Factors causing extinction of a freshwater pearl mussel, *Margaritifera laevis* in Japan (Bivalvia: Unionoida)

（日本におけるカワシンジュガイ *Margaritifera laevis* の絶滅要因（二枚貝綱イシガイ目））

YOSHIHIRO AKIYAMA

Laboratory of Environmental Medicine and Informatics

Division of Bioscience

Graduate school of Environmental Earth Science

Hokkaido University

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Contents

Summary

Freshwater bivalves are endangered nowadays the world over. One group of freshwater bivalve, Margaritiferidae is a family that is particularly endangered and is devised for conservation measure in many countries. Life history of unionoida including Margaritiferidae is very unique and their larvae known as glochidia are parasitic to fish and/or amphibians. Accordingly, host and population dynamics of Margaritiferidae are closely related. The major objective of the present study was to clarify the mechanism and cause of extinction of a Margaritiferid, *Margaritifera laevis*.

For the purpose of age estimation, marked mussels were reared in situ for one year and it was confirmed that internal and external growth bands of the shell increased annually. Both growth rings on the shell surface and growth bands on the cross-sectional surface of shell ligament could be used for determination of mussel age. For comprehending the status of growth and recruitment success, shell sizes and ages were examined for 14 populations in Japan. Irrespective of their reproductive potential, some populations lacked juveniles. These populations would become extinct if environment of habitat was not improved. The Lack of juveniles in mussel populations as described above is observed worldwide, and has been considered as the major cause of margaritiferid extinction. It was implied that the lack of juveniles in mussel populations was associated with dam and eutrophication. For clarifying the mechanism and cause of the lack of juveniles, I compared two

contrasting populations, i.e. the population in the Chitose River which consists of adult and juvenile mussels, and the population in Abira River which consists of adult mussels only. Adult mussels in the Abira River had full reproductive potential indicated by glochidial release from exhalant siphon, parasitism on host fish and growth of parasitic glochidia. Accordingly, the reproductive potential was not the cause of the lack of juveniles. Survival rate in the early life stage from free-living glochidia to juveniles was lower in the Chitose River. Free-living glochidia cannot survive without infection to hosts. Although viability in the free-living glochidial stage was superior in the Abira River, it was suggested that the survival rate of free-living glochidia in the Abira River was inferior due to a low density of host fish and a low rate of overlap of distribution between mussels and host fish. In the Abira River, the rate of glochidial infection to host was higher and the mean number of attached glochidia per host was larger. However the water temperature in the Abira River was colder than that in the Chitose River, which extended the duration of parasitic stage exposing attached glochidia to immune attack by host fish. In rearing juveniles in the rivers survival times were shorter in the Abira River, irrespective of the origin of glochidia. This suggested that the environment in the Abira River was not appropriate for juvenile mussels. Therefore, the lack of juveniles in *M. laevis* was caused by the low survival rate in early life stages irrespective of the mussel's reproductive potential. Furthermore, the population dynamics of host fish

was closely related to that of *M. laevis*.

In Margaritiferidae, sizes of glochidia were smaller than those in the other Unionoida and glochidium does not have a hook. Accordingly their host species and the suitable parts for their glochidia to attach on the host are extremely restricted. Survival time in free-living glochidia in Margaritiferidae is also shorter than that in the other Unionoida. These traits in Margaritiferidae make success rate of parasitism to keep low. The duration of the parasitic stage in Margaritiferidae is typically longer than that in the other Unionoida. In Margaritiferidae, attached glochidia are originally easy to be excluded by host immunity and their survival rate is typically low. In general, the survival of Margaritiferidae during the early life stages strongly depends on the distribution, population dynamics and physiological response of host fish.

I. Introduction

Decrease of freshwater mussel is a worldwide phenomenon. In IUCN Red List, 121 species were described as extinct or threatened species as of 2006 (IUCN, 2006). Most endangered species of freshwater mussels belong to Unionoida. Mussels of a family in the Unionoida, Margaritiferidae, are also endangered in the world. Long-term censuses of the freshwater pearl mussel, *Margaritifera margaritifera* show that this species has declined markedly since the beginning of the 20th century (Valovirta, 1977; Young & Williams, 1983). In Central Europe, the pearl mussel has decreased by more than 90% during the 20 centuries (Bauer, 1988). Accordingly, margaritiferid species have been designated as endangered in many countries, e.g. the freshwater pearl mussel that is classified by the UK Biodiversity Steering Group as a 'Priority Species' requiring the implementation of a Species Action Plan dedicated to its survival (Biodiversity Steering Group, 1995). On the other hand, *Margaritifera marrianae* was classified by the US Fish and Wildlife Service as a candidate for Endangered Species Act protection in 1999 (US Fish and Wildlife Service 1999). Detrimental factors for Margaritiferidae particularly are associated with human activities (Table 1). Various human activities have detrimental effects on Margaritiferidae. Life history in Unionoida is extremely unique. Their mussel larva which is called glochidium, is released from exhalant siphon of a female mussel and parasitizes gills and/or fin of a host (fish and/or amphibia) (Young & Williams,

1984a, b; Watters & O'dee, 1998) and thereafter detaches from the host and settle on river bed (Fig. 1). Accordingly, decline of Margaritiferidae may be affected by the dynamics of host fishes (Altaba, 1990). Even on susceptible hosts there may be considerable glochidial mortality (Fustish & Millemann, 1978; Young & Williams, 1984b; Bauer, 1987a) which must be attributed to the host response (Bauer, 1997). In addition, specific antibodies of host fish are produced leading to acquired immunity after infection of two or three times (Meyers *et al.*, 1980; Bauer & Vogel, 1987). On the other hand, Cunjak and McGladdery (1990) have suggested an increasingly detrimental impact of *M. margaritifera* glochidia on young-of-the-year Atlantic salmon as a function of time and the degree of infestation. However, the dense populations of salmonids occurring together with large *Margaritifera* populations (Young & Williams 1984b; Bauer, 1988) suggest that *Margaritifera* must be classified as a "benign" parasite. The impact on their hosts seems to be rather low because *Margaritifera* species are distributed in the limited parts of the distributional zone of their hosts (Fig. 2) (Bauer, 1997).

In Japan, two margaritiferid species, *Margaritifera laevis* and *M. togakushiensis* are found. *M. laevis* is distributed in the Sakhalin, the Kurile Islands, Hokkaido and Honshu (Okada & Koba, 1932), whereas *M. togakushiensis* is a newly described species found in Hokkaido and Honshu (Kondo & Kobayashi, 2005). Both species are reported to have declined

(Awakura, 1969; Matsuoka, 1979; Naito, 1989). Although the improvement in agricultural field and damming are regarded as causes of decrease of margaritiferid populations in Japan (Awakura, 1969; Yoshida, 1971, 1973), the mechanism of the decrease is not fully clarified. Predators which drastically affect the population of *M. laevis* or *M. togakushiensis* are not known. Margaritiferids were eaten by people in the old days (Satake *et al.*, 1984) but are not virtually consumed by people in recent years. There is a rumor among the local people that Hokkaido brown bear (*Ursus arctos yesoensis*) eats freshwater pearl mussel. Whether it is true or false remains unclear. Above mentioned facts indicate that the causes of margaritiferid decrease are anthropogenic impacts and population decrease of host fish, not the impacts of predators.

Lifespan and sizes of shells and the presence or absence of juvenile mussels in a population are also investigated because these factors are indicators of reproductive potential of mussels. In *Margaritifera margaritifera*, the maximum observed lifespan attained in a population varies from 30 to 132 years and the maximum shell length from 80 to 145 mm varying among populations (Bauer, 1992). Since the fertility of *M. laevis* seems to be depending on the shell length (Awakura, 1968) and since postreproductive period is absence in *M. margaritifera*, (Bauer, 1987c), a population with small-sized individuals may have shorter life-span and thereby lower reproductive potential, and may be more likely to become

extinct (Beasley & Roberts, 1999b). Lack of juveniles is often observed in margaritiferid populations (Altaba, 1990; Lucey, 1993; Beasley *et al.*, 1998; Costello et al., 1998; Alvarez *et al.*, 2000; Cosgrove *et al.*, 2000, Araujo & Ramos, 2000) which is considered as the precursor of margaritiferid extinction.

The main object in of this study was to clarify the cause of extinction in *M. laevis*. To achieve the objective, the population structures especially the proportion of juveniles and the reproductive potential were examined in Japan. Environmental factors which might affect the population structure were also examined. Furthermore, experimental analyses of mussel survival in early life stages were performed using populations with and without juveniles.

II. Materials & Methods

Margaritifera laevis was used as material for the present study. The biology and ecology of this species are known better than the other Japanese freshwater pearl mussel, *M. togakushiensis*. Basic characteristics of Margaritiferidae are listed in Table 2. Breeding season of *M. laevis* i.e. the duration in which female mussels bear eggs or glochidia in its marsupia is from late June to early August. Glochidial release occurs from Late July and mid-August (Awakura, 1968). Glochidia the sizes of which are only 50μm to 60μm, drifts in river water and attach on gills of host fishes: *Oncorhynchus masou masou*, *O. masou ishikawae*, *O. nerka*, *O. keta* and *Salmo gairdnerii* (Awakura, 1964, 1968; Naito, 1988). Glochidial parasitism on host fish is observed from late June to Spetember (Awakura, 1968). Young mussels detach from host fish after transformation from glochidia and settle on the river bed. Thereafter, they live as benthos for the rest of their lives. The longest lifespan recorded for freshwater mussel species in Japan is 79 years (Awakura, 1969). Basic characteristics of *M. laevis* are similar to those of *Margaritifera falcata*.

II. 1 Age estimation

II. 1-1 Research sites

The present study was conducted in two rivers belonging to different river systems in Hokkaido, the Shiribetsu River and the Chitose River. Lengths of

river channels were 679.2 km in the Shiribetsu River and 214.3 km in the Chitose River. Study sites in the Shiribetsu River were surrounded by deciduous forest, whereas in the Chitose River, study sites were surrounded by both forest and urban areas. In both rivers, there are abundant juvenile and adult mussels. The mussel population which includes juveniles is not common in Japan. Juvenile mussels are particularly appropriate materials for the study of growth because their growth rings on shells are easily observed.

II. 1-2 Observation of annual increment of growth rings on shell surface in a year

To assess the duration of no recruitment and the stability of reproductive potentials in a mussel population, estimation of age is required. Therefore, mussels were reared in situ after marking to confirm the annual increment of growth rings on shell surface. Since in the Chitose River, it has been studied that growth ring of *M. laevis* increase by one annually (Akiyama, 2003), the observation was only carried out for the population in the Shiribetsu River. A total of 107 mussels were collected on August 1st, 2003. Shell size of most samples was not over 40mm because shell surface of larger mussel tends to take on dark color and be low growth rate, therefore count of their growth rings on shell surface is difficult (Neves & Moyer, 1988; Akiyama, 2003). Their shell lengths were measured with a vernier caliper to

within 0.1 mm and the shell surface of the mussels were labeled with a mini drill (Rakugaki, Niigata-Seiki, Japan). Immediately thereafter, mussels were released to the original habitat. On August 2, 2004, labeled mussels were recollected and the number of growth rings on the shells was recorded.

II. 1-3 Comparison of the numbers of growth rings on shell surface and growth bands on cross-sectional surface of ligament

The method of counting growth bands on cross-sectional surface of ligament (Hendelberg, 1961) is often used for age estimation of long-lived Margaritiferidae (Hruska, 1992; Hastie *et al.*, 2000). To confirm that the age estimation methods in Hendelberg (1961) is applicable for *M. laevis*, the number of growth rings on shell surface was compared with the number of growth bands on cross-sectional surface of ligament. I collected 55 individuals from the Shiribetsu River and 75 individuals from the Chitose River. They were killed and the soft parts were separated from shells. The shell length and ligament length were precisely measured with a vernier caliper to within 0.1 mm. By using simple linear regression analysis, a regression equation for estimating ligament length from shell length was obtained and the major axis of the *n*th growth ring from the latest growth ring was measured. Using the regression equation, ligament length was estimated from the shell length. The length between *n*th growth band from the latest growth band on the cross-sectional surface of ligament and umbo

was measured. The difference between observed ligament length and the estimated ligament length from regression equation was tested by G-test. The number of samples was randomly determined for each sample.

II. 2 Status of *Margaritifera laevis* and the factors involved in the extinction of *M. laevis* populations in Japan

II. 2-1 Collection of mussels and empty shells

A total of 14 sampling rivers belonging to different river systems were selected from the map of mussel distribution (Kondo, 2002) except for the habitats of *M. togakushiensis* (Fig. 3). The distribution of *M. togakushiensis* was provided by Kondo & Kobayashi (2005) and Kurihara et al. (2005). To estimate parameter values of growth and maximum shell length from growth bands, live mussels and empty shells were collected randomly from each river for 1 hour. Since *M. laevis* is an endangered species in Japan, these empty shells were used for age estimation. Mussel collection was carried out between May 27 and June 11, 2004.

II. 2-2 Estimation of growth-function parameters

Parameters in von Bertalanffy growth model were calculated due to age estimation. von Bertalanffy growth model is often used for age estimation of Margaritiferidae (Bauer, 1983). The major axis of shell was measured in the field. For the sample of which growth rings on shell surface could be observed

clearly, the major axis of each growth ring was also measured and released immediately. Empty shells were brought to the laboratory for the examination of growth rings. The shells were boiled in potassium hydroxide solution to remove their periostracum from shell and to make examination of growth rings easier (Fig. 4). Thereafter shells were washed with brush in water and the lengths the major axes of respective growth rings on shells were measured. It was needed that growth parameters was estimated using not only empty shell but also live mussels because at several sampling sites, empty shells could not be collected. For the conservation of this species, it was required that growth parameters were estimated without killing mussels. Therefore, Ford-Walford plots (Walford, 1946) was applied for the estimation of L_∞ and k for von Bertalanffy growth function:

$$L_t = L_\infty \left\{1 - e^{-k(t-t_0)}\right\}$$

where L_t is shell length (in mm) at age t (in years), L_∞ is the asymptotic length of the average individual growth curve in a population, k is the growth constant (year^{-1}) which determines the curvature of the growth curve, i.e. the rate at which the asymptotic length is approached (von Bertalanffy, 1938), and t_0 is the hypothetical starting time at which an individual would have been zero-sized if it had always grown according to this function. By using this method, growth parameters could be estimated from the length of the major axes of each growth ring. Parameters which were derived from this method could be used for growth model (Anthony *et al.*, 2001). Mean size of

juvenile mussels just after the detachment from the gills of a host fish was 470 µm (Kobayashi & Kondo, 2005). Therefore, the time at which shell length attains 470 µm is defined as 0 year. The parameter t_0 was calculated from the following expression (Curtis & Vincent, 2006).

$$t_0 = \frac{1}{k}\ln\left(1 - \frac{0.47}{L_\infty}\right)$$

Maximum age in a population was also calculated from the growth formula. Maximum age and L_∞ were indicators of population reproductive potential and a population consisting of individuals with shorter life-spans and smaller sizes of shells, may be more likely to become extinct (Beasley & Roberts, 1999b). Ages of mussels were estimated from the inverse von Bertalanffy growth function:

$$t = -\frac{1}{k}\log\left(1 - \frac{L_t}{L_\infty}\right) + t_0$$

The shell length was substituted into the inverse function for estimating age of individual mussel in populations.

II. 2-3 Environmental factors influencing lack of juveniles

To examine the environmental factors influencing lacking juveniles in a population, 10 rivers (Abira, Shiribetsu, Chitose, Akka, Ukedo, Kuro, Chubu-nogu, Kawakami, Asahi and Takahashi rivers) were selected from 14 rivers studied in II. 2-1 as sampling sites because these populations were

distributed naturally without artificial introduction, and environmental data around these habitats were available. To select environmental factors influencing lack of juveniles in each population, multiple logistic regression analysis and model selection using the stepwise Akaike information criterion (AIC; -2 maximum log-likelihood minus the number of parameters in the model; Johnson & Omland, 2004) was used. Objective variables were presence and absence of juveniles. Presence and absence of juveniles were classified into 1 and 0 respectively. Definition of population without juveniles is absence of mussels shorter than 25mm (ca. 5 to 7 years). Explanatory variables were environmental factors: total phosphorus (TP) and total nitrogen (TN) concentrations in river water, annual mean air temperature, number of dams that are located downstream of mussel habitat. TP and TN are general indicators of eutrophication which is the cause of extinction for *Margaritifera margaritifera* (Bauer, 1986; 1988). The concentrations of TP and TN in the river water were analyzed by spectrophotometry after peroxodisulfate digestion (Japan Society of Analytical Chemistry, 1998). High water temperature is the major cause of low survival rate of juveniles in *Margaritifera margaritifera* (Buddensiek, 1995). High water temperature also limited the distribution of the host for *M. laevis*, *Oncorhynchus masou masou* (Inoue *et al.*, 1997). A dam is considered as a factor inducing the decline of host fish and *M. laevis* (Awakura, 1969). Water sample for data analyses of TP and TN was collected in a 200-ml polyethylene bottle each

river between May 5 and June 11, 2004. Collected water samples were kept at -20°C in a freezer and later these samples were analyzed. Groundwater temperature (GWT) was calculated from mean annual air temperature (AT) using the following equation (Nakano *et al.*, 1996):

$$GWT = 1.083 + 0.939AT$$

Mean annual air temperature around each sampling site for 5 years from 2000 to 2004 was calculated from the data at climate observatory of Meteorological Bureau in Japan. These data were corrected for -0.007°C m^{-1} applied to vertical residuals between the observatory and research site. Dams at downstream of mussel habitat which were indicated in the map of 1/25,000 magnification (Geographical Survey Institute, 2002) were checked and the number of dams was counted. The number of dams was ranked: 1 for lesser 10, or 2 for greater or equal of 10.

II. 3 Comparison of characteristics between two mussel populations with and without juveniles

II. 3-1 Research sites

To compare between mussel populations with and without juveniles, the Chitose River and the Abira River were selected. Both rivers are located in central Hokkaido northern Japan. Lengths of the Chitose and the Abira rivers are 107.9km and 49.8km respectively. The shortest distance between the mussel habitats in both rivers is ca. 10km. Therefore climate conditions

of both habitats were assumed to be similar. In the Chitose River, adult and juvenile mussels were found abundantly, whereas, in the Abira River, only adult mussels were found. In both mussel habitats, the river water was clear and the bottom substrates were composed of gravel and pebble in the Chitose River and gravel in the Abira River. Mean water temperature in the mussel habitat was 9.5℃ in the Chitose River and 7.2℃ in the Abira River (Fig. 5).

II. 3-2 Mussel densities in the Chitose and the Abira rivers

From a preliminary research, it was clarified that the extent of *M. laevis* habitat in the Chitose River was between the 4th power plant belonging to the Oji-Seishi Co. Ltd. (upper boundary) and the Neshikoshi-Bridge (lower boundary) (Fig. 6), whereas in the Abira River, mussels mainly lived between 3 km down the upstream border (upper boundary) and the site of the river crossing with the Dou-oh highway (lower boundary) (Fig. 7). Survey was carried out between September and December 2005. For the determination of mussel densities, six stations in the Chitose River and nine stations in the Abira River were set at intervals of ca. 2 km and 1 km, respectively. Each station had longitudinal length of 500 m and had three transects. Along each transect, 1 m×1 m quadrates were placed every 5 m in the Chitose and every 1 m in the Abira rivers. Density of visible or buried mussels was recorded within each quadrate. When the mussels lived in deep water, a 1 m scale was submerged onto the sediment surface and photographs of mussels were

taken to estimate the density of visible mussels emerged from the sediment. Total number of mussels in the quadrate was estimated from ratio of visible and buried mussels in the neighboring quadrates. The 95% confidence interval of density was calculated by standard bootstrap method (Manly, 1997) for each station. Number of bootstrap replicates was 10,000.

II. 3-3 Age estimation for mussel populations

For understanding age structure in each population, age estimation of mussels was carried out according to Hendelberg (1961). In this method, age is estimated from the number of annual layers on cross-sectional surface of ligament. Number of annual layers in corrosive part of ligament is corrected by using the relational diagram between ligament length and number of annual layers. Data for the diagram were available from young mussels of which shells were not damaged. Age estimation method in II. 2-2 using the Walford plots is less correct than the method by Hendelberg (1961) because the former omits available age information from shell and determines age from growth rate. Since juvenile mussels in the Abira River were absent, correction of number of annual layers in the eroded part of ligament was performed using the relationship between the number of growth bands and ligament length for the young mussels collected from the Chitose River. The relationship between age (t, year) and shell length (L_t, mm) was expressed by the Gompertz model:

$$L_t = L_\infty e^{-ae^{-kt}}$$

where L_∞ is the asymptotic length (in mm, theoretical final length), a is a constant and k is the growth rate (year^{-1}). Parameters were estimated by the nonlinear least-squares method. Gompertz model was the best-fitting equation for shell growth in *M. laevis* among three growth models Gompertz model, von Bertalanffy model and logistic model (Akiyama & Iwakuma, in preparation). The age of live mussel in each population was estimated by substituting shell length into inverse function of the Gompertz model:

$$t = -\frac{1}{k}\log\left\{-\frac{1}{a}\log\left(\frac{L_t}{L_\infty}\right)\right\}$$

II. 3-4 Breeding season

Mussels were collected randomly from the Abira River and the Chitose River every month between June 2005 and May 2006. Mussel valves were opened slightly with a shell opener and marsupia of the mussels were checked by eyes. Marsupia with brown color and relatively swollen were judged as gravid mussels. More than 50 individuals were observed monthly for each river to calculate the proportion of gravid mussels. The shell lengths were measured.

II. 3-5 Minimum and maximum ages and shell lengths of gravid mussels

To confirm ranges of shell length and age in gravid mussels in each

population, the minimum and maximum shell length and age in gravid mussels were determined using available data in II. 3-3. Shell lengths were measured for selected gravid mussels in each population and thereafter age estimation was carried out according to the method in II. 3-2.

II. 3-6 Total number of eggs in a population during a breeding season

To estimate the number of eggs from the shell length of the mussels in the Abira River, female mussels of various sizes were collected from both rivers in the breeding season, on July to August in 2005. The relationship between shell length and number of eggs for the population in the Chitose River was analyzed for the data by Awakura (1968). Collected mussels were kept separately in containers. The containers were gently aerated with airstones and air pump to induce release of glochidia from gravid mussels (Wellmann, 1943). After glochidial release, volume of water including eggs or larvae was measured with a graduated cylinder. The water in the container was well mixed to make a homogenous distribution of glochidia in the water. Mean densities of eggs or glochidia from the Abira River in containers were counted three times for up to 0.2 ml portion under a binocular microscope. Mean density of eggs or glochidia was calculated from the triplicate data. By multiplying density of offspring by water volume, total number of offspring was calculated. Relationship between shell length of gravid mussel and number of offspring was expressed by a simple linear regression formula for

each river. Mean offspring size for various sizes of mussels was calculated using the formula. According to the size-frequency distribution in each population (Fig. 15, 16), mean numbers of eggs in marsupia (R_E) was estimated as 2944 individuals for the Chitose River and 16 individuals for the Abira River. Total number of female mussels (N_F) was calculated from the following equation:

$$N_F = R_F N_T$$

where R_F is the rate of mean number of gravid female mussels which was calculated from the available data in II. 3-3 and N_T represents total number of mussels in a population which was calculated from the available data in II. 3-1. Total number of eggs (N_E) in the Abira River was calculated from the following equation:

$$N_E = R_E N_F = R_E R_F N_T$$

The data of shell length and number of eggs for the population in the Chitose River (Fig. 3 in Awakura, 1968) was used for the estimation of the number of offspring in the Chitose population.

II. 3-7 Viability of free-living glochidia at various temperatures

Gravid mussels were collected from the Chitose and Abira rivers on August 5, 2005 during their breeding period. Mussel valves were opened slightly for each individual with a shell opener in the field and the soft parts of the mussels were checked by eyes for marsupia condition. Mussels with

brown and relatively swollen marsupiums were judged as having glochidia. Ten incubative mussels were caught randomly from each river and brought to the laboratory. Glochidial release in both rivers started in late July and ended in early August (Awakura, 1964). Water temperatures in both rivers during the period were recorded at hourly intervals with temperature loggers (HOBO Temp Pro, Onset Co.,Ltd., USA). Mussels were reared separately for the Chitose population and the Abira population in two 1-l containers filled with 900 ml of respective river water that had been filtrated through glass fiber filters with pore size of ca. 0.7 μm (Whatman GF/F). The containers were gently aerated with airstones and air pump to induce release of glochidia from gravid mussels (Wellmann, 1943). Mussels were returned to respective river habitats after the completion of glochidial release in the laboratory.

The water in the container was well mixed to make a homogenous distribution of glochidia in the water: mean densities of glochidia from the Abira River and the Chitose River in containers were 4015 individuals ml^{-1} and 838 individuals ml^{-1}, respectively. Then 900ml of mixed water was sampled from each container and each 300ml portion of the sample was poured into a 525ml container. These six containers for the Abira and Chitose rivers were respectively kept in incubators at 10, 15 and 20°C under constant aeration. The survival of glochidia was observed nearly daily for 1 ml of water sampled from the container with a measuring pipette. The water was dropped on a counting glass chamber for which live and dead individuals

were counted one to three times for up to 0.2 ml portion under a binocular microscope. Larval death or survival was judged by the presence or absence of damage in cells.

