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Gonadal Changes and Serum Steroid Levels during the Annual Reproductive Cycle of the Pearl Oyster *Pinctada fucata* Gould

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Abstract: The annual reproductive cycle of pearl oyster *Pinctada fucata* was characterised by documenting gonadal development and changes in serum levels of estradiol-17β (E_2), testosterone (T) and progesterone (P) in wild bivalve caught in natural beds in the Persian Gulf throughout the year. Bivalve populations employed in this study spawn in June-July and November-December. The pearl oysters had group synchronous ovarian development with exogenous vitellogenesis during spring and summer for first spawning and during August - September for the second spawning. Serum E_2 levels in the females increased rapidly from low values in May to peak in June and also in October coinciding with the time of spawning. Serum T levels in male and female exhibited a bimodal pattern. However in the males it increased during the early spermatogenesis. P was detected in both females and males. It's concentrations start to increase during the early gametogenesis and reaching to peak during the spawning season. The obtained results indicated that vitellogenesis and spermatogenesis in pearl oyster are not completed by the mid-summer and early winter.

KEY WORDS: Steroid hormones, gametogenesis, *Pinctada fucata,* Persian Gulf

Introduction

The elucidation of the physiological mechanisms that control reproduction in the bivalve requires a basic understanding of gonadal and hormonal changes occurring during the different phases of reproductive cycle. In addition, such understanding can facilitate the development of methods for controlling the reproduction. Several studies have been conducted on various aspects of the annual reproductive cycle of marine invertebrates in commercially important species, including starfish *Asterias rubens* (Voogt and Dielman, 1984), molluscs (Aubry , 1962), gastropods (Voogt , 1973 ; Idler *et al.*, 1973), Lymnaea (Krush *et al.*, 1979) and *Mytilus edulis* (Reis-Henriques and Coimbra , 1990). In many species, specific hormonal changes, which occur during gametogenesis

and the spawning season have been documented (Dieleman *et al.* , 1979; Reis-Henriques and Coimbra , 1990 ; Schoenmakers and Dieleman , 1981).

The objective of our study on *P. fucata* was to characterise the gonadal development and the seasonal changes of sex steroid levels in male and female *P. fucata.*, which spawn both in spring and autumn and is a potentially important species for commercial aquaculture along the Iranian coast of the Persian Gulf (Tajalipour , 1983).

Materials and Methods

Classification of the gonad condition

At the end of the first sampling year which happened in Nakhiloo, Persian Gulf, gonadal development were classified into series of stages based on histological observation. The following classification for gonadal development was according to the Lubet (1957, 1959), Bayne (1976) and Reis-Henriques and Coimbra (1990).

Stage I	Resting stage	undifferentiated or neuter
Stage II	Developing stage	gametogenesis
Stage III	Ripe stage	ripe gametes
Stage IV	Spawning stage	reduction in sperm and ova density
Stage V	Spent stage	residual gametes remain gametogenesis after the first spawning

Radioimmunoassay

E_2, T and P levels were measured in the serum using an AMERLEX-M estradiol, testosterone and progesterone RIA Kit (Johnson and Johnson Clinical Diagnostics Ltd Amersham, UK). The AMERLEX-M steroid RIA kits procedure is a competitive radioimmunoassay, where in ^{125}I-labelled estradiol, testosterone and progesterone compete for a fixed time with E_2, T and P in the samples for antibody sites.

Statistics

Results are reported as mean±SD. Means were compared with sex and months by t-student test. Significance levels were set to $P<0.05$.

Results

During the first spawning season (late spring) gonads of most of the oysters became partly empty and the resting period lasted until July-August. Development of the resting gonad commences during October-November and gametogenesis occurs over the winter with its peak in March.

The effects of temperature, salinity and food availability, as well as the combination of these factors, showed that spawning was temperature dependent (Fig.1). A maximum spawning in late spring was coincident with higher temperature and salinity of the water. The activation of the gonads seemed to be originated by declining temperatures in November-December, progressing rapidly through the winter.

Serum E_2 levels were low in the spawned male and female pearl oysters, sampled in late July, and remained low until September. In June (Major) and October (Minor) serum E_2 levels (male and female) were at the highest level. From November through January, serum E_2 levels decreased steadily (Fig.2).

The patterns of the P levels in male and female are similar throughout the annual reproductive cycle, although it is apparently slightly higher in female (Fig.3).

Serum T levels showed a bimodal pattern in male and female pearl oyster, which dropped sharply before spawning and remained low from June throughout December (Fig.4).

