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RESEARCH ARTICLE

Gametogenic cycle of Monodonta canalifera (Lamark, 1801) at two rocky shores of Karachi coast, Pakistan

^{1, 2*} Nuzhat Afsar, ¹Ghazala Siddiqui and ¹Zarrien Ayub

1 Center of Excellence in Marine Biology, University of Karachi, Karachi-75270, Pakistan2 Institute of Marine Science, University of Karachi, Karachi

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*Corresponding Author

Nuzhat Afsar

Abstract Histological study was performed in order to observe gonadal cycle and gametogenic pattern of common top shell Monodonta canalifera at two rocky shores; Buleji and Manora Channel along the Karachi coast. Studies were taken during June 2005 to July 2006 followed by monthly intervals and samples were examined on histological basis. The oocyte diameter of M. canalifera collected from Manora was significantly higher than the specimens of M. canalifera studied from Buleji. The average oocyte diameter of M. canalifera at Manora ranged between 34.67±13.31 to 59.46±18.51µm and that at Buleji it was found between 41.19±11.55 to 49.35±13.98 µm. Generally cytolysis of unshed oocytes was not evident in

49.35 \pm 13.98 µm. Generally cytolysis of unshed oocytes was not evident in any of the specimen furthermore ripe females predominated the population throughout the year. The peak maturation activity in females was observed in June (77.78%) and February (90.00%) at Manora and Buleji respectively.

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Introduction

This paper describes the reproductive cycle of M. canalifera top shell, found at rocky shores along the coastal belt of Pakistan, bordering Northern Arabian Sea an outright part of the Indian Ocean. Histological examination of M. canalifera had never been attempted in past from Pakistani coastal waters. However there are few reports on gonadal maturation and spawning season of their kinfolk trochid species from different coastal regions across the globe (Underwood 1972; Bode et al., 1986; Takada 1996; Lee 2001).

Bode et al. (1986) have provided details of preliminary studies on the reproduction and population dynamics of Monodonta lineate and Gibbula umbilicalis on the central coast of North Spain. Takada (1996) reported vertical variation in fecundity of the intertidal gastropod M. labio from Japan. From England observations were made on spawning, larval development, settlement behavior and reproductive cycles of trochids M. lineate, G. umbilicalis and G. cineraria (Underwood, 1972). Similarly Lee (2001) from Korea has reported the gonadal development and reproductive cycle of one of the edible herbivore trochid gastropod "Omphalius rusticus". From Pakistan reports on taxonomy and biology of prosobranch gastropods (Ahmed 1980; Barkati and Ahmed 1983; Afsar et al 2012, 2013) are available in literature. However present study describes the gonadal cycle of trochid gastropod Monodonta canalifera from two rocky shores of Pakistan.

Materials and Methods

Sampling

Specimens of M. canalifera were obtained from Manora Channel (24'47' N, 66'58' E) the intensive shipping area of Karachi and Buleji rocky platform (24'50' N, 66'48' E) at ebb tides during August 2005 to July 2006. On the

northern side of the Channel, Karachi Fish Harbour is situated, while a shallow backwaters area covered with mangroves is located on the northeastern part of the channel. Fishing village of Buleji is situated Southwest of Karachi. Triangular platform of Buleji rocky ledge is facing the open Arabian Sea (Afsar et al., 2013) At monthly intervals about 16 to 27 specimens were randomly handpicked for each site. were analyzed on histological basis. Prior to preservation for histology all specimens were measured for their shell length and fixed in Davidson fluid for further as described previously by Afsar et al., (2013).

Stages of Gametogenesis and spawning season

The staging of histologically examined sections in the specimens of M. canalifera was carried out on the basis of presence of oogonia, primary oocytes, and oocyte coated with jelly layer in females and the presence of spermatogonia, spermatocytes and spermatids in males. The stages of gametogenesis are illustrated in Table 1. For the assessment of spawning season numeric ranks 0-4 were assigned to the gametogenic stages and for the assessment of spawning season the data of gametogenic stages 2 (ripe) to 4 (spawned out) was combined as detailed formerly (Afsar et al., 2013).

Measurement of oocyte diameter

Oocytes diameter in different developmental stages were measured from the histological sections by the help of an ocular micrometer across the widest part of the oocytes. For this purpose each section was divided into three sub samples of 2.5 mm area in 3-4 specimens of each month.