In the present experiment, the survival rate at each temperature was expressed as the ratio of the mean number of live glochidia at respective observation time (L_t) to the mean number of live glochidia in triplicate containers at the start of the rearing experiment (L_0). The number of dead glochidia at respective observation time (D_t) was obtained by $D_t = L_0 - L_t$. The mean numbers of live and dead glochidia observed daily were compared between two populations under the same temperature and between rearing water temperatures within the same population by the Pearson's χ^2 test. For the former case, the Yates' correction for continuity (Zar, 1999) was applied to the calculation of χ^2.

II. 3-8 Viability of free-living glochidia in the field

In order to compare the survival rate of free-living glochidia between the Chitose River and the Abira River, a rearing experiment was carried out for free-living glochidia during a period from August 2 to 8 in 2006. Mussels were randomly collected from each river and shells were slightly opened with a shell opener to check the existence of eggs or glochidia in marsupia. Further, a small portion of marsupia contents of selected gravid mussels were taken out with a syringe, put on a microscope slide and examined to confirm the existence of glochidia under a light microscope with a

magnification of 100×. A total of 10 mussels containing glochida were selected and placed in a container. Glochidial release from gravid mussels was induced by aeration with airstone and air pump at 18℃. The water with suspended glochidia from each river was divided into three equivalent portions, each of which was poured in a rearing chamber (Fig. 8). The top and bottom of the chamber were covered with nylon nets with mesh size of 40 μm. In each river, these containers were placed separately at the upper, middle and lower reaches within the habitat of the parent mussels of the glochidia(Fig. 6, 7). Some glochidia in containers were collected with a graduated pipette, fixed with 5% formalin solution and brought to the laboratory every day. Glochidial cells were observed under a light microscope with a magnification of 100× whether the cells were broken or not for the judgment of live or dead glochidia just before the formalin fixation. Numbers of live and dead glochida in each site were recorded daily.

II. 3-9 Host fish species

Fishes were collected by electrofishing in the mussel habitat of the Chitose and Abira rivers during 9 to 31, August, 2005. Collected fishes were preserved in 5% formalin solution and brought to the laboratory. Fork length of host fishes was measured and their gills were observed to confirm the existence of attached glochidia for each fish. The rate of parasitism and the number of glochidia in each host fish were recorded.

II. 3-10 Quantitative collection of host fishes

To calculate overall total number of host fishes in each population, host fishes were collected quantitatively by the removal method (Seber 1973) in the Chitose and Abira rivers during a period from August 9 to 31, 2005. Wadable sampling sites were selected at 12 sites in the Chitose River and 14 sites in the Abira River. Longitudinal length of the sampling site was 100 m and its width depended on the river width. Since the river width of the Abira River was narrow, the sampling site could be divided into upstream and downstream zones by a block nets. Since the Chitose River was too wide to divide, sampling was performed without using the block net in the river. Sampling was carried out by electrofishing three times for more than 30 minutes at 30 minutes intervals. Program CAPTURE (USGS) was used for the estimation of host fish density. The standard bootstrap method (Manly, 1997) was applied to estimate 95% CI by 10,000 bootstrap replicates if the frequency distribution of number of host fishes in each site was greatly different from normal distribution.

II. 3-11 Glochidial infection rates in host fishes

To compare the susceptibilities of host fishes against glochidia between two populations, rate of susceptibility of host fishes was surveyed. Collected host fishes in III. 3-9, was examined for glochidial infection. Less than 30 individuals were randomly selected for each site and fixed in 5% formalin

solution. The gills were examined for the presence of glochidia and the rate of infection in host fish was determined.

II. 3-12 Median number of attached glochidia on host fish

To compare between the susceptibilities of host fishes against glochidia between two populations, number of parasitic glochidia per host fish was counted and recorded for the specimens in II. 3-10. Mean number of attached glochidia on gills per host fish was calculated.

II. 3-13 Estimation of the total number of parasitic glochidia per population

To estimate the overall total number of glochidia in two populations, mean number of parasitic glochidia per host fish was multiplied by the number of host fishes in each site. The density of parasitic glochidia in each population was estimated by averaging the total numbers of parasitic glochidia divided by the areas of respective station.

II. 3-14 Number of detached juvenile mussels from host fish

To confirm that masu trout, *Oncorhunchus masou masou* behaves as host fish for *M. laevis* and to estimate the number of detached juvenile mussels from gills of host in each population, the following experiment was carried out. Before the parasitic period, four individuals and 12 individuals of masu trout were collected by electrofishing from the Abira and the Chitose rivers,

respectively between August and September 2005. Collected fishes were brought to the laboratory. They were reared separately in 16 containers and added with ca. 3000 glochidia per container during a day. Thereafter fishes were transferred separately to other tanks without glochidia. This experiment was performed with various combinations of growing rivers of glochidia and host fishes. Detached juveniles were collected with a plankton-net with 50μm mesh size and the number of glochidia in each tank was counted.

II. 3-15 Survival rates of juvenile mussels

To compare the durations of juvenile viabilities between the natal rivers, i.e., the Chitose and the Abira rivers, masu trout was collected by electrofishing before parasitic glochidia started to detach, i.e. late August to September 2005. They were brought to the laboratory and reared in tanks at 18℃. Detached juveniles from the fish gills were collected with a plankton-net with 50 μm mesh size, A total of 33 individuals from the Abira River and 72 individuals from the Chitose River were placed individually in rearing cages (Buddensiek, 1995) (Fig. 9). This cage system consists of three PVC plates of 270mm×230mm with 116 holes (5.0 mm in diameter). A sheet of plastic gauze (40 μm mesh) was first placed between two PVC plates. The juveniles were placed separately in the holes and then another sheet of plastic gauze and a PVC plate were covered and the hole plates were

clamped to hold juveniles inside. A cage containing 12 juveniles from the Abira River and 38 from the Chitose River was placed in the Chitose River, and another cage containing 11 juveniles from the Abira River and 34 from the Chitose River were placed in the Abira River. Their survival was observed every month.

III. Results

III. 1 Age estimation

III. 1-1 Annual increment of growth rings on shell surface

A total of 48 individuals of marked mussels were re-collected again in August 2005. Of these samples, growth rings of 38 individuals were increased by one after 1 year (Fig. 10, 11). For other individuals, which were mostly large-sized (Shell length ≧ 83 mm), increment of growth ring could not be identical precisely due to low growth rate and dark shell color (Fig. 11). Growth rings of some large samples were nearly overlapping each other. Disturbance lines were formed on many specimens due to the stress by the mini drill. However, these lines could be easily identified discriminated because these were less prominent than annuli and discontinuous (Fig. 10). In conclusion, number of growth rings on shell surface of *M. laevis* from the Shiribetsu River increased by one annually.

III. 1-2 Comparison of the numbers of growth rings on shell surface and growth bands on cross-sectional surface of ligament

Relationship between shell length (*Ls*, mm) and ligament length (*Ll*, mm) were expressed by linear regression equations (Fig. 11), i.e.,

Ll (mm) = 0.386Ls (mm) - 1.665 (R^2=0.97, F=2678.6, p<0.01, n=75)

for the Chitose River and

Ll (mm) = 0.384Ls (mm) - 0.711 (R^2=0.97, F=1574.3, p<0.01, n=55)

for the Shiribetsu River.

The length of existent ligament and that estimated from these equations were not significantly different for both rivers (G-test, $P<0.01$) (Fig. 13).

III. 2 Status of *Margaritifera laevis* and factors causing the extinction of *M. laevis* populations in Japan

III. 2-1 Size-frequency distribution

Observed size frequency profiles for fourteen mussel populations are presented in Figure 14, showing a general tendencies of predominance of medium- and large-sized mussels. The median of shell length in each population ranged from 42.2mm to 102.2mm and was significantly different among populations (Kruskal-Wallis test, corrected $H=486.1$, $p<0.01$). Ranges of the minimum and maximum sizes of shells in each site varied from 6.7 mm in the Akka River to 69.8 mm in the Abira River and from 74.6 mm in the Tomarinai River to 129.7mm in the Asahi River respectively. Of the 14 populations, eight lacked young mussels (shell length $\leqq$ 40mm) i.e., the Abira, the Tanabu, the Kuro, the Kawakami, the Nagara, the Musugo, the Asahi and the Taishaku. Moreover despite mussels in these populations belong to same species, size of maximum shell length is difference.

III. 2-2 Estimated parameters for von Bertalanffy growth function

Estimated values of k and L_∞ for Japanese populations are given in Table

3. The estimated value of L_∞ ranges between 63.5mm for the Tomarinai River and 220.1mm for the Kawakami River. The estimated value of k varies between 0.027 year^{-1} in the Chitose River and 0.143 year^{-1} in the Kuro River. The estimated value of t_0 ranges between -0.143 in the Tomarinai River and -0.024 in the Kuro River.

III. 2-3 Age structure

There are differences between the frequency distribution of mussel sizes (Fig. 14) and those of their ages (Fig. 15). The latter indicated the existence of young mussels (age < 10 years) in all populations. However, as Figure 15 clearly shows, in five populations (Tomarinai, Abira, Shiribetsu, Chubu-nogu. and Taishaku rivers) the predominance of young mussels expected from the age frequency had not been achieved. Observed age modes ranged from 5-10 to 15-20 years. There are apparent differences in the relative abundance of young mussels between the rivers. Above five rivers had relatively small numbers of mussels aged <5 years, suggesting a recent low recruitment success for these populations.

The median age ranged from 1.4 years for the Ukedo River to 17.1 years for the Tomarinai River. The maximum age varied from 4.3 years for the Ukedo River to 40.6 years for the Tomarinai River (Table 3).

III. 2-4 Environmental factors influencing lack of juveniles

In the Abira, the Kuro, the Kwawakami, the Asahi and the Takahashi rivers mussel populations lacked juveniles whereas in the Shiribetsu, the Chitose, the Akka, the Ukedo and the Chubu-nogu rivers, mussel populations contained juveniles. The minimum shell lengths of mussels and the values of environmental variables for these rivers are given in Table 4. The total phosphorus concentrations ranged from 0.0028 μg L^{-1} for the Chubu-nogu River to 0.0076 μg L^{-1} for the Shiribetsu River. The total nitrogen concentrations varied from 0.110 μg L^{-1} for the Chitose River to 0.731 μg L^{-1} for the Kuro River. The annual mean groundwater temperatures ranged from 7.8°C for the Abira River to 15.3°C for the Takahashi River. The number of weirs at downstream of the mussel habitat varied from 0 for the Shiribetsu River and 29 in the Asahi River. The probability of lacking juveniles in a population was higher in the rivers with more dams in the downstream of mussel habitat and with higher concentrations of total nitrogen (Table. 5).

III. 3 Comparison of characteristics between two mussel populations with and without juveniles

III. 3-1 Distribution of mussels

Habitat width of *M. laevis* was ca. 13km in the Chitose River and ca. 5km in the Abira River. Mussels were not distributed uniformly (Fig. 16, 17). The total number of mussels surveyed directly and the total areas of quadrates

were 3,327 individuals and 84m^2, respectively for the Chitose River and 16 individuals and 88m^2, respectively for the Abira River. Ratio of visible mussels to the buried mussels on the river bed were 39.6% for the Chitose River and 100% for the Abira River. Estimated total numbers of mussels and mean densities (95% CI in parenthesis) were 17,095,200 and 37 individuals m^{-2} (15.8 individuals m^{-2} to 76.6 individuals m^{-2}) for the Chitose River, 5,100 individuals and 0.13 individuals m^{-2} (0 individuals m^{-2} to 0.43 individuals m^{-2}) for the Abira River, respectively.

III. 3-2 Composition of shell lengths

Small juveniles to large mussels inhabited abundantly in the Chitose River. In contrast, adult mussels whose shell sizes were ≥ 60mm inhabited the Abira River (Fig. 18, 19). The maximum shell lengths were 131.7mm in the Chitose River and 131.4mm in the Abira River. The fact that the maximum shell sizes were subequal in both rivers indicated that the mussels in the Abira River had ceased reproduction or heterogenesis in recent years.

III. 3-3 Age composition

Growth models for the Chitose and the Abira rivers which were based on data from sub samples were as follows (Fig. 20). Sample sizes of mussels were 53 individuals in the Chitose River and 63 individuals in the Abira River.

The population in the Chitose River: $Lt = 107.9e^{-2.92e^{-0.086t}}$

The population in the Abira River: $Lt = 108.0e^{-2.78e^{-0.097t}}$

To estimate age from shell length, inverse functions were observed from the above growth models.

The population in the Chitose River: $t = -\frac{1}{0.086}\log\left\{-\frac{1}{2.92}\log\left(\frac{Lt}{107.9}\right)\right\}$

The population in the Abira River: $t = -\frac{1}{0.097}\log\left\{-\frac{1}{2.78}\log\left(\frac{Lt}{108.0}\right)\right\}$

The parameter L_∞ which potentially indicates reproductive potential in a population was nearly the same in two populations. Similarly, the parameter k which is the index of growth rate was nearly the same in two populations. Accordingly, reproductive potential of both populations differed little. The minimum ages of both populations, derived from the inverse functions were 0 year in the Chitose River and 16 years in the Abira River. This indicated that the mussel population in the Chitose River reproduced normally for years whereas in the Abira River, they had ceased reproduction since 16 years ago.

III. 3-4 Breeding season of mussels

Gravid mussels were observed during the period from the end of June to early August in both rivers. The maximum proportion of gravid female mussels (shell length ≥ 41.7mm) in adult mussels in both rivers were 18.0%

in the Chitose River and 48.5% in the Abira River (Fig. 21).

III. 3-5 Age and shell length of gravid mussels

The minimum and maximum sizes of gravid mussels in the Chitose River were 41.7 mm and 95.0 mm, respectively, those in the Abira River were 69.6mm and 138.4 mm respectively (Fig. 22). The individual of which shell size was smaller than 69.6 mm was only one, i.e., 62.5 mm in the Abira River. The minimum and maximum ages of gravid female mussels which were calculated from the functions in III. 3.3 were 13 years and 36 years for the Chitose River, respectively (Fig. 23). The minimum age of gravid mussels in the Abira River was 13 years (Fig. 23). The maximum age of gravid mussels from the Abira River could not be estimated because the shell length in the largest gravid mussels exceeded L_{∞}.

III. 3-6 Total number of eggs in a population during a breeding season

The number of eggs in marsupia of a female mussel during a breeding season in the Abira River increased with increasing shell length (Fig. 24). The linear regression equation between the number of eggs (E) and shell length of female mussel in the Abira River (SL, mm) was as follows:

$E = 159309SL - 7921417$ (R^2=0.85, F=28.41, p<0.01, N=6)

The estimated overall total number of eggs in the Abira population during a breeding season was approximately 23,149,210,000 individuals.

III. 3-7 Viability of free-living glochidia at various temperatures

Glochidia in the Abira and Chitose rivers survived for 1 day both at 15°C and 20°C but at 10°C, they survived much longer for 11 days in the Abira River and 4 days in the Chitose River respectively (Fig. 25). Survival time of glochidia in both rivers was longest at the lowest temperature of 10°C. Daily survival rates of each population differed with water temperature both in the Abira population (0-24h: χ^2=1615.2, df=2, p<0.01; 24-48h: χ^2=9071.4, df=2, p<0.01) and in the Chitose population (0-24h: χ^2=55.2, df=2, p<0.01; 24-48h: χ^2=728.8, df=2, p<0.01), but were not always higher at lower temperatures.

III. 3-8 Viability of free-living glochidia in the field

Glochidial extinction occurred 5 days after the initiation of experiment in the Chitose River, whereas in the Abira River, glochidia survived for 6 days (Fig. 26). The decrease of glochidial survival rate in the Chitose River was faster than that in the Abira River (Fig. 26). Therefore, viability of glochidia in the Abira River was higher than that in the Chitose River.

III. 3-9 Host fish for *M. laevis*

Petromyzontidae sp., *Salmo trutta*, *Oncorhynchus masou masou*, *Tribolodon* sp., *Noemacheilus barbatulus toni*, *Gasterpsteis aculeatus*, *Pungitius pungitius* and *Gymnogobius laevis* were collected from the Chitose

River. From the Abira River, Petromyzontidae sp., *O. mykiss*, *O. masou masou*, *Misgurnus anguillicaudatus*, *Noemacheilus barbatulus toni* and *Cottus nozawae* were collected (Table 6). Of these fish species, parasitic glochidia were only observed on gills of *Oncorhynchus masou masou* in both rivers. No glochidial parasitism on fins of host fish was observed. Therefore, the only host fish for *M. laevis* was *Oncorhynchus masou masou* in both rivers.

III. 3-10 Density of host fishes

In all sampling sites in the Chitose River, individuals of *O. masou masou* were collected at all sites of the Chitose River. In the meanwhile, they could be collected at a few sites of the Abira River (Fig. 27, 28). Occurrence of habitat for *O. masou masou* was significantly fewer in the Abira River than that in the Chitose River (Pearson's chi-squared test with Yate's continuity correction, $\chi^2=46.94$, df=1, $p<0.01$). Mean or median densities (95% CI in parenthesis) of *O. masou masou* were 1063.8 (421.7 - 1705.9) individuals ha^{-1} in the Chitose River and 71.9 individuals ha^{-1} (0 - 83.3 individuals ha^{-1}) in the Abira River. The density of *O. masou masou* in the Abira River was only 6.8% of that in the Chitose River.

III. 3-11 Glochidial infection rates in host fishes

The number of collected *O. masou masou* was 77 individuals in the

Chitose River and 19 individuals in the Abira River. Of these rate of infected fish was 53.2% in the Chitose River and 84.2% in the Abira River. Rate of infected fish was higher in the Abira River (Pearson's chi-squared test with Yates' continuity correction, $\chi^2=4.84$, df=1, $p<0.05$).

III. 3-12 Median number of glochidia on a host fish

The median numbers of glochidia per host fish were 1 and 28 individuals in the Chitose and Abira rivers, respectively. The maximum numbers of glochidia per host fish were 2093 and 358 in the Chitose and Abira rivers, respectively (Fig. 29, 30). The number of attached glochidia per host fish was significantly larger in the Abira River than that in the Chitose River (Wilcoxon rank sum test with continuity correction, $p<0.05$).

III. 3-13 Estimation of the number of parasitic glochidia in a population

The numbers of attached glochidia on host fishes for mussel population during a breeding season (95% CI in parenthesis) were calculated from the result of III. 3-10 and III. 3-12, and were approximately 49668.1 (19316.3 - 80020.1) individuals in the Chitose River and 132.0 (4.5 - 342.5) individuals in the Abira River.

III. 3-14 Number of detached juveniles from host fish

The mean number of detached glochidia from host fish was 27 individuals

in the Chitose River. On the other hand, mean number of detached glochidia from host fish was only two individuals in the Abira River (Table 7). The number of detached glochidia or juveniles from host fish was significantly larger in the Chitose River than that in the Abira River (G-test, G=5.10, df=1, p<0.05).

III. 3-15 Survival times of juvenile mussels

The maximum duration of survival of a juvenile mussel in the culture cage was 9 months in the Chitose River and 3 months in the Abira River (Fig. 31). The months in which the survival rate of juveniles was lowest except for the last month of existence of live mussels was October to November in the Chitose River and December to January in the Abira River (Fig. 31). Accordingly, the survival rate of juveniles was lower during the period from the time just after settlement at bottom to mid-winter. Although the duration of juveniles in the Chitose River was longer than that in the Abira River, mean survival times between two populations was not significantly different because in both populations, the juveniles were more feeble during a short phase just after the detachment from host fish than other periods (Exact Wilcoxon sign rank sum test, p>0.4).

III. 3-16 Survival times of juveniles in the Chitose and the Abira rivers

Duration of viability for juvenile mussels in the Chitose River was longer

than that in the Abira River irrespective of the origin of juveniles (Fig. 32). Monthly survival rate of fellow juveniles in the Chitose River was higher than that in the Abira River after October 2005 except for one occasion that was observed for the population from the Abira River in November 2005 (Fig. 32).

IV. Discussion

IV. 1. Age estimation method for margaritiferids

In the past studies on age estimation of *M. laevis* (Awakura, 1969, Ziuganov *et al.*, 2000), it has not been confirmed whether or not the growth band on shell surface actually increases by one annually. In general, a growth band of a bivalve is formed in winter due to the decrease of shell growth (Negus, 1966). Since shell growth in *M. laevis* ceases in winter (Okada & Ishikawa, 1959), the increment of growth band was recognized to occur in winter. Artificial or other exogenous ring has often been observed in many freshwater mussel species. However, it is easy to distinguish this from the annuli because the fake ring is narrower than the annuli and discontinuous (Adam, 1990). Neves & Moyer (1988) have proved that the number of growth rings on shell surface and the number of internal growth bands are equal in each unionid mussel species, *Fusconaia cor* and *Pleurobema oviforme*. However, it has not been known whether the number of growth rings on shell surface and the number of growth bands on cross section of the ligament in *M. laevis* are equal or not. The methods for age estimation using growth bands are counting of growth rings (Altnöder, 1926), counting of growth rings of a shell after boiling in potassium hydrate solution (Ekman, 1905; San Miguel *et al.*, 2004), counting of internal growth bands of a shell which has been cut from umbo to ventral margin with a saw unit (Neves & Moyer, 1988) and counting of growth bands on cross-sectional

surface of ligament (Hendelberg, 1961). Margaritiferid lifespan may reach 190 years (Ziuganov *et al.*, 2000). Counting of shell rings is not applicable to estimate exact age for mussels of over 40 years even if the periostracum is removed from the shell (Hendelberg, 1961). Methods by Hendelberg(1961) and Neves & Moyer (1988) have been used for age estimation in Margaritiferidae (Hruska, 1992; Hastie *et al.*, 2000; Howard & Cuffey, 2006). However, the method by Neves & Moyer (1988) is not suitable for some Margaritiferidae because the periostracum and the prismatic layer around umbo of a margaritiferid shell are often eroded. Okada & Ishikawa (1959) have also discussed that age estimation for *M. laevis* is difficult due to the erosion of umbo. The age estimation method by Hendelberg (1961) or Johnson & Brown (1998) compensates the number of growth bands in the damaged part of the shell. Therefore these methods are more suitable for Margaritiferidae.

IV. 2. Status of viability of *M. laevis* populations in Japan

In the present study, several populations lacked mussels younger than 7 years old and this fact indicated that the reproduction of mussels had ceased or recruitments had not been successful in the past years due to some factors. Little or no recruitment was observed for 5 populations. Some reports, have pointed out that juveniles are not observed in Japanese mussel populations (Hiruzen Education Board, 2004). The age estimated from the number of

growth bands of shell except severe eroded sample is usually more accurate than that estimated from shell length. The former methods are limited because the shells are often eroded around the umbo by hydraulic power. The ages of *M. laevis* should be also estimated without killing them because the species is designated as a protected species by the government of Japan. Consequently, the age of *M. laevis* was estimated from shell length.

The margaritiferid populations without juveniles are often observed in various countries (Araujo & Ramos, 2000, Cosgrove *et al.*, 2000). A population which has failed in recruitment for certain period of years is apt to extinguish. It has been pointed out that the recruitment in *M. togakushiensis* is associated with the abundance of host fishes (Awakura, 1969).

The total number of eggs and glochidia released from gravid mussels increased with increasing mussel size for the Abira River. For the verification whether or not the number of eggs in marsupia could be explained from the shell length of gravid mussel from the Chitose River, Awakura (1968) investigated the number of eggs in marsupia (N_E) in relation to the shell length (Ls, mm) of *Margaritifera laevis*. Using his data, the following regression expression was obtained:

$N_E = 4000Ls - 145800$ (adjusted R^2=0.87, F=88.94, p<0.01, N=28)

This indicates that the reproductive potential in gravid mussel increases with increasing shell length in *M. laevis*. Hence, a population with larger

mussels is advantageous in reproduction. In *M. margaritifera*, the tendency is not clear (Bauer, 1987c; 1998). A population which consisted of mussels with longer lifespans is able to exist under unfavorable conditions for juvenile mussels for a few decades due to the long duration of reproduction. Consequently, the population which consists of mussels attaining larger size and longer lifespan is considered to be more stable in theory (Fig. 33). However, indeed, a lack of juvenile mussels in a population occurred irrespective of size and lifespan of *Margaritifera* (Fig. 14, 15). In the rivers in which mussel populations lacked juveniles less than 25 mm in shell lengths, many dams had been constructed and the eutrophication had progressed. These two factors might be the major cause of the decline of Margaritiferidae in Japan.

IV. 3 Causes of margaritiferid decrease and extinction

Population size in the Chitose River was much larger than that in the Abira River. Although juveniles in the Chitose River were abundant, mussels under 10 years old were scarce in the Abira River (Fig. 15). Therefore, the population size in the Abira River was considered to be decreasing gradually. The lack of juveniles for a long time might induce the decline of mussels. Since mussels in the Abira River could reproduce in the field, cessation of reproduction was not considered as the cause of lack of juveniles. From the present incubation study of gravid mussels in the laboratory for the Abira

population, I observed the glochidial release from exhalant siphon, glochidial infection to gills of suitable host fish and the growth of parasitic glochidia on fish gills. Culturing of host fish in the laboratory, glochidia were observed to detach from the fish. However, since mussels less than 60 mm in size could not be collected in the Abira River (Fig. 19), juveniles might have died on the riverbed soon after the detachment from the host fish. Survival rates during the period from egg stage to juvenile stage were lower in the Abira River than that in the Chitose River throughout the initial life stages (Fig. 34). Accordingly, the lack of juveniles was induced not only by the low survival rate in the juvenile stage, but also by the low survival rate in the glochidial stages in the Abira River.

