A GONAD CONDITIONING STUDY OF THE GREENLIP ABALONE (*HALIOTIS LAEVIGATA*).

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Thesis presented for award of Masters Degree by Research.
University of Tasmania, 1992.

Declaration:

I hereby declare that this thesis has been composed by myself, that it has not been accepted in any previous application for a degree, that the work of which this is a record has been done by myself, that all quotations have been distinguished by quotation marks and the sources of information have been specifically acknowledged.

M. Lleonart.

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ABSTRACT

The Australian greenlip abalone Haliotis laevigata is of commercial importance in the abalone diving industry. The species is also believed to have culture potential and accordingly control over reproduction is considered valuable. The major purpose of the study was to accelerate gonad development resulting in spawning outside the natural spawning season.

Abalone were collected from Franklin sound in the Furneaux group of islands off the north-east tip of Tasmania. The important features of the conditioning tank were elevated water temperature, the provision of water movement within the tank to distribute feed to sedentary abalone and a diet of preferred red algae.

Animals collected on 27 April 1990 were induced to spawn on 21 August 1990, 112 days or 1750 degree days following commencement of gonad conditioning. The natural spawning season of the source population was found to be November to March.

A variety of methods for measuring reproductive development of abalone were used. This allowed the utility of individual methods to be examined and comparisons made between methods. Two gonad indices, the gonad bulk index (GBI) and the modified gonad bulk index (MGBI) were used as were a number of assessment methods with a histological component: oocyte size/frequency distribution, mean oocyte diameter, an ovarian phase method and percentage mature spermatozoa. The MGBI was considered more sensitive than the GBI, detecting first significant gonad growth following six weeks of gonad conditioning, compared to nine weeks for the GBI.

The gonad indices increased from initial values of 14.1 ± 4.4, n=10 and 0.4 ± 0.2, n=10 for GBI and MGBI respectively to 72.3 ± 9.2, n=10 and 7.0 ± 2.0, n=10 following 105 days of gonad conditioning. Mean oocyte diameter (µm) increased from 30.7 ± 2.0, n=5 initially to 109.7 ± 6.0, n=5 during the same time period. The percentage of male germ cells present as mature spermatozoa increased from zero to 90.6 ± 16.3, n=5 following 24 weeks of conditioning.

1. INTRODUCTION

Abalone are herbivorous marine gastropods of commercial importance. There are approximately one hundred species world-wide, all of which belong to the single genus Haliotis. Abalone are valued primarily for the meat of the large, powerful foot muscle. Twenty-two species of commercial importance are described by Hahn (1989). The majority of these species attain relatively large sizes and occur in temperate waters.

The major abalone fishing countries are Australia, Japan, Mexico, South Africa and the United States. Certain characteristics of abalones make them susceptible to overfishing, which has occurred to varying degrees in all abalone producing countries. These characteristics include the predictability of location and accessability of abalone populations and the lack of mobility shown by adults. In addition, growth to adult size is slow and recruitment is unpredictable (Tegner and Butler, 1989). Declining yields from the traditional fishery source have led to interest in abalone culture for reseeding programs and in captive growout to so called 'cocktail size' of between 5 cm and 8 cm.

The Japanese pioneered the use of hatchery reared juveniles to enhance wild stocks (Tegner and Butler,1989). This strategy appears successful and according to Hahn (1989) the domestic harvest is relatively stable although the demand is still high. In California a sharp decline in the abalone catch served as an impetus for fishery enhancement through aquaculture (Ebert and Houk, 1989). Unlike in Japan, the

emphasis in California has been on captive growout rather than reseeding. In Taiwan according to Chen (1989) increased demand and soaring prices led to the development of a successful culture industry.

Commercial culture of abalones is practiced in Japan, Korea, Taiwan, The United States, and has commenced in Australia and New Zealand. Haliotis discus Reeve and H.discus hannai Ino are produced in Japan and Korea; H.diversicolor Reeve is farmed in Taiwan, and H.rufescens Swainson and H.fulgens Philippi are cultured in the United States. The successful culture industry in Taiwan produced approximately 500 tonnes of abalone in 1986 (Chen, 1990).

There are three haliotid species of commercial importance in Australia: H.laevigata Donovan, H.rubra Leach, and the smaller H.roei Gray. The first two species are commonly called greenlip and blacklip abalone respectively. The export value of Australian abalone was 91.3 million dollars in the 1989/90 financial year (Australian Bureau of Statistics). Over three thousand tonnes of abalone were exported in live, chilled, frozen and canned forms to Hong Kong, Japan, and Singapore.

Tasmania is the largest abalone producing state contributing 46% of total tonnage. Management measures introduced to protect the fishery in this state resulted in quotas for professional divers reduced from 30.8 t in 1985 to 16.8 t in 1989. Such reductions in yield locally coupled with the known history of abalone fisheries overseas have contributed to interest in culturing local species. To date, research and farming effort have focused on H.laevigata and

H.rubra. Both abalones are believed to have relatively high growth rates (Shepherd and Hearn, 1983), especially the former species (Hone, 1989).

Control of the reproductive cycle is generally desirable for aquaculture. Induced maturation of captive abalone is referred to as conditioning or gonad conditioning and is generally practised where haliotids are cultured. The quantification of conditions required to produce gonad maturation of abalone e.g. time, temperature, diet and tank design have been established for the Japanese species H.discus (Kikuchi and Uki, 1974), H.gigantea Gmelin (Kikuchi and Uki, 1975) and H.discus hannai (Uki and Kikuchi, 1984).Control over the reproduction of the following North American species has also been achieved: H.fulgens (Leighton et al., 1981), H.rufescens (Ebert and Houk, 1984; Ault, 1985), H.corrugata Wood (Morse,1984) and H.cracherodii Leach (Morse, 1984). However full quantification of the conditions required for gonad conditioning of these species has generally not been reported.

The major advantage of gonad conditioning is the flexibility that can be introduced to production cycles. This is of particular advantage where culture species have restricted natural spawning seasons. The majority of haliotid species examined do in fact have an annual spawning season, typically of three or four months duration (section 4.16). Therefore, gonad conditioning may allow for more efficient use of hatchery resources through year round production. Additionally, production cycles can be initiated to take advantage of seasonal temperature variations. For example,

where species naturally spawn in late summer it may be advantageous to induce maturation several months early. This exploits the seasonal spring/summer rise in sea temperature, extending the time period post larvae are initially exposed to warm water and thus maximizing early growth.

A further advantage of gonad conditioning is that conditioned abalone produce more eggs than wild abalone of equivalent size (Ault, 1985). This allows relatively small abalone to be kept as broodstock reducing the quantity of hatchery resources such as feed and tank space allocated to such animals. Additionally, control over the reproductive cycle of abalones makes possible selective breeding programs to identify desirable traits such as superior growth rates. Hybridization of species which may be advantageous in terms of appearance and growth rate is also simplified by conditioning abalones. Indeed, where pairs of haliotid species do not share a spawning season hybridization cannot be achieved without gonad conditioning.

The major purpose of this study was to produce out of season spawning of the greenlip abalone H.laevigata through gonad conditioning. The spawning season of the species is the summer months in South Australia (Shepherd and Laws, 1974) and Victoria (McShane, 1988). Observation suggests this is also the case in Tasmania and a population of H.laevigata was monitored to provide confirmation. Therefore, it was planned to induce spawning in winter to demonstrate the effectiveness of the conditioning process. By this means the length of the reproductive cycle and feed requirements would be quantified.

A further aim of the research was to compare the usefulness of a variety of measures that have been used to monitor reproduction in abalone. In particular, two commonly used gonad indices were compared as were four methods involving a histological component. An examination of gonad maturity measures was considered important since a conditioning study by Ault (1985) showed the commonly used gonad bulk index to be incapable of detecting gonad maturation. Additional purposes of the research were to measure fecundity and determine seaweed preferences of H.laevigata.

