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RESEARCH ARTICLE

Comparison of Alteration in growth, Maturity and Spawning Ecology of Patella-gastropod, *Cellana karachiensis* (Winkworth, 1930) Deduced from Macro and Microscopic Techniques

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ABSTRACT

In the present study sex-ratio, gonadal maturation and temporal variation in the reproductive pattern of the limpet *Cellana karachiensis* was determined by the histological examination of the gonads. The specimens of *Cellana karachiensis* were collected from two rocky shores of Buleji and Paradise Point, located at the distance of 50km in the northwest of Karachi, Pakistan. Some 721 handpicked specimens were sampled from November 2007 to April 2009. The maximum total length of *C. karachiensis* was measured 50 mm and 40 mm at Buleji and Paradise Point respectively. This size variation might be due to adequate food-availability or space. Sex ratios at both sampling locations were male biased. The size-class distribution analysis showed that males and females in this species were equally represented in all the size-classes, which indicated that this species probably does not change sex. It was further validated by the histological study of gonad that no hermaphrodites were observed. The seawater temperature at both sites was low (average 23° C) in winter (northeast monsoon) with sudden rise of 5° C in spring inter-monsoon (average 28 °C) which remained high throughout the summer (south west monsoon). The highest gonado sometic index GSI = 18.1, 18.6 mean values calculated for males and 15.8, 16.3 for females at Buleji and Paradise Point during summer (south west monsoon) respectively. Three developmental stages of developing, ripe and spawning with no spent condition were deduced from microscopic gonad characteristics. The study showed an extended pattern of reproductive cycle with multiple spawning episodes and no resting stage in *C. karachiensis*. Although spawning in patella-gastropod occurred throughout the year but it was more intensified owing to the northeast monsoon characterized by low temperatures. It is hoped that this first-time study would provide underline information about an important gastropod species.

INTRODUCTION

The preponderance of limpets in the intertidal and subtidal habitats has a significant effect on the inhabiting ecosystem as they contribute to overall diversity of the shore by providing substrate to other species (Branch, 1981). Being an herbivore and a food source for carnivorous animals, limpets play an important role towards the energy transfer in the food chain of the intertidal rocky shore community (Hawkins and Hartnoll, 1978) and also affect community structure by influencing algal species composition and growth (Wright and Hartnoll, 1981). The limpets have gained economic importance and are exploited as food or bait in different parts of the world-such as, in India (Pillai and Menon, 2000), South Africa (Hockey and Bosman, 1986) Australia (Keough et al., 1993), North America (Pombo and Escofet, 1996; Lindberg et al., 1998) and Korea (Yoon and Kim, 2007).

The reproductive cycles of intertidal invertebrates have been extensively studied in order to provide information on spatial and temporal variations in spawning and fecundity (see review in Branch, 1981). Such studies are of prime importance for the estimation of timing and magnitude of the influx of recruits on the shore, determination of age at sexual maturity and sex

ratios of a population (Dunmore and Schiel, 2000). The limpets are important component in inter tidal zones of the rocky shores therefore, extensive investigations have been made to study the reproductive cycles of temperate water species, such as, *Patella vulgata.*, Linnaeus, 1758 (Orton, 1928; 1946; Das and Seshappa, 1947; Orton et al., 1956; Bowman and Lewis, 1977; Baxter, 1983; Bowman and Lewis, 1986; Guerra and Gaudêncio, 1986), *Patella depressa* (Orton and Southward, 1961; Bowman and Lewis, 1986; Guerra and Gaudêncio, 1986; Brazão et al., 2003), *Patella ulyssiponensis* (Thompson, 1979; Bowman and Lewis, 1986; Guerra and Gaudêncio, 1986); *Patella aspera* (Thompson, 1979); *Nacella concinna* (Picken, 1980; Picken and Allan, 1983; Brethes et al., 1994) and tropical water species: *Cellana radiata* (Rao, 1973); *Notoacmea petterdi* (Creese, 1980a; Parry, 1982); *Cellana tramoserica* (Parry, 1982; Fletcher, 1984); *Cellana radians* (Creese and Ballantine, 1983), *Patelloida alticostata* (Creese, 1980a; Parry, 1982; Fletcher, 1987), *Patella pygmaea* and *Cellana grata* (Liu, 1994); *Patella flexuosa* (Iwasaki, 1998) and *Cellana ornata* (Dunmore and Schiel, 2000).

Most limpets have short breeding seasons, and some have a single annual spawning season followed by a long resting stage such as *Patella vulgata* (Das and Seshappa, 1947), *Cellana nigrolineata* (Catalan and Yamamoto, 1993; *Cellana ornata* (Dunmore and Schiel, 2000). This situation in invertebrates has been reported to be typical among the broadcast fertilizers (Liu., 1994). Other patellids, such as *Cellana radiate* (Iwasaki, 1998), *Cellana radians* (Creese and Ballantine, 1983) or acmaeid, *Patelloida alticostata* (Fletcher, 1987) *Patella lutistrigutu* (Creese, 1980b) have been reproductively active during a large part of the year with multiple spawning episodes and little or no resting stage. The gonadal cycle is mainly under the influence of temperature (Das and Seshappa, 1947) and availability of food (Creese, 1980b). On the reproduction and spawning various authors worked from different parts of the world (Sutherland, 1970; Bosman and Hockey, 1988; Guallart et al. (2020); Reguera et al. (2018). Nhan and Ako (2019) explained the purpose of development of aquaculture technologies for the Hawaiian limpets. Vafidis et al., 2020 described correlation of ecological attributes with population, density, abundance and frequency.

This study is the first to document life history characters of an important gastropod sampled from two rocky shores of the Karachi coast, Pakistan. The reproductive cycle of the limpet was determined from the gonad index assessment and histological sections of the male and female gonad materials. Sexual maturity in males and females was also determined.

