ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of DEVELOPMENT RESEARCH

International Journal of Development Research
Vol. 06, Issue, 09, pp.9339-9343, September, 2016

Full Length Research Article

REPRODUCTIVE CYCLE AND HISTOLOGICAL CHANGES IN THE GONADS OF THE FRESHWATER CRAB, *BARYTELPHUSA GUERINI* (H. MILNE EDWARDS) (DECAPODA, POTAMIDEA)

***Vaishali Gangotri**

Department of Zoology, Shri Dhokeshwar Mahavidyalaya, Takali Dhokeshwar, 414304, Ahmendnagar, (M.S.) India

ARTICLE INFO

Article History:

Received 19th June, 2016
Received in revised form
22nd July, 2016
Accepted 19th August, 2016
Published online 30th September, 2016

Key Words:

Reproductive cycle,
Histological changes,
Freshwater crab,
Barytelphusa guerini.

ABSTRACT

The annual reproductive cycle of the crab, Barytelphusa guerini was studied during the period January 2015 to December 2015 by measuring monthly histological variations. Histological observations of the monthly gonads revealed that the ripening of the gonads started in the preparatory period of the reproductive cycle i.e. (January to April) or it may be extended upto the month of May. By that time, the gonads of the most individuals attained maturity and spawning began in the months of June and July. The spawning terminated by the end of September and the gonads entered a quiescent period, upto the month of December.

INTRODUCTION

The reproductive cycle of crustaceans has been widely studied mainly of those species that have commercial value or ecological potential. (Reigada and Negreiro Franzoso, 1999; Pinheiro and Franzoso, 2002; Castiglioni and Negreiro Franzoso, 2006). Histological observations of the monthly gonads in *Barytelphusa cunicularis* revealed that the ripening of the gonads started during the March and extended upto May. By June, the gonads of the most of the individuals attained full maturity and spawning began. Spawning terminated by the end of September and the gonads entered a quiscent period. (Diwan and Nagabhushanam, 1974). From the breeding behaviour and the annual variations in the gonadal indices of the freshwater crab, *Barytelphusa guerini*, the annual reproductive cycle in this crab divided into (i) repro-ductive period (May to August); (ii) post-reproductive period and quiescent period (September to December); and (iii) preparatory period for repro-ductive activity (January to April); (Gangotri *et al.,* 1978).

****Corresponding author: Vaishali Gangotri***
Department of Zoology, Shri Dhokeshwar Mahavidyalaya, Takali Dhokeshwar, 414304, Ahmendnagar, (M.S.) India.

The macroscopic characterization of the gonads among decapods has been commonly investigated by several authors. Most of the papers dealing with the determination of the most of the sexual physiological maturity or histological maturity, taking into account the degree of gonad development (Castiglioni and Santos, 2001; Swiney and Shirley, 2001; Castiglioni and Negreirosfransozo, 2006 among others). However, studies of histological description of gonads are less common, (Cronin, 1947; Adiyodi and Subramonian, 1983; Meusy and Charniaux Cotton, 1984; Wenner *et al.,* 1987, Krol *et al.,* 1992; Lopez *et al.,* 1997, Ando and Makioka, 1998). Since, little work has been done on the reproductive biology of the freshwater crabs in India and as already the phases of reproductive cycle has been investigated in the freshwater crab, *Barytelphusa guerini* (Gangotri *et al.,* 1978). Hence, the present study was undertaken with a view to investigate the histological changes during the reproductive cycle.

MATERIALS AND METHODS

The adult specimens with a carapace width of 45 mm of freshwater crab, *Barytelphusa guerini*, which are sexually mature and reproductively active (Gangotri, *et al.,* 1978) were collected from the field for a period of one year (January 2015

to December 2015). The male and female crabs were maintained in glass troughs containing sufficient water, specimens that were not healthy conditions as well as those that had just moulted were not used for analysis. Sufficient time was given for the animals to acclimate to the laboratory conditions. The male and female animals were sacrificed and the gonads were quickly dissected and fixed in Bouin's solution for 24 hours at 20^0C. Afterwards, the gonads were dehydrated through an alcohol series (80%, 90% and 95%, one hour in each). Gonads were placed and kept for two hours in pre-infiltration solution and transferred to an infilmation solution for three to four hours or an overnight. In the following day, blocks were made using blocking solution. (Paraffin wax M.P. 56^0 to 58^0C) and were sectioned at 6 to 8 µ. The sections were stained with Delafields Haematoxylin and Eosin.

