Study of Reproductive Cycle of Two Archaeogastropods, *Turbo coronatus* and *Monodonta canalifera*

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        Abstract.- Comparative studies were carried out to assess contamination impact related to shipping activity on reproductive fitness of two archaeogastropod species, *Turbo coronatus* and *Monodonta canalifera* from Manora Channel, a possible organotin contaminated site and Buleji, the reference site. This study was initiated in view of the fact that Manora Channel is a hub of shipping activity and shipping activity is concerned with organotin contamination which highly affects the marine organisms, especially gastropods. The gonadal histological examination of archaeogastropods, *T. coronatus* and *M. canalifera* was carried out to examine the possible correlation between reproductive fitness of the species and organotin contamination. In both the species ovo-testis development or ovarian spermatogenesis was not evident but spawning appeared to be better synchronized at Buleji as compare to Manora Channel the largest hub of vessel activity in Pakistan.

        Key words: Shipping, organotin contamination, Turbinids, Trochids, imposex.

INTRODUCTION

Reproductive biology of two species of archaeogastropods, *Turbo coronatus* and *Monodonta canalifera* was studied to examine the possible correlation between reproductive effort of both species and organotin contamination. Turbinids and trochids are common component of the littoral and sublittoral regions of all rocky shores. These macro- and microalgae grazing snails are widely distributed to rocky shore ecosystems of Pacific, Atlantic and Indian Oceans (Joll, 1980; Nakano and Nagoshi, 1984; Bode *et al*., 1986; Hickman and McLean, 1990; Takada, 1996). Many reports are available on basic biology, spawning and larval development of different turbinids and trochids (Ai *et al*., 1964; Ai, 1965). Trochids and turbinids are valued as food and ornamental items in many parts of the world. Their meat is edible and shells of both families are of commercial importance being utilized and commercially exploited as ornament and handicraft (Lasiak, 1991, 1992; Kwon *et al*., 1993; Appukuttan and Ramadoss, 2000). Several species belonging to these two families are fairly common on rocky shores of Sindh and Balochistan coast and commercially exploited by local coastal

inhabitants as ornament and handicraft for human consumption.

        In archaeogastropods the sexes are not differentiated by any superficial characters and it is not likely to find out any morphological evidence of tributyltin (TBT) contamination in female and male specimens. The evidence of ovo-testis development and disturbed reproductive cycle in abalone (archaeo-gastropods) species, *Haliotis madaka* and *H. gigantica* from Japan have been reported from TBT contaminated sites (Horiguchi and Shimizu, 1992; Horiguchi *et al.,* 2000b, 2001, 2002, 2005). This was further confined by laboratory experiments conducted with *H. gigantica* in which significant spermatogenesis was observed in ovaries of female abalones exposed to nominal concentration (100 ng^{-1}) of TBT and triphenyltin (TPhT) (Horiguchi *et al.,* 2002).

        The present work was designed in view of earlier studies conducted by Horiguchi *et al*. (2000b, 2001, 2002, 2005) focusing on the comparative histological studies of population of *Haliotis madaka* from clean and TBT and TPhT contaminated sites in Japan. For this purpose in the present study two species of trochids; *Monodonta canalifera* and *Turbo coronatus* were investigated from Manora Channel, a well known contaminated site (Ahmed, 1977, 1979; Saleem and Kazi, 1995; Afsar *et al*., 2010) and from Buleji the reference site. This study was also initiated in view of the fact that imposex and imposex and ovo-testis

development have been observed in number of meso- and neogastropods species from Manora Channel (Afsar, 2009). Manora channel is located at 24° 47′ N, 66° 58′ E near Karachi. On the northeastern side of Manora Channel lies the Chunna Creek, with shallow backwaters covered with mangroves, while on the northern side of the Channel Karachi Fish Harbour is situated. The Channel receives pollutants from Karachi Fish Harbour, shipyards, power plants and ships visiting Karachi Port (Saleem and Kazi, 1995). Karachi Port is the largest port in the country, where about 250-300 shipping vessels from all over the world including China, Japan, Singapore, Hong Kong, Malaysia, Indonesia, Europe, America, Russia and also those carrying the flags of PNSC (Pakistan National Shipping Corporation) visit the port every month (personal communication, KPT spokesman). The Buleji rocky ledge is situated at 24° 50' N, 66° 48' E, and southwest of Karachi near fishing village of Buleji. The Buleji rocky ledge is triangular platform, which extends into the open Arabian Sea.

