

# Survival, proximate composition, and proteolytic activity of *Artemia salina* bioencapsulated with different algal monocultures

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**Abstract.** This study focused on testing *Artemia* sp. zooplankton saturation with freshwater microalgae biomass and then using this method to correct the nutritional composition of *Artemia*. Accordingly, the influence of three species of microalgal monocultures was analyzed (2 freshwater – *Desmodesmus armatus* (Chod.) Hegew. and *Chlorella vulgaris* Veijerick; one halophilous – *Dunaliella viridis* Teodor.). The algal monocultures were applied once in a quantity of  $2-3 \times 10^6$  cells  $\times$  l<sup>-1</sup> for each 200,000 *Artemia* individuals hatched. The control group *Artemia* did not receive algae. The enrichment process lasted 24 h, and control measurements were performed every 6 h. The survival of *Artemia* nauplii, their proteolytic activity, and the content of proteins, lipids, and carotenoids were analyzed. The choice of algae species for *Artemia* enrichment was guided by the size of the algal cells and their biochemical composition. Selected algae contained about 50% protein, 20% lipid, and 12 mg of carotenoids per g of dry weight. Using algae to saturate the *Artemia* nauplii permitted reducing their mortality during the 24 h enrichment regime. The introduction of *C. vulgaris* biomass halved this value. The use of algae also increased the

proteolytic activity in *Artemia* nauplii and the content of proteins, lipids, and carotenoids in their biomass. The best results of the bioencapsulation of *Artemia* nauplii with algae was with the *C. vulgaris* biomass.

**Keywords:** *Artemia*, green algae, bioencapsulation, proteolytic activity, proteins, lipids, carotenoids

## Introduction

One important element of aquaculture is obtaining high quality fish stocks. This cannot be achieved without using nutrient-balanced feeds. Live feeds significantly increase the survival rates of early developmental fish stages (Watanabe et al. 1983, Aruho et al. 2020). The organisms cultivated are usually those that are part of the natural food base of fishes. These feed items have high nutritional value and high contents of irreplaceable nutrients such as essential amino acids, fatty acids, enzymes, vitamins, and minerals (Conceição et al. 2010).

*Artemia* nauplii are easy to produce, so they are often the first feed offered to larval fish as they make to shift to exogenous food (Conceição et al. 2010, Hamre et al. 2013). However, *Artemia* are an incomplete source of food as their composition lacks some

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basic elements, for example, polyunsaturated fatty acids that are often required for the successful development of larval fishes (Sorgeloos et al. 2001, Kolman et al. 2018). The level of essential compounds in live feed can be increased through bioencapsulation with various substances, including amino acids, proteins, polyunsaturated fatty acids, carotenoids, and even probiotics (Monroig et al. 2007, Viciano et al. 2015). Compounds incorporated into the diet of *Artemia* can be in commercial liquids or the organic matter of feed organisms (bacteria, yeast, or microalgae) (Harel et al. 2002, Prusińska et al. 2015). Using highly purified commercial supplements of essential compounds for bioencapsulation significantly increases the final cost of aquaculture products. The solution to this problem is to use the biomass of the producers, in particular microalgae, for both feeding zooplankton and the joint cultivation of these two groups of organisms (Cheban et al. 2018, Bernaerts et al. 2019). The successful use of algae as feed for crustaceans and plankton-feeding fishes, as well as for all stages of bivalve mollusks is well known (Ovie and Egborge 2002, Becker 2007, Van Der Meeren et al. 2007).

