96 h LC50 value of acute toxicity(12 mg L^{-1}) that was reported by Mohammadnejad-Shamoshaki & Shahkar (2010). A control group was also considered.

Preparation of tissue samples

Fish were sampled after 24 h and 96 h post-exposure, the remaining fish were shifted to pesticide-free brackish water (salinity increase was done gradually during 12 h) and the samples and weight were analyzed at 240 h after transfer to brackish-water. Fish were anesthetized using clove oil at 150 ppm and gill tissues were fixed in 10% buffered formalin (Roberts 1989). Tissues sections of 5 µm were prepared and stained with haematoxylin-eosin (Cruz & Pitogo 1989). The sections were examined using light microscope.

Statistical analysis

Analysis of variance (ANOVA) followed by Duncan' test using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA) software was applied to determine the significant difference ($P < 0.05$) between weight of different treatment means.

Results

The structural details of the gill tissues are shown in Fig. 1A. At 24 h post-exposure to 1 mg L^{-1} diazinon, the gills of experimental fish showed shortening and edema in secondary lamellae with destruction of epithelial layer (Fig.1B). Shortening of secondary lamellae, hemorrhage at primary lamella, epithelial lifting, collapse of secondary lamellae and club-shaped lamella was noticed after 96 h post-exposure to 1 and 2 mg L^{-1} (Fig. 1A-B; Fig. 2B). At 96 h post-exposure to 3 mg L^{-1} diazinon, the gills of experimental fish showed epithelial hyperplasia, lamellar fusion, curling of secondary lamellae and epithelial lifting (Fig. 2A). After 240 h post-exposure to brackish water, tissue damage was slightly decreased (Fig. 2C-D). The major observed changes in the gills were collapsed of secondary lamellae, oedema, epithelial lifting and shortening of secondary lamellae. The histological changes affected by diazinon are shown in Table1. No change was observed in the gill tissues of control group.

The final weight was measured at the end of the experiment, weight gain in the control group was significantly higher than other groups ($P < 0.05$) (Table 2).

Discussion

Histopathological results showed that gill was the main target tissue influenced by diazinon and can be used as a model for environmental impact (Mallatt 1985; McKim & Erickson 1991), because of being the main route for the arrival pesticides. In fish, gills

Table 1 Summarized histopathological effects in the gill tissues of Caspian roach exposed to diazinon at 25.3 °C

Concentration ($mg L^{-1}$)	Terms	Shortening of secondary lamellae	Destruction of epithelial lamella	Haemorrhage in primary lamella	Oedema and epithelial lifting	Collapsed of secondary lamellae	Epithelial hyperplasia and lamellar fusion	Curling of secondary lamellae	Club-shaped lamella
Control		-	-	-	-	-	-	-	-
Group 1	24 h	+	+	-	+	+	-	-	-
	96 h	+	+	+	++	++	-	+	-
Group 2	24 h	+	-	-	++	+	-	-	+
	96 h	++	++	+	++	++	+	+	++
Group 3	24 h	++	++	+	+++	++	-	++	++
	96 h	++	+++	++	++	+++	++	++	++
Diazinon free brackish water									
Control		-	-	-	-	-	-	-	-
Group 1	240 h	+	+	-	+	-	-	+	+
Group 2	240 h	+	++	+	+	+	-	+	++
Group 3	240 h	++	+	+	++	++	-	+	+

None (−), mild (+), moderate (++), and severe (+++).

