Original Article

Effects of Naphthalene on Plasma Cortisol and Thyroid Levels in Immature and Mature Female Klunzingeri Mulet, *Liza klunzingeri*

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ABSTARCT

Background: Polycyclic aromatic hydrocarbons (PAHs) such as naphthalene (NAP) are organic pollutants that have spread widely in littoral marine ecosystems. We aimed to study the effect of acute and prolonged exposure to naphthalene (NAP) on plasma cortisol and thyroid levels in mature and immature *Liza klunzingeri* mulet.

Methods: In acute stress experiment, the treatment group received 2µl g^{-1} sunflower oil containing NAP (50 mg kg^{-1}) intraperitoneally and the controls were injected with sunflower oil alone (2µl g^{-1}). Blood samples were obtained from both groups after 3 h. In prolonged stress experiment, 10 µl g^{-1} of coconut oil containing NAP (50mg kg^{-1}) was implanted and blood samples were obtained 72 h after injection.

Results: Both the acute and prolonged exposure induced significant increase in cortisol and a significant decrease (P<0.05) in thyroid hormone T4 levels. Thyroid T3 hormone levels only decreased significantly (P<0.05) after prolonged exposure. Acute exposure resulted in significant decrease (P<0.05) in T3/T4 ratio only in immature fish. On the other hand, prolonged stress increased T3/T4 ratio in immature fish.

Conclusion: Changes in the plasma levels of these hormones indicate low physiological capacity and survival potential of fish in waters polluted with naphthalene.

Keywords: Hydrocortisone, Naphthalenes, Polycyclic Aromatic Hydrocarbons, Thyroid Hormones, Triiodothyronine.

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) such as naphthalene (NAP) are organic pollutants spread widely in littoral marine ecosystems. These compounds commonly enter the water from the air, oil leaks as well as from industrial and domestic wastes [1]. PAHs are highly toxic to biological systems, and have considerable mutagenic and carcinogenic potential [2]. Furthermore, by induction of changes in the endocrine system, they may also affect physiological processes, which consequently disrupt essential processes like reproduction, metabolism, and the response to stress [3].

PAHs, as the ligands of aryl hydrocarbon receptors (AhRs), stimulate P450 1A cytochrome system, which consequently activate an enzymatic system responsible for removal of pollutants [4]. On the other hand, PAHs produce some intermediate compounds that interrupt the production and secretion of circulating hormones such as cortisol and thyroid hormones [5].These hormones play important roles in the responses of most animals such as fish to stress [5, 6]. Thyroid hormones (T_3 and T_4) and cortisol secretion are under the control of the hypothalamo-pituitary-thyroid and hypothalamo-pituitary-adrenal) systems [7, 8]. Thyroid hormones have crucial role in regulating the growth and balance of the hydro-mineral conditions of body fluids [9], while cortisol has a major role in regulating metabolic energy, stress responses, and immune system function [10]. Thyroid hormones and cortisol influence the metabolism of hydrocarbons [1]. Besides, they are used, in fish, as biomarkers [5]. Plasma levels of T_3, and T_4, and T_3/T_4 ratio can be used as suitable indices for estimating the metabolism state with respect to growth, rate of protein

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synthesis, and oxygen consumed by tissues [5, 11, 12].

In This research, levels of thyroid and cortisol hormones were used as biomarkers to evaluate chemical stress induced by NAP before and during maturation of mullet (*Liza klunzingeri*) a fish found worldwide in coastal temperate and tropical waters.

MATERIALS AND METHODS

Live female mullet fish (97 $\pm$ 3 g) were caught (November 2013) by trolling near Musa creek in the northern part of the Persian Gulf (Iran) and were transferred to the Imam Khomeini Fish Research Center. To adapt to the laboratory light and temperature conditions, they were kept in separate tanks (150 liter) for two weeks. To study the acute and prolonged effects of NAP exposure, two experimental protocols (injection and implantation of NAP dissolved in vegetable oil) were designed. Dosage selection was based upon published studies that evaluated the toxicological effects of PAHs on fish [13-15]. To study the acute effects of NAP, 70 fish were divided into the control and treatment groups. Under anesthesia (0.2% 2-phenoxyethanol), the fish were weighed and treated with sunflower oil (2µl g^{-1}) containing NAP (50 mg kg^{-1}) through peritoneal injection. The control group received sunflower oil alone. Three hours after injection, blood and gonad samples were taken. The procedure in the second experiment was the same as the first one except that the treatment group received a peritoneal implant of coconut oil (10µl g^{-1}) containing NAP (50 mg kg^{-1}). Blood and gonad samples were taken 72 h after NAP implantation.

