RESEARCH ARTICLE **(Open Access)**

Analysis of Several Indicators of Infection from Gyrodactylus spp. in one Year Rainbow Trout *(Oncorhynchus Mykiss)*

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Abstract

In this study are analyzed the dynamics of infection of rainbow trout from *Gyrodactylus spp*. one of most important fish parasite. We have proven the gradual increase of infection values from the first week of infection until the fourth week (the week with the highest number of parasites). The values of week five and six presented a reduction of parasites number. Comparing the values of parasites number (F%) in 12 areas of fish we can conclude that the most affected areas in the beginning of the infection were pectoral fins (F% = 46.2) and the abdominal fins (F% = 22.7%). Caudal fin showed a number of parasites value F = 9.6%. In the end of the period of the study (week six), compared with the beginning of the infection, the number of the parasites localized in the fins was reduced by 3.7 times, in the anal fin 3:09 times and abdominal fins for about 1.79 times. A different situation resulted for caudal fin and the cornea that after the six week showed a increase of the parasite number respectively 2.28 and 21.1 times. In this study is proved a negative correlation between the density of mucosal cells (Dcell / 0.6mm²) in a specific area of the tail.

Keywords: rainbow trout, *Gyrodactylus spp*, infection.

1. Introduction

The monogenea *Gyrodactylus spp.* is a fresh water parasite of trout and is one of the causes of health problems and big losses of production in trout cultivation. The gyrodactylosis disease is listed in List III of pathogen in the Fish Health Directive 91/67/EEC and is a notificable disease of OIE. Gyrodactylids attaches in the surface of their host and feed on their tissue. They are viviparous, and can reproduce both sexually and asexually. This study presents the results obtained from analyzing the dynamics of infection of yearling's rainbow trout with *Gyrodactylus sp.* This paper shows also the changes of parasite preference for the microenvironment during the evolution of the pathology and the change of the cells number in the localization area of the parasite as an indicator of parasite interactions with the host. The paper focuses on some aspects of "behavior" of *Gyrodactylus spp.* and can contribute to the knowledge of the particulars of the invasion in rainbow trout, with the aim of improving the prevention measures.

2. Material and Methods

The study was conducted in 2014 in an aquaculture farm of Zagorçan (Pogradec) which cultivates rainbow trout *(Oncorhynchus mykiss).* The farm is equipped with incubation unit and performs reproduction and fingerlings produce. The samples included individuals (0+) and tests were performed for a period of six weeks starting from the moment of infection with *Gyrodactylus spp.* About 250 infected individuals were divided in two smaller basins of the farm. The total number of *Gyrodactylus spp* was defined in 40 individuals under anesthesia [(MS 222 (ethyl 3-aminobenzoate methanesulfonate) in concentration 50 mg/L]. The body surface was controlled with Stereomicroscopes ZEISS STEMI SV 11, with and 20x and 50x magnifications and for six weeks are calculated the abundances values. To evaluate the parasites preferences for a specific parts of the fish body surface we have divided the fish exterior in 12 sections (Figure 1).

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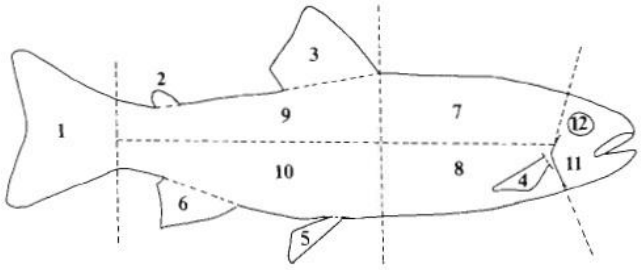

Figure 1. Body Section according parasites localization.

Section 1- caudal fin; Section 2- adipose fin; Section 3- dorsal fin; Section 4- pectoral fin; Section 5- ventral fin; Section 6- anal fin; Section 7- front dorsal part; Section 8- front ventral part; Section 9- caudal dorsal part; Section 10- caudal ventral part; Section 11- head; Section 12- cornea. [1]

The parasites count began immediately after the start of the infection in the 12 sections of the body for six weeks. The calculation of the mucous cells number was performed in order to estimate the burden of the parasites. Every ten weeks fish samples are collected and fixed in phosphate-buffered formaldehyde - 4 for seven days. After the fixation samples are rinsed with distilled water and stained with Alcian Blue (1%) in 3% acetic acid. After the performance of another rinse the sample is placed on the slide and mounted in AquaMount Mounting Medium for 48-72 hours. For the determination of the number of mucous cells is used a micrometer set in the ZEISS AxioScope A1 microscope using a 200 magnification. For each sample were counted the mucous cells in 5 squares of 0.6mm² areas.

3. Results and Discussion

In Figure No. 2 are presented the dynamics of infection values from the end of the first week of the infection of the rainbow trout with *Gyrodactylus spp.* until the conclusion of the test, six weeks after parasites detection.

