
SHORT
COMMUNICATIONS

Influence of Triiodothyronine (T₃) on the Reproduction and Development of the Green Terror *Andinoacara rivulatus* (Cichlidae)¹

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Abstract—It has been shown experimentally that hyperthyroidism, caused by prolonged treatment of triiodothyronine, influences on the development of secondary sexual characteristics, fertility and spawning behavior of the green terror *Andinoacara rivulatus*. In hyperthyroid fish with pronounced morphological consequences due to heterochronies, decreases the number of eggs in clutches and increases the time interval between spawnings. At the same time, hyperthyroidism in the parental pairs does not have any influence on the embryonic and early postembryonic development of offspring (hatching time, survival and frequency of developmental abnormalities). The obtained results give prospect for further studying the role of the thyroid signaling pathway in the regulation of ontogenesis in teleost fish.

Keywords: green terror *Andinoacara rivulatus*, thyroid hormones, triiodothyronine, heterochronies, reproduction, developmental abnormalities

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The thyroid hormones (TH) (prohormone thyroxine and biologically active triiodothyronine) are the most important signaling factor playing a decisive role in the ontogenesis of teleost fish. TH effect the activity of a wider range of tissues and biological functions than any other hormones, forming complexes with specific nuclear receptors they are able to control the timing and activity of transcription of numerous target genes (Janz, 2000; Power et al., 2001; Blanton and Specker, 2007).

Reproduction in teleost fish depends on the level of thyroid hormones. TH participate in the differentiation of gonads and influence the reproductive system of fish (Cyr and Eales, 1996; Swapna and Senthilkumaran, 2007; Duarte-Guterman et al., 2014). The action of TH can pass place at different levels of the hypothalamic-pituitary-gonadal axis, separating the signaling pathways with other hormones and influencing on other hormonal systems (Swapna and Senthilkumaran, 2007; Castañeda Cortés et al., 2014). Experimental studies have shown that TH play an important role in the maturation and functioning of gonads, influencing spermatogenesis in males and oogenesis in females. Increasing the level of TH to moderate concentrations leads to premature spermatogenesis in juveniles and an increase in the num-

ber of mature spermatozoa in adult fish (Timmermans et al., 1997; Lema et al., 2009). Treatment of adult males with high doses of TH negatively affects spermatogenesis and the functioning of seminal vesicles (Jacob et al., 2005). Treatment of females with TH, on the contrary, stimulates the growth of follicles and early oogenesis (Cyr and Eales, 1996; Swapna and Senthilkumaran, 2007), and also leads to an increase in the concentration of vitellogenin (Nelson and Habibi, 2016).

Most of the studies are devoted to the study of the influence of TH on the reproductive system of fish during a relatively short period of ontogeny. In addition, the effect of thyroid hormones on the reproduction of teleost fish and their offspring, grown in conditions of elevated TH levels, remains practically unexplored.

The purpose of this work is to study on the example of the green terror *Andinoacara rivulatus* (Cichlidae) the influence of triiodothyronine (T₃) on the reproduction and development of offspring of teleost fish grown in conditions of inducing hyperthyroidism.

MATERIALS AND METHODS

The clutch of fertilized eggs obtained from spawning of a pair *A. rivulatus* was divided into two groups (40 eggs each) that contained (from the beginning of

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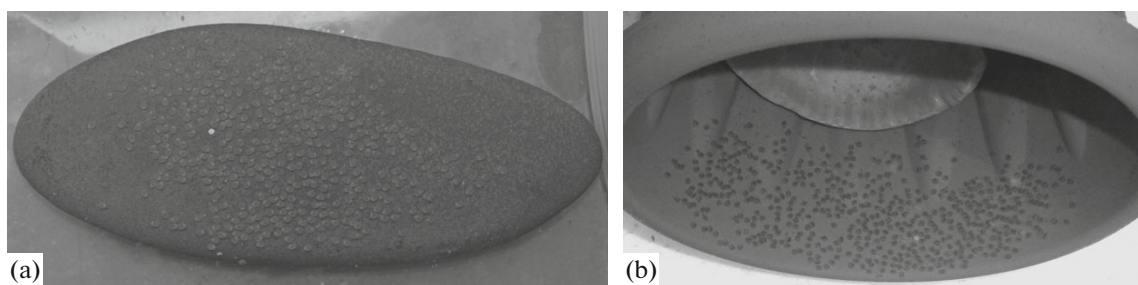


