網走湖におけるヤマトシジミの産卵及び初期生活史に関する生態学的研究[*1]

馬場　勝寿[*2]

Ecological study on spawning and early life stage of the brackish water bivalve *Corbicula japonica* in Lake Abashiri[*1]

Katsuhisa BABA[*2]

The spawning probability of *Corbicula japonica* is expressed with temperature and salinity by the following equation: (1) $1/\{1+\exp[-(-16.12+0.57T-12.64S+0.59TS)]\}$, (*T*: Temperature, *S*: Salinity). The occurrences of the planktonic larvae can be well predicted by the equation in Lake Abashiri. *C. japonica* failed to spawn when the temperatures and the salinity did not exceed the threshold environments for the spawning; the threshold environments are expressed by the equation(1). The failure of the spawning was not an exceptional phenomenon in Lake Abashiri; the failure probably occurred in 10 out of 21years for which data were available. The ovaries after the spawning season in the spawning-failure years were histologically characterized by massive atretic oocytes.

The vertical heterogeneities of abundances and size compositions of the planktonic larvae were observed in the water column. The most of larger (shell length: 170 to 200 μ m) larvae were observed near the surface of the lake. On the other hand, the smaller (shell length: 140 to 170 μ m) ones were mainly observed near the boundary between the well-oxygenated oligohaline upper stratum and anoxic polyhaline lower stratum. The salinity of the near the boundary was higher than shallower depths. I thought the heterogeneities probably related to the enhancement of the survivorships for the salinity-sensitive larvae. The larvae probably assemble to the higher salinity zone to gain sufficient salinity for the larval development in the early stages. The larvae migrate to the surface where the higher possibilities are expected to be transported to the marginal area of the lake, the habitat of *C. japonica.*

Few settled juvenile were observed on the muddy sediments. The favorable sits for the juveniles were sites where the content of silt-clay plus very fine sand was less than 36.9 %. Other restrictive sediment-environment factors, such as organic carbon, water content and total sulfide were highly correlated with the site-clay plus very fine sand; i. e. one factor can represent the favorable sediment conditions for the juvenile. Understanding the favorable conditions is very important for the management of fisheries grounds and it also facilitates the judgments about which sites are appropriate for the release of seed clams.

The juvenile growth of *C. japonica* in Lake Abashiri was extremely slow; it grew to the shell length of 0.7 mm at age 1 and 2.1 mm at age 2. I elucidated the proximal factor of the extremely slow growth by modeling between the environmental factors and the growth. The proximal factor was the less susceptible growth response to the environmental factors until the second winter. In many species of bivalve, populations from higher latitudes have a slower initial growth rate; but longevity and ultimate size in these populations are frequently greater than at lower latitudes. The extremely slow growth of *C. japonica* juvenile in Lake Abashiri is probably an extreme example of this phenomenon.

Key words: *Corbicula japonica*, spawning, early life stage, ecology

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GENERAL INTRODUCTION

The native range of brackish and fresh water bivalve *Corbicula* is from Africa to the Far East ranging from the tropical to the cold-temperate zone [1]. *Corbicula* extended its range to the cold-temperate zone only in the Far East (Fig. 1). Lake Abashiri lies near the northern limit of its range.

In Japan, there are three species of *Corbicula*, i.e. *C. japonica* Prime, *C. leana* Prime, *C. sandai* Müller [2]. The native range of *C. japonica*, a brackish-water species, is from the southern part of Sakhalin to Kyushu. The native range of *C. leana*, a fresh-water species, is from Honshu to Kyushu. *C. sandai*, a fresh-water species, is endemic to Lake-Biwa water system including Yodo River. Among *Corbicula*, only *C. japonica* successfully extended its range to the cold-temperate zone (Fig. 2).

The phylogenetic relationships among the three species were inferred from observations of their chromosomes [3] and from isozyme polymorphisms [4, 5]. These reports suggested that the lacustrine *C. sandai* had first speciated from the brackish *C. japonica* and then the fluvial *C. leana* had derived from *C. sandai*. The each species has a specific reproduction system. *C. japonica* is an oviparous dioecious species and the larva hatches out in the blastula stage. *C. sandai* is an oviparous dioecious species and the larva hatches out after it developed to D-shaped larva in the egg. The prolonged development in the egg is probably to adapt to freshwater environments. *C. leana* is a viviparous hermaphroditic species and it reproduces by the self-fertilization; and the fertilized eggs are incubated in the inner demibranchs through the trochophore and the pediveliger stage. The reproductive properties of *C. leana* probably adapt to lotic environments. The self-fertilization systems of *C. leana*, which is called spontaneous androgenesis, is very characteristic; i. e., the all of maternal genome of zygotes is extruded as two polar bodies and the only chromosomes derived from one male pronucleus constitute the metaphase of the first cleavages of zygotes [6]. Such spontaneous androgenesis is reported only in *C. leana* in the phylum Mollusca.

In the artificial and established range of *Corbicula*, especially in North America, it has become a major biofouling pest for industrial and domestic water supply systems [7, 8]. On the other hand, *Corbicula* spp. are popular food sources for human in Asia. The annual catch ranged from 19,000 to 27,000 t in 1995 to 2000 in Japan [9]. Among *Corbicula* spp., *C. japonica* is the main species for the fisheries.
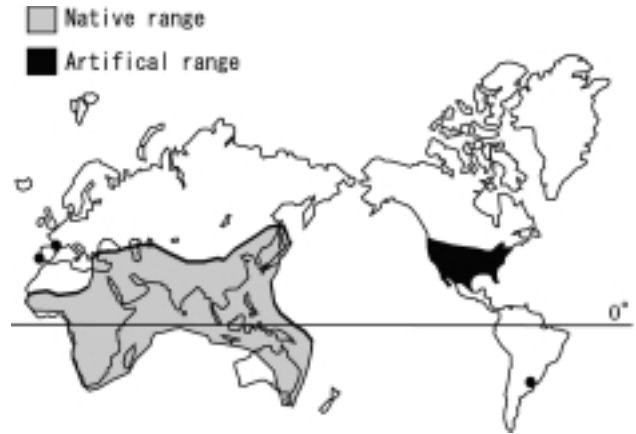

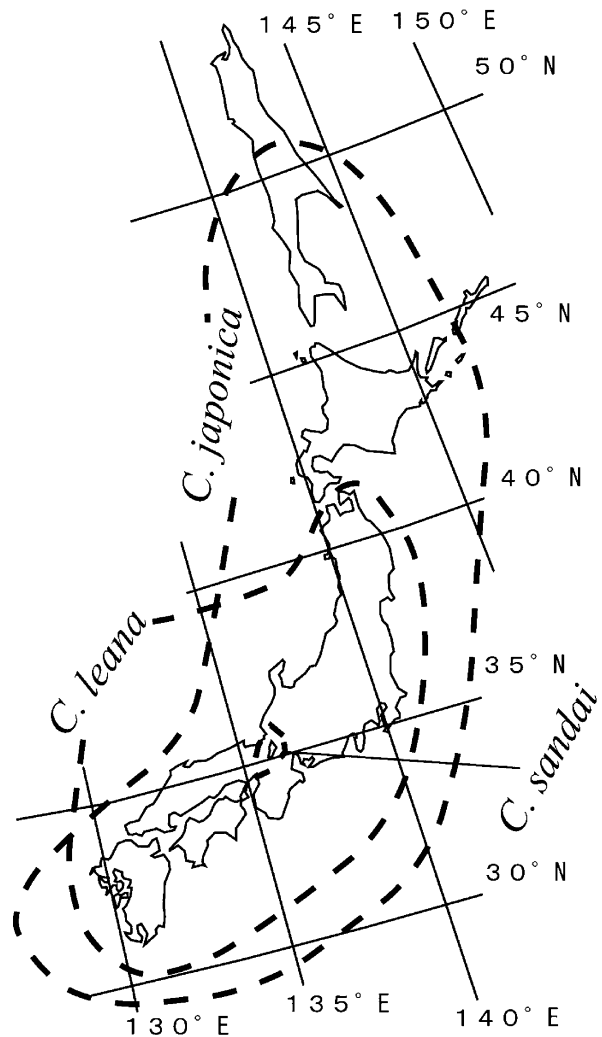Fig. 1　The distribution of *Corbicula*(Morton 1986).

Fig. 2　The distribution of *Corbicula* in Japan and Sakhaline

The habitats of *Corbicula* spp. are near the milieu of human being, therefore, the populations of *Corbicula* have been much influenced by developments such as land reclamations and irrigations. Actually some habitats of *Corbicula* were devastated and fisheries of it were diminished by such developments [10]. On the other hand, there are some resources, which were once devastated, drastically revived by its high reproductive potential and fast growth in southern habitats when limiting factors were mitigated. For example, the resource in Lake Hachiro, which was devastated by the irrigation developed in 1965, revived by a seawater inflow when a typhoon attacked the area in 1987 [11]. The limiting factor caused by the irrigation is that the salinity became too low for the reproduction. The production of *C. japonica* decreased to less than 100 t from about 1,000 t by the irrigation, and it was much enhanced to 10,900 t in 1990 by the seawater inflow. For other example, the resource in Lake Jinzai, which was much decreased by inflow of muddy sediment from the land around the lake, revived by an outflow of the muddy sediment when a flood attacked the area in 1973 [12]. The limiting factor of Lake Jinzai was that the sediment condition was too muddy for the settlement of *C. japonica*. The production was less than 30 t before the flood and it increased to 100-500 t after the flood.

All of these drastic revivals of the resources were reported in southern habitats of *C. japonica*. It is questionable whether such revival would occur in northern habitats because such revival has not been reported and ecological study of *C. japonica* is limited in northern habitats. Asahina [13] reported the spawning ecology, and gonadal and larval developments in Lake Mokoto, Hokkaido (northern habitat). Maru [14] studied the reproductive cycle by histological observations in Lake Abashiri, Hokkaido. Utoh [15] investigated the growth by the observations of annual rings formed at the surface of the shell in Lake Abashiri. These reports are not enough to judge whether drastic revival of the resources would occur in northern habitats because 1) the conditions, temperature and salinity, needed for the spawning are not clear, and 2) the growth of juvenile is still unknown. Asahina [13] reported the influences of temperature and salinity on the larval developments, but on the spawning. Utoh [15] also reported the differences between the actual shell lengths of the individuals that were thought as aged one year and the

shell length at the first annual ring; i. e. the actual juvenile growth has not been clarified.

I carried out present studies to clarify these unknown parts of the ecological properties of *C. japonica* and to considered important viewpoints, especially in the northern habitats, for the resources conservation and management.

In my thesis, I elucidate remarkable ecological properties of *C. japonica*, an economically important species, in Lake Abashiri; some of them are specific in the northern habitats. Chapter Ⅰ shows the favorable environments, temperature and salinity, for the spawning with field observations for six years and laboratory experiments including spawning inductions and histological observations. Chapter Ⅱ shows vertical heterogeneities of abundance and size of the planktonic larvae in the water column. I inferred the reasons of the heterogeneity from the vertical changes of environmental factors in the water column and incubation experiments of the planktonic larvae in the laboratory. The pre-settlement processes were hypothesized from the viewpoint of larval survivorship. Chapter Ⅲ shows the favorable sediment environments for the juvenile. The relationship between the sediment conditions and the juveniles are important information for the enhancement of *C. japonica* resources. For example, in Lake Jinzai, Shimane Prefecture, the resources of *C. japonica* were remarkably enhanced by the improvement of the sediment condition [12]. Chapter IV shows the extremely slow growth of the juvenile in Lake Abashiri. I inferred the proximal factor of the extremely slow growth by modeling the relationships between the juvenile growth and the environmental factors.

CHAPTER I

Effects of temperature and salinity on spawning of the brackish water bivalve *Corbicula japonica* in Lake Abashiri

Abstract

The abundance of planktonic larvae (veliger) of *Corbicula japonica* in Lake Abashiri, which is near the northern limit of this species' range, was extremely low in 1995 and 1996, compared to other years (1989, 1990, 1994 and 1997). In histological examinations, the female genital tubules were filled with the atretic oocytes after the

spawning season of 1995. Therefore, it was concluded that most *C. japonica* failed to spawn in 1995. The effects of temperature and salinity on the spawning of *C. japonica* were examined in a spawning induction experiment under laboratory conditions. Probability of spawning by *C. japonica* in the experiment was described by the equation: $1/\{1+\exp[-(-15.57+0.57T-12.64S-0.55Gam+0.69T\cdot S-0.10T\cdot S\cdot Gam)]\}$, ($T$: temperature; S: salinity; Gam: dummy variable of 1 or 2, i.e. 1 denotes each group to which the gamete suspension was added and 2 each group to which the gamete suspension was not added). The probability of of spawning within the group to which the gamete suspension was added(i.e.Gam=1) explained well both annual and seasonal fluctuations of larval occurrence in Lake Abashiri in past years. The spawning of *C. japonica* may have been extremely low in 1995 and 1996 because of low temperature and low salinity. Such unfavorable conditions for spawning are not exceptional and occurred in 10 out of 21 years for which data were available. In *C. japonica*, reproduction succeeds less frequently in northern than in southern populations because the probability of spawning depends markedly on the temperature.

Introduction

Corbicula spp. are harvested commercially in Japan. The annual catch ranged from 19,000 to 27,000 t in 1994 to 2000 [9], among which *Corbicula japonica* was the main species. *C. japonica* is distributed in brackish water lakes and tidal flats of rivers from the south of Japan (about 35°N) to south of Sakhalin (about 50°N) [16]. Lake Abashiri, which is near the northern limit of this species' range, is connected to the Sea of Okhotsk by 7.2 km of the Abashiri River. Because sea water flows back into the lake, depending on the tide, the lake has a well-oxygenated oligohaline upper stratum and an anoxic polyhaline lower stratum. The habitat of *C. japonica* is restricted to the upper stratum [17].

The maximum water temperature, the salinity of the upper stratum, and the depth of the boundary between water masses in Lake Abashiri differed markedly from year to year [18]. The depth of the boundary and the salinity of the upper stratum change mainly according to the amount of precipitation and wind strength [17]. When drought conditions continue for several years, the depth of the boundary becomes shallower. Strong winds, which

sometimes occur in spring or autumn, cause upwelling of the polyhaline water of the lower stratum. The upwelling of the lower water probably occurs more frequently under conditions with a shallower boundary. Upwelling of the polyhaline water causes the salinity of the upper stratum to increase and forces the boundary between the water masses deeper (Abashiri Local Office of Hokkaido Development Bureau unpubl. data).

Maru [14] reported the spawning season of *Corbicula japonica* to be July to September. However, my recent work revealed that recruitment of *C. japonica* was extremely low in 1995 [19]. To clarify the cause of such low recruitment, I examined the relationships between temperature, salinity and spawning by means of an experiment performed under laboratory conditions, and verified the relationships with field data. This report is very important for resource management because it is the first to elucidate the environmental conditions necessary for the spawning of *C. japonica*.

Materials and Methods
Occurrence of planktonic larvae during spawning season

Samplings of planktonic larvae (veliger) were carried out 3 to 9 times during the spawning seasons of 1989 to 1990 and 1994 to 1997 at 1 to 3 sites. In 1997, there were duplicate samples at each site. Sampling locations and dates are shown in Fig. 1-1 and Table 1-1. Larvae were collected by a plankton net hauled vertically from the depth of the boundary between the upper and the lower strata (depth from 5 to 7 m) to the surface. A 300 mm diameter plankton net of NXX-13 (mesh: $95 \times 95 \mu$m) was used. The numbers of planktonic larvae were counted after the samples had been fixed with 3 % neutralized formalin.

Environmental data

Temperatures and salinities at the water surface during the spawning season (July to September) were obtained for Lake Abashiri from unpublished data of Abashiri Local Office of Hokkaido Development Bureau from 1994 to 1997. The data were collected once or twice a week.

Additional data on monthly water surface temperatures and salinities (1977 to 1993), as well as annual maximum temperatures, were obtained from Results of Water Quality

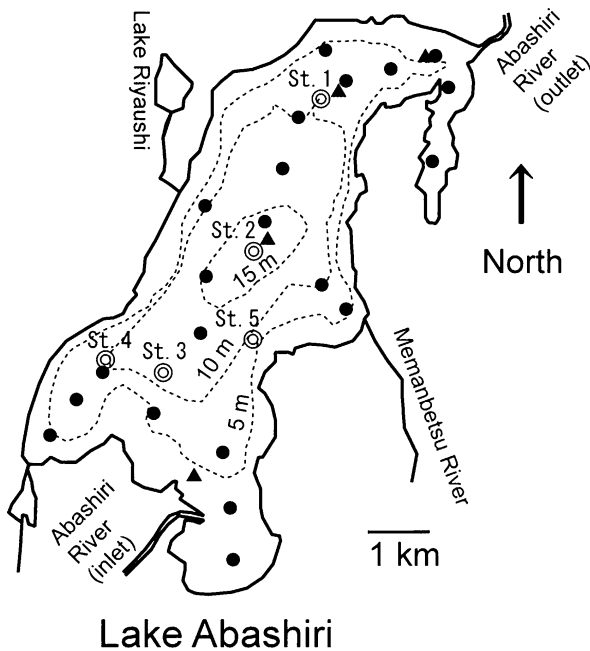Analysis in Public Waters'[20]. Collection locations for environmental data are shown in Fig. 1-1.

Fig. 1-1　Location of sampling sites in Lake Abashiri. Planktonic larvae of *Corbicula japonica* from different stations (◎)each year as follows: 1989, 1990 (Stn 5), 1994 (Stn 2), 1995 (Stn 2 and 4), 1996 (Stn 2, 4 and 5), 1997 (Stn 1, 2 and 3). Measurements of temperature and salinity were taken at (▲) 4 sites from 1977 to 1993 and (●) 21 sites from 1994 to 1997.

Histological examination

A histological examination was performed on the gonads of 10 to 20 individuals (20 to 30 mm shell length) sampled monthly in 1995 and 1997. Tissues were fixed in Bouin's solution and dehydrated through a graded alcohol series, embedded in Histprep 568 (Wako Pure Chemical Industries, Ltd.) and cut serially at 7 μ m thickness. The sections were stained with Mayer's hematoxylin-eosin double stain for examination under a light microscope.

Spawning induction experiment

The experiment on induction of spawning was carried out from 31 August to 6 September 1996. In 1996, few planktonic larvae were observed in Lake Abashiri. Twenty individuals were incubated in 10 L circular containers with 6 L of salinity- adjusted water. Salinity was adjusted to 0.0 (tap water only), 1.2, 2.3, 3.4, and 4.5 psu (practical salinity units) with seawater and tap water. Salinity was measured by an inductivity coupled salinometer (Model 601 Mk 1 V, YEO-KAL Environmental Electronics, Sydney). The containers were kept in incubators, with temperatures adjusted to 21.5, 22.5, 23.5, 24.5 and 26.0 ℃.

In half the groups, spawning was stimulated by adding 50 mL of gamete suspension from incubation water of groups previously spawned at 4.5 psu and 26 ℃. Success or failure of spawning induction was determined after 36 hours of incubation. The success or failure was easily distinguished because the incubation water became turbid with the gametes released by spawning groups. In the dilution procedure, not only salinity but also food, nutrients or other substances might be changed in incubation water. I thought these changes would not affect the results because of the short duration of the experiments.

The results of the experiment were analyzed by logistic regression calculated by the maximum-likelihood method. Logistic regression is useful for analysis of binary data [21]. The result of spawning induction was used as a binary dependent variable (i.e. a value of 1 was assigned to the each group in which spawning was induced and a value of 0 to a group without spawning). Temperature and salinity were used as continuous independent variables. Addition or exclusion of a gamete suspension was used as a categorical independent variable (i.e. a dummy variable of [1] was assigned to each group to which the gamete suspension was added and [2] was assigned to each group to which the gamete suspension was not added). A dummy variable is usually used in the logistic regression when a categorical variable is treated as an independent variable [22].

