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## Histological structure of thyroid gland and level of thyroid hormones in tadpoles exposed to oil and petroleum products

**Abstract.** Growing global demand and growth in oil production and refining lead to an increase in environmental pollution by waste from these industries. Huge territories of Kazakhstan are influenced by the activities of the oil and oil refining industries. The consequence of this is the deterioration of ecosystems in the oil-producing regions, the decline in biodiversity and deterioration of public health. In this regard, there is a need in an informative bioindicator for studying the state of ecosystems of oil producing regions. The aim of this research was to evaluate the effects of oil and petroleum products exposure on the function of thyroid gland in tadpoles of local amphibian species. The study revealed that chronic exposure to water-soluble fraction of oil, o-xylene or diesel fuel causes hypertrophy and hyperplasia of the thyroid follicular cells, a decrease in the colloid volume in the follicles, as well as a decrease in the content of thyroid hormones in the tadpoles of the marsh frog (*Rana ridibunda*) and green toad (*Bufo viridis*), which indicates a suppression of thyroid function.

**Key words:** thyroid histostructure, thyroid hormones, water-soluble fraction of oil, petroleum products, amphibians, tadpoles.

### Introduction

Deterioration of ecosystems, reduction of biodiversity and deterioration of human health is a well-known problem in the oil producing regions of Kazakhstan [1-2]. Oil spills on the water surface, as well as leak of chemical compounds of oil and petroleum products with the wastewater of enterprises through groundwater are dangerous to the aquatic environment. This poses a great risk to aquatic organisms, causing significant mortality among fish, amphibians and invertebrates [3]. Moreover, not only adults are sensitive to the action of pollutants, but also juveniles, larvae, and embryos, the death of which can lead to a decrease in biological resources. In this regard, it becomes relevant to study the toxic effects of oil and petroleum products on the growth and development of aquatic organisms. In this regard, amphibians are a convenient model system for assessing the state of both terrestrial and aquatic ecosystems under conditions of anthropogenic transformation and environmental pollution [4; 5]. For example, marsh frog (*Rana ridibunda*) and green toad (*Bufo viridis*) can serve as such ob-

jects, due to the wide distribution in Kazakhstan, including oil-producing regions [6-9].

It is known that the process of growth and development of vertebrates is controlled by thyroid hormones (TH) secreted by the thyroid gland [10; 11]. TH play a vital regulatory and conservative role in the development of many body systems of amphibians, like in all other vertebrate classes [12-14]. The main toxic components of oil, such as naphthenic acids (NA) and polycyclic aromatic hydrocarbons (PAHs) negatively affect the functioning of the endocrine system in amphibians. NAs can directly reduce the rate of metamorphosis in *X. tropicalis* and *R. pipiens*, and PAHs can significantly reduce the rate of metamorphosis in *X. laevis* [15; 16]. Since the thyroid gland stores produced TH extracellularly (in the follicles), changes in the histological structure of the thyroid gland are widely used to evaluate TH production during development and metamorphosis. Numerous studies of ontogenesis and activity of the thyroid gland in amphibians undergoing the process of metamorphosis confirmed the presence of a correlation between the activity of the thyroid gland and the duration of the larval period [11]. Histopathological changes in the thyroid gland are often the most

sensitive indicator of the adverse effects of chemicals on thyroid function [17]. It is believed that an increase in the follicular space of the thyroid gland, changes in follicular deformation and proliferation of follicular cells are associated with a decrease in the secretion of thyroid hormones [18; 19]. Unfortunately, there is a lack of knowledge on the functioning of the thyroid gland in developing amphibians of natural populations of Kazakhstan under oil pollution conditions. In this regard, the aim of our research was to study the effect of the chronic exposure to water-soluble fraction of crude oil (WSFO) and petroleum products (o-xylene or diesel fuel) on the thyroid gland of *R. ridibunda* and *B. viridis* tadpoles.

