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Reproductive biology of rock oyster, *Saccostrea cucullata* (Born, 1778) along Aare-Ware rocky shore of Ratnagiri, Maharashtra, India

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The present study regarding the reproductive biology of *Saccostrea cucullata* was carried out from May 2014 to April 2016. The observation has indicated that the peak spawning season is from the month of November to January along the Aare-Ware rocky shore of Ratnagiri. For females, the maximum values of Gonado Somatic Index (GSI) were observed in November 2014 (2.8890) and November 2015 (2.9910), whereas in males, GSI values were maximum in November 2014 (2.1964) and December 2015 (2.1681). During the study, the average male: female sex ratio of 1:1.4 was observed, with a minimum of 1:1 in the month of April 2015 and a maximum (1:1.8) during December 2014, indicating predominance of females in all the months. The size of the first maturity has been estimated to be 22.2 mm which can be utilized for signifying management measures for sustainable utilization of the resource.

[**Keywords**: First maturity, GSI, Rock oyster, *Saccostrea cucullata*, Spawning]

Introduction

Oysters are one of the most valuable organisms among the phylum Mollusca and are widely distributed in estuaries, backwaters, creeks, bays, harbors and can be found commonly near rocky areas or with other hard substratum[1]. Several oysters *viz.*, *Crassostrea madrasensis*, *C. gryphoides*, *C. discoidea* and *Saccostrea cucullata* had been reported from the Indian coast having economic importance[1]. *S. cucullata*, commonly known as the rock oyster, was recorded from both the east and west coasts of India but was more common along the west coast[2].

Reproductive organs of oysters are known as gonads, comprise a mass of tissue made up of minute tubules, connective tissue, and sex cells that encloses the digestive diverticula, stomach, and intestine fold making up a significant amount of the oyster body until maturity[3]. Generally, as sperm and eggs mature, gonads often get bigger and thicker[4]. Oysters are hermaphrodites, but dioecious behavior is also common in oysters[5].

Globally, more than 15 million tonnes of marine bivalves are produced each year for human consumption, accounting for 14 % of total global marine production. The major production of the marine bivalve (89 %) comes from the aquaculture sector, which has an economic worth of 20.6 billion US$ per year, while the rest 11 %, comes from the wild fishery[6]. The estimated bivalve production of India in 2018 was 132531 tons. The bivalve fishery was dominated by clams, mussels, and oysters consisting of 76.3, 15.3, and 8.4 %, respectively. In Maharashtra, the total landed bivalve was 1,318 tons in 2018 and fishery is dominated by the oysters (80 %) followed by clams (15 %) and mussels (5 %)[7].

Bivalve is natural bio-filter that purifies the ecosystem and also provides nutritional security to human beings. A bivalve's true economic worth arises from its multiple by-products and services like ornamental shell business, manufacture of ready-to-distribute products and shucking and packaging houses[8]. In addition to providing high amounts of protein and energy, bivalves also contain vitamins A and D, minerals (calcium, selenium, iodine), and omega-3 fatty acids that are beneficial to health[8-10].

Ratnagiri is one of the coastal districts of Maharashtra state along the west coast of India bound by the Arabian Sea. Generally, *S. cucullata* was reported from both east and west coasts but more common along the west coast of India, contributing

90 % to the oyster catch. Globally, the major portion of bivalve is coming from the aquaculture sector; however, it relies primarily on natural resources to supply its seed, therefore, seeds are frequently taken from the natural stocks[6]. In-depth knowledge about the reproductive biology of any species is necessary for thriving culture and management[11]. At present, *S. cucullata* has limited data on its reproduction biology. Hence, the present study estimates the reproductive biology of rock oyster (*S. cucullata*) along the rocky shore of Aare-Ware, Ratnagiri for further management and sustainability of this resource.

Materials and Methods

Aare-Ware rocky shore exhibit high tidal heights and wave action. Topographically it has more massive boulders and deeper rock pools. The intertidal zone, *i.e.*, the region between the high tide and low tide covering the area of around 10,000 m^2 was selected for conducting the proposed study.

