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Reproductive Cycle of the Edible Oyster *Crassostrea belcheri* in the Myeik Coastal Area of Southern Myanmar

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Abstract

This study investigated the reproductive cycle of the edible oyster, *Crassostrea belcheri*, harvested originally from creeks near Pedaing village in the southern part of Myanmar in September 2017-October 2018 through histological analysis. Five maturity stages were detected in the *C. belcheri* gonads: indifferent, developing, mature, spawning, and spent. The overall sex ratio of males to females of 1:0.98 showed no statistically significant difference from the expected sex ratio of 1:1 in any month. Hermaphrodites could not be observed in 250 oysters. Moreover, the quantitative monthly gonad index values varied from 1.5 in November and June to 2.75 in March. Histological analysis of *C. belcheri* revealed that mature male and female gonads were detected in almost all months, except in November-December 2017 and June 2018. Spawning occurred throughout the study period, except in January-March 2018, peaking at the beginning and end of the dry season in October-November and April-May, respectively.

Discipline: Fisheries
Additional key words: gonad maturity, histology, Ostreidae, spawning season, tropical oyster

Introduction

The locally edible oysters, called Ka-mar, in Myanmar comprise two species: *Crassostrea belcheri* (G. B. Sowerby II, 1871) and *Saccostrea cuccullata* (Born, 1778). Thi-Thi-Lay (1983) first determined the species based on personal communications with Dr. D. B. Quayle, former oyster culture specialist in the Fisheries Research Board of Canada, and his colleague Dr. F. R. Bernard. The identified species were collected in Setse, Mon State, Myanmar. The identification was supported partly based on a recent genetic study of oysters collected from the southern Tanintharyi Region (Li et al. 2017). Larger oysters up to 20 cm in height with thick and heavy shells without chomata on the internal surface are *C. belcheri*, and smaller oysters that rarely exceed 7 cm in height (Thi-Thi-Lay 1983) with chomata-bearing irregularly shaped shells are *S. cuccullata*. Li et al. (2017) reported a third species: *Crassostrea gryphoides*. The species has an elongated shell shape (Siddiqui & Ahmed 2002), and its abundance and distribution in Myanmar are unknown.

Crassostrea belcheri is a luxury seafood that is cultured in Thailand and Malaysia (Nowland et al. 2020). In these countries, *C. belcheri* is sold shell-on and consumed fresh (Songsaeng et al. 2010, Tan et al. 2014). In Myanmar, it is sold shell-on and consumed fresh or cooked. Myeik City is a major center of transportation, travel, and seafood production in the southern part of Myanmar, and it expects oyster culture in pristine mangrove forests to be a promising business for the local economy. Basic biological information, such as breeding periods and growth rates of the oyster, must be confirmed in this locality as Quayle (1980) mentioned that information obtained on a species in one area might not be applicable elsewhere.

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In Myanmar, studies on oyster culture began in Mon State in the 1970s (Thi-Thi-Lay 1983). Several studies have investigated the reproductive biology of edible oysters in the region. Thi-Thi-Lay (1983) reported a major spatfall peak of *S. cuccullata* in Setse, Mon State, in the northeastern part of the Myanmar coastline from March-May and a minor peak in October-November, with two peaks in the fully ripe gonad phase (November and April). Lwin-Lwin (1985) studied the spatfall of *C. belcheri* at Ye River, Mon State, and found similar results (heaviest spatfall in March and April and second peak in December). Htay-Aung (1987) continued the study of Thi-Thi-Lay (1983) and reported that fully ripe gonads of *S. cuccullata* were observed year-round, with two peaks in November and April. Investigating *C. belcheri* gonad maturity and spatfall in the Shwe-Thaung-Yan area in Ayeyarwady Region along the northern coastline of Myanmar, Htay-Aung (2011) observed that ripe gonads appeared in March-November, peaking in April and late October to mid-November, followed by mass spawning in May and October.

The study sites were all located on the northeastern Andaman coast or the eastern Bengal coast (15.2-17.1˚N). However, meteorological and oceanographic conditions differ in the southern Tanintharyi Region, including the Myeik coastal areas (12.1-12.4˚N).

