

... ЗООЛОГИЯ ТА ЕКОЛОГИЯ ... ZOOLOGY AND ECOLOGY ...

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Experimental study of changes in the intensity of infection of carp (*Cyprinus carpio carpio*) with blood parasites *Trypanosoma carassii* and *Cryptobia borelli* in the laboratory conditions
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Experimental studies have shown that in the body of carp yearlings infested with trypanosomes and *Cryptobia* in the water with a low temperature (4–12°C), intensive aeration and abundant feeding intensity of infection of blood parasites was low. Intensity of infection of the same fish that were kept in water with temperature 20–24°C, poor aeration of water and shortage of food increased. The sharp increase of the intensity of infection with *Cryptobia* in one of the individuals led to the fish death.

Key words: *carp, trypanosome, cryptobia, infection intensity.*

Экспериментальное исследование изменения интенсивности инвазии карпа (*Cyprinus carpio carpio*) кровепаразитами *Trypanosoma carassii* и *Cryptobia borelli* в лабораторных условиях содержания
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Экспериментальные исследования показали, что в организме годовиков карпа, инвазированных трипаносомами и криптобиями, и содержащихся в воде с низкой температурой (4–12°C), при интенсивной аэрации и обильном кормлении, интенсивность инвазии кровепаразитами падала. У тех же рыб, содержащихся в воде при температуре 20–24°C, слабой аэрации и дефиците корма, интенсивность инвазии повышалась. У одной из рыб наблюдалось резкое повышение интенсивности инвазии криптобиями, в результате чего рыба погибла.

Ключевые слова: *карп, трипаносома, криптобия, интенсивность инвазии.*

Introduction

In spite of a large number of observations over the flagellates in blood of fish we didn't note direct reproduction of parasites in them. However, while analyzing seasonal and age changes of infection of fish with blood parasites many researchers received numerous evidences of that in appropriate temperature conditions in fish organism there can be a noticeable increase in number of blood parasites, i.e. can take place spontaneous increase of intensity of infection (Ivasik, 1953; Guseinov, 2008a, 2008b, 2011; Khaibulayev, 1969; Khamnuyeva, 2004; Zintl et al., 2004). We made special experiments to check probability of increasing the number of flagellates in fish organism.

Materials and methods

As material for carrying out experimental studies served 16 individuals of carp yearlings caught from the Kur River in 2004 and 2005.

The blood was investigated on existence of flagellates, smears were made, dried up, then fixed in methanol and painted by azure-eosine by Romanovsky-Gimza (pH 7,2) method.

When carrying out experimental work on certain days we examined a drop of blood to find parasites. Blood was collected from the caudal portion of the fish, not killing them.

As indicators of infestation, we used the intensity of infection (I) and the specific intensity of infestation (SII) – the number of parasites in the blood smear.

Results and discussion

First experiment: at the beginning of November 2004, we investigated ten carp yearlings on infectiousness with blood parasites. For this purpose, without killing fish, we took from a tail artery one drop of blood and looked the crushed drop at big microscope magnification. In the blood of six carps two species of flagellates – *Trypanosoma carassii* and *Cryptobia borelli* were found (table 1).

Table 1.
Changes of specific intensity of invasion of six carps with flagellates *Trypanosoma carassii* and *Cryptobia borelli* in experimental conditions under temperature of water of 4–12°C, intensive aeration and abundant feeding

Fish №	Species of parasites	Days, passed from beginning of the experiment							
		1	7	15	30	60	75	105	120
1	<i>T. carassii</i>	2	2	1	1	1	1	1	1
	<i>C. borelli</i>	16	17	12	10	11	6	7	3
2	<i>T. carassii</i>	14	12	9	7	8	4	5	2
	<i>C. borelli</i>	29	26	21	17	14	5	4	1
3	<i>T. carassii</i>	21	23	22	12	9	5	3	4
	<i>C. borelli</i>	101	92	85	56	25	17	12	10
4	<i>T. carassii</i>	36	34	30	23	22	11	5	6
	<i>C. borelli</i>	41	42	34	21	21	12	6	7
5	<i>T. carassii</i>	8	9	7	8	6	3	2	2
	<i>C. borelli</i>	72	69	54	36	23	14	15	8
6	<i>T. carassii</i>	11	10	8	6	7	2	2	1
	<i>C. borelli</i>	19	17	15	9	6	8	2	2
From – to	<i>T. carassii</i>	2–36	2–34	1–30	1–23	1–22	1–11	1–5	1–4
	<i>C. borelli</i>	16–101	17–92	12–85	9–56	6–25	5–17	2–12	1–10
Average	<i>T. carassii</i>	15,3	15,0	12,8	9,5	8,6	4,3	3,0	2,7
	<i>C. borelli</i>	46,3	43,8	36,8	24,8	14,3	10,3	7,7	5,2

