RUSSIAN FEDERAL RESEARCH INSTITUTE
OF FISHERIES AND OCEANOGRAPHY (VNIRO)

Kovatcheva N., Epelbaum A., Kalinin A., Borisov R., Lebedev R.

EARLY LIFE HISTORY STAGES OF THE RED KING CRAB

Paralithodes camtschaticus (Tilesius, 1815)

BIOLOGY AND CULTURE

VNIRO Publishing 2006

MINISTRY OF AGRICULTURE OF THE RUSSIAN FEDERATION
FEDERAL AGENCY FOR FISHERIES
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2006

УДК 595.384.2 + 639.518

Reviewed by Dr. B.G. Ivanov, V.A. Shtrik

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K11 Early life history stages of the red king crab *Paralithodes camtschaticus* (Tilesius, 1815): biology and culture. Moscow: VNIRO Publishing, 2006. 116 p.

This book summarizes the results of the project on experimental cultivation of the red king crab *Paralithodes camtschaticus* (Tilesius, 1815) under laboratory and semi-industrial conditions.

The book consists of two chapters. In chapter 1 ("Biology") the results of the studies on the red king crab early development are described and compared with previous data. This knowledge of the red king crab biology at the early life history stages served as a basis for establishing feasible culture methods and techniques described in chapter 2 ("Culture").

Culture methods and techniques established in accordance with specific requirements of each stage allowed to obtain viable red king crab juveniles from the eggs in 2 months with overall survival equaling 30-35%. Therefore, artificial reproduction and rearing made it possible to increase the effectiveness of recruitment approximately 300 times compared to natural rates. Therefore, culture methods and techniques developed and described in this book may help in developing a red king crab restocking program through aquaculture, i.e. through releasing juveniles to the areas where red king crab populations are depleted.

The book is likely to be of interest for researchers in the fields of marine biology, ecology and aquaculture, as well as for biology students.

ISBN 5-85382-218-7

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INTRODUCTION

The red king crab *Paralithodes camtschaticus* (Tilesius, 1815) (Decapoda, Anomura, Lithodidae) is one of the most commercially used species of marine crustaceans. It is widely distributed in the North Pacific Ocean. Since the 1960s it has also become an important component of the biota of the Barents Sea where it was transplanted intentionally. Red king crab stocks are intensively exploited. Management strategies for this valuable marine resource include monitoring of natural populations, establishment of benchmarks for harvesting, setting of minimum legal crab size limits, adoption of strict requirements on crab fishing gear and conservation of certain areas to allow stock rebuilding, as well as other regulations and measures.

However, fishery statistics has shown that existing measures are not sufficient to maintain stable stock abundance and structure of red king crab populations. In recent years, sharp decline of red king crab stocks in the North Pacific Ocean has been observed, especially in the seas of the Russian Far East [Koblikov et al., 2002; Doljenkov and Koblikov, 2004]. Red king crab stock abundance in most of the areas of the Sakhalin and Kuril Islands has fallen to such levels that reproductive output is insufficient to produce large year classes. For example, due to overfishing and poaching, in the years of 1995-2000 the abundance of western Sakhalin population has declined from 2703 to 573 thousand individuals [Klitin, 2003]. Preventive measures, such as limitations and even complete prohibition of fishing in certain areas for long periods of time (e.g. in the southern Kuril Islands and Peter the Great Bay), did not result in considerable stock rebuilding.

In the Bristol Bay, the red king crab stock once supported one of the most successful fisheries in the world, but a sharp

decline of the stock over the last two decades has prompted fishery closures and a host of restrictive regulatory measures. However, these actions did not have sufficient effect on stock rebuilding either [Loher and Armstrong, 2001].

In the Barents Sea, the red king crab has formed sustainable, self reproducing population. It has now passed the first two stages of the acclimatization process [following the terminology of Karpevich, 1998] and reached the third stage – abundance explosion. However, in some areas of the Barents Sea the crabs are reported to be in adverse physiological state, having low meat content which could be a sign of a conflict of the transplanted species with native biota [Red king crab in the Barents Sea..., 2001]. Besides, during the surveys conducted in 1996 low abundance of juveniles was recorded, indicating possible future decrease in recruitment rates and stock replenishment [Hufthammer et al., 1997].

Thus, many commercially exploited populations of the red king crab are now depleted or are under the threat of depletion in the future. Under present conditions, it becomes important not only to reinforce existing management strategies, but also to consider and implement other approaches of resource management, such as stock enhancement by aquaculture.

Within the complex life cycle of the red king crab, as in other marine invertebrates, the early life history stages represent a physiological bottleneck, as they are generally more vulnerable to thermal, nutritional and other types of stress than conspecific adult forms [Anger, 2001]. In nature, less than 1% of red king crab larvae manage to survive up to the postlarval stage [Marukawa, 1933]. At the same time, culture techniques, based on the creation of easily controlled and regulated rearing conditions in accordance with specific requirements of each developmental stage, make it possible to increase overall survival and growth rates by dozens of times [Kovatcheva, 2002a,b]. Thus, artificial

reproduction and subsequent cultivation followed by the release of early juveniles to the ocean are considered as one of the possible ways of the red king crab stock enhancement. Laboratory cultivation is also a useful method to improve our knowledge of the early development of this species, which in turn makes it possible to help with establishing resource management strategies.

Research groups in several countries – Japan, the United States, Norway, and Russia – have been working on the establishment and improvement of existing culture methods for the red king crab. Experiments on laboratory cultivation of this species, aimed at clarifying various aspects of its early life history, have been carried out by Shimizu [1936], Sato and Tanaka [1949], Kurata [1959, 1960, 1964], Nakanishi et al. [1974, 1981, 1987], Mortensen and Damsgard [1996], Kovatcheva [2000], Zheltonozhko et al. [2000] and Kittaka et al. [2002]. Valuable information has been published on functional morphology and feeding habits of red king crab larvae [Paul et al., 1989; Abrunhosa and Kittaka, 1997a,b]. However, many aspects of the red king crab larval and juvenile biology remained largely unexplored. There also were a number of technical and methodological constraints in developing the technology of mass culture of the red king crab, as this coldwater species has strict environmental requirements, especially during the early life history phases. Therefore, extensive experimental work was required in order to establish the technology of the red king crab artificial reproduction and culture.

About this Book

This book summarizes the results of a project on experimental cultivation of the red king crab under laboratory and semi-industrial conditions. This project was carried out at the Laboratory of Crustacean Reproduction of the Russian Federal

Research Institute of Fisheries and Oceanography (VNIRO, Moscow) in 2000-2005.

Research staff of the Laboratory of Crustacean Reproduction. Front row (left to right): Dr. A. Kalinin; Dr. N. Kovatcheva, head of laboratory; Dr. A. Epelbaum. Back row (left to right): R. Lebedev; A. Parshin-Chudin; Dr. R. Borisov.

Laboratory cultivation experiments made it possible to conduct detailed investigations on the red king crab early development and establish feasible culture methods in accordance with specific requirements of each early life history stage.

This book consists of two main chapters. In chapter 1 ("Biology") we describe the results of our studies and experiments aimed at determining morphological and behavioral patterns, nutritional requirements and other biological features of red king crab early life history stages, and compare our findings with the data known from the literature. In this chapter we also provide

the data on developmental rates and growth of larvae and juveniles under laboratory conditions. The knowledge of the red king crab biology during early life history phases served as a basis for establishing the basic culture methods and techniques described in chapter 2 ("Culture").

The work was conducted within the framework of VNIRO Program "Elaboration of Normative and Methodical Bases for Artificial Reproduction of the Red King Crab in order to Restore its Natural Populations", which was an integral part of the Federal Program "Scientific and Technical Support for Fisheries Science in Russia" sponsored by Federal Agency for Fisheries of the Ministry of Agriculture of the Russian Federation.

Chapter 1. BIOLOGY

1.1 THE RED KING CRAB: BRIEF OVERVIEW OF THE SPECIES BIOLOGY

The red king crab *Paralithodes camtschaticus* (Tilesius, 1815) (Fig. 1) belongs to the order Decapoda, infraorder Anomura, family Lithodidae. It is one of the most commercially important species of crustaceans.

Figure 1. Red king crab, general view.

This boreal species has a broad distribution range in the North Pacific Ocean. In Asian waters, this species occurs from the Sea of Japan northward into the Sea of Okhotsk and along the shores of the Kamchatka Peninsula. In the northeast Pacific, distribution extends northward from the Queen Charlotte Islands, British Columbia, to Norton Sound in the Bering Sea. During the period of 1961-1969, as a result of USSR transplantation experiments, red king crabs were intentionally introduced into the North Atlantic [Orlov, 1962, 1994]. These crabs have naturalized and formed a viable, self-reproducing

population, which is now widely distributed along the Atlantic coasts of Russia and Norway, from the Gusinaya (Geese) Bank to the Lofoten Islands [Red king crab in the Barents Sea..., 2001].

The red king crab mainly inhabits continental shelf and upper slope areas, such as rocky biotopes rich in epibenthic fauna, sand and muddy sand bottoms [Vinogradov, 1941; Levin, 2001]. Its vertical distribution ranges from the shoreline and down to the depth of 400 meters, depending on size, age, and time of the year. Adult crabs undertake seasonal nearshore-offshore migrations. In spring and summer they exhibit a mating/molting migration to inshore, shallow waters (5-60 m) and commonly aggregate in large groups. In autumn the crabs undertake a feeding migration offshore, to deep waters (usually 120-300 m), where they spend the winter. Adult male crabs reportedly travel up to 170 kilometers round-trip and can cover more than 1.5 kilometers per day.

The crabs prefer salinity ranging from 32 to 35‰ and a habitat temperature range between +2 and +7°C. However, during seasonal and ontogenetic migrations they can be found at a wider temperature range, from -2 to +18°C [Pavlov, 2003].

The crabs feed on a wide assortment of marine life: benthic invertebrates (mainly mollusks, crustaceans, and polychaete worms), fish and algae [Fenyuk, 1945; Kulichkova, 1955; Tarverdieva, 1976]. During feeding migrations, the crabs move from one biocenosis to another, where they predate on dominant species. Their diets vary with their individual size and moulting cycle stage: for example, during the premoult period the crabs mainly consume food objects with high calcium content, such as mollusks and echinoderms [Logvinovich, 1945].

Like most Arthropod species, the red king crab must moult, or shed its old exoskeleton, in order to grow. The linear size of the crab remains constant during the intermoult period, and increases at moult. Growing juveniles and young crabs moult

several times a year in their first few years of life, then less frequently until they reach sexual maturity; adult crabs moult once a year on average. Females have to moult prior to mating while males do not. The crabs do not feed several days after the moulting occurs.

Several papers have been published on certain aspects of red king crab life history [e.g., Sato and Tanaka, 1949; Kurata, 1964; Makarov, 1966]. Therefore, here we will only briefly describe main phases of the red king crab life cycle (Fig. 2).

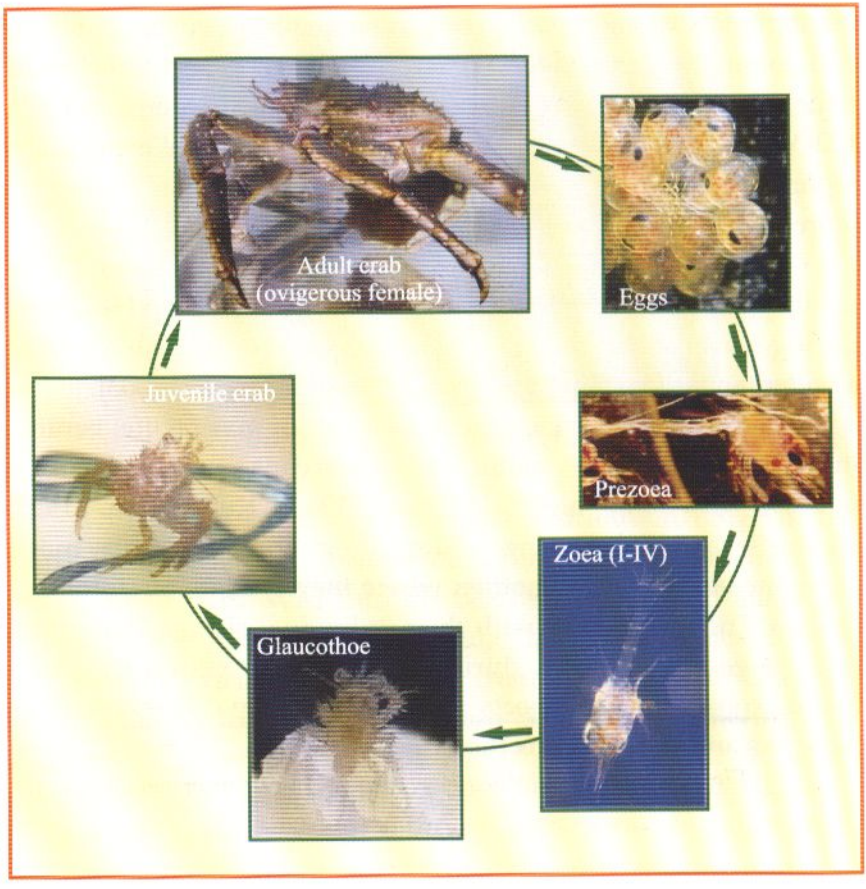


Figure 2. Phases of the red king crab life cycle.

The red king crab reproduces annually. Mating normally occurs in April or May, after the crabs migrate to shallow, inshore waters. The mating activity of the red king crab includes the grasping of females by males prior to and during copulation (Fig. 3). A male crab uses its chelipeds to grasp the upper meropodites of a female's chelipeds facing her, holds her until she molts, and then regrasps her until mating is completed [Marukawa, 1933]. After the female molts, the male passes spermatophores from his fifth pereiopods to the gonopores and coxae on the female's third pair of pereiopods. Several hours later the female extrudes the eggs, which, after being fertilized, attach to the female's pleopodal setae by the extension and twisting of the egg envelope. The eggs are physiologically independent from the female's body: the embryos utilize yolk reserves of the egg.

Figure 3. Precopulatory grasping of crabs (observed under laboratory conditions).

The female crabs brood eggs underneath their abdominal flap for about eleven months. A clutch of eggs is attached to the pleopods and is carried underneath the abdominal flap under its protection (Fig. 4).

Figure 4. Eggs attached to the female's pleopods (abdominal flap is unfolded).

A mature female crab may deposit from 25 000 to 500 000 eggs, depending upon its body size, physiological condition and geographical location. The individual absolute fecundity of a large female with carapace width of more than 16 cm ranges from 200 000 to 500 000 eggs. In the Barents Sea, fecundity of the females averages from 230 000 to 280 000 eggs [Red king crab in the Barents Sea..., 2001].

Embryonic development of the red king crab has been described by Marukawa [1933] and Nakanishi [1987]. Cell division was first observed on the 4th day after fertilization; the gastrula stage was formed in 20-25 days; in 50-52 days the nauplius stage of the embryos was observed; in 100-110 days the embryos reached the stage of a metanauplius; in approximately 200 days after fertilization they reached the zoeal stage: pigment

began to form in their compound eyes and all major embryonic parts were already present (Fig. 5). From day 211 onward, the increase in the embryo size corresponded to the decrease in the amount of yolk, but no major morphological changes occurred [Nakanishi, 1987].

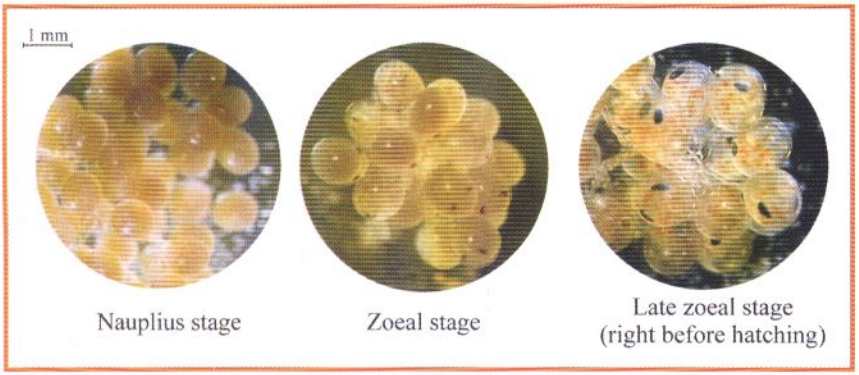


Figure 5. Main stages of the red king crab embryonic development.

The duration of the red king crab embryonic development depends on water temperature. In western Kamchatka, larval hatching is observed mainly between late April and early June [Makarov, 1966]. In southeastern Bering Sea, hatching takes place from the middle of April to the end of May [Shirley and Shirley, 1989]. In the Barents Sea, mass hatching occurs from the middle of March to early April [Bakanev and Kuzmin, 1999; Red king crab in the Barents Sea..., 2001; Matyushkin and Ushakova, 2002].

The larvae hatch out as prezoeae. The prezoea has a thorax covered by smooth carapace without rostral spine, compound eyes and long abdomen. Appendages of prezoea have the same morphological characteristics as those of the first stage zoea, but are covered by a thin envelope – an embryonic cuticle. The prezoea stage normally lasts for less than an hour [Kovatcheva, 2002; Borisov and Kovatcheva, 2003].

After moulting, the prezoea transforms into the first stage zoea. Red king crab larvae pass through 4 zoeal stages, commonly referred to as zoeae I, II, III and IV. The zoea has a thorax covered by smooth carapace with rostral spine and 2 posterior spines, compound eyes and long abdomen. Zoeae are merely planktonic and exhibit vertical annual migrations in the water column [Shirley and Shirley, 1988]. The duration of zoeal phase in the field ranges from 60 to 80 days [Takeuchi, 1962; Shirley ans Shirley, 1989; Bakanev and Kuzmin, 1999; Klitin, 2002, 2003].

The postlarval stage of the red king crab is called glaucothoe. The glaucothoe has spiny carapace which is more prominent than the one of juvenile and adult crabs; the abdomen remains elongated, as during the larval phase, and is not held beneath the carapace. Glaucothoes swim by means of pleopods and actively select the substrate suitable for settling [Stevens and Kittaka, 1998]. In approximately 20 to 30 days glaucothoes metamorphose into the first stage juveniles. Young-of-the-year juveniles are commonly found among sponge and bryozoan colonies [Sundberg and Clausen, 1977], hydroid colonies, stalked ascidians, and polychaete tubes [Stevens and MacIntosh, 1991], as well as among shell debris and cobble [McMurray et al., 1986]. These habitats are important until 1.5-2 years of age, when the crabs start to exhibit aggregative behavior. Young crabs are known to aggregate into protective "pods" that may total thousands of individuals. At 3-4 years of age, they generally move to deeper water and join adults in seasonal migrations to mating/molting and feeding grounds.

Female crabs generally reach sexual maturity at the age of 6-7 years, when their carapace width reaches 8-9 cm. Male crabs become mature in 7-10 years at 12.5-13 cm carapace width. Red king crabs are estimated to have a maximum lifespan of 20–30 years [Vinogradov, 1941].

1.2 EARLY LIFE HISTORY

In this section, we will describe our observations on morphology and behavior of the early life history stages of the red king crab, and the results of experiments aimed at clarifying their requirements and preferences.

1.2.1 LARVAL PHASE (PREZOEA, ZOEAE I-IV)

1.2.1.1 GENERAL MORPHOLOGY AND BEHAVIOR

Newly hatched prezoea (Fig. 6*A*) has typical zoeal appearance, but its body is covered with a thin envelope - an embryonic cuticle. The cuticle forms soft plumose processes with thin walls on the antennules, antennae, and telson (Fig. 6*B,F*). Appendages of the prezoea have the same morphological characteristics as those of zoea I, but are covered by an embryonic cuticle, and most setae are folded (unextruded) (Fig. 6*C,D,E*).

The prezoea swims up to the surface from time to time, and then slowly sinks back to the bottom. It does not have swimming setae on the exopods of maxillipeds and swims in zigzag movements by flexing and extending the abdomen. The telson, with its cuticular processes, acts as a primary blade, or paddle. When the prezoea stops abdominal movements, cuticle processes on antennules and antennas open like a fan and slacken the pace of sinking. Prezoeae are positively phototactic.

The main morphological feature found in prezoea, an embryonic cuticle covering prezoea's body, has also been described as a feature of prezoeae of many other decapod species [e.g. Gonor and Gonor, 1973; Konishi, 1987; Hong, 1988; Guerao and Abello, 1996]. This cuticle which forms long plumose projections in the antennal and telsonal areas is well modified for swimming. We assume that it would be impractical for the larva to have rigid fully developed setae when it is rolled up inside the egg, so it hatches out with its setae still folded. The presence of

embryonic cuticle projections allows it to move relatively quickly, and the larvae can thus disperse immediately after hatching. Another argument was offered by Gonor and Gonor [1973] in support of the interpretation of porcellanid crabs prezoea as a natural stage, not a laboratory artifact: a short-lived, rounded larva with few body projections would more effectively escape entangling detrital material on the bottom immediately after hatching.

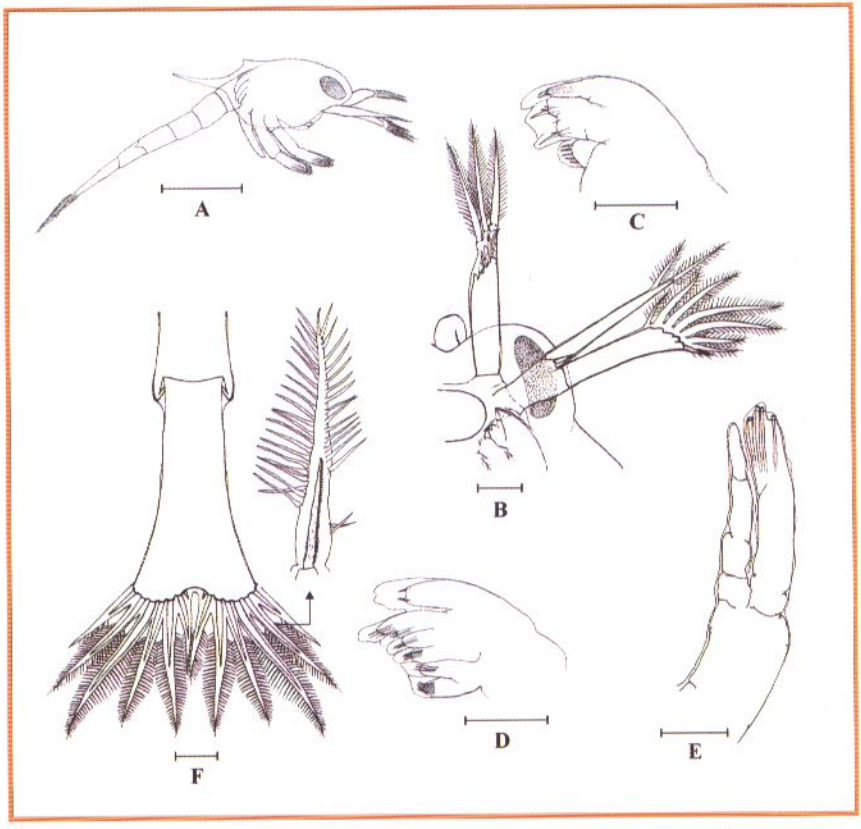


Figure 6. Prezoea: (A) – general view; (B) – antennule and antenna; (C) - maxillule; (D) – maxilla; (E) – second maxilliped; (F) – telson. Scale bars: (A) – 1 mm, (B)-(F) – 0.2 mm.

The experiments run at 7-8°C revealed that the prezoea stage normally lasts for less than an hour. The moulting occurs quickly, with the larva emerging from the prezoeal cuticle and the setae on its appendages turning out in several minutes.

After the prezoea sheds an embryonic cuticle, it transforms to zoea I (Fig. 7).

