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THE INFLUENCE OF PARASITIC INFECTION ON THE BLACK SEA WHITING *MERLANGIUS MERLANGUS EUXINUS* (GADIDAE) MORPHOPHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS

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The Influence of Parasitic Infection on the Black Sea Whiting, *Merlangius merlangus euxinus* (Gadidae), Morphophysiological and Biochemical Parameters. Skuratovskaya E. N., Yurakhno V. M., Zavyalov A. V. — Complex influence of infection by myxosporeans *Myxidium gadi* Georgevitsch, 1916; *Ceratomyxa merlangi* Zaika, 1966 and nematode *Hysterothylacium aduncum* (Rudolphi, 1802) on the morphophysiological and biochemical parameters of the Black sea whiting *Merlangius merlangus euxinus* (Nordmann, 1840) were studied. Significant decrease of spleenosomatic index and increase of hepatosomatic index in fish with average and high intensity of infection were found. The dependence of hepatic antioxidant enzyme activities on intensity of infection was observed. Increase of superoxidismutase, catalase, glutathion-S-transferase activities in fish with average and high intensity of infection and decrease of peroxidase activity in specimens with average intensity of infection were shown.

Key words: intensity, morphophysiological parameters, antioxidant system, fish.

Влияние паразитарной инвазии на морфофизиологические и биохимические параметры черноморского мерланга, *Merlangius merlangus euxinus* (Gadidae). Скуратовская Е. Н., Юрахно В. М., Завьялов А. В. — Исследовали комплексное влияние инвазии микроспоридиями *Myxidium gadi* Georgevitsch, 1916; *Ceratomyxa merlangi* Zaika, 1966 и нематодой *Hysterothylacium aduncum* (Rudolphi, 1802) на морфофизиологические и биохимические показатели черноморского мерланга, *Merlangius merlangus euxinus* (Nordmann, 1840). Обнаружено снижение индекса селезёнки и увеличение индекса печени у рыб со средней и максимальной степенью заражённости. Выявлена зависимость активности антиоксидантных ферментов печени от интенсивности инвазии рыб. Показано снижение активности супероксиддисмутазы, каталазы, глутатион-S-трансферазы у рыб со средним и высоким уровнем заражённости, повышение активности пероксидазы у особей со средней интенсивностью инвазии.

Ключевые слова: интенсивность инвазии, морфофизиологические показатели, антиоксидантная система, рыбы.

Introduction

The investigation of consequences of fish infection by parasites is one of the actual problems. Infection considerably worsens fish condition and weakens protective functions. Changes taking place in contaminated organism are connected with disturbances in metabolic processes, physiological state and development of the adaptive response reactions. Host adaptations to parasite are provided by protective systems, which activity is directed to lowering the negative influence and destruction of toxic metabolites. These are immune and antioxidant systems (Skuratovskaya, Zavyalov, 2008; Dautremepuits et al., 2003; Mertinez-Alvares et al., 2005; Sitja-Bobadilla et al., 2008).

Studies of parasites influence on fish organisms seems to be necessary for understanding the mechanisms of host response reactions, directed on maintenance of functioning of infected organs and the whole organism. This is especially important for fisheries and aquaculture, because infection can be the reason of mass fish death and lowers fish production (Rudneva et al., 2010; Dautremepuits et al., 2003; Mertinez-Alvares et al., 2005).

In most cases parasites in the host organism are represented by several species, attributed often to different systematic groups, which must be taken into consideration during the investigations. Myxosporeans and parasitic nematodes, often living together in the host organism take a special place among the parasites, sometimes reaching high intensity in commercial fish species. Myxosporeans are primitive multi-cell microparasite organisms located in different organs and tissues mostly of the Osteichthyes, but as well of

Chondrichthyes, marine and fresh water fish; sometimes they are found in amphibians, reptiles, birds and mammals (Dudin, 2010; Lom, Dykova, 2006). It is known that myxosporean in fish muscles can considerably deteriorate fish meat quality in results of post-mortem lysis of muscle tissue (milky fish condition or myoliquefaction) or macroscopic cysts forming, giving the resemblance of “wormy” meat. When Myxosporea locate in tissues and different inner organs of fish they can cause quite different diseases, leading even to mass mortality of their hosts.

