



**ASPECTS OF REPRODUCTION AND TRIPLOIDY
MANIPULATION IN THE PEARL OYSTER,
PINCTADA RADIATA (LEACH).**

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**A thesis submitted to the
University of Wales**

by

S A A KHAMDAN, (MSc, Wales)

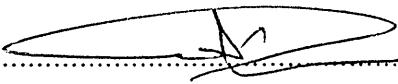
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DECLARATION

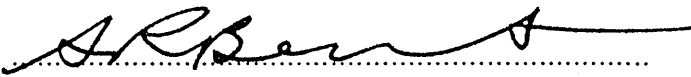
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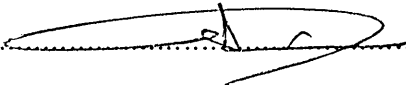
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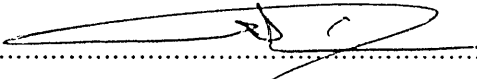
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PREFACE

In the past, the natural pearl industry in the Arabian Gulf in general and in Bahrain in particular, was a good example of what we call today "sustainable development".

It is my sincere belief that the industry can be revived only through scientific avenues of which pearl culture is one.

DEDICATION

"To my wife and daughters"

ACKNOWLEDGEMENT

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ABSTRACT

Pinctada radiata spawns in summer/autumn, but minor out of season spawnings may take place. Young oysters mature at 0.6mm - 1.25mm Heel depth (HD)/or 15 - 20mm dorsoventral measurement (DVM) which corresponds to an age of 3.5 - 7 months.

A standard pearl oyster of 50mm hinge length (HL) showed more convincing results of seasonal changes in weight than other standardized shell dimensions. Seasonal changes in weight are clearly related to the spawning cycle and recovery exhibited by the oysters. Most relationships between dimensions and weight studied here showed negative allometry in adults but isometry was more common in young oysters.

The structure of the biofouling community was basically simple in nature and consisted of 9 associated species of which the bryozoa, Bugula sp. and the alga, Hormophysa triquetra were dominant. Bucephalus sp. was found parasitizing and destroying the adult gonad of 4% of the pearl oysters sampled. There was no indication of seasonality in the appearance of the parasite nor any trend in the size of pearl oysters infected. No relationship was found between the occurrence of pearls and the infection with Polydora the tube boring polychaete worm. Natural pearls were very rare (1.93%) in the samples analysed and they were of small (< 2mm) diameters and of no commercial value. Polydora and Cliona the boring sponge were more common on the right than the left valve of the shell of the pearl oyster and they were most frequent in summer. The life history of P. radiata from egg through veligers and spat is described and shows the normal bivalve pattern. Cytochalasin B (CB) was used to induce triploidy in two experiments at two concentrations targeting meiosis I and meiosis II. Diploid eggs, which represent potential triploids were produced in treatments exposed to the CB and a best result (73%) was achieved at 0.5mg CBI⁻¹ targeting meiosis II. In Experiment 1, no significant difference in shell attributes was found between triploids and diploids. However, in Experiment 2, meiosis II culture showed a significant increase in anteroposterior measurement (APM) and dorsoventral measurement (DVM) over both meiosis I and the controls from week 1 to week 4. In week 5, there was no significant difference in the size of larvae among the three cultures. It is proposed that the overall percentage of triploids produced in meiosis II culture accounted for the significant difference in weeks 1 to 4.

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Chapter 1 : General Introduction.

Description of the family Pteriidae

The bivalve family Pteriidae is included in the superfamily Pteriacea and the order Pterioidea. The family consists of three genera, viz. Pinctada, Roding (1798), Pteria Scopoli (1777) and Electroma, Stoliczka (1871). The members of the family are truly marine byssate bivalves, mainly inhabiting the zones between the tropics. They are molluscs producing pearls and having a brilliant mother of pearl coating to their inner shells. Their hinge line is long, on which there are one or two tooth-like thickenings. Umbones are distinct, but only slightly elevated, and situated close to the anterior end of the hinge. Rays and spines radiate from the umbones. Adductor muscle scars and pallial muscle scars, which indicate that they are monomyarian bivalves, are present on the inside of both shells. The valves are unequal, and the left is deeper than the right. Furthermore, the nacreous area of the left valve is larger than that of the right, where it extends ventrally in the left valve. The non-nacreous area is, however, composed of prismatic structured constituents and consists of conchiolin and calcium. In the genus Pinctada, the line of the longest dorsoventral measurement is at right angles to the hinge line, while in Pteria and Electroma it is directed backwards and makes an obtuse angle with the hinge line.

Khamdan (1992) has reviewed the species of pearl oysters of the Arabian Gulf. Eight morphologically distinct species of the genus Pinctada have been found in Bahrain. They are, P.maculata (Gould), P.anomioides (Reeve), P.rutila (Reeve), P.sugillata (Reeve), P.nigra (Gould), P. sp., P.margaritifera and P.radiata. Almost all species of Pinctada other than P.radiata are rare. The genus Pteria is represented only by the species, P.penguin. Recently, the existence of Electroma zebra (Reeve) has been confirmed in Bahraini waters (Khamdan, in prep.).

The most colourful and widely publicised marine activity in the Arabian Gulf is pearl oyster fishing. Since time immemorial the Arabian Gulf has been noted for its pearl beds, and Bahrain Island has always been known for the best natural pearls (Bowen, 1951). The London-Bahrain Archaeological Expedition has recently revealed that shell remains of both P.margaritifera and P.radiata were found throughout the excavated deposit at Saar township of the early settlement period of the early second millennium BC (Glover, pers. comm.)

The Bahrain natural pearl industry produced about three million pounds (sterling) in 1933 and one hundred thousand pounds (sterling) in 1950, (Khamdan, 1988). Bowen (1951) maintains that close to 90% of the Gulf pearls pass through Bahrain while according to Dr Alexander (1947, cited by Bowen), only 35% of the Gulf pearls come from around Bahrain Island, the remainder coming from the Trucial Coast (present day United Arab Emirates) and from the pearl oyster banks near Kuwait.

Pearl fishing was the premier industry of the Arabian Gulf and it was the principal or only source of wealth among the residents of that region (Lorimer, 1915). In addition to the commercial and political aspects, some cultural aspects of Bahrain have been shaped by the pearl industry influencing its folklore through both poems and songs. However, the pearl-fishing industry was eclipsed in 1960 due to the discovery of oil, the introduction of cultured pearls, the effects of World War II, and world-wide economic depression.

A literature search during this study reveals that attempts to revive the Gulf's natural pearl industry (see chapter 3) based on P.radiata is not promising. The results of this study reaffirms that the natural pearl yield, besides being very low, consisted of pearls of < 2mm diameter which were of no commercial value.

Funakoshi (1992) has reviewed the differences between natural pearls (but see chapter 3) and cultured pearls.

1- Natural pearl

The mantle lobes, which cover the visceral mass, spread along the inner side of the shell. The outer epithelial cells of the mantle lobes produce the shell. If for some reason, a piece of outer epithelium drops into the soft tissue of a pearl oyster, it is changed into a bag-like structure separated from the mantle membrane. Shell nacre is produced within this bag-like structure known as the pearl sac. This globe-shaped nacre is a natural pearl. Apparently, then natural pearl formation is chance dependent.

2- Cultured pearl

Cultured pearls are classified as semispherical or spherical. The methods for producing these pearls differ.

2a- Semispherical pearl

Semispherical nuclei are placed between the mantle and shell valve. The outer epithelial cells of the mantle secrete shell nacre onto the surface of the nucleus to form the semispherical pearl. Semispherical pearls formed on the inner surface of valve are cut off. This method for producing semispherical pearls, which is based on the method for producing the Buddha pearl with Cristaria plicata (fresh water bivalve; see Cahn, 1949, for its world-wide distribution) during the 11th-13th century in China, was devised by Kokichi Mikimoto in 1893.

2b- Spherical pearl

The pearl sac originating from the outer epithelial cells of the mantle is necessary for spherical pearl formation. The pearl sac of a natural pearl is formed by chance, while that of cultured pearls is induced by a surgical procedure. The method for producing spherical pearls was devised by Tokichi Nishikawa and Tatsuhei Mise in 1907 (for debate concerning who was actually first to invent the technique, see Cahn, 1949). Presently, a procedure based on Nishikawa's method is used. The pearl sac is formed by grafting a small piece of mantle, with or without a nucleus, into the soft tissue of another oyster.

Although pearl production (see table 1-1) has expanded in many countries, eg China, French Polynesia, Myanmar, India, Colombia, Canada, the USA, Australia, the Solomon Islands, Mexico, the Cook Islands, Vietnam, New Caledonia, Bangladesh, the Philippines, France and Taiwan, another 20 nations are in the process of becoming pearl producers (Fassler, 1994), including Malaysia and Indonesia (Gervis & Sims, 1992). However, Japan remains the monopolizing force in the industry (Gervis & Sims, 1992). According to George (1994a) this monopoly is supported by the Japanese Government through "the three principles policy" of 1952 which states 1) pearl cultivating techniques should remain secret; 2) all pearls produced to be exported to Japan (but see table 1-1); 3) production amount to be regulated by Japan.

The "diamond" policy was successfully maintained by Japan for many years until Australia decided to establish a pearl industry in 1956 (George 1994b) followed by the French Polynesians in early 1980 (Intès, 1994). However, the technicians who implant bead nuclei are still Japanese (Gervis & Sims, 1992). The monopoly enjoyed by the Japanese is no longer controlled and this change has come about because of several significant factors, including, environmental degradation caused by pollution, the success of the Chinese in mass-production of both fresh water and marine pearls with a corresponding decline in the Japanese production (Fassler, 1994).

Pearl production offers a variety of economic scales and approaches, ranging from commercial companies to co-operatives, families or individuals.

Table 1.1: Japanese pearl imports from the major marine pearl producing countries for 1988 and 1989 (adapted after Gervis & Sims, 1992).

Country	Year	Nonprocessed		Stringed		Total	
		kg	US\$ (x 1,000)	kg	US\$ (x 1,000)	kg	US\$ (x 1,000)
Australia	88	1,068.45	45,209	0.99	163	1,069.44	45,372
	89	1,217.34	70,438	9.97	913	1,227.44	71,351
Fr. Polynesia	88	312.89	24,912		54	312.89	24,965
	89	496.63	40,759	4.99	692	503.62	41,451
Indonesia	88	339.96	8,454	0.00	0	339.98	8,454
	89	516.00	11,557	0.00	0	516.00	11,557
Philippines	88	81.40	5,096	-	6	81.40	5,075
	89	70.42	3,790	-	0	70.42	3,790
India	88	26.60	132	46.34	64	272.94	196
	89	31.52	604	307.18	2,085	338.70	2,689
Taiwan	88	55.47	55	207.28	2,041	325.75	2,096
	89	331.23	159	768.94	114	1,100.17	273
Thailand	88	36.80	589	4.99	786	41.79	1,375
	89	15.96	682	-	103	15.98	785
Malaysia	88	299.02	1,369	-	52	299.02	1,421
	89	25.83	701	0.00	0	25.83	701
Myanmar	88	0.65	75	0.00	0	0.65	75
	89	27.15	629	0.00	0	27.15	629
Cook Islands	88	0.00	0	0.00	0	0.00	0
	89	11.40	444	0.00	0	11.40	444

* Japan alone produces 70t of marine pearls worth (US\$ 476 million), Gervis & Sims (1992)

Many facets of production do not require a high capital input and are suitable for low technology village production (Gervis & Sims, 1992).

Pearls are the ideal export commodity, they are non-perishable, shipping costs are negligible and lucrative markets are already established (Gervis & Sims, 1992; for details; see abstract papers presented at Pearls '94; J. Shellfish Res., 13 (1): 325-354; 1994). Therefore, the development of pearl oyster culture offers great opportunities to developing as well as developed, countries. Many small island developing states which were until recently foreign-aid dependent are now pearl producers (see table 1-1).

The future of the pearl oyster culture as judged from the history of many nations (eg Bahrain and Kuwait) is promising but of course is best approached from a thorough scientific basis.

Some of these scientific approaches are the **aims of this study** and are listed below;

- 1- The reproductive biology of pearl oysters generally (Gervis & Sims, 1992) and P. radiata of the Arabian Gulf are poorly understood considering the importance of both pearls and shell fisheries (Chapter 2).
- 2- Characterisation of the structure of the biofouling community, disease (parasites) and infection with Polydora and Cliona, (Chapter 3).
- 3- The incidence of natural pearls, maximum pearl yield (Chapter 3).
- 4- The spawning, fertilization, larval rearing and genetic manipulation of P.radiata (Chapter 4).

The importance of the aims of this study stems from the fact that reliance on wild stocks of pearl oysters is bioeconomically risky for the pearl culture industry which is based on multi-million dollar investment (for example in Australia, Rose et al. 1990). In addition, operating costs associated with the collection and transportation of wild oysters for pearl production are likely to continue to increase above the current value as in Australia (from US\$10 to US\$ 13) for an individual P.maxima (Rose et al. 1990). Furthermore, ripe oysters are not good candidates for pearl culture since they either reject the beads inserted or do not usually survive the operation. Because of this, the Japanese pearl culturists have either to inhibit or accelerate gonad maturation (by low or high temperature) of the host oysters (Mizumoto, 1976). Therefore, a full understanding of the reproductive biology of the species of interest is of fundamental importance. More recent developments in aquaculture allow this procedure of inhibiting or accelerating gonad maturation to be overcome by producing sterile and/or abnormal triploid oysters which may be good alternatives for future pearl culture (Wada et al. 1989).

Chapter 2: Reproductive biology.

2-1 Introduction

There have been several studies conducted on the reproductive biology of different species of the genus Pinctada; P. radiata (Herdman & Hornell, 1906; Malpas, 1933; Tranter, 1959), P. margaritifera (Nicholls, 1931; Galtsoff, 1933; Tranter, 1958d), P. albina (Tranter, 1958a, b, c), P. maxima (Rose et al. 1990), P. martensii (Ojima & Maeki, 1955). The biology of pearl oysters is poorly understood, considering the importance of both natural and cultured pearl, and shell fisheries (Gervis & Sims, 1992).

No published work is available on the subject for the Arabian Gulf population. This chapter details some aspects of the reproductive biology of the Al-Jadoum, Bahrain, population of pearl oysters studied over a 2 year period. The results presented were determined from monthly histological sectioned gonads, and smears. The data were further supported by observations made on monthly batches of young oysters settled on the shells of adults.

Seasonal variation in weight of a "standard" bivalve (dimensions adjusted to a single size) has been found to be correlated with the period of spawning in a number of species for example; Tellina tenuis (Ansell & Trevallion, 1967); Donax vittatus (Ansell,

1972); Abra alba (Ansell, 1974a); Chlamys septemradiata (Ansell, 1974b); Nucula sulcata (Ansell 1974c); Pecten maximus (Comely, 1974); Aulacomya ater (Griffiths & King, 1979); Mercenaria mercenaria (Ansell & Lander, 1967, cited by Ansell, 1974c; Hibbert, 1977) Mytilus edulis (Dare & Edwards, 1975; Newell & Bayne, 1980; Bayne & Worrall, 1980; Kautsky, 1982a, b; Rodhouse et al. 1984a; b; MacDonald & Thompson; 1985a, b; NOAA, 1993). Seasonal variations in glycogen, lipid cholesterol and protein in P. fucata (= P. radiata) correspond to the reproductive cycle (Desai et al. 1979).

Apparently there are no publications which deal with seasonal variations in weight of a standard pearl oyster. This study details seasonal variations of weight or its derivatives (wet and dry weights) of muscle and remaining tissues demonstrated by a standard oyster.

There are very little published data on the allometric relationships of growth in the genus Pinctada (Galtsoff, 1931, 1933; Alagarwami & Chellam, 1977) and these are confined to small-sized samples taken only at one time of the year. This study details seasonal changes in relative growth patterns exhibited by young and adult oysters.

2-2 Materials and Methods

Samples of the pearl oyster Pinctada radiata, were collected regularly on monthly visits between January 1992 and December 1993, from a single shallow (5m depth)

population called Al-Jadoum, situated to the North of Bahrain, Long: 050 30 00 E; Lat: 26 20 00. Sample size was (on each sampling period) about 100 oysters (except September 1992 N=53). Oysters were collected by SCUBA diving and brought to the Laboratory of the Environmental Protection Committee.

2-2.1 Definition of terms (after Tranter, 1958c)

" Hermaphrodite: Containing both male and female reproductive organs within one individual.

Dioecious: Containing the male reproductive organ in one individual and the female in the other.

Bisexual: Containing both male and female gametes within the gonads at particular time.

Unisexual: Containing within the gonads at a particular time germ cells of one sex only.

Protandric: When gonads mature at first as males.

Protogynic: When gonads mature at first as females.

The last two terms also applied when;

1- Sex change from male to female, an individual undergoing such an event is said to be protandric.

2- An individual changing from female to male is said to be protogynic."

In Tranter's (1958a) " Hermaphrodite " refers to species and " Bisexual " refers to

individuals.

2-2.2 Macroscopic examination of the gonads

For each specimen, information on gonad development, sex, colour of gonad and biometrical attributes (see below) was recorded. Gonad development was estimated by taking a small sample of tissue from an area next to the urogenital pore using a clean glass pipette and smearing the sample on a slide. Smears were examined under a compound Zeiss microscope at X400 and/or X1600. Four stages were recognised; stage 0; designates indeterminate or resorbed, stage 1; developing, stage 2; ripe and stage 3; spawning, and a gonad index based on the overall mean of these numbers was calculated for each month.

2-2.3 Histological examination of the gonads

After being weighed the first fifty animals from the monthly sample were fixed in Bouin's fluid (75ml of Picric acid, saturated in filtered seawater; 25ml formalin:- 40 HCHO; 5ml glacial acetic acid) at room temperature for 24 hours prior to dehydration through a series of alcohols:- 30%, 50%, and 70%, allowing 2 hours between each change. Samples were then preserved in 70% alcohol for 1-2 weeks awaiting further treatment.

A piece of gonadal tissue, approximately 5mm X 5mm, from the gut loop, was

excised from each fixed animal with a clean sharp scalpel. This tissue was preserved in Methyl benzoate at room temperature over-night. It was then suspended in a small tube closed at the bottom by 2mm mesh and immersed in 1:1 of toluene-wax v/v in a 200ml glass beaker at 60 °C in an oven for 2 hours. The tube containing the tissue was given two changes of molten wax using a 200ml glass beaker held in the oven at 60 °C, for two hours at each transfer. The tissue was then embedded in fresh wax and allowed to cool.

Sections were cut at 7 μ m on a conventional Leitz rotary microtome fitted with a disposable blade. Ribbons of wax containing gonadal tissue were spread on a microscope slide and a few drops of glycerin-albumen solution (15 drops of concentrated stock in 100ml distilled water) were added to ease the spreading and to glue the tissue onto the slide. Each slide accommodated a double track ribbon of 10 - 20 sections. Two slides were made from each wax block, labelled with a diamond knife and placed on a hot plate at 35 °C for 6 - 8 hours, and then left standing at room temperature over-night.

For dewaxing, staining and counter-staining, slides were held in a slide holder immersed in a 250ml container of histo-clear solvent for 4 minutes; transferred into a series of alcohols; 100%, 70% and 50% for 2 minutes between each transfer. They were then placed in distilled water for 1/2 to 1 minute, followed by immersion in a concentrated solution of lithium carbonate for 1 minute, to

remove picric acid, and stained with Ehrlich's haematoxylin for 15 minutes. The slides were blued in tap water for 30 seconds, counter-stained with aqueous eosin for 30 seconds, and again placed under tap water for 30 seconds. The slides were then transferred immediately into a series of alcohols (90%, 100% and 100%), and finally, histo-clear, allowing 2 minutes at each change.

The slides were mounted with a few drops of DPX mountant and covered immediately but gently with a rectangular 64 X 22 mm (thickness = 1) cover-slip. The slides were placed on a hot plate at 35 °C for 8 hours and then left at room temperature over-night.

Rose et al. (1990) developed a simple staging scheme for Pinctada maxima based on several schemes first proposed by Tranter (1958 a,b,c,d; 1959) for several species within the genus Pinctada. Following initial observations Rose et al.'s (1990) scheme was found to be the most reliable staging procedure for P.radiata and it has been adopted without modification in this study. The scheme is as follows.

" Stage 0: Indeterminate or inactive

No evidence of gonadal development, except empty, collapsed follicles and connective tissue containing different types of granulocytes and phagocytes.

Stage 1: Early gametogenesis

MALE

Follicles initially small and lined with stem cells and spermatogonia. As spermatogenesis proceeds, primary and secondary spermatocytes rapidly proliferate, filling up the follicular lumen.

FEMALE

Follicles initially small, poorly formed and empty, with walls lined with stem cells and developing oocytes. Oogonia and early (or primary) oocytes each with a large nucleus, and often adhering to the follicular wall in clusters. As oogenesis proceeds, oogonia and young oocytes proliferate along the inside walls with a few larger oocytes beginning to elongate.

Stage 2: Actively developing to near-ripe gametogenesis

Follicles begin to enlarge with spermatogonia and spermatocytes proliferating along the periphery of the lumen and with spermatids and some spermatozoa filling the centre. Near-ripe follicles have enlarged greatly with developing sperm appearing. Except for isolated pockets of spermatocytes and spermatids, the follicular lumen is packed with spermatozoa.

Oocytes connected to the follicular wall and expand into the lumen, with a few free oocytes appearing in the centre. Near-ripe follicles are densely packed mainly with large elongated oocytes; these are still connected to the follicular wall by a long narrow stem.

Stage 3: Spawning-ripe

Follicles distended, confluent and almost entirely filled with spermatozoa. Spermatocytes and spermatid are restricted to lining the follicular walls which have become increasingly thinner with maturity.

Confluent follicles packed almost entirely with free, polygonal-shaped oocytes displaying both a nucleolus and nucleus.

Stage 4: Partially spawned to spent

Gonad containing follicles with partially empty lumen. Those which are still full have a gap between the follicular wall and the mass of spermatozoa remaining in the lumen. Partially spawned follicles contain phagocytes amongst the spermatozoa. Spent follicles are empty except for small packets of residual sperm and phagocytes inhabiting the lumen. Redevelopment can be seen along the walls of some follicles.

Follicles are empty, with small amounts of resorptive material occurring in the space between free oocytes which have become rounded or pear-shaped. Follicles which are almost completely spent have extensive redevelopment occurring along the inside follicular wall, with large amounts of resorptive material surrounding free oocytes undergoing cytolysis. Spent follicles are almost entirely empty with no sign of gametogenesis except for isolated regressing oocytes surrounded by resorptive tissue, phagocytes and interstitial connective tissue."

Sections were examined and photographed on a Zeiss photomicroscope II. A gonad index was produced based on the percentage frequency of each stage for each month.

2-2.4 Size at first maturity

Young oysters found settled on the shells of adults were also inspected macroscopically for gonadal development on a monthly basis. Biometrical data were also recorded for these young oysters. The pooled percentage frequencies of stage 0 (indeterminate) based on the observations made between July 1992 and November 1993, were plotted against the Heel Depth (HD) and the Dorsoventral Measurement (DVM) classes (see Fig 2.1). The position of an abrupt decline in the percentage frequency of stage 0 with size can be interpreted as the size (Age) at first maturity (Tranter 1958a,d;1959).

2-2.5 Sex ratio and sex change

The percentage frequencies of indeterminate, male and female adult oysters during the breeding periods (May - November; 1992 and 1993) were calculated and plotted against the heel depth (HD) to investigate any pattern of sex change with size (Tranter, 1959). Furthermore, the chi-square non-parametric test was employed on selected months data to examine whether sex ratios deviated from 1:1.

Figure 2-1: The right valve of P.radiata showing shell measurements, structures and characters referred to in the text.

Legend

DVM, dorsoventral measurement

APM, anteroposterior measurement

HL, hinge length

HD, heel depth

N, nacre

BN, byssal notch

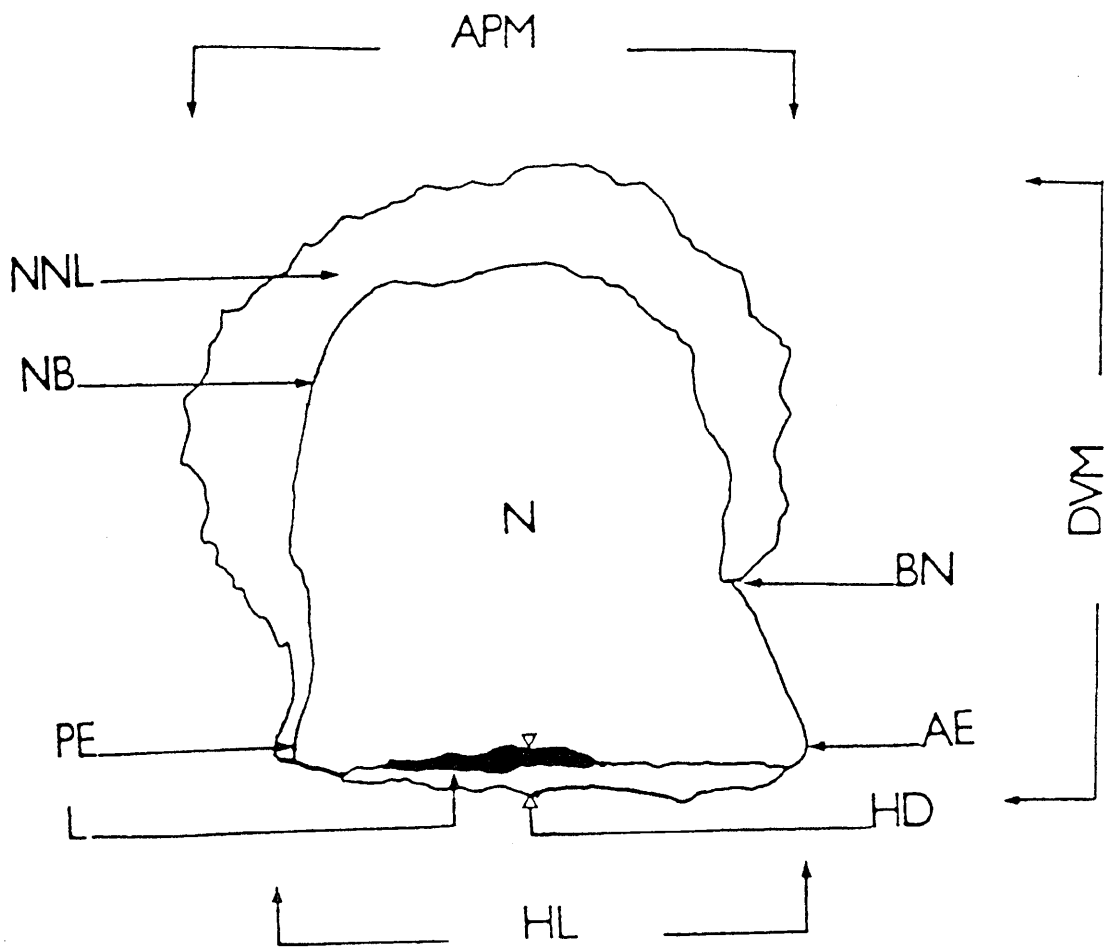
NB, distal border of nacre

NNL, non-nacreous layer

AE, anterior ear

PE, posterior ear

L, ligament



2-2.6 Biometry, Morphological attributes and terminology

Hynd (1955) , Tranter (1958a) and Khamdan (1988) clarified the expressions that may be used to describe the shell dimensions of the pearl oysters of the genus Pinctada and these are given below and illustrated in Figure 2.1.

Dorsoventral measurement (DVM)

This is the longest axis in the dorsoventral direction, and is equivalent to the term height used to describe this dimension in many other bivalves.

Anteroposterior measurement (APM)

This is the greatest horizontal distance between the anterior and posterior margins of the shell taken parallel to the hinge line. This is equivalent to the term length used for other bivalves.

Hinge length (HL)

This is the greatest distance between the anterior and posterior ears (auricles).

Heel depth (HD)

This is the thickness of the hinge line at the centre. It is the most reliable and indispensable aid in determining the age of the species of Pinctada (Tranter, 1958 a,d;1959). The most frequently used variable in growth studies of Pinctada is DVM (Gervis & Sims, 1992).

In this study, for each oyster, records were made for DVM, APM, HL and HD to the nearest 0.05mm using vernier callipers. Individuals smaller than or equal to 35mm DVM were classed as young oysters (= spat ;Alagarswami & Chellam , 1977) and those larger than 35mm as adults .

2-2.7 Weight

For each oyster, the total body wet weight was recorded to the nearest 0.1g on a self taring balance.

From the period October 1992 to December 1993, 20 from each monthly sample of 100 oysters were further analysed for wet and dry weights of adductor muscle separate from the remaining tissue.

The dry weights of the adductor muscle and remaining tissue were recorded by placing the respective tissues in labelled pre-weighed containers. Firstly the tissues were wet weighed, then the container was placed in a drying oven for 24 hours at 100°C followed by 5 minutes in a desiccator before dry weighing. Subtracting the container weight gives the net dry weight. From the 20 oysters, there are six sets of data;

- | | |
|-------------------------------|--------------------------------|
| 1. Total body wet weight (WT) | 2. Total body dry weight (WTD) |
| 3. Muscle wet weight (MWWT) | 4. Muscle dry weight (MDWT) |
| 5. Tissue wet weight (TWWT) | 6. Tissue dry weight (TDWT) |

The total wet weight data of 20 oysters were included with the other 80 oysters to give the total wet weight for 100 oysters each month.

2-2.8 The length/weight relationship

The simple exponential (= power function) non-linear regression model $y = a x^b$ was used to describe the relationship between the dependent variable (y) which is normally weight and its derivatives, and the independent variable (x), the shell dimension(s). This regression model becomes linear when the attributes in the equation are log-transformed and can be re-expressed as;

$$\text{Log } y = \text{Log } a + b \text{ Log } x;$$

where log is the logarithm to the base 10; a is the intercept and b is the slope. Raw data are given in appendix 2.3.

The most commonly used statistical techniques for describing the regression of the model above mentioned are; firstly; ANCOVA (Analysis of Covariance) and secondly, Least-squares regression of ANOVA (Analysis of variance).

The ANCOVA was employed, firstly, to test the homogeneity of the slopes of the monthly samples (=populations), and secondly, to determine the adjusted means (Ys; weights) at the grand mean of the covariate x (= grand population mean). It should be noted that one of the major pre-requisites for employing ANCOVA regression is equality of the slopes. Few of the ANCOVA regressions agreed with

the assumption of equality of slopes, suggesting that the populations under comparison exhibited real differences in their weight on length relationships. Therefore, ANCOVA was discontinued. For comparing size parameters (e.g. weight) using the weight on length relationship, however, the ANOVA regression is an appropriate statistic (NOAA, 1993). Instead of using the grand mean length of the populations determined by ANCOVA, the ANOVA regression gives the regression equation for each population. The adjusted weights (not the adjusted means of ANCOVA) were determined by substituting the length with a chosen standard oyster length (should be within the range of the monthly values of x).

In this study, the chosen standard oyster is given as 60mm DVM, X 60mm APM, X 50mm HL, X 4mm HD.

Monthly adjusted weight variations in respect to the shell dimension(s) were plotted and are illustrated graphically.

In all regression analyses, the departure from isometry (Seed & Richardson, 1990) was t-tested according to the formula

$$t_{\text{(observed)}} = (\text{slope} - \text{theoretical slope})/\text{s.e of the slope}$$

The theoretical slope for volumic or weight attributes is 3 and for the linear attribute is 1 (unity).

The t-observed was then compared with the t-tabulated at ($\alpha = 0.05$; two tails) with appropriate degrees of freedom ($n-1$).

If t-observed is less than the t-tabulated, this shows isometry (= not significant).

If t-observed is greater than the t-tabulated, this indicates significant departure from isometry.

Then if the estimated slope is greater than the theoretical slope showing that the increase in the dependent (y) variable is relatively greater than the increment in the independent (x) variable, this is termed positive (+ve) allometry. Where the opposite is encountered, the term negative (-ve) allometry is applicable.

2-3 Results

2-3.1 Morphology of the gonad

The gonad in P.radiata is not a discrete organ. The gonad is paired but assymetrical. The pair covers the stomach, liver and parts of the intestine. The byssal gland divides the gonad into a dorsal, larger part, and a ventral, smaller part. The gonad is bordered by the foot anteriorly and by the pericardium posteriorly both of which are free from gonad. No portion of the gonad extends into the foot or into the mantle, as is the case in Mytilus edulis (Seed & Suchanek, 1992).

Table 2.1 shows the relationship between the colour of gonads in adult oysters and their gender. The X^2 test confirms that the ratios of m:f:x are significantly different between colours and demonstrates that the gonad colour is an unreliable indicator of sex.

The reproductive follicles originate near the urogenital papilla proximal to the retractor muscle and proliferate within the connective tissue between the epithelium and visera. The morphology of the gonad in P.radiata resembles that reported for P.vulgaris (= P.radiata) from Sri Lanka [Ceylon], (Herdman, 1903), the Australian Pearl oysters; P.albina (Tranter, 1958 a,b,c), P.margaritifera (Tranter, 1958d), P.fucata (= P.radiata), (Tranter, 1959), and P.maxima (Rose et al. 1990).

2-3.2 Spermatogenesis

Plate I shows the sequence of events of spermatogenesis based on histological preparations of gonads of 50 oysters per month. Plate I (B & C) illustrate the early spermatogenesis of stage 1 where stem cells and spermatogonia (Sg) reside in the follicle wall. The volume of the follicle is gradually filled up and spermatogonia develop to spermatocytes (Sc). Plate I (C) shows that the spermatocytes are accommodated in a condensed dark band beneath the layer of spermatogonia which is attached to the follicle wall. There is a difference in the

Table 2.1: P. radiata: χ^2 contingency table, DF (3-1) x (3-1) on colour of gonad in relation to gender in adults.

