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## Reproductive Cycle and Shell Growth of the Tellin *Nitidotellina nitidula* (Dunker) in Hakodate Bay

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### Abstract

The developmental processes of gonads exhibit an annual cycle, as follows: recovery period (December-June), growing period (March-July), mature period (May-September), spawning period (May-October), and spent period (November-December). Rings are annually formed on the shell from August to November. The growth curve in shell length is estimated by external growth rings. The growth equation was calculated as follows:  $L_t = 24.72 (1 - \exp(-0.38(t + 0.47)))$ , where  $L_t$  is the theoretical shell length (mm) at time  $t$  (year). Shell length at the first sexual maturity defined as the length class at which 50% of the animals mature is 14-18 mm in both sexes. The age corresponding to the size at sexual maturity is 2 years old. The growth rate is better than, and the age at sexual maturity is a little older than those of other tellinid bivalves.

### Introduction

The tellin, *Nitidotellina nitidula*, which belongs to Tellinidae (Bivalvia) has wide distributional ranges in temperate and tropical regions from southern Hokkaido in Japan to Indonesia (Matsukuma, 1986). This species is a common member found in the muddy sand bottom from the intertidal region to 80 m in depth. Tellinid bivalves are generally the dominant species in the muddy sand benthic community; Salzwedel (1979) revealed that *Tellina fabula* comprised 28% of the biomass of the *Venus striatula* community living in the medium sand area of northeast Helgoland, and annual production of *Macoma balthica* was 50% of the estimated total macrofauna production in the Cumberland Basin (Cranford et al., 1985).

Several studies have been made on the reproduction and the growth of the tellinid bivalves (Stephen, 1929; Ansell and Trevallion, 1967; Trevallion and Ansell, 1967; Salzwedel, 1979, Goshima et al., 1991), yet little is known about *N. nitidula* in spite of being the dominant tellinid bivalves in the macrobenthos community in Hakodate Bay (unpublished data). This paper deals with the reproductive cycle, the size at sexual maturity, and the shell growth pattern in the northern part of Japan. Finally, the relationship between its reproduction and growth is discussed in relation to its life history pattern.

### Materials and Methods

Samples were collected using a small Smith-McIntyre grab sampler with 0.05 m<sup>2</sup>

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from 53 fixed stations, in an area where the depth was 2–10 m in Hakodate Bay (41°48'N, 140°43'E). Further details of the sites are given in Noda (1991). Samplings were carried out from December 1984 to February 1986 monthly, except for January both in 1985 and 1986 when the weather conditions were too rough. Bivalves were separated from the sediment by using a 1 mm mesh sieve and fixed in sea water with 10% formalin. In the laboratory, shell length was measured to the nearest 0.1 mm with a caliper.

To determine the seasonal gametogenic cycle, histological preparations were done. Each month, the soft body of about 30 adults which ranged above 20 mm was removed (detailed description will be given in the section concerning size at sexual maturity), fixed in aqueous Bouin's solution, and then dehydrated in a series of alcohols. After embedding in paraffin, the whole soft body was cut into 5–6  $\mu\text{m}$  cross-sections. The sections were stained in hematoxylin followed by eosin as a counterstain for histological observation. The developmental processes of male and female germ cells were divided into four stages, respectively, following the definition by Sastry (1979), Takahashi and Yamamoto (1970) and Goshima et al. (1991): in males they were spermatogonia, spermatocytes, spermatotids, and spermatozoa; in females they were oogonia, yolkless oocytes, yolk granule oocytes, and mature oocytes. The developmental stages of the gonads were classified according to the stage of germ cells which were dominant in the whole section.

The size at sexual maturity was determined by 84 bivalves in which shell length ranging from 6 to 26 mm were dissected out and embedded using the same method described above from July to September 1985. The size at sexual maturity was defined as the length class at which 50% of the tellins matured (Harvey and Vincent, 1989).