2. MATERIALS AND METHODS

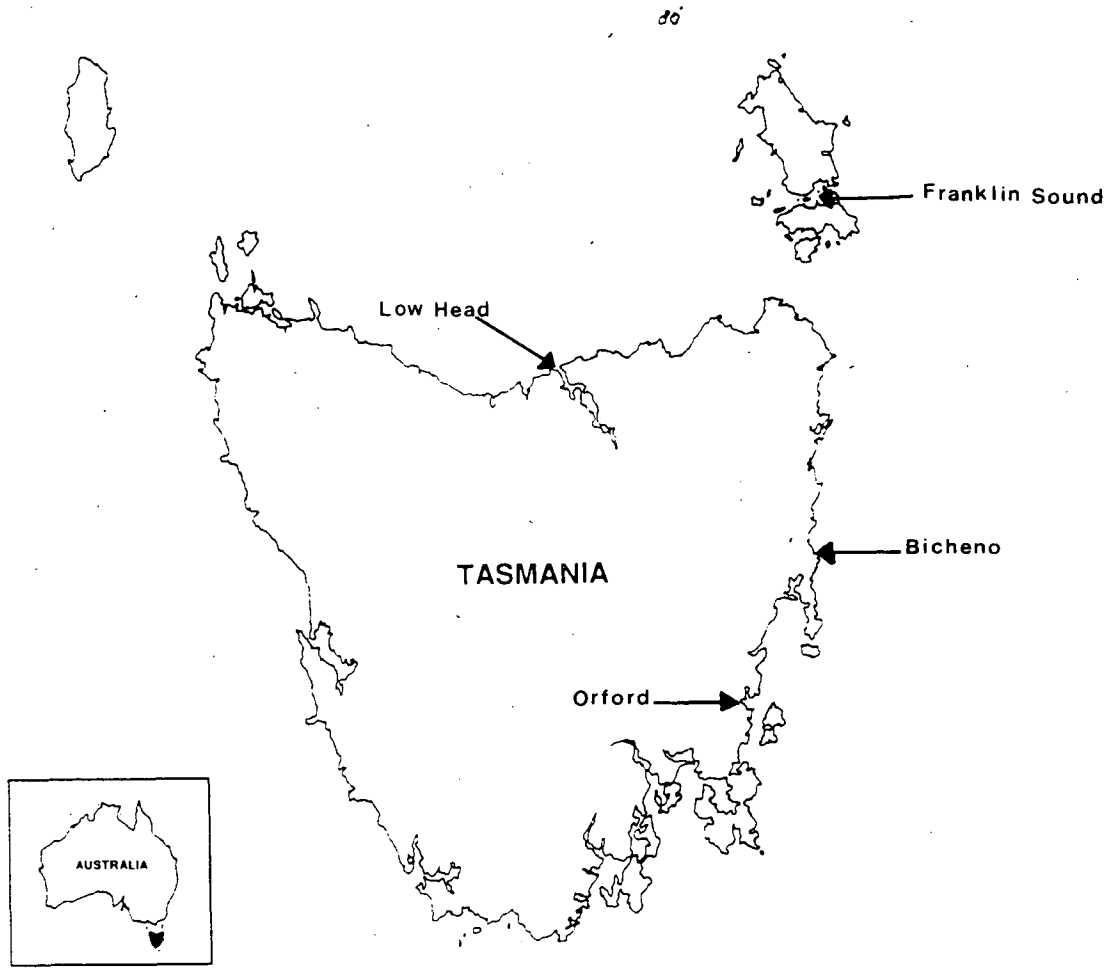
2.1
Experimental Animals

Specimens of H.laevigata (mean weight 359.9 g, s.d.=83.4 g; mean length 140 mm, s.d.=10 mm; n=128) were selected from a professional divers catch on 27 April 1990. The animals from Franklin Sound (Figure 1) were captured on a limestone reef at a depth of about five metres. Water temperature at the time was approximately 16°C. Franklin Sound is a stretch of water in the Furneaux Islands group located in eastern Bass Strait. The Furneaux group is a major contributor of H.laevigata to the Tasmanian fishery for the species (21% in 1987, Department of Primary Industry statistics).

The experimental animals were selected on the basis that they displayed no apparent sign of injury as a result of capture and that the first and second respiratory holes were free of obstruction to permit tagging. The legal minimum size for abalone in Tasmania is 132 mm and the pool of animals available for selection ranged from this size to approximately 180 mm. Because the feed requirements of abalone increase with size larger individuals were rejected. Every effort was made to select animals of the same size.

The reproductive cycle of the population from which the animals were selected had not been previously documented. Anecdotal evidence from local abalone divers suggests ripe individuals are found from November to at least January.

FIGURE 1
Collection Sites for Abalone and Seaweed


Franklin Sound
Low Head
Bicheno
TASMANIA
Orford
AUSTRALIA

At the time of capture gonad development was minimal and the sex of individuals could not be distinguished.

The abalone were transported in expanded polystyrene fish boxes packed with damp seaweed. Transport by air and road to Abalone Hatcheries Pty Ltd (Bicheno, Tasmania) where the research was conducted took five hours. No mortalities occurred during transport or immediately thereafter. The animals were allowed four days acclimatisation at ambient temperature (16°C) and natural photoperiod (10 hr 40 min light).

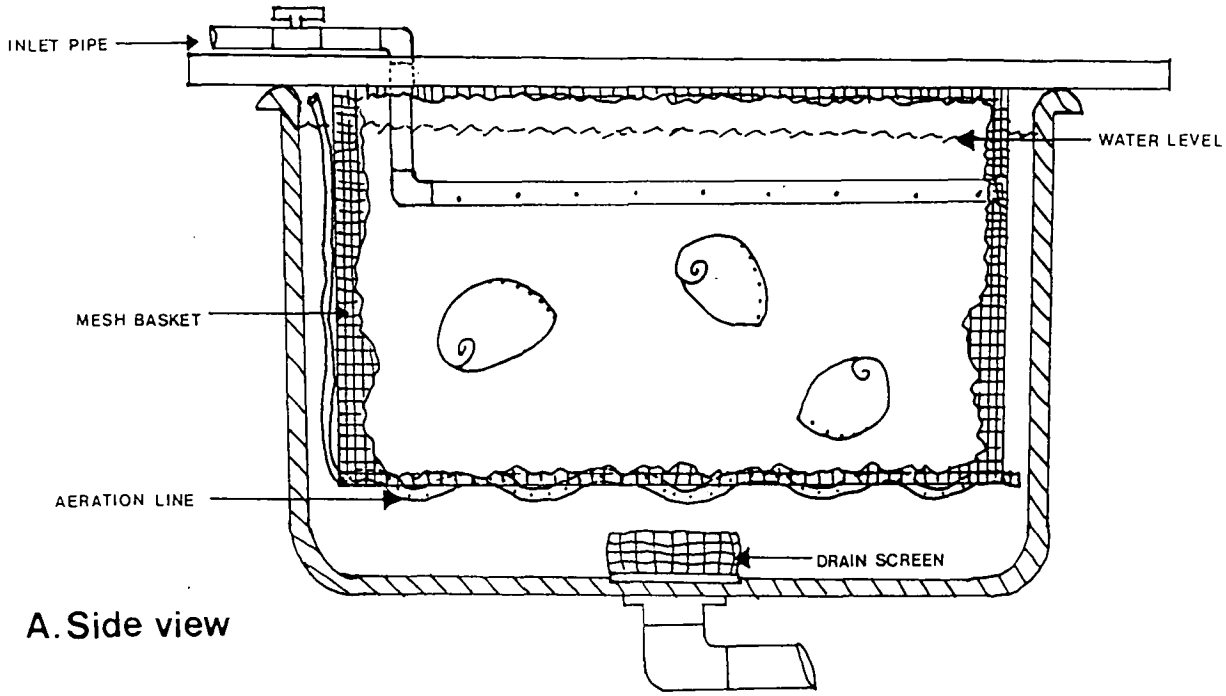
2.2
Holding Conditions

2.2.1
Abalone Hatcheries Pty.Ltd.

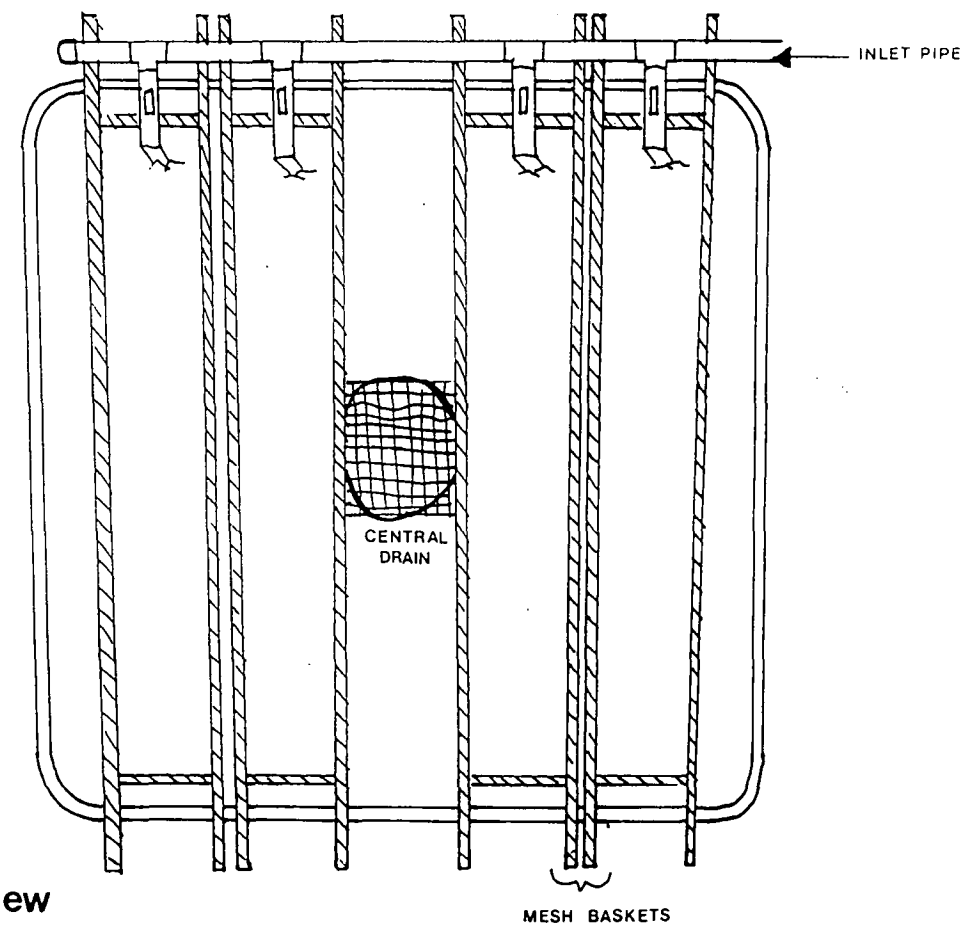
This aquaculture facility is located at Bicheno on the east coast of Tasmania (Figure 1). The site is south of the natural distribution of H.laevigata.