Two steps were applied to select independent variables for the logistic analysis. In the first, the main effects, i.e. temperature (T), salinity (S), adding gamete suspension (Gam), and their combinations were used as independent variables (7 cases). The best combination of the main effects was selected by the Akaike Information Criterion (AIC). In the second, the interaction terms, i.e. $T \times S$, $S \times Gam$, $T \times Gam$, $T \times S \times Gam$, and their combinations were added to the best combination of main effects (15 cases). The best model using both the main effects and the interaction terms was selected by the AIC.

The AIC was calculated as: AIC = -2 × ln(maximum-likelihood) + 2N, where N is the number of regression coefficients in the model. The AIC is useful for simultaneously comparing models with consideration for both maximum-likelihood and number of parameters [23]. In the AIC, a difference of 1 or more is regarded as statistically significant [24]. The equation of the logistic regression used in this study was: $f(T, S, Gam) = 1/(1+\exp(-g(T, S, Gam)))$,

where

$g(T, S, Gam) = a_0 + a_1 T + a_2 S + a_3 Gam + a_4 T \cdot S + a_5 S \cdot Gam + a_6 T \cdot Gam + a_7 T \cdot S \cdot Gam$,

and a_i (i = 0 to 7) are the regression coefficients, which are not simultaneously zero.

$f(T, S, Gam)$ represents the probability of spawning in *Corbicula japonica*. $g(T, S, Gam)$ is a linear component of the regression equation representing a log-odds ratio between the probabilities of a positive response and no response to spawning induction.

Salinity and larval development

Fertilized eggs obtained by the spawning induction experiment were incubated in water of the same salinity (i.e. 1.2, 2.3, 3.4, or 4.5 psu) at room temperature (21 to 23 ℃) for 24 hours to determine whether the eggs could develop to the D-shaped veliger at each salinity. Twenty-four hours after spawning, the number of D-shaped veligers among about 300 swimming larvae at each salinity was counted. Results were analyzed by logistic regression with the maximum-likelihood method. The logistic regression is useful for analysis of ratio data [21]. I did not feed the larvae during the experiment.

Results

Occurrence of planktonic larvae in each year are shown in Table 1-1. Numbers in 1995 and 1996 were extremely low compared with the other years (1989, 1990, 1994 and 1997).

Histological changes in female and male gonads during the spawning season in 1995 and 1997 are shown in Fig. 1-2. The female genital tubules were filled with mature oocytes at the beginning of the spawning season in both 1995 and 1997 (Fig. 1-2A, C). After the spawning season in 1995, female genital tubules were filled with atretic oocytes (Fig. 1-2B), but in 1997, it was observed that the genital tubules were either vacant or contained some oocytes degenerated by phagocytosis (Fig. 1-2D, E). Tubules filled with the atretic oocytes were observed in all the females examined in 1995, but none in 1997. The characteristics of the atretic oocytes of *Corbicula japonica* observed in 1995 were that the nucleus lost its basophilic properties, the cytoplasm became clear, the oocytes appeared terminally empty, and finally the cell membranes ruptured.

The male genital tubules were filled with mature sperm at the beginning of the spawning season in both 1995 and 1997 (Fig. 1-2F, H). After the spawning season in 1995, the genital tubules were filled with intensive degenerating sperm cells (Fig. 1-2G), whilst in 1997 small degenerating cells were observed and few sperm remained (Fig. 1-2I, J).

Results of the spawning induction experiment are shown in Table 1-2. Spawning or sperm release was observed in most individuals of the spawned groups. The results of logistic regression are shown in Table 1-3, where only the best models for each set using the same number of independent variables are indicated as representative examples. Model-5, which used the 3 main effects (T, S, Gam) and the 2 interaction terms ($T \times S$ and $T \times S \times Gam$), was the best in terms of AIC. Model-5 and model-6 were not significantly different in terms of AIC, but I selected model-5 as the best, because it showed the lowest AIC and used a lower number of independent variables. Model-5 was used to calculate the probability surfaces in Fig. 1-3.

Table 1-1 *Corbicula japonica*. Abundance of planktonic larvae during spawning season in Lake Abashiri.

Year (site)	Larval abundance (n/m³±SD) (Sampling date)								
1989 (Stn 5)	0 (14 Jun)	0 (13 Jul)	612 (21 Jul)	642 (7 Aug)	1217 (21 Aug)	698 (31 Aug)	0 (11 Sep)		
1990 (Stn 5)	0 (21 Jun)	0 (31 Jul)	1390 (21 Jul)	0 (9 Aug)	1820 (24 Aug)	1656 (6 Sep)	0 (23 Sep)		
1994 (Stn 2)	91 (1 Aug)	5832 (19 Aug)	17694 (30 Aug)						
1995 (Stn 2, 4)	0 (23 Jul)	15±15 (3 Aug)	2±2 (22 Aug)	0 (6 Sep)	0 (16 Sep)				
1996 (Stn 2, 4, 5)	0 (12 Jul)	0 (8 Aug)	0 (12 Aug)	7±2 (20 Aug)	0 (27 Aug)				
1997ᵃ (Stn 1, 2, 3)	0 (15 Jul)	26±21 (25 Jul)	479±47 (1 Aug)	608±65 (13 Aug)	3±2 (19 Aug)	74±46 (29 Aug)	141±75 (9 Sep)	95±40 (16 Sep)	0 (3 Oct)

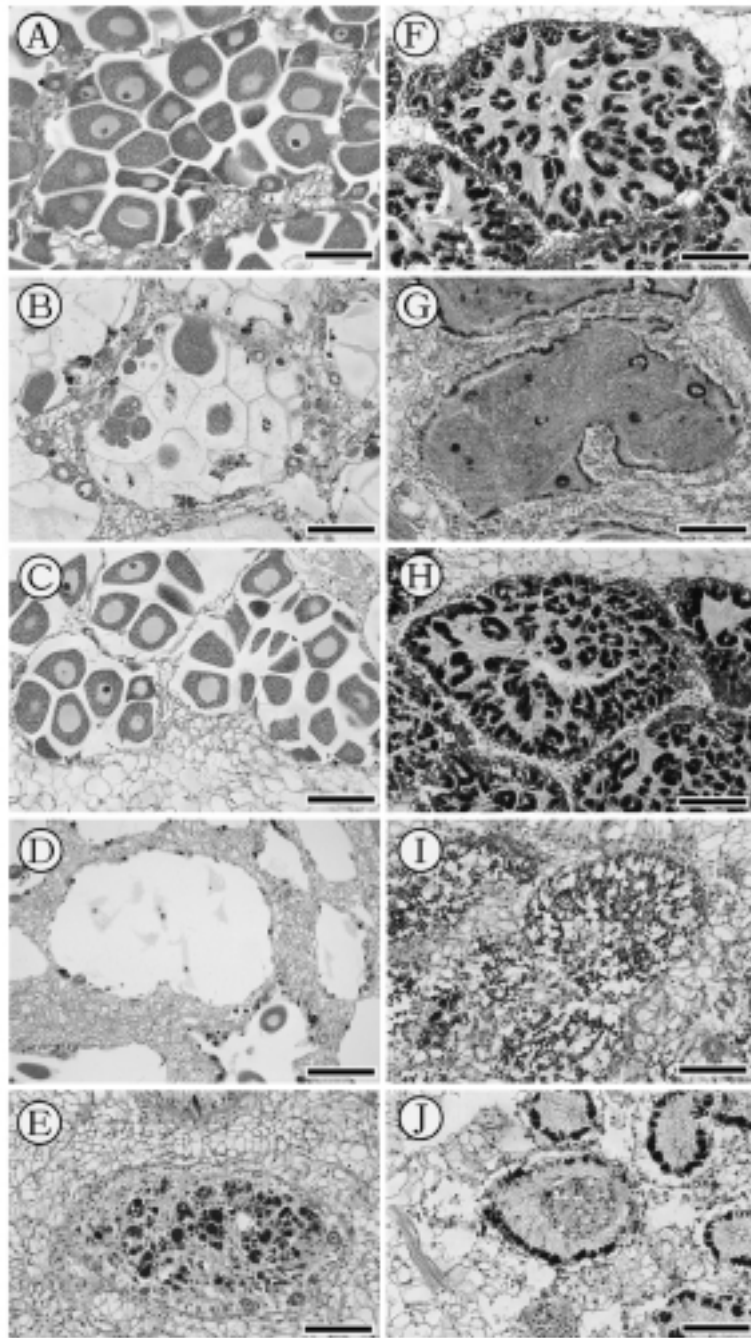[a] Sampling was duplicated at each site

Fig. 1-2 *Corbicula japonica.* Histological changes in gonads of *Corbicula japonica* in 1995 and 1997 (A-E: Female, F-J: Male). (A) Female gonad at the beginning of the spawning season in 1995 (3 August) showing genital tubules filled with mature oocytes. (B) Female gonad after the spawning season in 1995 (13 October) showing genital tubules filled with atretic oocytes.(C) Female gonad at the beginning of the spawning season in 1997 (1 August) showing genital tubules filled with mature oocytes. (D, E) Female gonad after the spawning season in 1997 (24 October) showing empty genital tubules (D), and some oocytes degener ated by phagocytosis (E). (F) Male gonad at the beginning of the spawning season in 1995 (3 August) showing genital tubules filled with mature sperm. (G) Male gonad after the spawning season in 1995 (13 October) showing genital tubules filled with many degenerating sperm. (H) Male gonad at the beginning of the spawning season in 1997 (1 August) showing genital tubules filled with mature sperms. (I, J) Male gonad after the spawning season in 1997 (24 October) showing few sperm remaining in the genital tubules (I), or some degenerated sperm (J). Bars = $100 \, \mu$m

Table 1-2 *Corbicula japonica.* Results of spawning induction experiments. Values are number of spawning-induced groups/ number of experimental groups.

	Gamete suspension added						Gamete suspension not added				
Salinity (psu)	Temperature (℃)					Salinity (psu)	Temperature (℃)				
	21.5	22.5	23.5	24.5	26.0		21.5	22.5	23.5	24.5	26.0
4.5	0/1	1/2	2/2	1/1	2/2	4.5	0/1	0/2	0/2	0/1	0/2
3.4	0/1	1/2	1/2	1/1	2/2	3.4	0/1	0/2	0/2	0/1	2/2
2.3	0/1	0/2	2/2	1/1	2/2	2.3	0/1	0/2	0/2	1/1	2/2
1.2	0/1	0/2	1/2	1/1	2/2	1.2	0/1	0/2	0/2	1/1	0/2
0.0	0/1	0/2	0/2	0/1	0/2	0.0	0/1	0/2	0/2	0/1	0/2

Table 1-3 *Corbicula japonica.* Logistic regression results from spawning induction experiment. Only the best models using the same number of independent variables are shown. *T*: temperature (℃), *S*: salinity (psu), *Gam*: adding gamete suspension (dummy variable 1 or 2)

Model no.	Used independent variables	Regression coefficients								AIC	p
		a_0	a_1	a_2	a_3	a_4	a_5	a_6	a_7		
Main effect only											
1	*T*	-18.7	0.75							85.8	1.2×10^{-5}
2	*T, Gam*	-25.8	0.95		2.54					71.8	2.4×10^{-8}
3	*T, S, Gam*	-32.5	1.17	0.71	3.02					63.7	8.4×10^{-10}
Main effect plus interaction term											
4	*T, S, Gam, S×Gam*	-35.1	1.34	2.29	0.62			-1.08		60.8	3.4×10^{-10}
5	*T, S, Gam, T×S, T×S×Gam*	-15.6	0.57	-12.6	-0.55	0.69			-0.10	56.3	6.1×10^{-11}
6	*T, S, Gam, T×S, S×Gam, T×S×Gam*	-18.0	0.66	-25.3	-0.53	1.22		9.33	-0.48	57.1	1.2×10^{-10}
7	*T, S, Gam, T×S, T×Gam, S×Gam, T×S×Gam*	-14.7	1.03	-23.8	-6.76	1.16	-0.25	8.12	-0.43	59.0	3.9×10^{-10}

Occurrence of planktonic larvae, temperature and salinity during the spawning seasons, and the spawning probability calculated by model-5 with the dummy variable *Gam*=1 in 1994, 1995, 1996 and 1997 are shown in Fig. 1-4. The occurrences of the planktonic larvae are well corresponded with the spawning probabilities calculated by the model-5. I did not have such intensive environmental data for 1989 and 1990 so I excluded these years.

Some delay was observed from a peak in the spawning probability to a peak of larval occurrence in 1995, 1996 and 1997. Very high density of planktonic larvae was observed at the end of August in 1994. This high density corresponds to increased salinity from about 2.2 to 2.5 psu. The spawning halted in mid August 1997 due to decreased temperature and resumed at the end of August when temperature increased. The densities of planktonic larvae were very low in 1995 and 1996 during the spawning season.

The isopleths of the spawning probability (0.1, 0.25, 0.5, 0.75, 0.9) calculated by model-5 with the dummy variable *Gam*=1, the larval occurrences in Lake Abashiri, and the temperature and salinity (mean ± SD) of the surface of the upper stratum at the maximum temperature in the lake in past years are compared in Fig. 1-5. The maximum water temperature and the salinity in the upper stratum of the lake differ markedly from year to year. Maximum water temperatures ranged from 19 to 22 ℃ in cold summer years and from 26 to 28 ℃ in warm summer years. Salinity at the maximum temperature ranged from 6.5 to 0 psu.

The isopleths can be regarded as an environmental border range for the spawning of *Corbicula japonica*, because they explained the larval occurrences of past years. Unfavorable conditions for spawning are not exceptional and occurred in 10 out of 21 years for which data were available.
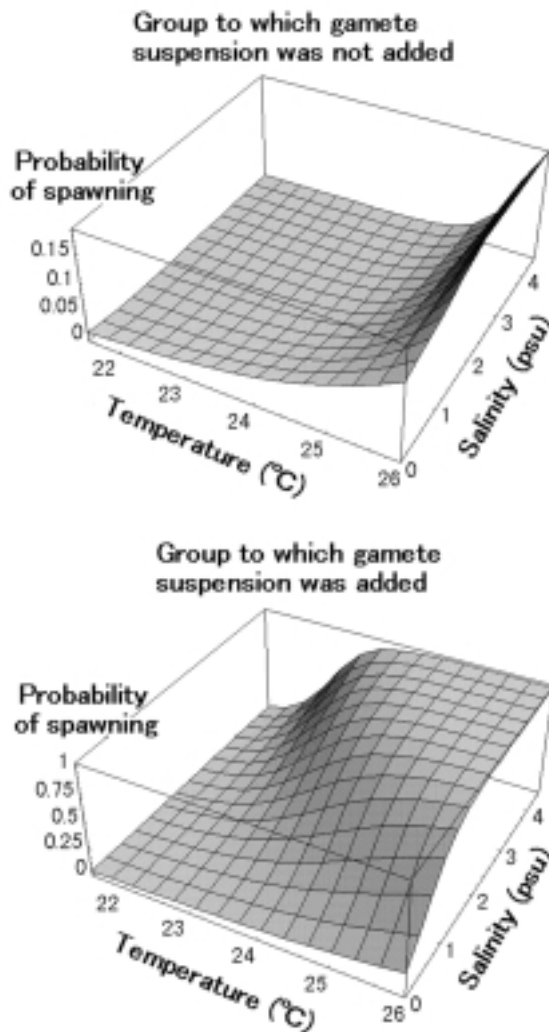
Fig. 1-3　Relationship between spawning probability, temperature and salinity in *Corbicula japonica* predicted by model-5 in Table 3. Upper panel shows the relationship between spawning probability, temperature and salinity of the groups to which the gamete suspension was not added (i.e. the dummy variable Gam =2 in model-5). Lower panel shows the relationship from groups to which the gamete suspension was added (i.e. the dummy variable Gam =1 in model-5).

Twenty-four hours after spawning, the mean percentages ±95% confidence intervals of the D-shaped veliger at each salinity are shown with the probability estimated from the logistic regression in Fig. 1-6. The equation for the logistic regression was $f(x) = 1/\{1+\exp[-(-6.42+2.37x)]\}$, where x is salinity (p < 0.01). The salinity at probability 0.5 of the logistic regression

was 2.7 psu. At 2.3 psu, 72.2 % of the swimming larvae did not reach the D-shaped veliger stage within 24 hours, and some were abnormal (swollen). Not only might their development have been delayed, but also some damage may have occurred at 2.3 psu. At 1.2 psu, most fertilized eggs developed to the morula stage 12 hours after spawning and many abnormal (swollen) blastulae were observed after 24 hours. No D-shaped veliger stage was observed at 1.2 psu.

Discussion

Temperature and salinity appear to regulate spawning in *Corbicula japonica* (Table 1-3, Fig. 1-3). More spawning occurs when environmental conditions exceed the border range (isopleths of spawning probability from 0.1 to 0.9), especially when favorable conditions persist (Figs. 1-4 and 5). Much less spawning occurs when conditions do not exceed the border range (Figs.1-4 and 5).

There is the potential that some of the interannual variation might be attributed to differences in sampling sites from year to year. In 1994, large numbers of planktonic larvae were observed in the central portion of Lake Abashiri, even though the habitat of *Corbicula japonica* is restricted to the margins. In 1995, 1996 and 1997, sampling was more intensive than in 1994. However, I found few planktonic larvae in 1995 and 1996. In 1997, there were fewer planktonic larvae than in 1994. Consequently, I believe my observations actually reflected the magnitude of population spawning in each year. Furthermore, Larval abundance seemed to be associated more with changes in the temperature and the salinity than the sampling location.

In years unfavorable for spawning, intensive oocyte atresia occurs in mature gonads of individuals after the spawning season. This is the first report of such intensive oocyte atresia in *Corbicula japonica*. Oocyte atresia is also regulated by environmental conditions in other marine bivalves such as *Pecten maximus*, in which the borderline water temperature is 15.5 to 16.0°C [25, 26]. I observed oocytes degenerated by phagocytosis after the spawning season in 1997. Degeneration of oocytes by phagocytosis was also reported by Maru [14].

