

SCIENTIFIC COMMUNICATIONS

UDC 595.34:591.13:582.26/.27

**PRODUCTION CHARACTERISTICS OF THE COPEPODS
ARCTODIAPTOMUS SALINUS AND *CALANIPEDA AQUAEDULCIS*
BEING FED WITH A MIXTURE OF MICROALGAE
DINOPHYCEAE AND PRYMNESIOPHYCEA**

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A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS, Sevastopol, Russian Federation

E-mail: la7risa@gmail.com

Received by the Editor 03.09.2019; after revision 14.02.2020;
accepted for publication 26.06.2020; published online 30.06.2020.

The ubiquitous copepod species *Arctodiaptomus salinus* (Daday, 1885) and *Calanipeda aquaedulcis* (Krichagin, 1873) are important components of food chains of numerous fresh- and saltwater areas. These copepods are suitable for feeding larvae of both marine and freshwater fish species; however, influence of nutrition on the production characteristics of these species is not well understood. Previously we determined that monocultures of microalgae Dinophyceae and Prymnesiophyceae are optimal feeding objects for egg production by females of *A. salinus* and *C. aquaedulcis*, survival rate, and development time of these copepods throughout ontogenesis. The aim of this work was to determine the production characteristics of copepods *A. salinus* and *C. aquaedulcis* under optimal temperature conditions depending on the model of the feeding with a mixture of microalgae Dinophyceae and Prymnesiophyceae. The highest survival rates of *A. salinus* from the naupliar stage to the adult one (93–95 %) were observed when copepods were fed with a monoculture of microalga *Isochrysis galbana* (Parke, 1949) or a mixture *I. galbana* + *Prorocentrum cordatum* (Ostenfeld) J. D. Dodge, 1975; the shortest development time (19 days) – when copepods were fed with a mixture of three microalgae *I. galbana* + *P. cordatum* + *Prorocentrum micans* (Ehrenberg, 1834). The shortest development time of *C. aquaedulcis* from the naupliar stage to the adult one (13 days) was observed when copepods were fed with a mixture of microalgae *I. galbana* + *P. cordatum*. The shortest duration of the naupliar stage of development of both copepod species was observed when their diet included *I. galbana* as a monoculture or one of mixture components. During the copepodit stage, the pattern remains the same, only with *P. cordatum*. The maximum absolute fecundity of *C. aquaedulcis* reached 24 eggs per female (*I. galbana*), of *A. salinus* – 16 eggs per female (*P. cordatum*). Egg hatching of *C. aquaedulcis* when being fed with both monocultures of microalgae *P. cordatum* and *I. galbana* and with their mixture reached 100 %. The highest egg hatching rate for *A. salinus* was reached only when copepod females were fed with a mixture of microalgae *I. galbana* + *P. micans*.

Keywords: copepods, *Arctodiaptomus salinus*, *Calanipeda aquaedulcis*, survival, development, reproduction, microalgae, mixture of microalgae, Dinophyceae, Prymnesiophyceae

For this experimental work, two Calanoida representatives were selected as model species: brackish water copepods *Arctodiaptomus salinus* (Daday, 1885) and *Calanipeda aquaedulcis* (Krichagin, 1873). One of the main advantages of using these species as food objects in aquaculture is the possibility of using them for feeding both marine and freshwater larvae of valuable fish species, since both copepod species can withstand a wide range of salinity (up to 50–60 ‰) [4]. Among other technological advantages

it can be distinguished that, unlike marine Calanoida (for example, those of genus *Acartia*), these species lack cannibalism (neither their own eggs nor early naupliar stages are eaten by adult copepods). As a result, naupliar, copepodit, and adult stages can be grown together.

The main criteria for microalgae nutritional value for copepods are: development time of individuals when being fed with the same microalgae species; survival rate during embryonic development (egg hatching rate); success of moults during the transition from one life stage to another; success of metamorphosis during the transition from the last naupliar stage to the first copepodit one; time of reaching sexual maturity; female fecundity (egg production rate); survival rate before feeding naupliar stage, and successful transition of nauplii to exogenous nutrition.

The results of our previous studies [1 ; 3] showed that monocultures of microalgae Dinophyceae and Prymnesiophyceae are optimal food objects for egg production by *A. salinus* and *C. aquaedulcis* females, as well as copepod survival and development rates throughout ontogenesis. We identified temperature optimal values of copepod cultivation (+20...+22 °C for *A. salinus* and +20...+26 °C for *C. aquaedulcis* [2]), at which the overall development time significantly reduces and the highest values of survival rate and fecundity of individuals are obtained.