Parasitic nematodes are distributed widely and met in vertebrate as well as in invertebrate animals. Separate species are the parasites with high ecological plasticity and wide specificity. They locate as a rule in fish stomach, pyloric appendices, intestine, and in body cavity and muscles tissue. Great number of parasitic nematodes leads to development of serious pathologies, considerably decreasing fish mass and other symptoms of the fish health deterioration. That is why studies of complex influence of myxosporeans and nematodes on the hosts organism is of great interest.

The Black sea whiting *Merlangius merlangus euxinus* (Nordmann, 1840) is one of numerous hosts for myxosporeans and nematodes. The Black sea whiting is a representative of cods, playing an important role in feeding of predatory fish, dolphins, as well as in transfer of parasites through the food chains. Whiting is of fishery importance. Near the shores of Crimea according to the data of fishery inspection the catches of *M. merlangus euxinus* make only a tenth part of a percent if compared with other ichthyofauna species. However, in Turkey, where representatives of this species are of greater size and its taste is traditionally estimated, whiting is a favorite object of fishery and its price exceeds that of salmon and many other fish species grown in mariculture.

In a whole whiting catches in the Black Sea at the end of 1960s — beginning of 1970-ties made 2.1 % (Ivanov, Beverton, 1985), in 1988 — 4.5 % (Prodanov et al., 1997). During those years *M. merlangus euxinus* was among four the most caught fish along with anchovy, sprat and horse-mackerel. From the end of 1980s four-fold decrease in the volume of the fish caught was observed in the Black Sea, which caused great losses in economics of all the Black Sea riparian countries. The reasons of this were disturbance of water exchange between the Azov, Black and Marmara seas as a result of decrease and over-regulation of rivers runoffs, complex pollution and eutrophication of waters, decrease in foodbase for fish-planktophages due to mass development of alien ctenophore *Mnemiopsis leidyi*, eating fish eggs and larvae, over-catches of fish due to insufficient regulation of intensive fishery, long-term climatic changes (Eremeev, Zuev, 2005).

Two most common species of myxosporeans inhabit the Black sea whiting gall bladder: *Myxidium gadi* Georgevitsch, 1916 and *Ceratomyxa merlangi* Zaika, 1966; they often are met in mixed infection. It has been stated earlier that high intensity of infection with the parasite *M. gadi* led to morphological and functional changes of the damaged host organ, described as myxidiosis of the Black sea whiting (Yurakhno, 2009). It is demonstrated by the change of strongly infected bladders color from transparent green to white, yellowish or brown — non-transparent, growing of their size, thickening of walls and dying of numerous cells of their epithelium, replacement of bile by the parasite spores and plasmodia. Histological studies revealed the fact that thickness of the subepithelial tissue of gall bladders of whiting strongly infected with *M. gadi* was 4–12 times larger than that of uninfected (0.021–0.056 mm contrary to 0.00175–0.014 mm, correspondingly). The thickness of epithelial tissue of strongly infected bladders was 2–3 times larger than that in uninfected (0.084–0.126 mm contrary to 0.028–0.056 mm correspondingly).

Thickening of the walls is accompanied with dying of numerous cells of bladder epithelium. Using the biochemical methods it has been stated that whiting myxidiosis causes total lipids decrease in liver 2–3 times and triacylglycerols 3–4 times exclusively in males during their spawning and fattening. In strongly infected females such differences were not revealed; any changes in liver and muscles lipid composition in strongly and weakly infected fish were not found as well (Shchepkina, Yurakhno, 2008). The recent studies showed that in some cases mass development of *C. merlangi* in whiting bladder also can lead to the painful conditions.

Nematode *Hysterothylacium aduncum* (Rudolphi, 1802) is another common parasite of the Black sea whiting. It is localized in the intestine, body cavity and sometimes in gall bladder. We have stated earlier, that *H. aduncum* can influence significantly on the antioxidant system of sprat muscle tissue (Skuratovskaya, Zavyalov, 2006) and it's blood (Skuratovskaya, Zavyalov, 2008).