RESULTS

The testes show changes during the reproductive cycle. Each testis is made up of numerous seminiferous tubules of varying size held together by connective tissue.

Spermatogenesis: The serial sections of the seminiferous tubules of the testis appear narrow while the lumen is full of sperms. The tubules are continuous and lead directly into the vas deferens. The different stages of spermatogenesis can be classified into four morphological entities:

- Spermatogonia
- Spermatocytes
- Spermatids and
- Spermatozoa

In the process of spermatogenesis, the spermatogonia are the first group of germ cells to appear and the distinction between primary and secondary spermatogonia is not possible under light microscopy. The spermatogonia are recognised by their size, nucleus, and position of nucleolus. These cells later on undergo meiotic divisions to give rise to primary and secondary spermatocytes. The primary spermatocytes are recognised by their eosinophilic cytoplasm, while the secondary spermatocytes by their poorly stainable cytoplasm. The spermatids which are formed as a result of secondary spermatocytes are small rounded bodies having deeply stained nuclei, while the cytoplasm stains grey with haematoxylin eosin. During spermatogenesis, they undergo morphological changes and finally become crescent shaped structure. During the period from January to April, the testes show marked changes in size. The testes appear turgid, its wall became thin and the vas deferens markedly coiled. This implies that during this period, the testis is prepared for spermatogenesis, Sperms are numerous in and full of the seminiferous tubules. The inter tubular spaces are decreased. Maturational changes do not occur simultaneously in all tubules of the testes. Most of the tubules are filled with spermatids and sperms. Various stages of spermatogenesis can observed in some of the seminferous tubules (Figure 1a). The second stage begins in May and continues upto the end of August. During this period, the size of the testes is decreased and it becomes thin. The number of spermatocytes and spermatids decreased and finally reached a minimum number. This indicates gradual cessation of spermatogenesis during this period. (Figure 1b). The third stage begin from September to December i.e. Post reproductive period. During this period, the testes became empty and quiescent (Figure 1c).

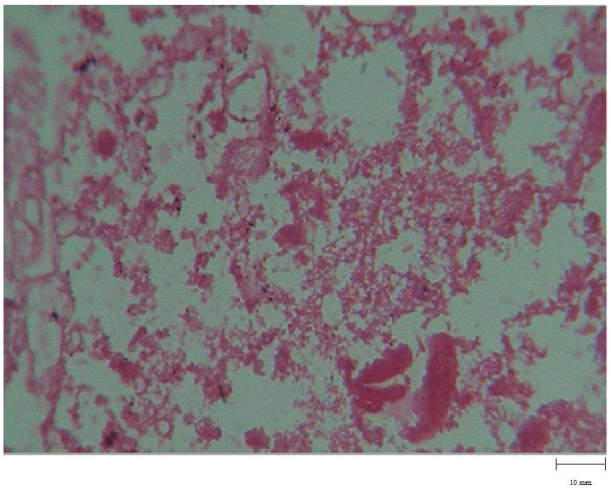

Figure 1a. April 2015 TS of testis during the pre-reproductive cycle of the male crab