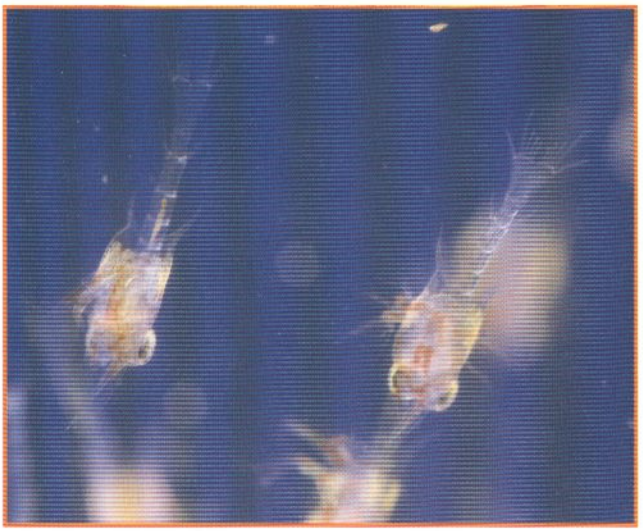


Figure 7. Zoeae I in the water column.

Red king crab larvae subsequently pass through 4 zoeal stages (hereinafter referred to as zoeae I-IV). Morphological distinctive features of the larvae of anomuran crabs have been described by Sato and Tanaka [1949] and Makarov [1966]. Based on those descriptions, Levin [2001] marked out the criteria for distinguishing red king crab zoeal instars. Here we provide a modified and more complete scheme for distinguishing red king crab zoeal stages (Table 1). In this scheme, not only the presence of pleopods and uropods, but also abdominal morphology (Fig. 8) and the number of setae on the exopodites of the third

pair of maxillipeds (Fig. 9) are considered as criteria. During our experimental work, the number of setae on the exopodites of the third pair of maxillipeds was found to be the best criterion for distinguishing zoea I from zoea II.

Table 1. Criteria for distinguishing red king crab zoeal stages.

Zoeal stage	Number of setae on the exopodite of maxilliped III	Pleopods	Uropods	Number of abdominal somites
I	0	absent	absent	5+telson
II	6	absent	absent	5+telson
III	8	absent	present	6+telson
IV	8	present	present	6+telson

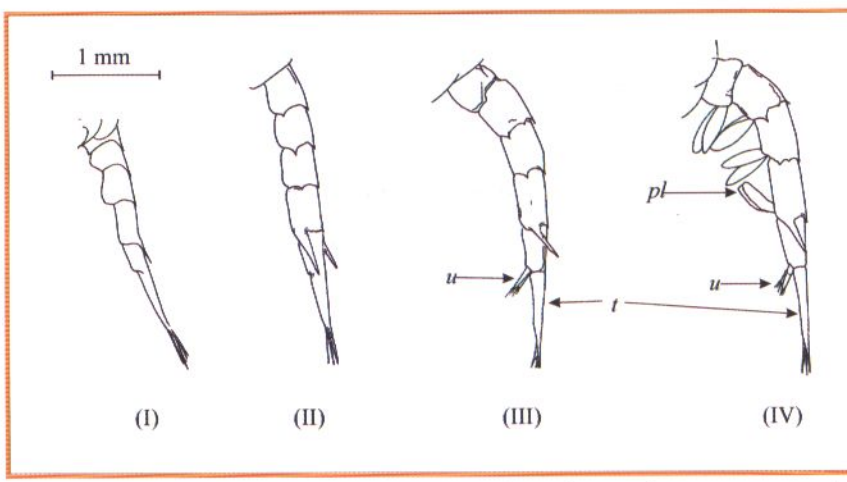


Figure 8. Abdomens of zoeae I-IV: pl – pleopod; t – telson; u – uropod.

The detailed description of the larval morphology is given in our recent paper [Epelbaum et al., in press]. Gross morphology of the body parts and appendages remains essentially the same throughout the whole larval period. With each molting, larval appendages become proportionally bigger, and some of them acquire additional setae and/or segments and parts.

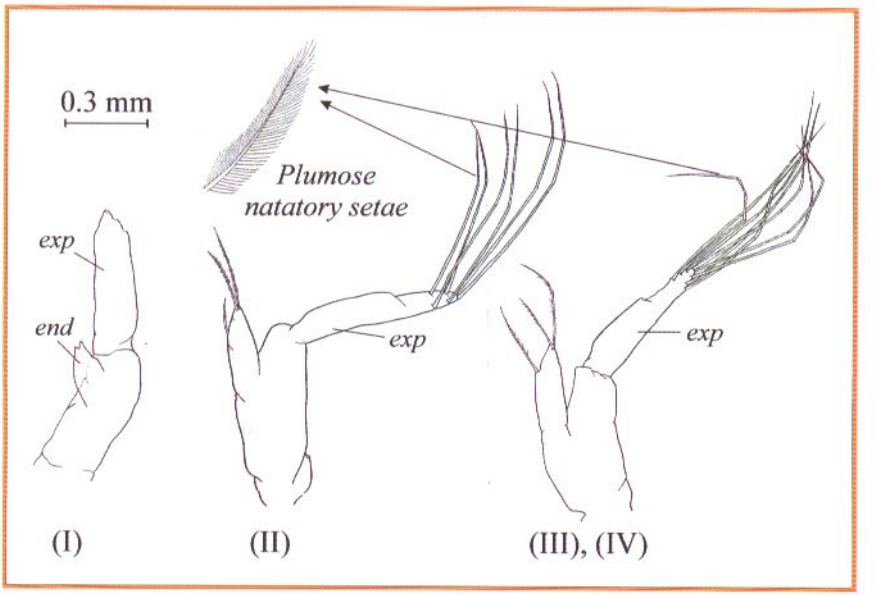
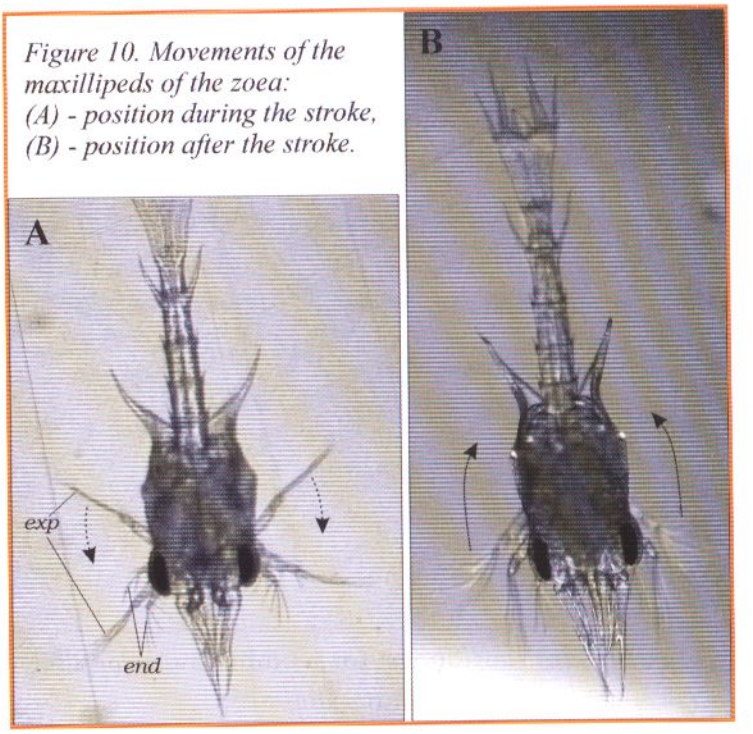


Figure 9. Third maxilliped of zoeae I-IV: end – endopodite, exp – exopodite.

Zoeae swim primarily by beating the maxillipedal exopodites that bear long natatory setae; these setae form somewhat concave blades. Rapid beating of exopodites downward and anteriorly (Fig. 10) creates a current directed toward the rostrum; this current sends the larva backward with its posterior carapace spines and telson leading. Therefore, by beating the exopodites the larva predominantly swims vertically up to the surface; with the exopodites motionless the larva descends in the water column (Fig. 11). Additionally, the larva can adjust the direction of swimming by changing the position of the maxillipeds and telson. Maxillipedal endopodites are held extended anteriorly (see Fig. 10*A*) and do not function in swimming.

Figure 10. Movements of the maxillipeds of the zoea: (A) - position during the stroke, (B) - position after the stroke.



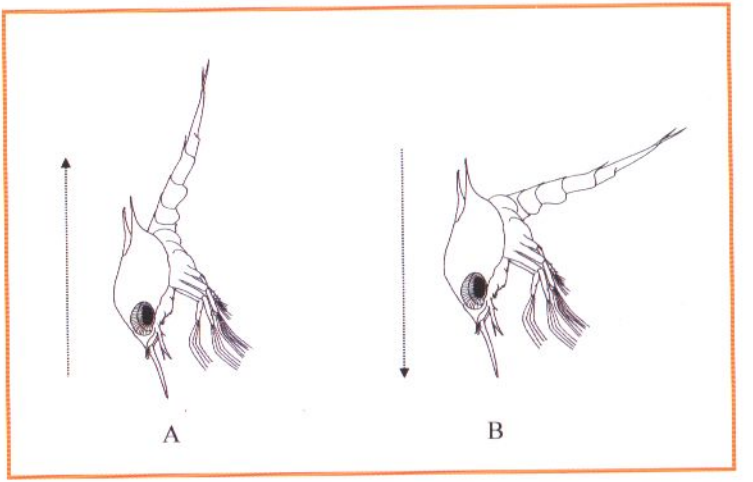


Figure 11. Swimming modes of the larva: (A) – zoea ascends by beating maxillipedal exopodites, (B) – zoea descends with exopodites motionless.

The larvae are positively phototactic [Shirley and Shirley, 1988]. Behavioral patterns remain essentially the same throughout the whole larval phase (zoeae I-IV).

1.2.1.2 FEEDING

It is generally accepted that there is extensive mortality in the early stages of organisms which produce large number of planktonic larvae endowed with little stored energy. Thus, for cultivation of the red king crab it is important to study various aspects of feeding of larvae, as food availability and quality are main limiting factors in larval survival [Paul and Paul, 1980; Paul et al., 1989].

Feeding Mode

As long as the setae of the prezoea are confined beneath the cuticle, there is no feeding during prezoeal stage. In zoeae I-IV, the appendages are well suited for feeding and bear specialized setae. The arrangement and general morphology of the appendages that are involved in feeding are shown in Fig. 12.

When the larva swims actively by beating maxillipedal exopodites, the current generated by the exopodites induces water circulation near the sides and ventral surface of the larva. Oral part of this circulation can be considered as a feeding current, directed anteriorly toward the labrum, parallel to the longitudinal axis of the body. Small food objects that pass near thoracic or abdominal region of the zoea are taken by the flow and delivered to the maxillipedal endopodites area. When the larva descends in the water column with its maxillipedal exopodites motionless, it passively approaches food items.

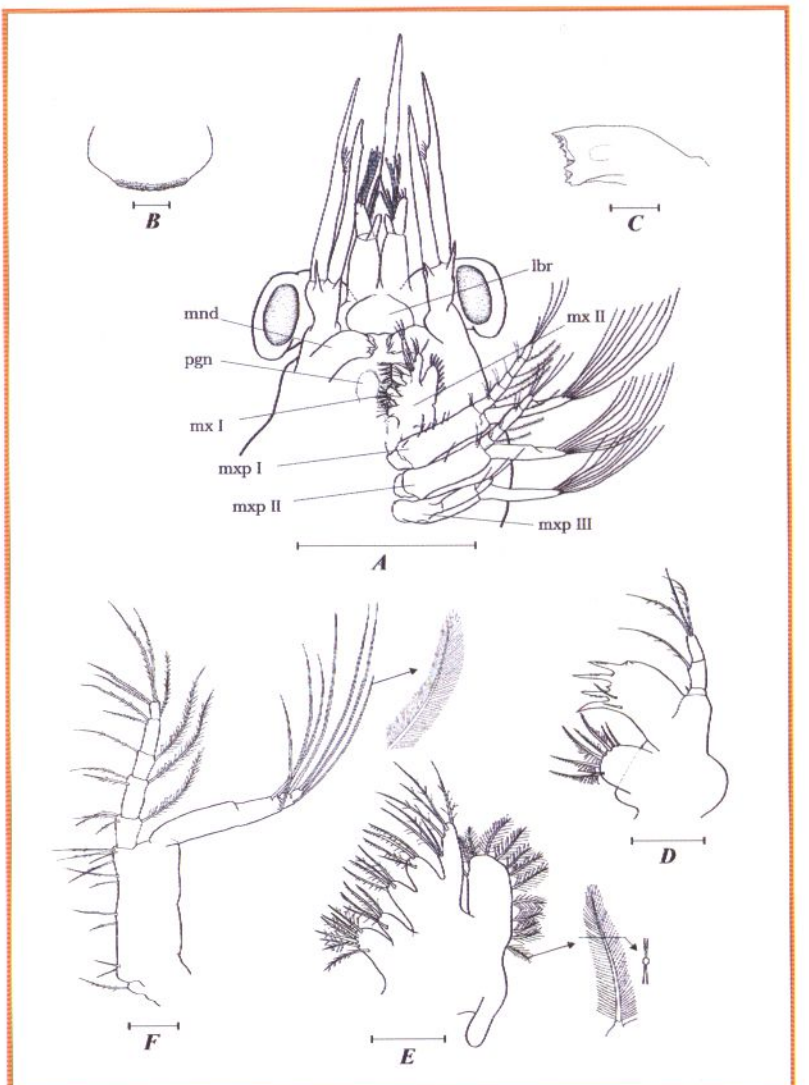


Figure 12. Fourth stage zoea: (A) – diagram of the ventral view showing appendages arrangement, (B) – labrum, (C) – mandibles, (D) – maxillule, (E) – maxilla, (F) – first maxilliped. Scale bars: A – 1 mm, B-E – 0.2 mm. Abbreviations: lbr=labrum, mnd=mandible, mx I=maxillule, mx II=maxilla, mxp I-III=maxillipeds I-III, pgn=paragnaths.

Therefore, we distinguish "active" and "passive" modes of capturing prey. The "active" mode is likely to be more effective while capturing small food objects that can not withstand the feeding current. While capturing large food items, comparable to the size of the larvae itself, and objects from the bottom, the "passive" mode must be more advantageous. Therefore, according to our observations under laboratory conditions, red king crab larvae show mixed feeding strategy: they are able to feed by capturing material suspended in the water column and by collecting food objects from the substratum (Fig. 13).

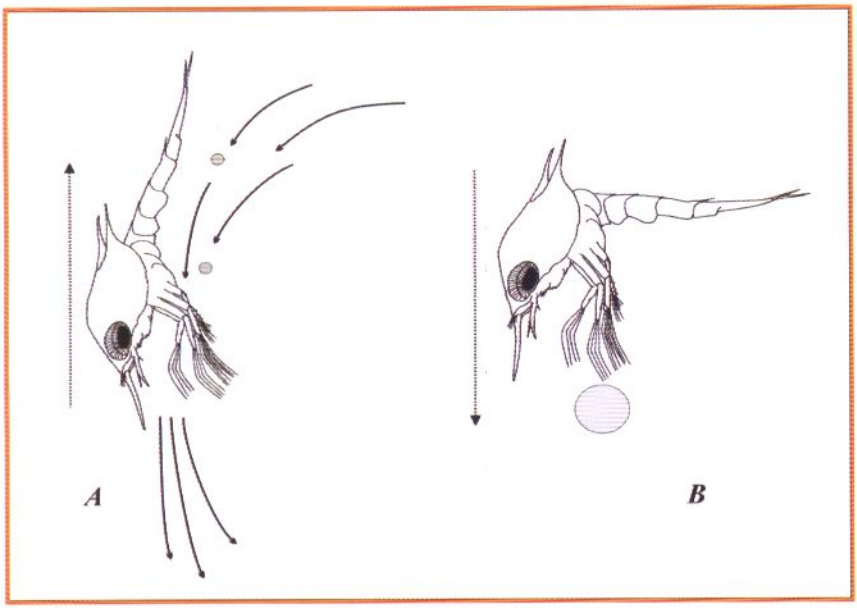


Figure 13. Two modes of prey capture: (A) – "active" (solid lines show the feeding current), (B) – "passive".

In both cases, a food item is captured by maxillipedal endopodites. The telson is likely to be used to help maxillipedal endopodites to scoop up the prey, quickly pushing it towards

oral appendages. The endopodites' setae are used to capture and pass the prey to maxillae and maxillules. A similar role of maxillipeds in capturing prey was described for the larval crabs of the family Porcellanidae [Gonor and Gonor, 1973]. However, this fact contradicts the data of Crain [1999] on the zoeae of the lithodid crab *Placetron wosnessenskii*, in which the prey manipulation and ingestion were unaffected by maxillipeds and telson, and maxillipeds were used for locomotion only. The setae of maxillae and maxillules help to pass the prey to the oral region; maxillules are also used to tear large food particles together with the mandibles. Paragnaths probably prevent the loss of food during the mandibular activity, and the labrum helps to swallow food along with the oesophageal peristalsis.

True hunting behavior reported for zoeae of some other decapod species [e.g. Knudsen, 1960] was never observed in our experiments. When food items were added to rearing reservoirs, zoeal locomotory behavior did not change significantly. When a potential food object suspended in the water column approached the thoracic region of the larva close to maxillipedal exopodites, the larva immediately captured the food object by maxillipedal endopodites and dragged its telson posteriorly across the mouthparts. However, when the food object touched the zoea's rostrum or any portion of the carapace first, the larva moved away from the object apparently showing avoidance behavior. Therefore, the act of food capture must be initiated by direct contact with the setae on maxillipedal endopodites and/or with the abdomen. These observations indicate that, in spite of the fact that zoeae are good swimmers and have well developed eyes, they do not use sight in locating and capturing the prey. Like the larvae of some porcellanid crabs [Gonor and Gonor, 1973], red king crab zoeae do not show true hunting behavior and appear to rely entirely on chance encounters with prey. Berkes [1975] termed this feeding mechanism as "encounter feeding".

Desirable Food Characteristics and Possible Feeds

McConaugha [1985] identified 3 criteria for suitability of prey items for food for larval crustaceans: (1) the appropriate size for capture and consumption, (2) the adequate concentration, and (3) the nutritional value essential to meet the larva's needs. Below we will consider the first two criteria in relation to red king crab zoeae.

Our experiments indicate that red king crab zoeae are able to capture and ingest a relatively wide range of particle sizes. The maximum size of a zoea's food objects must be comparable to the size of zoea itself and comprise about 2 mm, taking into account frequently observed cannibalistic behavior. The minimum size is likely to be limited by the distance between the setae on the maxillipedal endopodites and comprises approximately 100-150μ. This assumption was supported by the results of the experiments aimed at evaluating the consumption of agglomerated feeds on growth and survival of red king crab larvae under laboratory conditions [for details see: Epelbaum et al., 2005]. It was found that larvae are able to capture and consume artificial feeds Start 100 and Start 300 (Dana Feed A/S, Denmark), with particle sizes of 90-200 μm and 150-400 μm, respectively.

As long as the larvae do not use sight in locating and capturing food and rely on a chance to encounter food objects, larval survival is highly dependent upon appropriate density of food objects. Red king crab larvae can effectively capture feeds dispersed in water only up to certain concentration. The data on this minimum non-consumable food concentration for zoeae I-IV are given in subsection "Daily Food Intakes" below.

Historically, several items were tested as food for the red king crab larvae reared under laboratory conditions: several species of diatom algae, annelid trochophores, copepods, barnacle nauplii [Sato and Tanaka, 1949; Kurata, 1959; Paul et al., 1989], and recently agglomerated artificial feeds [Epelbaum et al.,

2005]. All these types of food turned out to be inappropriate, as none of them allowed high survival levels and successful development of red king crab larvae through metamorphosis. The testing of the new types of food that are able to fulfill nutritional requirements of larvae and improvement of existing feeding techniques still remain an interesting subject for future studies. To date, brine shrimp (*Artemia* sp.) nauplii are considered to be the best food for red king crab larvae [Kurata, 1960; Nakanishi, 1987; Epelbaum and Kovatcheva, 2005].

Artemia sp. is the most widely used diet in marine and freshwater larviculture: it is nutritious, convenient, and least labor-intensive live food [Lavens and Sorgeloos, 1996]. *Artemia* nauplii are well adapted for a wide range of environmental conditions, including those used for the red king crab culture. The nutritional quality of *Artemia* sp. can be further modified by bio-encapsulation of specific amounts of particulate or emulsified products rich in highly unsaturated fatty acids in the nauplii; this process is also called enrichment or boosting. The same bio-encapsulation method is now being developed for the delivery of vitamins, chemotherapeutics and vaccines [Lavens and Sorgeloos, 1996]. The studies of Kittaka et al. [2002] have demonstrated that the survival of *Paralithodes brevipes* larvae fed enriched *Artemia* nauplii was 1.8 times higher compared to the survival of those fed control *Artemia* nauplii without enrichment. Preferably, there should also be studies on using *Artemia* nauplii enriched with nutrients as food for red king crab larvae. These studies will help to understand feeding requirements for red king crab larvae further and increase the efficiency of artificial reproduction of this species.

Daily Food Intakes

It is very important to know feeding rations and food concentrations that are necessary for eliciting successful feeding

response of larvae but do not form excessive amount of food in rearing tanks, as this increases the risk of bacterial and fungal infections [Zheng and Fang, 1998].

Literature data on *Artemia* sp. nauplii consumption by red king crab larvae are rather scarce. Kurata [1960] studied daily feeding rhythms and food consumption of red king crab larvae and found out that the average number of nauplii consumed by a zoea through its whole life was about 760. Nakanishi [1987] determined daily food intakes for red king crab larvae at five different temperatures (from -1.8°C to +18°C), but in each experimental trial only one larvae and one nauplii concentration was used. No data on optimal concentrations of *Artemia* nauplii for red king crab larvae were available. Therefore, we conducted an experimental study aimed at determining daily food intakes, optimal feeding regimes, and concentrations of brine shrimp nauplii for each red king crab larval instar (zoeae I-IV).

After each regular moulting, some zoeae were scooped with a 0.5-mm-mesh net from rearing tanks and placed into plastic rectangular beakers containing 0.5 L of artificial seawater (32‰). 15-20 zoeae were placed in each beaker so that rearing density did not exceed 50 individuals per liter [Russian Federation Patent No. 2200386]. The temperature was maintained at 8.0±0.3°C throughout the experiment. A gentle air flow passing through an air stone at the bottom of each beaker was used to keep water aerated and food randomly dispersed; the air flow was adjusted so that it did not interfere with normal swimming of zoeae.

Fresh *Artemia* nauplii hatched at 28°C for 24 hours were used as food. *Artemia* cysts were harvested in the lakes of the Altai Region (Russia). Our previous experiments have demonstrated that while kept in seawater at 8°C and 32‰ all *Artemia* nauplii remain alive and active for 12-14 hours; the decrease of the nutritive value of the nauplii under these

conditions is less than 5% [Lavens and Sorgeloos, 1996]. Therefore, we have chosen a 12-hour interval between feedings. Feeding rates were determined by counting the initial number of nauplii and the number of nauplii still remaining after 12 hours of larval feeding. Nauplii were counted using the Bogorov chamber and a dissecting microscope. After 12 hours of feeding, zoeae were placed to new beakers with clear water and fresh nauplii. 4 control beakers containing nauplii but no larvae were used to check the accuracy of the counting method.

A total number of 75 experimental trials for zoea I-IV with various initial nauplii concentrations were carried out. Most trials were performed in the middle of each instar existence period (on the 4^{th} -5^{th} day after each molt). Consumption changes within each instar, which may occur shortly before or after molting in crustacean larvae [e.g. Kurata, 1960; Baylon et al., 2004], were not investigated in this study.

A daily food intake for each larval instar was calculated by using the following equation [Suschenya, 1975]:

{eqn 1} $r=v(K-K_t)24/nt$, where v is the water volume in a beaker (L), K is initial concentration of *Artemia* nauplii (nauplii/L), K_t is concentration of *Artemia* nauplii at the moment t (nauplii/L), n is the number of larvae used in the experimental trial (ind), and t is duration of the experiment (h).

Diagrams of a daily food intake for each larval stage were plotted and analyzed; non-linear least squares fitting procedure was performed using a modified Ivlev's equation [Suschenya, 1975]:

{eqn 2} $r=M[1-e^{(Ko-K)/l}]$, where r is a daily food intake (nauplii/ind per day), K is nauplii concentration (nauplii/L), M, l and K_0 are constants.

Four control trials set up to check the accuracy of the nauplii counting method demonstrated that the error in nauplii enumeration was insignificant (≤0.75%). It was determined that the wet weight of *Artemia* nauplii used for the experiments constituted 0.026 mg, while its dry weight was 0.0042 mg. The results of the experiments on daily food intakes of red king crab larvae at each developmental stage are shown in Figure 14 and Figure 15.