In Margaritiferidae, the number of eggs per mussel during a breeding season is generally more abundant than that in the other Unionoida. The maximum proportion of gravid female mussels in the population of the Abira River was higher than that of the Chitose River (Fig. 21). Within the same shell size, the mean number of eggs in a gravid mussel was larger for the Abira River than that for the Chitose River. Therefore, the reproductive potential might not be the cause of lacking juveniles in the Abira River. It is an obligate condition for free-living glochidia to parasitize host fish because glochidia cannot survive without parasitism (Jansen *et al.* 2001). The survival rate of glochidia until attaching to the host was lower in the Abira River (Fig. 34). This lower survival rate was induced presumably by the low

host density and the low habitat overlap between mussels and host fishes. In the Chitose River, the host fish was distributed throughout the mussel habitats, whereas in the Abira River, the host fish was distributed in half the mussel habitats. However, the viability of glochidia before parasitism was higher in the Abira River (Fig. 26). The survival time of free-living glochidia was longer in the Abira River at 10℃ (Fig. 25). These facts suggested that low glochidial survival rate in the Abira River was induced by the decreased chance of parasitism to host fish, irrespective of higher viability of glochidia.

The proportion of infected host fish was higher in the Abira River than that in the Chitose River and the mean number of attached glochidia on gills was larger in the Abira River. However, from the result of the glochidial infection-experiment in the laboratory, The ratio of attached glochidia from the Abira River was lower than that from the Chitose River (Table 7). Host fish acquire resistance to the parasitic glochidia (Dodd *et al.*, 2005, 2006), host produces specific antibodies against the glochidia some weeks after infection (Arey, 1932; Meyers *et al.*, 1980). *O. masou masou* can also acquire specific antibodies against the glochidia of *M. laevis* (Awakura, 1964), thereby reducing glochidial survival rate during the parasitic stage by immune reaction. Margaritiferidae are easy to be attacked by antibodies because their parasitic period was relatively longer than other Unionoida (Bauer, 1994) (Fig. 37). Since a host which had been infested by glochida in the past has more intense antibody response against glochidia, the survival

rate of parasitic glochidia on this fish becomes very low (Bauer, 1987a). However in the present study, 0-year fishes were used, which had never been infested by glochidia.

In the laboratory, the amount of water filtrated by host fish per unit time increased with fish size. Accordingly the number of parasitic glochidia on gills increased with fish size. However, in larger host fish, more glochidia were excluded by the host fish even if the age was the same among host fishes (Bauer & Vogel, 1987). In the Abira River, the mean fork length of *O. masou masou* was longer (Fig. 35), implying that they excluded more glochidia than in the Chitose River. Hence the survival rate of parasitic glochidia might become lower in the Abira River. The success of parasitism is suggested to be negatively correlated with the thickness of gill tissue of host fish, which changes with body size (Bauer, 1987b). In the glochidial parasitic stage from early-August to mid-September, water temperature was lower in the Abira River (Fig. 5). Accordingly, the survival rate of glochidia might be lower in the Abira River because the duration of glochidial parasitic stage was extended at lower temperature. Accordingly parasitic glochidia were easily be excluded by immune attack of host. Therefore, the survival rate of parasitic glochidia in the Abira River might become lower due to the larger size of host fishes and the lower water temperature which might enhance the exclusion of glochidia by host fish.

From the results of the in-situ rearing experiment, the survival rate of

juveniles was lower in the Abira River, irrespective of the juveniles' origin (Fig. 32), suggesting that their survival rate depended on habitat environment. Ecological characteristics of juvenile mussels are not known well because of the difficulty in sampling and treatment of them. Among twelve environmental factors including water temperature, dissolved oxygen, conductivity, pH and concentrations of ammonia, nitrite, nitrate, phosphate, sodium, potassium, calcium and magnesium (Buddensiek, 1995), water temperature was the most significant factor affecting viability of juveniles of *Margaritifera margaritifera*. The mean annual water temperature was lower in the Abira River than that in the Chitose River. The season in which the highest mortality was observed for juveniles was October-January when the mean water temperature was 1.0°C in the Abira River and 7.1°C in the Chitose River, which might be related to juvenile mortality. High concentrations of magnesium and ammonia induce decrease in survival rate of juveniles in *Margaritifera margaritifera* (Buddensiek, 1995). Water quality parameters might also affect juvenile survival.

Most of the factors causing decline of margaritiferids are related to the host fish. The present result suggested that the existence of *M. laevis* was greatly affected by the dynamics of host fish. In the Abira River, the recruitment of *M. laevis* never ocurred since ca. 1970 (Fig. 23). Eight dams had been constructed in the downstream of the Abira River during 1960 to 1985 and sea run-form of *Oncorhynchus masou masou* has not been able to

move upstream of the Abira River since ca. 1960 (K. Sakai, personal communication). Perhaps, dam construction may be one major cause of decline in freshwater mussels in this river (Table 5). The population of *M. laevis* in the Chitose River included abundant juveniles, whereas did fewer elder mussels as well as the lower proportion of gravid female mussels (Fig. 21, 22). Although the current population in the Chitose River has stable annual recruitment due to a large population size and plenty of host fish, recovery of population size may be difficult once a drastic decrease of mussels or host fishes occurs.

Size of margaritiferid glochidia is relatively small among the families of Unionoida (Bauer, 1994) (Fig. 36). Glochidia of *Margaritifera* do not have hook. Hookless glochidia are able to attach only to thin gills and soft tissues of suitable host fish thereby reducing their host range (Bauer, 1994; Table 8). Margaritiferid glochidia are only attachable to gills of host fish (Bauer, 1994) (Table 2). Survival times of free-living glochidia of *Margaritifera* are often shorter than that of other Unionoida (Akiyama, in press) (Fig. 37). For these traits in Margaritifera, chance of parasitism to host fish may be less than that in the other Unionoida. Accordingly survival rate during the period from free-living glochidia to the end of parasitic stages is originally lower in *Margaritifera*. The duration of parasitic stage in Margaritiferidae is longest among Unionoida (Fig. 38). Small glochidia are only poorly developed when they are released by the female mussel and they have to attach on gills of

host fish for a long period until metamorphosis to juvenile stage. Because Margaritiferidae are distributed in high latitudes, their glochidia grow in colder waters than other Unionoida families, which may further extend the duration of glochidial parasitic stage. Margaritiferid glochidia grow during the parasitic stage (Kobayashi & Kondo, 2005). Glochidial growth during parasitism is not common. Of the 18 species of Unionoida in Japan (Kondo, 2002; Kondo & Kobayashi, 2005; Kondo *et al.* 2006), only three species, *Margaritifera laevis*, *M. togakushiensis* and *Pseudodon omiensis* are known to grow during the period of attachment on host (Nakagawa *et al.*, 1998; Kobayashi & Kondo, 2005). Extension of parasitic period may increase the probability of exclusion of parasitic glochidia by the immune response of host fish. Consequently, the survival rate of parasitic glochidia in *Margaritifera* tends to become lower than that in the other families of Unionoida. These traits in Margaritiferidae indicate that their glochidia are originally more vulnerable in their life history and the survival of glochidia strongly depends on host fish. Therefore adverse impacts on glochidia determine margaritiferid population dynamics, sometimes causing extinction of Margaritiferidae worldwide.

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References

Adam M. E. (1990) Shell growth in some Nile bivalves. *Journal of Molluscan Studies*, **56**, 301-308.

Akiyama Y. (2003) Examination of methods for age and growth determination of a long-lived freshwater bivalve, *Margaritifera laevis* (L.). MSc Thesis, The Graduate School of Environmental Earth Science, Hokkaido University, Japan.

Altaba C. R. (1990) The last known population of the fresh-water mussel *Margaritifera auricularia* (Bivalvia, Unionoida) A conservation priority. *Biological conservation*, **52**, 271-286.

Altnöder K. (1926) Beobachtungen über die biologie von *Margaritana margaritifera* und über Ökologie ihres Wohnorts. *Archiv fur Hydrobiologie*, **17**, 423-491.

Alvarez C. C., Garcia-Roves P., Ocharan R., Cabal J. A., Ocharan F. J. & Alvarez M. A. (2000) A new record of the freshwater pearl mussel *Margaritifera margaritifera* L. (Bivalvia, Unionoida) from the River Narcea (Asturias, north-western Spain). *Aquatic Conservation: Marine and Freshwater Ecosystems*, **10**, 93-102.

Anthony J. L., Kesler D. H., Downing W. L. and Downing J. A. (2001) Length-specific grwoth rates in freshwater mussels (Bivalvia: Unionidae): extreme longevity or generalized growth cessation ? *Freshwater Biology*, **46**, 1349-1359.

Araujo R. & Ramos M. A. (2000) Status and conservation of the giant

European freshwater pearl mussel (*Margaritifera auricularia*) (Spengler, 1793) (Bivalvia: Unionoidea). *Biological Conservation*, **96**, 233-239.

Araujo R. & Ramos M. A. (2001) Life-history data on the virtually unknown *Margaritifera auricularia*. In "*Ecology and Evolution of the Freshwater Mussels Unionoida*" Ed by G Bauer, K Wachtler, Springer-Verlag, Berlin Heidelberg pp156-162.

Araujo R., Bragado D. and Ramos M. A. (2001) Identification of the river blenny, *Salaria fluviatilis*, as a host to the glochidia of *Margaritifera auricularia*. *Journal of Molluscan Studies*, **67**, 128-129.

Arey L. B. (1932) A microscopical study of glochidial immunity. *Journal of Morphology*, **53**, 367-378.

Awakura T (1964) On the parasitic glochidium of the fresh-water pearl mussel, *Margaritifera margaritifera* (L.) on salmonid fish. *Science Report of Hokkaido Fish Hatchery* **19**, 1-16.

Awakura T. (1968) The ecology of parasitic glochidia of the fresh-water pearl mussel, *Margaritifera laevis* (Haas). *Science Report of Hokkaido Fish Hatchery*, **23**, 1-21.

Awakura T. (1969) On the correlation between the age composition of the freshwater pearl mussel, *Margaritifera laevis* (Haas) and the population size of salmonid fishes. *Science Report of Hokkaido Fish Hatchery*, **24**, 55-88.

Bauer G. (1983) Age structure, age specific mortality rates and population trend of the freshwater pearl mussel (Margaritifera margaritifera) in

North Bavaria. *Archiv fur Hydrobiologie*, **98**, 523-532.

Bauer G. (1986) The status of the freshwater pearl mussel *Margaritifera margaritifera* L. in the South of its European range. *Biological Conservation*, **38**, 1-9.

Bauer G. (1987a) The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera* L.) II. Susceptibility of brown trout. *Archiv fur Hydrobiologie*, **76**, 403-412.

Bauer G. (1987b) The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera* L.) III. Host relationships. *Archiv fur Hydrobiologie*, **76**, 413-423.

Bauer G. (1987c) Reproductive strategy of the freshwater pearl mussel *Margaritifera margaritifera*. *Journal of the Animal Ecology*, **56**, 691-704.

Bauer G. (1988) Threats to the freshwater pearl mussel *Margaritifera margaritifera* L. in central Europe. *Biological Conservation*, **45**, 239-253.

Bauer G. (1992) Variation in the life span and size of the freshwater pearl mussel. *Journal of Animal Ecology*, **61**, 425-436.

Bauer G. (1994) The adaptive value of offspring size among freshwater mussels (Bivalvia; Unionoidea). *Journal of Animal Ecology*, **61**, 425-436.

Bauer G. (1997) Host relationships at reversed generation times: *Margaritifera* (Bivalvia) and Salmonids. In "*Vertical Food Web Interactions*" Ed by Dettner K., Bauer G. & Volkl W., Springer-Verlag, Berlin Heidelberg pp69-79.

Bauer G. (1998) Allocation policy of female freshwater pearl mussels. *Oecologia*, **117**, 90-94.

Bauer G. & Vogel C. (1987) The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera* L.) I. Host response to Glochidiosis. *Archiv fur Hydrobiologie*. **76**, 393-402.

Beasley C.R. & Roberts D. (1996) The current distribution and status of the freshwater pearl mussel *Margaritifera margaritifera* L. 1758 in North-West Ireland, *Aquatic Conservation: Marine and Freshwater Ecosystems*, **6**, 169-177.

Beasley C. R. & Roberts D. (1999a) Towards a strategy for the conservation of the freshwater pearl mussel *Margaritifera margaritifera* in County Donegal, Ireland. *Biological Conservation*, **89**, 275-284.

Beasley C. R. & Roberts D. (1999b) Assessing the conservation status of the freshwater pearl mussel in the north of Ireland relevance of growth and age characteristics. *Journal of Conchology*, **36**, 53-61.

Beasley C. R., Roberts D., Mackie T. G. (1998) Does the freshwater pearl mussel, *Margaritifera margaritifera* L., face extinction in Northern Ireland?, *Aquatic conservation: Marine and freshwater ecosystems*, **8**, 265-272.

Bertalanffy v. J. (1938) A quantitative theory of organic growth (inquiries on growth laws. II). *Human biology*, **10**, 181-213.

Biodiversity Steering Group (1995) *Biodiversity: The UK Steering Group*

Report. 2, Action Plans. HMSO: London.

Britton J. C. (1979) *The freshwater bivalve mollusca of the Sarannah River Plant*. Academy of Natural Sciences, Philadelphia.

Buddensiek V. (1995) The culture of juvenile freshwater pearl mussels *Margaritifera margaritifera* L. in cages: A comtribution to conservation programmes and the knowledge of habitat requirements. *Biological Conservation*, **74**, 33-40.

Clarke A. H. (1973) *The freshwater mollusks of Canada*. National Museum of Canada, Ottawa.

Clarke A. H. (1981) The freshwater mollusks of Canadian Interior Basin. *Malacologia*, **13**, 495pp.

Cosgrove P. J., Young M. R., Hastie L. C., Gaywood M. & Boon P. J. (2000) The status of the freshwater pearl mussel *Margaritifera margaritifera* Linn. in Scotland. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **10**, 197-208.

Costello M. J., Moorkens E. A., Larkin M., Kurz I. & Dowse J. (1998) Management priorities for the river Nore (Ireland) to conserve the pearl mussel, *Margaritifera durrovensis* Philips, 1928. *Journal of Conchology Specieal Publication*, **2**, 257-264.

Cunjak R. A. and McGladdery S. E. (1990) The parasite-host relationship of glochidia (Mollusca: Margaritiferidae) on the gills of youg-of-the-year Atlantic salmon (*Salmo salar*). *Canadian Journal of Zoology*, **69**, 353-358.

Curtis J. M. R., Vincent A. C. J. (2006) Life history of an unusual marine

fish: survival, growth and movement patterns of *Hippocampus guttulatus* Cuvier 1829. *Journal of Fish Biology* **68**, 707-733.

Dodd B. J., Barnhart M. C., Rogers-lowery C. L., Fobian T. B. & Dimock R. V. Jr. (2005) Cross-resistance of largemouth bass to glochidia of unionid mussels. *Journal of Parasitology*, **91**, 1064-1072.

Dodd B. J., Barnhart M. C., Rovers-Lowery C. L., Fobian T. B. and Dimock R. V. Jr. (2006) Persistence of host response against glochidia larvae in *Micropterus salmoides*. *Fish and shellfish immunology*, **21**, 473-484.

Ekman T. (1905) Undersökningar öfver flodpärlmusslans förekomst och lefnadsförhållanden I Ljusnan och dess tillflöden inom Härjedalen. *Meddelanden från Kungl*, **110**, 1-12.

Fuller S. L. H. (1974) Clams and mussels. *Pollution Ecology of Freshwater Invertebrates* (eds C. W. Hart & S. L. H. Fuller), pp. 215-273. Academic Press, New York.

Fustish C. A. and Millemann R. E. (1978) Glochidiosis of salmonid fishes. II. Comparison of tissue response of coho and chinook salmon to experimental infection with Margaritifera margaritifera (L.) (Pelecypoda: Margaritiferidae). *Journal of Parasitology*, **64**, 155-157.

Geographical Survey Institute (2002) *topographic map of 1/25,000 magnification*. Geographical Survey Institute.

Hastie L. C., Young M. R. and Boon P. J. (2000) Growth characteristics of freshwater pearl mussels, *Margaritifera margaritifera* (L.). *Freshwater Biology*, **43**, 243-256.

Hendelberg J. (1961) The fresh-water pearl mussel, *Margaritifera margaritifera* (L.). *Report of the Institute of Freshwater Research Drottningholm*, **41**, 149-171.

Heard W. H. (1975) Sexuality and other aspects of reproduction in Anodonta. *Malacologia*, **15**, 81-103.

Hiruzen Education Board (2004) *Report of the status of freshwater pearl mussel, Margaritifera laevis in the Asahi River Systems*. Hiruzen Education Board, Okayama, Japan.

Howard J. K. & Cuffey K. M. (2006) Factors controlling the age structure of Margaritifera falcata in 2 northern California streams. *Journal of the North American Benthological Society*, **25**, 677-690.

Hruska J. (1992) The freshwater pearl mussel in South Bohemia: Evaluation of the effect of temperature on reproduction, growth and age structure of the population. *Archiv fur Hydrobiologie*, **126**, 181-191.

Inoue M., Nakano S. & Nakamura F. (1997) Juvenile masu salmon (*Oncorhynchus masou*) abundance and stream habitat relationships in northern Japan. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 1331-1341.

IUCN (2006) *IUCN Red List of Threatened Species*. Available from http://www.redlist.org (accessed June 2007).

Jansen W, Bauer G & Zahner-Meike E (2001) Glochidial mortality in freshwater mussels. In "*Ecology and Evolution of the Freshwater Mussels*

Unionoida" Ed by G Bauer, K Wachtler, Springer-Verlag, Berlin Heidelberg pp185-211

Japan Society of Analytical Chemistry (1998) *Analysis of water*. Kagaku-Dojin, Kyoto. pp266-273.

Johnson P. D. & Brown K. M. (1998) Intraspecific life history variation in the threatened Louisiana pearlshell mussel, *Margaritifera hembeli*. *Freshwater Biology*, **40**, 317-329.

Johnson B. J. & Omland K. S. (2004) Model selection in ecology and evolution. *Trends in Ecology and Evolution*, **19**, 101-108.

Karna D. W. & Millemann, R. E. (1977) Glochidiosis of salmonid fishes. III. *Journal of Parasitology*, **63**, 728-733.

Kobayashi O. & Kondo T. (2005) Difference in host preference between two populations of the freshwater pearl mussl *Margaritifera laevis* (Bivalvia: Margaritiferidae) in the Shinano river system, Japan. *Venus*, **64**, 63-70.

Kohmoto T. (1928) Freshwater pearl mussel in the Yamaguchi prefecture. *Venus*, **1**, 15-18.

Kondo T. (2002) *Unionid mussels of Japan*. Osaka Kyoiku University.

Kondo T. & Kobayashi O. (2005) Revision of the genus *Margaritifera* (Bivalvia: Margaritiferidae) of Japan, with description of a new species. *Venus*, **64**, 135-140.

Kondo T., Tabe M. & Fukuhara S. (2006) Morphological differences of glochidia between two gnenetic types of *Anodonta* "*woodiana*" (Bivalvia:

Unionidae). *Venus*, **65**, 241-245.

Kondo T., Yamada M., Kusano Y. & Sakai K. (2000) Fish hosts of the freshwater pearl mussel *Margaritifera laevis* (Bivalvia: Margaritiferidae) in the Furebetsu River, Hokkaido. *Venus*, **59**, 177-179.

Kurihara Y., Sakai H., Kitano S., Kobayashi O. & Goto A. (2005) Genetic and morphological divergence in the freshwater pearl mussel, Margaritifera laevis (Bivalvia: Margaritiferidae), with reference to the existence of two distinct species. *Venus*, **64**, 55-62.

Kuroda T. (1931) Superfamily Naiadacea. *Venus*, **2**, 57-74.

Lucey J. (1993) The distribution of *Margaritifera margaritifera* (L.) in southern Irish rivers and streams. *Journal of Conchology*, **34**, 301-310.

Manly B. F. J. (1997) *Randomization, bootstrap and Monte Carlo methods in biology*. Second Edition, Text in Statistical Science, Chapman & Hall, London.

Matsuoka K. (1979) Conservation for freshwater pearl mussel, *Margaritifera laevis*. *Freshwater Fish*, **5**, 122-125.

Meyers T.R., Millemann R. E. & Fustish C. A. (1980) Glochidiosis of Salmonid fishes IV. Humoral and tissue response of Coho and Chinook salmon to experimental infection with *Margaritifera margaritifera*. *Journal of Parasitology*, **66**, 274-281.

Miyadi D. (1938) Terrestrial water animals in the Kunashiri Island. *Japanese Journal of Limnology*, **8**, 330-339.

Murphy G. (1942) Relationship of the fresh-water mussel to trout in the Truckee River. *California Fish and Game*, **28**, 89-102.

Naito J. (1988) A note on the breeding habit of the fresh-water pearl-mussel, *Margaritifera laevis* Haas (Pisces: Margaritiferidae), in Geihoku-Cho, Hiroshima Pref., near the Southern End in Japan. *Miscellaneous reports of Hiwa Museum for natural history*, **27**, 7-15.

Naito J. (1989) Conservation of freshwater pearl mussel, *Margaritifera laevis* in the Geihoku city, the Hiroshima prefecture. *Conservation of Freshwater Fish*, **89**, 99-101.

Nakagawa S., Deno T. and Kondo T. (1998) Growth and development of larvae of *Pseudodon omiensis*. *Memoirs of Osaka kyoiku University. III, Natural Science and Applied Science*, **46**, 181-186.

Nakano S., Kitano F., Maekawa K. (1996) Potential fragmentation and loss of thermal habitats for charrs in the Japanese archipelago due to climatic warming. *Freshwater Biology*, **36**, 711-722.

Negus C. L. (1966) A quantitative study of growth and production of Unionid mussels in the River Thames at reading. *Journal of Animal Ecology*, **35**, 513-532.

Neves R. J. & Moyer S. N. (1988) Evaluation of techniques for age determination of freshwater mussels (Unionidae). *American Malacological Bulletin*, **6**, 179-188.

Okada S. & Ishikawa Y. (1959) The biological study of fresh-water pearl

mussel, *Margaritifera margaritifera* (L.), the first report. *Scientific reports of the Hokkaido Fish Hatchery*, **14**, 73-81.

Okada S. & Koba K. (1932) Supplemental report for new distribution of *Margaritifera margaritifera*. *Venus*, **3**, 106-108.

Ross H. C. G. (1990) The potential role of Irish populations in the conservation of the freshwater pearl mussel (*Margaritifera margaritifera* L.) in Europe, *Environmental Encounters*, *Council of Europe*, **10**, 55-58.

San Miguel E., Monserrat S., Fernandez C., Amaro R., Hermida M., Ondina P. & Altaba C. R. (2004) Growth models and longevity of freshwater pearl mussels (*Margaritifera margaritifera*) in Spain. *Canadian Journal of Zoology*, **82**, 1370-1379.

Satake K., Sawada K. & Oohata Y. (1984) Decline of *Margaritifera laevis* in the Iwate prefecture after Jomon period. *Bulletin of the Iwate Prefectural Museum*, **2**, 1-11.

Seber G. A. F. (1973) *The estimation of animal abundance and related parameters*. Griffin, London. 506pp

Smith D. G. (1988) Notes on the biology and morphology of *Margaritifera hembeli* (Conrad, 1838) (Unionacea: Margaritiferidae). *The Nautilus*, **102**, 159-163.

Surber T. (1912) *Identification of the glochidia of freshwater mussels*. Bureau of Fisheries Document No 771. Government Printing Office, Washington.

Taki I. Margaritifera in Japan. *Venus*, **11**, 98.

US Fish and Wildlife Service (2004) Review of species that are candidates or proposed for listing as endangered or threatened. *Annual notice of findings on resubmitted petitions: Annual description of progress on listing actions*. *Federal Register* **69**, 24875-24904.

Valovirta J. (1977) Report of mapping of non-marine molluscs in Finland. *Malacologia*, **16**, 267-270.

Walford L. A. (1946) A New Graphic Method of Describing the Growth of Animals. *The Biological Bulletin*, **90**, 141-147.

Watters G. T. and O'dee S. H. (1998) Metamorphosis of freshwater mussel glochidia (Bivalvia: Unionidae) on amphibians and exotic fishes. *American Midland Naturalist*, **139**, 49-57.

Wellmann G (1943) Fischinfektionen mit glochidien der *Margaritifera margaritifera*. *Zeitschrift Fischerei* **41**, 385-390.

Yoshida Y. (1971) Distribution and conservation for *Margaritifera laevis*. *Animal and Nature*, **1**, 1-7.

Yoshida Y. (1973) Action about distribution and conservation of *Margaritifera laevis*. *Education for Biology in Gifu*, **18**, 19-26.

Young M. & Williams J. (1983) The status and conservation of the freshwater pearl mussel *Margaritifera magaritifera* Linn. in Great Britain. *Biological Conservation*, **25**, 35-52.

Young M. & Williams J. (1984a) The reproductive biology of the freshwater pearl mussel *Margaritifera margaritifera* (Linn.) in Scotland 1. Field studies. *Archiv fur Hydrobiologie*, **99**, 405-422.、

Young M. & Williams J. (1984b) The reproductive biology of the freshwater pearl mussel *Margaritifera margaritifera* (Linn.) in Scotland 2. Laboratory studies. *Archiv fur Hydrobiologie*, **100**, 29-43.

Young M. R., Cosgrove P. J. & Hastie L. C. (2001) The extent of, and causes for, the decline of a highly threatened naiad: Margaritifera margaritifera. In "*Ecology and Evolution of the Freshwater Mussels Unionoida*" Ed by G Bauer, K Wachtler, Springer-Verlag, Berlin Heidelberg pp337-357.

Zale A. L. & Neves R. (1982) Reproductive biology of four freshwater mussel species in Virginia. *Freshwater Invertebrate Biology*, **51**, 17-28.

