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ANALYSIS OF THE CONCENTRATIONS OF ZOOPLANKTON ORGANISMS IN THE PONDS OF THE STATE ENTERPRISE "EXPERIMENTAL FISH FARM "NYVKA" OF THE INSTITUTE OF FISHERIES OF THE NATIONAL ACADEMY OF AGRARIAN SCIENCES OF UKRAINE, TAKING INTO ACCOUNT THE POSSIBILITY OF REARING OF JUVENILES OF THE LARGEMOUTH BASS *(MICROPTERUS SALMOIDES)*

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ABSTRACT

The period from the largemouth bass larvae hatching to the moment, when fry reaching the size of 25-35 mm, can be attributed to the most critical in the aquaculture of this species. Unlike many other fish species, after switching to external feeding, largemouth bass larvae cannot immediately consume artificial pellets, therefore, the presence of groups of zooplankton organisms of different sizes in rearing ponds is a prerequisite for obtaining a sufficient number of fry and their rapid growth. Besides, a sufficient food base at the initial stages of growing of the largemouth bass fingerlings helps to reduce the degree of cannibalism among fish and increase fish productivity in the future. This article will consider the features of different feeding stages of the largemouth bass fry, the necessary concentrations of zooplankton organisms in the ponds of fish farms during its cultivation, generally accepted world methods of applying mineral and organic fertilizers to stimulate the natural food supply, as well as the results of a study, aimed to make an identification of the average indicators of the development of zooplankton in the ponds of the State enterprise «Experimental Fish Farm «Nyvka» of the Institute of Fisheries of the

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National Academy of Agrarian Sciences of Ukraine in terms of the possibility of growing of this species in aquaculture reservoirs at the initial stages.

Keywords: Largemouth bass, Micropterus salmoides, zooplankton, phytoplankton, aquaculture, cladocerans, copepods, rotifers

INTRODUCTION

The largemouth bass (*Micropterus salmoides* (Lac.) is a fairly common aquaculture species, which currently farmed on all continents except Antarctica and Australia (Rahel, 2007). If in 1976 this species could be found on the territory of 37 countries, then in 2007 it was present in at least 52 countries, and its range continues to increase, primarily due to the development of state programs to introduce largemouth bass into natural or fishery reservoirs (Heidinger, 1976; Rahel, 2007). The purpose of introducing of largemouth bass into water bodies of different countries of the world was to ensure the development of recreational fishing, enrichment of the local ichthyofauna and aquaculture.

Largemouth bass is a valuable species for aquaculture and recreational fishing. In particular, according to the 2011 National Fish, Game and Recreation Survey, each year 10.6 million fishermen in the United States spend 171 million days for largemouth bass fishing, making this species the most popular target of freshwater amateur fishing. Largemouth bass is also common in many countries of Europe and Asia, where its presence contributes to the development of sport fishing and international tourism.

Largemouth bass aquaculture in warm-water pond fisheries has some differences compared to the rearing of many other species, which must be taken into account when breeding and rearing of this fish. These differences include the preference of natural spawning over artificial with hormonal stimulation, the need to provide largemouth bass more than 10 cm long with a sufficient amount of forage fish, a rather complicated scheme of feed training, as well as the presence in rearing ponds appropriate amount of zooplankton organisms. The last requirement can be attributed to the most important in the implementation of largemouth bass aquaculture, given the fact that, unlike channel catfish *(Ictalurus punctatus)* and rainbow trout *(Oncorhynchus mykiss)*, largemouth bass larvae after switching to external feeding cannot be immediately accustomed to artificial feed (pellets) (Brandt *et al*., 1987; Williamson *et al*., 1993). Thus, the juveniles of largemouth bass are usually cultivated in fertilized rearing ponds until their total length will become 25-35 mm with weight about 1-2 g, and then the process of feed training begins (Tidwell *et al*., 2000).