MATERIALS AND METHODS

Site description

Two rocky ledges, Buleji and Paradise Point were selected for study, which lie adjacent to each other on Karachi coast lies at 24° 49’ N and 66° 50’ E of Karachi between Hawks Bay and Paradise Point, covering an area of about 800 meters. The ledge is triangular in shape and divisible into an exposed area which faces the open ocean and is under high wave action. The western protected side of the ledge is exposed to less wave action and is totally submerged on high tides. The ledge is luxuriant in algal vegetation. Buleji rocky ledge is a restricted area with no access to general public as is the other study site, Paradise Point. The Paradise Point is situated at 24° 50’ N and 66° 48’ E of Karachi between Buleji and Nathiagali. The slope of the shoreline of Paradise Point shows a sharp gradient towards the sea which results in narrow intertidal zone. The bottom of the shore is covered by boulders which are rather flat as compared to Buleji which showed elevations and depressions. On western side of this collection site has Rocky Mountains about 30 meters in height. In the supratidal zone a rocky mountain, 10 meters in height and 10 meters in length covered abundantly with limpets and barnacles can be seen. The other fauna in this area is scanty probably due to the location of Karachi Nuclear Power Plant which is operational since 1972. Paradise Point is also used by general public for recreational purposes.

Sex determination and gonad developmental stage

Patellid limpets have simple reproductive system and the gonad in males and females lies on the ventral surface of the visceral mass (Das and Seshappa, 1947). The sex and gonadal stages of the limpets were determined by detaching the foot from the visceral mass and the shell. The gonad was examined for the color, size and texture following the methodology described by earlier workers (Das and Seshappa, 1947; Shaw and Battle, 1957). The gonads of both males and females were of different colour in stages of maturation observed during the present study. The spawning and developing gonads were observed to be similar in size but can be distinguish on the basis of texture. The spawning gonads are flaccid and the developing gonads are firm. The ripe gonads are easily distinguishable as they markedly increase in size when they are ripe. The colour of male gonads was creamy peach during developing stage, peach or light orange in ripe stage and in, spawning males can be recognized by dark orangish tinge in the gonads. The gonad color was creamy green in developing females, olive green in ripe females and spawning females were identified by looseness of the eggs in the ovary and were dull green in appearance. The identification of sex and gonadal maturity was further confirmed by the histological examination of the gonads.

Histological characteristics of gonad materials

The animal was removed from their shell and weight of the whole tissue was taken to the nearest 0.01 gm. The gonad was removed from the tissue and weigh to the nearest 0.01 gm. For histological analysis of gonad, one gonad of each individual sample was fixed in Davidson's fluid (Humason, 1967) for 48 hours and then transferred to 70% alcohol till further processing. The gonads were passed through dehydration and infiltration and then embedded in paraffin wax and sectioned at a thickness of 7 µm by a rotary microtome (PR-5014) and dried in air. The sections were stained with Delafield's Haematoxylin and counter stained with Eosin (Gonor, 1972).

Gonado-somatic Index assessment

Gonado-somatic index (GSI) was calculated by using the formula:

GSI = wet gonad weight / total wet body weight × 100 (King, 1995).

The gonad indices were plotted against the months for males and females obtained from the sampling sites.

Gametogenesis

The gonads of males and females of *C. karachiensis* on the basis of histological examination were categorized into three stages of maturation 1. Developing 2. Ripe and 3. Spawning. The characteristics of each stage are described in Table 1.

Size at first sexual maturity

The limpets have no external sexual dimorphism therefore size of first sexual maturity was estimated by adopting the criteria of King (1995). According to him the size at which the gonad of 50% limpets in a given size class contained either spermatozoa or vitellogenic eggs is used as an index of first sexual maturity.

RESULTS

Sex-ratios (Buleji)

A total of 721 specimens of *C. karachiensis* were examined from Buleji to find the proportion of males and females in the population during the study period from November 2007 to April 2009. The over-all sex-ratios in the population of *C. karachiensis* deviated significantly from the Mendelian 1:1 ratio in favour of males ($X^2 = 13.6$; $P < 0.001$) at Buleji. Month-wise distribution of sexes at Buleji showed that the sex-ratio deviated significantly in favour of males in January 08 and March 09 while in the rest of the study period it did not deviate from 1:1 ratio ($P > 0.05$). In all the size-classes male and female limpets were present. The distribution of male and female *C. karachiensis* in different size-classes at Buleji revealed that the sex-ratios in size-classes 21-25 mm and 26-30 mm were significantly ($P < 0.05$) in favor of males, while in remaining size-classes the distribution of male and female was close to Mendelian 1:1 ratio (Table 2 & 4).

Paradise Point

A total of 720 specimens of *C. karachiensis* were examined during the study period at Paradise Point to identify their sex. The over-all sex-ratios in the population of *C. karachiensis* at Paradise Point, deviated significantly from 1:1 ratio in favour of males ($X^2 = 8.9$; $P < 0.01$). Month wise distribution of sexes showed that the sex-ratio followed the normal Mendelian distribution of 1:1 during the study period with the exception in November'07 and August'08, when they deviated significantly in favour of males.

The distribution of males and females of *C. karachiensis* in different size-classes at Paradise Point revealed that the sex-ratios in different size-classes followed the Mendelian distribution of 1:1 with the exception of the size-class 26-30 mm, the sex-ratios were significantly ($P < 0.01$) in favor of males (Table 3 & 4).

Size at first sexual maturity

First sexual maturity in *C. karachiensis* occurred at a shell length of 16-20 mm at Buleji and Paradise Point (Table 5).

Temporal variation in Gonado-Somatic Index (GSI)

Buleji: The mean GSI for males was always higher than that for females, however, both males and females showed a similar gonadal cycle. The gonad indices increased from February to August 2008 for males and from April to August 2008 for females. The gonado-somatic index showed a decrease from September 2008 to January 2009 in males, after which the GSI increased till April 2009. The gonado-somatic index in females decreased from September to December 2008 and then an increase in GSI was noted from January onwards till April 2009 (Fig. 1). Seasonal variation showed that the GSI values were comparatively higher in spring inter-monsoon and SW monsoon than in autumn inter-monsoon and NE monsoon at Buleji (Table 6).

Paradise Point

At paradise point the gonado-somatic index in males ranged from 6.7 to 18.6 and in females from 7.5 to 16.3. The males showed an increased GSI for a prolonged period from March to October 2008, with females showing a similar increased GSI in the same period from March to October 2008. In the rest of study period the GSI fluctuated with high values in December 2007 and March to April 2009 in males and in January 2008 and March 2009 in females (Fig. 2). Seasonal variation showed that the GSI value were comparatively higher in spring inter-monsoon and SW monsoon compared to autumn inter-monsoon and NE monsoon at Paradise Point (Table 7).

