УДК 639.517:591.526

ОПЫТ НЕПРЕРЫВНОГО ПОДРАЩИВАНИЯ СЕГОЛЕТКОВ ШИРОКОПАЛОГО РАКА *ASTACUS ASTACUS* ДЛЯ ПОТРЕБНОСТЕЙ АКВАКУЛЬТУРЫ

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Образец цитирования:
Голубев АП, Алехнович АВ, Бодиловская ОА, Улащик ЕА, Гопинатхан А. Опыт непрерывного подращивания сеголетков широкопалого рака *Astacus astacus* для потребностей аквакультуры . *Журнал Белорусского государственного университета. Экология.* 2022;2:12–25 (на англ.).
https://doi.org//10.46646/2521-683X/2022-2-12-25

For citation:
Golubev AP, Alekhnovich AV, Bodilovskaya OA, Ulashchyk EA, Gopinathan A. The experience of perennial growing up of fingerlings of noble crayfish *Astacus astacus* for aquaculture needs. *Journal of the Belarusian State University. Ecology.* 2022;2:12–25.
https://doi.org//10.46646/2521-683X/2022-2-12-25

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Разработан метод ускоренного искусственного выращивания в аквакультуре сеголетков *Astacus astacus* в климатических условиях Беларуси. Первоначально новорожденных личинок *A. astacus* выращивали с начала июля до начала октября (летний период) в открытых лотках при температуре 12–25 °C. В последующий зимний период (начало октября – начало апреля) подросшие особи выращивались в аквариумах в отапливаемом помещении при температуре 18–20 °C.

Средняя масса особей в конце летнего периода снижалась от 392 до 265 мг с увеличением конечной плотности посадки от 6 до 54 особей·м$^{-2}$. Средняя масса особей в конце зимнего периода незакономерно изменялась от 688 до 939 мг в градиенте конечной плотности посадки 58–122 особей·м$^{-2}$. Эти показатели близки к аналогичным данным для молоди в естественных водоемах в конце первого и второго лета жизни.

Уравнение зависимости между средней плотностью посадки (N, экз.·м$^{-2}$) за весь период выращивания и средней массой (W, мг) для особей в возрасте 3,5–4 мес., рассчитанное по результатам настоящего исследования и данным других авторов, имеет вид: W = 424–103·lgN. Аналогичное уравнение для особей в возрасте 9–10 мес. в конце зимнего выращивания имеет вид: W = 1376–278·lgN. Эти уравнения представляют собой альтернативные стратегии аквакультуры, позволяющие производить больше молоди с меньшей массой тела или меньшее количество более крупных особей.

В целом интродукция 9-месячных *A. astacus* в пруды позволяет получать в Беларуси экземпляры товарной массы (~10 г) в возрасте двух лет, или на один-два года раньше, чем в естественных водоемах. Максимальную начальную плотность (N_o) выращивания в аквакультуре новорожденных личинок *A. astacus* и особей в возрасте 3–4 мес. можно рассчитать по уравнению: $N_o = \frac{N_{lim}}{\sqrt{S}}$, где N_{lim} – плотность особей в конце периода выращивания, рассчитанная по приведенным выше уравнениям, при которой их масса будет соответствовать таковой для особей в естественных водоемах в конце их первого или второго лета жизни; S – выживаемость особей в аквакультуре, выраженная в долях единицы. Согласно этому уравнению, N_o для первой и второй возрастных групп составляет 423 экз.·м$^{-2}$ и 33 экз.·м$^{-2}$ соответственно.

Ключевые слова: *Astacus astacus*; высшие ракообразные; аквакультура; молодь раков; коммерческое рыболовство; плотность посадки.

Благодарность. Исследования выполнены в рамках двух международных научных проектов: Белорусско-Индийский проект «Эколого-биохимические подходы в регуляции роста и размножения у жаброногих и десятиногих раков в аспекте их потенциального использования в аквакультуре» (2015–2016 гг.) (DST/INT/BLR/P-6/2014 от 15 апреля 2015 г.), выполненный Международным государственным экологическим институтом им. А. Д. Сахарова Белорусского государственного университета (Минск, Беларусь) и Школой биологических наук и технологий Университета ВИТ (Веллуру, Индия). Проект финансировался Государственным комитетом по науке и технологиям Республики Беларусь и Департаментом науки и технологий правительства Индии. Белорусско-российский молодежный проект «Разработка подходов к созданию лабораторных культур эндемичных амфипод озера Байкал, аборигенных и инвазивных видов амфипод и десятиногих раков Беларуси» (Б19РМ-097: 2019–2021), выполненный Международным государственным экологическим институтом им. А. Д. Сахарова Белорусского государственного университета (Минск, Беларусь) и Иркутским государственным университетом (Иркутск, Россия). Проект финансировался Белорусским республиканским фондом фундаментальных исследований и Российским фондом фундаментальных исследований.

THE EXPERIENCE OF PERENNIAL GROWING UP OF FINGERLINGS OF NOBLE CRAYFISH ASTACUS ASTACUS FOR AQUACULTURE NEEDS

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The method of accelerated artificial rearing in aquaculture of *Astacus astacus* fingerlings in climatic conditions of Belarus has been elaborated. Initially, newborn *A. astacus* larvae were reared from early July to early October (*summer period*) in open-air trays at temperatures 12–25 °C. In successive *winter period* (early October – early April) grown up individuals were reared in aquaria in a heated room at temperatures 18–20 °C. The average weight of specimen at the end of summer period decreased from 392 to 265 mg with the increase of final stocking density from 6 to 54 ind.·m^{-2}. The average weight of individuals at the end of winter rearing changed irregularly from 688 mg to 939 mg within the final density gradient 58–122 ind.·m^{-2}. It is close to similar data for juveniles in natural reservoirs at the end of the first and second *summer* of their life.

The equation between geometric mean (N, ind.·m^{-2}) of density on the whole period of rearing and average body weight (W, mg) for individuals aged 3.5–4 months calculated from the results of present investigations and the relevant literature data as follows: W = 424–103·lgN. A similar equation for individuals aged 9–10 months after winter rearing is follows: W = 1376–278·lgN. These equations provide an alternative in aquaculture strategy to produce more juveniles with lower body weight or fewer larger ones.

Generally, the introduction of 9-month-old *A. astacus* into ponds makes it possible to obtain specimens of marketable weight (~10 g) in Belarus at the age of two years, or one-two years earlier than those in natural reservoirs.

