Abstract

Freshwater mussel (order Unionida) is one of the groups of freshwater molluscs having complex life histories that require temporary parasitization on host fish. They have important ecological functions in freshwater ecosystems but they became one of the most imperiled organisms worldwide. The identification and understanding of the causes and the processes of population declines or species extinction of freshwater mussels are imperative for effective conservation measures. Recent studies have shown that one of the major indications for the decline of populations of freshwater mussel is recruitment failure, i.e. low levels or no signs of recent joining of juvenile mussels. These populations will become extinct in the near future if recruitment will not restart. To prevent the extinction of freshwater mussel populations, the process of recruitment failure of freshwater mussels should be urgently determined. A series of observational and experimental studies determined the population status and demonstrated the processes of recruitment failure of long-lived endangered freshwater pearl mussel *Margaritifera togakushiensis* in the rivers of eastern Hokkaido, northern Japan.

The study first investigated the co-occurrence distributions of *M. togakushiensis* and *M. laevis* (Chapter 2). *Margaritifera laevis* is closely related and has very similar appearances with *M. togakushiensis* despite having ecological differences in host fish species and reproductive seasons. The two species were identified by gel electrophoresis banding patterns of 16S rRNA polymerase chain reaction products. The results showed that *M. togakushiensis* was likely to co-occur with *M. laevis* at river reach scale (within 50–100 m). In addition, co-occurrence distributions of two pearl mussel species and populations of *M. togakushiensis* relatively well confirmed in eastern Hokkaido (Chapter 2). In response to the high possibilities of co-occurrence distributions between two pearl mussel species, I developed simple non-lethal identification criteria of two pearl mussel species based on linear discriminant functions (LDFs) that were established considering intraspecific regional morphological differences from east and west regions at Hokkaido (Chapter 3). The two species were also identified using DNA-analysis. Regardless of region, the maximum length of *M. laevis* exceeded 100 mm, whereas all *M. togakushiensis* were < 100 mm in length. The LDFs revealed that the morphologies of the two species were clearly distinguished by the relationship between height and length. Identification accuracies of the established LDFs were high with 85–96% (mean: 92%) accuracy in the east and 67–96% (mean: 80%) in the west. Using these identification criteria, I investigated the status of 24 populations of *M. togakushiensis* in eastern Hokkaido (Chapter 4). A total of seven rivers were estimated as "functional" with more than 20% of ≤ 20-year-old juveniles contained whereas recruitment failure of *M. togakushiensis* was confirmed in four rivers where no juveniles were found. At maximum > 70 years remained until the extinction of those populations based on estimates deriving from age distributions. The estimated periods of recruitment cessation of non-recruiting populations well coincided with the timing of the development of the vast agricultural lands in the study region. Finally, I demonstrated the processes of recruitment failure of *M. togakushiensis* populations in eastern Hokkaido focusing on the major multiple reproductive life-stages (Chapter 5). The reduced survival rate of experimentally provided juveniles was associated with the amount of fine suspended sediment, dissolved nutrients, and their interaction. Although there were no clear relationships between suspended sediment levels and agricultural land proportions in catchments, the negative impacts of

catchment agriculture were inferred as a cause. In addition, the lack of the potential number of juvenile supplies at the parasitic stage can also cause recruitment failure interactively with juvenile survival rate. Furthermore, adult density and gravidity rate at adult stage can also indirectly affect mussel recruitment. These results suggest that the recruitment of *M. togakushiensis* can be limited by multiple life-stage processes.

This study is the first case that empirically demonstrated the processes of recruitment failure of long-lived freshwater mussel species by testing the multiple reproductive life-stages of *M. togakushiensis* comprehensively. In conclusion, the post-parasitic juvenile stage is the most sensitive to habitat deterioration among the life history of *M. togakushiensis* and can become a serious bottleneck for their recruitment. This study would provide perspectives for future conservation measures of freshwater mussels worldwide because the results suggest that greater attention should be paid to the order of conservation measures. To conserve populations of *M. togakushiensis*, the watershed management that reduces the inflow of excessive nutrients is likely to be an effective option for recovering the recruitment of *M. togakushiensis* in eastern Hokkaido. In addition, determining the origins of excessive fine sediments and reducing them is required. Furthermore, habitat improvement of host fish like installation of large wood and artificial propagations of *M. togakushiensis* may be also effective to enhance the potential numbers of juvenile supplies and thus recover natural recruitment.