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INFLUENCE OF A TROUT FARM ON MACROZOOBENTHOS COMMUNITIES OF THE TREŠNJICA RIVER, SERBIA

IVANA ŽIVIĆ[1], Z. MARKOVIĆ[2], and M. ŽIVIĆ[3]

[1]*Faculty of Biology, University of Belgrade,* 11000 Belgrade, Serbia
[2]*Faculty of Agriculture, University of Belgrade,* 11080 Belgrade, Serbia
[3]*Department of Biology, State University of Novi Pazar,* 36300 Novi Pazar, Serbia

Abstract — Trout farming poses an increasing threat to quality of the water of clean highland streams. Research of this problem has focused primarily on changes in physico-chemical composition of the water and structure of the river bottom, and less on the effects on living organisms. In the present work, we investigated influence of the farm with the highest trout production in Serbia, the "Riboteks" Trout Farm on the Trešnjica River, on its macrozoobenthos communities. Our investigations showed that the "Riboteks" Trout Farm wastewaters caused a clear and statistically significant change of moderate intensity in all measured parameters describing the composition and structure of macrozoobenthos communities. These changes were most pronounced in the part of the watercourse closest to the influx of waste water (locality III) but remained statistically significant even 500 m downstream (locality IV) and were lost only about 3.5 km away from the influx of the farm's wastewater (locality V). The most pronounced were changes in the participation in total abundance of the Baetidae, Chironomidae, and Plecoptera. Additionally, results of the present work confirmed that the mass of fish on the trout farm is a parameter that adequately defines the strength of its action, above all the intensity of its influence on structure of the macrozoobenthos community.

Key words: Trout farm, macrozoobenthos, fish mass, Trešnjica River, Serbia

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INTRODUCTION

Trout farming is constantly on the increase. Trout farms are usually built next to the sources or in the upper reaches of rivers because this species requires unpolluted water. These clean highland streams, due to relatively small water flows are especially sensitive to pollution. Trout farms with very intensive and relatively constant production pose a steady threat to water quality, above all in periods of low water from June to October (Mitrović-Tutundžić and Vidmanić, 1995; Marković and Mitrović-Tutundžić, 2003). The negative influence of trout farms on such streams is complex, but mainly caused by the release of fish food remains and fecal matter (Liao, 1970), which leads to deterioration of water quality and changes in structure of the stream bottom. Relatively extensive research conducted in the past three decades on the influence of trout farms on recipient streams has focused primarily on changes in physico-chemical composition of the water and structure of the riverbottom (Muñoz, 1989; Foy and Rosell, 1991; Boaventura et al., 1997; Pulatsu et al., 2004; Bartoli et al., 2007).

The effects of trout farms on forms of life in the recipient streams, e.g., their macrozoobenthos (Camargo, 1992a, 1992b; Loch et al., 1996) and ichthyofauna (Oberdorff and Percher, 1994), have been less thoroughly investigated. In general, wastewater from trout farms causes decline of diversity, replacement of more sensitive species by less sensitive ones, and changes of trophic structure due to increased abundance of collectors and reduced abundance of scrapers and shredders. However, despite similarities of the basic trend, the influence of fish farms on the forms of life in different watercourses varies significantly, primarily in regard to the strength of their effects and the rate of subsequent recovery of the benthocenosis (Camargo, 1992a, 1992b; Loch et al., 1996).

The diversity of effects of fish farms is a consequence of the great diversity of conditions determining their influence, e.g., farm size, the presence and kinds of water purification systems, the composition and structure of fish food used, and characteristics of the watercourse (slope, flow, presence of tributaries, etc.). In order to define proper legal measures required to ensure full protection of sensitive trout streams, it is necessary to investigate the influence of as many types of fish farms as possible on the habitat and forms of life in the recipient stream. This is especially important in developing countries with rapid growth of trout farming, but where legal regulation of the activity is inadequate or non-existent and relevant research is in its infancy. Such an area is the Balkan Peninsula, a predominantly highland region characterized by a large number of streams suitable for construction of trout farms. Thus, for example, the number of trout farms has doubled in the last 10 years in Serbia, where production now attains 2000 tons annually (Marković et al., 2007). Moreover, the shortage and high market price of freshwater fish in Serbia has stimulated the interest of investors in fish farming, which is continuing to grow at such a rate that the area of water under trout farms is predicted to increase three- to fivefold in the near future (Marković et al., 2007). A similar situation exists in other countries in the region. However, despite the intensive growth of trout farming, there have been few investigations of its influence on aquatic ecosystems of the Balkan Peninsula, and laws regulating emission of harmful substances from trout farms are still in the stage of formulation.