The maximal initial density (N_o) of rearing in aquaculture of newborn larvae *A. astacus* and specimen at the age 3–4 months can be calculated according to the equation: $N_o = \frac{N_{lim}}{\sqrt{S}}$, where N_{lim} is the density of individuals at the end of period of rearing calculated from the above equations at which their weight will correspond to those for individuals in natural reservoirs at the end of their first or second summer of their life; S – survival of individuals in aquaculture expressed in fraction of unity. According to this equation, N_o for the first and second age groups are 423 and 33 ind.·m^{-2} respectively.

Keywords: *Astacus astacus*; noble crayfish; aquaculture; crayfish juveniles; commercial fishery; planting density.

Acknowledgements. These studies were performed in the framework two International Scientific Projects: Belarusian-Indian Project «Ecological and biochemical aspects of the regulation of growth and reproduction in decapod crustaceans and anostracans for the revelation of their potentials in an aquaculture» (2015–2016) (DST/INT/BLR/P-6/2014 dated 15 April 2015) carried out by the International Sakharov Environmental Institute of the Belarusian State University (Minsk, Belarus) and the School of Biosciences and Technology, VIT University (Vellore, India). The project was funded by the State Committee on Science and Technologies of the Republic of Belarus and the Department of Science and Technology, Government of India. Belarusian-Russian Project «Development of approaches to the maintaining of laboratory cultures of endemic amphipods of Lake Baikal, indigenous and invasive species of amphipods and decapod crayfish in Belarus» (B19RM-097: 2019–2021) carried out by the International Sakharov Environmental Institute of the Belarusian State University (Minsk, Belarus) and the Irkutsk State University (Irkutsk, Russia). The project was funded by the Belarusian Republican Foundation for Fundamental Research and Russian Foundation for Fundamental Research.

Introduction

Noble crayfish *Astacus astacus* Linnaeus, 1758 is one of the most important commercial species of aquatic invertebrates in Europe, where it has been used as a fishery source for over 2000 years [1; 2]. Belarus was reported to be exceptionally rich in *A. astacus* until the mid-20th century. During the 1930–1950's, its annual catch in Belarus reached as high as 40 tons, a significant part of which was exported to Western Europe [3]. However, in the second half of the 20th century, the *A. astacus* population in Belarus began to decline rapidly, and its fishing came almost to a halt, due to economic unitability.

Reasons for the decline in the Astacus population in Belarus have been numerous, such as the uncontrolled fishing, large-scale hydromeliorative measures, and anthropogenic pollution of water bodies. Another significant reason has been the gradual replacement of *A. astacus* by a more aggressive and competitive species – the narrow-clawed crayfish *Astacus leptodactylus*, that penetrated into Belarus at the beginning of the 20th century from the reservoirs of the Northern Black Sea region [4]. A similar situation is reported to exist in other European countries as well, where *A. leptodactylus* has been widespread almost everywhere, although its abundance varies from region to region. Whereas *A. astacus* is a rare and even endangered species in most European countries, it still remains as a commercial species in Scandinavian and the Baltic states, although its catch is strictly regulated. In general, the *A. astacus* population in Western Europe decreased to the tune of 50–70 % during the past 20–35 years [2; 5].

Significantly, the market value of *A. astacus* from Western Europe is over 5 times higher than those of other crayfish species, such as the native *A. leptodactylus*, and the invasive *Pacifastacus leniusculus* and *Orconectes limosus* [6]. Nevertheless, the demand for *A. astacus* products remains stable and high. In this situation, the only way to meet the demand for *A. astacus* products is to promote its aquaculture, which is being attempted in a number of European countries [2].

In Swedish ponds, the productivity of sexually mature individuals reaches 60–430 kg·ha^{-1}, whereas in Germany, it scales up to even 600 kg·ha^{-1} [7]. Previous investigations have revealed that the high productivity of *A. astacus* seen in some European countries is entrained by several favorable climatic factors [8]. In the prevailing climatic conditions of France and southern Germany (Bavaria), for instance, the year-olds attain sexual maturity and commodity size by the end of the third summer of their life, i.e., at the age of 3 years [8; 9].

This is contrary to what is existing in Belarus, wherein the growth of *A. astacus* in natural water bodies is possible only during a short span of time of the year when the ambient temperature exceeds 10 °C [10; 11]. In the natural reservoirs of Belarus and adjacent territories (Baltic states, Poland, southern Scandinavia for example), the conducive temperature does not last for more than 5–5.5 months in a year – i. e., from April end/ early May to early October; for the yearlings born in the natural reservoirs of Belarus during late June - early July, the first season of vegetation will be even shorter, spanning only up to 3–3.5 months.

Compared with the situations in France, where the vegetation season in the natural reservoirs lasts for at least 6 months – from mid-April to mid-October, while the same is restricted to 3–3.5 months in Belarus. Resultantly, the Belarus population of Astacus reaches sexual maturity and commodity size only in the fifth season of vegetation, i. e. a year later than that in France [8], obviously, leading to increased financial expenditure.

Further, even under intensive pond cultivation, the young crayfishes show a perceptibly high mortality rate, due to various factors such as cannibalism, predators permeated in ponds diseases, and other extrinsic factors. Keeping these factors in view, we propose to develop ways to optimize aquaculture of the Belarus population of *A. astacus*, taking into account, the natural and climatic realities.

One of the potential strategies to offset the «unfavourable» environmental factors could be to grow the newborn larvae of *A. astacus* in open-air fishponds during summer up to the age of 3–3.5 months. The adolescent juveniles could subsequently be grown in autumn and winter for 5 to 6 months in an enclosed room under regulated temperature (18–20 °C), which could avoid slackened growth of juveniles during the prolonged autumn-winter period (~6 months). Growing the juveniles under controlled conditions could also significantly reduce mortality and, thus, could save the number of egg-bearing females used for producing planting material.

Stocking density has been identified as one of the most important parameters in intensive aquaculture inasmuch as it may greatly influence growth and productivity of the candidate species of aquaculture.

Earlier, we showed that the specific growth rate of *Astacus leptodactylus* before the age of 3 to 3.5 months decreases linearly with increasing planting density [12]. In the present paper, the results of our laboratory experiments have been processed through computational means, so as to bring out a mathematical expression that could be used as a tool for assessing the initial stocking density for the desired output.

