SEASONAL VARIATIONS AND DIVERSITY OF CLADOCERA (CRUSTACEA) IN NATURAL, DOMESTIC WASTEWATER TREATMENT LAGOONS

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SEZONSKA VARIJABILNOST I DIVERZITET CLADOCERA (CRUSTACEA) U LAGUNAMA ZA PRIRODNO PREČIŠĆAVANJE OTPADNIH VODA IZ DOMAĆINSTAVA

Apstrakt

Cladocera predstavljaju najznačajniju komponentu zajednice zooplanktona u većini stajaćih slatkovodnih ekosistema. Kao filtratori, hrane se algama, bakterijama, protozoama i organskim česticama, koje obiluju u netretiranim otpadnim vodama iz domaćinstava. Krupne Cladocera se proteklih decenija uveliko primenjuju u biomanipulaciji kao biološko sredstvo za prečišćavanje organski opterećenih otpadnih voda i smanjenje primarne produkcije u vodenim ekosistemima.

Istraživanje predstavljeno u ovom radu realizovano je na istraživačkom poligonu "Mali Dunav" Centra za ribarstvo i primenjenu hidrobiologiju, Poljoprivrednog fakulteta Univerziteta u Beogradu. U tri lagune, namenski napravljene na potoku Šugavac za prirodno prečišćavanje kanalizacionih voda poreklom iz uzvodno lociranog naselja, obavljeno je ispitivanje sastava i brojnosti zajednice zooplanktona. Pored bioloških ispitivanja, praćeni su hemijski parametri kvaliteta vode u trajanju od godinu dana, posmatranih kroz 4 perioda od po tri meseca. Istraživanje je sprovedeno sa ciljem ispitivanja dinamike populacije Cladocera u organski visoko zagađenim, plitkim, stajaćim vodenim ekosistemima i mogućnosti opstanka vrste *Daphnia obtusa* u datim uslovima sredine.

Prema parametrima kvaliteta vode, lagune su se značajno razlikovale samo u pogledu prosečnih vrednosti pH i rastvorenog kiseonika. Diverzitet Cladocera tokom jednogodišnjeg perioda istraživanja bio je relativno visok obzirom na smanjen kvalitet vode, sa ukupno 9 identifikovanih vrsta. Kruskal-Wallis test pokazao je da između laguna ne postoji statistički značajna razlika u ukupnoj brojnosti vrsta tokom perioda ispitivanja. Međutim, ukupna brojnost i diverzitet značajno su se razlikovali u svakoj laguni u zavisnosti od perioda posmatranja, što ukazuje na izražena sezonalna variranja u pogledu kvalitativnog i kvantitativnog sastava zajednice. Ova sezonska varijabilnost ogleda se u maloj brojnosti i potpunoj dominaciji sitne vrste *Chydorus sphaericus* tokom prvog perioda posmatranja (od kraja februara do sredine maja 2012.), praćenoj povećanjem brojnosti i diverziteta vrsta u drugom periodu (od sredine maja do sredine avgusta 2012) i dominaciji krupne vrste *Daphnia obtusa* u trećem periodu (od sredine avgusta do kraja novembra 2012.), čija je brojnost ponovo opala nakon dostizanja letnjeg maksimuma, uz ponovno pojavljivanje *C. sphaericus* u četvrtom periodu (od decembra 2012. do kraja februara 2013. godine). Vrsta koja je na svim tačkama na kojima je obavljeno ispitivanje dostigla najveću brojnost bila je *Daphnia obtusa*. Njeno prisustvo tipično je za vodene ekosisteme u kojima odsustvuju ribe kao predatori (Vadstein, 1993). Kruskal-Wallis test nije pokazao statistički značajne razlike u brojnosti ove vrste između laguna tokom jednogodišnjeg perioda istraživanja, dok su u svim lagunama utvrđene statistički značajne razlike u brojnosti posmatrane u različitim periodima. Rezultati pokazuju da je *D. obtusa* bila dominantna vrsta zooplanktona u lagunama (dostižući prosečnu maksimalnu brojnost od 1083 - 1444 ind/l tokom perioda ispitivanja), veoma tolerantna na niske vrednosti rastvorenog kiseonika (u proseku 0.32 – 6.90 mg/l) i visoko organsko opterećenje (BOD 26.65 – 33.09 mg/l).

Ključne reči: Cladocera, prirodno prečišćavanje otpadnih voda, Daphnia obtusa, organsko zagađenje

Keywords: Cladocera, natural wastewater treatment, Daphnia obtusa, organic pollution

INTRODUCTION

Currently worldwide, among the biggest ecological concerns are eutrophication and organic pollution coming from agriculture and urbanization. In recent decades, various biomanipulation methods for wastewater treatments have been developed, including constructed wetlands and wastewater stabilization ponds. Untreated domestic wastewaters contain different kinds of chemicals, high concentrations of phosphates and nitrates, organic matter, bacteria, protozoans and metazoans. Zooplankton, particularly Cladocerans, is capable of reaching high densities feeding on organic wastes and bacteria (Roche 1998, Nandini et al. 2004). Due to their high filtering rate, large daphnids such as *Daphnia magna* are commonly used for water purification in biomanipulation studies (Cauchie et al. 2002, Nandini et al. 2013).