The experimental animals were housed in a 3000 l fibreglass tank with a central drain. The tank was fitted with four easily removed mesh baskets, within which the abalone were located. The mesh base of each basket was left uncovered to allow fragments of uneaten food and faeces to fall through to the tank bottom approximately 10 cm below. Plastic sheet was fixed to the mesh on the sides of baskets to provide a suitable substrate for abalone (Figure 2).

FIGURE 2
Conditioning Tank Design



A. Side view



B. Overhead view

Water entering the conditioning tank was directed into the individual baskets through PVC pipe located below the water surface. The purpose of this was to create water movement within the baskets. Aeration was supplied to each basket by 13 mm polythene tubing threaded through the mesh of the base. A major purpose of the aeration was to keep seaweed fed to abalone in good condition and to circulate the weed affording all animals feeding opportunities. The importance placed on producing water movement in the conditioning tank was in part due to the observation of Shepherd (1973) that water movement elicits a characteristic feeding response in H.laevigata.

The conditioning tank was a flow through system with an approximate exchange rate of 700 l/h. This flow rate was considered adequate for removal of excretory products providing suitable conditions for gonad development. The flow was based on data cited in Hahn (1989) relating to H.discus hannai. Water entering the tank was sand filtered to 50 µm.

It was intended to maintain water temperature in the tank at 17°C. In the absence of data relating to temperature tolerance of H.laevigata, this figure was chosen as a conservative maximum based on typical summer water temperatures in the animal's natural range. In practice, as winter approached this temperature could not be reached without reducing water flow to undesirable levels. Heating was achieved by the use of one or more 2 kw heaters placed directly in the tank.

Light was excluded from the part of the hatchery containing the conditioning tank by the use of shadecloth. An artificial

photoperiod of 14 hr light: 10 hr dark was provided by a timer and 40 W light bulb suspended a metre above the water surface. The purpose of this was to simulate day length during the natural summer spawning season. This practice is in accordance with commercial conditioning techniques used in Japan (Hahn,1989). The light intensity at the water surface was 350 lux.

Experimental animals were fed a diet of fresh and/or frozen algae. Abalone used in the experiment showed a strong preference for red seaweeds which were supplied when ever possible. The majority of the algae were obtained by shallow water diving at Prosser Bay Orford, on the east coast of Tasmania (Figure 1). The major species obtained from this site were Hypnea sp., Rhabdonia coccinea Harvey, and Codium sp. (a green seaweed which proved palatable). Two further red seaweeds : Laurencia filiformis and Chiracanthea arborea Harvey were obtained from Flinders Island. Smaller amounts of the green seaweed Ulva sp. were fed as was the locally abundant brown seaweed Macrocystis pyrifera Linnaeus. Live algae were stored in 1000 l round, painted metal tanks located outside the hatchery building. The tanks were aerated and received approximately 100 l/h of unfiltered seawater. The maximum amount of algae present in a tank at one time was approximately 30 kg. In addition to the live food, a store of R.coccinea was frozen and found to be still palatable after four months. Each basket of abalone was generally given three days supply of food at a time. The animals were fed to slight excess and uneaten seaweed discarded if putrid. The algae remained in good condition for weeks at a time in the storage and experimental tanks with the exception of Hypnea sp.

The conditioning tank was cleaned twice weekly by siphoning the waste feed and faeces from the tank bottom. Seaweed of unpalatable appearance also was removed from the baskets at this time. Green turban shells, Subninella undulata Solander were kept in the conditioning tank to consume small pieces of feed descending from the baskets above.

A maximum/minimum thermometer was kept in the tank and the temperature range recorded at least every three days. Nitrite was measured with an Aquarium Systems Seatest kit and ammonia tested with a Sera products test kit. These analyses were performed four times while the abalone were housed in the conditioning system described above.

2.2.2

University of Tasmania, Launceston Campus

Those abalone that had not been sacrificed for gonad analysis were relocated to a tank at the University on 23 August 1990 following successful induced spawning. This tank was a recirculating system with a 1000 l holding section and a 600 l detached biofilter box including approximately 250 l of shell and gravel substrate. The holding section was of oval design with a central dividing wall two thirds the length of the tank. Construction was of marine plywood and fibreglass. The tank was lined with plastic sheet to provide a substrate from which the abalone could be removed easily.

The system was located within an insulated room and the temperature maintained at 17°C ± 0.5°C with a single 2 kw heater. The tank was aerated through a loop of polythene

tubing located on the tank bottom and water movement and additional aeration were created by water entering the tank through spray bars under pump pressure. The system was cleaned by siphoning waste seaweed and faeces from the tank twice weekly. Partial water exchanges of up to 20% were then performed to maintain water volume. In addition fresh water was added to the system as required to prevent the salinity rising. Action was taken to reduce salinity once it reached approximately 36 ppt. Ammonia levels were measured weekly with an Aquamerck 1117 test kit. Dissolved oxygen was also measured weekly using a Yeo-Kal model 603 DO/Temperature meter. Salinity was monitored at least once weekly with an Atago S-10 refractometer and temperature was recorded daily. An Activon model 209 pH/mV meter was used to measure pH.

The experimental animals were fed daily and to slight excess. The major seaweeds supplied were L.filiformis from Flinders Island and a variable mixture of red seaweeds deposited by the tide at Low Head, Northern Tasmania (Figure 1). In addition Gracilaria secundata was available from experimental cultures held at the University.

2.3 Experimental Design

The combination of temperature and photoperiod was intended to simulate conditions in the Tasmanian summer, the natural spawning season of the species. Also of importance in the experimental design was the provision of good quality feed at a minimum level of 4% abalone body weight/day. This feed level

is considered the minimum requirement for gonad conditioning in Japanese culture systems (Hahn,1989).

Five experimental animals of each sex were sampled at 21 day intervals throughout the conditioning period. Data from conditioned animals were compared with data from wild abalone remaining in the source population and with previous field studies concerning reproduction of the species. The field samples were obtained from an abalone processing facility in Smithton, north west Tasmania when specimens from the source population were available.

Gonad maturity was measured using two gonad indices (the gonad bulk index and modified gonad bulk index), oocyte size/frequency distribution, mean oocyte diameter, ovarian phase analysis, and percentage mature spermatozoa (Sections 2.10-2.18). Most previous studies on abalone reproduction have employed more than one method of assessing gonad maturity. Typically a gonad index and some form of histological measure have been used, Poore (1973); Shepherd and Laws (1974). The gonad maturity measures used in this study include nearly all measures described in the literature to monitor abalone reproduction. The use of such a range of measures in one study allows the validity of each method for determining reproductive maturity to be assessed and comparisons made between measures.

2.4

Length of the Reproductive Cycle

The metabolic rate of ectothermic animals such as abalone is driven by environmental temperature. For this reason time may usefully be expressed as degree days (i.e. the sum of daily temperature readings) when considering such animals. The time required for abalone to complete their reproductive cycle is of importance to the culturist. For example, where water must be heated to achieve conditioning the length of the reproductive cycle has a direct effect on hatchery costs. The length of the reproductive cycle is also important because it determines how many complete spawnings are achievable within a given time-frame. For instance, up to three individual spawnings may be achieved for specimens of H.rufescens in one year (Ault,1985). Whereas data presented by Kikuchi and Uki (1975) indicate specimens of H.gigantea may only be spawned once yearly. For these reasons the number of degree days to reach spawning condition was quantified in this study. However, it should be noted that the experimental animals were not collected immediately after spawning, but rather as shown by subsequent histological evidence (Sections 3.12 and 3.13) early in the recovery stage. The degree day estimation is therefore prone to error through understatement.