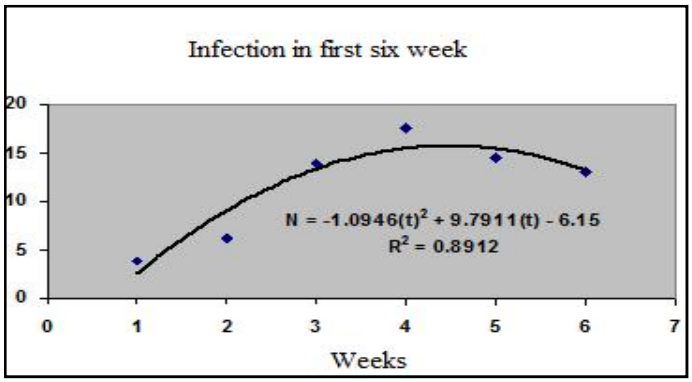

Figure 2. Changes of infection values in rainbow trout *(O.mykiss)* (0^{+}) during 6 weeks.

The calculation of the weekly infection (M ± SD) proved that in the first week, this index was 3.9 ± 1.101 parasite/fish (n = 40; Var% = 28.23). We have proven a gradual increase of infection values for the first week until the fourth week (17.5 ± 2.505; n = 40; Var% = 14:31). The fourth week was the week with the highest number of parasites one rainbow trout. The average infection value for week five and six, showed a slow reduction number of parasites in fish. The average infection value of samples taken in week six was 13.1 ± 2.331 parasites/fish (n = 40; Var% = 17.79). Interesting are also the interpretation of variance calculations (Var.%). We have proven that despite the gradual increase of the parasites number in one fish it is observed also a reduction of the average variance values. In the early stages of the infection was observed a significant difference of parasites number observed compared with the highest level of infection curve (Var.% 28.23% and 14.31%). In the beginning was observed an increase of the average

value of infection for all the fish population and after was observed a reduction of the parasites number found in rainbow trout. The reduction of variance values may be indicative of the fact that in the fish appears the ability to fight the infection.

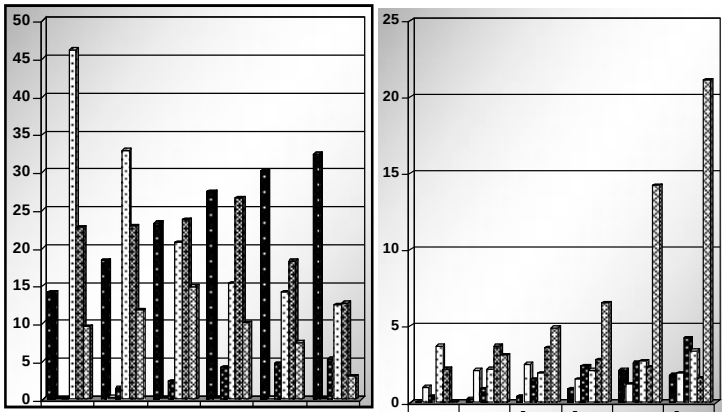

Figure 3. Changes of the Frequency No (%) of the parasites fixed in fins, body and head during 6 weeks of the test.

Specifically, we can highlight four forms of the frequency changes:

Form I: Progressive increase of parasites numbers; this form was characteristic for caudal fin (1), dorsal fin (3), caudal dorsal part (9), and cornea (12).

Form II: Progressive reduction of parasites numbers; this form was characteristic for pectoral fin (4).

Form III: Gradual increase of parasites number from the beginning to the middle of the period tested and the reduction of the values in the second half of this period; this form was characteristic for ventral fin (5), anal fin (6), caudal dorsal part (9), and the head (11).

Form IV: Gradual reduction of the frequency values of parasites from the beginning to the middle of the period of the study and an increase of the value in the second half of this period; this form was characteristic for the caudal ventral part (zone 10).

It is also calculated the coefficient of the regression for the four areas of the body with the typical forms mentioned.

Section 4 (pectoral fin): F% = -6571 (t) + 46.63; r = 0.923

Section 1 (caudal fin): F% = 3.72 (t) + 11:28; r = 0938

Section 6 (anal fin): F% = -0996 (t) ² + 5:54 (t) + 5:22; r = 0.903

Section 10 (caudal ventral part of the body): F% = 0243 (t) ² - 1665 (t) + 4.78; r = 0918

Where: F% is the frequency of the number (ratio in % between the numbers of parasites in one section/total number of the parasites in the 12 Sections).