Temporal variation in the stages of gonad development (Buleji)

Developing

The males of *C. karachiensis* in the developing stage occurred throughout the study period with the exception

Table 1: Stages of gonadal development in males and females of *Cellana karachiensis* (McCarthy et al., 2008; Rao, 1973).

Stages	Description
Developing	The follicles few in number and narrow in size, the inter-follicular connective tissue abundant. As gonadal maturation progresses the walls of gonadal follicles become thicker and the follicles increased, the intra-follicular space reduced. Female: During early development mostly oogonia and previtellogenic oocytes are present and with the gonad development vitellogenic oocytes begin to appear. Male: The tissue consists of spermatogonia, spermatocytes with few spermatids. The spermatids are present in the center of the gonadal follicle and the spermatogonia and spermatocytes are located at the periphery.
Ripe	Follicles completely fill the gonadal area and connective tissue nearly absent. Female: Mostly vitellogenic and ripe oocytes present very few oogonia and previtellogenic oocytes found close to the follicles borders. Male: Lumen of the follicles filled with spermatozoa; few spermatids and spermatocytes present at the periphery of the follicles.
Spawning	The follicles reduced in size and connective tissue appeared. Female: Decrease in numbers of ripe and vitellogenic oocytes. Some oogonia and previtellogenic present on outer border of follicles. Atretic oocytes observed in the follicles. Male: The follicles narrow and few spermatozoa present. Some spermatocytes present on outer border of follicles and atretic areas in the lumen.

Table 2: Monthly sex-ratios of *Cellana karachiensis* at Buleji during the study period.

Months	Total	No. of males	No. of females	Chi square	Probability
November'07	40	23	17	0.9	> 0.05
December	40	26	14	3.6	> 0.05
January'08	40	30	10	10.0	< 0.01
February	40	22	18	0.4	> 0.05
March	40	22	18	0.4	> 0.05
April	40	18	22	0.4	> 0.05
May	40	21	19	0.1	> 0.05
June	40	24	16	1.6	> 0.05
July	40	25	15	2.5	> 0.05
August	40	24	16	1.6	> 0.05
September	40	25	15	2.5	> 0.05
October	40	22	18	0.4	> 0.05
November	40	20	20	0.0	> 0.05
December	40	23	17	0.9	> 0.05
January'09	40	17	23	0.9	> 0.05
February	40	18	22	0.4	> 0.05
March	40	27	13	4.9	< 0.05
April	41	23	18	0.6	> 0.50
Total	721	410	311	13.6	< 0.001

Table 3: Monthly sex-ratios of *Cellana karachiensis* at Paradise Point during the study period

Months	Total	No. of males	No. of females	Chi square	Probability
November'07	40	30	10	10.0	< 0.01
December	40	22	18	0.4	> 0.05
January'08	40	25	15	2.5	> 0.05
February	40	22	18	0.4	> 0.05
March	40	21	19	0.1	> 0.05
April	40	22	18	0.4	> 0.05
May	40	22	18	0.4	> 0.05
June	40	22	18	0.4	> 0.05
July	40	21	19	0.1	> 0.05
August	40	27	13	4.9	< 0.05
September	40	23	17	0.9	> 0.05
October	40	14	26	3.6	> 0.05
November	40	24	16	1.6	> 0.05
December	40	24	16	1.6	> 0.05
January'09	40	19	21	0.1	> 0.05
February	40	23	17	0.9	> 0.05
March	40	22	18	0.4	> 0.05
April	40	17	23	0.9	> 0.05
Total	720	400	320	8.9	< 0.01

Table 4: The sex-ratios of *Cellana karachiensis* in various size-classes at Buleji and Paradise Point.

Buleji (n = 721)					
Size-classes (mm)	Total	No. of males	No. of females	Chi square	Probability
11-15	11	8	3	2.3	> 0.05
16-20	147	79	68	0.8	> 0.05
21-25	268	153	115	5.4	< 0.05
26-30	188	109	79	4.8	< 0.05
31-35	88	49	39	1.1	> 0.05
36-40	17	10	7	0.5	> 0.05
41-45	1	1	0	1.0	> 0.05
46-50	1	1	0	1.0	> 0.05
Paradise Point (n = 720)					
Size classes (mm)	Total	No. of males	No. of females	Chi square	Probability
11-15	3	1	2	0.3	> 0.05
16-20	174	99	75	3.3	> 0.05
21-25	320	171	149	1.5	> 0.05
26-30	163	99	64	7.5	< 0.01
31-35	50	25	25	0.0	> 0.05
36-40	10	5	5	0.0	> 0.05

Table 5: The first sexual maturity in *Cellana karachiensis.*

Buleji (n = 721)		
Size-class (mm)	Total number of limpets examined	Percentage of mature limpets
11-15	11	0.0
16-20	147	56.5
21-25	268	73.9
26-30	188	73.9
31-35	88	84.1
36-40	17	88.2
41-45	1	100.0
46-50	1	100.0
Paradise Point (n = 720)		
Size-class (mm)	Total number of limpets examined	Percentage of mature limpets
11-15	3	0.0
16-20	174	59.8
21-25	320	65.3
26-30	163	73.0
31-35	50	70.0
36-40	10	90.0

Table 6: Seasonal variations in the gonad index of *Cellana karachiensis* at two collection sites. The values are mean ± standard deviation.

Sex	Sites	Northeast monsoon	Spring inter-monsoon	Southwest monsoon	Autumn inter-monsoon	Northeast monsoon	Spring inter-monsoon
Males	Buleji	11.6 ± 2.9	16.6 ± 1.1	14.4 ± 3.0	11.5 ± 0.0	10.6 ± 3.0	15.1 ± 1.0
	Paradise Point	11.4 ± 4.0	15.4 ± 0.8	16.4 ± 2.0	15.0 ± 0.0	11.5 ± 2.8	16.1 ± 0.1
Females	Buleji	10.9 ± 0.8	12.9 ± 2.1	12.5 ±1.9	12.1 ± 0.0	11.2 ± 1.2	12.7 ± 0.9
	Paradise Point	12.4 ± 2.6	14.5 ± 1.8	14.0 ± 2.6	9.9 ± 0.0	10.2 ± 1.9	11.5 ± 2.3

Table 7: Seasonal variations in spawning of *Cellana karachiensis* at two collection sites. The values are mean±standard deviation.