As shown in Fig. 1-4, there was some delay between a peak in the predicted spawning probability and a peak in larval abundance in 1995, 1996 and 1997. It takes about 1

Larval abundance and spawning probability　　　Temperature (°C)　　　Salinity (psu)
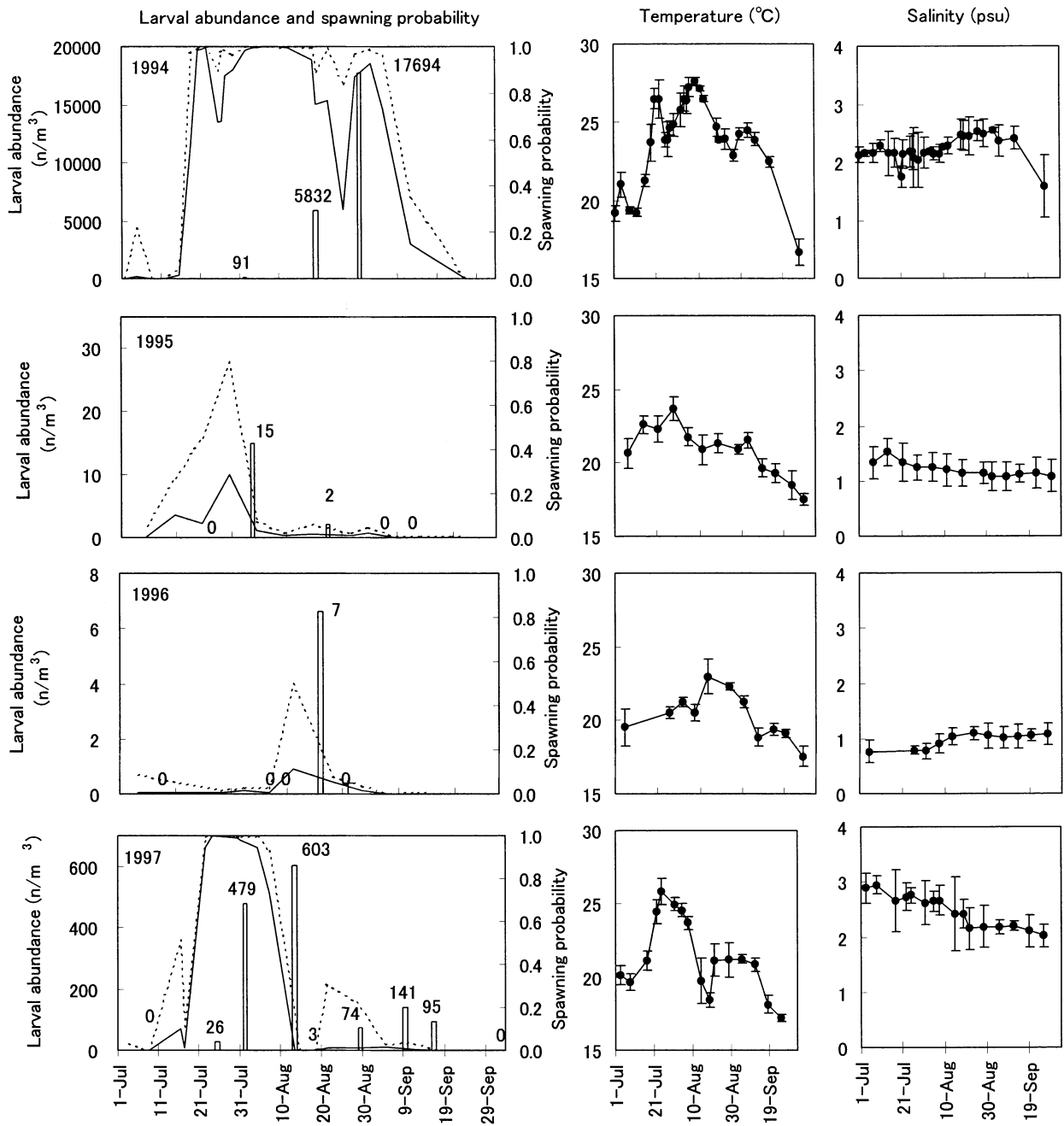

Fig. 1-4 Temperature and salinity changes (mean±SD), larval abundance (bars), and spawning probabilities (solid and dashed lines) calculated from model-5 with dummy variable Gam=1, during spawning season from 1994 to 1997. Solid line is the spawning probability calculated from average temperature and salinity. Dashed line shows the maximum probability on each day.

days to reach the veliger stage after fertilization, and the planktonic phase is about 6 to 10 days in *Corbicula japonica* [27]. The time required to pass through these phases may cause the observed delay.
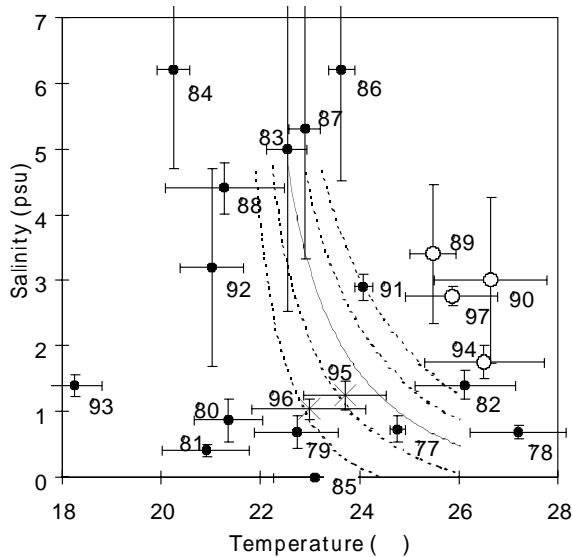

Fig. 1-5　Isopleths of spawning probability calculated from model-5 with the dummy variable Gam = 1, larval occurrence (○ abundant, × few, ● no data), and maximum surface temperature and salinity (mean ± SD) of Lake Abashiri in past years. Solid line shows the probability 0.5, dashed lines show probabilities 0.9, 0.75, 0.25, 0.1 from upper right to lower left respectively. The number indicates each year.
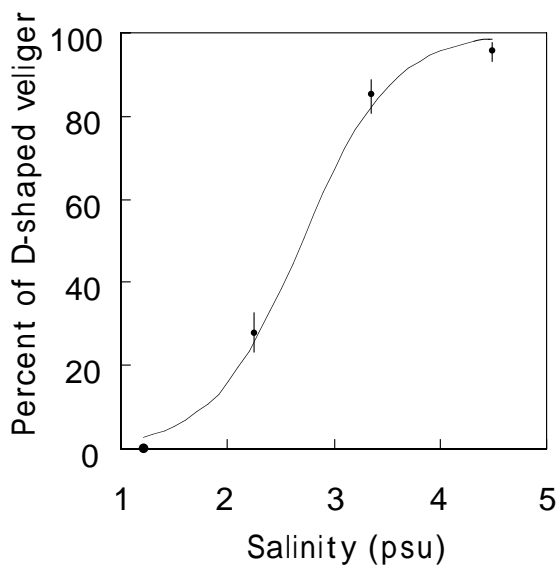

Fig. 1-6　Mean percentages ±95 % confidence intervals of D-shaped veliger of *Corbicula japonica* 24h after spawning at each salinity and probability of the logistic regression.

In 1997, spawning seemed to cease in mid August when temperature declined, but then resumed when temperature increased. The magnitude of the second spawning event was greater than one might have expected from the spawning probability estimate (Fig. 1-4). Model-5 with the dummy variable *Gam*=1 explained the timing of larval occurrences during the spawning season, but not the precise magnitude of the events. Perhaps time series models that include cumulative effects of environmental variables would be more useful in predicting the abundance of larvae.

In *Corbicula fluminea*, a fresh-water ovoviviparous hermaphroditic species, release of juveniles begins when temperature rises above 18 to 19 °C, but declines when temperatures rise above 26 °C [7]. In *C. japonica*, a brackish-water oviparous dioecious species, spawning began at temperatures above 22 °C, and inhibition of spawning by high temperature was not observed (Figs. 1-3 and 4). This suggests *C. japonica* may be more adapted to high temperatures than *C. fluminea*.

In the spawning induction experiment, some groups spawned at salinities of 1.2 or 2.3 psu (Table 1-2, Fig. 1-3), but these salinities were insufficient for larval development (Fig. 1-6). Asahina [13] reported that the development of the embryo could proceed at salinities from 3 to 28 psu in *Corbicula japonica*, and Sasaki [28] reported that the range was from 0.6 to 6.1 psu. Neither study determined whether the embryo could reach the veliger stage. In my experiment, the salinity that guaranteed embryonic and larval development to the D-shaped veliger stage was 3.1 psu, and the value at which half of the swimming larvae reached the veliger stage was 2.7 psu (Fig. 1-6). Under natural conditions, many planktonic larvae were observed in 1994 in the low-salinity upper stratum of Lake Abashiri (Figs. 1-4 and 5). A large portion of the planktonic larvae were found near the boundary between the oligohaline upper stratum and the polyhaline lower stratum in 1997 (unpubl. data). These observations suggest that larvae of *C. japonica* may be capable of salinity-related movement. A salinity-related response at the larval stage has also been reported in the marine bivalve *Mercenaria mercenaria* [29].

A substantial increase in abundance of planktonic larvae occurred between 1 August and 19 August 1994 and corresponded to an increase in salinity from 2.2 to 2.5 psu. Although this salinity change may seem small, it

occurred within a range at which there was a steep slope in the relationship between salinity and percentage of D-shaped veliger 24 hours after spawning (Fig. 1-6), suggesting that small changes within this salinity range could have important effects on larval survival and development.

Unfavorable environmental conditions for spawning are not exceptional, and in fact were observed in about half the years studied (Fig. 1-5). Reproduction appears to succeed less frequently in northern than in southern populations of *Corbicula japonica*, because the necessary environmental conditions for spawning depend heavily on temperature.

CHAPTER II

Pre-settlement processes in planktonic larva of the brackish water bivalve *Corbicula japonica* in Lake Abashiri

Abstract

I found the vertical heterogeneities of the planktonic larva (D-shaped veliger) densities and shell length compositions of *Corbicula japonica* in water column. A large portion of smaller size planktonic larvae (shell length 140 to 170 μm) were observed near the boundary between a well-oxygenated oligohaline upper stratum and an anoxic polyhaline lower stratum (depth about 6 m). On the other hand, most of larger planktonic larvae (shell length 170 to 200 μm) were observed near the surface of the lake (depth 0.2 m). To confirm the size of competent settling stage and the postponement of planktonic phase, I carried out an incubation experiment of the planktonic larvae under laboratory conditions. As the results, the planktonic larva reached the competent settling stage at the shell length of around 160 μm. The planktonic larva postponed their planktonic phase from 3.6 days to 4.4 days or from 6.3 days to 8.8 days when the bottom was changed from sand to seawater. From these results and my previous reports (Chapter Ⅰ in this thesis), I hypothesized the pre-settlement processes in the planktonic larva of *C. japonica*. First, the early-stage planktonic larvae assemble to the higher salinity zone, which formed near the boundary to gain the sufficient salinity for the larval developments. Second, the well-developed larvae migrate to near the surface, where higher possibility of being transported to the favorable habitat for the larval settlement is expected. These pre-settlement processes probably enhance the survival of the salinity-sensitive planktonic larvae in the specific environment of brackish-water lakes.

Introduction

Corbicula spp. are harvested commercially in Japan. The annual catch ranged from 24,000 to 27,000 ton in 1994-1996 [9], among which *Corbicula japonica*, a brackish-water oviparous dioecious species, was the main species. *C. japonica* is distributed in brackish water lakes and tidal flats of rivers from the south of Japan to the south of Sakhalin [16].

Lake Abashiri has a well-oxygenated oligohaline upper stratum and an anoxic polyhaline lower stratum because seawater flows back into the lake through 7.2 km of the Abashiri River depending to the tide. The salinity of the surface and the boundary depth between the two strata change from year to year, and they range from 0.0 to 7.0 psu (psu, practical salinity unit) and from 5 to 7 m respectively in recent years [17, 30]. Therefore, the habitat of *C. japonica* is restricted to the marginal area of the lake, shallow than about 5 m.

Baba et al.[30] reported spawning probability of *C. japonica* was expressed by the equation:
$1/\{1+\exp[-(-15.6+0.57T-12.6S-0.55Gam+0.69T\cdot S-0.10T\cdot S\cdot Gam)]\}$, (*T*: temperature; *S*: salinity; *Gam*: dummy variable of 1 or 2, i. e. 1 designated each group to which the gamete suspension was added and 2 each group to which the gamete suspension was not added), and this equation with *Gam*=1 can explain occurrences of the planktonic larvae in Lake Abashiri.

They also reported the probability to be D-shaped veliger 24h after spawning in *C. japonica* was expressed by the equation:
$1/\{1+\exp[-(-6.42+2.37S)]\}$
where *S* is Salinity. Figure 2-1 shows these two equations, the first equation with *Gam*=1 and the second equation. In the Fig. 2-1, the salinity was classified to three ranges for the larval development, i. e. lethal, limiting and non-limiting, according to Yamamuro et al. [31] in which these three ranges were proposed for a function expressing a limiting effect of an environmental factor to a macrobenthos. I used probability 0.05 and 0.95 as border points for the classification. As shown in Fig. 2-1, the spawning probability reaches maximum within the

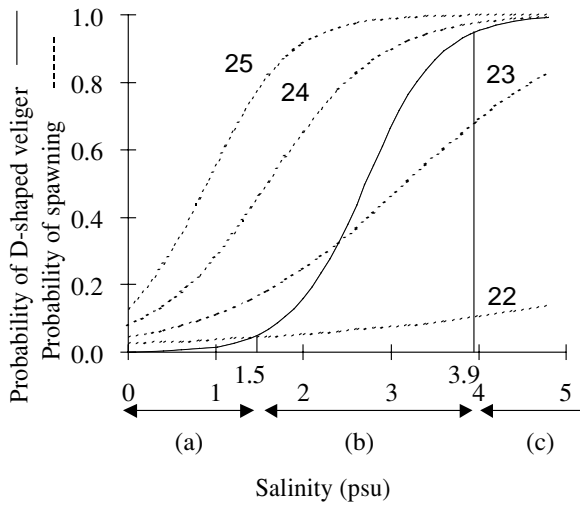limiting salinity range for the larval development.

Fig. 2-1　Relationships between salinity and the probability of the D-shaped veliger 24h after spawning (solid line) and relationships among salinity, temperature and the spawning probability (dashed line) in *Corbicula japonica* (Baba et al. 1999). Vertical lines show the salinity at the probability 0.05 (right) and 0.95 (left) of the D-shaped veliger. (a), (b), (c), ranges for the larval development; (a), lethal; (b), limiting; (c), non-limiting.

The anoxic polyhaline lower stratum covers about 78 % of the lakebed in Lake Abashiri. On the other hand, the planktonic larvae of *C. japonica* were observed in most the well-oxygenated oligohaline upper stratum [32].

Two questions were arisen from these previous reports. First, when *C. japonica* spawns in the limiting salinity range for the larval development, whether high mortality occurs on the planktonic larvae or the planktonic larvae have some mechanisms to alleviate the bad effects of the low salinity? Second, whether most the planktonic larvae locate above the anoxic polyhaline lower stratum die or the planktonic larvae have some behavior to enhance survivability? To answer these questions, I investigated the vertical change of the planktonic larvae distribution and environments at the midst of spawning season in 1997 and I confirmed postponement of planktonic phase and shell lengths in which *C. japonica* become the competent settling stage in a laboratory experiment.

Materials and Methods
Vertical distribution of planktonic larvae

Samplings of planktonic larvae were carried out at the central part of the lake in the daytime on August 7, 1997 (Fig. 2-2). Two hundreds litter (0.2 m³) of waters were pumped up from each depth of 0.2, 2, 4, 5, 6 and 6.8 m. The waters were filtrated with a plankton net NXX-13 (mesh: $95 \times 95 \mu$ m). The sampling was duplicated at the each depth. The samples had been fixed with 3 % neutralized formalin. The planktonic larvae were sorted and counted under a binocular microscope. The shell lengths of the planktonic larvae were measured under a profile projector (V-12, Nikon Ltd.) with 100 magnitude using a digital caliper (Digimatic caliper, Mitutoyo Ltd.).
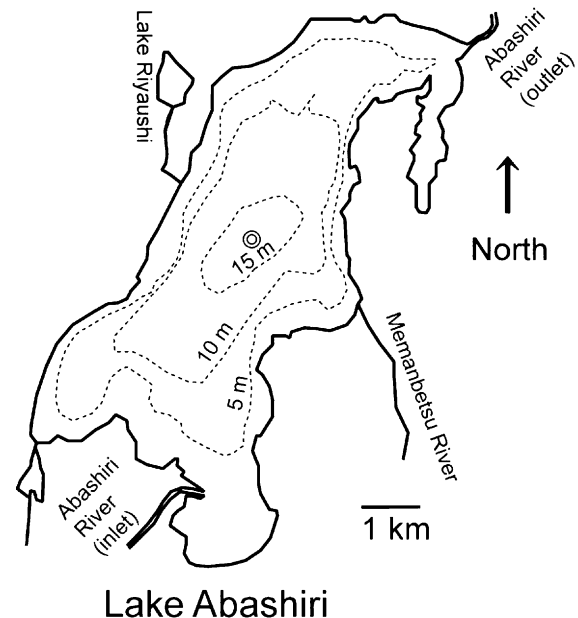

Fig. 2-2　Location of sampling site (○) in Lake Abashiri.

Environmental data

Salinities (psu, practical salinity unit), water fluorescence (equivalent to uranin density, μ g/L), turbidity (ppm, equivalent to kaoline density) and temperatures (°C) with interval of 0.1 m depth were obtained from unpublished data of Abashiri Local Office of Hokkaido Development Bureau. The environmental factors were measured by Memory Chlorotec (ACL-1180-OK, Alec Electronics Ltd.). The environmental factors were measured at the same site and the same day as the larval sampling.

Incubation experiment of planktonic larvae

Spawning was induced at 25 °C and 4 psu with 30 individuals, which have shell lengths from 20 to 30 mm, in 10 L circular containers. Twenty-four hours after spawning, 1 L of incubation waters, which contain veliger stage of *C. japonica*, were transferred to six aquariums (L × W × H, 585 × 280 × 340 mm) with 35 L of 4 psu incubation waters. The salinity of the incubation waters was adjusted by tap water and seawater. One day after start of incubation, the average density of the veliger was 4.1 ind./ mL. Before transfer the veliger, sand were laid on the bottom of the two aquariums (sand bottom group). Median diameter, skewness and kurtosis of the sand are 0.87 mm, 1.28 and 2.26 respectively. After transferred the veliger, 10 L of seawaters were calmly poured under the incubation water of the four aquariums with a rubber hose (seawater bottom group). Forty mL of a solution which contain about 107 cells / mL of *Pavlova lutheri* was added to the each aquarium everyday as foods for the larvae. Very little aeration had been performed at the surface of incubation water during the experiment. The incubation experiments were carried out under the room temperature (from 24 to 26 °C).

Sixty-five mL of incubation water was sampled from each aquarium everyday. The samplings were conducted with a 10 mm diameter glass tube with following methods. The tube was vertically inserted into the incubation water from the surface to the bottom of the aquarium, the top of the tube was sealed with a silicon rubber, and then water inside of the tube was harvested. These procedures were replicated four times at different sites of the each aquarium to minimize sampling error. The samples had been fixed with 3 % neutralized formalin. The planktonic larvae were sorted and counted under a binocular microscope. The shell lengths of the planktonic larvae were measured under a profile projector (V-12, Nikon Ltd.) with 100 magnitude using a digital caliper (Deigimatic caliper, Mitutoyo Ltd.).

Density (ind./65 mL) changes of the planktonic larvae were analyzed by logistic regression calculated by maximum-likelihood method. The logistic regression is useful for analysis of proportion [21]. Two types of equations were fitted to the density changes of the each group. The equations were as follows:

$f(t) = 1/\{1 + \exp[-(a_1 + a_2 t)]\}$ (model-1) and

$f(t) = a_0/\{1 + \exp[-(a_1 + a_2 t)]\} + (1 - a_0)/\{1 + \exp[-(a_3 + a_4 t)]\}$

(model-2)

where $f(t)$ = (planktonic larvae densities t days after spawning)/(initial planktonic larvae density),

t = days after spawning

a_0, a_1, a_2, a_3, a_4 = coefficients of the equation ($0 \leq a_0 \leq 1$).

The log-likelihood function for the both equations is as follow:

$$\log L = \sum_{t=ini}^{end} D_t \times \log (f(t))$$
$$+ \sum_{t=ini}^{end} (D_{ini} - D_t) \times \log (1 - f(t)),$$

where ini = initial day of larval density calculation, end = end day of larval density calculation, D_t = larval density t days after spawning.

The coefficients (a_0, a_1, a_2, a_3, a_4) were estimated as values that maximize the log likelihood. A day in which the maximum larval density was recorded was set as the initial day of the density calculation (*ini*). The initial day was two in the sand-bottom group and three in the seawater-bottom group respectively. A day in which the zero larval density was recorded was set as the end day of the larval density calculation (*end*). The end day was 8 in the sand-bottom group and 12 in the seawater-bottom group.

Results

Vertical changes of the planktonic larvae abundances and the environments are shown in Fig. 2-3. These mean abundances were significantly different for the total data set in SS-STP (SS-STP, sum of squares simultaneous test procedure; p<0.01). Shell length composition of the each depth is shown in Fig. 2-4. These shell lengths were significantly different among the groups in Kruskal-Wallis test (p <0.01). A large portion of smaller size planktonic larvae (shell length 140-170 μm), about 65 % of total number of the smaller size larvae in the samples, distributed at a depth of 6 m near the boundary between the well-oxygenated oligohaline upper stratum and the anoxic polyhaline lower stratum. The salinities drastically increased between the depth of 5 m and 6 m. The salinity at the depth 6 m reached the non-limiting range for the larval development. On the other hand, a large portion of the larger size planktonic larvae (shell length 170-200 μm), about 77 % of total number of the larger size larvae in

the samples, distributed at a depth of 0.2 m, where salinity was lower than deeper sites.

