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Biological Peculiarities of the Cultivation of Narrow-Clawed Crayfish Astacus Leptodactylus Eschscholtz, 1823 (Crustacea, Decapoda)

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Abstract. Crayfish *Astacus leptodactylus* Eschscholtz, 1823 is one of the largest commercial invertebrate internal waters of Ukraine. At the present time producers are unable to meet the needs of the people in this food. The largest number of crayfish (over 90%) is caught in natural reservoir as a result of illegal (poaching) production. Crayfish with spawn and larvae is caught which undermines the commercial reserves of this aquatic animal in reservoir. The article presents the results of growing crayfish under controlled conditions, taking into account their biological traits. We were investigated strength of currents on the development of crayfish in artificial conditions (closed water supply installation with a volume of 3240 l). We were analyzed the effect of surfactants, insecticides, fungicides, herbicides, and mineral fertilizers (nitrogen, phosphorus, and potassium) that enter natural and artificial reservoirs with wastewater on the organism of *A. leptodactylus*. We were used crayfish groups of 10 females and 10 males in each experiment. We have developed a methodology for 6 obtaining products in three years, namely keeping of the breeding stock in specialized tanks with temperature control, high landing density, sufficient number of caches, appropriate feeding and use of automatic devices for that, development and application of artificial caches of different sizes. It was found that the most optimal for crayfish was their keeping in closed water supply installation with a water exchange of 27 l/min. Their live weight was greater compared to individuals who grew up in the experiment with a flow rate of 0.5 l/min by 95%, 162 l/min – by 0.3%, and at 216 l/min by 1.3%. Optimal regime for crayfish growing was the dissolved oxygen content 6.0 mg/l, pH 7.3, NO_{2-}–130.03–0.35 mg/dm^3, NO_{3-}–0.35–0.37 mg/dm^3, CO_2–13–14 mg/dm^3. A number of integrated studies were also presented to determine the effect of surfactants, insecticides, fungicides, herbicides, and mineral fertilizers arriving into the reservoir with sewage are dangerous for aquatic animals and affecting the survival of experimental crayfish. Laboratory studies have shown that the lethal dose for crayfish is 2.5 g/l of washing powder Lotus. Invertebrates also died with the introduction of 0.05 ml/l of insecticide Bi-58 new. The lethal dose for crayfish are 5 g/l of the fungicide Rydomil and 0.08 ml/l of the herbicide Napalm. The lethal dose of ammonium nitrate, superphosphate and potassium salt are 0.4, 0.6 and 0.5 g/l, respectively.

Keywords: crayfish; aquariums; incubation; concentration; mineral fertilizers.

Introduction

River crayfish are one of the largest and most valuable industrial invertebrates in inlandwater bodies of Ukraine (Suprunovich & Makarov, 1990). For a long time they have been a favorite delicious food of man (Holdich, 2002a, 2002b; Harlioglu, 2004). The Danube crayfish Asteco's leptodactylus Esch. is one of the most common species used in aquaculture. It has a very important industrial significance due to its nutritional value (Holdich, 2002b). During autumn, the content of proteins, fats, carbohydrates and minerals in crayfish's meat is much higher than those in spring or summer.

Producers are not able to meet the needs of the population in this food product yet. The largest number of crayfish is sold in the summer, and then almost 90% of all products come from their catch from natural reservoirs, where they multiply themselves. The bulk of these products are obtained by poachers (Dovgal, 2009). The crayfish is caught with caviar and larva which results in irreparable losses in the number of adult individuals in the reservoir (Ackefors, 1998; Skurdal & Taugbl, 2002). Besides, such a situation harms the water body itself since the crayfish isknown to be a natural sanitary (Levadnyi, 2001; Rakhmanov, 2003). In natural conditions, small flow is the most favorable for crayfish (Brodskyi, 1981; V. I. Kozlov, 1998; A. V. Kozlov 2003). In turn, in recirculating aquaculture systems (RAS) there is a constant movement of water masses causing discomfort in crayfish and leading to constant stress.

In order to create comfortable conditions for the existence of crayfish and obtain the necessary products, we should take into account the power of water exchange in recirculating aquaculture systems (Lodge, Taylor, Holdich, & Skurdal, 2000). Data on the rate of water exchange in RAS for the maintenance of narrow-clawed crayfish are scarce. There are only some references to the content of other crustaceans, at least for the Australian freshwater crayfish Cherax quadrcarinatus. However, this species moderately differs in biology and in the way of cultivation (Ahyong & Yeo, 2007).