### Materials and methods

In our previous studies, the analysis of WSFO showed a significant content of o-xylene [20], in connection with which it was decided to study its effect on the functioning of the thyroid gland. Diesel fuel was chosen as a toxicant due to its use as a fuel in water transport. The concentrations were chosen according to the MPC value for petroleum products in water [21].

*Preparation of WSFO.* 100 ml of oil was mixed with 900 ml of distilled water, then placed in a 1 L flask with a tightly closed stopper and stirred in the dark for 18 hours, avoiding violation of the integrity of the oil film and its emulsification. The resulting mixture was left at room temperature for 6 hours. Next, the water-soluble fraction was extracted using a separatory funnel and stored at 4 °C. Before using in experiments, the water-soluble fraction was warmed to room temperature (21-23 °C) [22; 23].

*Obtaining of eggs and experimental design of chronic experiments.* Eggs and tadpole care were carried out according to the previously described methodology [20; 24]. Briefly, to obtain eggs, fifteen sexually mature individuals of *R. ridibunda* and *B. viridis* (nine males and six females) were caught from reservoirs of oil-contaminated regions of Kazakhstan, and then delivered to the Ecotoxicology laboratory, Faculty of Biology and Biotechnology, al-Farabi KazNU. After acclimatization of amphibians to laboratory conditions, hormonal stimulation of spawning using hormonal mixture AMPHIPLEX [25] was performed. The larvae that developed to active feeding stage were placed in 18 L aquariums filled with 15 L of pure dechlorinated aerated water (24-26°C). The tadpoles were divided into 4 groups of 15 tadpoles in each: control, 0.05 mg/L, 0.5 mg/L and 1.5 mg/L of WSFO or petroleum products in triplicate. Every

2 days, 50% of the water was replaced, after which a new dose of WSFO and petroleum products was introduced. At the end of the experiment, tadpoles that reached the stage of metamorphic climax were euthanized in a buffered anesthetic solution (3-aminobenzoic acid ethyl ester (MS-222; Sigma, USA), fixed in 10% formalin for histological examination or frozen in liquid nitrogen, and then stored at -80 °C until further TH analysis.

*Histological and morphometric examination of the thyroid gland.* After fixation, the material was washed in running tap water for 12 h, then dehydrated in a series of ascending alcohols, purified in xylene and placed in paraffin. Next, sections of 5 µm thickness were made on the MS-2 microtome. Sections were stained with hematoxylin and eosin and covered with Bio-Mount synthetic medium (Bio-Optica, Italy). During dehydration, pouring into paraffin and processing of paraffin sections, isopropyl alcohol was used [26]. Analysis of sections was carried out using a Leica DMLB2 light microscope with a Leica DFC 320 digital camera and microphotography at various magnifications: x200 and x400 were taken. Morphometric analysis of the obtained micrographs was carried out using special BioVision software (version 4.0). In this case, the follicle diameter and the height of the thyroid follicular cells were measured.

*Determination of thyroid hormone content in tadpoles.* Thyroid hormone extraction was performed based on a method developed by Brasfield *et al.* [27]. At the end of the chronic experiment, the tadpoles were frozen in liquid nitrogen immediately after euthanasia. Tissue manipulations were performed on ice. A homogenization buffer consisting of 1 mM 6-propyl-2-thiouracil (Sigma Aldrich, USA) in 95% ethanol was prepared before extraction and stored at -20 °C in a glass bottle. The tadpoles were weighed, ground in an equal amount of a homogenizing buffer and transferred to 16 mm x 100 mm glass tubes for cultivation. A second buffer volume equal to the tadpole volume was then added to the obtained homogenate before further homogenization using a Potter homogenizer. Then the samples were shaken for 1 min and kept on ice. Each sample was centrifuged at 2900 rpm at 4 °C for 10 minutes in a centrifuge. Next, the supernatant was transferred into a glass tube. The described procedure was repeated with the obtained precipitate. The resulting supernatant containing ethanol used in the extraction and thyroid hormones was combined with the first supernatant. Then this sample was evaporated in a stream of nitrogen in a water bath at 50 °C, so that the final volume was equal to the initial volume of the tadpole. The