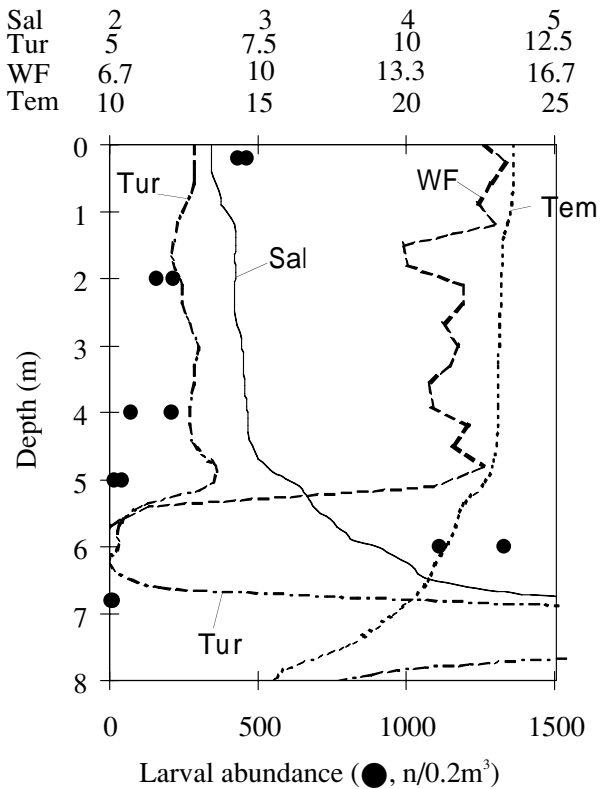| Sal	2	3	4	5
Tur	5	7.5	10	12.5
WF	6.7	10	13.3	16.7
Tem	10	15	20	25

Fig.2-3 Vertical changes of the planktonic larvae abundance of *Corbicula japonica* and environmental factors at the sampling site. The larval abundances were shown by ● (n/0.2m³). Each line showed the change of the environmental factor. Sal, Salinity (psu)(Solid line); Tur, Turbidity (mg/L) (chain line); WF, Water fluorescence (equivalent to uranin μ g/L)(long dashed line); Tem, Temperature (℃)(dashed line).

Density changes of the planktonic larvae and probabilities of the logistic regression during the planktonic larvae incubation experiment are shown in upper panel of Fig. 2-5. The results of logistic regressions are shown in Table 2-1. The model-2 was significantly better than the model-1 for the both sand-bottom and seawater-bottom groups with likelihood ratio test (Table 2-1). The slopes of the model-2 of the both groups are shown in lower panel of Fig. 2-5. In the sand bottom group, 78 % of the planktonic larvae settled around 3.6 days after spawning and 28 % of them settled around 6.3 days after spawning. On the other hand, 52 % of the planktonic larvae settled around 4.4 days after spawning and 48 % of them settled around 8.8 days after spawning in the seawater bottom group. The planktonic larvae postponed their planktonic phase from 3.6 to 4.4 and from 6.3 to 8.8 days when the bottom conditions were not suitable for the settlement.

Changes of the shell length composition during the experiment are shown in Fig. 2-6. Two days after spawning, the shell lengths and their variances were not significantly different between the two groups (shell length, $p > 0.05$ in Kruskal-Wallis test; variance, $p > 0.05$ in Bartlett's test). Three to seven days after spawning, the shell lengths were significantly different between the two groups ($p < 0.01$ in Kruskal-Wallis test). *Corbicula japonica* seemed to become the competent settling stage at shell lengths from 155 to 165 μ m because few individual with shell lengths more than 165 μ m was observed in the sand bottom group and some individuals with shell lengths 155-160 μ m were inferred as the planktonic phase postponing individuals in the seawater bottom group (Fig. 2-6).

Table 2-1 Results of modified logistic regression from larval incubation experiment in *Corbicula japonica.*

Model No.	Regression coefficients					AIC	p	Likelihood ratio test
	a1	a2	a3	a4	a5			
Sand bottom group								
1	5.94	-1.41				2623.0	<0.001	
2	18.79	-2.97	11.96	-3.29	0.22	2448.7	<0.001	**
Seawater bottom group								
1	4.23	-0.66				9920.4	<0.001	
2	11.61	-1.32	8.73	-1.98	0.48	9640.3	<0.001	**

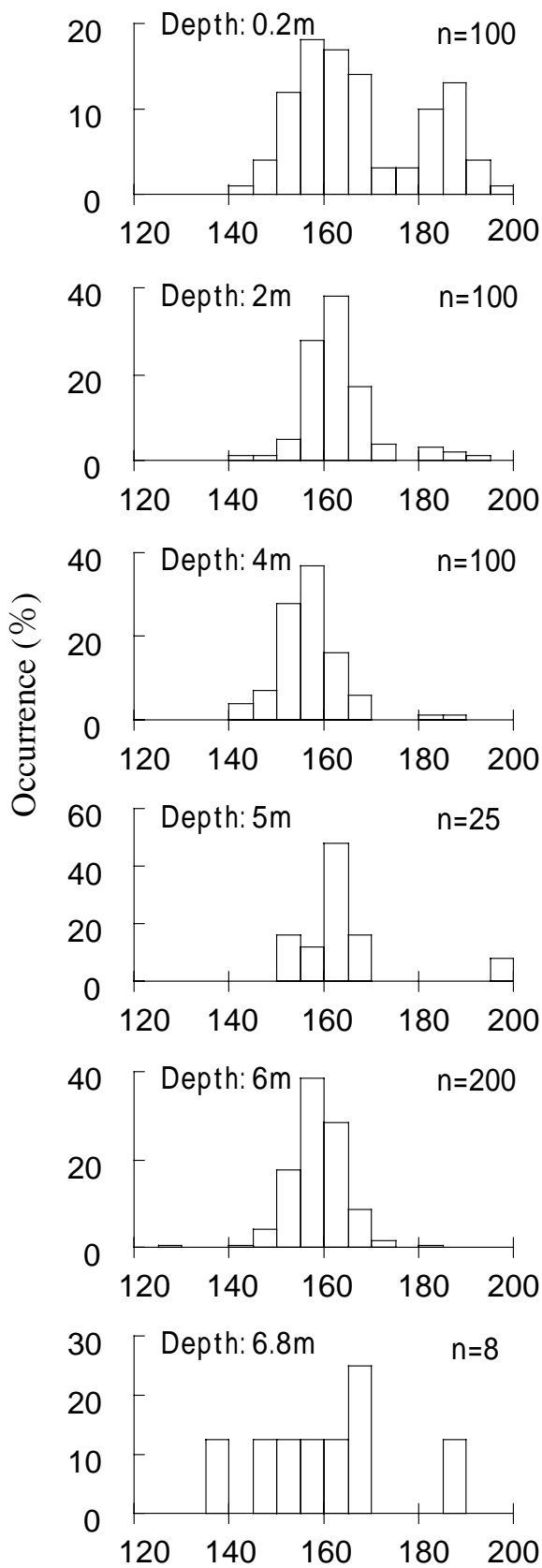**: significantly better model with $p < 0.001$

Fig. 2-4 Vertical change of shell length compositions of the planktonic larvae of *Corbicula japonica*. n, number of measured individuals.
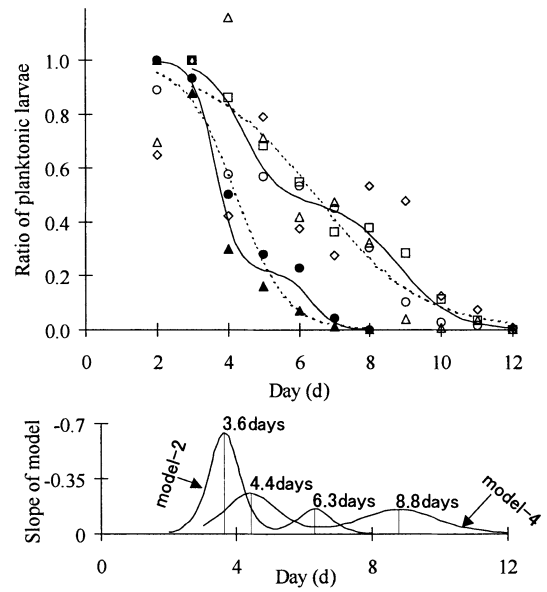

Fig. 2-5 Density changes of the planktonic larvae of *Corbicula japonica* during the planktonic larvae incubation experiment (upper panel). Filled marks (●) represent ratios between planktonic larvae densities at t days after spawning and the planktonic larvae density at the initial day of the density calculation (2 days after spawning) in the sand bottom group. Blanked marks (○) represent ratios between planktonic larvae densities at t days after spawning and the planktonic larvae density at initial day of the density calculation (3 days after spawning) in the seawater bottom group. Vertical lines represent standard deviations. Solid lines show the model-2 in the Table 2-1. Lower panel shows the slopes of the model-2.

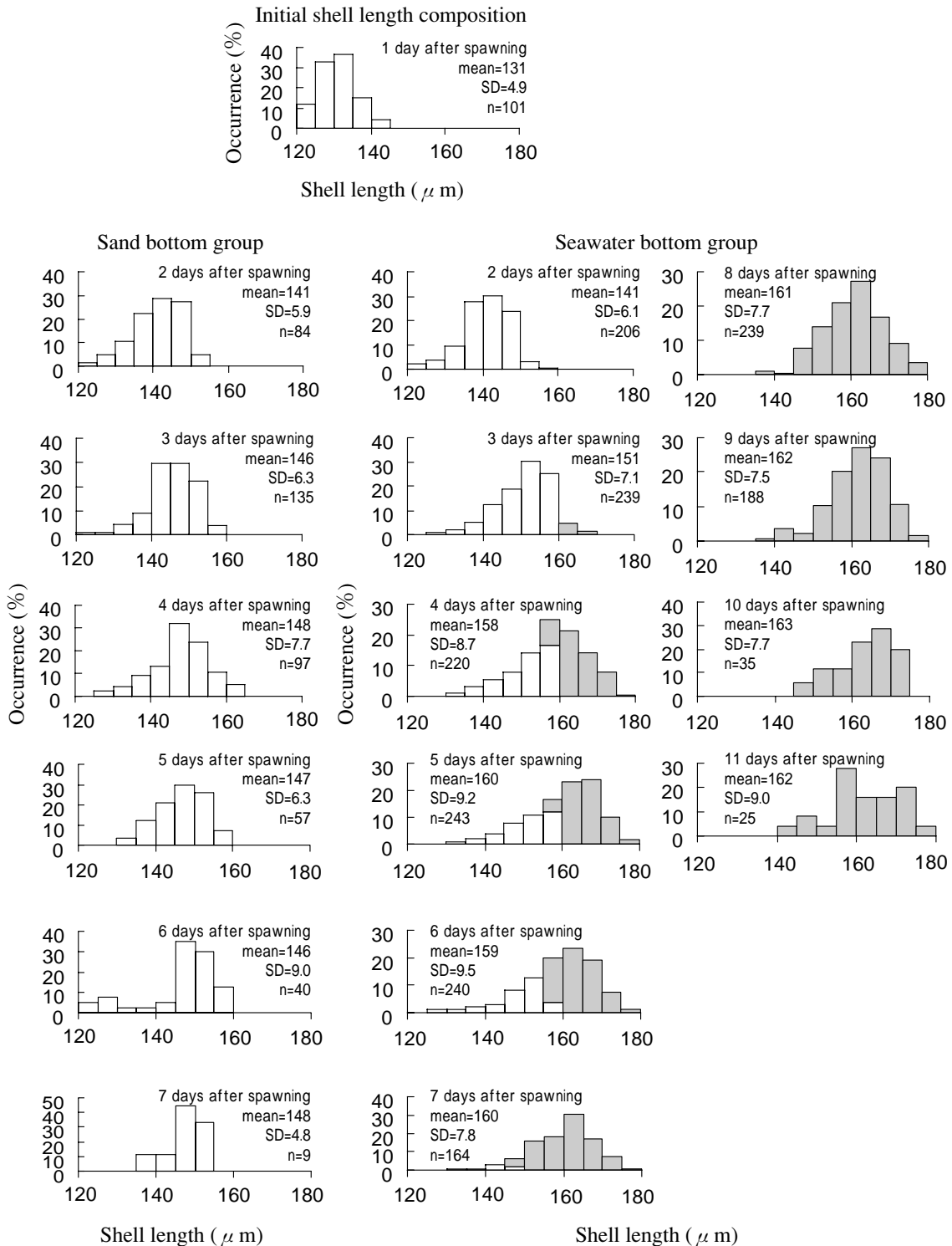Fig. 2-6　Changes of shell length compositions of *Corbicula japonica* during the planktonic larvae incubation experiment. The top panel shows the initial shell length composition, the shell length composition at one day after spawning. The filled bars show the planktonic phase postponing individuals inferred by the differences of the shell length compositions between the two groups. n, number of measured individuals.

Discussion

I schematized my hypothesis on the pre-settlement processes of *Corbicula japonica* in Fig. 2-7. I thought the early stage planktonic larvae assembled to near the boundary to gain sufficient ambient salinity for the larval development, i. e. the planktonic larvae have a mechanism to alleviate bad effects of the low salinity when *C. japonica* spawned in the limiting salinity range for the larval development. This behavior is important as one of the pre-settlement processes because Baba et al. [30] reported the spawning probability reaches maximum within the limiting salinity range for the larval development to D-shaped veliger (Fig. 2-1). As shown in Fig. 2-3, water fluorescence and turbidity drastically declined at around the depth of 6 m than the shallower depths. Therefore, the food availability was not factor for the assemblage of the smaller planktonic larva. I thought the salinity was probably the most important factor that induces the assemblage of the early stage planktonic larvae.

Most the larger size planktonic larvae were observed near the surface of the lake, where the salinities were lower than the deeper sites (Fig. 2-3, Fig. 2-4). In Lake Abashiri, about 78 % of the lakebed is covered by the anoxic polyhaline lower stratum, whereas planktonic larvae of *C. japonica* are observed in most the well-oxygenated oligohaline upper stratum [32]. In the lake, the migration toward the surface probably enhances the survival of the planktonic larvae. Larger disturbance is expected at the surface than deeper sites because the surface is more influential area of winds than deeper sites. The larger disturbance increases the chance to be transported to marginal area of the lake where sediments are not covered by the anoxic polyhaline lower stratum. The upward migration probably prolongs postponement of the planktonic phase more than it observed in the larvae incubation experiment because many planktonic larvae with shell lengths from 180 to 200 μm were observed near the surface of the lake whereas the maximum shell length in the planktonic phase postponing individuals of the experiment were less than 180 μm (Fig. 2- 4 and Fig. 2-6). The postponement of the planktonic phase also increases the chance to be transported to the marginal area of the lake.
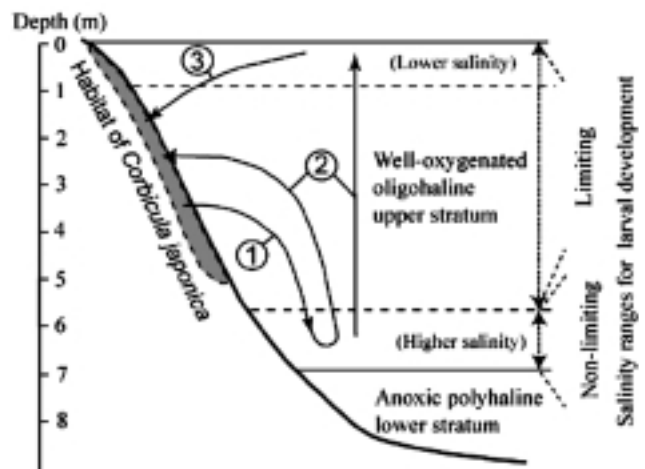

Fig. 2-7 Schematization of my hypothesis on the pre-settlement processes of *Corbicula japonica* when *C. japonica* spawned in the limiting salinity range for the larval development. First, the early stage planktonic larvae assemble the higher salinity zone, the nursery zone, to gain sufficient salinity for the larval development because the spawning probability reached maximum within the limiting salinity range for the larval development (Fig. 2-1, Fig. 2-3, Fig. 2-4). Second, the later stage planktonic larvae migrate upward may due to change of salinity preferences after they acquired tolerance to the low salinity. When the larvae encountered the favorable sediments for the settlement during the upward migration, the larvae would settle. When the larvae did not encounter the favorable sediments, the planktonic phase would be postponed and the larvae grow up to 200 μm at the surface of the lake. Third, the location of the larger size planktonic larvae (shell length 170-200 μm) at the surface may increase their chance to be transported to the habitat of *C. japonica*, the marginal area of the lake, because larger diffusion is expected at the surface than deeper sites due to influences of winds. When the larger size planktonic larvae were transported to the marginal area, the larvae would settle.

I thought the most possible factor that induces the upward migration of the larger size planktonic larvae is salinity, i. e. *C. japonica* changes its responses from salinity-positive in the early stage planktonic larvae to salinity-negative in the later stage after they acquire tolerance to the low salinity. The salinity difference

between the depth of 0.2 (2.7 psu) and 2 m (2.9 psu) may seem small. However, I thought *C. japonica* is sensitive to the range because it occurred within a range at which there was a steep slope in the relationship between salinity and the probability of D-shaped veliger (Fig. 2-1). The planktonic larvae of *C. japonica* do not have pallial eye, therefore, photo stimuli is probably not a factor for the upward migration. Salinity-related responses at larval stages have also reported in some marine bivalves [29, 33].

These distribution changes between the early stage and the later stage planktonic larvae of *C. japonica* were quite different from previous reports in marine bivalves. The early stage planktonic larvae of some marine bivalves swim upward as photopositive and/or geonegative and then the later stage larvae swim downward as photonegative and/or geopositive response [29, 33]. If I attributed the distribution changes in the planktonic larvae of *C.japonica* to the change of the salinity preference, the differences between *C.japonica* and the marine bivalves can be explained. Few planktonic larvae were observed at the depth of 6.8 m (Fig. 2-3). The anoxic conditions might become crucial at the depth.

The shell lengths just after spawning were 120-145 μ m (Fig. 2-6). On the other hand, few planktonic larvae with shell lengths less than 140 μ m was found at the central part of the lake (Fig. 2-4). I thought it took more than 3 days to be diffused from *C. japonica* habitat to the central part because the planktonic larvae reached shell lengths from 140 to 160 μ m at three days after spawning in the planktonic larvae incubation experiment (Fig. 2-6).

In the planktonic larvae incubation experiment, 78 % of the planktonic larvae settled around 3.6 days after spawning and 28 % of them settled around 6.3 days after spawning in the sand bottom group (Fig. 2-5). On the other hand, 52 % of the planktonic larvae settled around 4.4 after spawning and 48 % of them settled around 8.8 days after spawning in the seawater bottom group (Fig. 2-5). I could not find the factor, among experiment conditions, which caused the two phases of the settlements.

There are three species of *Corbicula*; *C. japonica*, *C. sandai* and *C. leana*, in Japan. *C. japonica* is a brackish-water oviparous dioecious species inhabiting in brackish water lakes and tidal flats of rivers from Hokkaido to Kyushu. *C. sandai* is a fresh-water oviparous dioecious species endemic to Lake Biwa and its water system. *C. leana* is a fresh-water viviparous hermaphroditic species inhabiting rivers and ponds from Honshu to Kyushu. It was reported that *C. japonica* diverged from the ancestral species first and *C. sandai* and *C. leana* subsequently differentiated from each other [3, 4, 5]. *C. japonica* hatches out at blastula stage [13] and needs substantial salinity for the development to be the D-shaped veliger (Fig. 2-1). On the other hand, other *Corbicula* species have different systems of larval developments. *C. sandai* hatches out at stage of D-shaped larvae [34]. *C. leana* hatches out and is incubated until pediveliger in the inner demibranchs [35, 36, 37]. *C. sandai* and *C. leana* probably acquired their reproductive system to adapt fresh water environment, i. e. low salinity, during the phylogenic processes.