Taking all these data into account, the main purpose of our study was to investigate the possibility of cultivating of the narrow-clawed crayfish in artificial conditions and to determine the impact of various environmental factors on their development and the state of the organism. It allowed us developing new and improve the existing methods of crayfish breeding, namely to get a viable larva, and to obtain a commodity crayfish in a short time.

Materials & Methods

A series of integrated studies was conducted to determine the optimal conditions for the reproduction of the narrow-clawed crayfish in order to establish the possibility of their cultivation in 61 recirculating aquaculture systems. The research was carried out in the laboratory of O. S. Tertyshnyi Department of Applied Biology of Water Bioresources and Hunting (Kharkiv State Veterinary Academy), as well as in the production conditions of the Lyman State Production Fishery Enterprise, Zmiyiv district (Kharkiv region). Investigations related to crayfish incubation were performed using existing techniques and somewhat improved technologies. In total, 35 individuals were used in our study. Animals were grown in six aquariums, with a capacity of 100 liters each, one modified tray-type incubator, two pools and seven heaters, nets, weights, etc.

Recirculating aquaculture system (3240 liters) was used for determining the influence of flow force on the development of crayfish in artificial conditions. To control the temperature regime, we used an alcohol thermometer, for water pumping – a pump NEKP-202, with a capacity of 12 000 liters per hour. Aeration was carried out using compressors with biological filters "Atman"™, chemical analysis of water was performed using a portable hydrochemical laboratory "Tetratest"™. For the determination of the influence of surfactants, insecticides, fungicides, herbicides, mineral fertilizers (nitrogen, phosphorus, and potassium) entering the reservoir with sewage, we choose crayfish of the same age and approximately the same size (9–10 cm). In each experiment, it was a group of 10 females and 10 males.

Prior to the experiment, crayfish were kept under the same conditions. They were placed in the RAS, with a water temperature of 18°C. Crayfish were fed with animal and vegetable feeds.

During the experiment, we used an aquarium plant of six identical aquariums 100 liters each. The illumination was artificial (Luxel lamps 20W – 100W). Atman pumps with a nominal capacity of 500 liters per hour were used for the enrichment of water with oxygen. The mass of crayfish and chemical preparations used in the experiment was carried out using electronic scales. The size of crayfish was calibrated with an electronic caliper MIOL 15–235.

The mathematical processing of the research results was carried out using a dispersion analysis according to Dospekhov B. A. (1985) and Goria V. S. (1978). The influence of effective microorganisms was conducted in the breeding laboratory conditions at the Department of Applied Biology and Aquatic Bioresources of the Kharkiv State Veterinary Academy. A special literature (Bezusyi, 2004; 2005), as well as the common research methods (Spott, 1983; Proskurenko, 2003) were used. In some cases, our own research elements were applied for the study.

Fig. 1. Caches for crayfish of branch pipes of different diameters

Results

We have developed a method for obtaining crayfish products in three years. For this purpose, we placed animals in optimal habitat conditions with an intensive and balanced feeding. This technique includes the following elements:

- farming in specialized containers under controlled temperature;
- growing in a densely landed area, providing a sufficient number of caches (Fig. 1);
- the usage of balanced feeds and automatic feeders;
- development and application of artificial caches of various sizes.

Crayfish were placed in one container for pairing. Given that one male can couple with 3–4 females, the ratio of males and females was 1:3. Crayfish were inspected every three days. The females with spermatophores near their sexual aperture were placed into a separate container. After pairing (which lasts for about a month in nature), females were kept in a short "artificial winter" to reduce the duration of dialysis, after which the optimal conditions were gradually returned.

The eggs were incubated on females, which facilitated the care. Females independently washed the eggs and took away the death ones. During incubation, females were housed in a specialized incubator, which was modified from the incubator Ukr NIIRH–MB (Fig. 2).

After hatching the larvae, they were placed in containers with caches having a soft structure. After the transition of juvenile crayfish to an active lifestyle, it is necessary to balance their diet because of cannibalism after the third molting. After reaching the size of 3 cm, crayfish were placed in equipped containers for cultivation (Fig. 3). Duringthe process of growing, it is necessary to sort crayfish constantly by size, because larger individuals eat smaller and weaker ones. The usage of intensive and advanced technologies in the development of the narrow-clawed crayfish allows to

Fig. 2. Modified incubatorUkr NIIRH–MB

Fig. 3. Second-year-old crayfish in the caches

breed them in the industrial conditions and to obtain the production throughout the year and in short time. The current profitability level of this technology is equal to 10%.