In the present work, we investigated influence of the farm with the highest trout production in Serbia, the "Riboteks" Trout Farm on the Trešnjica River, on its macrozoobenthos communities.

MATERIALS AND METHODS

The Trešnjica River arises on the southwestern side of Mt. Povlen (Western Serbia, Fig. 1) at an elevation of 1185 m a.s.l. The total drop of the river is 1005 m, its average incline 45.5%. The watershed of the Trešnjica is of a highland nature and cut by a large number of tributaries, the most important of which are the Tribuda and Sušica. The watershed has an area of 88.85 km^2. The Trešnjica empties into the Drina River (a second-order tributary of the Danube) in the village of Gornja Trešnjica at an elevation of 180 m a.s.l.

Investigations were performed at five sites on the Trešnjica River (Fig. 1): locality I, in the region of the Trešnjica's source, at 1000 m a.s.l.; locality II, 10 m upstream from the intake of water for the fish farm, at 253 m a.s.l.; locality III, 20 m downstream from the place where water from the fish farm is discharged into the Trešnjica, at 252 m a.s.l.; locality IV, about 500 m downstream from this place, at 226 m a.s.l.; and locality V, 3.3 km downstream from the fish farm, at 215 m a.s.l.

Breeding fish from the egg to market size, the "Riboteks" Trout Farm is located in the lower part of the Trešnjica River, which supplies it with water, within the boundaries of the protected "Trešnjica Gorge" Special Nature Reserve (Fig. 1).

With a production area of 42 ha, the trout farm consists of a dam and reservoir (situated on the Trešnjica River about 200 m upstream from the first battery of fish pools); a main input channel of the open type and a system of subsidiary input channels; 14 pools for nurturing of fry in the spawning building; 30 concrete pools for nurturing of older fry and consumer-grade trout; and a system of subsidiary output channels feeding into the main one. In the year 2004, production of the trout farm was 213,682.5 kg, with a total gain of 154,470.5 kg of fish. During the entire period of our investigation, water from the trout farm was released directly from the production pools into the Trešnjica River without preliminary sedimentation of excrement and unconsumed food in a settling tank.

Samples of macrozoobenthos were collected at five localities on the Trešnjica River during the year 2004 (on February 29, March 17, May 26, June 15, July 27, September 28, October 14, and November 30). Material was collected with a Surber net having

