World Journal of Fish and Marine Sciences 2 (1): 14-20, 2010
ISSN 2078-4589

Annual Cycle of Reproduction in *Turbo brunneus*, from Tuticorin South East Coast of India

[1]R. Ramesh, [2]S. Ravichandran and [2]K. Kumaravel

[1]Department of Zoology, Government Arts College, Salem, India
[2]Centre of Advanced Study in Marine Biology, Annamalai University, India

Abstract: This research work mainly focus on the reproductive and spawning season of Turbo brunneus a mollusk in the south east coast of India. Random samples from *Turbo brunneus* were collected from littoral tidal pools in Tuticorin coast, during May 2002 to April 2003. The number of male and females in the monthly samples was counted to determine the male: female ratio in the population and chi-square test was applied to test whether the population adheres to 1:1 ratio. The overall male and female ratio is found to be 1: 0.96 indicating only a slight variation in the evenness of male and female in the population. Both sexes of *T. brunneus* attain sexual maturity between 23 and 27mm. The mean gonadal index (G.I) was high (21.82%) in males during May, 2002 and then it decreased gradually and reached 15.52% during October 2002, which showed the low mean GI value in males for the whole study period. While for females it was high during May 2002 (23.09%) and low during September 2002(14.83%). The GI values for both the sexes were generally low until December 2002. The limited percentage of matured oocytes which exists even after spawning indicates the high possibility for partial spawning in *T. brunneus.* It was concluded that the reproductive behavior of *T.brunneus* is highly influenced by the seasonal factors

Key words: *Turbo brunneus* % Gonadal index % Spawning % Southeast coast

INTRODUCTION

The turbinids are much studied for their external mode of fertilization, which are in turn effected by simple structures, the gonad, gonoduct and right kidney opening. Moreover, studies on sex ratio, sexual maturity, breeding season and factors affecting the synchronicity of spawning of tropical and temperate regions were also studied.

Turbinids are helpful to find out their annual recruitment and the interpretation of their growth. This information can also be used to design efficient techniques for conditioning, spawning induction, larval development and settlement. A detailed population structural studies of a Turbinid species *Turbo* (*Callopoma*) *funiculosus* and its accompanying biota were elucidated recently from Socorro Island, Archipelago of Mexico [1].

Along the Indian coastal waters, only few works have been carried out so far on Turbinids and Trochids. Hence, the present study was concentrated on the reproductive biology of *T.brunneus*

MATERIALS AND METHODS

Random samples from of *T. brunneus* were collected from littoral tidal pools in Tuticorin coast, during May 2002 to April 2003. All collections were made at spring low tide. Individuals were measured along the columellar axis to the nearest 0.1mm before breaking open the shell and removing the soft parts.The number of male and females in the monthly samples was counted to determine the male: female ratio in the population and chi-square test was applied to test whether the population adheres to 1:1 ratio.

To study the monthly changes in gonadal conditions, the visceral mass immediately behind the spiral caecum was cut down and fixed in 10% formalin solution and routinely prepared for histology. The wax embedded material was sectioned at 6µm thickness and stained with Harris haemotoxylin and eosin.

Gonad Index: Month wise, more than 40 large matured individuals in shell length were taken for examining the gonad indices. The shell was cracked and the soft body

Corresponding Author: S. Ravichandran, Centre of Advanced Study in Marine Biology, Annamalai University, India

removed, operculum was detached from foot and the individuals were segregated sex wise. Then the somatic tissue and the gonad of each individual were dried in a hot air oven at 60°C for 24 hours. It was done for all male and female individuals with care so that the dried somatic tissue was represented by gonad of its own. The dried gonadal and somatic tissues were weighted separately to the nearest 0.1 mg in an electronic balance. Gonad index was calculated as

$$GI = \frac{\text{Dry weight of the gonad}}{\text{Dry weight of the total soft body}} \times 100$$

Percentage of matured Oocytes: Both ovaries and testis were prepared for microscopic studies, but only the ovaries were examined quantitatively as the ovaries could be more reliably quantified selected for oocyte counts, following the methods of Underwood [2] and Joll [3]. Oocytes were assigned into two classes mature or immature depending on the presence or absence of the layer of jelly around the oocytes. As the size of gonad is considerably large, counting of all the oocytes will be a great task. So, a standardized sub-sample was taken by making sections from the ovary in three different areas (i) near the gastric caecum (ii) middle part of the ovary and (iii) near the spiral end of the ovary. Two samplings were done in each part and the matured and immatured ova are counted under microscope. Counts from sub-samples of three sections from each ovary were taken and the values were averaged.