Sex	Sites	Northeast monsoon	Spring inter-monsoon	Southwest monsoon	Autumn inter-monsoon	Northeast monsoon	Spring inter-monsoon
Males	Buleji	41.8 ± 11.7	11.1 ± 15.7	25.2 ± 27.1	40.9 ± 0.0	43.4 ± 14.6	18.0 ± 0.8
	Paradise Point	46.9 ± 5.1	18.7 ± 7.1	27.4 ± 24.2	21.4 ± 0.0	47.3± 7.8	27.0 ±13.2
Females	Buleji	49.9 ± 22.3	14.6 ± 5.0	39.2± 21.5	22.2 ± 0.0	38.1 ± 12.5	19.9 ± 4.5
	Paradise Point	61.0 ± 20.7	43.4 ± 9.3	46.8 ± 28.6	11.5 ± 0.0	54.9 ± 11.2	22.0 ± 0.3

in January 2009. In samples obtained in 2008, two peaks of developing males, one in March-April and other in August-September were observed. In 2009 the peak of developing males was found in February-March period. Similarly, the females of *C. karachiensis* in this stage were found throughout the study period with the exception of June 2008. The females like males also showed two peaks, one in March-April and other in August-September in the year 2008 and in March-April during 2009 (Fig. 3).

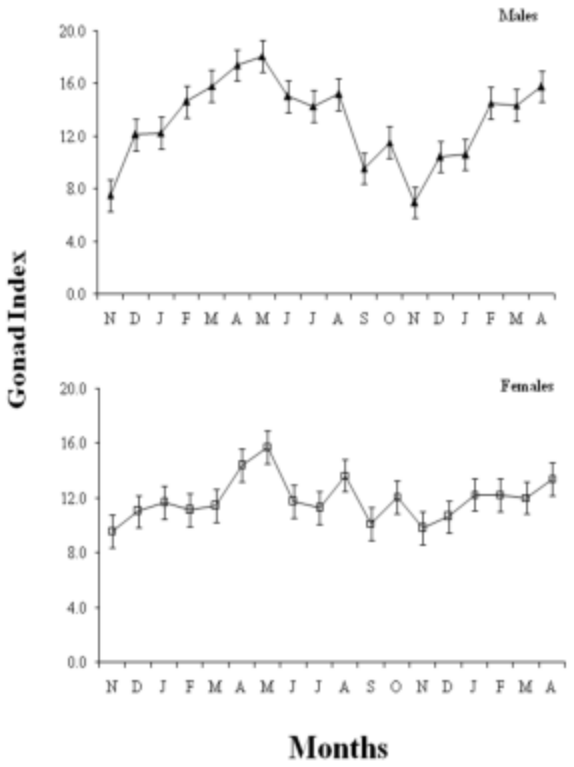

Figure 1: Monthly variations in gonad index of *Cellana karachiensis* at Buleji during the period from November 2007 to April 2009.

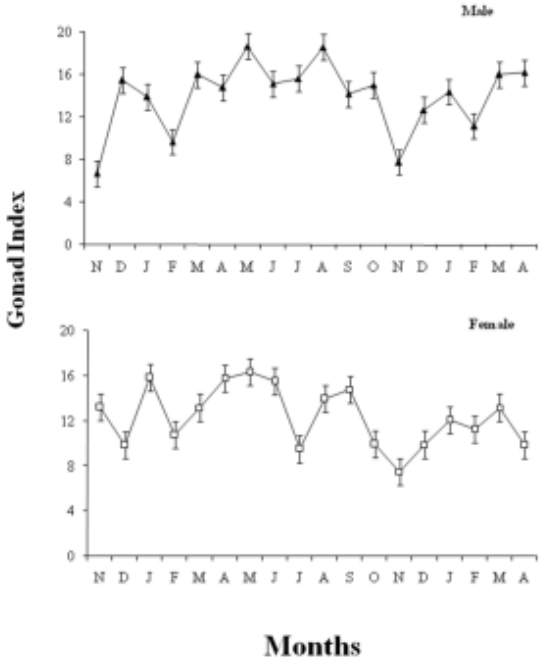

Figure 2: Monthly variations in gonad index of *Cellana karachiensis* at Paradise Point during the period from November 2007 to April 2009.

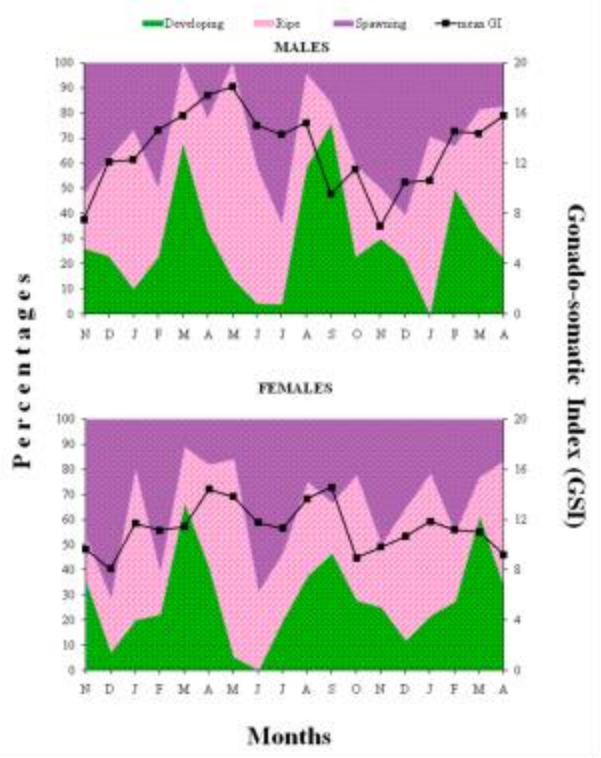

Figure 3: Monthly variations in the gametogenic patterns and gonad index of *Cellana karachiensis* (males and females) at Buleji.

