Journal of the Persian Gulf
(Marine Science)/Vol. 5/No. 15/March 2014/8/17-24

Seasonal Histological Comparison of Gonad and Gametogenesis in Female Pearl Oyster (*Pinctada radiata*) of the Persian Gulf

Mohammad Karami, Asma[1]; Khodabandeh, Saber[1*];
Rameshi, Hossein[2]; Hasanabadi Zadeh, Zohreh[1]

1- Faculty of Marine Science, Tarbiat Modares University, Noor, IR Iran
2- Mollusk Research Center, Bandar Langheh, IR Iran

Received: June 2013 *Accepted: December 2013*

Abstract

The pearl oyster, "*Pinctada radiate*", is a native species of Persian Gulf area, which has always been attractive for its ability of producing pearl. Thus, studying the gonadal maturation of the pearl oyster has been important for nucleus implantation to pearl production. In this study, samples were taken in winter and spring from Lavan and Kish Islands. The classic histological methods were used to describe the morphology of female gonad, and computer-based image analyzer (Image Tools 2) to measure oocytes areas. Results showed that female gonad was paired organ and asymetrical, situated between mantle and digestive gland. The follicles contained oocytes identifiable in previtelogenin, vitelogenin and postvitelogenin stages. In spring samples compared to winter samples, the connective tissue in follicles shrunk and greater part of follicles space was filled with postvitelogenin oocytes. The means of oocytes surface areas for spring and winter samples were 1276/71±228/6 μm^2 and 936/86±237/04 μm^2 , respectively. In conclusion, based on observation of female gonad from winter to spring, it was determined that upper stage of maturation occurred and results of previous studies which showed female gonad maturation happened from February to April, before summer spawning, were confirmed.

Keywords: *Pinctada radiata, Gonad maturation, Histology, Image analyzer*

1. Introduction

The Persian Gulf pearl oyster *Pinctada radiata* Leach, 1814, an Indo-Pacific species, is a member of the family Pteriidae (Southgate, 2008). Pearl oysters of this Family, because of their capacity of producing pearls, have always been attractive for commercial matters, since 2000 years ago in Persian Gulf area (Bowen, 1951). Therefore, studying the gonadal maturation of the pearl oyster has proven to be important, because the nucleus implantation is essential to pearl production, either in artificial seed production or in the preoperative procedures (Hwang, 2007).

Despite extensive studies conducted on the reproductive biology of the pearl oyster species of the Pteriidae family (Pouvreau et al, 2000; Moullac et al,

* *Email: skhoda@modares.ac.ir*

2011; Choi and Chang, 2003 and Hwang, 2007) pearl oysters from the Persian Gulf (Behzadi et al,1997; Jamili et al,1999 and Khamdan, 1998), because of the economic importance of both natural and cultured pearl and shell fisheries, still more investigation on the gametogenesis of *P.radiata* was necessary.

There are several ways of assessing gamete development in bivalves most of which are done via visual observation of the relative size, shape and color of the gonads or investigating developmental stages, based on histological characterization (Delgado and Camacho, 2005; Arjarasirikoon et al, 2004; Grande et al, 2001; Alagarswami, 1987; Coe, 1932; Steele and Mulcahy, 1999 and Assoi et al, 2004). In addition, the use of indices such as, gonad development index (GDI) for individuals, mean gonad index (MGI) for populations (Raleigh and Keegan, 2006 and Moullac et al, 2009) and mean oocyte diameter (Ferreira et al, 2006 and Lango-Reynosoa et al, 2000) have recently been used in many studies to determine gonad status. Since histological techniques provide extensive information about gonad development, they are always used to verify reproductive events (Lango-Reynosoa et al, 2000).

The aim of this research was to describe the morphology of *Pinctada radiata* gonad by taking oocytes and follicles (as parameters) in different stages of the gonadal cycle to show the differences of stages by means of comparative statistical analysis in winter and spring.