After the analysis of all available information on negative influence of the above-mentioned parasites on the host fish organism it will be interesting to study complex influence of complex infection by myxosporeans *M. gadi*, *C. merlangi* and nematode *H. aduncum* on morphophysiological parameters and antioxidant enzyme activities of the Black sea whiting liver.

Material and methods

Fish were collected in the Streletskaya Bay (Sevastopol), in winter period of 2009. Freshly caught non-living fish was brought to the laboratory in the morning. Females prevailed in a catch, therefore only females were studied. The biological analysis, including measuring of total (13.1 ± 0.9) and standard (11.75 ± 0.1) length (cm), determination of fish weight (15.99 ± 0.34), fish weight without internals (12.31 ± 0.23), liver (0.524 ± 0.03) and spleen (0.013 ± 0.001) weight (g), state of maturation (IV–V) and age (2–3 years) of fish, was made.

Table 1. Distribution of fish by the intensity of infection
Таблица 1. Распределение рыб по степени заражённости

Group, N	Myxosporeans	Nematodes	Number of fish, ex
I	No, or few or tens of spores and plasmodia	No, 1–2	16
II	Hundreds of spores and plasmodia	3–9	15
III	Thousands of spores and (or) great number of plasmodia	10–12	25

Parasitological analysis was performed by method of I. E. Bihovskaya-Pavlovskaya (1985) — by partial parasitological dissection of fish for revealing of myxosporeans in the gall-bladder and nematodes in the intestine, body cavity, stomach and in gall-bladder.

The specimens were subdivided in three groups, depending on intensity of infection. The first (I) group included free of Myxosporea and weakly infected (with low intensity) fish, with few or tens of myxosporean spores and plasmodia in the gall bladder smears and up to two nematodes in the body cavity, intestine and stomach. The second (II) group included specimens with average intensity of infection, with hundreds of myxosporean spores and plasmodia in the gall-bladder smears and up to 3–9 nematodes in the body cavity, intestine and stomach. The third (III) group included highly infected fish with thousands of Myxosporea spores and (or) great number of plasmodia in the gall bladder smears and 10–12 nematodes in the body cavity, intestine and stomach (table 1).

The morphophysiological parameters — spleenosomatic index (SI), hepatosomatic index (HI) and fatness index (FatI) were calculated using the following formulas:

$$SI = P_s / P_l * 100 (\%),$$

$$HI = P_l / P_l^* 1000 (\text{‰}),$$

$$FatI = P_f / L_{st}^3 * 100 (\%),$$

where P_f — fish weight without internals (g), P_l — liver weight (g), P_s — spleen weight (g), L_{st} — standard length (cm).

Activities of five antioxidant enzymes: superoxidismutase, catalase, peroxidase, glutathionreductase and glutathion-S-transferase were determined in fish liver according to the methods described previously, with some modifications (Rudneva, 1999).

Superoxide dismutase (SOD, EC 1.15.1.1) was assayed spectrophotometrically on the basis of inhibition of the reduction of nitroblue tetrasolium with NADH mediated by phenazine methosulfate under basic conditions.

Catalase (CAT, EC 1.11.1.6) was measured by the method involving the reaction of hydroperoxide reduction.

Peroxidase (PER, EC 1.11.1.7) activity was detected by spectrophotometric method using benzidine reagent.

Glutathione reductase (GR, EC 1.6.4.2) activity was assayed spectrophotometrically by reaction of the NADPH degradation.

Glutathione-S-transferase (GST, EC 2.5.1.18) activity was determined spectrophotometrically by the conjugate 1-chloro-2, 4-dinitrobenzene (CDNB) using as substrate at the presence of reduced glutathione.

The enzyme activity was calculated in terms of protein concentration. Protein content in fish liver was determined by the Lowry method.

Statistical differences were processed using Student's t-test which was applied for pair-wise testing for antioxidant enzymes between different groups. All numerical data are given as means (M) ± standard error (SE) (Halafyan, 2008). The significance level was 0.05.