The aim of this work was to determine production characteristics of copepods *A. salinus* and *C. aquaedulcis* under optimal temperature conditions when being fed with a mixture of microalgae Dinophyceae and Prymnesiophyceae.

MATERIAL AND METHODS

The experiments were carried out on laboratory cultures of copepods *A. salinus* and *C. aquaedulcis* at a temperature of (21 ± 1.5) °C. As food for *A. salinus*, a mixture of microalgae was used: Prymnesiophyceae (*Isochrysis galbana* Parke, 1949, of 3–6 µm) and Dinophyceae (*Prorocentrum cordatum* (Ostenfeld) J. D. Dodge, 1975, of 12–14 µm; *Prorocentrum micans* Ehrenberg, 1834, of 28–42 µm). As food for *C. aquaedulcis*, a mixture of microalgae *I. galbana* + *P. cordatum* was used. Concentration of food was maintained at the level of 0.02–0.08 mg of dry weight per ml (microalgae ratio in a mixture was equalized by the dry weight of its components). The microalgae used in the experiments were grown in a cumulative mode on the basis of sterilized Black Sea water saturated with Walne's medium [7], at a temperature of (24 ± 1.5) °C and round-the-clock illuminance with an intensity of 5000 lx. Adaptation of copepods to nutrition with a specific microalgae mixture was carried out for at least 2 to 3 weeks.

Black Sea water [(17.8 ± 0.2) ‰] was used as a culture medium (microalgae suspension in sterilized seawater) for copepods; this water was subjected to rough purification, settled, then mechanically purified by sequential filtration through cartridge filters (with pore sizes of 10, 5, and 1 µm), sterilized by ultraviolet, and pasteurized twice. For the experiments, 50-ml glass cylindrical vessels were used, which were illuminated around the clock with an intensity of 2000 lx. Complete replacement of culture medium in the experimental vessels was carried out every 2–3 days.

To determine copepod development time, survival rate, and percentage of males and females, we transplanted from laboratory cultures of *C. aquaedulcis* and *A. salinus*, being adapted to nutrition with a specific microalgae mixture, 15 hatched N1 nauplii (see the explanation below) (6 replicates for each microalgae mixture) of each copepod species into 50-ml vessels with culture medium. The experiments were carried out at a copepod density in vessels of 0.3 ind. per ml.

Copepod survival rate was assessed as the percentage of individuals that survived all stages from the first naupliar (N1) to adult one (C6). Copepod development time was identified as an average time interval for the development of individuals from N1 to reaching C6.

In experiments to determine reproductive characteristics of *A. salinus* and *C. aquaedulcis* from laboratory cultures of copepods adapted for being fed with a specific mixture of microalgae, 1 female (of each copepod species) with eggs was transplanted in 50-ml cylindrical glass vessels ($n = 25$ for each microalgae mixture). The number of eggs in the laying (absolute fecundity) and hatched viable nauplii (egg hatching rate) were calculated. All observations of copepods (every 1–3 days) were carried out intravitaly using MBS-12 microscope at a magnification of 2×8 and 4×8 .

For comparison, the data on the reproductive characteristics of *A. salinus* and *C. aquaedulcis* were used when copepods were fed with monocultures of microalgae Dinophyceae and Prymnesiophyceae. Those data were obtained in previous works [1 ; 3].

For all the data obtained, arithmetic means (M), confidence interval (95 % CI), standard deviations (SD), and reliability (p) of differences in sample means using Student's t -test were calculated.

RESULTS

The survival rate of *C. aquaedulcis* and *A. salinus* throughout moults from stage N1 to C6 varied depending on microalgae species the copepods were fed with. The survival rate of *C. aquaedulcis* was of 92.5 % when being fed with *P. cordatum*; of 83 % – with *I. galbana*; of 89 % – with a mixture *I. galbana* + *P. cordatum* (Fig. 1). The minimum survival rate of *A. salinus* throughout all the moults from stage N1 to C6 (68.6 %) was observed when being fed with *P. cordatum*; the maximum survival rate – when being fed with *I. galbana* (94.5 %) and with a mixture of microalgae *I. galbana* + *P. cordatum* (93 %) (Fig. 1).