extract was divided into 150 ml aliquots and stored at -80 °C until analysis. Thyroid hormones were quantified in an extract obtained from whole tadpoles using enzyme-linked immunosorbent assay kits (T3-BQ043T for triiodothyronine (T3) and T4-BQ044T for thyroxine (T4), BioQuant Inc., USA).

*Statistical analysis.* Data were analyzed for statistical significance using a one-way ANOVA followed by a Tukey test using SPSS software version 23 (IBM Inc., Chicago, USA) with  $\alpha$  set to 0.05. Before analysis, the data were analyzed for homogeneity of variance using the Levene's test [28].

All work with the animals was carried out in full accordance with the requirements of scientific ethics and international standards, and regulatory legal acts of the Republic of Kazakhstan [29-33].

## Results and discussion

*Histological structure of the thyroid gland R. ridibunda and B. viridis under chronic exposure to WSFO and petroleum products.* A microscopic examination of the thyroid gland of *R. ridibunda* and *B. viridis* tadpoles during chronic exposure to WSFO or petroleum products revealed pathological changes in the histological structure of the organ, such as a decrease in the volume of colloid in the follicles, hyperplasia and hypertrophy of thyrocytes.

The thyroid histostucture of *R. ridibunda* tadpoles in the control group was normal. Follicular cells formed a cuboidal epithelium. The nuclei of follicular cells were compacted and had an oval or round shape. The cytoplasm of these cells was acidophilic, homogeneous. Thyroid follicles contained an eosinophilic homogeneous colloid and were lined with a single layer of cuboidal follicular cells. Single foci of hyperplasia and hypertrophy of follicular cells, which may be present in the normal thyroid gland, were noted.

Changes during chronic exposure to WSFO, o-xylene, and diesel fuel were of a similar nature and were expressed in violation of the structure of the thyroid follicles. Moreover, the severity of pathological changes depended on the concentration of the active substance. Follicular cells under the influence of 0.05 mg/L WSFO, o-xylene or diesel fuel had a structure close to normal, however, cell hyperplasia and a decrease in the amount of colloid in some follicles were observed, which indicates atrophic processes in the thyroid gland.

Chronic exposure to 0.5 mg/L of WSFO or petroleum products caused a structural disorder of about 60% of thyroid tissue. Colloid was observed in only a

few follicles and its amount was noticeably less compared to the control. The thyrocytes of most follicles were prismatic and hypertrophied.

With chronic exposure to 1.5 mg/L of WSFO, o-xylene or diesel fuel, more than 80% of the thyroid tissue of the *R. ridibunda* tadpoles underwent pathological changes. Hyperplasia of follicular cells was observed, manifested in the appearance of stratification of the glandular epithelium. Hypertrophied thyrocytes had a prismatic shape; in most follicles, a lack of colloid was noted.

A study of the histological structure of the thyroid gland of *B. viridis* tadpoles during chronic exposure to WSFO or petroleum products revealed similar changes. In the tadpoles of the control group, the structure of the thyroid gland was normal. The severity of pathological changes also had a dose-dependent nature. Under chronic exposure to low doses (0.05 mg/L) of WSFO, o-xylene, or diesel fuel, the histostucture of the thyroid of the *B. viridis* tadpoles slightly differed from the norm. Foci of hyperplasia and a decrease in the size of follicles were observed. Most thyrocytes had a cuboidal shape, but there were follicles lined with prismatic epithelium.

With chronic exposure to 0.5 mg/L of WSFO and petroleum products on the tadpoles of *B. viridis*, atrophy of the thyroid gland was observed. A decrease in the number of follicles containing colloid was observed, while their size was smaller compared to the control, which indicated a decrease in thyroid function. The follicular epithelium became stratified, which indicated hyperplasia of thyrocytes.

