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FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

REPRODUCTION OF THE ANTARCTIC CLAM, *LATERNULA ELLIPTICA* COLLECTED FROM MARIAN COVE IN KING GEORGE ISLAND, ANTARCTICA

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DEPARTMENT OF MARINE BIOLOGY
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(Continued)

DEDICATION

This dissertation is dedicated to
my wife, Ae-Jung, and daughters, Seo-Hyun and Seo-Young,
my mother and father- and mother-in-law, and my family

국문 요약

1. 컴퓨터화상처리기법을 이용한 남극큰띠조개, *Laternula elliptica* (King & Broderip)의 생식소발달 정량을 위한 연구

1.1. 이 연구는 남극 환경변화에 따른 해양저서생물 변동에 대한 장기관측 연구의 일환으로서, 대한민국 세종기지가 위치한 킹조지섬의 마리안 소만 조하대에 서식하는 남극큰띠조개(*Laternula elliptica*)는 SCUBA를 이용하여 1996년 12월과 1998년 1월에 채집하였고, 조직학적 방법과 컴퓨터화상처리기법에 의해 생식소 발달과정 분석을 정량화 하였다.

1.2. 남극조개 난소소엽 내 난모세포의 면적비 지수(the percentage egg area within a follicle, FI)와 평균 난모세포 직경 지수(mean oocyte diameter, MOD)는 서로 다른 채집 시기인 1996년 12월과 1998년 1월의 생식소발달 차이를 유의하게 나타냈지만, 일반적인 성숙도 지수(maturity index, MI)와 전체육질부 면적에 대한 생식소 면적비 지수(percentage gonad area, PGA)는 두 시기의 남극조개에 관찰한 생식소 발달의 차이를 나타내지 못하였다. 이러한 결과는 남극조개 생식소 발달을 판정하는 지수로서 FI와 MOD는 신뢰성과 감도면에서 다른 두 지수보다 높았다.

1.3. 생식소 이미지를 이용한 생식세포의 면적측정방법(planimetric technique)은 사용이 용이할 뿐만 아니라 채집 개체수가 많을 경우에도 데이터 처리 속도가 빠르고, 일반적인 지수들보다 정량적인 정보를 제공하므로 남극큰띠조개 *L. elliptica* 번식 연구에 효율적으로 이용될 수 있다.

1.4. 특히 76-85 mm 크기의 남극큰띠조개 개체들은 다른 크기에 비해 높은 번식량을 나타내므로 남극큰띠조개 번식패턴 변동의 장기관측을 위해서는 이 크기의 개체들을 주로 사용하는 것이 보다 효율적일 것으로 판단된다.

2. 남극 킹조지섬의 마리안 소만 조하대에 서식하는 남극큰띠조개, *Laternula elliptica*의 연중 번식 특성

2.1. 남극큰띠조개의 연중 배우자형성과정과 계절별 번식특성을 연구하기 위해 1998년 2월부터 2000년 1월까지 2년 동안 SCUBA를 이용하여 개체들을 채집하였다. 남극큰띠조개 생식소발달, 성숙 및 산란 등은 위 Part 1에서 개발한 방법과 일반적인 방법을 이용하여 정성적, 정량적으로 측정하였다.

2.2. 생식소발달 지수인 FI와 MI는 1998년 10월부터 11월까지 유의하게 증가하여 12월에 최고치를 나타냈고, 1999년 1월과 2월에 급격하게 감소했다. 대형 성숙난의 크기(100-190μm)를 고려했을 때, 남극큰띠조개는 난황영양형(lecithotrophic) 유생과정을 거치는 것으로 판단된다. 산란 후 난소에 남아 있는 잔존난들은 혈구세포에 의한 식세포작용에 의해 연중 재흡수되었고, 2월과 3월에 집중적으로 나타났다. 이러한 재흡수 과정은 산란 후에 일어나는 체내 단백질원 재사용 기전의 일반적인 현상으로서, 연중 먹이공급이 여름철에 극히 제한된 남극 연안에 서식하는 남극큰띠조개의 효율적인 체내 에너지 관리를 위한 적응기전으로 판단된다.

2.3. 남극큰띠조개의 생식소 성숙은 1998년과 1999년 모두 마리안 소만 내 플랑크톤 대번식 시기와 맞물린 10월부터 12월까지 집중적으로 이루어졌다. 한편 수중으로부터의 먹이공급이 상대적으로 높았던 1998년 10월의 생식소 성숙이 1999년 10월보다 빠르다는 것을 고려했을 때, 수중의 먹이원은 남극큰띠조개의 생식소 성숙과 산란에 결정적인 역할을 하는 것으로 판단된다.

2.4. 남극큰띠조개, *L. elliptica*의 생식소 분석과 배우자형성과정을 2년간 관찰한 결과, 생식소의 성숙과 산란은 연중 일어나지 않고 남극 여름철의 짧은 기간에 이뤄지는 것으로 판단된다. 이러한 번식패턴의 뚜렷한 계절성은 배우자형성과정에서도 관찰됐다: 1) 흡수기와 휴지기의 주요 시기는 2월부터 3월, 2) 생식소발달과 성숙의 주요 시기는 10월부터 12월, 3) 산란은 12월부터 2월까지 주로 관찰됐다.

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SUMMARY

1. Quantitative assessment of reproductive condition of the Antarctic clam, *Laternula elliptica* (King & Broderip), using image analysis

1.1. The reproductive state of Antarctic clam, *L. elliptica*, collected from a small cove on King George Island in the spawning season was assessed quantitatively using computer-based image analysis.

1.2. The percentage egg area within a follicle (FI) and mean oocyte diameter (MOD) values differed significantly between December and January but conventional maturity index (MI) and percentage gonad area (PGA) values did not, suggesting that FI and MOD are the more reliable and sensitive indicators for differentiating reproductive condition.

1.3. This study also showed that a planimetric technique using computer-based image analysis is fast, convenient to use, and better than the conventional MI at providing reliable quantitative information about *L. elliptica* reproduction.

1.4. These indices varied with body size. The FI and MOD values peaked in clams of 76 to 85 mm shell length, indicating that clams of this size have the highest reproductive output at spawning time. Therefore, animals of a standard size (76 to 85 mm) should be monitored seasonally or over a longer term.

2. Annual gametogenesis of the Antarctic clam, *Laternula elliptica* from Marian Cove, King George Island

2.1. The annual gametogenesis of the Antarctic soft-shelled clam, *Laternula elliptica* inhabited in Marian Cove, King George Island was studied over a two-year period from February 1998 to January 2000. Annual changes in gonad maturation were investigated using histology-based techniques as previous mentioned.

2.2. In 1998, monthly mean FI and MI increased significantly from October to November and reached a peak in December. The FI and MI values dropped rapidly from December to January. In 1999, degenerated eggs were observed from the spent follicles in February and March. Degeneration and absorption of the residual eggs by phagocytosis occurred mostly in February and March in 1998 and 1999, although the resorption process was observed all year round. The gonad maturation during October to December in both years was corresponded with the annual chlorophyll maximum in the Cove, suggesting that food supply is crucial to gonad maturation and subsequent spawning.

2.3. Gametogenesis of *L. elliptica* at Marine Cove investigated for two years confirmed that the clam spawns annually, and the gonad maturation takes only a few months during the Austral summer. A distinct seasonality was also observed in the annual gametogenesis

as 1) absorbing and resting during February and March, 2) developing and maturation during October and December and 3) spawning during December and February.

INTRODUCTION

The Antarctic soft-shelled clam, *Laternula elliptica* (Fig. 1) is one of the most common marine organisms inhabiting the shallow subtidal area around Antarctic coastal waters (Ahn 1994a; Ansell and Harvey 1997; Sahade et al. 1998; Urban and Mercuri 1998). The clam is endemic to the Antarctic and is widely distributed in shallow waters around the continent and adjacent islands (Ahn 1994; Mercuri et al. 1998; Cattaneo-Vietti et al. 2000; Brockington 2001). This deep-burrowing, large (≈110 mm in shell length, Ahn 1994) filter-feeder occurs in dense patches (a few hundred/m^2) and appears to play a key role in the Antarctic nearshore marine ecosystem (Zamorano et al. 1986; Ahn 1993; Arntz et al. 1994; Ahn et al. 2001). Especially, the clam occurs in dense patches at a depth between 20 and 30 m at King George Island, South Shetland Islands and is considered to be one of the well adapted benthic animals in the harsh shallow Antarctic water (Zamorano et al. 1986; Ahn 1994b; Sahade et al. 1998). Despite its wide distribution and abundance, few studies have concerned the reproductive biology and ecology of *L. elliptica* (Pearse et al. 1991; Urban and Mercuri 1998; Bigatti et al. 2001; Ahn et al. 2000).

Laternula elliptica is a simultaneous hermaphrodite which produces unusually large encapsulated eggs (≈200 μm in diameter), which develop as encapsulated lecithotrophic larvae (Pearse et al. 1991;

Ansell and Harvey 1997). Spawning probably occurs during summer months along a latitudinal cline: mid-December at King George Island (Ahn et al. 2000) and from March to mid May at McMurdo Sound, King George Island (Bosch and Pearse 1988). The reproductive patterns and related characteristics of *L. elliptica*, however, need to be further elucidated with seasonal or long-term studies. Like other benthic animals in Antarctica, the clam has slow growth and slow gonad maturation (Pearse et al. 1991; Ansell and Harvey 1997; Urban and Mercuri 1998; Bigatti et al. 2001). Slow gonad maturation and pelagic non-feeding larval development of *L. elliptica* is considered to be a reproductive adaptation to the harsh Antarctic environment (Bosch and Pearse 1988; Pearse et al. 1991; Urban and Mercuri 1998). Like other benthic organisms inhabiting in the Antarctic, limited information is available on growth and reproduction of *L. elliptica* due to the technical difficulties involved in direct observation and sampling.

Recently studies have been conducted to investigate reproduction and growth of *L. elliptica.* These studies have reported that the clam spawns during austral summer although the spawning has a latitudinal cline; mid-December to February at Marian Cove (Ahn et al. 2000, 2003), and from March to mid May at McMurdo Sound, King George Island (Bosch and Pearse 1988; Pearse et al. 1991). Urban and Mercuri (1998) investigated annual gametogenesis of *L. elliptica* collected from Potter Cove, King George Island over a one year period

using histology. They reported that *L. elliptica* at Potter Cove spawn during February and March. Interestingly, they were unable to observe any early developing gametes in the gonad while they found ripe oocytes in the follicle all year round. Based upon histological observation, they concluded that the oocyte development cycle last longer than one year (Urban and Mercuri 1998). Bigatti et al. (2001) also analyzed the same clams sampled from Potter Cover by Urban and Mercuri (1998) by measuring the size of the oocytes. Contrary to Urban and Mercuri (1998), they concluded a complete oocyte growth cycle takes less then a year and the clam may be ready for spawning at any time during the whole year, although intensive spawning is believed to occur in February.

Numerous techniques have been used to assess the reproductive condition of marine molluscs. Histological methods have been the most widely and frequently used, since histology provides visual information about gonadal tissues. Reproductive condition is usually classified as one of several numerical codes on the basis of gonadal tissue characteristics viewed under a microscope (see review of Lango-Reynoso et al. 2000). The process of gonadal development is also monitored by measuring increases in oocyte diameter or the degree to which a microscopic field is occupied by gonadal cells (see a review by Heffernan and Walker 1989). With the aid of a computer and video camera, the microscopic image of the gonad is digitized into a

computer graphic file and software is used to calculate objectively the area of eggs or gonadal areas (Heffernan et al. 1989; Lango-Reynoso et al. 2000).

In general gametogenesis of bivalves is categorized into 1) formation of germ cells, 2) growth and enrichment of gametes known as vitellogenesis 3) spawning and discharging of the gametes, and 4) resorption of residual gamete (Loosanoff 1942; Kennedy and Battle 1964; Braley 1982; Olive 1984; Shafee 1989). Such cyclic changes in gametogenesis are in part governed by changes in environmental parameters such as water temperature, salinity and food (Bayne and Newell 1983; Soniat and Ray 1985; Heffernan et al. 1989; Hofmann et al. 1992; Kang et al. 2000). The amount of food in the water column for filter feeders often shows a strong seasonality in temperate region with water temperature playing a key role in governing the seasonality (Mann 1979; Clarke 1987; Heffernan et al. 1989; Hooker and Creese 1995; Saucedo et al. 2001). Numerous studies have reported that gonad development and subsequent spawning of marine bivalves is synchronized with seasonal change of available food in the water column (Hofmann et al. 1992; Grant and Creese 1995; Kennedy et al. 1996; Kang et al. 2000). Along with water temperature, the quantity of food in the water column determines the amount of gametes produced (i.e. reproductive output) and the timing of spawning (Kang et al. 2000; Llodra 2002; Park and Choi 2004). Primary production of coastal

Antarctic is extremely seasonal and the phytoplankton blooming occurs during a short period of austral summer (Chang et al. 1990, Rivkin 1990; Kang et al. 1997; Ahn et al. 2003). Therefore, available food is an important factor involving reproduction of Antarctic marine invertebrates (Urban and Mercuri 1998; Peck et al. 2000; Ahn et al. 2003; Clarke et al. 2004). *Laternula elliptica* are possibly adapted on reproductive strategy as reproductive output, spawning time and mode in relatively poor food condition.