Fig. 1. The clutches of eggs of females *Andinoacara rivulatus* control (a) and experimental (b) groups.

the embryonic period to before the first spawning) in different hormonal regimes: control group – natural TH status and hyperthyroid (experimental) group. Hyperthyroidism was induced by the addition of the active form of TH—3,5,3'-triiodo-L-thyronine (Sigma, United States) into the aquarium water up to the concentration 0.1µg/ml. The content of T₃ in the body of experimental fishes was not determined. The concentration of T₃ was selected empirically in such a way as to provoke heterochronies in development, the premature onset and acceleration of ontogenetic processes in comparison with the control group, herewith not to lead a significant increase in fish mortality. Other conditions of the experiment (temperature, aeration, the stocking density of fish, feeding, light regime, presence of shelters, etc.) were the same in both groups. Every two days, 1/3 of the water in the aquariums was replaced; in the aquarium with the experimental group was added of T₃ up to predetermined concentration.

In the experimental and control fish registered the time of appearance of features sexual dimorphism, the beginning of the first spawning and evaluated of reproductive efficiency. An autopsy of hyperthyroid specimens to assess the quality of the gonads was not performed in order to preserve model fish for subsequent experimental work.

The clutches of eggs by the producers on the substrate were photographed for further calculation of the number of eggs (Fig. 1), then they were moved for

incubate in a mesh container attached to the wall of the same aquarium. The number of unfertilized eggs was determined during the first 24 hours after spawning; the time of hatching of the offspring was recorded (duration of the embryonic period). In the early stages of postembryonic development (from hatching to resorption of the yolk sac), on the living material using the stereoscopic microscope Leica MS5 determined the presence of developmental disorders: body shape, external organs and functional abnormalities. Pictures of fish are made with a Canon EOS 100D photographic camera.

RESULTS

In *A. rivulatus*, grown under conditions of inducing hyperthyroidism, was observed of heterochronies in the development of various morphological features (in particular, in the development of the pelvic fins and the pigment pattern elements) and developmental abnormalities (shortening of the operculum, asymmetry and underdevelopment of the structures of the ventral fins, a reduction in the number of rays with their fusion in the dorsal fin, and others). In fish of the experimental group, compared with the controls, features of sexual dimorphism began to form earlier – by 180 days post-fertilization (dpf) versus 210 dpf. Heterochronies in the development of fish in the experimental group led to morphological changes in the definitive morphology and the formation of some sex features uncharacteristic of the normal phenotype

Table 1. Characteristics of the spawnings of hyperthyroid *Andinoacara rivulatus*

Spawning №	Female 1 (SL 65 mm)			Female 2 (SL 63 mm)		
	number of eggs in the clutch	proportion of unfertilized eggs, %	interval between spawnings, day	number of eggs in the clutch	proportion of unfertilized eggs, %	interval between spawnings, day
1	357	0.8	—	185	3.7	—
2	316	1.9	63	178	1.7	61
3	301	1.3	17	137	3.6	19
4	533	3	54	230	3.5	84
5	—	—	—	257	4.7	48