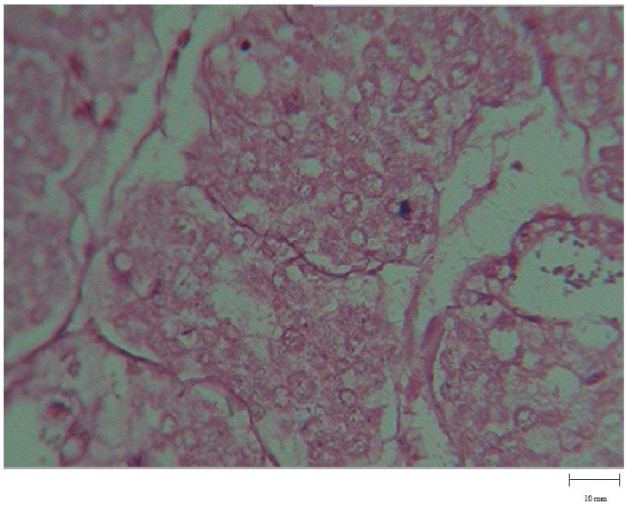

Figure 1b. June 2015 TS of testis during the reproductive cycle of the male crab

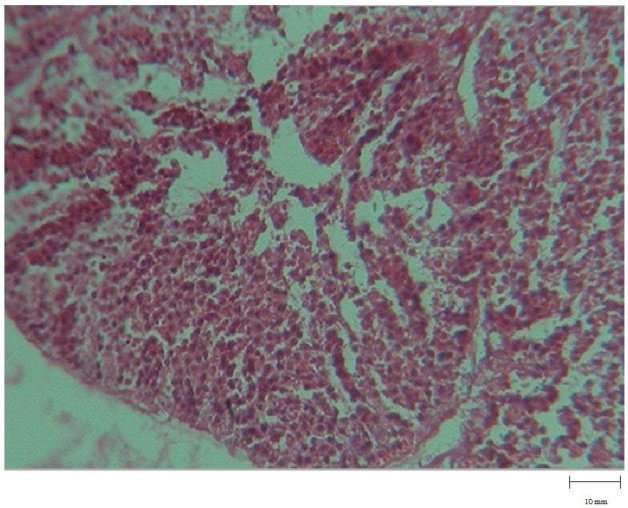

Figure 1c. December 2015 T.S. of testis during the post-reproductive cycle of the male crab

Oogenesis

The ovary in *Barytelphusa guerini* is covered with two membranes, an outer most epithelial layer and inner layer of germinative epithelium layer is lobulated. The germinative zone or zone of proliferation is distinguished by the presence of compact mass of oogonial cells which undergo meiotic division and give rise to primary oocytes. The cells grow in size and then mature into ova by meiosis in the vegetative phase. In a fully matured ovary, all the stages of oocyte growth is well as accessory cells (follicle cells) and nutritive phagocytes are concentrated. The oogonial cells grow and develop at the expense of nutritive cells into pre-vitellogenic organ.

Primary Oocyte (Previtellogenic)

These cells are found generally close to the oogonia. Early primary oocytes have a basophillic homogeneous cytoplasm without follicle cells. Late primary oocytes have a heterogeneous cytoplasm that presents small vacuoles. The cytoplasm of the late primary oocyte is less basophillic than the cytoplasm of early primary oocytes. Late oocytes are surrounded by follicle cells that are round in shape previtelloginic oocytes have a well defined nucleus with prominant nucleoli on the border.

Secondary Oocyte (Vitellogenic)

These cells are acidophilic showing high content of yolk and lipid drops. In these cells, nucleus cytoplasm ratio is very low. All vitellogenic oocytes are surrounded by the follicle cells that allowed the observation of the complete process of follicle formation. Degenerating oocytes are almost of the same size as the vilellogenic oocyte. They can be identified by the appearance of vacuoles. The degenerating ova are surrounded by nutritive phagocytes which increase in their size with growing vacuolisation. The freshwater crab, *Barytelphusa guerini* showed changes in the ovary during the course of reproductive cycle. The seasonal cycle of oogenesis is studied as follows. During January to April, the ovary shows changes in size. The ovary increased in size and is filled with vitellogenic oocyte due to accumulation of yolk material. Maturational changes do not occur simultaneously in all follicles of the ovary. In early January various stages of oogonia (oogonial cells, previtellogenic and vitellogenic oocyte) were observed. The oocytes are surrounded by follicular cells. In February and March, the ovary is fully developed and oocytes are compactly arranged. The oocytes were big and the ovarian wall appeared to corrogate against the developing oocyte. The beginning of April is characterised by the appearance of yolk globules along the periphery and oocytes towards the inner side of the ovary. This is the first and the major spawning season of the *Barytelphusa guerini*. (Figure 2a). The second stage begins in May and continues upto August. During this period, the size of the ovary decreased. The number of previtellogenic and vitellogenic oocytes is reduced. Oogonia cells and small oocytes appear to dominate during this period. This stage indicates the gradual cessation of the oogonia. (Figure2b). The third stage of oogenesis (September to December) ensures with gradual decrease in the size of the ovaries. The number of size of previtellogenic oocytes to vitellogenic oocytes also decreases. Few degenerating oocytes are also seen (Figure 2c).