In a certain range of nauplii concentrations, experimental curves form the plateau approaching the limit, i.e. the maximum ration (***M***). The maximum ration characterizes the physiological state of total satiation, when the food intake does not increase any more. If food concentration is sufficient, then the actual daily food intake can be assumed as equal to the maximum ration.

Optimal initial *Artemia* nauplii concentrations for feeding zoeae I-IV thus constitute 400-600, 600-800, 800-1000 and 1000-1200 nauplii/L, respectively (see Fig. 14 and 15). Daily food intakes for each zoeal instar fed *Artemia* nauplii at 8°C are summarized in Table 2.

Table 2. Daily food intakes of zoeae I-IV at 8°C.

Zoeal stage	Daily food intake (M)		
	nauplii/ind	mg (wwt)/ind	μg (dwt)/ind
I	11.3	0.294	47.46
II	22.4	0.582	94.08
III	33.2	0.863	139.44
IV	41.8	1.087	175.56

Abbreviations: dwt – dry weight, wwt – wet weight.

Thus, during the whole zoeal phase one larva consumes $10\times11.3 + 10\times22.4 + 9\times33.2 + 10\times41.8 = 1053.8$ *Artemia* nauplii of 0.026 mg each, i.e. 27.12 mg. Individual weight gain during the zoeal phase constitutes 1.81 mg (see Table 3).

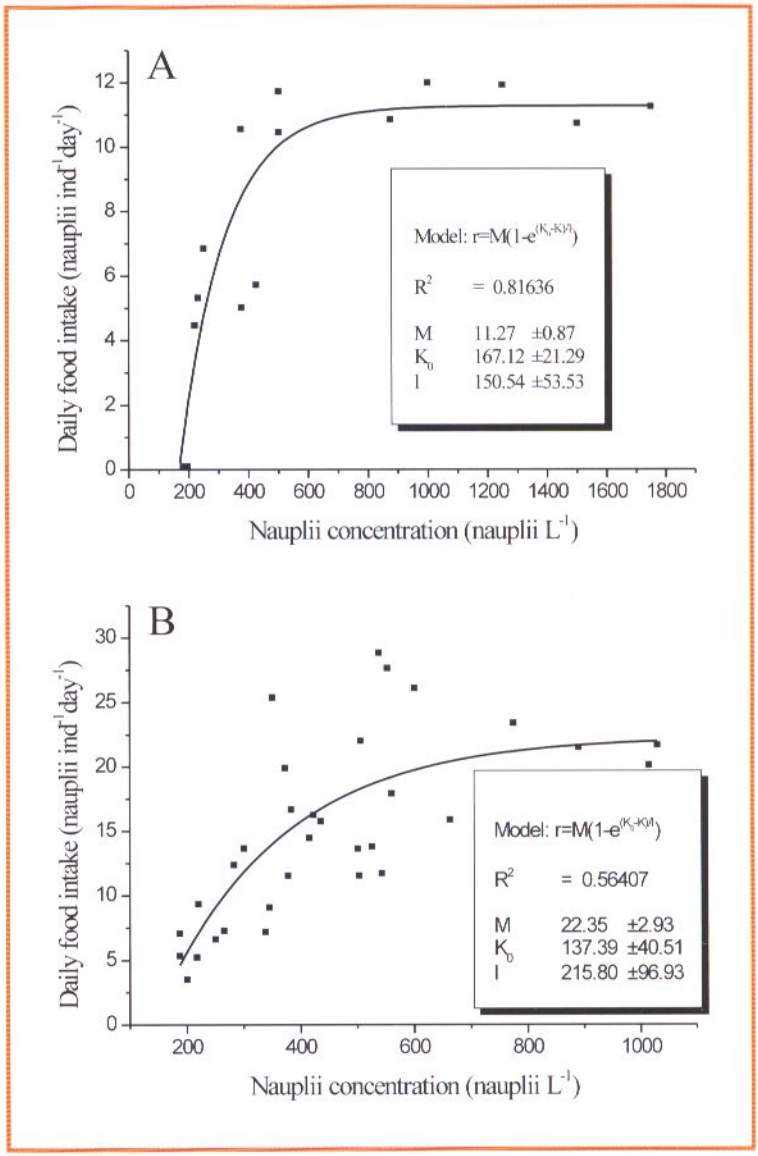


Figure 14. Daily food intakes of zoea I (A) and zoea II (B) at 8°C: r – actual ration (nauplii/ind per day), M – maximum ration (nauplii/ind per day), K – nauplii concentration (nauplii/L), K_0 – minimum non-consumable nauplii concentration (nauplii/L).

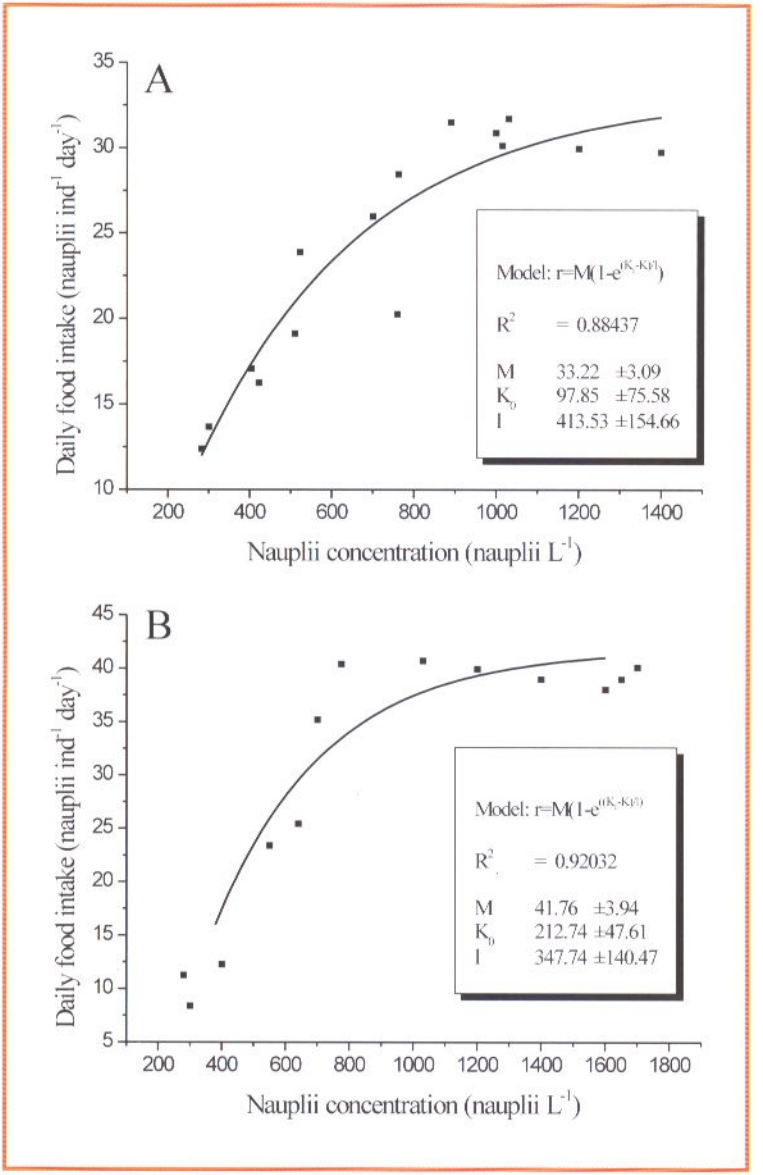


Figure 15. Daily food intakes of zoea III (A) and zoea IV (B) at 8°C (see Fig. 14 for parameters explanations).

The coefficient K_0 in the {eqn 2} stands for «minimum non-consumable food concentration», i.e. prey concentration at which the successful feeding of larvae ceases. For zoeae I-IV these minimum non-consumable food concentrations are 167.1±21.3, 137.4±40.5, 97.9±75.6 и 212.7±47.6 nauplii/L, respectively (see Fig. 14 and 15); thus, we can assume that the average value for the whole larval phase is 160 nauplii/L.

It should be noted that daily intakes may be slightly overestimated in laboratory feeding experiments with live food: larva may capture some nauplii, but consume them incompletely; these partly consumed nauplii will not be counted during subsequent recounts and considered as fully consumed. We believe, however, that this error may be considered negligible provided that a sufficient number of experimental trials was performed. Furthermore, the values of daily food intakes, though they may be slightly higher than the actual amount of the food consumed, indicate the amount of *Artemia* nauplii required for a successful feeding response of larvae in the laboratory.

1.2.1.3 DEVELOPMENT AND GROWTH

Like in other crustaceans, growth and development of red king crab larvae first of all depend upon thermal and nutritional regimes. The data on larval growth and development described in this section were obtained while the larvae hatched from the females caught in the Barents Sea were reared at 7-8°C under optimal feeding conditions: they were fed *Artemia* sp. nauplii at the daily rates given in subsection "Daily Food Intakes" above.

In order to take measurements, the larvae and their exuviae were fixed and preserved in 4% formalin. The measurements were made by means of an ocular micrometer used together with a binocular microscope. In all zoeal stages, the carapace length (from the rostrum base to the posterior edge of the carapace not

including posterior spines) and rostrum length were measured (Fig. 16).

Dry weights were determined by placing specimen on weighed pieces of tin foil, drying them to a constant weight at 60°C and then reweighing on torsion balance [Paul et al., 1989]. For measuring wet weights, the specimen were first placed on filter paper to remove surface moisture and then weighed. All the measurements were expressed as arithmetic averages and corresponding standard deviations.

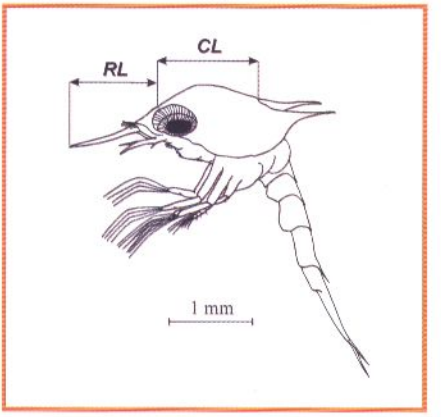


Figure 16. Zoea general view showing carapace measurements: CL – carapace length, RL – rostrum length.

Average values and standard deviations for growth and development parameters for each zoeal instar are given in Table 3.

Table 3. Development and growth of red king crab zoeae reared at 7-8°C.

Stage	Duration, days/degree-days	Carapace length (±SD), mm	Rostrum length (±SD), mm	Individual wet/dry weight, mg
Zoea I	10/66.0	1.39±0.029	1.29±0.038	0.86/0.110
Zoea II	10/68.7	1.63±0.027	1.52±0.089	1.41/0.165
Zoea III	9/69.3	1.83±0.044	1.53±0.121	2.00/0.250
Zoea IV	10/79.7	2.07±0.043	1.63±0.084	2.67/0.300

Average duration of the larval period constituted 39 days (approximately 284 cumulative degree days).

Our data on larval growth and development differ from those of Nakanishi [1978], who worked with the larvae obtained from the females caught in the North Pacific. In our experiments, the average carapace length of zoea I comprised 1.390 mm, whereas in the experiment of Nakanishi, only 1.099 mm. At the same time the data on carapace increments and wet weight gains during the whole larval period correspond very well: in our experiment, 48.9 and 210.5%; in the experiment of Nakanishi, 49.5 and 272.4%, respectively [see also: Kovatcheva and Epelbaum, 2003]. The only data on sizes of red king crab zoeae in the Barents Sea (collected during a plankton survey) are given by Bakanev and Kuzmin [1999], but these authors measured zoeal carapace length as a distance between the tip of the rostrum and the edges of posterior carapace spines. However, the lengths of the rostrum and the posterior carapace spine show considerable intraspecific variability [Nakanishi, 1987; our data, see Table 1], and therefore we can not directly compare our data with the data of Bakanev and Kuzmin [1999]. Thus, the comparison between the sizes of red king crab larvae from the native areas in the Pacific Ocean and from the Barents Sea remains a topic for future studies.

Our data on growing degree days required for larval development - about 284 cumulative degree-days - are consistent with the laboratory and field data reported in other studies: 422 [Marukawa, 1933], 282 [Shimizu, 1936], 337 [Sato, 1958], 285.5 [Nakanishi, 1987] and 350 degree-days [Klitin, 2002] [see also Kovatcheva 2002a,b; Kovatcheva and Epelbaum, 2003]. At the same time, an average duration of the larval phase in our experiments was considerably shorter than in nature: it comprised only 39 days, whereas Klitin [2002] reported that the duration of the red king crab larval period in nature averaged 73-79 days (the data obtained in West Sakhalin in 1991-1999). Thus, rearing red king crab larvae under laboratory conditions at 7-8°C

allowed shortening the duration of the larval development 1.8-2.0 times compared to natural rates.

1.2.1.4 SURVIVAL

Red king crab larvae exhibit a very high mortality rate in nature: according to Marukawa [1933], less than 1% of zoeae manage to survive up to the postlarval stage. Laboratory culture experiments [Nakanishi, 1987; Kittaka et al., 2002; Kovatcheva, 2000, 2002a,b] showed that red king crab larvae are very vulnerable and have strict environmental requirements; the studies reported high mortality rates.

The results of our study have demonstrated that rearing red king crab larvae under controlled laboratory conditions at 7-8°C and the rations shown above provides an opportunity to increase the survival of larvae up to 35% (Fig. 17).

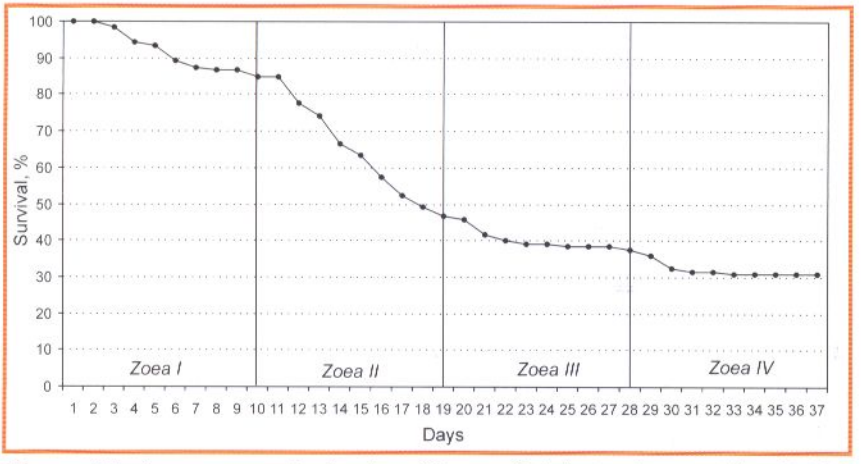


Figure 17. Average survival rates of larvae fed Artemia nauplii at 7-8°C (larvae were reared in 200 L tanks at the density of 50 ind/L).

In our experiments, a relatively high mortality rate was observed throughout larval development, especially during moulting periods. The highest mortality rates were observed after

the first moult and during the second zoeal stage (see Fig. 17). The most drastic increase in daily food intakes was also recorded after the first moult: zoea II consumes 98.2% more than zoea I, while after moulting to zoea III and IV consumption increases only by 48.2% and 25.9%, respectively (see Table 2). The biggest size increase was also observed after the first moult: zoea's wet weight increases by 64, 42, and 34% after each moult, respectively (see Table 3). These results indicate that the first moult is a critical period in the red king crab early development, and that the larvae must be especially vulnerable during the first and second zoeal stages.

We have identified the following main causes of larval mortality under laboratory conditions: (1) "unsuccessful moulting" (the larva dies during moulting process, see Fig. 18), (2) "cannibalism" (the larva's body parts and/or appendages are damaged or eaten, see Fig. 19), and (3) "unknown cause" (the larva does not have any apparent morphological deviations and shows no sign of damage).

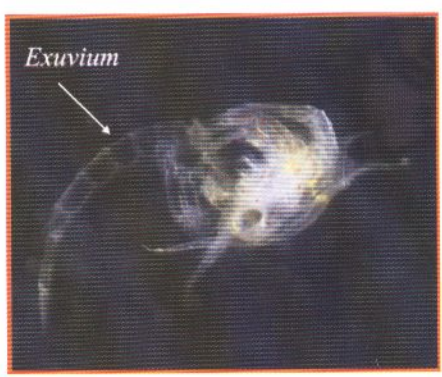


Figure 18. The larva which must have died due to unsuccessful moulting.

During moulting periods, the percentage of individuals who died due to unsuccessful moulting averaged 38.2%, the percentage of those who died due to cannibalism averaged 32.3%, and the percentage of those who died due to unknown reason, 29.5%. During intermoult periods, these values averaged 4.7%, 38.7% and 56.7%, respectively.

Interestingly, the percentage of the larvae which died due to cannibalistic behavior of their conspecifics remained largely constant during the whole larval phase and averaged 35.5%; among these larvae, 21.7% had their telsons eaten, 10.8% missed one or both eyes, and 3.0% were almost completely consumed [for details see Borisov et al., 2005].

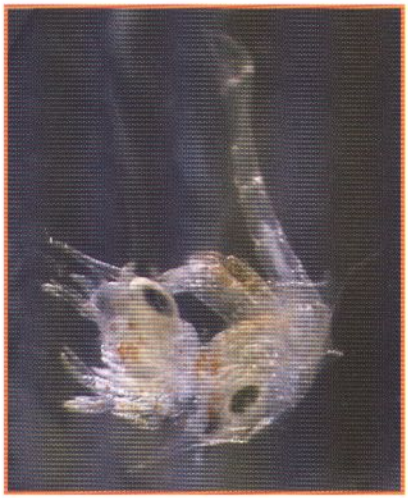


Figure 19. Cannibalistic behavior of the first stage zoea.

All four zoeal stages were observed to be highly cannibalistic when they were starved. During the experiment where the larvae received no food, 25% of all larvae were killed and partly consumed by their conspecifics [Epelbaum et al., 2005]. Dead individuals which had already become colorless were also observed being consumed.

A high level of cannibalism was also observed when the larvae crowded in certain parts of the rearing reservoirs, usually near the bottom. The level of cannibalism can thus be reduced by (1) providing adequate food supply, (2) adjusting water flow and aeration systems so that there are no "dead" areas, where stale water, food and larvae accumulate, and (3) using even illumination.

1.2.2 GLAUCOTHOE PHASE

1.2.2.1 GENERAL MORPHOLOGY AND BEHAVIOR

Basic morphological and behavioral patterns, which were generally stable throughout the zoeal period, undergo drastic changes at the glaucothoe stage. Unlike zoeae, the glaucothoe (Fig. 20*A*) swims with its rostrum leading. The glaucothoe has relatively small maxillipeds and swims by means of pleopods (Fig. 20*D*), with pereiopods extended forward.

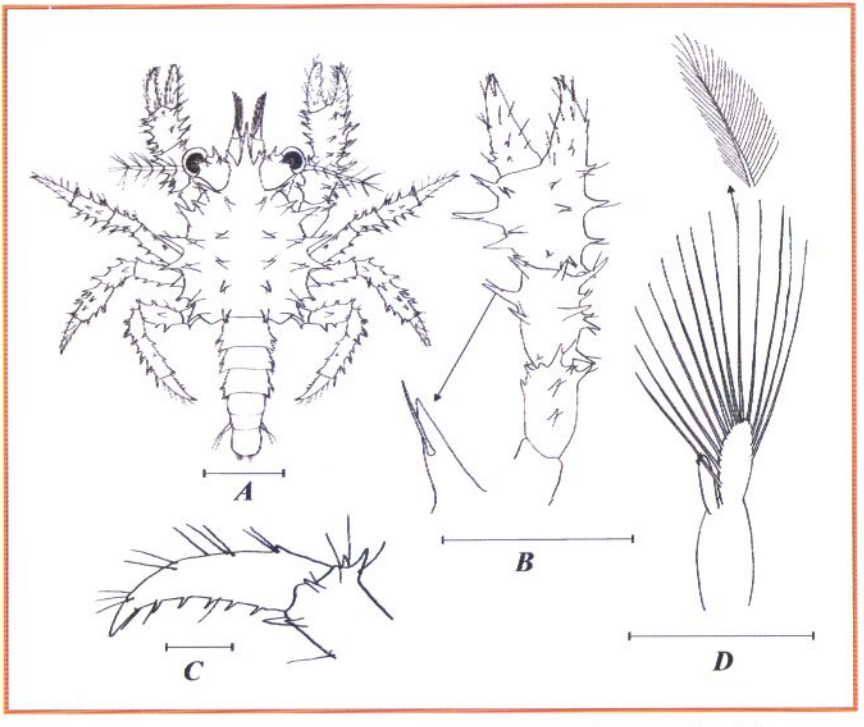


Figure 20. Glaucothoe: (A) – general view, (B) – right chela, (C) – dactilus of the second pereiopod, (D) – pleopod. Scale bars: A, B, D – 1 mm, C – 0.2 mm.

Locomotory behavior gradually changes throughout the glaucothoe stage: for the first 2 or 3 days most glaucothoes actively swim and settle on the substrate from time to time; in 3 days glaucothoes become more quiescent and primarily cling to the substrate or walk on it by means of pereiopods (Fig. 20*B,C*). Some glaucothoes do not change their positions throughout the whole postlarval period.

Glaucothoes were found to be positively phototactic. Photoresponse is stronger in newly molted individuals and becomes less pronounced by the time of metamorphosis to the first juvenile stage. Glaucothoes may form high density groups in more illuminated parts of a rearing reservoir; this increases the probability of their being captured and consumed by their highly cannibalistic conspecifics (i.e. individuals which are still at zoeal stages and/or have already moulted to the first juvenile instar due to asynchrony of moultings). Therefore, it is recommended to illuminate rearing tanks as evenly as possible, especially in cases of mass culture - it will favor even distribution of glaucothoes, reduce cannibalistic behavior, and consequently increase the effectiveness of cultivation.

1.2.2.2 FEEDING

Appendages of the glaucothoe become more adult-like in nature and resemble in form those of the juvenile. However, oral appendages of the glaucothoe are unsuitable for food processing (Fig. 21). Mandibles are relatively small and soft, mandibular palp lacks any kind of setae. Endites of maxillules and maxillae have sparse and weak setae, functionally useless for holding and processing the food. The third maxilliped is well developed, but lacks crista dentata. Oral appendages of the glaucothoe are much smaller than corresponding appendages of the first juvenile, although their carapace lengths are almost equal (see sections 1.2.2.4 and 1.2.3.3).

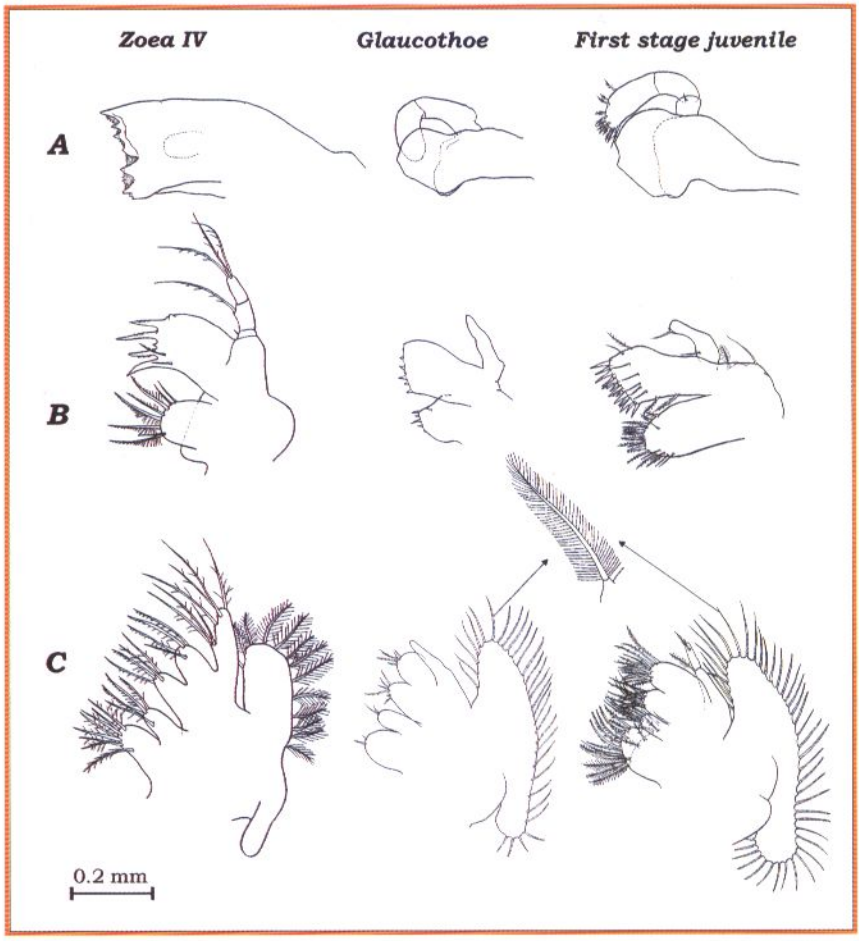


Figure 21. Oral appendages of zoea IV, glaucothoe and first stage juvenile: (A) – mandibles, (B) – maxillules, (C) – maxillae.