The key characteristics when choosing algae as feed substrates are their size, biomass growth rate, resistance to environmental fluctuations, nutritional value, and the absence of toxic compounds in their biomass (Villarruel-López et al. 2017, Cheban et al. 2018). Algal nutritional value is linked significantly to the characteristics of species and can be adjusted depending on growing conditions (Hu et al. 2008, González López et al. 2010). Thus, protein content in algal biomass fluctuates from 45 to 70%, while lipid content ranges from 10 to 30%, and changes of fatty acid profiles and the quantities of some fatty and amino acids are also observed (Salama et al. 2013, Samek et al. 2013). Therefore, carefully selected microalgal monocultures or mixtures are proposed as excellent feed substrate for fish introduced into the diet by direct feeding or indirectly by their bioencapsulation into feed zooplankton (Becker 2007, Duong et al. 2015). The joint cultivation of *Artemia* nauplii and their feed organisms, in particular algae, also solves the problem of nauplii death

when they are kept in automatic feeders. In addition to increasing survival rates, this also helps to reduce nauplii starvation and preserve or even increase their nutritional value. To facilitate implementing this idea, a study was devised to assess the impact of three microalgae monocultures, namely *Desmodesmus armatus* (Chod.) Hegew., *Chlorella vulgaris* Veijerinck, and *Dunaliella viridis* Teodor., on the survival rate and nutritional composition of *Artemia* sp. nauplii.

## Materials and Methods

### Conditions for algal cultivation

The research was conducted using pure cultures of the green algae *Desmodesmus armatus*, *Chlorella vulgaris*, and *Dunaliella viridis* obtained from the collection of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine (IBASH-A). Currently these cultures are maintained in the collection of the Institute of Biology, Chemistry, and Bioresources, Yuriy Fedkovych Chernivtsi National University. Wastewater from a recirculating aquaculture system (RAS) was used as the culture medium for *D. armatus* and *C. vulgaris* algae (Cheban et al. 2015, Khudiyi et al. 2016), while a NaCl in the amount of 1M was added to the *D. viridis* cultivation. All the manipulations of the cultures were conducted under the sterile conditions of a laminar flow hood. Cultivation was conducted in a climatic room with 16 h of illumination with fluorescent lamps at 2500–4000 lux and a temperature of  $28 \pm 2^\circ\text{C}$ . Microalgae cultivation lasted for 21 days until the culture reached a stationary growth phase. Then the number of cells in the algae suspension was counted with microscopy and a Goryaev chamber.

### *Artemia* cyst incubation and nauplii bioencapsulation

*Artemia* sp. cysts (Sepia Art *Artemia*, Ocean Nutrition, Belgium) were incubated in illuminated,

oxygenated 8 l Weiss flasks for 24 h at a temperature of 28°C. The salinity of the incubation medium for cysts was provided by adding of NaCl at a dose of 15 g × l<sup>-1</sup>. The release of nauplii under these conditions occurs within 24 h of placing the cysts into the incubation medium.

After sorting out cyst capsules and unhatched cysts, freshly hatched *Artemia* larvae at a density of 200,000 individuals were transferred to enrichment flasks filled with salt water. Biomasses of freshwater microalgae *D. armatus* and *C. vulgaris* and saltwater algae *D. viridis* was used for saturation, which was applied once in a quantity of 2-3 × 10<sup>6</sup> cells × l<sup>-1</sup>. *Artemia* from the control group did not receive algal biomass. Four experimental groups were formed: group I – *Artemia* nauplii without feeding (control group); group II – *Artemia* nauplii + *D. armatus*; group III – *Artemia* nauplii + *C. vulgaris*; group IV – *Artemia* nauplii + *D. viridis*.

Freshwater algae species are characterized by small cells, and they lack rigid cell wall outgrowths (Table 1). For comparison, saltwater algae *D. viridis* was used for zooplankton saturation, and it was also analyzed. This microalgal species does not have a polysaccharide cell wall, which is replaced by a thin elastic cell membrane. This fact probably suggests using given algal species as zooplankton feed. Algal size is also important; it should not exceed the size of the *Artemia* nauplii filter apparatus. The smallest among the proposed algae are the cells of *C. vulgaris*, which ranges from 5 to 10 μm. Due to the small cell size of 10-20 μm in length and 3.5-8 μm in width, *D. armatus* can also be used as a feed substrate for zooplankton bioencapsulation and for feeding fish fry.