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Like most other fish species, the largemouth bass larvae after hatching feed on with their yolk sac for several days. When the yolk sac is empty, the larvae of largemouth bass, whose total length at this time is about 6 mm, leave the nest and begin to swim. During the specified critical period, they must shift to external feeding. From the very beginning, the larvae feed on with small zooplankton, such as rotifers (Wickstrom and Applegate, 1989), but, with increasing length, they are quickly shift to larger zooplankton, which includes nauplii copepods, adult copepods and cladocerans, and a little later they begin feed on with young aquatic insects (Parmley *et al*., 1986). If the number of small crustaceans is large, and the larvae of the largemouth bass appeared earlier than the larvae of other fish species that can compete with them for zooplankton (for example, juveniles of Shad or other Centrarchidae), they grow quite quickly, up to 1,0-1,4 mm per day (Goodgame and Miranda, 1993). With a total length of about 25 mm, if there are a large number of small fish in the reservoir, largemouth bass fry gradually begin to prey on it, and can completely stop feeding on with zooplankton when they reach a total length of about 35 mm (Huskey and Turnigan, 2001). In waters with few other fish species, largemouth bass juveniles can feed on zooplankton for a longer time, until they reach a total length of 55-70 mm (Dorsey, 1997).

To stimulate the development of phyto- and zooplankton, in ponds, where it is planned to grow largemouth bass fry, mineral and organic fertilizers are usually applied. Organic fertilizers can accelerate the growth of zooplankton by providing a direct supply of nutrients to the water body before the mass development of phytoplankton begins. They also provide a source of carbon for potentially carbon-limited phytoplankton and heterotrophic feeding organisms (Boyd, 1990). To increase the production of zooplankton, organic fertilizers are particularly recommended for new or "sterile" ponds (Piper *et al*., 1982).

Organic fertilizers, which applied to ponds where the juveniles of largemouth bass are planned to be reared, are usually the most common local crops or by-products, e.g. soybean meal, rice bran, cottonseed meal (CSM), alfalfa grass meal (AGM), chopped hay (straw), or animal manure. As a rule, the choice of organic fertilizer depends on local availability, its cost, and past experience of use.

In 1996, a side-by-side comparison was made between AGM and CSM in nursery ponds with PVC film covered beds during the largemouth bass fry growing, with adding of the additional mineral fertilizers - nitrogen and phosphorus (Barkoh, 1996). Despite of similar zooplankton concentrations in the ponds, CSM provided more favorable hydrochemical parameters of the growing environment and higher fish productivity in the experimental ponds compared to AGM (Barkoh, 1996).

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According to different studies, earth-bed rearing ponds for largemouth bass, fertilized with CSM, rice bran and AGM, showed similar fish production and growth and survival level of juveniles, as well as zooplankton concentrations (Kurten *et al.*, 1999). In another study, CSM and mineral fertilizers, AGM and mineral fertilizers, and mineral fertilizers only were applied to the ponds, and in all three cases there was no significant difference in fish production (Kurten *et al.*, 1995). The authors hypothesized that eliminating the application of organic fertilizers can reduce the cost of fish rearing and increase the level of dissolved oxygen in the water without negatively affecting fish productivity.

In 1994, the studies on comparing plankton concentrations and numbers of largemouth bass fry in ponds fertilized with chicken manure or plant-based fertilizers containing equal parts of CSM, AGM and wheat meal were conducted (Barkoh *et al.*, 1994). Liquid mineral fertilizers were also applied to all ponds at a ratio of N : P = 470 : 750 µg/L per week. Wet chicken manure (moisture content 75%) was applied at a ratio of N : P = 219 : 67 µg/L during the first week, 158 : 49 µg/L during the second week, and 105 : 32 µg/L during the following weeks. A mixture of CSM, AGM and wheat meal was applied every week at a ratio of N : P = 2.442 : 149 µg/L for the first two weeks and at a ratio of 1.221 : 75 µg/L for the following weeks. Fish productivity for juvenile largemouth bass (mean length 51-55 mm) was significantly higher in ponds, fertilized with plant-based fertilizers (133.8 kg/ha) compared to ponds, fertilized with chicken manure (84 kg/ha). In ponds, fertilized with plant-based fertilizers, a higher concentration of chlorophyll-*a* and a lower concentration of dissolved oxygen in the water was observed compared to ponds, fertilized with chicken manure. Phytoplankton concentrations were also consistently higher in the ponds, fertilized with plant-based fertilizers (ground meal mixture), most likely due to the higher nitrogen content, applied to the ponds throughout the study period, compared to chicken manure. The density of zooplankton in the ponds during the study was not constant and fluctuated within 717-923 thousand ind./m^3.

