The short-neck clam, *Ruditapes philippinarum* (Adams & Reeve 1850), has as its natural habitat on the sandy shores from Hokkaido to Kyushu in Japan, and in the north Asian countries including Korea, North Korea, China, and Russia (Ponurovsky & Yakovlev 1992, Jie et al. 2001, Lee et al. 2001, Tsutsumi et al. 2002). This species immigrated with other imported bivalves such as oyster accidentally or was imported industrially to European and North American countries (e.g., Ireland, Italy, France, Canada and U.S.A., reviewed by Ponurovsky & Yakovlev 1992, Laruelle et al. 1994, Meneghetti et al. 2004, Drummond et al. 2006). This clam often occurs densely on sandy tidal flats (Oiba 1959, Magni et al. 2000, Bartoli et al. 2001, Hiwatari et al. 2002, Tsutsumi et al. 2002), and is important as a popular seafood resource. In 2007, approximately 36 thousand metric tons of the clams were harvested from the coastal areas of Japan (Ministry of Agriculture, Forestry and Fisheries, Japan 2007), and presumably a further larger amount of the clam was imported from the other Asian countries due to the marked decrease of the domestic harvest (Watanabe 2007).

We have recently studied the population dynamics of *R. philippinarum* in the coast of eastern Hokkaido, Japan, which is a subarctic region towards the northernmost extent of the geographical distribution of the clam. The winter climate would appear to be extremely hard on the survival of...
the clam since the shore is fully covered by ice in our study areas. However, according to the results of our recent study on the clam population on the tidal flats of Hichirippu Lagoon, the eastern part of Hokkaido, the clam population persisted, fluctuating stably around 8,000 ind. m$^{-2}$ in density and 10 kgWW m$^{-2}$ in biomass (Komorita et al. 2006) throughout the year, and 30 to 92 metric tons of the clam were harvested from the tidal flats of approximately 190 ha in area between 1998 and 2004 (based on the fishery statistics presented from Chirippu Fishery Cooperative Union). The adult clam, at least, does not appear to suffer from high mortality even during the cold winter in eastern Hokkaido.

In this study, we focus on the growth and survival processes in the post-settlement period of juvenile of the clam to understand how the clam is able to maintain its population on the tidal flats, enduring the severe winter in Hokkaido. In general, the juveniles of benthic animals suffer from extremely high mortality during the post-settlement period just after settlement on the substrate (Gosselin & Qian 1997). In the case of this species, it has a breeding season in autumn in the study areas (Yamamoto & Iwata 1997). Therefore, most of the physiologically weak juveniles in the post-settlement period tend to be exposed to the severe winter weather conditions on the tidal flats. It is seems likely that the juveniles in the post-settlement period would be subject to high mortality caused by the cold temperatures below freezing point, even if the adult clam could tolerate the cold conditions.

In this study, we conducted field surveys of the environmental conditions and quantitative samplings of R. philippinarum to monitor the population dynamics of the clam on the tidal flats of Hichirippu Lagoon in the eastern part of Hokkaido, Japan, between August 2006 and October 2007. We report the seasonal fluctuations of temperature at the surface of the sediment and the growth and survival processes of the clam that settled on the tidal flats in the autumn of 2006, and discuss how the cold winter in the eastern part of Hokkaido affects the recruitment of juveniles including growth and survival processes in the post-settlement period and the population persistence of the clam on the tidal flats.

**Materials and Methods**

**Study areas**

The study area, Hichirippu Lagoon, is located on the coast facing the Pacific Ocean in the eastern part of Hokkaido, Japan (44°03’41”N, 145°03’39”E, Fig. 1). It is approximately 3.56 km$^2$ in area and shallow (mean water depth: approximately 1 m). During low water in spring tide, approximately 190 ha of tidal flats appear, which is equivalent to approximately 5.3% of the whole area of Hichirippu Lagoon. The salinity of the water ranges between 30.0 and 33.5 psu throughout the year (unpublished data). Here, we established ten sampling stations (Stn 1 to Stn 10).