Results

A total of 284 (134 females and 150 males) and 273 (143 females and 124 males) specimens of M. canalifera were studied from Manora Channel and Buleji, respectively. During the present study the color variation in the gonads of male and female specimens of M. canalifera was observed. In both the species the gonad color patterns in female specimens appeared to be in shades of light to dark green and dark brown, whereas, in males it was pinkish, cream white to light yellow. Generally in spent females the gonad was light and dull green. In ripe and partially spawned females the color of gonad was found to be green, dark green and dark brown. In spent and developing males the testis color was pinkish and cream white, respectively, whereas, in ripe and partially spawned males light yellow color was noted.

Sex-ratios

At Manora Channel, the overall sex-ratios were close to 1:1 (X^2 0.90, P>0.05), except in December (X^2 4.26. P<0.05) when the ratio was in favor of females. Similarly at Buleji sex-ratios were also close to 1:1 throughout the year (X^2 2.29, P> 0.05), however, sex ratios in March (X^2 5.00, P<0.05) and May (X^2 3.00, P<0.05) were in favor of females. (Table 2).

Stages of gametogenesis

The gonadal histology of 284 (150 males and 134 females) specimens of M. canalifera from Manora Channel and of 273 (124 males and 149 females) from Buleji was performed during the study period. Following five (5) stages of gonadal development were observed in this species as illustrated in Table 1.

Temporal variation in gonadal development

Developing

At Manora Channel the developing females of M. canalifera were found from August to January. They reappeared in May and their number gradually increased till July. The peak (50%) of developing females was observed in November followed by January when 45.45% females were in developing stage. The males of M. canalifera in developing stage were found in ten months of the sampling period in fairly good numbers. However, no such individual was observed in February and July. The number of males in developing stage

increased to 50% in May which is the peak month and their lowest activity was recorded in September (12.50%) (Figure 2 & 4).

At Buleji developing females of M. canalifera were present throughout the year except, in November and February. Their greater activity was recorded in April to June period and reached the peak in May (65.00%). Such males appeared in the population during August to March and in June and July. They showed peak (60.00%) in March and their percentages declined considerably in October (6.67) and November (8.33) (Figure 3 & 4).

Ripe

In the population of M. canalifera at Manora the ripe females predominated the population throughout the year. During August to January period their percentages were in the range of 40.00 to 64.29. While in February their number declined to 33.33% and increased to 75.00 % in March, however, the peak was observed in June (77.78%). The ripe males of M. canalifera constituted 40.00 to 85.50% of the sample during August to December but their number declined to 21.43% in January. With the exception in March when no ripe male was found in the sample, the percentages of ripe males were 12.50 to 58.33 in February to July periods (Figure 2 & 4).

Fairly large number of ripe females of M. canalifera persisted year around at Buleji and reach the highest peak (90.00%) in February but their number considerably lowered down in May (15.00%). In the remaining months ripe females constituted 25.00 to 83.33% of the sample. The males of M. canalifera in ripe condition were found throughout the year except in March. During August to February period the percentage of ripe males were between 16.67 to 75.00%. April onward their percentages increased from 12.50 to 60.00 (Figure 3 & 4).

Partially spawned

The partially spawned females of M. canalifera at Manora Channel appeared in October constituting 31. 25% of the sample. Such females were present in the population in December, January, and March in fairly low numbers. Partially spawned males of M. canalifera were encountered in five months of the sampling period. Their highest peak was recorded in March (75.00%) and their lowest number (20.00%) occurred in December (Figure 2 & 4).

Partially spawned females of M. canalifera were found during August to January and in July at Buleji. Their highest percentage (68.75) was observed in September followed by July (30%) and lowest number (8.33%) was recorded in October. In males of M. canalifera at Buleji the partially spawned stage was recorded in five months of the sampling period, September to November, January, and July. The peak (44.44%) of such males was observed in July and their lowest number (16.67%) appeared in November (Figure 3 & 4).

Spawned out

In the females of M. canalifera collected from Manora Channel, spawned out condition was found in February to April period. The maximum number (44.44%) of such females was observed in February and their lowest number (6.25%) occurred in March. The males of M. canalifera in the spawned out condition appeared in the population during January, February and April. Their highest percentage 87.50 was recorded in April and the lowest (7.14%) in January (Figure 2 & 4).