Water temperature was recorded during the entire sampling period as per the standard methods given by APHA[12]. For the present study, twice a month, 20 – 40 samples of *S. cucullata* (Fig. 1) were randomly hand-picked during the lowest low tide of each month from May 2014 to April 2016 along the intertidal exposed rocky shore of Aare-Ware (Lat. 17°4′48.08″ N, Long. 73°17′00.31″ E), Ratnagiri (Fig. 2). A total of 766 specimens comprising 316 males and 450 females were examined for reproductive studies. In order to estimate the Gonado-Somatic Index (GSI), the gonads were carefully excised out, and the wet weight of the each oyster was recorded by an electronic micro-balance with a precision of 0.01 g. The following equation, as per Giese[13] was used in statistical calculations.

$$GSI = \frac{\text{Gonad weight (g)}}{\text{Wet weight of meat (g)}} \times 100$$

The mean length at sexual maturity (Lm) or mean length at first reproduction may be defined as a particular length at which 50 percent of the individuals are sexually mature. For estimation of the size at first sexual maturity of *S. cucullata*, an aggregate of 470 female samples were used. Using the friction of sexually mature individuals in different length classes, the logistic function is fitted using the following equation[14].

$$P = 1/(1= exp\ (-r(L-Lm)))$$

Where, P = proportion of mature individuals in length class L; r = the width of the maturity curve; and *Lm* = length at 50 % population attained maturity.

An attempt was made to study the sex ratio of *S. cucullata* with respect to month and size groups. Fresh gonadal smears were obtained on microscope

Fig. 1 — *Saccostrea cucullata* collected from Aare-Ware rocky shore of Ratnagiri