![Fig. 1 Study area and location of sampling stations (marked with circles). Temperature of the surface sediment was measured with a probe at Stn 7 indicated by an open circle.](image)

**Field surveys**

For monitoring the temperature of the surface sediment, we set a button type of probe (Thermochron G type, KN laboratories Inc.) in the sediment at a depth of approximately 5 cm at Stn 7. The probe measured the sediment temperature hourly, and stored the data in the memory. We exchanged the probe, and downloaded the temperature data stored in the memory of the probe to a personal computer bimonthly.

We conducted quantitative benthic samplings to monitor the population dynamics of the clam from August 2006 to October 2007 bimonthly. The density of the clam population was represented with the specimen collected at Stn 7, since the sediment and clam population was not seriously disturbed by the fishery activity to harvest the clam, but the density of the clam was relatively high. At this station, we collected five replicates of sediment samples using a stainless steel core sampler (10 cm$\times$10 cm$\times$10 cm), sieved them with a 1 mm opening mesh screen, and put the residues on the mesh screen in plastic bags. For determination of the density of juvenile settled on the sediment in the autumn of 2006, we collected five replicates of sediment sample using an acrylic core tube (3 cm in diameter) at the ten stations (Stn 1 to 10) to minimize to the extent possible the sampling error caused by patchy settlement of larvae on the tidal flats as much as possible. The surface layers of the sediment samples up to 2 cm in depth were sliced carefully, and stored in the plastic bags.

**Treatment of the samples**

In the laboratory, we fixed the residues after sieving the sediment samples for determination of the population density of the clam, and the sediment samples for determination of the density of juvenile settled in the autumn of 2006, using 10% formalin solution with a dye, Rose Bengal. At least a week later, we washed the fixed residues with water, and sorted out macro-benthic animals, which were visible to the naked eyes, from them. In case of the fixed sediment samples for determination of the density of juvenile, we...
sieved them with a 0.125 mm opening mesh screen, and sorted out only the juveniles of *Ruditapes philippinarum* under a stereoscopic microscope. We measured the shell length of the specimen of the clam using a digital calliper (Mitutoyo, Absolute coolant proof caliper) for large specimens or a microscope image analysing system for small specimens of less than 5 mm in shell length (cf. Tsutsumi et al. 2005) to draw the size frequency distribution of the population, to follow the growth and survival processes of the juvenile after settlement.

**Results**

**Sediment temperature**

Figure 2 indicates the seasonal fluctuations of the daily mean temperature of the surface sediment at Stn 7. It reached the highest daily mean temperature, 19.7°C±2.2°C (S.D.), on August 26, 2006. It decreased below 10°C on November 10, 5°C on December 2, 2006, and 0°C by the beginning of January, 2007, respectively, and reached the lowest daily mean temperature, −1.8°C±1.3°C (S.D.), on January 27, 2007. The surface sediment temperature fluctuated between −9.5°C and 8.0°C during the winter between December 2006 and March 2007. From April 2007, the surface sediment temperature increased, and reached the highest daily mean temperature in the summer of 2007, 18.9°C±2.7°C (S.D.), on August 30, 2007. Between April 22 and June 12, and August 27 and October 13, 2007, the temperature was not recorded normally due to trouble with the probe.

**Seasonal fluctuation of density and size frequency distribution of the clam population**

Figure 3 indicates seasonal fluctuations of the population density of the clam at Stn 7. The mean density of the clam decreased 6,460±4,020 (S.D.) ind. m⁻² on August 8, 2006, to 3,200±560 (S.D.) ind. m⁻² on June 13, 2007, repeating small fluctuations. However, the density increased to 4,480±800 (S.D.) ind. m⁻² on August 16, 2007, and 5,860±1,730 (S.D.) ind. m⁻² on October 16, 2007. According to the size frequency distribution of the clam population at Stn 7 (Fig. 4), a new juvenile cohort began to appear in the size frequency distribution of less than 10 mm in shell length from June 2007. Since the clam has a breeding season in the autumn in this study area (Yamamoto & Iwata 1956, Goshima et al. 1996), we deduced that the larvae of the cohort settled on the sediment in the autumn of 2006, the juvenile passed the winter in the shell size that was not retained in the sieve with 1.0 mm opening mesh (less than about 1.5 mm in shell length), grew to the size that was retained on the sieve from June 2007, and contributed to the increase of population density in August and October, 2007.

