

Seasonal changes in condition, hepatosomatic index and parasitism in sterlet (*Acipenser ruthenus* L.)

Mirjana LENHARDT^{1*}, Ivan JARIĆ², Predrag CAKIĆ¹, Gorčin CVIJANOVIĆ², Zoran GAČIĆ²,
Jelena KOLAREVIĆ²

¹Institute for Biological Research, Despota Stefana 142, 11000 Belgrade, SERBIA

²Institute for Multidisciplinary Research, Kneza Visislava 1, 11000 Belgrade, SERBIA

Received: 05.10.2007

Abstract: Measurements of biomarker responses in fish from contaminated sites could provide valuable data for environmental risk assessment. Biomarkers are sensitive to both environmental pollution and confounding factors that are not related to pollution (e.g., season, nutritional status, and population density). Unfortunately, data about the latter group are scarce. Therefore, juvenile sterlet (*Acipenser ruthenus* L.) specimens were collected on a monthly basis from the Danube River from September 2002 through August 2003 to determine if seasonal changes exist in condition, hepatosomatic index, and parasitism. Fulton condition factor (FCF range: 0.27 - 0.79), hepatosomatic index (HSI range: 1.14 - 6.67) and *Skrjabinopsolus semiarmatus* parasite prevalence (range: 37.9 - 85.7 %), intensity (range: 1 - 337 parasites per fish) and the mean intensity (range 9.6 - 89.2) in sterlet showed significant seasonal variations. This study pointed out that biomarker sampling on different locations has to be performed within the short time frame in order to avoid differences generated by confounding factors. Better understanding of the life cycle of *S. semiarmatus* and sensitivity of different phases to pollution is needed.

Key Words: Danube River, Fulton condition factor, hepatosomatic index, *Skrjabinopsolus semiarmatus* prevalence and intensity

Introduction

The extent of river habitat alterations and degradation, induced by industrial and communal wastewaters discharge, utilization of river for irrigation, power utility, drinking-water utility, sport, and recreational and commercial fisheries led to the development of the ecosystem health concept. Measurements of bioaccumulation and biomarker responses in fish from disturbed and contaminated

sites provide information that can contribute to environmental monitoring programs, designed for various aspects of environmental risk assessment. Confounding factors not related to pollution (e.g., season, nutritional status, reproductive and developmental status, and population density) should be carefully considered when interpreting biomarker data (1). Unfortunately, most available toxicity data rarely quantify the potency that confounding factors are likely to exhibit in natural environments (2).

* E-mail: lenhardt@ibiss.bg.ac.rs

Gross indices are sometimes indicative of toxicant effects. The condition of the liver and of the whole body, as measured with the hepatosomatic index (HSI) and Fulton condition factor (FCF), can provide information on potential pollution impacts. Although these parameters are not very sensitive, they may serve as an initial screening biomarker to indicate exposure and effects (1). Body condition is a practical tool for biologists and managers to gauge the overall health of fish population, and a good indicator of fish habitat quality and pollution levels (3-5). Hypothesis that metal contamination can lead to significantly lower values of FCF was strongly supported by some studies, although it was shown that that FCF can reach quite high values during the growth season in heavy metal contaminated lakes, reflecting increased feeding rate (6,7). While some authors (7,8) claimed that HSI might be useful as an indicator of chemical water pollution, others showed that it was inconsistent as a biomarker (3) and that it is dependant on the seasonal cycle (9). Prior to establishment of HSI as a pollution indicator, standard ranges should first be established, and the possible effects of internal biological rhythms and environmental factors on this parameter must be assessed (10).

Numerous studies have examined effects of anthropogenic environmental perturbations on parasitic organisms at both the population and the community level (11). As a general rule, infections with endoparasitic helminthes with complex indirect life cycles tend to decrease with increasing levels of pollution, while infections with ectoparasites tend to increase under these same conditions (12). It should be noted that the use of indices, which is recommended in the EC Water Framework Directive (WFD), requires collecting multiple parameters, including parasites (13).