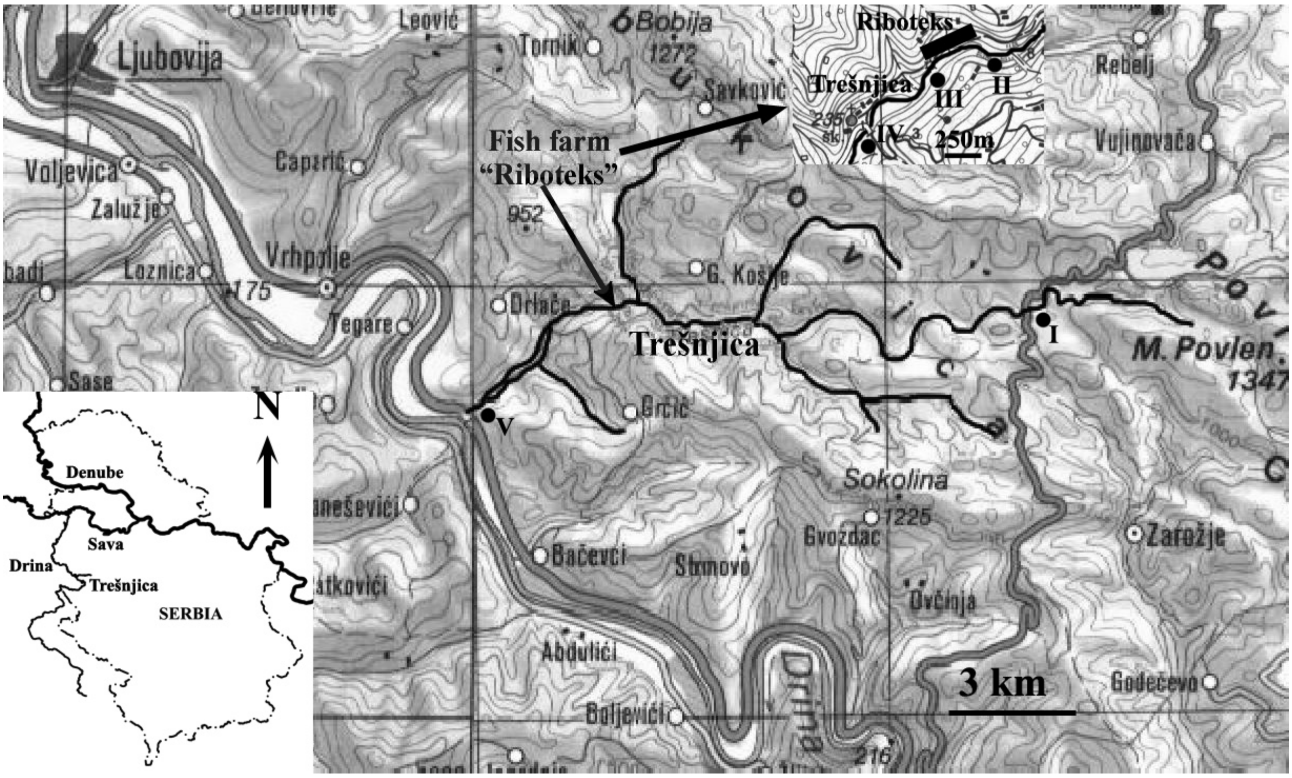

Fig. 1. Investigated sites (black dots) and location of the "Riboteks" Trout Farm (marked with an arrow) on the Trešnjica River. Insert lower-left: position of the Trešnjica River in Serbia. Insert upper-right: enlarged area around the "Riboteks" Trout Farm (black rectangle) with positions of localities II, III, and IV (black dots).

a catchment area of 300 cm^2 and mesh size of 250 μm, in addition to which organisms and other objects were harvested with forceps from the underside of rocks in the water. The collected material was put in plastic bottles and fixed with 96% alcohol in the field. Three samples were collected with the Surber net at each locality in every month of the investigation. A sample is defined as material obtained from the river by a single sweep of the Surber net.

The raw biomass of macroinvertebrates was measured with a Mettler analytical balance (AE 163) having a precision of 0.0001 g. Insect larvae and other collected macroinvertebrates were identified with the aid of pertinent literature (Hynes, 1977; Macan, 1979; Roskošny, 1980; Glöer et al., 1985; Waringer and Graf, 1997; Wallace et al., 2003) in the laboratory at the Biology Faculty's Institute of Zoology, where the material is stored. The "S" saprobic index (Pantle and Buck, 1955) was used to assess water quality. Saprobic values were taken from the list of bioindicators given in Moog (2002).

In order to measure diversity of benthocenoses, two diversity indices were used: the Shannon-Weiner index and the Berger-Parker dominance index. The Shannon-Weiner index (Shannon and Weiner, 1949) is the most commonly used diversity index. It is influenced both by species richness and by species equitability and is most sensitive to the abundance of rare species. The Berger-Parker dominance index (Southwood, 1978) is a measure of dominance that is strongly weighted towards the most abundant species. The value of the Shannon-Weiner index is directly proportional, while that of the Berger-Parker index is inversely proportional to the diversity value.

Diversity indices were calculated with the aid of the BioDiversity Professional Program (McAleece, 1997). The Mann-Whitney U-test with a $p<0.05$ level of significance was used for comparison of samples. In order to measure the strength of the association between pairs of variables, the Pearson product moment correlation, with a p<0.05 level of significance, was used. The Mann-Whitney U-test and determination of the Pearson product moment correlation were performed with the aid of the Sigma Stat program (version 2).

RESULTS

Analysis of the composition of macrozoobenthos communities along the investigated watercourse showed that except at locality I, where Plecoptera were distinctly dominant (38.51%), all localities were characterized by dominance of Ephemeroptera larvae, whose representation was between 33% (locality I) and 38% (locality IV) of total abundance. However, within the order Ephemeroptera, significant changes occurred in the participation of individual families in total abundance, the most pronounced of which were changes in abundance of the Baetidae (Fig. 2, insert 1). Their abundance increased from 11.74% at locality II to 28.63% at locality III, but subsequently declined to 21.83% at locality V. A reverse trend characterized the families