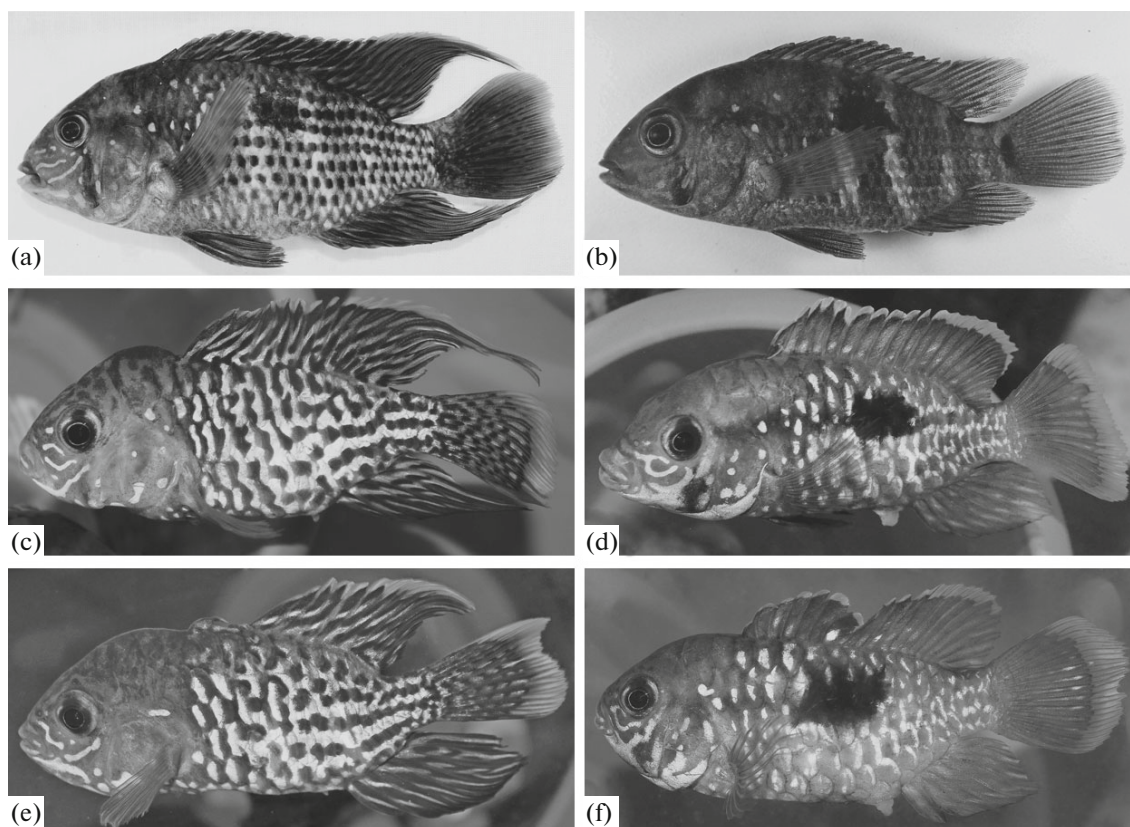


Fig. 2. The phenotypes of adult males (a, c, e) and females (b, d, f) *Andinoacara rivulatus*: (a, b) control and (c–f) experimental groups.

A. rivulatus (Fig. 2). These features were mainly manifested in the development of pigment patterns (combinations of pattern scales, melanophore spot on the trunk, fins coloration), differing in males (Figs. 2c, 2e) and females (Figs. 2d, 2f). In addition, the fish of both sexes had the same sizes—the standard length (*SL*) was 65 ± 0.5 mm, while in the control group the males were significantly larger than females (SL 79 ± 0.4 vs. 53 ± 0.5 mm). The differences in coloration between males (Fig. 2a) and females (Fig. 2b) from the control group were mainly manifested in the melanistic pattern elements—vertical bars on the trunk, spot in the base of the caudal fin and the infraorbital stripe.

The first spawning in *A. rivulatus* of the experimental group occurred at a later age than in the control group (270 vs. 230 dpf). Thus, the time interval between the appearance of external morphological sex differences (by coloration and shape of fins) and the first spawning in experimental fish was longer than in control fish (90 vs. 20 days). Of the nine adult hyperthyroid fish, systematic spawning was observed only in two pairs. Quantitative characteristics of spawnings are given in the table. For four spawnings, the average number of eggs in the clutch in the two females differed by a factor of two (376.7 and 182.5), and there

were also differences in fertility during short intervals between spawnings. In three control females, fertility was higher both in the first spawning (420–437 eggs) and on average for four spawnings (426.2–458.7 eggs). Herewith, the intervals between spawnings were shorter and amounted to 13–16 days.