Only sporadic information is available regarding the spawning seasons of edible oysters. Using repeated sampling at the mouth of the Kyauk-Phyar River from August 2013 to February 2014, Sint-Sint-Hlaing (2014) reported that spatfalls were observed throughout the period, and the abundance of settled spats was the lowest in September and the highest in January and February. The increase in spatfall was attributed to massive spawning during the dry season. However, the author did not identify the species of spats. Therefore, the data collected on these spats would not represent the abundance of *C. belcheri*. Yurimoto et al. (2019) histologically studied *C. belcheri* gonad maturity using 13 and 5 specimens purchased from local markets in Myeik City in December 2014 and March 2015, respectively, and observed oysters in the spawning stage in December, but not in March. As the datasets of these studies did not cover a whole year, edible oysters were obtained between September 2017 and October 2018 to collect their gonads and examine their seasonal reproductive cycle.

Materials and methods

Edible oysters (12-20 oysters) were purchased monthly from September 2017 to October 2018, except in September 2018, from the downtown market in Myeik City (Fig. 1). The purchased oysters were collected originally by diving into creeks near Pedaing village (Fig. 1). All oyster samples were alive in good condition, supposedly within several days of capture. The taxonomy of oysters followed the classification systems reported by Siddiqui & Ahmed (2002) and Li et al. (2017) based on shell shape and the presence of chomata on shells, as shown in Figure 2.

For histological analysis, a small cubic piece of the flesh was dissected from the middle part of the flesh where gonadal tissues were located, fixed in 10% seawater formalin, dehydrated through an upgraded ethanol series, and cleared in xylene (Yurimoto et al. 2014). The prepared tissue was embedded in paraffin, cut into 7-µm thick sections using a rotary microtome (RMT-20, Radical Instruments, Ambala, India), stained with hematoxylin and eosin, mounted in DPX mountant (Sigma-Aldrich, St. Louis, MO, USA), and finally examined under an optical microscope (YS100, Nikon, Tokyo, Japan) at 100× magnification. Gonadal maturation stages were classified based on the classification system reported by Yurimoto et al. (2014). The following criteria were used to distinguish between the different maturation stages.

Indifferent-sex stage I (Fig. 3 A): Undistinguished sex, few germ cells in the gonads, and tissues mainly occupied by empty genital tubes.

Developing stage II (Fig. 3 B, F): Male — Spermatogonia appeared along the follicle walls. Spermatocytes and a few spermatids could be observed. Female — Oogonia appeared along the follicle walls, and immature oocytes were attached to the tube wall.

Mature stage III (Fig. 3 C, G): Male — Follicles full of spermatozoa with tails pointing toward the center of the genital tubes. Female — Follicles full of mature oocytes with an irregular or polygonal shape.

Spawning stage IV (Fig. 3 D, H): Male — Some spermatozoa were released, and many spermatozoa remained in the genital tubes. Female — Mature oocytes decrease in number, form empty spaces in the follicle walls, and exhibit nuclear disappearance because of germinal vesicle breakdown. Many oocytes with late-developing mature stages remain in genital tubes.

Spent stage V (Fig. 3 E, I): Male — Genital tubes deformed, and some spermatozoa remained. Female — Deformed and degenerated genital tubes, with most of them empty.

In addition, the scoring of gonad maturation was conducted to assess the progress of the reproductive cycle using histological data, and the gonad index (*GI*) was calculated following the study by Kennedy (1977), with

