Study on larval mass mortality and its control in the seed production of portunid crabs

［課程博士・論文博士共通］

博士学位論文内容要旨
Abstract

専　攻 Major	応用生命科学 Applied Marine Biosciences	氏　名 Name	團　重樹 Shigeki Dan
論文題目 Title	Study on larval mass mortality and its control in the seed production of portunid crabs		

The portunid crabs constitute important fishery resources with a global production over 1,000,000 t according to FAO report. Since these species inhabit the coastal or brackish waters and have high economical values, the seed production has been intensively conducted in many countries for purposes of stock enhancement and aquaculture. The swimming crab *Portunus trituberculatus* and the mud crab *Scylla serrata* are important species which are the target species of stock enhancement programmes in Japan. In the seed production of these species, rotifers are generally supplied as food throughout the zoeal stages (four instars for *P. trituberculatus* and five instars for *S. serrata*), and *Artemia* are supplied from late zoeal to megalopal stages. As food for these livefoods, phytoplanktons such as *Chlorella vulgaris* and *Nannochloropsis oculata* are supplemented into larval culture tanks. The largest issue of the seed production is the low survival rate until to reach juvenile crab stage because of frequent larval mass mortality. Larval mass mortality occurred during zoeal and megalopal stages, and metamorphosis from zoeal to megalopal stages is known to be especially critical. In this study, three major causes of mass mortality were targeted; bacterial necrosis disease which causes mortality during zoeal stages, larval abnormal morphologies which induce mortality during metamorphosis into megalopae, and larval nutritional deficiency which causes mortality during megalopal to first crab stages.

The bacterial necrosis disease that exhibited symptom of tissue necrosis in the zoeal spines and appendages has been frequently observed during the seed production of *S. serrata*. Within a few days after the occurrence of this symptom, larval mortality occurred in almost all cases. To control this symptom without using antibiotics, the effect of probiotics was examined throughout the in vitro and mass larval culture experiments. The probiotic bacterial strain, which showed inhibitory effects against the causative bacterium (family Flavobacteriaceae), was screened from the seed production environment. Sterile culture medium supernatant of the probiotic bacteria inhibited the causative bacterium, suggesting that extracellular productions are the inhibitory factors. In large-scale (100-kl) larval culture experiments, the probiotics significantly suppressed necrosis symptom and improved larval survival during the early culture period. However, this effect disappeared after the fifth zoeal stage, corresponding to a decrease in probiotic bacterial counts in larval culture water. Almost all larvae had died by the first crab stage. The effectiveness of probiotics developed in vitro has not yet proved practical in commercial-scale seed production. The continuous proliferation of probiotic bacteria and extracellular production in larval culture water are suggested as the key factors in developing probiotics for commercial-scale seed production.

To clarify the occurrence of larval morphological abnormalities and its relation to mass mortality during the seed production of *P. trituberculatus*, larval survival data and larval morphological samples were collected from 111 seed production trials conducted in Japan. There were two distinct types of morphological abnormality. One was observed in the last stage zoeae having excessively advanced morphological features similar to a megalopa such as large

chelae and pleopods. Another was found in the megalopae retaining zoeal immature morphological features such as small dorsal spines and telson furcae. Both of these abnormalities were related to the moulting failure of larvae during metamorphosis into megalopae; the increase in size of last zoeal chelae and in retention of megalopal dorsal spine increased the frequency of megalopae retaining old exuviae on their carapace and/or appendages. The immature morphology of megalopae had a more potent negative effect on larval survival compared with last zoeal morphology; megalopal immature morphology was suggested as a major cause of mass mortality in *P. trituberculatus* seed production conducted in recent Japanese hatcheries.

To identify the extrinsic factors affecting the morphological abnormalities of last stage zoeae and megalopae, larval rearing experiments with different dietary and environmental conditions were conducted for *P. trituberculatus* and *S. serrata*. In addition, to understand the intrinsic controlling mechanism of larval morphogenesis, zoeal eyestalk ablation experiment was conducted for *P. trituberculatus*; eyestalks are known to include important neurosecretory endocrine system. The morphogenesis of the body parts, which are enlarged toward metamorphosis into megalopae such as chelae and pleopods, were continuously accelerated throughout the zoeal period by the control of eyestalk neurosecretory system. And such acceleration of morphogenesis was driven when larvae were reared with high dietary nutrition level and under appropriate environmental conditions such as higher docosahexaenoic acid content in rotifers, higher density of *Artemia* feeding, and higher salinity of culture water. To prevent the excessive morphogenesis of last stage zoeae, culturists should control the dietary nutritional level, feeding density, and environment to suppress the morphogenesis of these body parts under the threshold. On the other hand, the morphogenesis of the body parts, which are resorbed during metamorphosis into megalopae such as dorsal spine and telson furcae, were controlled instantaneously at the critical period by eyestalk neurosecretory system. The critical period was identified at premoult of penultimate zoeal stage (third zoeal stage for *P. trituberculatus*). The supplementation of phytoplanktons such as *Chlorella* and *Nannochloropsis* into culture water induced the retention of these body parts on megalopae via rotifers at this critical period, expressing abnormal morphology of megalopae. The phytoplankton appeared to have a factor disrupting the larval endocrine control of the resorption of body parts. To prevent the immature morphology of megalopae, culturists should avoid the larval intake of phytoplankton via rotifers during the critical period.

The nutritional deficiency of *Artemia* was also associated with the mortality occurred during the period from megalopal to first crab stages of *P. trituberculatus*. Newly hatched *Artemia* and starved *Artemia* had low eicosapentaenoic acid (EPA) contents, and larvae fed on these *Artemia* during the late zoeal development (third and fourth zoeal stages) died during megalopal period or moulted to unviable first stage crabs. The EPA has been known as an essential fatty acid for *P. trituberculatus* larvae. Furthermore, *Artemia* could not digest the phytoplanktons which have rigid cell walls such as *Chlorella* and *Nannochloropsis*. These phytoplanktons have been supplemented in the seed production as food for live foods in Japanese hatcheries, indicating that *Artemia* may starve easily in the larval culture water during the seed production. To prevent the nutritional deficiency of *Artemia*, nutritional enrichment of *Artemia* with EPA, and supplementation of digestible *Nannochloropsis* containing EPA into larval culture water was suggested to be effective.

To develop the technique overcome all of these mass mortality causes simultaneously, a new larval culture method for *P. trituberculatus* was developed. To prevent the immature morphology of megalopae, larvae were reared only with *Artemia* but without rotifer. To make larval feeding easy, small newly hatched *Artemia* nauplii were supplied

during first zoeal stage. Then, *Artemia* enriched with digestible *Nannochloropsis* were supplied from second zoeal stage, to provide EPA for larvae. The digestible *Nannochloropsis* were supplemented into a culture tank, to prevent *Artemia* starvation after being supplied and to maintain the nutritional condition. To control the excessive morphogenesis of last stage zoeae, *Artemia* feeding density should be maintained low and use of low salinity culture water was also effective. The larval culture water was not exchanged to reduce the inflow of pathogens. These measures achieved high survival rate until to reach first crab stage, demonstrating that *P. trituberculatus* larvae could be cultured only with *Artemia* but without rotifer for the first time.