During chronic exposure to 1.5 ml/L of oil and petroleum products in most tadpoles exposed to high concentrations of oil and petroleum products, the thyroid gland was hypertrophied. A decrease in the volume of follicles was noted, as well as a thickening of the glandular epithelium due to hyperplasia and hypertrophy of follicular cells. At the same time, in tadpoles, which had the largest developmental delay, an almost complete atrophy of the thyroid gland was noted. In this case, small follicles with a small content or complete absence of a colloid were observed, lined with one or several layers of cuboidal thyrocytes.

To confirm the observed changes in the histostucture of the thyroid gland of *R. ridibunda* and *B. viridis* tadpoles, a morphometric study was carried out, which included measuring the diameter of the follicles and the height of follicular cells.

The results of a morphometric study of the thyroid gland of *R. ridibunda* and *B. viridis* are presented in table 1 and 2.

**Table 1** – The follicles diameter and the follicular cells height of the thyroid gland of *R. ridibunda* tadpoles after chronic exposure to WSFO or oil products,  $\mu\text{m}$ 

Concentration	WSFO	O-xylene	Diesel fuel
	follicles diameter		
Control	53.14±17.21	49.45±12.43	56.33±15.58
0.05 mg/L	51.2±23.11	47.91±16.30	53.27±21.18
0.5 mg/L	39.64±14.70**	38.39±11.46***	43.72±13.66**
1.5 mg/L	24.82±8.36***	28.47±10.52***	28.53±11.23***
	follicular cells height		
Control	6.22±1.10	5.83±1.32	5.92±1.81
0.05 mg/L	5.91±2.04	5.78±1.40	5.96±1.73
0.5 mg/L	3.27±1.41***	3.61±2.52**	3.44±1.30***
1.5 mg/L	2.85±1.10***	3.19±1.60***	3.03±1.51***

Note: \*\*\* –  $P \leq 0.001$ , \*\* –  $P \leq 0.01$  compared to control

As can be seen from the tables, the thyroid follicles diameter in the tadpoles of *R. ridibunda* and *B. viridis* exposed to chronic exposure to WSFO or petroleum products was significantly smaller compared to control. At the same time, high concentrations of oil and petroleum products (1.5 mg/L) had the most

pronounced inhibitory effect, causing a 1.6-2.1 fold reduction in follicles compared to control values. Similarly, a decrease in the height of follicular cells was observed when exposed to WSFO or petroleum products. Changes in this parameter were more pronounced with increasing concentration of treatment compounds.

**Table 2** – The follicles diameter and the follicular cells height of the thyroid gland of *B. viridis* tadpoles after chronic exposure to WSFO or oil products,  $\mu\text{m}$ 

Concentration	WSFO	O-xylene	Diesel fuel
	follicles diameter		
Control	37.18±11.41	35.92±13.17	40.21±9.61
0.05 mg/L	38.56±13.20	33.56±12.22	39.44±12.10
0.5 mg/L	24.78±13.11**	27.63±9.81***	28.72±8.76**
1.5 mg/L	19.56±9.72***	22.45±10.12***	23.64±8.93***
	follicular cells height		
Control	5.44±1.31	5.56±1.07	5.29±1.26
0.05 mg/L	5.25±1.07	5.67±1.46	5.06±1.47
0.5 mg/L	3.09±1.14***	3.68±1.45**	3.26±1.09***
1.5 mg/L	2.62±1.18***	3.05±1.33***	2.77±1.05***