The length of the reproductive cycle in gonad conditioning systems has been previously quantified for H.discus (Kikuchi and Uki, 1974) H.gigantea (Kikuchi and Uki,1975), H.discus hannai (Uki and Kikuchi, 1984) and H.rufescens (Ault,1985).

Elapsed time to reach spawning condition from the commencement of conditioning expressed as degree days was

calculated by summing all day time temperature data. On days when temperature was not recorded it was estimated by examination of a maximum/minimum thermometer and the temperature readings for adjacent days.

2.5
Measurement of Growth

Shell growth of abalone is reduced while the gonad increases in size as the spawning season approaches (Poore, 1973). However, shell growth does not altogether cease and a minimum amount of such growth has been used as an indicator of conditioning tank suitability. According to Hahn (1989) shell growth of 50 µm/day is considered appropriate in commercial Japanese gonad conditioning systems.

Growth, expressed as increase in weight and shell length was calculated to provide data on the performance of H.laevigata in a conditioning system. The length and weight of all abalone were recorded as the animals were introduced to the holding tank. Such measurements also were taken from animals sacrificed at regular intervals for gonad analyses. These data were used to estimate the weight of animals in the conditioning tank so that the correct minimum amount of feed could be supplied (Section 2.6). Length was measured with a clear plastic ruler across the longest length of the abalone shell. When recording weight of abalone, the foot and shell of each animal first was dried with a towel, following which the mollusc was held and shaken until water ceased to drip from within the shell. This procedure was found necessary to avoid

errors in weight due to the presence of pockets of water within the mantle.

Growth was calculated as the difference in abalone population means between the commencement of conditioning and induced spawning. In addition, specific growth rates for both length and weight were calculated as set out below.

$$SGR = \frac{\ln \text{final} - \ln \text{initial}}{\Delta \text{ time}} \qquad \text{----- (1)}$$

Growth differences between the sexes were examined by calculating mean individual specific growth differences for animals that retained their tags until induced to spawn. Comparison of male and female growth was made using the unpaired t-test (Section 2.27). Differential growth between the sexes in H.laevigata has been reported by Shepherd and Hearn (1983) and has obvious implicatons for culture of the species. Weight versus length regressions, both before and after conditioning were plotted using the Statview 512+ statistical package.

Abalone were tagged so that individual growth could be calculated and to aid in identification of animals. Stainless steel wire was threaded through the first two respiratory pores and attached to numbered plastic tags. Numbering of tags was achieved by the use of a water proof marking pen and also by coding the tags with notches cut in the plastic. Respiratory pore tagging has been previously used by Harrison and Grant (1971) to tag H.rubra.

2.6

Measurement of Seaweed Consumption

An important component of all abalone conditioning systems is the provision of sufficient quantity of seaweed. Uki and Kikuchi (1984) demonstrated that a relationship between gonad conditioning time and temperature only was maintained when feed intake level was in excess of five percent abalone body weight daily for H.discus hannai. Data presented by Hahn (1989) show abalone food consumption may range from five to thirty per cent of body weight daily depending on algal species.

Average feed consumption was measured over three days and expressed as percentage feed consumed per body weight of abalone/day. This measurement generally was performed once weekly on animals in one of the four baskets housing abalone in the conditioning tank. On occasions, more than one basket was measured at a time and in these cases a mean was calculated. Overall mean daily feed consumption was calculated from the set of three day feed consumption means.

Seaweed used in feed consumption trials was placed in a mesh bag and swung vigorously until water ceased to spray from the bag. Following this the weed was spread out on a towel to drain for a further ten minutes, before being weighed and placed in the tank. The remaining seaweed was retrieved from the tank after three days and the procedure repeated. The mass of seaweed consumed was divided by the mass of abalone in the particular trial basket and converted to a percentage. Animal

weight was estimated by counting the abalone in the basket and multiplying by the latest mean weight estimate taken from sacrificed specimens.

2.7
Seaweed Preference

In a field study of H.laevigata, Shepherd (1973) found the species to be a selective feeder, preferring to consume species of red seaweed (Rhodophyta). In addition, gonad growth in this abalone correlates more closely with feed consumption than with environmental variables such as temperature (Shepherd and Laws,1974). For these reasons it was considered important to determine which of the available local seaweeds abalone preferred.

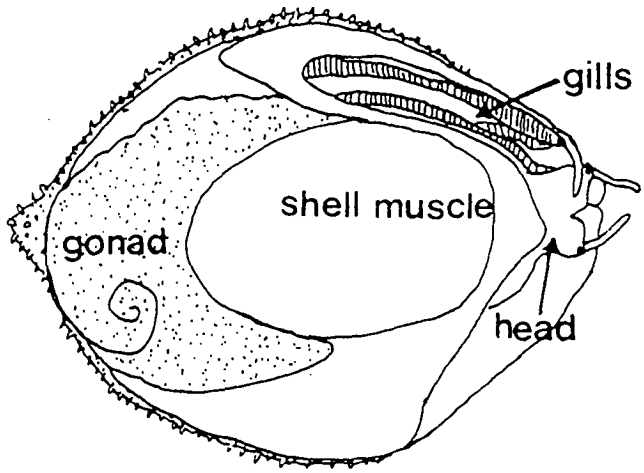
When sufficient quantities of certain seaweed species were available seaweed preference trials were performed. In these trials one species of macroalgae was placed in a test basket and daily consumption calculated as described in Section 2.6. The locally abundant seaweed species M.pyrifera and Codium sp. were used in combination with red seaweeds such as L.filiformis for everyday feeding, but were not trialed separately in preference experiments. This was because abalone appeared not to prefer these species and it was considered presentation of these seaweeds alone in preference experiments might compromise gonad growth. Observations of abalone feeding responses in the presence of various seaweeds were made and described in section 4.3.

2.8

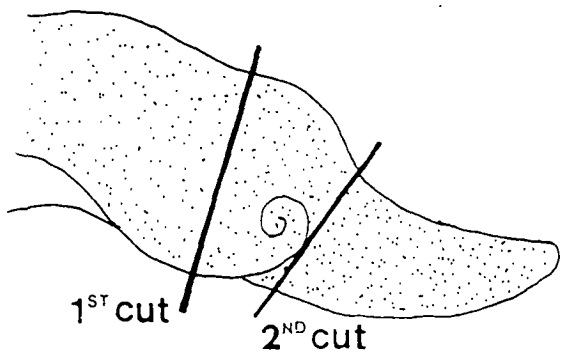
Structure of the Gonad

Abalone like other gastropod molluscs possess a single gonad. In haliotids, this organ lies superficially on the dorsal side of the visceral mass, including the visceral spire located directly under the shell apex. Gonad tissue also encloses the digestive gland or hepatopancreas on all sides in a structure projecting anteriorly from the base of the visceral spire and known as the conical appendage. The size of the gonad varies considerably throughout the reproductive cycle. A fully gravid specimen is illustrated in Figure 3a. In such specimens of H.laevigata the sexes are distinguished easily as the testis is cream-yellow in colour and the ovary is green. Early in the reproductive cycle, the gonad volume is negligible and the grey colour of underlying tissue can be observed through the thin layer of gonad. The conical appendage and its surface layer of gonad can be observed in live animals. This is achieved by holding the mollusc ventral side up and pushing the foot and mantle aside on the leading edge of the shell close to the shell apex.

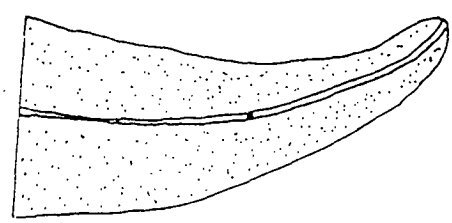
FIGURE 3
Preparation and Sampling of the Gonad



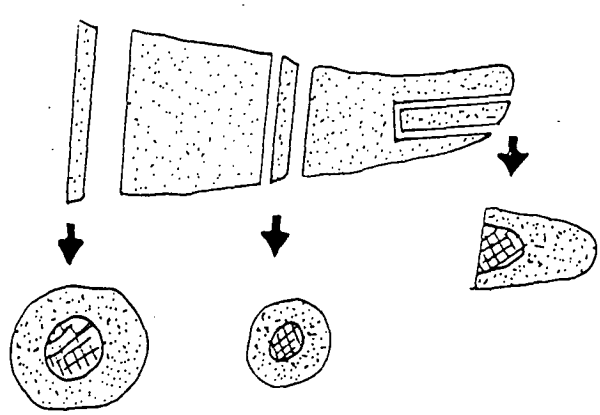
A) Dorsal view of abalone with shell removed



B) Preparation of the conical appendage



C) Determination of midpoint



D) Sampling for tissue processing

2.9

Sample Preservation and Histological Preparation

Animals for sampling were sacrificed by immersion in ten percent formalin solution or by freezing. The soft tissues were then removed from the shell and the stomach severed on the posterior side of the visceral coil (Figure 3b). The severed conical appendage was then preserved for at least three days in 10% neutralized formalin to harden the tissues. Following this the appendage was severed at the base of the visceral coil (Figure 3b). Calculation of the gonad indices (Sections 2.11 and 2.12) requires determination of the midpoint of the conical appendage. This was done using a similar method to that of Ault (1985) where fuse wire is bent to fit the curve of the conical appendage. The wire is then straightened, its length measured, the midpoint marked, and the wire refitted to the conical appendage yielding the midpoint (Figure 3c).