Mineral fertilizers are a relatively inexpensive source of nitrogen, phosphorus and sometimes potassium, which directly stimulate the growth of phytoplankton. Considering the known composition, it is easier to calculate the rates of application of mineral fertilizers to ponds compared to the rates of application of organic fertilizers. Dissolved inorganic nutrients stimulate the growth and reproduction of phytoplankton, which further supports the growth of the zooplankton population (Piper *et al.*, 1982). They can also accelerate the breakdown of organic matter, including organic fertilizers (Boyd, 1990). Excessive use of mineral fertilizers can lead to an increase in pH and ammonia levels, which can negatively affect the survival of fish (Barkoh, 1996).

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There are different schemes for applying phosphate fertilizers to ponds. According to one of them, after filling the rearing ponds for largemouth bass fry with water, superphosphate in the amount of 8 kg/ha (35 µg/l for ponds with an average depth of 1 m) and nitrogen in the amount of 9 kg/ha are added to the ponds with an interval of one week until a sufficient concentration of zooplankton is formed, which usually occurs after 3-4 fertilizations (Snow, 1970). Once zooplankton numbers have increased to desire levels, it is recommended to apply fertilizer once every two weeks to maintain zooplankton concentrations, and stop fertilizing approximately in 10 days before the planned catch of fish (Snow, 1970). During rearing of juveniles of the largemouth bass some authors recommend periodically apply 1 mg of phosphorus pentoxide (P_2O_5; 0,22 mg P/l) into ponds (Piper *et al.*, 1982).

In accordance with other schemes, when rearing largemouth bass fry, 1 mg of P_2O_5/l (0.22 mg P/l) is added to nursery ponds two to three times a week (Kurten, 1995). Alternatively, 1.0 mg of phosphoric acid H_3PO_4/l (0,32mg P/l) can be added to the ponds three times a week before and twice a week after stock rearing ponds with largemouth bass fry (Young and Flickinger, 1989). Consistent with other observations, the recommended application rates of phosphorus in rearing ponds for largemouth bass are 1,0 mg P/l (as orthophosphoric acid) for initial fertilization and 0.33 mg P/l three times per week to maintain phytoplankton concentrations (Hutson, 1990). There are also recommendations for lower fertilization rates of phosphorus, up to 30 µg P/l, with the aim to prevent pH rise and excess filamentous algae (Culver, 1993).

It should be noted that the life span of zooplankton organisms, consumed by largemouth bass fry in the initial stages of rearing, is not the same. Thus, the lifespan of generations of rotifers is only 2-3 days, while the lifespan of generations of cladocerans and copepods is 7 and 14 days, respectively (Allan, 1976). At a water temperature of 20°C, the time of peak concentration is 3.5 days for rotifers, 14-15 days for cladocera and about 24 days for copepods (Allan, 1976).

According to some studies, rotifers are consumed by largemouth bass larvae for a fairly short period of time, which is only 7-10 days (Applegate *et al.*, 1967). During the indicated period, their concentration should be at least 50 thousand ind./m^3 (Wickstrom and Applegate, 1989). After the larvae of largemouth bass begin to swim, they initially feed on with rotifers, but soon begin to consume copepods and cladocerans (Wickstrom and Applegate, 1989). Copepodia nauplii can also be attributed to important nutrient objects for largemouth bass larvae, which have recently switched to external feeding (Parmley *et al.*, 1986). Two weeks after the larvae began to swim, in order to obtain a sufficient number of largemouth bass fry, the average concentration of copepods should be not less, than 70 thousand ind./m^3 (Parmley *et al.*, 1986). One month after hatching, when the diet of juvenile largemouth bass mostly presented by cladocerans, its recommended average concentration in rearing ponds during the specified period and in the future should be >100 thousand ind./m^3 (Kurten *et al.*,1995). The concentration of cladocerans can rapidly increase, reach a maximum, and then decrease over the course of just a few days (Parmley and Geiger, 1985).