Studies on the anatomy of digestive tracts of larvae, glaucothoes, and juveniles [Abrunhosa and Kittaka, 1997a,b; Epelbaum, 2002] and laboratory observations [Kovatcheva, 2002a,b] confirm the conclusion that red king crab ***glaucothoe does not feed*** and ***represents a secondarily lecithotrophic stage***. The term "secondary lecithotrophy" was proposed by Anger

[1989] for non-feeding stages that develop with energy reserves accumulated during a preceeding larval phase. The phenomenon of secondarily lecithotrophy is described for a number of species of marine decapod crustaceans, such as hermit crabs (*Pagurus samuelis,* Coffin [1958]; *P. bernharus,* Bookhout [1964], Dawirs [1981], *P. alatus,* Bookhout [1972], *P. longicarpus* [Roberts, 1971]) and lobsters of the family Palinuridae [Nishida et al., 1990; Wolfe and Felgenhauer, 1991; Lemmens, 1994]. Secondarily lecithotrophy was also described for some other lithodid crabs, such as *Paralithodes platypus* and *P. brevipes* [Abrunhosa and Kittaka, 1997b]. However, Hoffman [1968] described the glaucothoe of *P. platypus* as a feeding stage; Nakanishi [1987] and Levin [2001] even mentioned cannibalistic behavior of glaucothoes. We suppose that the authors who described glaucothoe as a feeding stage based their assumptions on the decrease of food items and glaucothoes during the experiments. This decrease, however, can probably be explained by active feeding of individuals that are still at the zoeal and/or already at the first juvenile stage, due to asynchrony of moultings.

What are possible reasons underlying glaucothoe non-feeding behavior? Red king crab juveniles naturally mainly occur on course substrates covered with branchial epifauna, such as colonies of bryozoans, sponges, and hydroids, tubeworms and algae, and can rarely be found on open silt and sand bottoms [e.g. Sundberg and Clausen, 1977]. Experiments revealed that such distribution is a consequence of a selective glaucothoe settlement, i.e. substrate preferences [Stevens and Kittaka, 1998]. The glaucothoe is able to choose suitable substrate, which will provide future juveniles with food and shelters [Freese and Babcock, 1990]; therefore, the key role of the glaucothoe is microdistribution. Non-feeding behavior of the glaucothoe can thus be discussed as special early life history adaptation; lack of feeding provides the glaucothoe with the opportunity to continue

the planktonic lifestyle or settle once the suitable substrate has been found, without any dietary consequences [Stevens and Kittaka, 1998]. A similar idea was offered by Dawirs [1981] for non-feeding megalopa of the hermit crab *Pagurus bernhardus*: it is more advantageous for a larva to dispense with food in order to increase the probability of finding a protective shell to enter. Feeding behavior can also be a distraction for settling larvae, as feeding larvae may respond to stimuli not correlated with favorability of the substrate and therefore make a poor choice [Strathmann, 1985]. Main morphological and behavioral features of the glaucothoe, enabling it to effectively ensure transition between planktonic and benthic lifestyles, are summarized in Figure 22.

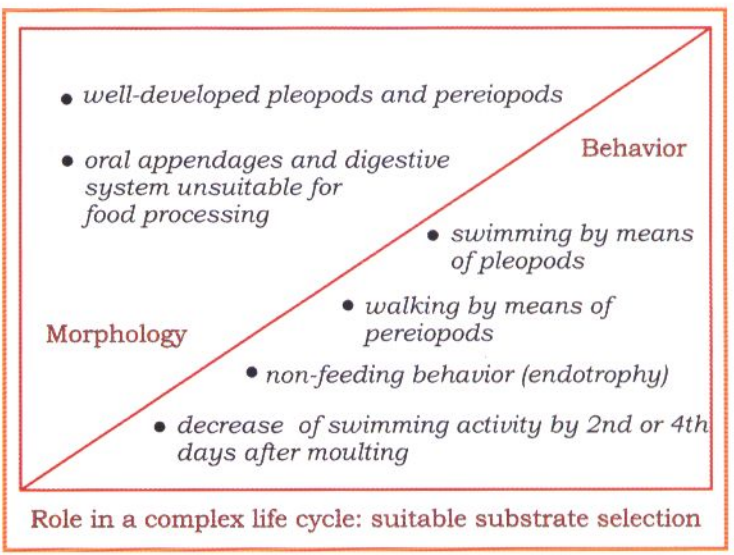


Figure 22. Main morphological and behavioral features of the glaucothoe.

According to our observations, the glaucothoe has sensory setae on the spines of the carapace and pereiopods (see Fig. 20*B*);

this trait holds true for juvenile and adult crabs as well. Such setae have also been described for the glaucothoe of *Paralithodes platypus* [Hoffman, 1968]. Many arthropod species have innervated receptive hairs on their body surfaces [Shelton and Laverack, 1970]. Therefore, these setae of the glaucothoe are likely to be mechano- and chemoreceptors, playing their role in the process of the suitable substrate selection.

1.2.2.3 SUBSTRATE PREFERENCES

Settling behavior and substrate preferences of the red king crab glaucothoe have been investigated by Stevens and Kittaka [1998]. These authors have tested course sand, quartz granules, and synthetic fiber mesh as substrates for glaucothoes. Glaucothoes showed a clear preference for the mesh substrate and appeared to reject sand substrate. The authors conclude that the settlement patters exhibited by glaucothoes are likely to be a response to the physical characteristics of the substrates, such as the size of particles or interstitial spaces, rather than to organic compounds. This study indicates that other artificial substrates lacking bioorganic constituents may be expected to be suitable for glaucothoe settlement.

The use of easy maintenance artificial substrates is more advantageous and convenient in mass-culture than the use of natural substrates. We have undertaken an additional study in order to test the suitability of 2 types of artificial substrates for rearing red king crab glaucothoes. Substrate preferences of 500 glaucothoes were investigated in a specially designed and equipped 80-L experimental aquarium with a recirculation water system. The water temperature was maintained at 10.0±0.5°C, salinity at 32‰. The aquarium was conditionally divided into 2 equal parts, left and right, where the substrates were arranged according to the "mirror-picture principle" (Fig. 23). The water flow and aeration systems in the aquarium were adjusted so that

the water circulation in both parts was even and basically identical.

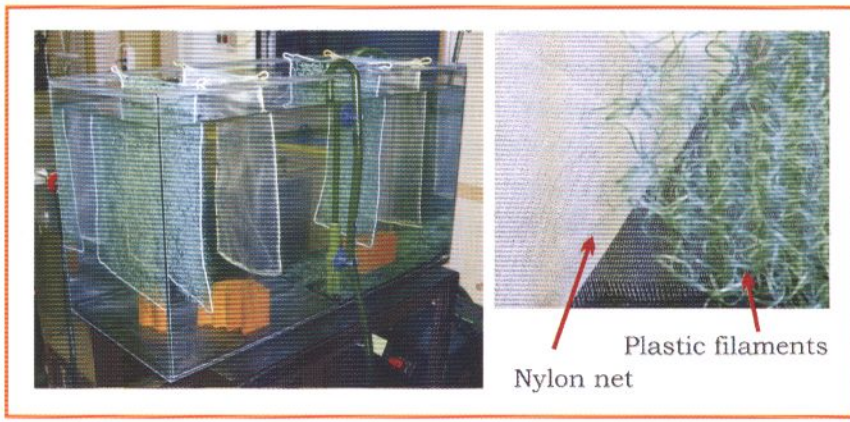


Figure 23. Experimental substrates for rearing glaucothoes.

The following types of substrates were tested:

- vertically oriented nylon net (0.5 mm) stretched over a plastic framework ("net 1");
- horizontally oriented nylon net (0.5 mm) stretched over a plastic framework ("net 2");
- vertically oriented flat mats of plastic filament (mechanical filter media) ("mat 1");
- horizontally oriented flat mats of plastic filament ("mat 2").

500 glaucothoes on the date of moulting from the fourth stage zoeae were transferred to the experimental aquarium with a large bore pipette and released 5 cm below the water surface in the center of the aquarium. The number of the swimming and settled glaucothoes was counted 1 hour after their introduction into the aquarium and daily thereafter. When metamorphosis to the first juvenile stage began, the aquarium was inspected daily

for moults and mortalities. The experiment was conducted until all glaucothoes molted to the first juvenile stage or died.

The results of this experiment are shown in Table 4 (3-day periods are numbered consequently as Period I, II, etc.).

Table 4. Results of the experiment aimed at determining substrate preferences of glaucothoes.

Substrate	Proportion of glaucothoes, %				
	Period I	Period II	Period III	Period IV	Period V
swimming individuals	0.7	0.2	0.0	0.0	0.0
net 1	2.8	2.5	0.7	0.7	0.4
mat 1	1.9	1.9	1.6	2.3	1.2
net 2	14.2	13.2	12.5	12.1	11.1
mat 2	79.1	79.5	82.8	81.7	83.8
other*	1.3	2.7	2.4	3.1	3.5

**surfaces of the pipes, bricks, and porous stone of the aeration system*

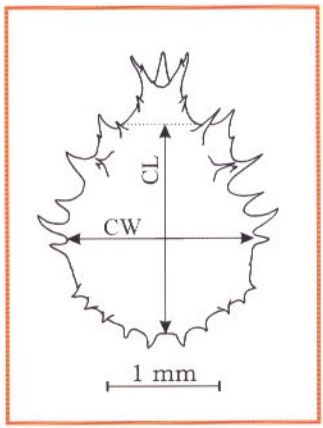
The glaucothoes began to settle immediately after being placed into the experimental aquarium. The proportion of swimming individuals was only 0.7% by day 3, declined to 0.2% by day 6, and no swimming glaucothoes were observed afterwards.

As shown in Table 4, the proportion of glaucothoes on each substrate remained fairly constant during the whole glaucothoe phase. Among the substrates tested, a horizontally oriented nylon net was chosen by 11.1-14.2% of the red king crab glaucothoes. 79.2-83.8% of the glaucothoes preferred horizontally oriented flat mats of plastic filaments. This substrate can be easily grasped by glaucothoes and provides a high degree of interstitial space. Therefore, safe and easy maintenance mats of plastic filaments can be recommended as suitable substrates for rearing red king glaucothoes.

1.2.2.4 DEVELOPMENT AND GROWTH

Here we provide the data on the size and development duration of glaucothoes reared from eggs under laboratory conditions. Thermal regime during the zoeal phase was 7-8°C; during the glaucothoe phase, 10-11°C.

For taking measurements, glaucothoes and their exuviae were fixed with 4% formalin for 2 hours, rinsed briefly in distilled water, and preserved in 70% ethanol [Konishi and Quintana, 1987]. In each glaucothoe, carapace length (from the eye notch to the posterior edge of the carapace) and carapace width (across the widest portion not including the spines) were measured (Fig. 24). Wet and dry weights were determined in the same way as those of the larvae (see section 1.2.1.3).



In our experiments, carapace length of the glaucothoes averaged 1.85±0.035 mm; carapace width, 1.63±0.05 mm. An individual wet weight averaged 3.77 mg; dry weight, 0.679 mg.

Figure 24. Carapace measurements of the glaucothoe: CL – carapace length, CW – carapace width.

The duration of the glaucothoe phase at 10-11°C was 18-20 days (177.7-200.0 degree-days).

1.2.2.5 SURVIVAL

When the glaucothoes were reared under the conditions described in sections 2.2 and 2.3.3 of this book, their survival comprised approximately 90%.

1.2.3 EARLY JUVENILE PHASE

1.2.3.1 MORPHOLOGY AND BEHAVIOR

After moulting to the first juvenile, the red king crab changes its locomotory behavior for the third and the last time. The juveniles are completely benthic and adult-like: they walk using 4 pairs of pereiopods (Fig. 25).

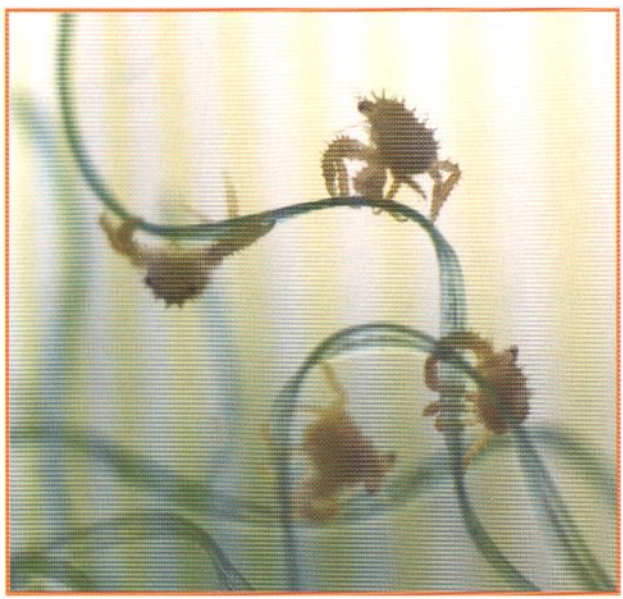


Figure 25. First stage juveniles on plastic filament substrate.

The clearest morphological distinctions were observed between the 1st and 2nd stage juveniles: a 1st stage juvenile can be distinguished by a pear-shaped carapace and the number of marginal spines located in the region between the lateral group of large spines and the posterior notch of the carapace (a 1st stage juvenile has 5 spines in this region, and a 2nd stage juvenile has 2-4 additional spines of smaller size there) (Fig. 26*A,B*).

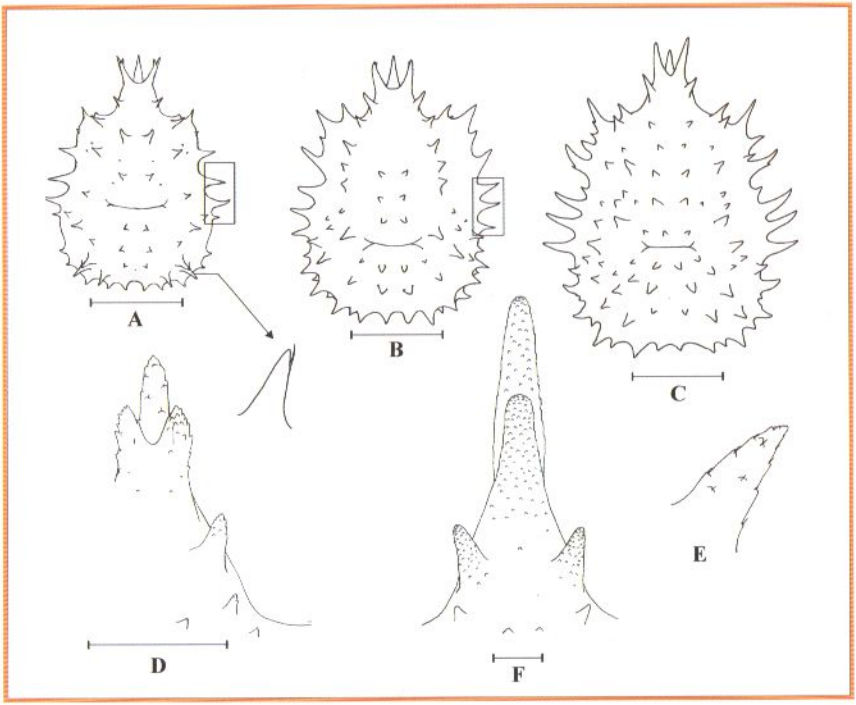


Figure 26. Juveniles: (A)-(C) – carapaces of 1st, 2nd, and 3rd stage juveniles; (D) – rostrum of 1+ juvenile; (E) – carapace spine of 1+ juvenile; (F) – rostrum of 2+ juvenile. Scale bars: 1 mm.

2nd stage juveniles do not have any pleopodal remnants observed among some of the 1st stage juveniles. The gender of a crab can be determined by looking at the abdominal tergites arrangement after 3 or 4 moultings; in addition, in females pleopods reappear by then [Borisov et al., 2004].

After each moulting, the number of setae on oral appendages increases; the ratio between carapace length and width changes; the number of carapace spines increases; relative length of the rostrum decreases, and the rostrum shape changes (Fig. 26*D,F*); denticulation on the chelae becomes more

pronounced; projections of gills gradually become more flat and lamella-like. The number of sensory setae on carapace spines gradually increases; ledges appear at the bases of these setae (Figure 26*E*). After several moultings, most carapace spines become less acute.

Under laboratory conditions, red king crab juveniles have shown weak positive phototaxis.

1.2.3.2 FEEDING

Feeding Behavior

Feeding behavior of red king crab juveniles may be called adult-like. Feeding appendages of the juveniles are well developed and functionally adapted for processing both soft and hard food (see Fig. 21). The juveniles grasp and tear food items using chelipeds and process it using maxillipeds and oral appendages.

Desirable Food Characteristics and Possible Feeds

Potential feeds for red king crab juveniles should meet the following requirements:

- ✓ be appropriate in size for the juveniles of a certain age and meet their nutritional requirements;
- ✓ have negative buoyancy;
- ✓ remain stable in seawater for at least 12 h and not alter water quality significantly.

In the laboratory, the juveniles appeared to feed successfully on raw mussels, squid, shrimps, and small mosquito larvae (*Chironomus* spp.).

1.2.3.3 DEVELOPMENT AND GROWTH

As during preceding larval phase, the juveniles must moult in order to grow, i.e. pass through ecdysis. During pre-ecdysis, demineralization of the old exoskeleton occurs: inorganic salts are

resorbed and stored internally. During ecdysis itself, the crab rapidly absorbs water which causes its tissues to swell and split the old exoskeleton open in between the carapace and the abdomen. The crab begins a slow process of backing out of its old exoskeleton, which is then discarded (Fig. 27).

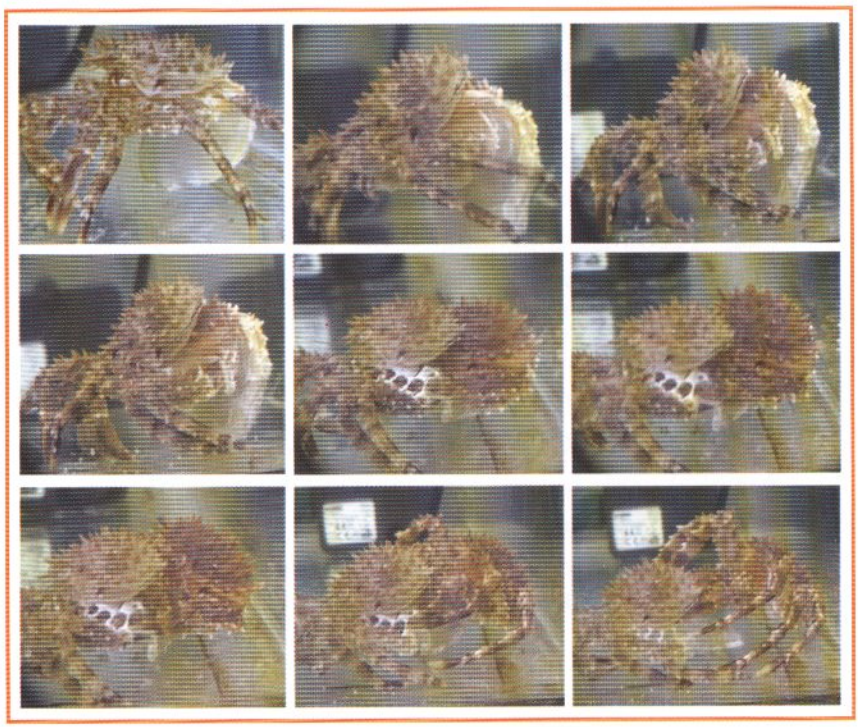


Figure 27. Moulting of the juvenile crab.

The newly molted crab pumps water into its tissues in order to inflate the new exoskeleton to its new size. The new carapace will be roughly one-fourth larger than the old one (Fig. 28).

The salvaged inorganic salts are rapidly redeposited to help thicken and harden the new exoskeleton. The hardening process takes approximately 2 to 4 days.

Figure 28. Newly molted juvenile crab and its exuvium.

Development and growth rates of the juveniles depend on thermal and nutritional regimes. In Table 5 we provide the data on the sizes of the juveniles reared from eggs under laboratory conditions. The thermal regime during the zoeal phase was 7-8°C; during the glaucothoe and juvenile phases, 10-11°C. The juveniles were fed meat of marine invertebrates, predominantly squid and mussel.

The juveniles and/or their exuviae were fixed with 5% formalin for 2 hours, rinsed briefly in distilled water, and preserved in 70% ethanol [Konishi and Quintana, 1987]. Carapaces were measured the same way as those of the glaucothoes (see Fig. 24). Aditionally, rostrum length was measured (from the tip of the rostrum to the eye notch). The growth parameters of the early juveniles at 10-11°C are given in Table 5.

Table 5. Growth of red king crab early juveniles under laboratory conditions at 10-11°C.

Stage	Carapace length (±SD), mm	Rostrum length (±SD), mm	Carapace width (±SD), mm
I	1.81±0.022	0.64±0.053	1.64±0.040
II	2.01±0.041	0.69±0.023	1.72±0.034
III	2.43±0.065	0.83±0.025	2.10±0.049
IV	2.85±0.114	0.88±0.045	2.56±0.077
V	3.41±0.118	1.04±0.067	3.08±0.097
VI	4.03±0.179	1.17±0.031	3.73±0.193

Individual wet weight of a 1st stage juvenile averaged 3.4/4.5 mg; dry weight, 0.9/1.0 mg (early and late in the stage, respectively). Individual wet weight of a 2nd stage juvenile averaged 5.6 mg; dry weight, 1.6 mg [Kovatcheva et al., 2005a].

In the juveniles 0-3 years old reared at 10-11°C, mean duration of intemoult periods comprised from 20 to 30 days (from 200 to 360 degree-days), while carapace width increment after each moulting comprised 15 to 29% on average.

1.2.3.4 SURVIVAL

Red king crab juveniles are highly cannibalistic. As a rule, large individuals attack smaller ones. Besides, the juveniles are often cannibalized during or soon after moultings, when they have soft exoskeleton and therefore are particularly vulnerable. The level of cannibalism mainly depends on the following factors: rearing density, availability and a type of shelter/substrate, and adequacy of feeding.

We have undertaken a study in order to test whether the use of artificial substrate (mats of plastic filament, see section 1.2.2.3) makes it possible to reduce the level of cannibalism in red king crab juveniles. Second stage juveniles were reared in the aquarium divided into 2 compartments: 20 juveniles in a

compartment with a thick (5 cm) mat of plastic filament and 19 juveniles in a compartment without any substrate, under otherwise constant and identical conditions (ca 10°C, 32‰, day-night regime 8:16). The juveniles received raw squid and mussels once a day. The compartments were monitored for moultings and mortalities on a daily basis. Dead individuals and exuviae were removed for measurements and morphological studies. The experiment was conducted in 3 replicates and lasted for 73 days, until the moulting to the 6th stage juvenile began. The survival of the juveniles in this experiment is shown in Figure 29.