The anoxic polyhaline lower stratum has been dealt as an abominable existence in brackish water lakes. Because sometimes upwelling of the anoxic polyhaline water are caused by strong winds, and the temporal anoxic conditions caused by the upwelling kill fishes and shrimps inhabiting in the well-oxygenated oligohaline upper stratum [17, 18]. On the contrary, I thought the polyhaline lower stratum has two important roles for the recruitment of *C. japonica*. First, it forms a nursery zone, i. e. a higher salinity zone, for the early stage planktonic larvae near the boundary between the oligohaline upper stratum and the polyhaline lower stratum. Second, the polyhaline stratum is one of the main sources for providing salinity to upper stratum via the upwelling caused by strong winds [18]. The substantial salinity is needed for the spawning and the larval development in *C. japonica.*

CHAPTER Ⅲ

Favorable sediment environments for juveniles of the brackish water bivalve *Corbicula japonica* in Lake Abashiri

Abstract

To determine the favorable sediment environments for juveniles of the brackish-water bivalve *Corbicula japonica*, the relationship between the presence of juveniles and limiting factors of the sediment environment that are negatively correlated with juvenile density was investigated in Lake Abashiri, one of the northernmost areas of this bivalve's distribution in Japan. Correlations between

juvenile density and sediment factors were estimated by Spearman's rank correlation coefficient. Logit models were made between the presence or absence of juveniles, as the dependent variable, and limiting sediment factors, as independent variables. The models were compared by the Akaike Information Criterion (AIC). The values at probability 50 % of the logit models (V50s) and the standard errors of the univariate logit models were calculated for each sediment factor. I defined favorable sites as those where any of these values are less than the V50s, which seem to provide good criteria to distinguish between favorable and unfavorable sediment conditions for juveniles. The V50s for limiting factors were: organic carbon 1.10 %, organic nitrogen 0.15 %, organic carbon/nitrogen ratio 7.43, ignition loss 4.45 %, water content 40.97 %, and silt-clay (particle diameter < 0.063 mm) plus very fine sand (0.063 - 0.125 mm) 36.88 %. A significant logit model could not be made for total sulfide. These criteria may provide important information for the management of *C. japonica* resources.

Introduction

The brackish-water bivalve *Corbicula japonica* is distributed in brackish lakes and tidal flats of rivers in Japan and Sakhalin [16]. Lake Abashiri is one of the northernmost areas of its distribution in Japan. *Corbicula* spp. are commercially harvested in Japan. The annual catches ranged from 24,000 to 37,000 tons during 1990-1994 [9], among which *C. japonica* was the dominant species.

The influence of environmental factors on the physiology of *C. japonica* has been studied, including temperature tolerance [38], salinity tolerance in adults [27, 38], salinity tolerance in juveniles [39], sulfide tolerance [40], tolerance to anoxic conditions [41], metabolic changes due to lack of oxygen [42], salinity effect on larval development [13], and free amino acid uptake [43]. However most of these were based on laboratory experiments.

In the fisheries management of brackish-water clams, it would be convenient to be able to assess whether the environmental conditions of a given site are suitable for the clams. In my preliminary analysis, I could not gain sufficient results with commonly used, and ordinary statistical methods such as multiple-regression analysis, principal component analysis, and their combinations, because they showed only non-significant differences and

sometimes did not fulfill the assumptions inherent in a regression analysis, e. g. unbiasedness or homoscedasticity. Transformations were not sufficient to ensure such preconditions were met.

Yamamuro et al. [31] reported the lethal factors of *C. japonica* in Lake Shinji, western Japan, based on field data. They suggested that the function expressing the limiting effect of each environmental factor ought to be S-shaped, take a positive value between 0 and 1, and be characterized by three ranges: lethal, limiting, and non-limiting. However, in fisheries management I would rather know simply whether the sediment environment is favorable or unfavorable for the clam, because fishermen could then avoid the areas with lethal environments. Therefore, I adopted logit models in the present study; they are similar to the function proposed by Yamamuro *et al.* [31], but can be treated more easily.

In this study, I employed logit models to analyze the relationships between juvenile presence and sediment variables and to clarify which sediment conditions are favorable for *C. japonica.* Understanding the favorable conditions is very important for the management of fisheries grounds and it also facilitates judgements about which sites are appropriate for the release of seed clams. My report is probably the first one demonstrating criteria for judging whether sediment conditions are favorable for juvenile *C. japonica.*

Materials and Methods

The field investigation was carried out on October 13, 1995, in Lake Abashiri, Japan. The lake is connected to the Sea of Okhotsk by the 7.2 km long Abashiri River. Since sea water flows back into the lake depending on the tide, the lake has a well-oxygenated, oligohaline upper layer and an anoxic, polyhaline lower layer, with a boundary at a depth of around 5 m [18]; *C. japonica* is restricted to the area above this boundary.

Sediment samples were collected from 32 sites laid out along seven lines (L1 to L7) at depths of 1, 2, 3, 4, and 5 m, except L5 which was sampled only at depths of 1, 2, and 3 m (Fig. 3-1). No sample was collected at a depth of 1 m along L 3 because the sediment was rocky.

A Smith-McIntyre grab was used to collect 0.05 m³ sediment samples twice at each site. The sediment in the first grab was washed on a 1 mm sieve, and *C. japonica* was sorted from the residue. The sediment in the second

grab was weighed, and about 1/10 of it was washed on a series of four sieves from 0.125 to 1 mm to sort the juveniles. Sorting was completed with a binocular microscope.
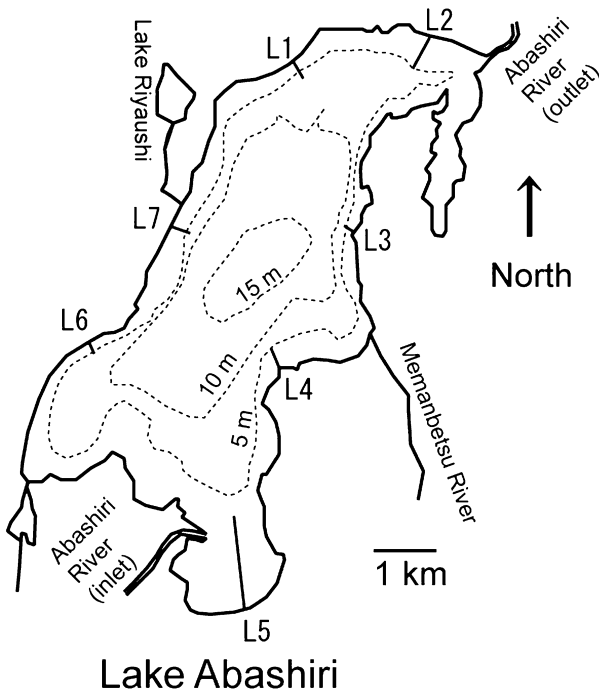

Fig. 3-1 Locations of sampling sites in Lake Abashiri.

Here I define juveniles as individuals that passed through the 1 mm sieve. Juvenile density was calculated according to the ratio of sorted sediment weight to total weight of the sediment sample obtained in the second grab. The minimum shell length of the juveniles was 0.48 mm. The juveniles were at least at age 1$^+$ because *C. japonica* did not spawn that summer due to the low salinity and low temperature of Lake Abashiri [19]. The spawning season is normally from July to September in Lake Abashiri [14]. The size of 1$^+$ individuals was much smaller than that previously reported by [15]. Some problems remain concerning the initial growth of *C. japonica* in Lake Abashiri (not discussed in this paper).

The other 90 % of the sediment from the second grab was used for laboratory analysis of the sediment factors described below. Sediment particle size composition was analyzed by a series of sieves: 2.00, 1.00, 0.50, 0.25, 0.125, and 0.063 mm mesh. Organic carbon and organic nitrogen were measured with a C-N analyzer

(Yanagimoto, C-N corder, MT-600) after treatment with 1N HCl for 24h to remove the $CaCO_3$. Water content and ignition loss were calculated by weight loss at 80 $^\circ$C for 24h from wet sediment and at 600°C for 1h from dry sediment, respectively. Total sulfide was measured with a detector tube (Gastec No 102L and No 102H, Kitazawa Sangyo Inc.).

Spearman's rank correlation coefficient was used to estimate correlations between the densities of *C. japonica* and sediment factors, and among sediment factors. Pearson's correlation coefficient can describe only the linear component of the relations [21], however, no such relation was observed in some of my data sets. With Kendall's coefficient of rank correlation, it is difficult to calculate probability if the data contain tied ranks [21], and since my data sets contained some tied ranks, I used Spearman's rank correlation coefficient.

Empirical logit models were made by the maximum-likelihood method using juvenile presence or absence as the dependent variable and each sediment factor and depth or their combinations as independent variables. Sites where juveniles were present were assigned a value of 1 as a dependent variable and sites where they were absent were assigned a value of 0.

The depth is further raised to the r-th power (r: real number; see results for the reason). I treated the r-th power as a parameter a_3 (not as a transformation). The parameter a_3 was also optimized by the maximum-likelihood method.

The proportion of silt-clay (particle diameter less than 0.063 mm) plus very fine sand (0.063 - 0.125 mm) fraction in the sediments was used as an independent variable, because a significantly better model, in terms of AIC, was obtained by this variable than either silt-clay or very fine sand alone in my preliminary analysis.

An empirical logit model is useful for analyzing binary data when the sample size is small [21]. The equations of the logit models used here are as follows:

$$f_1(x_1)=1/\{[1+\exp[-(a_0+a_1x_1)]]\},$$
$$f_2(x_1, x_2)=1/\{1+\exp[-(a_0+a_1x_1+a_2x_2)]\} \text{ and}$$
$$f_2(x_1, x_2)=1/\{1+\exp[-(a_0+a_1x_1+a_2x_2{}^{a3})]\}$$

where x_i ($i =1, 2$) are independent variables (x_1: each sediment variable, x_2: depth), and a_i ($i = 0, 1, 2,$ and 3) are regression coefficients.

The Akaike Information Criterion (AIC) was

calculated to compare the models. This criterion is useful to compare models that use the same dependent variables. In the AIC, one or more difference is regarded as a statistically significant difference [24].

The values at probability 50 % (V50s) and their standard errors in the univariate logit models were calculated for each sediment factor. In the case of the dose-response relationship of a poison, this value indicates the dose at which half of the examined animals probably die (lethal dose 50). In fact, the value can also be used for any condition which separates the results, i. e., death or survival [44]. In my study, I thought this value could be used as an indicator segregating sediment conditions into favorable or unfavorable ones. An advantage of this model is that it does not depend on the density. The density is not determined by the sediment conditions only, because larval supply, which varies depending on both biotic and abiotic environment factors, is also important [45-49].

Results
Corbicula japonica densities and sediment factors

As shown in Table 3-1, the average density of juveniles was much higher than the density of non-juveniles; however, there were more sites where juveniles were absent than sites where non-juveniles were absent. The absent sites were located along only three lines (L2, L4, and L5).

As shown in Table 3-2, seven limiting sediment factors were significantly negatively correlated with juvenile density. Juvenile density and non-juvenile density had similar correlations with the sediment factors; however, there were some exceptions. Juvenile density was significantly negatively correlated with total sulfide but non-juvenile density was not. Depth was not significantly correlated with either juvenile density or non-juvenile density. Juvenile density was not significantly correlated with non-juvenile density. All of the sediment factors were correlated with each other.

Logit models

The values at probability 50 % (V50s) and their standard errors are shown in Table 3-3. The significant logit models of limiting factors, the V50s, and the densities of juveniles are shown in Fig. 3-2. These figures show that sites with juveniles present predominated on the left side of the V50s. A significant logit model could not

be made with total sulfide. The V50s for the limiting factors were: organic carbon 1.10 %, organic nitrogen 0.15 %, organic carbon/nitrogen ratio 7.43, ignition loss 4.45 %, water content 40.97 %, and silt-clay plus very fine sand 36.88 %.

In a bivariate logit model, all combinations of sediment factors were tested as independent variables, but none of them was significantly better than the univariate logit models in terms of AIC except the combination with depth.

The logit models were significantly improved, in terms of AIC, when depth was added as the independent variable. But upon seeing the plots of Fig. 3-3, I thought a curved surface would be more appropriate than a plane surface for the bivariate logit model. In the logit model analysis, an interaction term (e. g., silt-clay × depth) is usually added to the independent variables to describe the curved surface. However, in my preliminary analysis, the bivariate logit models were not improved in terms of AIC by adding such an interaction term. Therefore, I instead raised the depth to the a_3-th power, which significantly improved the logit model in terms of AIC. The juvenile density scores and V50 isopleths of four of these bivariate logit models are shown in Fig. 3-3.

Discussion

I could not construct a significant logit model for total sulfide. In Lake Abashiri the sulfide probably has two origins, one related to sediment particle size and organic matter, and the other to water of the anoxic polyhaline lower layer, in which a lot of sulfide is accumulated. The latter is caused by temporary climate conditions, i. e., a strong wind in the *C. japonica* habitat (shallower than 5 m) in Lake Abashiri. This kind of temporary exposure to sulfide should not affect the survival of *C. japonica* because both juveniles and adults of *C. japonica* have a high tolerance for hydrogen sulfide. Half of the examined *C. japonica* survived for 23 days under a condition of 50 mg / L hydrogen sulfide at 18℃ [40]. Therefore, the total sulfide may not be an important factor in determining the most favorable sediment conditions for *C. japonica* in Lake Abashiri.

In this paper, I treated the density data as binary, i. e., present or absent. One may question whether the sites of purported absence are really so. To completely prove absence is very difficult because there remains the

Table 3-1 *Corbicula japonica* densities and sediment factors in Lake Abashiri.

No of line	Depth (m)	Juvenile (n/0.05m²)	Non-juvenile (n/0.05m²)	C (%)	N (%)	C/N	IL (%)	WC (%)	TS (%)	Silt-clay Silt(%)	VFS (%)
1	1	70	123	0.22	0.04	6.31	1.31	20.87	0.53	1.64	2.14
	2	842	70	0.06	0.01	4.50	1.36	23.73	0.00	1.32	1.02
	3	2065	57	0.05	0.01	4.90	1.22	22.53	0.00	0.78	0.54
	4	846	54	0.47	0.08	5.74	4.48	38.88	0.15	2.98	1.72
	5	1312	116	0.79	0.16	4.97	3.94	34.68	1.28	24.04	8.02
2	1	0	36	0.11	0.02	5.28	1.96	34.10	0.06	2.07	12.05
	2	0	45	1.55	0.19	8.15	4.64	50.14	0.30	29.87	17.69
	3	0	24	1.79	0.22	8.16	5.59	49.63	0.36	28.78	33.59
	4	0	11	2.76	0.31	8.89	8.00	60.80	0.32	53.28	30.41
	5	0	1	2.74	0.30	9.05	8.38	63.86	1.08	61.64	19.49
3	2	260	127	0.08	0.01	5.98	0.91	22.13	0.00	1.96	1.88
	3	395	107	0.17	0.03	5.69	1.47	25.95	0.03	2.50	0.99
	4	954	63	0.31	0.06	5.31	3.27	28.38	0.14	11.36	3.67
	5	2105	88	0.88	0.18	4.75	3.68	41.94	0.00	9.25	8.02
4	1	0	335	0.08	0.02	5.09	1.04	22.58	0.02	1.69	0.77
	2	0	158	0.12	0.02	5.69	1.17	23.97	0.02	2.95	0.83
	3	0	351	1.26	0.18	7.11	4.72	44.20	0.56	21.73	4.31
	4	0	73	1.16	0.15	7.82	4.25	40.81	2.40	24.88	14.13
	5	41	32	1.89	0.22	8.54	6.13	54.27	1.61	61.46	12.95
5	1	8	39	0.13	0.02	8.12	1.56	25.95	0.01	2.82	4.16
	2	0	0	2.52	0.23	10.99	9.66	62.64	0.07	96.71	2.38
	3	0	7	2.94	0.28	10.45	8.72	59.13	0.50	97.90	1.61
6	1	1267	110	0.16	0.02	6.56	1.04	21.42	0.03	1.85	3.52
	2	143	403	0.11	0.02	5.88	0.92	23.11	0.01	1.81	2.37
	3	2321	89	0.10	0.02	5.59	1.20	23.30	0.00	2.16	3.94
	4	195	87	0.09	0.02	5.61	0.94	25.71	0.00	2.38	4.20
	5		48	0.10	0.02	5.83	1.21	26.11	0.01	1.50	6.01
7	1	243	126	0.09	0.01	6.19	0.77	22.35	0.02	1.21	2.75
	2	80	54	0.32	0.05	6.57	2.18	26.64	0.01	2.70	4.87
	3	261	18	0.11	0.02	5.82	1.81	25.23	0.01	1.60	7.42
	4	2679	108	0.22	0.04	6.01	3.12	31.67	0.00	1.97	2.91
	5	906	798	0.10	0.02	4.90	1.60	14.25	0.00	0.81	0.58
Average		565	117	0.73	0.09	6.58	3.19	34.09	0.30	17.49	6.91
Standard deviation		785	158	0.94	0.10	1.68	2.61	14.30	0.55	27.39	8.26

C: Organic carbon, N: Organic nitrogen, C/N: Organic carbon/nitrogen ratio, IL: Ignition loss, WC: Water content, TS: Total sulfide
VFS: Very fine sand, Silt: φ≧4, Very fine sand: 4 ＞φ≧3

Table 3-2 Spearman's rank correlation coefficients between densities of *Corbicula japonica* and sediment factors and among sediment factors in Lake Abashiri.

	Juvenile *C. japonica*	Non-juvenile *C. japonica*	Depth	Organic carbon	Organic nitrogen	Organic carbon/ nitrogen ratio	Ignition loss	Water content	Total sulfide
Non-juvenile *C. japonica*	0.31								
Depth	0.31	-0.18							
Organic carbon	-0.45 **	-0.51 **	0.32						
Organic nitrogen	-0.39 *	-0.47 **	0.41 *	0.98 **					
Organic carbon/nitrogen ratio	-0.61 **	-0.50 **	-0.09	0.69 **	0.59 **				
Ignition loss	-0.38 *	-0.65 **	0.44 *	0.90 **	0.90 **	0.53 **			
Water content	-0.45 *	-0.69 **	0.43 *	0.85 **	0.84 **	0.55 **	0.90 **		
Toatal sulfide	-0.56 **	-0.29	0.11	0.76 **	0.73 **	0.57 **	0.62 **	0.60 **	
Silt-clay+very fine sand	-0.45 *	-0.68 **	0.33	0.84 **	0.81 **	0.66 **	0.80 **	0.88 **	0.62 **

*: Significant at P<0.05

**: Significant at P<0.01

Table 3-3 Empirical logit models and their equations. Sites where juveniles were present were assigned a value of 1 as a dependent variable and sites where they were not present were assigned a value of 0.