The objectives of the present study were to 1) establish standard methods for use in a seasonal or longer-term study of *L. elliptica* reproduction; 2) investigate the seasonal variations of oocytes growth and the gametogenic cycle, resorption process to the relict eggs and the correlation between available food and reproduction of *L. elliptica* collected from Marian Cove, King George Island in Antarctica.

Fig. 1. The Antarctic clam, *Laternula elliptica*.

Part I

Quantitative assessment of reproductive condition of the Antarctic clam, *Laternula elliptica* (King & Broderip), using image analysis

1. Abstract.

The reproductive state of Antarctic clam, *L. elliptica*, collected from a small cove on King George Island was assessed quantitatively in the spawning season. Using computer-based image analysis, percentage gonad area (PGA), percentage egg area within a follicle (FI), and mean oocyte diameter (MOD) were determined from gonadal cross-sections prepared for histological studies. These indices were then compared on the basis of scores from 1 to 6 on a conventional maturity index (MI), which is scored using subjective criteria determined by microscopic examination. Clams collected during two different spawning periods were analyzed. The FI and MOD values differed significantly between the two groups but MI and PGA values did not, suggesting that FI and MOD are the more reliable and sensitive indicators for differentiating reproductive condition. This study also showed that a planimetric technique using computer-based image analysis is fast, convenient to use, and better than the conventional MI at providing reliable quantitative information about *L. elliptica* reproduction. I also investigated whether these indices varied with body size. The FI and MOD values peaked in clams of 76 to 85 mm shell length, indicating that clams of this size have the highest reproductive output at spawning

time. Therefore, animals of a standard size (76 to 85 mm) should be monitored seasonally or over a longer term.

2. Materials and Methods

2.1. Sampling efforts

Marian Cove is a small embayment located in northeast Maxwell Bay (Ahn et al. 2000) (Fig. 2). Every summer for the past several years, *L. elliptica* has been collected from depths between 20 and 30 m at Marian Cove (62°13'S, 58°47'W) by SCUBA. For our study, clams collected during two austral summers (Dec 12th -22th, 1995 and Jan 12th 1998) were selected, as clams of a wide size range were available from the specimen sets collected. Shell length (SL, the longest dimension of the shell) was measured to 0.01 mm. Each clam's soft body was then removed and the soft tissues were fixed in 10% neutral formalin for further analysis.

2.2. Histological preparation and analysis of gonadal tissues

A 1-cm-thick longitudinal cross-section was removed from the center of the soft body mass of each clam for histological analysis (Fig. 3). Each cross-section contained gonads, visceral mass, a foot, gills,

and a mantle, all which are recommended for complete histological sectioning of marine bivalves (Howard and Smith 1983). The tissues were dehydrated in an alcohol, cleared in xylene, embedded in paraffin, sliced to 6 μm and stained with Harris' hematoxylin and eosin Y. The sections were examined under a light microscope equipped with a video camera connected to a personal computer.

L. elliptica is a hermaphrodite, gonadal development of testis and ovary were scored from 1 to 6 according to Table 1. Three microscopic fields on average were examined for each clam and the mean score was used as the maturity index (MI). Gonad development of ovary was also analyzed quantitatively using the following planimetric technique. A microscopic image of gonad was captured by a video camera and sent to a personal computer. Surface areas of ovary and testis in each cross-section were then measured from the digitized images using Image Pro® image analysis software. The ratio of gonadal/total cross-sectional area was also determined and expressed as percentage gonad area (PGA, μm^2) (Morales-Alamo and Mann 1989). The percentage area occupied by oocytes in a follicle, or follicle index (FI, %), was determined using three to six follicles that were randomly selected from the histological preparation of each clam. Mean oocyte diameter (MOD, μm) was determined using 40 to 50 oocytes in three to six follicles measured from the computerized microscopic image.

I investigated variation in these indices associated with body

size and age using specimens over a wide size range. In addition, some reproductive traits of *L. elliptica* are discussed.

3. Results

3.1. Quantitative assessment of *L. elliptica* reproductive condition

In mid December 1995, the reproductive condition of 51 clams was examined. Of these, 3.9% were sexually undifferentiated (stage I), 11.8% were developing (stage II, Fig. 4A), 51.0% were fully developed and sexually mature (stage III, Fig. 4B), 29.4% were spawning (stage IV, Fig. 4C); the remaining 4.0% were either spent (stage V, Fig. 4D) or absorbing (stage VI, Fig. 4E). In mid January 1998, 47 clams were examined. Of these, 23.4% were developing, 53.2% were fully developed, and 23.4% were spawning. No clams from this year had gonads that were in the early developing, spent, or absorbing stages. In both years, most clams were ready to spawn or already spawning.

Oocyte diameter *L. elliptica* from Marian Cove ranged from 20.0 to 192.1 μm. The diameter of fully developed eggs averaged 156.6 μm. Each fully developed egg was enveloped in a 9.1-μm membrane that stained strongly with Harris' hematoxylin. There were oocytes of various sizes within each follicle, which lowered the MOD of each clam. The mean MOD of oocytes was 57.79 μm in developing ovaries,

62.85 μm in fully developed ovaries, 49.30 μm in spawning ovaries, and 22.75 μm in spent ovaries. Mean values of PGA and FI were highest in fully developed ovaries, followed by spawning ovaries (Table 2). The area occupied by the testis was always larger than that of the ovary regardless of body size or reproductive stage.

In the developing stage of the testes, follicles expanded toward connective tissues and spermatogenic cells began to proliferate around follicle walls (Fig. 5A). Fully developed, spawning, and partially spawned testes were observed simultaneously in both years (Figs. 5B, C). Spent testes often exhibited relict spermatozoa (Fig. 5D). Gametogenesis of the testis progresses synchronously with that of the ovary.

3.2. Comparison of the quantitative indices in the clams from the two austral summers

Mean SL of the clams collected in December 1995 and in January 1998 was 65.6 mm and 64.9 mm respectively, i.e. no significant difference in SL was found between the two populations. Small oocytes of >60 μm diameter were dominant in the clams collected in December 1995, while relatively bigger oocytes of <71 μm diameter were dominant in January 1998 (Fig. 6). Since clams of a wide size range were used in this study, direct comparisons for MI,

PGA, FI and MOD values in the two austral summers could not be made from the mean values. The MI, PGA, FI, and MOD values of clams collected in each year, therefore, were plotted against clam SL (Fig. 7). Each of the four indices was compared using regression analysis (ANCOVA). There was no significant difference in the MI and PGA between the two years (Figs. 7A, B). However, FI and MOD did differ significantly between sampling years: FI and MOD were significantly higher in January 1998 than in December 1995 ($P < 0.05$ for FI, $P < 0.001$ for MOD) (Figs. 7C, D).

Distinct correlations of FI and MOD with body size (SL) were observed in clams collected in both years (Figs. 7C, D). In the case of MI, however, a significant size relation was found only for January 1998, and for PGA, none of the years showed a significant relation (Figs. 7A, B). The relations of these indices with body size were best fitted by quadratic regression curves that peaked between 76 and 85 mm SL.

4. Discussion

4.1. Computer-based image analysis of the reproductive condition of *L. elliptica*

Microscopic examination of histological sections of gonads has

been used to study marine bivalve reproduction over many decades. Reproductive status is often categorized according to a numerical scale that describes various stages of gonadal development (Loosanoff 1942; Kennedy and Krantz 1982; Shafee 1989; Barber 1996). While direct microscopic examination is relatively simple, scoring or grading gonadal condition using the numerical scale is rather subjective (see reviews in Lucas 1982 and Heffernan and Walker 1989). Reproductive condition is also often evaluated by using objective measurements such as gonad thickness or width, which are not applicable in most cases (Loosanoff 1965; Gauthier and Soniat 1989). Oocyte size, percentage area occupied by eggs in a follicle, and cross-sectional area occupied by gonads determined by stereology and planimetry have also been used widely to evaluate the status of gonadal development in bivalves (Morvan and Ansell 1988; Morales-Alamo and Mann 1989).

In our study, MI and PGA did not differ significantly between clams from the two years of collection, but FI and MOD did (Fig. 7). This finding suggests that planimetric indices, particularly FI and MOD, are more reliable and sensitive than the conventional MI in quantitative assessment of *L. elliptica* reproductive status. The planimetric technique used in this study employed computer-based image analysis and was fast and convenient to use; this technique would also be useful for processing large numbers of samples for seasonal or longer-term studies.

It is interesting to note that both FI and MOD values were higher in January 1998 than in December 1995. The higher MOD values in January 1998 indicate that oocytes were more mature (Fig. 6), probably at their developmental peak and ready to spawn, in January. The percentage of developing and mature oocytes (> 100 μm) in January 1998 (≈32%) was about double that in December 1995 (≈16%).

4.2. Reproductive traits of *L. elliptica* at Marian Cove

4.2.1. Oocyte size

As shown in Figure 2, ripe eggs were enclosed in a thick (9.1-μm) gelatinous layer. Mature eggs, measured using image analysis, averaged 156.6 μm in diameter, with a maximum of 192.1 μm in diameter, excluding the gelatinous layer. At Potter Cove, a few kilometers away from the study area, Bigatti et al. (2001) reported a mean mature oocyte size of 195.2 μm in diameter, with a maximum of 220.7 μm in diameter, including the gelatinous layer. The difference in oocyte diameter measured in this study and that by Bigatti et al. (2001) can be explained in part by variation in thickness of the gelatinous layer. I did not include the gelatinous layer in oocyte size measurements, since its thickness varies with developmental stage: on average, 34.8 μm in developing oocytes versus 9.1 μm in ripe eggs. Ansell and

Harvey (1997) reported that developing eggs (from non-preserved oocytes) were 162.3 ± 5.4 μm in diameter. This oocyte size is similar to our measurements when shrinkage of oocytes during fixation and dehydration for histology is taken into account.

4.2.2. Size and putative age at first reproduction

I estimated putative age of the clam using the *L. elliptica* growth curve reported by Ralph and Maxwell (1977); clams ranged from 3 years (34.4 mm SL) to 44 years old (106.0 mm SL). The 3-year-old clam collected in December 1995 contained fully mature eggs covered by a gelatinous layer and which were considered to be in the spawning stage. These data suggest that *L. elliptica* may achieve sexual maturity as young as three years old at Marian Cove. Bigatti et al. (2001) described an individual *L. elliptica* near Potter Cove (27 mm SL), estimated to be less than two years old, that had ovarian follicles and testicles. Their result pushes back the age at first reproduction to as early as two years. Due to the limited number of clams collected in this study, no clams with age of two or younger were included in our data set. It is very likely that *L. elliptica* at Marian Cove also achieve sexual maturity at the age of two as suggested by Bigatti et al. (2001) since our study area is geographically close Potter Cove.

4.2.3. Variation in reproductive output with body size

As shown in Figure 5, MI, FI and MOD were best fitted to a quadratic regression curve. These indices were significantly described by the curve with a peak between 76 and 85 mm SL. These data indicate that *L. elliptica* in this size range have the highest reproductive output. Several studies of reproduction in marine bivalves, including oysters, mussels, and hard clams, have shown that gamete production tends to increase with size and age and then plateau (Morvan and Ansell 1988; Levitan 1991; Choi et al. 1993; Franz 1996; Kang et al. 2003). Urban and Mercuri (1998) reported that gonad development in *L. elliptica* rapidly increased with size in individuals of 65 to 90 mm SL. The reproductive effort of older and larger clams (> 90 mm SL) may decrease after they have reached an optimal size for gamete production.