The influence of microalgae species on development time of copepod stages was identified (Fig. 2). When being fed with microalgae *I. galbana* and *P. cordatum*, development time of *C. aquaedulcis* was of 14 days; when being fed with a mixture *I. galbana* + *P. cordatum*, it was of 13 days. The shortest development time of naupliar stage (6 days) was obtained when copepods were fed with *I. galbana* and *I. galbana* + *P. cordatum*. The shortest development time of copepodit stage of *C. aquaedulcis* (C1–5) (7 days) was obtained when being fed with *P. cordatum* and *I. galbana* + *P. cordatum*.

Development time from the first naupliar stage to the adult one of another copepod species – *A. salinus* – turned out to be significantly longer than that of *C. aquaedulcis*, when being fed with all microalgae species proposed. The development time of *A. salinus* when being fed with monocultures *I. galbana* and *P. cordatum*, as well as a mixture *I. galbana* + *P. cordatum* was of 20 days. The shortest development time (19 days) was noted when copepods were fed with a mixture of three microalgae *I. galbana* + *P. cordatum* + *P. micans*. When being fed with a mixture *I. galbana* + *P. micans* and with a monoculture *P. micans*, the development time of copepods increased to 21 and 22 days, respectively. The longest development time (25 days) was observed when copepods were fed with a mixture *P. cordatum* + *P. micans*.

The shortest time of *A. salinus* naupliar stage of development was of 7 days (when being fed with *I. galbana* and mixtures of microalgae *I. galbana* + *P. cordatum* + *P. micans* and *I. galbana* + *P. micans*), and the longest was of 10 days (with *P. cordatum* + *P. micans*). The shortest time of *A. salinus* copepodit stage of development (C1–5) was of 12 days (when being fed with *P. cordatum* and mixtures of microalgae *I. galbana* + *P. cordatum* + *P. micans* and *I. galbana* + *P. cordatum*), and the longest was of 15 days (when being fed with *P. cordatum* + *P. micans*).

The percentage of males and females of *A. salinus* and *C. aquaedulcis* when reaching the adult stage of development also varied depending on microalgae species the copepods were fed with (Table 1). For *C. aquaedulcis*, the lowest percentage of males (21 %) was obtained (reliably) when copepods were fed with *I. galbana*; the proportion increased to 43 % when copepods were fed with *P. cordatum* and a mixture

I. galbana + *P. cordatum*. For *A. salinus*, the highest percentage of males (70 %) was obtained (unreliably) when copepods were fed with a mixture *I. galbana* + *P. micans*, and the lowest (45–48 %) – when being fed with monocultures *P. micans* and *P. cordatum*.