Every 6 h from the beginning of the experiment, the total number and the number of dead crustaceans in the experimental and control groups were counted. For this purpose 0.2 ml of culture medium was analyzed in Bogorov counting chambers after Lugol's solution had been added. Then the total number of *Artemia* was counted in the samples. The mortality rate was estimated by the Cumulative Mortality Index (CMI), which was calculated as the ratio of the sum of dead individuals at that and in previous hours to the total number of individuals in the sample at that and in previous hours:

$$CMI_i = \frac{\sum_0^{24} n_i}{\sum_0^{24} N_i} \times 100$$

$n$  – the number of dead individuals per  $i$  h;

$N$  – total number of individuals per  $i$  h;

$i$  – duration of feeding (0, 6, 12, 24 h).

### Biochemical analysis of algae and *Artemia* nauplii

Algal and *Artemia* nauplii biomass was concentrated by centrifugation at 5000 rpm for 15 minutes on a Heraeus Biofuge® Stratos®. The hydrated biomass was homogenized by adding 0.1 M phosphate buffer with a pH of 7.4. Ultrasonic Cleaner was used to destroy the biomass. The total protein content was determined with Lowry's method (Lowry et al. 1951). The determination of total lipids extracted with Folch's method (Folch et al. 1957) was conducted

**Table 1**

Characteristics of algal cells (Hosseini Tafreshi and Shariati 2009, Nermin 2011, Safi et al. 2014)

Alga species	Alga characteristics			
	Size	Cell wall	Cell wall outgrowths	Salinity tolerance
<i>D. armatus</i>	Length: 10-20 μm Width: 3.5-8 μm	Present	minor bulges	Freshwater species
<i>C. vulgaris</i>	Diameter: 6-8 μm	Present	Absent	Freshwater species
<i>D. viridis</i>	Length: 5.1-17.0 μm Width: 3.0-15.2 μm	Absent	Absent	Saltwater species

with the acid hydrolysis of the samples followed by the reaction between decomposition products and a phospho-vanillin reagent (Knight et al. 1972). The carbohydrate content was determined with a color reaction and an anthrone reagent (Roe 1955).

The carotenoids and chlorophyll were extracted with absolute acetone and centrifuged at 3000 rpm for 15 minutes. The spectra of pigments in the range of 450–690 nm were measured in the supernatant. The calculation of pigment concentration was performed according to conventional formulas (Tanaka 1978, Macías-Sánchez et al. 2008). The proteolytic activity was investigated with the modified Anson method (GOST 20264.2-88 1988). First, the nauplii were homogenized with universal buffer with pH of 4.8, 7.4, and 9.0. Casein was used as a substrate to determine neutral and alkaline proteases, and a hemoglobin solution was used as a substrate for acidic proteases. Enzyme activity was expressed in conventional units per mg protein, where 1 unit (U) corresponded to that amount of enzyme that catalyzes the conversion of 1 micromole of substrate in 1 m. All calculations were performed on dry weights. To determine humidity and dry weights, previously weighed samples were dried at 60°C for 24 h to a constant mass (Harris et al. 2000).

## Statistical Analysis

All data were presented as mean  $\pm$  SE. The significance of differences in the results was evaluated with one-way ANOVA. Statistical analysis was computed

using MS Excel and STATISTICA 6.0. Mean values were considered significantly different at  $P \leq 0.05$  according to Student's criterion.

## Results

### Biochemical characteristics

The lowest protein content among the species analyzed was observed in the biomass of *D. viridis* – about 38% of the dry cell biomass (Table 2). The same culture was characterized by the highest level of lipid accumulation (37%). Both species of freshwater algae (*D. armatus* and *C. vulgaris*) contained about 50% protein and about 25% lipid. All of the algal cultures analyzed had similar levels of carbohydrates. The content of the chlorophyll a and b pigments was similar in all three algae and was typical of green algae. Unexpectedly, the biomass of the freshwater algae *D. armatus* and *C. vulgaris* was characterized by fairly high contents of carotenoids at 10–15 mg  $\times$  g<sup>-1</sup> of dry weight.

### Mortality

Enriching *Artemia* with algal cultures had a statistically significant impact on mortality in comparison with the experimental groups (Table 3). Initially, the lowest *Artemia* mortality was noted in the control group and group III (*C. vulgaris*). *Artemia* mortality increased in subsequent hours of enrichment.