Results

Parasitological studies of three fish samples taken in January (15 specimens studied) and in February (41 specimens studied) 2009 in the Streletskaia Bay showed, that in the January sample there were no myxosporean-free fish; in February their number was 14 specimens. Prevalence of *M. gadi* and *C. merlangi* in the January sample was 50 % correspondingly; there was no mixed infestation. In February prevalence of *M. gadi* was 58 %, *C. merlangi* — 10 %, mixed infection by both species — 10 %. In January nematodes were found only in the intestine (prevalence 46 %). In February helminthes were found in the intestine, stomach and body cavity with prevalence 65 %.

The analysis of morphophysiological and biochemical parameters revealed differences between specimens from different groups.

Significant decrease of spleenosomatic index and increase of hepatosomatic index in fish with average (II group) and high (III group) intensity of infection as compared

with specimens from I group was found ($p \leq 0.05$). Fatness indexes of fish from three groups were not different (fig. 1).

Variations of the hepatic antioxidant enzyme activities in fish from different groups were observed. General tendency was determined for CAT, SOD and GST activities: CAT and SOD activities were significantly lower in fish from the group II, GST activity — in specimens from the groups II and III as compared with the group I ($p \leq 0.05$). For PER activity reversal tendency was observed: enzyme activity in fish with average