To ascertain the reliability of the rings as age characteristics, the time and frequency of ring formation were determined every month by observing the shell edges of 50 individuals, in accordance with Goshima et al. (1991) and Goshima and Noda (1992). Growth of *Nitidotellina nitidula* was analyzed by measuring shell length at each growth ring. A growth curve was obtained as follows: mean shell lengths at successive growth rings were fitted into the von Bertalanffy equation,

$$L_t = L_\infty(1 - \exp(-k(t - t_0)))$$

where  $L_t$  is the shell length at time  $t$ ,  $L_\infty$ ; the theoretical maximal shell length,  $k$ ; a growth constant, and  $t_0$ ; the theoretical time when shell length equals zero. The parameters of the equation were estimated by a computer program using the simplex method (Okumura, 1986).

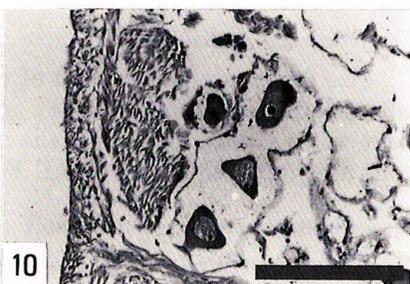
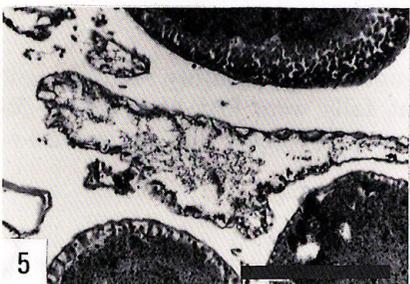
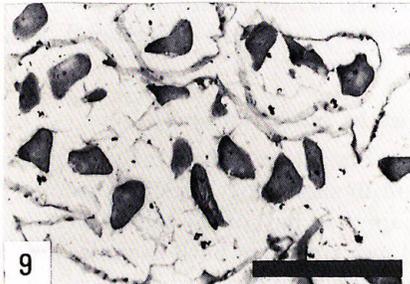
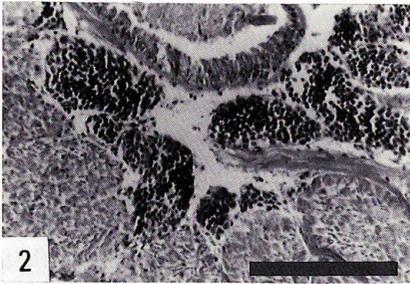
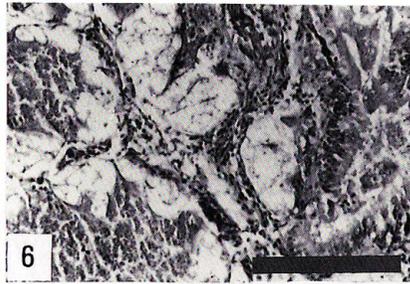
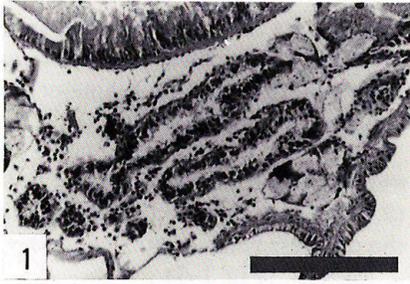
## Results

### *Developmental stages of the gonad*

In both female and male gonads, the following five stages were identified.

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Fig. 1. Histological microphotograph showing testis and ovary in various stages. Scale bar = 200  $\mu\text{m}$ . 1: Recovery stage of testis. 2: Growing stage of testis. 3: Mature stage of testis. 4: Spawning stage of testis. 5: Spent stage of testis. 6: Recovery stage of ovary. 7: Growing stage of ovary. 8: Mature stage of ovary. 9: Spawning stage of ovary. 10: Spent stage of ovary.