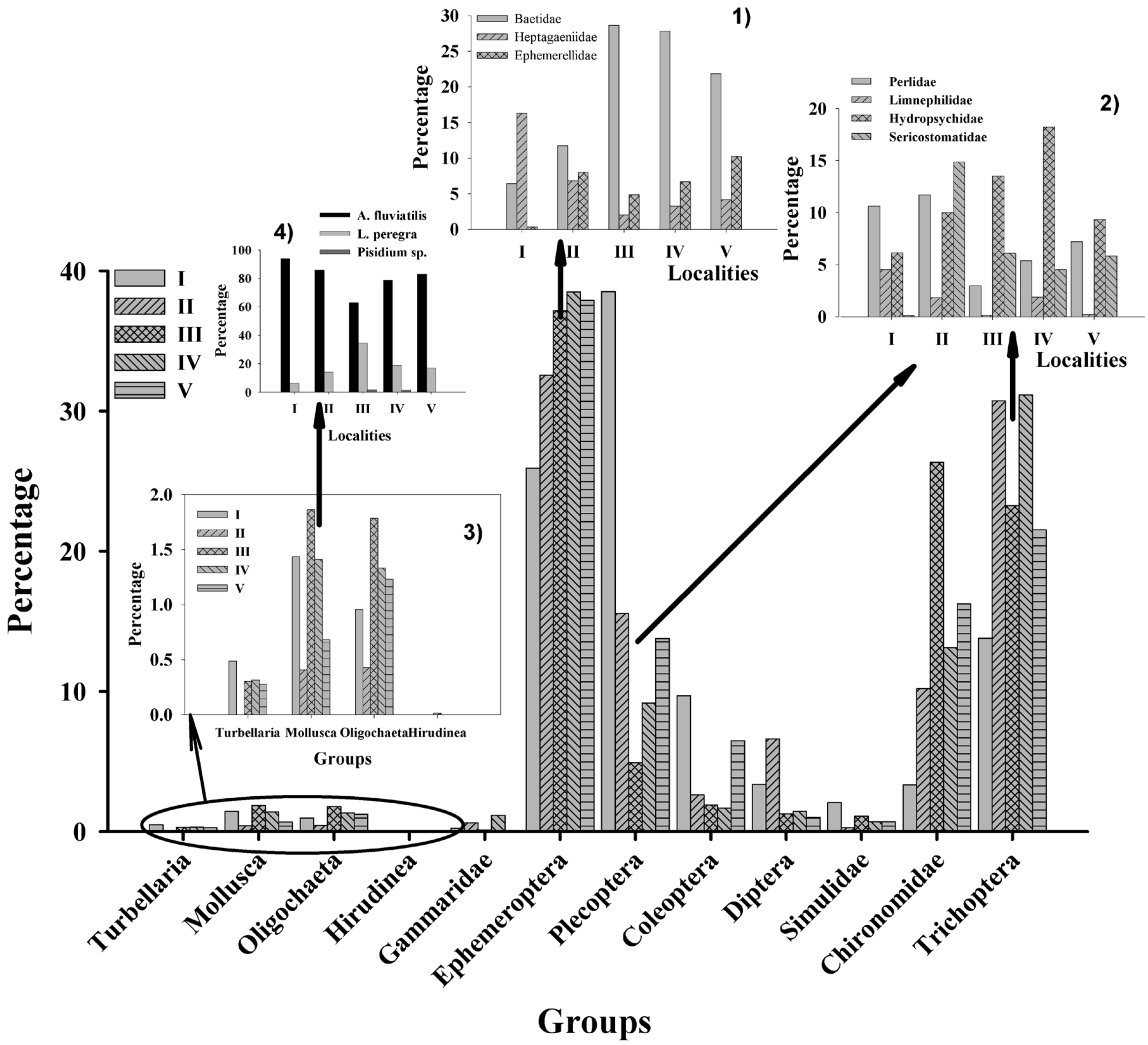

Fig. 2. Participation (%) of macrozoobenthos groups at the investigated localities. Insert 1) participation of Baetidae, Heptagaeniidae, and Ephemerellidae; insert 2) participation of Perlidae, Limnephilidae, Hydropsychidae, and Sericostomatidae; insert 3) participation of non-insect groups; insert 4) participation of species from the phylum Mollusca.

Heptagaenidae and Ephemerellidae, with decrease of relative abundance from 6.83% and 8.02% at locality II to 2.02% and 4.87% at locality III and subsequent increase to 4.17% and 10.25% at locality V, respectively. Except at localities I and III, Trichoptera larvae were subdominant. The most pronounced changes in relative abundance within Trichoptera characterized the families Hydropsychidae, Sericostomatidae, and Limnephilidae (Fig. 2, insert 2). Thus, relative abundance of the families Sericostomatidae and Limnephilidae significantly decreased from 14.6% and 1.84% at locality II to 6.10% and 0.12% at locality III, respectively. On the other hand, the family Hydropsychidae was represented with 10.0% in the total abundance at locality II, with 13.5% at locality III, with 18.2% at locality IV, and with 9.3% at locality V. Significant increase of relative abundance after emptying of trout farm wastewaters into the Toplica River also characterized by the Chironomidae larvae, with representation of 10.2% at locality II and 26.3% at locality III (Fig. 2). In fact, at locality III Chironomidae larvae were subdominant, while at localities IV and V Trichoptera became subdominant again. In addition to these changes, a significant decrease occurred in