Ripe

The males and females of *C. karachiensis* in the ripe stage occurred throughout the study period. In the year 2008 the ripe males showed peak (68.3%) in January which coincided with peak (60.0%) of ripe females in the same month. The second peak of ripe males was during April to June while the ripe females predominated in April to May and again in October and December. In 2009 the number of ripe males was considerably high in January and March to April period, while the ripe females dominated in January and April (Fig. 3).

Spawning

The spawning males of *C. karachiensis* were encountered throughout the study period with the exception, in March and May 2008, while, spawning females of *C. karachiensis* were almost present throughout the study period. The spawning in males and females was recorded in greater numbers during November to December 2007. However, in the limpets observed in 2008, the spawning in males was recorded in February, June to July and October to December and in females in February, June to July and November to December (Fig. 3).

Paradise Point

Developing

The males of *C. karachiensis*in the developing stage occurred throughout the study period at Paradise Point. Two peaks, one in March to April and other in August

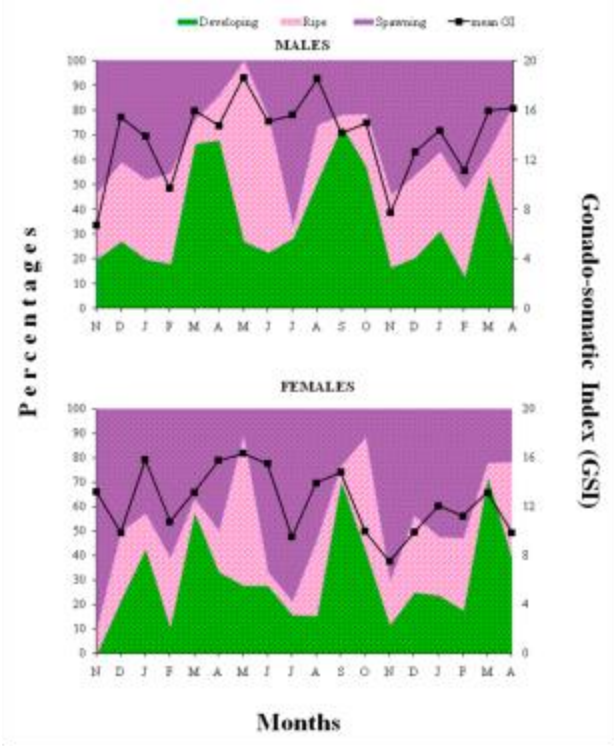

Figure 4: Monthly variations in the gametogenic patterns and gonad index of *Cellana karachiensis* (males and females) at Paradise Point.

to October were observed for developing males in the year 2008. In 2009, the peak of developing males was found in March. The developing females of *C. karachiensis* were found throughout the study period with the exception in November 2008. The developing females showed two peaks, one in March to April and other in September to October in the year 2008. In 2009, the peak of developing females was found in March to April (Fig. 4).

Ripe

The ripe males and females of *C. karachiensis* were encountered throughout the study period at Paradise Point. The ripe males in 2008 showed peak in May and June (72.7 and 54.5%, respectively), after which abrupt decrease in their numbers was observed till November when the ripe males amounted to 29.2% of the population. The ripe females were abundant in May (61.1%) and their number declined abruptly until October (46.2%) when second peak was recorded. In the following year, the ripe males and females appeared in large numbers in April and their percentages were 58.8 and 38.1, respectively (Fig. 4).

Spawning

The data of *C. karachiensis* shows that males had a prolonged spawning period at Paradise Point which was from November 2007 to February 2008, in July 2008 and from November 2008 to March 2009.

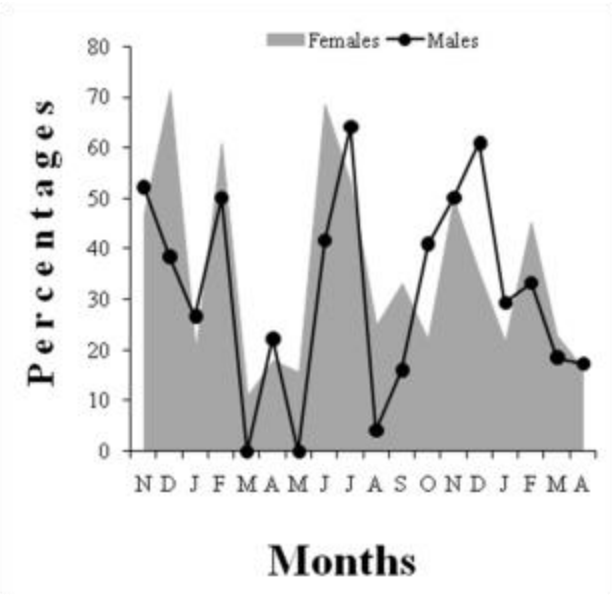

Figure 5: Spawning season of males and females of *Cellana karachiensis* at Buleji.

A similar pattern of spawning was observed in the females, when spawning females were encountered in greater numbers from November 2007 to April 2008, June to August 2008 and from November 2008 to February 2009 (Fig. 4).

Temporal variation in reproductive cycle

Buleji: The spawning season was assessed on the basis of percentages of spawning males and females in the population, the. The spawning in the males and females of *C. karachiensis* at Buleji showed almost a similar pattern during the study period. The spawning in males was intensified from November 2007 to February 2008, in June to July 2008 and from October 2008 to February 2009. The spawning in females showed a similar trend as in the males with most of the spawning occurred from November 2007 to February 2008, from July to December 2008 and then in February 2009 (Fig. 5). Seasonal variation showed that the spawning in males and females were comparatively more intensified in NE monsoon than in the other seasons. There was significant correlation in spawning between females and males ($r = 0.680$; $P = 0.002$; $n = 18$) at Buleji. The spawning females and males showed negative correlation with temperature (Table 7 & 8) though not significant. The spawning females and males showed no significant correlation with salinity and chlorophyll-a.