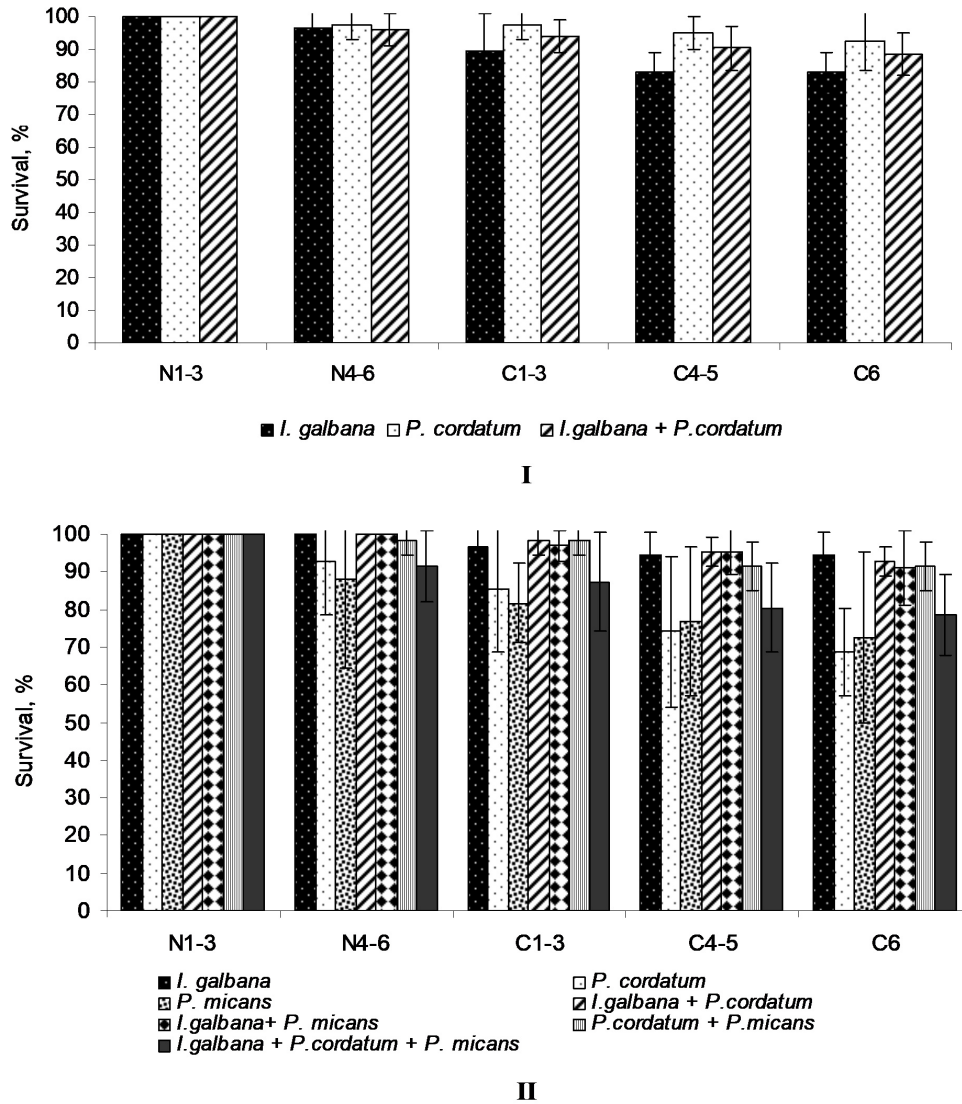


Fig. 1. Survival rate of *Calanipeda aquaedulcis* (I) and *Arctodiaptomus salinus* (II) in the experiment depending on being fed with various microalgae species (M ; 95 % CI; $n = 15$)

Table 1. Percentage of males (M) and females (F) of copepods *Calanipeda aquaedulcis* and *Arctodiaptomus salinus* when being fed with various microalgae species ($M \pm SD$; $n = 15$) (95 % CI)

Microalgae	<i>C. aquaedulcis</i>		<i>A. salinus</i>	
	M, %	F, %	M, %	F, %
<i>I. galbana</i>	20.8 ± 8.3	79.2 ± 8.3	56.1 ± 12.7	43.9 ± 12.7
<i>P. cordatum</i>	42.7 ± 6.3	57.3 ± 6.3	47.7 ± 10	52.3 ± 10
<i>P. micans</i>	–	–	45 ± 2.3	55 ± 2.3
<i>I. galbana</i> + <i>P. cordatum</i>	43.3 ± 9.3	56.7 ± 9.3	61 ± 5.9	39 ± 5.9
<i>I. galbana</i> + <i>P. micans</i>	–	–	70.1 ± 12.3	29.9 ± 12.3
<i>P. cordatum</i> + <i>P. micans</i>	–	–	59.1 ± 12.4	40.9 ± 12.4
<i>I. galbana</i> + <i>P. cordatum</i> + <i>P. micans</i>	–	–	52.8 ± 12.7	47.2 ± 12.7