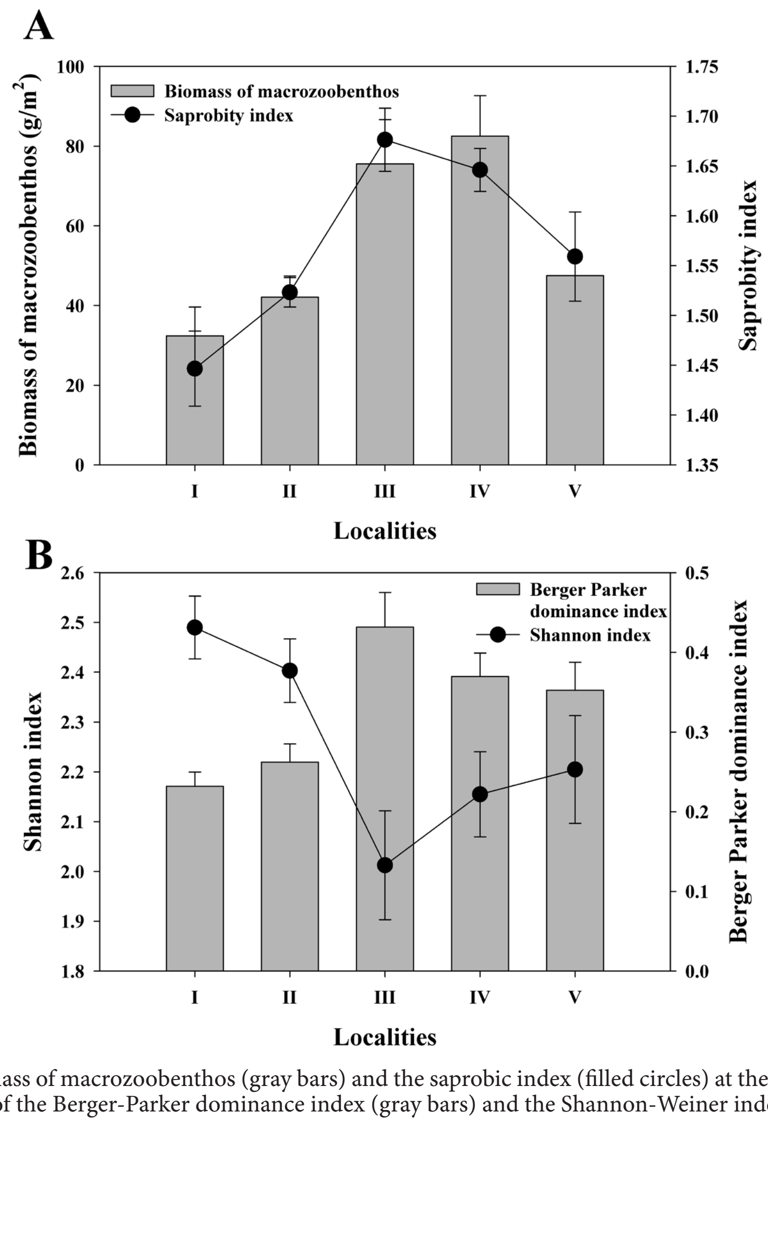

Fig. 3. A) Mean values of biomass of macrozoobenthos (gray bars) and the saprobic index (filled circles) at the investigated localities. B) Changes in average values of the Berger-Parker dominance index (gray bars) and the Shannon-Weiner index (filled circles) at the investigated localities.