Paradise Point

The spawning in males of *C. karachiensis* at Paradise Point was intensified from November 2007 to February 2008, in July 2008 and from November 2008 to March 2009). The females showed a prolonged spawning period from November 2007 to April 2008, then from

Table 8: Pearson's correlation between spawning females and males and temperature, salinity and chlorophyll-a at Buleji and Paradise Point. *Correlation is significant at the 0.01 level.

Sites		Temperature	Salinity	Chlorophyll-a
Buleji				
Spawning females	Pearson Correlation	-0.347	0.077	-0.161
	Sig. (2-tailed)	0.159	0.762	0.522
	N	18	18	18
Spawning males	Pearson Correlation	-0.411	0.090	-0.246
	Sig. (2-tailed)	0.090	0.724	0.326
	N	18	18	18
Paradise Point				
Spawning females	Pearson Correlation	-0.315	-0.249	-0.352
	Sig. (2-tailed)	0.203	0.319	0.152
	N	18	18	18
Spawning males	Pearson Correlation	-0.685*	0.231	-0.103
	Sig. (2-tailed)	0.020	0.357	0.685
	N	18	18	18

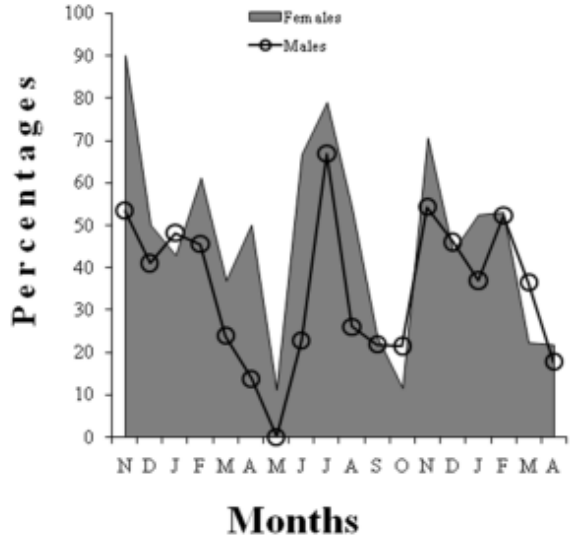

Figure 6: Spawning season of males and females of *Cellana karachiensis* at Paradise Point.

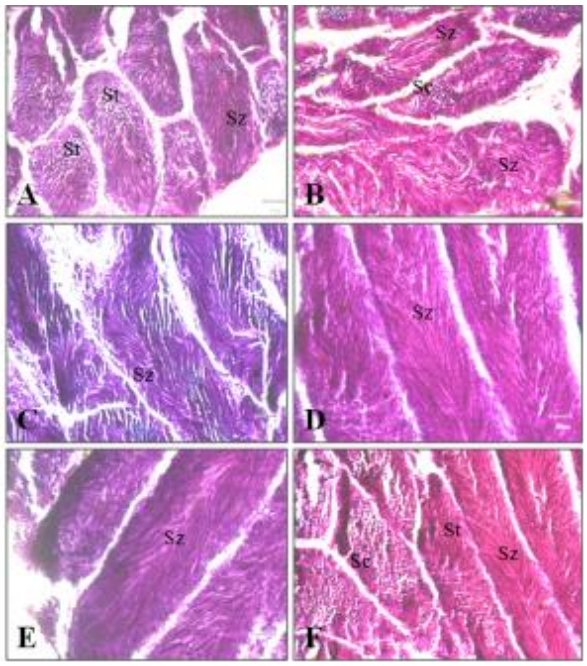

Figure 7: Photomicrographs showing developing stage of female gonad in *Cellana karachiensis*. Og - oogonia; Pvo - previtellogenic oocytes; Vo - vitellogenic oocytes; Mo - mature oocytes; Ct - connective tissue; N- nucleus. Scales: A, B & D = 50µm; C = 100µm

June to August 2008 and again from November 2008 to February 2009 (Fig. 6). Seasonal variation showed that the spawning in males and females were comparatively more intensified in NE monsoon at Paradise Point. There was significant correlation in spawning between females and males (r = 0.697; P = 0.001; n= 18) at Paradise Point. The spawning females and males showed negative correlation with temperature, being significant in males (r = -0.586; P = 0.002; n=18). The spawning females and males showed no significant correlation with salinity and chlorophyll-a (Table 7 & 8).

DISCUSSION

The present study provided the first description of the sex-ratio, gonad maturation and temporal variation in the reproductive pattern of the limpet *Cellana karachiensis* from the Karachi coast, Pakistan. The reproductive pattern was determined by the histological examination of the gonads which provides a precise assessment of different gonadal stages, as has been reported in *Patella ornata* from southern New Zealand (Dunmore and Schiel, 2000), *Collisella heroldi* from Hokkaido, Japan (Niu and Fuji, 1989), *Patella vulgata* from central region of the Portuguese coast (Brazão et al., 2003) and *Patella vulgata* and *Patella ulyssiponensis* from southwest coast of Ireland (McCarthy et al. 2008).

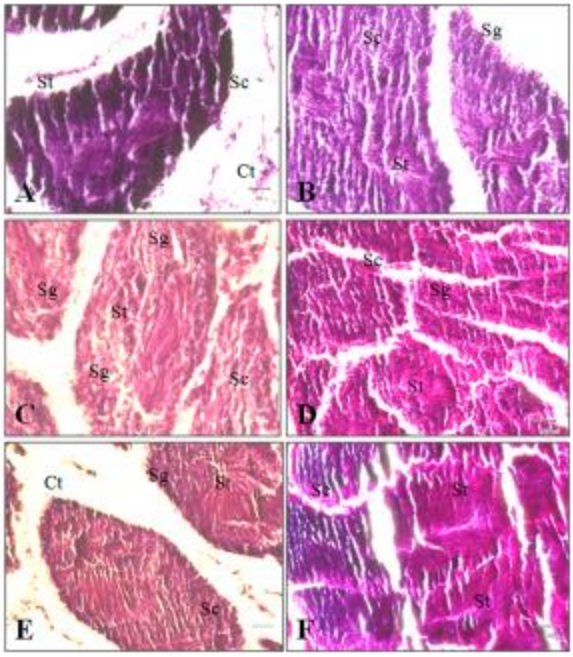

Figure 8: Photomicrographs showing ripe stage of female gonad in *Cellana karachiensis*. Og - oogonia; Vo - vitellogenic oocytes; Mo - mature oocytes; N - nucleus. Scales: A = 100µm; B & C = 50µm