2. Materials and Methods

2.1. Histology

Samples of pearl oyster *Pinctada radiata* were collected with scuba, from the Persian Gulf in around Lavan Island (26°49´N 53°9´E) in February, 2010 and from Kish Island (26°34´N 53°55´E) in May, 2011. The mean length of shells gathered from Lavan and Kish Islands were 5cm and 7cm, respectively. The soft part of the individuals were removed after opening the valves of the shells and fixed for 24 h in Bouin´s fixative for the histological studies. Then, samples were dehydrated through a graded series of ethanol solution and butanol, followed by xylene, prior to embedding in paraffin. Dehydrated samples were sectioned by MICRODS 4055 microtome at 4µm thickness, and collected on glass slides. All sections were stained by hematoxylin and eosin, prepared for examining, by using a light microscope (Martoja and Martoja-Pierson, 1967 and Khodabandeh et al, 2005).

2.2. Statistical Analyses

The total number of oocytes in samples gathered in each season were counted. Oocytes, existed in three stages: previtelogenin, vitelogenin and postvitelogenin (Quintana, 2005). By using a computer-based image analyzer (Image Tools 2), the area of 60 histologically sectioned through the nucleus oocytes were measured. Mean oocytes areas for Spring and Winter samples were 1276/71±228/6 μm^2 and 936/86±237/04 μm^2, respectively.

3. Results

It is known that the gonad in *P.radiata* is not a discrete organ. It is paired but asymmetrical (Figure 1). This tissue was situated between the digestive gland and the mantle (Fig. 2), and the pair covered the stomach, liver and parts of the intestine in mature form (Fig. 3).

The structure of gonad tissue is formed by a series of compact granular bags (follicles) that represent the structural units of gonad tissue; the follicles are surrounded by connective tissues (Fig. 4) and these "sacs" contain numerous gametes (Fig. 5).

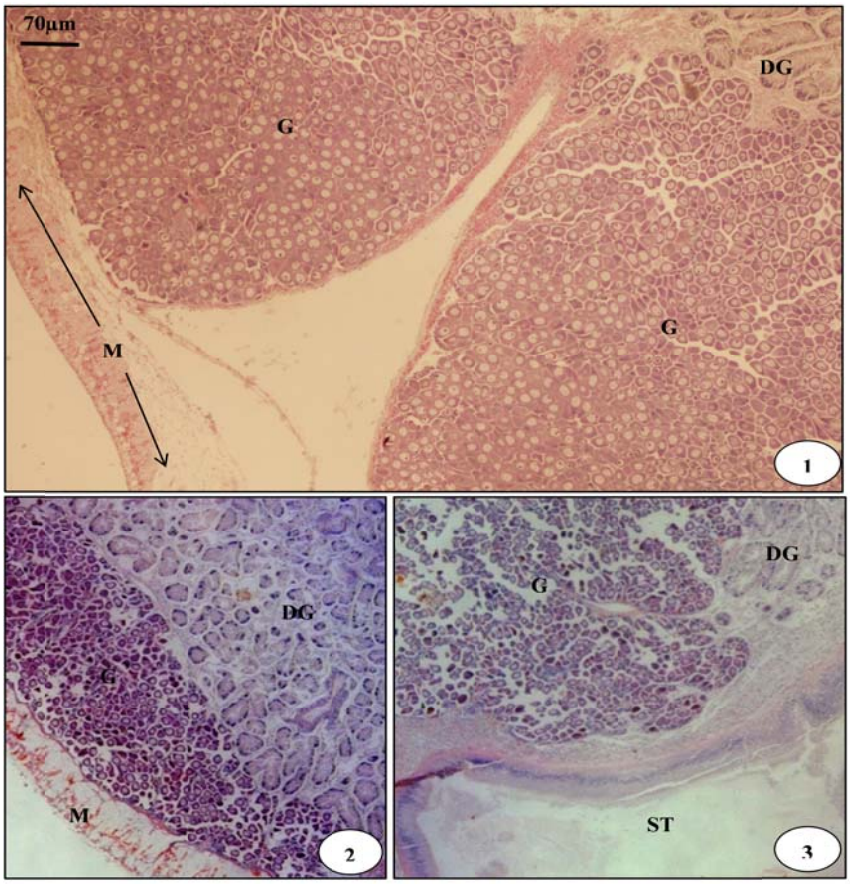

Fig 1, 2, 3: The female gonad organ in *Pinctada radiata* is paired and its situation in visceral mass is between digestive gland, mantle and digestive tract. DG: Digestive gland, G: Gonad, M: Mantle, ST: Stomach.