**Table 2**

Biochemical characteristics of the algae used to enrich *Artemia* nauplii (mean  $\pm$  SE, n = 4).

Indicators	<i>D. viridis</i>	<i>D. armatus</i>	<i>C. vulgaris</i>
Proteins (%)	37.5 $\pm$ 1.87 <sup>a</sup>	46.8 $\pm$ 2.15 <sup>b</sup>	49 $\pm$ 0.76 <sup>b</sup>
Lipids (%)	37.1 $\pm$ 1.85 <sup>a</sup>	23.3 $\pm$ 0.74 <sup>b</sup>	21.5 $\pm$ 0.63 <sup>b</sup>
Carbohydrates (%)	19.3 $\pm$ 0.72	18.2 $\pm$ 0.69	17.5 $\pm$ 0.59
Chlorophyll a (mg $\times$ g <sup>-1</sup> )	11.5 $\pm$ 0.57	11.17 $\pm$ 0.42	11.7 $\pm$ 0.58
Chlorophyll b (mg $\times$ g <sup>-1</sup> )	8.5 $\pm$ 0.42	7.07 $\pm$ 0.29	8.6 $\pm$ 0.43
Carotenoids (mg $\times$ g <sup>-1</sup> )	7.5 $\pm$ 0.37 <sup>a</sup>	12.65 $\pm$ 0.63 <sup>b</sup>	14.7 $\pm$ 0.73 <sup>b</sup>

Mean values in the same row with the same superscript were not significantly different ( $P > 0.05$ )

**Table 3**

Mortality (in %) of *Artemia* sp. fed algae (mean  $\pm$  SE, n = 4). Group I – *Artemia* nauplii without feeding (control group); group II – *Artemia* nauplii with *D. armatus*; group III – *Artemia* nauplii with *C. vulgaris*; group IV – *Artemia* nauplii with *D. viridis*

Experimental group	Experiment hours				
	0	6	12	18	24
Group I	7.8 $\pm$ 0.15 <sup>a</sup>	17.6 $\pm$ 0.36 <sup>b</sup>	28.9 $\pm$ 0.63 <sup>c</sup>	32.7 $\pm$ 0.89 <sup>b</sup>	23.6 $\pm$ 0.75 <sup>b</sup>
Group II	6.2 $\pm$ 0.17 <sup>b</sup>	17.9 $\pm$ 0.45 <sup>b</sup>	23.6 $\pm$ 0.58 <sup>b</sup>	36.3 $\pm$ 0.81 <sup>c</sup>	33.1 $\pm$ 0.68 <sup>c</sup>
Group III	8.4 $\pm$ 0.26 <sup>a</sup>	16.1 $\pm$ 0.41 <sup>a</sup>	13.6 $\pm$ 0.37 <sup>a</sup>	15.1 $\pm$ 0.36 <sup>a</sup>	13.1 $\pm$ 0.29 <sup>a</sup>
Group IV	6.4 $\pm$ 0.15 <sup>b</sup>	17.7 $\pm$ 0.39 <sup>b</sup>	23.1 $\pm$ 0.49 <sup>b</sup>	42.1 $\pm$ 0.98 <sup>c</sup>	57.3 $\pm$ 1.34 <sup>d</sup>

Mean values in the same column with the same superscript were not significantly different ( $P > 0.05$ )

*Artemia* enriched with *C. vulgaris* had the lowest mortality from 6 h of the experiment (Table 3,  $P < 0.05$ ). However, increased *Artemia* mortality in the groups enriched with *D. armatus* or *D. viridis* was noted after 18 h of the experiment.

### Proteolytic activity

The activity of proteases, depending on their optimal pH value (acidic, neutral, alkaline), was also determined (Fig. 1). It should be noted that for all feed substrates used, the proteolytic activity in *Artemia* nauplii at the time of transition to exogenous feeding (from 9 to 12 h) remained high enough for all isoforms compared with the control group. The exception was the option using *D. viridis* that correlated with high nauplii mortality under these saturation conditions. Of the freshwater algae, using *C. vulgaris* led to a sufficiently high background value of protease activity throughout all 24 h of enrichment. In almost all the saturation variants sufficiently high proteolytic activity in the homogenate biomass of *Artemia* nauplii occurred at 12 h, when the lowest mortality of nauplii was also noted.