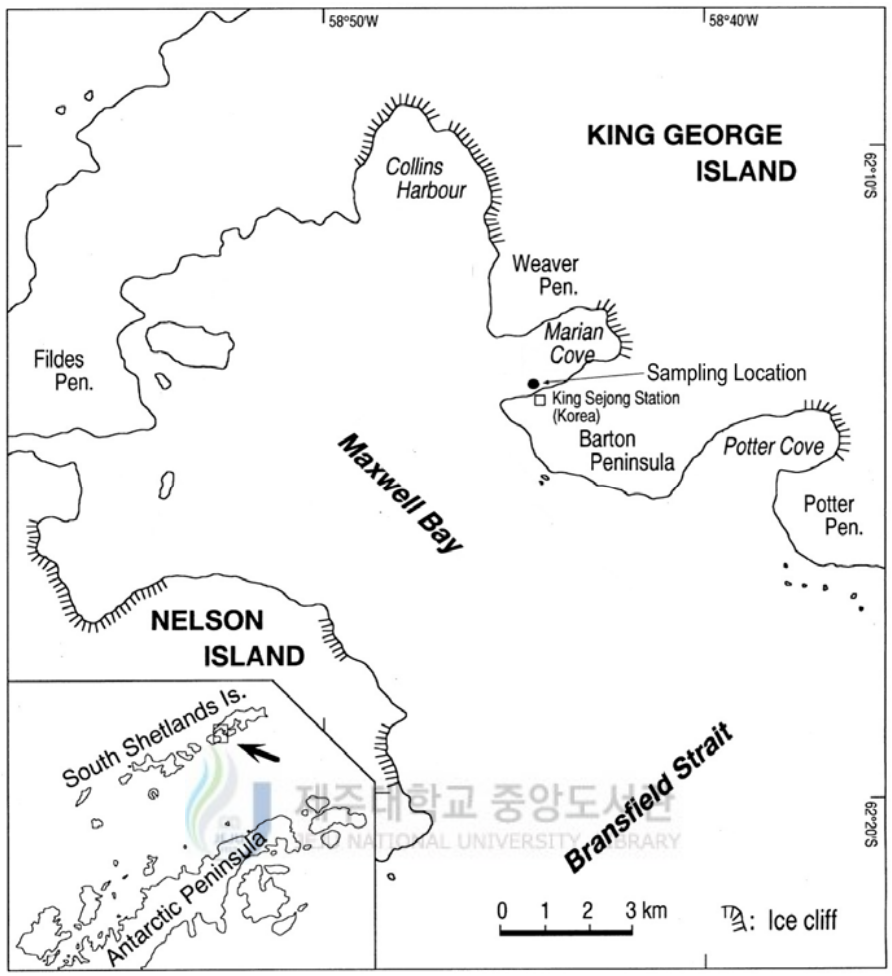

Fig. 2. Sampling location of *L. elliptica* at Marian Cove.

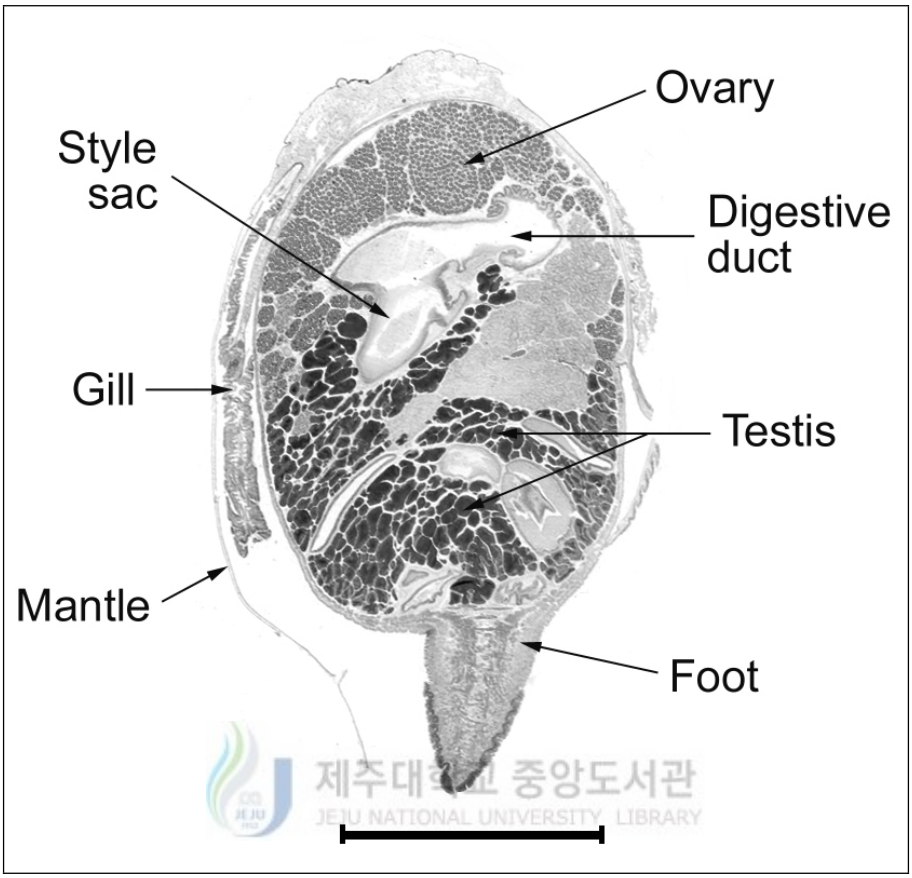

Fig. 3. Cross-section of *L. elliptica*. Scale bar = 1 cm.

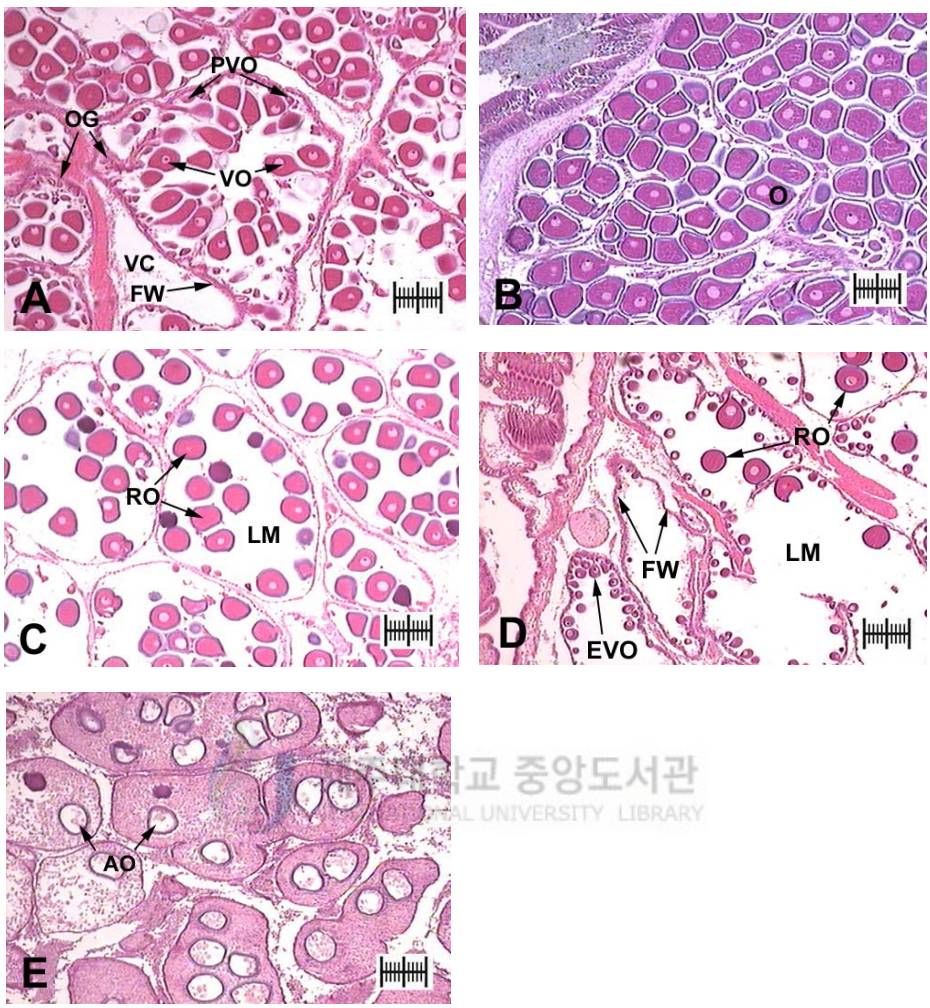

Fig. 4. Photomicrographs of ovaries from *L. elliptica* collected in December 1995 (scale bar = 200 μm). (A) Developing stage: ovary contains a small oogonium (OG), previtellogenic oocytes (PVO), and vitellogenic oocytes (VO) along the follicle wall (FW). Follicles expand into the vesicular connective tissue (VC) and few gaps are found between follicles. (B) Fully developed stage: large eggs are attached to germinal epithelium and the follicle is completely filled with ova (O). (C) Spawning stage: partially spawned ovaries contain loosely packed relict ova (RO) and spaces in the lumen (LM). The follicle wall is noticeably thinner. (D) Spent stage: follicle wall is withered and some relict ova still remain within follicles. Early vitellogenic oocytes (EVO) are attached to follicle wall. (E) Absorbing

stage: ovary degenerates and relict ova are reabsorbed (AO) into the thin follicle epithelium by phagocytic activities.

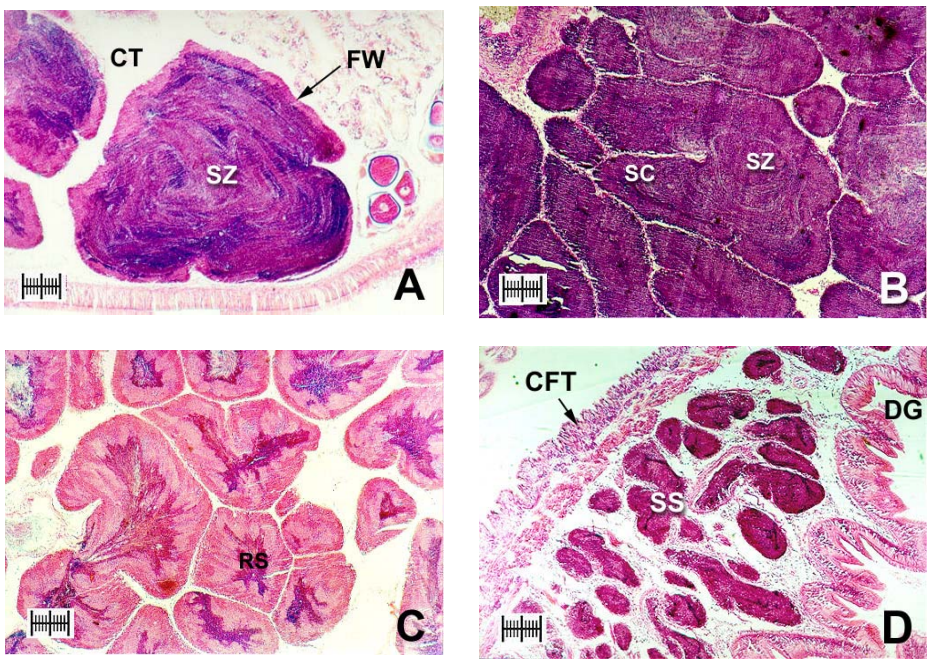

Fig. 5. Photomicrographs of testes (scale bar = 200 μm). (A) Developing stage: columns of spermatozoa (SZ) move to center of a growing testis with a thick follicle wall (FW). Most of the lumen is filled with dense radiating bands of spermatozoa (CT, connective tissue). (B) Fully developed stage: mature testes are filled with spermatocytes (SC) and spermatozoa, and are largely devoid of nutritive tissue. Dense bands of spermatozoa fill the majority of the lumen and often spiral into its center. (C) Spawning stage: partially spawned testes contain spaces vacated by spawned spermatozoa and nutritive phagocytes (RS, relict sperm). (D) Spent stage: spent spermaries (SS) are partially empty, although relict spermatozoa may be present. Follicle wall is thin and testis volume is small (CFT, connective fibromuscular tissue; DG: digestive gland).

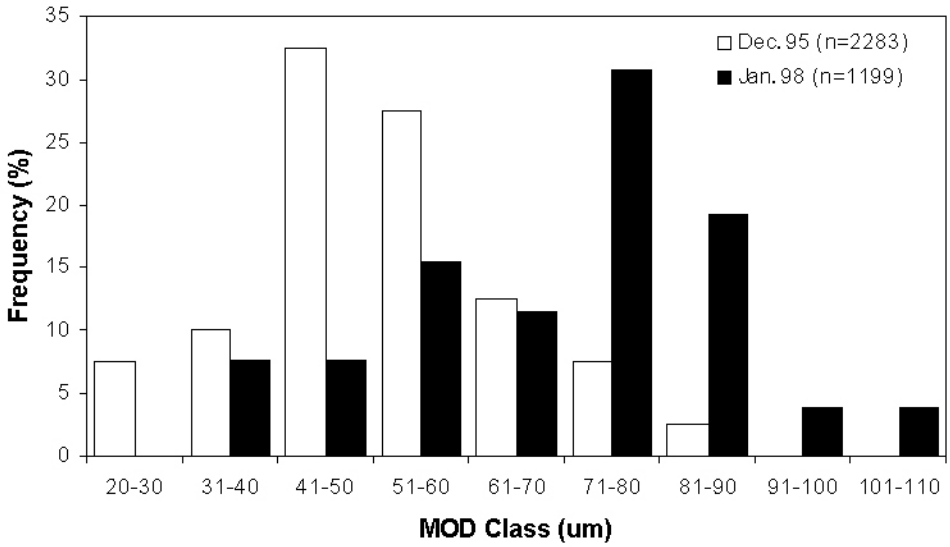

Fig. 6. Frequency distribution of MOD in December 1995 and January 1998 (n: number of oocytes measured).