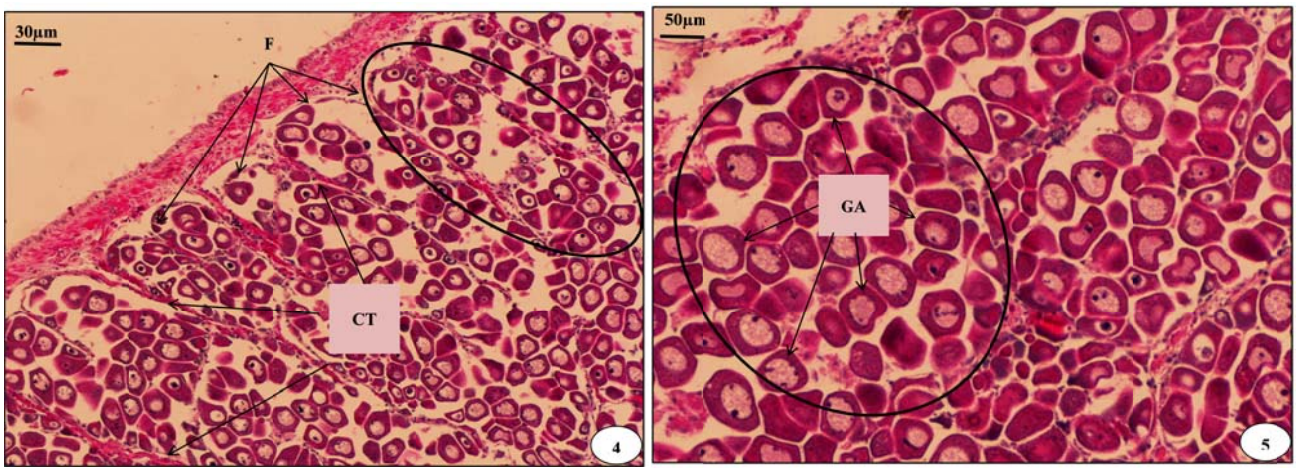

Fig 4, 5: The female gonad organ built of follicles, which is surrounded by connective tissues and contains gametes. F: Follicle; CT: Connective tissues; GA: Gametes.

The connective tissue of gonad, in winter samples, was shrunk but still remained as a thin layer. As it is seen, the follicles are filled with oocytes, but a wide interoocyte space still exist (Figs 6 and 7).

Oocytes from spring samples were mostly well-developed and full mature; the follicle wall was swollen and indefinite and oocytes were tightly connected to each other (Figs 8 and 9).