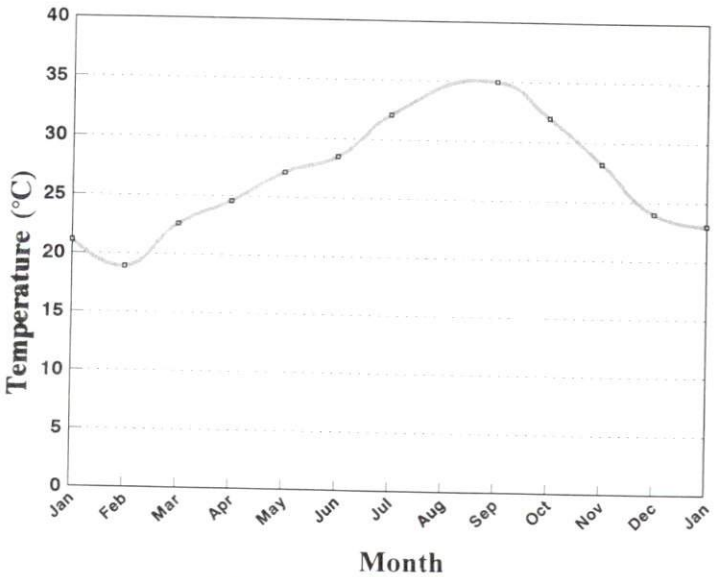

Fig. 1: Water Temperature in Nakhiloo

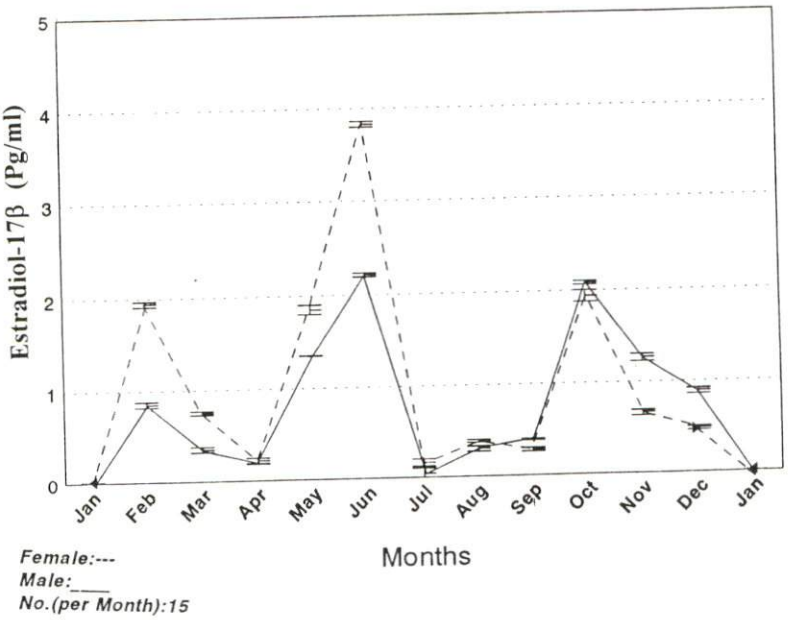

Fig. 2: Mean serum estradiol-17β concentration in monthly sampled pearl oyster in Nakhiloo

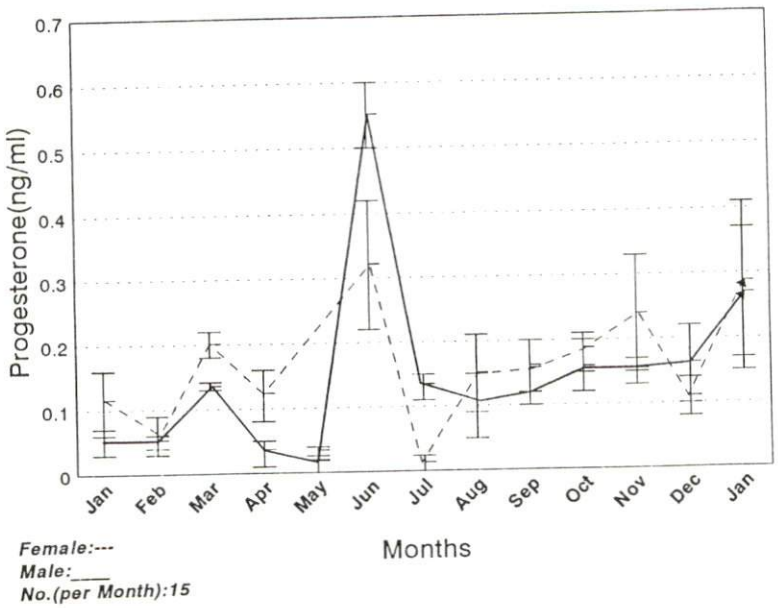

Fig. 3: Mean progesterone concentration in monthly sampled pearl oyster in Nakhiloo