Throughout its life cycle, sterlet (*Acipenser ruthenus* L.) is directly exposed to contaminants through water, sediments, and benthic prey. Therefore, it could be a good species for assessing the overall quality of their aquatic habitat. However, little is known about the seasonal changes of some parameters that could be used as biomarkers in sterlet and other sturgeon species. The importance of sturgeons comes from their high economic value and endangered state of their populations (14). This work was part of a complex investigation of sterlet, which

included age structure, growth, catch per unit effort, and morphological analyses (14-17). The purpose of this study was to examine seasonal changes in HSI, FCF, and parasite infection in wild sterlet population in order to more clearly interpret biomarker data collected from the Danube River sterlet population.

Materials and Methods

Sterlet specimens were caught by professional fishermen, using drift gill nets at 5 locations within a 50-km long stretch of the Danube River (1,123 – 1,173 river km; 44°39'–44°51' N, 20°24'–20°52' E) (Figure 1). Specimens were collected monthly from September 2002 to August 2003 (except for February and April 2003, when sterlet were absent from the catch). Analysis of 418 juvenile sterlet specimens was performed. Total length (L_t , 0.1 cm) and total mass (M , 0.1 g) were recorded to determine FCF as $FCF = (M \times L_t^{-3}) \times 100$, and liver mass (H , 0.01 g) to ascertain HSI = $(H \times M^{-1}) \times 100$. The entire digestive tract was removed and examined for the prevalence and intensity of parasites. According to Bush et al. (18), intensity of infection was calculated as the number of individuals of a particular parasite species in a single infected sterlet, and mean intensity was calculated as the average intensity of a particular species of parasite among the infected sterlet. Prevalence was calculated as the number of sterlet infected with 1 or more individuals of a particular parasite species, divided by the total number of sterlet examined for that parasite species (18).

Sterlet age determination was performed using pectoral fin spine sections according to Stevenson and Secor (19). Briefly, we cut the spines with a laboratory saw and then polished it with emery paper, until sections were transparent enough for microscopic observation. Age was estimated by counting annuli in the fin ray sections.

Statistical analyses included comparison of parameters between different months to determine if there were any variations throughout the year. Groups were compared using analysis of variance (ANOVA) and sequential Bonferroni correction for multiple comparisons, while groups with small sample sizes ($n < 30$) were compared using Mann-Whitney U Test (20).

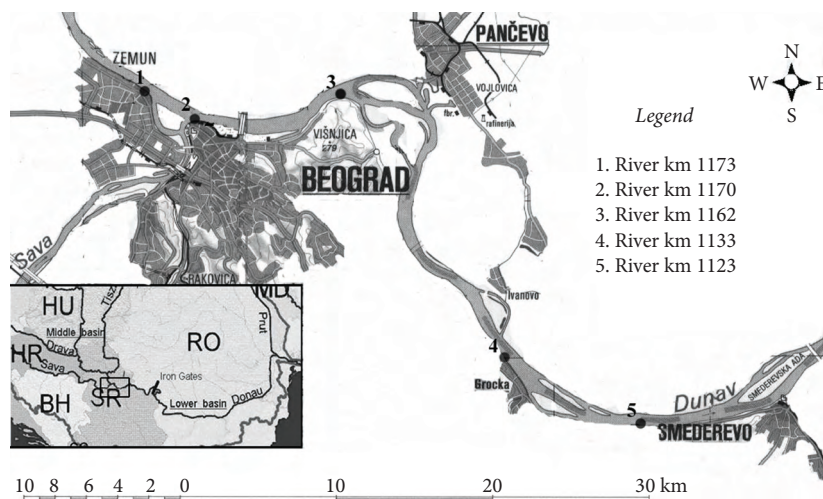


Figure 1. Map of the Danube River showing the sampling stretch. Locations 1 to 5 represent the sampling sites. Smaller map represents part of the Danube River where samples were collected.

Results

Sterlet specimens ranged in L_t and M from 14.2 to 42.5 cm and from 13.3 to 362.0 g, respectively. Pectoral fin ray analysis showed that all specimens were in age classes from 0^+ to 1^+ .

Intensity of parasite infection of all 418 fish were as follows: trematodes (1 - 337 *Skrjabinopsolus semiarmatus*, 1 - 2 *Azygia lucii*, and 1 *Posidiplostomum cuticula*), nematodes (1 - 59 *Hysterothylacium bidentatum*), acantocephalans (1 - 19 *Acantocephalus lucii*, and 1 *Pomphorhynchus laevis*) and cestodes (1 *Amphilina foliacea*), with an infection rate of 62.2%. Due to its dominance, only *S. semiarmatus* was included in statistical analysis.

