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EFFECTS OF THE APPLICATION OF POLYCULTURE WITH GRASS CARP TO CONTROL AQUATIC VEGETATION IN FISHPONDS ON THEIR PHYTOPLANKTON AND MACROZOOBENTHOS

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Abstract. Aquatic vegetation overgrowth is a serious problem for the fish farming. It is caused by combination of factors, such as rapid climate change, rising water temperatures and eutrophication. The excessive development o f aquatic macrophytes prevents the use of the fish ponds for different purposes and threatens the structure and functioning of the aquatic communities. In our experiment, in ponds stocked with two-year-old grass carp the aquatic vegetation (mostly *Ceratophyllum demersum* L.) was successfully reduced. In this way grass carp remained without its natural food and the improvement o f the light regime in the ponds led to bloom o f phytoplankton and changes in the species composition, which created a rich natural food base for the hybrid silver carp. High concentrations o f orthophosphates at the beginning of the experimental period coincided with the high rate o f development of aquatic macrophytes. Afterwards, during the period o f intense phytoplankton blooms their concentrations decreased, presumably due to the increased consumption by algae. The removal o f macrophytes led to high abundance o f macrobenthic organisms (more chironomids and less oligochates).

Key words: aquatic macrophytes, fish farming

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INTRODUCTION

Aquatic plants are an important part of the ecosystems and require appropriate control and conservation of their biotopes. They provide habitat for different species of fish and ensure optimal oxygen regime in the water basins. When nutrients are deposited in the ponds, they are difficult to remove. The accumulation of phosphorus, which is a major limiting factor for the development of macrophytes, can sustain their development for years. Different ecological problems are usually registered in shallow eutrophic ponds. P e t r (2000) notes that macrophytes can have a positive and negative impact on the aquatic biotopes. They directly affect oxygen saturation and convert ammonia into useful nitrates, and indirectly assist the recirculation of nutrients. Aquatic plants also affect benthic, planktonic and fish communities. Traditional methods of control of excessive aquatic vegetation, such as mechanical and chemical methods, are too labor-intensive and costly, and can have a negative impact on the aquatic ecosystems. Biological control can be an alternative to the traditional methods.

In order to effectively manage macrophytes it is needed to ensure long-term control without negative impacts on ecosystems and communities (Pipalová 2006; B o z k u rt e t a l. 2017). One of the most widely used species for control of macrophytes development is the grass carp ***(Ctenopharyngodon idella*** Val.). By using the higher aquatic vegetation as a major food source, it can be one of the solutions in managing aquatic vegetation overgrowth. The grass carp belongs to the family Cyprinidae with natural populations in Southeastern Russia and Northwest China. These herbivore species are introduced in many countries for the purposes of control of macrophytes development. ***C. idella*** is a component of polycultures in closed systems in most of Eastern European countries. After successful artificial reproduction in the former USSR in 1961, the grass carp was introduced in a number of Eastern European countries (Van Z o n 1977). In Bulgaria, according to our knowledge, it has been introduced in 1965 and a number of reproduction and breeding technologies have been carried out in the country. However, studies on its use in control of unwanted aquatic vegetation, its nutritional preferences and impact on aquatic ecosystems and communities are scarce. Dynamic climate change, as well as increased nitrogen and phosphorus concentrations in the water due to agricultural activity, increase the aquatic plants overgrowth and can cause serious problems in the fish farms. All mentioned environmental factors and ecological issues support the need for research of ***C. idella*** as a mean in the biological control of macrophytes overgrowth. The aim of the study is to develop a polyculture with grass carp as a mean in the biological control of the macrophytes overgrowth in fish ponds, by determining the effectiveness of the stocking density, age and size of the fish and their impact on the aquatic communities and environment.