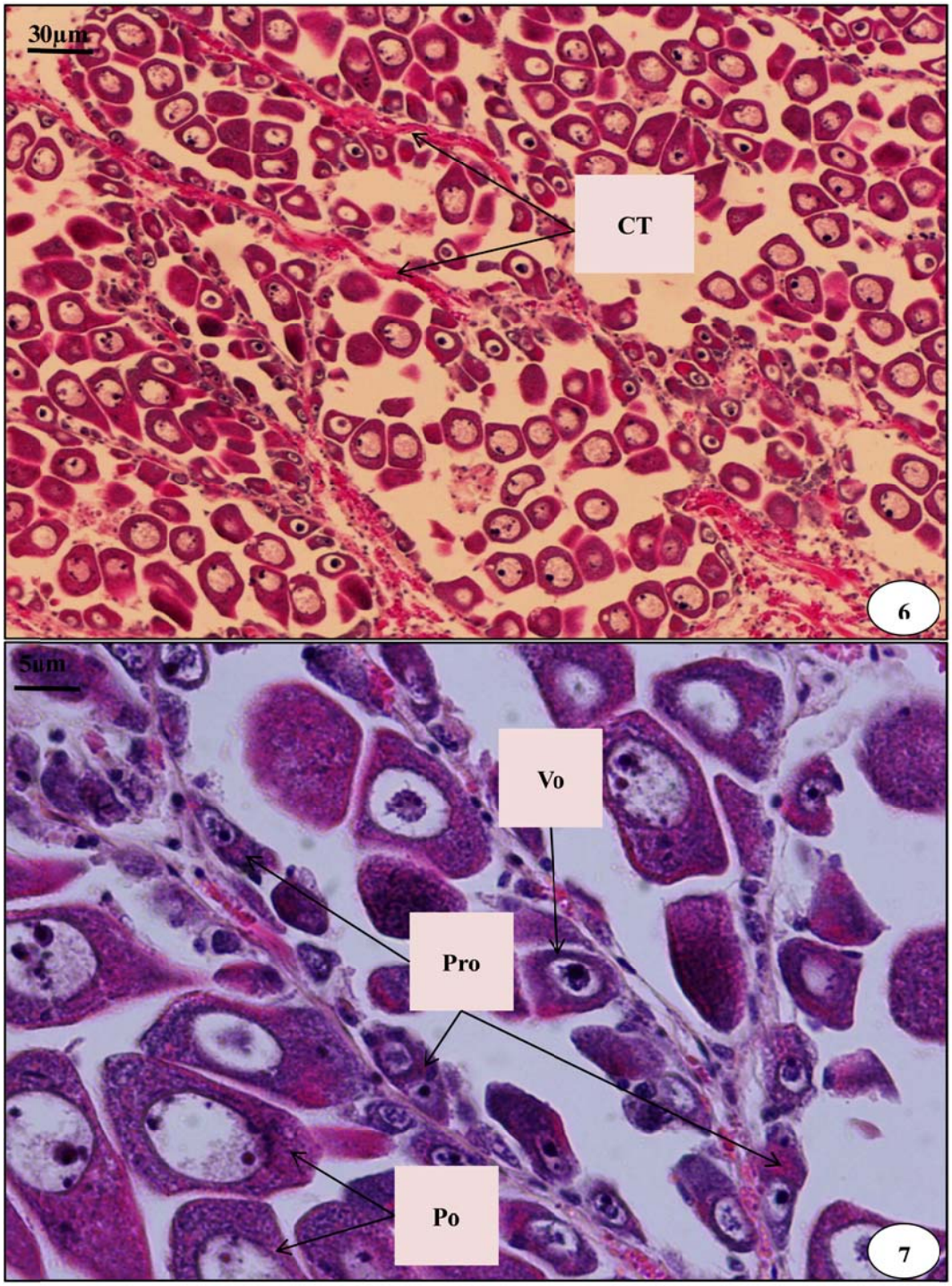

Fig 6: Gametes in female gonad organ in winter samples do not fill the follicles and the space between connective tissue and the follicles, also can be seen.

Fig 7: Three types of gametes during gametogenesis can be distinguished in these samples; CT: Connective tissues; Pro: Previtellogenic oocytes; Vo: Vitellogenic oocytes; Po: Postvitellogenic oocytes.

