学位論文題名

Population status and processes of recruitment failure of endangered freshwater pearl

mussels (*Margaritifera togakushiensis*) in eastern Hokkaido, northern Japan

（北海道東部における絶滅危惧淡水二枚貝

コガタカワシンジュガイの個体群の現状と再生産の停止機構）

1. Introduction

Freshwaters comprise only 0.01% of the water on Earth. Despite of relatively small spatial coverage, freshwater ecosystems are known as one of the most diverse systems on which about 9.5% of the described animal species on Earth (~125 000 species) including one-third of vertebrates. However, freshwater ecosystems have been rapidly degraded by human impacts in recent decades, and currently are considered to be the systems with the most serious decline in biodiversity. The identification and understanding of the cause and the processes of population declines or species extinction are imperative for effective conservation measures.

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 became one of the most imperiled organisms worldwide. Recent studies have shown that one of the major indications for the decline of populations of the freshwater mussels 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. Through a series of observational and experimental studies determined the population status, this dissertation demonstrated the processes of recruitment failure of long-lived endangered freshwater pearl mussel *Margaritifera togakushiensis* in the rivers of eastern Hokkaido, northern Japan.

2. **The co-occurrence of two endangered freshwater pearl mussels (*Margaritifera laevis* & *M. togakushiensis*) in rivers in Hokkaido, northern Japan**

The assessment of species distribution including sympatric co-occurrence of multiple species is helpful for the conservation and management of target species because it could provide knowledge that allows managers and researchers to know in priori whether this needs to be considered. The study in this chapter investigated the co-occurrence distributions of *M. togakushiensis* and *M. laevis* at the river reach scale (50–100 m) in a total of 53 rivers across 31 watersheds in Hokkaido, northern Japan. *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* were relatively well confirmed in eastern Hokkaido. These results can help to decide whether the co-occurrences of the two species should be taken into consideration when conducting the investigation and/or conservation practices. This led to the need to develop identification methods of two conservation-targeted mussel species inhabiting sympatrically with minimum damages, which was the main goal of Chapter 3.

3. **Simple non-lethal identification criteria for two endangered freshwater pearl mussels, *Margaritifera laevis* and *M. togakushiensis*, in Hokkaido, northern Japan**

Species identification is a fundamental process for ecological studies and conservation practices. Misidentification potentially causes data bias, which produces incomplete and/or false results. There is a great demand for identification methods and criteria that are simple, cost-effective, and not affected by inter-observer variability, also for freshwater mussels. The study in this chapter 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 in east and west regions of Hokkaido. The mussel specimens collected from a total of 52 rivers were measured for their morphology (length, height, and width) and identified using DNA-based analyses. Regardless of the 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. These criteria relied only on outer shell morphologies and identified nonlethally many collected pearl mussel individuals with high accuracies in Hokkaido. These criteria enabled the determinations of the detailed population status of *M. togakushiensis* in Chapter 4.

4. Population status of *Margaritifera togakushiensis* in eastern Hokkaido, northern Japan

The screening of the non-recruiting populations is important for determining the population viability and prioritization of conservation targets. The study in this chapter investigated the status of *M. togakushiensis* populations including age structure and the proportion of juveniles of *M. togakushiensis* populations to compile basic information regarding population viability for the conservation of the target species. The quantitative quadrat-based sampling was conducted in 24 rivers across eight watersheds in a total area of > 1500 km² of eastern Hokkaido. The collected mussels were identified using identification criteria developed in Chapter 3. The age of each mussel individuals was estimated using the most fitted functions among four non-linear functions. The results provided a first report of the population status of *M. togakushiensis* mainly focusing on recruitment, which was considered as one of the indicators of population viability. A total of seven rivers were estimated as "functional" with more than 20% of individuals being ≤ 20-year-old juveniles whereas recruitment failure of *M. togakushiensis* was confirmed in four rivers where no juveniles were found. The results suggested that the highest priority should be given to the preservation of the seven functional populations for the conservation of *M. togakushiensis* in the study region. 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. The identification of the causes of recruitment failure of *M. togakushiensis* populations is critically important to prevent the extinction of *M togakushiensis*, which was the purpose of Chapter 5.

5. **Processes of recruitment failure of long-lived endangered freshwater pearl mussel (*Margaritifera togakushiensis*)**

Recent studies have pointed out the threats of delayed extinction in various ecosystems (so-called "extinction debt"). Local populations and species can become extinct with a considerable long time (several months to several thousands of years) after any disturbances and perturbations. However, there is a lack of empirical understanding of the process of the extinction debt. Recruitment failure of freshwater mussels including *M. togakushiensis* can be considered to be an example of extinction debt because adult individuals can survive for a long time after recruitment ceased. The study in this chapter demonstrated the processes of recruitment failure of *M. togakushiensis* populations in 11 intensive rivers selected from 24 rivers targeted in Chapter 4, focusing on the major multiple reproductive life-history stages of *M. togakushiensis*. The reduced survival rate of experimentally provided juvenile abundances was associated with the amount of fine suspended sediment, the level of dissolved nutrients, and their interaction. Although there were no clear relationships between suspended sediment levels and agricultural land and urban proportions in catchments, the negative impacts of catchment agriculture and urban area were inferred as a cause. Furthermore, the lack of the potential number of juvenile supplies at the parasitic stage could also cause recruitment failure interactively with the juvenile survival rate. These results suggest that recruitment failure of long-lived freshwater mussels can be caused by habitat deterioration because adult individuals can persist in deteriorated habitats while individuals at the early life-history stage are not viable under the low habitat quality. In addition, the results also suggested that the recruitment of *M. togakushiensis* can be limited by multiple life-history stage processes.

6. **General discussion**

This dissertation provided the first set of quantitative data demonstrating the processes of recruitment failure of long-lived freshwater mussel species by testing the multiple reproductive life-history stages of *M. togakushiensis* comprehensively. In conclusion, the post-parasitic juvenile stage is the most sensitive to habitat deterioration among the life histories of *M. togakushiensis* and can become a serious bottleneck for their recruitment. This

study would provide pragmatic 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 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 improvements of host fish including practices of installations of large wood in river channels and artificial propagations of *M. togakushiensis* may be effective to enhance the potential numbers of juvenile supplies and thus recover natural recruitment.

These valuable findings from this dissertation and implementing proposed conservation measures could contribute to maintaining the functional integrity of freshwater ecosystems in the study region by recovering freshwater mussel recruitment. The long-term monitoring of the study populations and changes of key-environmental factors such as land-use and availabilities of host fish should be continued to better predict future changes in population dynamics. Furthermore, locating other remaining habitats of *M. togakushiensis* and monitoring them is also recommended.