**Recovery stage.** In the male gonads, there were no ripe gametes in the follicles, although there were spermatogonia (8–12  $\mu\text{m}$  in diameter) and spermatocytes (4–6  $\mu\text{m}$  in diameter) (Fig. 1-1). Oogonia (5–6  $\mu\text{m}$  in diameter) and yolkless oocytes (12–14  $\mu\text{m}$  in diameter) were observed in the ovary (Fig. 1-6). The follicle walls were thick.

**Growing stage.** In the testes, the cells were arranged in the following order; spermatogonia, spermatocytes, and spermatids (2.5  $\mu\text{m}$  in diameter) from the follicle wall to the lumen respectively (Fig. 1-2). In the ovaries, yolkless and yolk oocytes (32–37  $\mu\text{m}$  in diameter) were projected into the follicles (Fig. 1-7).

**Mature stage.** Mature sperm (the head of this cell was about 1  $\mu\text{m}$ ) formed dense masses in the follicles of animals (Fig. 1-3). The follicles in the ovaries were filled with mature oocytes (50–55  $\mu\text{m}$  in diameter) (Fig. 1-8).

**Spawning stage.** Partially spawned follicles still contained sperms, but they were less than those in the mature stage (Fig. 1-4). In the partially spawned gonads, a few mature oocytes were free in the lumen of the follicles (Fig. 1-9).

**Spent stage.** Most of the follicles contained no spermatozoa and if they did spermatozoa they were very few in number, and the lumina were empty (Fig. 1-5). In the spent ovaries, most of the follicles were devoid of ripe gametes, with few residual oocytes (Fig. 1-10). The follicle walls became thick.

#### *Reproductive cycle*

Histological examination revealed the seasonal maturation cycle in the population (Fig. 2). The recovery stage in males was seen from December to July. Females in the same stage were first encountered in January, and were continuously observed until July. Growing testes were observed from February to July. The ovaries in the growing stage were observed from March to July. Mature testes first appeared in May and the frequency of the stage increased in July. Mature ovaries showed the same trend as in the male testes. Spawning stages in the both sexes were parallel with mature stages. The spent stage in males was seen from August to February. On the other hand, females in the same stage were observed from May to January.

#### *Size at sexual maturity*

Size at sexual maturity is as shown in Fig. 3. Males and females from 6 to 14 mm in shell length were in recovery or growing stage. It is clear that individuals more than half of the tellins were in the mature, spawning and/or spent stages above 14 mm size class both in males and females. Therefore, the size at sexual maturity was about 14 mm in both sexes.

#### *Size frequency distribution*

Size frequency distribution of the shell length of the monthly samples showed polymodal distributions (Fig. 4). A few spat falls were first observed in May. There was a sudden increase in the number of spats in August, and a distinct 0-year group was followed at least until February.