Sections 3 mm thick were cut from the base, midpoint and tip of the conical appendage (Figure 3d) and placed in tissue capsules for automatic tissue processing. When gonad samples were sufficiently well developed to allow sex differentiation tissue slices were cut only from the midpoint of male samples. The tissue samples in their individual capsules were placed in an Autotechnicon tissue processor for automatic dehydration, clearing and infiltration with paraffin.
Following this the samples were embedded manually in paraffin wax and 8 µm slices cut from the resulting block with a rotary microtome. The slices were mounted on glass slides by the water bath method then processed by an automatic stainer for

staining with Gill's haemotoxylin and counterstaining with eosin. Haemotoxylin and eosin stain the nucleus and cytoplasm of the cell respectively. The methods used are set out in Lamberg and Rothstein (1978).

2.10
Gonad Indices

Reproductive cycles can be monitored by the use of histological techniques by which the gonad can be examined at the microscopic level and by the calculation of gonad indices. Gonad indices attempt to standardize the size of the gonad by comparing it to another measurement such as shell length, weight or size of the digestive gland. Such indices by their nature do not reveal as much information as histological measures but are simpler and less time consuming to use. The two gonad indices used in this study have successfully been used to identify the spawning seasons of a variety of abalone species (sections 4.4 and 4.5). A further index known as the simple gonad index (GI) which assumes the size of the digestive gland correlates with shell length has been excluded from the study. Hahn (1989) notes that this index is not a good criterion for monitoring the reproductive cycles of H.rufescens, H.kamtschatkana Jonas, and H.walallensis Stearns. The GI is the only commonly used gonad index excluded from this study.

2.11

Measurement of the Gonad Bulk Index (GBI)

This index has been used extensively in studies of abalone reproductive cycles (section 4.4). The method requires measurement of the areas of gonad and digestive gland tissue present in a cross section of the conical appendage and is expressed as gonad percentage of the total. The formula is given below.

$$GBI = \frac{\text{AREA OF GONAD IN CROSS SECTION}}{\text{AREA OF TOTAL IN CROSS SECTION}} \cdot 100 \quad ----(2)$$

A tracing of conical appendage tissue from the midpoint section and embedded in its paraffin wax block was made on transparent plastic with a fine tip marking pen. Calculation of the gonad cross sectional percentage was performed by placing the tracing over 1 mm graph paper and counting the squares. All tracings were made in duplicate and a mean determined for each sample. It was found simpler to perform the tracings on samples after they had been embedded in paraffin rather than trace the hardened tissue slices as performed by Young and DeMartini (1970). However, the latter method was used for some samples of wild abalone taken for comparative purposes.

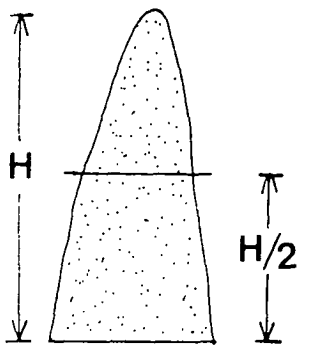
One way ANOVA (Section 2.27), treating the sexes separately, as recommended by Grant and Tyler (1983a), was used to determine whether a significant difference in GBI values occurred over the duration of the experiment.

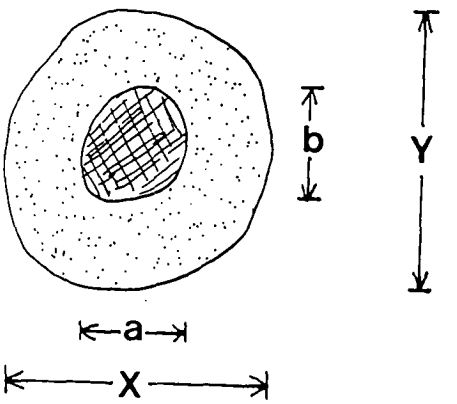
Individual sample means were compared using Fisher's PLSD test. Comparison of such means demonstrates the progress of gonad growth. Two way Anova was also performed using abalone sex and time as factors to determine whether the pattern of gonad development was the same in both sexes (Section 2.27). Where the interaction term of such an ANOVA is significant the pattern of development between the sexes is different (Tyler and Grant, 1983a). Because GBI data is expressed as a percentage, it was necessary to use the arcsine transformation. When percentage data are so transformed the distribution approximates the normal (Sokal and Rohlf, 1981), a necessary assumption for the application of ANOVA. Cochran's test for homogeneity of variance (Section 2.27) was also performed to ensure that this assumption for ANOVA could be met.

2.12
Measurement of the Modified Gonad Bulk Index (MGBI)

This index was developed by Tutschulte and Connell (1981) to provide a minimum estimate of gonad volume. These authors considered it impractical to routinely determine gonad weight by dissection and instead calculated the volume by taking linear measurements from frozen samples. The estimate is made by treating the conical appendage as a pair of concentric right circular cones and applying a correction factor to account for departures from the model. The method assumes that most of the gonad is contained within the conical appendage. The cross sectional measurements made and the formula used are shown in Figure 4a.

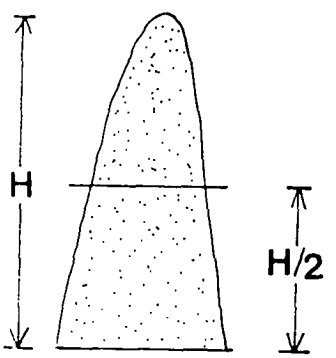
FIGURE 4
Calculation of Estimated Gonad Volume (EGV)

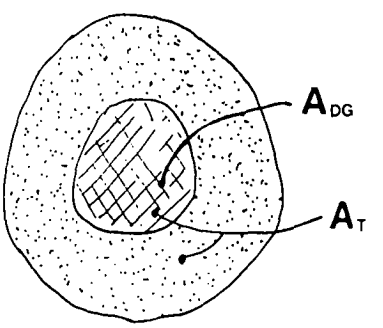




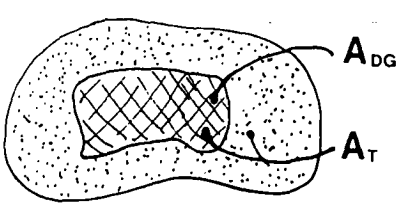
A. Method of Tutschulte and Connell

$$EGV = \frac{H.\pi}{96}\left[8(X+Y)^2 - \frac{(X+Y+a+b)}{(X+Y)}\right]$$





B. Modified Formula



$$EGV = \frac{A_T H}{6}\left[8 - \left(\sqrt{\frac{A_{DG}}{A_T}} + 1\right)^3\right]$$

The estimated gonad volume (EGV) is then divided by the body weight including the shell to yield the modified gonad bulk index (MGBI). The EGV formula presented by Tutschulte and Connel has been modified in this study to allow the use of tissue areas in cross section, rather than their linear measurements (Figure 4b). This allows for greater accuracy when the conical appendage is irregular in cross section. In addition the GBI and MGBI can be calculated from the same tissue area measurements, which is not the case if the original EGV formula presented in Figure 4a is used. The derivation of the formula is explained in Appendix A.

The measurements of gonad and digestive gland area were taken from the midpoint tissue slice of the conical appendage embedded in the paraffin wax block. This is done by the tracing method described for calculation of the GBI (Section 2.11). Since these measurements must be made to determine the GBI little extra work is required to calculate the MGBI. The conical appendage tissue area measurements along with the length of the conical appendage are substituted into the formula below to give the EGV. The MGBI is then obtained by dividing the EGV by abalone weight (shell included).

$$EGV = \frac{A_T H}{6}\left[8 - \left(\sqrt{\frac{A_{DG}}{A_T}} + 1\right)^3\right] \qquad ----(3)$$

WHERE: A_T = AREA OF TOTAL IN CROSS SECTION

A_{DG} = AREA OF DIGESTIVE GLAND IN CROSS SECTION

H = HEIGHT OR LENGTH OF CONICAL APPENDAGE

The two gonad indices (GBI and MGBI) have been used previously together in only one study: Ault (1985) used both

indices in a conditioning study on H.rufescens and found that the GBI was not capable of detecting gonad growth. By contrast, gonad growth was detected by the MGBI and histological examination. In the present study both gonad indices were used to assess their individual utility in monitoring reproduction and to allow comparison to be made.