Blood samples were obtained from the caudal vein using a heparinized syringe (2 ml), and were centrifuged (Hettich-D7200, Tuttlingen, Germany) at 6000 rpm for 6 min. The plasma was then rapidly frozen in liquid nitrogen, and stored at -80 ˚C for further analysis. To determine the sexual phase of the fish, the gonads were removed and fixed in Bouin's solution for 48 h. Then, samples were transferred to ethanol (70%) prior to histological studies. After histological preparation, paraffin blocks were cut (5 µm) and stained (hematoxiline-eosin). In the light microscope the previtellogenic and early vitellogenenic stages were identified (Olympus BH-Z- Japan) with Dinolite Digital Microscope software (AM211, ver. 3, AnMo Electronics Corp, Taiwan) [16].

Plasma Hormone Measurements

The amounts of cortisol, T_3, and T_4 hormones were measured by ELISA technique, according to the instructions of the manufacturer of a commercial kit (DIMETRA, Italy) [5, 17]. Absorbance in each well was measured at 450 nm in a microplate reader (IMMUNO-MINI; Nalge Nunc International) for hormone analysis. Samples from the fish with mature sexual oocyte and those with polygonal immature oocyte were used in the study.

Statistical Analysis

To compare cortisol, T_3 and T_4 hormones levels, as well as T_3/T_4 ratio in control and treatment groups at two sexual phases, two-way ANOVA was used. For multiple comparison the Student-Newman-Keuls test was used. The confidence coefficient was 95% (P<0.05). Moreover, Sigma plots (Systat Software, ver. 11, Inc., CA, USA) were used for analyzing the data and drawing the resulting diagrams.

Ethical Considerations

This research was performed according to convention of animal rights (approved by the Ethics Committee of Khoramshahr University of Marine Science and Technology). We tried to use animals (fish) without causing them unnecessary suffering if it could be avoided.

RESULTS

No abnormal behavior was observed in swimming, movements, and the rate of mortality during the experiments. Plasma cortisol levels were significantly increased (P<0.05) in acute (3 h) and prolonged (72 h) exposure to NAP in mature and immature fish groups as compared to control group (Figure1).

During the short-term exposure to NAP, the level of T_3 did not change, however in both mature and immature fish, T_4 levels significantly decreased (P<0.05). The ratio of T_3/T_4 showed significant decrease (P<0.05) in immature fish (Figure 2).

During prolonged exposure to NAP, T_3 and T_4 hormone levels reduced significantly (P<0.05) in mature and immature animals. Unlike to the short-term exposure, the ratio of T_3/T_4 showed a significant change (increase) (P<0.05) in immature fish (Figure 3).

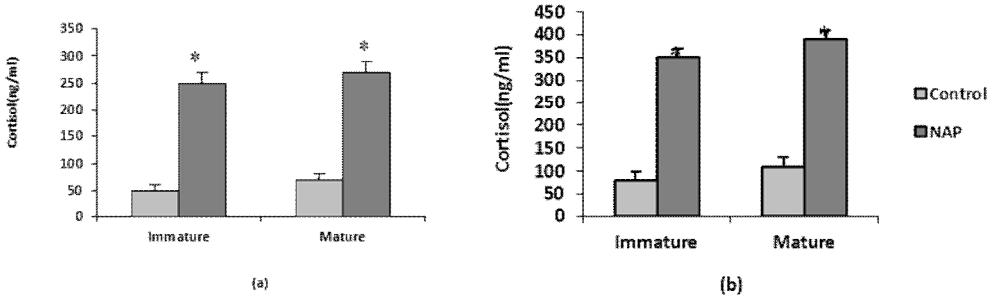

Figure 1. Effects of naphthalene treatment on the response of plasma cortisol levels in short term (a) and long term (b) exposure. Star symbol indicates significant difference in comparison with the control group (P<0.05).

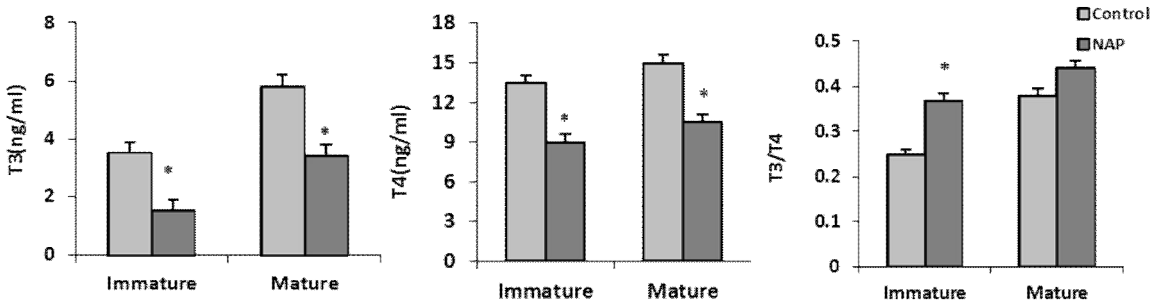

Figure 2. Effects of short-term exposure (3h) to naphthalene on the response of plasma T3, T4 and T3/T4. Star symbol indicates significant difference.