#### *Age determination by growth check and growth equation*

Time and frequency of the distinct white rings which were seen on the shell

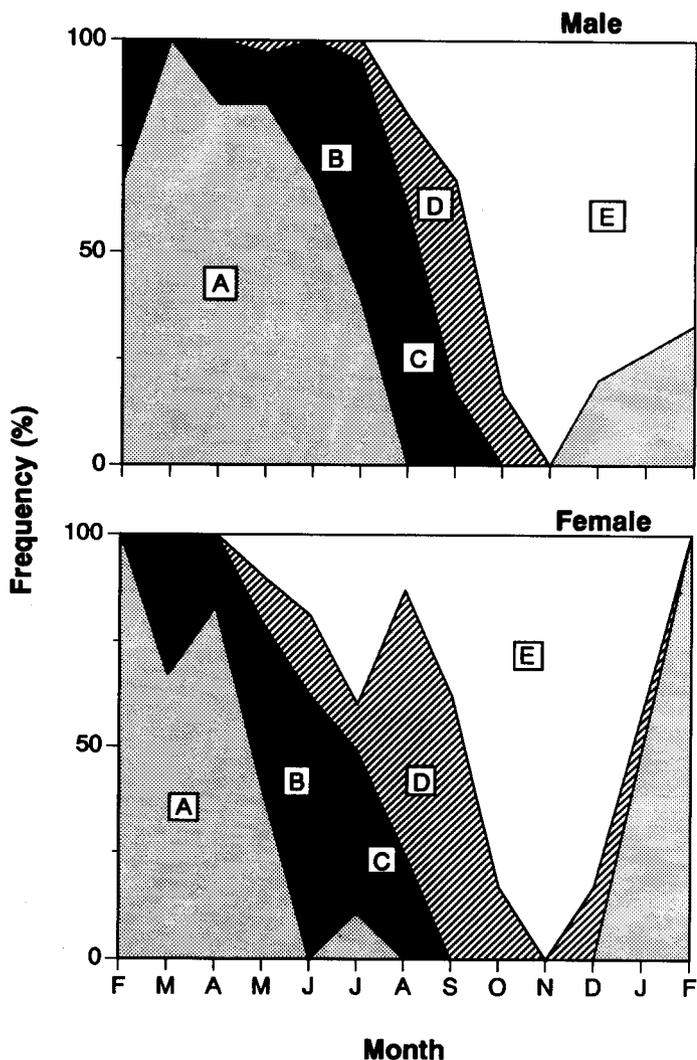


Fig. 2. Gonad stage frequency distribution of *N. nitidula* sampled from July to September 1985. A: Recovery stage. B: Growing stage. C: Mature stage. D: Spawning stage. E: Spent stage.

valves showed an annual cycle (Fig. 5). Approximately 20% of the tellins had the rings in May. The frequency of the rings increased continuously from August and reached at 100% in November, which indicated that the ring was formed annually.

After the age of an individual was determined by counting the number of rings, the tellinid bivalves were sorted out into different year classes. Six year classes were distinguished in this study (Table 1). Based on the mean shell length at each