**Mortality rate and growth in and after the post-settlement period**

Figure 5(a) shows the seasonal fluctuations of mean density and shell length of the juvenile of the clam that settled on the sediment at the ten stations in the autumn of 2006. The settlement of juvenile started at least from August 8, and the density of the newly settled juvenile reached a peak, 64,500 ind. m⁻², on October 24, 2006. Since then, it decreased to 5,500 ind. m⁻² on April 20, 2007. An extremely low density, 200 ind. m⁻², was noted on June 13, 2007, but this appears to be an underestimate due to sampling error. The density of the new cohort rebounded to 1,350 ind. m⁻² on August 16 and 1,200 ind. m⁻² on October 16, 2007. Thus, the seasonal fluctuations of mean density of the juvenile indicate that the juvenile suffered from high mortality in the first approximately nine months (late October, 2006, to end of July or early August, 2007) from the peak of the settlement, and entered a stable fluctuation period in density with much lower mortality (August to October, 2007). Therefore, in this study, we defined “the post-settlement period” of the juvenile of the clam as the period.
that the juvenile suffered from high mortality in the first approximately nine months from the peak of the density.

We determined the date when the post-settlement period of the juvenile had ended in Fig. 5(a), calculating the point of intersection of two regression lines of the mean density of the juvenile (1) between October 24, 2006, and April 16, 2007, and (2) between August 16 and October 16, 2007.

(1) \( M_1 = 71,661e^{-0.0139 \times D_1} \), \( r^2 = 0.974 \), \( M_1 = \) mean density, \( D_1 = \) number of days from October 24, 2006

(2) \( M_2 = 2,753e^{-0.0023 \times D_1} \), \( M_2 = \) mean density

These two regression lines intersect on August 1, 2007, and the mean density of juveniles on that day was 1,443 ind. m\(^{-2}\). From the growth rate of the juvenile between June 13, 2007, and August 16, 2007, we determined the mean size of the juvenile on the end of the post-settlement period as 3.0 mm in shell length \((SL = 0.0234 \times D_1 - 3.540, SL = \) Shell length).

Thus, the post-settlement period lasted for 281 days between October 24, 2006, and August 1, 2007. In this period, the mean density of the newly settled juveniles decreased from 64,500 ind. m\(^{-2}\) to 1,443 ind. m\(^{-2}\). Juvenile mortality in the post-settlement period was 97.8% (1.34% \( \text{d}^{-1} \)). (The mean daily mortality \((M)\) was calculated by the following formula. \( M = 100 \times \left\{ 1 - \frac{\text{Den}(r)}{\text{Den}(p)} \right\} \times \frac{\text{D}(r) - \text{D}(p)}{\text{D}(p)} \)\)

\( M: \) daily mortality rate (\% \( \text{d}^{-1} \)), \( \text{Den}(r) \) and \( \text{Den}(p) \): the density (ind. m\(^{-2}\)) of peak and recruit post-settlers, respectively. \( \text{D}(r) \) and \( \text{D}(p) \): the date of peak post-settlers and recruit density recorded, respectively.) Later from August 1, 2007, the juveniles entered the period after the post-settlement period. The mean density of juveniles decreased from 1,443 ind. m\(^{-2}\) to 1,200 ind. m\(^{-2}\) for 76 days between August 1 and October 16, 2007. The mortality in this period was 19.0% (0.24% \( \text{d}^{-1} \)), which was approximately 5.6 times lower than the post-settlement period (Table 1).

In the size frequency distribution of the clam population shown in Fig. 4, we could recognize the presence of another cohort clearly, whose mode of the size frequency distribution is indicated by an arrow. Judging from the growth rate of the juvenile settled in the autumn of 2004 (Fig. 4 and Fig. 5(a)), one brooding season in a year and the mode of the size of the other cohort in the size frequency distribution (around 16 mm in shell length on August 24, 2006), we
deduced that this cohort recruited to the population in the breeding season of 2004 (2-yr old cohort). We analyzed the size frequency distribution of the clam population shown in Fig. 4, using a software for generation analysis (Tsutsumi & Tanaka 1994), and determined the mode size of the size frequency distribution and density of the cohort that recruited to the population in the breeding season of 2004 (Fig. 5(b)). The mode of the shell size of the cohort was 15.8 mm on August 8, 2006, and the cohort grew to 27.7 mm in the mode on October 16, 2007. In this period for 384 days (from August 8, 2006 to August 27, 2007), the density

![fig5a.png](image)

Fig. 5 (a) Seasonal variations of (top) mean density and (bottom) shell length of the juvenile clam that settled in the autumn of 2006 at ten sampling stations (Stn 1 to Stn 10) at each station, (b) Seasonal variations of (top) mean density and (bottom) shell length of the clam that settled in the autumn of 2004 at Stn 7. Dotted lines indicate the fitting curve (detailed see in text).