Changes in FCF, HSI, and the mean intensity of *S. semiarmatus* infection during the year are given in Figure 2. The highest value for FCF was recorded in June, when only 1^+ -old individuals were in catch, while the lowest value was in December. Values for FCF varied between 0.27 and 0.79. The maximum value for HSI was recorded in October, while the minimum was in March. Values for HSI varied between 1.14 and 6.67. The greatest number of parasites per sterlet was found in November and December, with the maximum of 337 *S. semiarmatus* specimens recovered from a single individual in November. Mean intensity of *S. semiarmatus* varied

between 9.6 and 89.2 with the maximum value in November and the minimum in June. Statistical analysis found significant differences ($P < 0.05$) in HSI, FCF, and *S. semiarmatus* infection intensity among different months during the year. Prevalence of sterlet's infection by parasite *S. semiarmatus* in annual cycle showed normal distribution (Kolmogorov-Smirnov Test, $P = 0.20$) with the maximum value in January (Figure 3).

Discussion

Sterlet is the smallest species of sturgeons, reaching maturity between 3 and 8 years of age and with a life span of 22 - 24 years (21). Within the IUCN Red list, it is classified as endangered in principal rivers (Volga, Danube). Choudhury et al. (22) stated that knowledge on the biology of juveniles is critical for the successful management of threatened sturgeon species, and in that sense investigations of parasites in that life stage of sterlet are very important. This study involved only investigation of juvenile sterlet, so additional research is necessary for adult male and female specimens.

Morphological parameters that are often used in field research are the hepatosomatic index to identify possible liver diseases, and the condition factor to assess the general condition of fish (1). Although condition factor should be interpreted with caution,

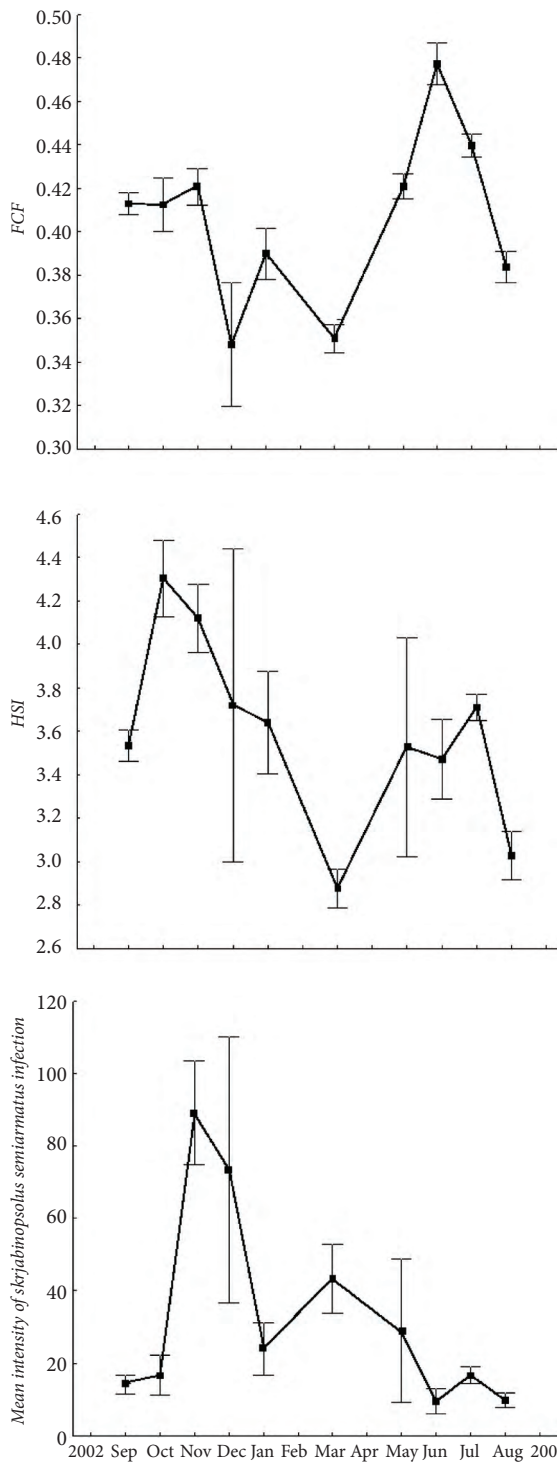


Figure 2. Mean value \pm S.E. for FCF, HSI, and the mean intensity of *S. semiarmatus* infection in sterlet during the annual cycle.