MATERIALS AND METHODS

The samples of two common archaeogastro-pods, *Turbo coronatus* (Turbinid) and *Monodonta canalifera* (Trochid) were collected at monthly intervals from the intertidal zones of Manora Channel and Buleji (Fig. 1) at ebb tides between August 2005 and July 2006. Approximately 35- 40 specimens of each species were collected every month from both the collection sites. A total of 474 (230 females and 244 males) specimens of *Turbo coronatus* from Manora Channel and 482 (221 females and 261 males) from Buleji were studied and 479 (219 females and 260 males) and 484 (230 females and 254 males) specimens of *M. canalifera* were studied from Manora Channel and Buleji, respectively.

Measurements and histological preparations

Animals were brought live to the laboratory and the specimens were measured along the columellar axis to the nearest 0.1 mm with the vernier caliper. The gonadal portion of 271 (144 males and 127 females) specimens of *T. coronatus* from Manora Channel and 274 specimens (149

males and 125 females) from Buleji were histologically examined. 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. For histological preparations animals were cracked open and the external features of the gonads were noted. A portion of the ovary/ testis was removed midway along the length of the gonad intact with the underlying digestive gland, from each of the specimen and fixed for histology in Davidson fluid. Sections were cut at 5-7μm and stained with eosin and haematoxylin.

Identification of sexes

Though the trochids and turbinids are dioecious gastropods their sexes cannot be distinguished by any superficial features: however, they show variation in gonad color during different stages of gonad maturation. Therefore, attempt was made to identify the sexes on the basis of coloration of gonads (Desai, 1959, 1966; Williams, 1964; Underwood, 1972). This was further confirmed by the histological examination of the gonads.

Gonadal developmental scores

Gonadal developmental scores were determined following the method described by Barkati and Ahmed (1989, 1990) and Horiguchi *et al*. (2002). For this purpose numeric ranks 0-4 were assigned to the gametogenic stages (0 = spent, 1 = developing, 2 = ripe, 3 = partially spawned, 4 = spawned out) as described by Afsar (2009) and then the number of specimens in each stage was multiplied by the numeric ranking of the stage and the sum of this product was divided by the total number of specimens for each month.

RESULTS

Although spawning individuals of both sexes were found throughout the year at both Manora Channel and Buleji but spawning appeared to be better synchronized at Buleji. In addition size of snails between sexes and localities was analyzed statistically to see any sexual dimorphism but there was no significant correlation was found except in
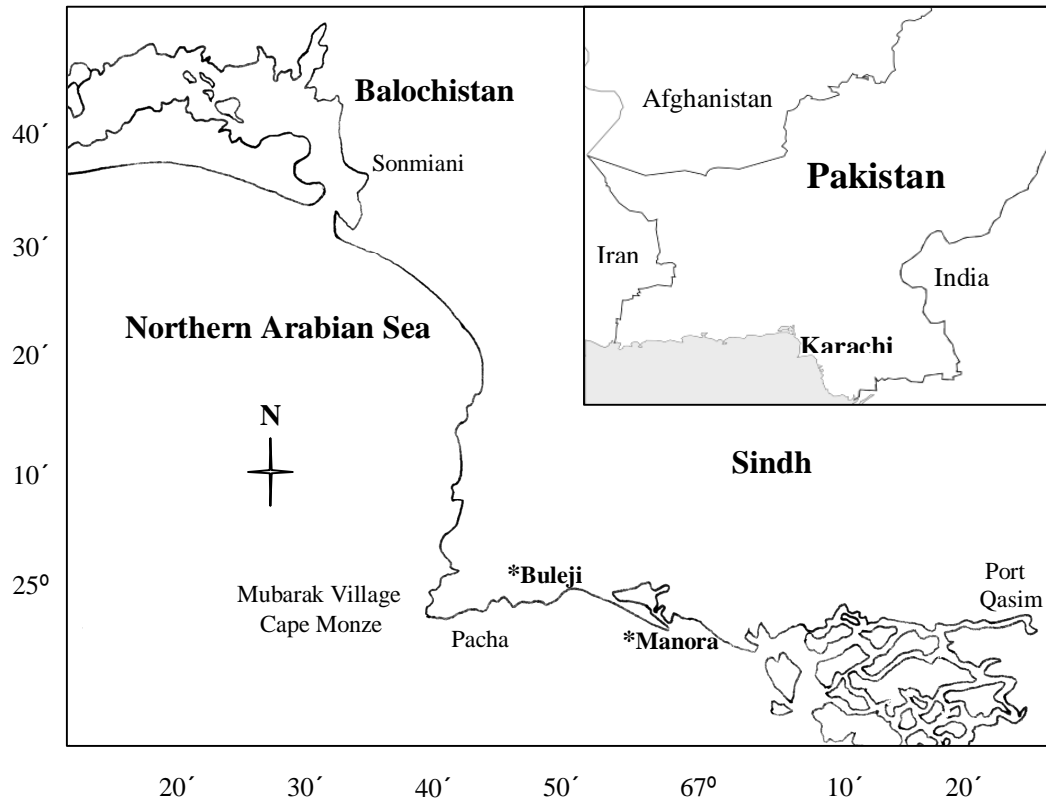
Fig. 1: Map showing collection sites*