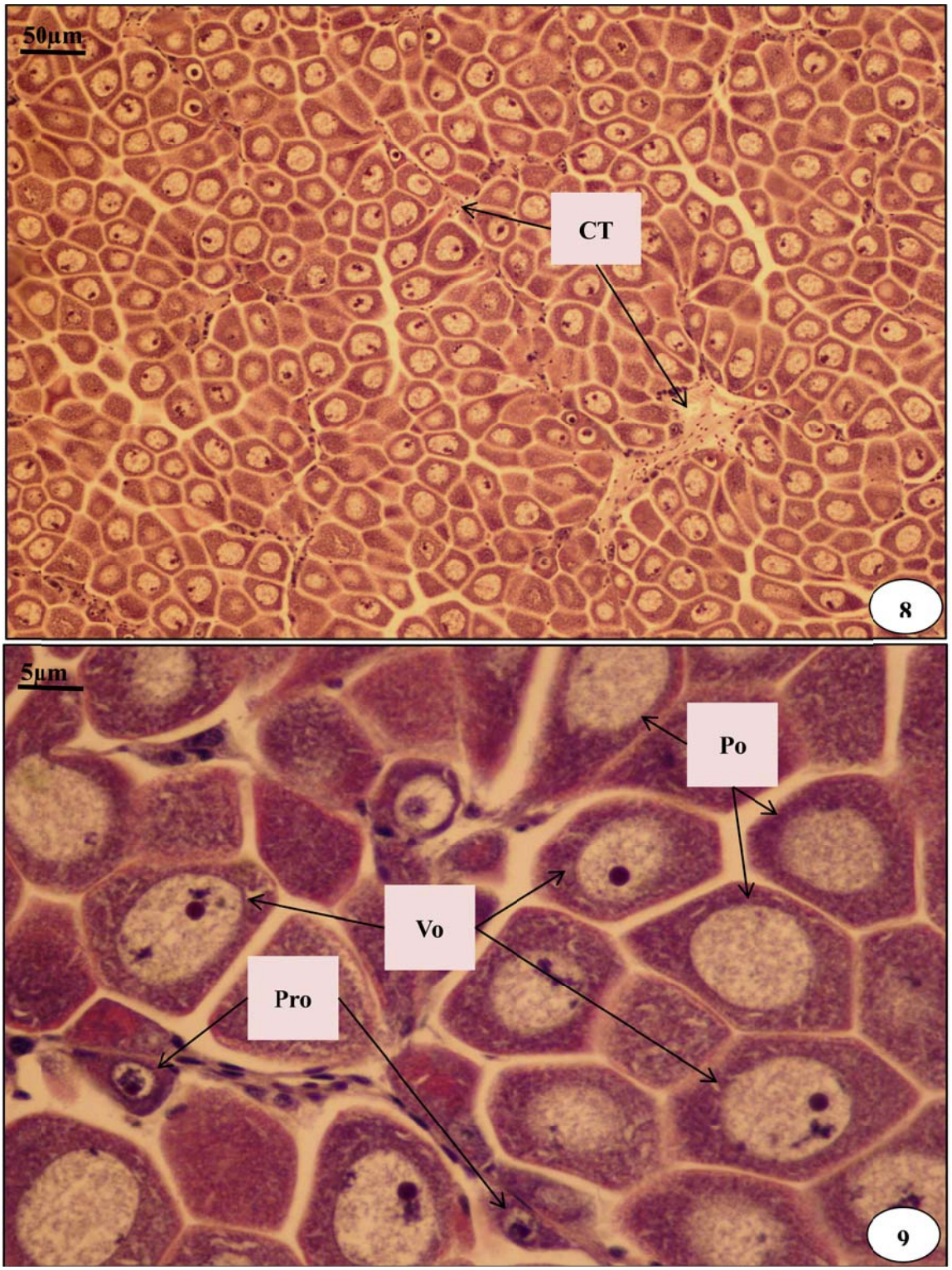

Fig. 8: Mostly ripe gametes in female gonad organ in spring samples fill the entire follicles' space and the connective tissues between follicles has shrunked.

Fig. 9: Three types of gametes during gametogenesis can be distinguished in these samples. CT: Connective tissues; Pro: Previtellogenic oocytes; Vo: Vitellogenic oocytes; Po: Postvitellogenic oocytes.

As mentioned before, characterization of gonad in these samples, were the presence of three distinguished types of oocytes in the brunches of follicles: previtellogenic (Pro), vitellogenic (Vo) and postvitellogenic (Po) (Figure 7). The mean of 50 measurements of histological sections and quantifying numbers of different types of oocytes for each season samples shown in Table 1. Although the three types of oocytes could be been seen in these samples, but the postvitellogenic oocytes were dominant in gonad space (Fig. 9).

Table1: The numbers of previtellogenic oocytes (Pro), vitellogenic oocytes (Vo) and postvitellogenic oocytes (Po) and the total number of oocytes have been determined in 100mm^2

Season	Pro	Vo	Po	Total
Winter	5	9	13	38
Spring	3	14	24	66

3. Discussion

Gonad development is a term that describes the changes that occur in the gonad throughout the inactive and active reproductive periods, which is a complex process occurring by means of seasonal changes, which prepare appropriate biological and physical conditions (Quintana, 2005).

There are several methods for assessing gonad development in bivalves, but the histological techniques have been suitable and used in this study.

The female gonad organ of *Pinctada radiata* is composed of two asymmetric lobes situated in visceral mass. This characterization have been expressed by other authors for Fam. Petriidea (Urban, 2000; Hwang, 2007; Pouvreau *et al*, 2000 and Choi and Chang, 2003) as well. Each lobe is filling with oogonial cells which get mature in gametogenesis process (Quintana, 2005).