MATERIAL AND METHODS

The study was carried on the territory of the Institute of Fisheries and Aquaculture, Plovdiv, Bulgaria. Water samples were collected in four experimental ponds (P12, P18, P19 and P23) from May to September 2018 during a research project G-146 „Development of new polyculture as a tool for adaptation to climate change“ (Agricultural Academy, Sofia, Bulgaria) according to the standard methods and normative requirements. Water temperature (T °C), dissolved oxygen (0 2), oxygen saturation (0 2%), electrical conductivity and pH were measured. The determination of the biogenic conditions involved the measurements of the following metrics: ammonium nitrogen (N-NH4), nitrite nitrogen (N-N02), nitrate nitrogen (N-N03), total inorganic nitrogen (TN), orthophosphates (P-P04) and transparency of water (Sd) by means of a Secchi disk. The concentration of the photosynthetic pigment chlorophyll ***a*** was measured by spectrophotometric method by extraction of chlorophyll in ethanol from phytoplankton. Phytoplankton samples were taken twice a month in 11 glass banks. The further preservation of the samples was carried out with formalin to a final concentration of 4%. Quantitative and qualitative analysis was performed in Burker's counting chamber following L a u g a ste (1974). Biomass was calculated using formulas for the corresponding stereometric forms (R o tt 1981; D eisin g er 1984). The identification of algae was done on standard European floras and of diatoms in particular, was done after Cox (1996). The total biomass for each sample was estimated as the sum of the biomass of all phytoplankton, summed up by individual taxonomic groups. Dominant species were determined according to the percentage of individual species to the total biomass. The species identification was performed with light microscope Carl Zeiss Axioscope 2 plus at a 400x magnification with critical use of Algae Base (G uiry & G uiry 2019).

The macrobenthos sampling was done twice a month with E ckm an & B irg g e grab with opening width of 225 cm2. Samples were washed through sieves with different mesh size (2 mm, 500 p,m, 250 ^m, 150 p,m and 63 p,m) and fixed in 95% ethanol. Further processing of the samples involved their washing with clean water, sorting by fraction and separation of the bottom invertebrates in sample tubes with 95% ethanol. The taxonomic identification was done on a binocular magnifying glass and a light microscope Carl Zeiss Axioscope 2 plus using keys and species descriptions mainly by O liv e r (1971), E p le r (2010), Oscoz e t a l. (2011). During the study, only macroinvertebrate fauna (> 500p,m) was recorded. In order to determine the structure and composition of the benthic communities, the density of the organisms (individuals m 2) was calculated. The percentage of oligochaetes (% Oligochaeta) and the percentage of chironomids (% Chironomus) were estimated.

The aquatic vegetation with its most common species ***(Ceratophyllum demersum*** L., ***Nuphar lutea*** (L.) Sm. and ***Typha angustifolia*** L.) were monitored bimonthly. The growth of macrophytes in the experimental ponds was calculated

as a percentage of the total water area. During the vegetation period, the ponds were stocked with polyculture of two-year old grass carp *(Ctenopharyngodon idella*

Valenciennes): 500 fish ha-1, common carp *(Cyprinus carpio* L.): 400 fish ha_1 and hybrid silver carp *(Hypophthalmichthys molitrix* Valenciennes x *Hypophthalmichthys nobilis* Richardson): 100 fish ha-1.

RESULTS AND DISCUSSION

After the application of polyculture grass carp stocking in density of 150 fish h a1 with fishes of the same age, the fastest and best results were achieved in removing of *Ceratophyllum demersum,* but it took longer time for the less affected *Nuphar lutea* and *Typha angustifolia.*

The obtained data on minimum, maximum and average values of the physicochemical parameters of the water of four studied ponds, are presented in **Table 1.** According to them, the dissolved oxygen in the water had optimal values. The registered maximum concentrations of phosphate ions in three of the studied ponds (P12, P18 and P19) were optimal for the carp species. The only higher phosphate amounts, above the technological requirements, were registered in P12 in May and June. These high concentrations of orthophosphates in May and June, especially in P12, coincided with the most intense period of macrophytes growth in the experimental ponds. Afterwards, from July to September, during blooms of phytoplankton and their intensive consumption, the concentrations of the orthophosphates decreased.

During the experiment, the peak of chlorophyll *a* concentration coincided with the maximum growth of the phytoplankton, in which algae from the divisions Cyanoprokaryota (known also as cyanobacteria or blue-green algae), Chlorophyta and Ochrophyta (class Bacillariophyceae) were identified. In May dominant species were the diatom *Aulacoseira granulata* (Ehrenberg) Simonsen, the green alga *Desmodesmus communis* (Hegewald) Hegewald and the cyanoprokaryote *Anabaena sphaerica* Bomet & Flahault. The biomass was lowest in P12 and highest in P19 **(Fig. 1).** In June, dominants in P19 and P12 were the blue-green algae *A.*
sphaerica and *Dolichospermum spiroides* (Klebahn) Wacklin, L. Hoffmann & Komarek with *D. communis* and *A. granulata* as co- dominants. The biomass significantly increased with the highest recorded values in P18 **(Fig. 1).** Generally, the biomass of phytoplankton and the concentration of chlorophyll a were more than 10 times higher in May but the phytoplankton biomass in PI 8 and P19 was significantly lower than the biomass in P23 and P12. In July the cyanoprokaryotes *Aphanizomenon flos-aquae* Ralfs ex Bomet & Flahault and *A. granulata* were dominating. In P18, P19 and P23 among the co-dominant species were *A.*
sphaerica, D. spiroides and *Oscillatoria limosa* C. Agardh ex Gomont, and in P18, P23 and P12 *Planktolyngbya limnetica* (Lemmermann) Komarkova-Legnerova & Cronberg. The phytoplankton biomass was the lowest in P18 and the highest

Table 1. Physicochemical parameters of the water and the concentration of chlorophyll *a* in the experimental ponds.