SII of fish with first species was 2–36 specimens (at average 15.3 spec.); with second species it was 16–101 spec. (at average 46.3 spec.). Then all six fish were placed into aquarium and kept at water temperature of 4–12°C, water intensive aeration and regular abundant feeding. For discernment of fish to the dorsal of each fish a tag with the number of individual was attached using the metal wire. All selected fish survived till the end of the experiment. In 7 days after beginning of the experiment we analyzed all fish and discovered that SII of almost all carps with both species of blood parasites decreased, but not much. So, for *T. carassii* it was 1–32 spec. (at average 15.0 spec.), for *C. borelli* – 14–98 spec. (at average 43.8 spec.).

On the 15th day of the experiment we discovered some reduction of SII with both species of parasites: for *T. carassii* it was 1–30 spec. (at average 12.8 spec.), for *C. borelli* – 12–85 spec. (at average 36.8 spec.).

The further examination of fish showed steady decline of infectiousness during all continuation of the experience. On the 30th day of the experiment SII of fish with trypanosomes was 1–23 spec. (at average 9.5 spec.), and with cryptobies – 9–56 spec. (at average 24.8 spec.), on the 60th day – respectively 1–22 spec. (at average 8.6 spec.) and 6–25 spec. (at average 14.3 spec.), on the 75th day – 1–11 spec. (at average 4.3 spec.) and 5–17 spec. (at average 10.3 spec.), on the 105th day – 1–5 spec. (at average 3.0 spec.) and 2–12 spec. (at average 7.7 spec.).

Experiments were finished on the 120th day. We dissected all 6 carps, examined blood and internals. SII of blood with trypanosomes was 1–4 spec. (at average 2.7 spec.), by cryptobies – 1–10 spec. (at average 5.2 spec.). No parasitic flagellates were found in internals.

Such steady falling of infection of the fish in water with quite low temperature, generally resembles the results of our seasonal researches of infection of fish (on the example of a pike) with blood parasites. According to them during winter time EI (extensiveness of an infection) and II of this fish by blood parasites are low.

In the above described experiment, the quantity of flagellates in blood of fish didn't increase and the assumption of reproduction of flagellates in organism of fish wasn't confirmed. Therefore, we carried out another experience in which conditions of fish keeping were most approached to natural conditions of the

summer period when, according to results of seasonal researches, intensity of invasion of fish by flagellates is higher.

The second experiment: in April 2005 we selected six yearlings of carp, approximately of the same size, as in the first experience. After testing of their infection with *Trypanosoma carassii* and *Cryptobia borelli*, they were placed in an aquarium and kept at the water temperature of 20–24°C, weak aeration of water and deficiency of feeding (table 2). For discernment of fish to the dorsal of each fish a tag with the number of individual was attached using the metal wire. Of six fish selected by us only five specimens survived till the end of experiment, one fish has died on the 112th day.

Table 2.
Changes of specific intensity of an invasion (spec.) of six yearlings of carp with flagellates *Trypanosoma carassii* and *Cryptobia borelli* in experimental conditions at water temperature of 20–24°C, weak aeration of water and deficiency of feeding

Fish №	Species of parasites	Days, passed from beginning of experiment							
		1	7	15	30	60	75	105	120
1	<i>T. carassii</i>	3	2	5	6	6	8	7	9
	<i>C. borelli</i>	4	8	7	15	20	19	33	31
2	<i>T. carassii</i>	2	2	3	2	8	9	12	11
	<i>C. borelli</i>	3	4	6	17	23	21	35	38
3	<i>T. carassii</i>	1	1	4	3	7	6	8	8*
	<i>C. borelli</i>	8	11	19	38	83	211	456	510*
4	<i>T. carassii</i>	4	6	6	11	14	12	11	17
	<i>C. borelli</i>	2	5	7	6	13	25	23	20
5	<i>T. carassii</i>	5	5	9	14	12	15	18	16
	<i>C. borelli</i>	9	11	10	10	15	16	14	12
6	<i>T. carassii</i>	1	2	1	4	3	3	2	2
	<i>C. borelli</i>	1	1	2	1	5	4	5	4
From – to	<i>T. carassii</i>	1–5	1–5	1–9	2–14	3–14	3–15	2–18	2–17
	<i>C. borelli</i>	2–9	1–11	2–19	1–38	5–83	4–211	5–456	4–510
Average	<i>T. carassii</i>	2.7	3.0	4.7	6.7	8.3	8.8	9.7	10.5
	<i>C. borelli</i>	4.5	6.7	8.5	14.5	26.5	49.3	94.3	102.5