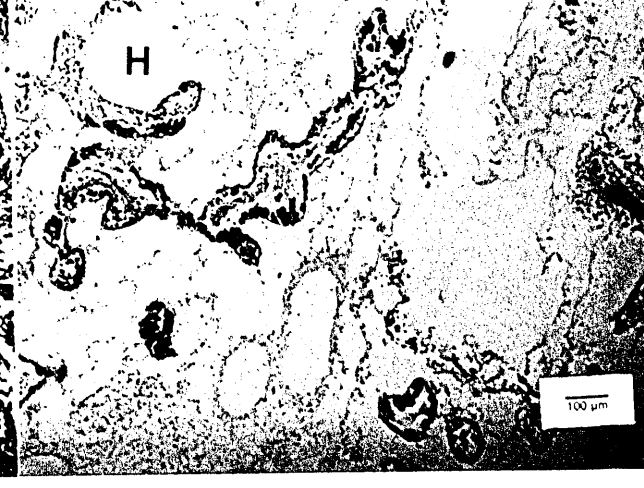
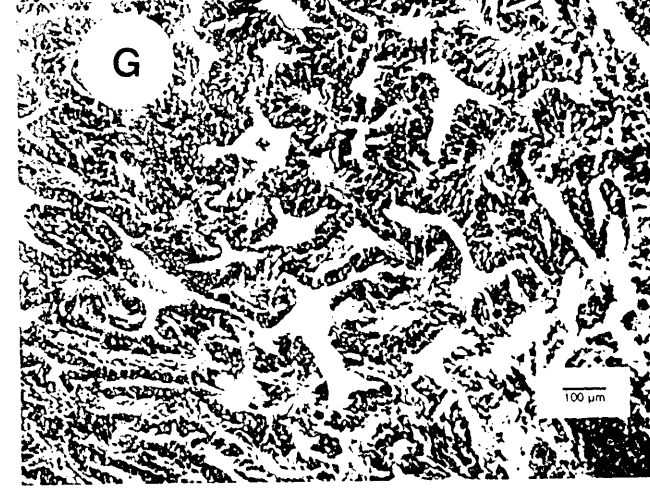
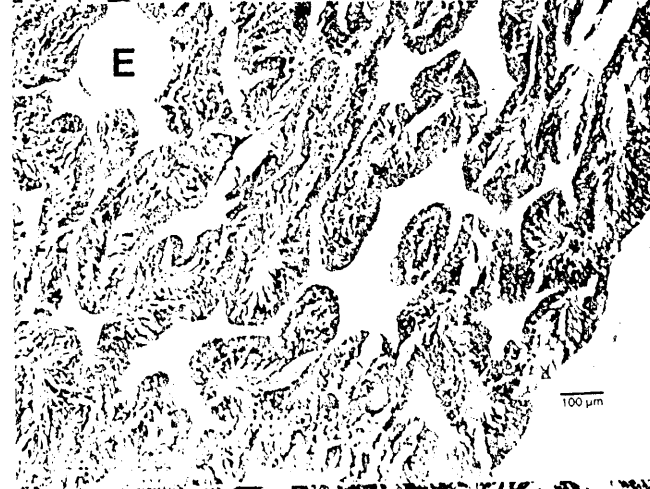
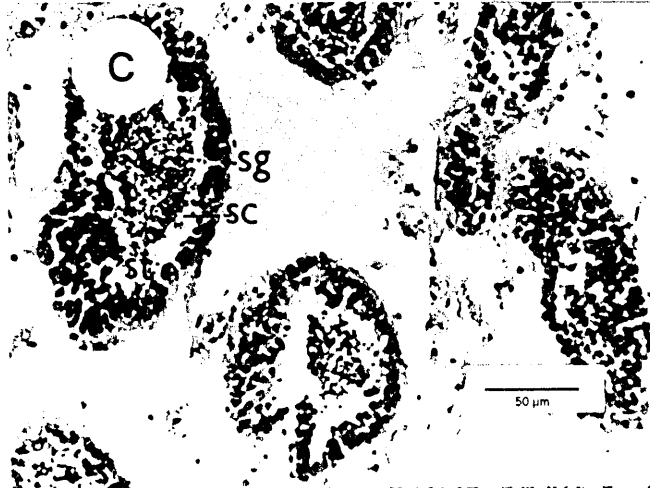
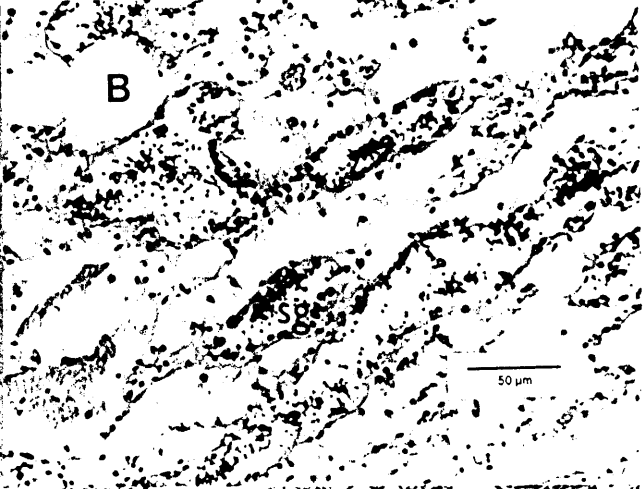
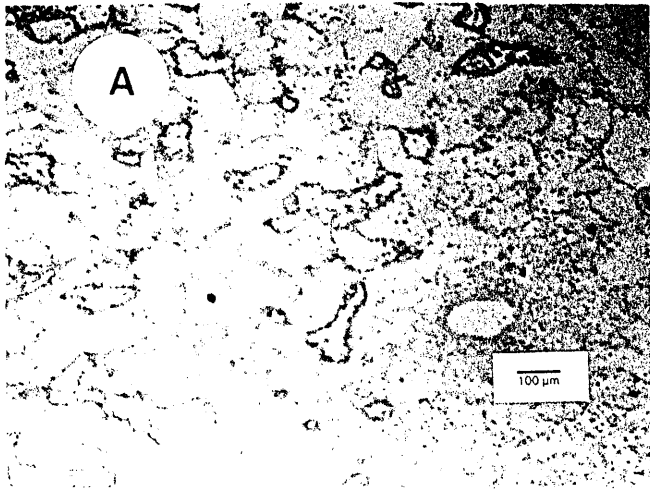
Abbreviations: c = cream; w = white; y = yellow;
 F = females; M = males; X = indeterminates.
 Figures in brackets are percentages (Pct).

	Colour			
Count Row Pct				
Sex	c	w	y	Row Total
F	464 (55.1)	109 (12.9)	269 (31.9)	842 (35.7)
M	491 (70.6)	81 (11.7)	123 (17.7)	695 (29.5)
X	455 (55.6)	317 (38.7)	47 (5.7)	819 (34.8)
Column Total	1410 (59.8)	507 (21.5)	439 (18.6)	2356 (100.0)

<u>Chi-Square</u>	<u>Value</u>	<u>DF</u>	<u>Significance</u>
Pearson	345.34874	4	<0.00001

PLATE I : Various stages of spermatogenesis in P. radiata.

(A) Indeterminate stage (0). (B) beginning of spermatogenesis (stage 1); (Sg), Spermatogonia. (C) advanced stage of early spermatogenesis, (SC) spermatocytes, (St) spermatids. (D) actively developing testis (stage 2), (Sz) Spermatozoa. (E) near ripe testis (stage 2). (F) spawning-ripe testis (stage 3). (G) partially spawned testis (stage 4). (H) spent testis (stage 4).



staining intensity (basophilic) within the band and this indicates that spermatocytes are undergoing maturation to give rise to secondary spermatocytes. Two major differences between the stem cells and spermatogonia are recognised. First, the nuclear chromatin staining is darker in spermatogonia, and second the spermatogonia are usually free from the follicle wall. The difference between the primary and secondary spermatocytes is distinguishable because the primary spermatocytes undergo cytoplasmic and nuclear growth producing a darker nucleus a suggestion of meiosis I prophase in the secondary spermatocytes. Plate I (D) shows actively developing testis of stage 2 where spermatids, which are in the centre with developed cytoplasm, are transformed to spermatozoa giving a characteristic river or stream-like pattern filling the core of the follicle. This event is followed by a corresponding decrease in the number of spermatogonia at the earlier stages and around the periphery of the follicle wall. Plate I (E) shows a near-ripe phase of stage 2 where the spermatozoa are condensed to a dark band in the lumen of the follicle. Plate I (F) illustrates that the follicles are enlarged as the spermatozoa continuously increase in number and this gives rise to stage 3 of spawning-ripe event. Plate I (G & H) show the partially spawned to spent phase of stage 4 where a gap between the follicular wall and the mass of spermatozoa has occurred indicating a spawning event. Thereafter, the follicle has shrunk with isolated pockets of spermatids. Plate I (A) illustrates stage 0 of indeterminate or inactive gonad where the follicles are collapsed and noticeably reduced in volume.

2-3.3 Oogenesis

Plate II illustrates the events of oogenesis. Plate II (A) shows early oogenesis of stage 1 where stem cells (St C) undergo mitotic division and give rise to oogonia (og) both of which reside on the wall of the follicle. Plate II (B) illustrates an advanced stage 1 where oogonia growing along the wall of the follicle are entering meiosis I "prophase" and it appears that some oogonia are at various stages of maturity while some are in the resting stage. Plate II (C) is an actively developing ovary of stage 2 where the connected oocytes, those which are still joined to the follicle wall, are undergoing cytoplasmic maturation. Mature free oocytes then accumulate in the lumen of the follicle. Plate II (D) is a near-ripe ovary of stage 2 where free oocytes are gradually filling the lumen of the follicle with a corresponding reduction in the numbers of earlier stage oogonia. Plate II (E) is a spawning-ripe ovary of stage 3 where free oocytes are increasing in their number and the follicle volume has expanded. A characteristic feature of the ova at this stage is their polygonal shape which results from the pressure exerted by the follicle wall.

Plate II (F) is a spawning-ripe ovary of stage 4 in a transitional phase where the shape of the ova has changed to pear-shape, an indication of relief from the pressure and commencement of partial spawning.

PLATE II : Various stages of oogenesis in P. radiata.

- (A) Beginning of oogenesis with stem cells (St C) and oogonia (Og).
- (B) Advanced stage of early oogenesis (stage 1) with larger oocytes (OC).
- (C) Actively developing ovary (stage 2) with connected oocytes (C OC) and free oocytes (F OC).
- (D) Near-ripe ovary (stage 3).
- (E) Spawning-ripe ovary (stage 3).
- (F) Partially spawned ovary (stage 4).
- (G) Advanced stage of partially spawned ovary.
- (H) Spent ovary (stage 4) with residual oocytes (r oc).



Plate II (G & H) are advanced stages of spawning stage 4 where the volume of the follicle has reduced and regressed or collapsed. It should be noted here that there are no yolk nuclei visible in the cytoplasm of the oocytes or ova as in P.margaritifera (Tranter, 1958d), P.maxima (Rose et al. 1990). The functions of these organelles which appear as circles, crescents or saucers are not yet understood (Rose et al. 1990).

To summarize, the timing of events of gametogenesis is similar in both sexes, i.e. commences in winter and continues through spring. However, as will be discussed in a later section on the breeding season, spawning of adult P.radiata is continuous in the sense that there are always at least a few spawning individuals present throughout the year. It follows that redevelopment, too, is continuous.

2-3.4 Breeding seasons

Figure 2.2 summarizes the histological data from monthly gonad preparations for the two years monitoring period. It can be seen in the figure that early gametogenesis, as judged by the development stage 1, took place during January, February, March, April and December 1992, and January through March and December 1993. During May and June 1992 the gonad underwent a rapid transition to ripe and spawning stages and then maintained a nearly 100% spawning stage through July 1992 followed by an abrupt decline in August of the

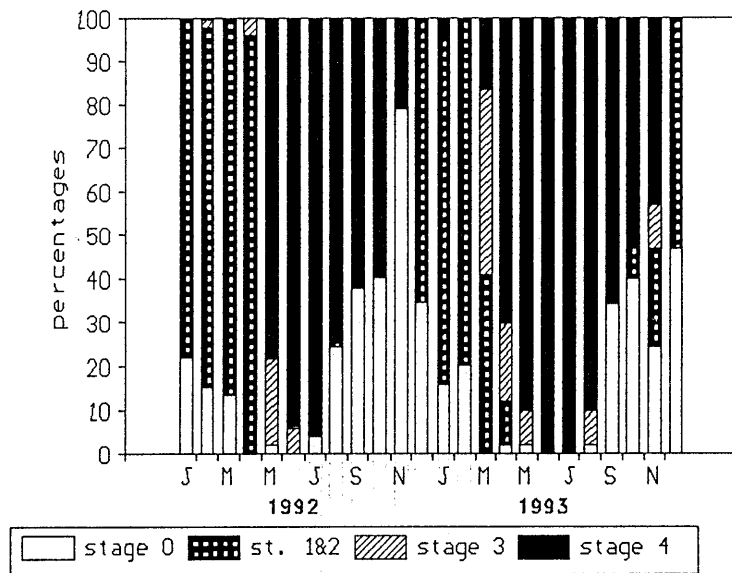


Figure 2-2: Annual reproductive cycle of *P. radiata* studied between January 1992 and December 1993. Abbreviations, Stage 0 = (spent); st.1&2 = Stages 1&2 (developing); Stage 3 = (ripe); Stage 4 = (spawning).

same year with a corresponding increase in the spent stage up to 25% suggesting the commencement of the summer peak spawning. In September 1993, there was a noticeable decline in the percentage of oysters in the spawning stage followed by a corresponding increase in the proportion of the spent individuals up to about 35%. The oysters continued to spawn intermittently but with redeveloping follicles and gametes. In November 1992, a noticeable increase to 80% in the percentage of spent individuals is evident while a similar pattern (50%) was found in December 1993 and this event signalled an autumn peak spawning. The majority of gonads after this second spawning were collapsed (Plate IA), and they entered a resting phase for 3 to 4 months during winter when early gametogenesis took place. However, insignificant partial spawning is noticeable in Figure 2.2 which had taken place in January and March 1993.

Figure 2.3 is an abstracted segment from the data in Figure 2.2 which shows the change with time in the percentage frequency of the combined stages of Ripe/Spawning. Two points should be noted in this figure. First, there are two major spawning peaks, one in summer and the other in autumn. Second, the area under the curve for the year 1993 is greater than for 1992 suggesting that the oysters had a rather more prolonged period of spawning in 1993, and the decline in September of that year was indeed very rapid. Furthermore, after the September 1993 major spawning, the gonad underwent a rapid re-development in October and November allowing an autumn spawning which took place in

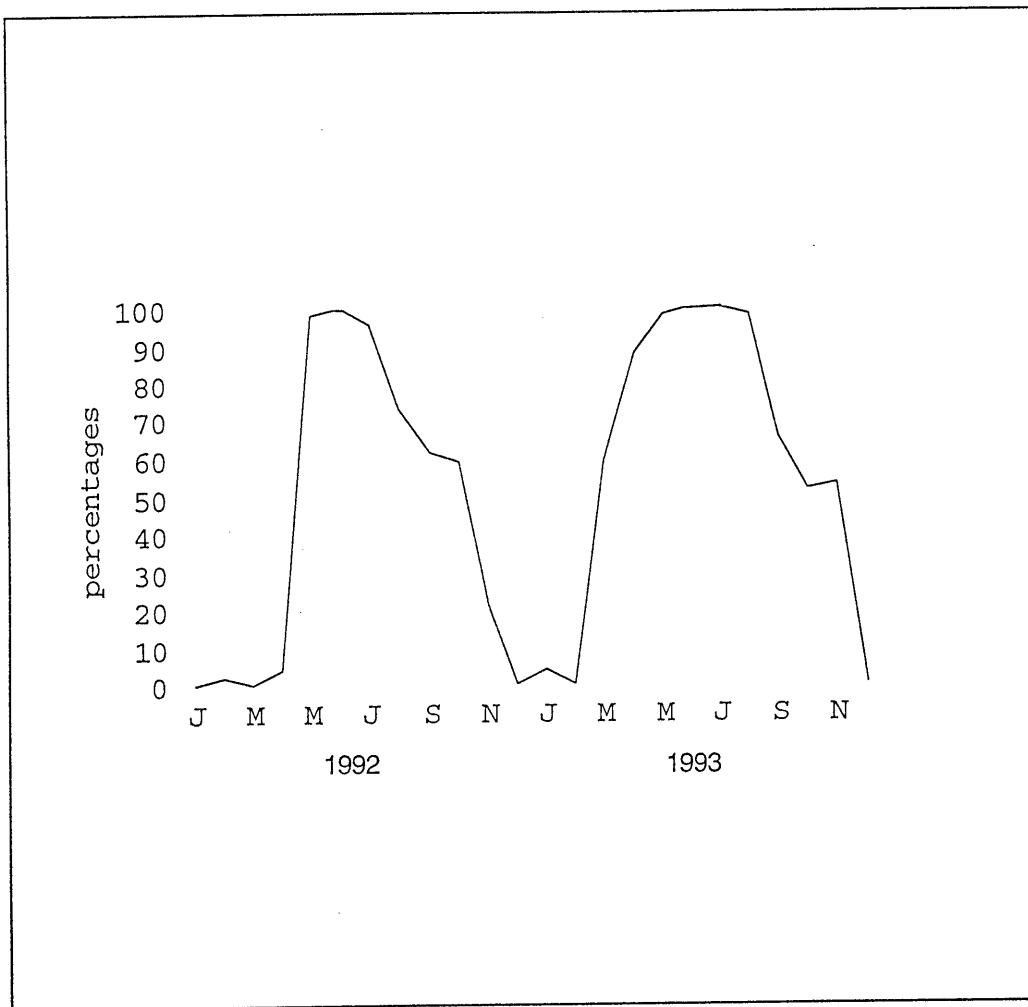


Figure 2-3: P.radiata: Mean monthly total percentages of individuals at stages 3 & 4 (ripe & spawning) over a 24 month period.

December.

Figure 2.4 shows the macroscopic gonadal maturation indices (GDX1 and GDX2) based on live smears. GDX1 is based on 4 values given to 4 stages as follows, spent = 0, developing = 1, ripe = 2, and spawned = 3. GDX2 is simpler than GDX1 where both spent and developing are given a combined value = 1, and the ripe and spawned stages are given a pooled value = 2.

Both indices give similar patterns to that obtained from histological preparations and are clear enough to suggest that there exist two spawning events, firstly, in summer; August/September and secondly, in autumn; November for 1992 and December 1993.

Figure 2.5 shows the mean monthly seawater temperature around the Island State of Bahrain for the years 1989, 1992 and 1993 (kindly supplied by the Meteorology office, Civil Aviation Directorate). Data for the years 1990 and 1991 had a substantial number of missing values and were excluded. This is because of difficulties in obtaining systematic data during the Gulf Crisis. The coldest months are December, January, February and March where the temperature is within the range 15 - 20°C. In April the temperature starts to rise to 25°C to reach its maximum above 30°C in August and then drops in September.

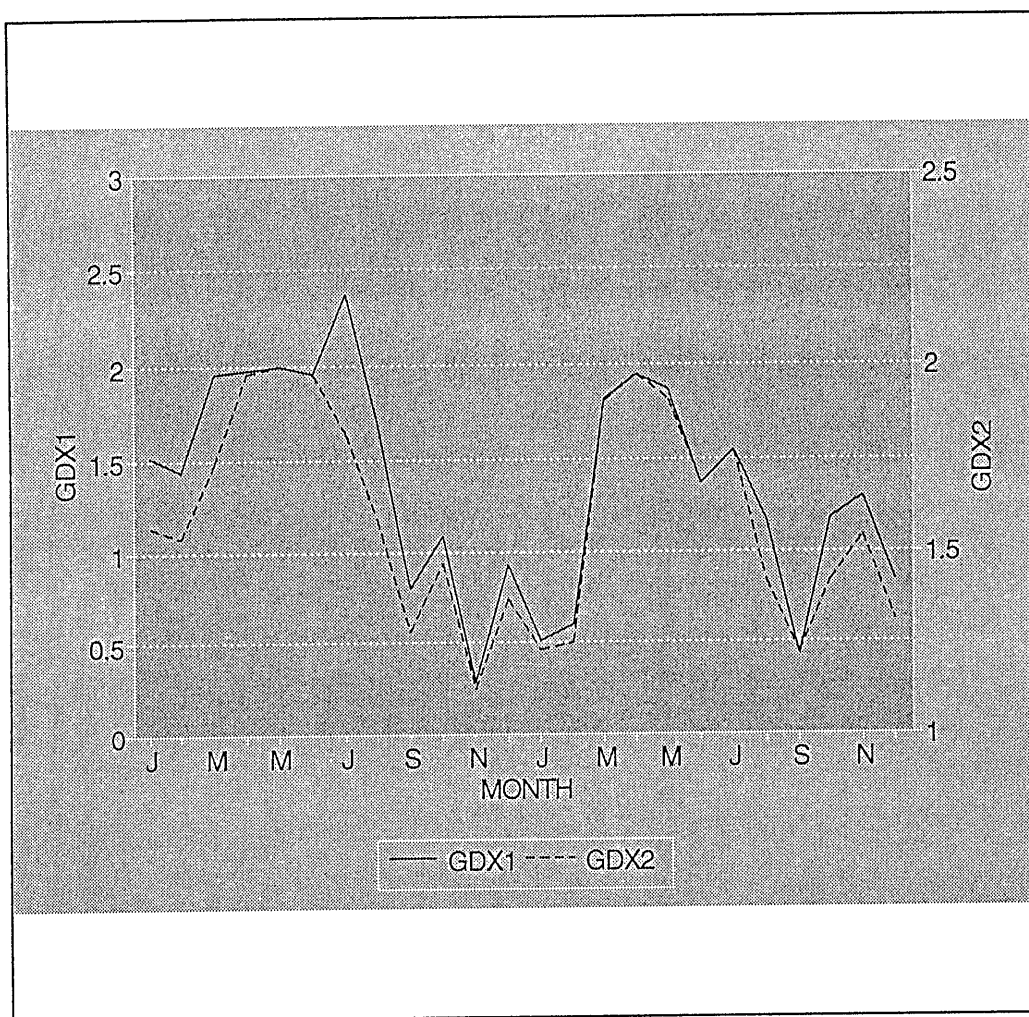


Figure 2-4: P.radiata: The macroscopic monthly gonadal indices (GDX1, GDX2, see page 27) based on examining live gamete smears for the period January 1992 to December 1993.

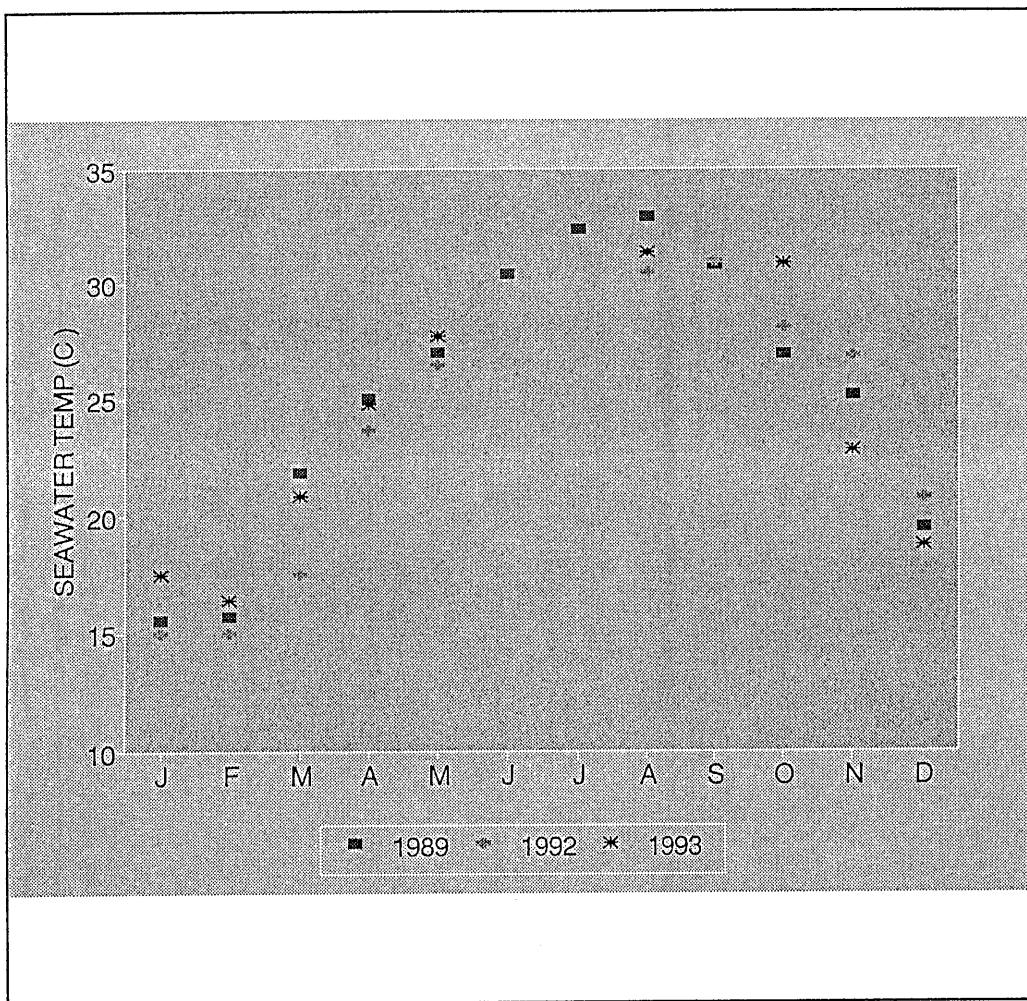


Figure 2-5: Mean monthly seawater temperature around the Island State of Bahrain (Arabian Gulf) for 1989, 1992 and 1993.

To summarize, the breeding cycle in this species is seasonal with two peak spawnings in summer (August/September) and autumn (November/December) and this correlates with the temperature regime prevailing in the Gulf region. However, partial spawning was found to have taken place in January and March 1993. Indices developed from both histological preparations and from simple gonad smears gave similar general annual patterns of gonadal maturation. There were slight but noticeable differences in the timings of the spawnings between years.

2-3.5 Size at First Maturity

Represented in figures 2.6 and 2.7 are the percentage frequencies of the indeterminate gonad stage (0) of young oysters during May - November of both 1992 and 1993 arranged according to class intervals of heel depth (HD) and dorsoventral measurement (DVM), respectively. The frequency of indeterminate stage gonads is a close approximation to the frequencies of immature gonads, and the point at which there is a sudden decrease in the percentage frequencies, may be regarded as the stage at which at least some of the individuals in the species reach sexual maturity (Tranter 1958a, d; 1959). This occurs between ($>0.6\text{mm} \leq 1.25\text{mm HD}$) and at the mid of class 3 ($> 15\text{mm} \leq 20\text{mm DVM}$).

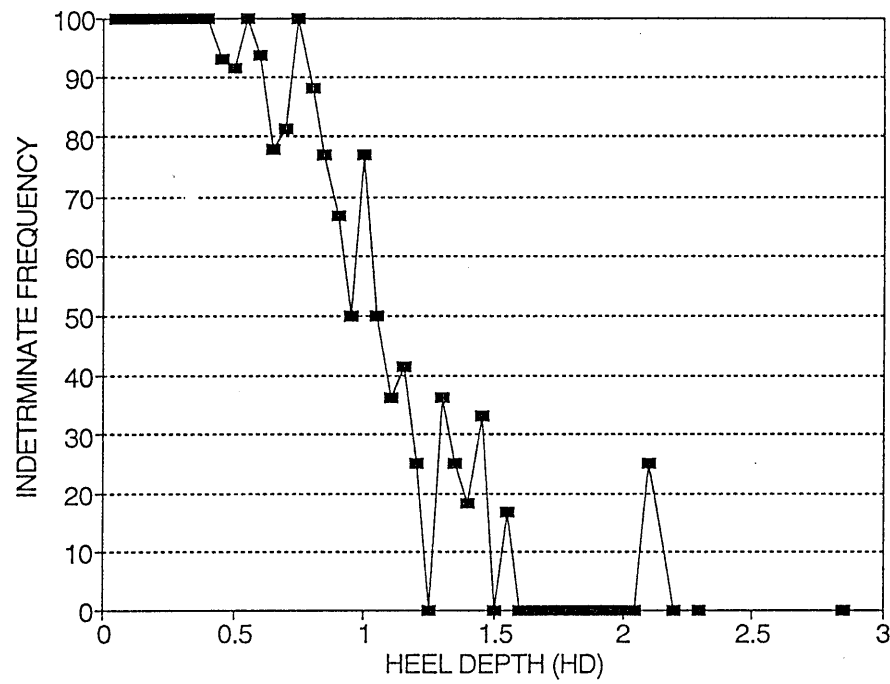


Figure 2-6: The percentage frequencies of indeterminates (stage 0) of young *P. radiata* plotted against their heel depth (HD, mm).

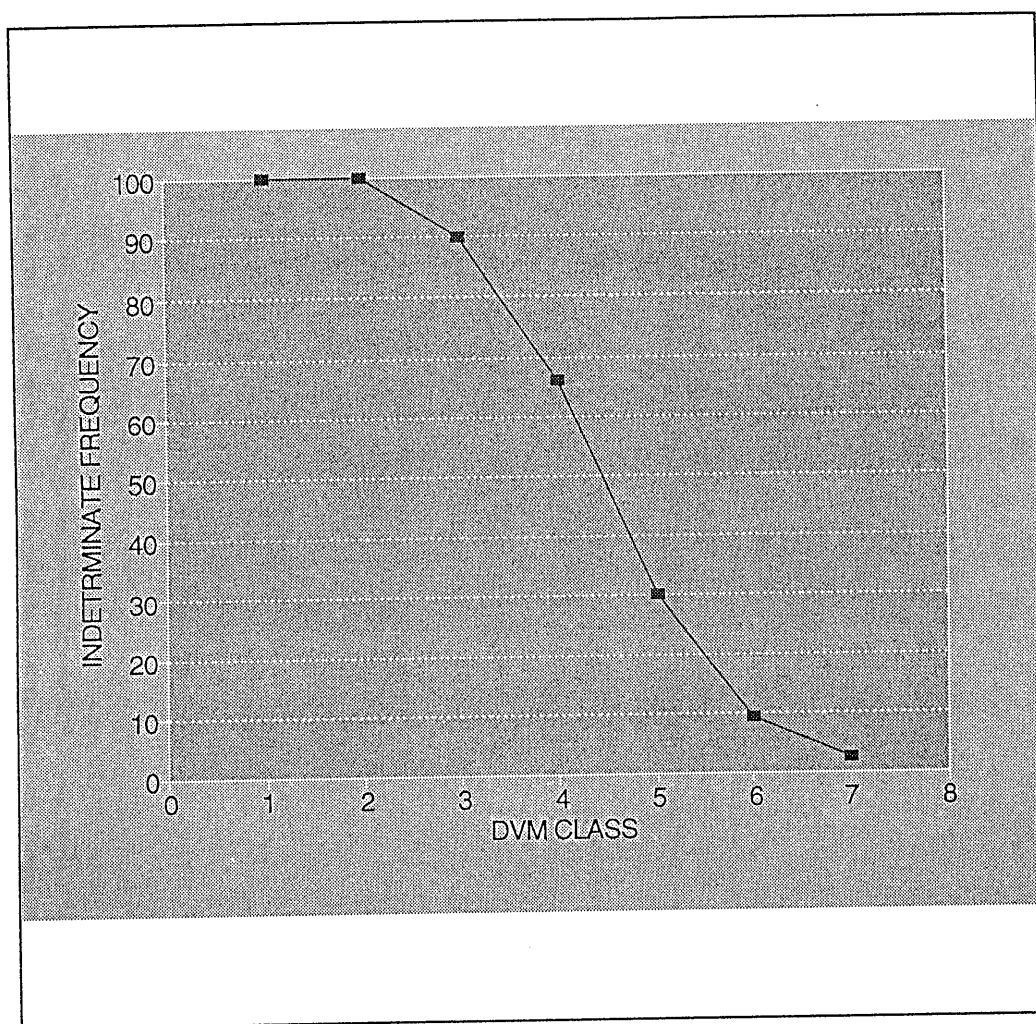


Figure 2-7: The percentage frequencies of indeterminates (stage 0) of young *P. radiata* plotted against their DVM classes. Class coding;

- | | |
|------------------------------|------------------------------|
| 1 = lowest $\leq$ 5mm DVM | 2 = $>$ 5mm $\leq$ 10mm DVM |
| 3 = $>$ 10mm $\leq$ 15mm DVM | 4 = $>$ 15mm $\leq$ 20mm DVM |
| 5 = $>$ 20mm $\leq$ 25mm DVM | 6 = $>$ 25mm $\leq$ 30mm DVM |
| 7 = $>$ 30mm $\leq$ 35mm DVM | |

According to Tranter (1958a, d; 1959) the heel depth is the most reliable measure of age and has proved to be an indispensable aid in aging young Pinctada spp. irrespective of any variations superimposed by the environment. Therefore, accepting Tranter's views (1959) on the linear model of experimentally defined age based on heel depth the young oysters mature at between 3.5-7 months of age. Unfortunately, Nayar (pers. comm.) did not study HD but indicates that young oysters of 15-20mm DVM are 2 months of age in Bahraini waters.

2-3.6 Sex ratio and sex change

Table 2.2 shows the percentage of males and females in early maturing young oysters excluding very minute individuals which were devoid of gonadal tissue (31.3%). The frequency of females is 1.6% (only 7 distinct females were observed out of 439 individuals), 13.7% males (60 individuals) and indeterminate 84.7% (372 individuals).

In July 1992, all young individuals were males. In other months the frequency of indeterminates was higher than both males and females making the use of X^2 , 2 x 2 contingency test difficult to confirm the sex ratio.

Examining gonadal smears from 2356 adult oysters, no cases of hermaphroditism were recognised. However, examining the same oysters, through sectioned

Table 2.2: Monthly percentages of females (F), males (M) and indeterminates (X) amongst young individuals sampled between the period July 1992 to December 1993. Figures in brackets are percentages.

Count Row Pct	SEX			Row Total
Month	F	M	X	
J 1992		12 (100.0)		12 (2.7)
A		4 (50.00)	4 (50.0)	8 (1.8)
S			4 (100.0)	4 (0.9)
O	1 (4.0)	4 (16.0)	20 (80.0)	25 (5.7)
N	1 (1.6)	1 (1.6)	62 (96.9)	64 (14.6)
D		3 (42.9)	4 (57.1)	7 (1.6)
J 1993		7 (29.2)	17 (70.8)	24 (5.5)
F			14 (100.0)	14 (3.2)
M		5 (10.9)	41 (89.1)	46 (10.5)
A		8 (15.7)	43 (84.3)	51 (11.6)
M		1 (12.5)	7 (87.5)	8 (1.8)
J		2 (25.0)	6 (75.0)	8 (1.8)
J		5 (55.6)	4 (44.4)	9 (2.1)
A		1 (20.0)	4 (80.0)	5 (1.1)
S	2 (6.9)	4 (13.8)	23 (79.3)	29 (6.6)
O	1 (1.7)	2 (3.3)	57 (95.0)	60 (13.7)
N	2 (5.3)	1 (2.6)	35 (92.1)	38 (8.7)
D			27 (100.0)	27 (6.2)
Column Total	7 (1.6)	60 (13.7)	372 (84.7)	439 (100.0)

materials, revealed one individual which seemed to be a transitional bisexual exhibiting male and female characteristics (Plate IIIA).

Table 2.3 and figure 2.8 shows the percentage frequencies of females, males and indeterminates categories in adult oysters after being plotted against their heel depth HD classes. There is a decline in male frequencies accompanied by a rise in female frequencies in the 2 - 8mm HD classes and this indeed is masked by a corresponding increase in the frequencies of the indeterminate group. Because of this, the data on numbers of males and females may be compromised making it difficult to assess the general pattern of sex change. Unfortunately, it was difficult to carry out direct observation on tagged oysters to monitor their sex with time in this study. However, the data are analysed by the non parametric chisquare test in order to elucidate any pattern of significant sex change with time. The significance of the results of these tests were Bonferroni adjusted and multiple table-wide (TW) comparisons on X^2 were undertaken.

Table 2.4 shows the general pattern of the monthly variations in the numbers of individuals in male, female and indeterminate categories exhibited by adults. In January and May 1992, the indeterminates were absent but they varied significantly between years and between corresponding months of the two years of study. Only 11 out of 24 months were selected on the basis of none or $\leq 25\%$ indeterminates (conservative approach) and they are included in assessing sex ratio

PLATE III :

- (A) Bisexual phase of P. radiata, ovary beginning oogenesis with residual spermatozoa (rsz), with concomitant connected oocytes (C OC).
- (B) Gonad infected with the parasite Bucephalus sp., follicle wall is damaged and the entire follicles are occupied with cercariae (p) developing in sac within the follicle (f). The colour of infected gonad is granulated orange.