Zar J. H. (1999) *Biostatistical Analysis*, 4th edn. Prentice Hall.

Ziuganov V., Miguel E. S., Neves R. J., Longa A., Fernandez C., Amaro R., Beletsky V., Popkovitch E., Kaliuzhin S. and Jonson T. (2000) Life span variation of the freshwater pearl shell: a model species for testing longevity mechanisms in animals. *Ambio*, **29**, 102-105.

Table 1 Detrimental factors implicated in the decline of Margaritiferidae populations. The numerals in the table indicate source literatures.

Factors	*Margaritifera margaritifera*	*M. laevis*	*M. auricularia*
Pearl fishing	1, 2, 3, 4, 5		11
Habitat modification	2		11
Pollution	2, 5, 6, 7		11
Hydro-electric schemes	5		11
Canalization	5, 6		
Salmon pool construction	5		
Deliberate rubbish dumping	5		
Forestry operation	5		
Improvement in farm field		9,10	
Sheep-dip effluent	5		
Acidification	5		
Damming	5, 6	8	11

Sources: 1, Young & Williams (1984a); 2, Ross (1990); 3, Beasley & Roberts (1996); 4, Beasley & Roberts (1999a); 5, Cosgrove *et al.* (2000); 6, Alvarez *et al.* (2000); 7, Bauer (1986); 8, Awakura (1969); 9, Yoshida (1971); 10, Yoshida (1973); 11, Altaba (1990)

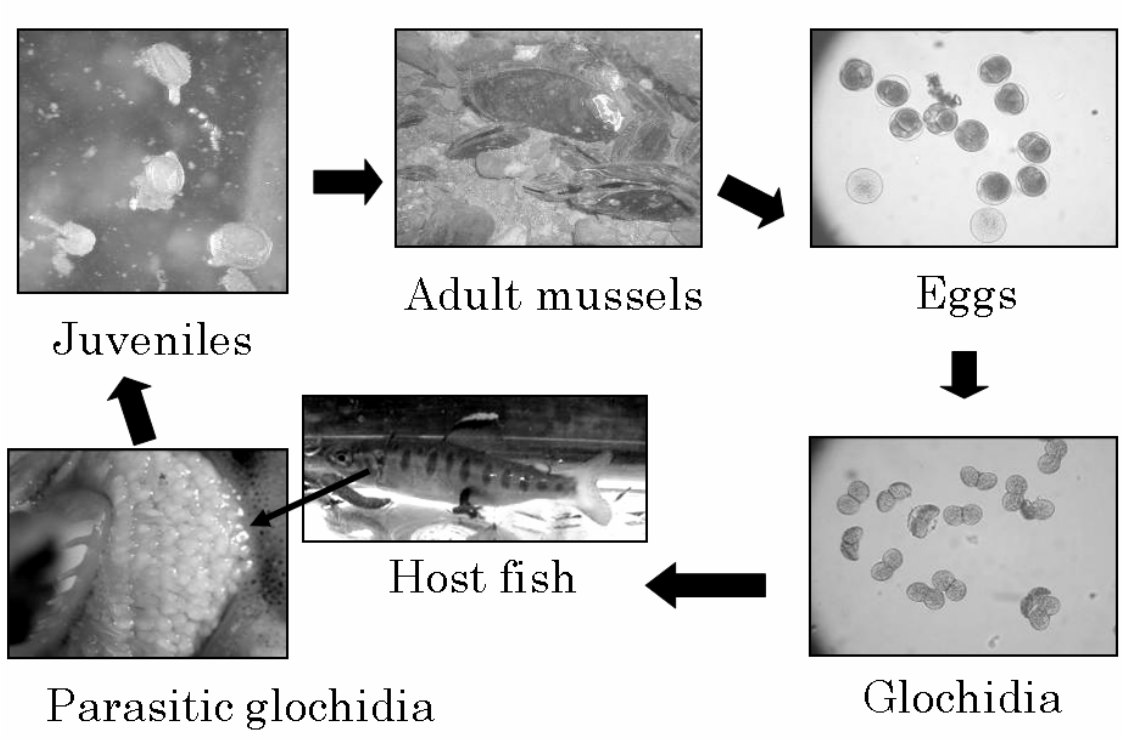

Fig. 1 General life history of Unionoida.

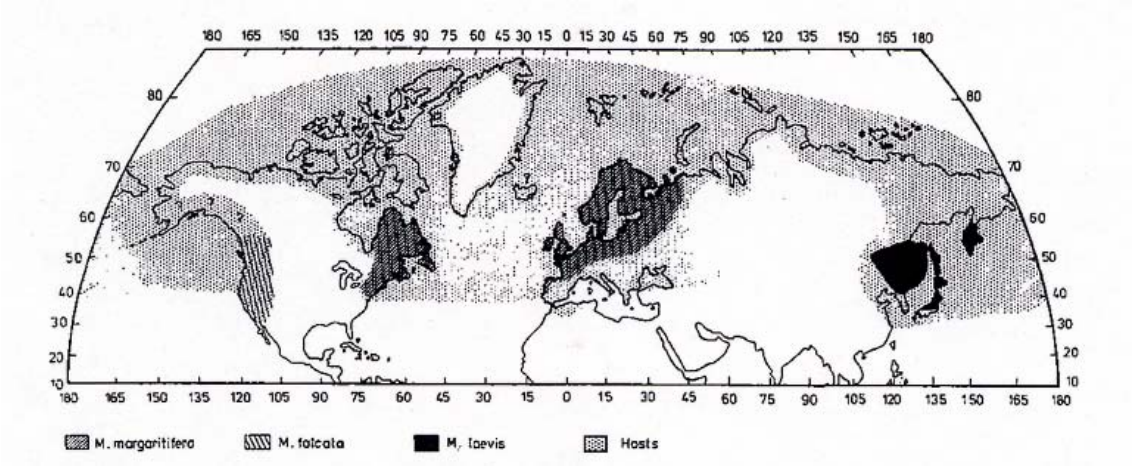

Fig. 2 Approximate distribution area of the three *Margaritifera* species *sensu stricto* and natural distribution of their hosts (Bauer, 1997).

Table 2 Traits of 6 species in margaritiferidae. – is no records

	Locality	Maximum shell size (mm)	Lifespan (year)	Adult age (year)	Breeding season	Glochidial releasing season
Margaritifera laevis	Japan, Kurile Islands, Sakhalin[1,2,3,4]	145.2[5]	79[5]	10[6]	Late June to early August[6]	Late July to mid-August[6]
M. togakushiensis	Japan[10]	122.2[5]	52[5]	-	-	-
M. margaritifera	Central and North Europe, Russia, North America[12]	162.0[13]	190[13]	12[14]	Late July to August[14]	Late July to mid-September[14]
M. falcata	West in North America[19]	92[19]	70-76[20]	-	May to late June[19]	Late June to early July[19]
M. auricularia	Europe, North Africa[21]	200[22]	44[21]	-	Mid-February[22]	February to March[22]
M. hembeli	Southeast in North America[17]	124.4[18]	75[18]	6-9[17]	November to January[17]	-

Table 2 (continued)

	Glochidial size (μm)	Host species	Region of glochidial attachment	Parasitic season	Duration of parasite on host (day)	Size of young mussel (mm)
Margaritifera laevis	50-60[6]	*Oncorhynchus masou masou, O. masou ishikawae, O. nerka, O. keta, Salmo gairdneri*[6,7,8]	Gill[7]	Late June to September[6]	ca. 45[6,7,9]	0.38-0.48[6,7,9]
M. togakushiensis	-	*Salvelinus leucomaenis, Salvelinus fontinalis*[9,11]	Gill[9]	Early July[11]	47-50[9]	0.4-0.47[9]
M. margaritifera	70[15]	*Salmo trutta, Salmo salar*[14,15]	Gill[14,15]	September to June in next year[14,15]	ca. 270[14,15]	0.33-0.5[15,16]
M. falcata	50-60[19]	*Salmo trutta, Salmo gairdneri*[19]	Gill[19]	Late June to early August[19]	36[19]	0.39-0.42[19]
M. auricularia	127-144[22]	*Acipenser baeri, Salaria fluviatilis*[23,24]	Gill[22]	February to April[22]	ca. 30[23]	-
M. hembeli	-	*Noturus phaeus*[18]	Gill[18]	Winter[18]	-	-

Sources: 1, Kohmoto 1928; 2, Taki 1930; 3, Kuroda 1931; 4, Miyadi 1938; 5, Awakura 1969; 6, Awakura 1968; 7, Awakura 1964; 8, Naito 1988; 9, Kobayashi & Kondo 2005; 10, Kondo & Kobayashi 2005; 11, Kondo *et al.* 2000; 12, Young *et al.* 2001; 13, Ziuganov *et al.* 2000; 14, Young & Williams 1984a; 15, Young & Williams 1984b; 16, Bauer 1997; 17, Smith 1988; 18, Johnson *et al.* 1998; 19, Murphy 1942; 20, Howard & Cuffey 2006; 21, Altaba 1990; 22, Araujo & Ramos 2001; 23, Araujo & Ramos 2000; 24, Araujo *et al.* 2001

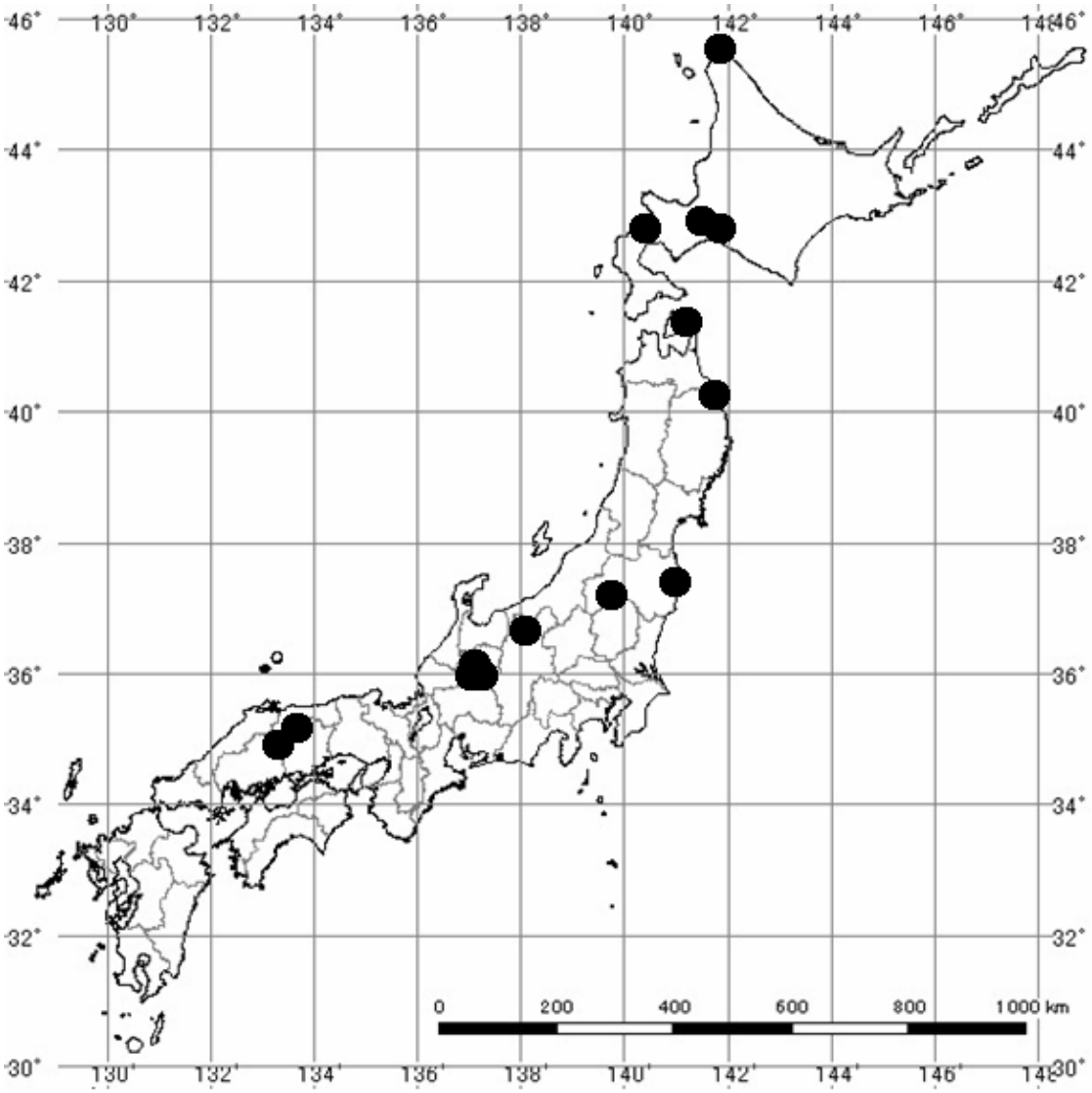

Fig. 3 Map of Japan showing 14 sampling sites of *Margaritifera laevis*.

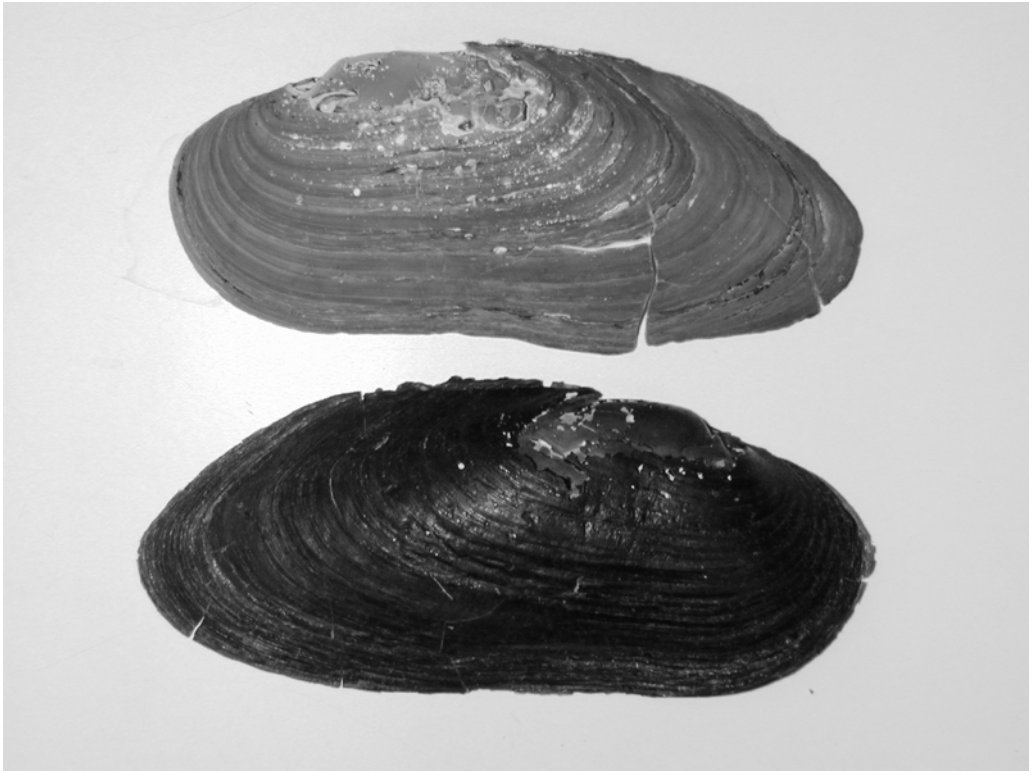

Fig. 4 A comparison of *Margaritifera laevis* shells treated (upper panel) and untreated (lower panel) with hot potassium hydroxide solution.

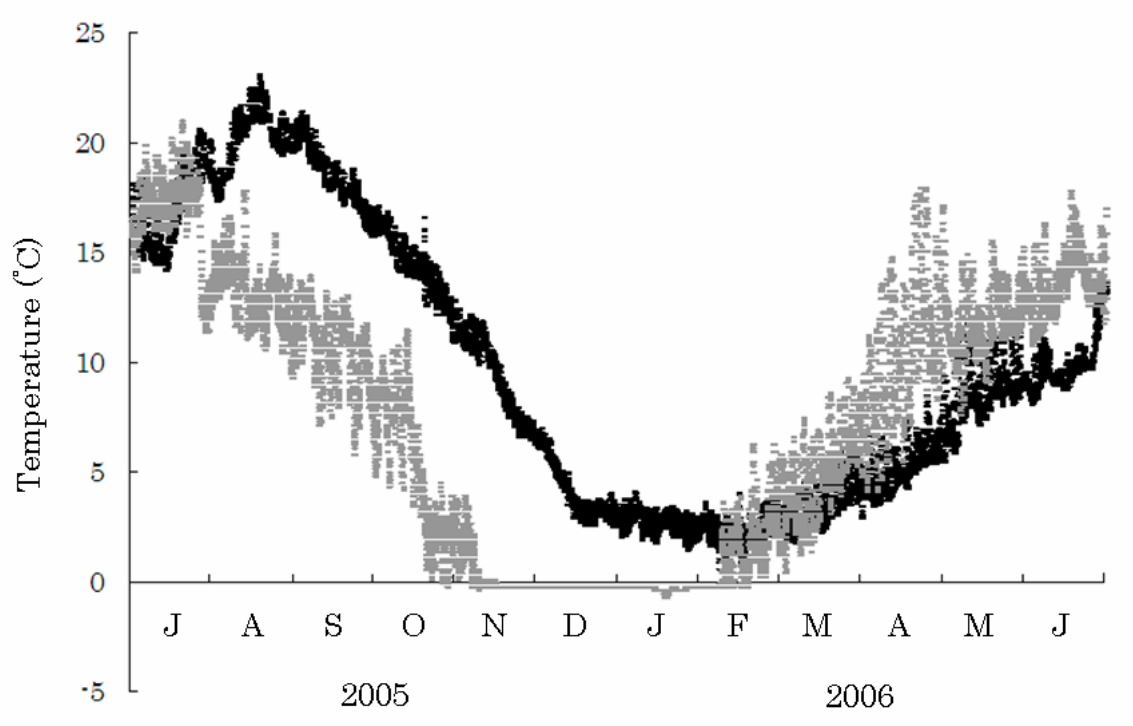

Fig. 5 Seasonal changes in water temperatures in the Chitose (black dots) and the Abira (gray dots) rivers.

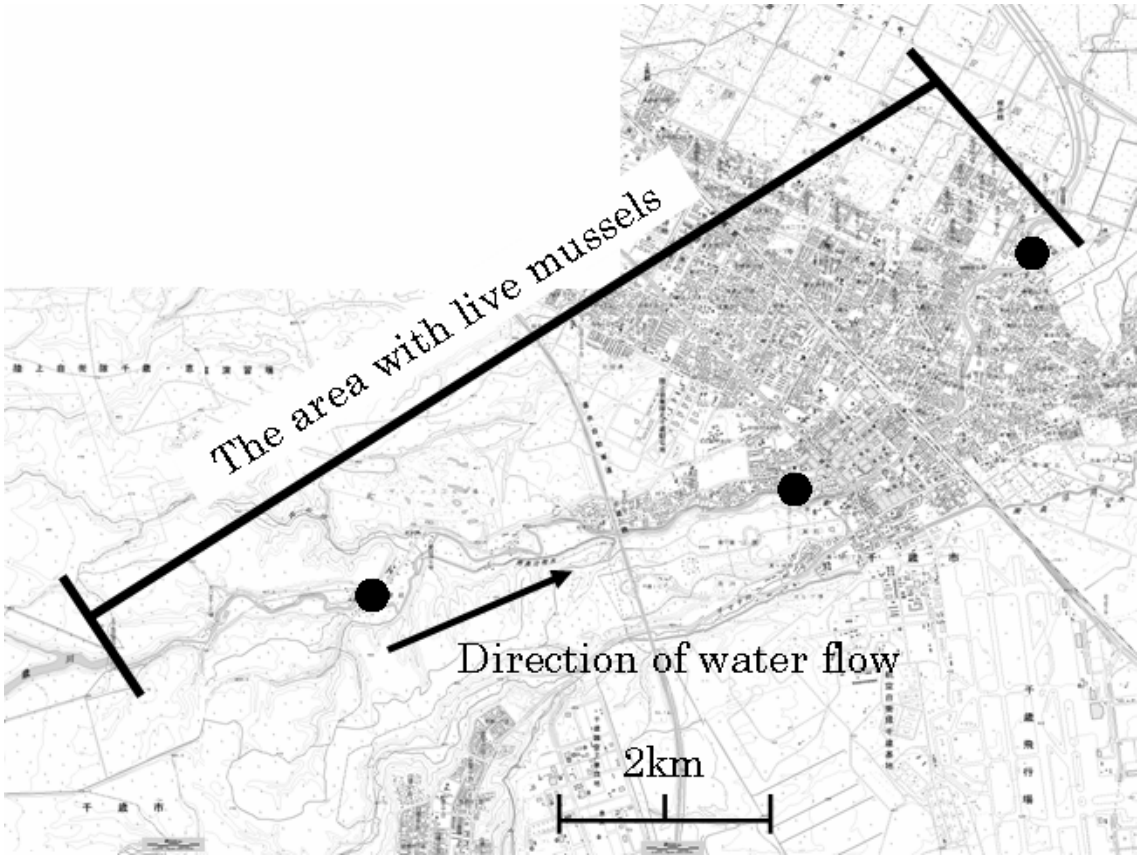

Fig. 6 Glochidial rearing sites (●) in the Chitose River.

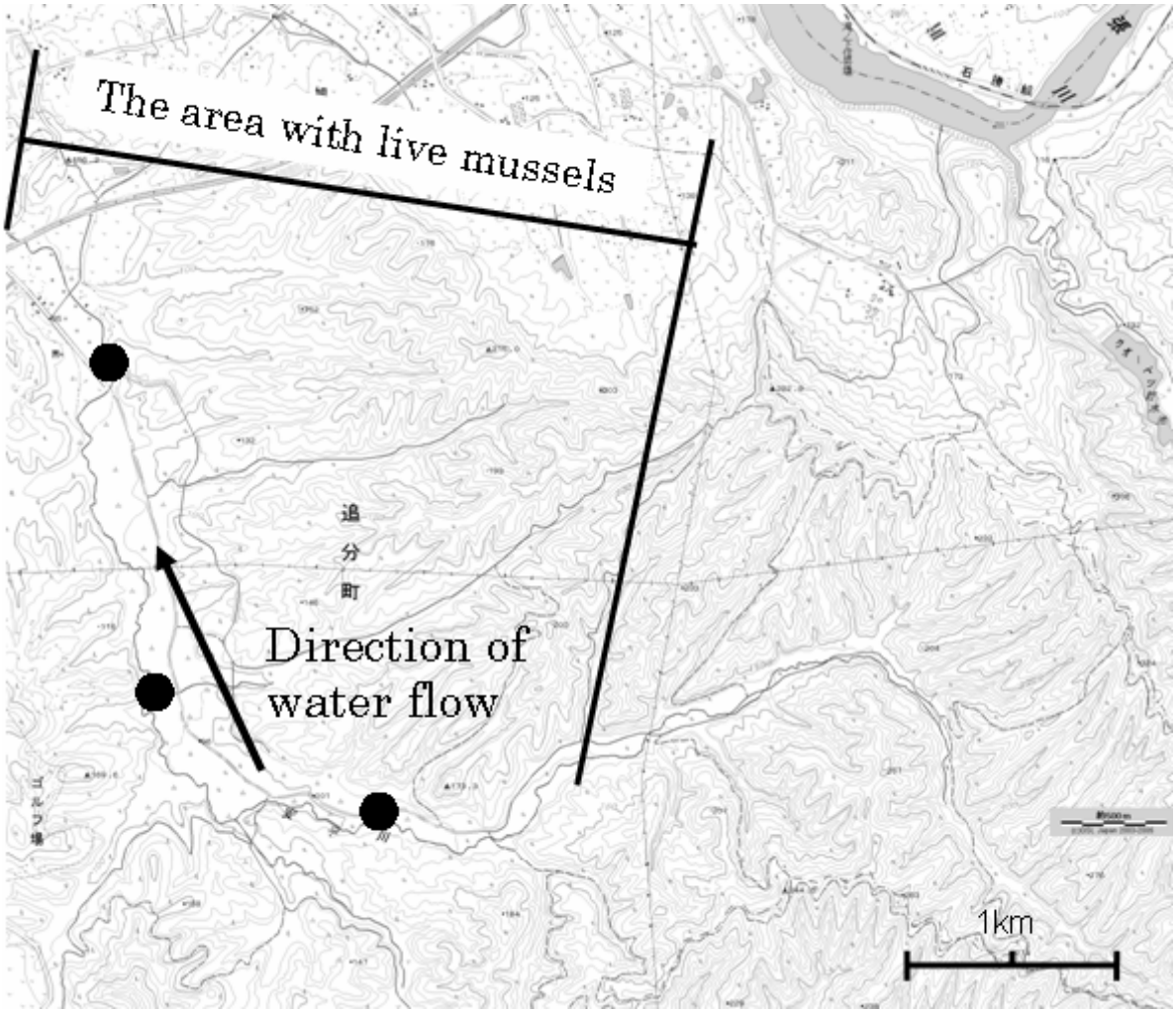

Fig. 7 Glochidial rearing sites (●) in the Abira River.