According to the comparison of the values of the frequency number of the parasites in the 12 Sections in the first week of the test the most affected areas were the pectoral fin (F% = 46.2), ventral fin (F% = 22.7%), for the anal fin this value was F = 14.9%. The frequency of the parasites number from the beginning of the infection until the week III showed a decrease for pectoral fin (F% = 20.7), for the ventral fin and anal fin the values were F% = 23.7% and F = 9.6%. In the end of the test week IV the parasites number decreased for the pectoral fin 3.7 times, anal fin 3.09, and for the ventral fin 1.79 times. According to the diagrams (Figure 3) in the fourth week the anal fin manifested the highest frequency of parasites number (F% = 27.4), the same situation was for the ventral fin (F = 26.6%). The fastest growth rates of the parasites number during all the test was observed in the cornea (F = 21.1% at week VI). In general, during the test was observed a migration of the parasites from the body fins and tail to the cornea. During the test the number of the parasites in the tail increased 2.28 times. From the second week until the week six the population of *Gyrodactylus sp*p in the cornea grew 6.8 times. At the beginning of the infection (first week) the fish showed a smaller number of helminthes in the tail, compared with the pectoral and caudal fin, while we have not found parasites in the cornea. In the Figure 4 are presented the changes of the mucous cells in 5 areas of the body. The smaller number of mucous cells is found in the tail (D = 20.86 ± 2745 qel / 0.6 mm²), it is observed also a negative correlation

between the density of mucous cells (Dqel / 0.6mm²) in a specific area of the tail and the parasites number (N), (D = -12 256 (N) + 102.23; r = -0949). With the reduction of the parasites number in the body the mucous cell number gradually increased.

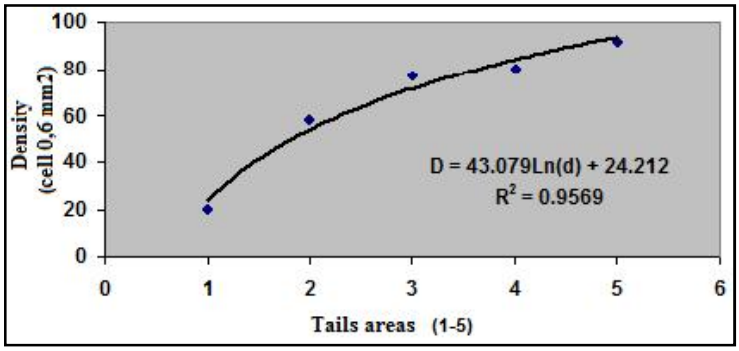

Figure 4. Changes of the mucous cells of the tail (D) during the test (from the corner to the caudal peduncle (r = 0.978).

Figure 5. The rupture of tail membranes due to the activity of Gyrodactylus spp.

The values obtained about the parasites frequency (F%) during the six weeks of the test showed a gradual shift of the helminthes from the section 4, 5 and 6 of the fish body, with the greatest density of population of parasites (until days 24-28 days) in sections 1, 12 and 3, where the highest number of parasites was found after the fourth week of the test. So the evolution of the F% values in the 12 sections of the body in the 40 trout samples analyzed for 6 week showed that the parasites migrate from areas positioned below the median line of the body above this area. In particular, it is observed an increase density of the parasites in the tail and cornea, a few numbers was observed in the dorsal fin and in the caudal dorsal part of the body.

The “migratory" behavior and the microhabitat selection may come as results of the improvements of the nutrition conditions, the local response of the “guest” and ensure of a successful contact during reproduction [1],[5]. This change can be also be explained by the fact that overpopulation acts as a signal for the start of the parasites migration and also by the parasites trends to leave the epithelium areas with immune reactions [1].Calculating the infection values we have proved that by the fourth week, when the parasite population density was in maximum (17.5 ± 2.505 paras./fish) until the end of the sixth week, this indicator decreased 1:34 times. This population reduction is associated with the parasites migration in the tail (at six weeks F% 2:28 times higher than at the end of the first week) and in cornea (at six weeks F% 6.81 times higher than at the end of the second week).

4. Conclusions

The infection evaluation in this study is considered as an indicator of the dynamics of yearlings rainbow trout. Our tests proved that the average maximum value of infection (17.5 ± 2.505 parasite./fish) was observed four weeks after the parasites fixation. In the following two weeks, this indicator decreased until the stabilized value at 13.1 ± 2.331 parasites /fish.

We proved that in the beginning of the infection *Gyrodactylus spp.* was present mainly in the pectoral fin (F = 46.2%), ventral fin (F% = 22.7%) and anal fin (F = 9.6%). These body parts are located in the lower half of the body and so are the first that come into contact with the end of the breeding tanks. *Gyrodactylus spp.* are viviparous parasites with a not specific phase of transmission and with no ability to swim [2]. The contact with parasites detached from the fish and fallen to the bottom of the tank is one of four possible routes of infection from Gyrodactylosis [3].

The microhabitat selection of the *G.derjavini* in the body surface of the rainbow trout varies significantly over the course of the infection and the most important part of parasites localization was pectoral fin, ventral fin and anal fin [4]. Few parasites are found in anal fin, tail, oral cavity and the gills. The only non infected body part was the nasal cavity.

5. Acknowledgements

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6. References

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