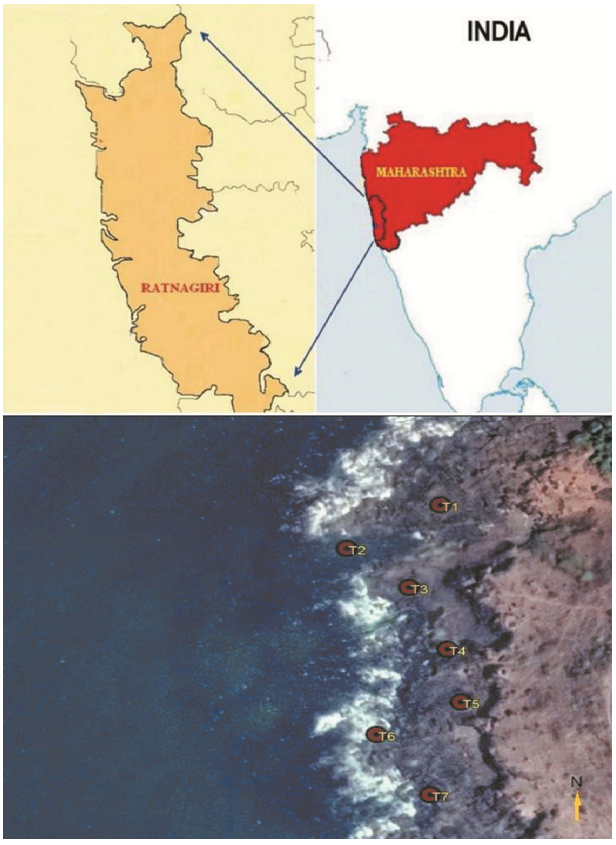

Fig. 2 — Sampling site at Aare-Ware intertidal rocky shore of Ratnagiri

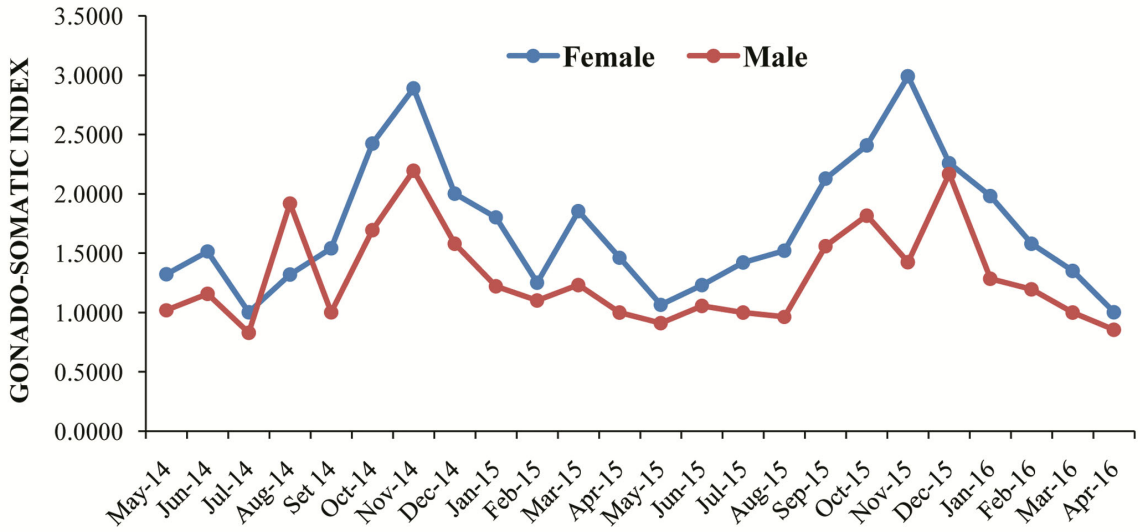

Fig. 3 — Monthly variation in the GSI values of males and females of *S. cucullata* at Aare-Ware rocky shore of Ratnagiri

glass slides and gonad histology was conducted to identify the sex[15]. Data on sex ratio were analyzed using χ^2 (chi-square) test to find the monthly domination of sexes if any[16]. $\chi^2 = (O - E)^2 / E$ is the common symbolization. To study the maturity stages gonad smears were observed under the microscope, and in different size ranges, gonads sections were also prepared as per standard histological procedures given by Howard *et al.*[17]. The stained slides were examined and photographed using a digital microscope to determine the sex and developmental stages of gonads. Based on the condition, gonads were categorized into four stages: maturing, mature, partially spent and spent[18].

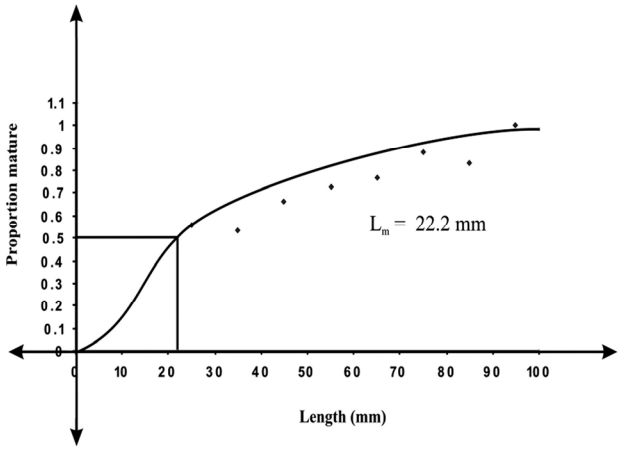

Fig. 4 — Size at first maturity of *S. cucullata* at Aare-Ware rocky shore of Ratnagiri

Results and Discussion

During the entire study period, water temperature ranged from 26.5 to 32.7 °C at Aare-Ware shore. In the present investigation, GSI value showed wide variations between both the sexes and throughout the study period GSI values were higher for females. GSI was highest for females during November 2014 (2.8890) and November 2015 (2.9910). For males, GSI was highest during November 2014 (2.1964) and December 2015 (2.1681). The average GSI of males and females was plotted against months to show monthly variations (Fig. 3). Through histological or quantitative measures, standard GSI measurements could be achieved[19-21]. Powell *et al.*[22] reported that gonadal production was lower when GSI value were low in the winter and mid-summer seasons. Choi *et al.*[23] stated that the GSI of *Crassostrea virginica* was maximum during the peak spawning in spring, while in the fall the rate of gamete production was at its peak.

In this study, the high GSI values were recorded during the months of November to January in both study years indicating beginning of spawning in November to January, and during this time, most of the oysters were ripe. By February, GSI decreased as the oysters were in the spent phase.