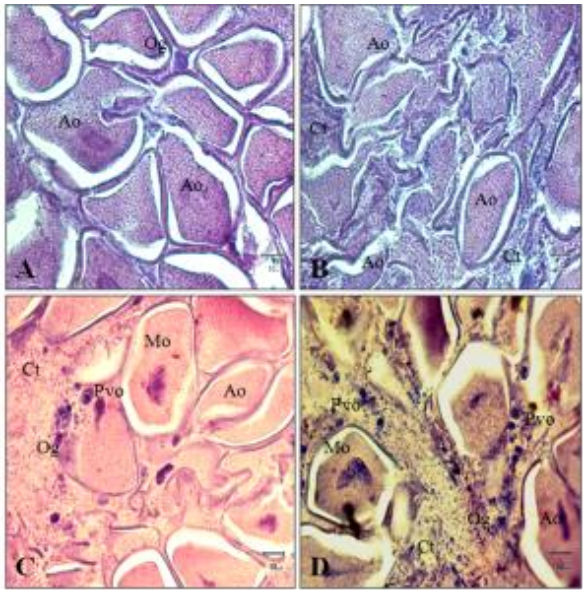

Figure 9: Photomicrographs showing spawning stage of female gonad in *Cellana karachiensis*. Og - oogonia; Pvo - previtellogenic oocytes; Mo - mature oocytes, Ct - connective tissue; Ao - atretic oocytes. Scales: A, B, C & D = 50µm.

The overall sex-ratio in the population of *C. karachiensis* at both sites was in favour of males. Earlier the abundance of males in the population of limpets has been reported by several authors such as in

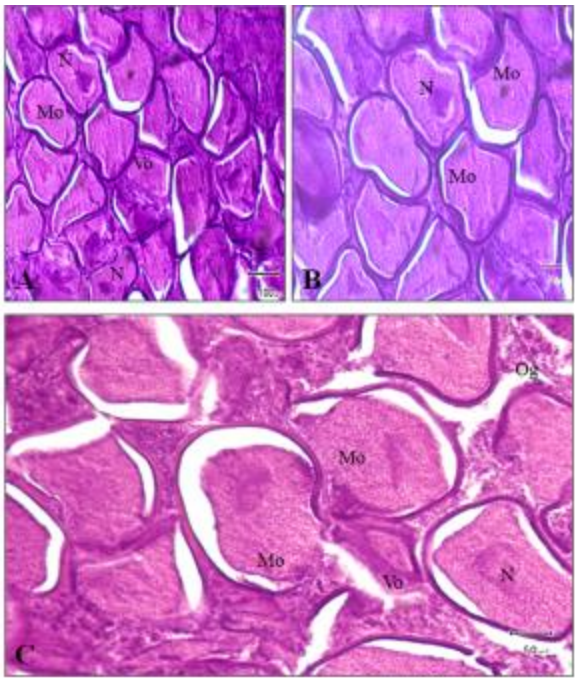

Figure 10: Photomicrographs showing the developing stage of male gonad in *Cellana karachiensis*. Sg - spermatogonia; Sc - spermatocytes; St- spermatids; Ct - connective tissue. Scales: A, B & C = 50µm; D, E & F =100µm

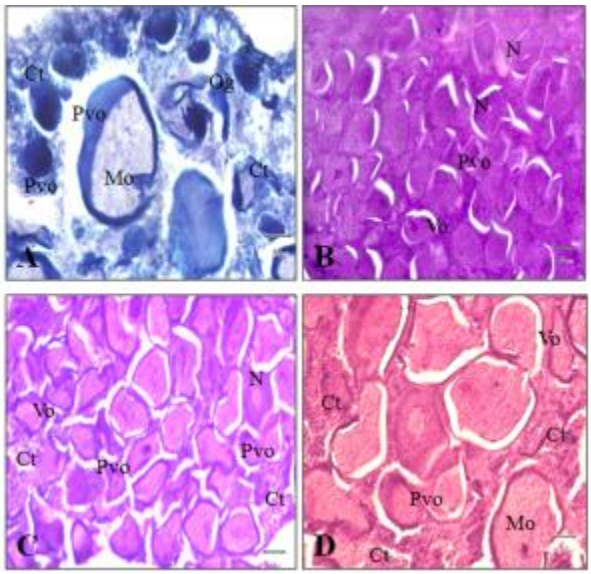

Figure 11: Photomicrographs showing the ripe stage of male gonad in *Cellana karachiensis.* Sc - spermatocytes; St - spermatids; Sz - spermatozoa. Scales: A, B & C = 100µm; D, E & F =50µm

Cellana radiata from India (Rao, 1973), *Cellana testudinaria* from Indonesia (Khouw, 2003), *P. vulgata* and *P. ulyssiponensis* from Ireland (McCarthy et al. 2008)

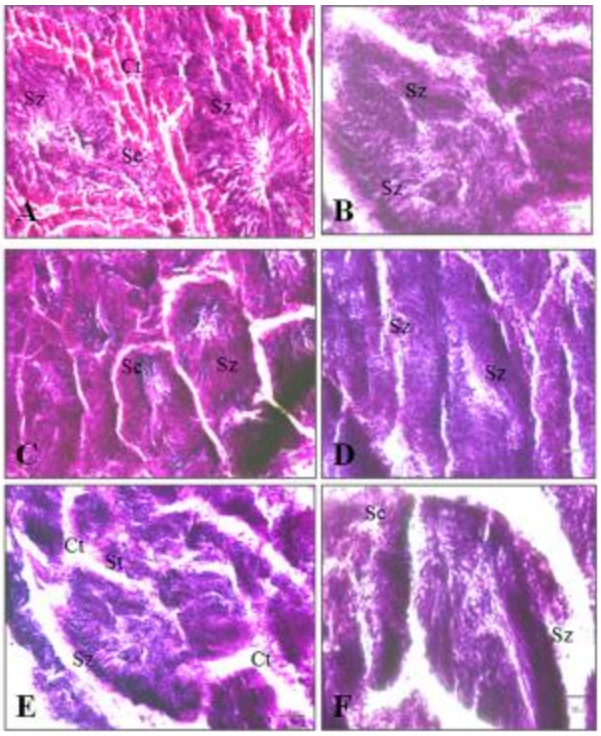

Figure 12: Photomicrographs showing the spawning stage of male gonad in *Cellana karachiensis*. Sc - spermatocytes; St- spermatids; Sz - spermatozoa; Ct - connective tissue. Scales: A, B, D & F = 50µm; C & E =100µm