Presumptive Fecundity: Presumptive fecundity of *T. brunneus* was calculated by following the method of Komatsu *et al*. [4] for *Turbo marmoratus.* Presumptive fecundity was calculated by counting the number of oocytes from three different parts of the gonad and then making it to the whole gonad. A small piece of ovary, approximately 10 mg in wet weight was dissected out from each three sections in an ovary (i.e.) just behind the gastric caecum (section a) and at the distance $^{1}/_{3}$ (section b) and ¾ (section c) of the length between the posterior end of the gastric ceacum whorl and the tip of the gonad. These small pieces of ovary were weighed with accuracy of 0.1 mg to assess the homogeneous distribution of oocytes within the same ovary and the numbers of oocytes were counted under a microscope. Then presumptive fecundity was calculated using the mean number of oocytes per 10 mg of those at the three sections and the values are averaged. Number of oocytes per milligram was calculated and multiplied with the total gonad weight (mg).

RESULT

Sex ratio: The number, percentage, sex ratio and chi-square values of 778 males and 749 females were evaluated. Of the 1527 individuals of *T. brunneus* examined, large numbers of females were collected during May, September and December. The male and female numbers are same or nearly same during July, November and February. The overall male and female ratio is found to be 1: 0.96 indicating only a slight variation in the evenness of male and female in the population.

The sex ratio of the population was calculated rank-wise for the period from May 2002 to April 2003 and chi-square was applied on monthly random samples to test whether the population follows 1:1 ratio. The 'P' values showed that the sex ratio does not vary significantly when compared to the expected 1:1 ratio.

Maturity Size: In an immature snail only the digestive gland is seen in the visceral coil. Both the sexes of *T. brunneus* attain sexual maturity between 23 and 27mm. The gonad is seen as a thin layer which overlaps the digestive gland. The first matured testis has a thin trabaeculae where few spermatogonial cells are seen over its surface and the newly formed spermatocytes are scattered in the intetrabaecular spaces.In a newly matured ovary, the oocytes are small and immature.

Spermatogenesis: After sexual development, the testis contained spermatogenic cells at all stages of development and this was observed throughout the study period. But, the percentage of mature spermatocytes with that of the maturing spermatogonial cells differs depending on the season. The mature spermatozoans are found in high proportion during pre-spawning months (May 2002, March and April 2003). The germinal cells which arise from the outer layer of tubules of trabaeculae and the inner walls of the testis differentiate into spermatogenic cells. Thus the layer near the tubules or trabaeculae is formed of spermatogonial cells and the next layer facing the lumen is formed of numerous spermatocytes. In early maturing stage, the space near the lumen is less dense and spermatogonial cells are accumulated near the trabaeculae. When snail reaches maturity conditions, the first stage in testis is fewer spermatogonial cells and spermatocytes which surround

Table 1: Number, percentage, sex ratio and chi-square of male and female of *T. brunneus* from May 2002 to April 2003

	Male		Female				
Month	No.	%	No.	%	M/F	Chi-square value	P
May -02	76	47.2	85	52.8	1:1.12	0.5030	>0.05
June	69	52.7	62	47.3	1:0.89	0.3798	>0.05
July	63	49.6	64	50.4	1:1.01	0.0078	>0.05
August	70	53.0	62	47.0	1:0.88	0.4848	>0.05
September	63	48.1	68	51.9	1:1.08	0.1908	>0.05
October	62	51.7	58	48.3	1:0.93	0.1332	>0.05
November	38	48.7	40	51.3	1:1.05	0.0512	>0.05
December	32	47.0	36	53.0	1:1.13	0.2352	>0.05
January- 03	63	55.3	51	44.7	1:0.81	1.2630	>0.05
February	78	49.4	80	50.6	1:1.02	0.0252	>0.05
March	87	53.4	76	46.6	1:0.87	0.6954	>0.05
April	77	53.4	67	46.4	1:0.87	0.6944	>0.05
Total	778	50.9	749	49.1	1:0.96	0.5404	>0.05

the lumen. The next stage shows the tubule sides are occupied by greater number of spermatogonial cells. In a matured testis, mostly spermatozoans occupy the intertrabaecular spaces and the spermatogenic cells in different stages of development. They are arranged in overlapping layers starting from the trabaeculae towards the lumen. Maturation is in high rate towards the area near the digestive gland and it proceeds from there to outer area.