The cumulative frequency analysis showed that the females of *S. cucullata* attained the first sexual maturity at 22.2 mm (Fig. 4), and the information can be used for the stock studies, and suggest management measures for the exploitation of the resource. *S. cucullata* has a short life span with a living of 2.5 years which might be due to

environmental conditions with exposure to pollution[24]. Nagabhushnam & Bidarkar[25] stated that the sexes are separate in *Crassostrea cucullata*, and they attained first sexual maturity at 10-12 mm in length along the Ratnagiri coast. But in the present study, the results showed that the oysters reached the first maturity at a slightly higher range, which could be due to climatic changes, the presence of distinct stocks, and environmental factors, including the availability of natural food. Similarly, Kripa[26] stated that males attained maturity at 18 – 22 mm and females at 20 – 22 mm length of *S. cucullata* in Ashtamundi Lake.

In the present study, a total of 766 specimens (316 males and 450 females) were examined for the sex ratio of *S. cucullata*. The average sex ratio of the males and females was recorded to be 1:1.4. Females were dominant in all months might be due to the higher availability of food. Teaniniuraitemoana *et al.*[27] studied the consequence of ecological combinations of food availability and temperature on gender determination in *Pinctada margaritifera*, and they noticed that a significant proportion of females changed to males at high temperature and less availability of food[27]. A higher number of females were observed in populations of pearl oysters when food availability conditions were favorable[28]. In the present study, the highest sex ratio (1:1.8) was observed during December 2014 and (1:1.7) during November 2015, and the lowest was recorded during April 2015 (1:1) (Table 1). Monthly, Chi-square value was calculated, and no significant difference ($p > 0.05$) was noticed in all studied months. Sawant & Ranade[29] stated that the overall sex ratio in *C. gryphoides* studied from the Bhatia creek, Ratnagiri showed that males formed 36.13 %, females 56.75 % and indeterminates 7.12 %. Further, Nagabhushanam & Bidarkar[25] reported the overall sex ratio for Indian rock oysters in which males formed 40.1 %, females 48.7 % and hermaphrodites 11.2 %. Generally, oysters are dioecious (sexes are separate) in nature, but hermaphrodites (both the sexes in particular individuals) were also reported. It was well noticed that young oysters primarily

Table 1 — Month wise sex ratio of *S. cucullata* at Aare-Ware rocky shore of Ratnagiri during May 2014- April 2016

Sr. No.	Month	Male	Female	Total	Sex ratio	Df	Chi square	
1	May-14	10	15	25	1:1.5	1	1	NS*
2	Jun-14	12	16	28	1:1.3	1	0.571	NS*
3	Jul-14	15	18	33	1:1.2	1	0.272	NS*
4	Aug-14	16	20	36	1:1.3	1	0.444	NS*
5	Sep-14	14	22	36	1:1.6	1	1.777	NS*
6	Oct-14	16	26	42	1:1.6	1	2.38	NS*
7	Nov-14	17	29	46	1:1.7	1	3.13	NS*
8	Dec-14	17	30	47	1:1.8	1	3.595	NS*
9	Jan-15	15	20	35	1:1.3	1	0.714	NS*
10	Feb-15	13	16	29	1:1.2	1	1.882	NS*
11	Mar-15	12	15	27	1:1.3	1	0.333	NS*
12	Apr-15	12	12	24	1:1	1	0	NS*
13	May-15	11	14	25	1:1.3	1	0.36	NS*
14	Jun-15	13	15	28	1:1.2	1	0.142	NS*
15	Jul-15	10	16	26	1:1.6	1	1.384	NS*
16	Aug-15	13	19	32	1:1.5	1	1.125	NS*
17	Sep-15	16	22	38	1:1.4	1	0.947	NS*
18	Oct-15	15	23	38	1:1.5	1	1.684	NS*
19	Nov-15	15	26	41	1:1.7	1	2.291	NS*
20	Dec-15	13	21	34	1:1.6	1	1.88	NS*
21	Jan-16	12	15	27	1:1.3	1	0.333	NS*
22	Feb-16	10	15	25	1:1.5	1	1	NS*
23	Mar-16	9	13	22	1:1.3	1	0.727	NS*
24	Apr-16	10	12	22	1:1.2	1	0.181	NS*
	Total	**316**	**450**	**766**	**1:1.4**		**28.152**	

* Not significant at 5 % level; Tabulated value 3.841

functioned as males (60 – 70 %) and later became females[5].