### Body composition

At the beginning of the experiment, *Artemia* body composition did not differ between the control group and the groups enriched with algal cultures

(Fig. 2;  $P > 0.05$ ). At this time, the protein content was about 50%, the lipid content was about 17%, and the carotenoid content was about 0.02%. Algal enrichment affected these parameters significantly and differently. In the first 12 h, the control group nauplii utilized about 30% of the protein present in their biomass and more than 50% of the available carotenoids, and this trend persisted for up to 24 h inclusive.

Saturation with *D. armatus* biomass permitted maintaining the amount of protein and lipid at values typical for nauplii at hatching. The introduction of *C. vulgaris* led to an increase in the amount of proteins in *Artemia* biomass compared to all other experimental groups (Fig. 2a). The level of proteins in the biomass of *Artemia* saturated with algae *D. viridis* was observed in the control values. However, under these conditions, the nauplii biomass was characterized by the highest lipid content of about 20%. The lipid contents in *Artemia* enriched with *D. armatus* (group II) or *C. vulgaris* (group III) were significantly statistically higher ( $P < 0.05$ ) in comparison with group I (control) from 6 h (group III) and 12 h (group II) until the conclusion of the experiment (Fig. 2b). Saturating *Artemia* with any of the algae tested permitted increasing the level of carotenoids in the crustaceans throughout the enrichment period up to 24 h inclusive (Fig. 2c). This process was the most pronounced with *D. armatus* and *C. vulgaris*. However, the introduction of *D. viridis* produced a similar effect. Obviously, this was due to the fairly high initial carotenoid levels in all three alga species.