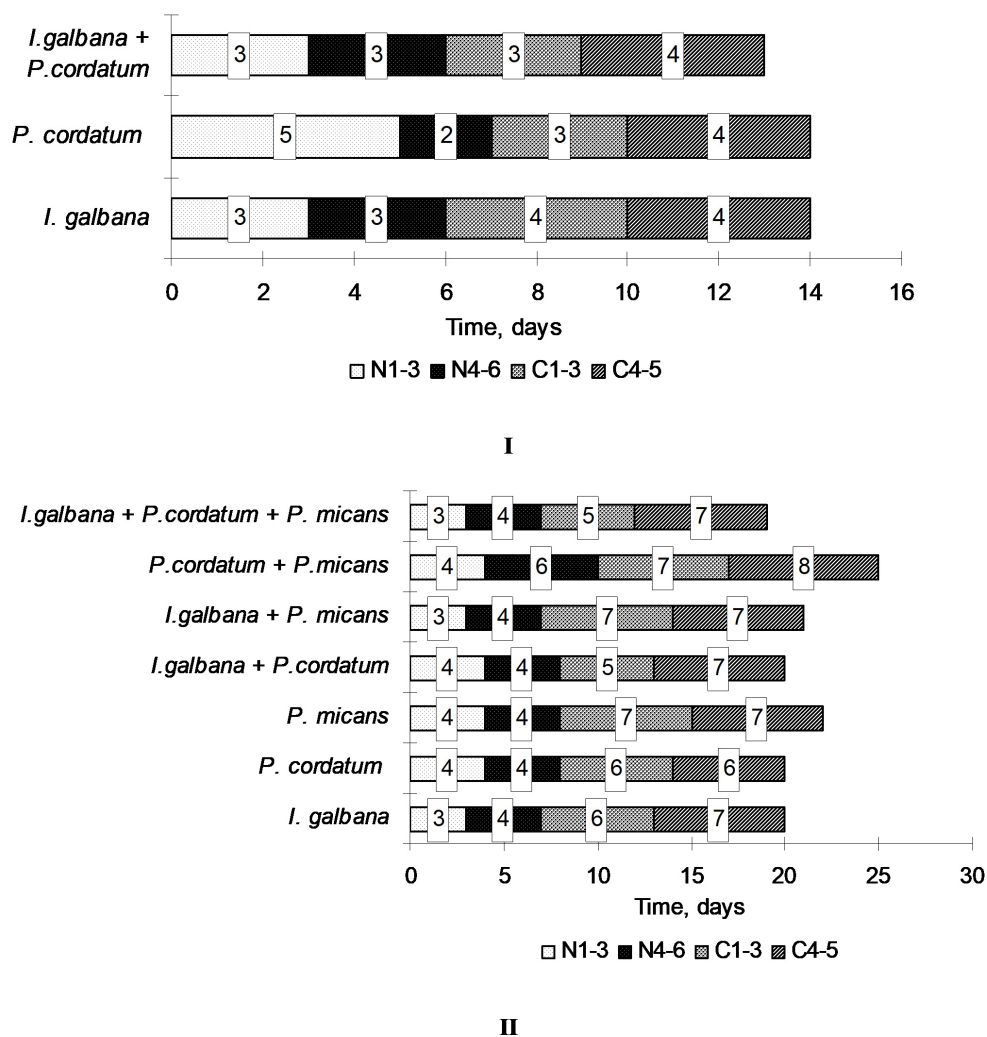


Fig. 2. Development time of *Calanipeda aquaedulcis* (I) and *Arctodiaptomus salinus* (II) in the experiment depending on being fed with various microalgae species (*M*; $n = 15$)

The average value of absolute fecundity of *C. aquaedulcis* slightly varied (19.3 ± 3.2) eggs per female (*I. galbana* + *P. cordatum*) to (24.2 ± 1.8) eggs per female (*I. galbana*). Egg hatching reached 100 % when copepods were fed with both monocultures of microalgae *P. cordatum* and *I. galbana* and with their mixture (Fig. 3).

Significant differences were identified in the effect of microalgae species on the absolute fecundity of *A. salinus*: the minimum [(8.8 ± 1.9) to (10.3 ± 1.3) eggs per female] was noted when copepods were fed with *P. cordatum* + *P. micans*; *I. galbana* + *P. cordatum* + *P. micans*; *I. galbana* + *P. micans*; *I. galbana*, and the maximum [(13.75 ± 2.3) to (16.4 ± 2.4) eggs per female] – when being fed with *P. micans*; *I. galbana* + *P. cordatum*; *P. cordatum*.

The trophic conditions (chemotaxonomic characteristics associated with a species and a class of microalgae which copepod females were fed with) had the most expressed effect on the embryonic development of *A. salinus*, the norm of which is characterized by the hatching rate from the eggs of viable nauplii. This index was reliably minimal [(62.63 ± 10) %] when *A. salinus* females were fed with *P. cordatum*. Then the value of the hatching rate of nauplii varied (unreliably) (84.9 ± 7.3) to (97.46 ± 2.7) %, reaching a maximum (100 %) when copepods were fed with *I. galbana* + *P. micans*.