Note: \*\*\* –  $P \leq 0.001$ , \*\* –  $P \leq 0.01$  compared to control

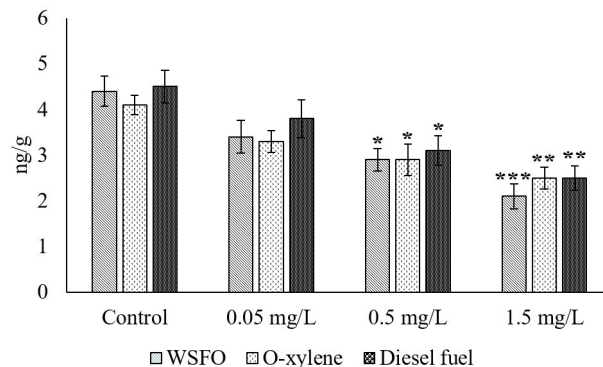
Disruption of thyroid gland function in the tadpoles of *R. ridibunda* and *B. viridis* shown by histological examination was confirmed with morphometrical measurements. The analysis of morphometric data revealed notable reduction of thyroid follicles diameter and decrease of follicular cells height in both studied species. However, there was no signifi-

cant difference between the species. It is generally accepted that histological examination of the thyroid gland is an important method for determining the ability of a chemical substance to affect the synthesis of thyroid hormones [18], as was shown on *X. laevis*, *X. tropicalis* and *R. rugosa* [34; 35]. Histopathological changes in the thyroid gland can be caused by various

chemicals [36; 37]. Typically, thyroid histopathology is characterized by a decrease in colloid, atrophy, gland hypertrophy, as well as cell hyperplasia and hypertrophy [17; 18]. A histomorphological study of the thyroid of *R. ridibunda* and *B. viridis* tadpoles under chronic exposure to WSFO or petroleum products showed a decrease in follicle size, hyperplasia and hypertrophy of thyrocytes. In tadpoles with the greatest developmental delay, thyroid atrophy was noted. According to foreign researchers, a decrease in the amount of colloid in the follicles indicates a predominance of secretion of thyroid hormones over their accumulation in the follicles of the thyroid gland. Thus, the size of the follicle may correlate with the level of thyroid hormones circulating in the blood [38]. In a study by Wang et al. [39] when studying the effect of copper on the growth and development of *B. gargarizans*, hyperplasia of follicular cells in the thyroid gland was detected. According to diagnostic criteria [18], follicular cell hyperplasia is associated with a decrease in the secretion of thyroid hormones in the thyroid gland. Similarly, in a recent study, exposure to lead also induced follicular cell hyperplasia and depletion of colloids in the thyroid gland. In addition, some follicles were markedly increased in thickness and height of the layer of epithelial cells, which led to an increase in the size of the thyroid gland [40]. It is known that the histological structure of the thyroid gland reflects its functional state, i.e. storage and release of thyroid hormones [41]. Therefore, the changes in the thyroid gland histostucture observed during chronic exposure to hypertension and petroleum products indicate a violation of the thyroid hormone homeostasis in tadpoles and, therefore, their effect on the growth and development of *R. ridibunda* and *B. viridis* tadpoles. This is confirmed by the fact that under the influence of WSFO or petroleum products a developmental delay, weight loss and a decrease in the body size of intoxicated tadpoles were found [20].

**The content of hormones T3 and T4.** The content of thyroid hormones in *R. ridibunda* tadpoles after chronic exposure to WSFO or petroleum products is shown on Figure 1. As can be seen in the figure, chronic exposure to 0.05 mg/L WSFO reduced the T4 content in tadpole by 1.2-fold. When exposed to 0.5 mg/L, the T4 content in the tadpoles was 1.5-fold lower compared to the control. Exposure to 1.5 mg/L WSFO caused a 1.8-fold decrease in T4 content. When exposed to 0.05 mg/L of o-xylene, the difference in the T4 content in the body of the tadpoles was insignificant. Compared with the control, when exposed to 0.5 and 1.5 mg/L of o-xylene, the T4 content was 1.2 and 1.4-fold lower, respectively. Simi-

larly, a chronic exposure of 0.05; 0.5 and 1.5 mg/L of diesel fuel led to a decrease in the content of T4 in the tadpoles of *R. ridibunda* by 1.2; 1.3 and 1.5-fold, respectively.