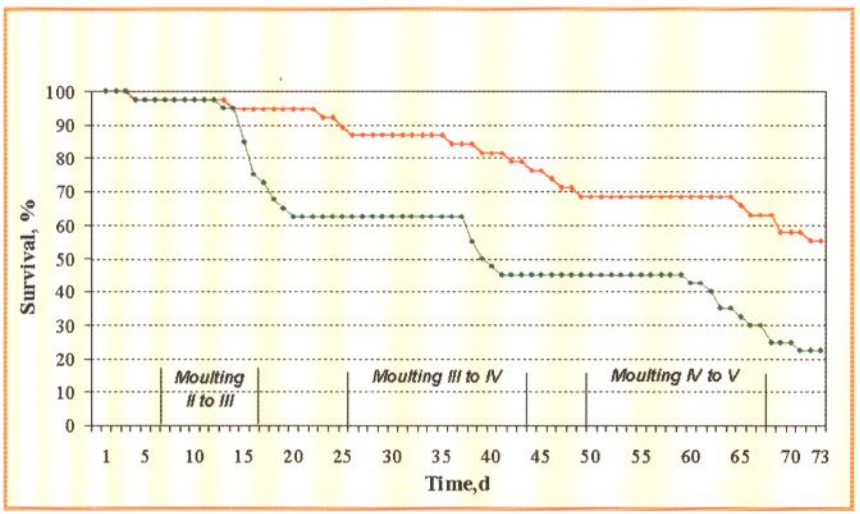


Figure 29. Survival of young-of-the-year juveniles reared at 10-11°C with substrate (orange line) and without substrate (green line).

During this experiment, cannibalism associated with moulting was the main cause of mortality: moulting and newly molted individuals were often cannibalized by their conspecifics. As shown in Figure 29, the moulting periods took from 10 to 18 days, due to the increasing asynchrony of moultings. Thus, at any point in time there were several moulting and/or newly molted

juveniles in the compartments, and the level of cannibalism was constantly relatively high. Mats of plastic filament provided a high degree of interstitial space, thus maximizing rearing reservoir volume usage: the moulting and newly molted juveniles had a better chance to hide, and their survival up to the 5th stage averaged 56%, which is 2.4 times higher compared to the survival of those reared without the substrate (see Fig. 29). Therefore, we recommend using the mats of plastic filament as substrate for the juveniles in order to reduce the level of cannibalism and maximize the survival of the juveniles.

Chapter II. CULTURE

Red king crab culture techniques are based on creation of easily controlled and regulated rearing conditions, taking into consideration specific requirements for each developmental stage, in order to maximize the overall survival and growth rates.

A full cycle of artificial reproduction and cultivation of the red king crab with the aim of repopulating depleted natural stocks consists of several stages, or phases, which are shown diagrammatically in Figure 30.

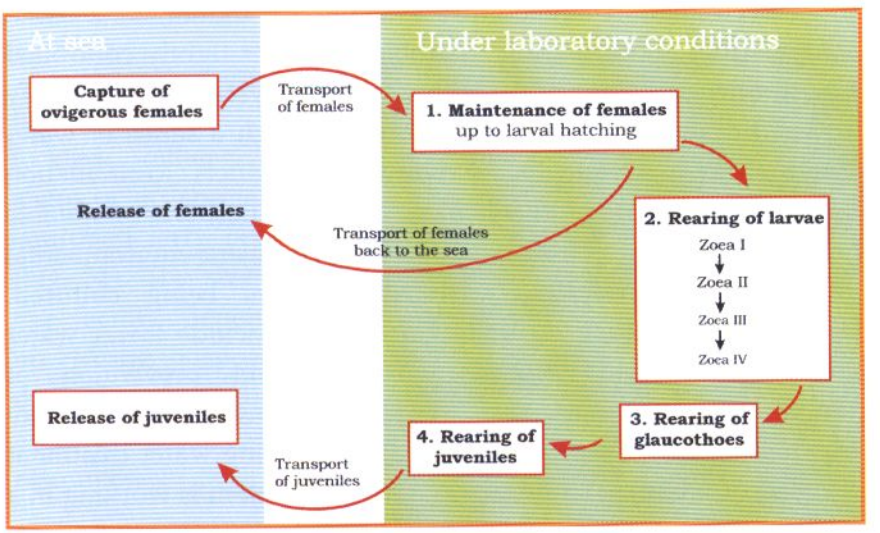


Figure 30. Diagram showing main phases of red king crab artificial reproduction and rearing under laboratory conditions.

When the red king crab is cultured for laboratory experiments aimed at clarifying certain aspects of its biology, the corresponding part of this scheme may be carried out independently.

2.1 CULTURE SYSTEMS AND EQUIPMENT

There are two major types of culture systems that can be used for red king crab rearing.

The flow-through culture system is based on regular water exchange, in order to reduce toxic substances accumulating in the rearing water. Advantages of using the flow-through system include lower costs, lower aeration requirements, and a possibility to effectively improve water quality by increasing flow rates.

Recirculation aquaculture systems are indoor, tank-based systems that allow rearing hydrobionts under tightly controlled environmental conditions. The water in the system is continually cycled through production tanks and through a series of water treatment equippment to remove waste products. Recirculation systems may be used in inland culture facilities, where suitable land or water supply is limited, or where ambient environmental conditions are not ideal for the species being cultured. Advantages of using recirculation systems include lower water consumption; heat economy; control and prevention of pollutants, infections and parasites. However, there are methodological constraints in effective recycling water treatment (first of all, in nitrogen removal) when the system is working in a low temperature regime [Summerfelt et al., 2004; Kovatcheva et al., 2004].

Both types of the culture systems can be used in rearing the red king crab, and they both have certain advantages and disadvantages. In this chapter, we will describe design and construction of flow-through and recirculation culture systems, as well as the basic laboratory equipment required for the red king crab culture.

2.1.1 FLOW-THROUGH CULTURE SYSTEM

For flow-through culture facilities, the choice of the facility location depends mainly on the availability of continuous natural sea water supply. The facility should preferably be situated far from cities, harbors, and activities which may pollute the water. The water should be free from heavy metals, herbicide residues, and biological contamination. Main physico-chemical characteristics of the water should comply with the red king crab requirements described in section 2.2.6. Characteristics of the coastal area, such as bottom topography, tidal movements and currents should also be taken into consideration.

A crab rearing facility may be located either on shore or on specially equipped ferry boats (Fig. 31). The size of the facility depends on rearing purposes, desired production and availability of resources.

Figure 31. Holding tanks of a crab rearing facility located on a ferry boat (Vidyaevo, Murmansk district, Russia).

A simplified layout of a flow-through red king crab culture system is shown in Fig. 32.

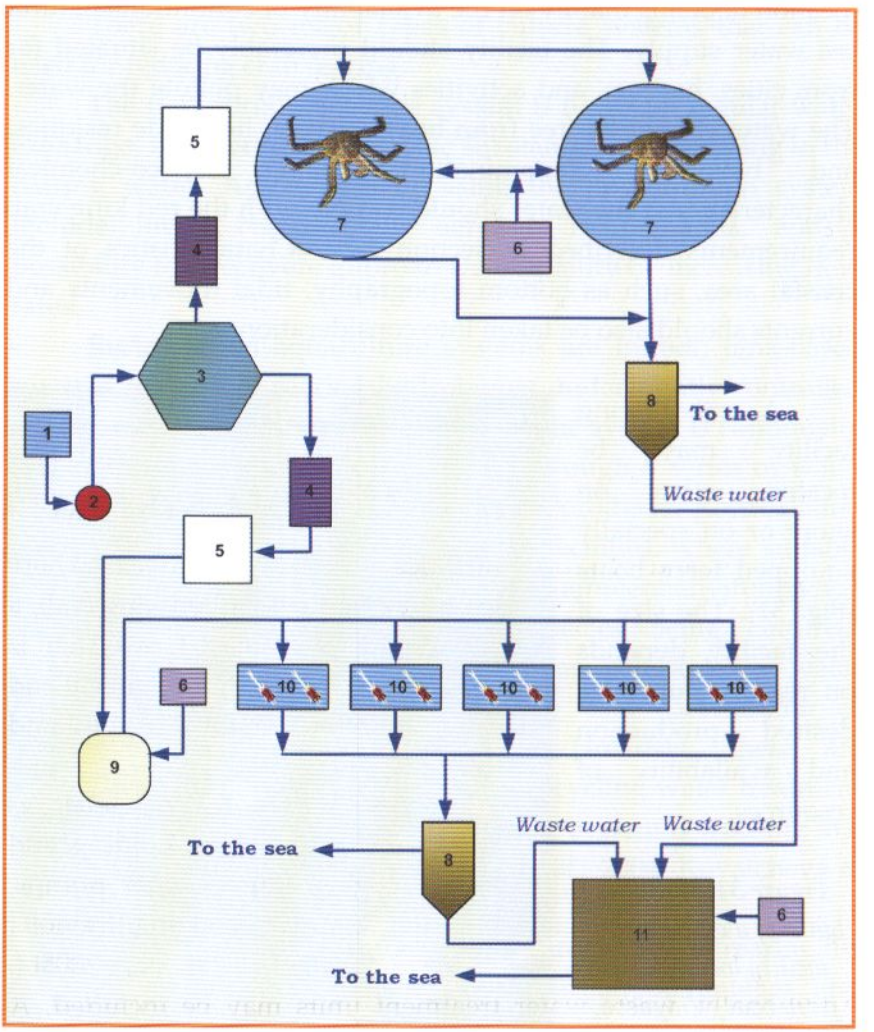


Figure 32. Layout of a flow-through culture system: 1 – Inlet (water intake area) , 2 – Sea water pump, 3 – Hydrodynamic filter, 4 – UV-sterilizer, 5 – Chiller, 6 – Air pump, 7 – Holding tanks, 8 – Sump tanks, 9 – Oxygen generator, 10 – Rearing tanks, 11 – Mineralizer.

Before entering the tanks, the water used in a flow-through culture system should first be processed, so that solid particles are removed. After that the water has to be cooled and disinfected (see section 2.1.3).

Drainage of holding and rearing tanks is usually achieved by gravity. Provided that the crabs are reared at appropriate rearing densities and are fed in accordance to their nutritional requirements at each developmental stage, so that there is no excess of food, the drained water will not cause contamination or any other environmental damage.

The water and bottom sediment siphoned from the tanks during cleaning procedures should undergo treatment in a mineralizer, allowing compacting excess sludge; after that the water may be discharged [Kovatcheva et al., 2005b].

2.1.2 RECIRCULATION CULTURE SYSTEM

In inland culture facilities or in the areas where environmental conditions are not optimal for the red king crab, a dynamic recirculation culture system may be used. This system is based upon continuous water circulation through mechanical and biological filters, thus providing continuous removal of solid and nitrogen wastes.

A closed recirculation water system used in *P. camtschaticus* culture includes the following main components: holding and rearing tanks, mechanical and biological filters, water pumps, cooling equipment (chillers), protein skimmers, and an ultraviolet disinfection unit (UV-sterilizer) [Kovatcheva et al., 2005b]. Additionally, waste water treatment units may be included. A layout of a recirculation system for culturing the red king crab is shown in Figure 33.

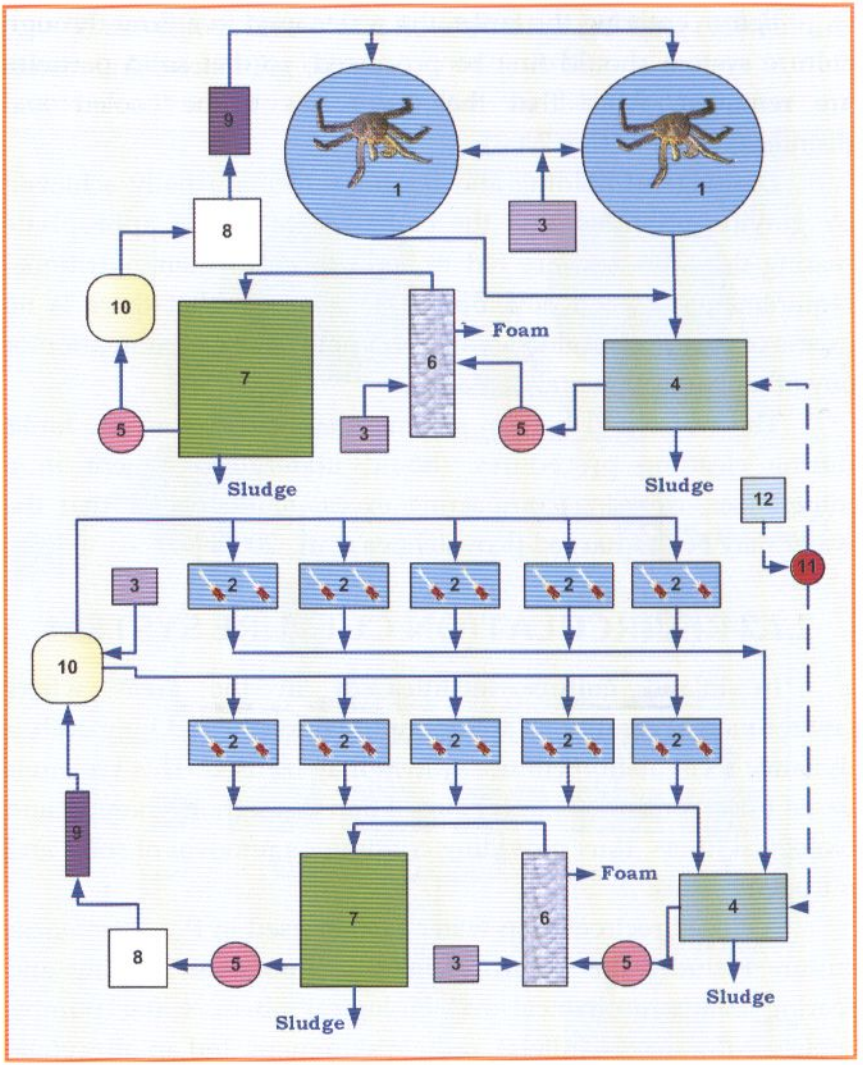


Figure 33. Layout of a recirculation culture system for rearing the red king crab: 1 – Holding tanks, 2 – Rearing tanks, 3 – Air pump, 4 – Sump tanks, 5 – Pump, 6 – Protein skimmer, 7 – Biological filter, 8 – Chiller, 9 – UV-sterilizer, 10 – Oxygen generator, 11 – Main sea water pump, 12 – Tank for water storage/preparation.

Figure 34 shows a fragment of a recirculation water system at the Crustacean Reproduction Laboratory (VNIRO, Moscow); this system was used for holding red king crab ovigerous females.

Figure 34. Fragment of a recirculation system (VNIRO, Moscow): 1 – Holding tank; 2 – Biofilter; 3 – Chiller.

System efficiency is dependent upon the filter and tank shape and size, the type of biofilter media, and the rate and pattern of water circulation. Both natural sea water and artificial water prepared from dry mixtures of marine salts may be used.

All recirculating systems require periodical water replacement in order to compensate for water losses. Therefore, the system should incorporate a tank for water storage or preparation (if artificial water is used) (see Fig. 33).

2.1.3 BASIC EQUIPMENT

The set of equipment used in the process of artificial reproduction and cultivation of the red king crab depends upon a specific facility design and culture system used. However, certain generalizations may be outlined.

The set of equipment should provide efficient water treatment in order to create and maintain optimal rearing conditions. Water treatment includes as follows:

1. Water disinfection/sterilization
2. Water aeration
3. Water temperature adjustment and maintenance
4. Water purification:
   - mechanical filtration in order to remove solid wastes from the incoming water and from the system;
   - biological filtration in order to remove nitrogen wastes (in recirculation culture systems);
   - foam fractionating (=protein skimming).
5. Waste water treatment.

Below we describe the tank systems and basic equipment required for water treatment processes stated above.

2.1.3.1 TANK DESIGN AND ACCESSORIES

Holding Tanks

Holding tanks may be oval, rectangular or square shaped. They may be constructed of glass, fiberglass or plastic. Tank capacity may range from 0.5 to 5 m^3, depending on the number of

crabs. The water depth in holding tanks should not be less than 0.5 m. The main criteria for choosing the type, shape and size of holding tanks are the expected number of crabs, the space available, and the ability to properly circulate and process water volume.

A water supply system should provide proper water circulation within the tanks to allow adequate removal of waste products and uneaten food. Water supply pipes should be provided with flow regulation armature, such as valves or faucets. Water circulation within the tank is also maintained through aeration, usually provided by the use of a standby blower; air distribution within the tank is ensured through the use of porous stones.

The drainage system should provide effective draining. Drainage pipes should be protected by nylon mesh screens (ca 750 μm) that retain hatching larvae.

It is advisable to use easily maintained portable siphon systems for cleaning the tanks. Smooth-sided tanks are better since rough-textured sides trap solids and may complicate tank cleaning operations.

Rearing Tanks

Larvae may also be reared in tanks but it is more convenient to rear them in glass or fiberglass aquaria of 150-200 L capacity [Kovatcheva, 2000], as it allows controlling rearing process more precisely (Fig. 35). The aquaria must be provided with water supply/drainage and aeration systems. Water supply and aeration systems should provide constant, but gentle water circulation within the aquarium so that water movements do not interfere with normal swimming of larvae (see sections 1.2.1.1 and 1.2.1.2). Drainage pipes should be thoroughly protected by nylon mesh screens (ca 750 μm), which let food objects pass through, but retain larvae.

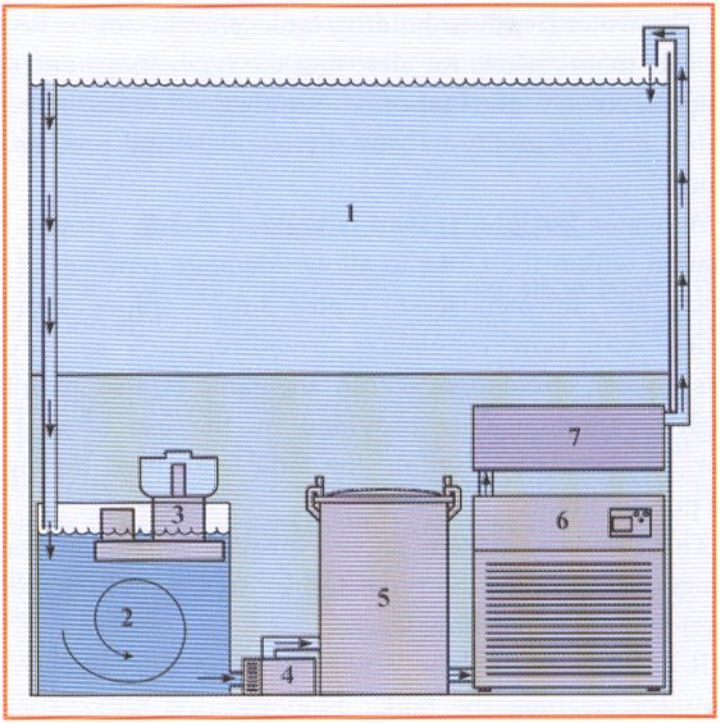


Figure 35. Scheme of a larval rearing tank (recirculation system): 1 – aquarium, 2 – sump tank, 3 – protein skimmer, 4 – water pump, 5 – biological filter, 6 – chiller, 7 – UV-sterilizer.

Additionally, for various experimental purposes, larvae, glaucothoes and juveniles may be reared in containers of various volumes and/or individual cells (see section 2.3.4.1).

2.1.3.2 WATER DISINFECTION EQUIPMENT

During all phases of the red king crab cultivation it is very important to provide effective disinfection of water - both the water from the ocean (when a flow-through culture system is used) and recycling water (in a recirculation system).

Traditional water disinfection methods, such as chlorination and ozonization, should not be used during the red

king crab cultivation due to formation of byproducts (residual chlorine, bromate ions) toxic for crabs. Therefore, the ultraviolet sterilization method is recommended. It is possible to use simple UV light sterilizers manufactured for drinking water sterilization. The size of a sterilizer should be chosen regarding the water flow rate in the system.

2.1.3.3 AERATION EQUIPMENT

A reliable 24-hour oil-free aeration system is required during all the phases of the cultivation process in order to maintain dissolved oxygen levels. It is advisable to use bubbler-type aeration. An air-blower should have sufficient capacity to simultaneously supply all operating tanks (water preparation, holding and rearing tanks, brine shrimp *Artemia* sp. rearing system) at a minimum level of 0.5-0.7 liter of air per hour for each liter of water.

Air distribution within the tanks may be provided through the use of air diffusers, usually porous stones, located on the bottom of the tank or suspended in water.

2.1.3.4 TEMPERATURE ADJUSTMENT AND MAINTENANCE EQUIPMENT

A water cooling system is essential for maintaining water temperatures at optimal levels for each early life history stage of the red king crab. It is recommended to use in-line chillers with an auto digital temperature control system. In-line chillers designed to maintain water temperatures in large aquariums or fish holding systems of the capacities from 200 to 7000 L may be successfully used for crab rearing facilities. The components of the chiller include a titanium evaporator, a steam condenser, a compressor and a digital temperature control system, typically in a corrosion-resistant case. In a recirculating water system facility,

it is advisable to place a water cooling system outside the main facility room, if possible.

2.1.3.5 WATER PURIFICATION EQUIPMENT

As most other coldwater aquatic species, the red king crab requires excellent water quality throughout the whole rearing period. For water purification, various types of filters may be used, depending upon the culture system design.

Mechanical Filters

Mechanical filters are used both in flow-through systems to remove solid wastes from the water taken from the ocean, and in recirculation systems to remove wastes produced in the system (faeces, uneaten food and bacterial debris). In recirculation systems mechanical filtration may be done in conjunction with biological filtration. Solids should also be removed daily by siphoning the tanks.

The filters should be properly sized to match the desired flow rates and the size of solids to be removed. Several types of mechanical filters, which differ in construction and types of filter media, may be used. The most common types of mechanical filters used in flow-through water systems are sand or gravel filters. Filters with quartz sand or shungizite (an artificial carbonaceous-clayey material) may also be used. The shortcomings of these filters are (1) a considerable loss of water flow pressure in the filter media and (2) the necessity of backwashing on a regular basis in order to prevent the accumulation of organic material, which causes clogging and growth of potentially pathogenic bacteria. For filtration of finer particles (up to 0.1 μm) diatom filters and screen filters composed of saltwater-resistant materials may be used. One of the best solutions is to use a highly efficient reliable self-cleaning screen filter (Fig. 36).

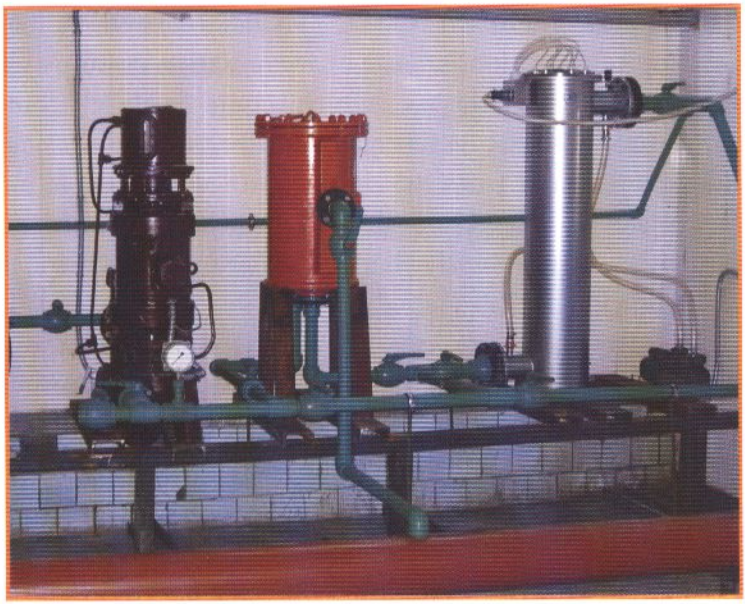


Figure 36. Self-cleaning screen filter (in the middle), of a crab rearing facility (Vidyaevo, Murmansk district, Russia).

This filter utilizes a simple hydraulic motor in a combination with a hydraulic piston to move cleaning nozzles across the screen during a cleaning cycle. When the screen becomes contaminated, a pressure differential across the screen is sensed causing the initiation of a cleaning cycle; even during the back-flush cycle, the clean water flow to the system is not interrupted. Self-cleaning screen filters may have various specifications, with the water flow ranging from 0.3 to 4000 cubic meters per hour and filtration ability ranging from 15 to 500 μm. These filters allow effective purification of water with initial solid wastes concentration of up to 5 g/L. Filtered water should not contain solid wastes in excess of maximum concentrations (see Table 6).

Biological Filters

Biological filtration is used in recirculation culture systems to remove nitrogen wastes, which come mainly from the ammonia produced by crabs and live feeds (e.g. *Artemia* sp.) during excretion and from the decomposition of organic matter. The nitrogen is converted from ammonia to nitrite and subsequently to nitrate by bacteria through the nitrification process.