Note: * Fish №3 has died on the 112th day of the experiment. Therefore here is provided data not for the 120th day as it was for other five experimental fish, but for the 112th day of the experiment.

In this experiment, as well as in the previous, we investigated the fish on the 1st, 7th, 15th, 30th, 60th, 75th, 105th and 120th days without killing them. On the first day of the experiment SII of carps with *T. carassii* was 1–5 spec. (at average 2.7 spec.), and with *C. borelli* – 2–9 spec. (at average 6.7 spec.). On the next days there was some increase in intensity of infection. And this increase was non-regular and not in the same degree for these two species.

SII of carps with *T. carassii* increased quite gradually, during all period of the experiment. So, on the 7th day it was 1–5 spec. (at average 3.0 spec.), on the 15th day – 1–9 spec. (at average 4.7 spec.), on the 30th day – 2–14 spec. (at average 6.7 spec.), on the 60th day – 3–14 spec. (at average 8.3 spec.), on the 75th day – 3–15 spec. (at average 8.8 spec.), on the 105th day – 2–18 spec. (at average 9.7 spec.), on the 120th day – 2–17 spec. (at average 10.5 spec.).

Approximately same was the growth of SII of carps with *C. borelli*: on the 7th day it was 1–11 spec. (at average 6.7 spec.), on the 15th day – 2–19 spec. (at average 8.5 spec.). On the 30th day of experiment SII was already in 1.7 times more and reached 1–38 spec. (at average 14.5 spec.), on the 60th day the increase continued to grow and it was already in 1.82 times more and was 5–83 spec. (at average 26.5 spec. , on the 75th day in 1.86 times, was 4–211 spec. (at average 49.3 spec.), on the 105th day in 1.91 times and was 5–456 spec. (at average 94.3 spec. .

It should be noted that such sharp increase of SII by cryptobies including average, noted by us from the 30th day of experience, occurred generally because of one fish which is shown in the table 2 under N3. Infection of other fish with cryptobies also increased, but not so sharp. The fish shown in the table under number 3 died on the 112th day of the experiment because of anemia, apparently, caused by the cryptobies.

Blood of this fish appeared as small amount of light-red plasma which contained very few erythrocytes and large quantity of cryptobies, more than 500 species in a blood drop, i.e. such was SII.

Such extraordinary case of sharp increase of SII of one experimental fish, at rather smooth increase of contamination of other five fish of the same species keeping at the same conditions would be surprising, if not one fact. The fact is that from six fish used in the experiment only in this specimen were registered strong indications of the trauma on the body, made, apparently, during catching and transporting.

Probably the reason of such sharp increase of intensity of an infection of this specimen with cryptobies was stress. It should be noted that other fish had stress, but might be not in such degree as died fish. Most likely that the fish specified under №3 was obviously weaker than other five experimental fish because of a trauma.

Taking into account the parasites registered in the fish, which died on the last day of the experiment, SII with cryptobies was 4–510 spec. (at average 102.5 spec.). However, if considering changes of SII with flagellates of *T. carassii* and *C. borelli* of five experimental fish without taking into account this died fish, we'll see a bit different picture.

From the data provided in table 2 we see that without considering data for fish number 3, increase of SII of five other experimental fish by flagellates in experimental conditions, is not such sharp as it is according to the table where average SII is given for all six experimental fish together. We should mark that if an average SII of fish №3 with cryptobies during the experience increase in many times more sharply than average SII of other five fish, then SII of fish №3 by trypanosomes during all experience is much lower, than, at average, in five other experimental fish. There is a possibility to do assumption that at very strong infection of fish with cryptobies, their infection with trypanosomes can't be very high.

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