and *Patella depressa* from Portugal (Ribeiro et al., 2009). On the contrary, Ribeiro et al. (2009) while studying three other species of limpets, *P. ulyssiponensis*, *P. vulgata* and *P. rustica* from Portugal reported the pre-dominance of females over males in the population. Generally, a larger number of females in the population are attributed to sex change in the species, differential growth or differential mortality between the sexes (Orton et al., 1956; Branch, 1974) while an increased number of males have been related to the fact that the sperm can be limiting in intertidal populations due to the wave intensity in the environment (Levitan, 1995). The increased numbers of males of *C. karachiensis* in the present study at both sites may be linked to this prediction (Levitan, 1995).

The size wise analysis of male and female *C. karachiensis* showed that males and females in this species were equally represented in all the size-classes, which indicated that this species probably does not change sex. This is further supported by the fact that no hermaphrodites were observed during the histological analyses of the gonads. Similar results have been observed in other limpets, *C. radiata* from India (Rao, 1973), *P. depressa* from north Cornish coast of Britain (Orton and Southward, 1961), *Fissurella barabadensis* from Gulf of Mexico (Ward, 1966). However, in hermaphrodite species, *P. vulgata* male predominate the smaller size groups and the proportion of females increases in the larger size groups (Garwood, 1987; Little and Kitching, 1996). The difference in sex-ratios in different size-groups was attributed to the fact that limpets first mature as males and later on change to female at certain stage in their life history (Orton et al., 1956).

In the present study the onset of maturity in *C. karachiensis* was observed in the limpets belonging to the size-class 16-20 mm at both sites. It has been proposed by Grahame and Branch (1985) that limpets which attain a smaller maximum size, reach sexual maturity at an earlier age. Therefore, it was expected that limpets at Paradise Point should have attained sexual maturity at an earlier age, as they attained smaller maximum size than the limpets at Buleji. Thus, in our study, the relationship between maximum shell length and the age at sexual maturity was reversed to that stated by Grahame and Branch (1985) as the limpets of Buleji which attained larger maximum size got sexually matured at an earlier age (approx. 6 months) than the limpets at Paradise point, which attained a smaller maximum size, got matured at an age of approx. 9 months.

In the present study, histologically the gonads in *C. karachiensis* were categorized into three developmental stages, that is, the developing, ripe and spawning stages with no resting phase in this species. All three gonadal stages were observed almost throughout the year in the population of *C. karachiensis* which is similar to the study of (Rao, 1973) from India who reported the presence of either developing or spawning gonads throughout the year and the absence of any resting phase in the populations of *C. radiata*. A similar observation has been reported from the population of *Cellana radians* from New Zealand (Creese and Ballantine, 1983). Most species of patellid limpets have shorter breeding seasons, with one clearly defined spawning per year and a long resting stage thereafter (Catalan and Yamamoto, 1993; Dunmore and Schiel, 2000). However, few species of patellid or acmaeid limpets have extended breeding season, such as, *C. radiata* which breeds from June to February or March in India (Rao, 1973).*C. tramoserica* breeds during June and October in New South Wales, and between July and December in Victoria, southeastern Australia (Underwood, 1974; Parry, 1978) , *C. radians* breeds from June to March of the following year in New Zealand (Creese and Ballantine, 1983) and *C. capensis* has several peaks in spawning activity from spring to autumn in South Africa (Lasiak, 1987). *Patella depressa* has also been reported to possess a tendency for multiple spawning (Lewis, 1986; Orton and Southward, 1961; Brazão et al., 2003). *Patelloida alticostata* and *P. lutistrigutu* are reproductively active

over an extended part of the year (Creese, 1980b). The present study also showed an extended pattern of reproductive cycle with multiple spawning episodes and no resting stage in *C. karachiensis*.

Environmental parameters such as temperature and salinity are among the important factors regulating of the gonadal cycle in many species of marine invertebrates. (Giese, 1959; Kinne, 1970; Giese and Pearse, 1974). Orton et al. (1956) and Orton and Southward (1961) reported that strong onshore winds and rough weather could act as a mechanical trigger to stimulate spawning in *P. vulgata* and *P. depressa*. In case of *C. radians*, it has been observed that an increased wave action due to storms stimulated spawning (Creese and Ballantine, 1983). Guerra and Gaudêncio (1986) are related to the spawning tendency in *P. depressa* to wind speed only. Brazão et al. (2003) reported that spawning peaks in *P. depressa* coincided with a rise in air temperature and with high wind speeds.

The coast of Pakistan lies slightly outside the tropics and does not experience much rainfall, with no major change in coastal salinity throughout the year. In the present study the salinity range reported at Buleji was 35 to 39 /parts per thousand /‰ and at Paradise Point, 35 to 40 ‰. The seawater temperature at both sites was low (average 23 °C) in winter (North East monsoon) with sudden rise of 5° C in spring inter-monsoon (average 28 °C) which remained the same throughout the summer (South West monsoon). In our study though spawning in *C. karachiensis* occurred throughout the year but it was more intensified in the NE monsoon when the temperature was comparatively lower than other seasons. A similar behaviour in spawning has been reported in *C. radiata* from Indian waters where temperature and salinity was reported to have no marked influence for inducing spawning in limpets, however, from April to May when the temperature was high, the spawning was comparatively lower and similarly from October to November when the salinity was low, the spawning was comparatively low (Rao, 1973). Rao (1973) suggested that high temperature and low salinity are unfavourable for spawning in Indian waters. In Pakistani waters the lower temperatures as compared to higher temperatures were found to be more favourable for spawning.

Authors' contributions

FHSZ performed laboratory work and drafted manuscript, ZA designed and supervised research, SKP helped to improve quality of the MS and final submission.

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