Parameter	T	O_2	O_2	pH	NH_4^+	NO_3^-	NO_2^-	TN	NH_4	COD	PO_4^{3-}	Cond.	Chl. a
Measure	°C	mg.l-1	%		mg.l-1	mg.l-1	mg.l-1	mg.l-1	mg.l-1	mg.l-1	mg.l-1	µS.cm-1	µg.l-1
Pond 12													
X	21.5	7.6	87	8.27	0.4	1.23	0.009	1.639	0.030	11.92	6.86	408	81.86
min	12.8	2.9	34	7.73	0.25	0.82	0.003	1.123	0.0130	0.11	0.36	349	3.7
max	27.5	10.3	126	8.88	0.53	1.45	0.020	1.910	0.062	24.75	24.75	570	203.62
n	30	30	30	30	10	10	10	10	10	10	10	30	10
s	4.21	1.8	20	0.25	0.1	0.19	0.007	0.231	9.718	8.77	63.36	63	71.38
Pond 18													
X	22.3	8.2	95	8.28	0.38	1.06	0.013	1.456	0.035	12.68	0.45	340	32.89
min	14.8	4.9	59	7.94	0.25	0.79	0.003	1.190	0.017	8.16	0.01	274	11.09
max	27	12.2	147	8.84	0.47	1.29	0.026	1.647	0.083	15.43	0.63	519	104.12
n	26	26	26	26	10	10	10	10	10	10	10	26	10
s	3.2	1.8	21	0.21	0.08	0.18	0.009	0.166	0.020	3	0.17	67	26.75
Pond 19													
X	22.7	7.3	86	8.22	0.45	1.16	0.016	1.618	0.026	11.83	0.61	367	31.4
min	14.9	3.2	40	7.84	0.21	0.63	0.003	0.903	0.012	6.94	0.01	283	4.94
max	27.3	12.3	134	8.69	0.66	1.64	0.040	1.990	0.039	16.59	1	505	107.7
n	26	26	26	26	10	10	10	10	10	10	10	26	10
s	3.1	2.1	23	0.18	0.16	0.27	0.012	0.331	0.01	2.71	0.29	65	30.51
Pond 23													
X	22.0	7.9	88	8.17	0.35	1.22	0.016	1.588	0.020	11.22	0.46	376	35.91
min	14.4	5.4	11	7.65	0.28	0.69	0.003	1.113	0.011	7.55	0.01	292	7.4
max	27.9	12.6	154	8.79	0.42	1.93	0.040	2.290	0.040	13.34	0.7	352	67.31
n	28	28	28	28	10	10	10	10	10	10	10	28	10
s	3.6	1.8	25	0.21	0.05	0.41	0.014	0.422	0.009	1.99	0.19	56	17.28

in P19 **(Fig. 1).** Phytoplankton blooms were registered, being most intensive in P19. Green algae were dominant in August and some euglenophyte algae ***(Euglena*** sp., ***Trachelomonas planctonica*** Svirenko) species also increased their abundance. Among the most abundant species were also ***A. sphaerica, D. spiroides, O. limosa*** and ***P. limnetica.*** During the study period ***A. granulata*** was found in all studied ponds. Compared to the previous month, no significant changes in the phytoplankton growth were recorded. Biomass values varied, with the lowest values in P12 and the highest in P19. Phytoplankton blooms continued to be most intensive in P18 and P19. In September, in all studied ponds, the dominant species was ***A. granulata.*** The cyanoprokaryotes ***Dolichospermum planctonicum*** (Brunnthaler) Wacklin, L. Hoffmann & Komarek and ***D. spiroides*** caused intensive blooms and were among the dominant species in P19, P23 and P12, with ***M icrocystis aeruginosa*** (Kützing)

Kutzing andP ***limnetica*** co-dominating in P18,P19 and P23. The highest biomass was recorded again in P18 and P19, and in P23 and P12 intensive blooms were registered with values of 16.356 and 24.440 mg **I 1 (Fig. 1).**

P12 P18 P19 P23

Fig. 1. Changes of total biomass (mg l-1) of phytoplankton in four experimental ponds (P12, P18, P19 and P23) with grass carp polyculture from May to September.