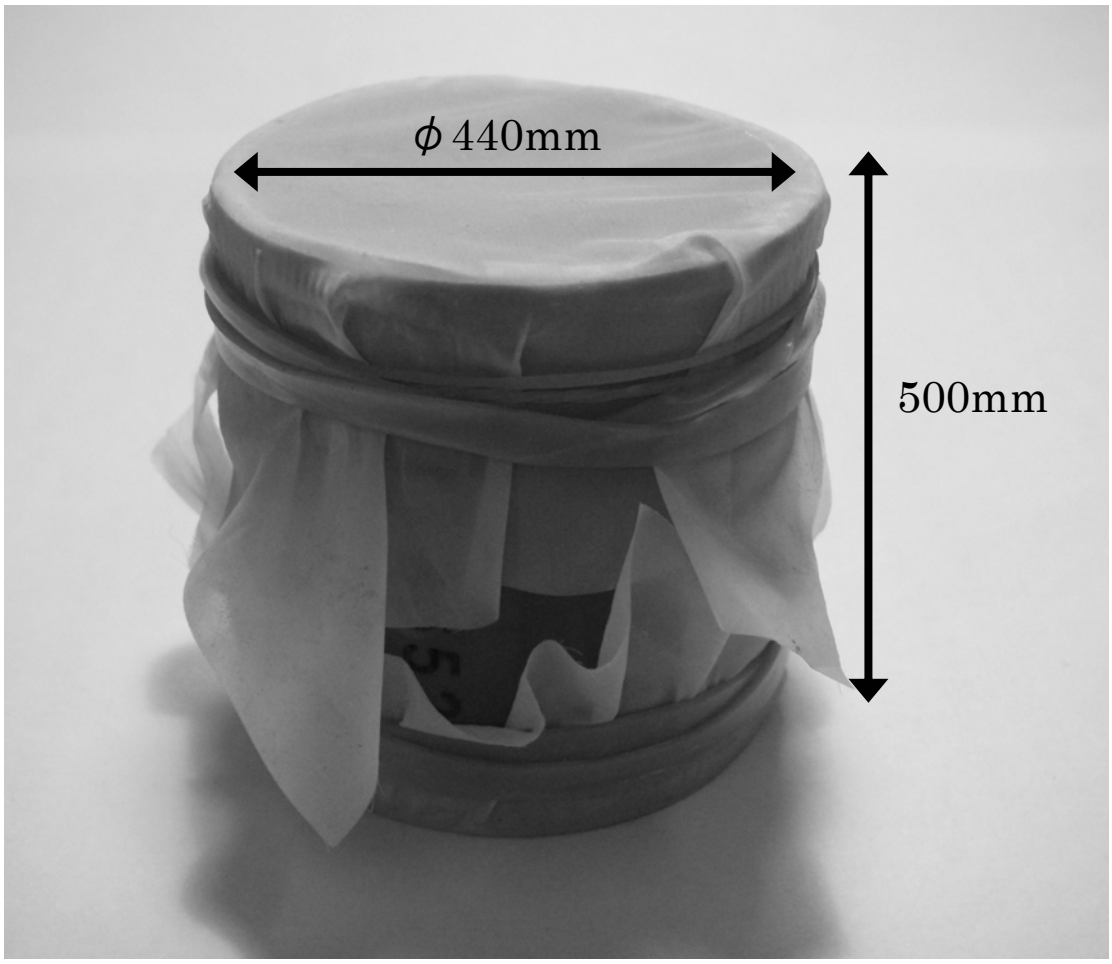

Fig. 8 A chamber for glochidial rearing in the river. The top and bottom of the chamber were covered with nylon nets with mesh size of 40μm.

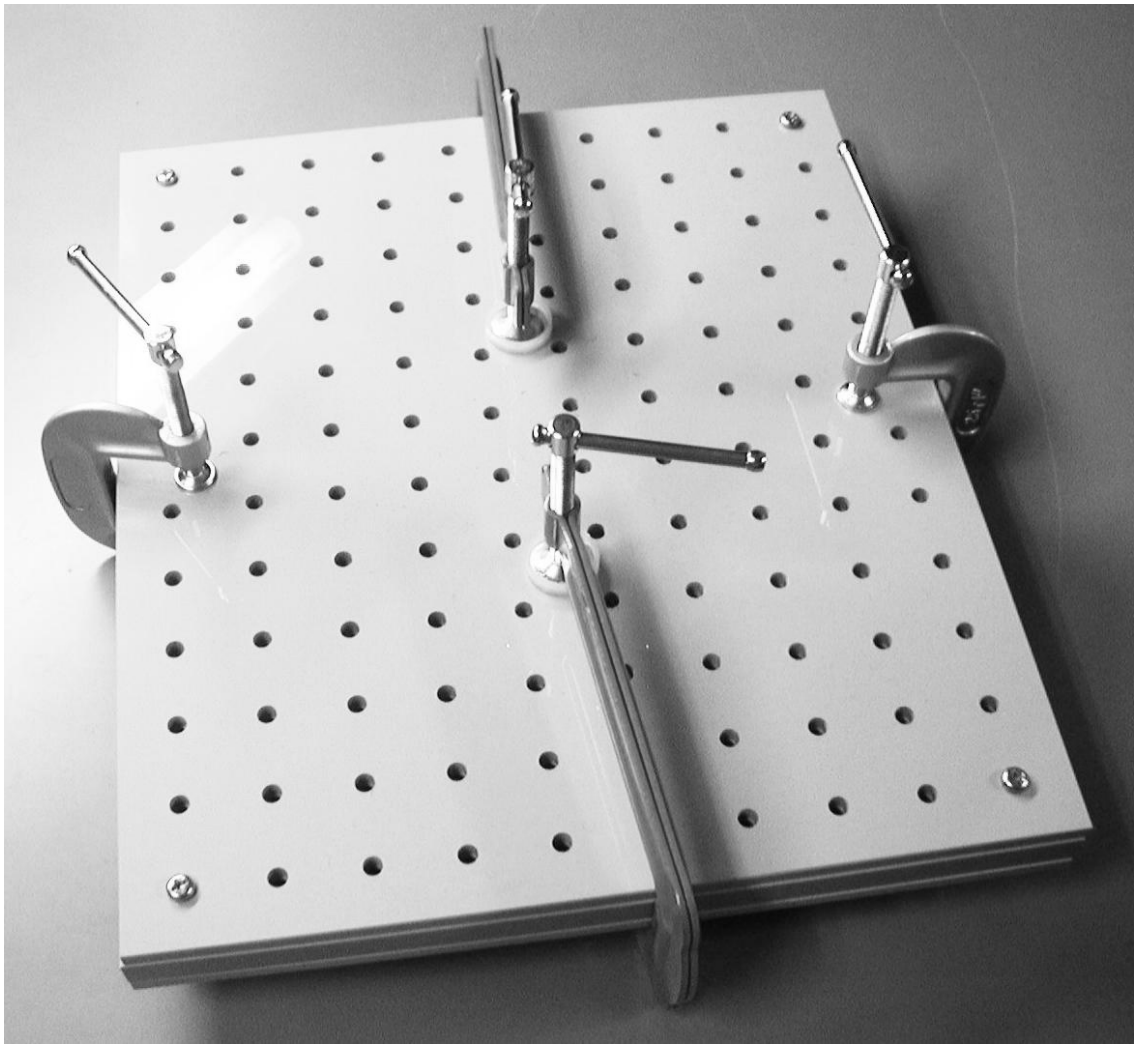

Fig. 9 A culture cage for juvenile mussels.

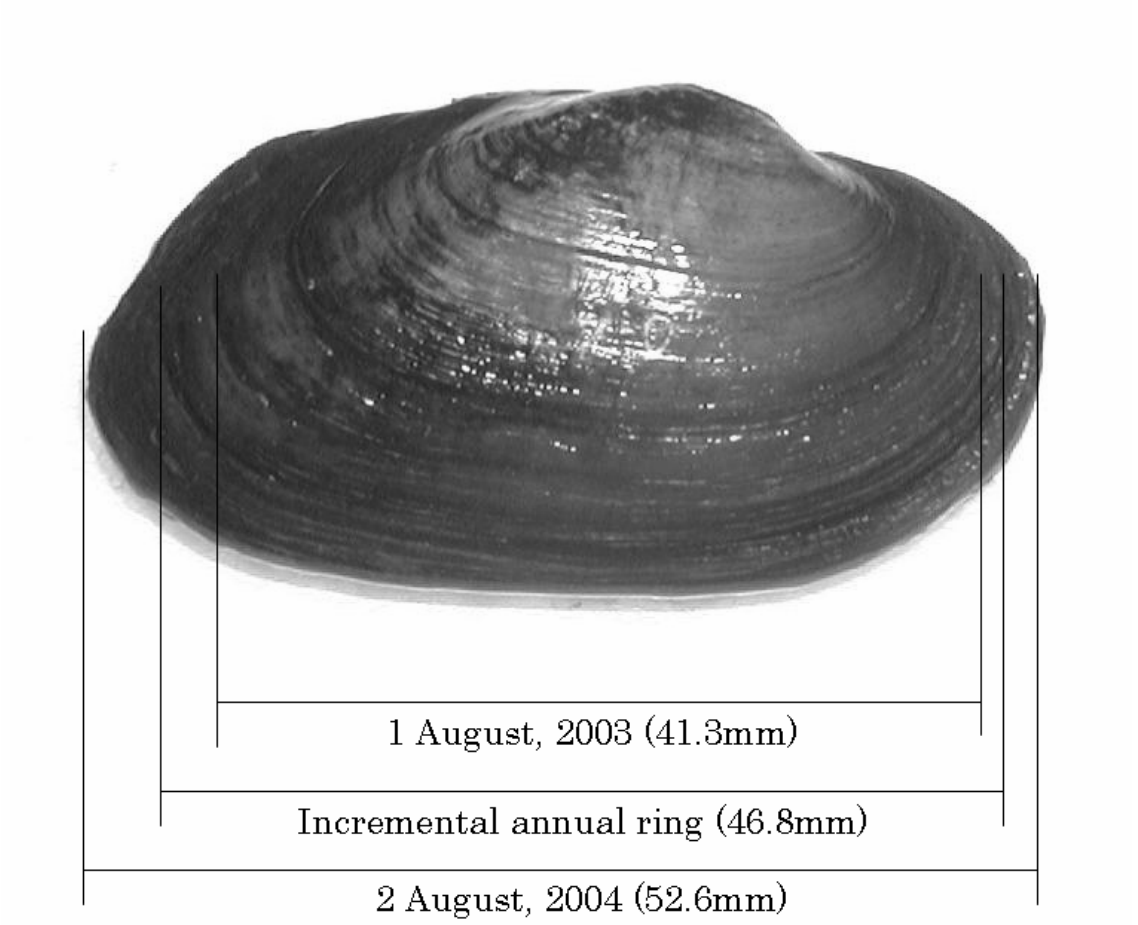

Fig. 10 Increase of annual growth ring on shell from the Shiribetsu River. Some stress lines were formed after the mussel was labeled by the mini drill on August 1, 2003.

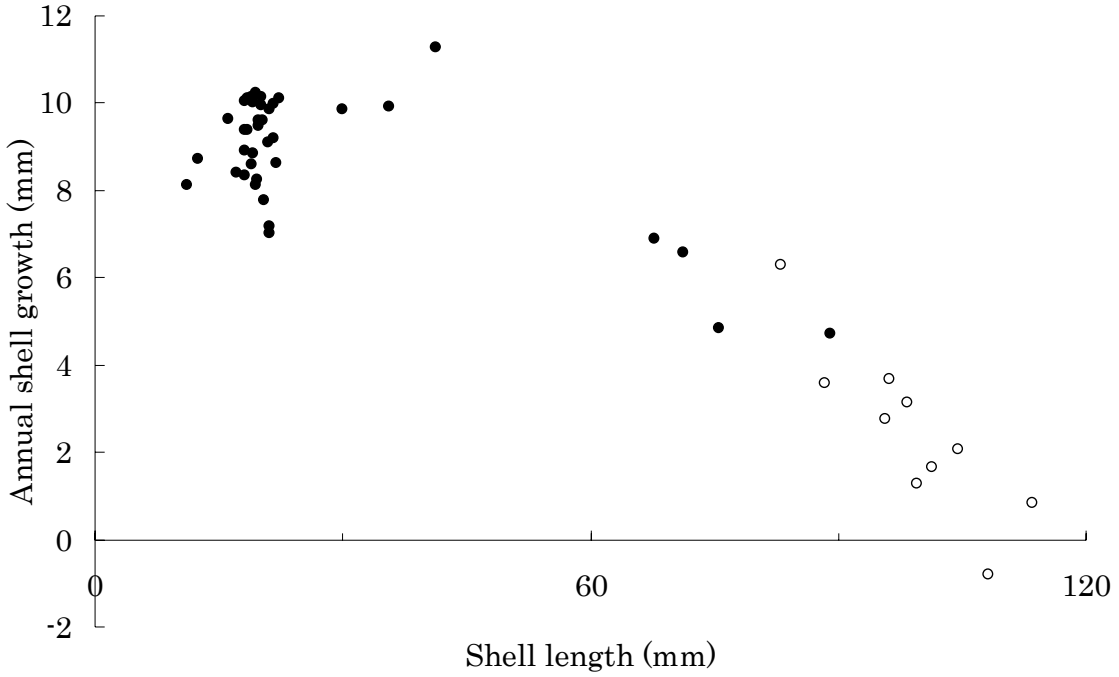

Fig. 11 Annual shell growths of mussels in the Shiribetsu River against initial shell lengths on August 1, 2003. Mussels for which incremental annual growth band was confirmed are expressed by filled circles, whereas mussels whose incremental growth ring could not be identified are represented by open circles.

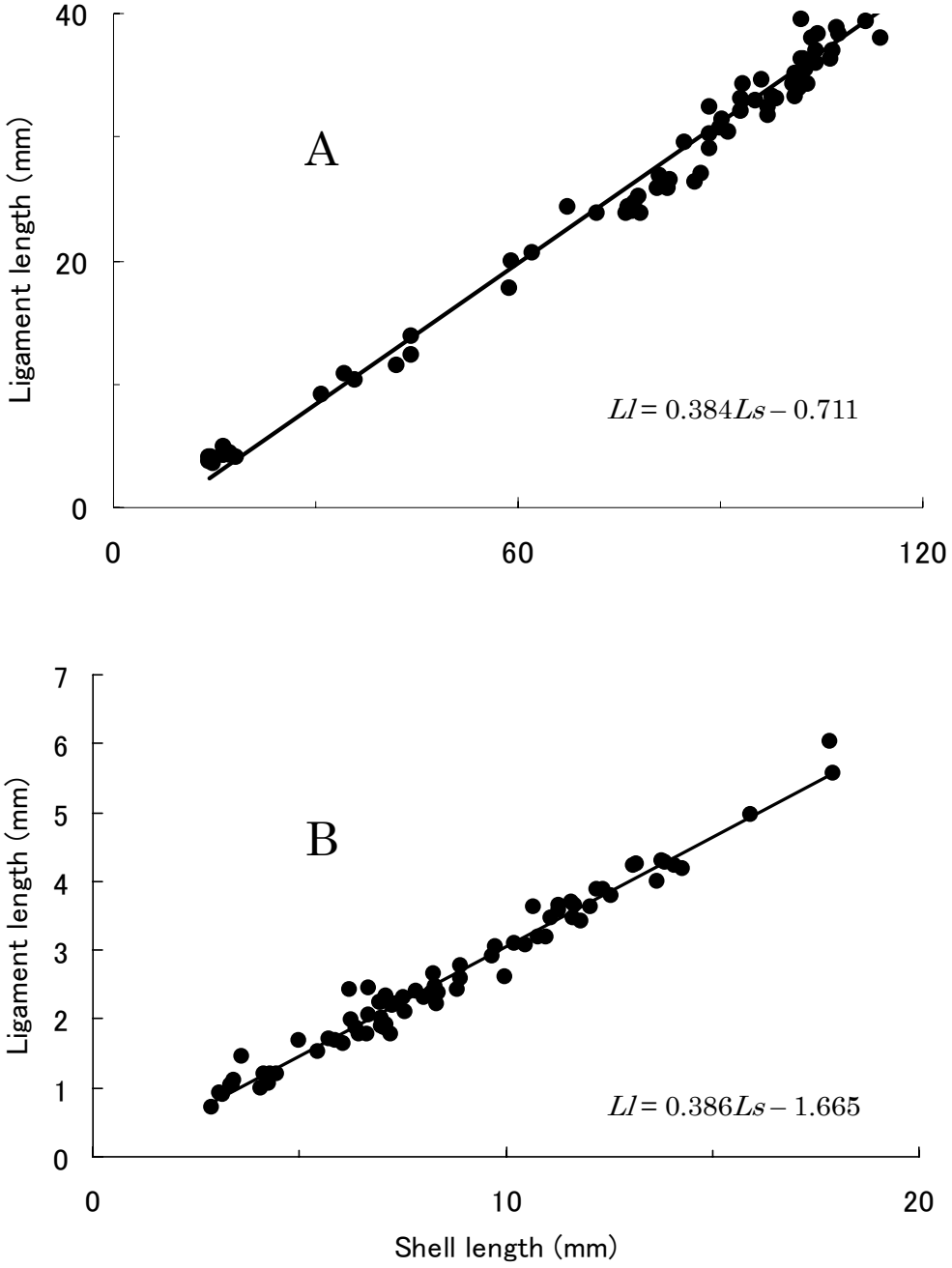

Fig. 12 Relationships between shell length (*Ls*, mm) and ligament length (*Ll*, mm) of *Margaritifera laevis* from the Shiribetsu River (A) and the Chitose River (B). Solid lines indicate regression lines, $Ll = 0.384Ls - 0.711$ for the Shiribetsu River and $Ll = 0.386Ls - 1.665$ for the Chitose River.

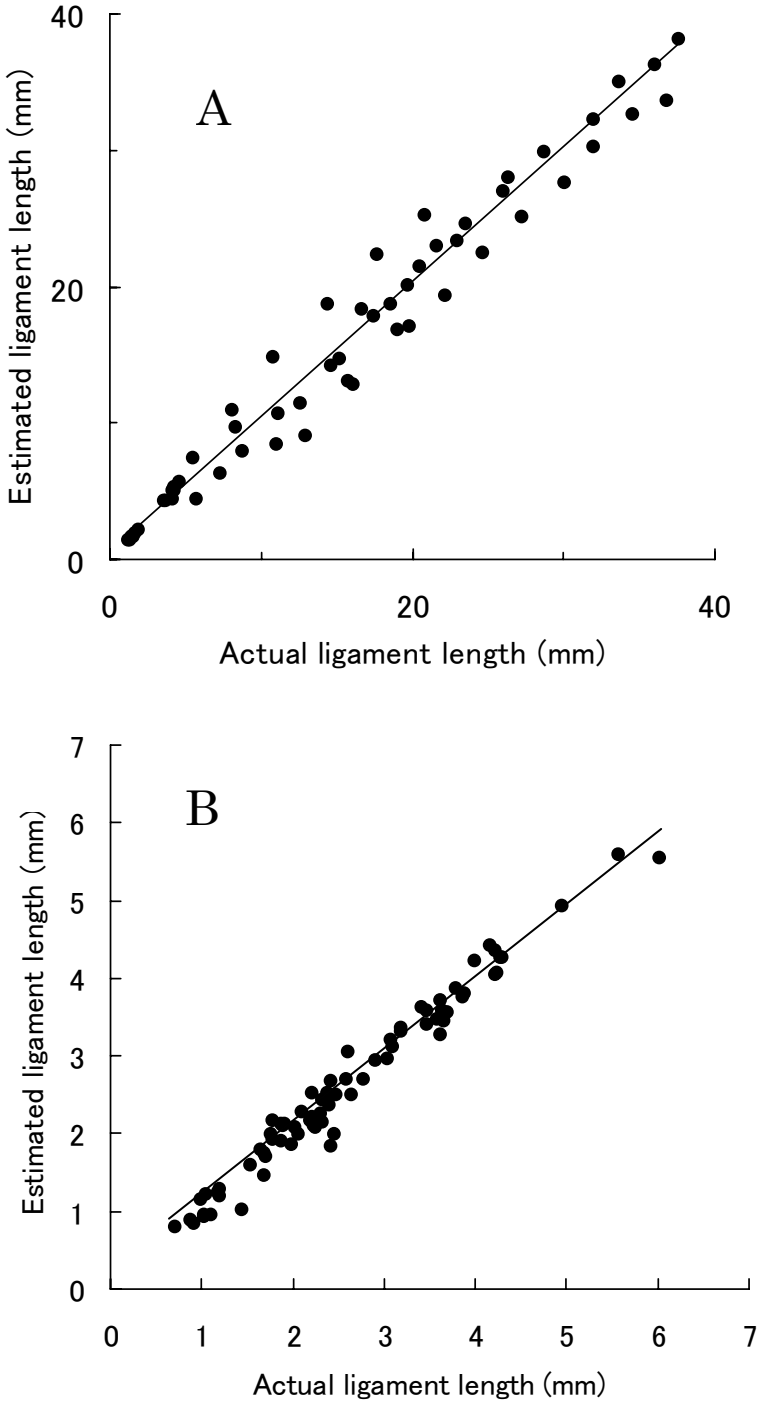

Fig. 13 Relationships between actual and estimated ligament lengths of *Margaritifera laevis* from the Shiribetsu River (A) and the Chitose River (B). Diagonal lines represent predicted values when actual and estimated ligament lengths are equivalent.

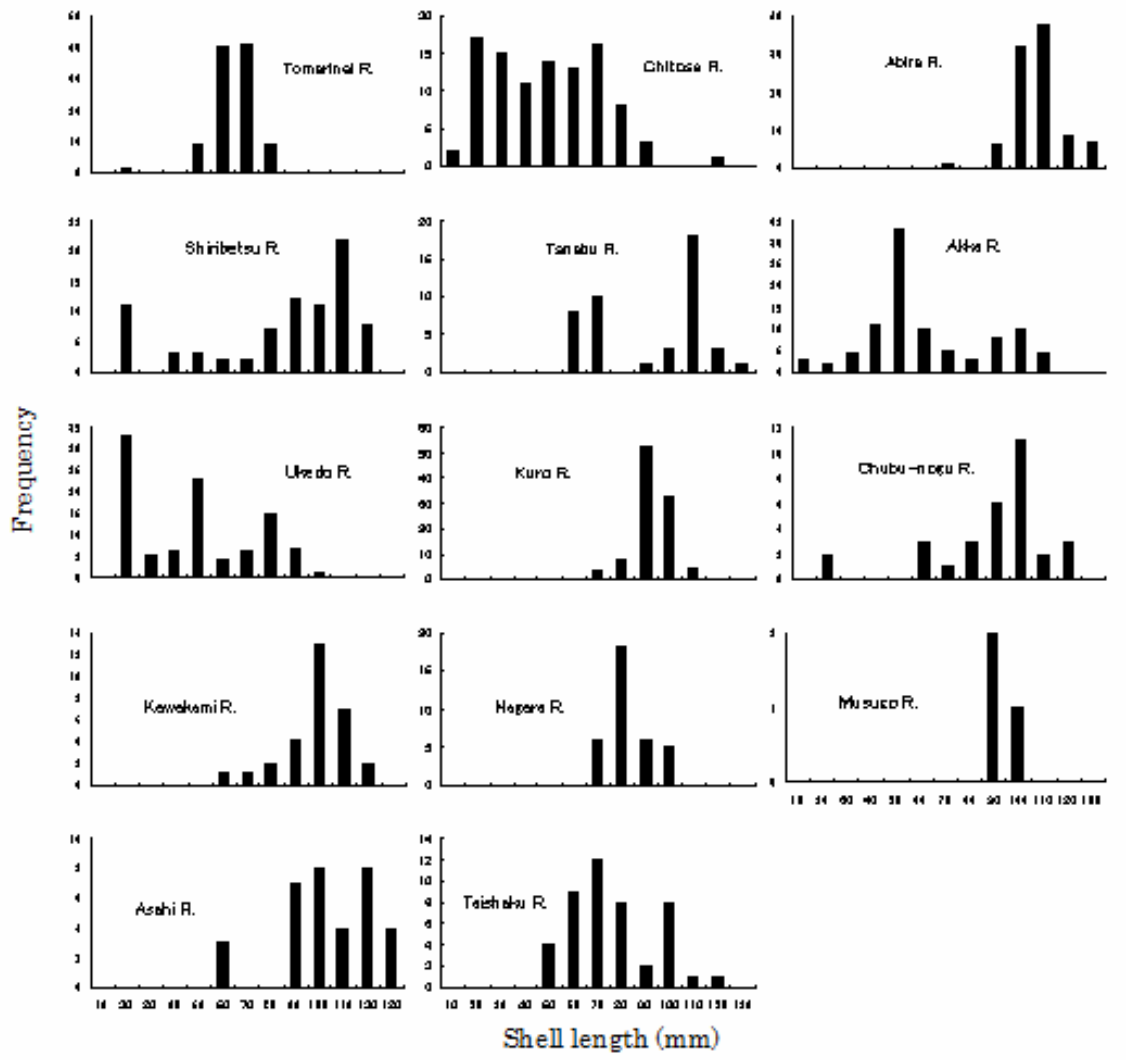

Fig. 14 Size-frequency distribution of *Margaritifera laevis* from 14 sites in Japan.

Table 3 Parameters values for von Bertalanffy growth function and the maximum ages for *Margaritifera laevis* at 12 sites in Japan. L_∞: the theoretical maximum shell length (mm), k: a growth constant (year^{-1}), t_0: a hypothetical starting time at which the size of an individual would have been zero-sized if it had always grown according to this growth function and A_{max}: .the maximum age of mussel in each population.