In order to investigate the effect of current on the living parameters of crayfish in the RAS, we conducted a series of experiments with a decrease and increase its strength. The pump works in the following regime: 2.7 l/sec (166.7 l/min); it is equal to three times of water exchange per hour.

We continuously monitored the chemical composition of water in the system and controlled the state of animals at the pumping rate of 0.5 l/sec. (Table 1). The data presented in the Table 1 showed that the chemical parameters of water changed at such rate of the water exchange. The dissolved oxygen content in the upper basin, where there were 83 crayfish, decreased to 6 mg/dm^3 (7.3%) on 11th day of the experiment and up to 3,5 mg/dm^3 on 15–23 days. The amount of nitrates in the water increased by 33.3% (19–23 days), their content reached 0.05 mg/dm^3 or increased by 12666.1%. A similar increase was observed for the content of nitrates (NO_{3-}), the amount of carbon dioxide (CO_2) increased to 18–19 mg/dm^3 or by 28.5–35.7%, respectively.

Under these conditions (low dissolved oxygen content, high content of carbon dioxide, nitrates and nitrites), the ethological rhythm of crayfish was disturbed: animals tried to leave the pools, grouped near the water supply, which in our opinion caused by hypoxia and resulted in the deaths of three and four individuals on the 19th and 23rd days of the experiment.

In the second series of experiments, when the speed of water current was 27 l/min, the chemical parameters of water remained within the limits of the MPC (Table 2). The dissolved oxygen content remained in the range of 6.5 mg/dm^3, the content of nitrites does not exceed 0.03–1350.035 mg/dm^3, and nitrates 0.35–0.37 mg/dm^3, carbon dioxide –13–14 mg/dm^3. Under these conditions, crayfish were located along the entire bottom of the pool, actively eating the food.

The third series of experiments included the water supply at a speed of 162 l/min. According to the data presented in the Table 3, it was established that there no changes were recorded in the chemical composition of water, but the crayfish were placed unevenly along the bottom. In the fourth series of experiments, water exchange was l/min. In this case, more than 60% crayfish individuals tried to keep away from the water supply, as a strong current forced them to resist the flow. After five days of the experiment, the death of two individuals was recorded. It has been established an increase of dissolved oxygen up to 8 mg/l (Table 4).

The data presented in Table 5 indicate that the most appropriate regime for crayfish in the RAS occurred at the water exchange of 27 l/min. Their weight was greater compared to individuals keeping with a current rate of 0.5 l/min by 95%, l/min – by 0,3% and with l/min – by 1,3%.

Thus, the current rate of water should not exceed twice. Crayfish actively consume the food and evenly distributed throughout the bottom when the dissolved oxygen is near6.0 mg/l, pH 7.3, NO_{2-} is 0,03–0,35 mg/dm^3, NO_{3-} 0.35–0,37 mg/dm^3, CO_2 not higher than 13–14 mg/dm^3.

A series of experiments was conducted to identify the influence of surfactants, insecticides, fungicides, herbicides and mineral fertilizers on the narrow-clawed crayfish, as well as their fatal doses (FD). In the first experiment, we used the washing powder "Lotos" (active components – sodium sulfate >30%, sodium chloride 15–30%, carbonates 5–15%, anionic surfactants <5%, sodium silicate 5%, antifoaming agent 5%, aromatic additives) in five different doses (the tables only show the doses that had a negative effects). The sixth aquarium was a control where crayfish were kept under similar conditions, but without adding the washing powder. In total of 10males and 10 females remained alive even after 14 days after the adding this powder at 2.0 g/l.

Their behavior almost does not differ from normal, crayfish continued to consume food during the experiment. The fatal dose of the powder for the narrow-clawed crayfish is 2.5 g/l.

In the second experiment, the resistance of crayfish to insecticides was determined, in particular to the "BI-58 new" (active component – dimetoate 400 g/l), which is used in agronomy to protect

Table 1. The dynamics of the chemical indicators of water

Parameters	Day							
	1	5	11	15	17	19	21	23
Dissolved oxygen, mgO_2/dm^3	6,5	6,5	6.0	5,5	5,5	5,0	4,0	3,5
pH	7,2	7,2	7,2	7,2	7,4	7,4	7,6	7,8
Nitrites(NO_2), mgN/dm^3	0,03	0,03	0,03	0,04	0,03	0,04	0,05	0,05
Nitrates (NO_{3-}), mgN/dm^3	0,35	0,37	0,36	0,37	0,40	0,42	0,45	0,45
Carbon dioxide(CO_2), mgC/dm^3	14	14	16	16	16	18	19	19

Table 2. The dynamics of sanitary-hygienic indicators of water at a speed of 27 l/min*