Oogenesis: Oogonial cells are found attached to the ovarian trabaeculae and the inner lining of the gonad. Developing oocytes were found throughout the study period. But, the percentage of matured oocytes with maturing oocytes depends upon the ripeness of the gonad and that of the season. In *T. brunneus,* high percentages of matured oocytes were observed during May 2002, June 2002, March and April 2003.

The developing oocytes (Previtellogenic) are found attached to trabaeculae or inner side of the ovarian wall by a stalk. These developing oocytes have clear nuclei with a deeply staining nucleolus. At later stages of development, granular due to the depositions of yolk granules (post vitellogenic).The well-developed oocytes are generally covered by a jelly layer. The size of the matured oocytes ranges from 280 to 360 µm in diameter, where the thickness of the jelly layer ranges from 60 to 80 µm.

Spent Gonad: From the total samples collected, only very few are found in spent condition. Spent gonads are observed only during the months of September, October and November 2002. The spent testis is brown in colour and is slightly shrunken, while in females the spent gonad acquires a brownish green colour and forms a loose layer over the digestive gland. Histology of the spent testis shows unutilized spermatozoans and few spermatocytes near the tubule. Spent ovary contains degenerating oocytes and the trabeculae are arranged close to one another.

Gonad Index (GI): The seasonal changes in the GI of males and females are presented in (Figs. 1 a, b).The mean gonadal index (GI) was high (21.82%) in males during May, 2002 and then it decreased gradually and reached 15.52% during October 2002, which shows the low mean GI value in males for the whole study period. While, for females it was high during May 2002 (23.09%) and low during September 2002(14.83%). The GI values for both the sexes were generally low until December 2002. The mean gonad index of female was always more than 14%. But high GI, above (20%) was observed during May, June 2002 and March, April 2003. The GI declined gradually and reached a mean value of 14% during October 2002. In the male, the gonad index fluctuated within an average 15 to 22% throughout the study period.

Percentage of Matured Oocytes (PMO): The mean percentage of matured oocytes during different months of study period is given in (Fig. 2). The mean percentage of matured oocyte (PMO) was high 79.41) during the month of May. Then the mean PMO started decreasing from June onwards and it reached a minimum value of 35.89% during the month of September. The decrease in the value