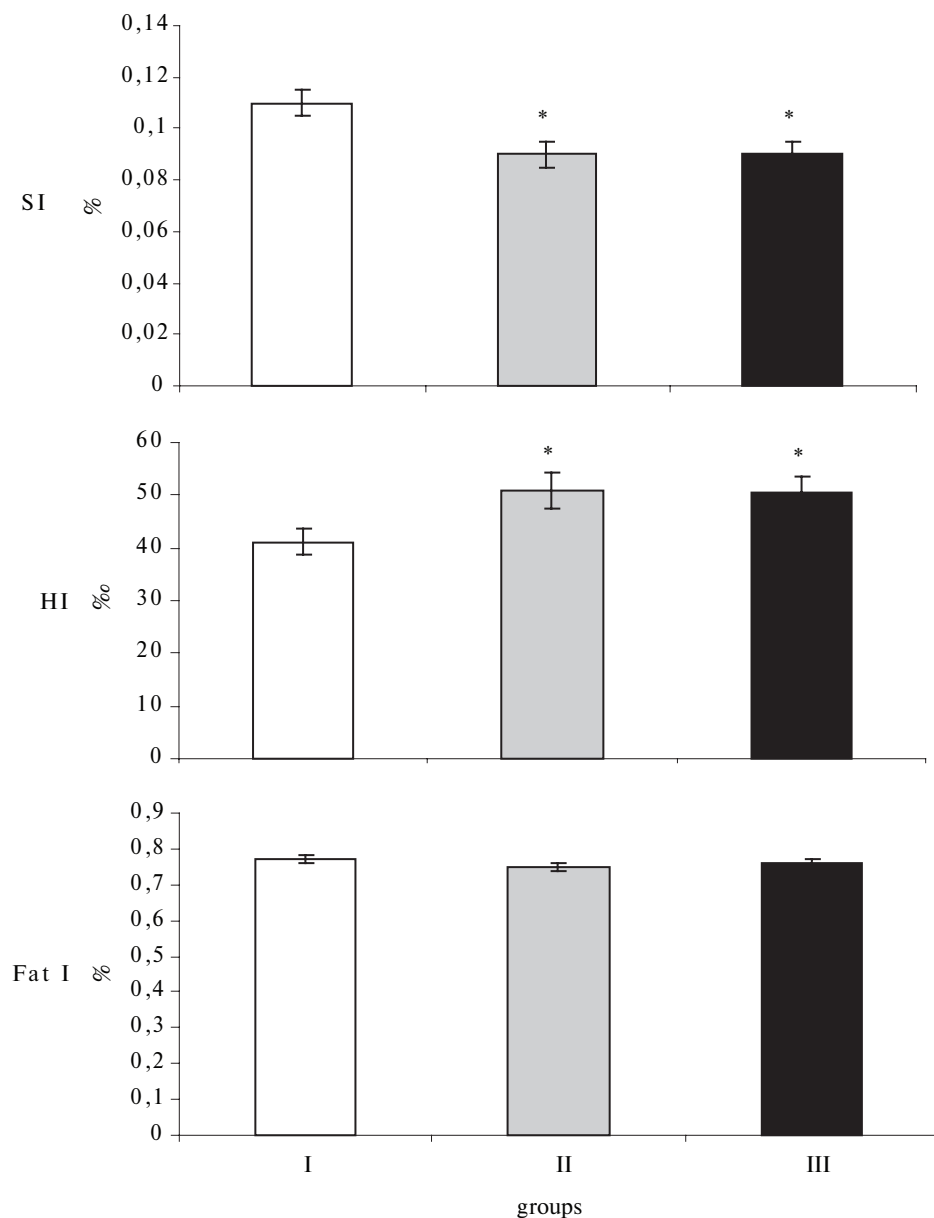


Fig. 1. Morphophysiological parameters of whiting with different intensity of infection ($M \pm SE$; $n_I = 16$, $n_{II} = 15$, $n_{III} = 25$); * — differences are significant as compared with fish from the group I ($p \leq 0.05$).

Рис. 1. Морфoфизиологические показатели мерланга с разной степенью заражённости ($M \pm SE$; $n_I = 16$, $n_{II} = 15$, $n_{III} = 25$); * — различия достоверны по сравнению со значениями рыб из I группы ($p \leq 0,05$).