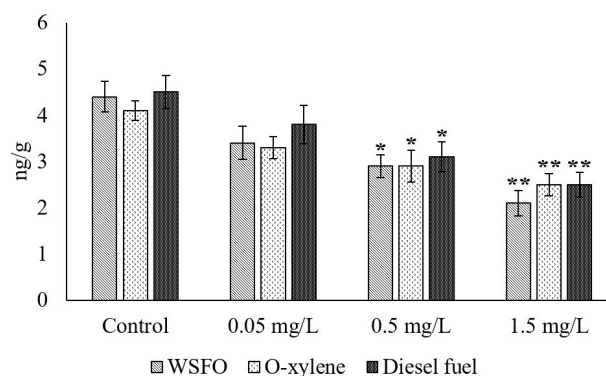


**Figure 1** – The content of T4 hormone in *R. ridibunda* tadpoles after chronic exposure to WSFO, o-xylene or diesel fuel.

Note: \*\*\* –  $P \leq 0.001$ , \*\* –  $P \leq 0.01$ ,

\* –  $P \leq 0.05$  compared to control

Similar changes in T4 content were observed in *B. viridis* tadpoles (Figure 2). Chronic exposure to 0.05 mg/L of WSFO caused a decrease in T4 content by 1.3-fold, 0.5 mg/L by 1.5-fold, 1.5 mg/L by 2.1-fold. With chronic exposure to 0.05 mg/L of o-xylene, a decrease in T4 content by 1.2-fold was noted. In the tadpoles exposed to 0.5 mg/L o-xylene, the T4 content was 1.4-fold lower than in the tadpoles of the control group. When exposed to 1.5 mg/L of o-xylene, the decrease was 1.6-fold. The impact of diesel fuel also led to a reduced T4 content in the *B. viridis* tadpoles: at 0.05 mg/L – 1.2-fold, at 0.5 mg/L – 1.5-fold, at 1.5 mg/L – 1.8-fold.



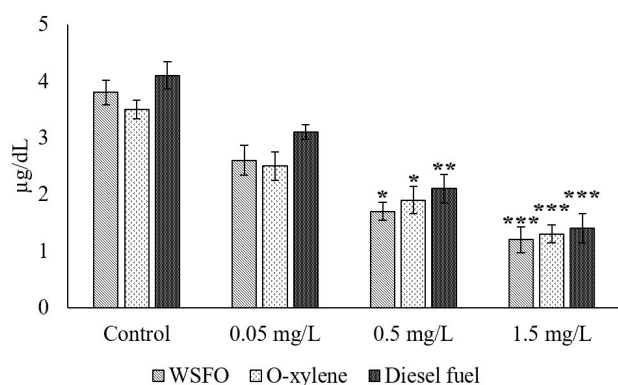
**Figure 2** – The content of T4 hormone in *B. viridis* tadpoles after chronic exposure to WSFO, o-xylene or diesel fuel.

Note: \*\*\* –  $P \leq 0.001$ , \*\* –  $P \leq 0.01$ ,

\* –  $P \leq 0.05$  compared to control

Analysis of the T3 content in *R. ridibunda* tadpoles showed significant changes (Figure 3). Expo-

sure to WSFO at a concentration of 0.05 mg/L caused a 1.5-fold decrease in T3 content.