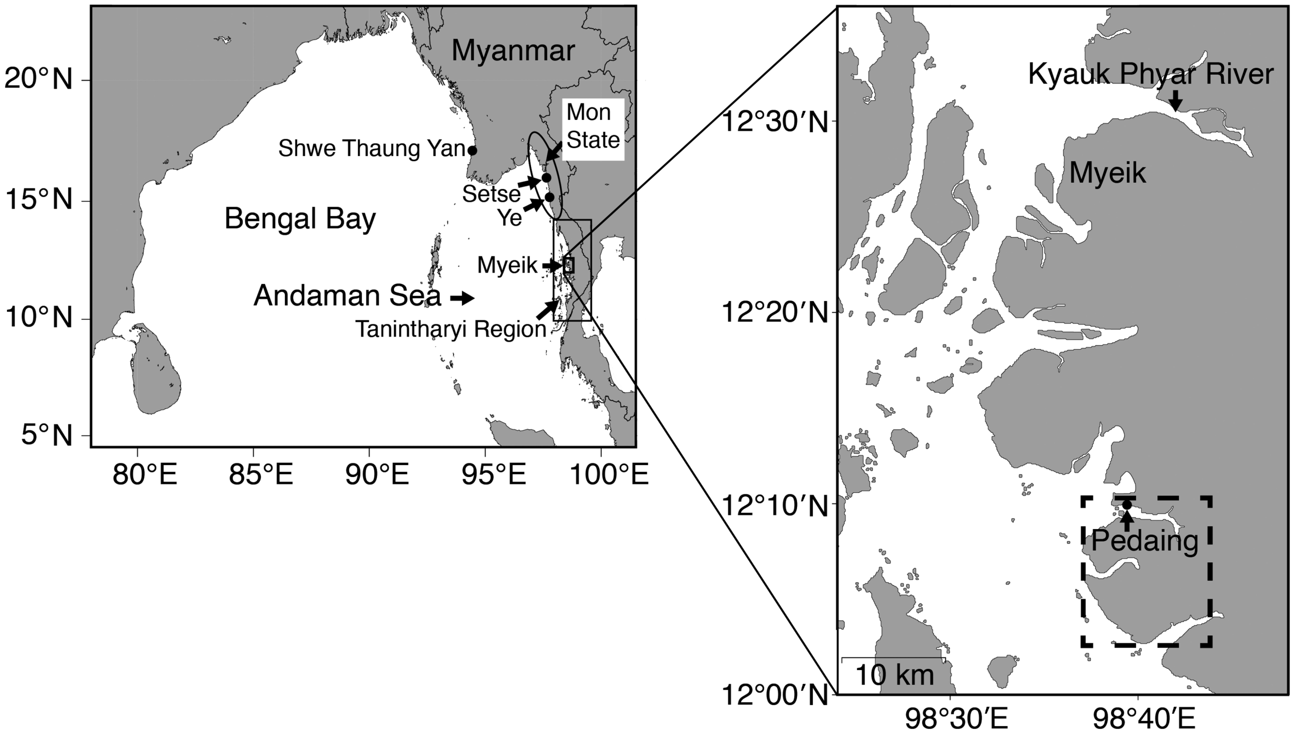

Fig. 1. Map showing the sites examined in previous studies in Myanmar (Setse and Ye in Mon State, Shwe-Thaung-Yan coastal area in Ayeyarwady Region, and Kyauk-Phyar River and Myeik in Tanintharyi Region) and the sample collection site of the present study near Pedaing village

Oysters from Pedaing village were harvested in the region enclosed by the dashed line.

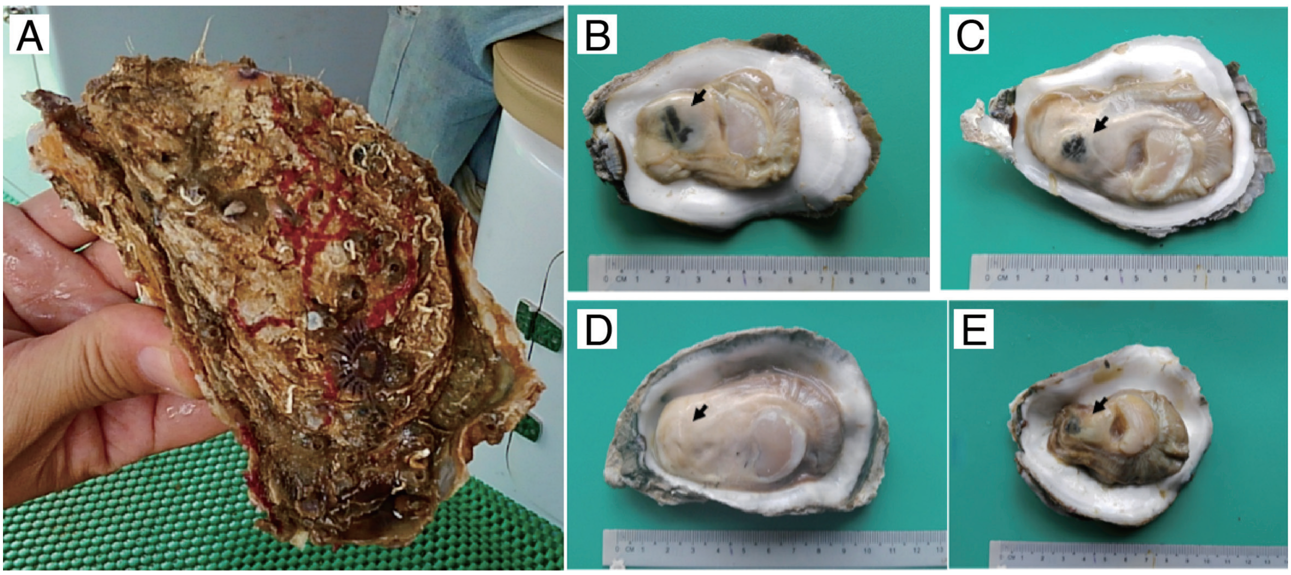

Fig. 2. *Crassostrea belcheri*

Outlook of a live specimen A and shucked samples with a scale that shows the size and approximate gonadal maturation stages determined by the naked eye B-E:

B: indifferent-sex stage, C: developing stage, D: white gonad growing large in mature stage, and E: spawning and spent stages. Arrows indicate the part of the flesh body where gonadal tissues are located.

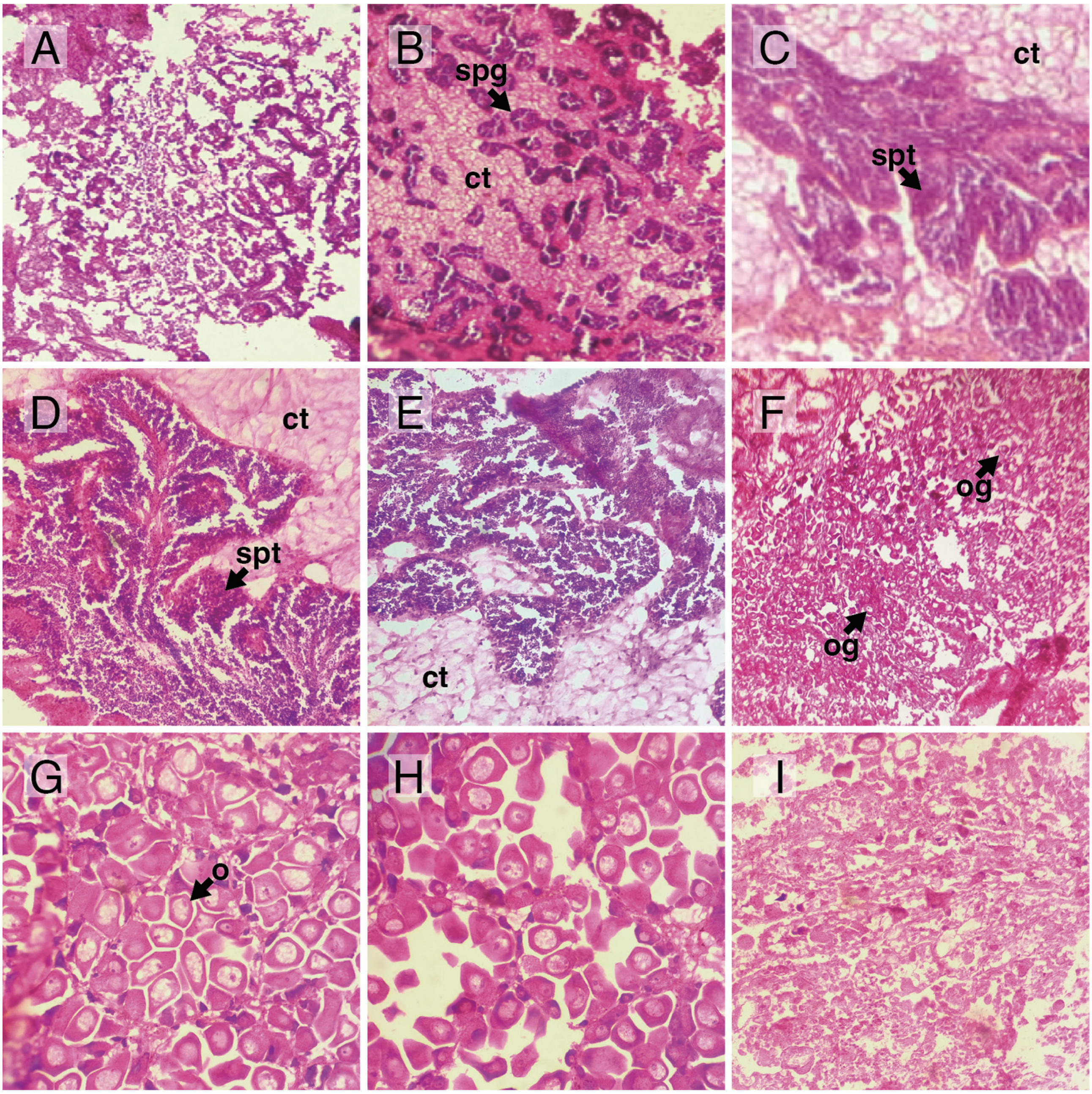

Fig. 3. Histological sections of the gonads of *Crassostrea belcheri* showing sex and gonadal maturation stages
A: indifferent-sex, B-E: males, and F-I: females. B, F: developing, C, G: mature, D, H: spawning, and E, I: spent stages. Abbreviations: ct, connective tissue; og, oogonia; o, oocytes; spg, spermatogonia; and spt, spermatids

three ranks assigned to the determined stages: indifferent-sex (I) and spent (V) assigned rank 1, developing (II) and spawning (IV) assigned rank 2, and mature (III) rank 3. The *GI* was calculated every month.

$$GI = \sum_{1}^{3} (i \times N\mathrm{i}) \;/\; N$$

where *i* is the rank 1-3, *N*i is the number of specimens at rank *i*, and *N* is the total number of oyster specimens analyzed in the month.

The sex composition was recorded monthly. The sex ratio was tested using a binomial test to check whether the ratio significantly ($P < 0.05$) differs from 0.5, considering a male:female ratio of 1:1 (Siegel & Castellan 1988). The probability that the number of males or females was lower than the observed number in the samples was calculated monthly, assuming an even sex ratio of 1:1 and binomial distribution using R version 4.2 software (R Core Team 2022).

Monthly average air temperature and rainfall data from Myeik between January 2017 and December 2018 were obtained from the Department of Meteorology and Hydrology of Myanmar.

Results