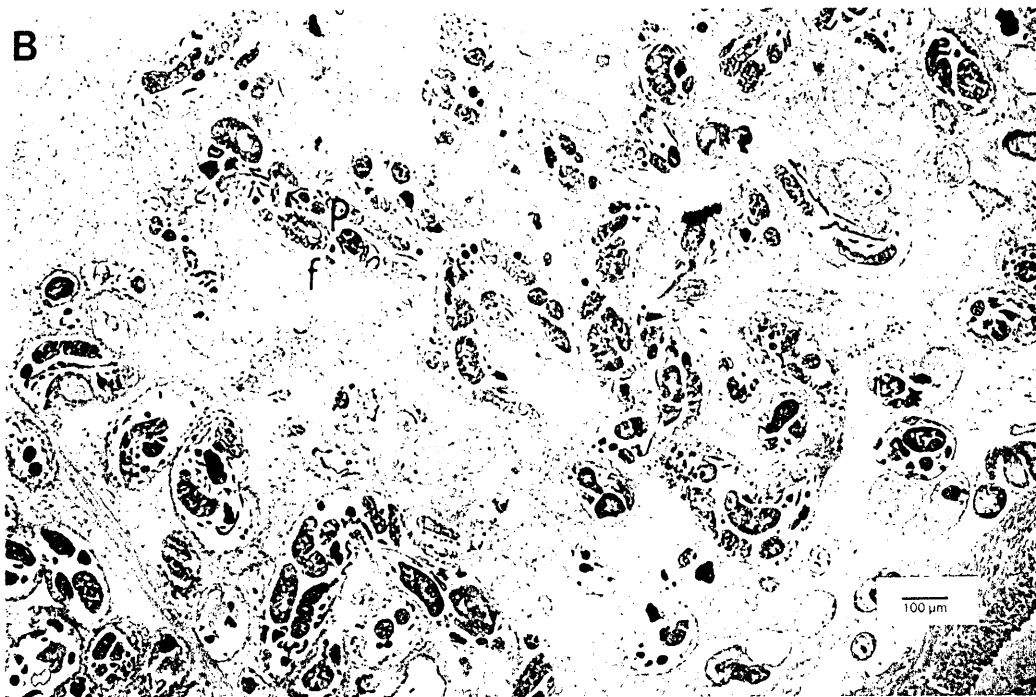
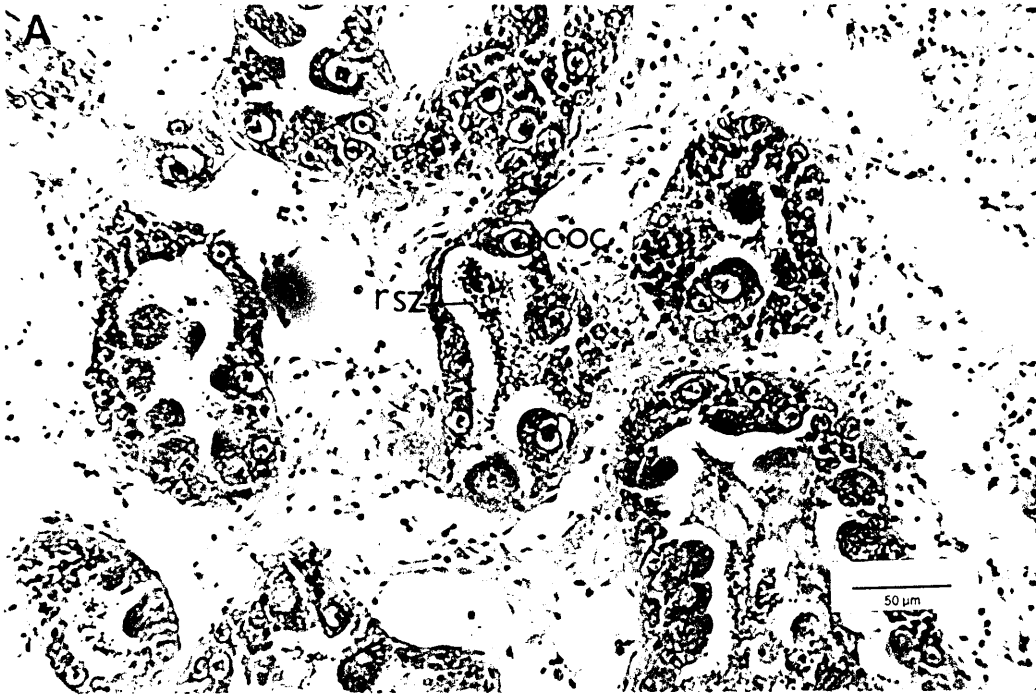


Table 2.3: *P. radiata*: Percentages of adult males (M), females (F) and indeterminate individuals (X) in relation to mean heel depth (HD mm) class

HD	X	M	F
1.92	0.00	100.00	0.00
2.50	33.87	37.65	28.46
3.47	31.02	32.81	36.19
4.48	36.84	24.96	38.21
5.48	50.80	17.70	31.49
6.48	54.79	13.60	32.06
7.44	49.16	24.72	26.11
8.43	28.57	28.57	42.86
9.25	0.00	0.00	100.00
10.35	0.00	100.00	0.00

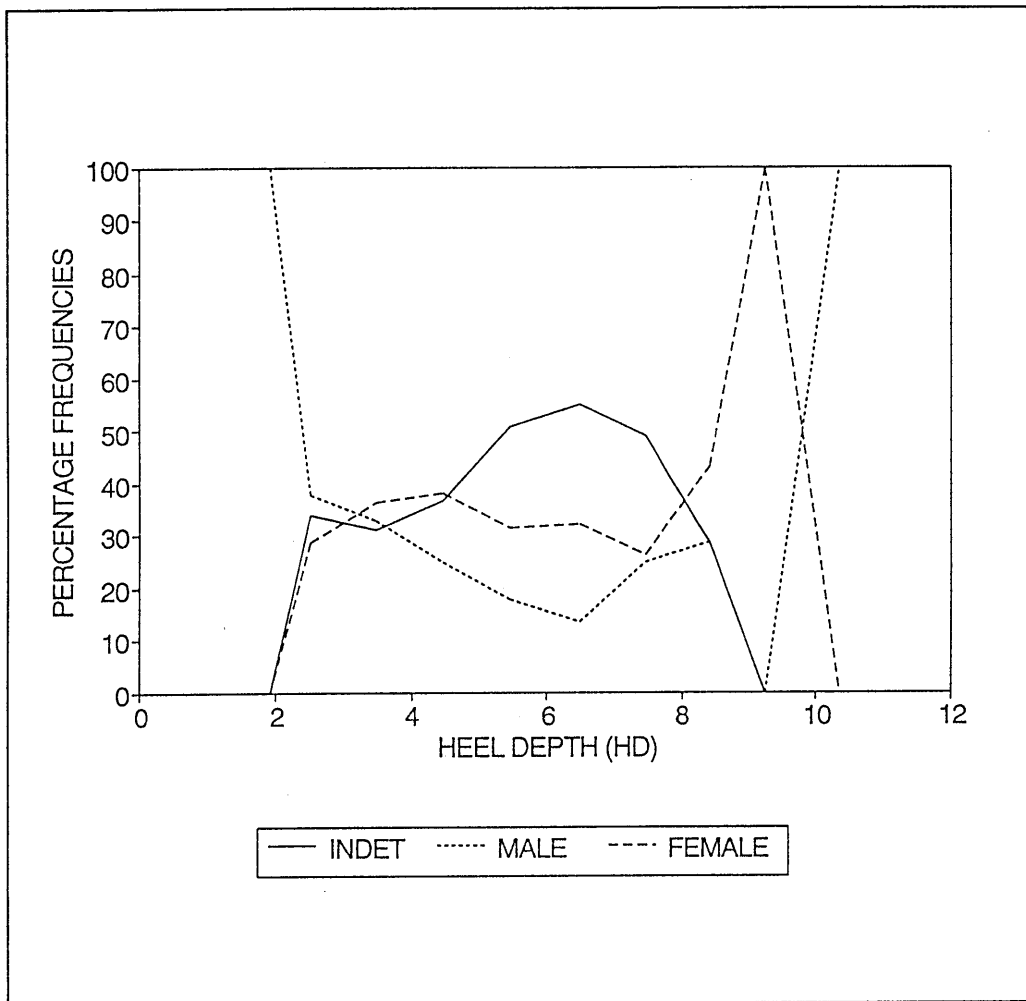


Figure 2-8: The percentage frequencies of indeterminates, males and females of adult *P. radiata* plotted against HD mm (Data from table 2-3).

Table 2.4: The monthly variations in numbers of individuals of male (M), female (F) and indeterminate (X) categories of adult P. radiata monitored over 24 months.

Month	SEX		
	F	M	X
J 1992	67	33	0
F	65	30	5
M	52	23	25
A	62	36	2
M	72	28	0
J	73	25	2
J	65	18	17
A	40	21	39
S	4	12	37
O	28	20	52
N	5	7	88
D	11	26	63
J 1993	6	22	71
F	14	18	68
M	40	51	9
A	47	50	3
M	29	62	8
J	27	41	32
J	23	54	23
A	25	18	57
S	32	27	47
O	22	20	58
N	25	29	45
D	8	24	68

changes (Table 2.5). Therefore, an attempt to generate conclusions from the monthly sex ratios data by applying the Chisquare (χ^2) test to examine the 1:1 ratio will be confirmed by three approaches below;

- 1- On samples where indeterminates are absent.
- 2- Indeterminates will be combined with males. This is to be considered an approach which works against protandry and/or to balance the sex ratio to 1:1.
- 3- Indeterminates will be pooled with females. This is opposite to the second approach mentioned above, i.e to work against protogyny and/or to balance the sex ratio to 1:1.

Then the logic of testing 1:1 sex ratio becomes as follows:

- 1- If a sample exhibited a significant deviation from 1:1 sex ratio in the first approach, then this can be interpreted as a significant preponderance of the sex in excess of its expected number.
- 2- If a sample showed significant departure from 1:1 sex ratio (in the same direction) in both the second and the third approaches, then this is also a confirmed deviation from the 1:1 sex ratio.

Table 2.5: Summary results of the non-parametric chi-square test on the sex ratio for selected 11 months based on $\leq 25\%$ indeterminates.

Year	1992							1993			
Month	J	F	M	A	M	J	J	M	A	M	J
INDETs	0	5	25	2	0	2	17	9	3	8	23
MALES	33	30	23	36	28	25	18	51	50	62	54
FEMALES	67	65	52	62	72	73	65	40	47	29	23
N (sample size)	100	100	100	100	100	100	100	100	100	99	100
NO INDETs	Fs **				Fs ***						
INDETs pooled with MALES	-	Fs **	ns	Fs *	-	Fs ***	Fs **	Ms *	ns	Ms ***	Ms ***
INDETs pooled with FEMALES	-	Fs ***	Fs ***	Fs **	-	Fs ***	Fs ***	ns	ns	Ms *	ns
FINAL concl- usion (Singles)	Fs	Fs	ns	Fs	Fs	Fs	Fs	ns	ns	Ms	ns (see Appendix 2.1)
FINAL concl- usion (on TW χ^2)	Fs	Fs	ns	ns	Fs	Fs	Fs	ns	ns	ns	ns (see Appendix 2.2)

Abbreviations: Fs = significant excess of females; Ms = significant excess of males; ns = not significant
 * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

- 3- If a sample demonstrated significant deviation from 1:1 sex ratio in either the second or the third approach, but not both, then the safest interpretation is to regard it as not significant.

The final conclusions based on X^2 single tests (Appendix 2.1) are summarized in table 2.5 of which 6 samples demonstrated significantly more females than expected under a 1:1 sex ratio, 1 sample showed significantly more males and 4 samples exhibited no significant differences from the 1:1 sex ratio.

However, as suggested by Hochberg (1988; cited by Lessios, 1992) Bonferroni adjustments for significance X^2 on table-wide (TW) have been carried out (Appendix 2.2) and the final conclusions are summarized in Table 2.5. The results show that indeed the female:male, 1:1 sex ratio in 5 months exhibited significantly more females than expected. Furthermore, the significant more males under 1:1 sex ratio found in March and May 1993 (Single X^2 0.05) when the indeterminates are pooled as males and females respectively, are no longer significant when adjusted for significance table-wide. Therefore, the safest interpretation of such results is to be considered as not significant (Lessios, 1992). Based on the final conclusions of TW multiple comparisons of significant X^2 , the species is less likely to be regarded as dioecious.

2-3.7 Seasonal changes of weight(s) of a standard oyster 60mm DVM x 60mm APM x 50mm HL x 4mm HD

The seasonal trend in the overall 1) total body wet weight (WT; Table 2.6 and Figure 2.9), 2) muscle wet weight (MWWT), 3) muscle dry weight(MDWT), 4) remaining tissue wet weight (TWWT), 5) remaining tissue dry weight (TDWT) and 6) total body dry weight (WTD) of the standard oyster in relation to DVM (Table 2.7 and Figure 2.10), APM (Table 2.8 and Figure 2.11); HL (Table 2.9 and Figure 2.12), HD (Table 2.10 and Figure 2.13) which generally show a fall during spawning maxima and fluctuated increase during gametogenesis in winter and early spring.

However, anomalous (= deviated from the expected or in doubt; Ansell, 1974 a, c; Comely, 1974; or due to chance alone) results in various categories of weights in relation to 4 standardized shell size dimensions were reported in table 2.11 which shows the direction of increase (+), decrease (-) and unchanged (0) of weight category relative to the preceeding month. If the change is "real" it should be detected equally in the 4 standardized shell sizes. If it follows some other pattern, i.e an increase in weight for one standardized dimension and a decrease for another then these are better considered either as anomalous results or as an indication that the standardized shell dimensions are not reliable.

Table 2.6: Total body wet weight (WT) monthly variations of a standard pearl oyster 60mm DVM x 60mm APM x 50mm HL x 4mm HD, monitored from January 1992 to December 1993. (See also Fig. 2.9).

Abbreviations: **WT1** = wt of 60mm DVM sd oyster;
 WT2 = wt of 60mm APM sd oyster;
 WT3 = wt of 50mm HL sd oyster;
 WT4 = wt of 4mm HD sd oyster.

Month	WT1	WT2	WT3	WT4
J	11.38	11.76	12.08	12.04
F	11.47	11.82	11.97	11.99
M	9.44	9.01	9.89	9.83
A	10.27	10.19	10.97	10.59
M	9.30	8.68	8.70	8.69
J	8.50	8.48	8.77	8.30
J	9.37	9.21	9.67	9.11
A	7.39	7.60	8.10	7.36
S	8.86	9.49	10.30	9.93
O	8.66	9.16	8.63	8.27
N	9.02	8.81	8.75	8.85
D	10.26	9.33	8.26	7.58
J	11.45	10.82	8.66	8.13
F	10.63	9.07	7.97	7.90
M	11.57	11.57	10.83	9.91
A	12.75	10.66	9.44	9.48
M	12.65	11.68	10.46	10.00
J	10.60	10.54	9.06	8.86
J	11.10	10.20	9.34	9.11
A	9.77	9.40	8.05	7.72
S	7.43	7.26	6.62	5.59
O	8.73	8.66	7.78	7.10
N	10.90	9.67	8.82	8.55
D	10.36	9.99	9.28	7.54

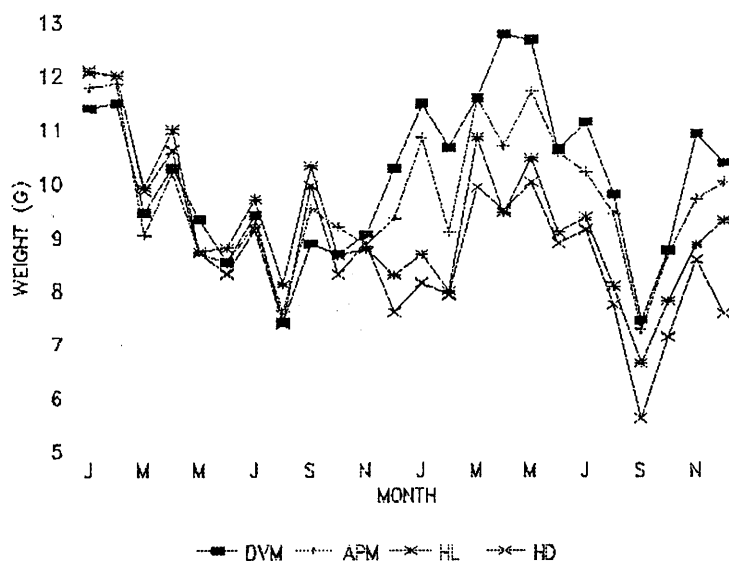


Figure 2-9: Monthly variations of total body wet weight of a standard pearl oyster, 60mm DVM X 60mm APM X 50mm HL X 4mm HD, monitored from January 1992 to December 1993.

Table 2.7: Monthly variations of dry and wet weights of adductor muscle and the remaining tissue of a standard oyster 60mm DVM, monitored from October 1992 to December 1993. (See also Fig. 2.10)

Abbreviations: MWWT = muscle wet weight;
 MDWT = muscle dry weight;
 TWWT = remaining tissue wet weight;
 TDWT = remaining tissue dry weight;
 WTD = total dry weight.

Month	MWWT	MDWT	TWWT	TDWT	WTD
O	2.19	0.51	5.84	0.88	1.45
N	1.90	0.43	5.98	0.82	1.30
D	2.39	0.56	6.48	1.13	1.65
J	3.16	0.84	9.10	1.36	2.24
F	3.11	0.80	7.37	1.21	1.94
M	3.03	0.70	7.53	1.30	2.05
A	2.86	0.77	7.88	1.36	2.13
M	3.60	0.83	8.23	1.29	2.18
J	2.88	0.68	6.42	0.93	1.65
J	2.50	0.63	6.67	1.04	1.67
A	2.44	0.61	6.16	0.92	1.50
S	1.76	0.30	5.59	0.67	0.98
O	2.13	0.44	5.87	0.89	1.38
N	2.51	0.64	7.74	1.15	1.80
D	2.01	0.50	6.76	0.96	1.48

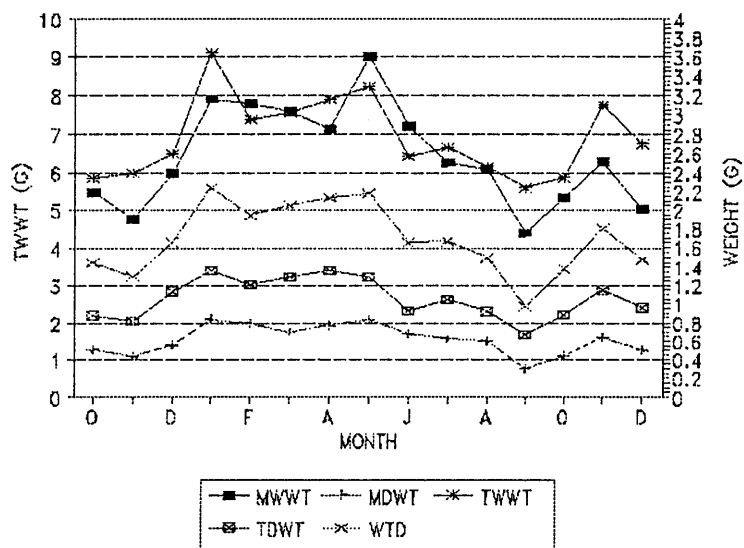


Figure 2-10: Monthly variations of wet and dry weights of adductor muscle and the remaining tissue of a standard oyster, 60mm DVM monitored from October 1992 to December 1993 (Data from table 2.7). Abbreviations see table 2.7.

Table 2.8: Monthly variations of dry and wet weights of adductor muscle and the remaining tissue of a standard oyster 60mm APM, monitored from October 1992 to December 1993. (See also Fig. 2.11)

Abbreviations: See Table 2.7.

Month	MWWT	MDWT	TWWT	TDWT	WTD
O	2.19	0.53	6.01	0.93	1.47
N	1.71	0.41	5.74	0.82	1.23
D	2.37	0.55	6.14	0.97	1.52
J	2.66	0.69	8.19	1.20	1.88
F	2.77	0.66	6.40	1.02	1.68
M	2.88	0.67	7.41	1.26	1.92
A	2.66	0.76	7.60	1.28	2.04
M	3.21	0.76	7.78	1.22	1.95
J	2.62	0.64	6.07	0.91	1.55
J	2.47	0.59	6.33	0.99	1.55
A	2.42	0.60	6.54	0.94	1.54
S	1.46	0.23	5.02	0.59	0.83
O	2.09	0.43	5.59	0.87	1.34
N	2.33	0.58	6.81	1.04	1.62
D	2.04	0.48	6.48	0.90	1.43

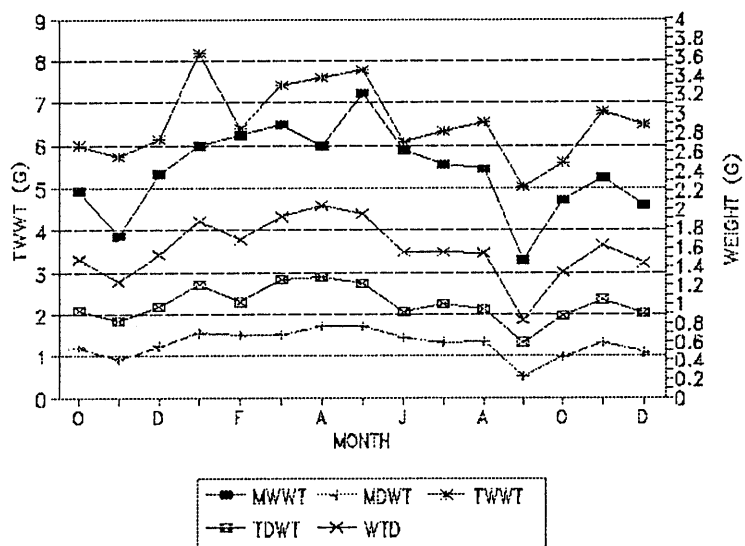


Figure 2-11: Monthly variations of wet and dry weights of adductor muscle and the remaining tissue of a standard oyster, 60mm APM monitored from October 1992 to December 1993 (Data from table 2.8). Abbreviations see table 2.7.

Table 2.9: Monthly variations of dry and wet weights of adductor muscle and the remaining tissue of a standard oyster 50mm HL, monitored from October 1992 to December 1993. (See also Fig. 2.12)

Abbreviations: See Table 2.7.

Month	MWWT	MDWT	TWWT	TDWT	WTD
O	2.15	0.53	6.03	0.88	1.41
N	1.89	0.44	5.84	0.85	1.29
D	2.24	0.53	5.79	0.86	1.40
J	1.63	0.39	5.22	0.84	1.23
F	2.32	0.54	5.47	0.92	1.46
M	3.09	0.72	7.65	1.28	2.03
A	2.25	0.60	6.40	1.12	1.70
M	2.94	0.68	7.20	1.15	1.81
J	2.43	0.59	5.90	0.85	1.47
J	2.16	0.53	5.72	0.86	1.36
A	2.09	0.54	5.59	0.80	1.34
S	1.55	0.25	4.86	0.60	0.88
O	2.12	0.42	5.69	0.90	1.36
N	2.22	0.54	6.23	0.95	1.52
D	1.86	0.48	5.97	0.84	1.30

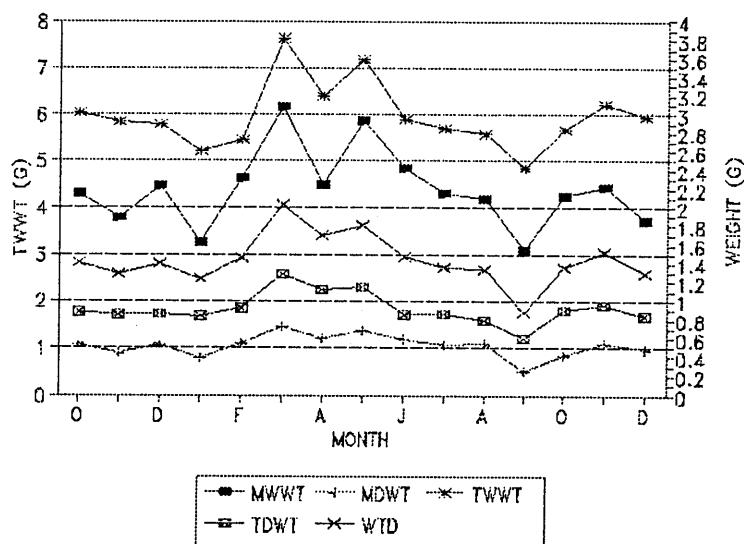


Figure 2-12: Monthly variations of wet and dry weights of adductor muscle and the remaining tissue of a standard oyster, 50mm HL monitored from October 1992 to December 1993 (Data from table 2.9). Abbreviations see table 2.7.

Table 2.10: Monthly variations of dry and wet weights of adductor muscle and the remaining tissue of a standard oyster 4mm HD, monitored from October 1992 to December 1993. (See also Fig. 2.13)

Abbreviations: See Table 2.7.

Month	MWWT	MDWT	TWWT	TDWT	WTD
O	2.22	0.52	5.83	0.89	1.43
N	2.08	0.47	5.92	0.84	1.34
D	1.82	0.43	4.97	0.77	1.21
J	2.07	0.50	6.35	0.96	1.48
F	2.33	0.56	5.37	0.88	1.45
M	2.51	0.59	6.55	1.15	1.74
A	2.11	0.55	6.05	1.08	1.61
M	2.55	0.60	6.45	1.05	1.65
J	2.31	0.57	5.49	0.83	1.42
J	2.15	0.53	5.71	0.88	1.43
A	1.95	0.49	5.07	0.75	1.23
S	1.26	0.21	4.30	0.53	0.76
O	1.63	0.34	4.34	0.72	1.09
N	2.26	0.53	6.28	0.95	1.51
D	1.84	0.44	5.16	0.80	1.03

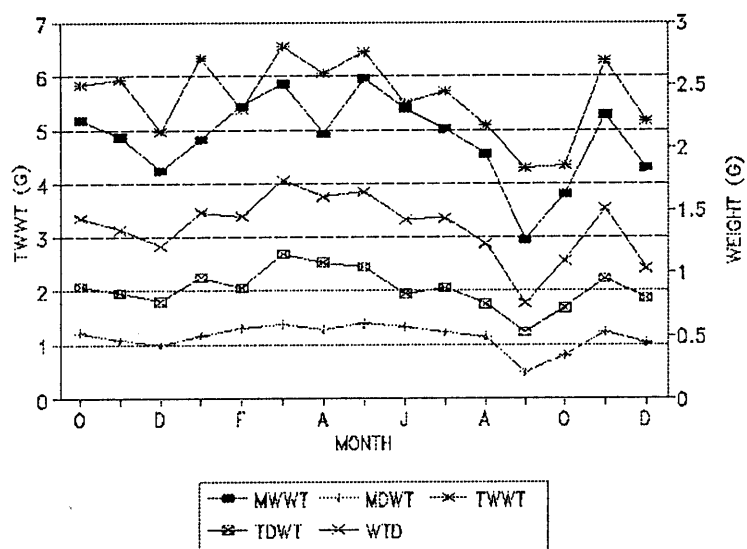


Figure 2-13: Monthly variations of wet and dry weights of adductor muscle and the remaining tissue of a standard oyster, 4mm HD monitored from October 1992 to December 1993 (Data from table 2.10). Abbreviations see table 2.7.

Table 2.11: Results on the direction of weight changes exhibited by the standard oyster 60mm DVM x 60mm APM x 50mm HL x 4mm HD.

Abbreviations: + = increasing relative to the preceeding month,
- = decreasing relative to the preceeding month; 0 = unchanged relative to the preceeding month.

Month Comparison	1992				1993							
	F	J	N	D	J	F	M	A	M	J	O	D
WT/DVM	+	-	+	+				+	-	+		-
WT/APM	+	-	-	+				-	+	-		+
WT/HL	-	+	+	-				-	+	+		+
WT/HD	-	-	+	-				-	+	+		-
MWWT/DVM			-		-		-	-	+	-		
MDWT/DVM			-		-		-	+	+	-		
TWWT/DVM			+		+		+	+	+	+		
TDWT/DVM			-		+		+	+	-	+		
WTD/DVM			-		+		+	+	+	+		
MWWT/APM						+		-	+	-	-	
MDWT/APM						-		+	0	-	+	
TWWT/APM						-		+	+	+	+	
TDWT/APM						-		+	-	+	-	
WTD/APM						-		+	-	0	+	
MWWT/HL										-	-	
MDWT/HL										-	+	
TWWT/HL										-	-	
TDWT/HL										+	-	
WTD/HL										-	-	
MWWT/HD			-			+			+	-		
MDWT/HD			-			+			+	-		
TWWT/HD			+			-			+	+		
TDWT/HD			-			-			-	+		
WTD/HD			-			-			+	+		

Table 2.11 shows the direction of weight changes for 24 comparisons made on 4 standardized shell dimensions and the months in which conflicting results were reported. The standard oyster relating both DVM and APM showed conflicting results in 10 months whilst that related to both HL and HD produced anomalous results in 9 months. Generally, the results of the total body wet weight (WT) of the common standardized oyster was found to be anomalous in 8 months. This may indicate that total body wet weight is not a reliable assessment to monitor seasonal changes in weight as it is affected by water content (Ansell 1974 a, b, c; Comely, 1974). With the exception of aforementioned results the HL becomes a reliable shell dimension as it is the only dimensional character which produced consistent results and it is reasonable to assume that there are plausible reasons for the anomalous results for July and October 1993. In July 1993, only TDWT was increasing while other weights were decreasing. This may be related to gonad enlargement. In October 1993, after summer spawning, only the MDWT showed an increase in weight and this may be related to energy build up and preparation for autumn spawning which took place in December of the same year.

Generally, a standardized 50mm HL pearl oyster exhibited more uniform seasonal changes than the other standardized oysters in keeping with the reproductive cycle of the species. It follows that the lowest minima of weight were reported during peak spawning events and the highest maxima of weight during winter and spring during gametogenesis.

2-3.8 Patterns of allometric growth.

The intercepts and slopes of the regression equations of adults and young oysters are given in Appendix 2.3. Table 2.12 and Figure 2.14 show that adult oysters exhibited a universality of 8 out of 10 cases of negative allometry in the comparisons investigated in 19 out of 24 months. However, a maximum of 9 cases was reported in November 1992 and this was due to APM/DVM relationship which showed a negative allometry. In the period between January to April 1993 a drop in the overall negative allometry is evident and this is correlated to increase/decrease of the negative allometry exhibited by the relationships of WT relative to both DVM and HL. Could the maximum (November 1992) and the minimum values (January to April) be related to spawning? Table 2.13 and Figure 2.15 show the results of 24 allometrical comparisons and suggest that there is a general trend in elevated negative allometry in late autumn and summer with corresponding decrease in winter and early autumn.

Table 2.14 and Figure 2.16 show the results of 6 allometrical comparisons in young pearl oysters which suggest that there exist a general trend of elevated negative allometry in autumn and late spring and summer. This may suggest that young oysters are approaching the adult characteristics in being more negatively allometric in their growth in the periods mentioned above and possibly these young/adult merge into adult population during breeding seasons.

Table 2.12: Summary of results of isometric/allometric growth patterns exhibited by adult P. radiata sampled at monthly intervals for 2 years (see Figure 2.14).

Comparison	Month	J	F	M	A	M	J	J	A	S	O	N	D	J	J	A	S	O	N	D
WT/DVM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WT/APM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WT/HL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WT/HD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
APM/DVM	N	N	-	N	N	N	N	N	N	N	N	-	N	N	N	N	N	N	N	N
HL/DVM	-	-	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HD/DVM	N	+	N	N	N	N	N	+	+	N	+	+	+	N	N	+	+	+	+	N
DVM/HD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
APM/HD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HL/HD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Abbreviations: N denotes not significant = isometric; - = negative allometric; + = positive allometric

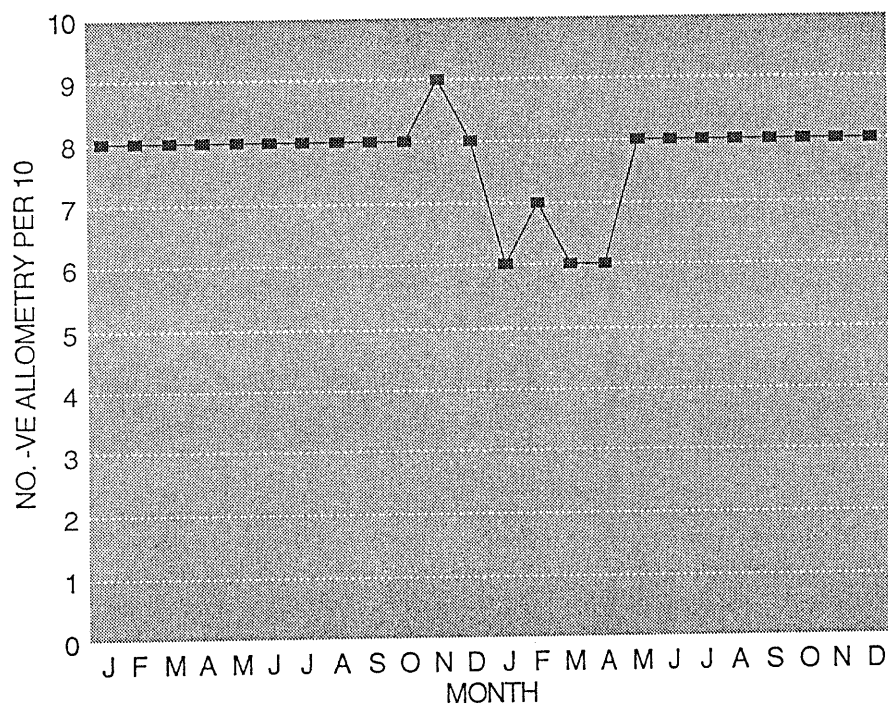


Figure 2-14: Adult P.radiata: monthly variations of allometric growth based on 10 allometric comparisons over a 24 month period (Data from table 2-12).

Table 2.13: Summary results of isometric/allometric growth pattern showed by adult P. radiata at monthly intervals from the period October 1992 to December 1993 (see Figure 2.15).

Month Comparison	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
MWWT/DVM	-	N	-	N	N	N	N	N	N	N	-	N	N	N	-
MWWT/APM	-	N	-	N	N	N	N	N	N	N	N	N	N	-	-
MWWT/HL	-	-	-	N	N	N	N	N	N	N	-	N	N	-	-
MWWT/HD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MDWT/DVM	-	N	N	N	N	N	N	N	N	N	-	N	N	N	-
MDWT/APM	-	N	-	N	N	N	N	N	N	N	N	N	N	-	-
MDWT/HL	-	N	N	-	N	N	N	N	N	N	N	N	N	N	N
MDWT/HD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TWWT/DVM	-	-	-	N	N	N	N	N	N	N	-	N	N	N	-
TWWT/APM	-	-	-	N	N	-	-	-	-	-	-	-	N	-	-
TWWT/HL	N	-	-	N	N	N	N	N	N	N	-	N	N	N	N
TWWT/HD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Abbreviations: N, - and + (see Table 2.12)

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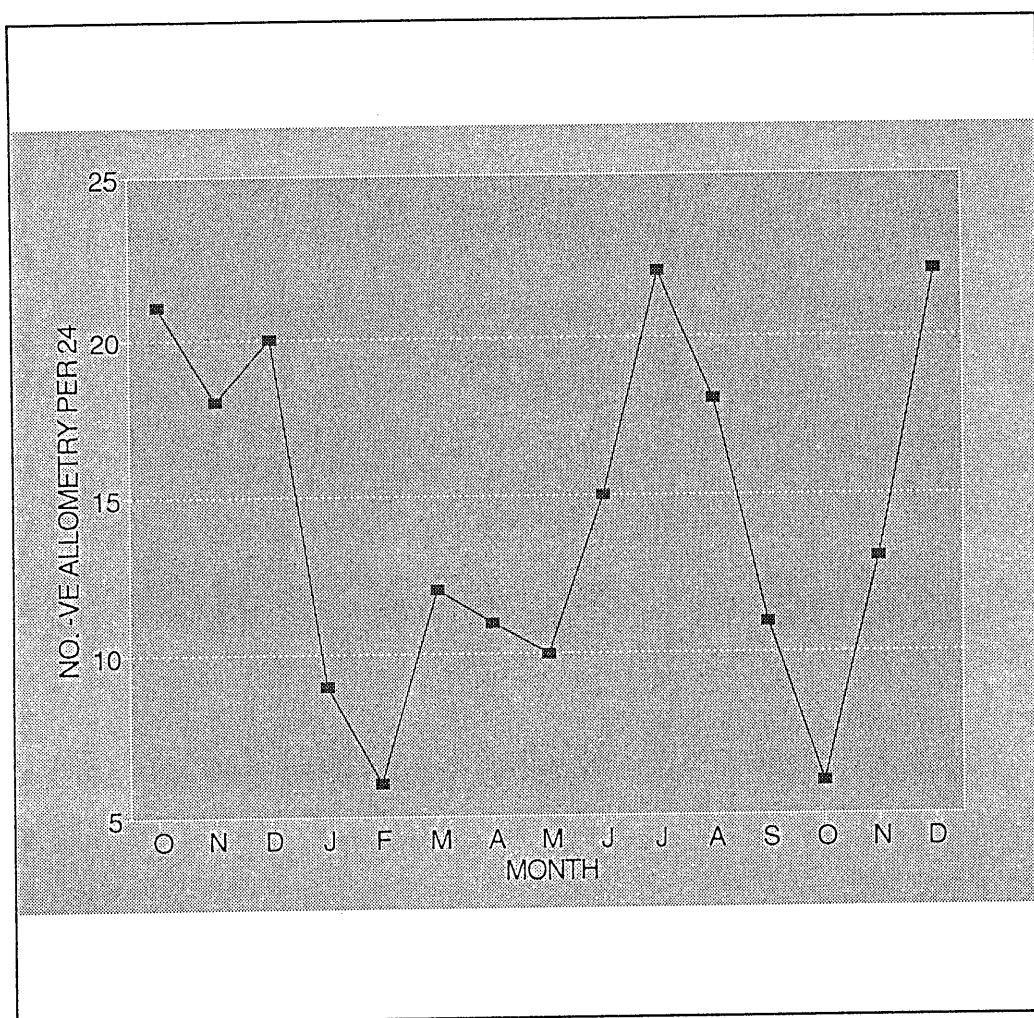


Figure 2-15: Adult *P. radiata*: monthly variations of allometric growth based on 24 comparisons over a 15 month period (Data from table 2-13).