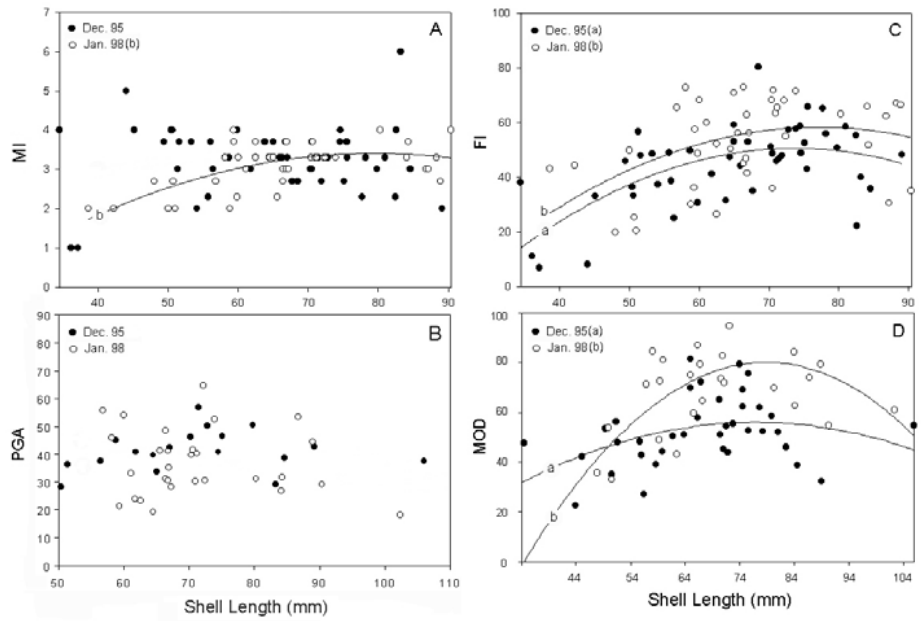

Fig. 7. Comparisons of MI, PGA, FI and MOD between *L. elliptica* collected in December 1995 and January 1998. There was no significant difference in the MI and PGA between the two years (A and B), while the FI and MOD values were significantly higher in January 1998 than in December 1995 (C and D). Regression lines: (A) MI (Jan. 98) = $-0.000864SL^2 + 0.142SL - 2.40$, $r^2 = 0.41$, n=42, $P < 0.001$; No significant size relation was found for Dec. 95. (B) PGA; No significant size relation was found for both years. (C) FI (Dec. 95) = $-0.0133SL^2 + 2.26SL - 43.35$, $r^2 = 0.36$, n=49, $P < 0.001$; FI (Jan. 98) = $-0.0158SL^2 + 2.58SL - 46.88$, $r^2 = 0.19$, n=42, $P < 0.05$. (D) MOD (Dec. 95) = $-0.0132SL^2 + 2.024SL - 21.82$, $r^2 = 0.15$, n=38, $P < 0.05$; MOD (Jan. 98) = $-0.0405SL^2 + 6.40SL - 172.2$, $r^2 = 0.39$, n=26, $P < 0.01$

Table 1. Classification of *Laternula elliptica* gonad developmental stages (I–VI) with numerical scores (1–6).

Developmental stages	Numerical score	Histological description
Sexually undifferentiated (I)	1	Little or no gonadal tissue visible.
Developing (II)	2	Follicle expanded; growing ovary with vitellogenic oocytes and premature oocytes. Some mature gamete present.
Fully developed (III)	3	Most gametes mature; little connective tissue remaining.
Spawning (IV)	4	Fully mature eggs detached from the follicle. Spawning ovaries have thin follicle wall.
Spent (V)	5	Reduced number of gametes; some mature gametes still remaining; evidence of renewed reproductive activity.
Absorbing (VI)	6	Apparent phagocytic activities around gonadal tissues; ragged eggs or spermary.

Table 2. Mean follicle index (FI) and mean percentage gonad area (PGA) and at various stages of gonadal development in *L. elliptica*. Data from December 1995 and January 1998 are pooled. –, data are not available.

		Mean FI (%)	PGA		
			Testis	Ovary	Total
Developmental stages	I	0	0	0	0
	II	–	–	–	–
	III	52.75	23.43	16.65	40.08
	IV	37.70	16.04	12.05	28.09
	V	8.17	5.46	0.07	5.53
	VI	–	18.17	7.22	25.39

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Part II

Annual gametogenesis of the Antarctic clam, *Laternula elliptica* from Marian Cove, King George Island

1. Abstract

The annual reproductive cycle of the Antarctic soft-shelled clam, *Laternula elliptica*, in Marian Cove, King George Island, was studied over a 2-year period from February 1998 to January 2000. Annual changes in gonad maturation were investigated using histological techniques, including numerical scoring of the microscopic appearance of the gonad (maturity index, MI), measuring the percentage of area occupied by oocytes in a follicle (follicle index, FI), and assessing the oocyte size. In 1998, the monthly mean FI and MI increased significantly from October to November, peaked in December, and dropped rapidly from December to January. In February and March 1999, degenerated eggs were observed in the spent follicles. Degeneration and absorption of residual eggs by phagocytosis occurred mostly in February and March in 1998 and 1999, although the resorption process was observed year-round. Gonad maturation and spawning of *L. elliptica* have significant year to year differences; the gonad maturation and spawning occurred rapidly when food levels in the water column become sufficient, suggesting that food supply is critical to gonad maturation and subsequent

spawning. *L. elliptica* spawns annually and gonad maturation takes only a few months during the austral summer. There was a distinct seasonality to the annual gametogenesis, with absorption and resting in February and March, development and maturation in October and December, and spawning from December to February.

2. Materials and Methods

2.1. Sample collection

Clams were collected by SCUBA divers from depths of 20 to 30 m near King Sejong Station (62°13' S, 58°47' W) in Marian Cove, King George Island (Fig. 1). Twenty to thirty clams were collected on a monthly basis between February 1998 and January 2000, except in April, July, and September 1998, when we were unable to collect clams because of inclement weather. Collected clams were preserved in 10% buffered formalin *in situ* for histological analyses. Daily total chlorophyll concentration and water temperature data were obtained from a long-term monitoring program conducted at the station (KORDI 1999). Total Chlorophyll (Chlorophyll *a* + phaeopigments) concentration was determined *in vivo* using a fluorometer (Turner 10-AU-005). Water temperature was measured using a conductivity meter (YSI 610-D).

2.2. Histological Preparation

Upon arrival to the laboratory, shell length and height was measured to 0.01 mm. The soft body of the clams was then removed and wet weight of the tissue recorded to 0.01 g. Clam tissues were preserved in 75% ethanol. A longitudinal cross section of 1 cm thickness was made along the central part of the soft body. After dehydration, clearing and embedding in paraffin, the block was sliced to 5 μm and stained with Harris' hematoxylene and eosin Y.

Degeneration of relict ova by hemocytes (i.e., phagocytosis) was examined using the hemocyte-specific Giemsa staining for paraffin sections. Briefly, the dewaxed section was rinsed in ethanol and distilled water. The section was stained in a glass jar in a mixture of Giemsa stock and distilled water in a water bath at 56ºC for 40 min. After a rinse in distilled water, staining was contrasted using acetic acid. After blotting dry, clearing and mounting, phagocytosis was observed by activities of hemocyte stained blue color and phagocytosed granules stained eosinophilic red.

2.3. Analysis of annual gametogenesis

The reproductive condition of *L. elliptica* was examined under

a light microscope equipped with a video camera connected to a personal computer. Since *L. elliptica* is a hermaphrodite, gonadal development of testis and ovary were scored according to Table 3. Four microscopic fields were randomly selected from each slide and graded from 1 to 6 according to the criteria in Table 3. The qualitative reproductive condition of each clam was determined with an average score of the four microscopic fields, and the average score was used as the maturity index (MI).

Gonad and oocytes development of the ovary was also analyzed using a planimetric technique (Kang et al. 2003). From the digitized images of gonadal tissue, oocyte diameter and area in a follicle were measured using an image analyzing software. An oocyte diameter was measured from a microscopic image of the ovary, and mean oocyte diameter (MOD, μm) was calculated using 40 to 50 oocytes selected in follicles. Then, the frequency distribution of the oocytes was analyzed to identify the monthly variation of oocyte size and growth. The normality of the frequency distribution of oocytes was tested the symmetric and/or asymmetric distributions using the Kolmogorov–Smirnov tests (Sokal and Rohlf 1981). One-way ANOVA test and Pearson's correlation were conducted to compare similarity and/or difference of the frequency distributions in the sampling period. Various sizes of oocytes were classified according to the different oogenic stages. The percentage area occupied by

oocytes in a follicle, or follicle index (FI, %), was determined using three to six follicles selected from the histological slide of each clam. The percentage between follicle area and total area of oocytes was then calculated. The average FI per month and standard deviation of each clam were calculated to analyze the temporal fecundity and spawning time of the clam. One-way ANOVA was used to analyze the annual and interannual differences of the FI among sampling dates. Two samples t-test was used for comparisons after one-way ANOVA analysis. Regression analysis was performed to test the correlation between monthly FI and total chlorophyll concentrations in the water column at a significance level higher than 99 %.

3. Results

3.1. Seawater temperature and total chlorophyll

Water temperature and total chlorophyll concentrations in surface seawater varied monthly (Fig. 8). Water temperature varied from –1.8°C in August 1998 to +4.5°C in January 1999. Water temperature dropped from February to August, and gradually increased from October to January. Daily total chlorophyll concentrations varied widely from 0.1 to 12.2 μg/L, with an irregular seasonal pattern year-round. Algal blooms (1.07–12.2 μg/L) occurred

from late September to early October 1998 in the early austral spring, and the algal blooms in the time was highly increased relative to the same time in 1999. In addition, another bloom occurred from December 1999 to January 2000 in the austral summer. Blooms were most intense in early spring. Daily water temperature and total chlorophyll concentrations were averaged in each sampling month. The averaged values were then correlated with the reproductive indices (MI, FI and MOD).

3.2. Microscopic observation of gonad

Seasonal changes of the female part of gonadal tissues are shown in Fig. 9. Figure 9A shows resting stage, and shrunken or vacated follicles are commonly observed in this stage. Primary oocytes (PO) and relic ova (RO) can be infrequently observed along the follicle walls. Figure 9B shows developing stage, with large number of early vitellogenic oocytes (EVO), previtellogenic oocytes (PVO) and vitellogenic oocytes (VO) are dominant along the germinal epithelium resulting in expansion of the follicles. Developing stage was observed from March to September, and a few ripe eggs are often present in the follicles in this stage. A fully developed stage is characterized by follicles which are greatly expanded and completely

filled with fully mature eggs (Fig. 9C). The proportion of mature females increased in December when the mature gonad enlarged into the connective tissues and occupied much of the gonad space. The outer margin of ripe eggs was covered with a gelatinous membrane with 12-20 μm in thickness. A few number of PO are often found along the germinal epithelium. The spawning stage of the clams collected in January 1999 (Fig. 9D). Partly spawned ovaries were loosely packed with eggs in the follicles. Spent ovaries were observed in late February to March in 1999 (Fig. 9E). Small numbers of RO and VO can be observed in the follicles, and the follicles tend to decrease the size. In the absorbing stage, ROs are reabsorbed by phagocytic activities in the follicles (Fig. 9F). Some degenerating ova are surrounded by the hemocytes, indicating that the eggs were phagocytosed by hemocytes. Reabsorbing eggs in the ovaries were observed throughout the year (2-28%).

Figure 10 shows seasonal changes of male part of gonadal tissues. In resting stage, most of the follicle walls were thin and lumens were empty (Fig. 10A). In the developing stage, spermatocytes (SC) began to proliferate to the center of the lumen, and maturation of spermatozoa (SZ) rapidly progressed in the center of follicle. Thick differentiated bands between SC and SZ were observed in the follicles (Fig. 10B). In mature spermaries, the mass of mature SZ increased in volume (Fig. 10C). Most of the SZ was observed in the center of the

lumen. In spawning spermaries, follicles have numerous gaps with spawning SZ and relict spermatogenic cells still occupy the lumen (Fig. 10D). Figure 10E shows spent testes, and the follicle walls were constricted while lumens are empty except for relict SZ. In the absorbing stage, shrunken follicles were observed with relict or degenerating spermatogenic cells (Fig. 10F).