Seasonal changes in air temperature and rainfall in Myeik, January 2017-December 2018, are shown in Figure 4. During the study period, the monthly mean daily maximum and minimum temperatures were 27.9°C - 34.8°C and 22.8°C - 25.2°C, respectively. Thus, the seasonal variation in monthly mean daily minimum and maximum temperatures was 2.4 and 7.9°C, respectively. In general, the temperature was low during the rainy season and high during the dry season. In contrast, the monthly rainfall showed marked seasonal changes because of the seasonal monsoons. The monthly rainfall was >400 mm until October 2017 and June-September 2018, with a maximum of 1,425 mm in August 2018. Monthly rainfall of >400 mm can be referred to as the rainy season. The monthly rainfall from November 2017 to May 2018 was <300 mm, which could be considered the dry season.

The edible oysters used in the present study ranged from 120 to 150 mm in shell height. The monthly percentages of male, female, and indifferent-sex *C. belcheri* samples collected from September 2017 to September 2018 are shown in Figure 5 A. Among the 250 sampled oysters examined microscopically, 46.4% (116), 45.6% (114), and 8% (20) were male, female, and individuals carrying indifferent-sex cells, respectively. Hermaphrodites could not be observed through microscopic examination. The number of indifferent-sex *C. belcheri* samples was high (30%) in June 2018. No indifferent-sex *C. belcheri* samples were observed in October 2017, March-May 2018, and July-October 2018. The overall sex ratio was 1:0.98 (males:females). The sex ratio was not significantly different (P > 0.05) from the even sex ratio (1:1) for any of the sampled months.