The macrobenthic fauna was represented by class Oligochaeta and genus ***Chironomus*** **(Table 2).** The oligochaetes predominantly belong to the family Tubificidae, with highest abundance registered in July in P12, P18 and P23 and in August in P19. The chironomids were represented mainly by ***Chironomus*** cf. ***plumosus*** with a maximum registered density in July in P12 and P18, and in P19 and P23 in August. During the study period, chironomid larvae at II and III stage of development were recorded. The dynamics of macrobenthic populations varied between ponds, with the highest values registered from June to August. At the beginning of the experimental period the density of the macrobenthic fauna was characterized by low values. Peaks in the total abundance of the individuals were recorded in June and August in P23, and in July inP18. In September a sharp decline in the density was registered in all studied ponds. The average density for the whole experimental period was the highest in P19 **(Fig. 2).**

Itis widely known thatmacrophytes play an importantrole in the aquatic ecosystems, with some species being the main source of nutrients for different aquatic organisms and that aquatic plants improve water quality and increase biodiversity. On the other hand, their overgrowth can obstruct the use of ponds for various fish farming purposes and endanger the structure and functioning of the biological communities (B o z k u r t e t a l .

Table 2. Average abundance (individuals m-2) of *Chironomus* and Oligochaeta individuals.

	Chironomus	Oligochaeta
Month	Average abundance (individuals m-2)	Average abundance (individuals m-2)
May	95.3±5.9	45.3.3±11.4
June	117.7±24.5	72.7±16.5
July	138.0±24.5	97.3±12.0
August	153.7±15.5	94.7±16.3
September	94.3±9.5	66.7±4.5

Pond №	Average abundance (individuals m-2)	Average abundance (individuals m-2)
P12	102.8±38.3	48.0±9.0
P18	112.0±29.3	74.4±25.5
P19	122.8±28.0	81.8±22.5
P23	124.6±30.9	69.8±23.2

2017). Macrophytes density is a factor that affects not only the different parameters of the aquatic ecosystem, but also the nature of the interactions between them. However, registering only the average levels of the density of aquatic vegetation is insufficient, as the impact of the macrophytes varies according to the time they reach their maximum

0 50 100 150 200 250 individuals m -2

Fig. 2. Total abundance (individuals m-2) of macrobenthic fauna in four experimental ponds (P12, P18, P19 and P23) with grass carp polyculture, represented in average val ues for the studied period (for details see text).

density (N ikolova e t a l. 2013). According to these authors, macrophytic overgrowth impacts the rearing of fish and the interactions of other environmental factors, but this influence changes its strength and direction depending on the type and the stage of development of the macrophytes. According to these authors, the relationship between macrophytes, water transparency and biomass of zoobenthos and zooplankton is negative, and between the biomass of phytoplankton and chlorophyll ***a*** is positive. In the present study, the peaks in chlorophyll concentration coincided with the peaks in phytoplankton growth. According to N ikolova e t a l. (2013) the density of the aquatic vegetation affects negatively all biotic components, with the most significant impact on the biomass of zoobenthos and zooplankton, with weaker impact on the phytoplankton biomass. In our study also the highest zoobenthos density was registered during the active summer period after the elimination of the macrophytes and the abundance of chironomids was higher than the abundance of oligochaetes.

According to our results, the abundance of macrophytes coincided with the lowest biomass of phytoplankton and, by contrast, its highest biomass was registered in the period after the removal of the aquatic vegetation by the grass carps. This is on conformity with the results of Abdel-Tawwab (2006) and P e tr (2000), who reported a negative relationship between macrophyte density and chlorophyll ***a*** and phyto- and zooplankton abundance. When interpreting the complex relationships between aquatic plants and various organisms, these authors noted that plants provided shelter for the larger zooplankton organism, which increased the zooplankton density and inhibited the growth of the smaller zooplankton organisms, thus preventing negative changes in the structure of the phytoplankton.