Table 2.14: Summary results of isometric/allometric growth pattern demonstrated by young P.radiata at monthly intervals from the period February 1992 to December 1993 (see Figure 2.16).

Comparison	Month	F	M	A	M	J	J	D	N	O	A	J	M	A	M	J	J	S	O	N	D
APM/DVM		N	N	N	N	N	N	N	-	N	N	N	N	N	N	N	N	N	N	N	-
HL/DVM		N	N	N	N	N	N	N	-	-	-	N	N	-	N	N	N	-	N	-	-
HD/DVM		N	N	N	N	-	N	N	-	+	N	-	-	N	N	N	N	+	+	N	N
DVM/HD		N	N	N	N	-	N	N	-	-	N	N	N	-	N	N	N	-	-	-	-
APM/HD		N	N	N	N	-	N	N	-	-	-	N	N	-	N	N	N	-	-	-	-
HL/HD		N	N	N	N	-	N	N	-	-	-	N	N	-	N	N	-	-	-	-	-

Abbreviations: See Table 2.12.

January and June 1992, and August 1993 samples are excluded due to small sample size.

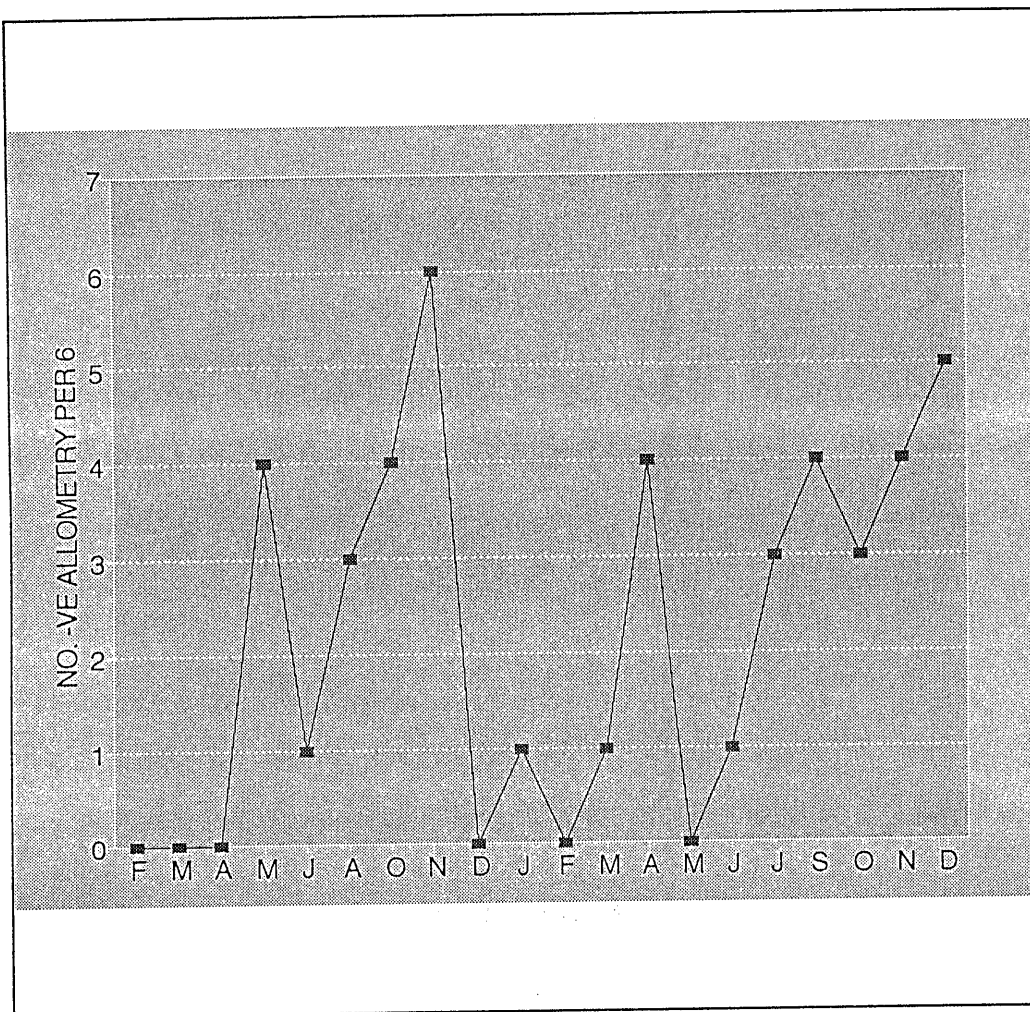


Figure 2-16: Young *P. radiata*: monthly variations of allometric growth based on 6 comparisons over a 20 month period (Data from table 2-14).

To summarize, young and adult oysters sampled at monthly intervals from the same population exhibited different growth patterns being more isometric in young oysters and generally negatively allometric in adults in the comparisons investigated. The seasonal variation in allometrical growth is perhaps not related to spawning per se but most likely to be correlated to the merging of young/adult into adult population to commence spawning.

2-4 Discussion

2-4.1 Morphology of the gonad

Sexual dimorphism is absent in P. radiata and the colour of the gonad is an unreliable aid in sex determination. Gonad colouration distinguishes sex in the Australian P. margaritifera being pinkish, creamy or yellow in females and white in males, with an error of less than 5% (Tranter 1958 d). Gonad colour has also been found to be reliable in determining sex in P. albina (Tranter, 1958 a) and P. maxima (Rose et al. 1990). In this study and also that of Herdman (1903) on P. vulgaris (= P. radiata), and Tranter (1959) on P. fucata (= P. radiata) gonad colour is an unreliable character in sex determination. The morphology of the gonad of this population of P. radiata is similar to other populations of this species and other species of the genus (Herdman, 1903; Tranter 1958 a, b, c, d; 1959; Rose et al. 1990).

2-4.2 Gametogenesis

A ripe oyster is identifiable superficially by the size of the gonad and macroscopically by examining live gamete smears, and microscopically by examining histological preparations of gonads. Rose et al. (1990) simplified Tranter's (1958 a, b, c, d; 1959) several schemes on histological gonad indices and

in this study, Rose et al's (1990) scheme was found to be reliable. Furthermore, the sequence of events of gametogenesis depicted, in this study (plates I and II) is identical to that produced by Rose et al.(1990).

2-4.3 Breeding seasons

In Sri Lanka, Herdman and Hornell (1906) studied P. vulgaris (= P. radiata) and found that spawning occurs all year round with two distinct maxima; May/July and November/January. Malpas (1933) re-examined the species in Sri Lanka and found it to spawn both in summer (July/August) and winter (December/January). The summer peak coincided with the south-west monsoon when there is influx of high salinity water from the Indian Ocean into the Gulf of Mannar, where major pearl oyster beds are situated. The winter spawning coincided with the north-east monsoon which produces significant rainfall and a reduction of salinity. He suggested that spawning is controlled by salinity.

The Australian P. fucata (= P. radiata) was studied by Tranter (1959) and the species was found spawning all year round with two distinct peaks being in the southern hemisphere summer (January/February) and autumn (April/May). Gametogenesis took place in winter and ripening in spring. In this study, young P. radiata have been found settled on the shells of adults all year round, very little spawning activity out of season was noticeable (in January, March 1993) and two

distinct spawning maxima were found in summer/autumn. Gametogenesis took place in winter and spring and this corresponds well with the Australian and the Sri Lankan populations of the same species of pearl oysters.

In Hawaii, P. galtsoffi (= P. margaritifera) spawns once a year in July/August (Galtsoff, 1933). In New Zealand direct observation of spawning in the field was reported in August by Bullivant (1962). Nicholls (1931) during the Great Barrier Reef expedition (1928-1929) reported that P. margaritifera spawns twice a year once in November and again in May. However, Tranter (1958 d) criticized the work of Nicholls (1931) as being incomplete because there were no consecutive samples for a full year and stated instead that in Australia, the species spawns all year round with two spawning maxima being in summer (January) and winter (July). Crossland (1957) studied the P.margaritifera in the Sudanese Dongonab Bay, the Red Sea, and suggested that spawning took place between July to October with gonad development from March to June. Nicholls (1931), Galtsoff (1933), Crossland (1957), Tranter (1958d) and Bullivant (1962) considered that the temperature (26-28°C) was the spawning inducer in P.margaritifera. Tranter (1958a,b,c) studied P.albina from Australia and found it breeding continuously all year round but with one peak spawning in winter (May/April) coinciding with the drop in seawater temperature.

Recently Rose *et al.* (1990) studied the reproductive biology of the silver or gold-lipped pearl oyster, P.maxima, from Australia and found it breeding in winter/spring (September/October) and summer/autumn (March/April). They suggested temperature as the major spawning controller.

Considering the biogeographical distribution of the species of Pinctada being between 30° north and south of the equator (Galtsoff, 1933; Tranter, 1958d) and the experience gained in this study from the spawning, fertilization and larval rearing (see chapter 4) it seems that the temperature is more likely to be the controlling factor for spawning at least for the summer peak. However it is of interest to ascertain the cause(s) of the autumn peak which perhaps is more likely controlled by food availability as judged from redevelopment of gonad after summer spawning and variation of weight of a standard oyster, which will be dealt with at a later stage in the discussion.

2-4.4 Size at first maturity

Tranter's (1959) observations on the gonad development in young oysters accompanied by growth studies enabled him to conclude that P.fucata (= P.radiata) reaches sexual maturity at 0.6mm HD and 20mm DVM which corresponded with 3.5-7 months. He suggested that the young oysters breed twice a year.

In this study, similar approaches to Tranter's (1959) were undertaken and the same conclusions were drawn based on a drop in the number of indeterminates young oysters which coincided with 0.6mm-1.25mm HD and 15-20 DVM. It seems highly probable that the species in Bahrain's waters and, perhaps in the Gulf region as a whole (area 226,000 km² ; UNESCO,1976) behaves in a similar way to Australian pearl oyster populations are separated by 1000 miles from each other (Tranter, 1959).

2-4.5 Sex ratio and sex change

Wada & Wada (1939) reported sex reversal in sexually predetermined and tagged P.maxima, and Ojima & Maeki (1955) observed several bisexual transitional stages in sectioned gonads of P.martensii in Japan. In this study, a bisexual gonad (plate IIIA) was found in histological preparations similar to that found by Tranter (1959) on P.fucata (= P.radiata) and Rose *et al.* (1990) on P.maxima. Furthermore, in this study, P.radiata has often been found to depart from the 1:1 sex ratio and this was demonstrated statistically through X² based on both single and multiple testing. The conclusion reached was that this species should not be regarded as simple dioecious species as suggested by Herdman (1904), and Herdman & Hornell (1906). In fact Herdman was influenced by the early work made by Kelaart (1857) in Sri Lanka P.radiata who found 3 to 4 males out of 100 oysters and believed this is a normal sex ratio for that species, and Galtsoff (1933), too,

was influenced by the work of Kelaart. Galtsoff (1933, Page 32) examined 160 adult P.margaritifera and found 65 or (40.6%) were males and 95 or (59.4%) females.

I have X^2 tested Galtsoff's (1933) sex ratio on P.margaritifera (Appendix 2.4) ($X^2 = 5.625$, $P = 0.018$) and this suggests significantly more females than expected are found in the sample. Therefore, Galtsoff's (1933) conclusion that the 1:1.462 male to female as normal is not upheld.

However, recent studies on the species of the genus Pinctada (Tranter 1958a,b,c,d; 1959; Rose et al. 1990) are reproducible. Therefore, the present author accepts their views and in this study the Bahrain P.radiata is regarded as a transitional or consecutive hermaphrodite (Sastry, 1979).

2-4.6: Seasonal changes of weight(s) of a standard oyster, 60mm DVM X 60mm APM X 50mm HL X 4mm HD.

The total body wet weight (WT) of a standard oyster in relation to 4 standardized shell dimensions produced anomalous results. Similar conflicting results in other species have been related to the effect of water content (Ansell, 1974a,b,c; Comely, 1974), fluctuation in skills (Ansell, 1974b) or a difference in sexes (Ansell, 1974a). This study confirms that total body wet weight (WT) is an unsatisfactory

method of assessment of seasonal changes. Furthermore, DVM, APM and HD linear measurements are unreliable shell dimensions for a standardized pearl oyster and Comely (1974) has warned against the use of unreliable standardized shell dimensions in Pecten maximus .

Hilbish (1986) studied M.edulis population at the Long Island Sound, N.Y., and found that the mussels spawn in September and the dry weight of a standard mussel did not show reciprocal decrease in weight during spawning but instead an increase. He therefore, regarded the use of the "standard mussel" as inadequate since growth in length and weight was uncoupled. He further criticized the works of Kautsky (1982a) and Rodhouse (1984a) on M.edulis and claimed that his conclusion was also valid for these two studies. Finally Hilbish (1986) warned against the use of the "standard mussel" as it is vulnerable to the effect of artefacts.

As stated earlier studies on bivalves, up to date (Hilbish, 1986 excepted), have related the decrease in weight of a standard bivalve to spawning and in such studies occasional anomalous results are not uncommon. For example, Ansell (1974a) found anomalous results in the dry weight of male and female Abra alba increasing in the males at a time when that of the females was decreasing. Ansell (1974a) regarded this as a simple anomaly and continued with his analysis on the females alone. Similar examples on biochemical components of various parts of

organs in bivalves did not show pronounced seasonal variation and deviated from the expected in relation to spawning and these are considered explicitly as anomalous results (Comely 1974, Dare & Edwards 1975). Ansell (1974c) found a decrease in phaeophytin content in a standard Nucula sulcata which was not related to spawning and it was not clear to him why there should be a reduction at that time.

Examining the papers of Kautsky (1982a,b) and Rodhouse et al. (1984a,b) on Mytilus edulis it should be noted that the studies of both Kautsky (1982b) and Rodhouse et al. (1984b) are more relevant to the subject of relating changes in weight of a standard mussel to spawning and both studies produce conflicting results.

Kautsky (1982b) studied the gonad cycle, fecundity, reproductive output and recruitment of M.edulis in the Baltic Sea. He found reduced weights of a standard mussel persisting from mid June to the end of February. The increase in weight of a standard mussel was noticeable only during March-May. He attributed the decline in weight to the period of spawning. He could not find any significant elevations in weights of mussels collected from 4m and 15m of depth spawned "some weeks later". Apparently then, since there was no significant difference between weights but a difference in spawning timings this could be an anomalous result due to chance alone?

Rodhouse et al.(1984b) studied gametogenesis and ash-free dry weight (AFDW) in wild and cultured M.edulis from the west coast of Ireland. The wild population spawns in late winter and early spring (= February/April) followed by a second spawning in May/June. The spring spawning was confined to the larger length >40mm in length mussels only!

The Irish cultured population of M.edulis spawns in May/June followed by a second spawning in August/September. The seasonal cycle of AFDW varied with size in the wild population. Most noticeable is the gain in weight amongst the larger but not the smaller mussels in winter. It should be recalled here that Hilbish (1984b) used 17.4 mm in length standard M.edulis. Rodhouse et al.(1984b) attributed the decline in AFDW to the fact that larger mussels spawn in spring. During September of both years there was a drastic loss in AFDW that was not associated to spawning. They correlated this with a period of sharply diminished salinity. Could there be simply anomalous results due to chance alone?

Rodhouse et al.(1984b) related the difference in gametogenesis between wild and cultured M.edulis to conservative / opportunistic spawnings. They concluded based on this evidence that the physiological phenotype of M.edulis is a very plastic character and extremely susceptible to food availability.

Seed (1971) working on Mytilus spp from Rock in S.W. England identified two species within his samples; M.edulis and M.galloprovincialis based on

morphological characters and a clear difference in reproductive biology. Most studies on Mytilus from Britain and Ireland (For example Seed, 1974, 1990; Gosling & Wilkins, 1981; Gosling, 1984; Gosling, 1992, for review) confirmed the co-existence of both M.edulis and M.galloprovincialis around the South & West coasts of Ireland. Then the question arises why might the larger wild M.edulis spawn differently from the smaller sized mussels of the same "population"!

Based on extensive experience of the reproductive biology of mussels (Seed pers.comm) differences between spawning timings of large and small mussels are uncommon. However, studies on the reproductive biology of Irish mussels are scarce (Seed pers.comm). Seed's (1990) figure 3 suggests that M.galloprovincialis spawns partially in spring in the Camel estuary in S.W. England.

The results of this study are in general agreement with results from other boreal species but with some minor differences. Below is given an outline of the features of the seasonal cycle exhibited by 50mm HL standard pearl oyster. P.radiata compared with Ansell & Trevallion's (1967) scheme for boreal bivalve species.

1- The minima and maxima of tissue and muscle weights differed from year to year, between the corresponding months and between the corresponding spawning peak episodes, and this is in accordance with the pattern of spawning and recovery demonstrated earlier. However, the difference in pattern between years is not an unusual phenomenon in bivalves (Comely, 1974; Ansell, 1974c; Dare & Edwards,

1975).

2- The lowest weight minima for all components of the standard oyster were reported in September 1993 and this coincided with a greatest maximum spawning peak.

3- Oysters are relatively inactive during winter and spring when energy build up in both gonadal and muscle tissues commences. Further increases in these tissues are apparent after the summer spawning peak and are noticeable in the autumn which may be related to food availability. However, this assumption needs to be substantiated by evidence from routine chlorophyll concentrations studies by the Environmental Protection Committee as the knowledge at present is lacking.

4- In winter and early spring gametogenesis proceeds and proliferation of gonad and increase in body weight take place. Conversely, boreal bivalves utilize energy stored in the adductor muscle in winter for routine metabolic requirements.

5- Gonad renewal is continuous all year round following partial spawning and after major spawning peaks. In contrast, boreal bivalves commence renewal in spring.

6- Reproduction and spawning during summer is triggered by temperature (Figure 2.5) while the autumn peak spawning, perhaps, is controlled by both food

availability and temperature (= balance environmental factors; Ansell, 1974b; = opportunistic reproduction; MacDonald & Thompson, 1985b).

2-4.7 Allometric growth patterns.

There are very few published studies on growth in the genus Pinctada and in most cases the total weight of oyster (both the shell and meat) are regressed on linear dimensions, and often the deviation of the slope(s) is not tested in respect to isometrical relationships. Exceptions are studies of Galtsoff (1931, 1933) Alagarwami & Chellam (1977) and Chellam (1978) but in these studies the sample is often taken from a single visit to the collection site. It was impractical to weigh the shells of P.radiata in this study due to the excessive biofouling and the fragility of the valves which were easily damaged even though handled with extreme care. Therefore, shell weight was not recorded to avoid biased results.

Ricker (1975) states that in a regression of weight on any linear dimension a value of b for the slope equal to 3 (= volumic) describes isometric growth, such as would characterise a fish having an unchanging body form and unchanging specific gravity. Many species seem to approach this "ideal" though weight is affected by such factors as time of year, stomach contents, and spawning conditions. On the other hand, some species have b -values characteristically greater or less than 3, a condition described as allometric growth (Lent, 1967; Ricker, 1975). There are

sometimes marked differences between the same species and between the same population at different time of the year, presumably associated with their nutritional and gametogenetic condition (Hickman, 1979; Seed & Richardson, 1990; NOAA, 1993).

The results of this study are in general agreement with previous works in the genus of Pinctada (Galtsoff, 1931, 1933; Tranter, 1959; Alagarwami & Chellam, 1977; Chellam, 1978; Nasr, 1984; Coeroli *et al.* 1984; Sims, 1994) which showed extraordinarily fast growth demonstrated by young pearl oysters. Lent (1967) states that "in some species the negative allometry is a universality".

It was demonstrated that in the comparisons made in this study the negative allometry is an almost universal in adults (Figures 2.14 & 2.15) and that young oysters generally exhibited negative allometry in late spring, summer and autumn (Figure 2.16).

According to Alagarwami & Chellam (1977) the difference between young and adults in their growth patterns is a natural phenomenon in keeping with growth of the soft parts. Based on the results of this study, Alagarwami & Chellam's (1977) aforementioned explanation is so simple and it was demonstrated that the difference in allometric direction is caused by the effect of merging of young but mature individuals into the adult population during the breeding season. This may suggest that the cessation of growth is an adaptive requirement for the developing of gonad and other organs to enable these young but mature individuals to

commence breeding.

Alagaraja (1962) and Alagarswami & Chellam (1977) could not explain the heterogeneity of slopes of spat (= young oysters ; in this study) and adult P.fucata (= P.radiata) taken from different oyster banks (Alagarswami & Chellam, 1977) in India in 3 different years (Alagaraja, 1962). Perhaps, the causative factor in the heterogeneity of slopes is the influx of young but mature individuals which have different growth patterns from the young and/or adult oysters.

Chapter 3: Biofouling community, parasitic infection and incidence of natural pearls.

3-1: Introduction

During the reproductive biology study (chapter 2) it was observed that the trematode Bucephalus sp infected adult P.radiata. It was decided therefore, to document the incidence of Bucephalus infection and this seems to be the first record of "such a study" in the Gulf region. Several species of Bucephalus have been reported in several bivalves, in the pearl oysters, P.albina and P.martensii (Tranter, 1958a; Sakaguchi, 1966a,b; Funakoshi, 1992), Ostrea edulis and Crassostrea virginica (Galtsoff, 1964; Cheng & Burton, 1965), C.madransensis (Thangavelu & Sanjeevaraj, 1987); M.edulis and other mytilids (Seed, 1969; Lasiak, 1992), Cerastoderma edule and C.glaucum (Boyden, 1971) and fishes; the whiting (Johnston & Halton, 1981) the caranids (Sakaguchi, 1966a,b). Schaefer & Hoffmann (1985) attributed the incidence of fishkills in Main River in Germany to B.polymorphus. In the Arabian Gulf fishkills have been reported but the reasons for their occurrence are still mysterious.

Polydora vulgaris a boring polychaete worm has been reported earlier in the Kuwaiti Gulf, populations of P.radiata (Mohammad, 1972, 1976) and was correlated with the incidence of natural pearls. This study documents both the incidence of Polydora and Cliona a boring sponge in Bahrain's population of P.radiata and compares the incidence

of both biofouling species with that found in M.edulis (Ambariyanto & Seed, 1991), M.galloprovincialis (Tkachuk, 1988), the abalone, Haliotis tuberculata (Clavier, 1989) and the limpet, Patella vulgata (Baxter, 1984). The study also investigates the incidence of natural pearls in P.radiata and compares the incidence with previous similar studies in Arabian Gulf populations of P.radiata (Mohammad, 1972; Almatar et al. 1994; Al-Rumaidh, 1994) and North Wales M.edulis populations (Fernandes & Seed, 1983).

The structure of the biofouling community was studied to characterise the pearl oyster population in the light of the categorisation suggested by Kawahara (1961).

3-2: Materials and methods

The study reported in this chapter is based on the monthly samples taken from the Al-Jadoum population which were used principally in the study of pearl oyster reproductive biology. Full details of their collection are given in the materials and methods of chapter 2. During the sampling period from November 1992 to December 1993 the occurrences of pearls and biofouling organisms were registered qualitatively (i.e. presence and absence) for each shell valve of the oyster. The incidences of parasites were provided for 24 months, from January 1992 to December 1993. The abbreviations used throughout the study are listed below;

BP = Blisters; Polydora vulgaris (spionid polychaete)

PS = Parasite; Bucephalus sp. (trematode)

PL = pearl; cyst pearls

RCM = right valve; Chama aspara (chama oyster)

RA = right valve; Hormophysa triquetra (alga)

RO = right valve; P.radiata (young & adults)

RZN = right valve; Bugula sp. (bryozoa)

RG = right valve; Cliona vestifica (sponge)

RW = right valve; Vermetus sulcata (gastropod tube)

RM = right valve; Malleus regula (hammer oyster)

WIN = polychaete worm Nereis sp. inside the oyster.

The letter L denotes the respective species is found on the left valve, e.g. LCM Chama aspara on the left valve of the oyster.

The species of molluscs listed below were found very rarely and they were therefore excluded from the study;

Plicatula sp., Anomia laqueata, Cronia konkanensis and Chlamys lividis.

Variations in the percentage frequencies of parasites, pearl and biofouling incidences in relation to month and size (DVM) were summarized graphically.

3-3: Results

Throughout this study young pearl oysters up to a size of 35mm DVM (= spat, Alagarswami & Chellam, 1977) appear to be devoid of parasites, pearl and biofouling. However, these young oysters are part of larger conspecifics because they were settled on larger oysters.

3-3.1: Composition of the biofouling community

There were 9 associated species in total settled on the shells of adult P.radiata; 3 species of bivalves (Chama aspara, Malleus regula and young P.radiata); 1 gastropod species (Vermetus sulcata); 2 worm species (Polydora vulgaris and Nereis sp.); 1 bryozoa (Bugula sp.); 1 sponge species (Cliona vestifica) and 1 alga (Hormophysa triquetra).

The bryozoa Bugula sp. and the alga H.triquetra showed higher percentages of occurrences on monthly samples (Appendix 3.1) and on both shell valves (Appendix 3.2) than any of the other macro organisms.

3-3.2 Parasitic infection of the adult gonad by Bucephalus sp.

Plate III (B) shows a parasitized gonad of an adult pearl oyster where the follicle wall is damaged, suggesting that this parasitized oyster will not initiate gametogenesis and this is an indication of full destruction of gonad.

Figure 3.1 shows the monthly percentage occurrence of unidentified species of Bucephalus infecting the gonad of the pearl oyster between the period January 1992 to December 1993. The values ranged between 0 and 4% being relatively higher in 1992 than 1993. The occurrence of Bucephalus does not appear to follow any seasonal pattern nor spawning events. Zero incidences have been encountered in every season of both years except autumn 1992. There is no infection pattern, the parasite is common in some monthly samples and completely absent in others. There is, perhaps, an indication of the sudden appearance of the final host in large numbers such as a group of fish or possibly the appearance of the second intermediate host in vast numbers and subsequent infection of the oyster.

Figure 3.2 shows that the maximum infection (4.5%) with the parasite was in class 8 ($>69\text{mm} \leq 74\text{mm}$ DVM) but there appears to be no general trend between the infection and the size of oyster.

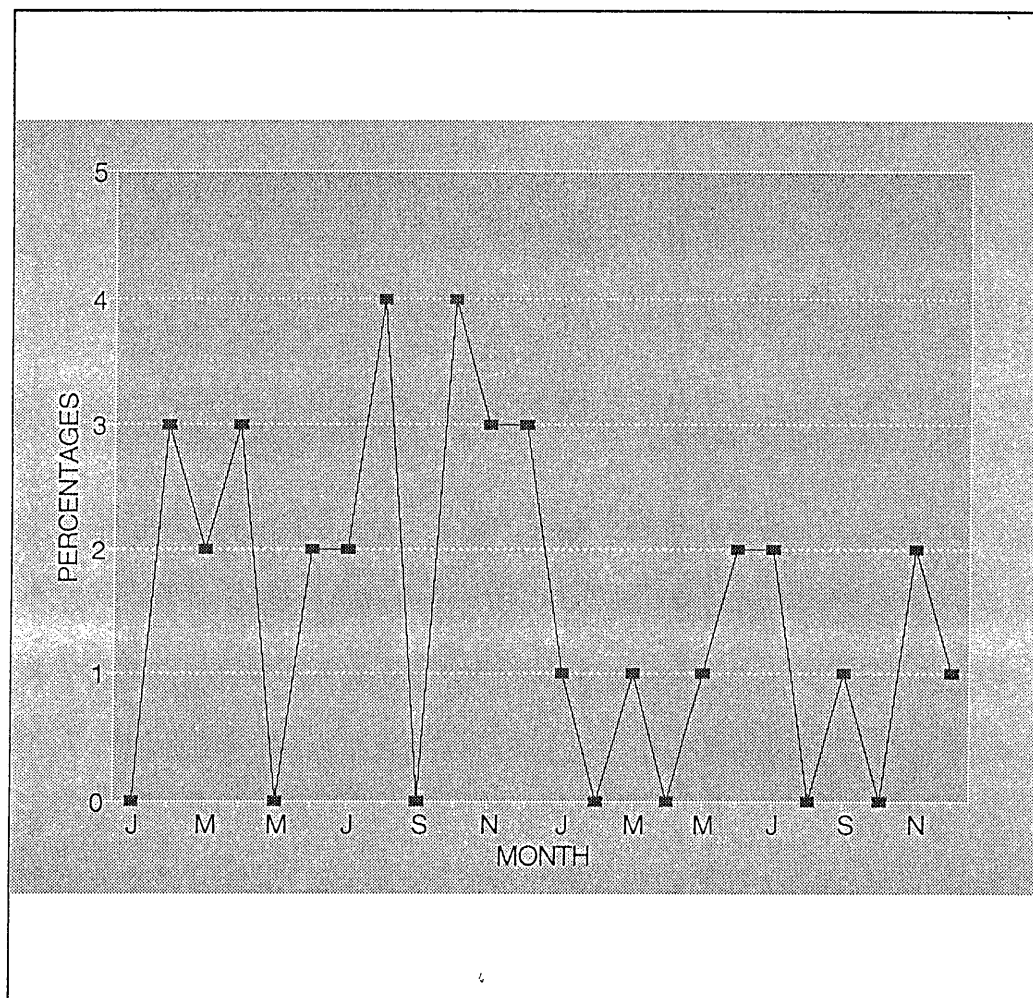


Figure 3-1: the percentage of *P. radiata* infected with *Bucephalus* sp. in the gonad, between the period January 1992 to December 1993.

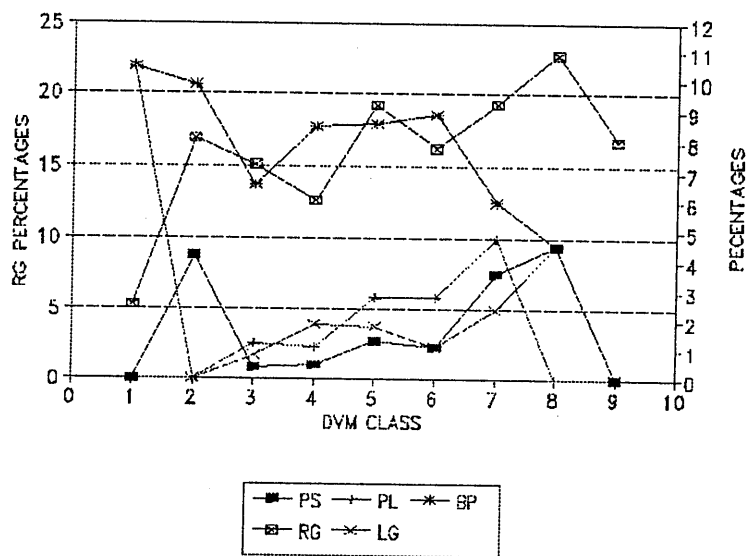


Figure 3-2: The percentage of *P.radiata* bearing pearls (PL), and infected with *Bucephalus* sp. (PS), *Polydora* (BP), and *Cliona* on the right (RG) and the left (LG) valve of the shell.

For class abbreviations see Appendix 3-2.

3-3.3 Incidence of natural pearls

Natural pearls not more than 2mm in their greatest diameter are of the cyst (=free) type and are to be found at the mantle edge facing the hinge. Table 3.1 shows that 27 oysters out of 1398 (= 1.93%) bore 1 to 15 pearls. Furthermore, table 3.1 shows no apparent trend of association between the incidence of pearls and the infection with the polychaete Polydora vulgaris which produces blisters.

Figure 3.2 shows that the incidence of natural pearls was confined to size classes 3 to 7 (= 44mm to 69mm DVM), and that the maximum 4.8% occurred in class 7 (>64mm ≤69mm DVM). This may suggest the size of oysters at which the maximum pearl yield can be obtained.

3-3.4 Infestation with Polydora and Cliona.

Tables 3.2 and 3.3 document the infestation rate with Polydora and Cliona, respectively, reported in this study along with other relevant studies on several mollusc species.

The results of this study suggest that both Polydora and Cliona were more frequently present in summer and on the right valve of the oyster rather than the left one (figure 3.3).

Table 3.1: Cross-tabulation of pearl frequency with the incidence of Polydora in adult P. radiata. Values in brackets are percentages.

Count Row Pct	Infected with <u>Polydora</u>		Row Total
Number of Pearls	No	Yes	
0.00	1263 (92.1)	108 (7.9)	1371 (98.1)
1.00	17 (85.0)	3 (15.0)	20 (1.4)
2.00	3 (100.0)		3 (0.2)
6.00	1 (50.0)	1 (50.0)	2 (0.1)
13.00		1 (100.0)	1 (0.1)
15.00	1 (100.0)		1 (0.1)
Column Total	1285 (91.9)	113 (8.1)	1398 (100.0)

Total incidence of pearls = 27. Thus the percentage (27/1398) = 1.93%

Table 3.2: The maximum infestation percentages with Polydora species in several species of molluscs.

Mollusc species	<u>Polydora</u> species	Infestation rate (percentages)	Authority
<u>P. radiata</u>	<u>P. vulgaris</u>	total 113/1,398 = (8.1 %) see Table 3.1 Monthly maximum (17 %) see Appendix 3.1 Class 1; (10.5 %) 35.05mm to 39mm DVM see Figure 3.2 and Appendix 3.2 Very rare on left valve	This study
<u>P. margaritifera</u> (= <u>P. radiata</u>)	<u>P. vulgaris</u>	(3.20 %) right valve (1.41 %) left valve (0.07 %) both valves total = 971/20,733 = (4.68 %) Class 9+; (14.77 %) 90mm to 100mm DVM	Mohammad (1972)
<u>P. fucata</u> (= <u>P. radiata</u>)	<u>P. ciliata</u>	not specified rare in oysters < 40mm DVM	Dharmaraj & Chellam (1983)
<u>M. galloprovincialis</u>	<u>P. ciliata</u>	(11-100 %) larger sized	Tkachuk (1988)
<u>M. edulis</u>	<u>P. ciliata</u>	estuary population (60-65 %) larger sized > 40mm in length < (10 %) other population	Ambariyanto & Seed (1991)
<u>Haliotis tuberculata</u> (abalone)	<u>P. ciliata</u>	(100 %) larger sized absent below 50mm in length	Clavier (1989)
<u>Patella vulgata</u> (limpet)	<u>P. ciliata</u>	(24.9 %) larger sized absent in < 22mm in length	Baxter (1984)

Table 3.3: The maximum infestation percentages with Cliona species in several species of Mollusca.

Abbreviations: see Figures 3.2 & 3.3 and Appendices 3.1 & 3.2.