At Buleji, the spawned out females of M. canalifera appeared in February and remained in the population until June in fairly low numbers (10.00 to 20.00%). The spawned out males of M. canalifera at Buleji were found

from February to July but their percentages increased from 6.67 in February to 62.50 in June, however, their number declined to 11.11% in July (Figure 3 & 4).

Spent

The spent females of M. canalifera at Manora were found in November, December, February and May. Their highest percentage (22.22) was observed in February and the lowest (7.14) was recorded in December. The males of M. canalifera in spent stage appeared in very small numbers in November, January, and February. Their highest number (10%) was recorded in February and the lowest (7.14%) in January (Figure 2 & 4).

Very small number of females of M. canalifera in spent condition occurred at Buleji only in December and May and constituted 20 and 10% of the sample, respectively. Whereas, the males of M. canalifera in spent condition were present only in February, amounting to 16.67% (Figure 3 & 4).

Spawning season

At both Manora Channel and Buleji the spawning in males and females of M. canalifera was observed throughout the year. At Manora Channel during February to April period all the females (100%) were in spawning condition. In the remaining months their percentages were 54.55 to 92.86. Likewise, more than 50% males of M. canalifera were in spawning condition in ten months of the study period, however, in February and April spawning was observed in 100% males (Figure 1).

At Buleji the spawning seems to be synchronous till January. However, in February and March the number of spawning males of M. canalifera was almost half that of females, whereas, their number was almost double in April and May when all the males were in spawning condition. Among females of M. canalifera during August to March period, 82.35 to 100% were in spawning condition. Their percentages declined in April to June period (35 to 60%) (Figure 1).

Oocyte diameter

The oocyte diameter of M. canalifera collected from Manora was significantly greater (ANOVA: F=6.18; P<0.05) than the specimens of M. canalifera studied from Buleji. The average oocyte diameter of M. canalifera at Manora was in the range of 34.67 ± 13.31 to $59.46\pm18.51\mu$ m and that at Buleji it was between 41.19 ± 11.55 to $49.35\pm13.98 \mu$ m.

Table 1. Description of gametogenesis stages in Monodonta canalifera.

Female	Male	Stages
		0
Acini contracted few in numbers. Few	Acini few in numbers and contracted. Few	Spent (0)
residual ova may be seen but in M. canalifera	residual spermatozoa can be seen.	
2	1	
autolycic of aggs not apparent		
cytorysis of eggs not apparent.		

Developing (1)	Acini small in size, spermatogonia and	Acini small in size, oogonia and primary
	spermatocytes abundant. Very few	oocytes abundant.
	spermatids or spermatozoa present in the	
	acini.	
Ripe (2)	Acini increase in size, completely filled	Acini large in size, filled with ripe ova that
	with spermatids and spermatozoa and fewer	forming a honey comb like structure.
	spermatocytes.	Gonadal area completely filled with ripe
		ova. Oogonia, primary oocytes and
		secondary oocytes rarely present.
Partially spawned	Acini large but partially empty. Gonadal	Acini large but partially empty. Gonadal
(3)	area loosely filled with unspawned	area loosely filled with unspawned ripe ova.
	spermatozoa.	
Spawned out (4)	Contracted acini, mostly empty, few acini	Contracted acini, mostly empty. Few acini
	containing residual spermatids and	containing residual oocyte.
	spermatozoa.	

Table 2. Monodonta canalifera: Sex ratios at Buleji and Manora Channel. M-male; F-female

Site	Month	Total	F	Μ	Chi-square	Male proportion
Manora Channel	Aug	28	11	17	1.29	0.61
	Sep	25	9	16	1.96	0.64
	Oct	28	16	12	0.57	0.43
	Nov	27	10	17	1.81	0.63
	Dec	19	14	5	4.26	0.26
	Jan	25	11	14	0.36	0.56
	Feb	19	9	10	0.05	0.53
	Mar	24	16	8	2.67	0.33
	Apr	25	9	16	1.96	0.64
	May	18	12	6	2.00	0.33
	June	26	9	17	2.46	0.65

	July	20	8	12	0.80	0.60
Buleji	Aug	21	17	4	8.05	0.19
	Sep	33	16	17	0.03	0.52
	Oct	27	12	15	0.33	0.56
	Nov	24	12	12	0.00	0.50
	Dec	25	10	15	1.00	0.60
	Jan	17	5	12	2.88	0.71
	Feb	16	10	6	1.00	0.38
	Mar	20	15	5	5.00	0.25
	Apr	26	10	16	1.38	0.62
	May	25	20	5	9.00	0.20
	June	20	12	8	0.80	0.40
	July	19	10	9	0.05	0.47



Figure (1) Temporal variation in spawning season of Monodonta canalifera at Manora Channel and Buleji. M- males; F- females.