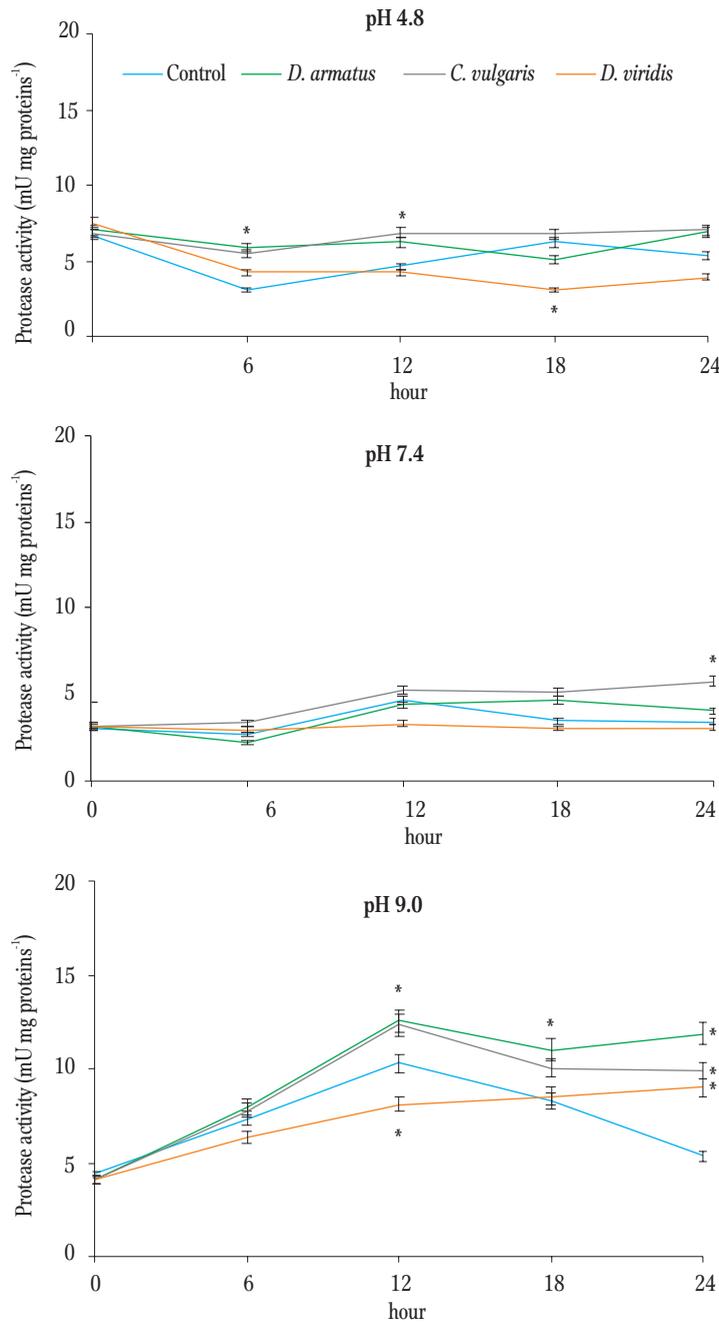


Figure 1. Proteolytic activity of *Artemia* sp. homogenate under bioencapsulation with algae biomass. \*mean values with star notation differed significantly from the control group ( $P < 0.05$ ).

## Discussion

Algae used as a feed substrate should be small, i.e., 1–15  $\mu\text{m}$  for zooplankton that filter feed and 10–100  $\mu\text{m}$  for those with mouthparts capable of sucking (Brown 2002). These algae must also grow fast

enough and contain enough nutrients. Saltwater algae such as *Dunaliella viridis* are appropriate for feeding saltwater zooplankton. Because of the peculiar structure of the cell surface, algae can change their shape, and they are easily digested and assimilated in the zooplankton alimentary tracts. *D. viridis*