Further, it is a matter of concern that since 1984, *A. astacus* has been included in the Red Data Book of Belarus, where it is currently classified as Category III («Vulnerable» species, but not directly under the threat of extinction). Hence optimization of *A. astacus* aquaculture, as described in the paper, is also aimed at the improvement of the stock from conservational perspectives.

Materials and methods

Summer rearing up of newborn larvae of *A. astac*us was carried out in 2015 in a private enterprise located in the village Korovchino (54°02′54″ N; 30°49′22″ E, Dribin district, Mogilev Province, Belarus) on the shore of a small artificial reservoir. Here, an experimental base was created of four rectangular plastic trays (4.3×0.74×0.37 m), with a bottom area of 3.18 m^2. The trays were filled with water from the reservoir using an electric pump, so as to maintain an excellent flow-through system.

At the bottom of the trays was placed a layer of clay (3–5 cm thick), wherein the year-olds could build burrows. In addition, short (10–14 cm in length) plastic tubes of 5 cm diameter were placed on the bottom of the trays as shelters for egg-bearing females and subsequently for housing their larvae. To prevent the crayfish from exposure to direct sunlight, a wooden canopy was constructed, covered with branches of trees, and the trays themselves were covered with slate sheets for about 2/3 of their length.

During April 24–28, 2015, 30 egg-bearing females of *A. astacus* with embryos at the final stage of development (ocellus stage) were sampled from one of the water bodies in the Dribin district. Two to three hours after the capture, the females were planted in one of the trays to complete the incubation of eggs. Between 21 and 30 June 2015, there was a massive outbreak of larvae (Stage I) from the eggs, which still remained attached to the pleopods of the mother crayfish. These larvae were relying on the yolk materials as alimentary resources, and hence did not need any feeding from an external source. After 5–8 days, the larvae were molted and transformed to Stage II. At this stage, except for the telson structure, the larvae looked quite comparable with the adult individuals. The Stage II larvae, although switched over to external nutrition, remained attached to the females.

On July 4, 2015, the larvae were removed from the females very carefully through a shaking process within the water tank. The females, soon after the removal of the larvae, were returned to the natural water body.

The larvae (Stage II) were then placed in four trays as described below:

I. 50 individuals per tray, with initial density of planting – 15.7 ind.·m^{-2}.

II. 100 individuals per tray, with initial density of planting – 31.4 ind.·m^{-2}.

III. 450 individuals per tray, with initial density of planting – 141.5 ind.·m^{-2}.

IV. 500 individuals per tray, with initial density of planting – 157.2 ind.·m^{-2}.

During the experimental period, the larvae in the trays were regularly fed *ad libitum* on concentrated zooplankton from the nearby reservoir, as well as on finely chopped boiled carrots and potatoes. Further, a periphyton community of filamentous algae, formed on the walls of the trays by mid-July, was also well consumed by the larvae. Interestingly, the filamentous algae were also functioning as a biological filter, which helped maintain water quality. The temperature during the growing period in the trays varied between from 20–25 °C in July – August and 12–15 °C in late September – early October 2015.

During the experimental period, samples of 15 specimens were chosen at random from each tray every fortnight to determine the size and body weight. Measurements of size were carried out using a caliper through standard procedure – beginning from the tip of the rostrum up to the end of the telson to the nearest 1 mm. Weighing was carried out on electronic balance ALS-220 (KERN, Germany) to the nearest 0.1 mg. After the measurements, the individuals were returned to the trays. At the end of the experimental period (October 5, 2015), the surviving specimens in each tray were counted and the size- body weight measurements of 15 20 crayfish specimens from each tray were made as described previously.

Winter cultivation. At the end of the summer rearing, 44 specimens were chosen at random for further experimentation. The next day (6 October 2015), these specimens were taken to the laboratory premises of the International Sakharov Environmental Institute (Minsk). On the same day, these individuals were randomly placed in laboratory containers maintained as another four variants, as shown below:

V. 5 individuals in an aquarium with an area of 690 cm^2; initial density of planting – 72.5 ind.·m^{-2}.

VI. 10 individuals in an aquarium with an area of 690 cm^2, the initial density of planting is 145 ind.·m^{-2}.

VII. 19 individuals in an aquarium measuring 900 cm^2; initial density of planting of 211 ind.·m^{-2}.

VIII. 10 individuals singly in cylindrical vessels with a diameter of 8.0 cm and a bottom area of 50 cm^2; planting density of 200 ind·m^{-2}.

All the vessels and the aquaria were filled with standing tap water to the height of 6–7 cm. Fragments of plastic tubes, 5–7 cm in length, and 2 cm in internal diameter were used as shelters for crayfishes. Their number corresponded to the number of crayfishes contained in vessels and aquaria. The individuals were fed *ad libitum* on live larvae of *Chironomus sp*. and *Daphnia sp*.; the uneaten remains of the feed were removed by changing the water, 2–3 times a week in the vessels, and twice in tanks in the aquaria.

The duration of winter cultivation was a few more than 6 months, from October 6, 2015 to April 9, 2016. The water temperature in the vessels and aquaria during this period varied within 18–21 °C, close to the optimum temperature for *A. astacus* [13]. At the beginning and soon after the termination of the experiment, the length (L) – body weight (W) measurements of all the individuals were made by the methods described earlier. Measurements were also taken before and after molting (as applicable). The mean of size – body weight measurements and standard deviation (σ) for these indices were determined. The reliability of the differences in mean values was estimated from the nonparametric Mann – Whitney criterion. For winter growth, data were collected from the juveniles (aged from 3.5–4.5 to 10–11 months) grown under laboratory conditions at temperature close to 20 °C, grown in aquaria and individual vessels. In all the experiments, *A. astacus* juveniles were provided with optimal conditions for its maintenance and feeding.

Statistical analysis

The present study involved statistical analysis such as the conduct of «t» test, calculation of regression equations and Spearman correlation coefficients (r) for them. In all these instances, the calculations were performed using the software package *Statistica 8.0*.

Results

The body length of Stage II larvae separated from females (as described above) and placed in trays at the age of 8–12 days varied within 8–10 mm (mean length, 8.53 mm), and body weight within 28–43 mg (mean weight, 36.9 mg). After the second molt, at the age of 15–20 days, the larvae completed their metamorphosis and individuals were outwardly quite identical with the sexually mature individuals. From this time onwards, until the end of their growing season in trays they were referred to as «fingerlings». The average weight of fingerlings in different trays after the end of summer cultivation varied between 268.7 and 391.9 mg (table 1; fig. 1, variants I–IV).