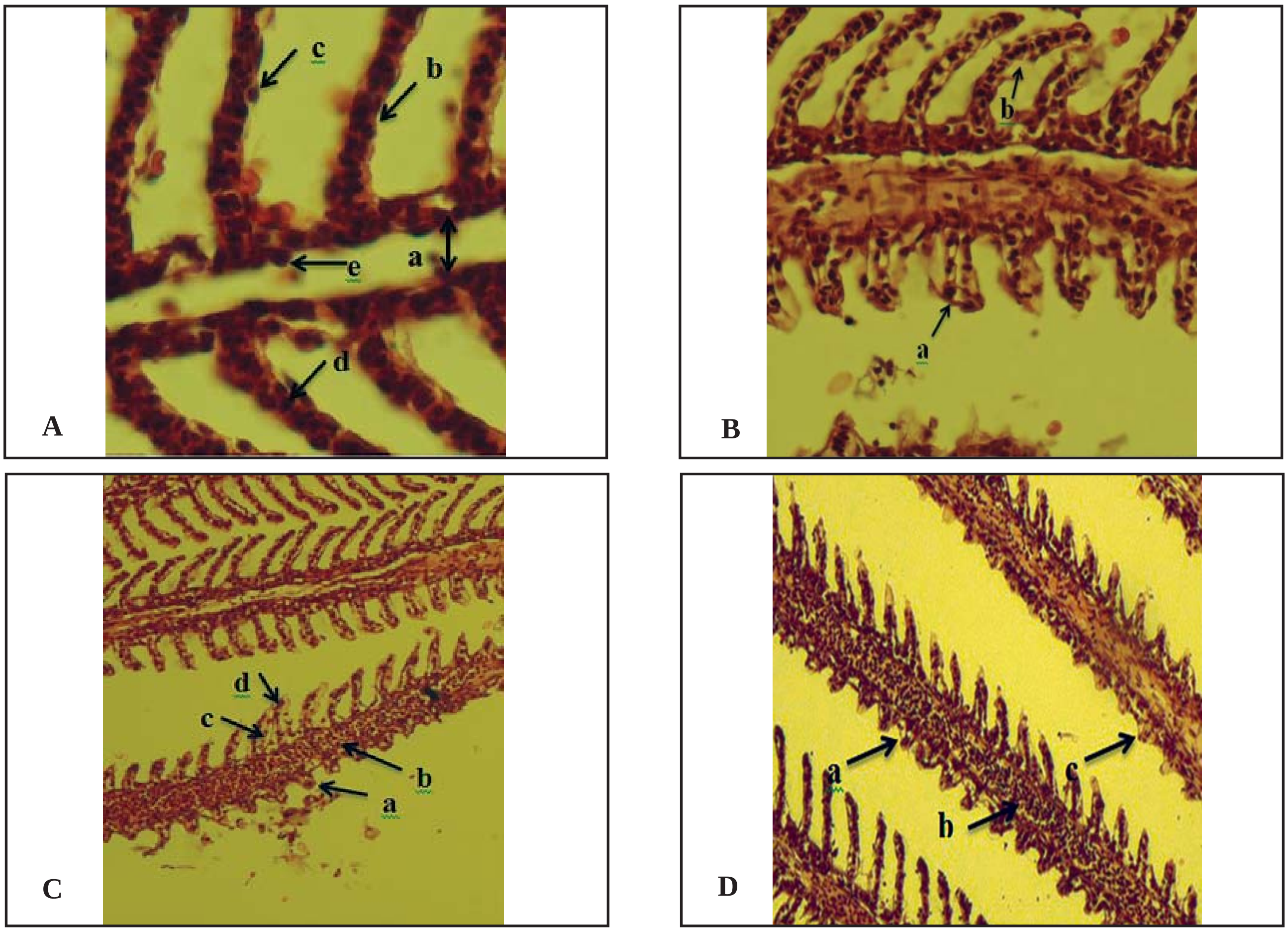

Figure 1 (A) Gill structure of control fish: (a) gill filament or primary lamella, (b) secondary lamellae, (c) epithelial cell, (d) pillar cell and (e) chloride cell. H&E, x 400. (B) Gill tissue of Caspian roach exposed to 1 mg L^{-1} diazinon for 24 h: (a) Shortening of secondary lamellae and oedema, (b) Destruction of epithelial lamella. (C) Gill tissue of Caspian roach exposed to 2 mg L^{-1} diazinon for 96 h: (a) Shortening of secondary lamellae, (b) Haemorrhage at primary lamella (c) Epithelial lifting and (d) Oedema. (D) Gill tissue of Caspian roach exposed to 1 mg L^{-1} diazinon for 96 h: (a) Shortening of secondary lamellae and oedema, (b) Haemorrhage at primary lamella and (c) Collapsed of secondary lamellae (H&E, x 100).