the size class 21-25 of *M. canalifera* at Buleji, as shown in Table I. Size range 16-37 mm and 14-33 mm (minimum-maximum) for *T. coronatus* at Manora channel and Buleji was recorded respectively. Where as individuals of *M. canalifera* were found with 8-30 and 11-29 mm (minimum-maximum) respectively at Manora Channel and Buleji with mature size.

Turbo coronatus

The overall sex-ratio in specimens of *Turbo coronatus* were close to 1:1 theoretical ratio at Manora Channel ($X^2 = 0.41$, P>0.05). During the entire study period at Manora Channel the sex-ratio was significantly (P>0.05) close to 1:1 except, in December ($X^2 = 8.10$, P<0.05) when the ratio was in favour of females. Similarly at Buleji the overall sex-ratio was also close to 1:1 ratio ($X^2 = 3.32$, P>0.05), however, in June the number of males was significantly higher than the females ($X^2 = 7.04$, P<0.05) (Table II).

The mean gonadal developmental scores in female *T. coronatus* from Manora Channel were fairly constant (2.91-3.20) in the period between August and October. The highest score of 3.27 was observed in December 2005. Lowest score of 0.00 was recorded in February 2006. Similarly in males *T. coronatus* higher gonadal developmental scores (2.14-2.50) were seen in the period between August and December 2005; from January onwards the scores ranged between 0.43 and 1.20 (Fig. 2) to those recorded from Manora.

Mean gonadal developmental scores in females of *T. coronatus* from Buleji were in the ranging from 1.82 to 3.20 between August and November 2005 and reach in a peak (3.22) in December 2005. Between January and June the scores ranged between 0.33 and 1.86 and the highest peak was recorded in July (3.25). In male *T. coronatus,* however, the highest score (3.18) was recorded in September. In the remaining months the scores ranged between 0.80 and 2.44 (Fig. 2).

Table I.-   **Sex-ratios in different size-classes of *T. coronatus* and *M. canalifera* at Manora Channel and Buleji.**

Species	Site	Month	Total	Proportion of males	Males	Females	Chisquare
T. coronatus	M. Channel	11-15	0	0	0	0.00	0.00
		16-20	62	27	35	0.44	1.03
		21-25	224	120	104	0.54	1.14
		26-30	167	85	82	0.51	0.05
		31-35	19	10	9	0.53	0.05
		36-40	2	2	0	1.00	2.00
	Grand Total		474	244	230	0.51	0.41
	Buleji	11-15	8	6	2	0.75	2.00
		16-20	130	62	68	0.48	0.28
		21-25	254	143	111	0.56	4.03
		26-30	87	48	39	0.55	0.93
		31-35	3	2	1	0.67	0.33
		36-40	0	0	0	0.00	0.00
	Grand Total		482	261	221	0.00	3.32
M. canalifera	M. Channel	11-15	5	3	2	0.60	0.20
		16-20	150	80	70	0.53	0.67
		21-25	237	130	107	0.55	2.23
		26-30	87	47	40	0.54	0.56
	Grand Total		479	260	219	0.54	3.51
	Buleji	11-15	12	7	5	0.58	0.33
		16-20	240	119	121	0.50	0.02
		21-25	166	96	70	0.58	*4.07
		26-30	66	32	34	0.48	0.06
	Grand Total		484	254	230	0.52	1.19