Among fingerlings grown at low densities (variants I and II), the mean body weight at 3–4 months old was significantly higher ($p < 0.05$) than in individuals grown at higher densities (variants III and IV) (table 1, fig. 1). Differences between the average weight of the fingerlings in variants I and II, as well as in III and IV, were statistically insignificant ($p > 0.1$). The survival rate of fingerlings in all rearing variants varied between 28–38 %. This is significantly higher than similar indicators in natural water bodies – usually not more than 3–5 %.

Table 1

The growth and survival of *Astacus astacus* fingerlings in summer-autumn period at rearing in experimental conditions from larvae II stage till the age of 3–4 months

Conditions of rearing	Terms of rearing	Duration of rearing, days	Initial density (N_o), ind.·m^{-2}	Average initial body weight (W_o), mg	Final density (N_d), ind·m^{-2}	Average final body length, mm	Average final body weight (W_d), mg	Survival, %	Author
Group rearing									
Open-air fishing trays, area 3.18 m^2; average ≈19 °C	04.07.2015 – 06.10.2015	90; I**	15.7	21.0	5.9	24.2	391.6	38.0	Present study
		90; II**	31.4	21.0	9.1	23.1	327.9	29.0	
		90; III**	141.5	21.0	51.5	21.5	265.4	36.4	
		90; IV**	157.2	21.0	54.1	22.2	268.7	34.4	
Two glass aquaria total area 0.31 m^2, indoor; 18.0 °C	27.06.1966–31.10.1966	126	1468.0	20.0	297.4	19.0***	182.2	25.5	[14]
Concrete basin area 3.9 m^2, indoor; 19.1 °C	29.06.1966–23.10.1966	116	256.0	22.7	166.0	20.0	212.0	64.9	
Wooden cages, installed on the bottom of lake littoral zone; area 1.9 m^2: 18.5–19.7 °C	Summer 1966	95	22.4	21.6	9.0	24.1	348.1	40.2	[15]
	Summer 1967	87	30.3	21.6	16.0	21.9	258.6	52.8	
	Summer 1968	90	≈ 100	21.6	49.0	19.4	170.7	≈49.0	
Aquaria, area 0.06 m^2, indoor: 20.6 °C	May – October	112	666	44.4	300.0	21.0	229.9	83.7	[16]
Aquaria, area 0.16 m^2, indoor, 8–16 °C	August – October	90	187.5	38.0	143.1	13.9***	76.3	76.3	[17]
Trough, area 0.16 m^2, indoor; 20.5 °C	10 May – 7 September	120	400	59.0	76.4	21.3	305.0	19.1	[18]
Tank, area 1.6 m^2, indoor; ≈20.0 °C.	September – November	91	270.0	21.0	140.4	17.9***	153.3	52.0	[11]
Plastic basin, area 4 m^2, indoor	Summer	100	100		58.5	15.3***	100.0	58.5	[19]
		100	300		150.9	14.4***	84.0	50.3	
Open-air pond with natural food, area 15 m^2	Summer	100	100		67.5	20.2***	221.0	67.5	[19]
		100	300		97.2	18.3***	166.0	32.4	
Aquaria, area from 0.06 to 0.12 m^2, indoor; 3.5–21,5 °C (average – 13.4 °C)	15.07.1985 – 15.11.1985	125	250	63.7	16.8–100.0	19.3–22.3***	190.0–290.0	6.7–40.0	[20]
		125	500	63.7	33.5–83.0	17.7–20.2***	150.0–220.0	6.7–16.6	
		125	1000	63.7	50.0–150.0	16.3–18.8***	120.0–180.0	3.3–15.0	
Individual rearing									
Holding cells area 0.0024 m, indoor, 20.5 °C	Summer 2004	90	416.6	21.3	416.6	19.9	188.8	44.4	[21]
Compartments, area 0.00275 m^2, indoor, ≈20.0 °C	Mid July – Mid October	78	363.6	29.0	363.6	18.2***	170.0	66.6	[22]
		83	363.6	34.0	363.6	20.1***	220.0	79.2	
		82	363.6	38.0	363.6	19.9***	210.0	58.3	

Note. *age of specimen at the end of experiments; **I–IV – variants of experiments, given in the section «Material and Methods»; ***recalculated by equation (1).

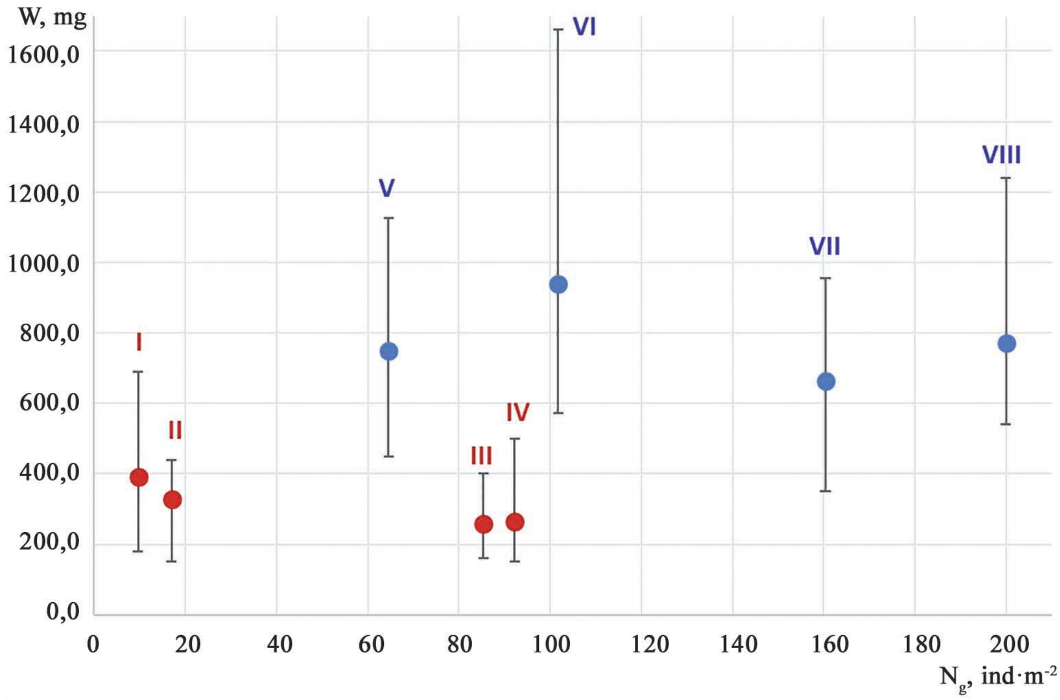

Fig. 1. Variation in body weight (W, mg) in juveniles *Astacus astacus* at the end of the summer (variants I–IV) and winter (variants V–VIII) periods of rearing at different values of geometric mean of their density (N_g, ind.·m^{-2}) per cultivation period. Features of separate variants are given in the «Material and Methods» Chapter. «Whiskers» – limits of changes in body weight, points – their average values