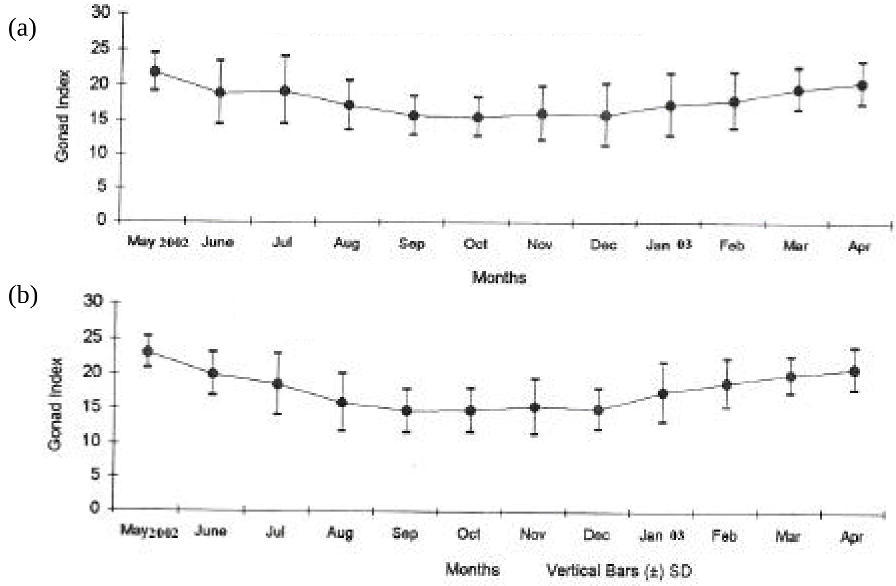

Fig. 1a,b: The seasonal changes in the G.I of Males and Females

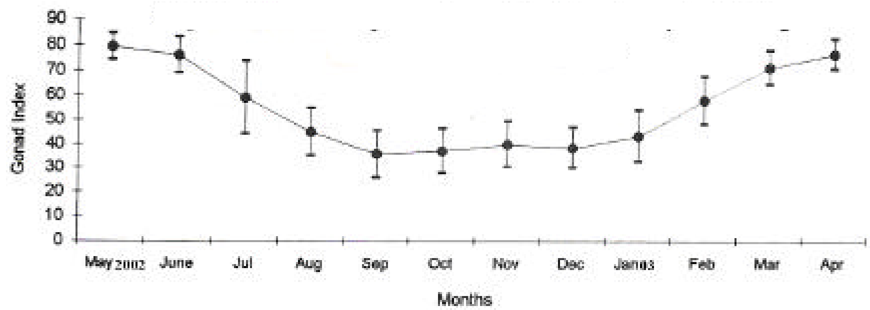

Fig. 2: The mean percentage of matured Oocytes during different months

Table 2: Presumptive fecundity in *Turbo brunneus*

S.No	Shell length (mm)	Gonad wet weight (mg)	Mean no. of oocytes per 10 (mg)	No. of oocytes per animal
1	51	2023	787 (± 47)	159210
2	37	720	612 (± 59)	44064
3	40	1008	610 (± 52)	61488
4	48	1690	753 (± 60)	127257
5	45	1020	670 (± 95)	68340
6	50	1440	689 (± 33)	99216
7	49	1582	791 (± 67)	125136
8	54	1688	753 (± 102)	127162
9	53	2038	700 (± 65)	142660
10	46	1477	780 (± 63)	115206
11	35	506	563 (± 72)	28531

Table 3: Correlation coefficient ('r') values between shell length, gonad Weight and number of oocytes

Parameters	Shell length (mm)	Gonad wet Weight (mg)	Mean no. of oocytes	No. of oocytes per animal
Shell length (mm)	1	-	-	-
Gonad wet weight (mg)	0.92794[b]	1	-	-
Mean no. of oocytes	0.80029[b]	0.83426[b]	1	-
No. of oocytes per animal	0.91213[b]	0.98912[b]	0.89773[b]	1

may be due to the spawning activity. Then the mean PMO increased in the value may be due to the spawning activity. Then the mean PMO increased steadily from February onwards. The minimum PMO was never less than 22.74% (September) and this showed that the matured oocytes were present throughout the year and its percentage decreased due to the spawning activity.

The correlation coefficient ('r') values calculated between the gonad index (female) and percentage of matured oocytes is highly significant (P < 001).

Presumptive Fecundity: The shell length, gonads weight and number of eggs per gonad are given in (Table 2). The number of oocytes varied with different individuals and within different regions of same ovary itself. But it was directly related to the size of the gonad and its maturity index.

The correlation coefficient analysis carried out between shell length, gonad weight and number of oocytes. The 'r' values were highly significant and the results are given in (Table 3).

DISCUSSION

The external observation of *T. brunnues* shows no sexual dimorphism in its shell shape or in other morphological features except that of the coloration of gonads and the right kidney opening. But, the analysis of covariance applied to find out the allometric relationships that exists between the shell and body character of both the sexes showed differences. Size dimorphisms between sexes have not been generally reported in archaeogastropoda [5]. In *Haliotis*, no different has been found between average sizes of males and females [6, 7]. However, when studying the shell characters of *Turbo cornalis* from different localities, only the female shells had tubercles and the males were devoid of it [8]. In larger individuals of *Tegula*, the foot of males is distinctly lighter in coloration than that of females [9]. But, no such variation in morphological features has been observed in male and females of *T. brunneus.*

Data on sex ratio of *T. brunneus* shows that the male and female members are more or less same in numbers in the population studied. The sex ratio of trochid *Monodonta lineate* never deviated the 1:1 ratio [10]. The 1:1 ratio in *Turbo intercostalis* outnumbered the females in *T. cornutus* from Transkei coast in South Africa [3, 11].

Gametogenesis process is continuous and the matured gametes have been observed throughout the study period. Thus, all stages of developing gametes are found along with matured gametes during all the seasons. The fact that females with matured oocytes, which existed year round, have been reported for *Haliotis rufescens* [12], *H. Hamischatkann* [13]. *Gibbula cineraria* [2], *Turbo coronutus* [11,14], *Turbo intercostalis* and *T. torquatus* [3] and *T. marmoratus*, [4].

From the observation on the reduction in percentage of mean number of mature ova to that of the immature ova and that of the decreasing gonad index, it is clear that spawning in *T. brunneus* occurs to be a prolonged activity from June to September. Peak spawning activity of *T. coronutus* was observed between December and February [14] from Transkei coast, S. Africa. In Port Elizabeth, *T. sarmaticus* spawns during the summer autumn period (December-April) [15]. Prolonged breeding season has been reported in three Australian Turbinids, *Subninell undulate* [16], *T. intercostalis* and *T. torquatus* [3]. However, the Newzealed turbinid *Lunella smargada* has a short spawning period of only two months [17]. While, the spawning season for *Lunella granulate* was from September to December in Okinawan waters [18].