The structure of zooplankton communities has been the subject of many studies, but only few research have been performed in polluted environments (Utz et al., 2008). This type of studies have an important role in obtaining knowledge on species diversity, variability and dynamics in stressed aquatic ecosystems. The aim of presented research was to investigate the dynamics of zooplankton communities, with emphasis on cladoceran species developing within shallow natural wastewater treatment lagoons.

MATERIAL AND METHODS

This research was conducted in three earthen lagoons at the experimental site „Little Danube" of the CEFAH, Faculty of Agriculture, University of Belgrade. The lagoons cover a total area of 0,4 ha, with average depth ranging from 0.4 to 0.5 m. Ponds are fed by surface water from the stream Sugavac and domestic wastewater coming from upstream households through an inlet pipe. The wastewater runs through lagoon one (L1), two (L2) and three (L3), respectively. In two lagoons (L2 and L3), a gravel layer 60 cm wide is built laying 1.5 m from the inlet, so that the inflow water from the previous lagoon runs through this filtering barrier while entering the pond. In each lagoon, emergent vegetation dominated by *Typha spp.* is planted and is present from the end of April until the beginning of December. Field sampling was conducted biweekly, from February 2012 till February 2013, at 4 sampling points: two points were located in L1 and L2 each, while two were located in L3: at the inlet and the outlet point. Environmental variables (temperature, pH and dissolved oxygen DO) were measured using a water field kit WTW MULTI 340/i (WTW, Germany) while total phosphorus (TP), total ammonia nitrogen (NH_4^+) and biological oxygen demand (BOD_5) were analyzed according to APHA (1998). Zooplankton samples for quantitative analysis were taken by collecting 30 l of water at each point using a plastic bucket, and filtering through sieve with 23 µm mesh size. Qualitative samples were taken by pulling plankton net (mesh size 75 µm) through surface layer in the centre of each lagoon. Samples were preserved using 4% formalin and examined under optical microscope Leica Galen III with maximal magnification of 160x. Zooplankton was identified to the level of species according to standard key for identification (Šramek-Hrušek et al., 1962). Quantitative samples were analyzed using subsampling technique, where the number of identified species was recalculated to the volume of 1 liter. Statistical analysis of obtained results was carried out using software STATISTICA v.6.0. Water quality parameters were logarithmically transformed ($\log_{10}$ x for BOD and NH_4^+, $\log_{10}$ (x+0.5) for temperature, $\log_{10}$ (x+1) for TP, $\log_{10}$ (x+1.5) for DO) except for pH (coefficient of variation < 30%) and evaluated using ANOVA and Tukey test. Biological parameters were evaluated using Kruskal-Wallis and Mann-Whitney U tests. In order to analyze the data easier, the investigation year was divided into four periods each lasting three months: period 1 – end of February till mid May of 2012, period 2 – mid May till mid August of 2012, period 3 – mid August till end of November of 2012 and period 4 – from December of 2012 till end of February of 2013.

RESULTS AND DISCUSSION

Analysis of variance of the environmental variables showed statistically significant difference between lagoons for pH ($p = 0.001$) and DO ($p = 0.023$), while other chemical parameters examined were not significantly different. Tukey test showed statistically significant difference between sampling points 1 and 3 ($p = 0.019$) 1 and 4 ($p = 0.001$) for pH, as well as 1 i 4 ($p = 0.018$) for DO. The results are presented in Table 1.