	L_∞	k	t_0	A_{max}
Tomarinai R.	63.5	0.054	-0.143	40.6
Chitose R.	148.7	0.027	-0.121	25.4
Abira R.	110.8	0.061	-0.073	40.5
Shiribetsu R.	139.0	0.081	-0.043	10.2
Akka R.	218.2	0.036	-0.062	8.0
Ukedo R.	178.0	0.081	-0.034	4.3
Kuro R.	144.8	0.143	-0.024	3.9
Chubu-nogu R.	112.0	0.110	-0.040	18.8
Kawakami R.	220.1	0.050	-0.044	6.0
Nagara R.	130.9	0.095	-0.039	6.6
Asahi R.	126.7	0.127	-0.031	14.3
Taishaku R.	97.7	0.055	-0.091	29.8

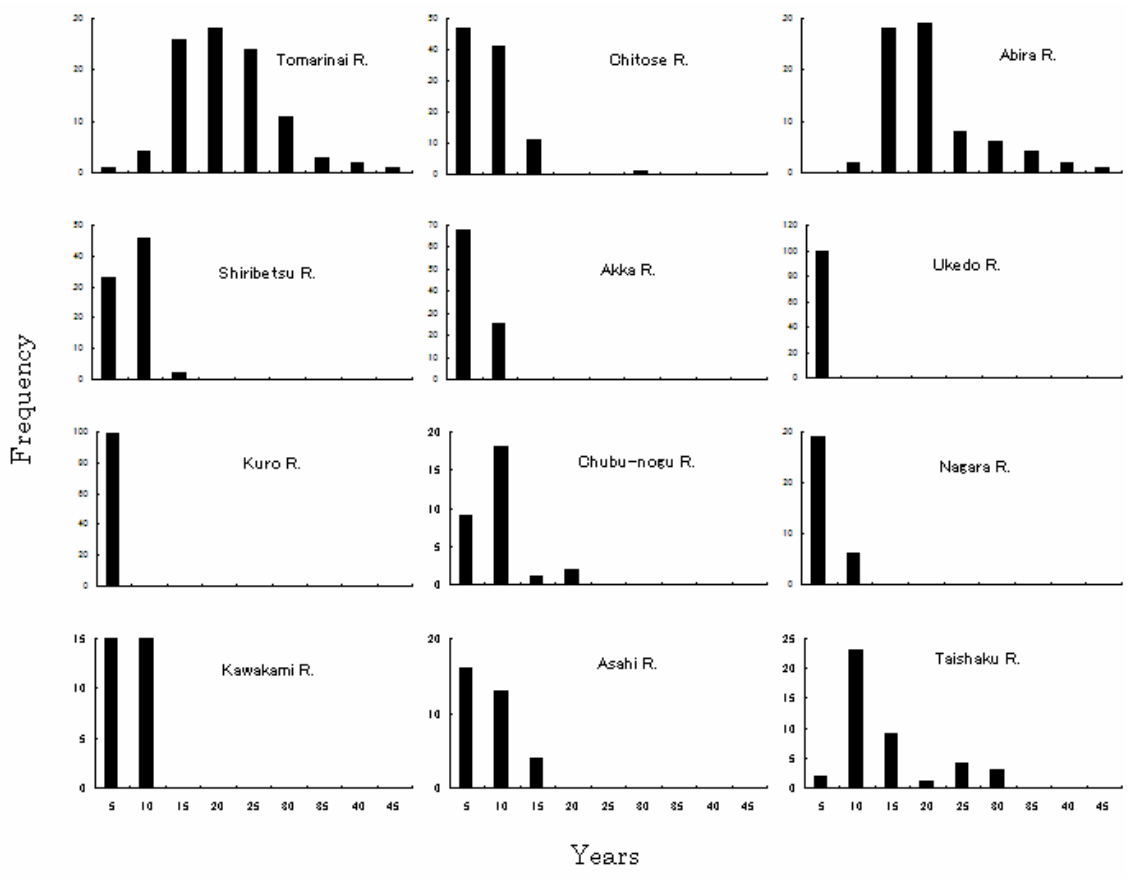

Fig. 15 Age frequency distribution of *Margaritifera laevis* from 12 sites in Japan.

Table 4 Minimum shell lengths of *Margaritifera laevis* and the environmental variables in each habitat.

	Minimum shell length (mm)	Total phosphorus concentration (μ g L^{-1})	Total nitrogen concentration (μ g L^{-1})	Annual mean groundwater temperature (℃)	Number of weirs (N)
Abira R.	76.0	0.0039	0.463	7.8	7
Shiribetsu R.	12.7	0.0076	0.545	8.4	0
Chitose R.	4.5	0.0046	0.110	8.3	4
Akka R.	8.8	0.0052	0.192	11.5	1
Ukedo R.	15.4	0.0059	0.453	14.2	1
Kuro R.	67.2	0.0056	0.731	13.8	4
Chubu-nogu R.	10.8	0.0028	0.164	11.4	6
Kawakami R.	57.1	0.0059	0.124	12.8	14
Asahi R.	50.5	0.0074	0.388	14.6	29
Takahashi R.	25.5	0.0054	0.355	15.3	7

Table 5 Odds ratio and its 95% CI for selected explanatory variables

Explanatory variable	Odds ratio	95% CI
Number of dam	0.002	0.00006-0.130
Total nitrogen concentration	0.16	0.08-0.33

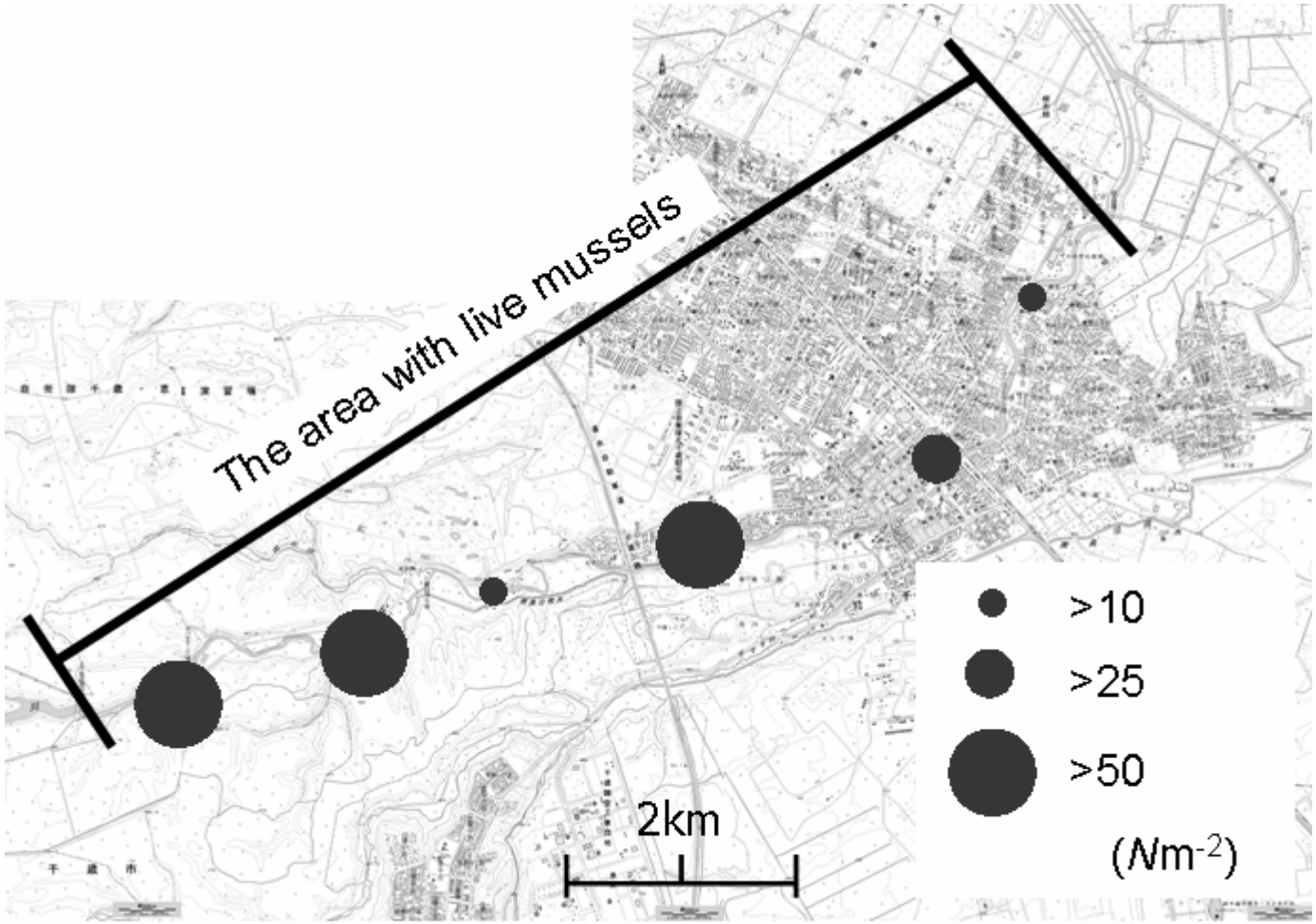

Fig. 16 Distribution of *Margaritifera laevis* in the Chitose River.

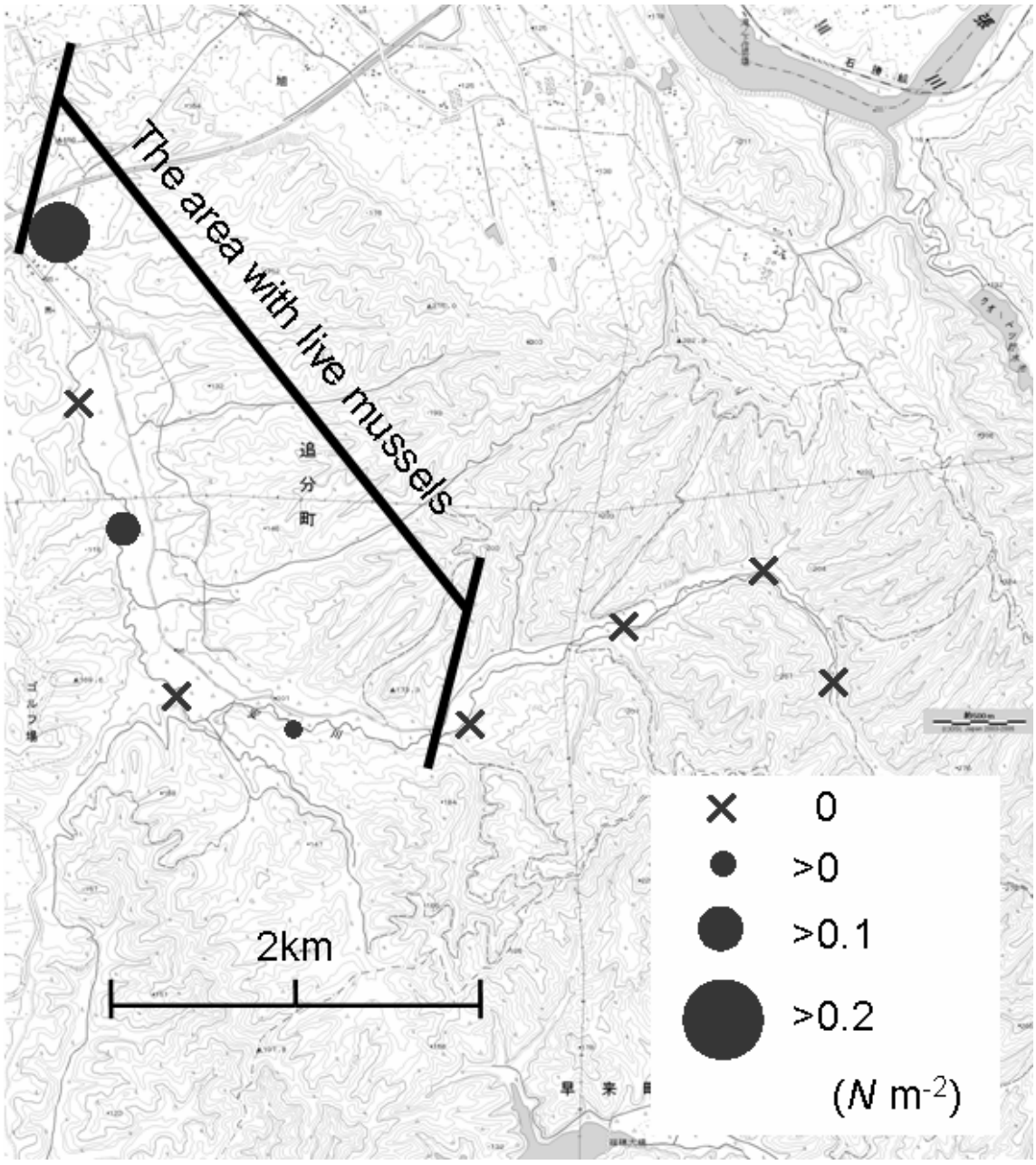

Fig. 17 Distribution of *Margaritifera laevis* in the Abira River.

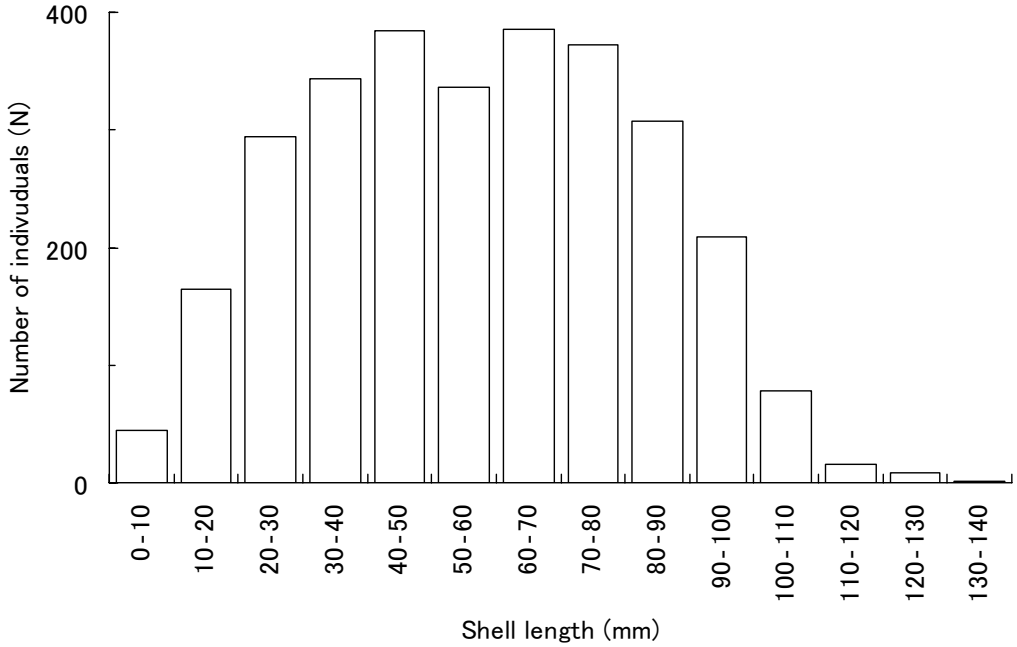

Fig. 18 Size-frequency distribution for *Margaritifera laevis* in the Chitose River. Total number of samples was 2944 individuals. The mean, the minimum and the maximum shell lengths were 54.7 mm, 3.9 mm and 131.7 mm, respectively.

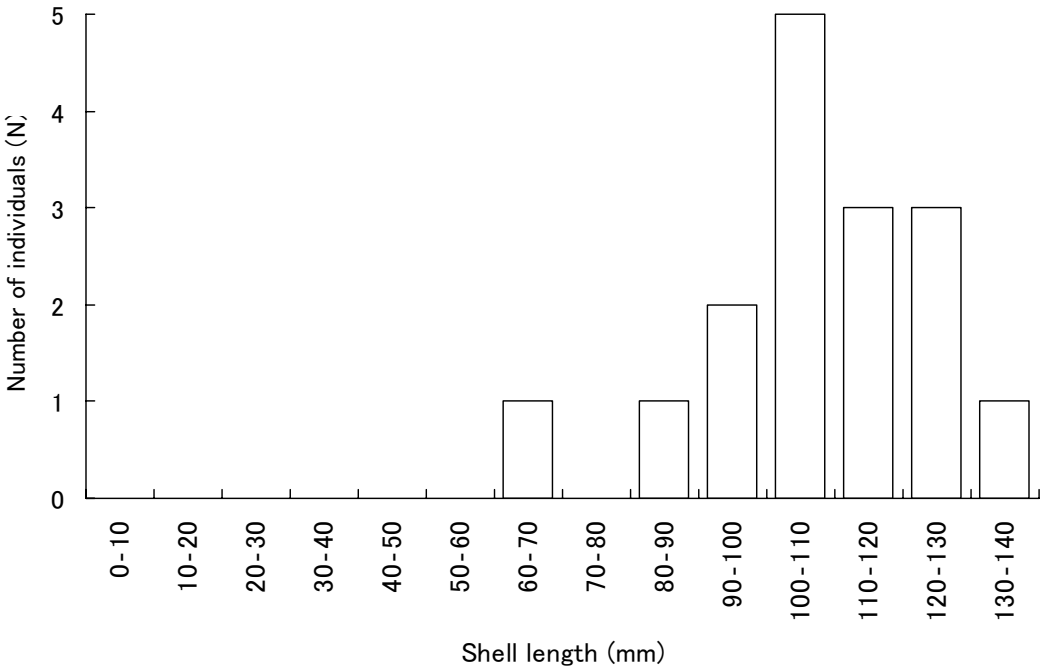

Fig. 19 Size-frequency distribution for *Margaritifera laevis* in the Abira River. Total number of samples was 16 individuals. The mean, the minimum and the maximum shell lengths of mussels were 106.5 mm, 61.6 mm and 131.4 mm respectively.

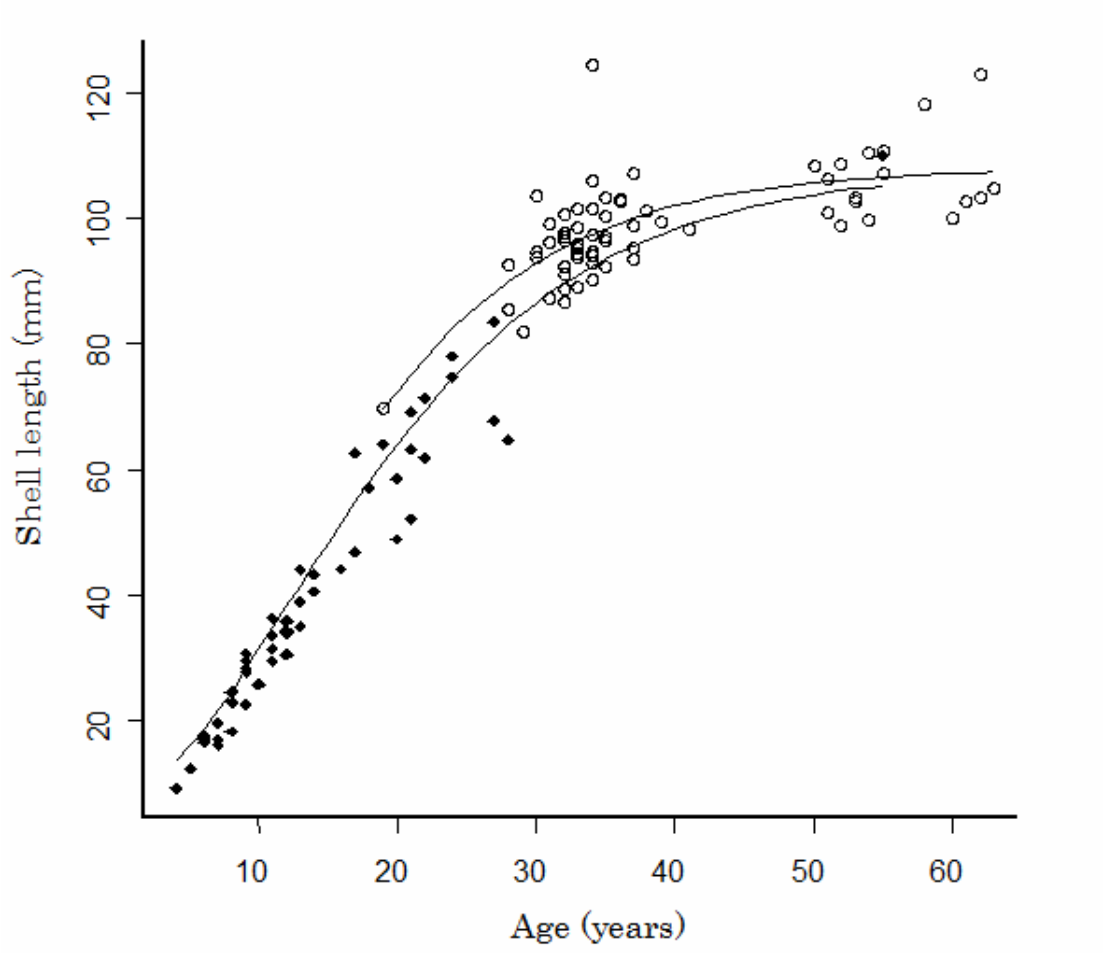

Fig. 20 Estimated Gompertz growth curves with scatter plots for *Margaritifera laevis* populations from the Chitose River (●) and the Aibra River (○). Number of samples was 53 from the Chitose River and 64 from the Abira River.

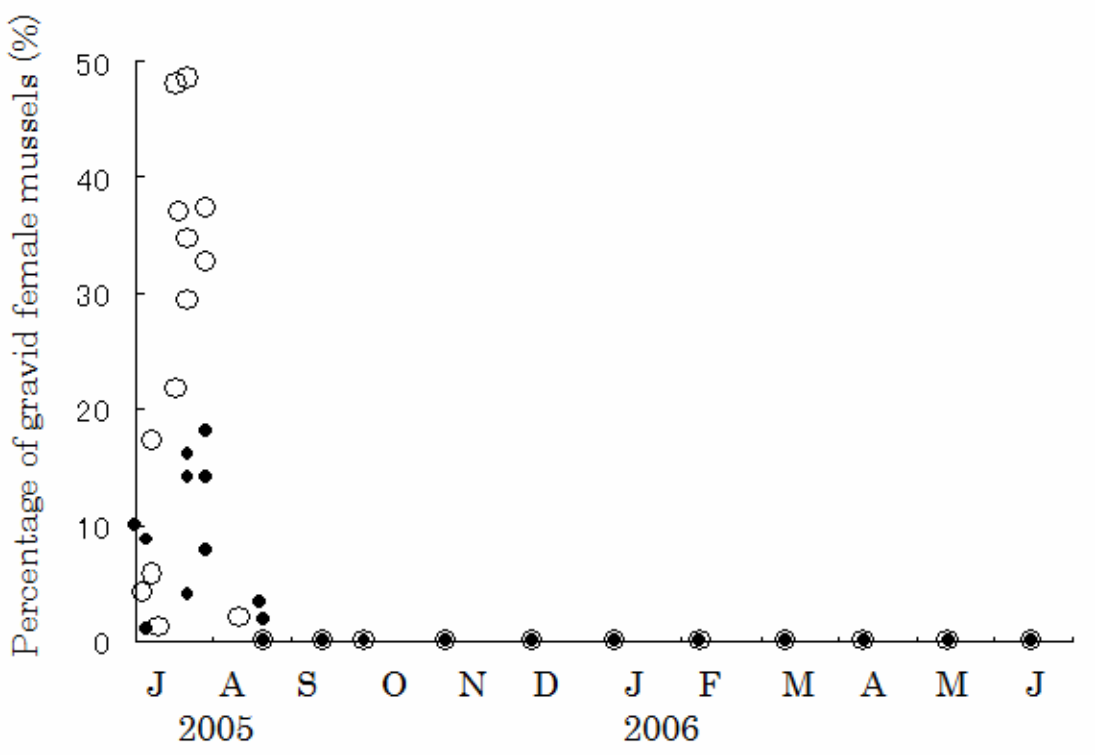

Fig. 21 Seasonal changes of the proportion of gravid female mussels in adult mussels for the Chitose River (●) and the Abira River (○) between June 2005 and May 2006.