Table 2 Weight obtained after 240 h in brackish water

Fish weight (g)	The experimental groups			
	Control	Group 1	Group 2	Group 3
Initial weight	1.66 ± 0.05	1.66 ± 0.05	1.66 ± 0.05	1.66 ± 0.05
Final weight	1.95 ± 0.03^{c}	1.85 ± 0.02^{b}	1.87 ± 0.04^{b}	1.80 ± 0.02^{a}

Values in a row lines with different superscripts denote a significant difference ($P < 0.05$).

are vital organs for their respiratory, osmoregulatory and secretory functions. Respiratory decrease is one of the early signs of pesticide toxicity (Cengiz 2006) that may influence the physiological function or cause the death of fish.

In this study, some histological changes in 96 h after exposure to diazinon were shortening of secondary lamellae, oedema, destruction of epithelial lamella, epithelial lifting, curling of secondary lamellae, epithelial hyperplasia and lamellar fusion. After 240 h in brackish water, histological destructions were slightly reduced. Several other studies have shown similar effects of pesticides in gills of different fish species. For example, histopathological effects due to deltamethrin on the gills of Nile tilapia (*Oreochromis niloticus*) were reported by Yeldirim Benlı, Selvı, Özkul, Erkoç & Koçak (2006). In a similar experiment after exposure *Gnathonemus petersii* to atrazine, degenerative effects in the gill epithelium have been reported (Alazemi, Lewis & Andrews