Parameters	Day						
	1	5	11	15	17	19	21
Dissolved oxygen, mgO_2/dm^3	6,5	6,5	6,5	6,5	6,5	6,5	
pH	7,3	7,3	7,3	7,3	7,3	7,3	7,3
Nitrites(NO_2), mgN/dm^3	0,03	0,035	0,035	0,03	0,03	0,035	0,03
Nitrates (NO_{3-}), mgN/dm^3	0,37	0,37	0,35	0,36	0,36	0,36	0,37
Carbon dioxide(CO_2), mgC/dm^3	14	15	14	14	14	13	14

* At the water temperature of +18 °C

Table 3. The dynamics of sanitary-hygienic indicators of water at a speed of 162 l/min

Parameters	Day						
	1	5	11	15	17	19	21
Dissolved oxygen, mgO_2/dm^3	6,5	6,5	6,5	6,5	6,5	6,5	6,5
pH	7,3	7,3	7,2	7,2	7,3	7,3	7,4
Nitrites(NO_2), mgN/dm^3	0,37	0,37	0,35	0,030	0,036	0,035	0,030
Nitrates (NO_{3-}), mgN/dm^3	0,37	0,37	0,35	0,36	0,36	0,36	0,37
Carbon dioxide(CO_2), mgC/dm^3	14	15	12	12	12	13	14

Table 4. The dynamics of sanitary-hygienic indicators of water at a speed of 216 l/min

Parameters	Day						
	1	5	11	15	17	19	21
Dissolved oxygen, mgO_2/dm^3	5,0	6,0	8,0	8,0	8,0	8,0	8,0
pH	7,3	7,3	7,3	7,3	7,3	7,3	7,3
Nitrites(NO_2), mgN/dm^3	0,030	0,030	0,032	0,033	0,036	0,040	0,045
Nitrates (NO_{3-}), mgN/dm^3	0,037	0,037	0,039	0,040	0,042	0,042	0,043
Carbon dioxide(CO_2), mgC/dm^3	14	12	12	12	14	12	12

plants from pests. The results obtained during the experiment are shown in Table 6.

All individuals were alive after 14 days after adding the insecticide at the concentration of 0.04 ml/l. The fatal dose of "BI-58 new" is 0.05 ml/l. In the third experiment, we studied the survival of crayfish after the adding of the fungicide "Ridomil" (active components – mannose 64%; mephenoxam 4%), which is used to protect of various crops against fungal diseases. The results obtained during the experiment are presented in Table 6. The fatal dose calculated for crayfish was 5 g/l.

The purpose of the fourth experiment was to determine the crayfish resistance to water-169soluble herbicides, in particular "Napalm" (active component – isopropyl amine glyphosate 480 g/l in acid equivalent 360 g/l), which is used in agriculture. When the concentration of the herbicide was 0,07 g/l, allindividuals remained alive after 14 days,and the fatal dose is 0,08 g/l (see Table 6).We also determined the fatal dose of nitrogen fertilizers, in particular ammonium nitrate (Table 7). In the variant with the concentration of thechemical at 0.4 g/l in 14days, all individuals remained alive, but the higher dose (0,5 g/l) of the ammonium nitrate was fatal for crayfish.

Results of the influence of superphosphate dissolved in water on survival of crayfish are represented in Table 8. When the concentration of this fertilizer was at the level of 0.5 g/l, the death of animals was not observed during 14 days of the experiment.

The fatal dose for crayfish was 0.6 g/l of superphosphate. We also estimated the effect of potassium fertilizers (potassium sulfate) on the crayfish survival (Table 8). The fatal dose of this chemical for crayfish is 0.5 g/l.

Generalized data of all our studies are represented in Table 9.

It should be noted that there were no deaths of animals in the control variant of all experiments.

Discussion

Anthropogenic factors adversely affecting the number of crayfish are the sharp deterioration of environmental conditions, first of all the wastewater entering to the river. A significant amount of surfactants is used for washing, cleaning and housing. Mineral fertilizers, pesticides, insecticides and other chemicals are additional sources of wasting the water bodies (Sarikaya, Sepici-Dinçel, Cağlan Karasu Benli, Selvi, & Erkoç, 2010; Ehsani & Jasour, 2014).