*Significant at 5% level

Monodonta canalifera

In specimens of *M. canalifera* at Manora Channel, except for September the sex-ratios in all the months were significantly (P>0.05) close to 1:1 while, at Buleji also the sex-ratios were close to 1:1 throughout the year except in May when the proportion of females was higher than the males (χ^2 =6.40, P< 0.05) (Table II).

At Manora Channel, in the female population of *M. canalifera* high gonadal developmental scores (2.00 to 3.13) were observed during the entire study period except, in February (1.00). In males of *M. canalifera*, the gonadal developmental scores ranged between 2.36 to 3.17 in August to January period and in July highest score (3.42) was recorded in males. Whereas, during February to June period inconsistent scores were observed (Fig. 2).

At Buleji in females of *M. canalifera* high scores of gonadal development were noted from August to March and July (value ranging between 2.50 to 3.63). Slight decrease in scores was observed in April to June. While in males of *M. canalifera* the gonadal developmental scores remained high during August to January and in July (2.60 to 3.33) and in rest of the months their values ranged between 0.60 to 1.80 (Fig. 2).

DISCUSSION

In the present study two species of archaeogastropods, *T. coronatus* and *M. canalifera* were studied for their reproductive performance at Manora Channel and Buleji. Both the species were found to be gonochoristic and no significant variation in the proportion of males and females at the two sites was observed. The overall sex-ratios in both the species at Manora Channel and Buleji were significantly in agreement with 1:1 ratio. However, deviation from expected 1:1 ratio was observed in certain months at both the sites. This could be attributed to the disparity in sizes of the animals collected in these months as variations in male and

Table II.- **Monthly sex ratios in *Turbo coronatus & Monondonta canalifera* at Buleji and Manora Channel**

Species	Site	Month	Total	Proportion of Males	Males	Females	Chisquare
Turbo coronatus	Manora Channel	Aug	40	0.53	21	19	0.10
		Sep	36	0.56	20	16	0.44
		Oct	40	0.45	18	22	0.40
		Nov	40	0.58	23	17	0.90
		Dec	40	0.28	11	29	*8.1
		Jan	40	0.45	18	22	0.40
		Feb	40	0.60	24	16	1.60
		Mar	40	0.48	19	21	0.10
		Apr	40	0.60	24	16	1.60
		May	37	0.51	19	18	0.03
		June	41	0.56	23	18	0.61
		July	40	0.60	24	16	1.60
	Buleji	Aug	41	0.39	16	25	1.98
		Sep	36	0.47	17	19	0.11
		Oct	39	0.49	19	20	0.03
		Nov	39	0.49	19	20	0.03
		Dec	40	0.53	21	19	0.10
		Jan	40	0.63	25	15	2.50
		Feb	41	0.51	21	20	0.02
		Mar	40	0.53	21	19	0.10
		Apr	40	0.65	26	14	3.60
		May	40	0.58	23	17	0.90
		June	46	0.70	32	14	*7.04
		July	40	0.53	21	19	0.10
Monodonta canalifera	Manora Channel	Aug	41	17	24	0.59	1.20
		Sep	42	13	29	0.69	*6.10
		Oct	40	20	20	0.50	0.00
		Nov	40	19	21	0.53	0.10
		Dec	40	20	20	0.50	0.00
		Jan	40	15	25	0.63	2.50
		Feb	40	20	20	0.50	0.00
		Mar	40	22	18	0.45	0.40
		Apr	40	18	22	0.55	0.40
		May	36	18	18	0.50	0.00
		June	40	17	23	0.58	0.90
		July	40	20	20	0.50	0.00
	Buleji	Aug	34	18	16	0.47	0.12
		Sep	40	18	22	0.55	0.40
		Oct	40	18	22	0.55	0.40
		Nov	40	22	18	0.45	0.40
		Dec	40	17	23	0.58	0.90
		Jan	40	14	26	0.65	3.60
		Feb	40	17	23	0.58	0.90
		Mar	40	24	16	0.40	1.60
		Apr	40	14	26	0.65	3.60
		May	40	28	12	0.30	*6.40
		June	50	21	29	0.58	1.28
		July	40	19	21	0.53	0.10