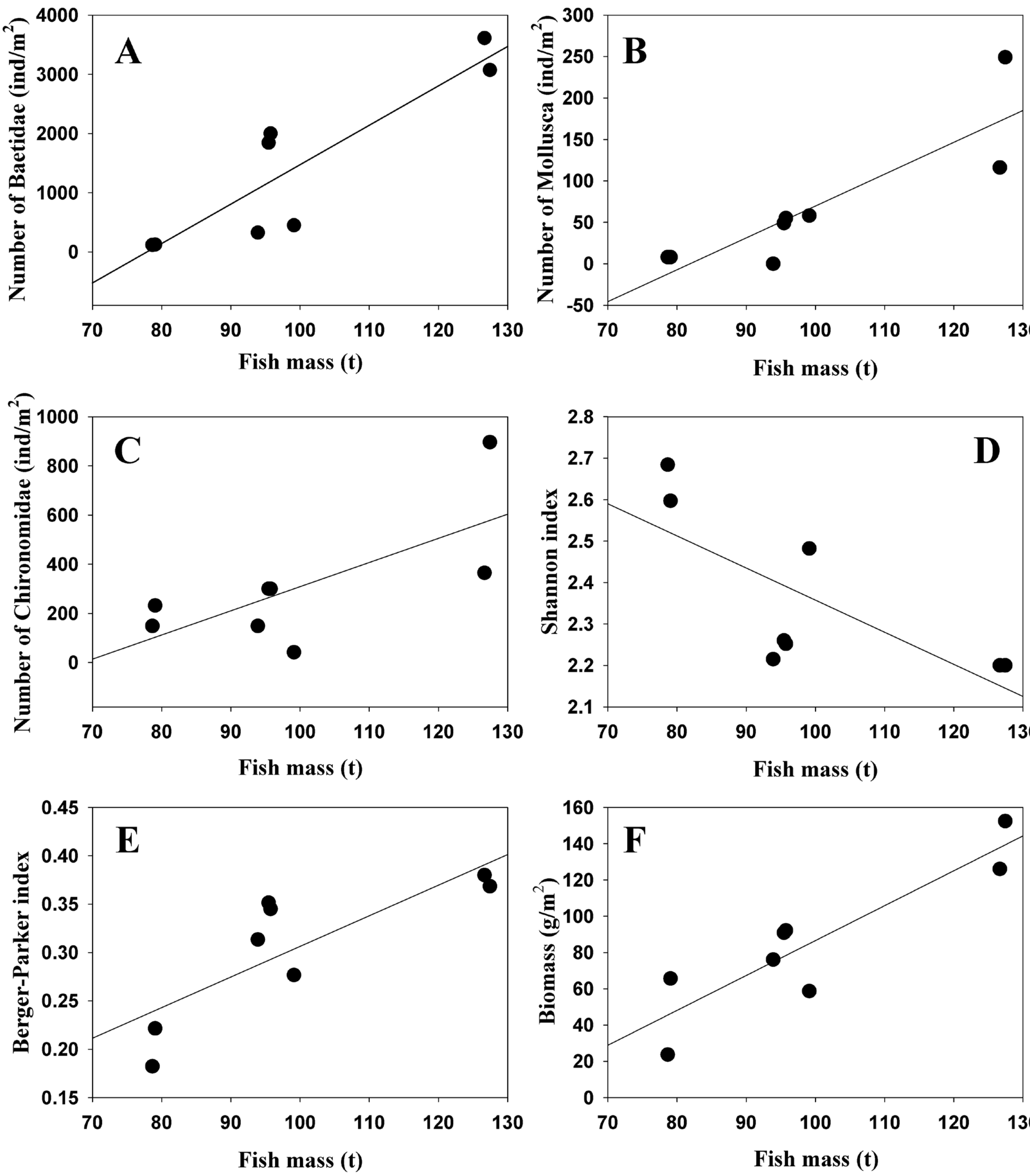

Fig. 4. Effect of changes in fish biomass at the end of the month at the "Riboteks" Trout Farm during the investigation period on A - Number of Baetidae larvae, B - Number of Mollusca, C - Number of Chironomidae larvae, D - Shannon-Weiner index, E - Berger-Parker dominance index, and F - biomass of macrozoobenthos at the third locality on the Trešnjica River. Solid lines represent linear fit of the data.

the representation of Plecoptera larvae at locality III, where it comprised 4.9% (versus 15.6% at locality II), after which it rose to 9.2% and 13.8% at localities IV and V, respectively. The non-insect group, generally less sensitive to pollution (Mollusca, Oligochaeta, Turbellaria, and Hirudinea), was not abundant in the Trešnjica River (Fig. 2, insert 3). However, members of the group were significantly more represented at locality III compared to locality II, with subsequent decrease in relative abundance at localities IV and V. That was especially characteristic of Hirudinea, which appeared only at locality III (Fig. 2, insert 3). It can be seen (Fig. 2, insert 3) that these groups, especially Mollusca, were present in a similar percentage at locality I, but their structure was completely different (Fig. 2, insert 4). Thus, the species *Ancylus fluviatilis* was dominant among Mollusca with 94%, while *Lymnaea peregra* was poorly represented with 6% at locality I, whereas the participation of *A. fluviatilis* declined to 63% and that of *L. peregra* increased to 34.6% at locality III, where members of the tolerant genus *Pisidium* appeared with a representation of 1.6%. At locality V, the species *A. fluviatilis* and *L. peregra* attained percentages of total abundance similar to those at locality II, e.g., 83% and 17%, respectively.