Statistical analysis of MGBI data was performed in the same way as that for GBI data (Section 2.11). It was necessary to use the logarithmic transformation (Sokal and Rohlf, 1981) on MGBI data to meet the requirement for ANOVA of homogeneity of variance. The assumption made for calculation of the MGBI that the conical appendage contains most of the gonad tissue was examined. This was performed by dissecting all gonad tissue from animals considered to be ripe and calculating the percentage by weight of gonad located in the conical appendage.

2.13
Gonad As Percentage Body Weight

The simplest indicator of reproductive state is the percentage of total body weight contributed by the gonad (Grant and Tyler, 1983a). In studies of abalone reproductive development this measure has rarely been used, two exceptions being Webber and Giese (1969) and Rho and Park (1975). This is because the abalone gonad is located superficially around the digestive gland and other tissues (Figure 3a) making it very time consuming to remove by dissection for weight analysis.

Dissection of gonad tissue and calculation of percentage gonad by weight was performed in the present study on samples of experimental and wild abalone with ripe gonads. This was done to allow comparison of maximum gonad size with published data on other species and to compare wild and conditioned animals. The method was not, for the reason explained previously, used for routine monitoring.

2.14
Subjective Gonad Indices (Visual Assessment)

Subjective gonad indices where the gonad is scored by visual assessment based on certain characteristics of size, shape and colour have been used in conditioning studies by Kikuchi and Uki (1974, 1975) and Uki and Kikuchi (1984). Such indices do not require specimens to be sacrificed and are used in commercial hatcheries. In the present study, the appearance of the developing gonad in both sexes is described and compared to published data referring to subjective gonad indices for other haliotid species.

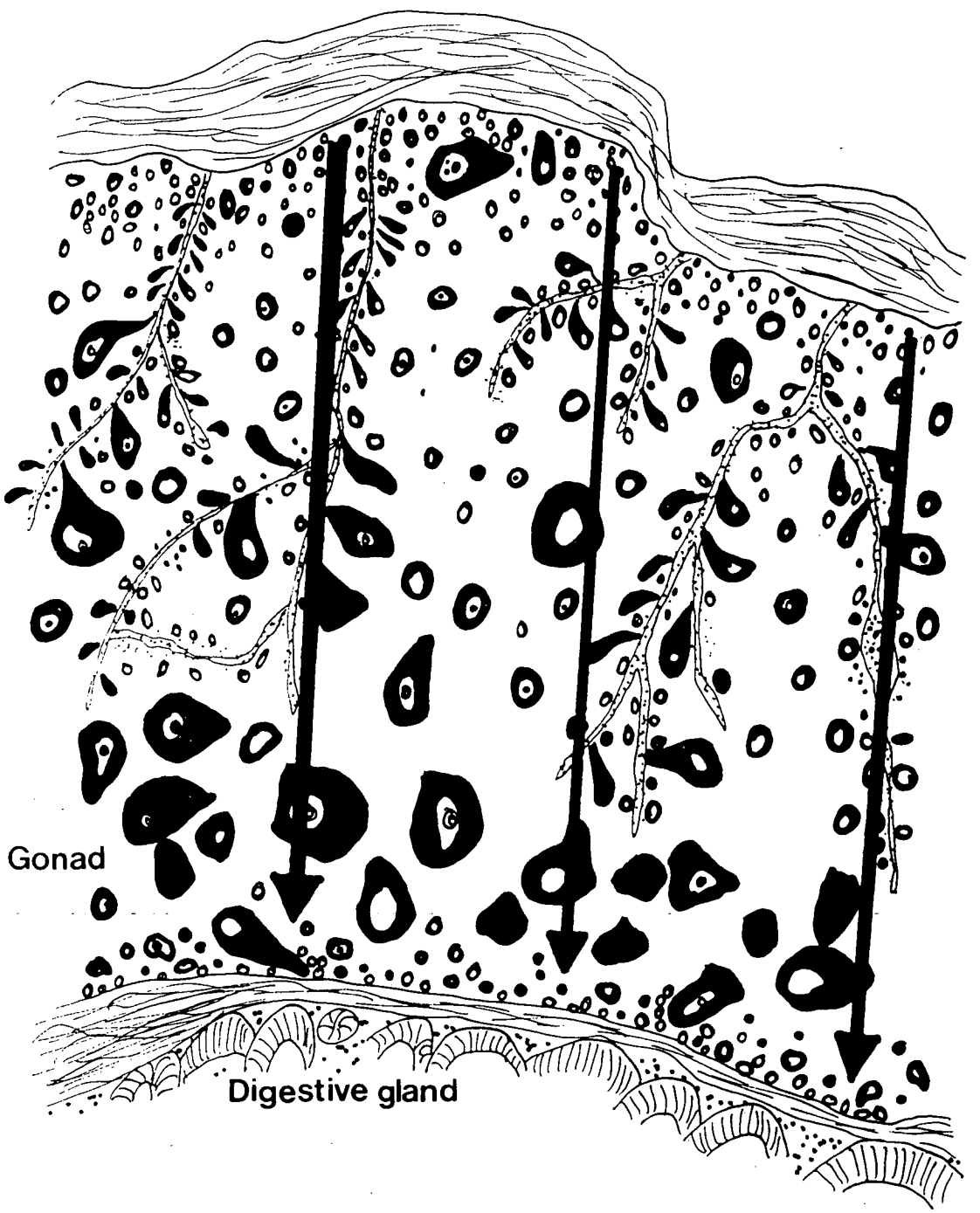
2.15
Measurement of Oocyte Size/Frequency Distribution

Grant and Tyler (1983b) state that the measurement of oocyte sizes is the best method for examination of the reproductive cycles of molluscs and echinoderms. Contingency table analysis allows the growth of oocytes to be studied in detail and differences in developmental stage between samples

identified. The method is independent of the size of specimens, which may not be the case when using some gonad indices (Boolootian et al.,1962)

Oocyte size/frequency distribution analysis was performed on five female abalone at 21 day intervals. Slides were prepared (Section 2.9) from three sections cut from the conical appendage (Figure 3d). The practice of measuring oocyte sizes from samples taken from different areas and along different axes of the ovary is to guard against heterogeneity of development within an ovary (Grant and Tyler, 1983b). From each prepared slide 100 oocytes sectioned through the nucleus were measured and assigned to 20 µm size classes. The mean distribution for each specimen was calculated by averaging the counts from the three sections. Measurements were made using an eyepiece graticule calibrated against a stage micrometer. The method of Hayashi (1980) where oocytes longer than 120 µm are measured along their major and minor axes and the mean diameter determined was used. Smaller oocytes were measured along the major axis only. This method allows large immature teardrop shaped oocytes to be distinguished from the more rounded mature oocytes. When performing counts on more mature specimens, it was found that small oocytes tended to be found adjacent to the inner and outer gonad walls and trabeculae. Conversely, larger oocytes tended to lie distant from the walls in the ovarian lumen. In an effort to obtain representative counts, oocytes occurring on imaginary lines perpendicular to the inner walls of the ovary were measured (Figure 5). Oocytes which were obviously necrotic were not measured since the nucleus cannot be distinguished in such cells.

FIGURE 5
Oocyte Counting Method


Gonad
Digestive gland

Specimens exhibiting a large proportion of necrotic oocytes were excluded from counts.

Statistical analysis was performed by the contingency table method described by Grant and Tyler (1983b). The oocyte size/frequency distribution data for the sample means was treated as an (r x c) contingency table with r as the number of samples (taken at 21 day intervals) and c the number of size classes. The expected frequency of each cell, e_{ij} , (assuming all samples are at the same developmental stage) was calculated as:

$$e_{ij} = (R_i \times C_j) / \sum_j C_j \qquad \text{----(4)}$$

where: R_i is the total of oocytes in the i^{th} size class summed over all samples

C_j is the total number of oocytes counted in the j^{th} sample

$\sum C_j$ is the total number of oocytes measured

Next the following statistic was calculated:

$$G = \sum \left(\frac{(o_{ij} - e_{ij})^2}{e_{ij}} \right) \qquad \text{----(5)}$$

where : o_{ij} are the observed oocyte frequencies for each cell

Assuming samples are at the same stage of development, G is distributed χ^2 with (r-1)(c-1) degrees of freedom. Where the computed chi-squared value is greater than the critical value given in standard tables then the samples are not at the same

developmental stage. The size classes and samples contributing most to the chi-squared value were also calculated as described by Grant and Tyler (1983b). This was done by examining standardized residuals, calculated as:

$$r_{ij} = (o_{ij} - e_{ij}) / \sqrt{e_{ij}} \qquad \text{----(6)}$$

where : r_{ij} is the residual, which is standardized through division by its expected variance

V_{ij} is the expected variance, calculated:

$$V = [1 - (R_i/n)][1 - (C_i/n)] \qquad \text{----(7)}$$

n is the total number of oocytes measured.