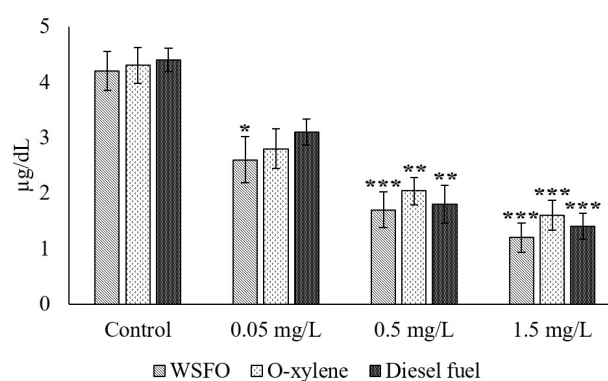


**Figure 3** – The content of T3 hormone in *R. ridibunda* tadpoles after chronic exposure to WSFO, o-xylene or diesel fuel. Note: \*\*\* –  $P \leq 0.001$ , \*\* –  $P \leq 0.01$ , \* –  $P \leq 0.05$  compared to control

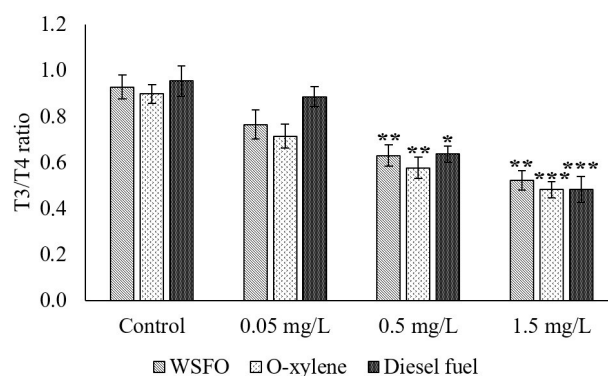
Higher concentrations reduced the T3 content by 2.2-fold when exposed to 0.5 mg/L and by 3.2-fold when exposed to 1.5 mg/L. With chronic exposure to o-xylene at a concentration of 0.05 mg/L, the T3 content was 1.5-fold lower. Exposure to 0.5 mg/L of o-xylene led to a 1.8-fold decrease in the T3 content. When exposed to 1.5 mg/L of o-xylene, the decrease was 2.7-fold compared with the control. When exposed to diesel fuel at a concentration of 0.05; 0.5 and 1.5 mg/L, a decrease in the content of T3 in *R. ridibunda* tadpoles by 1.3-fold; 2.0-fold and 2.9-fold, respectively, was also observed.

The content of T3 in *B. viridis* tadpoles was reduced by 1.6-fold when exposed to 0.05 mg/L of WSFO (Figure 4). T3 content in 0.5 mg/L WSFO treated tadpoles was 2.5-fold lower than the control. The largest decrease of 3.8-fold was found when exposed to 1.5 mg/L of WSFO. Exposure to o-xylene led to a decrease in the T3 content in *B. viridis* tadpoles at 0.05 mg/L – 1.5-fold, at 0.5 mg/L – 2.4-fold, at 1.5 mg/L – 3.1-fold. Under chronic exposure to 0.05 mg/L diesel fuel, a 1.4-fold decrease in T3 content was observed. Exposure to higher concentrations (0.5 and 1.5 mg/L) caused a decrease in T3 content by 2.4 and 3.4-fold, respectively, compared with the control.

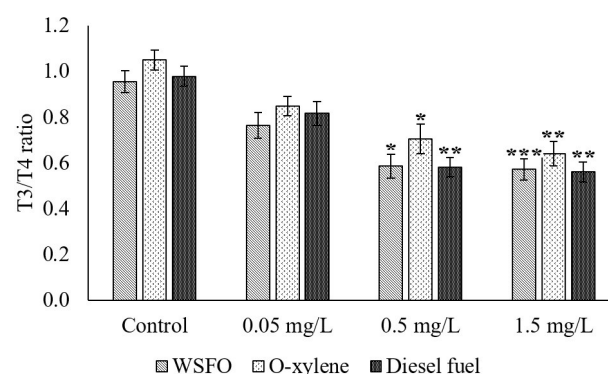
The ratio of the content of T3 to T4 in the body of tadpoles shows the completeness of the conversion of T4 into a more active form of the hormone – in T3. Studies have shown that the lowest ratio of triiodothyronine to thyroxine was in tadpoles exposed to high concentrations of WSFO or petroleum products (Figures 5 and 6).