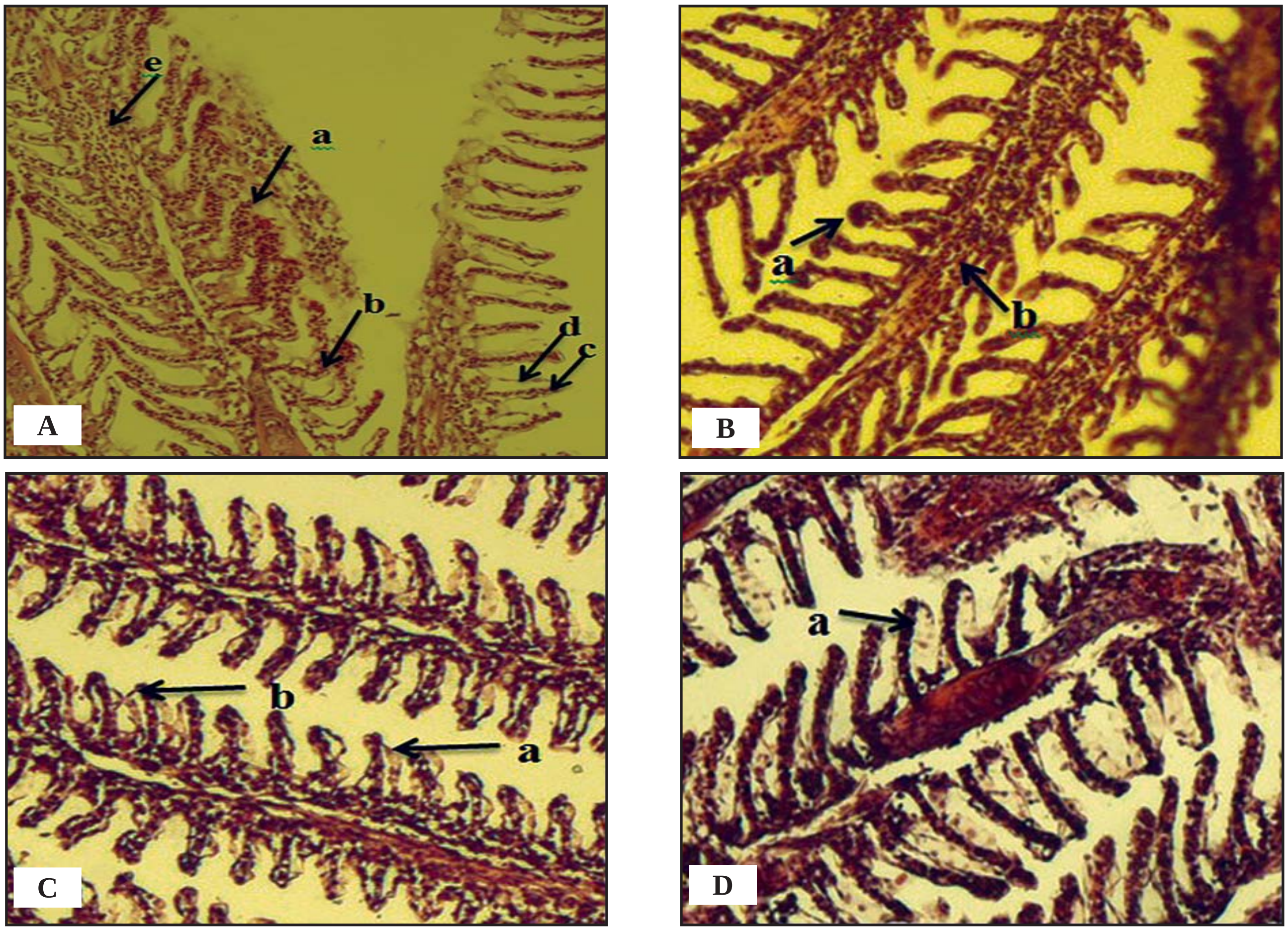

Figure 2 (A) Gill tissue of Caspian roach exposed to 3 mg L^{-1} diazinon for 96 h: (a) Epithelial hyperplasia and lamellar fusion, (b) Curling of secondary lamellae, (c) Oedema, (d) Epithelial lifting and (e) Haemorrhage at primary lamella. (B) Gill tissue of Caspian roach exposed to 2 mg L^{-1} diazinon for 96 h: (a) Club-shaped lamella and (b) Haemorrhage at primary lamella. (C) Gill tissue of Caspian roach in brackish water without diazinon for 240 h: (a) Oedema and (b) Epithelial lifting. (D) Gill tissue of Caspian roach in brackish water without diazinon for 240 h: (a) Epithelial lifting (H&E, x 100).

1996). Epithelial hyperplasia, aneurism, epithelial necrosis, desquamation, epithelial lifting, oedema and lamellar fusion were observed in mrigal (*Cirrhinus mrigala*) after exposure to sublethal concentrations of lambda-cyhalothrin (Velmurugan *et al*. 2007).

Dutta *et al*. (1993) found that Atlantic salmon (*Salmo salar*) exposed to diazinon showed variable changes, such as lifting of the epithelial layer, hyperplasia and necrosis, shortening of the lamellae and frequent epithelial rupture, lamellar fusion, mucous cells hypertrophy, extensive fusion, and clavate lamellae. Also when common carp (*Cyprinus carpio*) was exposed to diazinon, lamellar fusion and epithelial lifting of gills were seen (Oruç &Usta 2007).

Gill tissues injuries can be divided into two categories (Richmonds & Dutta 1989). For example, the observed epithelial damage of the gill is direct responses induced by the action of pesticide. The defense responses noticed are lifting up of the epithelium and lamellar fusion. The lifting of the epithelium can enhance the distance via the toxicant in the blood stream. Lamellar fusion is a rejoinder that it reduces the amount of sensitivity of gill surface area. Gill hyperplasia a type of defensive mechanism that leading to diminish in the respiratory area and an enhancement in the toxicant-blood diffusion distance (Cengiz 2006); such a defense response that occurs at the gills will cause a disorder in the respiratory activity (McGeer, Szebedinszky, McDonald & Wood 2000; Yeldirim *et al*. 2006).