During another study, which was conducted in 197 rearing ponds with largemouth bass over 6 years to produce 28 mm fingerlings, the relationship between fish production, fry stocking density and zooplankton concentration was examined (Kurten, 2001a). During the catching of fish, it was found that the total mass of fingerlings and their length increased with the extension of the interval between filling the ponds with water and fish stocking. It should be noted that the concentration of zooplankton at the time of fish stoking and 7 days later was a relative indicator of future fish productivity. The low degree of relationship between future fish production and changes in zooplankton abundance, as well as the data, obtained about increase of the total mass and length of fingerlings while extending the time between filling ponds with water and fish stocking, indicate, that measures, aimed to increasing the number of large spineless can improve fish productivity, compared to activities, aimed solely at the development of zooplankton (Kurten, 2001a).

Successful rearing of juveniles of the largemouth bass largely depends on the composition and density of zooplankton in nursery ponds for 4-6 weeks after the transition of larvae to external feeding (Geiger and Turner, 1990). The number and age of the fish affect the amount of zooplankton, required for their successful rearing. In general, during the cultivation the total number of zooplankton organisms in pond water should be not less, than 250-500 thousand ind./m^3 (Geiger and Turner, 1990). As a rule, during the first few months of life, largemouth bass fry and fingerlings mostly consume the largest zooplankton, which they can catch and swallow. Such actions may lead to a situation, where the proportion of smaller zooplankton increases over time as larger zooplankton is selectively removed. An increase in the number of small zooplankton usually indicates excessive pressure of the fish on the natural food base, which may be a signal to start catching fish from the ponds (Morris and Mischke, 1999). From that moment on, further fertilization of the ponds is usually not advisable.

MATERIALS AND METHODS

Insufficiency of the required concentration of zooplankton in the rearing ponds of fish farms can lead to high mortality among juvenile largemouth bass, increased levels of cannibalism and reduced fish productivity during the first and subsequent rearing seasons.

Thus, the study of zooplankton development indicators and the implementation of measures, aimed to increase the natural food supply in nursery ponds by applying mineral and organic fertilizers, is a mandatory component of the largemouth bass fry cultivation.

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Given the fact that the natural food base has a direct impact on the cultivation of aquaculture objects in fish farms, the study of the concentrations of zooplankton organisms in ponds using the intensification technologies recommended in the world scientific literature makes it possible to assess the quantitative indicators of zooplankton and determine its taxonometric affiliation, as well as to correct the scheme of applying organic and mineral fertilizers in order to obtain the maximum indicators of fish productivity in the event of a possible future cultivation of largemouth bass in full-system warm-water pond fish farms on the territory of Ukraine during the first year.

The purpose of this scientific work is to study the average indicators of zooplankton development in the ponds of the State enterprise "Experimental Fish Farm "Nyvka"" of the Institute of Fisheries of the National Academy of Agrarian Sciences of Ukraine during the hydrobiological analysis of the aquatic environment over the period from the third decade of May to the second decade of July 2019 and comparing the results with generally accepted recommendations for rearing of juveniles of the largemouth bass in aquaculture.

During the period from the third decade of May to the second decade of July 2019, hydrobiological studies of the ponds of the State enterprise "Experimental Fish Farm "Nyvka" of the Institute of Fisheries of the National Academy of Agrarian Sciences of Ukraine were carried out. The main aim of this studies were to define the average indicators of zooplankton development in order to determine the suitability of these water bodies for rearing juvenile largemouth bass. During the study, samples with zooplankton were taken from two ponds every 10 days to determine the species composition and number of zooplankton organisms. The period of sampling coincides with the average periods of consumption of zooplankton organisms by largemouth bass fry within the natural range.

Zooplankton sampling was carried out by filtering water through a plankton net. During the study, the water was taken by plastic buckets with a volume of 10 liters. In this case, all the water from the bucket pours out through the walls of the plankton net cone, and the desired zooplankton settles in the plankton trap. For each sample, 15 buckets of water were passed through the plankton net.

Two weeks before the start of the research, the water supply of the ponds was stopped, with the next mineral and organic fertilization of the reservoirs according to the following scheme. First, mineral fertilizers (superphosphate) were applied to the ponds at the rate of 150 kg/ha. Later, within 10 days, organic fertilizers (soybean meal) were applied in the amount of 200 kg/ha. After that, in 2 weeks, superphosphate was re-applied in the amount of 75 kg/ha. Subsequently, organic and mineral fertilizers were not introduced into the ponds.