Table 5. Growth, development and preservation of crayfish

Parameters	Water exchange, l/min			
	0,5	27	162	216
Numberof crayfish	83/69	159/69	152/150	150/149
Total weight, g	3748/3477	7342	7320/7328	7274
Average length of the crayfish, mm	114/119	–	–	–
The death of crayfish:				
at 5th day	–	–	–	4
at 19th day	3	–	–	–
at 23rd day	4	–	–	–

Table 6. The influence of surfactants (washingpowder "Lotos"), insecticide "BI-58 new", fungicide "Ridomil", and herbicide "Napalm" on the narrow-clawed crayfish

Research variants	Time and the number of dead crayfish				
	9 hours	24 hours	48 hours	5 days	14 days
"Lotos"					
2,0 g/l	0	0	0	0	0
2,5 g/l	0	4♀ 6♂	1♀	5♀ 4♂	–
"BI-58 new"					
0,04 ml/l	0	0	0	0	0
0,05 ml/l	0	3♀ 8♂	1♀	7♀ 2♂	–
"Ridomil"					
4 g/l	0	0	0	0	0
5 g/l	0	1♀	9♀ 7♂	3♂	–
"Napalm"					
0,07 g/l	0	0	0	0	0
0,08 g/l	0	1♂ 4♀	4♂ 6♀	5♂	–
Control	0	0	0	0	0

Table 7. Growth, development and preservation of crayfish

Research variants	Time and the number of dead crayfish				
	9 hours	24 hours	48 hours	3 days	14 days
0,4 g/l	0	0	0	0	0
0,5 g/l	0	7♂ i 5♀	2♂ 5♀	1♂	0

Table 8. The influence of superphosphate and potassium sulfate on the survival of the narrow-clawed crayfish

Research variants	Number of dead crayfish (control – every 5 hours)				
	1	2	3	4	5
Superphosphate					
0,4 g/l	0	0	0	0	0
0,5 g/l	0	5♂ 8♀	2♂ 2♀	3♂	0
Potassium sulfate					
0,4 g/l	0	0	0	0	0
0,5 g/l	0	0	2♂ 7♀	3♂ 8♀	0
Control	0	0	0	0	0

Table 9. The influence of surfactants, pesticides, herbicides, fungicides and mineral fertilizers on 61the survival of the narrow-clawed crayfish

Research variants	Active component	Concentration	Fatal dose (FD)	Time of death
Washingpowder “Lotos”, g/l	anionic surfactants	45	2,5	9 hours
“Bi-58 new”, ml/l	dimetoate	40	0,05	3 days
“Ridomil”, g/l	mancozeb	64	5,0	5 days
“Napalm”, ml/l	glyphosphate	48	0,08	3 days
Ammonium nitrate, g/l	N	34,4	0,4	2 days
Superphosphate, g/l	P	40	0,6	4 days
Potassium sulfate, g/l	K	40	0,5	3 days

The influence of these substances on crayfish has not been fully explored. Only some pesticides, in particular DDT, which has been banned for use for several decades, are mentioned in the literature (Yildiz & Benli, 2004; Kouba, Buřič & Kozák, 2010).

Biotic factors negatively affecting on crayfish are parasites (e.g. Branchiobdella pentodonta) and pathogens, e.g. Ramularia astaci (Beitinger &Huey, 2002).

Laboratory studies have shown that the lethal dose for crayfish is 2.5 g/l of washing powder Lotus. Invertebrates also died with the introduction of 0.05 ml/l of insecticide Bi-58 new. The lethal dose for crayfish are 5 g/l of the fungicide Rydomil and 0.08 ml/l of the herbicide Napalm. The lethal dose of ammonium nitrate, superphosphate and potassium salt are 0.4, 0.6 and 0.5 g/l, respectively.

Conclusion

Visual observations on crayfish during the experiments described above showed that the adding of the washing powder “Lotos” at a dose of 2.0 g/l does not changed the animal behavior, 187and only slightly differs from the control variant. The fatal dose of this surfactant for crayfish is 2.5g/1. The insecticide (“Bi-58 new”) has the most negative effect on the studied animals (FD 0.05 189 ml/l). Besides, adding the fungicide “Ridomil” into the water medium with crayfish caused the mass death of individuals at the concentration of 5 g/l. The herbicide “Napalm” (FD 0.08 ml/l) was also active for crayfish. Ammonium nitrate, superphosphate and potassium sulfate had a fatal effect on crayfish at the concentrations of 0.4, 0.6, and 0.5 g/l, respectively.

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Highlights

1. A new methodology for obtaining crayfish products in three years is presented.

2. The most optimal for crayfish was their keeping in RAS with a water exchange of 27 l/min.

3. The optimal dissolved oxygen content for crayfish growing is 6.0 mg/l.

4. The effect of various chemical agents on the survival of crayfish was studied.

5. The fatal dose of surfactants, insecticides, fungicides, herbicide and fertilizers is estimated.

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