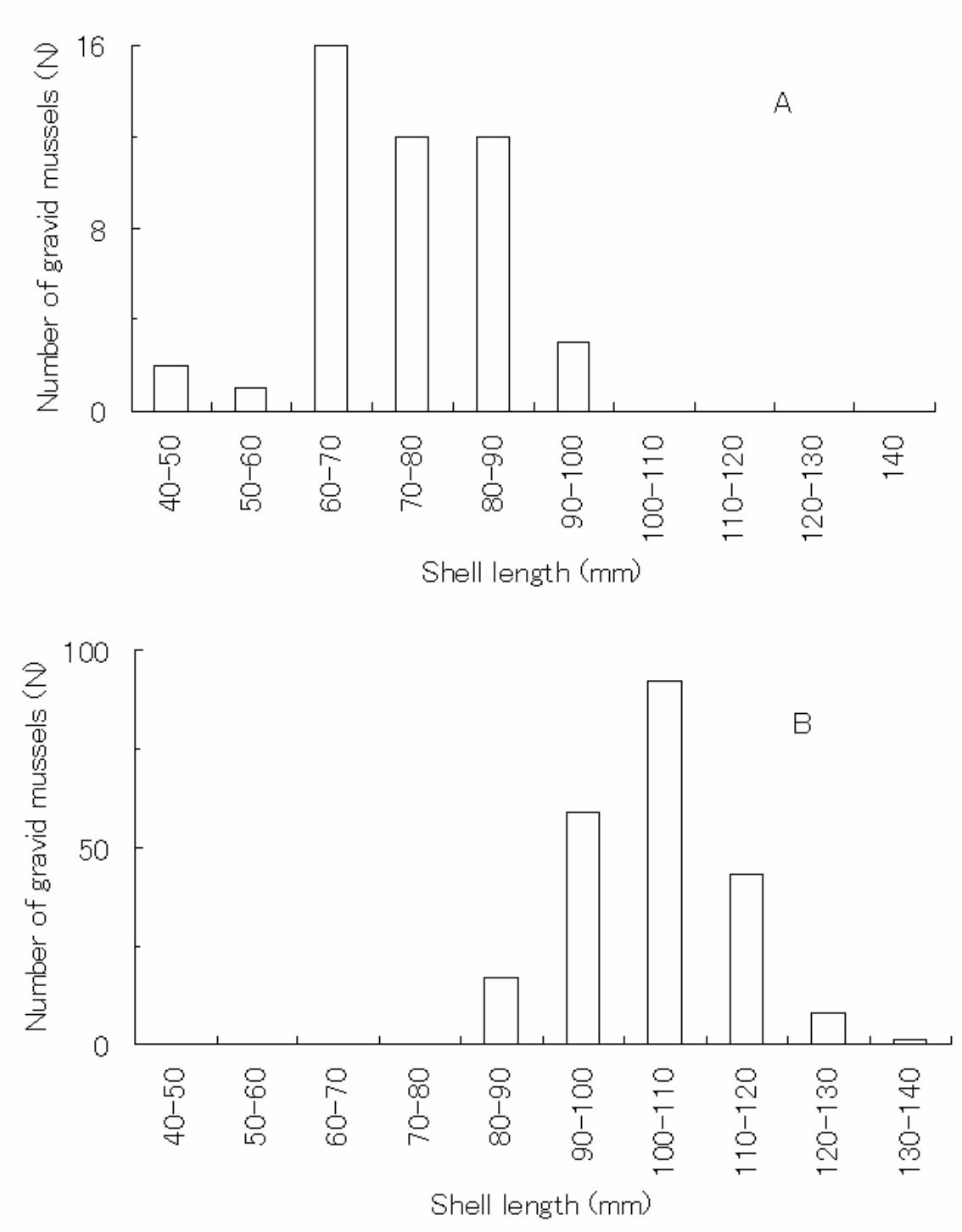

Fig. 22 Size-frequency distribution of gravid mussels from the Chitose River (A) and the Abira River (B).

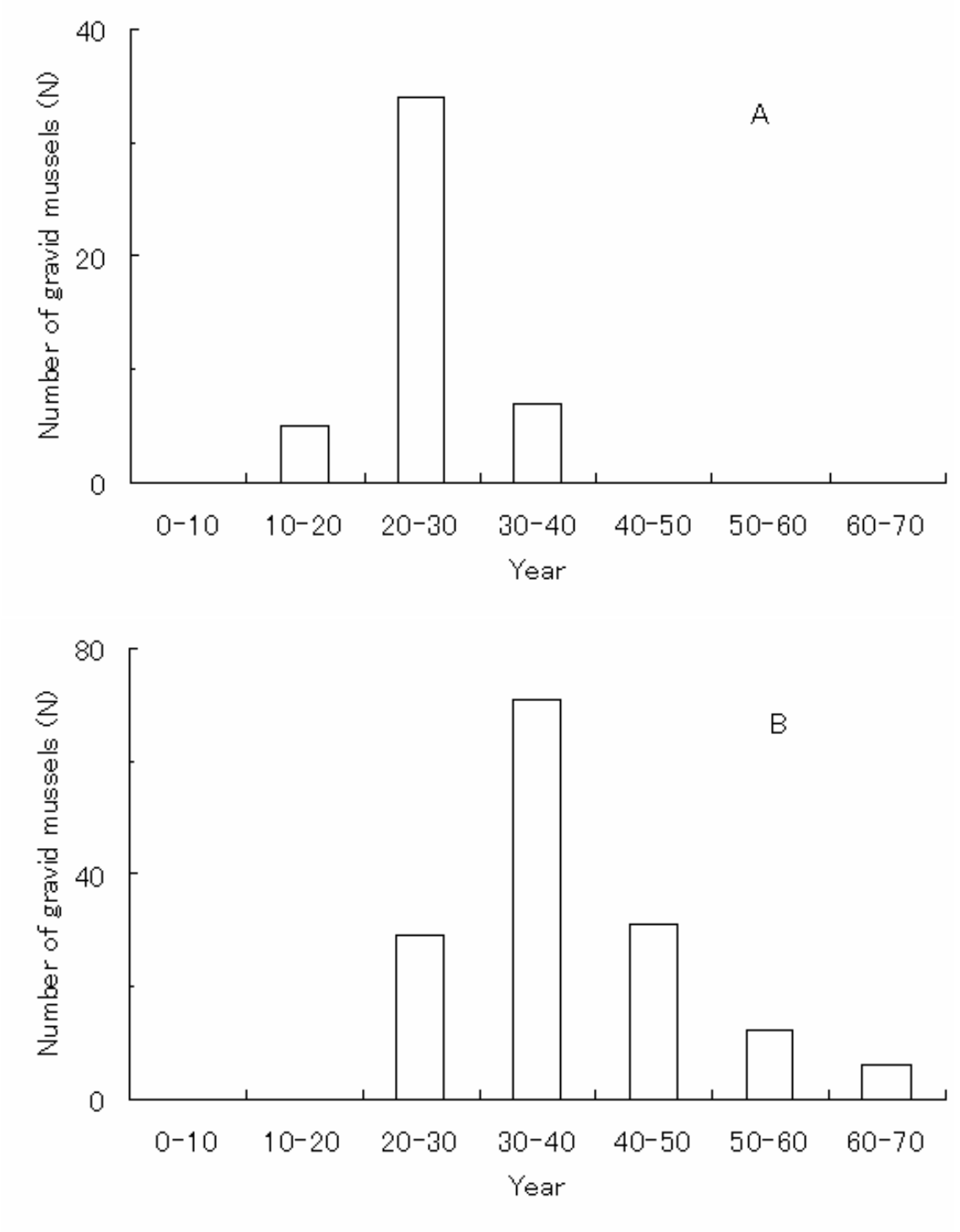

Fig. 23 Age-frequency distribution of gravid mussels from the Chitose River (A) and the Abira River (B) in 2005.

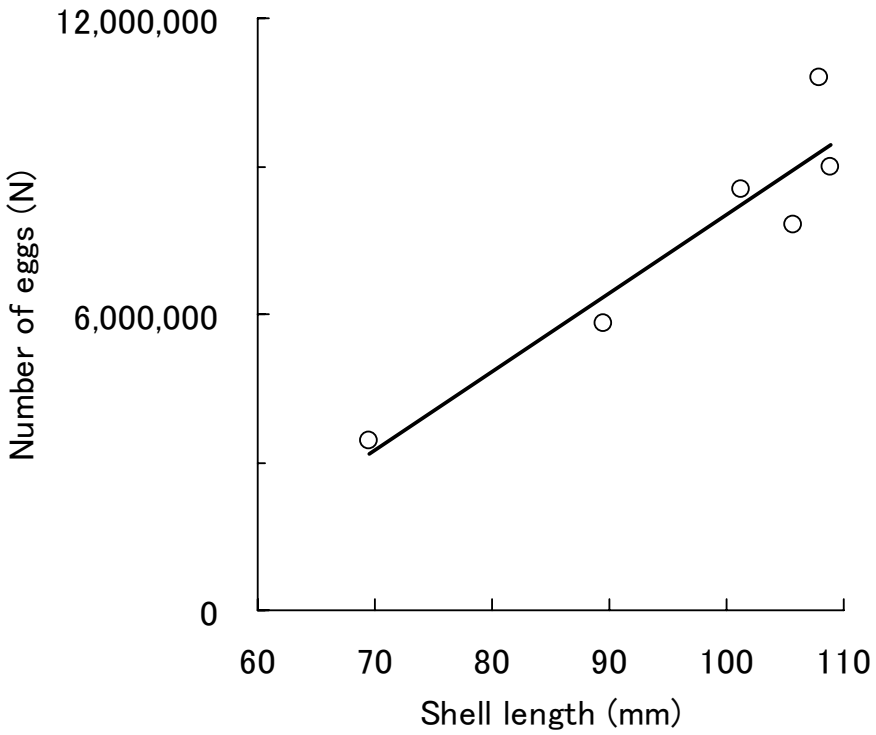

Fig. 24 The relationship between the shell length of gravid mussel and the number of eggs in the marsupia of *Margaritifera laevis* from the Abira River.

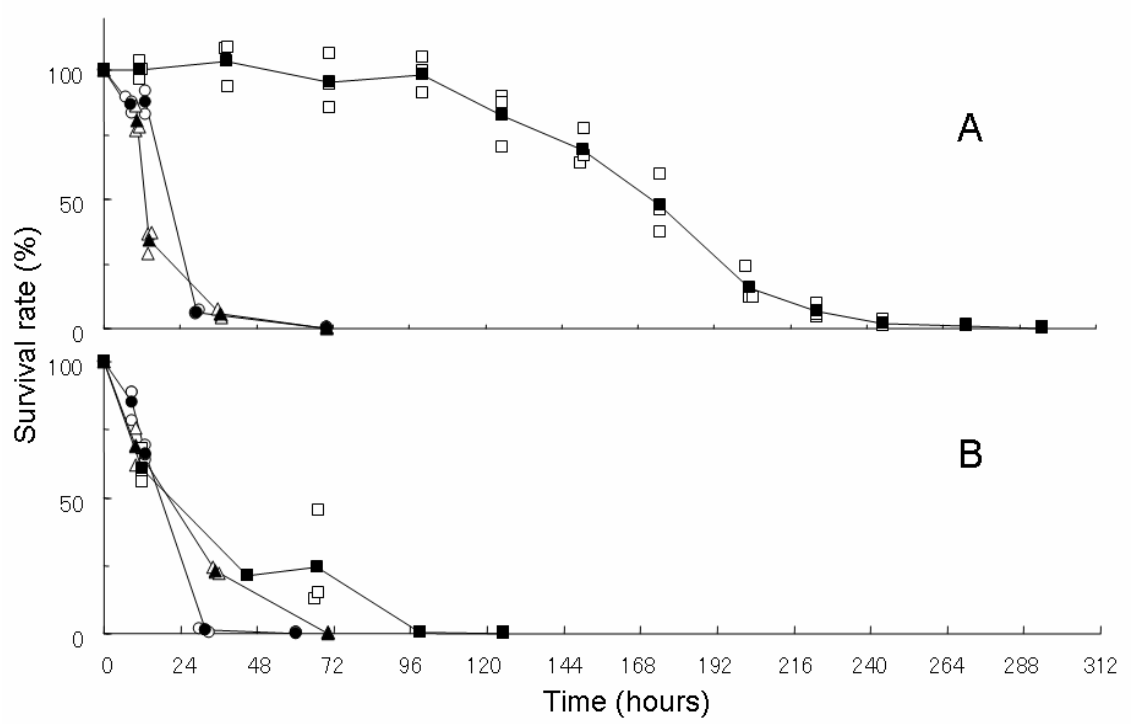

Fig. 25 Survival rates of glochidia of *Margaritifera laevis* from the Abira River (A) and the Chitose River (B) at three levels of water temperature (□: 10˚C, △: 15˚C, ○: 20˚C). Mean survival rate at each observation time is indicated by a filled mark (■: 10˚C, ▲: 15˚C, ●: 20˚C).

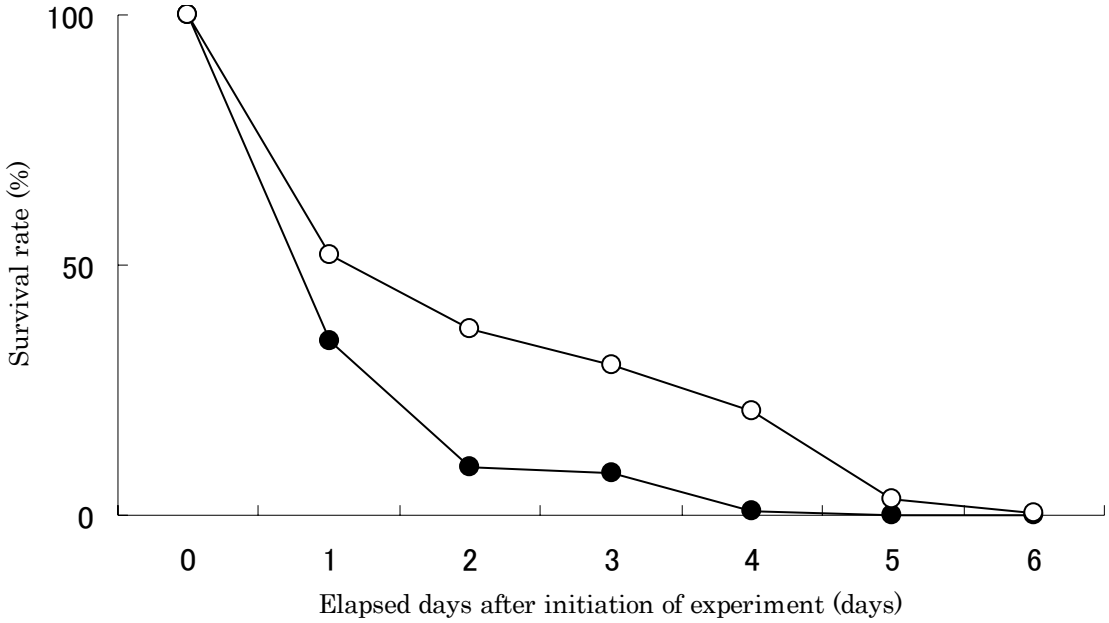

Fig. 26 Mean glochidial survival rates of *Margaritifera laevis* from the Chitose (●) and the Abira River (○) reared in the respective rivers.

Table 6 Number of samples and range of fork length of collected fishes from the Chitose and Abira rivers.

	Petromyzontidae	*Salmo trutta*	*Oncorhynchus mykiss*	*O. masou masou*	Tribolodon sp.	*Mugil cyprinoides*	*Hemibarbus barbus barbus*	*Gnathopogon caerulescens*	*Pungtungia herzi*	*Gymnogobius taranetzi*	*Cottus reinii*
Chitose R.	1 (150)	9 (62–93)	–	77 (51–178)	9 (21–73)	–	1 (28)	5 (28–70)	6 (34–57)	4 (55–74)	–
Abira R.	6 (50–140)	–	68 (36–282)	19 (35–131)	–	1 (61)	28 (45–147)	–	–	–	28 (28–127)

Number of samples (range of fork length (mm))

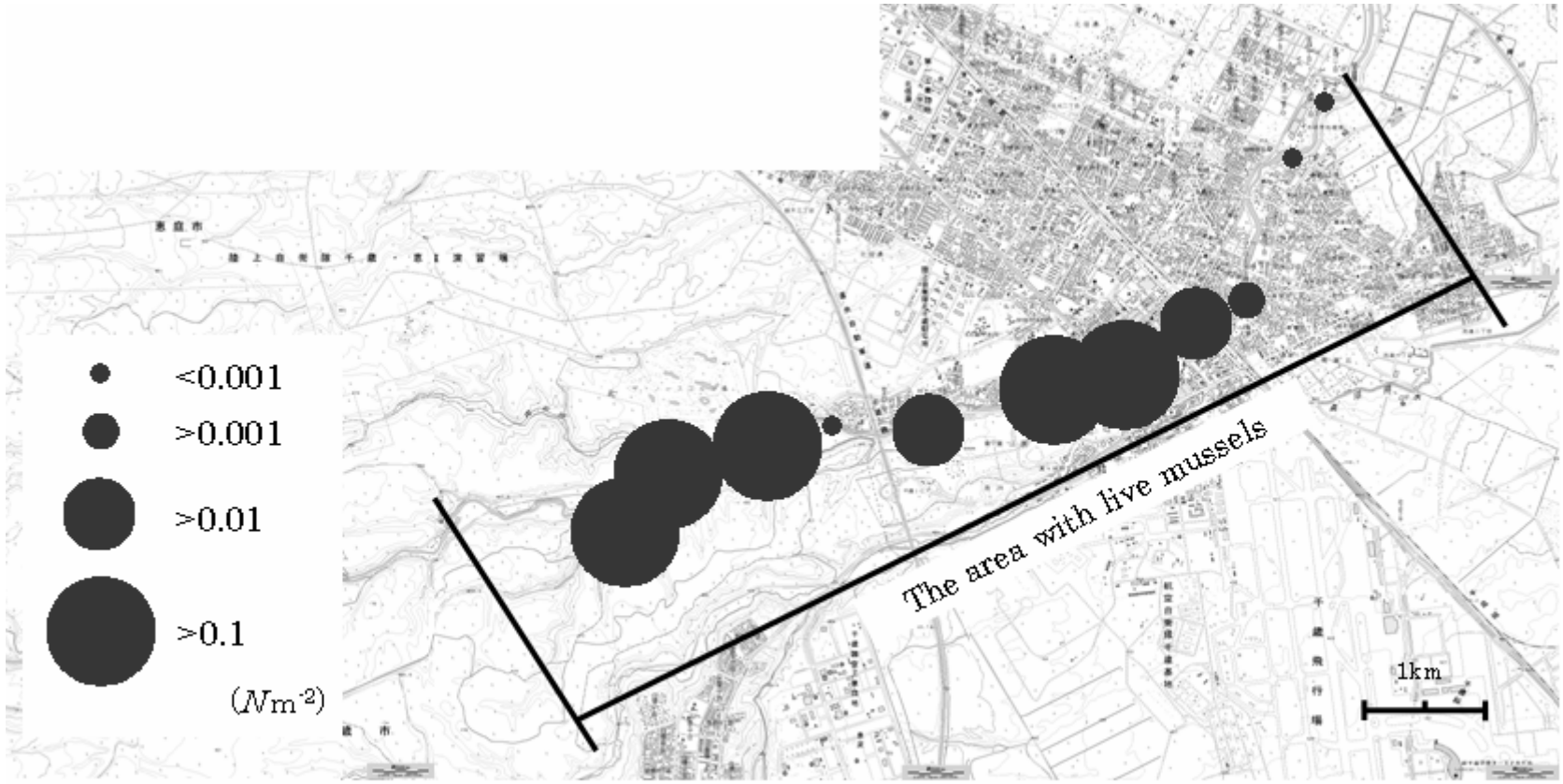

Fig. 27 Distribution of host fishes for *Margaritifera laevis, Oncorhynchus masou masou* in the Chitose River.

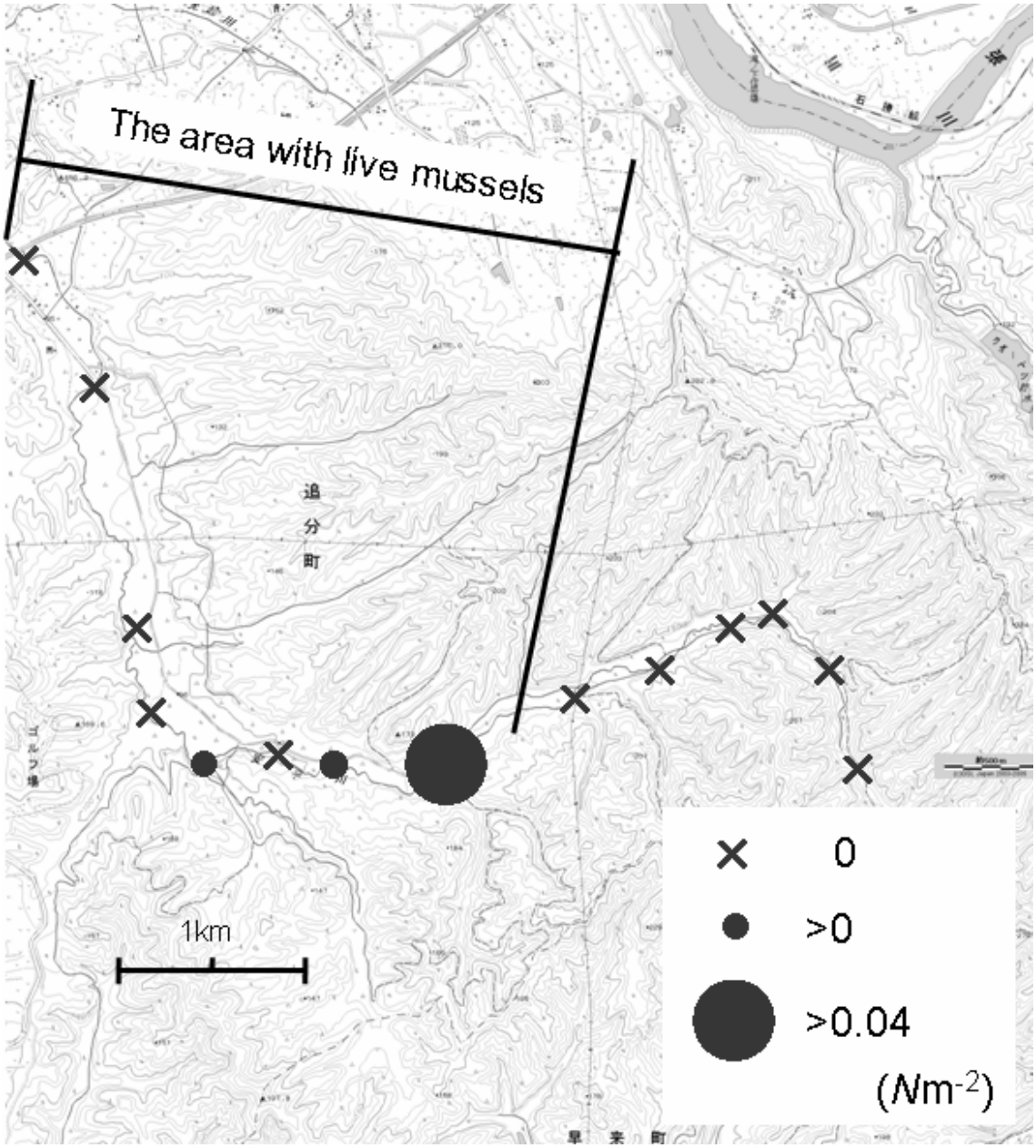

Fig. 28 Distribution of *Oncorhynchus masou masou*, a host fish for *Margaritifera laevis*, in the Abira River.

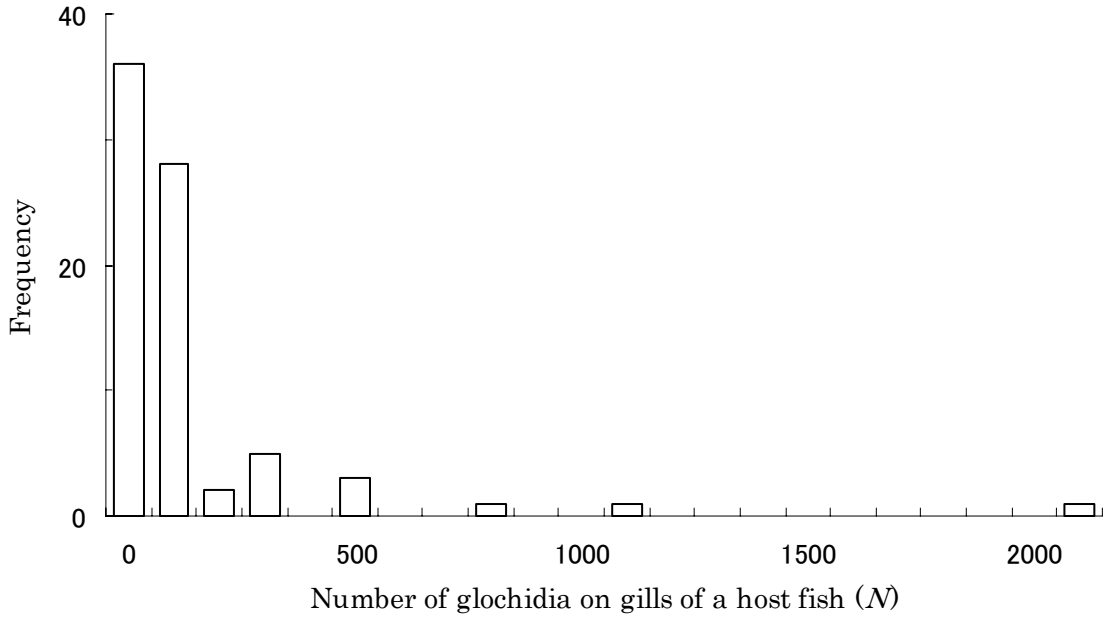

Fig. 29 Frequency distribution of the number of glochidia attached on the gills of an individual host fish, *Oncorhynchus masou masou* in the Chitose River.

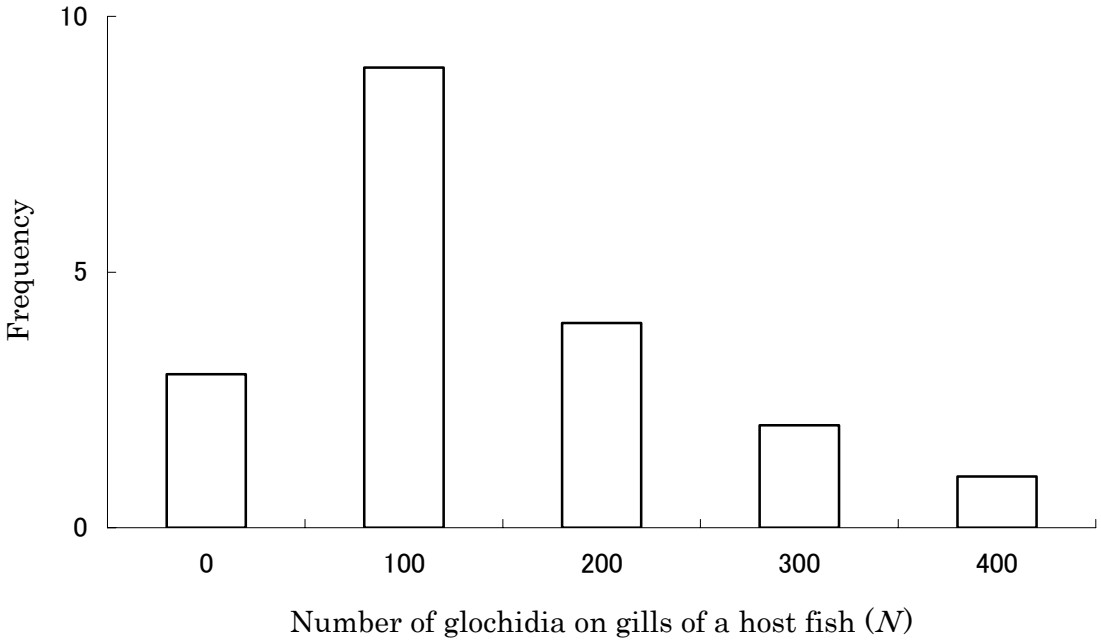

Fig. 30 Frequency distribution of the number of glochidia attached on the gills of an individual host fish, *Oncorhynchus masou masou* in the Abira River.