During winter cultivation, the average weight of individuals at group rearing (table 2, fig. 1; variants V–VII) at the end of the experiment differed statistically insignificantly ($p > 0.5$).

Table 2

The growth and survival of *Astacus astacus* yearlings in autumn – winter period at rearing in experimental conditions from the age of 3–4 months till the age of 9–10 months

Conditions of rearing	Terms of rearing	Duration of rearing, days	Initial density (N_o), ind.·m^{-2}	Average initial body weight (W_o), mg	Final density (N_d), ind·m^{-2}	Average final body length, mm	Average final body weight (W_d), mg	Survival, %	Author
1	2	3	4	5	6	7	8	9	10
Group rearing									
Aquarium, area 0.069 m^2, indoor; 17–19 °C	07.10.15–09.04.16	185^{V*}	72.0	258.1	58.0	31.1***	749.3	80.6	Present study
Aquarium, area 0.069 m^2, indoor; 17–19 °C		185^{VI*}	142.0	348.6	73.0	33.7***	939.1	51.4	
Aquarium, area 0.090 m^2, indoor; 17–19 °C		185^{VII*}	211.0	262.3	122.0	29.9***	668.3	57.8	

Ending table 2

1	2	3	4	5	6	7	8	9	10
Fiberglass basins, area 0.86 m^2, indoor, 18–19 °C	11.09.1987–11.03.1988	182	40.0	279.4	5.1	32.2	525.8	50.0	[23]
		182	86.0	279.4	9.5	33.2	573.0	40.0	
		182	86.0	279.4	8.6	33.3	580.7	40.0	
		182	86.0	279.4	12.0	34.0	613.4	45.0	
		182	86.0	279.4	14.6	34.8	655.2	45.0	
		182	86.0	279.4	25.8	32.4	535.2	50.0	
		182	86.0	279.4	20.6	38.9	898.2	35.0	
		182	86.0	279.4	27.5	36.1	726.5	30.0	
		182	86.0	279.4	24.1	32.6	544.6	40.0	
Plexiglas aquaria, area 0.16 m^2, indoor, 22 °C	May – July 2002	70	125	420.0	100.0	32.5***	840.0	80.0	[24]
		70	125	420.0	100.0	29.3***	630.0	80.0	
Concrete basin 3.9 $м^2$, indoor; 19,1 °C	March – August	127	≈120.0	234.5	≈60.0	32.9	960.1	≈50.0	[14]
Individual rearing									
Glasses, area 0.005 m^2, indoor; 17–19 °C	07.10.15–09.04.16	185 ^{VIII*}	200.0	283.0	200.0	30.8	778.1	90.0	Present study
Cages, area 0.00663 m^2, indoor; ≈20 °C	01.12.1990–30.05.1991	181	151.0	153.3	151.0	28.0	553.1	no data	[11]
		181	151.0	153.3	151.0	30.0	672.4	no data	
Compartments, area 0.00275 m^2, indoor; ≈20 °C	Mid October – Mid April	172	363.6	170.0	363.6	27.0***	500.0	75.0	[22]
		162	363.6	220.0	363.6	29.3***	630.0	94.5	
		148	363.6	210.0	363.6	29.2***	625.0	75.0	

Note. *V–VIII – variants of experiments, given in the section «Material and Methods»; ** recalculated by equation (1).

By the end of the winter growing period, we obtained individuals with body sizes at 26–34 mm range (mean size 30.3 mm), and with the mean body weight in different variants reaching up to 668–931 mg, while the weight of the largest individuals reached 1400 mg. In individual rearing (variant VIII), where the area per individual was significantly higher than in groups, the average weight of individuals at the end of experiment rearing was within the same limits – 778.1 mg.

Generally, the continuous growth of *A. astacus* newborns for 9 months (early July to the succeeding April) i. e., slightly over three months under «summer rearing up» exposed to natural conditions, followed by six months of «winter cultivation» under controlled conditions.

The survival rate of *A. astacus* period was 50–80 %; no definite relationship was found to exist between this index and the planting density. Interestingly, the survival rate of individuals kept singly in separate containers (variant VIII) was significantly high – 90 %. The number of molts in specimens at individual rearing (variant VIII of the experiment) varied within 2–4 in the 6-month period of the experiment, with no instances of molt or postmolt mortality. The total number of molts in all the 10 individuals grown under variant VIII was seen to be 26. Significantly, all 9 out of the 10 individuals under variant VIII survived the entire duration of the experiment. The death of a single crayfish (from variant VIII), which occurred shortly after the commencement of the experiment, was most likely due to the injury inflicted up on her during weighing. However, to our observation, the major causes for mortality among the individuals reared in groups could be postmolt cannibalism, and/or the inevitable clashes between individuals.