A positive residual indicates the frequency of oocytes in that size class is greater than expected and a negative value indicates a lower than expected frequency. Thus the change in distribution of oocyte size can be determined over time.

2.16

Measurement of Mean Oocyte Diameter

The measurement of mean oocyte diameter provides another sensitive indicator of female reproductive development. In this study the data were obtained directly from the oocyte size/frequency distribution data. A mean diameter was calculated for each individual specimen from the three prepared slides. Sample means were then calculated from the data set of individual means. There is a loss of detail compared to size/frequency distribution analysis but the interpretation of graphical displays is simpler. Mean oocyte diameter also may be calculated without first determining

size/frequency distribution which would decrease the time required to collect the data. The method has been previously used by Harrison and Grant (1971) and Shepherd and Laws (1974).

Statistical analysis was by the one way ANOVA technique (section 2.27) having first transformed the data using the logarithmic function to ensure homogeneity of variance. Comparison of sample means was performed by Fisher's PLSD test contained on the Statview 512+ statistical package.

2.17
The Ovarian Phase Method

The simplest method of assessing tissue sections is to subjectively place them in a number of developmental stages (Grant and Tyler,1983a). Such methods are commonly referred to as maturity indices. The advantage of such methods is that assessment can be performed relatively quickly.

In this study reproductive maturity of females was assessed by determining the percentage of gonadal area in cross section occupied by certain defined stages of ovarian development. Five readily determined ovarian phases were defined after examination of all histological preparations. Prepared tissue sections from the base and midpoint of the conical appendage of five individuals from each 21 day sample were photographed to quantify the percentage of phases present. Areas of each ovarian phase were calculated by tracing photographic prints on to transparent plastic and positioning the tracing obtained

on 1 mm graph paper. Each ovarian phase area was then converted to a percentage of the total and the means calculated from the pair of prints produced from each female abalone. Sample means were then calculated from the set of individual means. It should be noted that areas of photographs devoid of oocytes were omitted from total area calculations. Quantification of maturity phases in a conditioning study has been previously performed by Ault (1985) and Wells and Keesing (1989).

2.18
Quantification of Testis maturation

Quantification of male gonad development by histological methods rarely has been attempted in studies of abalone reproduction. Possibly this is due to the small size of male gametes. The head of a mature abalone sperm cell is 6 µm long and 1-1.5 µm wide with the flagellum just visible at a magnification factor of 1000 (Hahn,1989). Despite this difficulty Webber and Giese (1969) and Takashima et al. (1978) studied the development of the testis by measuring the percentage of sex cells present as mature sperm. This technique was used in the present study to accurately assess male gonad development and to provide a benchmark against which the sensitivity of gonad indices could be compared.

The method used was essentially the same as that described previously for quantifying ovarian phases (section 2.17). Although the individual cell types involved are very small, distinct areas consisting of large numbers of like cells are

easily identified allowing quantification. Two photographs were taken per individual of representative areas in a section prepared from the midpoint of the conical appendage.
The photographic prints obtained were traced on clear plastic and placed over graph paper so that the area occupied by spermatozoa could be compared with that occupied by less mature male germ cells (spermatogonia, spermatocytes, spermatids).

Statistical analysis was performed using the one way ANOVA method (section 2.27) and comparison of sample means was made with Fisher's PLSD test. The data were treated using the arcsine transformation so that the assumption of normal distribution, required for ANOVA analysis could be met.

2.19
Comparison of Gonad Development Measures

The variety of methods used in this study to measure gonad development allows considerable scope for comparison between methods and assessment of individual utility. In particular the sensitivity of the gonad indices in detecting gonad growth can be compared to that of the more informative histological methods. To examine the relationships between the various methods for measuring abalone reproductive development the Spearmann rank correlation coefficient (section 2.27) was calculated. This measure of correlation was chosen because it is suitable for use with data that do not conform to the normal distribution e.g. data expressed as percentages such as the GBI and percentage spermatozoa data. Correlations of

sample means between the two gonad indices, and between both indices and the mean oocyte diameter and percentage spermatozoa data were performed. The significance of the calculated correlation coefficient was assessed by examination of standard significance tables provided by Zar (1984). Since the oocyte size/frequency distribution method does not yield a single value for each sample, but rather a distribution in the form of a histogram it could not be paired with the other measures to calculate correlation coefficients. For the same reason the ovarian phase method (Section 2.17) also was excluded from correlation coefficient analyses.

Ault (1985) found the GBI incapable of detecting gonad growth in H.rufescens though such growth was detected by the MGBI. This was an important finding since the GBI is the most widely used measure of abalone reproductive maturity (section 4.4). Accordingly, one of the aims of the present study was to examine the relationship between these gonad indices. This was done by calculating a Spearmann rank correlation coefficient using the data pairs for each individual specimen sampled. Young and DeMartini (1970) used the same method to examine the relationship between the GBI and the gonad index (area of gonad in conical appendage mid-point section divided by shell length and converted to a percentage) and found no significant correlation.

The sensitivity of the maturity measures in detecting gonad development may be assessed by the use of Fisher's PLSD test. This test identifies significant differences between sample means.

Therefore it may provide the elapsed time from the beginning of the conditioning period until a given maturity measure records a value significantly higher than its initial value.

2.20
Comparison With the Reproductive Cycle of the Source Population

Research on the reproductive cycle of H.laevigata in South Australia (Shepherd and Laws, 1974) and Victoria (McShane, 1989) has shown the species to be a summer spawner. This also is believed to be the case in Tasmania though there is a lack of published data. In order to test whether the experimental conditions had advanced gonad maturity, samples of wild abalone from Franklin Sound were examined. These specimens were collected from an abalone processing facility (Tasmanian Seafoods, Pty Ltd) when animals from the appropriate locality were available. Gonad indices were calculated and comparison with conditioned samples made by the unpaired t-Test method (Section 2.27).

2.21
Methods for Fecundity Estimation

There is a lack of published information on the fecundity of H.laevigata though such studies have been performed on a number of other abalone species (Section 4.15). Consequently, one of the aims of this study was to provide such data. Ault (1985) found that conditioned specimens of H.rufescens were

relatively more fecund than animals obtained in spawning condition from the wild. Fecundity estimates of conditioned and wild abalone were made since this result is of potential importance to the abalone culture industry.

Several methods for estimating the fecundity of abalone are available. Generally, only the fecundity of females is measured. The simplest method is to induce spawning of ripe abalone and estimate egg number by sampling. This technique has been used by Kikuchi and Uki (1974, 1975) and Ault (1985). The method may underestimate the actual fecundity since not all the eggs may be expelled at one spawning. Also, some individuals, although ripe may not spawn when required. Other methods of estimating female fecundity involve the sacrifice of animals so that ovarian samples of known weight or volume can be taken. The number of eggs in ovarian subsamples are then estimated and total fecundity calculated.
The advantage of these methods is that estimates can be derived from all individuals in a sample regardless of spawning condition. The weight subsample method has been used by Poore (1973) and Wells and Keesing (1989), while the volume subsample technique was developed by Tutschulte and Connell (1981). In the present study estimates of spawned eggs were made where possible and the other methods used when it was not possible to induce spawning.

The relationships between length, weight and fecundity were examined using Spearmann's rank correlation method and regression analysis (Section 2.27).

The nature of the relationship between fecundity, length and weight of haliotids has been previously examined by Poore (1973), Pena (1975), Hayashi (1980), Ault (1985) and Wells and Keesing (1989).

In addition the relationship between fecundity and the two gonad indices was examined. It was felt that the MGBI in particular might provide a useful estimate of fecundity since this index involves a measure of gonad volume (EGV) in the numerator. Accordingly Spearmann rank correlation coefficients (Section 2.27) were calculated between fecundity and each of GBI, MGBI and also EGV. Such correlations were performed for both field and conditioned specimens. The relationship between the two sacrificial fecundity estimation techniques also was examined using the Spearmann rank correlation coefficient.

2.22
Estimation of Number of Spawned Eggs

Spawned eggs were siphoned from the spawning vessel into a bucket containing 15 l of water. The eggs were stirred and five subsamples were taken from the bucket with a 1 ml pipette. The number of eggs in each sample were then counted on a petri dish. Additional fecundity estimates made at Furneaux Aquaculture (Lady Barron, Flinders Island, Tasmania) were performed by siphoning eggs into 10 l buckets containing 9 l of water. As before five 1 ml samples were then taken and distributed on petri dishes for counting.

2.23

Fecundity Estimation by Known Weight Subsample Method

Abalone used for fecundity estimation were weighed then sacrificed and the soft parts preserved in 10% buffered formalin except for the muscular foot. At least three days were allowed for the tissue to harden following which the gonad was dissected from the underlying viscera.