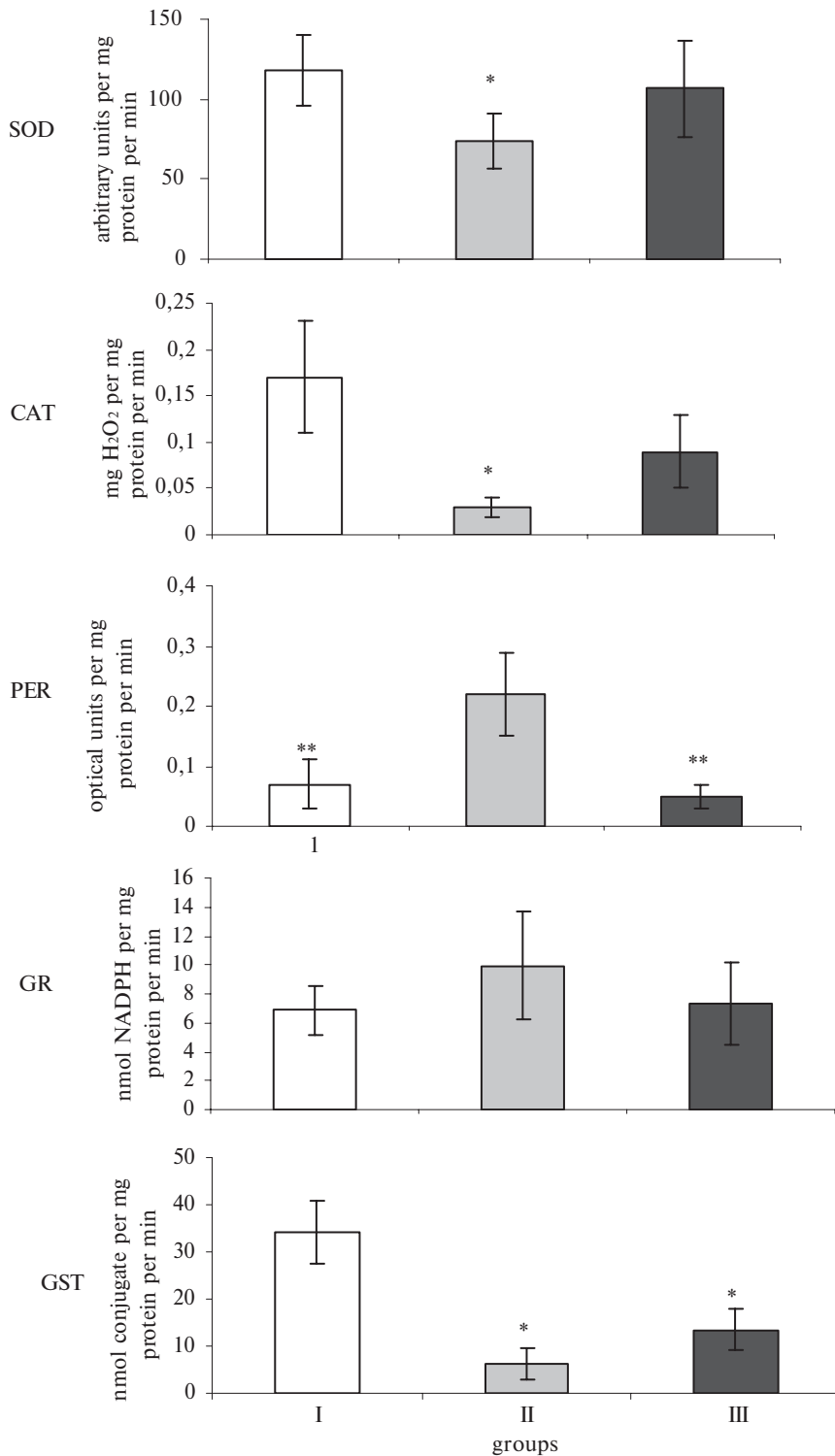


Fig. 2. Antioxidant enzyme activities in whiting liver with different intensity of infection ($M \pm SE$; $n_I = 16$, $n_{II} = 15$, $n_{III} = 25$); * - differences are significant as compared with fish from group I; ** - as compared with fish from group II ($p \leq 0.05$).

Рис. 2. Активность антиоксидантных ферментов в печени мерланга с разной степенью заражённости ($M \pm SE$; $n_I = 16$, $n_{II} = 15$, $n_{III} = 25$); * - различия достоверны по сравнению со значениями рыб из I группы; ** - то же по сравнению со значениями рыб из II группы ($p \leq 0,05$).

intensity of infection (group II) was three times lower than in the specimens from other groups ($p \leq 0.05$). No significant changes in GR activity were found (fig. 2).

Discussion

Parasite uses the host as a habitat and nutrition source and adapts to its peculiarities. As a result of infection pathological processes develop in fish organism, leading in some cases to their death. Changes in the host organism can display at different levels of biological organization, including the molecular one. Infection causes biochemical reorganization of metabolism in infected tissues, directed to selective uptake of nutritional substances and can lead to serious structural and functional changes in organs. Due to this host response reaction develops; it is directed to support of the infected organs functioning as compensation of parasites effect (Dautremepuits et al., 2003; Vasilyeva et al., 2010). That is why the diagnostics of parasitic infection using indicators of different biological level is necessary for evaluation of the infected host condition.

The organs' indices reflect fish physiological status and can characterize stress. Index parameters depend on different factors: habitat conditions, substances in environment, infective diseases (Kuzminova, 2006; Zhidenko, 2008). Our investigations have shown that spleenomatic index in fish with average and high degree of intensity was lower as compared with the I group, which, possibly, is conditioned by increased income of blood from the spleen into blood channel and functional exhaustion of this organ as a result of inflammatory process caused by the presence of parasites and discharge of their metabolites (fig. 1).

Fish spleen is immune-competent and blood producing organ, it participates in providing the mechanisms of urgent adaptation, throwing into blood current "deposited" erythrocytes (Davydov et al., 2006). It is known that under stress factors stocks of blood get from the spleen into blood channel, blood exhaustion and its size reduction take place, in heavy cases dystrophy develops (Lapirova et al., 2004). Possibly, high infection intensity is one of such stress-factors causing size decrease and, consequently, the decrease of spleen index of the host-whiting.

Various liver functions (synthesis of proteins and carbohydrates, blood production, detoxication etc.) cause lability of mass and variations in this organ index. Liver index is widely used in the ichthyomonitoring as a sensitive indicator for evaluation of fish condition and its habitat (Kuzminova, 2006; Zhidenko, 2008). Increase of liver index in fish with average and high infection level can testify to the organ hypertrophy due to the tissue transformation and strengthening of the function of parasites metabolites detoxication, as well as due to the products of peroxidation and free-radical oxidation (fig. 1).