In the present study, the highest sex ratio was observed during December 2014 and November 2015. It might seem due to the vigorous gonad development from June to October as most of the oysters observed were in the mature stage, and from November to January, spawning took place. The oysters entered into an indeterminate sexual stage during the month of February to May, and the sex ratio decreased.

Different stages of gonad development of *S. cucullata* were identified from the histological studies (Fig. 5) of the gonads as illustrated in Figures 6 & 7. Onwards June 2014 (first sampling year) and June 2015 (second sampling year), there was an incessant development of the gonads, with an increasing number of oysters entering the maturing stage. The vigorous gonad development was observed during June to October in both the studied years, with a rapid increase in the mature gonads. During the same period, water temperature gradually increased from 26.5 to 32.7 °C at Aare-Ware shore. The findings of the present investigations were in accord with the study conducted by Sreedevi *et al.*[30], and where they observed that rising temperature leads to a positive effect on the gonadal maturation of *P. viridis*. Males were found to have spermatozoa filling their follicles, while in females great quantity of rounded oocytes were noticed in October 2014 and October 2015. November onwards, the spawning activities of the oysters have been increased, which could be due to a rise in the temperature after the monsoon

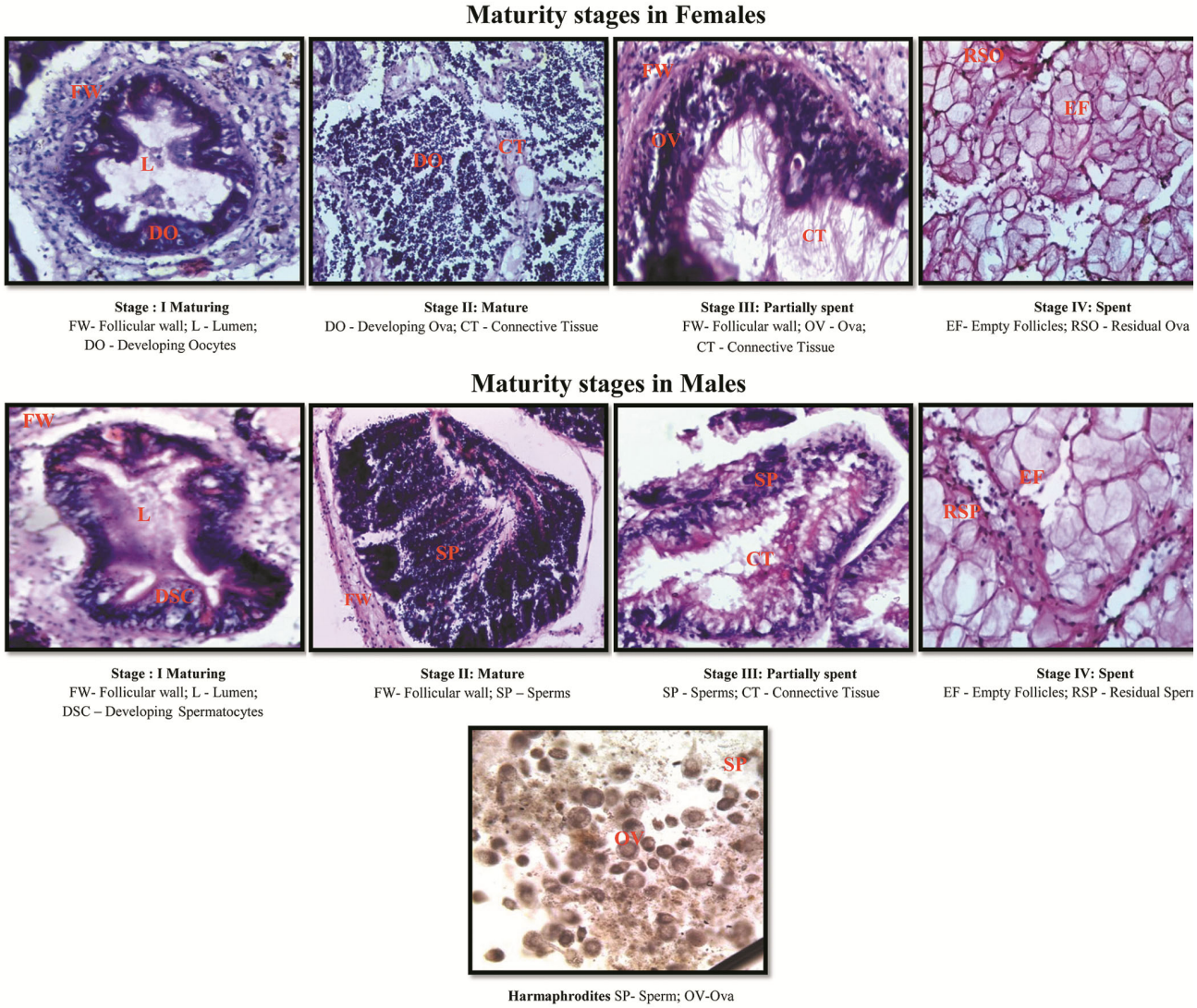

Fig. 5 — Maturity stages in *Saccostrea cucullata*

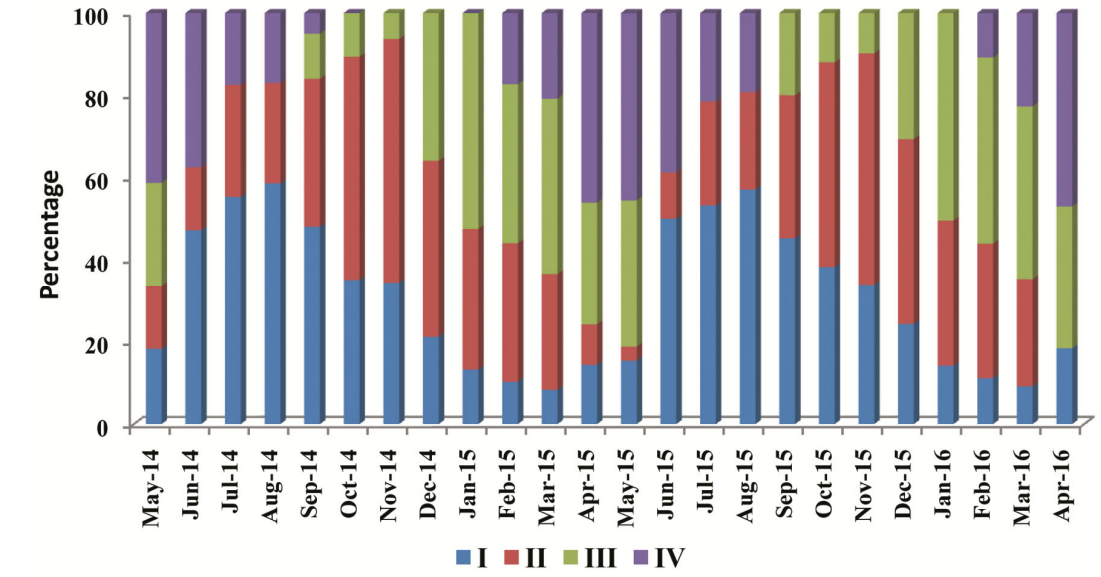

Fig. 6 — Monthly variation in the maturity stages in male *S. cucullata* at Aare-Ware rocky shore of Ratnagiri