<table>
<thead>
<tr>
<th>Study period</th>
<th>Duration (days)</th>
<th>Developmental stage</th>
<th>Shell length (mm)</th>
<th>Density (indiv. m⁻²)</th>
<th>Mortality (%)</th>
<th>Daily mortality (% d⁻¹)</th>
<th>Daily mean temperature of the surface sediment (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hichirippu Lagoon, Hokkaido (this study)</td>
<td>Oct. 24, 06–Aug. 1, 07</td>
<td>281</td>
<td>Juvenile in the post-settlement period</td>
<td>0.3 to 3.0</td>
<td>64,500 to 1,443</td>
<td>97.8</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td>Aug. 1, 07–Oct. 16, 07</td>
<td>76</td>
<td>Juvenile after the post-settlement period</td>
<td>3.0 to 3.5</td>
<td>1,443 to 1,200</td>
<td>19.0</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Aug. 8, 06–Aug. 27, 07</td>
<td>384</td>
<td>Juvenile to adult</td>
<td>15.8 to 27.7</td>
<td>5,240 to 2,720</td>
<td>48.1</td>
<td>0.17</td>
</tr>
<tr>
<td>Kikuchi River Tidal Flat, Kumamoto (Tsukuda 2008)</td>
<td>Dec. 1, 04–May 26, 05</td>
<td>176</td>
<td>Juvenile in the post-settlement period</td>
<td>0.3 to 1.9</td>
<td>778,319 to 43,931</td>
<td>94.4</td>
<td>1.62</td>
</tr>
<tr>
<td></td>
<td>May 25, 02–May 20, 03</td>
<td>360</td>
<td>Juvenile</td>
<td>3.6 to 17.2</td>
<td>52,648 to 9,118</td>
<td>82.7</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>May 20, 03–May 22, 04</td>
<td>368</td>
<td>Juvenile to adult</td>
<td>17.2 to 24.0</td>
<td>9,118 to 1,807</td>
<td>80.2</td>
<td>0.44</td>
</tr>
</tbody>
</table>

of the cohort decreased from 5,240 ind. m\(^{-2}\) to 2,720 ind. m\(^{-2}\) (Fig. 5(b)). We estimated the mortality rate of this cohort in the same manners as the juvenile settled in the autumn of 2006 to 48.1% (0.17% d\(^{-1}\)). In daily mean mortality, it is almost equivalent to that of the juvenile after the post-settlement period that grew larger than 3.0 mm in shell length in 2007, 0.24% d\(^{-1}\) (Fig. 5(a)). Therefore, it seems that the clams tend to possess much higher resistance against the mortality factors, once they have passed the post-settlement period.

**Discussion**

Temperature used to be considered one of the critical environmental factors that control breeding activity of the clam. Mann (1979) showed that sexual maturation of *Ruditapes philippinarum* (synonym of *Ruditapes philippinarum*) did not proceed at water temperature below 12°C from the laboratory experiments. Toba & Miyama (1995) also noted that the rate of sexual maturation tended to decrease as lower water temperature in the range between 10 and 21°C, and estimated that the maturation did not occur below at least 5°C in the laboratory experiments.

The delay of the maturation due to low water temperature reflects the restriction of the number of breeding in a year or the duration of the breeding season. On the tidal flats in Ariake Bay, Kyushu, the western Japan, where the water temperature ranged between 12°C and 28°C throughout the year, the clam had an extended breeding season and peaks of juvenile recruitment were observed three times between April and November in a year (Tsutsumi et al. 2002). On the tidal flats in Tokyo Bay, central Japan, where the water temperature ranged between 8°C and 28°C, major spawning occurred twice in a year between May and November (Toba et al. 2007). The results of this study showed that the spawning and larval settlement concentrated on a short period between September and November once in a year (Fig. 5). Temperatures above 12°C, which Mann (1979) noted as the lowest temperature required for sexual maturation, lasted for only about four months between June and October (Fig. 2). It seems that the clams occurring in this study area store the reproductive matter during the short warm season, and breed once at the end of the warm season. Therefore, the juveniles that succeeded the settlement on the sediment of the tidal flats must experience the long cold season just after the settlement.