Independent variable (x_1, x_2)	Regression coefficient				Log-likelihood	AIC[*1]	P[*2]	Value at provability 50% (V0.5) and SE[*3] or equations for isopleth of provability 50%		
	a_0	a_1	a_2	a_3				V50	SE[*3]	
Organic carbon	2.10	-1.91			-13.5	29.1	0.00020 **	1.10	0.30	% in dry weight
Organic nitrogen	2.16	-14.70			-14.7	31.3	0.00060 **	0.15	0.04	% in dry weight
Organic carbon/nitrogen ratio	6.70	-0.90			-15.0	32.0	0.00080 **	7.43	0.57	
Ignition loss	2.67	-0.60			-14.7	31.4	0.00060 **	4.45	0.88	% in dry weight
Water content	4.91	-0.12			-13.6	29.1	0.00020 **	40.97	4.46	% in wet weight
Silt + very fine sand	1.99	-0.05			-14.1	30.2	0.00030 **	36.88	10.73	% in dry weight
Organic carbon, depth	-0.58	-3.74	1.440		-9.7	23.4	0.00002 **	$-0.581-3.738x_1+1.44x_2=0$		
Organic nitrogen, depth	-0.65	-35.36	1.730		-9.6	23.3	0.00002 **	$-0.652-35.361x_1+1.73x_2=0$		
Ignition loss, depth	0.52	-1.54	1.816		-9.7	23.3	0.00002 **	$0.521-1.544x_1+1.816x_2=0$		
Water content, depth	4.78	-0.26	1.717		-8.8	21.6	0.00001 **	$4.780-0.255x_1+1.717x_2=0$		
Silt + very fine sand, depth	-0.34	-0.09	1.184		-10.9	25.8	0.00006 **	$-0.341-0.093x_1+1.184x_2=0$		
Organic carbon, $(depth)^{a3}$	1.96	-7.42	0.012	4.42	-8.0	22.1	0.00001 **	$1.964-7.423x_1+0.012x_2^{4.42}=0$		
Organic nitrogen, $(depth)^{a3}$	2.24	-62.81	0.033	3.76	-8.1	22.1	0.00002 **	$2.237-62.81x_1+0.033x_2^{3.76}=0$		
Water content, $(depth)^{a3}$	13.33	-0.52	0.048	3.64	-7.0	19.9	0.00001 **	$13.331-0.517x_1+0.048x_2^{3.64}=0$		
Silt + very fine sand, $(depth)^{a3}$	2.91	-0.36	0.013	4.69	-7.2	22.4	0.00001 **	$2.911-0.358x_1+0.013x_2^{4.69}=0$		

[*1]: Akaike Information Criterion

[*2]: Calculated by likelihood ratio test in which the log-likelihood of only intercept was -20.592 and degree of freedom was number of regression coefficient minus 1.

[*3]: Standard error

**: Significant at P<0.01

possibility of finding some individuals if the sample size is expanded greatly. But I consider my judgments of absence to have been adequate, because there is at least assuredly a much lower density (undetectable) than at the present sites.

Tsutsumi & Sekiguchi [47] proposed three possible processes leading to the absence of benthic bivalves, i. e., avoidance of settling until the larvae encounter preferable sediment, high mortality at the unfavorable sites, and subsequent transportation after settlement. In my analysis, I could not elucidate which processes have led to the absence of *C. japonica* juveniles at some sites in Lake Abashiri. Juveniles of *C. fluminea* have been reported to prefer fine and coarse sand but not mud for settlement [7, 50]. Thus, it is likely that the first process, avoidance of settlement, also applies to *C. japonica* in Lake Abashiri.

As shown in Fig. 3-3, the V50s increased with depth. The reason for this is unknown, but there might be some survival-promoting or dispersion-restricting (i. e., retention-promoting) factors such as higher salinity in the deeper sites. *Corbicula japonica* requires a salinity of at least 3.1 psu (psu: practical salinity unit) for the development of its planktonic larva [13]. The favorable

salinity for juveniles (shell length about 0.2 mm) is more than 1.7 psu [39]. On the other hand, the salinity of the oligohaline upper layer in Lake Abashiri varies from 1 to 1.5 psu during June to November in 1995 [19]. Salinity does not vary within the oligohaline upper layer (variation was between 0.1 and 0.5 psu), but it increases to around 15 psu near its lower boundary [51]. On windy days the polyhaline lower layer rises to shallower depths than its usual upper boundary in Lake Abashiri [13]. The anoxic conditions and hydrogen sulfide, which temporarily coincidentally increase along with a rise in salinity, may not affect the survival of *C. japonica*, because *C. japonica* has a high tolerance for them [40-42].

Yamamuro et al. [31] found that high mud content is the most limiting factor for the distribution of *C. japonica* among several environmental factors: dissolved oxygen saturation, chlorinity, pH of bottom water, ignition loss and mud content of sediment. They found the lethal mud level was 91.5 %. In this study, the highest silt-clay content at sites where non-juveniles were found was 97.9 %, whereas the highest silt-clay content at sites where the juveniles were present was 61.5 %, and the V50 of
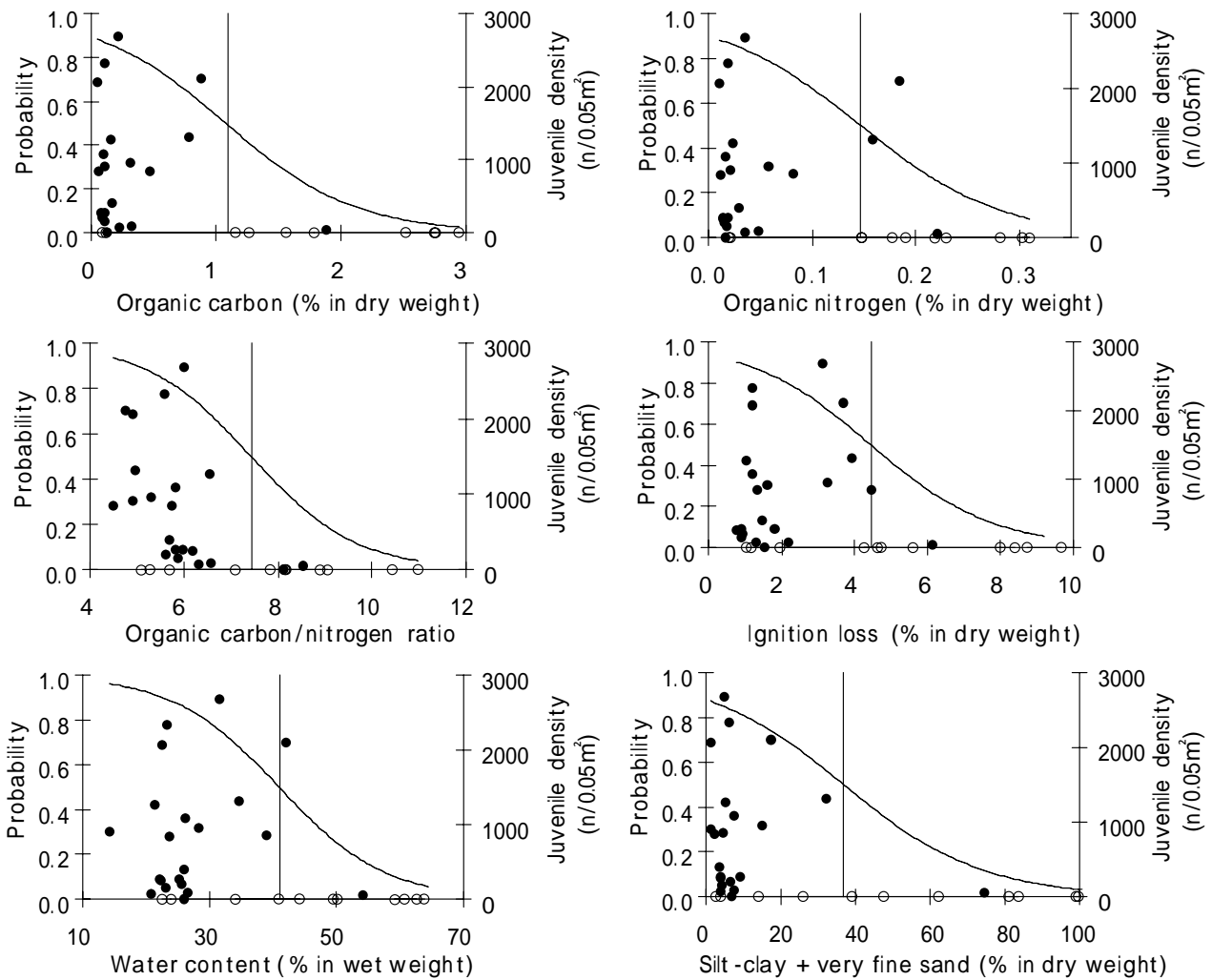
Fig. 3-2　Logit models made with limiting sediment factors for the presence of juveniles and densities of *Corbicula japonica* juveniles. Closed circles indicate sites where juveniles were present. Open circles indicate sites where juveniles were absent. The S-shaped curve represents the logit model. The vertical line indicates the V50 (the value at probability 50 %) of the logit model.

silt-clay plus very fine sand was 36.9 % (if silt-clay or very fine sand was separately used as the independent variable of the logit model, the V50 was 27.2 % and 10.7 % respectively). These findings suggest that large individuals have a higher tolerance for different sediment conditions.

As shown in Fig. 3-2, the V50s seem to be good criteria for judging whether a site is favorable for juveniles because the juvenile presence sites were predominately on the left side of the V50 among the limiting factors. Failure of *C. japonica* recruitment, maybe due to poor sediment conditions, has been reported in several places in Hokkaido, Japan (Rumoi Hokubu Fisheries Technology District Training Center, unpublished data; Nemuro Hokubu Fisheries Technology District Training Center, unpublished data); therefore, these criteria should be useful in plans to enhance *C. japonica* resources.

Here, I have clarified the favorable sediment conditions for juveniles of *C. japonica*. On the other hand, there remain some unresolved problems concerning the distribution of *C. japonica*, i. e., the relationship to the larval supply or mortality not related to sediments. I need further investigations to clarify these problems.
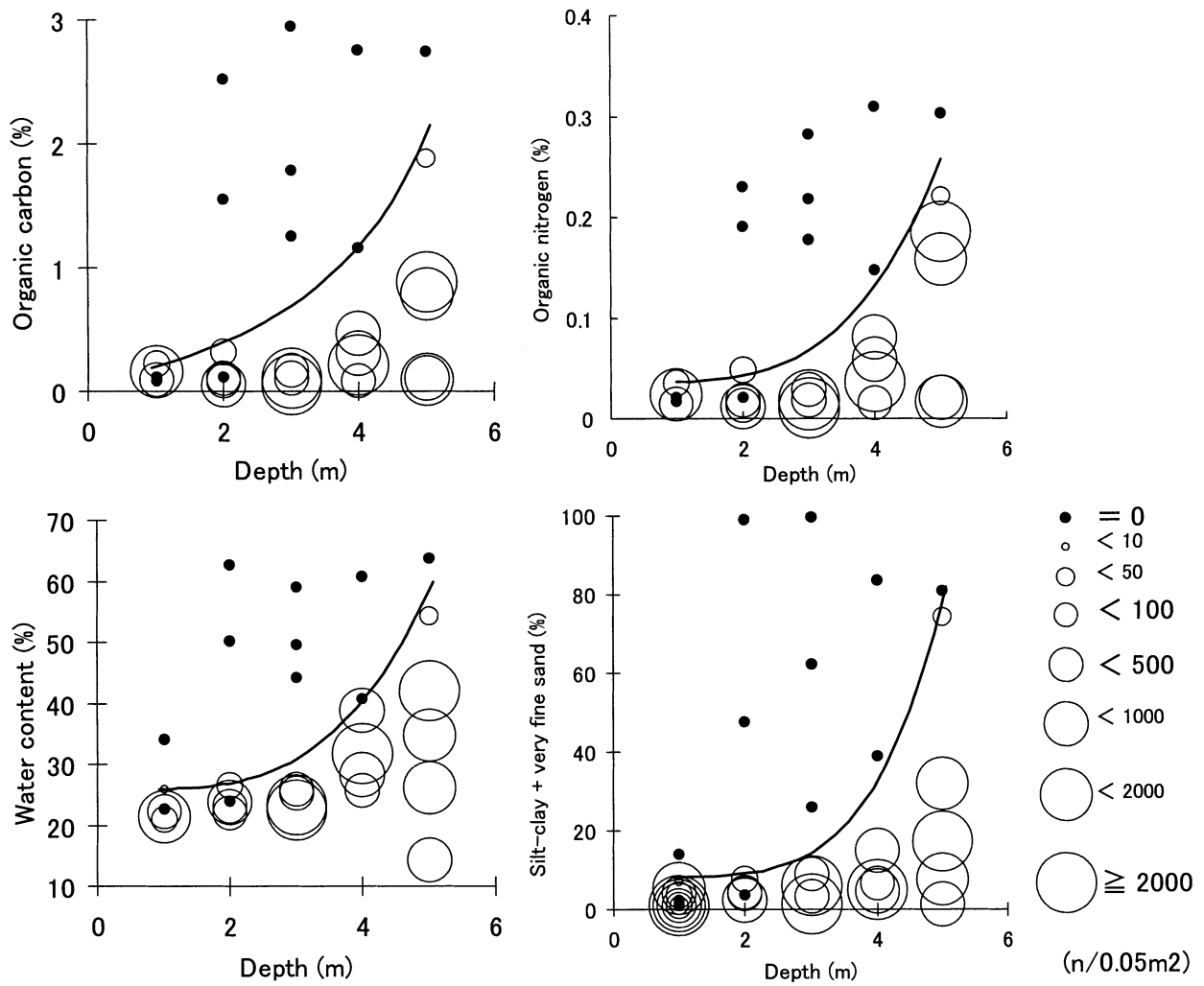
Fig. 3-3 Plots of *Corbicula japonica* juvenile density scores and an isopleth of V50 of the logit model made with depth and the other sediment factors. Open circles indicate sites where juveniles were present, and their size indicates the density of the juveniles. Closed circles indicate sites where juveniles were absent.

CHAPTER IV

Growth of *Corbicula japonica* juveniles in Lake Abashiri: application of an environmentally based growth model that uses finite difference calculus with maximum likelihood method

Abstract

I present a growth analysis model that combines large amounts of environmental data with limited amounts of biological data. The model uses the maximum-likelihood method with the Akaike Information Criterion (AIC), which provides an objective criterion for model selection. An adequate distribution for describing a single cohort is selected from available probability density functions, which are expressed by location and scale parameters. Daily relative increase rates of the location parameter (dRIRL) are expressed by a generalized multivariate logistic function using environmental factors for each day and categorical variables indicating animal ages as independent variables. Daily relative increase rates of the scale parameter are expressed by an equation describing the relationship with the dRIRL. *Corbicula japonica* grows to a modal shell length of 0.7 mm during the first year in Lake Abashiri. Compared with the attainable maximum size of about 30 mm, the growth of juveniles is extremely slow; this is due to less susceptible growth response to environmental factors until the second winter. The extremely slow growth in Lake Abashiri could be a geographical genetic variation within *C. japonica*.

Introduction

The extreme fluctuations, both short-term and seasonal, in food availability (e.g., phytoplankton density) make it difficult to derive relationships between the growth of filter-feeding bivalves and environmental factors [52]. On the other hand, it is becoming easier to acquire large amounts of environmental data through the use of data loggers, submersible fluorometers, or remote-sensing satellites. The development of these devices could solve difficulties in data collection, because the devices enable environmental monitoring at daily or shorter intervals. However, analytical methods of combining large amounts of environmental data with limited amounts of biological data (e.g., shell length) are not yet well developed. I present an environment-growth model that combines such unbalanced numbers of data sets. This model is useful in elucidating relationships between environmental factors and growth of filter feeders from field data.

Complex box models, ecophysiological models, can derive the relationships between environmental factors and the growth of filter-feeding bivalves [53-55]. These models are useful to estimate impacts of cultivated species on an ecosystem and/or carrying capacity [56-58]. However, they are suitable for animals that have been widely studied, such as *Mytilus edulis*, because they were derived by integrating a huge amount of ecophysiological knowledge acquired mainly from laboratory experiments. Furthermore, extrapolation of such knowledge to natural conditions is still controversial [52,59]. On the other hand, my model treats complicated ecophysiological processes as a black box; I constructed it directly from fluctuations in environmental factors and growth rates. My approach is reasonable for animals for which ecophysiological knowledge is limited, especially when the main purpose of investigation is to derive the relationships between environment and growth.

I applied the model to a single cohort of *Corbicula japonica* juveniles spawned in August 1997. I did not need to consider bias caused by adjacent cohorts because *C. japonica* failed to spawn in 1995, 1996, and 1998 in Lake Abashiri due to low water temperatures during the spawning season [30]. Such investigations provide important basic information, such as the shape of the distribution of a single cohort, and the relationship between growth rate and expansion rate of size variation in a single cohort.

Corbicula fluminea is an invasive pest and a fast-growing short-lived species in North America, growing to a shell length of 16-30 mm in the first year and living 1.5-3 years [7]. On the other hand, other *Corbicula* species are harvested commercially in Japan. The annual catch of *C. japonica* ranged from 770 - 800 metric tons during the years 1991 to 2000 in Lake Abashiri [9]. The species is distributed in brackish lakes and tidal flats of rivers from the south of Japan to the south of Sakhalin [16]. Lake Abashiri lies near the northern limit of its range. Its life span is quite long, at least ten years in Lake Abashiri [15]. I found extremely slow growth of juveniles compared with the maximum attainable size of about 30 mm, and interpreted this factor by my model.

Materials and Methods
Model formulation
Selection of adequate function to describe a single cohort

An adequate function to describe a single cohort of each animal should be selected to avoid biases caused by any inadequacies of the function. Probability density functions of many distributions are applicable for the purpose. Characteristics of many distributions are well described by Evans et al. [60]. I used three distributions: normal distribution, largest extreme value distribution, and smallest extreme value distribution. The normal distribution is symmetric. The largest extreme value distribution is asymmetric with a longer tail toward the larger side. The smallest extreme value distribution is asymmetric with a longer tail toward the smaller side. All are expressed by a location parameter and a scale parameter.

To use all the information inherent in data, parameters of the distribution functions are estimated from raw data (e.g., lengths), not from summarized data such as length frequency. This estimation method is described by Sakamoto et al. [24]. The most adequate distribution is selected by the Akaike Information Criterion (AIC) [23]. Log-likelihood functions of the distributions take the following forms:

normal distribution;

$$\log_e L_{normal}(a,b)$$

$$= \sum_{i=1}^{n} \log_e \left\{ \frac{1}{\sqrt{2\pi b^2}} \exp\left[-(l_i - a)^2 / 2b^2 \right] \right\} \quad (1),$$

largest extreme value distribution;

$$\log_e L_{l\,\mathrm{arg}\,est}(a,b)$$

$$= \sum_{i=1}^{n} \log_e \left\{ (1/b) \exp\left[-(l_i - a)/b \right] \right\}$$

$$\times \exp\left\{ -\exp\left[-(l_i - a)/b \right] \right\} \quad (2),$$

smallest extreme value distribution;

$$\log_e L_{l\,\mathrm{arg}\,est}(a,b) =$$

$$\sum_{i=1}^{n} \log_e \left\{ (1/b) \exp\left[(l_i - a/b) \right] \right\}$$

$$\times \exp\left\{ -\exp\left[-(l_i - a)/b \right] \right\} \quad (3),$$

where n = number of data;
l_i = length of ith individual;
a = location parameter; and
b = scale parameter.

The location parameter is a mean in the normal distribution. The location parameter is a mode in the largest and the smallest extreme distributions. The scale parameter is a standard deviation in the normal distribution.

The AIC is calculated by

AIC = -2 log$_e$ (*maximum likelihood*) + 2*m* (4),

where m = number of parameters to be estimated. The AIC is an information-theoretic criterion extended from Fisher's likelihood theory and is useful for simultaneous comparison of models [23, 61]. The model with the minimum AIC is the best model. A difference of more than 1 or 2 is regarded as significant in terms of AIC [24].

Introduction of finite difference calculus into growth model

Values of the location and scale parameters usually increase with the growth of an animal. The relative increase rate in a certain time step is defined as

$$r_i = (P_i - P_{i-1}) / P_{i-1} \quad (5),$$

where r_i = relative increase rate of a parameter in the ith time step; and P_i = parameter value after the ith time step.