*Significant at 5% Level

female sizes have been observed in both the species. Variability in sex- ratios has been used to elucidate female mortality in *Nucella lapillus* populations from the TBT contaminated areas (Evans *et al.,*

1991). However, in the present study no significant increase in the proportion of males versus females was found in *T. coronatus* and *M. canalifera* at the TBT contaminated site, Manora Channel indicating that the TBT has not affected the population dynamics of *T. coronatus* and *M. canalifera.* Similarly, Bech (2002b) was not able to detect any mortality and significant increase in the number of males in the population of the neogastropod *T. distinguenda* at TBT contaminated site in Phuket, Thailand. Bode *et al.* (1986) have also reported significant agreement to 1:1 ratio in the natural population of *Monodonta lineata* and *Gibbula umbilicalis* from Spain.

In histological sections of gonads of *T. coronatus* and *M. canalifera at* both the sites, five gonadal stages were recognized and the populations reproductive development scores were studied to quantify the temporal variation in the reproductive cycle of males and females in the population. Archaeogastropods are broadcast spawners and it essential that gametogenic cycles and spawning are well synchronized in both sexes throughout the populations to increase fertilization. In the present study, the gonad development in males and females of *T. coronatus* seems to be synchronous at Buleji (control site), whereas, at Manora Channel it appears to be slightly asynchronous because of more frequent testicular development in males as compared to oocyte development in females. Similarly Houriguchi *et al.* (2000b) reported asynchronous release of male and female gametes in the population of abalone species *Haliotis madaka* from TBT contaminated site in Japan and attributed this to the faster testicular development in males at TBT contaminated site.

More females in ripe condition were found at Manora Channel than at Buleji in the two species examined, which could be due to longer retention of ripe oocytes. In the ovaries of two species studied from Manora Channel and Buleji, pre- and post-vitellogenic oocytes were found and normal oogenesis was observed. However, in histological sections of *M. canalifera* in some months the pre-vitellogenic oocytes were seen between the mature oocytes at both the sites. In *M. canalifera* complete resorption of ripe oocytes was not evident in any of the specimen and females with ripe oocyte were
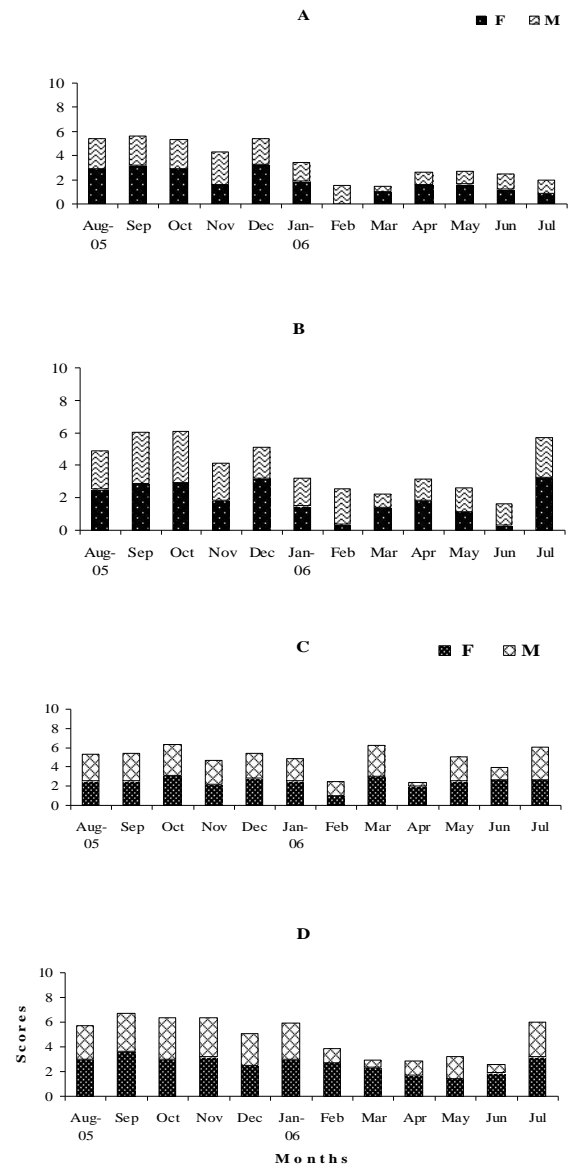