Histological studies cannot prove that whether *T. brunneus* is a complete spawner or it follows partial spawning. The limited percentage of matured oocytes after spawning indicates the high possibility for partial spawning in *T. brunneus*. Turbinids might spawn partially at the beginning and complete by towards the end of the breeding cycle [4]. So that, if some individuals only partially spawn, it would appear that mature oocytes are retained for the next breeding season. Thus partial spawning throughout the breeding period may well maximize the potential reproductive success.

Despite the advantages supposedly gained by synchronization of reproductive activity, several protracted breeders have been shown to exhibit asynchronous spawning [19-21]. From the present study, it was not possible to draw any conclusion on how synchronous is spawning among individual *T.brunneus* within a population. From the observation the gonad index and the percentage of matured oocytes it was found that many individuals of the population spawn from June to September. The possibility to define how often or exactly when spawning takes place is not clearly demarcated in turbinids [3].The number of immature oocytes increases markedly during spawning. This indicates an increase in the rate of oocytes production. Contrastingly, the number of mature oocytes decreases continuously and thus indicating that spawning occurs more rapidly than oocyte production.Gonadal maturity depends on the availability of nutrients, either in the form of nutrient reserve or food ingested. Spawning in turn is dependent on the completion of gonad maturity.

The chances of reproductive success are undoubtedly dependent on both the density and proximity of mature adults as well as the extent of water movement experienced during spawning. The congregation of adults in groups clearly ensures the proximity of the sexes during spawning [19]. Aggregations of mature *Monodonta lineate* in tide pools, which also contained eggs at early cleavage stages were also noticed [10]. In the three species of patellids and a trochids, males and females have been reported to be close together during emission of gametes [22]. Pairing has also been described in broadcast spawners e.g., *Patina pellucida* and *P.caerulea* [23], *P.lusitania* [24] and *Gibbula tumida* [25]. Pair formation or grouping has widely been observed in areas with thick algal growth, under small boulders or inside the crevices of dead coral mass during low tides. Thus, the aggregation observed here is mainly due to its feeding or hiding behaviour and this may indirectly favours them in their reproductive activity. Reproductive aggregations in turbinids are less studied because of external mode of fertilization. Pairing or grouping of animals but not strictly say it as spawning aggregations or aggregations due to the favorable conditions of the habitat is observed [3].

The exogenous and endogenous factors important in regulating reproductive activity in prosobranchs are poorly understood [4]. Several reproductive cycles have been investigated [7, 26, 27], however, few have demonstrated causal relationships. Increasing temperature enhances spawning in trochids such as *Trochus* [28, 29]. In *T. brunneus,* spawning occurs during June to September when the seawater temperature is comparatively higher.

From the result it is concluded that the spawning period and reproductive behavior of *T.brunneus* changes with respect to the seasonal factors and as well as internal physiology.

ACKNOWLEDGEMENT

Authors are greatful to the Director, Centre of Advanced Study in Marine Biology, Annamalai University, for rendering a great support for my research.