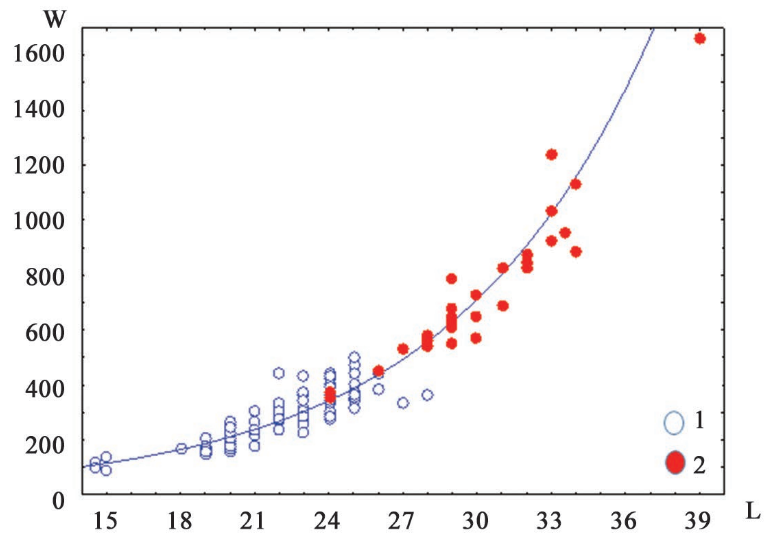

Fig. 2. Dependence of wet body mass in juvenile *Astacus astacus* (W, mg) on their body length (L, mm).
1. Individuals under the age of 3 months. 2. Individuals aged from 3 to 9 months. Curve 1 is the line of equation (1)

The body length (L, mm) – body weight (W, mg) relationship of *A. astacus* juveniles from 2 weeks to 9 months of age is shown in fig. 2. The functional relationship between W and L follows the equation:

$$W = 0{,}0441L^{2{,}832}. \quad (1)$$

The Spearman correlation coefficient (r) for the equation (1) is +0.960, implicating a high degree of correlation between the two variables. Thus, the equation (1) enables to calculate the body weight of juvenile *A. astacus* by their body size, or vice versa.

Discussion

As noted earlier, the growth of *A.astacus* in natural water bodies stops at water temperatures below 10–12 °C. Therefore, the cultivation of this species in open-air aquaculture (fishing trays, concrete basins, small ground ponds, etc.) is possible only in a relatively short period of the year (growing season), when the air temperature stably exceeds 10–12 °C.

In Belarus and the adjacent regions, its duration does not exceed an average of 5 months (May – September). For newborn *A. astacus* larvae hatching in late June – early July, the growth period in its first summer of life becomes even shorter – no more than 3 months. Hence, the further growth of the specimen is possible only in a closed premises, in which the temperature is maintained within the range of 18–20 °C, which is close to the average temperature in the littoral zone of water bodies in temperate latitudes in the growing season. The growing period under such conditions can be carried out until mid-late April when individuals reach the age of 9–10 months. Thus grown juveniles can be transferred to further cultivation to marketable sizes in open-air reservoirs, for example, in earthen ponds, the water temperature in which at the end of April already exceeds 10–12 °C.

The biotechnology of pond growing of yearlings and older age groups of *A. astacus* to marketable sizes is quite well developed [9; 25]. However, many issues of growing yearlings in enclosed spaces remain clearly not studied enough, first of all, the influence of planting density on their growth and survival.

To this end, we have analyzed the results of our own research and the available literature data on the cultivation of *A. astacus* juveniles at different densities under experimental conditions – in fish trays, laboratory aquarium and individual vessels, open wooden cages established in the littoral zone of the lake, concrete pools and small earthen ponds, both for single and group keeping.

Two age groups of *A. astacus* were selected for analysis: I. Newborn stage II larvae growing up to the age of 3–3.5 months; II. Yearlings aged 3 to 5 months, bred to the age of 9 to 11 months.

These age groups correspond to those in our experiments on summer and winter growing, respectively. Data on the size and average weight of individuals, as well as their planting density in both groups at the beginning and end of the growing period are presented in tables 1 and 2.

In experiments conducted on individuals of group I (table 1), the initial density of stage II larvae changed by almost two orders of magnitude – from 16 to 1468 ind.$\cdot$m^{-2}. The survival of individuals by the age of 3–3.5 months varied over a wide range – 3.3 to 83.7 %, which had a significant impact on their growth rate. That's why, as the average arithmetic mean of planting density could give a significant overestimate of the density at the end of the sprouting period, when the density effect impacts the growth of larger individuals most strongly [26]. Therefore, the planting density of individuals for the entire period of their growth in both the series of experiments was determined through geometric mean (N_g):

$$N_g = (N_o \cdot N_d)^{0.5}, \qquad (2)$$

where N_o and N_d represent the density of individuals respectively at the beginning and end of their growing period.

Pertinently, correlation analysis revealed the existence of a significant negative correlation between increased values of N_g and a decline in the average body weight of fingerlings at the end of their growing season (fig. 3). At minimal N_g values (up to about 16 ind.$\cdot$m^{-2}), the average weight of fingerlings (3–4 months old) reached 350–390 mg (table 1; fig. 3). Approximately the same weight is reached by fingerlings of *A. astacus* at the end of their first growth season in natural reservoirs of Belarus and adjacent territories with similar natural and climatic conditions [4; 27].

On the other hand, with an increased N_g to 100 ind.$\cdot$m^{-2}, the average weight of the individuals declined to 160–260 mg. Survival rate during the period of summer growth in different versions of the experiment, varied within the limits of 29–38 %. A clear dependence of the survival rate on the density of planting of individuals, however, was not established ($p > 0.5$).

The relationship between the average body weight of individuals aged 3–4 months (W_d, mg) and their geometric mean density (N_g, ind.$\cdot$m^{-2}) during the cultivation period at group rearing is best approximated by the empirical equation:

$$W_d = A - \beta \cdot \lg N_g, \qquad (3)$$

where A – is an empirical constant corresponding to the average weight of individuals at the age of 3 to 4 months when grown at a density close to zero; β – is an empirical constant characterizing the rate of decrease W_d with increase of $\lg N_g$.