In this study, we examined the growth and mortality of the juvenile of the clam after settlement, measuring the temperature of the surface sediment that the clam inhabited. Here, we compared the growth and mortality in the post-settlement period of juvenile clam to those in the warmer habitats to clarify the negative impact of the extremely cold temperature on them. However, to collect the juveniles in the post-settlement period, it is necessary to sieve the sediment samples, using a sieve with a 125 μm opening mesh. In most of the previous studies on the clam, such a fine mesh was not used. Therefore, the information on the post-settlement period of the clam is very restricted. Now, the only data from the population study of the clam that was carried out on Kikuchi River Tidal Flat in Kumamoto are available (Tsukuda 2008) (Table 1).

From the available data on the juveniles that were produced by the autumn breeding in 2004 on Kikuchi River Tidal Flat (based on the original data of Tsukuda 2008), we set the duration of the post-settlement period of the juveniles as 176 days between December 1, 2004 and May 26, 2005. In this period, the density of juvenile just after settlement (0.3 mm in shell length) reached a peak (778,319 ind. m\(^{-2}\)) on December 1, 2004, and the juveniles passed the winter, growing very slowly (0.4 mm in mean shell length on March 25, 2005), initiated a rapid growth from April, and reached 1.9 mm in mean shell length by May 26, 2005. Temperature data in for this period are not available. However, the temperature of the surface sediment was measured at the same place on the tidal flat every 30 min. between August 2005 and June 2006, using the same probe with this study. The range of the temperature of the surface sediment in the same season (December in 2005 to May 2006) as the juveniles settled on the sediment in the early winter of 2004 and passed the winter was between 5.7°C and 20.9°C in daily mean temperature, which was approximately 6°C higher in the daily mean temperature than that of this study areas.

We calculated the mortality in the post-settlement period of the juvenile produced by the autumn breeding on Kikuchi River Tidal Flat as 1.62% d\(^{-1}\). It is rather higher than that of this study, although the sediment temperature on Kikuchi River Tidal Flat was approximately 6°C warmer (Table 1). We also calculated the mortality of the juveniles after the post-settlement period on Kikuchi River Tidal Flat, using the original data of the population study of the clam by Tsukuda (2008). The mortality of juvenile in the growth process from 3.6 mm to 17.2 mm in mean shell length for 360 days between May 25, 2002, and May 20, 2003, was 0.49% d\(^{-1}\). That of juvenile in the growth process from 17.2 mm to 24.0 mm in mean shell length for 368 days between May 20, 2003, and May 22, 2004, was 0.44% d\(^{-1}\) (Table 1). Therefore, the mortality of the juvenile after the post-settlement period over 3.6 mm in shell length was much lower (less than one third) than that in the post-settlement period, as we reported in this study. However, the mortality of the juvenile after the post-settlement period on Kikuchi River Tidal Flat was approximately twice that in this study. Of course, we need to consider the influence of other environmental conditions such as food conditions, density of the population, etc. on the mortality of the clam. But, these facts indicate that the extremely low temperature of the winter in this study area (the eastern part of Hokkaido) does not bring large-scale mortality to the clams, and that this is true not only of the juveniles after the post-settlement period but also the small ones of less than 3 mm in shell length in the post-settlement period on the tidal
flats.
Now, only two studies including this study are available to examine the mortality of the juvenile of *R. philippinarum* in the post-settlement and the influence of temperature conditions on the mortality. To further clarify the influence of temperature on the growth, mortality and breeding of the clam, we need to accumulate the information from various habitats of the clam in Japan and other eastern Asian countries. We are continuing the population study of the clam on Hichirippu Lagoon, and have initiated field surveys on several tidal flats where the clam occurs densely in Kumamoto, Kyushu, Japan, and the western coast of Korea, measuring the sediment temperature continuously. The results of these studies will be reported elsewhere.

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### References