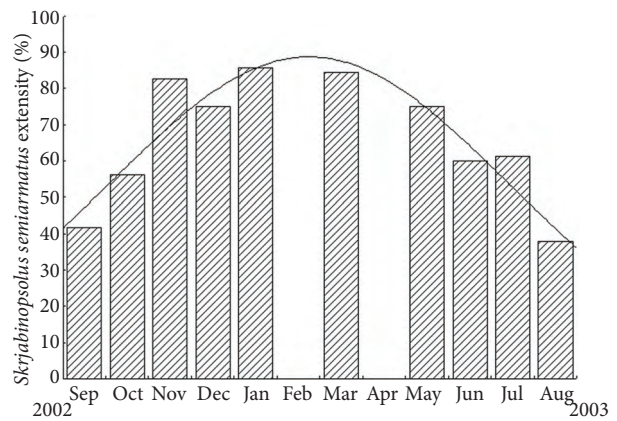


Figure 3. The prevalence (%) of *S. semiarmatus* in sterlet during different months in the annual cycle; the line represents fitting with the normal distribution function.

body condition is very commonly used in fisheries biology because it is an inexpensive, non-lethal alternative to proximate analysis of tissues (5). In a study by Craig et al. (5), FCF of lake sturgeon (*Acipenser fulvescens*) had an overall mean of 0.64, while in studies cited by Beamish et al. (23) it had a range of 0.28 - 0.54. In sterlet analyzed in this study FCF had a little bit extended range (0.27 - 0.79). Work presented here shows that significant changes occurred in FCF in juvenile sterlet during the year, which could be associated with reduced food intake during winter (3). The lowest values were recorded in December and March, which is probably connected with the fact that sterlet, like other sturgeons, aggregates in bottom holes during the winter and exhibits little activity.

HSI is associated with liver energetic reserves and metabolic activity (7). HSI had a maximum value in October and November in the studied sterlet. According to Lapkina et al. (24), food of juvenile sterlet on investigated locations in the Danube River consisted mainly of chironomids during July, 70% of food consisted of leech in August, while leeches were present 100% in sterlet food in September. Daily growth in weight in July was 1.8 g day^{-1} , while in September it had the value of 2.2 g day^{-1} . It could be a possible explanation for an increase in HSI from August until October. The minimum value for HSI in sterlet was in March. This is in accordance with the finding in juvenile lake sturgeon, in which HSI had

lower value in March than in June, July, and August (23). On the other hand, HSI did not differ between June and October in lake sturgeon, while it did in Danubian sterlet. Yang and Baumann (9) stated that HSI depends on the seasonal cycle. In the study carried out by Hung et al. (25), HSI differed among juvenile white sturgeon (*Acipenser transmontanus*) that were fed with different carbohydrates, ranging between 1.99 and 3.71. Differences in HSI that were found in this study (ranging between 1.14 and 6.67), were probably also caused by sterlet diet in natural conditions that differed throughout the year.

Skrjabinopsolus semiarmatus has been found to infest all anadromous sturgeons in the Mediterranean, Black, and Caspian seas and it appears also in sterlet. The intensity of infestation declines between April and September (26), which is comparable to the data obtained in this study. The highest value for mean intensity of *S. semiarmatus* in sterlet caught in the Danube was in November (89.2) and it is significantly different from all other months. Reported mean intensity for other sturgeons is below values obtained in this work. Mean intensity of *S. semiarmatus* was 2.8 in *A. gueldenstaedtii*, 47.9 in *A. nudiventris* (27), 7.7 in *A. stellatus*, and 6.1 in *A. persicus* (28). There is no data about the intermediate host of *S. semiarmatus* in the Danube River, but according to Sattari and Mokhayer (27), Oligochaeta are known to be the intermediate hosts of *S. semiarmatus* in the Caspian Sea. In the search for indicators to monitor the effects of pollutants, there are good reasons for focusing on parasites, because of their complex life cycle with the widely differing requirements for different stages, so that each stage must be assessed separately, thereby greatly increasing the number of potential indicators

(12). The relationship between pollution and parasitism in aquatic organisms and the potential role of parasites as water quality indicators have received increasing attention during the last 2 decades (29). Ability of some macroparasites, like *P. laevis*, to significantly accumulate higher levels of different pollutants than their hosts, indicate that some fish parasites could be more sensitive indicators of pollution in aquatic ecosystems than their hosts (29,30).