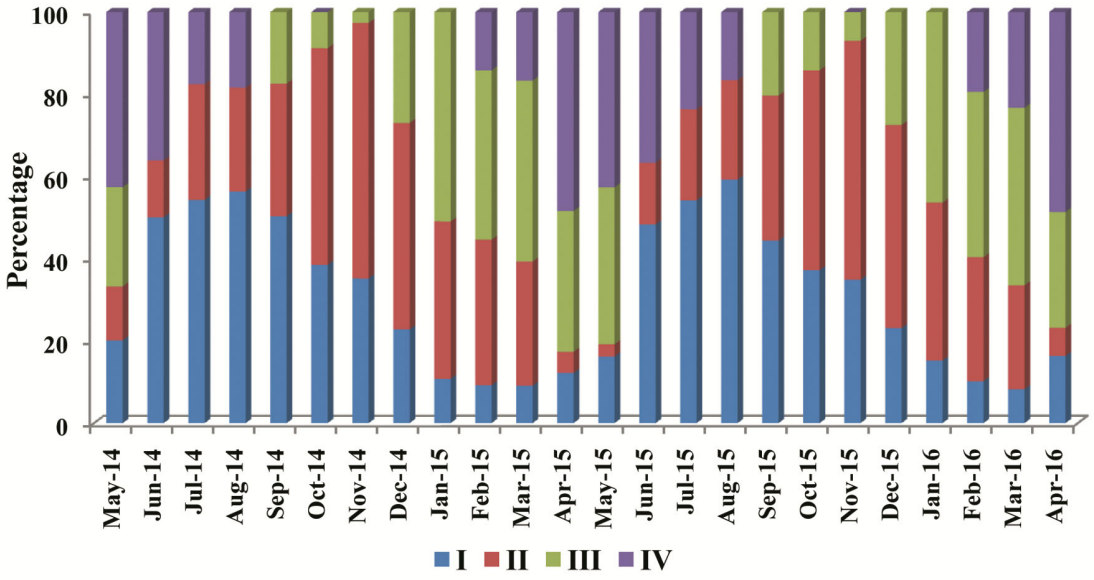

Fig. 7 — Monthly variation in the maturity stages in female *S. cucullata* at Aare-Ware rocky shore of Ratnagiri

season. Sreedevi *et al.*[30] also stated that gametes development has accelerated due to a surge in temperature and reducing the sexual cycle. Bhattacharya *et al.*[31] also observed that *S. cucullata* released gametes during a period of high water temperature, salinity, and pH. The temperature might interact with various environmental parameters, such as pH, salinity, and availability of food, as a key factor in defining growth and survival[24,32]. In the present study, hermaphrodites were also observed in the years 2014 and 2015. The spawning process continued till January, with stray cases of oysters in ripe condition. Generally, the spawning activities were gradually decreased from January. January 15 to March 15 and January 16 to March 16 showed partially spent stages of oysters. In the month of February, numerous oysters were in the spent stage, while gonad development was initiated in some oysters.

The time it takes for sex cells to develop varies depending on how frequently and when spawning occurs. The follicular walls were quite thin in mature

males and females, and the lumen was full of mature sex cells. In most cases, mature males had spermatozoa-filled follicles with tails pointing towards the lumen centre, while mature females had follicles filled with big mature oocytes. The number of mature sexual cells was lower during the spawning stage than for mature oysters. Fully spawned oyster lumina often contained mature sexual cells that can undergo cytolysis, depending on the oyster species. The higher salinity and temperature values increased after monsoon[33] due to this reason, gametogenesis and maturation progressed slowly during the months of March to July, while the spawning peaked in November. The present study reveals that *S. cucullata* has a prolonged spawning season, as shown in various bivalves[34,35]. The peak spawning of *S. cucullata* studied by Awati & Rai[36] at the Mumbai coast was in March-June, whereas in the Ratnagiri coast, it was during October-January[37]. Sukumar & Joseph[38] reported the spawning of *S. cucullata* from the Someshwar coast in June-September and November-December. Along the southwest coast of India, *S. cucullata* recruits round the year with two major peaks during April and September[24]. Moreover, Kripa[26] observed the spawning of rock oysters in Ashtamudi Lake during November-February and May-June. Thus the peak spawning period varied in geographically separated areas due to various exogenous and endogenous influences that control gametogenesis, growth, and maturation of gonads.

Conclusion

The present study provides extensive information regarding the reproductive biology of *S. cucullata* along the Ratnagiri coast. The females of *S. cucullata* attained the first maturity at a size of 22.2 mm and will be used for the stock assessment and signifying management steps to ensure the sustainable harvest and utilization of the resources. The GSI was high from November to January, which indicated the peak spawning, and in February the GSI decreased as the oysters were in the spent phase. Finally, it can be stated that the present study will provide a basic idea toward the fishery and biology of *S. cucullata*, which will be helpful for further research studies and cultural practices for sustainable management of this species along the studied region.

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Conflict of Interest

The authors declare no competing financial interest.

Author Contributions

PKP: Conceptualization, formal analysis, sampling and data analysis, and writing - original draft; SAM: Overall supervision; ST: Writing - review & editing; and URG: Sample analysis and software.

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