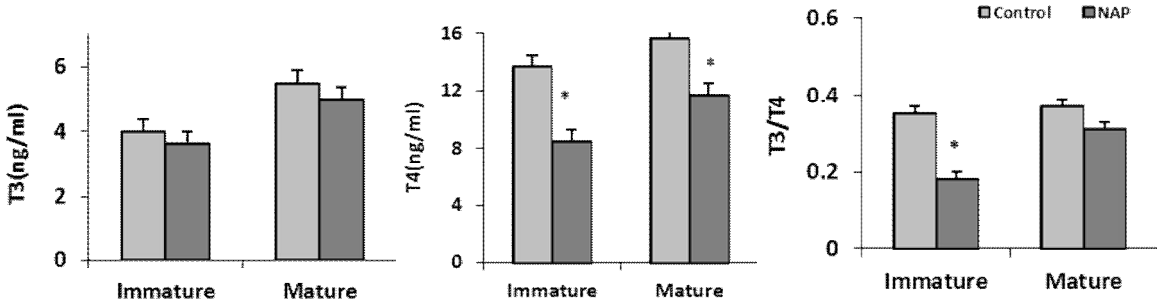

Figure 3. Effects of long-term exposure (72 h) to naphthalene on the response of plasma T3, T4 and T3/T4. Star symbol indicates significant difference.

DISCUSSION

Acute and prolonged exposure to NAP induces increase of plasma cortisol in both mature and immature mullets. These results are similar to those reported by Thomas et al. [18], Kennedy and Farrell [19], and Tintos et al. [20] on *Mugil cephalus*, *Clupea harengus*, and *Oncorhynchus mykiss* respectively. NAP activates AhRs [21], although the mechanism of action of AhRs is not fully understood but activation of these receptors affect hypothalamo-pituitary-adrenal axis, probably increases the expression of corticotropin releasing factor (CRF) gene [22] and consequently, increases the secretion of ACTH and cortisol. On the contrary, Teles et al. [6] reported that PAH resulted in decreased plasma cortisol level but they concluded that it could be the result of hypothalamo-pituitary-adrenal axis inefficiency or loss of mitochondrial cortisol-synthesizing enzymes.

Thyroid hormones are important in growth, metabolism, and homeostasis. Secretion

of thyrotropin-releasing hormone from hypothalamus releases thyroid-stimulating hormone (TSH) from pituitary gland into the plasma, which stimulates the release of thyroid hormones. The main difference between T3 and T4 is their ability to bond to the receptors. T3 bonds to receptors ten times more than that T4; therefore, it is considered as the main active biological form [5]. Acute exposure to NAP decreased plasma levels of T_4 in mature and immature fish. However, in prolonged exposure, both T_3 and T_4 levels were decreased in mature and immature animals. Changes in T_3/T_4 ratio were just observed in the immature fish in each experiment, which was due to their greater sensitivity. Organic pollutants may decrease thyroid hormones production and change their circulating concentrations [6, 23, 24]. However, the exact mechanism of effect of these pollutants is not clear.

Alkindi et al. [25] observed that petroleum hydrocarbons might decrease plasma levels of thyroid hormones, and increase plasma cortisol, in flounder fish. Stephens et al. [26] reported similar results in *O. mykiss*. Decreased thyroid hormones and T_3/T_4 ratio variations in regions polluted with crude oil were reported earlier [6]. Increased deiodination, biliary excretion of thyroid hormones [12], negative feedback in the hypothalamo-pituitary-thyroid axis [25], and disruption in the function of the hepatic 5'-monodeiodinase [27] would decrease T_3 and T_4 in plasma, and change T_3/T_4 ratios. Furthermore, NAP might affect CYP1A1 and AhRs gene expression [4]. Expression of these genes may inhibit the expression of genes related to thyroid hormones receptors and even the genes responsible for thyroid hormones synthesis [5].

CONCLUSION

It seems that acute and prolonged exposure to NAP increase plasma cortisol level and decrease thyroid hormones concentrations. These changes were more intense in immature fish. It appears that in female fish, immature ovary is more sensitive to pollutants. Considering the key role of thyroid hormones and cortisol in osmotic regulation and metabolism, changes in plasma levels of these hormones may cause low physiological capacity and survival potential of fish in polluted environment.

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