REFERENCES

1. Oscar E. Holguin Quiñones and Jesús E. Michel-Morfín, 2006. Population structure and accompanying biota of the snail *Turbo* (*Callopoma*) *funiculosus* (Gastropoda: Turbinidae), on Socorro Island, Revillagigedo Archipelago, Mexico. Rev. Biol. Trop. (Int. J. Trop. Biol.) 54: 1079-1084.
2. Underwood, A.J., 1972. Observation on the reproductive cycle of *Monodonta lineate, Gibbula umbilicalis* and *G. cineraria.* Mar. Biol., 17: 33-340.
3. Joll, L.M., 1980. Reproductive biology of two species of Turbinidae (Mollusca: Gastropoda). Aust. J. Mar. Fresh water Res., 31: 319-335.
4. Komatsu, T., M. Murakoshi and R. Nakamura, 1995. A study of the reproduction of the green snail, *Turbo marmoratus* in the Ryukyu Islands, Southern Japan. Jpn. Aqua. Soc. Suisanzoshoku, 43: 297-304.
5. Webber, H.H., 1977. Gastropoda: Prosobranchia. In: Reproduction in marine invertebrates Vol. IV. Moluscs: gastropods and cephalopods (Eds.,) A.C. Giese & J.S. Pearse, Academic press. pp: 1-97.
6. Newman, G.G., 1967. Reproduction of the South African abalone *Haliotis midae.* Invest Rep. Div. Sea. Fish. S. Africa. 64: 1-24.
7. Webber, H.H. and A.C. Giese, 1969. The reproductive cycle and gametogenesis in the black abalone *Haliotis cracheroidii* (Gastropoda: Prosobranchia). Mar. Biol., 4: 152-159.
8. Amio, M., 1955. Growth and development of spines on the top shell *Turbo brunneus.* J. Shimonoseki college fisheries, 4.
9. Frank, P.W., 1969. Sexual dimorphism in *Tegula funebralis.* Veliger, 11: 440
10. Desai, B.N., 1966. The biology of *Monodonta lineata* (da costa). Proc. Malac. Soc. Lond., 37: 1-17.
11. Lee, J.J., 1983. Histological study in the reproductive cycle of Jeju Island’s turban shell *Turbo corntus.* Bull. Mar. Resour. Res. Int. Jeju Natl. Univ., 7: 29-51.
12. Boolootian, R.A., A. Farmanfarmaian and A.C. Giese, 1962. On the reproductive cycle and breeding habits of two western species of *Haliotis.* Biol. Bull., 122: 183-193.
13. Sloan, N.A. and P.A. Breen, 1988. Northen abalone, *Haliotis kamtchatkana,* in British Columbia: Fisheries and synopsis of life history information. Canadian special Publ. Fisheries and Aquatic Science, 103: 46.
14. Lasiak, T., 1986. The reproductive cycle of intertidal gastropod *Turbo cornutus Gmelin* 1971, on Transkei coast. S. Afr. J. Zool., 21: 153-155.
15. Mc Lachlan, A. and H.W. Lombard, 1981. Growth and production in exploited and unexploited populations of a rocky shore gastropod, *Turbo sarmaticus*. Veliger, 23: 221-229.
16. Underwood, A.J., 1974. The reproductive cycles and geographical distribution of some common eastern Australian prosobranchs (Mollusca: Gastropoda). Aust. J. Mar. Fresh water Res., 25: 63-88.

17. Grange, K.R., 1976a. Larval development in *Lunella smargada* (Gastropoda: Turbinidae). New Zealand J. Mar. Fresh water Res., 10: 517-525.
18. Fuse, T., 1993. Annual cycle of reproduction in a Turban snail *Lunella granulate,* at Chinen, Okinawa Galaxea, 11: 135-142.
19. Simpson, R.D., 1977. The reproduction of some littoral molluscs from Macquarie Island (Sub. Antartic). Mar. Biol., 44: 125-142.
20. Joska, M.A.P. and G.M. Branch, 1983. The reproductive cycle of the trochid gastropod *Oxystele variegata* (Anton, 1839). Veliger, 26: 47-51.
21. Lasiak, T., 1987. The reproductive cycle of three trochid gastropods from the Transkei coast, Southern Africa. J. Moll. Stud., 56: 69-81.
22. Fretter, V. and A. Graham, 1962. *British prosobranch mollusks.* Ray society, London, XVI+755pp. 317 figs.
23. Ankel, W.E., 1936. Prosobranchia. Tierwelt Nord-Ostsee, 11: 223-295.
24. Von Medem, F.G., 1945. Untersuchungen uber die Ei-und spermawirkstoffe bei marinen mollusken. Zool. Jahrb. Abt. Anat. Ontog., Tiere., 61: 1-44.
25. Gersch, M., 1936. Der Genitalapparat und die sexual biologie der Nordeet Tochiden. Z. Morph. Okol. Tiere. 31: 106-150.
26. Sutherland, J., 1970. Dynamics of high and low populations of the limpet *Acmaea scabra* (Gould). Ecol. Monogr., 40; 169-188.
27. Pearse, J.S., 1978. Reproductive periodicities of Indo-pacific invertebrates in the Gulf of Suez. IV. The chiton *Acathopleura haddoni winckworth* and *Onithochiton lyelli* (sowerby) and the abalone *Haliotis pustulata* Reeve. Bull. Mar. Sci., 28: 92-101.
28. Ducros, C., 1957. Induction de l 'e' vacuation desproduits sexuals chez gibbules. C.R. Seances Biol. Ses fil., 151: 285.
29. Ai, T., 1965. Spawning and early development of the top shell *Turbo* cornutus Solander. II. Induction of spawning and larval development. Bull. Jpn. Soc. Sci. Fish., 31: 105-112.