**Figure 4** – The content of T3 hormone in *B. viridis* tadpoles after chronic exposure to WSFO, o-xylene or diesel fuel. Note: \*\*\* –  $P \leq 0.001$ , \*\* –  $P \leq 0.01$ , \* –  $P \leq 0.05$  compared to control



**Figure 5** – The content of T3 and T4 hormones ratio in the *R. ridibunda* tadpoles after chronic exposure to WSFO, o-xylene or diesel fuel. Note: \*\*\* –  $P \leq 0.001$ , \*\* –  $P \leq 0.01$ , \* –  $P \leq 0.05$  compared to control



**Figure 6** – The content of T3 and T4 hormones ratio in the *B. viridis* tadpoles after chronic exposure to WSFO, o-xylene or diesel fuel. Note: \*\*\* –  $P \leq 0.001$ , \*\* –  $P \leq 0.01$ , \* –  $P \leq 0.05$  compared to control

A study of the effects of various concentrations of WSFO and petroleum products revealed a significant



decrease in the content of T3 and T4 in *R. ridibunda* and *B. viridis* tadpoles compared to the control. The T3:T4 ratio, which reflects the rate of conversion of T4 to T3 [42], was the lowest among tadpoles exposed to high concentrations of WSFO or petroleum products. A lower T3: T4 ratio in tadpoles from groups exposed to 0.5 and 1.5 mg/L of WSFO or petroleum products may result from changes in the normal physiology of tadpoles by contaminants. Similar to the changes in histostructure of thyroid gland, difference in alteration of T3 and T4 levels between *R. ridibunda* and *B. viridis* tadpoles was insignificant.

Two possible normal physiological phenomena that pollutants might have acted, as Shi [12] suggested, are the incomplete conversion of all T4 to T3, as well as the conversion of T3 to reverse T3 (rT3) by the enzyme deiodinase 3. Both of these processes can lead to decrease in the concentration of T3 in relation to T4. These changes in the functional state of the thyroid gland are probably responsible for the delay in the metamorphosis of the tadpoles. An impaired metabolism of thyroid hormones can have other consequences for frogs, since thyroid hormones are necessary for normal, competent immunological function and growth, along with other metabolic processes. Thyroid hormones have similar functions in a large number of vertebrates, including regulation of growth and development. Thyroid hormones are crucial for amphibian metamorphosis [12] and have complex functions that control multiple events, such as tail resorption and development of the forelimbs. Metamorphosis changes in the absence of normal levels of thyroid hormones, although stopped metamorphosis can be resumed if thyroid hormones are introduced [43]. The endocrine destructive potential of these compounds is apparently quite stable in the environment since the ratio of T3/T4 changed significantly in the tadpoles of *R. sylvatica* exposed to water from ponds of wetlands located near reclaimed oil sands, and also changed the rate of metamorphosis depending on the age of the reclaimed wetlands [44]. Thus, it was shown that the study of histological structure and hormones of thyroid gland in tadpoles of amphibians may serve as an informative indicator of petroleum contamination.

### Conclusion

Our results indicate a violation of the thyroid gland of the tadpoles of anuran amphibians in oil pollution conditions. Hypertrophy and hyperplasia of follicular cells, as well as a decrease in the volume of colloid in the follicles, found in the thyroid gland his-

tostructure of the tadpoles of amphibians, representatives of natural populations of Kazakhstan, indicate a violation of its function and TH homeostasis. As a result of the study, it was found that during chronic exposure to the water-soluble fraction of crude oil or petroleum products (o-xylene, diesel fuel), the content of triiodothyronine and thyroxine in the tadpoles of *R. ridibunda* and *B. viridis* decreases, which also indicates a suppression of thyroid function.

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