3.3. Annual variations of MI

Of the 637 clams examined, the smallest clam analyzed was 34.5 mm, estimated to be three years old (Ralph and Maxwell 1977), and exhibited a mature gonad. Microscopic observations of the gonad revealed that gonads of *L. elliptica* showed cyclic changes year round (Table 4). Mean MI of the clams varied from 1.7 in March 1998 to 4.5 in February 1999, and reached a peak when water temperature was near 1.3ºC. The mean MI from May to August was similar in both years. However, we found significant interannual variation in the MI value during the austral summer periods. The mean MI was significantly higher in October and November 1998 than the MI at the both months in 1999 ($P < 0.01$). The seasonal variation of MI was significantly correlated to the variation of total chlorophyll concentration ($P < 0.001$) (Table 5).

Most of the clams displayed in early or mid-developing stages

in February 1998 (72.6%). The proportion of fully matured and spawning stages increased from October (60.0%, 25.3% respectively) while the spawning stage steadily increased from November (32.0%) to December (38.0%). In 1999, an extended spawning stage was observed in January (47.4%), and the ovaries also displayed a spent stage (30.3%). Spawning gradually decreased from February (12.9%). Occurrence of the absorbing stage was observed in year round while the stage was highest in February (27.9%). In March, the gonad displayed a developing stage (88.6%) and the stage increased up to October (88.5%). Sexually ripe clams were observed from November 1999 (25.0%) to January 2000 (60.8%) when water temperatures reached on 0.9 and 1.8°C, respectively. In addition, the clams collected from December-January were also in spawning (19.5-22.6%). Gonadal development of the clams showed certain cyclic changes in this study despite a protracted development time, March to October.

3.4. Annual variations of oocyte diameters and growth

Monthly frequency distributions of the oocyte size class are shown in Fig. 11. The class cohorts showed a clear growth pattern of small oocytes. The small oocytes (< 60 μm in diameter) were dominant in March in both years. The oocytes size class of 61-100 μm moved up to the size class of 81-120 μm from May to October 1998.

The high frequencies of the oocyte of 81-150 μm in diameters were observed in October and November 1998 ($P < 0.05$). The size class of 121-150 μm reached a peak in December 1998 (48%). The size of oocyte was decreased in January 1999 while the size class of 101-120 μm increased about 20% in February 1999. In March 1999, the female gonad resumed oogenesis with the small oocytes with 21-60 μm in diameter (65%). The size class of 61-100 μm began to increase from April-July 1999. The size class of 81-120 μm moved up to the size class of 121-150 μm from November 1999 to January 2000. The size class of 120-150 μm reached a peak (35%) in January 2000. The asymmetric distributions (i.e. bimodal distributions) of oocyte size were observed from February to June 1998 and from March to May 1999 when the small oocytes were mainly developed in these months. The symmetric or less asymmetric distributions (i.e. unimodal distributions) were showed in December 1998, January to February 1999 and June 1999 to January 2000.

Seasonal variations of the different size classes of oocytes are shown in Fig. 12. The small oocytes, such as the PO and PVO with 21-40 μm in diameter, were about 60% of the oocytes in March 1999 (Fig. 12a). The proportion of oocytes of 41-60 μm (i.e. EVO) was about 35% in April-May 1999 (Fig. 12b). The oocyte size with 61-80 μm (i.e. VO) reached a peak at 34% in October 1999 while the lowest rate (1.3%) was observed in March 1999 (Fig. 12c). Figure 12d shows

the oocytes with 81-100 μm in diameter (i.e. premature oocytes) reached a peak at 25% in February 1999 and the lowest frequency was about 1% in March 1999. The thick gelatinous membrane was associated with the 81-100 μm size class (Fig. 13), indicating that the membrane-covered oocytes were close to maximum growth and ready to spawn. The oocytes of size class with 101-120 μm reached a peak at 34.5% in February 1999 (Fig. 12e). The oocytes decreased rapidly from February-March. The number of large oocytes (> 121 μm) increased from October-December while the oocytes decreased suddenly from December to January (Fig. 12f), indicating that the clam spawned ripe eggs in the time.

Figure 14 shows seasonal variations of the monthly FI from February 1998 to January 2000. The highest FI was recorded in December 1998 (70%), and was similar with the FI (68%) in December 1999. The minimum FI was estimated in March of both years (19%) when the clams were in spent and absorbing stages. The FI were not differed from May to August 1998 and from May to September 1999. The FI values significantly increased from October (38%) 1998 ($P < 0.01$). The maximum FI was observed in December 1998 (70%) when the clams were sexually mature or ready to spawn. The FI value significantly decreased to 35% in January 1999, and also decreased about 20% from February to March 1999 ($P < 0.01$). The main spawning of clams commenced from December 1998 to January

1999 and continued through February and March 1999, and 90% of the ripe eggs were released for spawning in two spawning peaks. In addition, the variation of FI was strongly correlated to the variation of total chlorophyll concentration in the water column ($P < 0.01$).

3.5. Resorption process of the relict ova

The absorbing stage was observed year round, while degenerating and absorbing the residual eggs was mainly observed in February-March (Table 4). Figure 15 shows typical degenerating ovaries of *L. elliptica* collected in February 1999. Invaded hemocytes began phagocytic activity to the relict ova (RO) with wounding gelatinous layers (Fig. 15A). The resorption process was started with the degeneration of the gelatinous membranes. The membranes and germinal vesicle of degenerative ova were phagocytosed by hemocytes, although some RO remain in the follicular lumen. Invading of hemocytes into the follicular lumen, the hemocytes began the phagocytosis to the remaining yolk granules (Fig. 15B). Hemocytes were round in shape with biased basophilic oval nucleus (Fig. 15C). After phagocytosis, resorption was completed in the inner membrane. Hemocytes were also observed around the follicular epithelium (Fig. 15D). At the end of the process, relics of phagocytosed materials were observed at follicular epithelium as

rounded opaque and eosinophilic corps (Figs. 15E, F).

4. Discussion

4.1. Large lecithotrophic eggs of *L. elliptica*

Laternula elliptica eggs, excluding the gelatinous layer, varied from 120 to 150 μm in size, and were much larger than eggs of the Antarctic scallop, *Adamussium colbeki*, which exhibits planktotrophic larval development (approximately 55 μm in diameter; Berkman et al. 1991). The relatively large egg size of *L. elliptica* suggests that these clams produce lecithotrophic eggs (Bosch and Pearse 1988; Berkman et al. 1991; Arntz et al. 1994; Ansell and Harvey 1997). Pearse et al. (1991) suggested that pelagic lecithotrophy may be an adaptation to poor food availability in Antarctic waters. Ansell and Harvey (1997) reported that trochophore larvae of the clam were observed 5 days after fertilization, and the larvae had developed to shelled larvae of about 200 μm in size, 18 days after fertilization. Larval settlement of *L. elliptica* has been mainly observed in late April and May (Bosch and Pearse 1988). The size of shelled larval *L. elliptica* as previous study is somewhat larger than the size of mature eggs (120–150 μm in diameter) in the present study, indicating that fertilized eggs rapidly developed to pelagic larvae just after the spawning season (January–

February). The large size of the eggs is believed to be an important reproductive strategy in *L. elliptica* for successful recruitment of offspring.

4.2. Food availability and the clam reproduction

Ahn et al. (2001) suggested that the low metabolic rate of *L. elliptica* was selected to maintain low energetic costs in Antarctic water. As a result of accumulating energy in storage tissues during phytoplankton blooms (Brockington 2001; Ahn et al. 2003), *L. elliptica* allocates a relatively large proportion of energy to reproduction (Clarke 1987). The mass of Antarctic bivalve (i.e., *Yoldia eightsi* and *L. elliptica*) gonads was significantly correlated with austral summer plankton blooms (Peck et al. 2000; Ahn et al. 2003). Metabolic rates and tissue energy concentrations of *L. elliptica* were high in this season (Brockington 2001). The blooms led to high somatic growth rates, and then a peak in gonad maturation occurred in the austral summer (Ahn et al. 2003). Oocytes steadily grew to maturity between June and December. Most of the ripe eggs occurred in December and January. However, oocyte growth and gonad maturation were intensive during the period from October to December in both years ($P < 0.01$). Microscopic examination of the gonads revealed that the clams became sexually mature just before the summer phytoplankton bloom. Gonad maturation rapidly increased

from October in both years, as a consequence of optimal nutritional conditions in the same months. Bimodal oocyte frequencies were observed from February to June 1998 and from March to May 1999, whereas unimodal oocyte frequencies were observed in December 1998, January to February 1999, and June 1999 to January 2000 (Fig. 5). The annual development of various oocytes has an extremely seasonal rhythm, and small oocytes 21–40 μm in diameter dominated in March of both years. The seasonal levels of total chlorophyll in the water column were significantly correlated with oocyte growth and gonad maturation (Table 3, $P < 0.01$). Ahn et al. (2003) reported that clam gonad mass increased significantly in October 1998 relative to the same time in 1999, indicating food availability is a key factor regulating gonad maturation. Thompson (1972) reported that the energetic transfer of ingested food from the digestive gland to the gonad in mussel occurred within approximately 7 days when adequate food was presented. This rapid conversion could support the maintenance of gametogenesis. Gonad maturation in *L. elliptica* occurred rapidly, within 3 months from October to December 1998 when food levels in the water column became earlier sufficient than the same time in 1999; the peak of gamete production coincided with maximum food availability. In the present study, total chlorophyll level was not corresponded with water temperature cycle during the period from late September to early October 1998. The MI, FI and

MOD were strongly correlated with the total chlorophyll concentration than water temperature in the present study. As a result, the MI and MOD in these times could be significantly higher than the two indices recorded in 1999. The food level in these times also significantly affected to the growth of tissue mass and gonad maturation relative to the effect of water temperature (Ahn et al. 2003). As previous study (Ahn et al. 2003), these unusual spring blooms were obvious evidence concerning how available food in the water column effect to clam's reproduction. These results indicated that *L. elliptica* are sensitive in relation to their gametes production when available food is sufficient in the water column. This suggests that the clams can promote larval or juvenile survival by spawning when there is an early nutrient source of suspended or deposited phytoplankton on a substrate.

Several studies have estimated the spawning time of *L. elliptica* by investigating the reproductive condition of individuals collected from different Antarctic nearshore areas using direct and histological techniques (Table 6). These studies reported that the major spawning of *L. elliptica* occurred during the summer months. In Potter Cove, a small bay only a few kilometers from Marian Cove, the major spawning of *L. elliptica* occurred in January and February; up to 75% of the clams collected in these months exhibited spent gonads (Urban and Mercuri 1998). At Marian Cove, spawning of the clam has

been observed in December, January, and February (Ahn et al. 2003). At Signy, in the South Orkney Islands, *L. elliptica* collected in February were accidentally caused to spawn at the laboratory, possibly because of variable temperatures after changing an incubator (Ansell and Harvey 1997). However, south of Marian Cove in McMurdo Sound, the spawning activity of *L. elliptica* was highest in late March to early April, and continued to mid-May (Bosch and Pearse 1988), indicating some latitudinal differences in spawning time.

I found that spawning of *L. elliptica* occurs from December to February after phytoplankton blooms, and about 90% of the mature eggs in follicles were discharged at this time (Fig. 14). In addition, gonad mass also decreases by about 50% at this time (Ahn et al. 2003). Soniat and Ray (1985) reported that oyster fecundity was closely correlated with food availability during the annual gametogenic cycle. Park and Choi (2004) also suggested that the fecundity and spawning frequency of *Ruditapes philippinarum* would track the frequency of plankton blooms. I observed two spawning peaks: during the period from December 1998 to January 1999, and from January to February 1999 (Figs. 12, 14). Ahn et al. (2003) also reported that the major spawning season of *L. elliptica* occurred when proteins and lipids in the storage tissues of clams were decreasing.