Especially pronounced were changes of macrozoobenthos biomass. Zoobenthos biomass increased from 42.00±5.32 g/m^2 at locality II to 75.53±11.15 g/m^2 at locality III, remained at the same level at locality IV, then declined significantly to 47.47±5.32 g/m^2 at locality V (Fig. 3A). Similar changes characterized the saprobity index (Fig. 3A), with statistically significant increase at locality III (1.68±0.03) compared to locality II (1.52±0.01) and decrease at locality V (1.56±0.04) compared to locality IV (1.65±0.02). It was characteristic of both of the diversity indices used (Fig. 3B) that statistically significant differences did not exist between localities I and II and that their values at locality III and IV significantly decreased (the Shannon-Weiner index) or increased (Berger-Parker index) and showed the greatest variability there. In contrast to biomass and the saprobity index, changes of diversity indices at the fifth locality were not statistically significant compared to locality IV.

Entirely as expected, the effect of wastewater from the trout farm was greatest at locality III. This locality was therefore selected for testing of the farm's influence on the composition of macrozoobenthocenoses with the passage of time. The mass of fish at the end of each month during the period of investigation was selected as the core parameter for quantification of strength of the influence exerted by the "Riboteks" Trout Farm on the ecosystem of the Trešnjica River. This parameter was selected because it directly determines intensity of the two main sources of pollution, e.g., the amount of food consumed and the amount of excrement produced. The mass of fish on the trout farm (m_f) at the end of the month increased constantly during the period of investigation from February (when it was about 70 tons) to October (when it was 130 tons). A sudden drop (to about 100 tons) occurred in November due to significant sales of fish.

The results of determining the Pearson product moment correlation indicated that the mass of fish shows a statistically significant positive correlation with the values of abundance of Baetidae, Chironomidae, and Mollusca, total biomass of macrozoobenthos, and the Berger-Parker diversity index at locality III, and a negative one with values of the Shannon-Weiner index at that locality (Fig. 4). The mass of fish at the end of each month shows the strongest correlation with the number of Baetidae (correlation coefficient = 0.892, p = 0.003) and total biomass of macrozoobenthos (correlation coefficient = 0.896, p = 0.003), and the weakest one with the number of Chironomidae (correlation coefficient = 0.710, p = 0.045).

DISCUSSION AND CONCLUSIONS

In the present investigation, we monitored influence of the "Riboteks" Trout Farm on macrozoobenthos communities in the Trešnjica River. This is the largest trout farm in Serbia, and its production puts it on the boundary between large and middle-sized fish farms (Engle et al., 2005). In view of its high production, the given trout farm could be expected to exert significant influence on the Trešnjica River. Indeed, influence of the "Riboteks" Trout Farm wastewaters caused a clear and statistically significant change in all measured parameters describing the composition and structure of macrozoobenthos communities at localities downstream from the farm.

The strongest changes in structure of benthocommunities were recorded at locality III, which was to be expected, since it was located only 20 m downstream from the influx of wastewater from the fish farm. Those changes were mainly a consequence of considerable increase in the abundance of individuals from the families Baetidae and Chironomidae, and decrease in abundance of Plecoptera larvae. It is known that species of the family Baetidae (Ephemeroptera) are tolerant to a moderate level of pollution and turbidification of the substrate (Hilsenhoff, 1988). Increase in abundance of Baetidae was also recorded downstream from other fish farms (Adamek and Sukop, 1996; Camargo, 1992a; Loch et al., 1996). The great abundance of Baetidae, relatively small abundance of pollution resistant groups (especially Oligochaeta and Hirudinea), and the presence of Plecoptera larvae imply that the trout farm wastewaters have a clear impact on structure of benthocommunities at locality III, but one that was of moderate intensity.

Except for significant lowering of the percentage representation of Chironomidae, the composition of benthocommunities at locality IV was not changed compared to locality III. However, the structure of macrozoobenthos communities in the Trešnjica River was significantly improved at locality V. This is indicated by changes in the Mollusca community, whose composition was the same at localities II and V, although the abundance of *Lymnaea peregra* in relation to *Ancylus fluviatilis* increased and species of the tolerant genus *Pisidium* were recorded at locality IV and especially at locality III. In addition, the participation of Plecoptera increased at locality V, while the percentage representation of Hydropsychidae declined to attain values characteristic of this group at locality II.