Mollusc species	<u>Cliona</u> species	Maximum infestation rate (percentages)	Authority
<u>P. radiata</u>	<u>C. vestifica</u>	(34%) right valve/summer (RV) (7.2%) left valve/autumn (LV) see Figure 3.3 and Appendix 3.1 (22.7%) Class 8 (RV) 69 to 74mm DVM see Figure 3.2 and Appendix 3.2 (10.5%) Class 1 (LV) 35.05 to 39mm DVM see Figure 3.2 and Appendix 3.2	This study
<u>M. galloprovincialis</u>	<u>C. vestifica</u>	(60%) Cultured (100%) Wild	Tkachuk (1988)
<u>H. tuberculata</u> (abalone)	<u>C. celata</u>	(80%) larger sized absent in smaller sized	Clavier (1989)
<u>P. vulgata</u> (limpet)	<u>C. celata</u>	(3.1%) larger sized absent in smaller sized < 22 mm in length	Baxter (1984)

Table 3.4: Maximum incidence of natural pearls in the Arabian Gulf P. radiata and North Wales M. edulis populations

Species (State)	Maximum Percentage	Authority
<u>P. radiata</u> (Bahrain)	1.93%	This study
<u>P. radiata</u> (Bahrain)	4.74%	Al-Rumaidh (1994)
<u>P. radiata</u> (Kuwait)	4.39%	Mohammad (1972)
<u>P. radiata</u> (Kuwait)	2.17%	Almatar <u>et al.</u> (1994)
<u>M. edulis</u> (North Wales, UK)	<13%	Fernandes & Seed (1981)

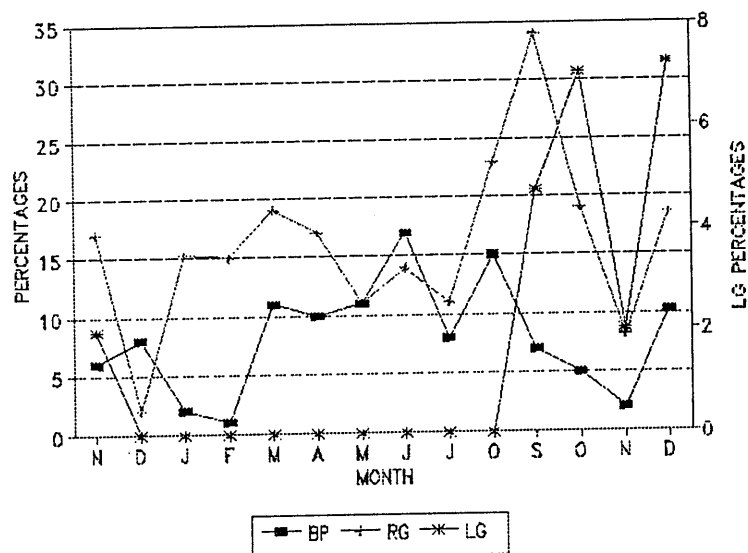


Figure 3-3: The percentage of P.radiata infected with Polydora (BP) and Cliona on the right (RG) and the left (LG) valve of the shell, between the period, November 1992 to December 1993.

For details see Appendix 3-1.

To summarize, nine associated species of biofouling organisms were found to characterise the community structure of this shallow pearl oyster population. The bryozoa Bugula sp. and the alga H.triquetra were the common biofouling species. An unidentified species belonging to the trematode genus Bucephalus was found parasitizing the adult gonad of a small proportion of the pearl oysters sampled. There was no indication of seasonality in its appearance nor any trend in the size of pearl oysters infected.

Natural pearls were very rare in the sampled oysters (1.93% of oysters bore pearls) and they were of a small (<2mm) diameter and of no commercial value. There was no apparent relationship between the incidence of pearls and the presence of P.vulgaris in these oysters. The maximum pearl yield of 4.8% occurred in class 7 (>64mm ≤69mm DVM) in this population.

Polydora and Cliona were more common on the right valve than the left valve of the pearl oysters and they were most frequent in summer.

3-4 Discussion

3-4.1 Biofouling community

Kawahara (1961) studied the spatial composition of fouling communities in pearl oyster farms at Ago Bay, Japan. There were 26 species in total. Kawahara (1961) divided the community structure into two categories (1) a complex deeper water community where barnacles dominate, and (2) a simple shallower water community where worms and bryozoans prevail. He attributed the significant difference in community structure of the fouling biota to differences in the water depth and availability of food and temperature.

In this study the pearl oyster population was shallow (5m depth) and the dominant biofouling organisms were the bryozoa Bugula sp. and the alga H.triquetra. Therefore, it can be considered as falling into the second category of Kawahara (1961) in being simple in its community structure and shallow in its depth.

3-4.2 Parasitic infection of the adult gonad with Bucephalus sp.

Plate III(B) shows a parasitized gonad of a pearl oyster in which the follicle wall is damaged suggesting that this parasitized oyster will not initiate gametogenesis; this situation leads to full destruction to the gonad noted earlier. The presence of Bucephalus in some monthly samples and its absence in others suggest that infection with Bucephalus sp. is spasmodic and maybe correlated with the sudden appearance of the final host, most probably a group of fish or possibly the appearance of the second intermediate host in vast numbers (Rothschild, 1936).

The monthly maximum infection with Bucephalus was 4.5% reported in class 8 (>69mm ≤74mm DVM). No general trend is associated with seasonality nor with the size of oyster.

Similar observations made by other authors have suggested that various species of the trematode of the genus Bucephalus are implicated in the castration of the gonad of bivalves. In the pearl oysters P.albina (Tranter, 1958a) P.martensii was found to be infected with B.varicus (Sakaguchi, 1966a,b; Funakoshi, 1992). B.haimeanas is occasionally found in Ostrea edulis and Crassostrea virginica (Galtsoff, 1964), and Thangavelu & Sanjeevaraj (1987) found B.haimeans infected (17.7%) C.madransensis. Bucephalus sp. infected (35%) C.viginica (Cheng & Burton, 1965). B.mytili infected (1.23%) M.edulis (Seed, 1969), and Lasiak (1992) reported Bucephalus sp. in the

mytilids, Perumytilus purpuratus (1%) and Semimytilus alcosus (20 to 32%) Boyden (1971) found digenetic trematodes (= perhaps, Bucephalus sp.) in the cockles Cerastoderma edule (13.1%) and C.glaucum (0.41%).

However, both Wolf & Sprague (1978) and Nasr (1982) have implicated an unknown parasite of spherical shape as a cause of mortality in the pearl oysters, P.maxima and P.margaritifera, respectively. Johnston & Halton (1981) suggested that in the Irish Sea Abra alba harbouring cercariae of B.gracilescens and being the first intermediate host, contributed to the infection of the whiting which feeds on the benthic organisms. Moreover, Schaefer & Hoffmann (1985) attributed fishkill incidence in Main River in Germany to B.polymorphus.

According to Sakaguchi (1966a,b) B.varicus infects the Japanese pearl oyster P.martensii; the first intermediate host, and subsequently infects the caranid fishes Caranx sexfaciatus and C.ignobilis which are caught in the pearl farms.

If Schaefer and Hoffmann's (1985) suggestion that some species of Bucephalus may cause fishkills is correct then could fishkills which took place in July 1993 in Bahrain, and in September of the same year in UAE, Iran and Kuwait be linked to Bucephalus?

Bucephalus sp. reported in P.radiata in this study appears, to the best of the author's knowledge, to be the first and only documented record of this species in the Gulf area. The lack of knowledge on similar occurrences of Bucephalus in fishes of the

Gulf make Schaefer & Hoffmann's (1985) idea difficult to test at present but nonetheless reinforces the fact that research in this field is urgently needed. According to Funakoshi (1992) who states that the only practical treatment against B.varicus, which invades cultured pearl oysters in Japan in August and September, is to move the oysters to non-infected waters. In this study, however, the results showed no apparent seasonality in the infection with Bucephalus sp. in P.radiata and finding non-infected waters would therefore be difficult.

3-4.3 Incidence of natural pearls

Mohammad (1972) found a 4.39% incidence of pearls in P.margaritifera (= P.radiata) from Kuwait, of which only 19.43% were infected by P.vulgaris. The percentage of pearl carriers was higher among older oysters than young ones. Mohammad (1972) found an inverse correlation between the percentage of infestation and weight of pearls yielded per oyster. He attributed this to the fact that infested oysters must use a considerable amount of nacre to wall out the intruding pest.

Amongst Mohammad's (1972) oysters, pearl carriers were more prevalent among oysters infested by P.vulgaris and he attributed this to the effect of detritus propelled toward the oyster by the ciliary-feeder worm which might have played a part in the introduction of pearl-inducing particles into the oysters.

In contrast, Ambariyanto & Seed (1991) studied M.edulis infested with P.ciliata and found no significant relationship between the incidence of pearls and the intensity of infestation.

In this study, crosstabulation of P.vulgaris by pearl incidences (table 3.1) revealed no uniform pattern to support Mohammad's (1972) theory. However, fewer differences exist between the Arabian Gulf populations of P.radiata in the percentage of those bearing pearls (table 3.4). A considerably higher frequency of pearls is found in M.edulis from North Wales (Ambariyanto & Seed, 1991).

Other views concerning pearl formation do exist and they are presented here for information only. Cheng (1967) criticized the theory proposed by Herdman (1903-1906) who believed that the cestode, Tettrahyncus unionifactor was the causative agent of natural pearls in the Sri Lankan P.radiata, and suggested the genus as Tylocephalum. Funakoshi (1992) states that in the Japanese P.matensii, a natural pearl is formed when a piece of outer epithelium drops into the soft tissue of the oyster. This is then it is changed into a bag-like structure separated from the mantle membrane. Shell nacre is produced within this bag-like structure known as the pearl sac.

In contrast, Seed (1969; Lutz, 1980 for review) suggest that pearl formation in M.edulis might be caused by the trematode, Gymnophallus sp. and the encysted metacercarial stages of Parorchis acanthus which are found in the mantle tissues.

In this study, the maximum incidence of pearls (4.8% was reported in class 7 (>44mm ≤69mm DVM) and the absence of pearl bearing oysters in classes 8 and 9 > 69mm DVM may be due to expulsion or rejection of pearls from larger individuals. This process is encountered in pearl culture operations (Gervis & Sims, 1992). Therefore, for this particular population of pearl oysters, the maximum pearl yield is in class 7 and the targeted size should be between classes 2 and 7, that is from 39 to 69mm DVM.

The result agrees up to a point with Mohammad's (1972) data for the same species and the study by Fernandes & Seed (1983) on M.edulis in which the incidence of pearls is higher in larger sized animals. However, above a certain size expulsion of pearls by oysters may occur.

It should be noted, however, that the size (diameter) of pearls is important in determining their commercial values. This study and others (Mohammad, 1972; Almatar, 1994; Al-Rumaidh, 1994) have confirmed that in addition to their rare occurrence, the majority of obtainable natural pearls from Gulf P.radiata are less than 3mm diameter and of little or no commercial value.

3-4.4 Infestation with Polydora and Cliona

In this study, very few infestations with Polydora were found on the left valve and the data were therefore combined with those of the right valve. Mohammad (1972) also

found (table 3.2) a difference in the infestation with Polydora on the right and the left valves of P.radiata from Kuwait. Mohammad (1972) believed that the right valve in P.radiata, being immovable and closer to the substrate, is more suitable for infestation by the crawling stage of young spionids than the upper left valve.

The highest infestation (10.5%) with Polydora was reported in class 1 (= 35.05 to 39mm DVM) and this contrasts with the result presented by Mohammad (1972) who found a higher infection rate (14.77%) in class 9 + (= 90mm to 100mm DVM) and other mollusc species (table 3.2) where the incidence increased with size.

The sponge Cliona vestifica infested almost all classes of the right valve being a maximal 23% in class 8 (>69 ≤74mm DVM) but favoured the left valve of class 1 (35.05 - 39mm DVM) being a maximal 11%. Both valves were infected at a higher rate than by C.celeta in the limpet (Baxter, 1984) but less than that found in M.galloprovincialis infected by C.vestifica (Tkachuk, 1988) and the abalone (Clavier, 1989), as reported in table 3.3. It seems, therefore, that the same species of Polydora and/or Cliona behave differently on different targets (shell valves) of the host and also in relation to different size classes. Moreover, Polydora and Cliona interact differently with different host species or the same species at different localities. This may be due to the overall difference of the community structure (Kawahara, 1961; and this study) between populations of hosts.

Chapter 4: Reproduction and production of triploid pearl oysters.

4-1 Introduction

The pearl culture industry comprises six main steps and they are as follows: 1) The preparation of recipient pearl oysters. 2) Bead insertion. 3) Convalescence. 4) Nurture. 5) Harvest. 6) Products (Mizumoto, 1976). The most important and difficult procedure is the preparation of a host oyster. The host oyster will determine the quality of the pearl that will be produced. The best site to accommodate a bead or nucleus, a future pearl, is the gonad (Mizumoto, 1976). Ripe oysters are not good candidates for bead operation since they either reject the beads or do not usually survive the operation. Because of this, the Japanese pearl culturists have either to inhibit or accelerate gonad maturation (by low or high temperature) of the host oysters. This procedure has been problematic and time consuming. Therefore sterile triploid pearl oysters may be good alternatives for pearl culture.

A triploid embryo, is an individual whose genomic content possesses three sets ($3n$) of chromosomes, rather than the normal two sets, diploid ($2n$). The gonadogenesis of triploids has been found to be either reduced (Tabarini, 1984; Allen & Downing, 1986; Jiang *et al.* 1990; Komaru & Wada, 1994) or absent (Allen *et al.* 1986). Triploidy can be induced by the application of thermal shock (low or high temperature), pressure shock or the chemical action of cytochalasin

B (CB) during the egg maturation division. Recently, 6-dimethylaminopurine (6-DMAP) has been found to be less toxic and inexpensive substance and a good alternative to CB (Desrosiers et al. 1993; Beaumont, pers. comm.).

Cytochalasin B (CB), a fungal metabolite, has been found to prevent cytokinesis, polar body formation but not karyokinesis, the movement of chromosomes. The commonest procedure to induce triploidy in bivalves is the use of CB. If meiosis I is targeted and the first polar body extrusion is prevented, meiosis I triploids will be produced, whereas, targeting meiosis II by inhibiting the formation of the second polar body, will yield meiosis II triploids.

Triploidy has been induced in several edible bivalves such as Crassostrea virginica (Stanley et al. 1981), C.gigas (Quillet & Panelay, 1986) Mya arenaria (Allen et al. 1982; Mason et al. 1988), Argopecten irradians (Tabarini, 1984), Pecten maximus (Beaumont, 1986), Chlamys nobilis (Komaru et al. 1988; Komaru & Wada, 1989, 1991), C.varia (Baron et al. 1989), Tapes semidecussatus (Beaumont & Contaris, 1988; Gosling & Nolan, 1989; Utting & Child, 1994), Mytilus edulis (Yamamoto & Sugawara, 1988; Beaumont & Kelly, 1989) Ostrea edulis (Gendreau & Grizel, 1990), and the pearl oyster Pinctada martensii (Jiang et al. 1987, 1990, 1993; Uchimura et al. 1989; Wada et al. 1989; Komaru et al. 1990 a,b; 1991; Komaru & Wada, 1994; Li et al. 1992; Yi et al. 1993) (see Beaumont & Fairbrother, 1991, for review).

Stanley et al. (1984) and Beaumont & Kelly (1989) postulated that the significance of increased early growth found in meiosis I triploids, compared to meiosis II triploids or diploids is related to the overall increase in heterozygosity in meiosis I triploids. Increased triploid performance at a later stage is due to sterility since sterile animals which have problems with pairing of homologous chromosomes at meiosis divert the metabolic energy allocated for gametogenesis and reproduction to somatic growth (Tabarini, 1984). It is interesting to note that sterile animals are also favoured in situations where exotic species are to be introduced since they cannot breed and out-compete related indigenous species.

This study reports on preliminary aspects of the reproduction of triploid pearl oysters and it is the first of its nature in the Arabian Gulf region.

4-2 Materials and methods

4-2.1 Collection and maintenance of a pearl oyster sample

On 12 April 1990, a batch of 150 individuals of Pinctada radiata was collected by SCUBA diving from a newly discovered Pearl Oyster bed off Al-Busaiteen village (Muharaaq, State of Bahrain, Arabian Gulf). The oysters were wrapped in damp cloths, packed in a polystyrene box and air-freighted live to Manchester, UK thence by car to the University College of North Wales, Marine Science Laboratories, Menai Bridge, Gwynedd. On arrival the oysters were held alive in 100 l indoor controlled temperature ($23 \pm 1^{\circ}\text{C}$) aquarium with a continuously circulating seawater flow. the oysters were held under a quarantine system which involved retaining the drainage water from the aquarium for 24 hours in a 200 l bin for chlorination treatment before allowing the water to enter drains. 'Chloros', a commercial solution of sodium hypochlorite, was used at a concentration of 0.05% v/v.

4-2.2 Fertilization, triploidy induction and larval rearing

Preliminary trials were carried out on induced spawning (4 attempts in total. using 10 oysters in each trial) through injection of 0.5-1 ml of 0.5M KCl and/or 2 mM serotonin (Braley, 1985) into the adductor muscle of the oyster using a syringe or thermal shocks, by transferring oysters from 25°C to 37°C. These trials resulted in liberation of inactive sperm from males but no gametes at all from females. Therefore, the gonad stripping technique as suggested by Wada & Wada (1953); Wada (1961, 1963) and Kuwatani (1965), on P.martensii was adopted without modification.

4-2.3 Handling the eggs and treatment with cytochalasin B.

Experiment 1

Twenty oysters were removed from the tank. Each oyster was opened and its gonad excised with a clean scalpel. Eggs from 14 females and sperm from 6 males were placed in 2 separate 1 l glass dishes containing ammoniacal (0.5×10^{-3} M NH_4) UV irradiated, $0.2 \mu\text{m}$ filtered seawater at a temperature of 28.5°C. The gametes were held separately until all glassware and chemicals were prepared in order to avoid commencement of fertilization too early. The pooled eggs were screened through an $80\mu\text{m}$ and a $40\mu\text{m}$ sieve to remove tissue remains and debris but to retain eggs. The eggs were then finally collected on a $20\mu\text{m}$ mesh sieve.

The eggs were thoroughly rinsed with fresh filtered seawater and placed in a 1l graduated cylinder. Eggs were maintained in suspension using a plunger and 5 random samples of 20 μ l were examined under a compound microscope to count the number of eggs.

One mg of Cytochalasin B (CB) was dissolved in 200 μ l of the carrier, dimethyl sulphoxide (DMSO). The resultant solution was placed in a 2l conical flask and topped upto 2l with filtered seawater giving a final concentration of 0.05 mg CB l⁻¹ in 0.01% DMSO in filtered seawater. One hundred μ l of DMSO was added to 1 l of filtered seawater, this gave 0.01% DMSO in filtered seawater. Two hundred ml of 0.5 CB l⁻¹ in 0.01% DMSO in filtered seawater was added to 800 ml of 0.01% DMSO in filtered seawater giving a final concentration of 0.01 CB l⁻¹ in 0.01% DMSO in filtered seawater.

Eggs suspended in a 1 l graduated cylinder containing ammoniacal filtered seawater 0.5 X 10⁻³M NH₄ were activated by the addition of 100 ml of concentrated sperm. The suspension was stirred by the plunger to ensure complete mixing of eggs and sperm.

The activated eggs were exposed to the chemical action of the CB at two concentrations, 0.1mg CB l⁻¹ in 0.01% DMSO in filtered seawater, each at two time intervals after activation; one to target meiosis I, between 2-20 minutes post

activation and the other to target meiosis II, between 15-30 minutes.

Seven 1 l crystallizing dishes were set up. Three of these were controls. One control had filtered seawater and was designed for sampling embryos at 5 minute intervals between 0 to 90 minutes after activation. This dish received 400ml of the activated eggs (800×10^3 eggs) and was discarded after 90 minutes. The second control dish contained filtered seawater and the third contained 0.01% DMSO. Neither of these dishes were sampled during embryo development. The fourth and fifth dishes were of meiosis I treatment using 0.1 and 0.5 mg CBI^{-1} whereas the sixth and seventh dishes were for meiosis II treatment using 0.1 and 0.5 mg CBI^{-1} .

Each dish other than the "control sampling" received 100 ml of the activated eggs (200×10^3 eggs). The activated eggs were held in their dishes in a 20 μm nylon mesh sieve (cut from PVC piping, 14.5 cm diameter X 6 cm depth) during treatment.

Eggs allocated for the control and meiosis I treatment were placed in their dishes after 5 minutes from insemination whereas those of meiosis II treatment were held in "stand-by" dishes until 15 minutes had elapsed from insemination. After treatment with the CB eggs were rinsed in 0.01% DMSO in filtered seawater for 15 minutes and then transferred to fresh filtered seawater.

Samples taken from control dishes of both experiments at 5 minute intervals, between 0 and 90 minute post-activation, were used to establish and to illustrate the meiotic or maturation division.

Samples of embryos from meiosis I treatments were taken at 15, 30, 45 and 90 minutes post-activation and from meiosis II treatments at 30, 45 and 90 minutes post-activation. This was done by raising the sieve out of the seawater and washing the embryos to one end of the sieve. A plastic pipette was used to transfer early embryos to a small vial containing Carnoy's fixative (1 part glacial acetic acid and 3 parts alcohol). Embryos taken at 90 minutes after activation, however, were transferred to small PVC tubes closed at the bottom by 20 μ m nylon mesh. These tubes containing embryos were held in separate 100 ml beakers containing 0.2% Colchicine in seawater for 30 minutes to arrest cell divisions at metaphase. Embryos were then transferred into diluted seawater (1 part seawater and 3 parts distilled water) for 20 minutes to aid spreading of the chromosomes on the spindle. They were finally fixed in Carnoy's fixative in labelled vials for chromosome analysis.

The temperature at various stages of treatment ranged from 28.5°C (maximum) during egg handling and fertilization to 26°C (minimum) at the rearing phase.

After incubation of the embryos in the dishes for 24 hours, larvae from the two

control dishes were pooled to give a control culture. Similarly, larvae from meiosis I (0.1 and 0.5 mg CBl⁻¹) and meiosis II (0.1 and 0.5 mg CBl⁻¹) were combined giving meiosis I and meiosis II cultures respectively.

Each culture produced in this experiment contained D-shaped larvae which were reared in a 2l plastic container at a density of 30 larvae ml⁻¹. No aeration was provided. The larvae were fed with Rhinomonas reticulata (Lucas) Novarino and Pavlova lutheri (Droop) Green at 10 and 40 cell μl^{-1} , respectively. At 72 hours, 25 individuals from each culture were measured on two axes, the longer anteroposterior (APM) axis which is the shell length and the shorter dorsoventral (DVM) axis, the shell height, using a micrometer eyepiece.

Experiment 2

This experiment was similar to experiment 1 except that ten individuals (4 females and 6 males) were dissected. Each treated dish received 156×10^3 eggs. Embryos from this experiment grew into "D" larvae and were cultured through the veliger stage to spat. They were measured weekly for their APM and DVM for a five week period. The larvae were fed on the same diet provided for the larvae of the first experiment but three times a week. The larvae in their third week of age were suspended in a PVC tube (measuring 10.5 diameter X 14cm depth closed at the bottom with 80 μm mesh), this tube provided a settlement substratum for spat

and was placed in the 2 l container.

After five weeks, spat were settled and were fed once weekly with Rhinomonas reticulata (Lucas) Novarino and Pavlova lutheri (Droop) Green at 20 and 80 cell μl^{-1} , respectively, and reared for more than five months to confirm growth and survival on laboratory conditions.

4-2.4 Chromosome analysis

Fixed samples of eggs and embryos were removed from the sample bottles using a plastic pipette. These were placed on a microscope slide and evenly spread using a pin. Excessive fixative was allowed to evaporate and a few drops of aceto orcein stain in acetic acid (Appendix 4.1) were added to the eggs and left for 2 minutes before heating from below to near boiling with a naked flame. The slide containing the stained eggs was covered with a square 22 X 22 mm (thickness = 0) cover-slip. A little thumb pressure was applied onto the cover-slip to spread the chromosomes.

A minimum of thirty eggs from each vial were assessed for chromosomes number and presence or absence of polar bodies. Eggs and embryos were examined and photographed on a Zeiss Photomicroscope II.

4-2.5 Statistical analysis

It was intended to use ANCOVA to compare the general growth patterns exhibited by the 3 cultures from the 5 weekly sets of data of experiment 2. However, since one of the assumptions of the use of ANCOVA, i.e, the homogeneity of variances exhibited by the 3 cultures was violated, even when the data were log 10 or square root transformed, it was decided to use 5 separate one way ANOVA tests of the null hypothesis that the mean shell attributes, either APM or DVM are equal between the control, meiosis I and meiosis II cultures. The 5 weekly data sets were tested separately for their homogeneity of variance by the use of the Levene homogeneity test using the SPSS statistical package (1992).

This test is not heavily dependent on the assumption of normality. When the Levene test showed heterogeneity of variances, then the weekly data were log 10 transformed. If heterogeneity still persisted, then the data were square root transformed. When square root transformation failed to remove the heterogeneity, the non-parametric (NPAR) Kruskal-Wallis (K-W) test, analogous to one way ANOVA, was employed.

The single larval size data set of experiment 1 was analysed by NPAR (K-W). The null hypothesis of this test states that the mean rank of treatments is equal

data sets showed homogeneity of variances. Therefore, ANOVA was employed and in all pairwise multiple comparisons between the 3 groups, either Scheffe's (for unequal sample sizes between groups) or Tukey's (for equal sample sizes) tests were used to ascertain which group(s) mean(s) differed significantly from which others.

4-3 Results

4-3.1 Fertilization, triploidy induction and larval rearing

a - spawning

Preliminary trials on induced spawning by the use of 0.5M KCl or serotonin through injection into the adductor muscle with a syringe, or thermal shock (25°C to 37°C) resulted in liberation of inactive sperm but no gametes at all from females. Therefore, the stripping technique developed by the Japanese scientists (Wada & Wada, 1953; Wada, 1961, 1963; Kuwatani, 1965) on P.martensii was adopted. The results reported in this chapter are derived from two experiments after direct stripping of gonads.

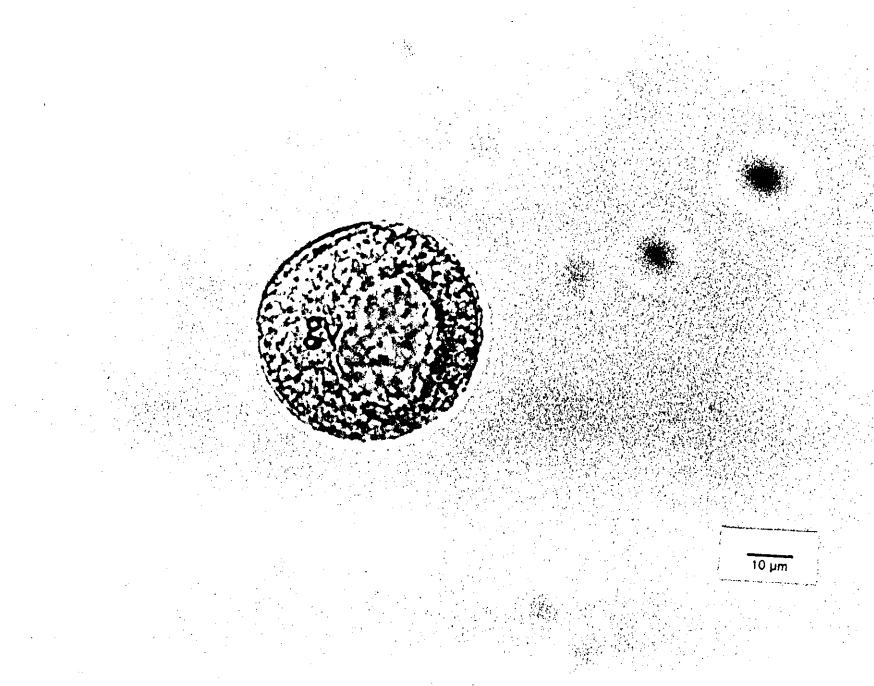
b - eggs

Eggs stripped directly from a female gonad and kept in plain seawater possess a germinal vesicle (g.v) in the centre (Fig 4.1). Eggs underwent germinal vesicle breakdown when they were exposed to the ammoniacal seawater (Fig 4.2).

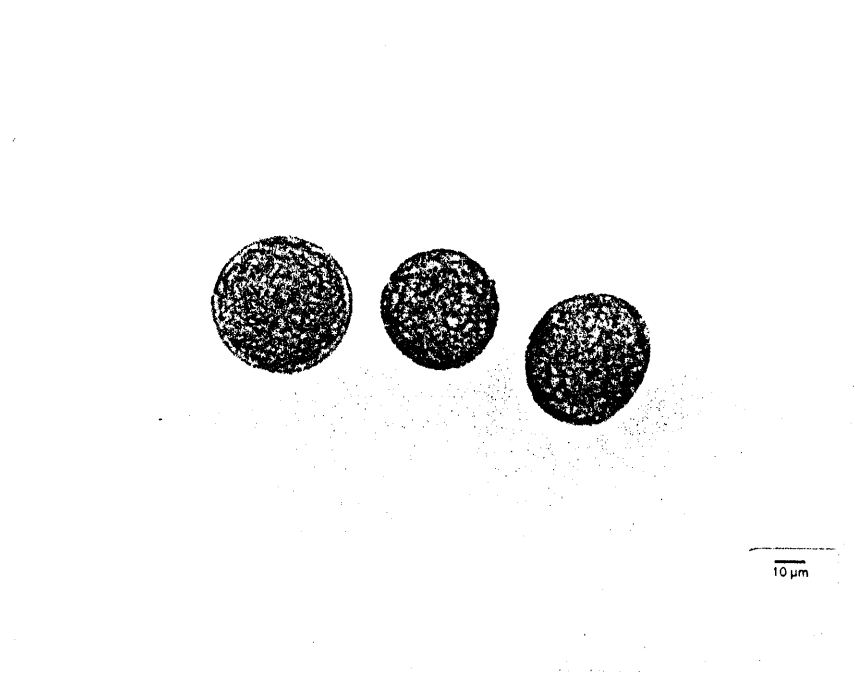
(1) Figure 4-1: Shows an egg stripped directly from a female gonad. The egg possesses germinal vesicle (g.v).

(2) Figure 4-2: Eggs exposed to ammoniacal seawater and germinal vesicle breakdown.

1



2



c - Sperm

Sperm stripped from male testis are initially sluggish or immotile but become intensely active and capable of fertilization when placed in ammoniacal seawater.

d - Fertilization and life history

The average size of activated eggs is 45 μm and the range is 42-48 μm .

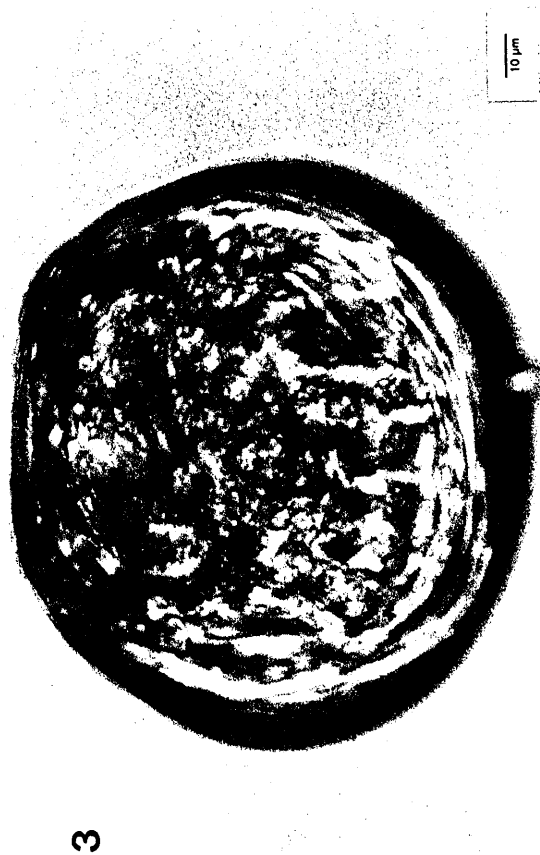
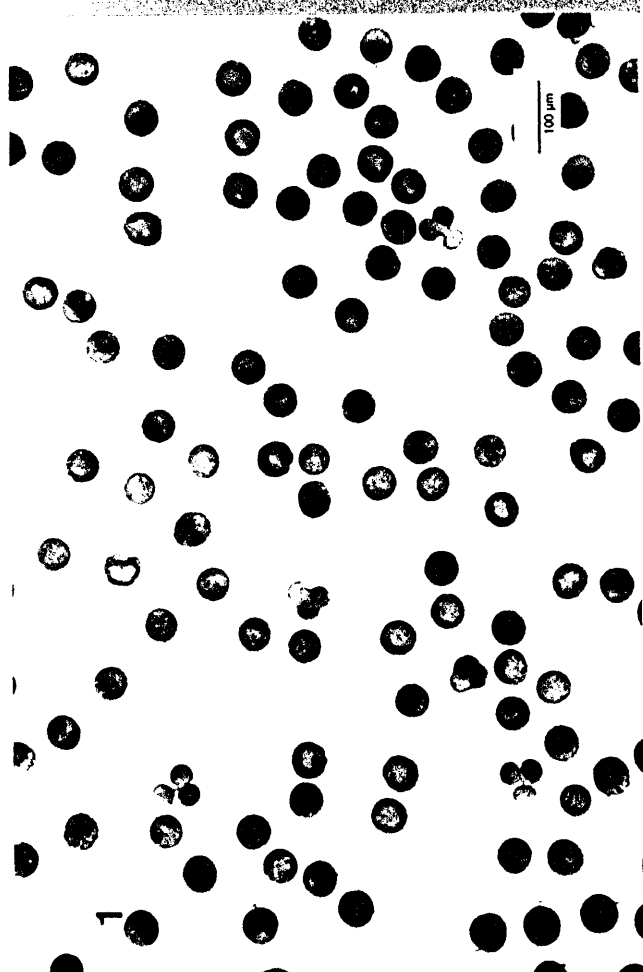
Plate IV(1) shows the first cleavage which is along the polar axis so that the polar bodies come to reside in the cleavage furrow. The resulting body of the 2 cell stage is a trefoil form where one cell of the pair has a large polar lobe pinched off from it. This could be mistaken for a three-cell stage, but is a normal stage in development of bivalve embryos (Raven, 1966).

Straight-hinge stage

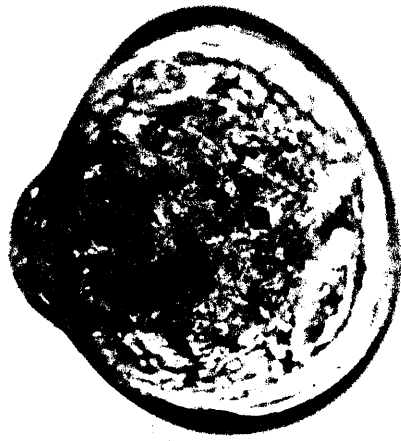
The trochophore develops to a straight-hinge or "D-shaped" veliger larva by 24 hours after insemination at a temperature of 26°C. Plate IV(2) pictures an early D-shaped larva measuring 80 X 70 μm . This larva swims by means of the velum, using ciliary locomotion. The larva is characteristically transparent but exhibits a granulated interior.

PLATE IV

- 1 Eggs at first cleavage, trefoil form at 80 min Post activation.
- 2 An early straight-hinge veliger; size 80 x 70 μm .
- 3 Early umbo stage; size 130 x 125 μm .
- 4 A) 17 - day umbo larva; size 170 x 170 μm .
- 5 22 - day eye spotted pediveliger, size 220 x 200 μm .
- 6 A) 29 - day plantigrade; size 250 x 235 μm .
B) 29 - day spat; size 350 x 320 μm .
- 7 Spat 42 days old; size 529 x 429 μm .
- 8 Spat - 5.5 months old.



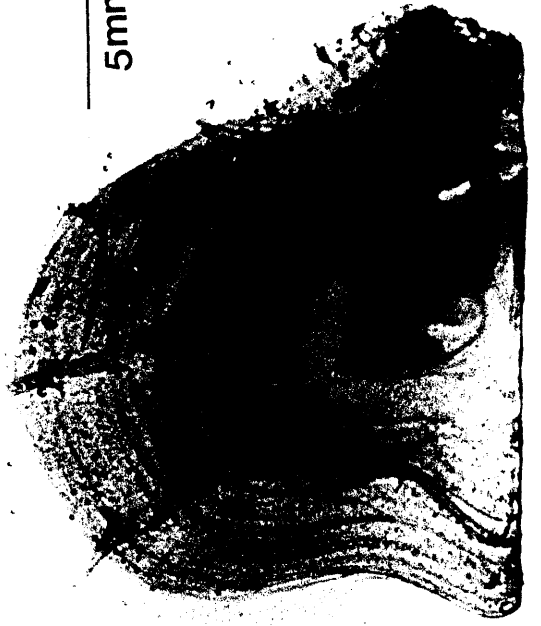
5



7



8



6



Umbo stage

As it grows the straight-hinge larva gradually develops an elevation at the umbonal region. Plate IV(3) shows an early umbo larva measuring 130 X 125 μm and Plate IV(4A) shows a 17 day old umbo larva measuring 170 X 170 μm . The valves are equal. The yellowish digestive apparatus is distinctly visible.

The eye spot / pediveliger stage

Toward the end of the veliger development, the larva tends to keep to the bottom, having passed through the swimming stage to a crawling "pediveliger" phase. Plate IV(5) shows a 22 day old pediveliger measuring 220 X 200 μm with an eye spot. This pediveliger, as its name implies, has developed a foot by which it crawls and its velum is reduced.