The monthly occurrence and percentage of different

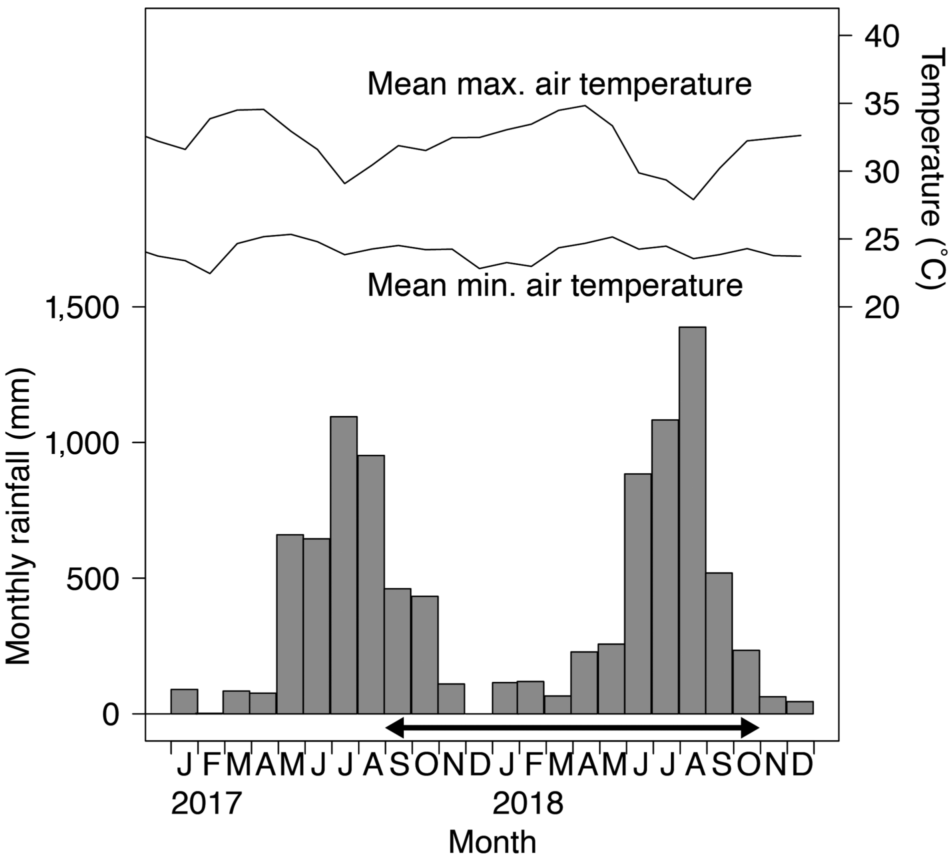

Fig. 4. Monthly rainfall and air temperature in Myeik from January 2017 to December 2018
The double arrow line indicates the study period of this study.

maturity stages in females and males are described in Figure 5 B and C, respectively. Oysters in developing stage (II) were observed first in December 2017. Percentage of developing stage was highest both in females (83%) and males (80%) in January 2018. Male and female oysters in mature stage (III) were observed in almost all months, except in November-December 2017 and June 2018. Oysters in spawning stage (IV) were