Kang et al. (2003) reported that FI measurement using a planimetric technique provided reliable quantitative information on *L.*

elliptica reproduction. Seasonal variation in FI indicates variation in the reproductive effort of *L. elliptica*. Monthly changes in FI varied seasonally (Fig. 14), indicating that gametogenesis has a seasonality within an annual gonad growth pattern. Urban and Mercuri (1998) suggested that cyclic oogenesis took more than 1 year. In contrast, Bigatti et al. (2001) reported that the complete growth of small oocytes was possible in about 7 months. I found, however, that the FI increased significantly from October 1998 and 1999, and peaked in December 1998 and 1999 ($P < 0.01$). In addition, the total chlorophyll minimum in the water column from March to August was significantly correlated with the annual minimum FI ($P < 0.01$). The maximum FI was observed in December 1998, 1 month after the total chlorophyll maximum in the water column, indicating that the chlorophyll maximum caused rapid gonad growth in the clams. Our data suggest that the time required for eggs to mature for spawning at Marian Cove is 3 months under phytoplankton bloom conditions. Ahn et al. (2003) reported that oocyte development and the increase in gonad mass proceeded at a much slower rate from March to August 1999. After October, however, gonad mass began to increase rapidly, and the mass peaked in December 1999. *Laternula elliptica* spawning occurred from December 1998 to March 1999 and coincided with the sudden decrease in FI (90%) in this season.

4.3. Resorption process for regulating nutritional energy source

The annual gametogenic cycle in marine bivalves is often categorized into several stages, according to the microscopic appearance of the ovary or testis. In brief, the cycle can be summarized in four phases: a resting phase, when gonadal tissues are absent; the development and growth of ovary or testis; spawning to discharge eggs or sperm; and an absorbing phase, when residual or relic eggs are degenerated and absorbed (Loosanoff 1942; Seed and Brown 1977; Sastry 1979, see Table 1). After major spawning periods, residual eggs are often degenerated by hemocytes and absorbed to recycle the nutrient-rich cells. Such absorption activity is considered a reproductive strategy of marine bivalves (Loosanoff 1942; de Jong Brink et al. 1983; Dorange et al. 1989; Ituarte 1997). I observed resorption in the gonad of *L. elliptica*. Relict eggs rapidly shrunk and were degenerated by hemocytes (Fig. 15); degenerating eggs were observed throughout the year (Table 4). Dorange et al. (1989) suggested that the reuse of phagocytosed material from degenerated eggs was important in regulating valuable nutrients in the scallop, *Pecten maximus*, in which ripe eggs can be observed in the gonads year-round (Poulet et al. 1988; Mackie and Ansell 1993; Strohmeier et al. 2000) and the eggs are absorbed at any time in the reproductive cycle (Dorange et al. 1989). These materials allocate sufficient

energetic resources for gametogenesis (Thorson 1950; Strathmann 1990, 1993).

Urban and Mercuri (1998) observed that 60–80% of ovaries were ripe throughout the year in clams collected from Potter Cove, King George Island. Bigatti et al. (2001) re-examined Urban and Mercuri's (1998) histological data and concluded that *L. elliptica* at Potter Cove possibly prepared to spawn at any time in suitable conditions because degenerated eggs were not observed year-round. Contrary to the observations of Urban and Mercuri (1998) and Bigatti et al. (2001), the spawning activity of clams collected from Marian Cove was rather confined to spring and summer (from October 1998 to February 1999 and from December 1999 to January 2000). I also found a number of clams in the absorbing stage that exhibited degenerating ovaries (Table 4). The absorbing stage could be identified year-round, although intensive degeneration and absorption of relic or residual ova mostly occurred in February and March (Table 4). The degeneration and absorption process was detected on serial sections of soft tissues after observing some trace of phagocytic activity in the first tissue section. Phagocytic activity was further confirmed using the hemocyte-specific Giemsa staining technique, and the degeneration of residual eggs was visualized (Fig. 15). It is unclear why the absorption process was not observed in previous studies at Potter Cove, located only a few kilometers from Marian

Cove. Because Potter Cove and Marian Cove are in close proximity, it is unlikely that different environmental conditions between the two locations, such as water temperature and food availability, resulted in the reported differences. It is also unlikely that the absorption stage was absent in previous studies because of year to year variation in gametogenesis in the clam, because the gametogenic pattern in the reproductive strategy of marine bivalves is thought to be a consequence of long-term evolutionary adaptation (Clarke 1987; Pearse et al. 1991; Poulin et al. 2002). Alternatively, the discrepancy could be the result of different processes used in the preparation of the histological slides. Often, only a small part of the gonadal tissue is taken from the body of marine bivalves for preparation of histological slides; thus, some cells or tissues may be overlooked.

Because the eggs of *L. elliptica* are large and lecithotrophic, egg production may require abundant nutritional energy to support vitelline synthesis. Thus energy is a limited resource for adult *L. elliptica*. Phagocytic activity by hemocytes in *L. elliptica* affected only relict eggs, and phagocytosed materials derived from relict eggs should be deposited in follicular cells (Dorange et al. 1989; Ituarte 1997). This process benefits the clam by increasing energy availability in follicular cells after spawning, for use in subsequent vitelline synthesis at a low energetic cost. Absorbed vitelline granules deposited in follicular cells play an important role in providing

improved initial gametogenic conditions for the development of primary oocytes in periods when there are no phytoplankton blooms. Therefore, degeneration and absorption of relict eggs appears to occur after spawning to allow for energy resorption in *L. elliptica*. This process is fitting as an adaptation to the harsh environment of *L. elliptica* to avoid an excess energy budget.

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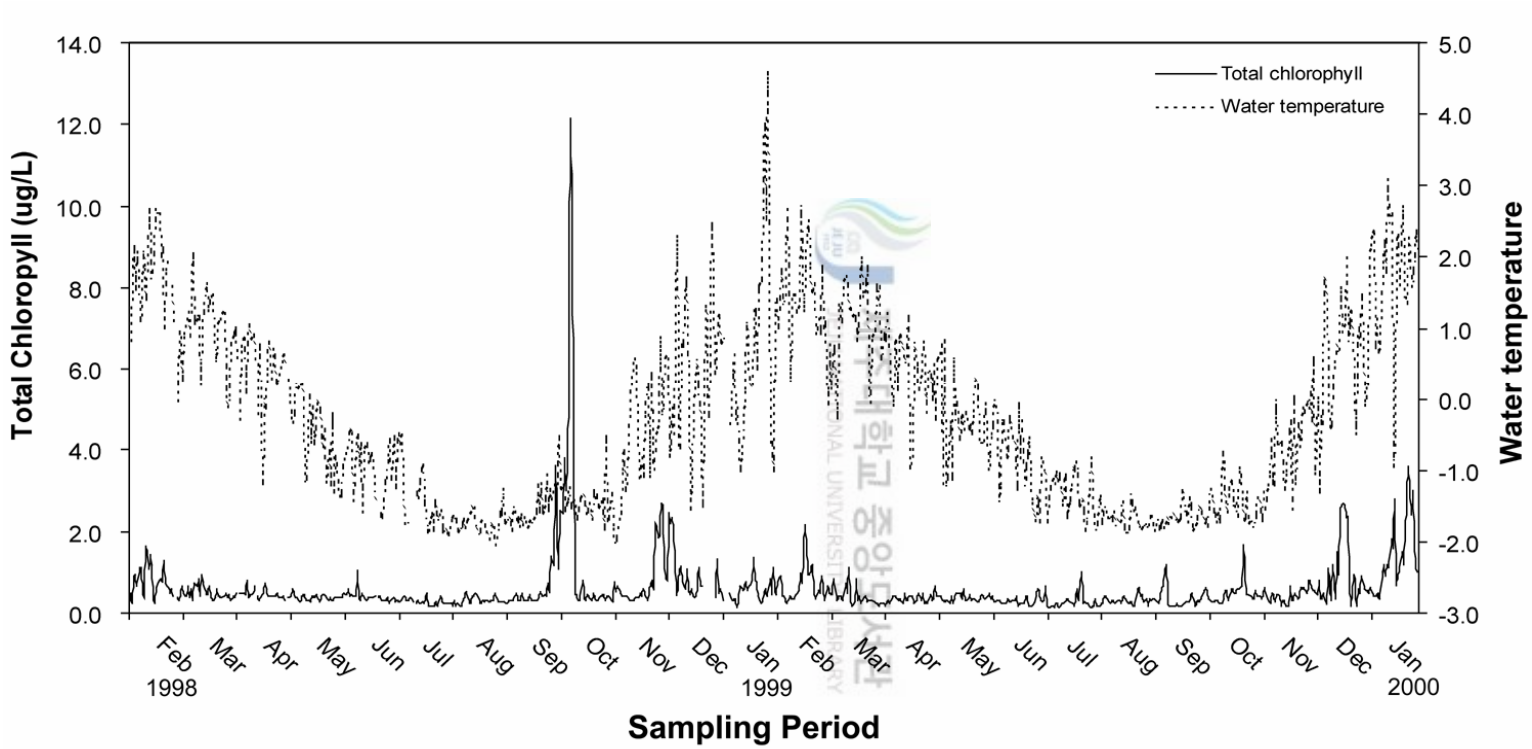

Fig. 8. Seasonal variations in water temperature and total chlorophyll *a* at Marian Cove.

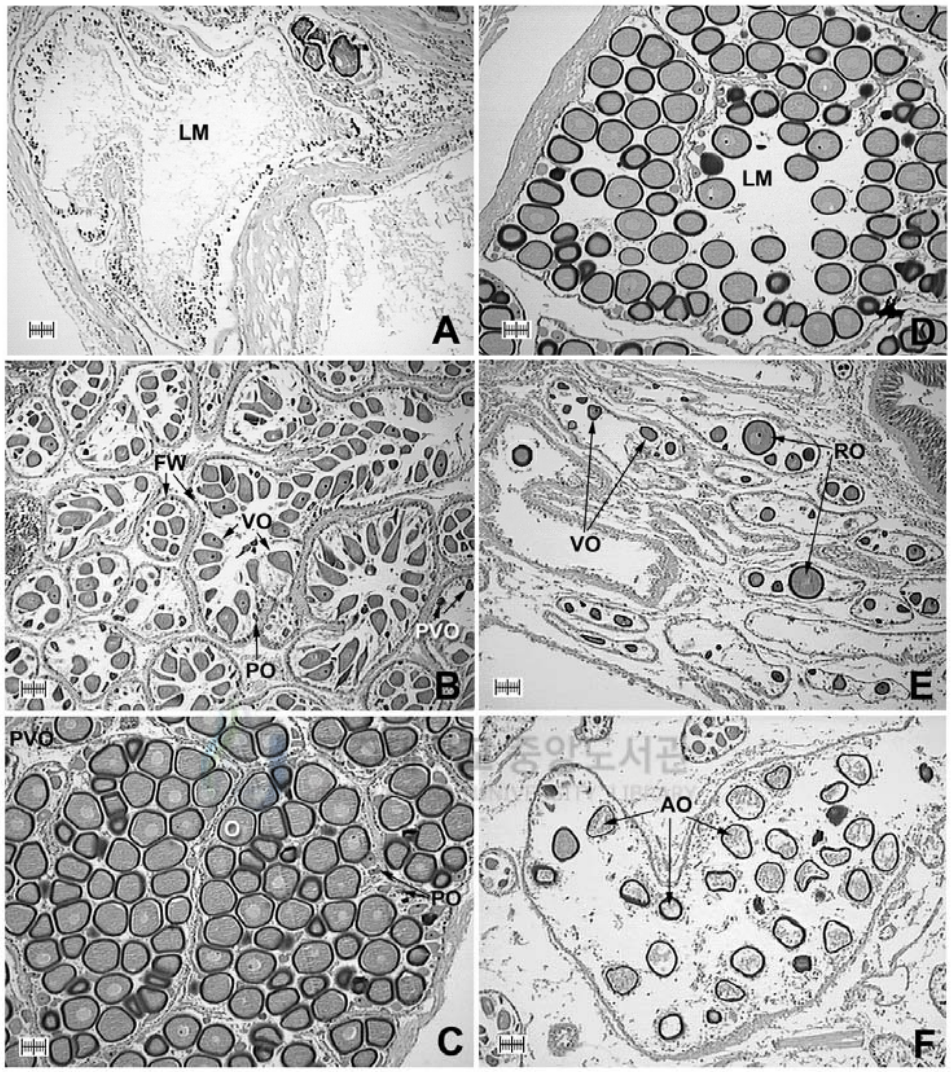

Fig. 9. Photomicrographs of ovaries. (A) Resting stage: gonadal tissues are little or no visible along the thin follicle wall. (B) Developing stage: growing ovary with primary oocytes (PO), previtellogenic oocytes (PVO) and vitellogenic oocytes (VO) along the follicle wall (FW). (C) Fully developed stage: mature ovaries packed with ova (O) and a thin layer along follicle wall. In germinal epithelium, very small PO is present. (D)

Spawning stage: partly spawned ovaries with loosely packed ova and the spaces vacated in the lumen (LM). (E) Spent stage: ovaries largely devoid of ova and shrinking ovary with VO and relict ova (RO). (F) Absorbing stage: relict oocytes will be reabsorbed (AO) and a thin follicle epithelium. Scale bar: 100 μm.