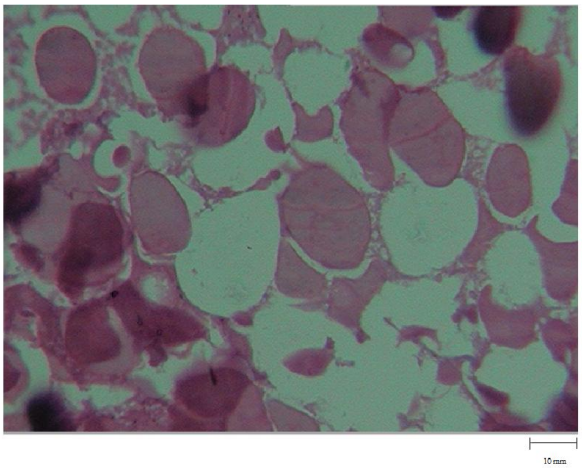

Figure 2a. April 2015 T.S. of ovaries during the pre-reproductive cycle of the female crab (X_{40})

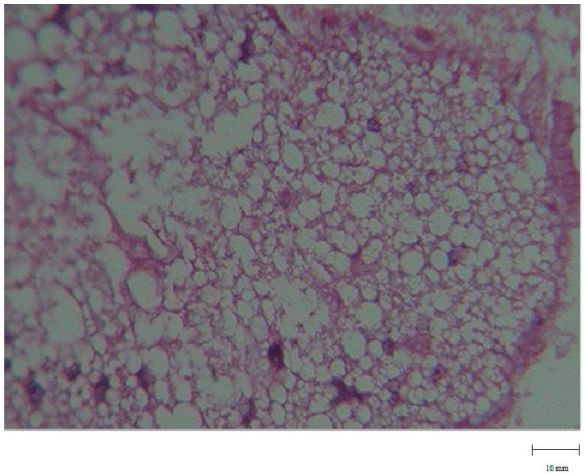

Figure 2b. August 2015 TS of ovaries during the reproductive cycle of the female crab (X_{40})

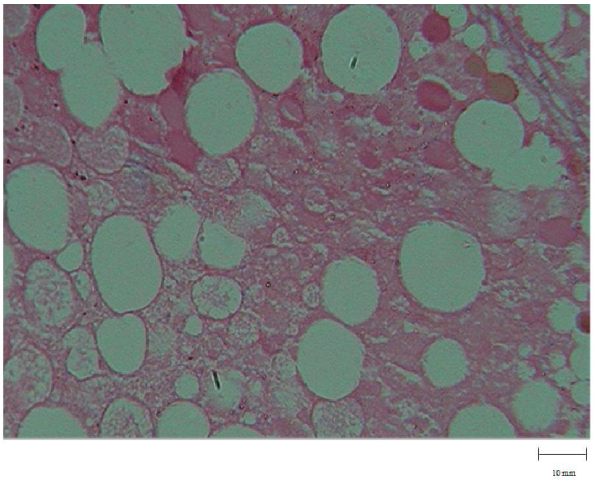

Figure 2c. December 2015 Various stages during the Post-reproductive period (December 2015)

This stage ensured with gradual decrease in the size of the ovaries. The number of size of prevvitellogenic oocyte to

vitellogenic oocytes also decreased. Few degenerating oocytes were also seen. (X_{40})

DISCUSSION

The reproductive cycle in the freshwater crab, *Barytelphusa guerini* was studied by determining the gonadal indices. From the annual variations in the gonadal indices, the annual reproductive cycle in the crab, *Barytelphusa guerini* divided into - (i) Preparatory period for reproductive activity i.e. January to April (ii) Reproductive (breeding) period i.e. May to August and (iii) Postreproductive and quiscent period i.e. September to December (Gangotri, *et al.,* 1978). From the present study, involving the size of gonads, breeding behaviour, number of eggs in females, total number of eggs and young ones in the abdomen of females during breeding season, it is clear that the crab, *Barytelphusa guerini* attains sexual maturity on reaching a CW of about 45 mm in both the sexes. The data also indicate that the intermediate sized animals with CW of 45-60 mm are reproductively more active and the reproducive activity declines in older animals with CW of 60 mm and above. Different species of crabs breed during different periods. The breeding periods are prior to spring season for *Paratelphusa jacquemontii* of Salsetteis Island (McCann, 1937), June to September for the same species occuring at Bombay (Ali, 1955), February to April for *Paratelphusa jacquemontii* of Peninsular India (Chacko and Thyagarajan, 1952) and June to September for *Barytelphusa cunicularis* of Aurangabad (Diwan, 1973). The present study revealed that *Barytelphusa guerini* breeds from May to August with the peak period during June and July. It was also reported earlier in this species that high gonadal indices in females during April and May are due to the presence of large number of ripe eggs in the ovaries. The decrease in gonadal index during June and July is due to the release of eggs and it is supported by the presence of eggs and young ones in the abdominal region during these two months. High gonadal indices occur in males with a time lag during the period May to August (Gangotri, *et al.,* 1978).