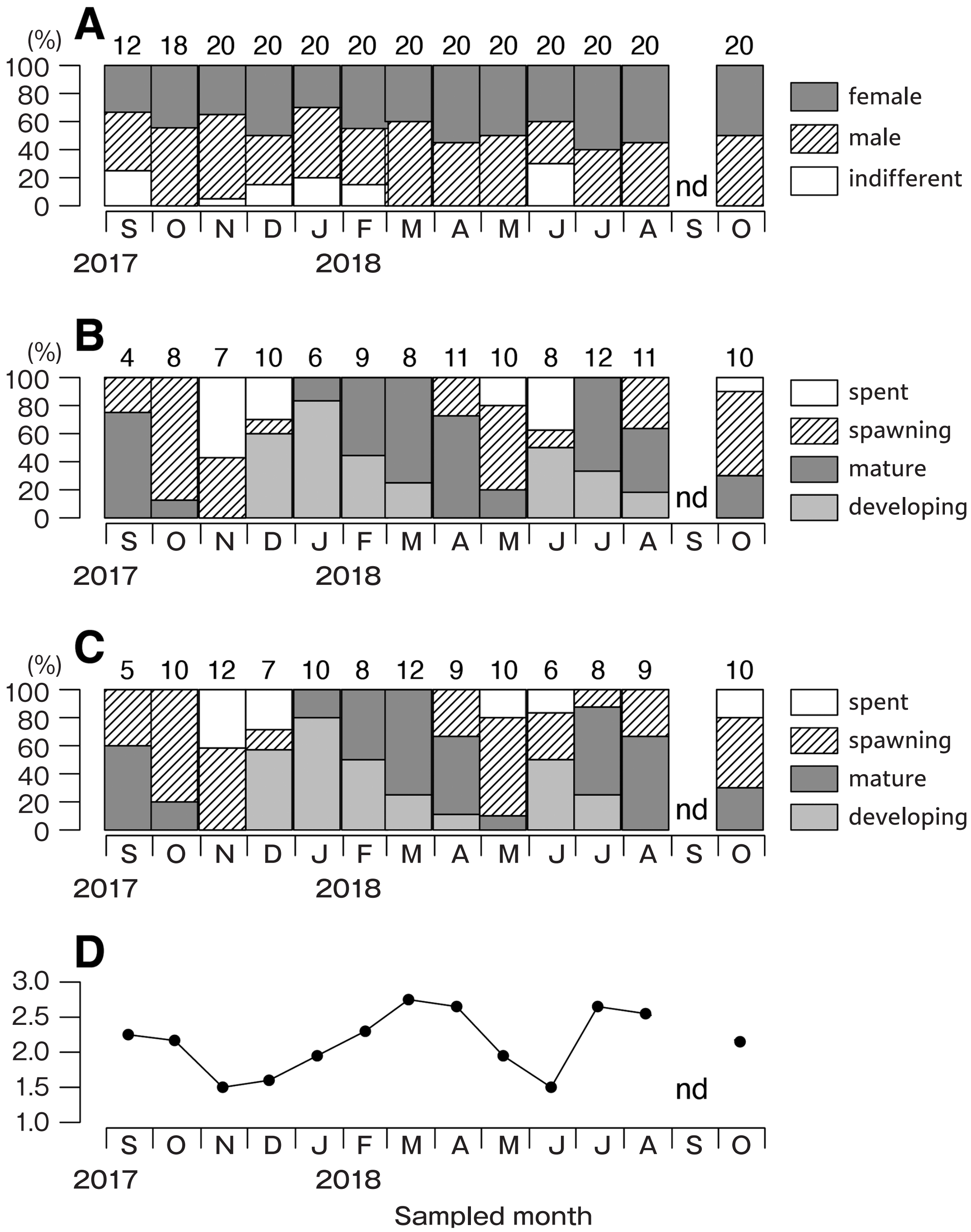

Fig. 5. Gonadal maturation stages of *Crassostrea belcheri* in Myeik coastal area from September 2017 to October 2018

A: sex ratios; B: gonadal maturation stages in females; and C: gonadal maturation stages in males. D: gonad index values (see text). Numbers of samples are shown on top of the graphs (A-C). *nd* denotes no data on the month.

present nearly throughout the year, except for January through March 2018 for both sexes and July 2018 for females. The percentage of females and males with spawning stage gonads peaked in October-November 2017 and April-May 2018. The spent stage (V) occurred from November to December 2017, May to June 2018, and October 2018.

The monthly variation in the *GI* values of female and male oysters is presented in Figure 5D. Quantitative assessments of *GI* values ranged from 1.5 (November and June) to 2.75 (March). The *GI* values showed two peaks throughout the study period, indicating a high percentage of mature gonads. There were two drops in *GI* value in October-December 2017 and April-June 2018, showing the high occurrence of spent and spawning stage gonads.

Discussion

A total of 250 *C. belcheri* individuals examined during the study period showed no seasonal change in sex ratio and no statistically significant difference ($P > 0.05$) from the expected sex ratio of 1:1. Htay-Aung (2011) reported that the monthly percentage of *C. belcheri* females was greater than that of males in the Shwe-Thaung-Yan coastal area of Myanmar. However, the sex ratio did not seasonally change. For the same species, Dangwatanakul (1992) reported an almost equal sex ratio and no seasonal change in the sex ratio of the oyster population in Thailand. Seasonal changes in sex ratios, which indicate sex changes in oysters, have been reported by Angell (1986). Sex change is influenced by size difference and oyster age, where the sex ratio favors males in younger and smaller oysters and females in older and larger oysters (Castaños et al. 2009). Barman et al. (2022) also reported a size-based male–female–male sexual transition of *Crassostrea* (*Magallana*) *saidii* from Malaysia. There is no information on sex changes in *C. belcheri* based on size and age group (Dangwatanakul 1992, Htay-Aung 2011). In addition, this study could not conclude the relationship between size and the sexual transition phase of oysters because of the small sample size and arbitrary selection of large oysters (120 mm-150 mm shell height). Thus, further studies should focus on emphasizing the periodicity and phase of sex change based on the oyster size group.

No hermaphrodites were observed in the 250 *C. belcheri* samples examined in this study. The presence of hermaphroditic specimens of tropical *Crassostrea* species has been reported by Angell (1986) and Mena-Alcantar et al. (2017). The rare occurrence of hermaphrodites in *C. belcheri* populations in Thailand and Myanmar was reported by Dangwatanakul (1992) and Htay-Aung (2011), respectively. Rao (1956) stated that the rare occurrence of hermaphrodites is a purely transitional phase, showing a change in sex from male to female or female to male. Joseph and Madhyastha (1984) suggested that the transition phase from one sex to another depends on environmental stress, and Narasimham and Kripa (2007) stated that oyster species of the genus *Crassostrea* are usually nonfunctional hermaphrodites. Thus, hermaphroditism in *C. belcheri* in Thailand and Myanmar might be caused by the sex change.