It should be noted that female 1 (Fig. 2d) had significantly fewer abnormalities in development as compared with female 2 (Fig. 2f), which had multiple abnormalities in the development of fins and skull bones. In both males participating in spawnings, the number of abnormalities in morphology caused by heterochronic shifts was the same. Between the two pairs of experimental fish, during all spawnings, there were differences in reproductive behavior. In the first pair (Fig. 3) the aggressive behavior was manifested by the female, while the male exhibited typical behavior for *A. rivulatus*, consisting in the protection of the territory both during spawning and after it. In the second pair (Fig. 4), the male and female did not manifest aggressive behavior, and after spawning, when the female was engaged in aeration and protection of egg clutch, the male did not participate in the protection of the spawning territory.

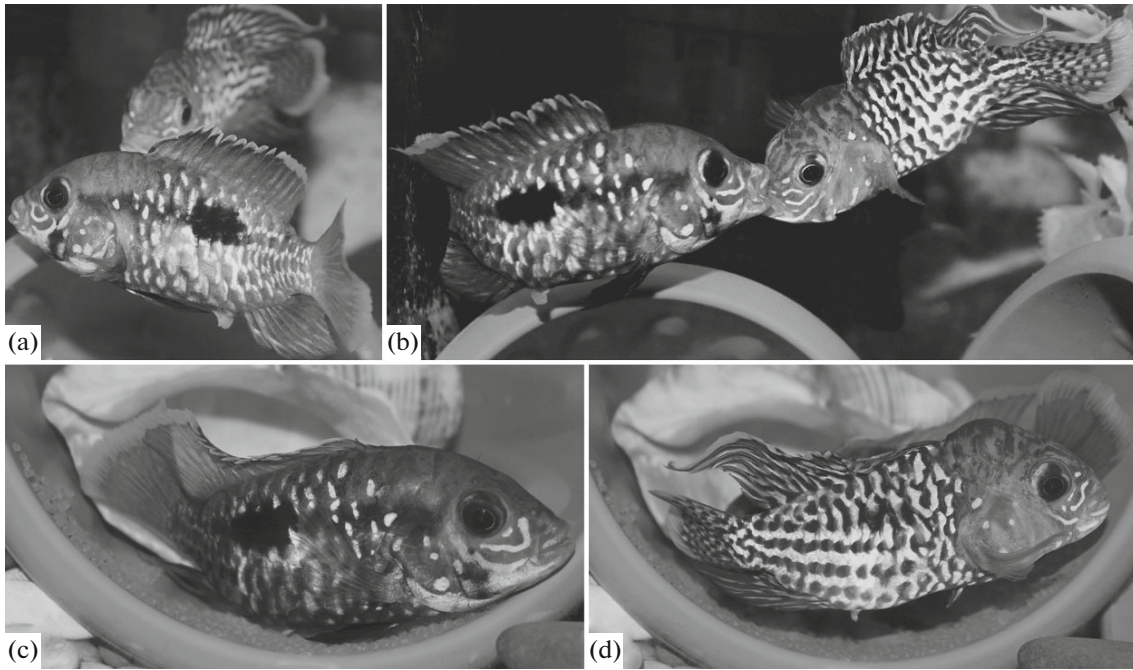


Fig. 3. Fragments of spawning of the first hyperthyroid pair *Andinoacara rivulatus*: (a) female at the beginning of spawning, (b) aggressive female behavior, (c) female lays eggs, (d) male fertilizes the eggs.



Fig. 4. Fragments of spawning of the second hyperthyroid pair *Andinoacara rivulatus*: (a) male during spawning, (b) female lays eggs, (c) female protects the clutch of eggs.