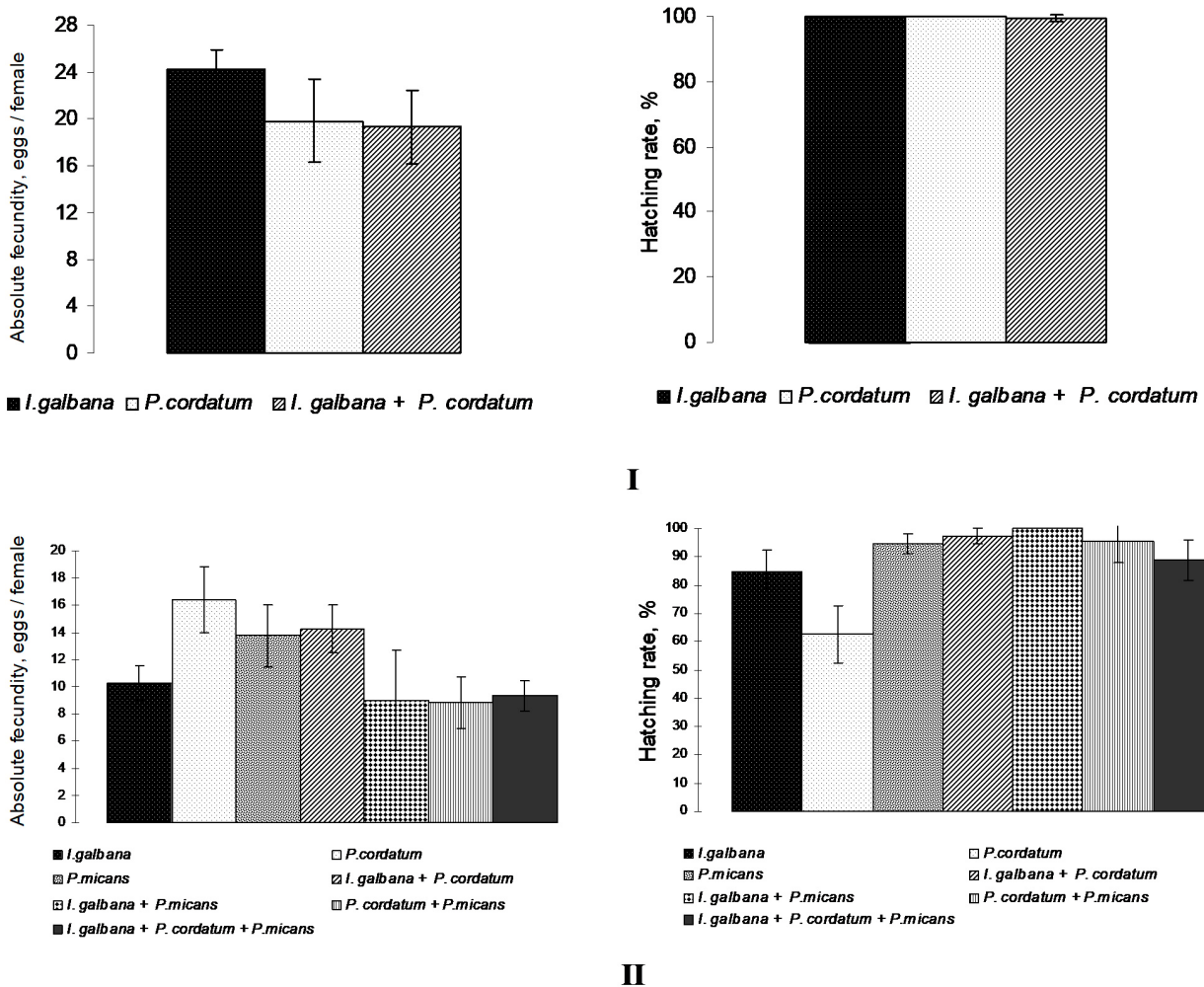


Fig. 3. Comparative diagrams of absolute fecundity and hatching rate of viable nauplii from the total number of eggs of females of copepods *Calanipeda aquaedulcis* (I) and *Arctodiaptomus salinus* (II) when being fed with various microalgae species ($M \pm SD$; $n = 15$) (95 % CI)

DISCUSSION

When comparing values of copepod survival rates and development time, a similarity of the influence of some microalgae species on indices of *C. aquaedulcis* and *A. salinus* is found. The shortest duration of the naupliar stage of development of both copepod species was observed when their diet included *I. galbana* as a monoculture or one of mixture components. During the copepodit stage, the pattern remains the same, only with *P. cordatum*.