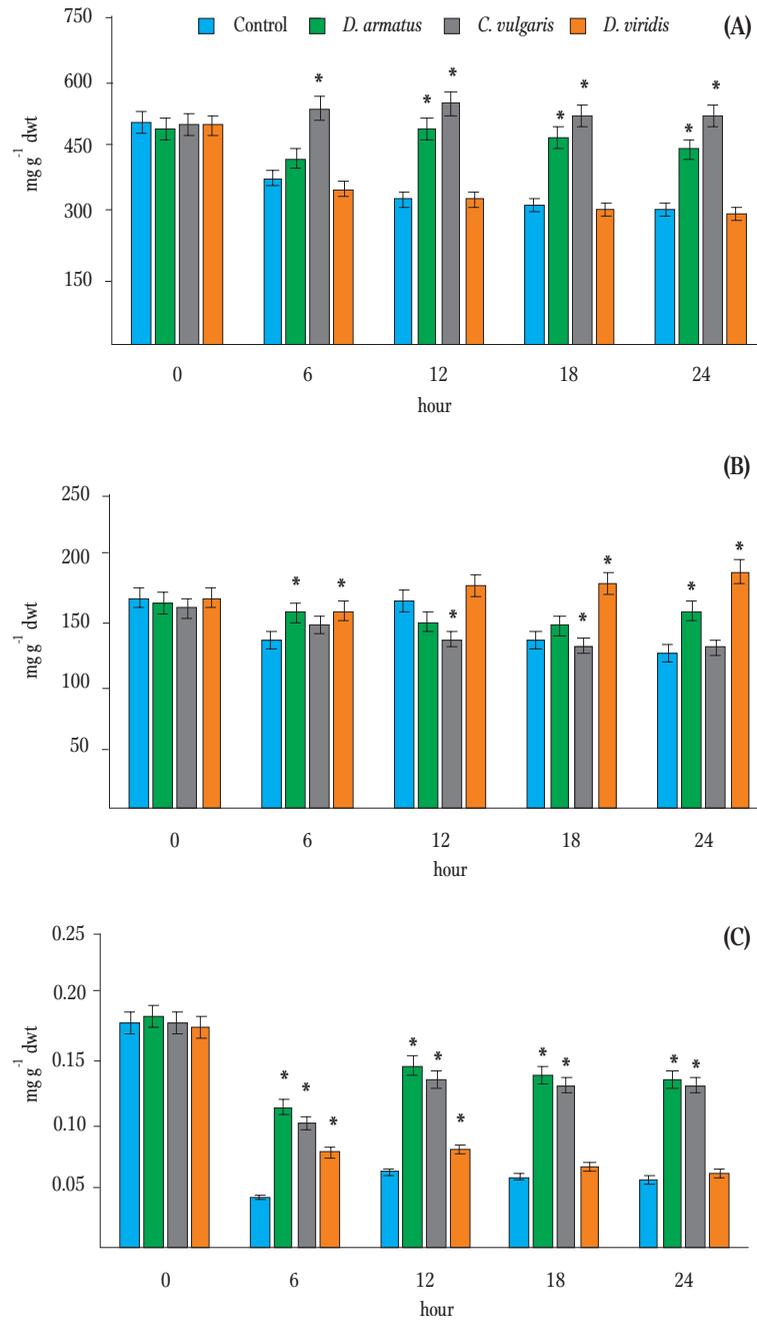


Figure 2. Content of proteins (A), lipids (B), and carotenoids (C) in *Artemia* sp. biomass following algal bioencapsulation. \*mean values with star notation differed significantly from the control group ( $P < 0.05$ ).

is a halophilous species and can survive in environments with high salinity from 0.2% to 35%. Additionally, it is able to grow at high light intensities, at elevated temperatures, and in wide ranges of pH, and it is a natural source of  $\beta$ -carotene. In high salinity, this algae is able to accumulate large amount of lipids (Ben-Amotz et al. 2009, Hosseini Tafreshi and

Shariati 2009). In comparison with this species, freshwater algae biomasses were also used to saturate *Artemia*. *Chlorella vulgaris* is a freshwater unicellular green microalga, the cells of which have a spherical shape. One of the important characteristics of *Chlorella* is the protein content, which ranges from 42 to 58% of dry biomass depending on

cultivation conditions; it is believed that the qualitative composition of these proteins is quite high. *Chlorella* is also rich in minerals and B-vitamins, especially vitamin B12 (Safi et al. 2014). *D. armatus* is also a freshwater green protocoalga. *D. armatus* is valuable forage object as it has a fairly high content of proteins, polyunsaturated fatty acids, vitamins, pigments, including carotenoids, and a balanced composition of amino acids (Nermin 2011). In terms of suitability for enriching *Artemia*, the algae selected meet the basic nutritional requirements. They have high protein and carotenoid contents and small cells.

In first few hours after hatching *Artemia* nauplii do not feed, but there is an active transport of substances through their integuments. For the first 6 to 9 h, *Artemia* feeds endogenously and utilizes nutrients from the yolk sac (Simon 2016). The transition to active exogenous feeding occurs after the first moult, when nauplii become metanauplii, which is when this crustacean begins to actively filter small food objects (algae, bacteria, protozoa). This is a rather critical stage in the *Artemia* life span. If a food substrate is not introduced in time, all of the hatched zooplankton will die. Guided by our previous results (Kushniryk et al. 2015, Cheban and Grynko 2017, Cheban et al. 2018), algae were introduced into the cultivation system immediately after the nauplii hatched.

Since the size of methanauplii usually does not exceed 50  $\mu\text{m}$ , foraging objects that do not exceed a size of 3–8  $\mu\text{m}$  are optimal for all stages of *Artemia* development (Makridis and Vadstein 1999). Between the two freshwater algae, the best results in reducing the mortality of *Artemia* nauplii throughout the experiment were achieved using *C. vulgaris* as compared to *D. armatus*. Obviously, these results depended on the cell size and physiological characteristics of the algae cultures. The size of *D. armatus* cells is from 10 to 20  $\mu\text{m}$  in length and 3.5 to 8  $\mu\text{m}$  in width, and there are no outgrowths on their cells. However, in addition to single cells, *D. armatus* is able to form conglomerates of 2–4 cells, which prevents their capture by *Artemia* (Nermin 2011).

