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Determination of the Optimal Conditions for Bioassay Using Embryo of a Mussel, *Mytilus galloprovincialis*

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Introduction

Mussels were widely used in the assessment and the monitoring of marine environmental quality (Beiras and Albentosa, 2004; Wedderburn et al., 2000). There was a standard protocol for conducting developmental bioassay using mussels (ASTM, 1994). However, studies on mussels in Korea mainly focused on the biological characteristics in aquacultural aspects, especially for post-developmental stages (Hur and Hur, 2000). Biological information on early development of *Mytilus galloprovincialis* is still lacking. As a preliminary step for developing bioassay protocols using the embryo of *M. galloprovincialis*, some of the crucial conditions (i.e. temperature, salinity, density of embryo) for successful development were determined in this study.

Materials and Methods

Adults of *M. galloprovincialis* were collected at a rocky coast of Jangmok, Geoje Island, in February 2004. Spawning was induced by exposing mussels to air for more than 6 hr and then dipping them into filtered seawater (FSW, salinity: 32 psu). Embryos were rinsed with FSW 5 times before use. Temperature experiments were conducted using 1-L polycarbonate bottles as incubation chambers. The embryo suspension was prepared for the initial density to be 100 embryos/mL, and then embryos were incubated at 5, 10, 15, 20, and 25℃. Subsamples were taken at a 3-hr interval. Experiments for determining optimal salinity and density were conducted using 50-mL culture flasks at 15℃, and incubation time was 48 hr. Salinity treatments were set from 5 to 50 psu with 5 psu interval. Density treatments were set as 25, 50, 100, 200, 400, 800 and 1,600 embryos/mL. After incubation, samples were fixed with 5% formaldehyde. For temperature

experiments, embryos developing each stages (morula, blastula, hatching, trochophore, veliger) were counted. With the results from temperature experiments, the biological minimum temperature (BMT) and cumulative water temperature (CWT) for each stage were estimated. For salinity and density experiments, the proportion of normal veliger larvae were calculated.

Results and Discussion

Temperature affected greatly the developmental time of *M. galloprovincialis*. The developmental time to the veliger larva at 10, 15, and 20℃ were 90, 48, and 30 hr, respectively. The proportion developed to veliger larvae was low at 5℃ (4.3%), increased higher than 90% at 10℃ (90%) and 15℃ (96%), decreased at 20℃ (58%), and further decreased at 25℃ (1.8%). The larval normality could be guaranteed when temperature was from 10 to 15℃. But, if we consider the developmental time and proportion of normal larvae together, the optimal temperature for embryonic development of *M. galloprovincialis* was 15℃. From the relationship between the inverse of developmental time and water temperature, the BMT was estimated as 0.6℃. The CWTs for morula, blastula, hatching, trochophore, and veliger were calculated as 104, 189, 353, 469, and 708 hr, respectively. The range of salinity in which the rate of normal development was more than 70% was 30-35 psu. When initial density of embryo was 25-100 embryos/mL, the proportion of normal larvae were higher than 80%. However, it decreased less than 70% as the density increased higher than 200 embryos/mL, and zero when density was 1,600 embryos/mL. Therefore, the optimal salinity and density of embryo were 30-35 psu and 100 embryos/mL, respectively.

References

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