The nitrification process is a sequential action of two groups of nitrifying bacteria. The first group oxidizes ammonia to nitrite and is represented by the genera *Nitrosomonas* and *Nitrosococcus*. The second group oxidizes nitrite to nitrate and includes the genera *Nitrobacter* and *Nitrococcus*. The use of biofilter media with large carrying capacity (high surface area to volume ratio) provides more area for bacteria to grow and thus favors the achievement of sufficient bacterial load.

Biofilters with their bacterial populations are "living" systems that must be activated, fed, and maintained. The performance of biofilters is known to depend on several factors. One of these factors is salinity: bacteria on filter media may die as a result of an abrupt salinity change. Another important factor is pH; in sea water closed recirculation systems, nitrifying bacteria act more efficiently in a range of pH from 7.5 to 8.2. Finally, water temperature is a very important parameter in the process of nitrification. In seawater systems the nitrification process is most efficient at 30-35°C. This fact should be taken into account while planning and designing filtration systems for red king crab rearing, as it requires low water temperatures; for example, coldwater systems will require the use of filter media with larger bacterial carrying capacity and a longer procedure of biofilter activation (i.e. prolonged start-up period).

In closed recirculation water systems for red king crab rearing it is advisable to use the submerged biolfilters supplied

with an aeration system (Fig. 37). In these filters, media surface area to volume ratio should be at least 300.

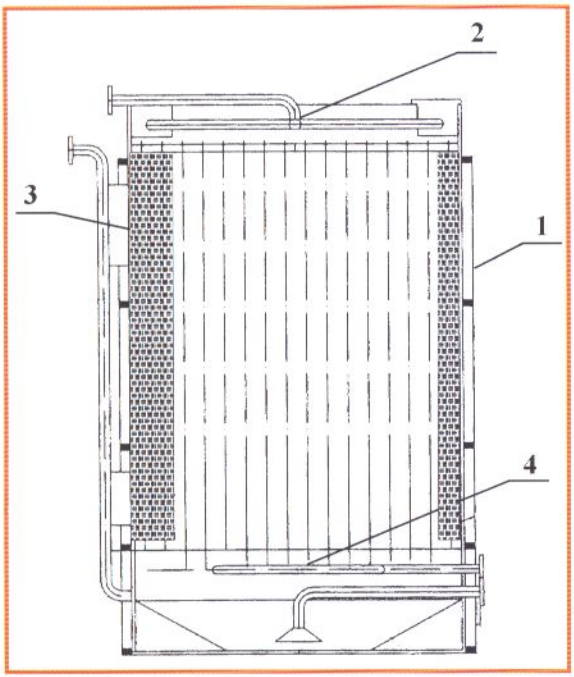


Figure 37. Working scheme of a submerged biofilter: 1 – filter case; 2 - sprinkler, 3 – filter media; 4 – aeration system.

The stabilization of new biofilter performance in a coldwater recirculation system commonly occurs in 75-80 days after the start of a filtration process. In a stabilized, balanced system ammonia concentrations do not exceed 0.1 mg/L [Kovatcheva and Kalinin, 2005; Kalinin et al., 2005].

Protein Skimmers

Foam fractionating units, or protein skimmers, are very important components of a coldwater recirculation water system. Protein skimmers are used in order to reduce the load of a

biological filtration system, stabilize its performance and thus maintain high water quality.

A protein skimmer unit works by fractionating water and causes protein, dissolved organic carbon waste (DOC) and fine particles, which are suspended in water, to be extracted in the form of foam. A protein skimmer unit contains a vessel in which a water flow is injected with tiny air bubbles; these bubbles have a weak electrical surface charge. Small bubbles adhere to a dissolved organic matter which then foams up a column and overflows into a collection cup. Concentrated wastes in a collection cup appear to have salinity of 40-42‰ and concentration of nitrogen wastes 1.7-2.5 times higher compared to the water in the tanks [Kovatcheva et al., 2005; Kovatcheva and Kalinin, 2005].

2.1.3.6 WASTE WATER TREATMENT EQUIPMENT

When holding and rearing tanks are cleaned, uneaten feeds, debris and other wastes (e.g. larval exuviae during molting periods) are siphoned with a small amount of water and removed from the system. It is possible to treat waste water with hypochlorite before discharge, in order to minimize environmental damage. Another option is to process this water in a mineralizer for compacting excess sludge; after that the water may be discharged.

2.1.3.7 SUPPLEMENTARY EQUIPMENT

In addition to the major facilities and equipment described above, many other items are required for the red king crab culture: a means of measuring salinity (preferably a refractometer), a pH-meter, dissolved oxygen meter, dissecting and compound microscopes, a balance, a freezer and a refrigerator for food storage, various types of pipes, pipettes, screens and containers.

2.1.4 GENERAL REQUIREMENTS AND EQUIPMENT MAINTENANCE

All equipment needs to be suitable for use in seawater systems and to be free from potential contamination from the leaching of metals such as copper, brass, or zinc.

The pipework system should preferably be made of plastic. Water and air supply pipes must be provided with flow regulation armature, such as valves or faucets. The drainage pipes system should provide for effective draining and be constructed in such a way that in case of emergency termination of water supply a minimal necessary amount of water still remains in the tank. At the same time the drainage pipes system design must provide for an opportunity to empty the tanks completely upon completion of the rearing cycle.

Where power supply is unreliable, an emergency generator should be available.

Maintaining the equipment, especially pumps, blowers and generators, in good working conditions is critical and, therefore, should include at least a weekly check-up.

2.2 CULTURE CONDITIONS

Creation and maintenance of optimal rearing conditions for each developmental stage are the very important prerequisites for a successful red king crab cultivation process. In this chapter, we describe main culture parameters, such as temperature, dissolved oxygen concentration, salinity, pH and water quality, in relation to specific requirements of red king crab larvae, postlarvae, and juveniles.

2.2.1 TEMPERATURE

The red king crab, like other crustaceans, is poikilothermic. Thus, the temperature of water directly affects the rate of all biological processes: metabolism, development, and growth.

Different life history stages have different water temperature requirements. When a recirculation culture system is used, the water temperature should be automatically controlled and maintained within an optimal range for a given life history stage (±1°C) because fluctuating temperatures are stressful for the crabs.

Embryonic Phase

The red king crab has a very long period of embryonic development (approximately 11 months), and therefore it is reasonable to begin artificial reproduction with the capture of ovigerous females when the eggs are at the late zoeal stage of the embryogenesis ("eyed embryo", see Fig. 5), i.e. shortly before hatching. However, it is also possible to capture the females earlier and keep them under laboratory conditions up to larval hatching [Kovatcheva, 2005a,b]. In the latter case, careful control and maintenance of constant water temperatures is especially important. The water temperature during embryonic development should preferably be as follows:

- from fertilization to nauplius stage (approx. 120 days) - 8°C;

- from nauplius stage to zoeal stage (approx. 200 days) – 6°C;
- from zoeal stage through late zoeal stage ("eyed embryo") and up to hatching – 3°C.

Thus, several weeks prior to hatching and during the hatching period the females should be kept at 3-4°C. After hatching is complete, the water temperature may gradually (1°C per day) be raised up to 7-8°C, which is an optimal temperature range for rearing red king crab larvae in a recirculation water system [Kovatcheva, 2002b].

Larval and Glaucothoe Phases

Our experimental data have proved that rearing red king crab larvae under laboratory conditions at 7-8°C allows shortening the duration of the larval development 1.8-2.0 times compared to natural rates and increasing survival of larvae up to 35% [for details see sections 1.2.1.3 and 1.2.1.4; Kovatcheva and Epelbaum, 2003; Epelbaum and Kovatcheva, 2005]. The effectiveness of biofilter production is much higher at 7-8°C compared to 3-4°C. Thus, we recommend maintaining the water temperature at the level of 7-8°C during the whole larval period (35-40 days) [Russian Federation Patent No. 2200386; Kovatcheva and Kalinin, 2005].

After the larvae moult to the postlarval (or glaucothoe) stage, the water temperature should gradually, 1°C per day, be raised up to 10-11°C. At this temperature, the glaucothoe stage lasts for approximately 18 days (177.7 degree-days) and results in the successful metamorphosis to the first juvenile stage.

Juvenile Phase

The optimal temperature for rearing red king crab juveniles in a recirculation system was found to range from 10 to 12°C. At this temperature, the juveniles' growth rate is approximately 2 times higher than that at 7-8°C, due to the shortening of intermoult periods. Short-time behavioral experiments indicate

that at water temperatures lower than 5°C and higher than 19°C, juveniles' moving and feeding activity drastically decreases [Borisov et al., 2004].

2.2.2 DISSOLVED OXYGEN CONCENTRATION

Oxygen is required for respiration, a physiological process in which cells oxidize carbohydrates and release the energy needed to metabolize nutrients from the feed. The dissolved oxygen level should be maintained at or above 70% during all phases of red king crab rearing. 74-88% saturation of water with dissolved oxygen is considered to be optimal for red king crab early life history stages. The solubility of oxygen in water is known to be the function of temperature, salinity, and altitude. At the temperature of 7-9°C and salinity of 32-35‰, 74-88% saturation corresponds to 7.0-8.4 mg/L; 3.3 mg/L is a lethal dissolved oxygen concentration for red king crab larvae.

The maintenance of the necessary dissolved oxygen concentration is provided by water flow and aeration systems. These systems must be carefully designed to avoid "dead" areas, where stale unsaturated water can accumulate. All operating tanks should be supplied with air at a minimum level of 0.5-0.7 liter of air per hour for each liter of water. Dissolved oxygen concentrations should be closely monitored at least once a day.

2.2.3 SALINITY

The red king crab is a stenohaline species adapted to a relatively narrow range of salinities, from 32 to 35‰. The tolerance to salinity changes throughout its life cycle, being relatively high at early life history stages: eggs before hatching can tolerate salinity ranging from 23 to 47‰, larvae and postlarvae - from 27 to 40‰, early juveniles - from 30 to 40‰

[Nakanishi, 1987]. At the salinity of 23‰ adult crabs die within 3 hours [Orlov, 1998].

During the whole cycle of the red king crab culture water salinity should preferably be kept at a range of 32.0-34.0‰ [Russian Federation Patent No. 2200386], which corresponds to natural seawater salinities in this species' area of distribution in the North Pacific Ocean and the Barents Sea.

While using flow-through culture systems, a natural water intake area should be carefully selected, so that it is not too close to estuaries, where the salinity is lowered.

In a recirculation culture system, artificial seawater is often used. Artificial seawater is prepared from dry salt mixtures. Unfortunately, not all commercially available salt formulations are equally efficacious and properly formulated for larval crustaceans. For red king crab rearing, artificial salt mixtures "HW Marinemix Professional" (Wiegandt GMBH, Germany) and SERA Premium (Sera, Germany) proved to be most effective [Russian Federation Patents No. 2200386 and No. 2261594].

For artificial water preparation, a salt mixture is diluted with previously filtered freshwater, preferably at 24-26°C until the desirable salinity of 32-34‰ is attained. Salinity may be measured by means of a densitometer, conductivity meter or a refractometer. Water should be thoroughly agitated with stirrer or through intense aeration. The final solution should be left in a preparation tank for about 2 weeks. Before use, the water should be maintained under strong aeration for 24 hours and checked for salinity.

Salinity in culture tanks may change during the cultivation process due to evaporation. Thus, salinity should be constantly monitored and periodically adjusted, if necessary. Each time new water is added to the system, its salinity should first be adjusted in water preparation tanks.

2.2.4 HYDROGEN ION CONCENTRATION (pH)

For the red king crab culture, pH of 7.5-8.2 is recommended, especially when using a closed recirculation system. In this range of seawater pH biofilters function more effectively, as most nitrifying bacteria are adapted to this range. Fluctuations in pH should be monitored periodically, at least once a week.

2.2.5 LIGHT INTENSITY AND DURATION

Red king crab females were observed to avoid direct natural and artificial light. Therefore, it is advisable to rear them at low light intensities, preferably around 1000 lux; shaded areas in the rearing tank should also be provided.

Larvae, postlarvae, and early juveniles of the red king crab may be reared at the 1000-2000 lux light intensity. The data of Nakanishi [1987] suggest that at 1000 lux the photoperiod (i.e. periods of darkness and light exposure) does not affect the survival and growth of red king crab larvae. However, in our experiments a cyclic light regime led to more effective consumption of live feeds, which in turn led to better water quality in rearing tanks and enhanced larval survival. Therefore, we recommend the cyclic light regime (8 h light, 16 h dark) during the red king crab early life history stages culture.

As long as red king crab larvae are positively phototactic [Shirley and Shirley, 1988], it is advisable to illuminate rearing tanks/aquaria as evenly as possible. The bottoms of transparent aquaria should preferably be blacked out. Luminescent lamps of 1000-2000 lux may be mounted 1.5-2 meters above each rearing tank. These measures will reduce the probability of larvae crowding in more illuminated areas and/or on the bottom of the tank and thus reduce the intensity of cannibalism (see section 1.2.1.4).

2.2.6 WATER QUALITY

The suitability of water for aquaculture is determined by the concentration of dissolved inorganic ions, dissolved gases, suspended solids, dissolved organic compounds and microorganisms. Water quality directly affects the survival, growth and health of cultured individuals. The quality of water is a dynamic characteristic, changing over time as a result of environmental factors and biological processes.

Initial water quality is related to the source of water. When using natural seawater, water analyses in a potential water intake area should be done first, in order to profile the chemical composition and detect any abnormalities or potential toxins. Basic water quality requirements of the red king crab are listed in Table 6. Chemical characteristics of water should comply with the existing water quality standards [List of fisheries standards..., 1999].

Most water quality parameters can not be economically modified, so the water in the intake area should preferably be within acceptable limits initially. The presence of toxic compounds, such as pesticides, herbicides, and heavy metals, disqualify the site as a potential water source.

Table 6. Water quality requirements for the red king crab culture.

Water quality parameter	Recommended range
Suspended solids	≤0.25 mg/L
Floating oils, fats	Not allowed
Temperature	7-11°C
pH	6.5-8.5
Salinity	30-35‰
Dissolved oxygen	≥6 mg/L
Biochemical oxygen demand (BOD)	≤3.0 mg/L

When a recirculation system with artificial seawater is chosen, the risk of pollution is considerably reduced. However, in recirculation systems ***the maintenance of water quality*** during the culture process is more challenging. Water quality may be altered by physical processes, biological processes, such as respiration and metabolic wastes excretion, and even management strategies, such as overfeeding that leads to eutrophication of the system. Thus, water quality variables should be monitored on a regular basis (at least once a week). Nitrogenous waste product concentrations should be monitored most carefully. Safe levels of nitrogenous waste products for the larval crustacean culture are given in Table 7.

Table 7. Safe levels of nitrogenous products for the red king crab culture (all values are given in mg/L).*

Unionized ammonia (NH_3)	Ammonium ions (NH_4)	Nitrite ions (NO_2)	Nitrate ions (NO_3)
0.05	0.26-0.5	0.08-0.2	20-40

** Specific toxicity tests with red king crab larvae have never been conducted, and thus safe levels of nitrogenous products for the red king crab remain largely unknown to date. In this table, the ranges of safe levels known for the larvae of some other marine decapod crustacean larvae are given (penaeid shrimps, Chinese mitten crab) [Jayasankar, Muthu, 1983a,b; Chen, Chin, 1988; Ostrenzky, Poersch, 1992; Gao, Zou, 1994; Zou, Gao, 1994; Zhao et al., 1997; Tsai, Chen, 2002].*

Even with well-established biofilters, high ammonia and/or nitrite levels may occasionally develop in culture tanks. When this occurs, the water should be partially or fully replaced. In addition, the cause of the problem should be determined (e.g. overfeeding, declined biofilter efficiency) and corrective measures taken. Otherwise, nitrogenous product concentrations will quickly return to high levels [Kovatcheva and Kalinin, 2005].

2.3 CULTURE OPERATIONS AND TECHNIQUES

In this chapter, we describe main culture operations and methods that proved to be effective and convenient during our experimental work. Operations and techniques are described for each stage of the culture process (see Fig. 29), in the consecutive order.

2.3.1. CAPTURE AND HANDLING OF OVIGEROUS FEMALES

2.3.1.1 CAPTURE

It is expedient to start the artificial reproduction with the capture of ovigerous females when the eggs are at the late zoeal stage of the embryogenesis (see Fig. 5), i.e. several weeks before hatching. In the North Pacific Ocean, the females should be caught in late March or early April, whereas in the Barents Sea it is better to catch the females no later than at the end of February, when the embryos are about 300 days old.

Female crabs may be collected by scuba divers or captured by standard king crab pots (rectangular or conical) set at a depth from 30 to 70 meters.

After being caught, the females have to be carefully sorted: those that are too young, too old or already have newly extruded clutches of eggs should be rejected and released. The females should be thoroughly examined with regard to apparent disease infections and to the presence of visible parasites, especially on egg masses. Inactive females and those with any sort of bodily injury or damage should also be rejected as unsuitable and released back into the sea.

2.3.1.2 TRANSPORTATION

Figure 38. Isothermal container for crabs' transportation.

After selection, healthy ovigerous females can be delivered to the shore by boat, preferably in containers with flow-through water supply.

If the females have to be delivered to a laboratory facility located far from the port, they can be transported in isothermal containers supplied with a water aeration system (Fig. 38). For transporting 2 or 3 females, a container of 60-80 L capacity may be used. Ideally, transportation time should not exceed 20 hours.

Ovigerous females and their eggs are very sensitive to water temperature fluctuations. Therefore, water temperature during transportation should not differ significantly from the water temperature in the area, where the females were caught, and should preferably be held relatively constant in a range from 1.5 to 3.0°C. It is possible to use small amounts of ice to keep the temperature constant.

2.3.1.3 ACCLIMATION

After the females are delivered to the laboratory, they should be transferred to holding tanks. The water temperature in the tanks should be adjusted to be the same as in the transportation container, in order to minimize stress. Afterwards it is recommended to keep the water temperature at 3-4°C. The

drainage pipes of the tanks should be protected by nylon mesh screens (ca 750 μm) that will retain hatching larvae. Reliable 24-hour water aeration is required, usually provided by the use of a standby blower; air distribution within the tank is ensured through the use of porous stones.

Rearing density for the females during the acclimation period should not exceed 4 individuals per m^2. However, individual holding of females is advisable, if possible.

During the acclimation, it is desirable to take bacteriological samples in order to diagnose and prevent possible diseases. The acclimation period takes from 3 to 5 days.

2.3.1.4 HANDLING UP TO LARVAL HATCHING

After the end of the acclimation period it is recommended to gradually increase the water temperature, 1°C per day, up to 7–8°C. Dissolved oxygen concentration should not drop below 7-8 mg/L. If the duration of the handling does not exceed 2 weeks, the females typically do not have to be fed; otherwise they may be fed with mussels, squid or fish meat at a daily rate of 1% of total biomass.

Approximate timing of hatching can be predicted by carefully observing the female's behavior. Two or three times a day a female ventilates the eggs by slightly pulling down the abdomen and moving the pleopods; these movements provide a steady flow of oxygen to the developing eggs (Fig. 39). Shortly before hatching the female can be observed ventilating the eggs for 1 or 2 minutes several times an hour. Monitoring of the females' behavior thus provides an opportunity to predict the beginning of hatching, which is very important in terms of larval survival [Kovatcheva, 2002b].

Figure 39. The female ventilating the eggs.

When the females are held under optimal conditions, the hatching rate comprises 95-100%.

2.3.1.5 RELEASE BACK TO THE OCEAN

Once hatching has occurred, the females should be released back to the area where they were caught. Prior to transportation the females should be acclimated to the water temperature in their natural habitat at the time of release, as it may differ from the handling conditions. The females can be transported in the same containers and under the same conditions as described in section 2.3.1.2.

2.3.2 LARVAL CULTURE

Red king crab larvae are especially vulnerable to stress, i.e. fluctuations of environmental factors. Therefore, during the whole larval phase there should be monitoring and maintaining at optimal levels of culture conditions and parameters, first of all:

- ✓ water quality parameters
- ✓ rearing densities
- ✓ feeds quality and availability.

2.3.2.1 COLLECTION, ENUMERATION, TRANSFER TO REARING RESERVOIRS

Larvae may be reared in the same tanks where they hatched, or they may be transferred to special rearing tanks or aquaria. It is advisable to use glass/fiberglass aquaria of 150-200 L capacity for the larval culture, as it allows a more precise control of the rearing process (see Fig. 35). When small amounts of larvae are reared for experimental purposes (e.g., behavioral observations), it is convenient to use glass beakers of various volumes positioned in a temperature-controlled aquaria.

A collection procedure is based on the larval positively phototactic nature. When small and precise amounts of larvae have to be collected for experimental purposes, it proved to be convenient to catch and transfer the larvae by means of a large-bore pipette or a short glass tube. In order to successfully collect and transfer a big amount of larvae for mass-culture, the following sequence of actions is recommended:

1. Before transferring the larvae to a new reservoir, adjust the water temperature in this reservoir, so that it is equal to the temperature in the holding tank (usually around 4°C)
2. Turn on the light source, so that the central area of the holding tank becomes most illuminated
3. Turn off the main lights in the room and wait until the larvae concentrate in the spotlight
4. Carefully scoop the larvae with a glass or a soft mesh net (mesh size 0.5 x 0.5 mm) and transfer them to a light-colored collecting container of the 2-3 L capacity; count the number of the larvae in the container and carefully release them into the rearing reservoir
5. Repeat step 4 until the desired larval density in the rearing reservoir is attained; it is recommended to count all the

larvae in the collecting container for 3 or 4 times; afterwards, the number of the larvae can be roughly assessed by eye

6. Gradually increase the water temperature in the rearing reservoir up to 7-8°C (1°C per day).

Red king crab larvae are highly cannibalistic even at the first zoeal stage [Borisov et al., 2005]. Therefore, ideally in mass-culture the rearing density should not exceed 50 larvae per liter [Russian Federation Patent No. 2200386].

2.3.2.2 FEEDING

As described in section 1.2.1.2, brine shrimp (*Artemia* sp.) nauplii are up to date the best food for laboratory reared red king crab larvae. This most widely used diet in marine and freshwater larviculture, being nutritious, convenient, and the least labor-intensive live food [Lavens and Sorgeloos, 1996], successfully elicits feeding response of red king crab larvae and meets their nutritional requirements. Therefore, in this section we describe feeding techniques when *Artemia* nauplii are used as food.

Hatching of *Artemia* nauplii

Artemia nauplii are obtained by incubation of dormant embryos, the so-called cysts. These cysts are harvested in large quantities from natural and semi-natural biotopes and, after processing and drying, are made available as commercial storable, "on-demand" feed. The cysts should be stored in a refrigerator, preferably at 0-4°C.

Although incubation of *Artemia* cysts is basically very simple, several parameters need to be taken into consideration for the successful hatching, such as:

- ✓ water temperature 25-27°C
- ✓ salinity 30-50‰
- ✓ dissolved oxygen concentration ≥ 6-7 mg/L.

The best hatching results are achieved in cylindrical containers with a conical bottom, aerated from the bottom. Flat-bottom containers have "dead spots" in which *Artemia* cysts and nauplii accumulate and suffer from oxygen depletion. The volume of the containers depends on desired production and may range from 2 to 100 L; they should preferably be transparent to facilitate inspection of the hatching suspension. In order to have constant supply of freshly hatched nauplii, it is required to have 2 groups of hatching containers loaded at 12-h intervals.

Incubation of *Artemia* cysts includes as follows (Fig. 40):

- preparation of incubation solution (1)
- stocking hatching containers with the cysts (2)
- incubation of the cysts under vigorous aeration for 24-36 h (3)
- settling of hatching suspension components, allowing the separation of nauplii from cyst shells (4)
- rinsing and restocking hatching containers.