Different spawning strategies of oysters for a limited season of the year and almost year-round have been observed in temperate and tropical regions, respectively. Spawning in oysters in the tropics may occur through most of the year, with peaks usually before and after the rainy season (Quayle 1980). Antonio et al. (2021) stated that low variation in temperature might be the main factor in continuous spawning in tropical regions. There were variations in the spawning season and spawning peaks between and within the tropical oyster species depending on habitat localities (Table 1). Choo (1983) reported that rain triggers the peak spawning period of oysters in Malaysia. Based on the rainfall amount in Myeik from January 2017 to December 2018, the rainy season prevailed from May to October in 2017 and from June to September in 2018. The results of this study showing the two spawning periods of *C. belcheri*, October-November 2017 (after the rainy season) and April-May 2018 (before the rainy season), are in good agreement with those reported by Quayle (1980), Dangwatanakul (1992), and Htay-Aung (2011). Yurimoto et al. (2019) also suggested that the main spawning period of edible bivalves in Myeik occurs during the dry season, with phytoplankton blooms. In the Tanintharyi River estuary along the coastal Myeik, the abundance of diatoms and dinoflagellates was higher during the dry season than the wet season (Lett-Wai-Nwe et al. 2021). The rainy season exhibited the lowest amount of primary production owing to the extensive discharge of turbid water from the rivers, which lowered the euphotic layer depth (Saw-Htoo-Thaw et al. 2017). High primary production in the estuary during the dry season would have provided suitable food condition for bivalves and promoted their sexual maturation.

The spawning period is determined by the season of favorable food condition, and the main spawning periods of other competing organisms coincide with the spawning period of the target species *C. belcheri*. Htay-Aung (2011) also reported that the spawning period of *C. belcheri* coincides with that of other competing organisms, including *S. cuccullata* and barnacles. Thus, finding a

Table 1. Two spawning periods of tropical *Crassostrea* species in different localities

Species	Localities	Spawning period		Sources
		1st peak	2nd peak	
C. belcheri	Ban Don Bay, Prachuap Khiri Khan Coastal Aquaculture Development Centre and Manao Bay, Thailand	April	September	Dangwatanakul (1992)
C. belcheri	Shwe-Thaung-Yan coastal area, Myanmar	May	October	Htay-Aung (2011)
C. belcheri	Myeik coastal area, Myanmar	April-May	October-November	This study
C. corteziensis	Coastal lagoon in northwest, Mexico	May-June	September-November	Rodríguez-Jaramillo et al. (2008)
C. madrasensis	Mulki estuary, India	December-January	April-May	Joseph & Madhystha (1984)
C. madrasensis	Moheshkhali Channel, Bay of Bengal	October	May	Alam & Das (1998)
C. (*Magallana*) *saidii*	Sungai Muar estuary, Johor, Malaysia	April	November	Barman et al. (2022)

favorable period in a year for exclusively collecting *C. belcheri* spats may be difficult because other sessile organisms constantly settle on the substrate and compete for space and food. The spatfall of small-sized oyster species *S. cuccullata* showed two peaks, a major peak in March-May and a minor peak in October-November, annually in Setse, Myanmar (Thi-Thi-Lay 1983), which agrees with the assumed spawning period of *C. belcheri* in the Myeik coastal area. Among the two spatfall peaks of edible oysters, Htay-Aung (2011) reported that the average *C. belcheri* settlement rate was higher in the premonsoon period (prerainy season) than in the postmonsoon period (postrainy season) in the Shwe-Thaung-Yan coastal area, Myanmar. However, the mortality rate in *C. belcheri* was higher in the prerainy season than in the postrainy season. Thus, further studies should specify the settlement peak preferable for spat collection to help set up local oyster farmers who are currently experimenting with trial and error.

In conclusion, histological observations of *Crassostrea belcheri* gonads in Myeik, Myanmar, showed a high percentage of mature males and females from March to April and July to September (rainy season). The main spawning period of the species is assumed to be from October to November and April to May. Dual annual spawning peaks of the species are common phenomena consistent with previous reports. However, the monthly range varied locally.

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