Also, the weight of the fish was severely limited in this study (Table 2). This is because of the affected fish are not fed. These unfed fish also spent energy to compensate stress. Previous studies showed some inhibitory effects on fish growth (Arunachalam, Jeyalakshmi & Aboobucker 1980; Arunachalam & Palanichamy 1982; Roex, Vries, Gestel & Van 2002). Arunachalam *et al*. (1980) showed that when two species of Mystus exposed to carbaryl, the amount of growth rate was reduced. They concluded that the growth was limited because food energy is spent with an increased activity, unfavorable respiratory and opercular beating. In other study by McGeer *et al*. (2000), rainbow trout (*Oncorhynchus mykiss*) exposed to the pollution showed a decrease in growth rate due to the increased respiratory movements. In the present study there was no significant difference in mortality ($p< 0.05$). However, the amount of final weight in experimental groups significantly was reduced after 240 h post-exposure. All histopathological changes seen in this study indicated that exposure to sublethal concentrations of diazinon caused destructive effects in the gills of Caspian roach fingerlings. These changes were continued to several days after transfer of fish to free-pollution brackish water. This can cause stress or even mortality occurrence in the next phase of fingerlings lives in the sea. Also, in this study we found that after transferring fish to the brackish water, their growth rate was significantly less than the control group. This poor growth rate in the early stages of fish life may not compensate later in life which may result in failure of "Sea Reaching Project".

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اثرات تحت حاد سم دیازینون بر بافت آبشش و رشد بچه ماهیان کلمه (*Rutilus rutilus*) در آب شیرین و آب لب شور

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چکیده

ماهی کلمه (*Rutilus rutilus*) یکی از ماهیان مهم اقتصادی در دریای خزر بوده که ذخایر مربوط به آن درحال کاهش است. سالانه میلیون‌ها بچه ماهی از این گونه به صورت مصنوعی تولید و در دهانه رود خانه قره سو واقع در جنوب شرق دریای خزر رها می‌شوند که در معرض آفت کش‌های ناشی از مزارع برنج و باغات مرکبات قرار گرفته لذا اثرات این در معرض قرار گیری در تنظیم اسمزی این بچه ماهیان حائز اهمیت می‌باشد. در این مطالعه در ابتدا بچه ماهیان کلمه به مدت ۹۶ ساعت در معرض غلظت‌های مختلف دیازینون قرار گرفتند (مشابه غلظت‌های گزارش شده در مصب) و در مرحله بعد به مدت ۲۴۰ ساعت در آب لب شور قرار گرفتند و تغییرات بافت آبشش، رشد و بقای آن‌ها مورد بررسی قرار گرفت. بیشترین تغییرات مشاهده شده در ماهیان در معرض قرار گرفته شامل تخریب، تورم، تغییرات اپی تلیالی و کوتاهی تیغه‌های ثانویه آبشش بود. نتایج نشان داد که میزان تخریب بافت آبشش پس از انتقال به آب لب شور اندکی کاهش یافته در حالی که نرخ رشد در مقایسه با گروه شاهد به طور معنی‌داری کاهش داشت($p<۰/۰۵$). نتایج این آزمایش نشان داد که در معرض قرار گیری بچه ماهیان با دیازینون در آب شیرین می‌تواند قابلیت سازگاری آن‌ها را کاهش داده و به دنبال آن بقاء این ماهیان در مراحل بعدی زندگی در آب لب شور تحت تاثیر قرار گیرد که می‌تواند باعث کاهش کارایی پروژه بازسازی ذخایر ماهی کلمه شود.

واژه‌های کلیدی: ماهی کلمه، بافت شناسی، آبشش، رشد، دیازینون.

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