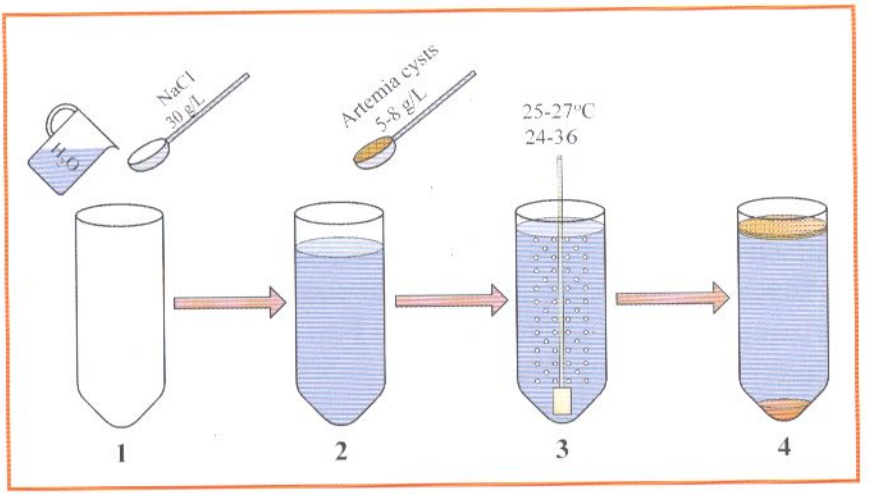


Figure 40. Incubation of Artemia cysts.

Before incubation, a disinfection procedure, i.e. the soaking of the cysts for 30 min in 2000 mg/L hypochlorite solution [Lavens and Sorgeloos, 1996], needs to be applied, unless commercially disinfected cysts are used.

The incubation solution is commonly prepared with sodium chloride (non-iodized table salt) and should have the salinity of 30-50‰. Natural seawater may also be used as the incubation solution. The temperature of the incubation solution should preferably be kept in the range of 25 to 28°C; otherwise, below 25°C the cysts hatch more slowly and above 33°C the cyst metabolism is irreversibly stopped. The aeration intensity must be sufficient to maintain oxygen levels above 5 mg/L. The optimal aeration rate is a function of the hatching container size and the density of the cysts incubated. Excessive foaming can be reduced by adding a few drops of a non-toxic antifoam agent (e.g. silicone antifoam). Strong illumination (not less than 2000 lux at the water surface) is very important, especially during the first hours after complete hydration, in order to trigger the start of embryonic development. The cyst density may be as high as 4-8 g/L in small volumes provided that hatching conditions are carefully maintained and controlled.

After hatching is complete, the nauplii need to be separated from the hatching wastes. By switching off the aeration in hatching containers, the separation of suspension components is attained: in approximately 10 minutes, cyst shells will float on the surface, while nauplii will concentrate near the bottom. This way the nauplii can be siphoned off to a special separation device (Fig. 41).

The method of *Artemia* nauplii separation from cyst shells is described in detail in our patent [Russian Federation Patent No. 40841]. The method is based on attracting the nauplii by a light stimulus, as they are strongly positively phototactic. It helps to

obtain a pure suspension of active nauplii in a relatively easy way.

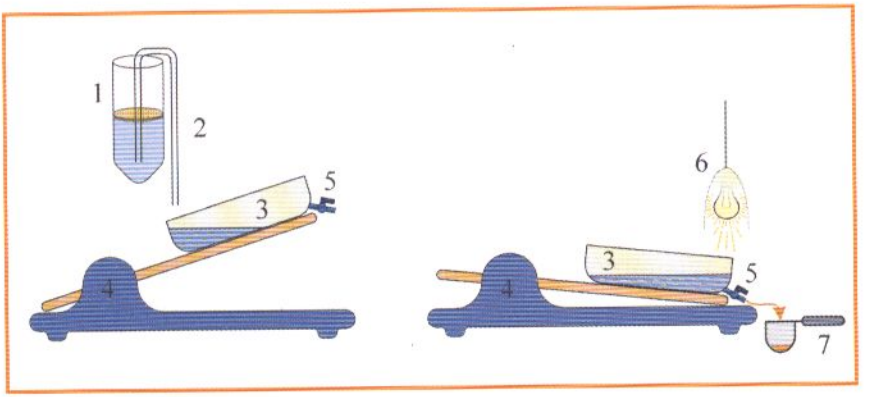


Figure 41. Nauplii separation device: 1 – hatching container, 2 - siphon, 3 – separation container, 4 - stand, 5 - faucet, 6 – light source, 7 – net.

Considerable variations in the hatching rate and maximum output have been observed among cysts of various origins. Attention should be paid to selecting cysts with a good hatching rate (less than 38 hours, preferably 24) and hatching synchrony. An important factor affecting the nutritional value of *Artemia* is the content of essential fatty acids, eicosapentaenoic acid (EPA: 20:5n-3) and even more importantly docosahexaenoic acid (DHA: 22:6n-3). Most marine organisms do not have the capacity to biosynthesize these EFA from lower chain unsaturated fatty acids, such as linolenic acid (18:3n-3), and thus they need to receive these EFA with food [Watanabe et al., 1991; Tamaru, 1999; Suprayudi, 2004]. In *Artemia* nauplii incubated for 48 hours, the content of these acids is 2 times lower than in those incubated for 24 hours due to the energy loss. Therefore, freshly hatched nauplii with a high hatching rate and hatching synchrony should preferably be used as food for larvae. If the use of freshly hatched nauplii is not possible, they may be stored at a temperature

below 10°C in densities of up to 8 million per liter in aerated containers with seawater. In this way, the nauplii can be stored for periods up to 24 h without significant mortalities and a reduction of energy for less than 5% [Lavens and Sorgeloos, 1996]. This procedure helps to maintain a constant reserve of live feed which may be used when freshly hatched nauplii are not available.

Feeding Regime

The recommendations on feeding red king crab larvae given below were outlined taking into consideration the larval feeding behavior and daily food intakes described in section 1.2.1.2.

The feeding should start on the first day of hatching. Ideally, the larvae should be fed twice a day, with 12-hour intervals between feedings. The number of *Artemia* nauplii required for one feeding can be calculated with the following equation:

$$X = V(\frac{MN}{2w} + 160), \text{ where:}$$

X is the number of nauplii required for one feeding;
V is the volume of a rearing reservoir (L);
M is the maximum daily food intake for a certain zoeal stage, expressed as dry weight (47.46, 94.08, 139.44 and 175.56 μg/ind per day for zoeae I-IV, respectively);
N is larval rearing density (ind/L);
w is *Artemia* nauplii individual dry weight (μg).

This equation makes it possible to calculate the amount of nauplii required for one feeding, depending on the tank volume, larval stage and rearing density.

Maximum daily food intakes of the larvae (***M***) reared at 7-8°C are 47.46, 94.08, 139.44 and 175.56 µg/ind per day for zoeae I-IV, respectively (for details, see section 1.2.1.2, Table 2).

Individual dry weight of *Artemia* nauplius (***w***) may be determined by the direct weighing of the counted number of the nauplii. Biometrical characteristics of the nauplii vary significantly between strains, but generally remain rather constant within the strain. Data on nauplii dry weight from many *Artemia* cyst sources around the world are available in published literature [e.g, Gusev, 1990; Mikulin, 1994; Lavens and Sorgeloos, 1996].Therefore, when it is not possible to determine nauplius weight directly, but the cyst source is known, the literature data may be used. For example, according to the data of Gusev [1990], average dry weight of *Artemia* nauplii from lakes in Russia comprises 3.0 µg.

Table 8 shows sample calculation of the number of the nauplii required for one feeding of the larvae reared in a 200-L aquarium at a density of 50 ind/L.

Table 8. Sample calculation of the number of Artemia nauplii required for one feeding of red king crab zoeae reared at 8°C (V=200 L, N=50 ind/L, w=4.2 µg).

Larval stage	Number of nauplii required for 1 feeding	Nauplii initial concentration in a rearing reservoir, nauplii/L	Number of nauplii consumed by larvae in 12 h	Nauplii concentration by the time of next feeding, nauplii/L
Zoea I	88 500	442.5	56 500	160
Zoea II	144 000	720.0	112 000	160
Zoea III	198 000	990.0	166 000	160
Zoea IV	241 000	1205.0	209 000	160

As a result of using this calculation mode, by next feeding the concentration of the nauplii will approximately be 160 nauplii/L, i.e. equal to the minimum non-consumable concentration, when successful feeding ceases (see section 1.2.1.2 "Daily Food Intakes")

The number of the nauplii used for feeding should be adjusted after each moulting, in accordance with the maximum food intake (*M*) of a corresponding larval stage. During the culture process, larval density is gradually decreasing due to mortality (see Fig. 17). Thus, the number of the nauplii used for feeding should also be regularly adjusted in accordance with the changes in larval rearing density. This periodical adjustment of feeding rations is very important, as a considerable excess of food concentrations results in the increase of toxic nitrogenous products in the water, leading to high mortality.

Feeding Procedure

When the larvae are mass-cultured in large reservoirs, the feeding procedure should preferably include the following sequence of actions:

1. Turn on the light source, so that the upper water level is illuminated in the best way
2. Turn off the main lights in the room
3. Stop water circulation through the rearing reservoir
4. Place a required amount of nauplii into the rearing reservoir, so that they are distributed as evenly as possible
5. In 10 to 15 minutes restart water circulation, turn off the light source and turn on the main lights in the room.

2.3.2.3 CLEANING OF REARING RESERVOIRS

Bottom debris (excessive food and exuviae) should be removed from rearing reservoirs by siphoning at least once a day,

prior to feeding. It is convenient to siphon the debris to a shallow, preferably light-colored container of 5-10 L capacity. Live larvae can then be transferred back to a rearing reservoir. Dead larvae and exuviae may be examined under the microscope in order to get an idea of possible mortality causes, determine developmental stages, take measurements or conduct relevant morphological studies.

During the phases when mortality is higher than usual (e.g. after transferring the larvae to new tanks), during moulting periods, and when overfeeding occurs, the reservoirs should be cleaned 2 or 3 times a day, depending on the amount of bottom debris. Protective mesh screens on drainage pipes should also be cleaned, when necessary.

2.3.3 REARING OF GLAUCOTHOES

Glaucothoes may be reared in the same reservoirs where the larvae were cultured. In this case, it is recommended to place appropriate substrates into the rearing reservoirs in advance (1 or 2 days prior to the beginning of moulting to the glaucothoe stage). The asynchrony of moultings increases during the larval phase, and the period of moulting to the glaucothoe stage may last for up to 7 days. Thus, during this period the larvae and glaucothoes occur simultaneously in the rearing reservoir. Well-timed placing of suitable substrates allows for considerable reduction of glaucothoes mortality due to larval cannibalistic behavior.

2.3.3.1 PREPARATION OF SUBSTRATES

The substrates for glaucothoes should meet the following requirements:

- ✓ they should be non-toxic

✓ they should not significantly impede reservoir cleaning procedures

✓ their texture should allow the glaucothoes to grab it and hold on it easily.

The substrates that were found to better meet these requirements are a nylon mesh (0.5 mm) stretched over a plastic framework and flat mats of plastic thread (e.g. used as mechanical filter media). These substrates may be oriented both horizontally and vertically; during a substrate preference experiment, around 80% of glaucothoes preferred horizontally oriented flat mats of plastic thread (for details see section 1.2.2.3). Therefore, safe and easy maintenance nylon mesh screens and mats of plastic thread (see Fig. 23) can be recommended as suitable substrates for rearing red king glaucothoes. Stones, bricks, corals, artificial water plants and other items meeting the requirements stated above may be used as additional substrates.

2.3.3.2 TRANSFER TO REARING RESERVOIRS

In cases when it is planned to rear glaucothoes in other reservoirs, it is advisable to transfer them gradually as they appear. New reservoirs should be supplied with the substrates; the water temperature should be adjusted so that it is equal to the temperature in a larval rearing reservoir (7-8°C). Glaucothoes can be carefully siphoned or caught by means of a large-bore pipette or glass pipe. After all glaucothoes are transferred to the new reservoir, the water temperature should be increased step-by-step up to 10-11°C (1°C per day).

2.3.3.3 FEEDING

As glaucothoes are fully lecithotrophic (see sections 1.2.2.1 and 1.2.2.2), they should not be fed.

2.3.3.4 CLEANING OF REARING RESERVOIRS

Regular cleaning of rearing reservoirs is not required during the glaucothoe phase, because food is not provided. When necessary, the bottom of the rearing reservoir should be cleaned following the same procedure as that during the larval phase.

2.3.4 REARING OF JUVENILES

First-stage juveniles are considerably more viable than zoeae and glaucothoe, but they exhibit strong cannibalistic behavior and thus are highly prone to mortality due to cannibalism, especially when reared in mass culture at high densities. Therefore, when the red king crab is cultured with the aim of repopulating depleted natural stocks, it is expedient to release juveniles into the ocean when they are at the first developmental stage.

2.3.4.1 REARING RESERVOIRS, SUBSTRATES, AND TRANSFER METHODS

When it is planned to keep red king crab juveniles in the laboratory for the experimental purposes that do not require mass-culture (e.g. behavioral observations, studies of feeding rations, morphological studies), we recommend rearing of the juveniles in individual compartments, or cells (Fig. 42). This rearing mode completely eliminates the possibility of cannibalism and therefore favors a high survival rate, up to 100%.

An important factor for the successful rearing of the juveniles is the availability of the suitable substrate which the juveniles can easily grasp and walk on. The juveniles may be simply reared in cells with rugged bottom (as in Fig. 42). Another option is to use cells with smooth bottom, but place additional substrates into them (e.g. flat mats of plastic filament, see Fig. 23).

When the juveniles are reared in mass-culture, we recommend using the substrates that maximize the rearing reservoir volume usage, e.g. loosely arranged thick mats of plastic thread. This will ensure more even distribution of the juveniles and reduce the level of cannibalism.

Figure 42. Cells for rearing the juveniles (individual water supply).

Individuals may be transferred to rearing reservoirs both before the metamorphosis (at the stage of glaucothoe) and after the metamorphosis. It is possible to transfer them together with pieces of the substrate on which they settled, or by capturing them with a wide-bore pipette or a glass tube of an appropriate diameter (≥ 5 mm).

2.3.4.2 FEEDING

As described in section 1.2.3.2, raw meat of marine invertebrates (squid, shrimp, and mussels) was found to be the most appropriate food for the red king crab juveniles reared under laboratory conditions. Feeding should be started on the first day after the metamorphosis. The juveniles should preferably be fed twice a day, with 12-hour intervals between feedings. Prior to a feeding, the food should be cut in small pieces.

2.3.4.3 CLEANING OF REARING RESERVOIRS

Rearing reservoirs should be cleaned once a day, prior to the first feeding. Uneaten food, dead individuals and exuviae should be siphoned into a cleaning container. Dead individuals and exuviae may then be examined under a microscope for taking measurements, determining possible mortality causes, and conducting morphological studies. After that, the exuviae should preferably be placed back to rearing reservoirs, as the juveniles can partly consume them in order to regain the necessary calcium lost in the molting process.

2.3.4.4 TRANSPORTATION AND RELEASE TO THE OCEAN

The juveniles can be transported in isothermal containers supplied with a water aeration system of the same type as that for female crabs' transportation (see Fig. 7). Prior to the transportation, the juveniles should be acclimated to the water temperature in their natural habitat at the time of release, as it may differ from the rearing conditions. The juveniles may be transferred (1) together with pieces of the substrate on which they settled, (2) by carefully siphoning them at a low-speed water flow, or (3) by capturing them with a wide-bore pipette or a glass tube of an appropriate diameter (≥ 5mm). In any case, the transportation container should be filled with appropriate substrates to maximize its volume usage: pieces of nylon mesh, plastic thread mats, etc. The transportation should preferably take less than 24 hours.

The juveniles may be released at previously selected sites, which should have enough natural shelters or prepared artificial substrates (collectors, reefs, etc.).

2.4. BIOLOGICAL AND TECHNICAL PARAMETERS FOR MASS-CULTURE OF THE RED KING CRAB FROM THE EGG TO VIABLE JUVENILE STAGES (Summary)

№	PARAMETER	VALUE	COMMENTS
Phase 1. Capture and Handling of Ovigerous Females			
		1a. Transportation	
1.	Capture time, month	Late February*; Early April**	i.e. 10 to 15 days prior to expected hatching
2.	Transportation duration	≤ 20 h	
3.	Transportation density	≤ 40 kg/m^3	
4.	Transportation container volume	40-60 L	For transportation of 2-4 individuals
		1b. Handling	
5.	Handling density	≤ 4 ind/m^2	
6.	Handling duration	10-15 d	
7.	Water flow rate	≥ 5 L/min	
8.	Water exchange rate	300 L/volume per hour	
9.	Water temperature	3-4°C	
10.	Salinity	32-35‰	
11.	Daily feeding ration	None	1% of biomass per day when handling duration exceeds 2 weeks
12.	Hatching rate	95-100%	i.e. 200 000 larvae on average
Phase 2. Larval Culture			
13.	Hatching time	Middle March* Early April**	

№	PARAMETER	VALUE	COMMENTS
14.	Rearing density	≤ 50 ind/L	
15.	Zoeal phase duration: Zoea I Zoea II Zoea III Zoea IV	 9-10 9-10 8-9 10-11	Total zoeal phase duration: 36-40 days/284 degree-days
16.	Daily ration per zoea (*Artemia* nauplii): Zoea I Zoea II Zoea III Zoea IV	 11.3 nauplii/d 22.4 nauplii/d 33.2 nauplii/d 41.8 nauplii/d	
17.	Number of feedings per day	2	
18.	Water temperature	7-8°C	
19.	Salinity	32-35‰	
20.	Light intensity	1000-2000 lux	Even illumination is desirable
21.	Water flow rate	≥ 1 L/min	
22.	Water exchange rate	120 L/volume per hour	
23.	Survival up to glaucothoe stage	35-40%	
Phase 3. Rearing of Glaucothoes			
24.	Rearing density	25 ind/L	Substrate is required
25.	Glaucothoe phase duration	18-20 days/177-200 degree-days	
26.	Daily ration per glaucothoe	None	Glaucothoe is fully lecithotrophic
27.	Water temperature	10-11°C	

№	PARAMETER	VALUE	COMMENTS
28.	Salinity	32-35‰	
29.	Light intensity lux	500-1000 lux	Even illumination is desirable
30.	Water flow rate	≥ 1 L/min	
31.	Water exchange rate	120 L/volume per hour	
32.	Survival from first stage zoea to first stage juvenile	30-35%	
Phase 4. Rearing and Release of Juveniles *4a. Rearing*			
33.	Rearing density	1000-1500 ind/m^2	Rearing in individual cells is recommended, if possible. For mass-rearing substrates are required.
34.	Duration of the first juvenile stage	20-25 days	
35.	Daily feeding ration (squid, mussels, shrimp meat)	15-20% of biomass	
36.	Number of feedings per day	2	
37.	Water temperature	10-12°C	
38.	Salinity	32-35‰	
39.	Water flow rate	≥ 5 L/min	
40.	Water exchange rate	300 L/volume per hour	
41.	Survival from first stage zoea to second stage juvenile	25-30%	For individual rearing-up to 35%

№	PARAMETER	VALUE	COMMENTS
	4b. Transportation and release to the ocean		
42.	Time of release	Early July*; Early August**	
43.	Transportation container volume	≤ 1 m^3	Substrate is required
44.	Transportation duration	≤ 10 h	In aerated containers – up to 24 h
Juveniles may be released at previously selected sites, which should have enough natural shelters and food or prepared artificial substrates (collectors, artificial reefs, etc.).			

*Notes: * - for the Barents Sea; ** - for the Sea of Japan.*

CONCLUSION

The investigations on the red king crab early development described in this book made it possible to establish feasible culture methods and techniques which allowed us to obtain viable juveniles from eggs in approximately 2 months, with the overall survival of 30-35%. This implies that artificial reproduction and rearing of red king crab larvae provides for increasing the effectiveness of recruitment approximately 300 times compared to natural rates. Therefore, the culture methods and techniques developed and described in this book may form a basis for a red king crab restocking program through aquaculture, i.e. through releasing the juveniles reared from eggs to the areas where red king crab populations are depleted. Such program is planned to be implemented at red king crab raising facilities in the Russian Far East and the Barents Sea [Kovatcheva, 2005a]. In order to enhance the effectiveness of restocking activities, future studies should be directed at selection of sites and habitats suitable for release, development of reliable methods for monitoring the juveniles and investigation of their behavioral patterns in their new environments.

The data obtained in the course of culture experiments have also broadened the knowledge of the red king crab early development. Laboratory culture experiments represent a useful method for studying various aspects of the early development, as they allow precise determination of stage duration. Unlike field studies, they provide an opportunity to accurately relate morphological, physiological and biochemical changes during a life cycle to results of a specific factor influence, which can be modified while other conditions remain controlled and constant. Some of our data obtained in the laboratory may further be extrapolated to field conditions. For example, the data on feeding

rations of the larvae, coupled with the data on spawning time and plankton production in certain areas of the ocean, may help to assess and analyze the trophic role of red king crab larvae in natural ecosystems. Advances in the knowledge of the red king crab early development may aid in the development of fisheries strategies and efficient conservation management plans.

ANKNOWLEDGEMENTS

The authors are indebted to Dr. Boris N. Kotenev, the director of the Russian Federal Research Institute of Fisheries and Oceanography (VNIRO) for his help and support of the research program.

We are very grateful to Dr. Urii Orlov, the supervisor of the project of red king crab acclimatization to the North Atlantic in 1961-1969, for inspiring ideas; Dr. Bradley G. Stevens (Kodiak Fishery Research Center, NMFS, NOAA) and Dr. Jiro Kittaka (Research Institute for Marine Biological Science, Science University of Tokyo) for helpful discussions and sending rare literature sources to us.

We would like to thank A. Parshin-Chudin, N. Kryakhova, A. Tertitskaya and others at the Laboratory of Crustacean Reproduction of VNIRO for assistance in the course of culture experiments; Drs. V. Sapojnikov, N. Lapina, O. Luk'yanova and others at the Laboratory of Marine Ecology of VNIRO for chemical analyses of culture water. We are also grateful to everyone who collaborated with our laboratory during the studies on biology and culture of the red king crab.

REFERENCES

Abrunhosa F.A. and Kittaka J., 1997a. Morphological changes in the midgut, midgut gland and hindgut during the larval and postlarval development of the red king crab, *Paralithodes camtschaticus*. *Fisheries science (Tokyo)*, **63** (5), 746-754.

Abrunhosa F.A. and Kittaka J., 1997b. Functional morphology of mouthparts and foregut of the last zoea, glaucothoe and first juvenile of the king crabs *Paralithodes camtschaticus, P. brevipes* and *P. platypus*. *Fisheries science (Tokyo)*, **63** (6), 923-930.

Anger K., 1989. Growth and exuvial loss during larval and early juvenile development of the hermit crab *Pagurus bernhardus* reared in the laboratory. *Marine Biology*, **103**, 503-511.

Anger K., 1996. Physiological and biochemical changes during lecithotrophic larval development and early juvenile growth in the northern stone crab, *Lithodes maja* (Decapoda, Anomura). *Marine Biology*, **126**, 283-296.

Berkes F., 1975. Some aspects of feeding mechanisms of euphausiid crustaceans. *Crustaceana*, **29** (3), 266-270.

Bakanev S.V. and Kuzmin S.A., 1999. First results of the investigation on red king crab (*Paralithodes camtschaticus*) larvae distribution in the Barents Sea. Pp. 99-101 in: Proceedings of the International Conference "*Fishery Research of the World Ocean*", Vladivostok, September 1999. Vladivostok: Dalribvtuz [in Russian].

Baylon J.C., Bravo M.E.A. and Maningo N.C., 2004. Ingestion of *Brachionus plicatilis* and *Artemia salina* nauplii by mud crab *Scylla serrata* larvae. *Aquaculture Research*, **35**, 62–70.

Bookhout C.G., 1964. Salinity effects on the larval development of *Pagurus bernhardus* (L.) reared in the laboratory. *Ophelia*, **1** (2), 275-294.

Bookhout C.G., 1972. Larval development of the hermit crab *Pagurus alatus* reared in the laboratory. *Crustaceana*, **22**, 215-238.

Borisov R.R. and Kovatcheva N.P., 2003. Prezoea stage of the red king crab (*Paralithodes camtschatica*). Pp. 125-126 in: Reports and Abstracts of the International Symposium *Cold Water Aquaculture: Start*

in the XXI century, Saint-Petersburg, September 2003. Moscow: Rosinformagrotech [in Russian].