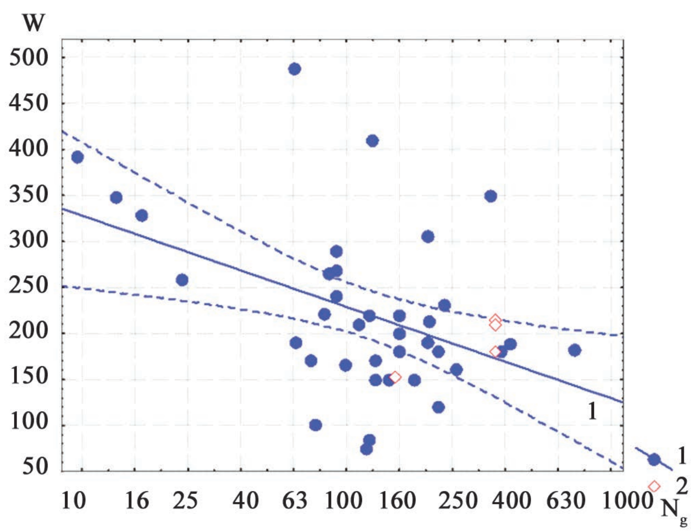

Fig. 3. The dependence of the body weight of fingerlings *Astacus astacus* (W, mg) by the age of 3–4 months on the geometric mean of their density (N_g, ind.$\cdot$m^{-2}) during the cultivation period. N_g values are presented on a logarithmic scale. 1. Group rearing; 2. Individual rearing. Straight line 1 is the regression line of equation (3); broken lines – significance level *P = 0.95*

In numerical form, equation (3) calculated on the results of our research and the relevant data of other authors (table I) for individuals aged 3–4 months is as follows:

$$W_d = 424–103 \cdot \lg N_g. \qquad (4)$$

The correlation coefficient (r) between W_d and $\lg N_g$ for equation (4) is equal to –0.5210, implicating a weak negative correlation existing between the variables.

The equation (4), if applied to the cultured population of *A. astacus*, during summer rearing would ensure juveniles of better quality, compared to those in the wild. In water bodies of Belarus and the Baltic states, their sizes at the end of the first growing season, were 15–22 mm [1; 4], which, according to the equation (3), corresponds to their body weight ranging between 100 and 280 mg.

According to available experimental data (table 1; fig. 3), a similar weight of fingerlings at the end of summer rearing could be achieved if the N_g is maintained at the range 150–300 ind.$\cdot$m^{-2}.

From equation (2), it is easy to derive formula (5) for calculating the maximum stocking density of Stage II larvae at the beginning of rearing period:

$$N_{max} = \frac{N_{glim}}{\sqrt{S}}, \qquad (5)$$

where N_{glim} – limited density of rearing is equivalent to 300 individuals·m^{-2} when the average weight of juveniles at the end of the summer season reaches 170–230 mg; S – survival of individuals during the growing period, expressed in fraction of unity.

The average survivability of fingerlings over a period of summer growth is equivalent to 50 % (i.e. S = 0.5), which corresponds to the available experimental data (table 1). Hence, the maximum limit for the optimal planting density for Stage II larvae for summer cultivation will be close to

$$N_{max} = \frac{300}{\sqrt{0,5}} \approx 423 \text{ ind.}\cdot\text{m}^{-2}. \quad (6)$$

Thus, this mathematical expression has a predictive value inasmuch as it offers us a cue for the initial planting density to obtain the desired output. On the other hand, this dependence provides an alternative in aquaculture strategy to produce more juveniles with lower body weight or fewer larger ones.

In individuals at the age of 9–11 months, despite a considerable scatter of the experimental data, a definite tendency for a decreased average weight, in consonance with an increase in their planting density was observed (fig. 4).

The dependence of the final weight of individuals (W_d, mg) aged 9–11 months, on their planting density (N_g, individuals·m^{-2}) under group rearing can be approximated by equation (3), the numerical form of which is:

$$W_d = 1376–278 \cdot \lg N_g. \quad (7)$$

The correlation coefficient (r) between W_d and $\lg N_g$ for equation (5) is equal to -0.4517.

At planting densities less than 100 ind.·m^{-2} in group rearing, the maximal weight of the specimen reached 1,400 mg by the end of the rearing period (table 2, fig. 4).

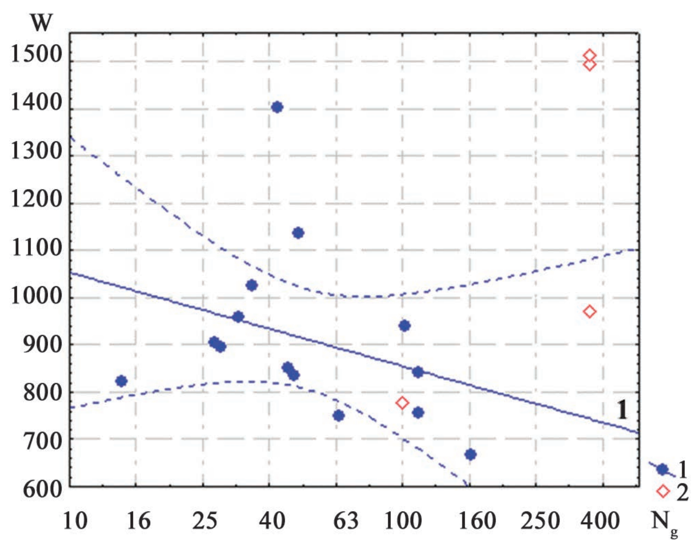

Fig. 4. The dependence of the body weight of juvenile *Astacus astacus* (W, mg) by the age of 9–11 months on the geometric mean of their density (N_g, ind.·m^{-2}) during the cultivation period. N_g values are presented on a logarithmic scale.
1. Group rearing; 2. Individual rearing. Straight line 1 is the regression line of equation (5); broken lines – significance level P = 0.95

In natural reservoirs of Belarus, the body weight of *A. astacus* juveniles by the end of the second summer of their life reaches an average of 1000 mg. According to (5) and fig. 4 at cultivation in laboratory conditions, this weight corresponds to the average rearing density of 25 ind.·m^{-2}. At maximal density in experiments in group rearing for the winter growing season exceeded 200 ind.·m^{-2}. But maximum body weight by the end of rearing at such densities only reached 700 mg.

The survival rate of juvenile *A. astacus* at the end winter rearing period reached 30–95 % (table 2). It is slightly higher than in summer cultivation but far superior to that in natural water bodies [4]. The average survival value of juveniles over a period of winter growth season is equivalent to 60 % (i. e. S = 0.6). Hence, the maximum limit for the optimal initial planting density for *A. astacus* at the age of 3–4 months for winter cultivation, will be close to

$$N_{max} = \frac{25}{\sqrt{0,6}} \approx 33 \text{ ind.}\cdot\text{m}^{-2}. \quad (8)$$

If the average survival rates of the experimental individuals were approximately 35 %, and 63 % during summer and winter rearing respectively the overall survival of juveniles over the entire 9-month period will be 0.35×0.60×100 % = 22 %. On the other hand, in natural water bodies, the survival of yearlings, according to rough estimates has never exceeded 5–8 % of the total number of newly born larvae [4], thus making it explicit the advantage of crayfish culturing under controlled conditions. To a considerable extent, the results of our studies, in unison with those from other authors (table 2) would offer valuable clues to optimizing the aquaculture of *A. astacus* fingerlings at industrial levels.