Table 1. Basic statistical data and results of ANOVA and Kruskal-Wallis tests

Parameter	Sampling point	Mean* ± SE	Min	Max	Cv (%)	*P* value**
Total count (ind/l)	1	$226.32^{a} \pm 83.22$	0.00	1546.91	180.13	0.999
	2	$231.63^{a} \pm 88.40$	0.00	1628.20	186.96	
	3	$443.87^{a} \pm 146.61$	0.00	2914.00	161.81	
	4	$348.96^{a} \pm 104.80$	0.00	1422.60	147.13	
Daphnia obtusa (ind/l)	1	$346.00^{a} \pm 136.48$	0.49	1307.78	136.64	0.829
	2	$366.54^{a} \pm 122.41$	0.52	1195.70	110.76	
	3	$452.04^{a} \pm 143.38$	0.97	1444.00	114.36	
	4	$478.87^{a} \pm 146.47$	0.18	1083.00	101.44	
pH	1	$7.74^{aA} \pm 0.02$	7.53	7.88	1.18	**0.001**
	2	$7.78^{ab} \pm 0.02$	7.55	7.90	0.97	
	3	$7.82^{b} \pm 0.03$	7.57	8.20	1.59	
	4	$7.85^{B} \pm 0.02$	7.62	8.10	1.36	
T (°C)	1	$11.13^{a} \pm 1.34$	2.70	23.70	58.87	0.960
	2	$11.74^{a} \pm 1.45$	3.50	25.70	60.57	
	3	$12.23^{a} \pm 1.50$	3.40	26.60	60.05	
	4	$12.46^{a} \pm 1.55$	3.40	27.90	61.12	
DO (mg/l)	1	$2.11^{a} \pm 0.23$	0.32	5.73	52.59	**0.023**
	2	$2.30^{ab} \pm 0.26$	0.47	6.90	54.61	
	3	$2.53^{ab} \pm 0.27$	0.74	6.80	51.60	
	4	$3.06^{b} \pm 0.23$	1.43	6.30	36.99	
NH_4^+ (mg/l)	1	$6.28^{a} \pm 0.49$	2.33	9.8	38.35	0.785
	2	$6.03^{a} \pm 0.43$	2.09	9.9	34.78	
	3	$5.67^{a} \pm 0.40$	2.46	9.8	34.15	
	4	5.62 ± 0.41	2.3	9.7	36.10	
TP (mg/l)	1	$3.61^{a} \pm 0.30$	0.65	7.20	41.07	0.894
	2	$3.35^{a} \pm 0.26$	0.60	5.50	37.40	
	3	$3.24^{a} \pm 0.27$	0.64	5.40	41.18	
	4	$3.29^{a} \pm 0.25$	0.42	5.00	37.94	
BOD_5 (mg/l)	1	$33.09^{a} \pm 3.18$	16.22	59.81	41.92	0.462
	2	$30.56^{a} \pm 2.98$	15.37	55.30	42.55	
	3	$29.34^{a} \pm 3.01$	14.38	56.00	44.78	
	4	$26.65^{a} \pm 2.81$	13.90	54.40	45.91	

*significant difference between values marked by different letters: a, b ($P < 0.05$), and A, B ($P < 0.01$); There is no significant difference between values marked by the same letter ($P > 0.05$), **bolded values are significant

Species diversity of Cladocera was relatively high throughout the examination period in all three lagoons taking into account the poor water quality. In total 9 species were identified in the samples (*Daphnia obtusa, Moina micrura, Scapholeberis aurita, Chydorus schaericus, Simocephalus vetulus, Alona quadrangula, Bosmina longirostris, Ceriodaphnia cornuta* and *Pleuroxus striatus)*. Kruskal-Wallis test showed no significant difference for total count of cladoceran species determined between the lagoons (p = 0.9998). However, significant statistical difference was found in each lagoon for total count of cladocerans between the periods (sampling point 1 p = 0.0057, 2 p = 0.002, 3 p = 0.0030, 4 p = 0.0114). Similar results were obtained for presence of *Daphnia obtusa*: no significant difference was found between the lagoons (p = 0.8288), while statistically significant differences were shown between the periods in each lagoon (sampling point 1 p = 0.0065; 2 p = 0.0039; 3 p = 0.0039; 4 p = 0.0065). Significance of differences in average total count as well as *Daphnia obtusa* count between the periods were tested by U test and the results are presented in Table 2.

Table 2. Effect of the period on the average total count and *D. obtusa* count in the lagoons

Period	*P*-level*							
	Total count (ind/l)				***Daphnia obtusa*** (ind/l)			
	1	2	3	4	1	2	3	4
1 and 2	0.260	0.228	**0.050**	0.319	0.317	0.317	0.317	-
1 and 3	**0.004**	**0.004**	**0.003**	**0.004**	**0.002**	**0.002**	**0.002**	**0.002**
1 and 4	**0.025**	**0.010**	**0.003**	**0.016**	**0.007**	**0.022**	**0.007**	**0.007**
2 and 3	0.055	**0.016**	0.261	0.261	**0.003**	**0.003**	**0.003**	**0.002**
2 and 4	0.873	0.873	0.873	0.873	**0.049**	0.074	**0.021**	**0.007**
3 and 4	**0.006**	**0.004**	**0.004**	**0.006**	**0.006**	**0.004**	**0.004**	**0.006**

*bolded values are significant

The seasonal variations were characterized by absolute domination and low abundance of small cladoceran *Chydorus schaericus* in the period 1, followed by diversity and production increase in the period 2, leading to maximum production values and domination of large *Daphnia obtusa* in the period 3. After reaching summer peak of production, gradual decrease of abundance occurs, followed by reappearance of *Chydorus schaericus* in the period 4.

CONCLUSIONS

Taking into account the poor water quality in all three natural wastewater treatment lagoons, species diversity of Cladocera was relatively high throughout the examination period. Strong seasonal variations for both abundance and species diversity were found between the three-month periods which were evaluated. Maximum diversity was reached during the period 2 (from the mid May till the mid August of 2012), with production peak in the period 3 (from the mid August till the end of November of 2012). *Daphnia obtusa* was shown to be well adapted to environmental conditions in these shallow wastewater lagoons, tolerant to low DO of 0.32 – 6.90 mg/l, reaching maximum production of 1444 – 1083 ind/l throughout the investigation period. Due to the potential for growing in domestic wastewater, *D. obtusa* may be adequate for use in biomanipulation studies.

ACKNOWLEDGMENTS

The present study was supported by the Ministry of Education, Science and Technological Development, through the project Improvement of production capacities of carp (*Cyprinus carpio)* through nutrition and selective breeding programs (TR 31075).

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