Pathare and Patil (2010), reported earlier that *Barytelphusa cunicularis* is the continuous breeder. During the month of July to September, the maximum numbers of ovigerous females were observed and that breeding peak was correlated with maximum development in gonads in the crabs. In July and September, maximum numbers of males with mature gonads and the maximum carapace length were observed. Histological section of the gonad at monthly interval of *Barytelphusa guerini* showed oocytes of many sizes and spermatogenic cells in various stages including spermatozooa only during the month of May to August. Full grown ova and spermatozooa were generally noticed in gonads during the breeding season. After spawning the size of the gonads was decreased and developing oocytes and spermatogenic cells were found to be of normal size. There is a paucity of information regarding the seasonal histological changes in the testes of crustaceans. Both continuous and discontinuous spermatogenic cycles occur in different species of crustaceans studied. Such variations in the testicular activity may be dependent on several factors. In the crab, *Carcinus maenas* (Spalding, 1942) and *Paratelphusa sanguinolentus* (Ryan, 1967), the sperms are produced throughout the year. In the shrimp *Penaeus setiferous* (King, 1948), spermatogenesis seems to be continuous once the individual attain sexual maturity. In the histological analyses of the ovaries, the modifications observed in the oocytes during the process of gonad maturation are similar to the descriptions in the literature for other females of decapod crustaceans (Adiyodi and Subramonian, 1983; Lopez, *et al.,* 1997; Oliveira, *et al.,* 1999; Ando and Makioka, 1998; Elorza and Dupre, 2000). The similarities are specially found in the morphological changes in the reproductive cells, and also in the presence and arrangement of follicle cells during the process of ovary maturation. Thus, the morphology and development of females gonad has intensively been utilized as a tool for reproductive cycle studies. In conclusion, the results obtained on histological observations in the three different periods of reproductive cycle i.e. (i) Preproductive period (January to April) (ii) Reproductive period (May to August) and (iii) the Post-reproductive period (September to December) in *Barytelphusa guerini* suggests that these three periods are characterised by the variations in histological composition.

Acknowledgement

The authors express their grateful thanks to the Hon. President, Ahmednagar Jilha Maratha Vidya Prasarak Samaj, Ahmednagar for providing the laboratory facilities and constant encouragements during the tenure of present study.

REFERENECES

Adiyodi, K.G., and Subramonian, T., 1983. Arthropode-crustacea. In : Adiyodi, K.G., ed. Reproductive biology of invertebrates : Oogenesis, Oviposition and Oosorption. London, John < Wiley and Sons, p. 365-443.

Ali, J.A., 1955. "Hunting the land crab, *Paratelphusa guerini*", *J. Bombay Nat. Hist. Soc., 52* : 941-945.

Ando, H., and Makioka, T., 1998. "Structure of the ovary and mode of oogenesis in a freshwater crayfish", *Procambarus Clarkii* (Girard), *Zoological Science*, *15*: 893-901.

Castiglioni, D.S., and Santos, S., 2001. "Reproductive aspects of *Crytograpsus angulatus* Dana, 1851 (Brachyura, Grapsidae) in the lagoa do Peixe, Rio Grande do Sul. State, Brazil", *Nauplius*, *9 (1)* : 11-20.

Castiglioni, D.S., Negreiros-Fransozo, N.L., and Cardoso, R.C.F., 2006. "Breeding season and molt cycle of the fiddler crab, *Uca rapax* (Brachyura Ocypodidae) in a subtropical estuary, Brazil, South America", *Gulf and Carubbean Research*, *19* : 11-20.

Chacko, P.I., and Thyagarajan, S., 1952. "On the development and parental care in the Potamonid crab, *Paratelphusa jacquemontii*", *J. Bombay Nat. Hist. Soc.*, *52* : 289-291.

Cronin, L.E., 1947. "Anatomy and histology of the male reproductive system of *Callinectes sapidus Rathbun*", *Journal of Morphology*, *81 (2)* : 209-239.