Plantigrade / spat stage

During "metamorphosis" a number of morphological changes occur in the larva between attachment and the formation of the post-larval, or dissoconch shell (Sastry, 1979). A metamorphosed plantigrade, or spat measuring 250 X 235 μm is shown in Plate IV, 6(A). The velum has disappeared and dissoconch shell is

made up of transparent and uniformly deposited conchiolin.

Plate IV(6B) pictures an early 29 day old spat measuring 350 X 320 μm . The spat begins to take on the appearance of the adult shell, or dissoconch. The shell of the spat assumes a distinct character; the left valve is slightly more concave than the right. The spat attaches itself to the substratum by byssus threads. Surprisingly, the spat were often found climbing upwards and occasionally even to the top edge of the rearing container which was out of water. Little is known of the behaviour of bivalve spat, in general. and future studies on this aspect of the life cycle of pearl oysters will have relevance not only to oysters but also to other bivalves.

4-3.2 Chromosome number, meiosis and triploidy induction.

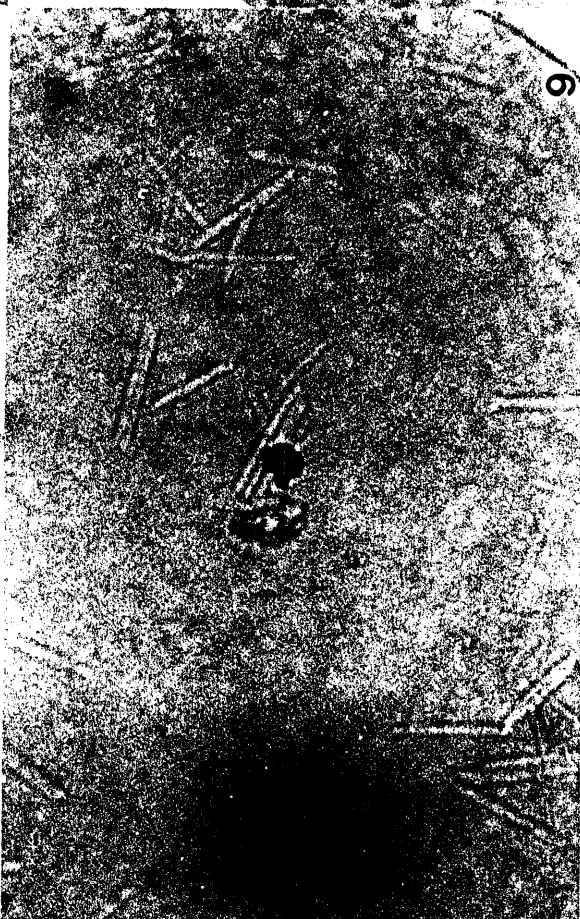
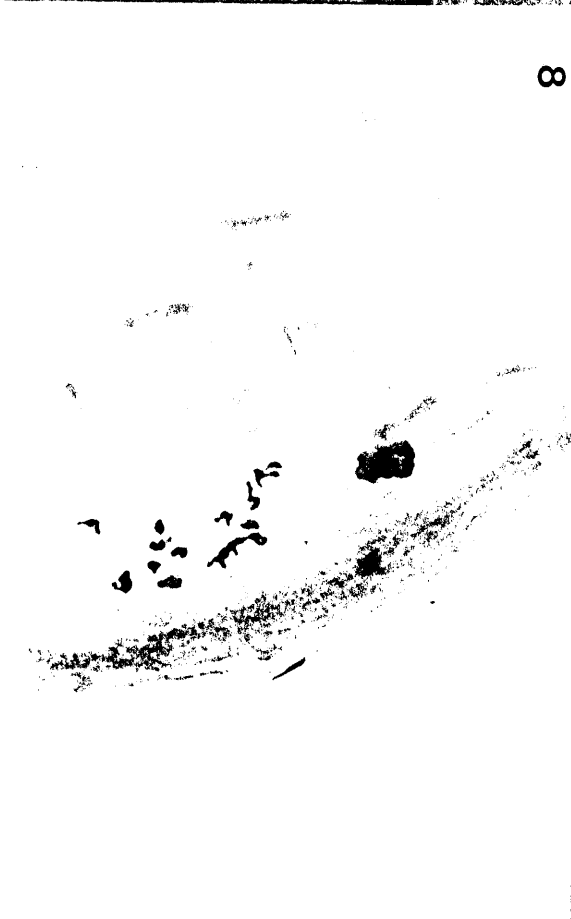
The haploid number of chromosomes reported from hundreds of unfertilized eggs was fourteen pairs (bivalents) of chromosomes at metaphase of meiosis I. In Plate V (1) however, a range of 11 to 17 bivalent chromosomes of the metaphase I were observed in a small number of unfertilized eggs (Plate V (2)). Unactivated eggs sampled at various time periods remained unchanged at the metaphase I stage.

Appendix 4.2 documents the results of the inspections made to the control samples and show that the first polar body (Plate V 5) and the second polar body

PLATE V: P. radiata, Meiosis, reduction division events

Magnification x 800 throughout.

	<u>Condition</u>	<u>Time</u>	<u>Event</u>
1	Metaphase I		14 bivalent chromosomes
2	Metaphase I		17 bivalent chromosomes
3	Late Metaphase I		
4	Late Metaphase I/Anaphase I		
5	Telophase I	20 min	First polar extrusion
6	Metaphase II		
7	Anaphase II		2N+Pb
8	Telophase II	30 min	N+2Pb, second polar body extrusion



1



2



3



4



(Plate V 8) began to be extruded at around 5 minutes and 20 minutes post activation, respectively. Meiosis I took place between 5-20 minutes post-activation Plate V 1-5) between 20-30 minutes (Plate V 6-8).

Chromosome counts of mitotic division, in both experiments, in embryos sampled at 90 minutes post-activation could not accurately be assessed because embryos were commonly in interphase where chromosomes could not be seen thus making definitive judgement of triploid production difficult.

Appendix 4.3 shows the results of the condition of the eggs and/or embryos treated with the cytochalasin B at 15, 30 and 45 minute post-activation recorded in the two experiments. Some treated eggs fell into two unique classes. The first class, was characterised by the possession of two groups of 14 chromosomes but no polar body and this type of eggs was produced in samples of eggs treated during meiosis I (Plate VI 1 and 2). The second class of eggs consisted of two sets of 14 chromosomes and the first polar body, but no second polar body, and these eggs were produced after treatment during meiosis II (Plate VI 3 and 4).

Eggs belonging to either class are termed "diploid eggs" (Beaumont & Kelly, 1989). These diploid eggs have the potential to produce triploid embryos after syngamy with a sperm pronucleus.

PLATE VI: Diploid eggs.

Magnification x 800 unless otherwise stated.

1 and 2: Meiosis I diploid egg

2 sets of approximately 14 chromosomes.

3 and 4: Meiosis II diploid egg

2 sets of approximately 14 chromosomes and first polar body.

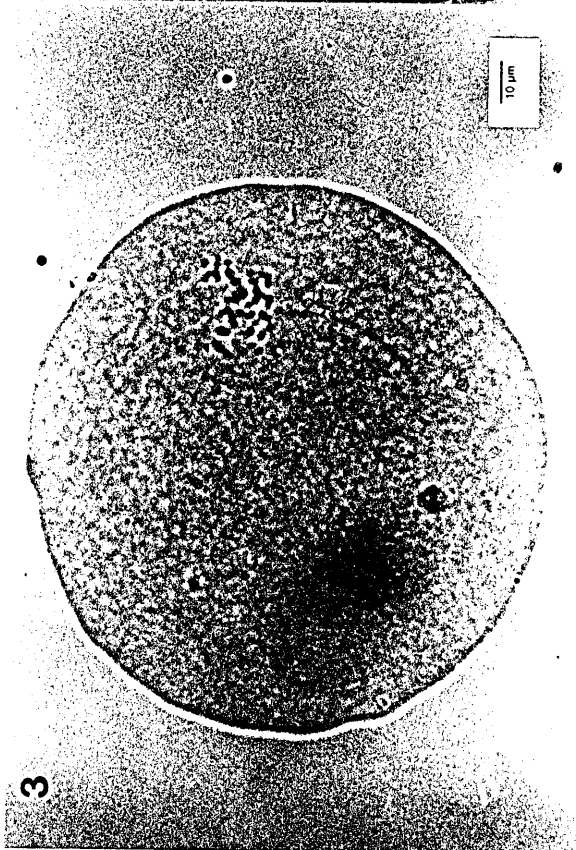
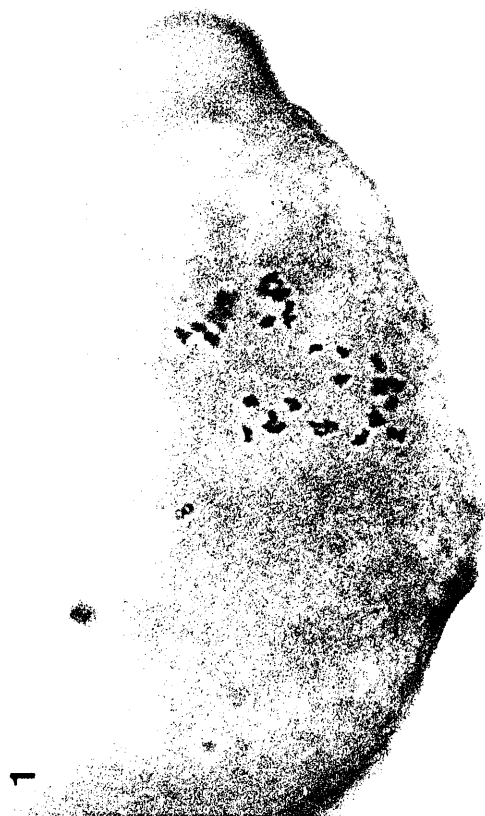
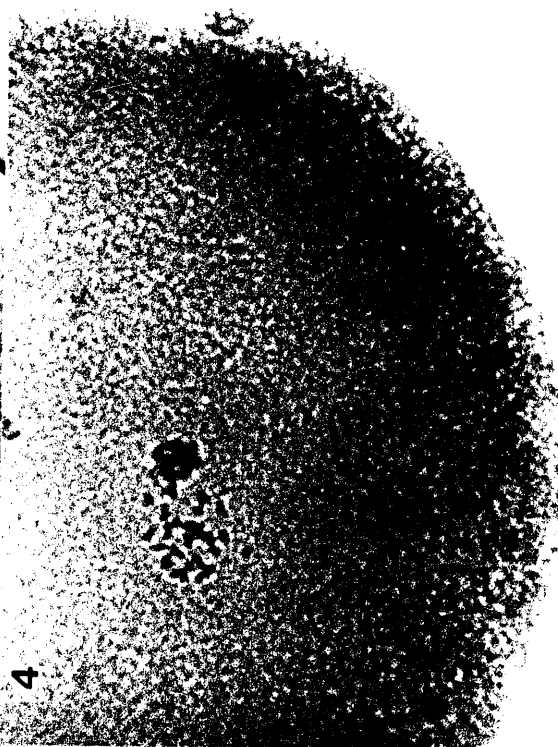
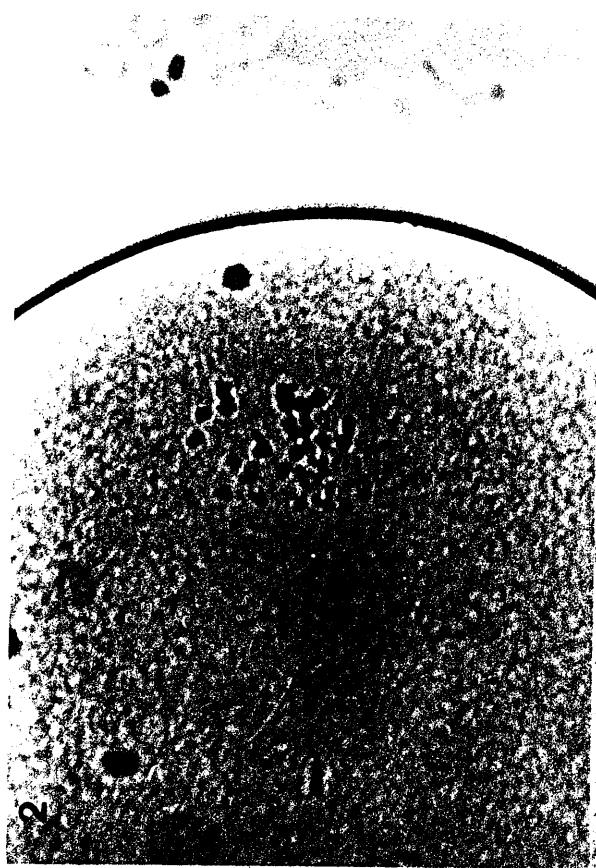


Table 4.1 summarizes the results reported in Appendix 4.3 and lists the mean value of percentages of diploid eggs in different treatments by the cytochalasin B in the two experiments. No diploid eggs were observed in the control of either experiment.

Both meiosis I and meiosis II diploid eggs were observed in samples of treated eggs in both experiments and the highest percentage estimate of diploid eggs resulted from the treatment with 0.5 mg CBl⁻¹ in experiment 2 (73%). In experiment 1, (16-28%) diploid eggs were produced, and the highest percentage values were observed in treatments targeted at meiosis I. However, in experiment 2, (43-73%) diploid eggs were recorded and the overall combined value of percentages in treatments targeted at meiosis II (36.5 and 73%) is higher than the pooled value of percentages in treatments targeted at meiosis I (43 and 47%).

4-3.3 Size of shell attributes, APM and DVM

Detailed in Appendix 4.4 and summarized in tables 4.2 and 4.3 are the means $\pm$ s.e of APM and DVM larvae of experiment 1 (3 days after insemination) and experiment 2 respectively (5 weekly sets of data).

Table 4.1: Mean percentages of diploid eggs produced by treatment with cytochalasin B targetting meiosis I and meiosis II. (based on results reported in Appendix 4.3)

Condition		Experiment	
		1	2
Control		0	0
Meiosis I	0.1mg CBl ⁻¹	28.5	47
Meiosis I	0.5mg CBl ⁻¹	28	43
Meiosis II	0.1mg CBl ⁻¹	16.5	36.5
Meiosis II	0.5mg CBl ⁻¹	26.5	73

Table 4.2: *P. radiata* embryos and larvae, mean APM in $\mu\text{m} \pm$ (s.e.) in three different cultures of EXP 1 and EXP2.
Sample size (n) = 25 unless otherwise stated*.
Other details: refer to Appendix 4.4.

EXP	Week	Control	MI	MII	ANOVA		NPAR K-W ANOVA	
					F	P	χ^2	P
EXP 1	- WK1	70.9 (1)	68.6 (1.1)	68.6 (2)* n = 10			1.7261	0.4219
EXP 2	- WK2	74.4 (1.2)	78.7 (1.1)	82.9 (2.1)	7.36	0.0012		
	- WK2	76.9 (1.85)	77.2 (1.2)	100.7 (3.8)	28.61	<0.0001		
	- WK3	97.6 (2.5)* n = 26	95.3 (3.1)* n = 12	133.8 (6.1)	22.43	<0.0001		
	- WK4	130.1 (2.6)	134.9 (2.3)	173.6 (12.2)	10.53	0.0001		
	- WK5	140.9 (3.4)	152.2 (4.2)	145.2 (5.3)	1.66	0.1971		

Table 4.3: *P. radiata* embryos and larvae, mean DVM in $\mu\text{m} \pm$ (s.e.) in three different cultures of EXP1 and EXP2.
Sample size (n) = 25 unless otherwise stated*.
Other details: refer to Appendix 4.4.

EXP	Week	Control	MI	MII	ANOVA		NPAR K-W ANOVA	
					F	P	χ^2	P
EXP1	- WK1	57.5 (1.2)	56.9 (1.1)	59.3 (1.5)* n = 10			0.7189	0.698
EXP2	- WK1	61.7 (1.4)	66 (1.1)	70.6 (2.3)	6.59	0.0023		
-	WK2	65.5 (1.8)	63.8 (0.9)	87.8 (3.7)	30.37	<0.0001		
-	WK3	85.5 (2.6)* n = 26	84 (3.1)* n = 12	124.7 (5.7)	27.22	<0.0001		
-	WK4	121.2 (2.9)	126.4 (2.2)	136.4 (4.7)	11.91	<0.0001		
-	WK5	134.1 (3.1)	145.3 (3.8)	161.3 (10.4)	2.28	0.11		

The Levene test showed heterogeneity of variances in the experiment 1 data and neither log 10 nor square root transformation removed this heterogeneity. Therefore, the non-parametric Kruskal-Wallis test was employed to test the null hypothesis that the 3 groups, control, meiosis I and meiosis II cultures have the same mean rank. The result obtained for the APM ($X^2 = 1.726$, $P = 0.421$) and for DVM ($X^2 = 0.718$, $P = 0.698$) of the three cultures showed no significant differences in their mean ranks (Tables 4.2, 4.3, and 4.4).

Tables 4.4 gives the summary results of the multiple comparison tests (Scheffe/Tukey) made between the 3 treatment cultures (= groups) for 5 weekly sets of data for experiment 2. The final conclusion based on the results (table 4.2 and 4.3) are summarized below;

1- Both APM and DVM gave similar direction of significant/non-significant results.

2- In experiment 2 week 1, the mean shell attributes of the meiosis II triploid culture were significantly larger than those of meiosis I triploids which were not significantly different from control cultures. This is contrary to the results obtained from Experiment 1 where there were no significant differences at all between the 3 cultures.

3- This week 1 pattern remained for weeks 2 to 4, in experiment 2.

4- In experiment 2 week 5, however, the mean shell attributes of the 3 groups showed no significant differences.

Clearly, then, based on week 1 results of both experiment 1 and experiment 2 though with similar experimental design, two opposing results were obtained. Furthermore, the persistent significant higher shell size of meiosis II triploid culture, found in week 2 through week 4, meiosis I and control disappeared in week 5 making a clear generalized conclusion difficult.

Based on the general observations made during the weekly measurement of APM and DVM it was noticed that mortalities were highest in the meiosis I culture and lowest in the control, particularly in week 3 (see table 4.2; it was difficult to find larvae to measure in week 3 without concentration in weeks 4 and 5). Unfortunately no systematic data were taken on the mortality rate at that time due to quarantine and space constraints.

4-4 Discussion

4-4.1 Spawning

There are few published works on the controlled spawning of pearl oysters (Yamaguchi, 1957; Alagarswami *et al.* 1983). Preliminary trials on spawning induction by the chemical action of KCl and serotonin or thermal stimulation resulted in liberation of inactive sperm for males but no gametes at all from females.

Nevertheless, histological examination of the gonads revealed that the oysters were at their fully developed or ripe stage. Sperm obtained by chemical or thermal shocks and stripping were initially inactive. Furthermore, eggs obtained from excised gonads possessed germinal vesicles (Figure 4.1).

According to Wada & Wada (1953); Wada (1961, 1963) and Kuwatani (1965) the use of ammoniacal seawater, at an optimum concentration of 0.5×10^{-3} M NH_4 , will enhance the motility of sperm and disrupt the germinal vesicle of the egg of many species of the genus Pinctada.

In this study, the use of ammoniacal seawater has shown itself as an indispensable technique for increasing the ripeness of the inactive sperm and breaking down the

germinal vesicle in the egg (figure 4.2).

It is recommended, however, that further research should be conducted into the controlled spawning of this species.

4-4.2 Fertilization and life history

In this study, the average size of activated egg was 45 μm and the range was 42-48 μm . The results of this study agree with other similar studies on the species of the genus Pinctada. Cahn (1949) gives 48 μm for P.martensii, Minaur (1969) 59-60 μm for P.maxima, and Alagarwami et al. (1983), 47.5 μm for P.fucata (= P.radiata).

There have been a few attempts at rearing pearl oysters. Herdman (1903) in his first attempt to study the life-history of P.vulgaris (= P.radiata) could not take D-shaped larvae further than three days and he ultimately bridged the gap by sampling different stages of larvae and spat from nature. Herdman (1903) suggested that settling takes place five days after fertilization. Cahn (1949), reviewed the Japanese work on pearl oysters and gave brief information on the aspects of breeding. Minaur (1969) reared larvae of P.maxima but his veligers did not metamorphose.

The few works on the larval rearing given by Kobayashi & Yuki (1952), Ota (1957), Alagarwami et al. (1983) agree with the details reported in this study.

4-4.3 Chromosome number

Ojima & Maeki (1955) reported the haploid number (n) in spermatocytes of P.martensii as $n = 18$ and Tranter (1959) gives $n = 6$ for P.fucata ($P = P.radiata$). More recently, Wada (1976, 1978); Wada & Komaru (1985), Komaru & Wada (1985) studied the chromosome number in many species of the genus Pinctada and suggested the haploid number $n = 14$.

Thiriot-Quievreux (1994) has reviewed the modal haploid chromosome number in Mollusca in general and states that in the Pteriidae (7 species) as well as in the Mytilidae (11 species), the haploid number is 14.

In this study, the majority of unfertilized eggs possessed 14 bivalent chromosomes, confirming the work of Wada and his associates. However, a small number of unfertilized eggs were found to have between 11 and 17 bivalent chromosomes suggesting occasional instances of aneuploidy in this species.

4-4.4 Meiosis

The first polar body (Plate V 5) and the second polar body (Plate V 8) are extruded at around 5 and 20 minute post-activation, respectively, at 28.5°C. Meiosis I (Plate V 1-5) and meiosis II (Plate V 6-8) took place between 5-20

minute post-activation, respectively.

The sequence of meiosis reported in this study, for P.radiata, is similar to the Japanese pearl oyster P.fucata (= P.martensii) (Wada, 1976; Komaru et al. 1990a, b).

The systematic status of the Japanese pearl oyster is discussed in Khamdan (1988) and indeed more research on a direct electrophoretic comparison between both the Bahranian and the Japanese pearl oysters would be scientifically very interesting.

Chromosome counts from the mitotic division, in both experiments, in embryos sampled at 90 minute post-activation, could not be accurately made, because embryos were generally in interphase and chromosomes could not be seen. This unfortunately precluded a definitive decision on triploid production at the early embryo stage. Beaumont & Kelly (1989) suggested the "diploid egg" count as an alternative estimate for ploidy assessment.

4-4.5 Triploidy induction

In this study, the assessment of triploidy is derived from the diploid eggs which were produced in treated eggs only suggesting that they are, indeed, potential

triploids. Although triploidy has been induced and much studied in the Japanese pearl oyster, P.martensii (Jiang et al. 1987, 1990, 1993; Uchimura et al. 1989; Wada et al. 1989; Komaru et al. 1990a, b, 1991; Komaru & Wada, 1994), there is no published work on polyploidy in any other pearl oyster species. This study is the first to report on aspects of triploidy in the pearl oyster, P.radiata of the Arabian Gulf region.

The range of triploid yield in experiment 2 was between 43% and 73% and meiosis II treatments produced more triploids than meiosis I treatments. These results conflict with that obtained previously from experiment 1 and this observed difference between two different experiments but with similar design is not uncommon in laboratory situations and many explanations have been advanced to account for such variation, e.g, egg or sperm density at fertilization (Gruffydd & Beaumont, 1970), water quality (Martin et al. 1981; Utting & Helm, 1985) or genetic differences between progenitors (Beaumont & Budd, 1983). Downing & Allen (1987) attributed initial mortalities in triploid C.gigas to the combined effects of strip-spawning and CB treatment. Strip-spawned female C.gigas released eggs at all stages from immature to mature. In this study, however, egg quality variation could not be ruled out and it may have contributed some of the difference reported between the two experiments. Recently Beaumont & Fairbrother (1991) have reviewed ploidy manipulation in bivalves. Yields of triploids vary considerably amongst studies which have used cytochalasin B. Allen

et al.(1981) achieved 85% triploidy in Crassostrea virginica at 0.5 mg CBl⁻¹ whilst Wada et al.(1989) achieved 100% meiosis II triploids at 0.5 mg CBl⁻¹ in the same species. Beaumont & Contaris (1988) produced nearly 82% meiosis II triploids of Tapes semidecussatus with 0.5 mg CBl⁻¹ whilst Dufy & Diter (1990) achieved 75% triploids with 1 mg CBl⁻¹ and Utting & Child (1994) obtained 70-77% meiosis II triploids with 0.5 mg CBl⁻¹ in T.philippinarium. According to Jiang et al.(1987), Wada et al.(1989) and Komaru et al.(1990a,b) targeting meiosis II is usually much more productive than targeting meiosis I and this seems to be a general trend toward the higher production of meiosis II triploids compared to the low yield on meiosis I triploids. Furthermore, evidence suggests that triploids of meiosis I are much more vulnerable and higher larval mortalities occur in meiosis I cultures (Beaumont & Fairbrother, 1991). In this study , in experiment 2 higher mortalities were found in meiosis I than meiosis II, and in both these cultures compared with the control in experiment 2, the overall estimated percentage of meiosis II triploids (36.5 and 73%) is higher than meiosis I triploids (43 and 47%) suggesting that meiosis II culture contained more triploids than the meiosis I culture, and if triploidy itself is important, this could account for the significant increase in shell sizes, APM and DVM for the meiosis II culture in early stages of larval rearing.

Results on adult meiosis I triploids of Crassostrea virginica (Stanley et al. 1984), larval meiosis I triploids of Mytilus edulis (Beaumont & Kelly, 1989; Beaumont,

pers.comm), Pecten maximus (Beaumont, pers.comm) and P.martensii (Jiang et al. 1987), suggest that they indeed, have grown faster and larger than their diploids or meiosis II counterparts. It has been postulated that this is a result of the higher overall genomic heterozygosity expected in meiosis I triploids because homologous chromosomes normally separate at meiosis I but if this division is initially blocked all the heterozygosity of the female is retained in the targeted egg and the subsequent addition of the haploid sperm set will increase overall heterozygosity beyond that of either meiosis II triploids or diploids. However, it is now apparent that the level of heterozygosity accruing to meiosis I or meiosis II triploids at a locus is dependent on how frequent gene-centromere crossing over differs the locus (Beaumont, 1994).

It is not unknown, however, for significant differences in increase of shell dimensions to occur between laboratory cultures of apparently identical populations of bivalve larvae. Maybe this is just another example of that phenomenon. Furthermore the non-significant difference between the 3 cultures found in week 5 of experiment 2 is not uncommon in bivalve studies, e.g. Chlamys varia (Baron et al. 1989) C.gigas (Downing & Allen, 1987) Tapes philippinarum (Utting & Child, 1994) Ostrea edulis (Gendreau & Grizel, 1990). It could be that the high mortalities found in meiosis I and meiosis II cultures after week 3 in experiment 2 reduced the overall percentages of "real" triploids in week 5 and accounted for this non-significance.

As triploids reach maturity gonadogenesis is either reduced, as in the scallop, Argopecten irradians (Tabarini, 1984), the oyster, Crassostrea gigas (Allen & Downing, 1986), Chlamys nobilis (Komaru & Wada, 1989), the pearl oyster, P.martensii (Jiang *et al.* 1990, 1993; Li *et al.* 1992; Komaru & Wada, 1994), or absent, as in the clam, Mya arenaria (Allen *et al.* 1986). This is thought to be because homologous chromosomes cannot synapse in meiosis I rendering the individual sterile. From the aquaculture point of view, sterility is advantageous, since energy not utilized for reproduction may be available for somatic growth. Whether or not meiosis I triploid larvae grow best, therefore, triploids overall will have an advantage at a later stage.

The pearl culture industry is a highly specialised aqua-farming business in which the ultimate product is not food but a high value-luxury item. Pearl production involves complicated and also extremely professional manipulation. The most suitable part of a pearl oyster tissue or organ into which a bead or nucleus, the future pearl, is to be inserted, is the gonad. This is why the Japanese pearl culturists either induce their oysters to spawn or inhibit spawning by delaying maturation because mature animals are not good candidates for the operation (Mizumoto, 1976; Alagarwami *et al.* 1983). Wada *et al.* (1989) suggested that sterile pearl oysters produced by triploidy induction may be a better way of overcoming the intricate procedures of spawning induction or delaying maturation. Recent work on P.martensii has confirmed that triploid gonads are either sterile (Jiang *et al.* 1990) or abnormal (Komaru & Wada, 1994).

CHAPTER 5: General Discussion and Conclusions.

This thesis provides fundamental information regarding the reproductive biology, biofouling, incidence of natural pearls, larval production and rearing, and ploidy manipulation of the Arabian Gulf pearl oyster P.radiata.

In this study and also that of Herdman (1903) and Tranter (1959), the gonad colour is shown to be an unreliable character for sex determination in P.radiata. The morphology of the gonad of P.radiata from Bahrain is similar to other populations or other species of the genus (Herdman, 1903; Tranter, 1958a, b, c, d; 1959; Rose et al.1990). The sequence of events of gametogenesis reported in this study is identical to that observed by Rose et al.(1990) on P.maxima. Gametogenesis takes place in winter and spring.

Young P.radiata have been found settled on the shells of adults all year round, very little spawning activity out of season was noticeable and two distinct spawning maxima were found in summer/autumn. These observations are in agreement with those of Herdman (1903), Malpas (1931) and Tranter (1959) on the same species.

Tranter's (1959) observation on gonad development in young oysters accompanied by growth studies enabled him to conclude that P.radiata reaches sexual maturity at 0.6 mm HD and 20 mm DVM, which corresponded with 3.5-7 months of age. In this study, similar approaches to Tranter's (1959) were undertaken and the same conclusion was drawn based on a drop in the number of "indeterminate stage" young oysters which coincided with 0.6 mm - 1.25 mm HD and 15 - 20 mm DVM. It seems highly

probable that the species in Bahrain waters and, perhaps, in the Arabian Gulf region as a whole behaves in a similar pattern to Australian pearl oyster populations.

In this study, a bisexual gonad was found in histological preparations from a single oyster similar to that found by Tranter (1959) for the same species and that of Rose *et al.*(1990) for *P.maxima*. Furthermore, samples of adult oysters have often been found to depart significantly from a 1:1 sex ratio. The conclusion reached was that *P. radiata* may not be dioecious, the condition proposed by Kelaart (1857), Herdman (1904) and Herdman and Hornell (1906). Moreover, recent studies on this and other species of the genus *Pinctada* (Tranter, 1958a, b, c, d;1959; Rose *et al.*1990) suggest that they are transitional or consecutive hermaphrodites (Sastry, 1979).

Pearl oysters standardised against a base of 50 mm HL exhibited more uniform seasonal changes of weight than those standardised against 3 other morphometric characters (DVM, APM and HD) in keeping with the reproductive cycle of the species. It follows that the lowest minima of weight were reported during peak spawnings and highest maxima of weight during gametogenesis in winter and spring.

Young and adult oysters sampled at monthly intervals from the same population exhibited different growth patterns being generally more isometric in young oysters and universally negatively allometric in adults. The seasonal variation in allometric growth is perhaps not related to spawning *per se* but most likely can be correlated to the merging of young oysters which commence spawning at an early age into adult populations.

The studied pearl oyster population was shallow (5 m depth) characterised by 9 associated species of biofouling organisms of which the bryozoa Bugula sp. and the alga Hormophysa triquetra were dominant. Therefore, the population can be considered as falling into the second category of Kawahara (1961) in being simple in its community structure and shallow in its depth.

An unidentified species belonging to the trematode genus Bucephalus was found parasitizing and destroying the adult gonad of a small proportion of the pearl oysters sampled. There was no indication of seasonality in its appearance nor any trend in the size of pearl oysters infected.

Natural pearls were very rare in the sampled oysters (1.93%) and they were of small (< 2 mm) diameter and of no commercial value. There was no apparent relationship between the incidence of pearls and the presence of Polydora in these oysters.

The maximum pearl yield of 4.8% occurred in Class 7 (64 mm ≤ 69 mm DVM) in this population.

Polydora and Cliona were more common on the right than the left valve of the shell of the pearl oysters and they were most frequent in summer.

The life history from egg through veligers and spat of P.radiata is similar to P. martensii (Cahn, 1949) and that described by Alagarwami et al.(1983) on P.fucata (= P.radiata).

Cytochalasin B (CB) was used to induce triploidy in two experiments at two concentrations targeting meiosis I and meiosis II. Diploid eggs, which represent

potential triploids, were produced by treatments with CB, and a best result (73%) was achieved at 0.5 mg CBI⁻¹ targeting meiosis II.

Meiosis II triploid larvae produced in Experiment 2 showed significant increase in shell size (APM and DVM) over meiosis I and the control during the early stages of larval growth . This was attributed to the overall percentage of triploids in the culture changing with time.

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Appendix 2.1: Results of χ^2 tests on the sex ratio for selected 11 month based on ≤ 25 indeterminates. Abbreviations: 0=indeterminates, 1=males, 2=females (refer to Table 2.5).