The high mortality of *Artemia* nauplii during enrichment with *D. viridis* demands discussion. Algae of the genus *Dunaliella* are part of the natural food base of *Artemia*, and because they lack solid cell walls they are easily digested in the zooplankton intestinal tract. Nevertheless, *Dunaliella* remains virtually inaccessible for crustacean nauplii as it has large cell sizes from 5.1 to 17.0  $\mu\text{m}$  in length and from 3.0 to 15.2  $\mu\text{m}$  in width (Brown 2002). Thus, among all algae used, the saturation of *Artemia* nauplii with freshwater biomass of *C. vulgaris* reduced their mortality by almost half. This trend persisted for the 24 h during which the *Artemia* were kept in the feeders. Digestion in fish occurs mainly by hydrolytic enzymes of consumed live feed during the transition to exogenous feeding (Kolkovski 2001). Proteolytic enzymes of feed organisms are used to activate zymogens of digestive enzymes in fish larvae. The use of algal biomass to saturate *Artemia* significantly affected its enzymatic activity. In the present study it was found that the introduction of green algae *C. vulgaris* and *D. armatus* into the zooplankton diet stimulated an increase in the protease activity of all the enzyme isoforms in the food organisms. Sufficiently high indicators of protease activity when feeding *Artemia* nauplii with freshwater algae biomass permitted asserting the efficiency of zooplankton saturation with the biomasses of these algae.

One of the problems with the long-term storage of nauplii is they can lose their nutritional value. *Artemia* nauplii can contain from 50 to 65% protein, and storing *Artemia* for more than 12 h without feeding can lead to them using proteins, lipids, and carotenoids, which will significantly affect their value as feed. This should be avoided since the use of poor feed does not facilitate obtaining viable, reared fish larvae at an early stage or increasing their growth rates. Using the freshwater algae *C. vulgaris* and *D. armatus* avoided wasting valuable metabolites. Both algae contain sufficiently high contents of proteins in their own biomass, which helped to fully compensate for the crustaceans' protein requirements during their transition to exogenous feeding. The high lipid content in the original biomass of *D. viridis* explained the rather high lipid content in the *Artemia* nauplii

biomass during the 24 h of enrichment with this algae. However, not only is the amount of lipids important, but the amount and ratio of fatty acids in the feed is also significant (Brett et al. 2006).

A positive result of the scheme applied to saturate nauplii with freshwater algae was the fairly high content of carotenoids. Crustaceans cannot synthesize these in their own bodies, so they must be consumed with feed organisms capable of carotenogenesis (algae, yeast, etc.) (Guedes et al. 2011). The sufficiently high content of carotenoids in the biomass of the freshwater algae *C. vulgaris* and *D. armatus* permitted maintaining a sufficiently high content in the biomass of nauplii throughout the saturation period. These values were significantly higher than values of both the control group of *Artemia* and the group saturated with *D. viridis*. It was obvious that the *Artemia* digestive tract digested and assimilated *Chlorella* biomass easily. In fact, the size and shape of *Chlorella* cells were optimal for *Artemia* to capture. Therefore, the possibility of feeding *Artemia* sp. with freshwater algae biomass was demonstrated and confirmed by the results of determining the survival of nauplii and their proteolytic activity. The most effective scheme was to saturate *Artemia* nauplii with the biomass of the freshwater algae *C. vulgaris*. The *Artemia* sp. individuals obtained can be used as a starting live feed for commercially important fish species.

## Conclusions

1. The possibility and conditions of *Artemia* sp. saturation with freshwater algae *C. vulgaris* and *D. armatus* biomass were demonstrated by introducing 200,000 individuals of *Artemia* sp. nauplii into flasks for saturation with the simultaneous, one-time introduction of algae monoculture suspensions at rates of  $2-3 \times 10^6$  cells  $\times$  l<sup>-1</sup> and exposure for 24 h at a temperature of 24°C.
2. According to the survival rates of the nauplii and their high proteolytic activity, the *Artemia* spp. saturation scheme with freshwater algae *C. vulgaris* biomass was determined to be optimal.
3. The high content of total protein and carotenoids in *Artemia* nauplii biomass obtained as a result of saturation with freshwater algae *C. vulgaris* permitted designating them for use as starting live feed for commercially important fish species.

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