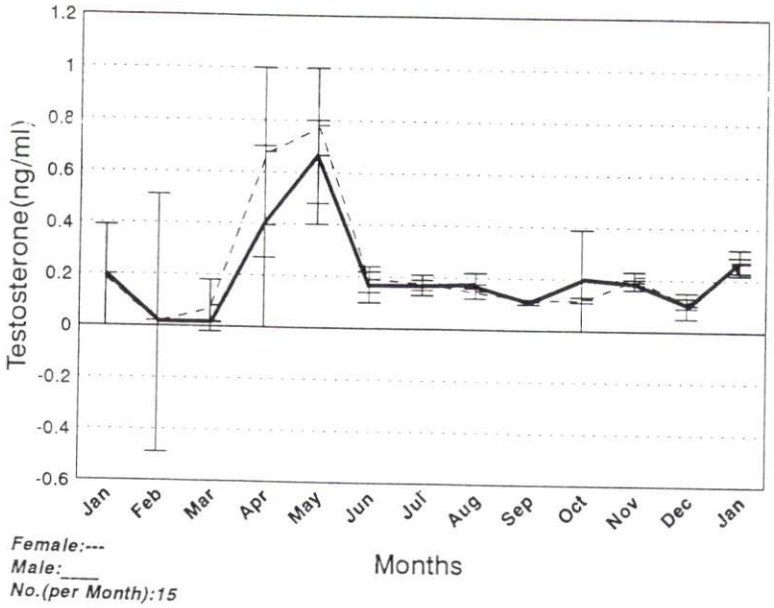

Fig. 4: Mean Testoserone concentration in monthly sampled pearl oyster in Nakhiloo

Discussion

Coe (1943) was the first researcher, who noted the occurrences of various types of sex change in bivalves. Protandric hermaphrodism has been reported in several species, *P. fucata* (Tranter , 1959), *Chlamys varia* (Lucas , 1965) and *Argopecten irradiens* (Sastry , 1968). These researchers pointed out that genetics sex determination in bivalves was very unstable and could be modified by changes in nutritional conditions. The sequence of events forming the reproductive cycle of invertebrates is known to be strongly affected by physical and biological environmental factors.

In our present study, gametogenesis started in late autumn and proceeded to the winter. Histological studies (Behzadi *et al.,* 1997) confirm that bivalve, like many invertebrates from temperate waters, have an annual reproductive cycle with group synchronous gonads development (Coe , 1943). In most specimens oocyte growth occured in late winter continuing throughout the spring following a relatively long period of gonadal quiescence, and is environmentally regulated by extending the photoperiod and/or water temperature. Like other temperate spring-spawning bivalves, however, final gamete maturation and spawning in *P. fucata* appear to be regulated by increasing water temperature and photoperiod.

In males, the period of gonadal quiescence was shorter than in females. Oocyte growth accrued in the mid-winter through spring. The timing and duration of the reproductive cycle of *Mytilus edulis*, from activation of the gonad through and gametogenesis to spawning and subsequent regression of the gonads, is controlled by an interaction of environmental factors like temperature, salinity, food availability, as well as endogenous factors like the hormonal cycle and energy reserves (Sastry , 1979 ; Lubet and Mathieu , 1982 ; Kaustsky , 1982). The protracted breeding season of tropical bivalve often feature one or two peak reproductive period during one year (Baron , 1992 ; Choi *et al.*, 1994 and Robinson , 1992).

Like most molluscs investigated to date, there was a dramatic increase in serum E_2 levels in bivalve at the first vitellogensis, which is consistent with the hypothesis that stimulate the synthesis of hepatic yolk precursors (Idler *et al.*, 1981). In P. *fucata* serum E_2 levels rose rapidly from May to June. Simultaneously, there was increase in the serum levels of T one month prior to spawning, suggesting that a drop in E_2 and/or rise in T is required for final oocyte maturation and spawning. Perhaps the elevated levels of T simply reflect an overall increase in the steroid secretory activity of the gonad, with T serving principally as a precursor for E_2 production (Dedual and Pankhurst , 1992). Alternatively, T may regulate some events during oocyte development, such as stimulating yolk globule formation, or increase cerebral ganglia production.

T increased dramatically prior to spawning, probably because of a shift in the steroidogenic pathway form C_{19} to C_{21} steroid synthesis coincident with spawning (Barry *et al.* , 1992). In males, two periods of increasing serum T levels were observed over the annual reproductive cycle. The first (Major) rise in T occurred just prior to the spawning month. A similar rise in serum T level is seen in fish and it seems to regulate male germ cells differentiation (Schreck *et al.* , 1972 ; Billiard *et al.* , 1978). The second (Minor) occurred between the months of September and October.

There was a dramatic increase in P level at the spawning period which is consistent with the hypothesis that P stimulated the spawning. Then, P levels decreased over an one-month period prior to final gamete maturation, coincident with second spawning. This pattern of reproduction is similar to that demonstrated in *M. edulis* by Seed (1975), Kelley (1982) and Reis-Henriques and Coimbra

(1990). Maximum progesterone in *M. edulis* was 0.4-0.5 ng/mg fresh weight, even if the spawning periods are not exactly the same.

The pattern of the P level in the serum of bivalve of both sex during the year supported the idea that P is involved in final gamet mutration in both sexes. The high levels of P correspond to the levels of other steroid hormones like esterogens (Reis-Henriques and Coimbra , 1990). Rises in P levels are thought to regulate the process of spermiation (Ueda *et al.* , 1985 ; Baynes and Scott. , 1985), and control male spawning behaviour (Liley and Stacey , 1983).

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