Fig. 2. *Turbo coronatus* (A-B); *Monodonta canalifera* (C-D): Seasonal variations in reproductive developmental score in specimens of Buleji (B, D) and Manora Channel (A, C).

present throughout the year. Similarly, the males with mature sperms predominated the population. Underwood (1972) in British trochid, *M. lineata* also reported the presence of ripe oocytes in the ovary which have not undergone any cytolysis or resorption and retained in the following season.

Similar pattern of gonadal development was also reported by Webber and Giese (1969) in archaeogastropod species *Haliotis carcheroidii*. The prolonged occurrence of ripe females and males in the populations of *M. canalifera* and *T. coronatus* could also be attributed to the extended spawning season as suggested by Ahmed (1980) in species of invertebrates which are low tidal or sub tidal in occurrence and were accordingly termed as year round spawners.

Imposex or ovo-testis development in meso- and neogastropods (Fioroni *et al.,* 1991; Stroben *et al.,* 1992; Bauer *et al.,* 1995; Bettin *et al.,* 1996; Vishwakiran and Anil, 1999; Vishwakiran *et al.,* 2006) is typically induced by organotins leached out from antifouling paints used on boat hulls and other submerged structures such as oil rigs supports, buoys and fish cages (Hung *et al.,* 1998; Horiguchi *et al.,* 2001, 2006; Duft *et al.,* 2007). Many studies have shown a positive correlation between imposex and the presence of tributyltin (TBT) in sediments (Axiak *et al.,* 1995), seawater (Stroben *et al.,* 1992) and tissue of the gastropods (Gibbs *et al.,* 1990) implicate TBT as a major cause of imposex (Evans *et al.,* 2000). Tributyltin acts as an endocrine disruptor which impairs ovo-testicular development in archaeogastropod species such as, *Haliotis gigantean* and *H. madaka* (Horiguchi *et al.,* 2000a, 2000b, 2001, 2002, 2006) and also in many neo-gastropods, including *N. lapillus* (Gibbs *et al.,* 1988), *Ocenebra aciculate* (Oehlmann *et al.,* 1996), *Babylonia japonica* (Houriguchi *et al.,* 2001, 2006) such impacts have been reported at many TBT contaminated sites and have been confirmed by exposure experiments of gastropods to tributyltin. During the present study development of ovo-testis or spermatogenesis was not evident in any female specimen of *T. coronatus* and *M. canalifera* from Manora Channel. Although the imposition of spermatogenesis as ovo-testis in the females of *Babylonia spirata* has been observed in specimens examined from the same locality (Afsar, 2009). Houriguchi *et al*. (2001, 2006) have reported the ovarian dysmaturity and suppressed ovarian maturation, decrease in spawning and consequently decrease in commercial landing of *B. japonica* due to TBT contamination. Horiguchi *et al* (2002) have also reported the presence of contracted oocytes in ovaries of female abalone *species* collected from the contaminated site. However, in the present study no ovarian dysmaturity or suppressed ovarian maturation and contracted oocytes were found in the two species, *T. coronatus* and *M. canalifera* examined from Manora Channel, the TBT contaminated site.

CONCLUSIONS

It is concluded in the present study that although ovarian spermatogenesis was not evident in both the species moreover spawning appeared to be better synchronized at Buleji the cleaner site as compared to Manora Channel the largest hub of vessel activity in Pakistan. It appears that in Pakistani waters the TBT contamination has not reached to a point to affect the population dynamics of *T. coronatus* and *M. canalifera.* Reproductive success of these snails at sites in Pakistan where organotin pollution have recently been highlighted is found to be more resistant than other coexistent muricoides found in same habitat.

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