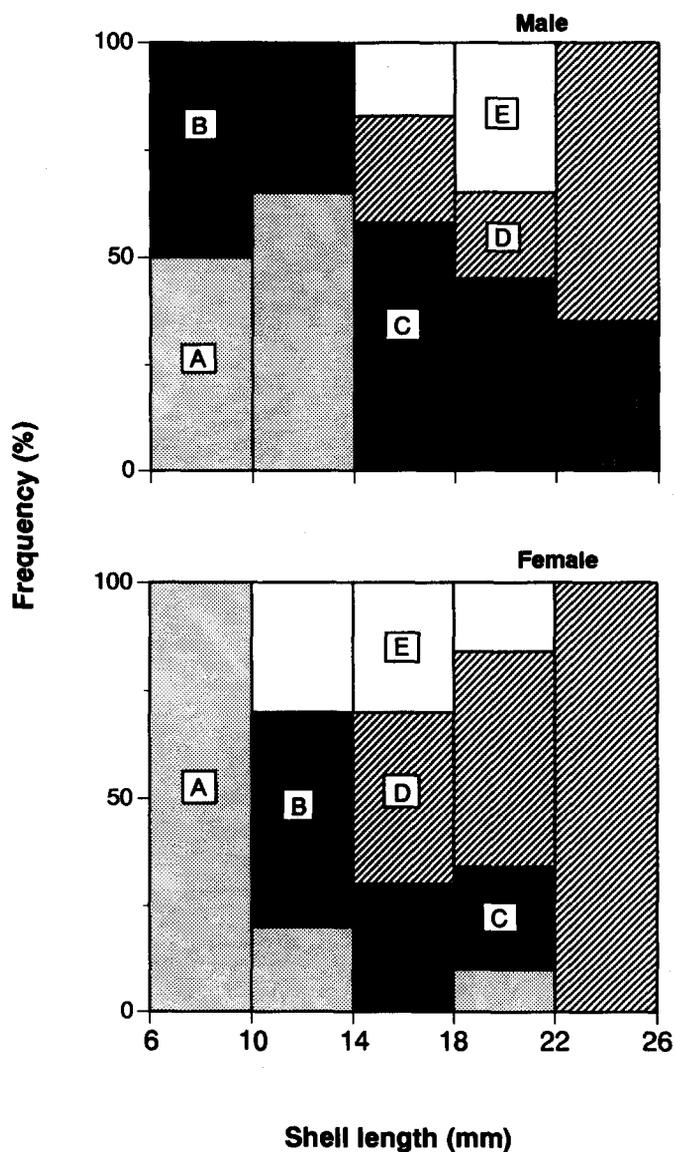


Fig. 3. Diagram showing the relative proportion (%) of tellin in each gonad stage. A: Recovery stage. B: Growing stage. C: Mature stage. D: Spawning stage. E: Spent stage.

ring, the growth equation was calculated as follows (Fig. 6):

$$L_r = 24.72(1 - \exp(-0.38(r + 0.80))) \quad (1)$$

where  $L_r$  is the shell length (mm) at  $r$ -th ring.

Main spawning was observed from July (Fig. 2), and the rings were formed in

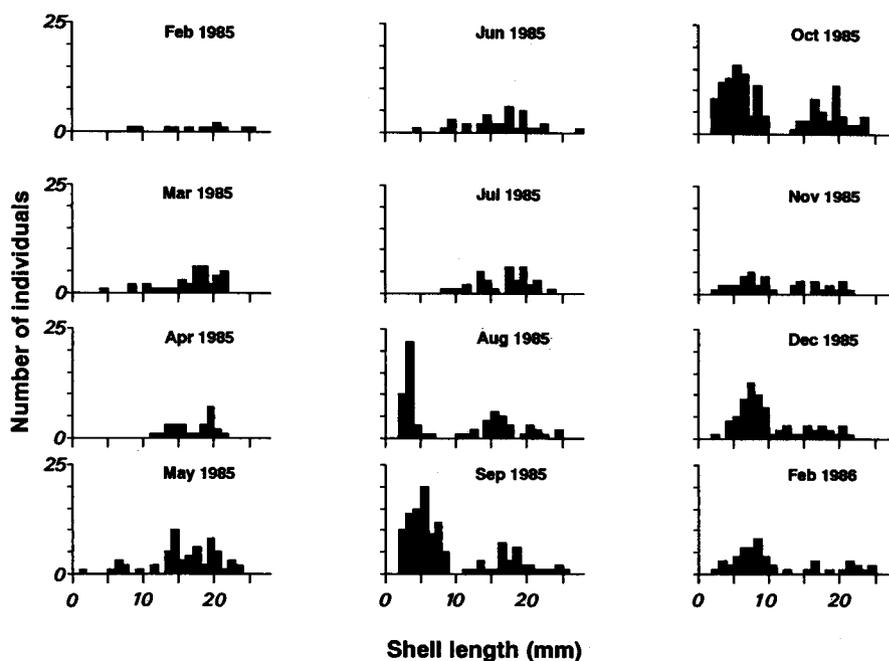


Fig. 4. Size frequency distribution for *N. nitidula* population sampled from February 1985 to February 1986.

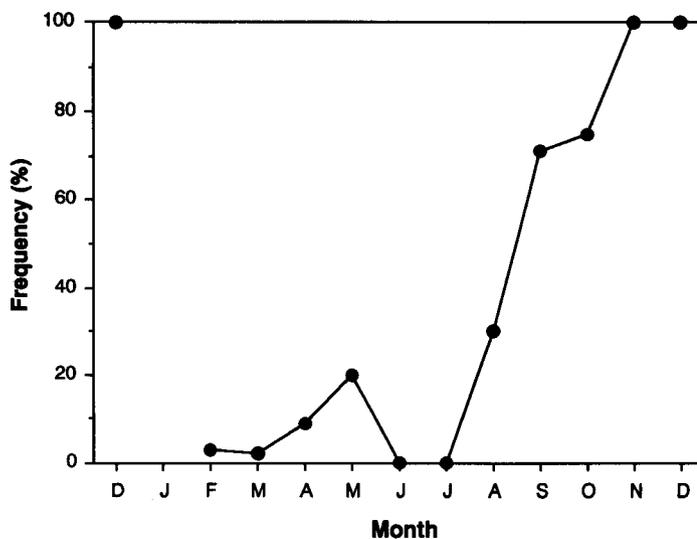


Fig. 5. Cumulative percentage of individuals with new growth rings along shell margin.