DATA FOR JANUARY 1992

Cum Value Label Percent	Value	Frequency	Percent	Valid Percent
33.0	1.00	33	33.0	33.0
100.0	2.00	67	67.0	67.0
		-----	-----	-----
	Total	100	100.0	100.0

- - - - - Chi-square Test

Category	Cases Observed	Expected	Residual
1.00	33	50.00	-17.00
2.00	67	50.00	17.00

Total	100		
Chi-Square		D.F.	Significance
11.560		1	.001

DATA FOR FEBRUARY 1992

Cum Value Label Percent	Value	Frequency	Percent	Valid Percent
5.0	.00	5	5.0	5.0
35.0	1.00	30	30.0	30.0
100.0	2.00	65	65.0	65.0
		-----	-----	-----
	Total	100	100.0	100.0

INDETERMINATES POOLED WITH MALES

- - - - - Chi-square Test

Category	Cases Observed	Expected	Residual
1.00	35	50.00	-15.00
2.00	65	50.00	15.00

Total	100		
Chi-Square		D.F.	Significance
9.000		1	.003

INDETERMINATES POOLED WITH FEMALES

- - - - - Chi-square Test

Category	Cases Observed	Expected	Residual
1.00	30	50.00	-20.00
2.00	70	50.00	20.00

Total	100		
Chi-Square		D.F.	Significance
16.000		1	.000

DATA FOR MARCH 1992

Cum Value Label Percent	Value	Frequency	Percent	Valid Percent
25.0	.00	25	25.0	25.0
48.0	1.00	23	23.0	23.0
100.0	2.00	52	52.0	52.0
		-----	-----	-----
	Total	100	100.0	100.0

INDETERMINATES POOLED WITH MALES

- - - - - Chi-square Test

Category	Cases Observed	Expected	Residual
1.00	48	50.00	-2.00
2.00	52	50.00	2.00

Total	100		
Chi-Square		D.F.	Significance
.160		1	.689

INDETERMINATES POOLED WITH FEMALES

- - - - - Chi-square Test

Category	Cases Observed	Expected	Residual
1.00	23	50.00	-27.00
2.00	77	50.00	27.00

Total	100		
Chi-Square		D.F.	Significance
29.160		1	.000

DATA FOR APRIL 1992

Cum Value Label Percent	Value	Frequency	Percent	Valid Percent
2.0	.00	2	2.0	2.0
38.0	1.00	36	36.0	36.0
100.0	2.00	62	62.0	62.0
	Total	100	100.0	100.0

INDETERMINATES POOLED WITH MALES

- - - - - Chi-square Test

Category	Cases Observed	Expected	Residual
1.00	38	50.00	-12.00
2.00	62	50.00	12.00
Total	100		
Chi-Square	5.760	D.F. 1	Significance .016

INDETERMINATES POOLED WITH FEMALES

- - - - - Chi-square Test

Category	Cases Observed	Expected	Residual
1.00	36	50.00	-14.00
2.00	64	50.00	14.00
Total	100		
Chi-Square	7.840	D.F. 1	Significance .005

DATA FOR MAY 1992

Cum Value Label Percent	Value	Frequency	Percent	Valid Percent
28.0	1.00	28	28.0	28.0
100.0	2.00	72	72.0	72.0
	Total	100	100.0	100.0

- - - - - Chi-square Test

Category	Cases Observed	Expected	Residual
1.00	28	50.00	-22.00
2.00	72	50.00	22.00
Total	100		
Chi-Square		D.F.	Significance
19.360		1	.000

DATA FOR JUNE 1992

Cum Value Label Percent	Value	Frequency	Percent	Valid Percent
2.0	.00	2	2.0	2.0
27.0	1.00	25	25.0	25.0
100.0	2.00	73	73.0	73.0
	Total	100	100.0	100.0

INDETERMINATES POOLED WITH MALES

- - - - - Chi-square Test

Category	Cases Observed	Expected	Residual
1.00	27	50.00	-23.00
2.00	73	50.00	23.00
Total	100		
Chi-Square	21.160	D.F. 1	Significance .000

INDETERMINATES POOLED WITH FEMALES

- - - - - Chi-square Test

Category	Cases Observed	Expected	Residual
1.00	25	50.00	-25.00
2.00	75	50.00	25.00
Total	100		
Chi-Square	25.000	D.F. 1	Significance .000

DATA FOR JULY 1992

Cum Value Label Percent	Value	Frequency	Percent	Valid Percent
17.0	.00	17	17.0	17.0
35.0	1.00	18	18.0	18.0
100.0	2.00	65	65.0	65.0
	Total	100	100.0	100.0

INDETERMINATES POOLED WITH MALES

- - - - - Chi-square Test

Category	Cases Observed	Expected	Residual
1.00	35	50.00	-15.00
2.00	65	50.00	15.00
Total	100		
Chi-Square	9.000	D.F. 1	Significance .003

INDETERMINATES POOLED WITH FEMALES

- - - - - Chi-square Test

Category	Cases Observed	Expected	Residual
1.00	18	50.00	-32.00
2.00	82	50.00	32.00
Total	100		
Chi-Square	40.960	D.F. 1	Significance .000

DATA FOR MARCH 1993

Cum Value Label Percent	Value	Frequency	Percent	Valid Percent
9.0	.00	9	9.0	9.0
60.0	1.00	51	51.0	51.0
100.0	2.00	40	40.0	40.0
	Total	100	100.0	100.0

INDETERMINATES POOLED WITH MALES

- - - - - Chi-square Test

Category	Cases Observed	Expected	Residual
1.00	60	50.00	10.00
2.00	40	50.00	-10.00
Total	100		
Chi-Square		D.F.	Significance
4.000		1	.046

INDETERMINATES POOLED WITH FEMALES

- - - - - Chi-square Test

Category	Cases Observed	Expected	Residual
1.00	51	50.00	1.00
2.00	49	50.00	-1.00
Total	100		
Chi-Square		D.F.	Significance
.040		1	.841

DATA FOR APRIL 1993

Cum Value Label Percent	Value	Frequency	Percent	Valid Percent
3.0	.00	3	3.0	3.0
53.0	1.00	50	50.0	50.0
100.0	2.00	47	47.0	47.0
	Total	100	100.0	100.0

INDETERMINATES POOLED WITH MALES

- - - - - Chi-square Test

Category	Cases Observed	Expected	Residual
1.00	53	50.00	3.00
2.00	47	50.00	-3.00
Total	100		
Chi-Square		D.F.	Significance
.360		1	.549

INDETERMINATES POOLED WITH FEMALES

- - - - - Chi-square Test

Category	Cases Observed	Expected	Residual
1.00	50	50.00	.00
2.00	50	50.00	.00
Total	100		
Chi-Square		D.F.	Significance
.000		1	1.000

DATA FOR MAY 1993

Cum Value Label Percent	Value	Frequency	Percent	Valid Percent
8.1	.00	8	8.1	8.1
70.7	1.00	62	62.6	62.6
100.0	2.00	29	29.3	29.3
	Total	99	100.0	100.0

INDETERMINATES POOLED WITH MALES

- - - - - Chi-square Test

Category	Cases Observed	Expected	Residual
1.00	70	49.50	20.50
2.00	29	49.50	-20.50
Total	99		
Chi-Square		D.F.	Significance
16.980		1	.000

INDETERMINATES POOLED WITH FEMALES

- - - - - Chi-square Test

Category	Cases Observed	Expected	Residual
1.00	62	49.50	12.50
2.00	37	49.50	-12.50
Total	99		
Chi-Square		D.F.	Significance
6.313		1	.012

DATA FOR JULY 1993

Cum Value Label Percent	Value	Frequency	Percent	Valid Percent
23.0	.00	23	23.0	23.0
77.0	1.00	54	54.0	54.0
100.0	2.00	23	23.0	23.0
	Total	100	100.0	100.0

INDETERMINATES POOLED WITH MALES

- - - - - Chi-square Test

Category	Cases Observed	Expected	Residual
1.00	77	50.00	27.00
2.00	23	50.00	-27.00
Total	100		
Chi-Square	29.160	D.F. 1	Significance .000

INDETERMINATES POOLED WITH FEMALES

- - - - - Chi-square Test

Category	Cases Observed	Expected	Residual
1.00	54	50.00	4.00
2.00	46	50.00	-4.00
Total	100		
Chi-Square	.640	D.F. 1	Significance .424

Appendix 2.2: Table-wide (TW) multiple testing of 1:1 sex ratio based on Bonferroni adjustment for significance demonstrated by single χ^2 results (Appendix 2.1 and Table 2.5), in accordance with the suggestion of Hochberg (1988 cited by Lessios, 1992).

χ^2 test Month	χ^2 Single	$\overline{\chi^2}$ TW $\overline{\alpha}_1 = 0.0025$	$\overline{\chi^2}$ TW $\overline{\alpha}_2 = 0.004$	$\overline{\chi^2}$ TW $\overline{\alpha}_3 = 0.006$	Conformity of TW with χ^2 Single	TW Final Conclusion
J 92	0.001		**		Yes	Fs
F (M)	0.003		**		Yes	Fs
F (F)	<0.001	***			Yes	
M (M)	0.689			ns	Yes	ns
M (F)	<0.001	***			Yes	
A (M)	0.016			ns	Yes	ns
A (F)	0.005		**		Yes	
M	<0.001	***			Yes	Fs
J (M)	<0.001	***			Yes	Fs
J (F)	<0.001	***			Yes	
J (M)	0.003		**		Yes	Fs
J (F)	<0.001	***			Yes	
M 93 (M)	0.046			ns	No	ns
M (F)	0.841			ns	Yes	
A (M)	0.549			ns	Yes	ns
A (F)	1.000			ns	Yes	
M (M)	<0.001	***			Yes	ns
M (F)	0.012			ns	No	
J (M)	<0.001	***			Yes	ns
J (F)	0.424			ns	Yes	
Total = 20		$\overline{\alpha}_1 = 0.05/20$	$\overline{\alpha}_2 = 0.05/12$	$\overline{\alpha}_3 = 0.05/8$		
	$\overline{\alpha}$	0.0025	0.004	0.006		
Subtotal significant cases		8	4	None		

(M) = Male; (F) = Female

Abbreviations: TW, *** = $0.0025 > \overline{\alpha}_1 > 0.0001$
 TW, ** = $0.004 > \overline{\alpha}_2 > 0.0025$
 TW, ns = $\overline{\alpha}_3 \geq 0.006$
 Fs = significant excess of females

Appendix 2.3: Regression coefficients $\pm$ s.e. of the allometric formula $\log y = \log a + b \log x$ (where a is the intercept and b is the slope), for the adult pearl oysters sampled at monthly intervals from January 1992 to December 1993. The letter 'l' denotes $\log_{10}$ of the attribute in the equation.

Regression coefficients of the lwt/ldvm

M	N	a	ase	b	bse
J	100.00	-1.22	.29	1.28	.16
F	100.00	-1.91	.25	1.67	.14
M	100.00	-2.03	.35	1.69	.20
A	100.00	-2.10	.34	1.75	.19
M	100.00	-2.57	.30	1.99	.17
J	100.00	-2.36	.33	1.85	.19
J	100.00	-2.94	.36	2.20	.20
A	100.00	-2.83	.36	2.08	.20
S	53.00	-3.16	.44	2.31	.24
O	100.00	-2.21	.28	1.77	.16
N	100.00	-2.05	.39	1.69	.22
D	100.00	-3.47	.26	2.52	.15
J	99.00	-4.08	.22	2.89	.13
F	100.00	-3.81	.25	2.72	.15
M	100.00	-3.88	.19	2.78	.11
A	100.00	-4.14	.25	2.95	.15
M	99.00	-3.29	.19	2.47	.11
J	100.00	-2.78	.27	2.14	.15
J	100.00	-3.24	.24	2.41	.14
A	100.00	-3.10	.27	2.30	.16
S	106.00	-2.97	.21	2.16	.12
O	100.00	-3.54	.27	2.52	.16
N	99.00	-3.39	.22	2.49	.13
D	100.00	-2.95	.28	2.23	.16

Regression coefficients of the lwt/lapm

M	N	a	ase	b	bse
J	100.00	-.69	.23	.99	.13
F	100.00	-1.15	.24	1.25	.13
M	100.00	-1.41	.32	1.33	.18
A	100.00	-1.25	.31	1.27	.17
M	100.00	-1.80	.26	1.54	.15
J	100.00	-1.81	.31	1.54	.17
J	100.00	-2.45	.36	1.92	.20
A	100.00	-2.64	.34	1.98	.19
S	53.00	-2.33	.41	1.86	.22
O	100.00	-2.15	.27	1.75	.15
N	99.00	-1.90	.38	1.60	.21
D	100.00	-2.96	.24	2.21	.14
J	99.00	-3.66	.23	2.64	.13
F	100.00	-2.51	.23	1.95	.13
M	100.00	-3.40	.16	2.51	.10
A	100.00	-3.40	.17	2.49	.10
M	99.00	-3.04	.18	2.31	.10
J	100.00	-2.48	.27	1.97	.15
J	100.00	-3.01	.26	2.26	.15
A	100.00	-2.69	.27	2.06	.16
S	106.00	-2.98	.20	2.16	.11
O	100.00	-3.49	.25	2.49	.14
N	99.00	-2.98	.20	2.23	.11
D	100.00	-2.93	.27	2.21	.16

Regression coefficients of the lwt/lhl

M	N	a	ase	b	bse
J	100.00	-.26	.33	.79	.19
F	100.00	-.23	.29	.77	.17
M	100.00	.01	.22	.58	.13
A	100.00	-.20	.37	.73	.22
M	100.00	-1.66	.36	1.53	.21
J	100.00	-.62	.35	.92	.21
J	100.00	-1.58	.47	1.51	.27
A	100.00	-1.47	.44	1.40	.25
S	53.00	-1.06	.47	1.22	.27
O	100.00	-2.19	.37	1.84	.22
N	99.00	-1.98	.50	1.72	.29
D	100.00	-2.43	.51	1.97	.31
J	99.00	-2.97	.74	2.30	.44
F	100.00	-1.97	.60	1.69	.36
M	100.00	-4.30	.41	3.14	.25
A	100.00	-4.19	.41	3.04	.24
M	99.00	-3.33	.36	2.56	.21
J	100.00	-1.88	.43	1.67	.25
J	100.00	-3.09	.49	2.39	.29
A	100.00	-2.90	.46	2.24	.28
S	106.00	-2.73	.28	2.09	.17
O	100.00	-2.83	.41	2.19	.24
N	99.00	-3.03	.39	2.34	.23
D	100.00	-2.77	.45	2.20	.27

Regression coefficients of the lwt/lhd

M	N	a	ase	b	bse
J	100.00	.93	.05	.25	.07
F	100.00	.82	.04	.43	.07
M	100.00	.89	.04	.17	.06
A	100.00	.82	.05	.34	.08
M	100.00	.59	.06	.58	.10
J	100.00	.66	.05	.43	.08
J	100.00	.55	.05	.68	.07
A	100.00	.53	.07	.56	.10
S	53.00	.75	.09	.41	.13
O	100.00	.55	.06	.61	.09
N	99.00	.70	.06	.41	.09
D	100.00	.44	.05	.73	.10
J	99.00	.29	.05	1.03	.10
F	100.00	.38	.07	.86	.13
M	100.00	.37	.05	1.04	.10
A	100.00	.20	.05	1.29	.10
M	99.00	.44	.04	.93	.07
J	100.00	.49	.06	.76	.11
J	100.00	.46	.05	.83	.08
A	100.00	.49	.06	.66	.11
S	106.00	.23	.05	.86	.08
O	100.00	.43	.07	.70	.11
N	99.00	.42	.05	.85	.08
D	100.00	.51	.07	.61	.11

Regression coefficients of the lapm/ldvm

M	N	a	ase	b	bse
J	100.00	.05	.15	.97	.08
F	100.00	.02	.12	1.00	.07
M	100.00	.39	.16	.79	.09
A	100.00	.17	.15	.91	.08
M	100.00	-.11	.11	1.07	.06
J	100.00	.45	.15	.75	.09
J	100.00	.11	.09	.95	.05
A	100.00	.18	.10	.90	.06
S	53.00	.11	.18	.93	.10
O	100.00	.05	.06	.97	.03
N	100.00	.34	.12	.81	.07
D	100.00	-.03	.08	1.02	.05
J	99.00	.03	.07	.99	.04
F	100.00	-.19	.12	1.12	.07
M	100.00	-.09	.07	1.05	.04
A	100.00	-.20	.08	1.13	.05
M	99.00	-.03	.06	1.03	.03
J	100.00	.12	.09	.93	.05
J	100.00	.11	.07	.94	.04
A	100.00	.14	.10	.92	.06
S	106.00	.13	.07	.93	.04
O	100.00	.10	.08	.94	.04
N	99.00	-.03	.08	1.03	.05
D	100.00	.13	.07	.93	.04

Regression coefficients of the lhl/ldvm

M	N	a	ase	b	bse
J	100.00	1.01	.17	.38	.09
F	100.00	.66	.18	.58	.10
M	100.00	.31	.29	.77	.16
A	100.00	.92	.19	.44	.11
M	100.00	.51	.13	.67	.08
J	100.00	.99	.20	.39	.11
J	100.00	.64	.14	.60	.08
A	100.00	.63	.14	.60	.08
S	53.00	.45	.27	.70	.15
O	100.00	.60	.10	.62	.06
N	100.00	.91	.13	.45	.07
D	100.00	.78	.11	.52	.06
J	99.00	1.09	.09	.33	.06
F	100.00	.96	.11	.41	.07
M	100.00	.60	.08	.62	.04
A	100.00	.56	.09	.66	.05
M	99.00	.57	.09	.65	.05
J	100.00	.75	.12	.54	.07
J	100.00	.82	.10	.49	.07
A	100.00	.76	.10	.53	.06
S	106.00	.42	.10	.72	.06
O	100.00	.56	.11	.65	.07
N	99.00	.63	.11	.61	.06
D	100.00	.79	.11	.51	.06

Regression coefficients of the lhd/ldvm

M	N	a	ase	b	bse
J	100.00	-1.42	.43	1.14	.24
F	100.00	-2.33	.40	1.66	.22
M	100.00	-.81	.72	.79	.40
A	100.00	-2.00	.50	1.46	.28
M	100.00	-1.70	.32	1.29	.18
J	100.00	-1.79	.46	1.36	.26
J	100.00	-3.28	.37	2.21	.21
A	100.00	-2.27	.38	1.65	.21
S	53.00	-1.74	.63	1.34	.35
O	100.00	-2.03	.30	1.50	.17
N	100.00	-2.31	.39	1.67	.22
D	100.00	-2.48	.30	1.77	.17
J	99.00	-2.17	.27	1.58	.16
F	100.00	-1.49	.28	1.18	.17
M	100.00	-2.26	.24	1.61	.14
A	100.00	-2.26	.21	1.64	.12
M	99.00	-2.74	2.30	1.92	.13
J	100.00	-1.13	.31	.99	.18
J	100.00	-2.48	.29	1.76	.17
A	100.00	-1.79	.30	1.37	.17
S	106.00	-2.26	.22	1.66	.13
O	100.00	-2.18	.29	1.61	.17
N	99.00	-2.49	.28	1.77	.16
D	100.00	-1.35	.32	1.15	.18

Regression coefficients of the ldvm/lhd

M	N	a	ase	b	bse
J	100.00	1.69	.02	.17	.03
F	100.00	1.65	.02	.22	.03
M	100.00	1.76	.02	.05	.02
A	100.00	1.70	.02	.15	.03
M	100.00	1.61	.02	.26	.04
J	100.00	1.68	.02	.16	.03
J	100.00	1.63	.02	.24	.22
A	100.00	1.64	.02	.24	.03
S	53.00	1.70	.03	.17	.04
O	100.00	1.59	.02	.30	.03
N	100.00	1.64	.02	.22	.03
D	100.00	1.55	.02	.29	.03
J	99.00	1.53	.02	.32	.03
F	100.00	1.55	.02	.29	.04
M	100.00	1.54	.02	.36	.03
A	100.00	1.49	.02	.39	.03
M	99.00	1.52	.02	.36	.02
J	100.00	1.60	.03	.25	.04
J	100.00	1.56	.02	.30	.03
A	100.00	1.56	.02	.29	.04
S	106.00	1.50	.02	.37	.03
O	100.00	1.56	.02	.30	.03
N	99.00	1.55	.02	.31	.03
D	100.00	1.57	.03	.25	.04

Regression coefficients of the lapm/lhd

M	N	a	ase	b	bse
J	100.00	1.70	.03	.14	.05
F	100.00	1.65	.02	.23	.04
M	100.00	1.77	.02	.06	.03
A	100.00	1.73	.02	.11	.04
M	100.00	1.64	.03	.22	.05
J	100.00	1.70	.02	.14	.04
J	100.00	1.64	.02	.25	.03
A	100.00	1.63	.02	.24	.03
S	53.00	1.69	.04	.17	.05
O	100.00	1.59	.02	.28	.04
N	100.00	1.67	.02	.18	.03
D	100.00	1.54	.02	.32	.03
J	99.00	1.53	.02	.34	.03
F	100.00	1.55	.03	.31	.06
M	100.00	1.52	.02	.38	.04
A	100.00	1.46	.02	.48	.04
M	99.00	1.52	.02	.39	.03
J	100.00	1.62	.03	.21	.05
J	100.00	1.58	.02	.29	.03
A	100.00	1.57	.02	.27	.04
S	106.00	1.52	.02	.36	.03
O	100.00	1.58	.02	.28	.04
N	99.00	1.56	.02	.33	.03
D	100.00	1.56	.03	.27	.04

Regression coefficients of the lhl/lhd

M	N	a	ase	b	bse
J	100.00	1.66	.02	.06	.04
F	100.00	1.62	.03	.12	.04
M	100.00	1.68	.03	.02	.04
A	100.00	1.66	.02	.07	.04
M	100.00	1.60	.03	.14	.04
J	100.00	1.63	.02	.07	.04
J	100.00	1.61	.02	.15	.03
A	100.00	1.63	.03	.12	.04
S	53.00	1.65	.04	.11	.06
O	100.00	1.57	.02	.21	.03
N	100.00	1.64	.02	.10	.03
D	100.00	1.60	.02	.10	.03
J	99.00	1.62	.02	.07	.03
F	100.00	1.61	.02	.10	.04
M	100.00	1.56	.02	.20	.03
A	100.00	1.53	.02	.27	.03
M	99.00	1.55	.02	.23	.03
J	100.00	1.63	.03	.09	.04
J	100.00	1.60	.02	.15	.03
A	100.00	1.61	.02	.13	.03
S	106.00	1.50	.02	.28	.03
O	100.00	1.55	.02	.21	.03
N	99.00	1.59	.02	.17	.03
D	100.00	1.57	.02	.15	.03

Appendix 2.3: Continued: Regression coefficients $\pm$ s.e. of the allometric formula $\log y = \log a + b \log x$ (where a is the intercept and b is the slope), for the adult pearl oysters sampled at monthly intervals from October 1992 to December 1993. The letter 'l' denotes $\log_{10}$ of the attribute in the equation.

Regression coefficients of the $\log \text{mwwt} / \log \text{ldvm}$

M	N	a	ase	b	bse
O	20.00	-2.95	.68	1.85	.38
N	19.00	-1.34	2.09	.91	1.17
D	20.00	-3.16	.71	1.99	.42
J	20.00	-5.51	.91	3.38	.54
F	20.00	-4.61	.97	2.87	.57
M	20.00	-4.64	.40	2.88	.23
A	20.00	-4.70	.69	2.90	.41
M	20.00	-5.24	.66	3.26	.38
J	20.00	-4.43	.76	2.75	.44
J	20.00	-2.66	.85	1.72	.49
A	20.00	-3.08	.67	1.95	.38
S	20.00	-5.89	1.26	3.45	.72
O	20.00	-3.94	.84	2.40	.48
N	20.00	-2.73	1.16	1.76	.66
D	20.00	-1.60	.98	1.07	.57

Regression coefficients of the $\log \text{mwwt} / \log \text{lapm}$

M	N	a	ase	b	bse
O	20.00	-3.11	.61	1.94	.34
N	19.00	-3.11	2.14	1.88	1.19
D	20.00	-2.95	.48	1.87	.28
J	20.00	-4.66	.83	2.86	.49
F	20.00	-3.79	.96	2.38	.56
M	20.00	-4.27	.42	2.66	.24
A	20.00	-3.95	.60	2.46	.36
M	20.00	-4.65	.60	2.90	.34
J	20.00	-3.53	.79	2.22	.46
J	20.00	-2.63	.82	1.70	.47
A	20.00	-3.35	.85	2.10	.49
S	20.00	-3.66	1.29	2.15	.74
O	20.00	-4.00	.78	2.43	.45
N	20.00	-2.30	.83	1.50	.47
D	20.00	-1.45	.92	.99	.53

Regression coefficients of the lmwwt/lhl

M	N	a	ase	b	bse
O	20.00	-2.03	1.21	1.39	.70
N	19.00	.13	2.35	.09	1.37
D	20.00	-2.47	.90	1.66	.55
J	20.00	-.11	1.74	.19	1.10
F	20.00	-2.37	1.46	1.61	.88
M	20.00	-6.17	.95	3.92	.57
A	20.00	-4.37	.77	2.78	.47
M	20.00	-5.07	1.25	3.26	.74
J	20.00	-3.03	2.00	2.01	1.20
J	20.00	-.55	1.28	.52	.76
A	20.00	-2.28	1.15	1.53	.68
S	20.00	-4.94	1.02	3.02	.61
O	20.00	-5.06	1.26	3.17	.77
N	20.00	-1.25	1.35	.94	.79
D	20.00	-.97	1.40	.73	.80

Regression coefficients of the lmwwt/lhd

M	N	a	ase	b	bse
O	20.00	.04	.16	.51	.24
N	19.00	.65	.34	-.55	.52
D	20.00	-.07	.13	.55	.21
J	20.00	-.37	.15	1.14	.29
F	20.00	-.24	.16	1.01	.29
M	20.00	-.25	.11	1.08	.21
A	20.00	-.44	.10	1.27	.19
M	20.00	-.19	.12	.99	.17
J	20.00	-.25	.15	1.02	.25
J	20.00	.05	.11	.47	.20
A	20.00	.08	.15	.35	.24
S	20.00	-.35	.20	.75	.33
O	20.00	-.51	.25	1.20	.41
N	20.00	.06	.17	.49	.27
D	20.00	.29	.19	-.04	.29

Regression coefficients of the lmdwt/ldvm

M	N	a	ase	b	bse
O	20.00	-3.33	1.71	1.71	.49
N	19.00	-2.27	2.99	1.07	1.67
D	20.00	-3.70	1.02	1.94	.59
J	20.00	-7.33	.91	4.08	.54
F	20.00	-5.98	1.42	3.31	.83
M	20.00	-4.83	.57	2.63	.33
A	20.00	-5.27	.99	2.90	.59
M	20.00	-6.00	.70	3.33	.40
J	20.00	-4.61	.83	2.50	.48
J	20.00	-3.08	1.62	1.62	.62
A	20.00	-3.77	.78	2.00	.45
S	20.00	-7.17	2.50	3.74	1.45
O	20.00	-4.52	.66	2.34	.38
N	20.00	-4.48	.81	2.41	.47
D	20.00	-2.20	1.12	1.07	.65

Regression coefficients of the lmdwt/lapm

M	N	a	ase	b	bse
O	20.00	-3.32	.85	1.71	.48
N	19.00	-3.68	3.17	1.85	1.76
D	20.00	-3.76	.71	1.97	.41
J	20.00	-6.01	.95	3.29	.56
F	20.00	-5.14	1.34	2.79	.78
M	20.00	-4.51	.56	2.44	.32
A	20.00	-4.94	.73	2.71	.43
M	20.00	-5.38	.64	2.96	.36
J	20.00	-3.68	.85	1.96	.49
J	20.00	-2.79	1.08	1.44	.62
A	20.00	-3.92	1.00	2.08	.58
S	20.00	-3.75	2.33	1.75	1.33
O	20.00	-4.28	.70	2.20	.40
N	20.00	-3.72	.54	1.96	.30
D	20.00	-2.20	1.04	1.06	.60

Regression coefficients of the lmdwt/lhl

M	N	a	ase	b	bse
O	20.00	-2.60	1.35	1.37	.78
N	19.00	.97	3.35	-.78	1.96
D	20.00	-3.28	1.13	1.77	.69
J	20.00	-1.12	2.00	.42	1.21
F	20.00	-2.63	1.97	1.39	1.20
M	20.00	-6.36	1.04	3.66	.63
A	20.00	-4.96	1.04	2.79	.63
M	20.00	-5.89	1.28	3.37	.76
J	20.00	-3.42	1.96	1.88	1.17
J	20.00	-1.06	1.47	.46	.87
A	20.00	-3.63	1.17	1.98	.70
S	20.00	-5.55	2.14	2.91	1.30
O	20.00	-5.39	1.09	2.95	.65
N	20.00	-4.62	.82	2.56	.48
D	20.00	-3.16	1.40	1.67	.83

Regression coefficients of the lmdwt/lhd

M	N	a	ase	b	bse
O	20.00	-.55	.18	.44	.27
N	19.00	.14	.49	-.77	.74
D	20.00	-.69	.16	.53	.27
J	20.00	-1.08	.17	1.30	.34
F	20.00	-.99	.21	1.23	.39
M	20.00	-.83	.12	1.00	.22
A	20.00	-.93	.16	1.11	.31
M	20.00	-.82	.13	.99	.19
J	20.00	-.82	.15	.96	.25
J	20.00	-.58	.13	.51	.22
A	20.00	-.53	.16	.37	.27
S	20.00	-1.09	.33	.68	.55
O	20.00	-1.14	.23	1.11	.37
N	20.00	-.70	.13	.71	.22
D	20.00	-.42	.21	.10	.33

Regression coefficients of the ltwwt/ldvm

M	N	a	ase	b	bse
O	20.00	-2.95	.59	2.09	.33
N	19.00	-.61	.78	.78	.44
D	20.00	-2.46	.52	1.84	.30
J	20.00	-4.98	.78	3.34	.46
F	20.00	-4.20	.69	2.85	.40
M	20.00	-3.80	.34	2.63	.20
A	20.00	-3.78	.60	2.63	.40
M	20.00	-3.53	.48	2.50	.27
J	20.00	-2.66	.61	1.95	.35
J	20.00	-2.11	.46	1.65	.27
A	20.00	-2.82	.36	2.03	.21
S	20.00	-4.16	.79	2.76	.45
O	20.00	-4.05	.38	2.71	.22
N	20.00	-4.41	.53	2.98	.31
D	20.00	-2.78	.59	2.03	.34

Regression coefficients of the ltwwt/lapm

M	N	a	ase	b	bse
O	20.00	-3.08	.50	2.17	.28
N	19.00	-.93	.82	.95	.45
D	20.00	-2.11	.36	1.63	.21
J	20.00	-4.51	.56	3.05	.33
F	20.00	-3.55	.69	2.45	.40
M	20.00	-3.54	.32	2.48	.18
A	20.00	-3.28	.44	2.34	.26
M	20.00	-3.11	.42	2.25	.24
J	20.00	-2.56	.46	1.88	.26
J	20.00	-2.15	.42	1.66	.24
A	20.00	-3.31	.46	2.32	.27
S	20.00	-2.66	.84	1.89	.48
O	20.00	-3.84	.45	2.58	.26
N	20.00	-2.99	.49	2.15	.28
D	20.00	-2.62	.53	1.93	.30

Regression coefficients of the ltwwt/lhl

M	N	a	ase	b	bse
O	20.00	-2.55	1.11	1.96	.64
N	19.00	-.27	.91	.61	.53
D	20.00	-1.31	.80	1.22	.49
J	20.00	-1.44	1.58	1.27	.95
F	20.00	-2.15	1.24	1.70	.75
M	20.00	-5.08	.90	3.51	.54
A	20.00	-3.56	.65	2.57	.39
M	20.00	-3.39	.94	2.50	.55
J	20.00	-2.27	1.44	1.79	.86
J	20.00	-.67	.90	.84	.54
A	20.00	-2.43	.86	1.87	.51
S	20.00	-3.17	.70	2.27	.42
O	20.00	-5.14	.85	3.47	.51
N	20.00	-3.30	.97	2.41	.57
D	20.00	-2.35	1.09	1.84	.65

Regression coefficients of the ltwwt/lhd

M	N	a	ase	b	bse
O	20.00	.29	.13	.79	.20
N	19.00	.67	.14	.17	.21
D	20.00	.51	.12	.31	.20
J	20.00	.02	.11	1.30	.22
F	20.00	.20	.14	.88	.26
M	20.00	.19	.09	1.04	.17
A	20.00	.08	.09	1.17	.16
M	20.00	.31	.07	.83	.10
J	20.00	.30	.11	.73	.19
J	20.00	.51	.08	.41	.14
A	20.00	.35	.11	.59	.18
S	20.00	.17	.12	.77	.20
O	20.00	-.29	.17	1.54	.27
N	20.00	.28	.13	.86	.21
D	20.00	.49	.17	.37	.26

Regression coefficients of the ltdwt/ldvm

M	N	a	ase	b	bse
O	20.00	-2.49	.92	1.37	.51
N	19.00	-2.22	1.06	1.20	.59
D	20.00	-4.41	1.20	2.51	.70
J	20.00	-4.90	.74	2.83	.44
F	20.00	-4.88	.79	2.79	.46
M	20.00	-4.19	.44	2.42	.26
A	20.00	-3.85	.51	2.24	.30
M	20.00	-3.66	.42	2.12	.24
J	20.00	-2.27	.69	1.26	.39
J	20.00	-2.99	.64	1.69	.37
A	20.00	-3.52	.54	1.96	.31
S	20.00	-3.84	1.00	2.06	.58
O	20.00	-3.82	1.04	2.12	.60
N	20.00	-4.33	.67	2.47	.38
D	20.00	-2.58	.81	1.44	.47

Regression coefficients of the ltdwt/lapm

M	N	a	ase	b	bse
O	20.00	-2.70	.86	1.50	.48
N	19.00	-1.97	1.18	1.06	.66
D	20.00	-3.02	1.11	1.69	.65
J	20.00	-4.19	.67	2.40	.40
F	20.00	-4.08	.81	2.30	.47
M	20.00	-3.99	.39	2.30	.23
A	20.00	-3.20	.48	1.86	.28
M	20.00	-3.31	.37	1.91	.21
J	20.00	-2.07	.61	1.14	.35
J	20.00	-2.83	.65	1.59	.37
A	20.00	-3.83	.71	2.14	.41
S	20.00	-3.09	.85	1.61	.49
O	20.00	-3.44	1.08	1.90	.62
N	20.00	-3.29	.51	1.86	.29
D	20.00	-2.41	.77	1.33	.44

Regression coefficients of the ltdwt/lhl

M	N	a	ase	b	bse
O	20.00	-3.15	1.18	1.82	.68
N	19.00	-.82	1.29	.44	.76
D	20.00	-1.58	1.58	.89	.96
J	20.00	-1.64	1.40	.92	.85
F	20.00	-3.18	1.25	1.85	.76
M	20.00	-5.11	1.01	3.07	.62
A	20.00	-3.33	.66	1.99	.40
M	20.00	-3.66	.78	2.19	.46
J	20.00	-1.99	1.29	1.13	.77
J	20.00	-.83	1.09	.45	.65
A	20.00	-3.12	.98	1.78	.58
S	20.00	-3.43	.78	1.89	.47
O	20.00	-4.94	1.48	2.88	.89
N	20.00	-4.00	.82	2.34	.48
D	20.00	-2.01	1.21	1.14	.72

Regression coefficients of the ltdwt/lhd

M	N	a	ase	b	bse
O	20.00	-.38	1.67	.55	.25
N	19.00	-.23	.19	.26	.29
D	20.00	-.30	.21	.31	.35
J	20.00	-.60	.12	.97	.24
F	20.00	-.58	.15	.87	.28
M	20.00	-.53	.09	.98	.17
A	20.00	-.55	.08	.97	.15
M	20.00	-.40	.06	.70	.09
J	20.00	-.41	.10	.55	.19
J	20.00	-.34	.09	.47	.16
A	20.00	-.43	.13	.51	.21
S	20.00	-.63	1.30	.59	.21
O	20.00	-.79	.28	1.08	.46
N	20.00	-.37	.14	.58	.23
D	20.00	-.15	.18	.09	.27

Regression coefficients of the lwtd/ldvm

M	N	a	ase	b	bse
O	20.00	-2.40	.72	1.44	.40
N	19.00	-2.11	1.54	1.25	.86
D	20.00	-3.50	.55	2.09	.32
J	20.00	-5.41	.70	3.24	.42
F	20.00	-4.94	.95	2.94	.56
M	20.00	-4.08	.44	2.47	.25
A	20.00	-4.01	.58	2.44	.34
M	20.00	-4.25	.47	2.58	.27
J	20.00	-2.93	.67	1.77	.39
J	20.00	-2.73	.70	1.66	.40
A	20.00	-3.29	.55	1.95	.32
S	20.00	-4.40	1.16	2.47	.67
O	20.00	-3.79	.86	2.21	.50
N	20.00	-4.10	.65	2.45	.37
D	20.00	-2.16	.88	1.31	.51

Regression coefficients of the lwtd/lapm

M	N	a	ase	b	bse
O	20.00	-2.57	.66	1.54	.37
N	19.00	-2.40	1.66	1.40	.92
D	20.00	-2.84	.48	1.70	.28
J	20.00	-4.51	.69	2.69	.40
F	20.00	-4.13	.94	2.45	.55
M	20.00	-3.86	.40	2.33	.23
A	20.00	-3.46	.48	2.12	.28
M	20.00	-3.80	.42	2.30	.24
J	20.00	-2.44	.64	1.48	.37
J	20.00	-2.53	.71	1.53	.41
A	20.00	-3.53	.74	2.09	.43
S	20.00	-2.91	1.10	1.59	.62
O	20.00	-3.43	.91	2.00	.53
N	20.00	-3.15	.46	1.89	.26
D	20.00	-2.05	.82	1.24	.47

Regression coefficients of the lwtd/lhl

M	N	a	ase	b	bse
O	20.00	-2.62	1.01	1.63	.59
N	19.00	-.16	1.82	.16	1.06
D	20.00	-1.86	.93	1.18	.57
J	20.00	-1.20	1.55	.76	.94
F	20.00	-2.69	1.50	1.68	.88
M	20.00	-5.18	.97	3.23	.59
A	20.00	-3.54	.71	2.22	.43
M	20.00	-4.21	.93	2.63	.55
J	20.00	-2.28	1.50	1.44	.88
J	20.00	-.63	1.13	.45	.67
A	20.00	-3.00	.97	1.84	.58
S	20.00	-3.74	.94	2.17	.56
O	20.00	-4.88	1.26	2.95	.76
N	20.00	-3.93	.75	2.42	.44
D	20.00	-2.13	1.22	1.32	.72

Regression coefficients of the lwtd/lhd

M	N	a	ase	b	bse
O	20.00	-.14	.14	.49	.21
N	19.00	.15	.27	-.04	.41
D	20.00	-.14	.13	.37	.22
J	20.00	-.48	.13	1.08	.26
F	20.00	-.43	.16	.98	.30
M	20.00	-.35	.09	.98	.18
A	20.00	-.40	.10	1.01	.18
M	20.00	-.27	.08	.81	.12
J	20.00	-.28	.11	.72	.19
J	20.00	-.14	.09	.49	.16
A	20.00	-.18	.13	.45	.22
S	20.00	-.48	.16	.60	.26
O	20.00	-.62	.25	1.09	.41
N	20.00	-.20	.13	.63	.21
D	20.00	-.04	.18	.09	.28

Appendix 2.3: Continued: Regression coefficients $\pm$ s.e. of the allometric formula $\log y = \log a + b \log x$ (where a is the intercept and b is the slope), for the young pearl oysters sampled at monthly intervals from February 1992 to December 1993. June and September 1992, and August 1993 samples are excluded due to small sample size. The letter 'l' denotes $\log_{10}$ of the attribute in the equation.