Thus, it is necessary to carefully consider the infection status of animals used for toxicological and ecotoxicological studies, as parasites may alter the uptake and accumulation of toxins in host tissues. Interactions between the environment and host-parasite systems are complex and not easily interpreted, since they are dependent on a wide variety of factors.

This study pointed out that significant changes exist in FCF, HSI, and prevalence and intensity of *S. semiarmatus* sterlet infection among different months throughout the year. This fact must be kept in mind when using these parameters as biomarkers, and any biomarker sampling on different locations has to be performed within a short time frame, in order to avoid differences generated by confounding factors. Use of *S. semiarmatus* as a biomarker needs better understanding of its life cycle, including sensitivity of different phases to pollution.

Acknowledgements

This study was supported by grant #143045 of the Serbian Ministry of Science and Environmental Protection.

References

1. van der Oost, R., Beyer, J., Vermeulen, N.P.E.: Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ. Toxicol. Phar.*, 2003; 13: 57-149.
2. De Kruijf, H.A.M.: Extrapolation through hierarchical levels. *Comp. Biochem. Physiol. C*, 1991; 100: 291-299.
3. Khan, R.A.: Length-mass relationship, histopathology, and parasitism in winter flounder (*Pleuronectes americanus*) living near a PCB-contaminated naval facility in Newfoundland. *Can. J. Zool.*, 1999; 77: 381-388.
4. Oliva-Paterna, F.J., Vila-Gispert, A., Torralva, M.: Condition of *Barbus sclateri* from semi-arid aquatic systems: effects of habitat quality disturbances. *J. Fish Biol.*, 2003; 63: 699-709.
5. Craig, J.M., Thomas, M.V., Nichols, S.J.: Length-weight relationship and a relative condition factor equation for lake sturgeon (*Acipenser fulvescens*) from the St Clair River system (Michigan, USA). *J. Appl. Ichthyol.*, 2005; 21: 81-85.