Feeding with small-sized *I. galbana* turned out to be optimal for the development of copepod naupliar stages. Meanwhile, such nutrition delayed the development of copepodit stages compared to nutrition with large-sized *P. cordatum*. The shortened development time during the naupliar stage when copepods are fed with *I. galbana* is leveled out by the shortened development time during the copepodit stage when they are fed with *P. cordatum*. Thus, the total development time (naupliar stage plus copepodit stage) was the shortest for *C. aquaedulcis* when copepods were fed with a mixture *I. galbana* + *P. cordatum* (13 days), and for *A. salinus* – when they were fed with *I. galbana* + *P. cordatum* + *P. micans* (19 days).

It is known that in most Calanoida copepods, females are always larger than males; smaller sizes of Calanoida males are usually associated with their faster development [6]. According to hypothesis [11], under the influence of unfavorable environmental conditions, a shift in the sex ratio towards the predominance of males can be expected; when the conditions are favorable, a shift towards females can be expected. In case of *C. aquaedulcis* and *A. salinus*, males are smaller than females. Therefore, if the hypothesis is correct, then a shift in the sex ratio towards males can testify non-optimal environmental conditions: extreme temperatures and/or high salinity, or inadequate food availability. It is likely that food biochemical composition can also affect sex differentiation of developing copepods, as defined for other hydrobionts [13]. Concentration and quality of food affect numerical sex ratio of adult *Calanus* spp.: an increase in the proportion of females is observed with an increase in the concentration of food in the medium, in which copepods develop [8]. However, the data obtained on the supposed influence of microalgae chemotaxonomic characteristics on the sex ratio in copepod experimental populations require additional comprehensive studies of their biology, combined with studies of the biochemical composition of microalgae and copepods being fed with them.

The chemotaxonomic composition of microalgae, that female copepods are fed with, certainly affects their reproductive characteristics and especially viability of nauplii of both species throughout hatching. In our experiments, the maximum egg hatching rate (100 %) of nauplii was observed when female *C. aquaedulcis* were fed with monocultures *I. galbana* and *P. cordatum*, as well as a mixture of the same microalgae. However, for *A. salinus*, some differences were found in the influence of microalgae species on the survival rate of nauplii while hatching. Thus, the minimum hatching rate of *A. salinus* nauplii and their females' maximum absolute fecundity were obtained when copepods were fed with *P. cordatum*.

Microalgae Dinophyceae are characterized by a high content of highly unsaturated fatty acids with a predominance of docosahexaenoic acid (hereinafter DHA) over eicosapentaenoic one (hereinafter EPA) [12]. Prymnesiophyceae are characterized by an increased DHA content with a low EPA content [10]. Content and ratio of DHA and EPA in microalgae composition, supposedly, are among the main chemotaxonomic factors, which influence the reproductive characteristics of Calanoida copepods [5 ; 9]. Balanced EPA and DHA presence in microalgal nutrition throughout copepod ontogenesis has a positive effect on the development rate of *C. aquaedulcis* and *A. salinus*. At the same time, the nutrition of copepod females with a mixture of microalgae Dinophyceae and Prymnesiophyceae determines maximum nauplii hatching rate of both copepod species.

Conclusion. The highest survival rates of *A. salinus* from the naupliar stage of development to the adult one (93–95 %) were obtained when copepods were fed with a monoculture of microalga *I. galbana* and a mixture *I. galbana* + *P. cordatum*, and the shortest development rate (19 days) – when copepods were fed with a mixture of three microalgae *I. galbana* + *P. cordatum* + *P. micans*. The shortest development rate of *C. aquaedulcis* from the naupliar to the adult stage (13 days) was obtained when copepods were fed with a mixture of microalgae *I. galbana* + *P. cordatum*.

For *C. aquaedulcis*, the lowest percentage of males (21 %) was obtained reliably when copepods were fed with *I. galbana*; their proportion increased to 43 % when being fed with *P. cordatum* and a mixture *I. galbana* + *P. cordatum*. No significant differences in the percentage of *A. salinus* males and females when reaching the adult stage depending on microalgae species were identified.

The shortest duration of the naupliar stage of development of both copepod species was observed when their diet included *I. galbana* as a monoculture or one of mixture components. During the copepodit stage, the pattern remains the same, only with *P. cordatum*.