Relationships between the parameter value and the relative increase rate of the parameter can be expressed by

$$P_1 = P_0(1 + r_1)$$
$$P_2 = P_1(1 + r_2) = P_0(1 + r_1)(1 + r_2)$$
$$P_3 = P_2(1 + r_3) = P_0(1 + r_1)(1 + r_2)(1 + r_3) \quad (6),$$
$$\vdots$$

$$P_n = P_0 \prod_{i=1}^{n} (1 + r_i)$$

where P_0 = parameter value at the first sampling;
P_i = parameter value after the ith time step; and
r_i = relative increase rate of the parameter in the ith time step.

I used one day as the time step in this study. In my environment-growth model, I assumed that the daily relative increase rate of location parameter (dRIRL) depends on the age of the animal and on environmental factors for each day. Sigmoid functions that take values

between 0 and a certain maximum are empirically appropriate for expressing the relationships between the dRIRL and independent variables, especially for measures such as shell length that do not show negative growth. Therefore, using categorical variables indicating animal ages and environmental factors for each day as independent variables, I express the dRIRL by the generalized multivariate logistic function,

$$s_i =$$

$$s_{\max} / \left\{ 1 + \exp\left[-\left(\sum_{j=1}^{n_A} \alpha_j A_j + \sum_{k=1}^{n_E} \beta_k E_{ki} \right) \right] \right\} \quad (7)$$

where

s_i = dRIRL on the ith day from the first sampling;

$s_{\max}$ = potential maximum dRIRL of the animal;

α_j, β_k = coefficients of each independent variable;

A_j = categorical variable (a dummy variable indicating animal ages) that takes the value 1 or 0;

E_{ki} = the kth environmental factor on the ith day from the first sampling;

n_A = number of age categories; and

n_E = number of environmental factors.

The categorical variable takes the value of 1 when the animal comprises the category, otherwise it takes 0. A method of giving a value to the categorical variable is described by Zar [62].

The daily relative increase rate of scale parameter (dRIRS) and dRIRL must be correlated because the dRIRS is larger when the dRIRL is larger. Therefore, I estimated the dRIRS from an equation expressing the relationship to the dRIRL. I tested two functions,

$$t_i = \begin{cases} \gamma_1 + \gamma_2 s_i & (\gamma_1 + \gamma_2 s_i > 0) \\ 0 & (\gamma_1 + \gamma_2 s_i \leq 0) \end{cases} \quad (8),$$

and

$$t_i = \begin{cases} (s_i - \gamma_1)^{\gamma_2} & (s_i - \gamma_1 > 0) \\ 0 & (s_i - \gamma_1 \leq 0) \end{cases} \quad (9)$$

where t_i = dRIRS on the ith day from the first sampling;

γ_1, γ_2 = coefficients of the equations; and

s_i = dRIRL on the ith day from the first sampling.

The location and scale parameters at the first sampling (a_0 and b_0), the coefficients of equation (7) ($s_{\max}$, α_j and β_k), and the coefficients of equations (8) and (9) (γ_1 and γ_2) are estimated as values that maximize total

log-likelihood. The total log-likelihood is evaluated by the adequate probability density function selected in the first step. The log-likelihood functions take the following forms:

Normal distribution;

$$\log_e L_{normal}(a_0, b_0, s_{\max}, \alpha_j, \beta_k, \gamma_1, \gamma_2)$$

$$= \sum_{q=1}^{N} \sum_{i=1}^{n_q} \log_e \left\{ \frac{1}{\sqrt{2\pi \hat{b}_q^2}} \exp\left[-(l_{qi} - \hat{a}_q) / 2b_q^2 \right] \right\}$$

(10),

Largest extreme value distribution;

$$\log_e L_{l\arg est}(a_0, b_0, s_{\max}, \alpha_j, \beta_k, \gamma_1, \gamma_2)$$

$$= \sum_{q=1}^{N} \sum_{i=1}^{n_q} \log_e \left\{ (1 / \hat{b}_q) \exp\left[-(l_{qi} - \hat{a}_q) / \hat{b}_q \right] \right.$$

$$\times \exp\left\{ -\exp\left[-(l_{qi} - \hat{a}_q) / \hat{b}_q \right] \right\} \right\} \quad (11),$$

Smallest extreme value distribution；

$$\log_e L_{l\arg est}(a_0, b_0, s_{\max}, \alpha_j, \beta_k, \gamma_1, \gamma_2)$$

$$= \sum_{q=1}^{N} \sum_{i=1}^{n_q} \log_e \left\{ (1 / \hat{b}_q) \exp\left[(l_{qi} - \hat{a}_q) / \hat{b}_q \right] \right.$$

$$\times \exp\left\{ -\exp\left[(l_{qi} - \hat{a}_q) / \hat{b}_q \right] \right\} \right\} \quad (12),$$

where a_0, b_0 = values of the location and scale parameters respectively at the first sampling,

$s_{\max}$, α_j, β_k = coefficients of equation (7),

γ_1, γ_2 = coefficients of equations (8) and (9),

N = number of samplings,

n_q = number of data at the qth sampling,

$\hat{a}_q$ = location parameter at the qth sampling estimated by equation (6) ($r_i = s_i$),

$\hat{b}_q$ = scale parameter at the qth sampling estimated by equation (6) ($r_i = t_i$), and

l_{qi} = length of the ith individual at the qth sampling.

AIC is used to select significant environmental factors, the age categorization, and the equation to express the relationship between dRIRL and dRIRS, i.e., equation (8) or (9).

Estimation of confidence interval of coefficient

To evaluate uncertainties of coefficients' values and model selection, I estimate the 95% confidence intervals

of all coefficients―i.e., a_0, b_0, s_{max}, α_j, β_k, γ_1, and γ_2―based on profile likelihood. For example, the 95% confidence interval of $a_0 - a_{0,95}$-is estimated as an interval that suffices in the following equation:

$$2\left\{ \max \log_e L\left(\hat{a_0}, \hat{b_0}, \hat{\alpha}_j, \hat{\beta}_k, \hat{\gamma}_1, \hat{\gamma}_2\right) - \right.$$
$$\left. \max \log_e L\left(\hat{a_0}, \hat{b_0}, \hat{\alpha}_j, \hat{\beta}_k, \hat{\gamma}_1, \hat{\gamma}_2 \middle| a_0 = a_{0,95}\right)\right\}$$
$$\leq \chi_1^2 (0.05) \quad (13)$$

where $x_1^2(0.05)$ = value of a chi-squared distribution at an upper probability of 0.05 with 1 degree of freedom, i.e., 3.84.

The characteristics of the interval are explained by Burnham and Anderson [61].

I used Microsoft Excel as the analysis platform, and Solver as the non-linear optimization tool.

Field study and model selection
Sampling of *C. japonica* juveniles

To collect juveniles of *C. japonica* spawned in August 1997, sediments were sampled with a 0.05 m² Smith-McIntyre grab once or twice a month during the period from September 1997 to July 1999 at a depth of 3.5 to 4.0 m in Lake Abashiri (Fig. 4-1). The habitat of *C. japonica* is restricted to areas shallower than 6 m depth because the deeper area is covered by anoxic polyhaline water, that is the lower stratum of the lake. The selectivity of the sampling gear on *C. japonica* juvenile is probably negligible because the gear grabs the juveniles with the sediment. Because the magnitude of spawning in 1997 was relatively small [30], I selected a sampling site where I found abundant settled juveniles in my preliminary investigations. Samples could not be obtained during winter because of ice cover. Sediments were washed with tap water on 2- mm and 0.125 mm mesh sieves during the period from September 1997 to October 1998, and on 4.75 mm and 0.125 mm mesh sieves during the period from April to July 1999. To separate the juveniles from the retained sediments, I treated the sediments with zinc chloride solution as described by Sellmer [63]. Then I sorted the juveniles under a binocular microscope. Identification of the cohort spawned in 1997 was quite easy, because *C. japonica* failed to spawn in 1995, 1996, and 1998 due to low water temperatures during the spawning season [30]. I regarded all the individuals which passed through the larger-mesh sieves and retained on the smaller-mesh sieve

as the cohort spawned in 1997. Shell lengths were measured under a profile projector (V-12, Nikon Ltd.) at ×50 magnification with a digital caliper (Digimatic caliper, Mitsutoyo Ltd.), which has a 0.02 mm precision.

Environmental factors

Values for water temperature (°C), water fluorescence (fluorescence equivalent to uranin density, μ g·L⁻¹), salinity (psu, practical salinity unit), and turbidity (equivalent to kaolin density, ppm) were obtained for 0.1 m intervals from unpublished data of the Abashiri Local Office of the Hokkaido Development Bureau. The variables were measured by a submersible fluorometer (Memory Chlorotec, ACL-1180-OK, Alec Electronics Ltd.) at four sites in Lake Abashiri at intervals of about one week (Fig. 4-1). The average values of each variable between the depths of 1 m and 6 m were used for later analyses. Values between the measured dates were interpolated linearly for subsequent analysis with the environment-growth model. The water fluorescence reflects the density of phytoplankton.

Model selection for application to *C. japonica*

I used three procedures of model selection to achieve the best model. First, I constructed an *a priori* set of candidate base models from the point of view of biological reasonability; and then I selected the best base model. Second, I excluded insignificant factors from the best base model. Third, I checked the significance of environmental factors that were not included in the base models. If one was significant, I included it in the best base model. All of these procedures were performed by AIC. The construction of the *a priori* set of candidate models is partially subjective, but it is an important part of the model construction [61].

Seasonal growth in bivalves is influenced by water temperature and food supply [64]. The growth rate of *Corbicula fluminea* changes with age [7]. Therefore, I constructed base models combining water temperature, water fluorescence, and categorical variables indicating age for the independent variables of equation (7). I tested two types of categorization of age. The first segregates ages based on real age, i.e. two categories: 0⁺ or 1⁺. The second segregates ages relative to winter, i.e. three categories: before the first winter, from the first to the second winter, and after the second winter. For the
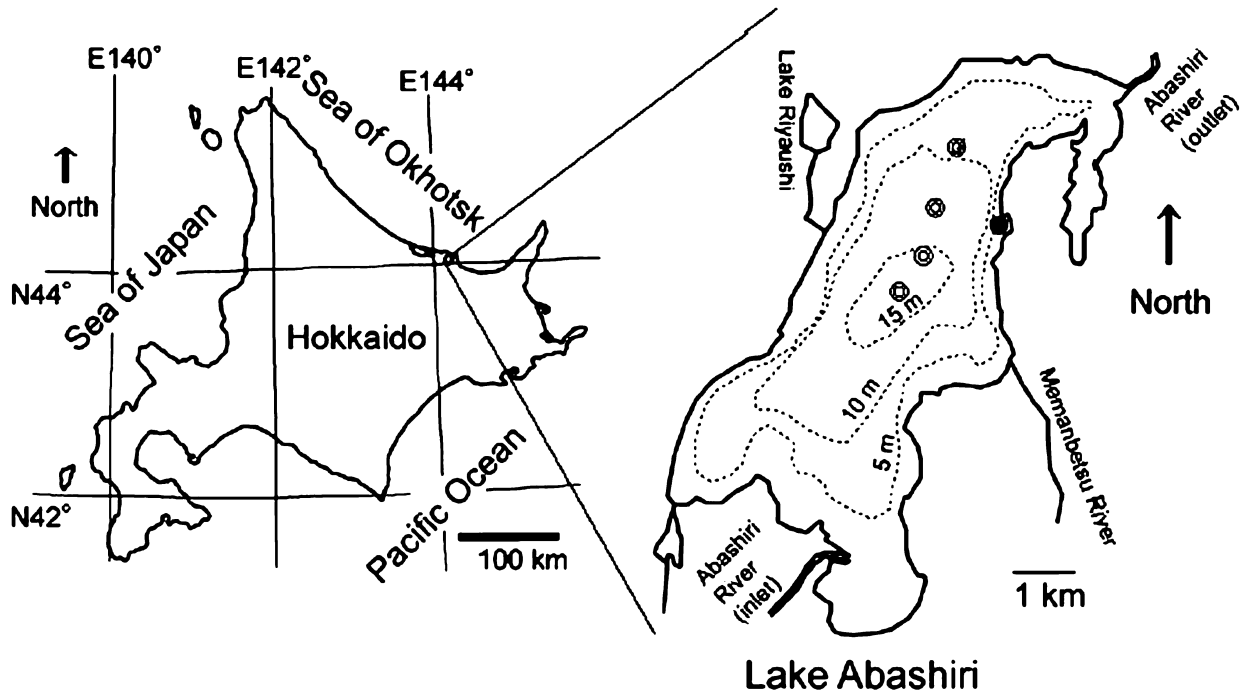
Fig. 4-1 Location of sampling site of *Corbicula japonica* juveniles in Lake Abashiri, Japan ●. Environmental factors-water temperature, water fluorescence, salinity, and turbidity-were measured at four sites, designated by ◎.

real-age categorization, age was segregated based on 1 September, because the spawning season was in August 1997. For the winter-base age categorization, I segregated ages based on 1 January. No biases should occur due to the segregation date of the winter-base categorization because the growth of *C. japonica* is negligible during winter. Four base models were constructed combining the two types of age categorization and two types of equations expressing the relationship between the dRIRL and the dRIRS, i.e. equation (8) or (9). I selected the best base model by AIC.

To check the significance of each environmental factor and age categorization, I removed the independent variables one by one from the best base model and re-optimized the model. When the model was significantly improved by the removal in terms of AIC, the effect of the variable was insignificant on the model, so I excluded it.

To check the significance of salinity and turbidity, which were not included in the base models, I included them one at a time into the best base model and re-optimized the model. When the model was improved by the inclusion, the effect of the variable was significant on the model, so I included it.

Results
Selection of adequate function to describe a single cohort

The largest extreme value distribution was the best in terms of AIC except for data sampled on 13 May 1998 (results are not shown). The exception is due probably to the small sample size (n = 38) on that date. The largest extreme value distribution was therefore used to evaluate likelihood in later analyses: I selected equation (11) from equations (10), (11), and (12). The result of fitting the three distributions to the shell lengths sampled on 22 April 1999 is shown in Fig. 4-2 as a representative example. The largest extreme value distribution is apparently the most suitable for describing the single cohort of *C. japonica* spawned in 1997.

Model selection and application

Model 4 was the best in terms of AIC among four base models (Table 4-1, Model 1 to 4); ages were categorized based on winter; and the relationship between dRIRL and dRIRS was expressed by equation (9).

Four models were made by removing each independent variable from model 4 (Table 4-1, Model 4-1 to 4-4). The effect of one age categorization－segregation of ages between the first and second winters－was

Table 4-1 Values of location and scale parameters at the first sampling, coefficients, log-likelihood and AIC of constructed models. The best AIC among four base models (Models 1 to 4) is enclosed by a single line. The best AIC of all models is enclosed by a double line. dRIRL = daily relative increase rate of location parameter, dRIRS = daily relative increase rate of scale parameter, Temp = water temperature, WF = water fluorescence, Sal = salinity, Turb = turbidity, C1 = before the 1st winter, C2 = from the 1st to the 2nd winter, C3 = after the 2nd winter.

Model no.	Parameters at 1st sampling a_0	b_0	Max. dRIRL s_{max}	Age categorization α_1	α_2	α_3	Environmental factors β_1	β_2	β_3	β_4	Expressing relationship between dRIRS and dRIRL γ_1	γ_2	Eq. No	Log-L	AIC
				0+	1+										
1	0.299	0.04	0.012	-62.6	-23.7		0.16	2.61			0	1.69	(8)	850.4	-1683
2	0.297	0.04	0.011	-56.1	-22.1		0.2	2.44			0.0001	0.89	(9)	852.3	-1687
				C1	C2	C3									
3	0.299	0.042	0.011	-16.8	-16.7	-9.1	0.61	0.41			-0.0076	2.90	(8)	950.4	-1881
4	0.299	0.042	0.011	-17.5	-17.6	-9.6	0.65	0.42			0.0034	0.76	(9)	952.2	-1884
4-1	0.299	0.042	0.011	-18.3[1]		-10	0.68	0.44			0.0034	0.76	(9)	952.2	-1886
4-2	0.297	0.038	0.005	-127.9	-26.8[1]		0.34	4.15			0	0.90	(9)	735	-1452
4-3	0.295	0.037	0.008	-47.3	-16.3	-8.8		1.47			0.0033	0.77	(9)	848.9	-1680
4-4	0.299	0.041	0.013	-4.9	-8.9	-4.9	0.4				0.002	0.81	(9)	909.6	-1801
4-5	0.299	0.042	0.011	-16.7[1]		-9.1	0.62	0.42	-0.25		0.0033	0.76	(9)	952.4	-1885
4-6	0.299	0.042	0.011	-18.5[1]		-10.2	0.68	0.44		0.007	0.0034	0.76	(9)	952.2	-1884

(1): One common coefficient for the two categorical variables.

Table 4-2 95% confidence limits of location and scale parameters at the first sampling and coefficients of the best model (Model 4-1 in Table 1) estimated by profile likelihood method. dRIRL = daily relative increase rate of location parameter, dRIRS = daily relative increase rate of scale parameter, Temp = water temperature, WF = water fluorescence, Sal = salinity, Turb = turbidity.

	Parameters at 1st sampling a_0	b_0	Max. dRIRL s_{max}	Age categorization α_1	α_2	α_3	Environmental factors Temp β_1	WF β_2	Sal β_3	Turb β_4	Expressing relationship between dRIRS and γ_1	γ_2
Lower 95%	0.294	0.039	0.01	-26.6[1]		-14.6	0.41	0.27			0.0027	0.734
Upper 95%	0.304	0.045	0.013	-11.5[1]		-6.4	1.00	0.64			0.0039	0.793

(1): One common coefficient for the two categorical variables.

insignificant on the model, because the model was significantly improved by its removal in terms of AIC. The effects of the other independent variables were significant on the model, because the model was significantly worsened by their removal in terms of AIC. The effects of salinity and turbidity were insignificant on the model, because adding each variable significantly worsened the model in terms of AIC (Table 4-1, Models 4-5 and 4-6). Consequently, model 4-1 was the best model to describe the relationships among environmental factors, ages, and growth of *C. japonica* juveniles spawned in 1997.

The coefficient value for age categorization of before the 2nd winter (-18.3) is much smaller than that of after the 2nd winter (-10.0) (Table 4-1). This difference suggests that the growth response of *C. japonica* juveniles is much less susceptible to environmental factors before the second winter than after.

Peaks of the dRIRL corresponded with peaks of water fluorescence, when the water temperature was warmer than about 10 ℃, especially before the second winter (Fig. 4-3B, C). Therefore, food supply is the most influential factor when the water temperature is above about 10 ℃. The slow or no growth during winter is due

to the low water temperatures. The dRIRL reached a plateau after 30 May 1999. This is due to two factors: Water fluorescence was relatively large after 30 May 1999 (Fig. 4-3B); and the growth response of *C. japonica* to the environmental factors was more susceptible after the second winter than before.

The largest extreme value distributions estimated by model 4-1 fitted the shell lengths of *C. japonica* juveniles very well (Fig. 4-4).
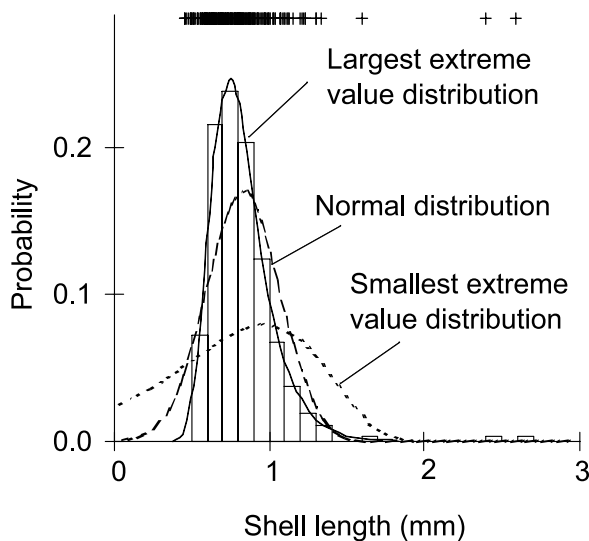

Fig. 4-2 Three distributions fitted by the maximum-likelihood method to the shell lengths of *Corbicula japonica* juveniles spawned in 1997 and sampled on 22 April 1999. Raw data are shown by +. The shell length composition is shown by histogram.