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During the study the water temperature in the ponds was 21-26°C. The average concentration of dissolved in water oxygen was 5.6–5.7 O_2/dm³, the average daily values of dissolved in water oxygen were in the span of 4.7–7.5 O_2/dm³. The maximum value of free ammonia nitrogen was 0.05 mg N/dm³, which corresponded to the upper acceptable norm for this indicator. Also, we can highlight the increase of the sodium + potassium complex to 127.0 mg/dm³ with the upper allowable level up to 50 mg/dm³. Most of all, the normative value exceeded the concentration of chlorides with the level of 163.9 mg/dm³ and a standard value up to 70 mg/dm³. Indicators of other mineral and organic substances in the ponds water during the study were within the normal range.

RESULTS AND DISCUSSION

Analyzing the presence of zooplankton organisms in the aquatic environment of ponds during the period, when they form the basis of the food base for juveniles of the largemouth bass, it can be concluded, that the detected hydrobionts belong to representatives of rotifers, as well as cladocerans and copepods. Among the discovered representatives of rotifers, one can note such species as *Keratella quadrata*, *Brachionus angularis*, *Br. diversicornis*, *Asplanchna priodonta*, *Notholca acuminate* and *Polyarthra vulgaris*. The species diversity of cladocerans was presented by *Daphnia longispina*, *D. magna*, *Moina rectirostris*, *Bosmina longirostris* and *Diaphonosoma brachyurum*. Among copepods, such species as *Cyclops miles*, *C. ovalis*, *C. pelagicus*, *Diaptomus castor* and *D. linus* were mainly recorded. In the structure of zooplankton, in accordance with the biomass of organisms, the main part was represented by genera Cladocera and Copepoda.

The number of cladocerans and copepods, as well as rotifers, during the period from the third decade of May to the second decade of July, was subject to periodic changes, without changing the general structure of distribution. Thus, in the period from the beginning of the third decade of May to the end of the first decade of June, the average zooplankton biomass was 4.27 g/m^3 with a total number of organisms of 411.4 thousand ind./m^3. At the same time, the proportion of Cladocera representatives in the total zooplankton biomass was 57.31%, Copepoda – 28.42%, Rotifera – 14.27%. During the period from the beginning of the second decade of June to the end of the third decade of June, the average biomass of zooplankters was 6.59 g/m^3 with a total number of organisms of 570.5 thousand ind./m^3. The percentage of different toxonometric groups in the composition of the total zooplankton biomass also changed - the proportion of Cladocera representatives was 62.27%, Copepoda – 30.28%, Rotifera – 7.45%. In the period from the beginning of the first decade of July to the end of the second decade of July, the average biomass of zooplankters was 5.63 g/m^3 with a total number of organisms of

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542.6 thousand ind./m^3. In this case, the proportion of Cladocera representatives in the total zooplankton biomass was 59.78%, Copepoda – 34.86%, Rotifera – 5.36% (Table 1).

Table 1: Average indicators of zooplankton development in the ponds of the State enterprise "Experimental Fish Farm "Nyvka" of the Institute of Fisheries of the National Academy of Agrarian Sciences of Ukraine during hydrobiological studies (2019)

Groups of zooplankton organisms	Period of hydrobiological research					
	III decade of May - I decade of June		II decade of June - III decade of June		I decade of July - II decade of July	
	Average biomass, g/m^3	Total number, thousand ind./m^3	Average biomass, g/m^3	Total number, thousand ind./m^3	Average biomass, g/m^3	Total number, thousand ind./m^3
Cladocera	2.45	235.8	4.11	355.2	3.37	324.3
Copepoda	1.21	116.9	1.99	172.8	1.95	189.2
Rotifera	0.61	58.7	0.49	42.5	0.31	29.1
Total	4.27	411.4	6.59	570.5	5.63	542.6

It should be noted, that the species composition of zooplanktonic organisms, such as cladocerans, was not constant during the sampling period and was subject to periodic changes. Thus, during the third decade of May - the first decade of June, species such as *Moina rectirostris* and *Daphnia longispina* dominated in the composition of the Cladocera. During the second and third decade of June the domination of such species, as *Daphnia magna* and *Daphnia longispina* were observed. During the first and second decade of July, *Daphnia magna*, *Bosmina longirostris* and *Diaphonosoma brachyurum* were the main part of cladocerans.