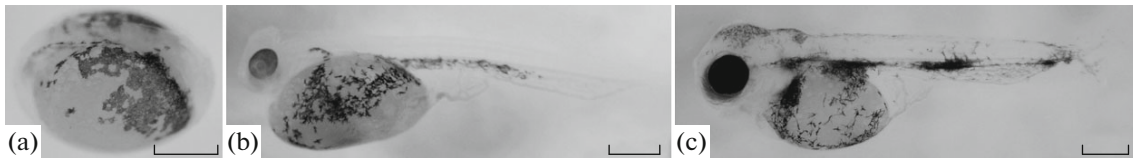


Fig. 5. Offspring obtained from the producers of *Andinoacara rivulatus*, grown in conditions of inducing hyperthyroidism: (a) completion of the embryonic period; (b, c) larvae. Scale: 0.5 mm.

The duration of the embryonic period (from the moment of fertilization to hatching) in the experimental and control groups did not differ and amounted to 68–70 hours (at 26°C). Observations of the development of embryos (Fig. 5a) and larvae before the completion of the yolk sac resorption (Figs. 5b, 5c)

revealed no significant abnormalities in morphology. The proportion of morphological abnormalities in the early stages of postembryonic development in offspring obtained from nine spawnings of two pairs of *A. rivulatus* was the same and varied within 3.2–3.6%. Among the most common abnormalities marked cur-

vatures of the chord (1.8–2.1%), edemas of yolk sac and pericardium (1.4–1.6%).

DISCUSSION

The results obtained indicate the influence of hyperthyroidism on the development of secondary sexual characteristics and the reproduction of *A. rivulatus*. Heterochronies in the experimental group of fish led to an accelerated formation of sexual dimorphism and a later development of the reproductive system. In females the fertility has changed depending on the degree of severity of the consequences of heterochronies in definitive morphology. In female 2 with multiple developmental abnormalities in comparison with female 1, whose morphological deviations less were pronounced, the total fertility and number of eggs in the clutch were smaller (Table 1). Decreased fertility may be due to the negative influence of increased concentrations of TH on ovarian development. The low number of unfertilized eggs in the clutches of two pairs of *A. rivulatus* (2.7% on average) indirectly indicates the normal functioning of the testes of males grown in conditions of inducing hyperthyroidism.

Thus, despite the influence of hyperthyroidism and the presence of morphological consequences caused by heterochronies in the parental pairs *A. rivulatus*, the fish spawned and gave viable offspring. At that the average frequency of all forms of developmental abnormalities in the offspring did not exceed the background threshold of occurrence of abnormalities—5% (Kirpichnikov, 1979; Borkin et al., 2012; Prazdnikov, 2015).

Previously, it was shown that in fish treated with thyroid hormones, TH accumulate in oocytes during their development in the ovaries and have a positive influence on the development of offspring, including the survival of larvae (Brown et al., 1989, 2014; Ayson and Lam, 1993). In another study, despite the high content of TH in eggs, their influence on fertility, survival and frequency of developmental abnormalities during hatching was not revealed. However, in larvae that switched to exogenous feeding, a higher frequency of occurrence of skeletal abnormalities was noted than in the control group (Mylonas et al., 1994). Contradiction of the obtained data on the influence of an increased level of maternal TH on offspring can be due to different methods of carrying out the experiments (different doses and methods of introducing TH) and the species specificity of the fish studied. The follicles of the thyroid gland in different species of fish appear at different stages of ontogenesis. In the larvae of cichlids the thyroid follicles are absent, and appear in later periods of development (Nacario, 1983). Therefore, before the start of production of own TH, developing embryos and larvae can use only hormones obtained from the mother.

Our experimental data show that hyperthyroidism in females of *A. rivulatus* does not influence embryonic and postembryonic development of offspring, including hatching time, survival and frequency of developmental abnormalities. This may be due to both the accessibility of maternal TH for developing embryos and larvae, and the low hormonal dependence of these periods of ontogeny in *A. rivulatus*.

The obtained experimental results on the influence of T₃ on fertility, development of secondary sexual characteristics, spawning behavior give new prospects for further study of the role of the endocrine system in the regulation of ontogeny, including participation of TH in the hypothalamic-pituitary-thyroid and hypothalamic-pituitary-gonadal axes. In addition, the individual TH-sensitivity of *A. rivulatus* of experimental group and their ability to reproduction, despite heterochronies, make it possible to use this species of cichlids as a model for experimental works on evolutionary biology.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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