Notwithstanding the previous demonstrations on the existence of a negative relationship between the planting densities and the body mass in some crayfish species such as *Astacus leptodactylus* and *Pasifactacus leniusculus* [12; 28], the present study is the first of its kind where a quantitative estimate is made to define the correlation between planting density and optimal growth rate among crayfishes.

The phenomenon of growth inhibition in conditions of increased density was confirmed in experiments of so-called «battery culture», i. e. the growth of the juveniles, one by one in separate small cells [29], thus eliminating a number of negative effects of increasing density in aquaculture, such as intraspecific competition for food resources and postmolt cannibalism. However, contrarily, it would demand a concurrent increase in the area of cells as the individuals grow, which may not always be profitable from economic standpoints. Therefore, in most cases, rearing of juvenile crayfish in aquaculture is done in groups, with ample consideration of the effect of limiting factors on the growth and survival of individuals. At this juncture, the established dependencies, as per equations (3) and (5) would be very useful as it provides options for either getting a larger number of smaller individuals or a smaller number of larger ones per unit area.

Another important aspect to be considered at this juncture is molting, a hormone-driven phenomenon for growth among crustaceans, even after attaining puberty [30]. Post-molting cannibalism is one of the most important mortality factors in crayfish aquaculture, especially at high crayfish rearing densities. And the molting itself is reported to create immense stress and anxiety in crayfishes due to the high energy costs for it [31].

In crustaceans, the duration of intervals between successive molts increases in parallel with their increasing body weight and with decreasing temperature [32]. Among *A. astacus* fingerlings, the number of molts in natural water bodies is known to depend on the duration of the vegetation season and the average temperature. Its number varies between five and seven for smaller age groups, though it does not exceed three for yearlings [1; 10]. Pertinently, asynchrony of molt would increase with the age of the juveniles.

Therefore, when they are grown in high density, with limited shelters, the early postmoult specimens with their soft exoskeleton could become easy prey for other individuals with harder a exoskeleton (at the intermolt stage, for instance). Since the number of molts in larvae is higher than that of juveniles, the probability of death of the latter due to postmolt cannibalism is substantially lower than those of fingerlings, a contention that was confirmed by our studies and those of other authors (tables 1 and 2).

This part of the study reveals that the survival rate of juveniles over the period of winter growth under controlled conditions (within the range of 30.0–94.5 %) was substantially higher than that of autumn-winter period in natural reservoirs (not more than 10–15 %) thus reinforcing the relevance of the present study; significantly, maximum survival was observed at the instances of individual rearing (variant VIII). As per the data from the present study, the mortality of individuals during the process of exuviation has been generally very low. Among the individuals of variant VIII, mortality was not observed at all both at exuviation proper and immediate post-exuviation.

A very serious limiting factor for *A. astacus* aquaculture is relatively late terms for the appearance of planting material of fingerlings in nature, namely at the end of June – beginning of July, i.e. approx. in the middle of the growing season. To overcome this limiting factor, a method has been proposed for the early production of *A.astacus* fingerlings in aquaculture [33], which is as follows. The egg-bearing females are to be transferred from natural reservoirs to basins/trays located indoors as early as February – March. In the first 15 days after transfer, females are to be kept at 2–3 °C, and in the next 45 days, at 8–9 °C. During this time, the release of eggs from embryonic diapause would take place. After this, the eggs are to be incubated at 18–20 °C before the release of the larvae directly on females or, after removing them from the females, in special incubation devices. It is also obvious that early transfer of the gravid females to controlled indoor conditions would not only shorten the span of time required for the individuals to reach marketable size, it could as well ensure a better quality of the stock.

This method was previously tested by us successfully on closely related crayfish species *Astacus lepdodactylus* [34]. We obtained the larvae at Stage II as early as in the first decade of May, i. e. 1.5–2 months earlier than in reservoirs with a natural thermal regime. The success of this experiment on *A. leptodactylus* encourages us to recommend the same procedure in *A. astacus* as well. Resultantly, it can be expected that by using this method the period of growth of *A. astacus* fingerlings in open-air aquaculture could be prolonged by the same 1.5–2 months. This makes it possible to obtain aquaculture at the end of the summer season *A. astacus* fingerlings of a larger size than those in natural reservoirs. In its turn with their further winter rearing indoors, it will be possible to obtain larger underyearlings. If these grown-up individuals are introduced into fish ponds in the spring, it is quite possible that they will reach marketable sizes by the end of this growing season, as how it shows the experience of breeding crayfish in aquaculture in Germany [9].

Conclusion

To conclude, the results of our present study demonstrated that culturing of *A. astacus* larvae under controlled conditions would accelerate its growth to reach commodity size, one year earlier than the controls; while the wild population required 4 years, the experimental ones attained comparable size and body weight in 3 years. Significantly, this growth rate corresponds to that of the Astacus populations in Germany and France with a warmer climate and longer season of vegetation.

The culture methods under the controlled conditions (adopted in the present study) offer proper protection to the *A. astacus* juveniles at a time as they pass through the most vulnerable (to factors such as predation, abrasion, and infection) stages of postembryonic development. Maintenance of optimal environmental conditions for the juveniles could thus be a major contributory factor for its higher survival rate (reaching up to 90 %; table 2) in comparison with natural water bodies.

The present study is also relevant in terms of designing the optimal stocking densities for culture. The series of experiments with varying densities under the summer and winter growth periods have helped us obtain valuable clues regarding the optimized stocking densities under a given situation. This would indeed help a farmer to predict the density of the individual and the total space required to attain maximum productivity within the minimum span of time.

Finally, but importantly. The optimized pattern of culture under a natural environment (summer growth) interspersed with the culture under controlled conditions (winter growth), as envisaged in the present study, would, not only help us to have grown-outs of *A. astacus* with improved quality, and optimization of its commercial fishery, but it would as well foster the restoration of the natural reserves of the species within the country, with the prospect of its conservation leading to its possible withdrawal from the Red Data Book of Belarus.

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Статья поступила в редколлегию 15.04.2022.
Received by editorial board 15.04.2022.