The classification of the gametogenic cycle of bivalve mollusc is varied and many authors examined different methods to reach to this purpose. Recent studies used histological and quantitative methods together. (Choi and Chang, 2003 and Raleigh and F.Keegan, 2006).The basis of gonad development classification, used in this study, was Rose et al (1990) staging scheme, which have been developed for *Pinctada maxima*, based on several schemes, first proposed by Tranter (1959) for several species within the genus Pinctada. This pattern was found to be the most reliable staging for *P.radiata* species gonadal development which was studied by Khamdan (1998) as a report from Offshore Environment of the ROPME Sea Area Project in Bahrain waters.

Although the study of reproduction cycle of *P.radiata* was not our purpose in this research, but according to our findings about gonad development in two different times, we compared our results to Rose et al (1990) scheme too. In this method, the gametogenic stage was divided to five stages: multiplicative stage, growing stage, mature stage, spawning stage and resting stage (Choi and Chang, 2003).

According to our observation at histological sections of the Winter samples, the connective tissues between the gonad follicles were shrunk, oocytes on the germinal epithelium grew and the three stages of vitelogenesis were present in oocytes, so it seemed to be on the second stage of gametogenesis: "Growing stage".

As gametogenesis proceeded, connective tissues disappeared and the previtelogenin oocytes got dominant in follicles area, they filled the interoocytes spaces and connected tightly to each other. These evidences, related to "Mature stage" which was obvious in histological sections of Spring samples.

Quantitative results (Table 1) showed the mean oocyte area in spring samples was higher than winter samples, that could be the result of the higher reproduction stage of these samples, a fact shown by Raleigh and Keegan (2006) on the reproduction cycle of *Scrobicularia plana*. Furthermore, results in Table 1, confirmed that winter samples in comparison to spring samples, were at lower stage because of more previtelogenin oocetys and the lower number of

oocytes in total that is related to "growing stage".

Growing observation in gonad from winter to spring, can confirm the results of Behzadi *et al* (1997) and Jamili *et al* (1999), which showed bimodal gametogenic pattern for *Pinctada fucata* in the Persian Gulf area and showed gonad maturation happened during February to April, before summer spawning.

References

Alagarswami, K., 1987. Pearl Culture. Indian Council of Agriculture Research. Bulletin 39

Arjarasirikoon, A., Kruatrachue, M., Sretarugsa , P., Chitramvong, Y., Jantateme, S., Suchart, E., 2004. Gametogenic prosses in the pearl oyster, *Pteria penguin* (Roding, 1798) (Bivalvea, Mollusca). Journal of Shellfish Research. 23(2): 403-409

Assoi Etchiana, O., Pellerina, J., Audeta, C., Mathieu, M., 2004. Sexual maturation and related changes in aspartate transcarbamylase activity of gonad tissues in the soft shell clam (*Mya arenaria*). Comparative Biochemistry and Physiology. Part B 139: 287 – 297

Behzadi, S., Parivar, K., Roustaian, P., 1997. Gonad cycle of pearl oyster, *Pinctada fucata* (Gould) in Northeast Persian Gulf, Iran. Journal of the Shellfish Research. 16: 129–135

Bowen, R.Leb., 1951. The pearl fisheries of the Persian Gulf. Middle East Journal. 5 (2): 161-180

Choi, Y., Chang, Y., 2003. Gametogenic cycle of the transplanted-cultured pearl oyster, *Pinctada fucata martensii* (Bivalvia: Pteriidae) in Korea. Aquaculture. 220: 781–790

Coe, W., 1932. Development of the gonads and the sequence of the sexual phases in the California oyster (*Ostrea lurida*). Technical Series. 3 (6):119-144

Delgado, M., Camacho, A., 2005. Histological study of the gonadal development of *Ruditapes decussates* (L.) (Mollusca:Bivalvia) and its relationship with available food.