Values of the saprobic index and biomass also indicate that influence of the trout farm was confined to localities III and IV, and that statistically significant changes in structure of the macrozoobenthos community occurred at locality V, since statistically significant differences in relation to the bottom fauna community at locality II disappeared. As far as water quality is concerned, the saprobic index indicates that the oligosaprobic water at localities I and II passed over into the β-saprobic class at localities III and IV, while water quality at locality V was on the boundary between these two categories. Similar values of the saprobic index were recorded in the case of the Punkva River (Adamek and Sukop, 1996). Prior to the present work, the effects of fish farms on total biomass was investigated in two other studies. In that of Camargo (1992a), increase of biomass was noticed only in the summer months, not in the spring of the year, whereas such a seasonal difference was not observed in the study of Adamek and Sukop (1996). Increase of biomass can be attributed to the fact that under conditions of moderate pollution with no drastic decline of diversity (see below), greater amounts of available food (originating from the trout farm) in the form of suspended and sedimented organic particles as well as microalgae made greater macrozoobenthos production possible.

Analysis of structure of the benthocenosis in the Trešnjica River showed that it was significantly altered even 500 m downstream and that only at a distance of about 3.5 km did it for the most part return to the state characteristic of it prior to the influx of wastewater. Analysis of similar investigations showed that no definite boundary of action of fish farm wastewater on benthocenosis structure was established in most of them. Thus, on the Río Tajuña River (Camargo, 1992b) and three streams in the watershed of the Little Tennessee River (Loch et al., 1996), the negative influence of fish farms was still significant even at the most distant localities (1 and 1.5 km downstream from the farms), although a certain improvement in structure of the zoobenthos community was recorded there.

Results of the present work confirmed that the mass of fish on the trout farm is a parameter that adequately defines the strength of its action, above all the intensity of its influence on structure of the macrozoobenthos community. To be specific, temporal changes of the majority of strongly farm-influenced parameters describing structure of macrozoobenthos communities also showed statistically significant correlation with seasonal changes in the mass of fish (Fig. 4).

In conclusion, the “Riboteks” Trout Farm’s wastewaters caused only moderate deterioration of the structure and composition of macrozoobenthos communities despite the lack of additional water purification by means of settling tanks at the outlet from the farm. However, their construction is planned in the near future. That measure gave excellent results in the case of fish farms in Virginia (Selong and Helfrich, 1998). To test the effect on the Trešnjica River, investigations of macrozoobenthos at localities downstream from the farm will be carried out after construction of the settling tanks. If the results prove to be positive, e.g., if (as expected) the influence of wastewater from the fish farm on the macrozoobenthos community is reduced to a statistically insignificant level, then it will be possible to take the "Riboteks" Trout Farm as a model for further development of trout farms on the territory of Serbia, as well as for formulation of appropriate legal regulation.

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УТИЦАЈ ПАСТРМСКОГ РИБЊАКА НА ЗАЈЕДНИЦЕ МАКРОЗООБЕНТОСА РЕКЕ ТРЕШЊИЦЕ, СРБИЈА

ИВАНА ЖИВИЋ[1], З. МАРКОВИЋ[2] и М. ЖИВИЋ[3]

[1]*Биолошки факултет, Универзитет у Београду,* 11000 Београд, Србија
[2]*Пољопривредни факултет, Универзитет у Београду,* 11080 Београд, Србија
[3]*Департман за биологију, Државни универзитет у Новом Пазару,* 36300 Нови Пазар, Србија

Узгој пастрмки представља растућу претњу за квалитет воде чистих брдско-планинских потока. Истраживања овог проблема су се фокусирала пре свега на промене у физико-хемијским особинама воде и структури речног дна. У овом раду истраживан је утицај рибњака са највећом продукцијом пастрмке у Србији („Риботекс“), смештеног на реци Трешњици на заједнице макрозообентоса те реке. Истраживања су показала да су отпадне воде из пастрмског рибњака „Риботекс“ изазвале јасну, статистички значајну, промену умереног интензитета свих мерених параметара који описују састав и структуру заједница макрозообентоса. Ове промене су биле најизраженије у делу водотока најближег испусту отпадне воде из рибњака (локалитет III), али су остале статистички значајне и 500 м низводно (локалитет IV), да би се изгубиле тек око 3.5 км низводно од улива рибњачких отпадних вода (локалитет V). Највеће су биле промене у учешћу бројности Baetidae, Chironomidae и Plecoptera у укупној бројности макрозообентоса. Поред тога резултати су потврдили да је маса риба у рибњаку параметар који на одговарајући начин дефинише интензитет његовог деловања, пре свега степен утицаја на структуру заједница макрозообентоса.