6. Couture, P., Rajotte, J.W.: Morphometric and metabolic indicators of metal stress in wild yellow perch (*Perca flavescens*) from Sudbury, Ontario: a review. *J. Environ. Monit.*, 2003; 5: 216-221.
7. Pyle, G.G., Rajotte, J.W., Couture, P.: Effects of industrial metals on wild fish populations along a metal contamination gradient. *Ecotoxiol Environ. Saf.*, 2005; 61: 287-312.
8. Sloof, W., Van Kreijl, C.F., Baars, A.J.: Relative liver weights and xenobiotic-metabolizing enzymes of fish from polluted surface water in the Netherlands. *Aquat. Toxicol.*, 1983; 4: 1-14.
9. Yang, X., Baumann, P.C.: Biliary PAH metabolites and the hepatosomatic index of brown bullheads from Lake Erie tributaries. *Ecol. Indicat.*, 2006; 6: 567-574.
10. Lenhardt, M.: Seasonal changes in some blood chemistry parameters and in relative liver and gonad weights of pike (*Esox lucius* L.) from the River Danube. *J. Fish Biol.*, 1992; 40: 709-718.
11. Marcogliese, D.J.: Parasites of the superorganism: are they indicators of ecosystem health? *Int. J. Parasitol.*, 2005; 35: 705-716.
12. Mackenzie, K.: Parasites as pollution indicators in marine ecosystems: a proposed early warning system. *Mar. Pollut. Bull.*, 1999; 38: 955-959.
13. Berge, D., Dahl-Hansen, G.: Management of Freshwater Fisheries on Bordering Rivers - EU - regulations in the field of water and fish management. Norwegian Institute for Water Research, Report 24274, 2005.
14. Lenhardt, M., Cakić, P., Kolarević, J.: Influence of the HEPS Djerdap I and Djerdap II dam construction on catch of economically important fish species in the Danube River. *Ecohydrol. Hydrobiol.*, 2004; 4: 499-502.
15. Kolarević, J., Lenhardt, M., Cakić, P., Jarić, I.: Population study of the Danube sterlet (*Acipenser ruthenus* L.). In: Otterstad, O., Ed. Releasing development potentials at the Eastern Adriatic. NTNU, 2004; (<http://www.easternadriatic.com>).
16. Lenhardt, M., Cakić, P., Kolarević, J., Micković, B., Nikcević, M.: Changes in sterlet (*Acipenser ruthenus* L.) catch and length frequency distribution in the Serbian part of the Danube River during the twentieth century. *Ecohydrol. Hydrobiol.*, 2004; 4: 193-197.
17. Lenhardt, M., Kolarević, J., Jarić, I., Cvijanović, G., Poleksić, V., Micković, B., Gacić, Z., Cakić, P., Nikcević, M.: Assessment concepts for river ecosystems characterization based on sterlet (*Acipenser ruthenus* L.) population research. Proc. 5th Int. Symp. Ecohydraulics, IAHR, Madrid. 2004; 153-156.
18. Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W.: Parasitology meets ecology on its own terms: Margolis et al. revisited. *J. Parasitol.*, 1997; 83: 575-583.
19. Stevenson, J.T., Secor, D.H.: Age determination and growth of Hudson River Atlantic sturgeon, *Acipenser oxyrinchus*. *Fish. Bull.*, 1999; 97: 153-166.
20. Rice, W.R.: Analyzing tables of statistical tests. *Evolution*, 1989; 43: 223-225.
21. Peterson, D., Vecsei, P., Hochleithner, M.: Threatened fishes of the world: *Acipenser ruthenus* Linnaeus, 1758 (Acipenseridae). *Environ. Biol. Fish.*, 2006; Published online, DOI: 10.1007/s10641-006-6659-1.
22. Choudhury, A., Bruch, R., Dick, T.A.: Helminths and food habits of lake sturgeon *Acipenser fulvescens* from the Lake Winnebago System, Wisconsin. *Am. Midl. Nat.*, 1996; 135: 274-282.
23. Beamish, F.W.H., Jebbink, J.A., Rossiter, A., Noakes, D.L.G.: Growth strategy of juvenile lake sturgeon (*Acipenser fulvescens*) in a northern river. *Can. J. Fish. Aquat. Sci.*, 1996; 53: 481-489.
24. Lapkina, L.N., Streljnikova, A.P., Chuiko, G.M., Lenhardt, M., Kolarević, J.: Leeches as the objects of fish feeding. Int. Conf. "Structural - Functional Characteristics of the Biosystem of the North", Petrozavodsk State University, Russia. 2005; 214-217.
25. Hung, S.S.O., Groff, J.M., Lutes, P.B., Fynn-Aikins, F.K.: Hepatic and intestinal histology of juvenile white sturgeon fed different carbohydrates. *Aquaculture*, 1990; 87: 349-360.
26. Bauer, O.N., Pugachev, O.N., Voronin, V.N.: Study of parasites and diseases of sturgeons in Russia: a review. *J. Appl. Ichthyol.*, 2002; 18: 420-429.
27. Sattari, M., Mokhayer, B.: Occurrence and intensity of some parasites in five sturgeon species (Chondrostei: Acipenseridae) southwest of Caspian Sea. *Curr. Sci. India*, 2005; 89: 259-263.
28. Rajabpour, M., Malek, M., MacKenzie, K., Aghlmandi, F.: Helminth parasites of stellate sturgeon *Acipenser stellatus* Pallas, 1771 and Persian sturgeon *Acipenser persicus* Borodin, 1897 (Pisces: Acipenseridae) from the South-East Caspian Sea. *Parasitol. Res.*, 2008; 102: 1089-1091.
29. Sures, B.: Environmental parasitology: relevancy of parasites in monitoring environmental pollution. *Trends Parasitol.*, 2004; 20: 170-177.
30. Schludermann, C., Konecny, R., Laimgruber, S., Lewis, J.W., Schiemer, F., Chovanec, A., Sures, B.: Fish macroparasites as indicators of heavy metal pollution in river sites in Austria. *Parasitology*, 2003; 126: 61-69.