Regression coefficients of the lapm/ldvm

M	N	a	ase	b	bse
F	14.00	.66	.06	.94	.06
M	12.00	-.16	.09	1.11	.08
A	12.00	-.05	.05	1.02	.04
M	25.00	.01	.06	.99	.05
J	15.00	-.27	.35	1.17	.28
A	33.00	.02	.03	.98	.02
O	25.00	-.05	.03	1.02	.02
N	64.00	.08	.03	.89	.03
D	7.00	.02	.13	.96	.11
J	24.00	-.04	.04	1.01	.03
F	14.00	.07	.06	1.04	.05
M	46.00	.02	.03	.97	.02
A	51.00	-.02	.04	1.01	.03
M	8.00	-.03	.06	1.02	.05
J	8.00	.12	.21	.92	.15
J	9.00	.05	.37	.97	.25
S	28.00	.05	.03	.97	.03
O	60.00	.05	.05	.94	.06
N	38.00	.04	.03	.95	.03
D	27.00	.19	.08	.80	.08

Regression coefficients of the lhl/ldvm

M	N	a	ase	b	bse
F	14.00	.18	.10	.90	.09
M	12.00	-.03	.17	1.04	.14
A	12.00	.23	.09	.85	.08
M	25.00	.21	.11	.87	.10
J	15.00	.01	.11	1.05	.09
A	33.00	.17	.04	.91	.03
O	25.00	.13	.05	.91	.04
N	64.00	.16	.03	.90	.03
D	7.00	.10	.17	1.00	.15
J	24.00	.10	.05	.98	.04
F	14.00	.11	.05	.97	.04
M	46.00	.10	.04	.97	.04
A	51.00	.19	.05	.91	.04
M	8.00	.09	.07	.98	.06
J	8.00	.67	.32	.55	.22
J	9.00	.32	.99	.81	.66
S	28.00	.20	.03	.87	.03
O	60.00	.08	.03	1.00	.04
N	38.00	.15	.03	.93	.03
D	27.00	.28	.08	.81	.07

Regression coefficients of the lhd/ldvm

M	N	a	ase	b	bse
F	14.00	-1.10	.14	.83	.13
M	12.00	-.92	.19	.70	.16
A	12.00	-.12	.13	.93	.12
M	25.00	-.61	.25	.50	.22
J	15.00	-1.08	.08	.87	.06
A	33.00	-1.14	.08	.97	.07
O	25.00	-1.67	.10	1.31	.09
N	64.00	-1.00	.07	.81	.08
D	7.00	-.94	.21	.75	.19
J	24.00	-1.02	.07	.83	.06
F	14.00	-1.04	.19	.86	.15
M	46.00	-1.00	.10	.78	.08
A	51.00	-1.21	.11	.94	.09
M	8.00	-1.20	.18	.89	.14
J	8.00	-.98	.42	.77	.29
J	9.00	-2.55	1.20	1.82	.80
S	28.00	1.48	.06	1.16	.05
O	60.00	-1.49	.06	1.17	.08
N	38.00	-1.46	.09	1.13	.09
D	27.00	-1.01	.15	.72	.14

Regression coefficients of the ldvm/lhd

M	N	a	ase	b	bse
F	14.00	1.26	.05	.93	.15
M	12.00	1.27	.03	.94	.21
A	12.00	1.20	.02	.93	.11
M	25.00	1.14	.04	.38	.16
J	15.00	1.24	.01	1.08	.08
A	33.00	1.18	.02	.90	.06
O	25.00	1.25	.02	.69	.05
N	64.00	1.12	.03	.79	.08
D	7.00	1.21	.05	1.02	.25
J	24.00	1.22	.02	1.07	.08
F	14.00	1.21	.02	.85	.15
M	46.00	1.25	.02	.87	.09
A	51.00	1.28	.01	.73	.07
M	8.00	1.33	.03	.98	.15
J	8.00	1.34	.04	.69	.27
J	9.00	1.46	.02	.23	.10
S	28.00	1.26	.02	.82	.04
O	60.00	1.17	.03	.68	.04
N	38.00	1.24	.03	.73	.06
D	27.00	1.21	.05	.70	.14

Regression coefficients of the lapm/lhd

M	N	a	ase	b	bse
F	14.00	1.26	.04	.91	.13
M	12.00	1.23	.04	1.00	.27
A	12.00	1.17	.02	.94	.13
M	25.00	1.14	.04	.43	.16
J	15.00	1.18	.05	1.29	.32
A	33.00	1.17	.02	.88	.06
O	25.00	1.24	.02	.72	.04
N	64.00	1.09	.02	.72	.07
D	7.00	1.19	.05	.99	.26
J	24.00	1.19	.02	1.11	.08
F	14.00	1.20	.04	.92	.15
M	46.00	1.23	.02	.85	.09
A	51.00	1.26	.02	.73	.08
M	8.00	1.33	.04	.98	.18
J	8.00	1.37	.05	.44	.34
J	9.00	1.48	.03	.19	.14
S	28.00	1.28	.02	.81	.04
O	60.00	1.16	.04	.65	.06
N	38.00	1.22	.03	.70	.06
D	27.00	1.16	.04	.59	.13

Regression coefficients of the lhl/lhd

M	N	a	ase	b	bse
F	14.00	1.32	.06	.82	.17
M	12.00	1.29	.04	1.00	.27
A	12.00	1.24	.02	.82	.10
M	25.00	1.20	.04	.33	.17
J	15.00	1.30	.02	1.14	.12
A	33.00	1.24	.02	.83	.06
O	25.00	1.28	.02	.65	.04
N	64.00	1.18	.03	.72	.07
D	7.00	1.32	.06	1.04	.29
J	24.00	1.29	.02	1.06	.08
F	14.00	1.29	.04	.82	.15
M	46.00	1.31	.02	.87	.09
A	51.00	1.34	.02	.62	.08
M	8.00	1.39	.04	.93	.19
J	8.00	1.44	.04	.32	.27
J	9.00	1.51	.05	.16	.25
S	28.00	1.31	.02	.72	.04
O	60.00	1.25	.04	.68	.05
N	38.00	1.31	.03	.69	.05
D	27.00	1.27	.04	.64	.12

Appendix 2.4: χ^2 test on Galtsoff's (1933) sex ratio on
P.galtsoffi (= P.margaritifera).

Cum Value Label Percent	Value	Frequency	Percent	Valid Percent
40.6	MALES	65	40.6	40.6
100.0	FEMALES	95	59.4	59.4
	Total	160	100.0	100.0

- - - - - Chi-square Test

Category	Cases Observed	Expected	Residual	
MALES	65	80.00	-15.00	
FEMALES	95	80.00	15.00	
Total	160			
Chi-Square	5.625	D.F. 1	Significance .018	

Appendix 3.1: Percentages of incidence of pearl and biofouling on adult P.radiata, studied from November 1992 to December 1993.

The abbreviations used throughout the study are listed below;

BP = Blisters; Polydora vulgaris (spionid polychaete)
 PS = Parasite; Bucephalus sp. (trematode)
 PL = pearl; cyst pearls
 RCM = right valve; Chama aspara (chama oyster)
 RA = right valve; Hormophysa triquetra (alga)
 RO = right valve; P.radiata (young & adults)
 RZN = right valve; Bugula sp. (bryozoa)
 RG = right valve; Cliona vestifica (sponge)
 RW = right valve; Vermetus sulcata (gastropod tube)
 RM = right valve; Malleus regula (hammer oyster)
 WIN = polychaete worm Nereis sp. inside the oyster.

The letter L denotes the respective species is found on the left valve, e.g. LCM Chama aspara on the left valve of the oyster.

M	PL	BP	RCM	RA	RO
N	4.00	6.00	7.00	3.00	9.00
D	.00	8.00	2.00	2.00	4.00
J	.00	2.00	3.10	20.40	22.00
F	.00	1.00	1.00	32.00	36.00
M	.00	11.00	2.00	41.00	30.00
A	.00	10.00	7.00	53.00	43.00
M	3.00	11.00	19.20	56.60	13.10
J	3.00	17.00	20.00	40.00	14.00
J	.00	8.00	19.00	51.00	19.00
A	1.00	15.00	13.00	78.00	16.00
S	4.70	7.00	8.50	27.40	8.50
O	5.00	5.00	20.00	38.00	13.00
N	2.00	2.00	13.30	33.70	17.30
D	2.10	10.30	23.70	33.00	28.90

M	RZN	RG	RW	RM	WIN	LA
N	1.00	17.00	.00	5.00	.00	8.00
D	.00	2.00	.00	.00	.00	5.00
J	1.00	15.30	.00	3.10	.00	35.70
F	2.00	15.00	1.00	2.00	3.00	30.00
M	34.00	19.00	5.00	8.00	4.00	74.00
A	31.00	17.00	5.00	10.00	2.00	87.00
M	53.50	11.10	2.00	5.10	.00	69.70
J	70.00	14.00	.00	.00	.00	52.00
J	87.00	11.00	.00	1.00	.00	62.00
A	96.00	23.00	1.00	1.00	1.00	90.00
S	97.20	34.00	12.30	2.80	2.80	78.30
O	93.00	19.00	1.00	.00	4.00	80.00
N	93.90	8.20	3.10	1.00	1.00	51.00
D	96.90	18.60	1.00	.00	5.20	83.50

M	LO	LZN	LG	LW	LCM
N	2.00	1.00	2.00	.00	20.00
D	1.00	.00	.00	.00	1.00
J	11.20	.00	.00	.00	1.00
F	37.00	.00	.00	1.00	.00
M	20.00	3.00	.00	2.00	10.00
A	35.00	4.00	.00	1.00	10.00
M	14.10	28.30	.00	1.00	12.10
J	9.00	62.00	.00	.00	20.00
J	14.00	88.00	.00	.00	12.00
A	14.00	96.00	.00	2.00	10.00
S	10.40	95.30	4.70	4.70	13.20
O	14.00	96.00	7.00	5.00	19.00
N	12.20	93.90	2.00	2.00	11.20
D	16.50	97.90	7.20	2.10	9.30

Appendix 3.2: Percentages of parasites, pearls and biofouling in relation to dvm classes (after the scheme of Mohammad, 1972) of adult P.radiata monitored from November 1992 to December 1993.

code dvm classes 35.05mm throu 39mm=class 1,
 >39mm ≤ 44mm=class 2, >44mm ≤ 49mm=class 3,
 >49mm ≤ 54mm=class 4, >54mm ≤ 59mm=class 5,
 >59mm ≤ 64mm=class 6, >64mm ≤ 69mm=class 7,
 >69mm ≤ 74mm=class 8, >74mm=class 9.

CLAS	PS	PL	BP	RCM	RA	RO
1.00	.00	.00	10.50	5.30	42.10	36.80
2.00	4.20	.00	9.90	12.70	36.60	25.40
3.00	.40	1.20	6.60	8.90	38.80	22.90
4.00	.50	1.10	8.50	13.50	37.90	21.40
5.00	1.30	2.80	8.60	10.10	34.80	17.70
6.00	1.10	2.80	8.90	12.80	33.00	14.50
7.00	3.60	4.80	6.00	9.60	30.10	15.70
8.00	4.50	.00	4.50	18.20	45.50	9.10
9.00	.00	.00	.00	16.70	66.70	.00

CLAS	RZN	RG	RW	RM	WIN	LA
1.00	52.60	5.30	.00	.00	.00	42.10
2.00	29.60	16.90	4.20	8.50	2.80	50.70
3.00	46.50	15.10	3.90	1.90	1.20	56.60
4.00	55.50	12.60	1.90	3.00	1.10	57.10
5.00	65.90	19.20	2.00	2.30	2.00	61.90
6.00	54.70	16.20	2.20	2.80	2.20	59.20
7.00	39.80	19.30	.00	1.20	2.40	48.20
8.00	36.40	22.70	.00	4.50	.00	59.10
9.00	66.70	16.70	.00	16.70	.00	66.70

CLAS	LO	LZN	LG	LW	LCM	LM
1.00	31.60	52.60	10.50	.00	.00	.00
2.00	21.10	26.80	.00	1.40	4.20	1.40
3.00	17.80	36.00	.80	1.60	9.70	.00
4.00	15.70	51.10	1.90	1.60	10.70	.50
5.00	14.40	57.80	1.80	1.50	10.90	.30
6.00	10.60	51.40	1.10	1.70	11.70	.00
7.00	8.40	34.90	2.40	1.20	13.30	.00
8.00	13.60	18.20	4.50	.00	27.30	.00
9.00	.00	66.70	.00	.00	16.70	.00

APPENDIX 4.1 : Preparation of aceto-orcein stain

1 gram of orcein (Sigma Chemicals Ltd) was dissolved in 45 ml glacial acetic acid plus 55 ml distilled water. The mixture was heated in a microwave oven for 3 minutes at low temperature, then allowed to stand overnight at room temperature. The mixture was filtered through Whatman No. 3 filter paper to remove undissolved stain.

Appendix 4.2: Percentage of eggs at various stages of development at different time periods.

(Experiment 1)

Time Class	Meiosis I			Meiosis II	
	Metaphase I	Anaphase I	Telophase I/ Metaphase II	Anaphase II	Telophase I
	N	2N	N+Pb	2N+Pb	N+2Pb
0	100				
5	20		80		
10	23		77		
15	*				
20			13		87
25	20		3		77
30	7			73	20
35	21			37	42
40	17				83
45	13		7		80
90	**				

* Poor Sample

** Could not be assessed

Continued...../

(Experiment 2)

Time Class	Meiosis I			Meiosis II		
	Metaphase I N	Anaphase I 2N	Telophase I/ Metaphase II N+Pb	Anaphase II 2N+Pb	Telophase II N+2Pb	Others
0	100					
5	37		63			
10	58	25	18			
15	31	26	44			
20	22	44	34			
25	*					
30	17		13	17	53	
35	27		7	7	53	7
40	37		17	10	37	
45	23			10	67	
90	**					

* Poor Sample

** Could not be assessed

Appendix 4.3 Results of the condition of eggs and embryos treated with CB at 15, 30 and 45 minute post-activation recorded in two experiments.

Percentage of eggs at various stages of development observed at 15 minutes post-activation

(Experiment 1)

Treatment/Class	Unfertilized egg	Telophase I/Metaphase II
Control *	77	23
MI, 0.1mg CBI ⁻¹	20	80
MI, 0.5mg CBI ⁻¹	23	77

*Based on sample 10 min. post-activation

(Experiment 2)

Treatment/Class	N	Anaphase I	Diploid eggs 2N	Telophase I/ Metaphase II n+Pb
Control	31	26	0	44
MI, 0.1mg CBI ⁻¹	43	0	47	10
MI, 0.5mg CBI ⁻¹	43	0	7	50

Continued...../

**Percentage of eggs at various stages of development
observed at 30 minutes post-activation**

(Experiment 1)

Treatment/Class	Unfertilized eggs N	Anaphase II 2N+Pb	Diploid eggs 2N+Pb	Telophase II N+2Pb
Control	7	73		20
MI, 0.1mg CBI ⁻¹	23		37	40
MI, 0.5mg CBI ⁻¹	7		33	60
MII, 0.1mg CBI ⁻¹	57		20	23
MII, 0.5mg CBI ⁻¹	23		40	37

(Experiment 2)

Treatment/Class	N	Diploid eggs (2N)	N+Pb	Anaphase II 2N+Pb	Diploid eggs 2N+Pb	N+2Pb	Others
Control	17	0	13	17	0	53	
MI, 0.1mg CBI ⁻¹	27	0		0	47	27	
MI, 0.5mg CBI ⁻¹	10	10	7	0	60	13	
MII, 0.1mg CBI ⁻¹	10	0	53	0	20	13	3
MII, 0.5mg CBI ⁻¹	10	0		0	86	3	

Continued...../

Appendix 4.3 Continued...../

**Percentage of eggs at various stage of development
observed at 45 minutes post-activation**

(Experiment 1)

Treatment/Class	N	Telophase I Metaphase II n+Pb	Diploid egg + Pb 2N+Pb	Telophase II n+2Pb
Control	13	7		80
MI, 0.1mg CBI ⁻¹	33	7	20	40
MI, 0.5mg CBI ⁻¹	17	17	23	43
MII, 0.1mg CBI ⁻¹	7	13	13	67
MII, 0.5mg CBI ⁻¹	3	27	13	57

(Experiment 2)

Treatment/Class	N	Anaphase II 2n+Pb	Diploid Eggs 2N+Pb	N+2Pb	Others
Control	23	10		67	
MI, 0.1mg CBI ⁻¹	2	0	47	47	
MI, 0.5mg CBI ⁻¹	10	0	53	37	
MII, 0.1mg CBI ⁻¹	7	0	53	37	3
MII, 0.5mg CBI ⁻¹	3	0	60	37	

Appendix 4.4: Non parametric Kruskal-Wallis (K-W) oneway anova results of apm and dvm for Experiment 1 on the induction of triploidy in P.radiata studied for one week period.

Abbreviations used throughout.

GROUP 1=CONTROL; GROUP 2=MI; GROUP 3=MII.

Variable APM
By Variable GROUP

Group	Count	Mean	Standard Deviation	Standard Error
Grp 1	25	70.9280	5.0220	1.0044
Grp 2	25	68.6400	5.4609	1.0922
Grp 3	10	68.6400	6.0294	1.9067
Total	60	69.5933	5.4064	.6980

Group	Minimum	Maximum
Grp 1	57.2000	85.8000
Grp 2	57.2000	71.5000
Grp 3	57.2000	78.6500
Total	57.2000	85.8000

Levene Test for Homogeneity of Variances

Statistic	df1	df2	2-tail Sig.
2.7462	2	57	.073

Variable DVM
By Variable GROUP

Group	Count	Mean	Standard Deviation	Standard Error
Grp 1	25	57.4980	6.0102	1.2020
Grp 2	25	56.9140	5.2542	1.0508
Grp 3	10	59.3450	4.8259	1.5261
Total	60	57.5625	5.4950	.7094

Group	Minimum	Maximum
Grp 1	42.9000	71.5000
Grp 2	42.9000	71.5000
Grp 3	57.2000	71.5000
Total	42.9000	71.5000

Levene Test for Homogeneity of Variances

Statistic	df1	df2	2-tail Sig.
.2351	2	57	.791

	N	Mean	Std Dev	Minimum	Maximum
APM	60	69.59333	5.40642	57.20	85.80
DVM	60	57.56250	5.49498	42.90	71.50

- - - - - Kruskal-Wallis 1-way ANOVA

APM
by GROUP

Mean Rank	Cases	
33.96	25	GROUP = 1
28.42	25	GROUP = 2
27.05	10	GROUP = 3
	--	
	60	Total

CASES	Chi-Square	Sign	Corrted for Ties Chi-Square	Sign
60	1.7261	.4219	3.1537	.2066

- - - - - Kruskal-Wallis 1-way ANOVA

DVM
by GROUP

Mean Rank	Cases	
30.86	25	GROUP = 1
28.68	25	GROUP = 2
34.15	10	GROUP = 3
	--	
	60	Total

CASES	Chi-Square	Significance	Corrtd. for Ties Chi-Square	Sign
60	.7189	.6980	1.4750	.4783

Appendix 4.4: Continued: Oneway Anova results of apm and dvm
 for Experiment 2 on the induction of triploidy
 in P.radiata studied over a 5 week period.

Abbreviations used throughout:

GROUP 1=CONTROL;GROUP 2=MI;GROUP 3=MII.
 IF (GROUP=1 AND WEEK=1) G=1.
 IF (GROUP=2 AND WEEK=1) G=2.
 IF (GROUP=3 AND WEEK=1) G=3.
 IF (GROUP=1 AND WEEK=2) G=4.
 IF (GROUP=2 AND WEEK=2) G=5.
 IF (GROUP=3 AND WEEK=2) G=6.
 IF (GROUP=1 AND WEEK=3) G=7.
 IF (GROUP=2 AND WEEK=3) G=8.
 IF (GROUP=3 AND WEEK=3) G=9.
 IF (GROUP=1 AND WEEK=4) G=10.
 IF (GROUP=2 AND WEEK=4) G=11.
 IF (GROUP=3 AND WEEK=4) G=12.
 IF (GROUP=1 AND WEEK=5) G=13.
 IF (GROUP=2 AND WEEK=5) G=14.
 IF (GROUP=3 AND WEEK=5) G=15.

GROUP 1=CONTROL;GROUP 2=MI;GROUP 3=MII.

LAPM=LOG10 (APM), LDVM=LOG10 (DVM).
 WEEK1 G1 TO G3

- - - - - O N E W A Y - - - - -

Variable APM						
By Variable G						
Analysis of Variance						
Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.	
Between Groups	2	920.2050	460.1025	7.3636	.0012	
Within Groups	72	4498.7800	62.4831			
Total	74	5418.9850				
Group	Count	Mean	Standard Deviation	Standard Error		
Grp 1	25	74.3600	6.1921	1.2384		
Grp 2	25	78.6500	5.4609	1.0922		
Grp 3	25	82.9400	10.9218	2.1844		
Total	75	78.6500	8.5574	.9881		
Fixed Effects Model			7.9046	.9127		
Random Effects Model				2.4768		

WEEK1 G1 TO G3

- - - - - O N E W A Y - - - - -

Variable DVM
By Variable G

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	2	982.9153	491.4576	6.5945	.0023
Within Groups	72	5365.8176	74.5252		
Total	74	6348.7329			

Group	Count	Mean	Standard Deviation	Standard Error
Grp 1	25	61.7760	7.4075	1.4815
Grp 2	25	66.0660	5.5690	1.1138
Grp 3	25	70.6420	11.7341	2.3468
Total	75	66.1613	9.2625	1.0695

Fixed Effects Model 8.6328 .9968

Random Effects Model 2.5598

Random Effects Model - Estimate of Between Component Variance
16.6773

Group	Minimum	Maximum
Grp 1	42.9000	78.6500
Grp 2	57.2000	71.5000
Grp 3	50.0500	92.9500
Total	42.9000	92.9500

Levene Test for Homogeneity of Variances

Statistic	df1	df2	2-tail Sig.
6.7198	2	72	.002

Multiple Range Test

Tukey-HSD Procedure
Ranges for the .050 level -

Random Effects Model - Estimate of Between Component Variance 15.9048

Group	Minimum	Maximum
Grp 1	57.2000	85.8000
Grp 2	64.3500	85.8000
Grp 3	71.5000	107.2500
Total	57.2000	107.2500

Levene Test for Homogeneity of Variances

Statistic	df1	df2	2-tail Sig.
11.3892	2	72	.000

Multiple Range Test

Tukey-HSD Procedure

Ranges for the .050 level -

3.38 3.38

The ranges above are table ranges.

The value actually compared with $\text{Mean}(J) - \text{Mean}(I)$ is..

$5.5894 * \text{Range} * \sqrt{1/N(I) + 1/N(J)}$

(*) Denotes pairs of groups significantly different at the .050 level

		G G G
		r r r
		p p p
Mean	Group	1 2 3
74.3600	Grp 1	
78.6500	Grp 2	
82.9400	Grp 3	*

Homogeneous Subsets (Subsets of groups, whose highest and lowest means do not differ by more than the shortest significant range for a subset of that size)

SUBSET 1

Group	Grp 1	Grp 2
Mean	74.3600	78.6500

SUBSET 2

Group	Grp 2	Grp 3
Mean	78.6500	82.9400

3.38 3.38

The ranges above are table ranges.

The value actually compared with $\text{Mean}(J) - \text{Mean}(I)$ is..

$$6.1043 * \text{Range} * \sqrt{1/N(I) + 1/N(J)}$$

(*) Denotes pairs of groups significantly different at the .050 level

		G G G
		r r r
		p p p
Mean	Group	1 2 3
61.7760	Grp 1	
66.0660	Grp 2	
70.6420	Grp 3	*

Homogeneous Subsets (Subsets of groups, whose highest and lowest means do not differ by more than the shortest significant range for a subset of that size)

SUBSET 1

Group	Grp 1	Grp 2
Mean	61.7760	66.0660
- - - - -		

SUBSET 2

Group	Grp 2	Grp 3
Mean	66.0660	70.6420
- - - - -		

WEEK2 G4 TO G6

- - - - - O N E W A Y - - - - -

Variable APM
By Variable G

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	2	9279.7562	4639.87	28.6109	.0000
Within Groups	72	11676.3790	162.17		
Total	74	20956.1352			

Group	Count	Mean	Standard Deviation	Standard Error
Grp 4	25	76.9340	9.2950	1.8590
Grp 5	25	77.2200	5.8380	1.1676
Grp 6	25	100.6720	19.1321	3.8264
Total	75	84.9420	16.8283	1.9432

Fixed Effects Model 12.7347 1.4705

Random Effects Model 7.8654

Random Effects Model - Estimate of Between Component Variance
179.1082

Group	Minimum	Maximum
Grp 4	64.3500	100.1000
Grp 5	64.3500	85.8000
Grp 6	64.3500	143.0000
Total	64.3500	143.0000

Levene Test for Homogeneity of Variances

Statistic	df1	df2	2-tail Sig.
8.1161	2	72	.001

Multiple Range Test

Tukey-HSD Procedure

Ranges for the .050 level -

3.38 3.38

The ranges above are table ranges.
 The value actually compared with $\text{Mean}(J) - \text{Mean}(I)$ is..
 $9.0048 * \text{Range} * \text{Sqrt}(1/N(I) + 1/N(J))$

(*) Denotes pairs of groups significantly different at the .050 level

		G G G
		r r r
		p p p
Mean	Group	4 5 6
76.9340	Grp 4	
77.2200	Grp 5	
100.6720	Grp 6	* *

Homogeneous Subsets (Subsets of groups, whose highest and lowest means
 do not differ by more than the shortest
 significant range for a subset of that size)

SUBSET 1

Group	Grp 4	Grp 5
Mean	76.9340	77.2200

SUBSET 2

Group	Grp 6
Mean	100.6720

WEEK2 G4 TO G6

- - - - - O N E W A Y - - - - -

Variable DVM
By Variable G

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	2	8981.2008	4490.600	30.3711	.0000
Within Groups	72	10645.7494	147.857		
Total	74	19626.9502			

Group	Count	Mean	Standard Deviation	Standard Error
Grp 4	25	65.4940	9.1565	1.8313
Grp 5	25	63.7780	4.5782	.9156
Grp 6	25	87.8020	18.4058	3.6812
Total	75	72.3580	16.2859	1.8805

Fixed Effects Model 12.1597 1.4041

Random Effects Model 7.7379

Random Effects Model - Estimate of Between Component Variance
173.7097

Group	Minimum	Maximum
Grp 4	57.2000	92.9500
Grp 5	57.2000	71.5000
Grp 6	57.2000	135.8500
Total	57.2000	135.8500

Levene Test for Homogeneity of Variances

Statistic	df1	df2	2-tail Sig.
15.8241	2	72	.000

Multiple Range Test

Tukey-HSD Procedure
Ranges for the .050 level -

3.38 3.38

The ranges above are table ranges.
 The value actually compared with $\text{Mean}(J) - \text{Mean}(I)$ is..
 $8.5982 * \text{Range} * \text{Sqrt}(1/N(I) + 1/N(J))$

(*) Denotes pairs of groups significantly different at the .050 level

		G G G
		r r r
		p p p
Mean	Group	5 4 6
63.7780	Grp 5	
65.4940	Grp 4	
87.8020	Grp 6	* *

Homogeneous Subsets (Subsets of groups, whose highest and lowest means do not differ by more than the shortest significant range for a subset of that size)

SUBSET 1

Group	Grp 5	Grp 4
Mean	63.7780	65.4940

SUBSET 2

Group	Grp 6
Mean	87.8020

The ranges above are table ranges.

The value actually compared with $\text{Mean}(J) - \text{Mean}(I)$ is..

$$14.7599 * \text{Range} * \text{Sqrt}(1/N(I) + 1/N(J))$$

(*) Denotes pairs of groups significantly different at the .050 level

		G G G
		r r r
		p p p
Mean	Group	8 7 9
84.0125	Grp 8	
85.5250	Grp 7	
124.6960	Grp 9	* *

WEEK3 G7 TO G9

- - - - - O N E W A Y - - - - -

Variable APM
By Variable G

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups .0000	2	20627.3195	10313.65	22.4330	
Within Groups	60	27585.2553	459.75		
Total	62	48212.5748			

Group	Count	Mean	Standard Deviation	Standard Error
Grp 7	26	97.6250	12.7811	2.5066
Grp 8	12	95.3333	10.7069	3.0908
Grp 9	25	133.8480	30.4414	6.0883
Total	63	111.5627	27.8859	3.5133

Fixed Effects Model 21.4419 2.7014

Random Effects Model 13.6525

Random Effects Model - Estimate of Between Component Variance
491.9145

Group	Minimum	Maximum
Grp 7	71.5000	121.5500
Grp 8	78.6500	114.4000
Grp 9	100.1000	207.3500
Total	71.5000	207.3500

Levene Test for Homogeneity of Variances

Statistic	df1	df2	2-tail Sig.
14.5065	2	60	.000

Multiple Range Test

Scheffe Procedure
Ranges for the .050 level -

3.55 3.55

The ranges above are table ranges.

The value actually compared with $\text{Mean}(J) - \text{Mean}(I)$ is..

$$15.1617 * \text{Range} * \text{Sqrt}(1/N(I) + 1/N(J))$$

(*) Denotes pairs of groups significantly different at the .050 level

		G G G
		r r r
		p p p
Mean	Group	8 7 9
95.3333	Grp 8	
97.6250	Grp 7	
133.8480	Grp 9	* *

WEEK3 G7 TO G9

- - - - - O N E W A Y - - - - -

Variable DVM
By Variable G

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups .0000	2	23723.7557	11861.87	27.2243	
Within Groups	60	26142.5915	435.70		
Total	62	49866.3471			

Group	Count	Mean	Standard Deviation	Standard Error
Grp 7	26	85.5250	13.6384	2.6747
Grp 8	12	84.0125	11.0452	3.1885
Grp 9	25	124.6960	28.9759	5.7952
Total	63	100.7810	28.3601	3.5730

Fixed Effects Model 20.8737 2.6298

Random Effects Model 14.6487

Random Effects Model - Estimate of Between Component Variance
570.4030

Group	Minimum	Maximum
Grp 7	57.2000	114.4000
Grp 8	71.5000	100.1000
Grp 9	85.8000	185.9000
Total	57.2000	185.9000

Levene Test for Homogeneity of Variances

Statistic	df1	df2	2-tail Sig.
9.4093	2	60	.000

Multiple Range Test

Scheffe Procedure
Ranges for the .050 level -

3.55 3.55

WEEK4 G10 TO G12

- - - - - O N E W A Y - - - - -

Variable APM
By Variable G

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	2	28368.2161	14184.10	10.5380	.000
Within Groups	72	96911.9008	1345.99		
Total	74	125280.1169			

Group	Count	Mean	Standard Deviation	Standard Error
Grp10	25	130.1300	13.0541	2.6108
Grp11	25	134.9920	11.5512	2.3102
Grp12	25	173.6020	61.1078	12.2216
Total	75	146.2413	41.1458	4.7511

Fixed Effects Model 36.6879 4.2363

Random Effects Model 13.7521

Random Effects Model - Estimate of Between Component Variance
513.5244

Group	Minimum	Maximum
Grp10	114.4000	171.6000
Grp11	121.5500	171.6000
Grp12	114.4000	429.0000
Total	114.4000	429.0000

Levene Test for Homogeneity of Variances

Statistic	df1	df2	2-tail Sig.
6.4253	2	72	.003

Multiple Range Test

Tukey-HSD Procedure
Ranges for the .050 level -

3.38 3.38

The ranges above are table ranges.
The value actually compared with $\text{Mean}(J) - \text{Mean}(I)$ is..
 $25.9422 * \text{Range} * \text{Sqrt}(1/N(I) + 1/N(J))$

(*) Denotes pairs of groups significantly different at the .050 level

Mean	Group	G G G	r r r	p p p	1 1 1	0 1 2
130.1300	Grp10					
134.9920	Grp11					
173.6020	Grp12					* *

Homogeneous Subsets (Subsets of groups, whose highest and lowest means do not differ by more than the shortest significant range for a subset of that size)

SUBSET 1

Group	Grp10	Grp11
Mean	130.1300	134.9920

SUBSET 2

Group	Grp12
Mean	173.6020

WEEK4 G10 TO G12

- - - - - O N E W A Y - - - - -

Variable DVM
By Variable G

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	2	24084.8322	12042.41	11.9070	.0000
Within Groups	72	72818.8890	1011.37		
Total	74	96903.7212			

Group	Count	Mean	Standard Deviation	Standard Error
Grp10	25	121.2640	14.9524	2.9905
Grp11	25	126.4120	11.0613	2.2123
Grp12	25	161.5900	51.8478	10.3696
Total	75	136.4220	36.1871	4.1785

Fixed Effects Model 31.8021 3.6722

Random Effects Model 12.6714

Random Effects Model - Estimate of Between Component Variance
441.2417

Group	Minimum	Maximum
Grp10	100.1000	164.4500
Grp11	107.2500	157.3000
Grp12	107.2500	378.9500
Total	100.1000	378.9500

Levene Test for Homogeneity of Variances

Statistic	df1	df2	2-tail Sig.
5.6282	2	72	.005

Multiple Range Test

Tukey-HSD Procedure
Ranges for the .050 level -

3.38 3.38

The ranges above are table ranges.

The value actually compared with $\text{Mean}(J) - \text{Mean}(I)$ is..
 $22.4875 * \text{Range} * \text{Sqrt}(1/N(I) + 1/N(J))$

(*) Denotes pairs of groups significantly different at the .050 level

		G G G
		r r r
		p p p
		1 1 1
Mean	Group	0 1 2
121.2640	Grp10	
126.4120	Grp11	
161.5900	Grp12	* *

Homogeneous Subsets (Subsets of groups, whose highest and lowest means
do not differ by more than the shortest
significant range for a subset of that size)

SUBSET 1

Group	Grp10	Grp11
Mean	121.2640	126.4120
- - - - -	- - - - -	- - - - -

SUBSET 2

Group	Grp12
Mean	161.5900
- - - - -	- - - - -

WEEK 5 G13 TO G15

- - - - - O N E W A Y - - - - -

Variable APM
By Variable G

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	2	1584.4953	792.24	1.6613	.197
Within Groups	72	34335.3172	476.87		
Total	74	35919.8125			

Group	Count	Mean	Standard Deviation	Standard Error
Grp13	25	140.9980	17.0854	3.4171
Grp14	25	152.1520	21.2023	4.2405
Grp15	25	145.2480	26.2525	5.2505
Total	75	146.1327	22.0319	2.5440

Fixed Effects Model 21.8376 2.5216

Random Effects Model 3.2501

Random Effects Model - Estimate of Between Component Variance 12.6147

Group	Minimum	Maximum
Grp13	114.4000	178.7500
Grp14	121.5500	193.0500
Grp15	114.4000	200.2000
Total	114.4000	200.2000

Levene Test for Homogeneity of Variances

Statistic	df1	df2	2-tail Sig.
4.0383	2	72	.022

No two groups are significantly different at the .050 level

WEEK 5 G13 TO G15

- - - - - O N E W A Y - - - - -

Variable DVM
By Variable G

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	2	1768.1569	884.07	2.2848	.109
Within Groups	72	27859.7176	386.94		
Total	74	29627.8745			

Group	Count	Mean	Standard Deviation	Standard Error
Grp13	25	134.1340	15.8917	3.1783
Grp14	25	145.2880	18.9980	3.7996
Grp15	25	136.1360	23.3955	4.6791
Total	75	138.5193	20.0094	2.3105

Fixed Effects Model 19.6708 2.2714

Random Effects Model 3.4333

Random Effects Model - Estimate of Between Component Variance 19.8855

Group	Minimum	Maximum
Grp13	114.4000	164.4500
Grp14	121.5500	178.7500
Grp15	100.1000	185.9000
Total	100.1000	185.9000

Levene Test for Homogeneity of Variances

Statistic	df1	df2	2-tail Sig.
3.1906	2	72	.047

No two groups are significantly different at the .050 level