Table 7 The mean number of detached glochidia from host fish in laboratory. The number of host fish for each experiment is shown in respective parenthesis.

		Juvenile mussels	
		Chitose R.	Abira R.
Host fish	Chitose R.	14.8 (5)	0 (7)
	Abira R.	0 (2)	2.0 (2)

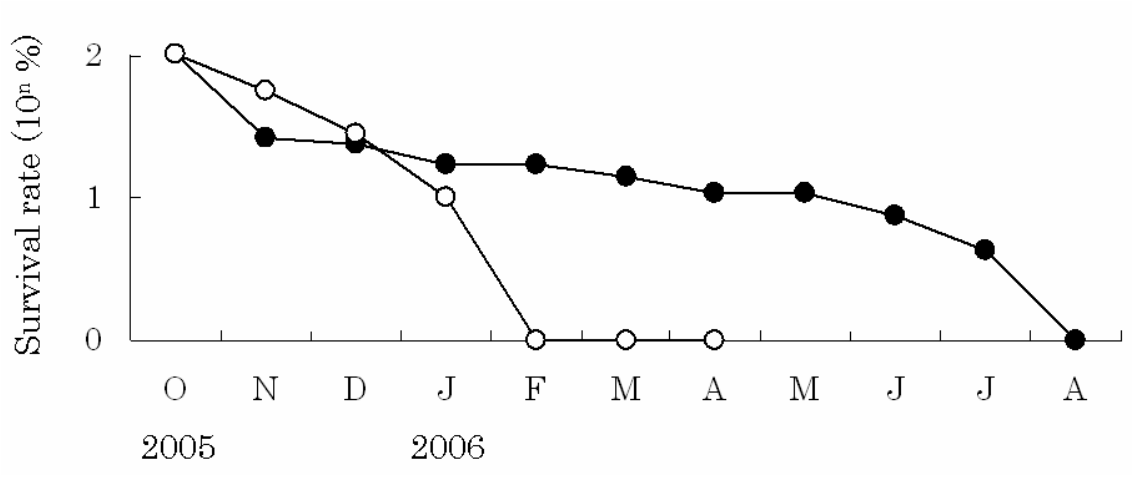

Fig. 31 Changes in the Survival rate of juvenile *Margaritifera laevis* reared in the Chitose River (●) and the Abira River (○). The numbers of individuals at start of the experiment were 31 in the Chitose River and 11 in the Abira River.

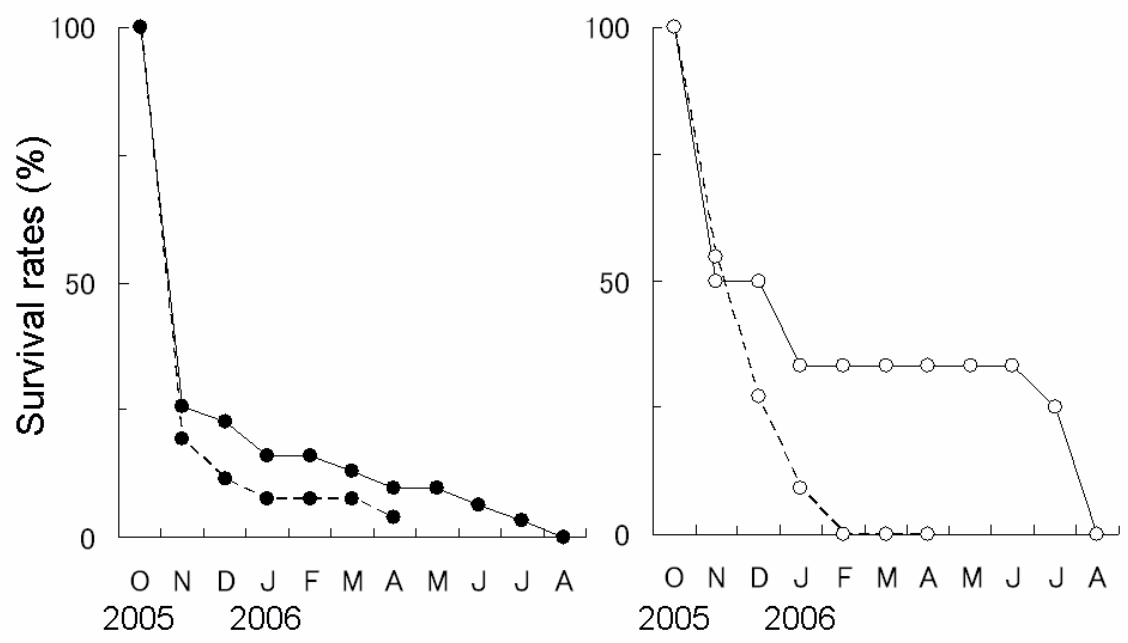

Fig. 32 Changes in the glochidial survival rates for the populations of the Chitose River (●) and the Abira River (○). Rearing locations are indicated by solid lines (Chitose River) and dash lines (Abira River). The numbers of individuals at start of the experiment are: 31 for the Chitose population reared in the Chitose River, 26 for the Chitose population reared in the Abira River, 12 for the Abira population reared in the Chitose River and 11 for the Abira population reared in the Abira River.

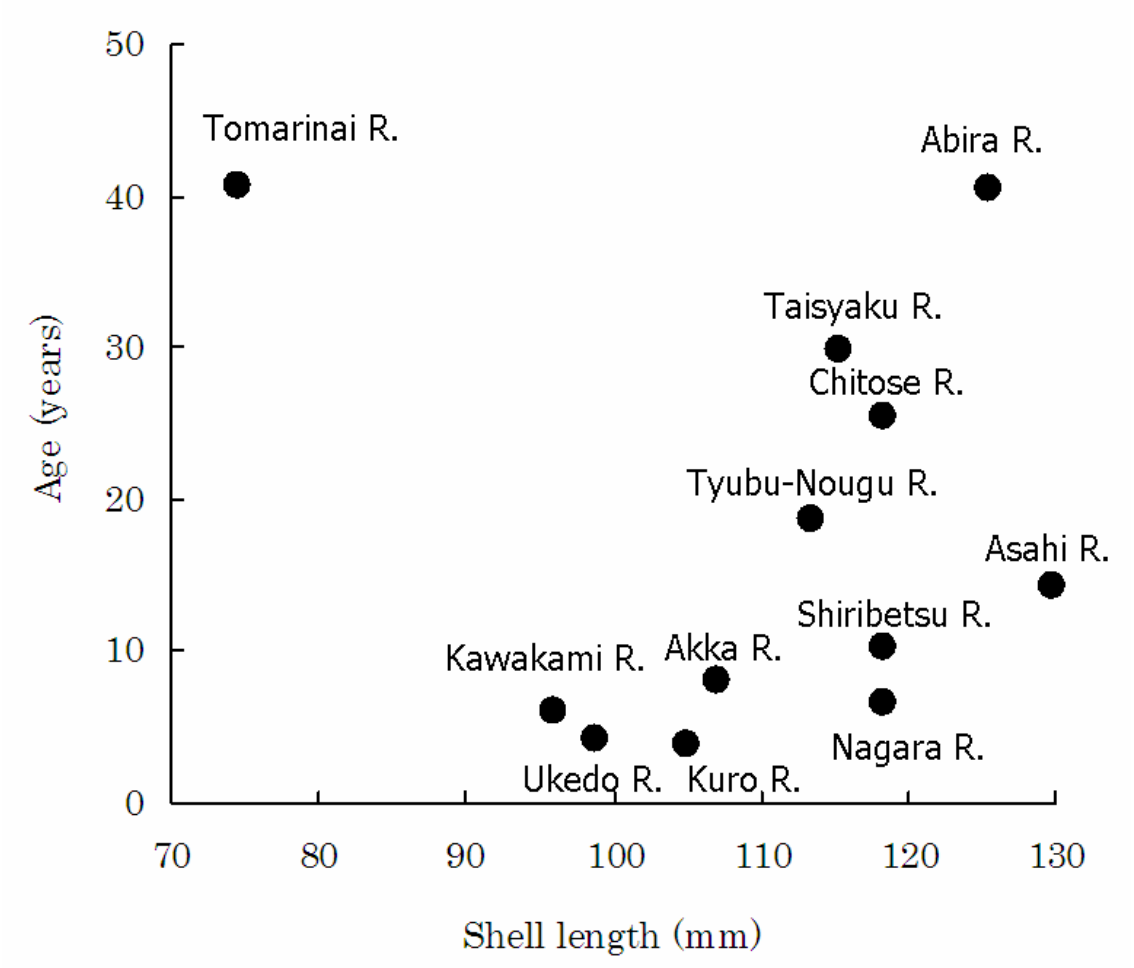

Fig. 33 A scatter diagram of lifespan against maximum shell length in *Margaritifera laevis*.

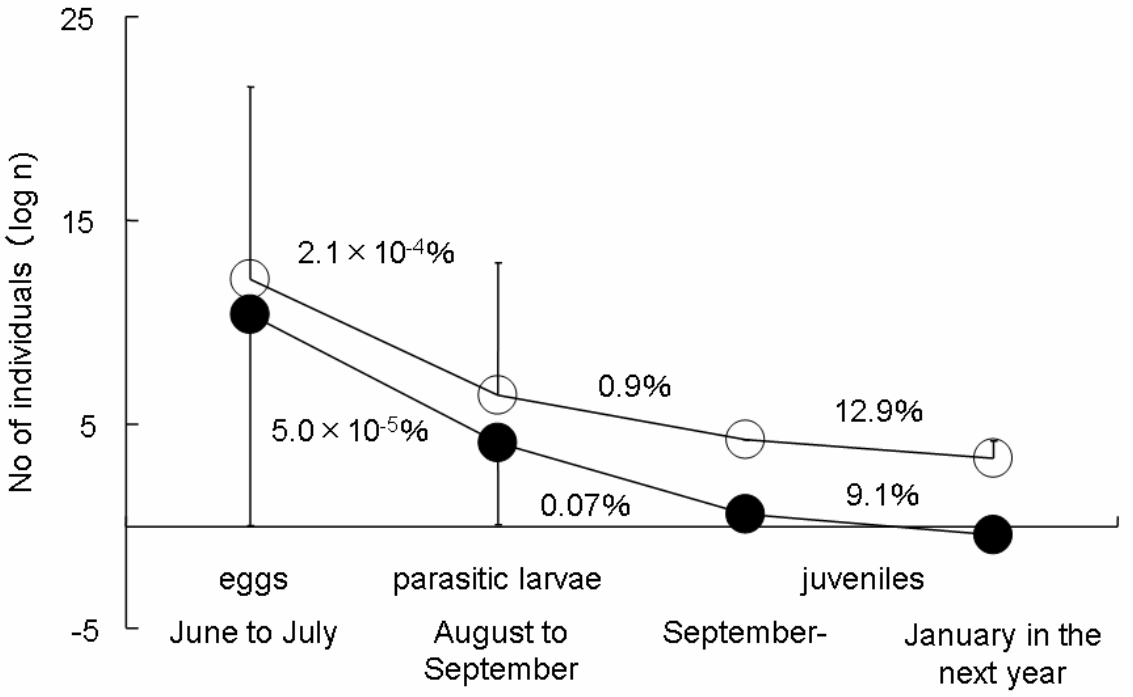

Fig. 34 The overall total number of eggs, parasitic glochidia and juveniles with ranges of number of estimated individuals for *Margaritifera laevis* in the Chitose River (○) and the Abira River (●) between June 2005 to January 2006. The numeral (%) between consecutive life stages indicates their survival rate between the stages.

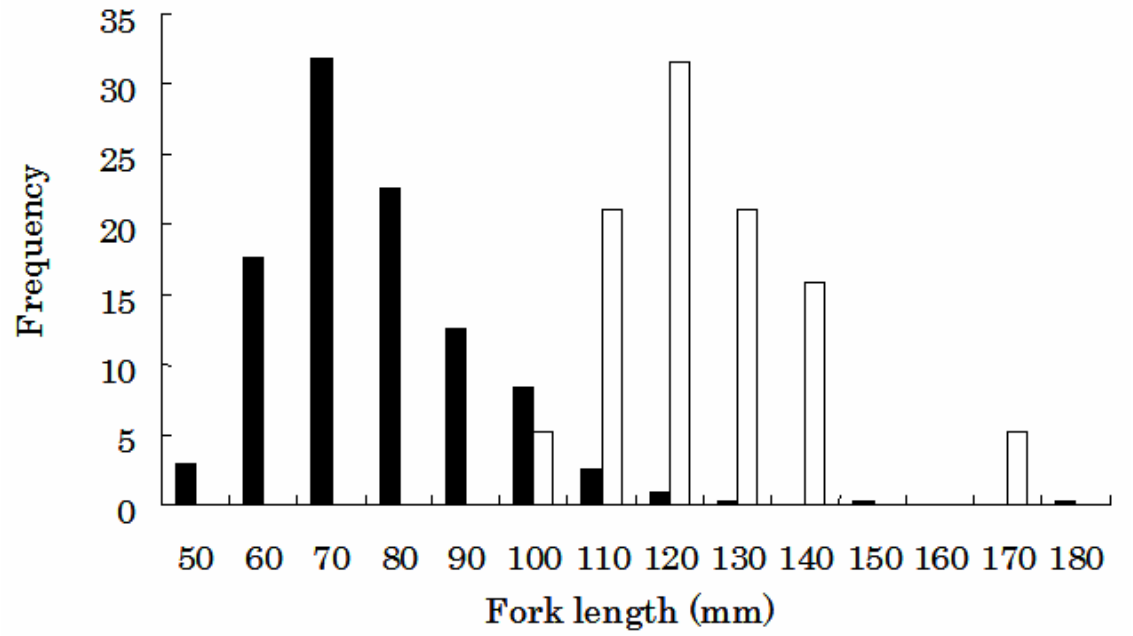

Fig. 35 Frequency distributions of fork length of host fish for *Margaritifera laevis*, *Oncorhynchus masou masou*. Host fishes from the Chitose River are represented by filled bars. Whereas, host fishes from the Abira River are expressed by open bars.

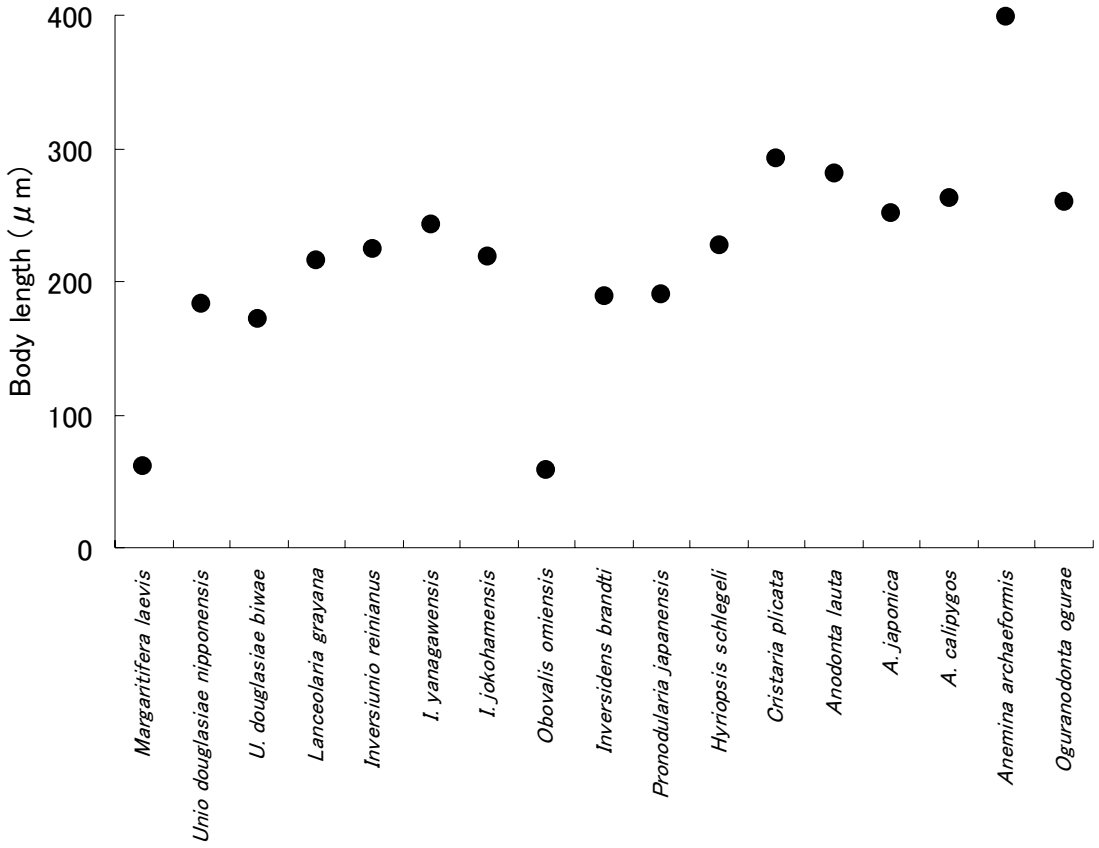

Fig. 36 Body lengths of free-living glochidia in Japanese Unionoida (Kondo, 2002).

Table 8 Reported number of families of hosts for Unionoida

Scientific name	Number of host families
Margaritifera laevis	1[1]
Margaritifera togakushiensis	1[2,3]
Margaritifera margaritifera	1[4]
Margaritifera falcata	1[5]
Margaritifera auricularia	2[6,7]
Margaritifera hembeli	1[8]
Fusconaia ebena	2[9]
Elliptio complanata	1[10]
Elliptio icterina	4[10]
Elliptio dilatata	2[11]
Anodonta beringiana	2[12]
Alasmidonta viridis	2[11]
Lampsilis ovata	3[13]
Lampsilis radiate siliquoidea	3[13]
Leptodes fragilis	1[14]
Villosa nebulosa	2[15]
Villosa vanuxemi	1[15]
Obovaria olivaria	2[11]
Proptera laevissima	2[9]

Sources: 1, Awakura (1968); 2, Kondo *et al.* (2000); 3, Kobayashi & Kondo (2005); 4, Bauer (1987b); 5, Karna & Milleman (1977); 6, Araujo & Ramos (2000); 7, Araujo *et al.* (2001); 8, Johnson *et al.* (1998); 9, Surber (1912); 10, Britton (1979); 11, Clarke (1981); 12, Heard (1975); 13, Clarke (1973); 14, Fuller (1974); 15, Zale & Neves (1982).

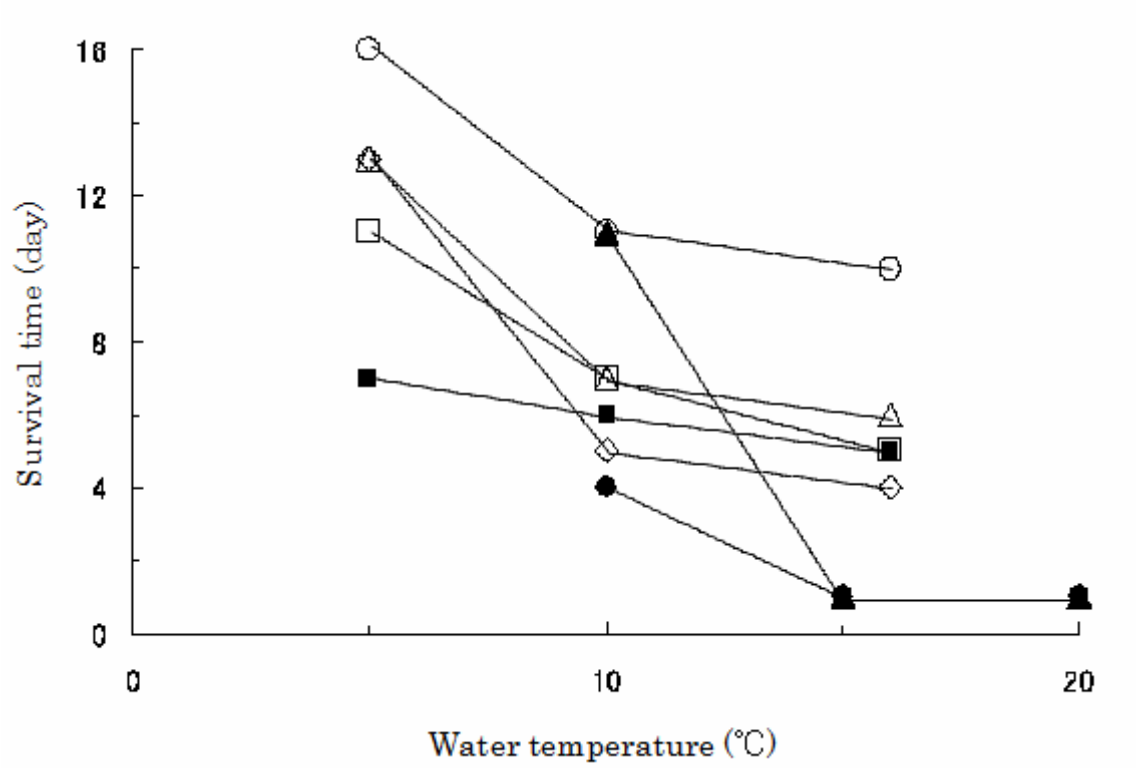

Fig. 37 Comparison of viabilities of glochidia among Unionoida species: *Margaritifera laevis* in Abira River (▲); *Margaritifera laevis* in Chitose River ; (●); *Margaritifera margaritifera* (■); *Unio crassus* (◇); *Unio pictorum* (○); *Anodonta cygnea* (□); *Anodonta anatina* (△) at various water temperatures. Data for *Margaritifera laevis* are from Akiyama (in press) and for other species from Jansen *et al.* (2001).

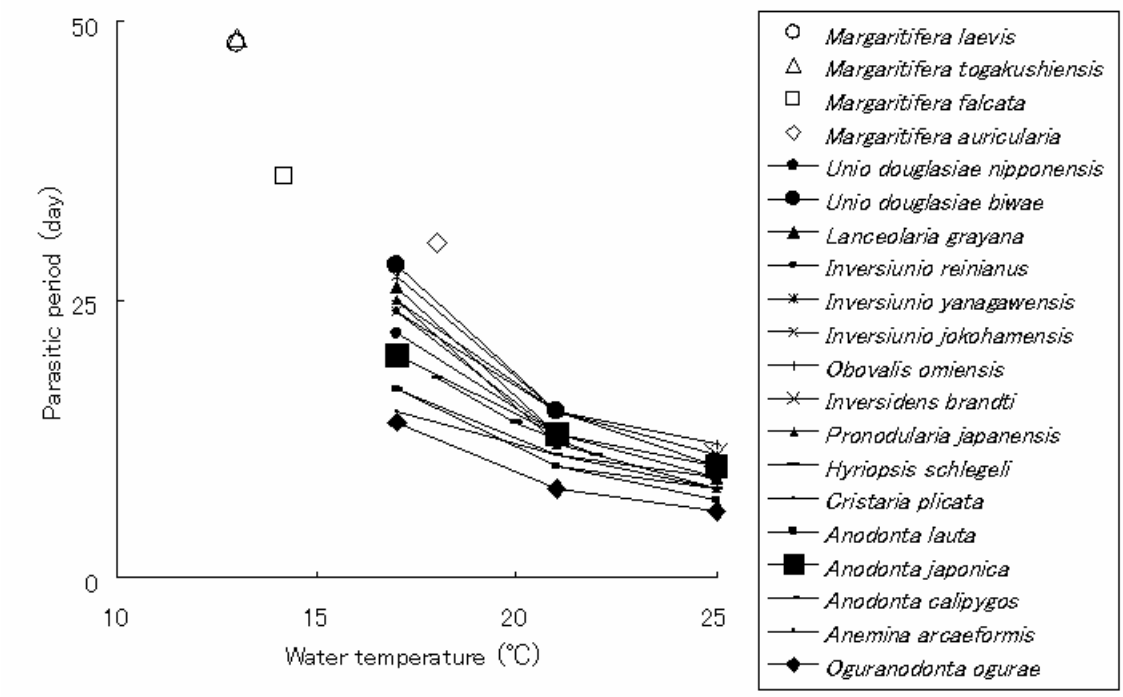

Fig. 38 Duration of parasitic stage of glochidia in Unionoida at various water temperatures. Open symbols indicate Margaritiferidae. Filled symbols represent Unionoida except for Margaritiferidae. Sources: *Margaritifera laevis* and *M. togakushiensis*: Kobayashi & Kondo (2005); *M. falcata*: Murphy (1942); *M. auricularia*: Araujo & Ramos (2000); Unionoida except for Margaritiferidae: Kondo (2002).