Scientia Marina.69(1):87-97

Ferreira, M.A.P.; Paixão, L.F.; Alcântara-Neto, C.P.; Santos, S.S.D., Rocha, R.M., 2006. Morphological and Morphometric Aspects of *Crassostrea rhizophorae* (Guilding, 1828) Oocytes in Three Stages of the Gonadal Cycle. International Journal of Morphology. 24(3): 437-442

Grande, C., Araujo, E, Ramos, M.A., 2001. The gonads of *Margaritifera auricularia* (Spengler, 1793) and *M. margaritifera* (Linnaeus, 1758) (Bivalvia:Unionoidea). Journal of Molluscan Studies. 67:27-35

Hwang, J., 2007. Reproductive cycles of the pearl oysters, Pinctada fucata (Gould) and Pinctada margaritifera (Linnaeus) (Bivalve: Petriidae) in southwestern Taiwan waters. Journal of Marine Science and Technology. 15 (2): 67-75

Jamili, S., Amini, G., Oryan, S., 1999. Gonad changes and serum steroid levels during the annual reproductive cycle of the pearl oyster Pinctada fucata Gould. Iranian Journal of Fisheries Science, 1 : 67–75.

Khamdan, S.A.A., 1998. Aspects of reproduction in the pearl oyster, *Pinctada radiata* (Leach). In: Offshore Environment of the ROPME Sea Area after the War-Related Oil Spill. Eds. Otsaki et al., Terra Scientific Publishing Company. 203-214

Khodabandeh, S., Kutnik, M., Charmantier-Daures, M., Charmantier, G., 2005 Ontogeny of the antennal glands in the Crayfish *Astacus leptodactylus* (Crustacea Decapod). Immunolocalization of Na^+/K^+-ATPase. Cell Tissue Research. 319: 153-16

Lango-Reynosoa, F., Chavez-Villalbaa, J., Cochard, J., Le Pennec, M., 2000. Oocyte size, a means to evaluate the gametogenic development of the Pacific oyster, Crassostrea gigas Thunberg. Aquaculture. 190: 183–199

Martoja, R., Martoja-Pierson, M., 1967. Initiation Aux Techniques de l histologe animale, Paris: Masson et Cie.345

Moullac, G., Hui, B., Vonau, V., Levy , P., Cochard , J., 2009. Effect of food conditioning on gonadic activity in the oyster Pinctada margaritifera. 11th Pacific Science Inter-Congress, 02-06 March, Tahiti, French Polynesia.

Moullac, G., Tiapari, J., Teissier, H., Martinez, E., Cochard, J., 2011. Growth and gonad development of the tropical black-lip pearl oyster, Pinctada margaritifera (L.), in the Gambier archipelago (French Polynesia). Aquaculture International, DOI 10.1007/s10499-011-9460-x

Pouvreau, S., Gangnery, A., Tiapari, J., Lagarde, F., Garnie, M., Bodoy, A., 2000. Gametogenic cycle and reproductive effort of the tropical blacklip pearl oyster, *Pinctada margaritifera* (Bivalvia: Pteriidae), cultivated in Takapoto atoll (French Polynesia). Aquatic. Living Resources. 13 (1): 37−48

Quintana, R., 2005. Effect of Estradiol-17β on the gonadal development of diploid and triploid female eastern oysters, PhD Thesis, Universidad Autonoma de Baja California

Raleigh, J., F.Keegan, B., 2006. The gametogenic cycle of *Scrobicularia plana* (Mollusca :Bivalvia) in Mweeloon Bay (Galway, west coast of Ireland). J. Mar. Biol. Ass. U. K, 86, 1157-1162

Rose, R.A., Dybdahl, R., Harders, S., 1990. Reproductive cycle of the Western Australian silverlip pearl oyster, *Pinctada maxima* (Jameson) (Mollusca: Pteriidae), Research Shelfish Journal of 9: 261–272

Southgate, P., Lucas, J., 2008. The P earl Oyster. Elsevier. 574P.

Steele, S., Mulcahy, M.F., 1999. Gametogenesis of the oyster Crassostrea gigas in southern Ireland. Journal of the Marine Biological Association of the UK. 79: 673 -686

Tranter, D.J, 1959. Reproduction in Australian pearl oysters (Lamellibranchia). V.Pinctada fucata (Gould). Australian Journal of Marine & Freshwater Research. 10: 45–66

Urban, H.J., 2000. Culture potential of the pearl oyster Pinctada imbricata from the Caribbean.I. Gametogenic activity, growth, mortality and production of a natural population. Aquaculture. 361–373.