One of the protective systems is an antioxidant that protects the organism against oxidative stress caused by biotic and abiotic factors. Parasites affecting the metabolism of infected fish stimulate oxidative stress manifested by increasing free radical and peroxide processes modulating the host antioxidant status (Skuratovskaya, Zavyalov, 2006, 2008; Bello et al., 2000; Dautremepuits et al., 2003; Mertinez-Alvarez et al., 2005).

The literature data testify to changes of antioxidant enzyme activities in infected fish. Character and direction of these changes depend on the species of host and parasite, and on the life cycle stage of parasite. As a result of infection synthesis of the reactive oxygen forms increases in the host organism causing parasites elimination, which can lead to the inhibition of the host antioxidant enzyme activities (Mikrjakov, Silkina, 2006; Skuratovskaya, Zavyalov, 2006, 2008; Bello et al., 2000; Dautremepuits et al., 2003; Mertinez-Alvarez et al., 2005).

In this study, decrease of the SOD, CAT and GST activities in the liver of fish with average and high intensity of infection (II and III groups) can be the result of their inhibition with reactive oxygen species of host macrophages and the high content of

parasites metabolites, causing oxidative stress and presenting great danger for fish health. At the same time insignificant increase of the SOD, CAT and GST activities in fish from the group III as compared with the group II can be the host adaptive response directed to the organism functioning maintenance at the high intensity of infection (fig. 2).

PER activity increase in the liver of fish from the group II at the background of low SOD, CAT and GST activities can testify to a compensatory effect of the antioxidant system by unfavorable factors, which was found by us earlier (Skuratovskaya, Rudneva, 2008). It demonstrates adaptive response, which is able to neutralize the oxidative stress. But PER activity decrease in the liver of fish from group III testifies to inhibition of the enzyme activity at high intensity of infection (fig. 2).

Similar results have been obtained by us earlier, when we studied muscle antioxidant enzyme activities in the Black sea sprat *Sprattus sprattus phalericus*, depending on intensity of infection by the nematode *Hysterothylacium aduncum* larvae. CAT activity was significantly reduced in infected sprat as compared with healthy fish. The lowest CAT activity was in specimens with high intensity of infection. PER activity decreased with increase of infection intensity. The data obtained testified to the fact of inhibition of the antioxidant enzyme activities with high infection by larvae of nematodes; these data reflected suppressing effect of parasites on the host protective reactions and appearance of toxic response (Skuratovskaya, Zavyalov, 2006).

The parasitic infections can lead to the inhibition of antioxidant enzyme activities associated with the production of reactive oxygen species by macrophages at the infection site in order to eliminate the parasite. Such response was also noticed in fish by Bello et al. (2000) who suggested that the freshwater fish *Rhamdia quelen* response to infection by *Clinostomum detrunctum* could involve reactive oxygen intermediates and, therefore, induced an oxidative stress, but without inducing significant differences in the SOD and CAT activities between healthy and parasitized fish (Bello et al., 2000). The other authors found that breams *Abramis brama* infected with plerocercoids of *Ligula intestinalis* were characterized by high content of malondialdehyde and low antioxidant activities, which is associated with an increase of under the influence of parasites and the development of oxidative stress (Mikrjakov, Silkina, 2006).

In another study, the antioxidant enzyme activities measured in liver and head kidney of *Ptychobothrium* sp. — infected carp *Cyprinus carpio* were significantly higher as compared to the healthy fish. This result could indicate that infection did not lead to an oxidative stress and that it indeed is associated with an improvement of the antioxidant status of the parasitized fish (Dautremepuits et al., 2003).

Thus, the results of the study have shown that infection by myxosporeans *Myxidium gadi*, *Ceratomyxa merlangi* and nematode *Hysterothylacium aduncum* affects the functional status of the Black sea whiting, which is manifested by the change of morpho-physiological parameters and antioxidant enzyme activities. The nature of these changes depends on an intensity of infection.

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