Discussion
Model formulation and application

Largest extreme value distribution is apparently better than the other distributions to describe the single cohort of *C. japonica* spawned in 1997. This distribution has a mode and a longer tail toward the larger side. I thought that the shell length distribution of the cohort was already asymmetric just after settlement. Such a distribution might be influenced by fluctuations in larval settlement during the spawning season; and larval settlement would be influenced by fluctuations in larval supply from the water column. During the spawning season of 1997, the average planktonic larval density gradually increased from 26 ind·m^{-3} on 25 July to a

maximum of 603 ind·m^{-3} on 13 August. Then it sharply decreased to 3 ind·m^{-3} on 19 August [30]. Such a pattern of larval density fluctuation might have caused the asymmetric distribution of shell lengths of the settled juveniles. An other possible factors that influenced the shapes of the shell length distributions and the relationship between dRIRL and dRIRS is size-dependent mortality, e.g. predations and fisheries. Size-dependent mortality has been reported in several marine bivalves [65]. Potential predators of *C. japonica* are fishes, such as Japanese dace *Leuciscus hakonensis*, far eastern dace *L. brandti*, carp *Cyprinus carpio*, and mullets *Liza haematocheila* (Kawasaki). In my study, the size-dependent mortality is negligible because the range of the shell lengths observed in this study is very narrow.

The shape of the distribution to describe a single cohort should be determined from the data. In contrast, single cohorts are usually separated from multi-cohort data by assuming a normal distribution of lengths in a single cohort [66]. Therefore, it is possible that multi-cohort analysis done without selection of an adequate distribution to describe a single cohort causes substantial bias in estimations of various stock features of animal populations, such as age composition, growth, mortality, and recruitment.

Relatively large confidence intervals were obtained in the coefficients of the linear component of equation (7), i.e., α_j, and β_k (Table 4-2). The relatively large confidence intervals may indicate that the number of estimated coefficients is somewhat larger than the number of samplings. Therefore, to estimate these coefficients more precisely, I may need to investigate more cohorts spawned in other years in future investigations.

Growth of *C. japonica*

I identified extremely slow growth of *C. japonica* juveniles, which grew to a modal shell length of 0.7 mm during the first year in Lake Abashiri, which lies at 43.7°N. Spats of *C. japonica* collected during 1992 to 1997 in Lake Shinji, which lies at 35.5°N, grew to a mean shell length of 6.7 mm in natural conditions by the first winter [67]. Using environmental factors measured in Lake Shinji during 1990 to 1998 at monthly intervals [68], I simulated the growth of *C. japonica* by model 4-1. It grew to a mean shell length of 1.4 mm (standard error, 0.37) by the first winter in the simulations. Therefore, the large difference in
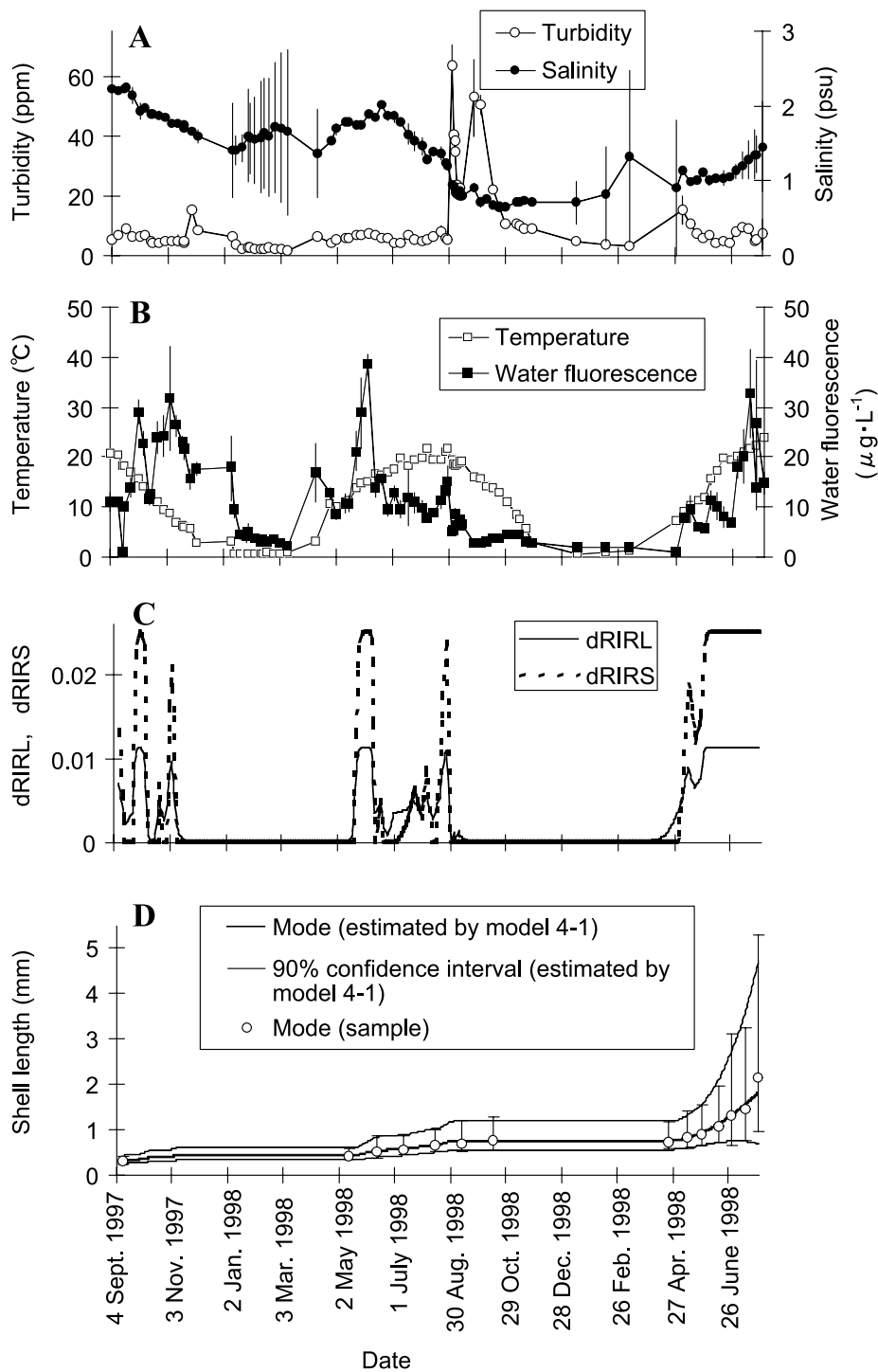
Fig. 4-3 Environmental fluctuations and prediction of the growth of *Corbicula japonica* juveniles spawned in 1997 in Lake Abashiri by the best model (Model 4-1 in Table 4-1). (A) Insignificant environmental factors (excluded factors by model selection), turbidity (equivalent to kaolin density, ppm) and salinity (psu, practical salinity unit). (B) Significant environmental factors (included factors by model selection), temperature (℃) and water fluorescence (equivalent to uranin density, μ g·L^{-1}). (C) Daily relative increase rate of location parameter (dRIRL) and daily relative increase rate of scale parameter (dRIRS) estimated by the model. (D) Growth of *Corbicula japonica*; vertical bars represent 90-% confidence interval of the shell length of the samples.
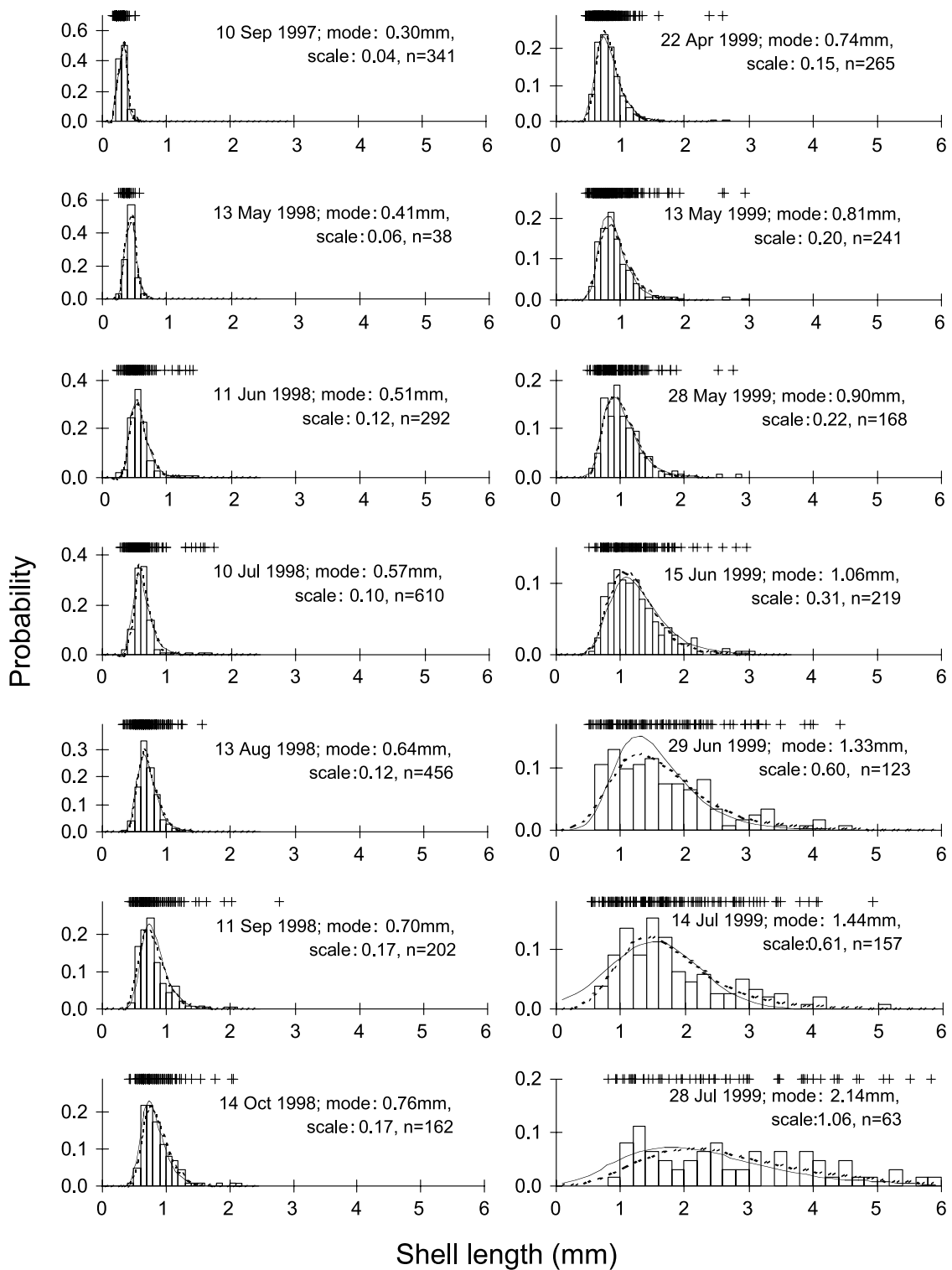
Fig. 4-4　Shell length compositions of a single cohort of *Corbicula japonica* spawned in 1997. The raw data (shell lengths) are shown by +. The largest extreme value distribution estimated by the best model (Model 4-1 in Table 4-1) is shown by a solid line. The largest extreme value distribution independently fitted by the maximum likelihood method is shown by a dashed line. The sampling date and values of location parameter (mode) and scale parameter independently fitted by the maximum likelihood method are shown in each panel

juvenile growth between the two habitats cannot be explained by environmental differences, because the results of the simulation were apparently an underestimate. I conclude that the extremely slow growth of the juveniles (prolonged phase of meiobenthic development) in Lake Abashiri is a geographical variation, which is probably genetically determined, within *C. japonica*. Prolonged phases of meiobenthic development have been reported in some marine bivalves [65, 69]. However, a prolonged phase of meiobenthic development as a geographical variation is rarely reported.

In many species of bivalve, populations from higher latitudes have a slower initial growth rate; but longevity and ultimate size in these populations are frequently greater than at lower latitudes [70, 71]. The extremely slow growth of *C. japonica* juveniles in Lake Abashiri may be an extreme example of this phenomenon.

The growth response of *C. japonica* juveniles is much less susceptible to environmental factors before the second winter than after. This unsusceptible growth response is the proximate factor for the extremely slow growth rate. *Nuculoma tenuis*, a detritus feeder, develops its palp proboscides, its feeding apparatus, during the prolonged phase of meiobenthic development [69]. The changes of growth susceptibility to environmental factors in young ages may suggest that some functional morphological changes occur in *C. japonica*, also a filter feeder. In my preliminary analyses, I could not find a better model when I used different values of s_{max} in equation (7) between ages instead of categorical variables indicating ages. Therefore, I conclude that the difference in growth rates between ages is not due to a difference in potential maximum growth rate.

Utoh[15] investigated the growth of *C. japonica* in Lake Abashiri using a resting zone formed on the shell surface that is regarded as an annual ring. He concluded that *C. japonica* grows to a mean shell length of 5.7 mm in the first year. This is apparently an overestimate, owing to difficulties in recognizing the resting zone on small individuals. I inferred the mean shell length of the 5.7 mm reported by Utoh[15] may be the mean shell length of a cohort aged 3 years because some shell lengths of aged 2 years in my research overlapped with the shell lengths of the first year reported by Utoh [15]. To understand overall growth of *C. japonica* in Lake Abashiri, I integrated my results of the juvenile growth and the report of Utoh[15]

and draw a growth curve in Figure 4-5. The growth curve of *C. japonica* in Lake Abashiri is S-shaped, and attainable maximum mean shell length is 31 mm. Maru[14] reported *C. japonica* matures at shell length of 15 mm in Lake Abashiri. Therefore, it is inferred that *C. japonica* matures at 5 years old in Lake Abashiri.

GENERAL DISCUSSION

Spawning

The spawning season of Corbicula japonica is summer in general [13, 14, 30, 72, 73]. Before the report of Baba et al. 1999 (Chapter I), neither the threshold temperature for the spawning nor the phenomena of the spawning failure in the natural conditions had been known. In Lake Abashiri, lies near the northern limit of this species range, *C. japonica* fails to spawn when the summer temperature is lower than the threshold temperature. The failure of the spawning is not exceptional phenomenon; it is estimated that the failure occurred in 10 out of past 21 years. The plans of resources managements are usually worked out based on the magnitudes of the new recruitments. Growth analyses without the information of the spawning conditions causes crucial biases because the failure of the spawning causes lacks of cohorts. Therefore, whether the spawning had occurred or not-occurred is important information for the resources managements and the ecological studies of *C. japonica*.

The spawning failure is histologically characterized by massive atretic oocytes in the female gonads after the spawning season [30] (Chapter I). The atretic oocyte is apparently different from the degenerating oocyte, which commonly observed after spawning season in many bivalves. The degenerating oocyte is characterized by extensive phagocytosis, whereas the atretic oocyte do not accompany with the extensive phagocytosis in *C. japonica*. In some marine bivalve, mainly in the scallops (Pectinidae), the atretic oocytes are also observed during the phases of oogenesis [26, 74-76]. The annual fluctuations of recruitments are quite large in these species [77, 78]. However, the relationships between the recruitment fluctuations and ovarian conditions had not been studied. I thought the ratio of the atretic oocyte would be a good criterion for the evaluation of the ovarian conditions; and it would be one of important physiological clues for the prediction of recruitment magnitudes.

Pre-settlement processes

The anoxic polyhaline lower stratum in brackish water lakes has been dealt as an abominable existence because the anoxic water sometimes upwelled by strong wind and the temporal anoxic conditions killed fishes and shrimps inhabiting the well-oxygenated oligohaline upper stratum. To vanish the anoxic stratum or make the boundary deeper, some developments have been planed in some brackish lakes. However, I revealed that the boundary zone is probably important for the early stage of the planktonic larvae of *C. japonica*, especially when the salinity of the upper stratum is not enough for the larval development (Chapter II). From viewpoint for the conservation of *C. japonica* resources, not only the salinity of the upper stratum but also the boundary depth should be considered when development of brackish water lakes is planed.

Sediment environments

Muddy sediment conditions are apparently one of the most restrictive factors for *C. japonica* habitat because few juveniles are observed on the muddy sediments (Chapter III). Such muddy sediments distribute near the mouths of the inlet and the outlet rivers around Lake Abashiri (Baba and Takahashi 199b). Therefore, the conservations of natural environment, such as forestry and reed fields, around the lake are also important to protect the habitats because the silt and clay are provided from the upper streams of the rivers. As a concrete example, juveniles of *C. japonica* have not been observed in Lake Panke (Horonobe, Hokkaido) since 1986 in spite of the abundant planktonic larvae were observed in some years (Sakai 2000). It is probably due to the muddy sediment conditions. The farm land constructions, which started from 1974, are suspected as the factor that make the sediment conditions muddy.

In Lake Abashiri, the muddy sediments occupy about 30 to 40 % of the shallower area than depth 5 m, where the sediment are not covered by the anoxic polyhaline lower stratum. In the southern habitat, the resources enhancements were succeeded by the sediment improvements. For example, the abundances of *C. japonica* changed from 400 ind. / m^2 to 1,580 ind. / m^2 in two years after the muddy sediments were covered by 30 cm-thick sand layer in Lake Jinzai, Shimane, Japan

(Nakamura 2000b). However, the effects of the sediment improvements on the resources enhancements are probably limited in the northern habitat because the spawning dose not occur in some years (Chapter I) and the juvenile growth is extremely slow (Chapter IV) in the northern habitat. Therefore, the conservation of the natural environments around the habitat is much more important in the northern habitat than the artificial resources enhancement efforts.

Juvenile growth

The juvenile growth of *C. japonica* in Lake Abashiri is extremely slow; it grows to the shell length of 0.7 mm at age 1 and 2.1 mm at age 2. The extreme slow growth is due to the less susceptible growth responses to environmental factors until the second winter (Chapter IV). The population growths of *C. japonica* are very rapid in the southern habitat. For example in Lake Hachiro, the fisheries' harvest of *C. japonica* devastated after land reclamation; and it decreased to less than 100 t in 1980. In August 1987, seawater flowed back into Lake Hachiro when a typhoon attacked there. After the typhoon, the population rapidly enhanced from less than 5 ind. / m^2 (before the typhoon) to 3,000 ind. / m^2 (shell lengths from 3 to 5 mm) in July 1988; and the fisheries harvest drastically increased to 10,900 t in 1990 and it quickly decreased to 281 t in 1994 and 58 t in 1995 (Satoh 2000). Therefore, the southern populations are characterized by strong reproductive ability, rapid individual growth in the early life stage. On the contrary, the northern populations are characterized by unstable reproduction (they do not spawn in the low temperature summer year) (Chapter I), extremely slow juvenile growth (Chapter II), and quite long life span. For example, the fisheries harvest had been kept in the range of 100 to 150 t during 1992 to 1996 in spite of that few juvenile have observed since 1986 in Lake Panke, Horonobe, Hokkaido. Only in recent years, the fisheries harvest decreased to 63 t in 1999 in Lake Panke; i. e. it took more than 10 years until the fisheries harvest decrease from the reproduction systems were destroyed. In the northern habitat, the fisheries harvest is stable due to the long life span; however, different from the southern populations, the rapid population growth can not be expected if the populations were once devastated. Therefore, environment conservations are much more important in the northern habitat than the southern ones.

There are two important points for the conservations. 1) The salinity of the upper stratum should be monitored whether the range is within the appropriate for the spawning. 2) The environments around lakes and inlet rivers, especially woods and reed field, should be conserved not to increase the influx of muddy sediment.

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