Borisov R.R., Kovatcheva N.P. and Parshin-Chudin A.V., 2004. Laboratory observations on the growth of red king crab *Paralithodes camtschaticus* (Tilesius, 1815) juveniles. Pp. 164-167 in: Materials of the V Scientific Conference "*Conservation of biological diversity of Kamtchatka and adjacent seas*", Petropavlovsk-Kamchatskiy, October 2004. Petropavlovsk-Kamchatskiy: KamchatPress [in Russian].

Borisov R.R., Epelbaum A.B., Kryakhova N.V. and Kovatcheva N.P., 2005. Cannibalism of the red king crab at the early life history stages reared under laboratory conditions. Pp. 120-122 in: Materials of the Second International Scientific and Practical Conference "*Marine Coastal Ecosystems: Seaweeds, Invertebrates and Products of their Processing*" (Kotenev et al., eds.). Arkhangelsk, October 2005. Moscow: VNIRO Publishing [in Russian].

Chen J-C. and Chin T-S., 1988. Acute toxicity of nitrite to tiger prawn, *Penaeus monodon*, larvae. *Aquaculture*, **69** (3-4), 253-262.

Coffin H.G., 1958. The laboratory culture of *Pagurus samuelis* (Stimpson) (Crustacea, Decapoda). *Walla Walla College Publications*, **22**, 1–5.

Crain J.A., 1999. Functional morphology of prey ingestion by *Placetron wosnessenskii* Schalfeev zoeae (Crustacea: Anomura: Lithodidae). *Biological Bulletin*, **197**, 207-218.

Dawirs R.R., 1981. Elemental composition (C, N, H) and energy in the development of *Pagurus bernhardus* (Decapoda: Paguridae) megalopa. *Marine Biology*, **64**, 117-123.

Doljenkov V.N. and Koblikov V.N., 2004. Condition of the western kamtchatka population of the red king crab has declined to critical level. *Rybnoe khozyaistvo (Moscow)*, **5**, 42-44 [in Russian].

Epelbaum A.B., 2002. Non-feeding behavior of the glaucothoe of the red king crab *Paralithodes camtschaticus* (Tilesius, 1815). Pp. 67-69 in: *Reports of VI All-Russian Conference on Commercial Invertebrates*, Kaliningrad, September 2002. Moscow: VNIRO Publishing [in Russian].

Epelbaum A.B., Borisov R.R. and Kovatcheva N.P. Early development of the red king crab *Paralithodes camtschaticus* from the

Barents Sea reared in the laboratory: morphology and behaviour. *Journal of the Marine Biological Association of the United Kingdom* (in press).

Epelbaum A.B. and Kovatcheva N.P., 2005. Daily food intakes and optimal food concentrations for red king crab (*Paralithodes camtschaticus*) larvae fed *Artemia* nauplii under laboratory conditions. *Aquaculture Nutrition*, **11** (6), 455-462.

Epelbaum A., Kovatcheva N. and Kryakhova N., 2005. Use of artificial feeds for red king crab larvae cultivation. Pp. 175-178 in: Materials of the Second International Scientific and Practical Conference "*Marine Coastal Ecosystems: Seaweeds, Invertebrates and Products of their Processing*" (eds. Kotenev B.N. et al.). Arkhangelsk, October 2005. Moscow: VNIRO Publishing [in Russian].

Fenyuk V.F., 1945. Analyses of stomach content of the red king crab. *Izvestiya TINRO*, **19**, 71-78 [in Russian].

Freese J.L. and Babcock M.M., 1990. The utility of artificial substrate collection devices to determine time and location of red king crab (*Paralithodes camtschatica*) glaucothoe settling in Auke Bay, Alaska. Pp. 119-131 in: Proceedings of the International Symposium on King and Tanner Crabs, Anchorage, Alaska, 28-30 November 1989. University of Alaska Sea Grant College Progress Report 90-04.

Gao S. and Zou D., 1994. Toxicity of nitrite to the larvae of *Penaeus penicillatus*. *Journal of Oceanography, Taiwan Strait*, **13** (3), 236-239.

Gonor S.L. and Gonor J.J., 1973. Feeding, cleaning and swimming behavior in larval stages of porcellanid crabs (Crustacea: Anomura). *Fishery Bulletin*, **71** (1), 225-234.

Guerao G. and Abello P., 1996. Morphology of the prezoea and first zoea of the deep-sea spider crab *Anamathia rissoana* (Brachyura, Majidae, Pisinae). *Scientia Marina (Barcelona)*, **60** (2-3), 245-251.

Gusev E.E., 1990. Hypergaline aquaculture. Moscow: Agropromizdat. 159 p. [in Russian].

Hoffman E.G., 1968. Description of laboratory-reared larvae of *Paralithodes platypus* (Decapoda, Anomura, Lithodidae). *Journal of the Fisheries Research Board of Canada*, **25** (3), 439-455.

Hong S.Y., 1988. The prezoeal stage in various decapod crustaceans. *Journal of Natural History*, **22** (4), 1041-1075.

Hufthammer M.K., Kuzmin S.A. and Olsen S., 1997. Investigations on the red king crab acclimatized in the North Atlantic. *Akvakultura: OI VNIERKh*, **8**, 3-14 [in Russian].

Jayasankar P. and Muthu M.S., 1983a. Toxicity of ammonia to the larvae of *Penaeus indicus*. *Indian Journal of Fisheries*, **30** (1), 1-12.

Jayasankar P. and Muthu M.S., 1983b. Toxicity of nitrite to the larvae of *Penaeus indicus*. *Indian Journal of Fisheries*, **30** (2), 231-240.

Kalinin A.V., Parshin-Chudin A.V., Kovatcheva N.P. and Lebedev R.O., 2005. Closed recycling water system unit for red king crab cultivation. Pp. 148-151 in: Materials of the Second International Scientific and Practical Conference "*Marine Coastal Ecosystems: Seaweeds, Invertebrates and Products of their Processing*" (eds. Kotenev et al.), Arkhangelsk, October 2005. Moscow: VNIRO Publishing [in Russian].

Karpevich A.F., 1998. Acclimatization of aquatic organisms and scientific bases of aquaculture. *Selected Works of VNIRO*, Vol. 2. Moscow: VNIRO Publishing. 871 p. [in Russian].

Kittaka J., Stevens B.G., Teshima S. and Ishikawa M., 2002. Larval culture of the king crabs *Paralithodes camtschaticus* and *P. brevipes*. Pp. 189-212 in: *Crabs in cold water regions: biology, management, and economics* (eds. A. J. Paul et al.). Fairbanks, Alaska: University of Alaska Sea Grant.

Klitin A.K., 2002. Distribution and development of red king crab larvae off the West Sakhalin. *Trudi SakhNIRO*, **4**, 212-228 [in Russian].

Klitin A.K., 2003. Red king crab (*Paralithodes camtschaticus*) along the shores of Sakhalin and Kuril Islands: biology, distribution and functional structure of the area. Moscow: FGUP "National Fish Resources". 253 p. [in Russian].

Knudsen J.W., 1960. Reproduction, life history and larval ecology of the California Xanthidae, the pebble crabs. *Pacific Science*, **14**, 3-17.

Koblikov V.N., Doljenkov V.N., Rodin V.E., Boldirev V.Z. and Solodovnikov S.A., 2002. The present condition of commercial crustacean resources (Decapoda) in the waters of Russian Far East. Pp. 20-22 in: Theses of Reports of *VI All-Russian Conference on Commercial Invertebrates*, Kaliningrad, September 2002. Moscow: VNIRO Publishing [in Russian].

Konishi K., 1987. Larval development of spiny of sand crab *Lophomastix japonica* (Durufle, 1889) (Crustacea, Anomura, Albuneidae)

under laboratory conditions. *Publications of the Seto Marine Biological Laboratory*, **32** (1/2), 123-139.

Konishi K. and Quintana R., 1987. The larval stages of *Pagurus brachiomastus* (Thallwitz, 1892) (Crustacea, Anomura) reared in the laboratory. *Zoological Science*, **4**, 349-365.

Kovatcheva N.P., 2000. Artificial reproduction of the red king crab (*Paralithodes camtschaticus*) using artificial seawater in "Aquatron". *Marikultura: OI VNIERKh*, **4**, 14-26 [in Russian].

Kovatcheva N.P., 2002a. Observations on rearing red king crab, *Paralithodes camtschaticus* zoea and glaucothoe in a recycling water system. Pp. 273-282 in: Proceedings of the Symposium "*Crabs in Cold Water Regions: biology, management, and economics*", Anchorage, January 2001 (eds. Paul, A.J. et al.). University of Alaska Sea Grant: Fairbanks.

Kovatcheva N.P., 2002b. Biotechnology of red king crab artificial reproduction in a recycling water system. Pp. 300-308 in: Materials of the International Scientific Conference "*Coastal Fishery – XXI Century*", Yuzhno-Sakhalinsk, September 2001 (eds. V.I. Radchenko et al.).Yuzhno-Sakhalinsk: Sakhalin Press [in Russian].

Kovatcheva N.P. and Epelbaum A.B., 2003. Comparison of development and growth of the early life stages of the red king crab (*Paralithodes camtschaticus*) in the process of artificial rearing and in nature. Pp. 135-143 in *Bottom Ecosystems of the Barents Sea* (ed. V.I. Sokolov). Moscow: VNIRO Publishing [in Russian].

Kovatcheva N.P., Kalinin A.V. and Jigin A.V., 2004. Preliminary results on recirculating water purification during red king crab rearing under laboratory conditions. Pp. 191-194 in: Materials of the V Scientific Conference "*Conservation of biological diversity of Kamtchatka and adjacent seas*", Petropavlovsk-Kamchatskiy, October 2004. Petropavlovsk-Kamchatskiy: KamchatPress [in Russian].

Kovatcheva N.P., 2005a. Red king crab as a new species for marine aquaculture. *Pribrejnoe Ribolovstvo i Akvakultura: OI VNIERKh*, **1**, 1-40.

Kovatcheva N.P., 2005b. Recent developments in aquaculture production methods of red king crab (*Paralithodes camtschaticus*) in Russia. Pp. 280-281 in: Extended Abstracts and Short Communications of contributions presented at the International Conference "*Aquaculture*

Europe 2005". Trondheim, Norway, August 2005. EAS Special Publication No. 35.

Kovatcheva N.P. and Kalinin A.V., 2005. Development of hatchery technology for the red king crab (*Paralithodes camtschaticus*) in artificial conditions. Pp. 282-283 in: Extended Abstracts and Short Communications of contributions presented at the International Conference "*Aquaculture Europe 2005*". Trondheim, Norway, August 2005. EAS Special Publication No. 35.

Kovatcheva N.P., Kalinin A.V., Epelbaum A.B., Borisov R.R. and Lebedev R.O., 2005a. Red king crab cultivation. Part 1. Early ontogenesis. Techniques and recommendations for artificial reproduction. Moscow: VNIRO Publishing. 78 p. [in Russian].

Kovatcheva N.P., Kalinin A.V., Tkatchenko V.P., Parshin-Chudin A.V. and Borisov R.R., 2005b. Handling and fattening of the red king crab in flow-through water systems. Pp. 151-153 in: Materials of the Second International Scientific and Practical Conference "*Marine Coastal Ecosystems: Seaweeds, Invertebrates and Products of their Processing*" (eds. B.N. Kotenev et al.). Arkhangelsk, October 2005. Moscow: VNIRO Publishing [in Russian].

Kulichkova V.A., 1955. Feeding of the red king crab in spring and summer near the shores of Kamtchatka and Sakhalin. *Izvestiya TINRO*, **43**, 21-42 [in Russian].

Kurata H., 1959. Studies on the larva and postlarva of *Paralithodes camtschatica*. I. Rearing of the larvae, with special reference to the food of the zoea. *Bulletin of Hokkaido Regional Fisheries Research Laboratory*, **20**, 76–83.

Kurata H., 1960. Studies on the larva and postlarva of *Paralithodes camtschatica*. II. Feeding habits of the zoea. *Bulletin of Hokkaido Regional Fisheries Research Laboratory*, **21**, 1-8.

Kurata H., 1964. Larvae of decapod Crustacea of Hokkaido. 6. Lithodidae (Anomura). *Bulletin of the Hokkaido Regional Fisheries Research Laboratory*, **29**, 49-66.

Lavens P. and Sorgeloos P., 1996. Manual on the production and use of live food for aquaculture. FAO Fisheries Technical Paper. FAO, Rome. 295 p.

Lemmens J.W.T.J., 1994. Biochemical evidence for absence of feeding in puerulus larvae of the western rock lobster *Panulirus cygnus* (Decapoda, Palinuridae). *Marine Biology*, **118** (3), 383-391.

Levin V.S., 2001. Red king crab *Paralithodes camtschaticus*: biology, fisheries, aquaculture. Saint-Petersburg: Ijitsa. 198 p. [in Russian].

List of fisheries standards: safe levels and tentatitive safe exposure levels of toxic substances for commercially valuable hydrobionts, 1999. Moscow: VNIRO Publishing. 304 p. [in Russian].

Logvinovich D.N., 1945. Aquarial observations on red king crab feeding. *Izvestia TINRO*, **19**, 79-97 [in Russian].

Loher T. and Armstrong D.A., 2001. Spatial stock structure in Bristol Bay (Alaska) red king crab and its influence on long-term recruitment trends. *Journal of Shellfish Research*, **20** (3), 1197.

Makarov R.R., 1966. The larvae of Macrura, Anomura, and Brachyura of the west Kamchatka shelf and their distribution. Moscow: Nauka. 164 p. [in Russian].

Marukawa H., 1933. Biological and fishery research on Japanese king crab, *Paralithodes camtschatica* (Tilesius). *Journal of the Imperial Fisheries Experimental Station*, **37** (4), 1-152.

Matyushkin V.B. and Ushakova M.V., 2002. Characteristics of larval cycle of the red king crab (*Paralithodes camtschaticus*) and the hermit crab (*Pagurus pubescens*) in the Western Murman fiords. Pp. 125-136 in: *Biological resources and aquaculture in coastal waters of the White and Barents Seas: PINRO proceedings*. Murmansk: PINRO Press [in Russian].

McMurray G., Vogel A.H., Fishman P.A., Armstrong P.A. and Jewett S.C., 1986. Distribution of larval and juvenile red king crabs (*Paralithodes camtschatica*) in Bristol Bay. *US Department of Commerce, NOAA, OCSEAP, Final Report*, **53**, 267-477. OCSEAP Alaska Office, Anchorage.

McConaugha J.R., 1985. Nutrition and larval growth. Pp. 127-154 in: *Crustacean Issues 3: Larval growth* (ed. A.M. Wenner). Rotterdam: A.A. Balkema.

Mikulin A.E., 1994. Live feeds. Moscow: Delfin. 104 p. [in Russian].

Mortensen A. and Damsgard B., 1996. Growth, mortality, and food preference in laboratory-reared juvenile king crab (*Paralithodes camtschaticus*). Pp. 665-674 in: *High latitude crabs: biology, management,*

economics. Alaska Sea Grant College Progress Report 96-02. Fairbanks: University of Alaska.

Nakanishi T., 1987. Rearing conditions of eggs, larvae, and postlarvae of king crab *Paralithodes camtschaticus*. *Bulletin of the Japan Sea Regional Fisheries Research Laboratory*, **37**, 57-161.

Nakanishi T., Kuwatani Y. and Kahata H., 1974. The relationship between carapace length and body weight of the larvae and post larvae of *Paralithodes camtschaticus*. *Bulletin of Hokkaido Regional Fisheries Research Laboratory*, **40**, 32-37.

Nakanishi T. and Naryu M., 1981. Some aspects of large-scale rearing of larvae and post-larvae of the king crab (*Paralithodes camtschatica*). *Bulletin of the Japan Sea Regional Fisheries Research Laboratory*, **32**, 39-47.

Nishida S., Quigley B.D., Booth J.D., Nemoto T. and Kittaka J., 1990. Comparative morphology of the mouthparts and foregut of the final-stage phyllosoma, puerulus and postpuerulus of the rock lobster *Jasus edwardsii* (Decapoda, Palinuridae). *Journal of Crustacean Biology*, **10**, 293-305.

Orlov J.I., 1962. On the problem of red king crab acclimatization into the Barents Sea. *Proceedings of the All-Union Hydrobiological Society*, **XII**, 400-409 [in Russian].

Orlov J.I., 1994. Acclimatization of commercial crabs in the North East Atlantic Ocean: biological substantiation and preliminary results. *Akvakultura: OI VNIERKh*, **1**, 1-55 [in Russian].

Orlov J.I., 1998. Tranoceanic transplantation of the red king crab *Akvakultura: OI VNIERKh*, **3**, 24-32.

Ostrensky A. and Poersch L.H., 1992. Acute toxicity of nitrite on larval rearing of pink shrimp *Penaeus paulensis* Perez-Farfante, 1967. *Neritica*, **7** (1-2), 101-107.

Paul A.J. and Paul J.M., 1980. The effect of early starvation on later feeding success of king crab zoea. *Journal of Experimental Marine Biology and Ecology*, **44** (2-3), 247-251.

Paul A.J., Paul J.M. and Coyle K.O., 1989. Energy sources for first-feeding zoea of king crab *Paralithodes camtschatica* (Tilesius) (Decapoda: Lithodidae). *Journal of Experimental Marine Biology and Ecology*, **130** (1), 55-70.

Pavlov V.J., 2003. Life history of the red king crab *Paralithodes camtschaticus* (Tilesius, 1815). Moscow: Moscow Publishing. 110 p. [in Russian].

Red king crab in the Barents Sea (results of PINRO research in 1993-2000), 2001 (ed. B.I. Berenboim). Murmansk: PINRO Press. 189 p. [in Russian].

Roberts P.E., 1971. Zoea larvae of *Pagurus campbelli* Filhol. 1985 from Perseverance Harbor, Campbell Island (Decapoda: Paguridae). *Journal of the Royal Society of New Zealand*, **1**, 187-196.

Russian Federation Patent No. 2200386. Kovatcheva N.P. *Method of* crustacean *reproduction (red king crab)*. Declared 27.12.2001. Published 20.03.2003. Bull. 8 [in Russian].

Russian Federation Patent No. 40841. Kovatcheva N.P., Jigin A.V., Kalinin A.V. and Lebedev R.O. *A device for Artemia salina incubation.* Declared 09.06.2004. Published 10.10.2004 [in Russian].

Russian Federation Patent No. 2261594. Kovatcheva N.P., Jigin A.V., Epelbaum A.B., Borisov R.R., Kalinin A.V., Parshin-Chudin A.V. and Lebedev R.O., 2005. *Method for crustacean reproduction (red king crab).* Declared 27.05.2004. Published 10.10.2005. Bull. 28 [in Russian].

Sato S., 1958. Studies on larval development and fishery biology of king crab, *Paralithodes camtschatica* (Tilesius). *Bulletin of Hokkaido Regional Fisheries Research Laboratory*, **17**, 1-103.

Sato S. and Tanaka S., 1949. Study on the larval stage of *Paralithodes camtschatica* (Tilesius). I. About morphological research. *Hokkaido Fisheries Experimental Station Research Reports*, **1**, 7-24.

Shelton R.G.J. and Laverack M.S., 1970. Receptor hair structure in the lobster *Homarus gammarus*. *Journal of Experimental Marine Biology and Ecology*, **4** (3), 201-210.

Shimizu J., 1936. Outline of the rearing experiment on king crab zoea. *Hokkaido Prefectural Fisheries Experimental Station Ten-day Report*, no. 327, 408 pp.

Shirley S.M. and Shirley T.C., 1988. Behavior of red king crab larvae: phototaxis, geotaxis and rheotaxis. *Marine Behaviour and Physiology*, **13**, 369-388.

Shirley S.M. and Shirley T.C., 1989. Interannual variability in density, timing and survival of Alaskan red king crab, *Paralithodes camtschatica*, larvae. *Marine Ecology Progress Series*, **54**, 51-59.

Stevens B.G. and MacIntosh R.A., 1991. Cruise Results Supplement OH-91-1. A survey of juvenile red king crabs with dredge and beam trawl, in Bristol Bay, Alaska. NMFS, Kodiak, AK, USA.

Stevens B.G. and Kittaka J., 1998. Postlarval settling behavior, substrate preference, and time to metamorphosis for red king crab *Paralithodes camtschaticus* // *Marine Ecology Progress Series*, **167**, 197-206.

Strathmann R.R., 1985. Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. *Annual Review of Ecology and Systematics*, **16**, 339-361.

Summerfelt S.T., Wilton G., Roberts D., Rimmer T. and Fonkasrud K., 2004. Developments in recirculating systems for Arctic char culture in North America. *Aquacultural Engineering*, **30** (1-2), 31-71.

Sundberg K.A. and Clausen D., 1977. Post-larval king crab (*Paralithodes camtschatica*) in Kachemak Bay, Lower Cook Inlet, Alaska, 1976. Pp. 1-36 in: *Environmental studies of Kachemak Bay and Lower Cook Inlet* (eds. L.L. Traskey, L.B. Flagg and D.C. Burbank), Vol. 5.

Suprayudi M.A., Takeuchi T. and Hamasaki K., 2004. Essential fatty acids for larval mud crab *Scylla serrata*: implications of lack of the ability to bioconvert C18 unsaturated fatty acids to highly unsaturated fatty acids. *Aquaculture*, **231** (1-4), 403-416.

Suschenya L.M., 1975. Quantitative aspects of feeding of crustaceans. Minsk: Nauka i tekhnika. 208 p. [in Russian].

Takeuchi I., 1962. On the distribution of zoeal larvae of king crab, *Paralithodes camtschatica*, in the southeastern Bering Sea in 1960. *Bulletin of Hokkakido Regional Fisheries Research Laboratory*, **24**, 163-170

Tamaru C.S., Ako H., Paguirigan R. Jr. and Pang L., 1999. Enrichment of *Artemia* for use in freshwater ornamental fish production. Center for Tropical and Subtropical Aquaculture publ. No. 133. 24 p.

Tarverdieva M.I., 1976. Feeding of the red king crab *Paralithodes camtschatica* and the tanner crabs *Chionoecetes bairdi* and *C. opilio* in the south-east Bering Sea. *Biologiya Morya (Vladivostok)*, **1**, 41-48 [in Russian].

Tsai S. and Chen J., 2002. Acute toxicity of nitrate on *Penaeus monodon* juveniles at different salinity levels. *Aquaculture*, **213** (1-4), 163-170.

Vinogradov L.G., 1941. The red king crab. Vladivostok: TINRO Press 94 p. [in Russian].

Watanabe T., Lavens P., Sorgeloos P., Jaspers E. and Ollevier F., 1991. Importance of docosahexaenoic acid in marine larval fish. European Aquaculture Society. Larvi'91 Special Publication, No. 15. 19 p.

Wolfe S.H. and Felgenhauer B.E., 1991. Mouthparts and foregut ontogeny in larval, postlarval, and juvenile spiny lobster *Panulirus argus* (Decapoda, Palinuridae). *Zoologica Scripta*, **20**, 57-75.

Zhao J-H., Lam T.J. and Guo J-U., 1997. Acute toxicity of ammonia to the early-stage larvae and juveniles of *Eriocheir sinensis* H. Milne-Edwards, 1853 (Decapoda: Grapsidae) reared in the laboratory. *Aquaculture research*, **28** (7), 517-525.

Zheltonozhko O.V. Zheltonozhko V.V. and Levin V.S., 2000. Elaboration of the technology of obtaining larvae in order to restock red king crab *Paralithodes camtschatica* on East Kamtchatka shelf. Pp. 26-28 in: Proceedings of Regional Workshop "*Optimization of utilization of marine biological resources and management of the coastal zone of the Barents Sea*", Murmansk, November 1999. Murmansk: MMBI [in Russian].

Zheng Y. and Fang H., 1998. Technique for prevention and treatment of common disease in seed-rearing of river crab. *Shandong Fisheries Yantai*, **15** (5), 31-34.

Zou D. and Gao S., 1994. Toxicity of ammonia to larvae of *Penaeus penicillatus*. *Journal of Oceanography, Taiwan Strait*, **13** (2), 133-137.

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Borisov R., Lebedev R.

EARLY LIFE HISTORY STAGES OF THE RED KING CRAB *Paralithodes camtschaticus* (Tilesius, 1815): BIOLOGY AND CULTURE

Issued in print 30.01.2006 Format 60×84 1/16
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