Diwan, A.D., 1973. "Neuroendocrine regulation of glycogen and fat content in the hepatopancreas and muscle tissue of the freshwater crab, *Barytelphusa cunicularis*", *Marathwada Univ. J. Sci.*, *12* : 279-294.

Diwan, A.D., and Nagabhushanam, R., 1974. "Reproductive cycle and Biochemical changes in the gonads of the freshwater crab, *Barytelphusa Cunicularis*", (Westwood), *Indian. J. Fish.*, *21 (1)*: 164-176.

Elorza, A., and Dupre, E., 2000. "Arquitectura del ovario dela langosta de Juan Fernandez", *Investigaciones Marinas*, *28*: 175-194.

Gangotri, M.S., Vasantha, N., and Venkatachari, S.A.T., 1978. "Sexual maturity and breeding behaviour in the freshwater crab, *Barytelphusa guerini*", Milne Edwards, *Proc. Indian Acad. Sci.*, *87B (9)* : 195-201.

King, J.E., 1948. "A Study of the reproductive organs of the Common Shrimp, *Penaeus Setiferus* (Linnaeus)", *Biological Bulletin*, *94* : 224-262.

Krol, R.M., Hawking, W.E., Overstreet, R.M., 1992. "Reproductive Components in *Harrison*, F.W. and Humes, A.G. eds. *Microscopic Anatomy* of Invertebrates", 10 New York Wiley Liss, p. 295-344.

Lopez, L.S.G., Stella, V.S., and Rodriguez, E.M., 1997. "Size at onset of sexual maturity in (*Chasmagnathus granulata*)", (Decapoda, Brachyura), *Nauplius*, *5 (2)* : 65-75.

McCann, C., 1937. "Notes on the common land crab, *Paratelphusa* guerini of Salsetle Island", *J. Bombay Nat. Hist. Soc.*, *39* : 531-542.

Meusy, J.J., and Charniaux-Cotton, H., 1984. "Endocrine control of Vitellogenesis in Malacostraca Crustaceans. In Engels W. ed. *Advances in invertebrate reproduction*", Amsterdam, *Elsevier Science*, p. 231-232.

Oliveira, P.S.P., Santos, G.V., and Correa, A.N.A., 1999. "O desemvolvemento Ovocitario em *Penaeus Penicillatus* (Alcock, 1905)", Submetidos a monoablacao do *Pedunculp Ocular. Nauplius*., *7* : 53-64.

Pathare, R.F., and Patil Meena, 2010. "Breeding cycle and Fecundity of the freshwater Crab, *Barytelphusa Cunicuaris* (Decapoda, Potamidea)", *World Journal of Zoology*, *5 (2)* : 96-102.

Pinheiro, M.A.A., and Fransozo, A., 2002. "Reproductive dynamics of the speckied swimming crab, *Arenaeus Caribrarius* (Lamarck, 1818) (Brachyura, Portunidae) on the North Coast of Sao Paulo State, Brazil", *Journal of Crustacean Biology*, *22 (2)*: 416-428.

Reigada, A.L.D., and Negreiros-Fransozo, M.L., 1999. "Maturidae Sexual em *Hepatus pudibundus* (Decapoda, Brachyura, Calapppidae)", *Iheringia Serie. Zoologica*, *86* : 159-164.

Ryan, E.P., 1967. "Structure and function of the reproductive system of the crab, *Portunus Sanguinolentus* (Herbst, Brachyura, Portunidae) II, The Female System", *Proc. Symp. Crustacea*, 1965, Ernakulami Cochin, (Part-II): 522-544 (Mar. *Biol. Ass.* India).

Spalding, J.S., 1942. "The mature and formation of spermatophore and spermplug in *Carcinus maenas*", *Quart. J, Micro. Sci.*, *183*: 399-422.

Swiney, K.M., and Shirley, T.C., 2001. "Gonad development of South-eastern Alaskan Durgeness Crab, *Cancer magister* under laboratory conditions", *Journal of Crustacean Biology*, *21 (4)*: 897-904.

Wenner, E.L., Ulrich, G.F., and Wise, J.B., 1987. "Exploration for Golden Crab, *Gerion fenneri* in the South Atlantic Bight: Distribution, Population, Structure and Gear Assessment", *Fishery Bulletin*, *85 (3)*: 547-560.

****