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The average biomass of representatives of rotifers during the period from the third decade of May to the first decade of June (inclusive) was 0.61 g/m^3, the total number was 58.7 thousand ind./m^3, which corresponds to the indicators, recommended for growing the juveniles of the largemouth bass (> 50 thousand ind./m^3). Over the next two experimental periods, the average biomass of Rotifera representatives decreased to 0.49 and 0.31 g/m^3, the total number - to 42.5 and 29.1 thousand ind./m^3. However, according to specialized scientific literature, largemouth bass larvae consume rotifers for 7–10 days after switching to external feeding, and a further decrease in their number cannot significantly affect the growth of juveniles.

During the period from the second decade of June to the third decade of June (inclusive), the average biomass of representatives of the genus Copepoda was 1.99 g/m^3, the total number was 172.8 thousand ind./m^3. Taking into account the fact, that during the second, third and fourth weeks of life copepods form the basis of the food base for juvenile largemouth bass, and after comparing the obtained results with the recommended concentrations of copepods during the specified rearing period (>70 thousand ind./m^3), it can be concluded, that the number of representatives of the genus Copepoda was sufficient for rearing largemouth bass fry. It should also be noted, that cladocerans during this period were mainly represented by a relatively small species, *Daphnia longispina*, which can also be consumed by largemouth bass fry and enrich their diet.

During the period from the second decade of July to the third decade of July (inclusive), the average biomass of the genus Cladocera, represented mainly by relatively large organisms, for example, *Daphnia magna*, was 3.37 g/m^3, the total number was 324,3 thousand ind./m^3. Taking into account the fact, that during the second month of life in natural water bodies, the main part of the diet of largemouth bass juveniles is the largest zooplankton organisms, represented, mainly, by cladocerans, it can be concluded, that the food supply during this period in ponds will be sufficient for growing of the largemouth bass fry. This information is confirmed by the fact, that the recommended average concentration of cladocerans in rearing ponds during the second month of rearing should be > 100 thousand ind./m^3, while during the study, the total number of representatives of the genus Cladocera in the ponds during the third period was 324,3 thousand ind./m^3, which significantly exceeds the recommended parameters.

It should be noted that the total number of zooplankton organisms during the first, second and third experimental periods was 411.4 thousand ind./m^3, 570.5 thousand ind./m^3 and 542.6 thousand ind./m^3, respectively. Taking into account the recommendations regarding the presence of zooplankton organisms in nursery ponds with largemouth bass in the amount of 250-500 thousand ind./m^3, it can be argued, that the concentration of zooplankton in aquaculture reservoirs was sufficient for rearing fish during all periods of the study.

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CONCLUSION

Analyzing the data, obtained during the research, we can make the following conclusions. In general, the indicators of zooplankton development, obtained during the hydrobiological studies of the aquatic environment of the ponds of the State enterprise "Experimental Fish Farm "Nyvka" of the Institute of Fisheries of the National Academy of Agrarian Sciences of Ukraine, can be considered as favorable for growing largemouth bass juveniles during the first months of life.

Thus, it can be concluded that the ponds of the indicated full-system warm-water fish farm with usage of appropriate intensification methods, such as mineral and organic fertilization, can be used for growing of largemouth bass fry at the initial stages.

However, the concentrations of zooplankton organisms in the ponds during the growing of largemouth bass require further study to select the optimal amount of organic and mineral fertilizers in order to minimize the level of cannibalism and maximize the fish productivity.

Also, given the fact that when using some intensive technologies in the aquaculture of largemouth bass, for example, out-off-season spawning, that, due to artificial changes in the duration of lighting and water temperature, provide opportunities for reproduction of this species in spawning pools throughout the whole year, with further possible rearing in nursery ponds, there is a need to conduct further studies on zooplankton concentration and species during the growing season periods, that do not coincide with the periods of natural spawning of largemouth bass in full-system pond fisheries, as well as to explore the effect of fertilization on the development of zooplankton in months, not covered by the study.

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