The maximum absolute fecundity of *C. aquaedulcis* reached 24 eggs per female (*I. galbana*); of *A. salinus* – 16 eggs per female (*P. cordatum*). Egg hatching reached 100 % when copepods were fed with both monocultures of microalgae *P. cordatum* and *I. galbana* and with their mixture. The highest egg hatching rate for *A. salinus* was reached only when females were fed with a mixture of microalgae *I. galbana* + *P. micans*.

Therefore, mixtures of microalgae *P. cordatum* + *I. galbana* (for *C. aquaedulcis*) and *I. galbana* + *P. cordatum* + *P. micans* (for *A. salinus*), due to balanced presence of eicosapentaenoic and docosahexaenoic acids, were determined as optimal food objects for copepod survival rate and development time throughout ontogenesis. Also, these microalgae mixtures determined the maximum hatching rate of viable nauplii.

This work was carried out within the framework of government research assignment of IBSS “Investigation of the mechanisms of controlling production processes in biotechnological complexes with the aim of developing the scientific foundations for the production of biologically active substances and technical products of marine genesis” (No. AAAA-A18-118021350003-6).

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**ПРОДУКЦИОННЫЕ ХАРАКТЕРИСТИКИ КОПЕПОД
ARCTODIAPTOMUS SALINUS И CALANIPEDA AQUAEDULCIS
ПРИ ПИТАНИИ СМЕСЬЮ МИКРОВОДОРОСЛЕЙ
DINOPHYCEAE И PRYMNESIOPHYCEAE**

Л. О. Аганесова

Федеральный исследовательский центр «Институт биологии южных морей имени А. О. Ковалевского РАН»,
Севастополь, Российская Федерация

E-mail: la7risa@gmail.com

Убиквитные виды копепод *Arctodiaptomus salinus* (Daday, 1885) и *Calanipeda aquaedulcis* (Krichagin, 1873) — важные компоненты пищевых цепей многочисленных пресных и солёных водоёмов. Данные копеподы пригодны для кормления личинок как морских, так и пресноводных видов рыб, однако влияние питания на продукционные характеристики этих видов копепод изучено недостаточно. Ранее нами было определено, что монокультуры микроводорослей Dinophyceae и Prymnesiophyceae являются оптимальными кормовыми объектами для продуцирования яиц самками *A. salinus* и *C. aquaedulcis*, выживаемости и скорости развития этих копепод на всём протяжении их онтогенеза. Цель данной работы заключалась в определении продукционных характеристик копепод *A. salinus* и *C. aquaedulcis* в оптимальных температурных условиях в зависимости от варианта питания смесью микроводорослей Dinophyceae и Prymnesiophyceae. Наиболее высокие значения выживаемости *A. salinus* от науплиальной до взрослой стадии развития (93–95 %) отмечены при питании копепод монокультурой микроводоросли *Isochrysis galbana* (Parke, 1949) и смесью *I. galbana* + *Prorocentrum cordatum* (Ostenfeld) J. D. Dodge, 1975; наименьшая продолжительность развития (19 суток) — при кормлении смесью из трёх микроводорослей *I. galbana* + *P. cordatum* + *Prorocentrum micans* (Ehrenberg, 1834). Наименьшая средняя продолжительность развития *C. aquaedulcis* от науплиальной до взрослой стадии развития (13 сут.) зафиксирована при питании смесью микроводорослей *I. galbana* + *P. cordatum*. Наименьшая продолжительность науплиального периода развития копепод обоих видов отмечена тогда, когда в состав их диеты входила *I. galbana* в качестве моно- или одного из компонентов смеси. Такая же закономерность сохраняется для копеподитного периода, только уже с *P. cordatum*. Максимальная абсолютная плодовитость *C. aquaedulcis* достигала 24 яиц на самку (*I. galbana*), *A. salinus* — 16 яиц на самку (*P. cordatum*). Выклев *C. aquaedulcis* при питании как монокультурами микроводорослей *P. cordatum* и *I. galbana*, так и их смесью достигал 100 %. Для *A. salinus* только питание самок смесью микроводорослей *I. galbana* + *P. micans* обуславливает максимальный процент выклева.

Ключевые слова: копеподы, *Arctodiaptomus salinus*, *Calanipeda aquaedulcis*, выживаемость, развитие, размножение, микроводоросли, смесь микроводорослей, Dinophyceae, Prymnesiophyceae