Table 1. Mean shell length  $\pm$  standard deviations (SD) at each shell ring.

No. of rings	Shell length (mm)						
	N	L1	L2	L3	L4	L5	L6
1	35	7.08 $\pm$ 4.29					
2	7	7.50 $\pm$ 2.23	15.15 $\pm$ 7.83				
3	28	6.58 $\pm$ 2.35	12.65 $\pm$ 4.25	16.23 $\pm$ 4.00			
4	17	6.15 $\pm$ 2.51	12.29 $\pm$ 3.34	15.74 $\pm$ 2.39	18.45 $\pm$ 2.09		
5	2	6.14 $\pm$ 7.26	12.04 $\pm$ 0.01	17.15 $\pm$ 0.00	19.67 $\pm$ 0.85	23.67 $\pm$ 2.69	
6	2	5.02 $\pm$ 0.17	10.09 $\pm$ 3.43	16.40 $\pm$ 0.04	18.33 $\pm$ 0.47	21.04 $\pm$ 1.22	23.25 $\pm$ 7.41
mean $\pm$ S.D.		6.69 $\pm$ 3.10	12.71 $\pm$ 4.93	16.13 $\pm$ 2.96	18.59 $\pm$ 1.78	22.36 $\pm$ 2.86	23.25 $\pm$ 7.41

$L_r$  ( $r=1\sim 6$ ) indicates shell length at the  $r$ -th ring. N: number of samples.

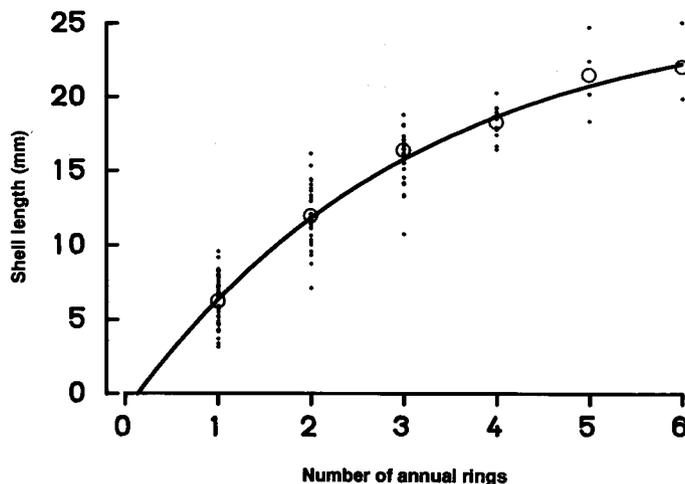


Fig. 6. Mean shell length (open circles) of *N. nitidula* at the time when each growth ring was formed along the shell margin and growth curve calculated using the von Bertalanffy growth equation. Each dot is the measured shell length on each ring of the tellins.

November (Fig. 5), which means that *N. nitidula* was 0.33 year old when the first ring was formed on the shell. The growth equation (1) was, therefore, recalculated follows:

$$L_t = 24.72(1 - \exp(-0.38(t + 0.47))) \quad (2)$$

where  $L_t$  is the shell length (mm) at time  $t$  (year).

### Discussion

Histological examination of the gonad sections during the study period allowed us to determine that the reproductive cycle of *Nitidotellana nitidula* is a biological

event with annual periodicity. A mature stage was observed from May to September in males and from May to August in females, and spawning extended from May to October in both males and females. Spat falls were first observed in May, and main spat falls settled from August to October. These results suggest that the *N. nitidula* population had an extended reproductive period from May to October, and the main spawning occurred from July to October. Such extended spawnings were also observed in other tellinid species. *Tellina fabula* had an extended reproductive period from March until September in German Bight (Salzwedel, 1979). Warwick et al. (1978) indicated that the main spat fall of *T. fabula* took place in May in the British west coast and perhaps another small one in March. It is not known whether the extended spawnings were due to successive spawning of different individuals or the same individuals. We could not determine when the spats had settled. An investigation concerning the spat fall season of individuals is still necessary.