Figure (2) Temporal variation in gametogenesis stages of Monodonta canalifera in Males (M) and Females (F) at Manora Channel. 0=spent; 1=developing; 2=ripe; 3=partial spawned; 4=spawned out



Figure (3) Temporal variation in gametogenesis stages of Monodonta canalifera in Males (M) and Females (F) at Buleji 0=spent; 1=developing; 2=ripe; 3=partial spawned; 4=spawned out



Figure (4) Photomicrographs showing developmental stages in histological sections of gonads of female (A-C) and Male (D-F). A-B, ripe; C, spawning; D, developing; E, ripe; F, spawning.

Abbreviations: jl- jelly layer; lu- lumen; mo-mature oocyte (post- vitellogenic); N- nucleus; n- nucleolus; oc- ripe oocyte; sg-spermatogonia; sc- spermatocyte; st- spermatid; sz-spermatozoa.

Discussion

In the present study two populations of archaeogastropod M. canalifera were examined for gonadal development and temporal variation in gemetogenesis from Manora Channel and Buleji. The histological analysis revealed the gonochoristic sexual mode in candidate species at both sites. No hermaphrodites were found in the targeted populations. The dominant possible sexual modes for most prosobranchs have long been established as gonochoristic with small proportion hermaphrodites in the population (Fretter and Graham 1964; Fretter 1984; Beninger et al., 2001). Morphologically ovary of M. canalifera appeared green to olive green and the testes was creamy to creamy pink same as it has been previously described by Underwood (1972) for British trochids G. umbilicalis and M. lineata respectively in females and males.

At both sites spawning in males and females was observed throughout the year consequently gonadal maturity was apparent year round. Though ripe females predominated the population throughout the year. The peak of gonadal maturation in Manora population occurred in summer (June and July) and in Buleji population during early spring (February). Variation in populations of M. labio from Japan has been reported from Japan. In Northern Japan population the reproductive season occurred during July to August (Kojima, 1962), whereas in the population of South Japan it extend from April to October for the same species (Sumikawa, 1958, 1963; Takada 1996).

At Manora Channel during February to April increased spawning was observed and all females were found in spawning state though more than 50% females were spawning in rest of the month .. Likewise, in February and April all the males (100%) were found to be in spawning condition. Other than that at Buleji the spawning appears to be synchronous till January. While, in February and March the number of spawning males was almost half as compare to females, and subsequently in April and May increased to 100%. Peak spawning activity in females of M. canalifera was found during August to March period, amounted 82.35 to 100% spawning followed by a declined in April to June period when it was ranged between 35 to 60%. Gonadal maturation and spawning in intertidal molluscan communities is rather coupled with increasing temperatures, as this prototype for gonadal maturation has been reportedly found in many species of prosobranchs (Yamamoto and Yamakawa 1985; Brousseau 1995; Kim et al., 1999; Lee (2001) also found rising sea water temperature and coinciding spawning period in both sexes of trochid Omphalius rusticus, from west coast of Korea. Upon revelation of study result a slight swing is evident among populations spawning rhythm at Manora in comparison to Buleji is showing slight variation that could be possibly due to exogenous environmental factors (Kim et al., 1999; Lee 1999; Lee 2001) and corresponding endogenous endocrine system (Euler and Heller, 1963; Afsar et al., 2013) as described by earlier workers. Reproductive biology of some prosobranchs inhabiting Pakistan coast, have been studies in past decades. Reproductive season of four prosobranchs; namely Thais rudolphi, T. carinifera, T. bufo and T. tissoti based on the occurrence of egg capsules was carried out. Respective breeding seasons were pragmatic during June to September, February, April to August and March to August respectively (Barkati and Ahmed 1983). Spawning in four species was found to be associated with rising temperature of springs and summer when a lowering of salinity also occur due to the south west monsoon rains (Ahmed 1980; Barkati and Ahmed 1983).

Conclusion

The information collected in this study will provide the baseline information for future detailed studies on reproductive patterns and biological aspects of native marine prosobranch communities of trochid kinfolk for better scientific understanding. Lastly, this could be possibly serve for biodiversity management interference in future under certain conditions.

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