While exploring the impact of certain technological factors on the growth of carp fish, N ikolova (2013) found that one-year old grass carp grows faster than the two-year old individuals, noting that the organic fertilization of the ponds has a significant impact on the growth rate. In a different study N ikolova (2004) reported a considerable impact of the pond area on the growth rate of the grass carp. The results of N ikolova e t a l. (2008) demonstrated that a polyculture with two-year old grass carp with density of 100 fish h a1 leaded to effective control of the macrophytes overgrowth, resulting in their further development being stopped. Besides the size, vegetation has a significant effect on the studied carcass slaughter characteristics of other species reared in polyculture with grass carp. For example, the macrophytic growth of the ponds had a positive effect on the slaughter characteristics of silver carp (N ikolova & D ochin 2017). However, although the grass carp rears under similar conditions and aquatic vegetation is its main source of food, it has been found that macrophytic overgrowth has a negative effect on its slaughter characteristics and it has been supposed that this is most likely related to the specific plant species in the different ponds and to the food selectivity of grass carp (N ikolova & D ochin 2011), the last studied in detail by C atarin o e t a l.

(1997). V in o g rad o v & Z o lo to v a (1974) reported the disappearance of 36 species of aquatic plants after two years of rearing of grass carp. Its impact on the species diversity of aquatic vegetation depends on the stocking densities and the size of the fish, the

presence or absence of preferred plant species, the survival rate and the duration of its impact (PiPALOVA 2006). Higher density of grass carp can have serious consequences for the functioning of the aquatic systems. Ecological changes may be associated with alterations in the structure and abundance of the plant communities ***(e.g.*** Catarino et al. 1997), as well as different transformations in the habitat, such as shifts in water transparency, sedimentation, and increased levels of biogenic waste as result of fecal deposition (PiPALOVA 2006; Dibble & Kovalenko 2009).

However, we have to underline that rearing of grass carp, as well as the use of other species, does not eliminate the factors that cause excessive aquatic plant overgrowth, which is often associated with human activity. Shallow ponds, eutrophication, climate change and the occurrence of invasive species can favor development of macrophytes. When intensive plant development is associated with long-term nutrient loadings, grass carp can help convert them into fish biomass and phytoplankton (PiPALOVA 2006; V olpert 2010). At high stocking density, the grass carp can eliminate the vegetation, and the released nutrients cause an increase in phytoplankton. As it was described above, a similar effect was recorded in the present study: after the removal of aquatic vegetation, a significant increase in the phytoplankton biomass and chlorophyll ***a*** concentration was observed. Moreover, their elimination significantly improved the light regime in the pond, which is one of the necessary conditions for phytoplankton blooms. Thus, our data confirmed that changes in the biomass and species composition of phytoplankton are depending on the presence or absence of aquatic macrophytes. On turn, high phytoplankton biomass can cause macrophyte suppression, and winds decrease water transparency due to sediment movement (Bonar e t al. 2002). According to Richard et al. (1984) three years after the introduction of the grass carp, a significant increase of Chlorophyta and Bacillariophyceae, and a decrease in Cyanoprokaryota has been observed. Similarly, H oldren & P o rter (1986) reported on changes in the dominant phytoplankton species after the introduction of the grass carp. In taxonomic aspect, we detected another set of events: at the beginning of the experiment, algae had relatively high biodiversity, while at the end of the study period Cyanoprokaryota were predominantly found in the samples.

The elimination of macrophytes affects the fish growth rate, especially when no additional feeding is received by the fish, as is the case of our experimental study. Despite the detailed description of the growth rates of different fishes in this polyculture is out of the scope of the present paper, we would like to note that during the experiment there were differences in the development of the fishes. For example, the elimination of the macrophytes in the ponds which led to improved light regime and phytoplankton blooms, created a rich food base for the hybrid silver carp and in all experimental ponds it grew almost 10 times its initial weight, while the growth of the carp was mostly insignificant. Moreover, negative growth rate and lack of growth rate of grass carp in two of the studied ponds was detected. In one of these ponds, where only ***Ceratophyllum demersum*** was found shortly at the beginning of the study period, no growth and no reduction of weight were observed. Considering the lack of additional feeding of the grass carp during the experiment, we could suppose that the growing of grass carp would be higher in the presence of sufficient vegetation and additional feeding. However, since overgrowth by macrophytes in undesirable, it is necessary to find a balance between their development and fish production. One possible solution for this is to optimize the polycultures with a focus on the age structure and the number of grass carp used.

CONCLUSION

In conclusion, based on the results from the present study, the two-year old grass carp with a stocking density of 150 fish h a 1 was sufficient to reduce aquatic vegetation overgrowth and had effects on composition and abundance of both phytoplankton and zoobenthos. This study can serve as a base for further research on the uses of polyculture with grass carp as a mean in the biological control of unwanted growth of macrophytes, with focus on the impact on the aquatic ecosystems and their biological communities.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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