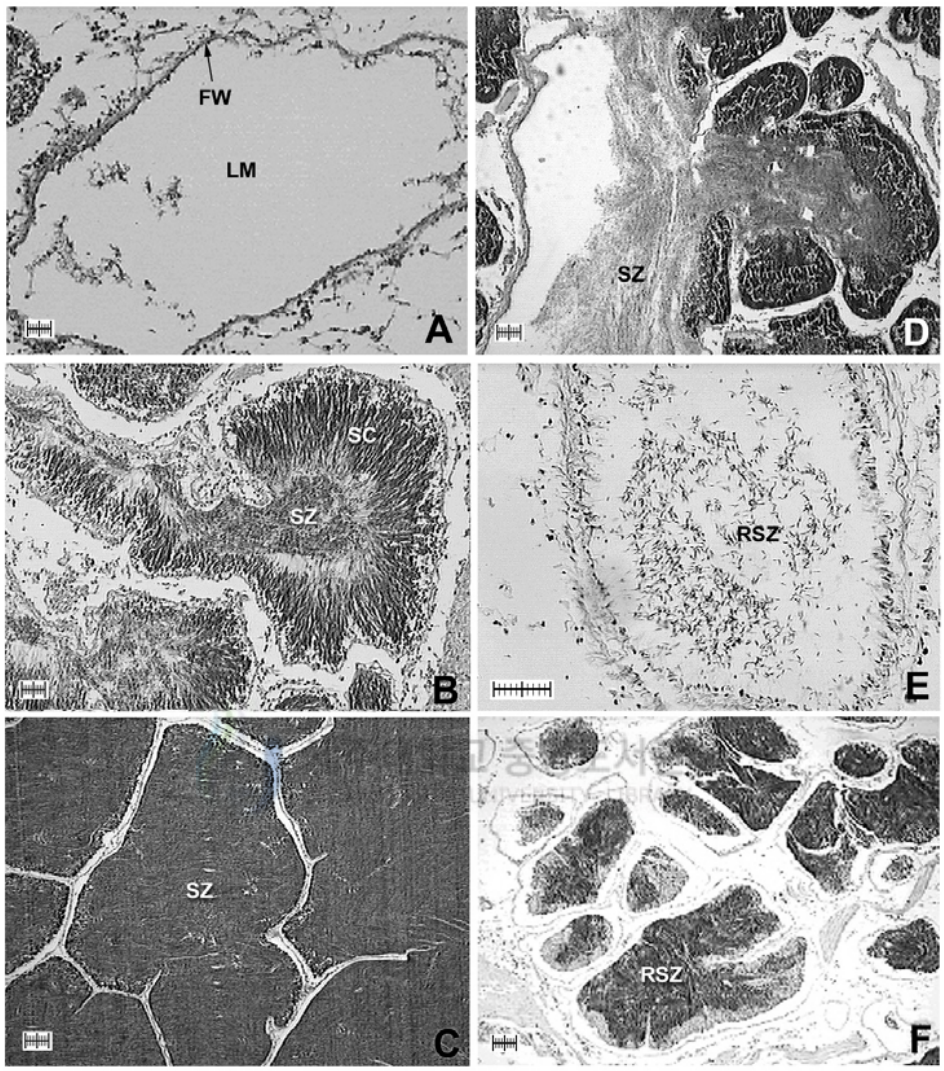

Fig. 10. Photomicrographs of testes. (A) Resting stage: resting ovaries are vacated with lumen (LM) and thin follicle wall (FW). (B) Developing stage: columns of spermatocytes (SC), spermatozoa (SZ) move to central part in growing testes. (C) Fully developed stage: mature testes filled with SZ and largely devoid of nutritive tissue. (D) Spawning stage: partly spawned testes with spaces vacated by spawned SZ. (E) Spent stage:

partly devoid of content, although relict spermatozoa (RSZ) may be present. (F) Absorbing stage: shrinking testes with thin follicle wall. Scale bar: 100 μm.

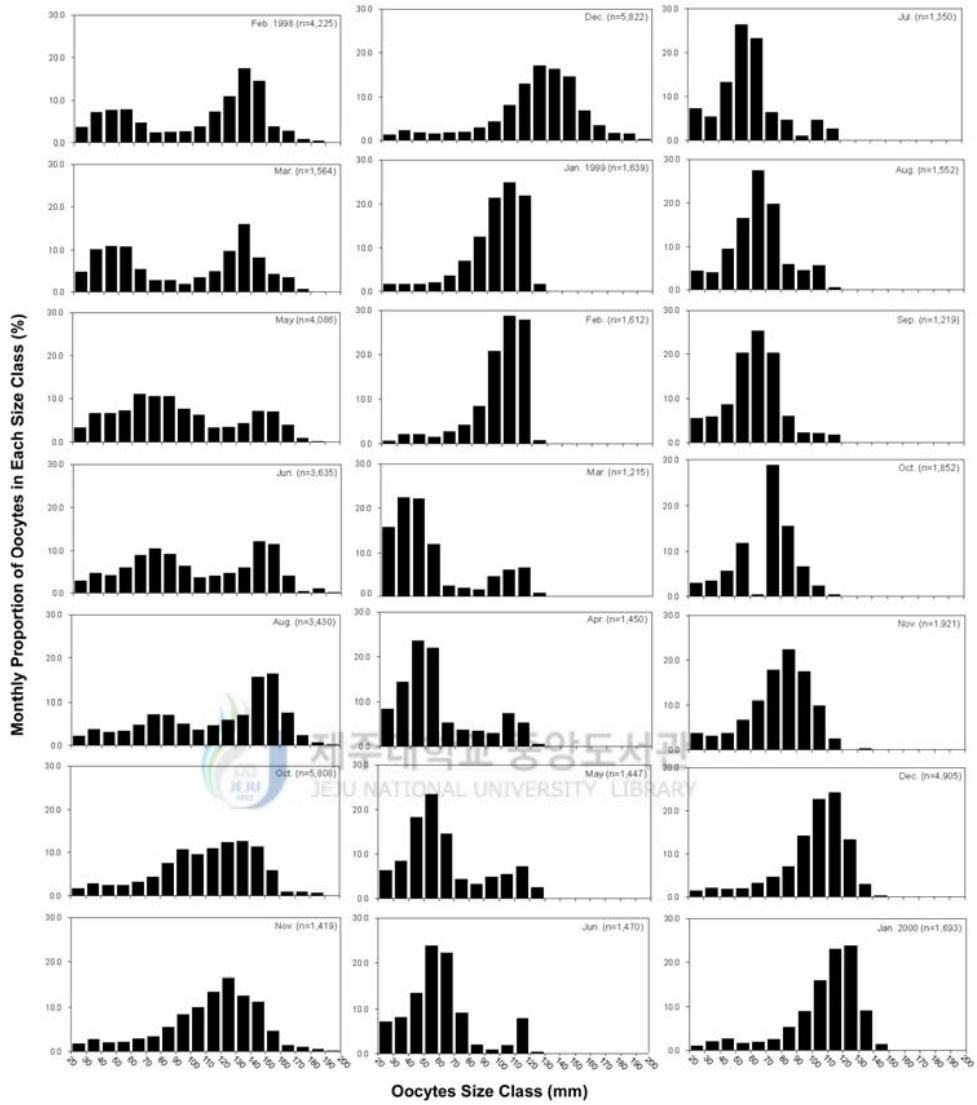

Fig. 11. Temporal changes in proportional occurrence of various oocyte with size-frequency distributions in MOD.

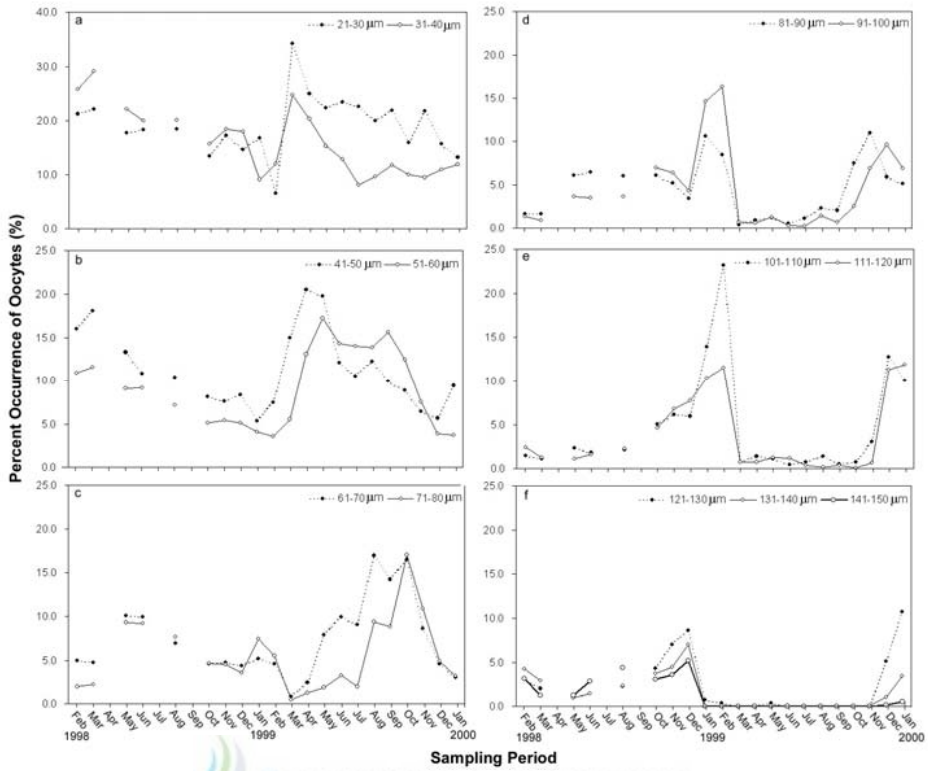

Fig. 12. Temporal changes in respective percentages in each oocyte classes characterizing the developmental size of oocytes distinguished in this study.

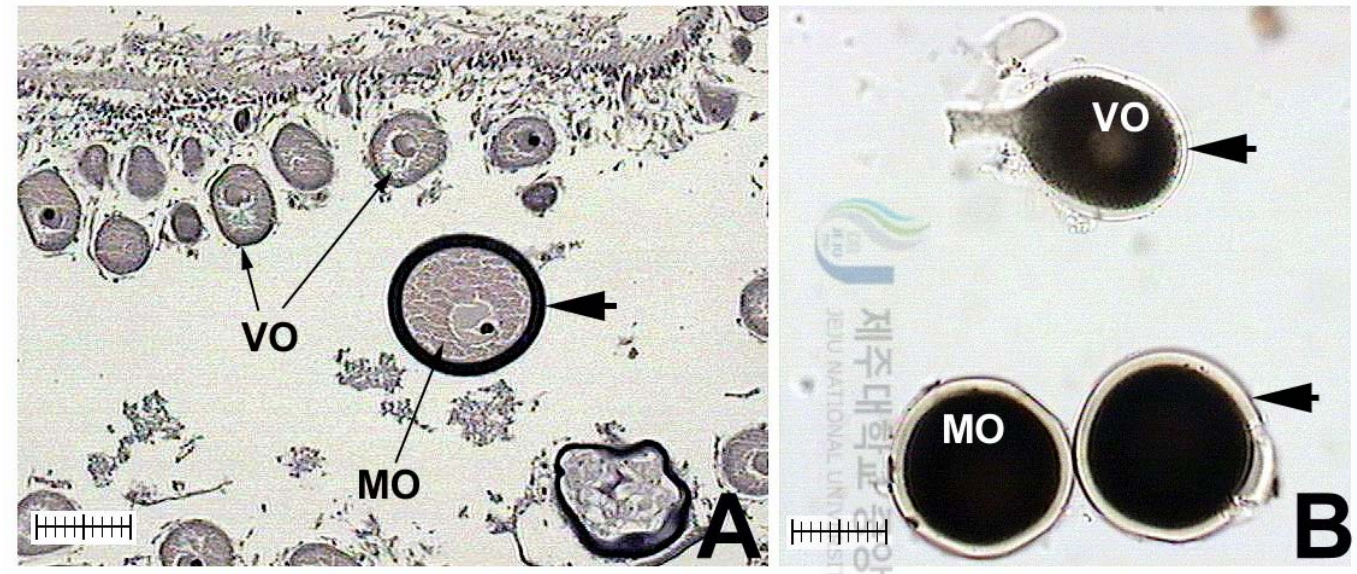

Fig. 13. Photomicrographs of different developmental stages of oocytes between vitellogenic oocytes (VO) and mature oocytes (MO). A: histological feature of the oocytes in gonad; B: extracted oocytes from gonad. Thick arrows indicate the gelatinous layers in each developmental stages of oocyte. Scale: 100 μm.