In this study, the growth check method was used in measuring age determination. It was recognized that shells obtained from August to February possessed new rings at the shell margin. The percentage of individuals with new rings reached 100% in November. In general, the annual rings were formed during the reproductive period, cold or hot temperature period (Salzwedel, 1979; Goshima et al., 1991). For *N. nitidula*, the rings were formed annually on the shell from autumn to winter. The period coincided with the spawning season and also with the following cold period (6°C in February). The causes might be different according to the size of the tellin; the size at sexual maturity was 14 mm, while individuals smaller than that size already had a first ring at 6.7 mm shell length on the average, which indicated that the first ring formation of the younger ones was not concerned with reproduction. On the other hand, *T. fabula* formed such rings during reproductive, cold and hot periods, respectively (Salzwedel, 1979). Although the factors of the ring formation might differ according to the size and the species, the distinct annual rings observed on the shells are quite useful for age determination.

Estimated shell length of one year old of *N. nitidula* was 10.6 mm which was bigger than those of other tellinid species; *Tellina fabula* was 1.5 mm (Muus, 1973), 1.8 mm (Warwick et al., 1978), 3.0 mm (Salzwedel, 1979) long and *Macoma balthica* was about 3 mm long (Harvey and Vincent, 1990) at one year old. *T. fabula* was distributed in the colder regions from Norway to the Black Sea (Abbott and Dance, 1985). Salzwedel (1979) reported that the shell and the soft tissues of *T. fabula* grew well at 6–16°C, but the growth stopped in the period above 17°C, when summer rings were formed. On the other hand, *N. nitidula* was distributed in the temperate and tropical regions, and the temperature in the present study area ranged from 15 to 22°C during the period from settlement time (August–October) to ring formation (November). Zero year animals grew up to 6.7 mm in shell length in November, which showed a more rapid growth rate, compared to *T. fabula* and *M. balthica*. It was suggested that a higher temperature was an important factor for younger *N. nitidula* to grow well in this area.

Using the growth equation (2), the age corresponding to the size at sexual maturity for *N. nitidula* was estimated as 2 years (14 mm in shell length) in both sexes. The size and age at sexual maturity were reported for other tellinid species: 10 mm and 1.5 year for *Tellina fabula* (Salzwedel, 1979), 7 mm (Stephen, 1929) and

2 years (Ansell and Trevallion, 1967; Trevallion and Ansell, 1967) for *T. tenuis*, and 6 mm and 1.7 year for *Macoma balthica* in Canada (Harvey and Vincent, 1989), 9 mm and 1 year in America (Gilbert, 1978), 3 mm and 1 year in Holland (Lammens, 1967), respectively. The growth rate of *N. nitidula* was faster than, and the size at sexual maturity was larger than those of other tellinid bivalves, while the age at sexual maturity was slightly older than those of other tellinid species. Such a larger size and later age at sexual maturity in *N. nitidula* might couple with the higher survival rate during the younger stage. Salzwedel (1979) reported that only 4% of *T. fabula* survived at 1.5 year when maturing during four years of its lifespan, while there were older individuals in *N. nitidula* up to six years (Table 1), which suggests a higher survival rate in *N. nitidula* than in *T. fabula*. *N. nitidula* could therefore devote much energy towards somatic and shell growth during the younger period and make increased reproductive output by being of a bigger size at sexual maturity, because an increase in reproductive output according to an increased size or age is usual phenomenon for marine invertebrates (Menge, 1974; Thompson, 1984). Such a life history pattern in the present species might be subject to the trade-off between reproduction and survival (Stearns, 1976; Goshima, 1982).

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