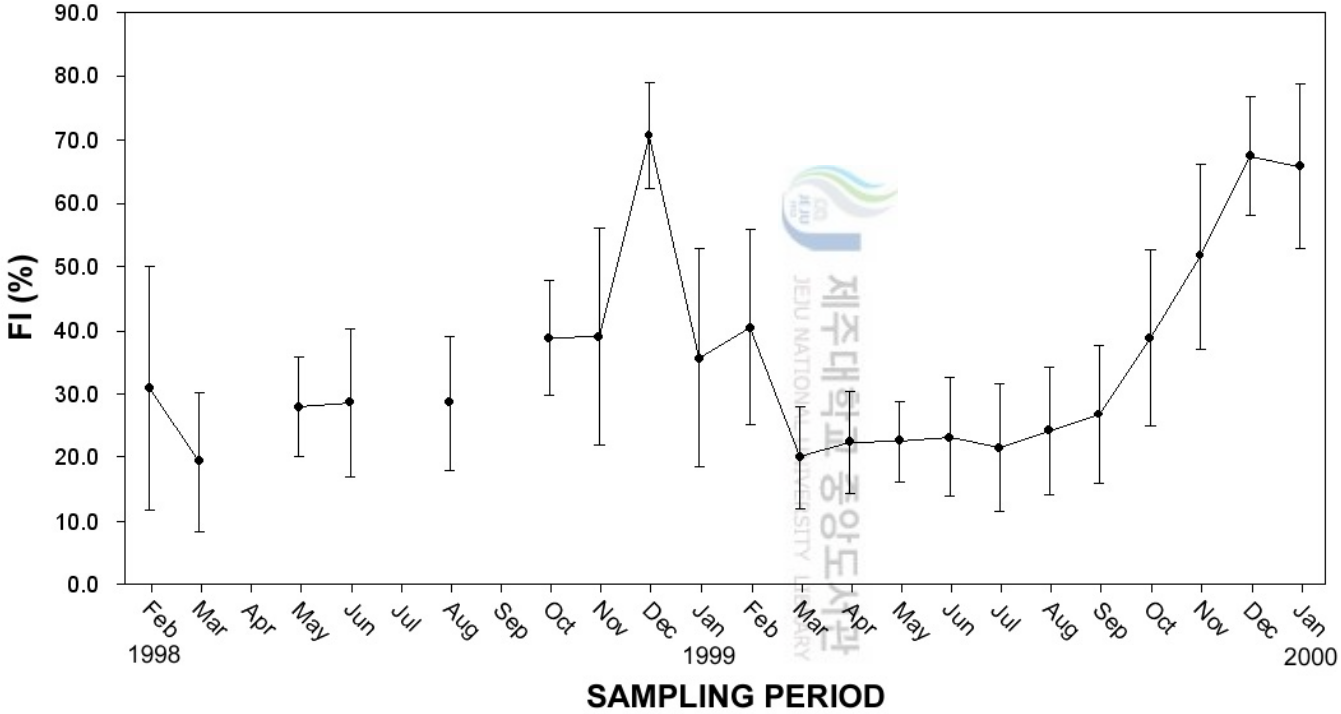

Fig. 14. Monthly changes in FI of *L. elliptica*.

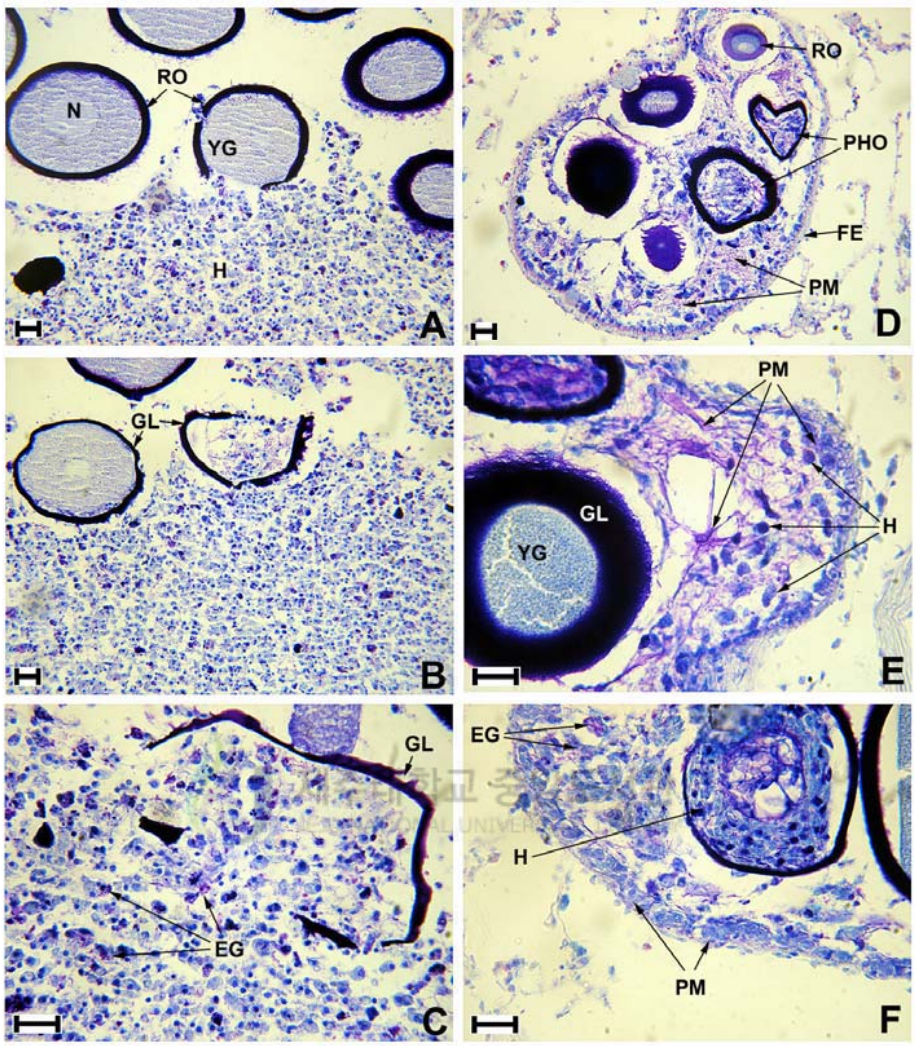

Fig. 15. Photomicrographs show resorption process in degenerative ovaries of *L. elliptica*. A-B: invaded hemocytes (H) in the follicular lumen, and the process of phagocytosis began to relict ova (RO) with nucleus (N), yolk granules (YG) and wound gelatinous layers (GL); C: transported eosinophil granules (EG) by H with fluttered GL; D: RO and phagocytosed ova (PHO) remaining in follicle and transported phagocytosed materials (PM) to follicular epithelium (FE); E: magnified image of

transported PM by H; F: magnified image of deposited PM on follicle. Scale: 20 μm.

.

Table 3. Developmental stage of *L. elliptica* gonad.

Developmental stages	Scale	Histological description
Resting	Ⅰ	Gonadal tissues are little and thin ovarian follicles
Developing	Ⅱ	Follicle walls consisting of primary oocytes, early vitellogenic oocytes, relict ova and absorbing ova. Ovarian follicles are gradually expanded and ovaries contain early vitellogenic oocytes and clusters of developing and vitellogenic oocytes along the follicle wall. Some mature gametes are present.
Fully developed	Ⅲ	Most gametes are mature; prespawning ovaries are filled with closely packed ova and little connective tissues are remaining.
Spawning	Ⅳ	Partly spawned ova are loosely packed with spaces vacated by spawned ova: reduced number of gametes; some mature gametes still remaining.
Spent	Ⅴ	Spent ovaries have thin walls and ova still remaining. Evidence of renewed reproductive activity.
Absorbing	Ⅵ	Apparent phagocytic activities around gonadal tissues, ragged eggs.

Table 4. Shell length (SL), tissue wet weight (TWW) and percentage of gonadal maturation with maturity index of *L. elliptica* used in this study.

Sampling Period	Clams used in analysis			Percentage of gonad developmental stages						Maturity index (Mean±SD)
	n	SL (mm, Mean±SD)	TWW (g, Mean±SD)	Resting	Developing	Fully Developed	Spawning	Spent	Absorbing	
1998										
Feb	21	76.4±10.9	31.7±8.9	8.3	72.6	14.3			4.8	1.9±0.6
Mar	9	76.8±10.6	32.1±9.4	25.0	58.3	11.1			5.6	1.7±0.7
May	19	72.7±11.3	26.6±7.6	5.3	76.3	18.4				2.1±0.3
Jun	30	70.9±10.0	26.5±8.9	5.0	70.8	24.2				2.2±0.3
Aug	22	74.8±10.7	27.8±8.5	6.8	60.2	33.0				2.3±0.5
Oct	30	76.6±9.5	30.0±8.7		1.7	60.0	25.3	3.0	10.0	4.0±0.7
Nov	29	73.3±13.6	28.1±10.9			45.0	32.0	10.0	13.0	4.4±0.9
Dec	58	66.6±12.7	24.5±10.5	1.4	10.0	33.9	38.0	6.9	9.8	3.3±1.3
1999										
Jan	19	72.3±7.1	30.7±7.8	3.9	1.3	3.9	47.4	30.3	13.2	4.4±0.6
Feb	35	67.9±10.9	24.9±8.8	4.3	16.4		12.9	38.6	27.9	4.5±0.8
Mar	34	73.8±8.6	36.5±10.3	7.6	88.6				3.8	2.1±0.4
Apr	25	71.8±7.6	28.8±8.7	5.0	82.0	2.0			11.0	2.4±0.9
May	19	78.5±10.8	32.8±9.3	11.1	83.3				5.6	2.2±0.8
Jun	21	75.1±10.5	29.4±8.0	1.2	95.2				3.6	2.1±0.5
Jul	48	70.7±9.2	25.6±6.2	4.3	85.3				10.3	2.4±0.9
Aug	36	69.3±7.9	23.2±7.3	7.6	86.8		2.8		2.8	2.1±0.5
Sep	25	71.8±7.7	25.0±8.7	7.0	82.0		4.0	1.0	6.0	2.3±0.6
Oct	26	66.6±8.6	21.3±5.3	1.0	88.5	8.7			1.9	2.2±0.3
Nov	19	73.2±9.0	30.2±10.6		69.7	25.0	1.3		3.9	2.4±0.5
Dec	59	65.9±11.9	26.3±9.7		12.7	62.2	19.5	2.2	3.3	3.2±0.6
2000										
Jan	53	74.3±11.7	36.4±11.7	2.4	6.6	60.8	22.6	5.2	2.4	3.3±0.6

Table 5. Partial correlation coefficients between total chlorophyll, water temperature and reproductive indices of *Laternula elliptica* used for analysis. *MI* Maturity index, *FI* Follicle index, *MOD* Mean oocyte diameter, *GI* Gonad index. Significance: ** $0.001 < P < 0.01$, * $0.01 < P < 0.05$

	Total chlorophyll	Water temperature	References
Water temperature	0.354		
MI	0.716 **	0.390	In the present study
FI	0.569 **	0.387	In the present study
MOD	0.704 **	0.360	In the present study
GI	0.596 *	-0.426	Ahn et al. (2003)

Table 6. Reproductive characteristics of *L. elliptica* reported from various studies.

Collected year	Sampling sites	Methods	Descriptions for spawning time	Author(s)
Jan.-Dec. 1993	McMurdo Sound (77°51'S, 166°40'W)	Direct observation	Spawning activity was high in March-early April	Bosch and Pearse (1988)
Summer in 1994-95	Signy Island (60°43'S, 45°36'W)		Fertilized eggs were observed in March	Ansell and Harvey (1997)
Sep.1993-Aug.1994	Potter Cove (62º15'S, 58º44'W)	Histological observation	More than 75% of the clams collected in January-February had spawned gonads.	Urban and Mercuri (1998)
Mid-Dec. 1995	Marian Cove (62°13'S, 58°45'W)		More than 80% of the clams were ready to spawn or were at spawning in mid-December	Ahn et al. (2000)
Jan.-Dec. 1993	Potter Cove (62º15'S, 58º44'W)		Clams would be prepared to spawn at any moment depending on environmental conditions	Bigatti et al. (2001)
Dec.1995, Jan.1998	Marian Cove (62°13'S, 58°45'W)		More than 73% of the clams were in fully developed in summer season	Kang et al. (2003)
Mar.1998-Dec.1999			More than 80% of calms collected in January had fully developed gonads or were in spawning	Ahn et al. (2003)
Feb.1998-Jan.2000			Two spawning peaks were observed in December-January with eggs of 121-150 μm in mean diameter and February-March with eggs of 101-120 μm in mean diameter	In this study

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