The gonad was divided into two parts : a part contained within the conical appendage severed at the base of the visceral coil and a second part consisting of the remaining gonad mass. This was done to test the validity of the assumption made for the modified gonad bulk index ie. that most of the gonad is contained within the conical appendage (Section 2.12). The gonadal tissue was dissected from the underlying tissues in both sections and weighed. Samples of approximately 0.2-0.4 g were taken from the two parts of the gonad to account for possible heterogeneity of egg density. The samples were then placed in a petri-dish with a drop of water and all eggs teased out with a dissecting needle and small paint brush. Care was taken to reduce the size of egg clumps. Eggs were rinsed from the petri dish into a glass beaker and the volume made up to 100 ml. The beaker was placed on a magnetic stirrer and three 1 ml samples (with replacement) were taken. The samples were then deposited on a dry petri-dish and eggs greater than 160 µm in diameter counted under a dissecting microscope. Estimates of eggs per gram of gonad for each 1 ml sample were calculated by multiplying the count by 100 and dividing by the weight of the particular gonad subsample. The final fecundity estimate for

each abalone was then obtained by calculating the mean egg density (eggs/gram) from the two subsampled areas of the gonad and multiplying by the total gonad weight. Heterogeneity of egg density was checked for using one way ANOVA (Section 2.27).

2.24

Fecundity Estimation by Known Volume Subsample Method

This estimate is based on the method developed by Tutschulte and Connell (1981). The method uses the difference between minimum and maximum estimated gonad volume (EGV) values to calculate fecundity from egg density estimates per unit volume.

The two gonad subsamples taken from each individual animal for fecundity estimation by weight also were used for this analysis. All subsamples removed were of a regular geometric shape (either a cube or a flat cylinder) allowing volume estimation based on linear measurements to the nearest half-millimetre. The counts made as described previously for fecundity estimation by weight were converted to a density figure in eggs/cubic millimetre and the mean of the two subsamples determined as before.

The sample mean was subsequently substituted in the following formula :

$$F = D.(MGBI_s - MGBI_m).W. (100/G) \qquad ----(8)$$

Where : F is fecundity estimate by volume.

D is mean eggs/mm^3 for the sample.

$MGBI_s$ is the sample modified gonad bulk index.

$MGBI_m$ is the mean minimum modified gonad bulk index calculated from females before conditioning began and has the value 0.5

W is abalone weight in grams.

G is the percentage of gonad weight located in the conical appendage for the sample.

It should be noted that gonad dissection revealed most of the gonad weight was not located in the conical appendage (Section 3.5). For this reason the term "G" is included in the formula to account for the entire ovarian volume.

2.25

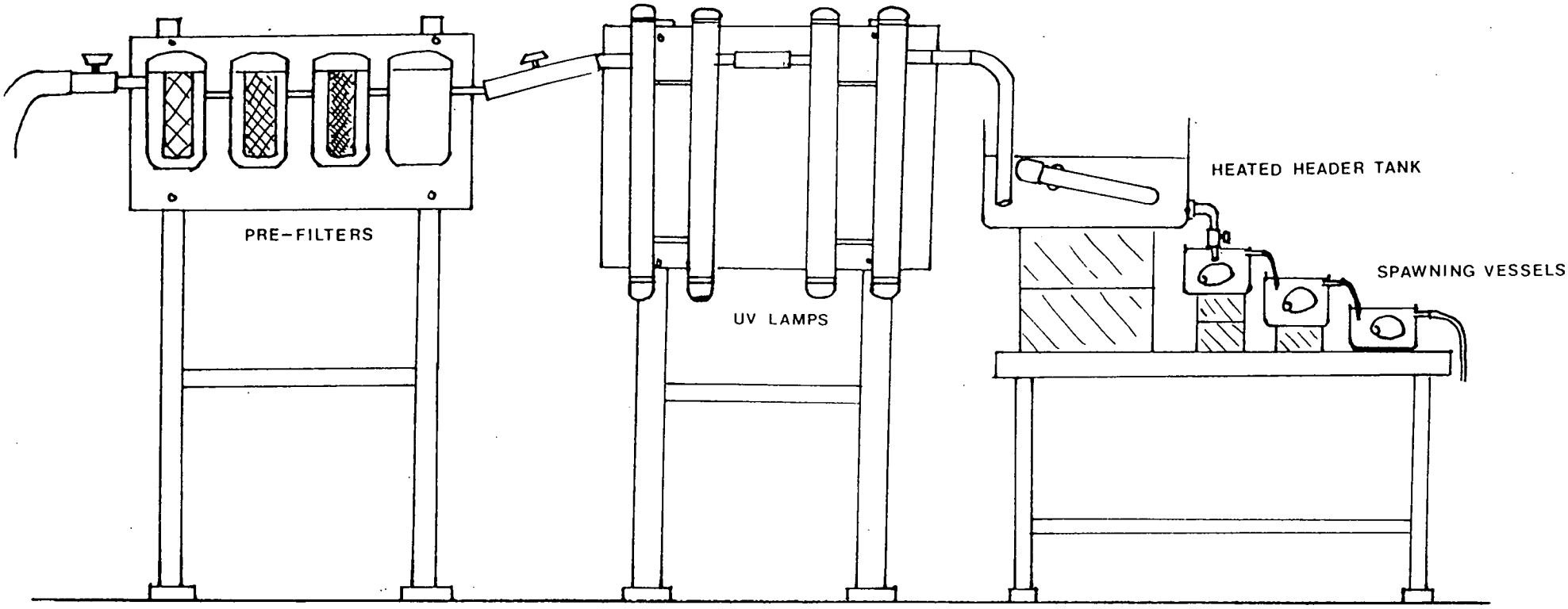
Induction of spawning

Spawning was induced by the ultra-violet irradiated seawater method. This spawning technique was a serendipitous discovery by Japanese researchers Shogo Kikuchi and Nagahisa Uki in 1972 (Hahn,1989).

The mechanism by which spawning is induced is believed to involve the decomposition of water molecules to yield free radicals which activate haliotid prostoglandin synthesis.

Ultra-violet light systems were used for spawning attempts at Abalone Hatcheries Pty Ltd, the University and Furneaux Aquaculture Pty Ltd. In some cases desiccation at room temperature and thermal shock (varying water temperature between 15 and 20°C) were used in combination with ultra violet irradiated water. This combination of spawning stimuli is used in Japanese abalone culture facilities as described by Hahn (1989). Abalone were exposed to spawning stimuli for up to thirteen hours at a time. Figure 6 shows the spawning system used on Campus. Spawned gametes were fertilized and the larvae reared for four days at 20°C before settlement on diatom covered plates.

FIGURE 6
Spawning Apparatus Used at the University


PRE-FILTERS
UV LAMPS
HEATED HEADER TANK
SPAWNING VESSELS

2.26

Sex Ratio

Male abalone outnumber female in populations of H.laevigata according to Shepherd and Laws (1974). An unbalanced sex ratio in favour of males also has been reported in five other haliotid species (Section 4.17). Unequal sex ratios, especially if females are less numerous may have important implications for stock recruitment of commercially exploited species. The nature of the sex ratio in H.laevigata was also considered of some importance for conditioning and spawning of the species. This is because if broodstock are collected early in their reproductive cycle the sex of individuals cannot be determined. Thus, a sex ratio imbalance, particularly where females are less numerous could lead to suboptimal results at eventual spawning.

The null hypothesis that the sex ratio of H.laevigata is equal was tested on the population of animals collected for the conditioning experiment. The statistical method used was the Chi-Square analysis (Section 2.27).

2.27
Statistical Methods

The one and two way ANOVA, Fisher's PLSD test, Chi-square analysis, Spearmann rank correlation coefficient and unpaired t-test statistical methods were performed using the Statview 512+ statistical package. For explanation of the performance of two way ANOVA by the statistical package refer to the manual (Cuneo, et al., 1986). All tests were performed at the alpha = 0.05 level of significance. Formula for the various tests are as follows:

1. Chi-square analysis

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

Where: O = observed values
E = expected values

2. Spearmann rank correlation coefficient

$$Rho = 1 - \frac{6\sum D^2}{N(N^2-1)}$$

$$Z = Rho\sqrt{N-1}$$

Where: N = number of matched pairs
R_x = Rank of X_i
R_y = Rank of Y_i
D = $R_x - R_y$ for each matched pair

3. Unpaired t-test

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{\left[\sum (X_1)^2 - \frac{(\sum X_1)^2}{N_1}\right] + \left[\sum (X_2)^2 - \frac{(\sum X_2)^2}{N_2}\right]}{N_1+N_2+2} \cdot \left(\frac{N_1+N_2}{N_1 N_2}\right)}}$$

Where: N_1 = number of observations in group 1
N_2 = number of observations in group 2
$\bar{X}_1$ is the mean of group 1
$\bar{X}_2$ is the mean of group 2
X_1 designates the first group
X_2 designates the second group
DF = N_1+N_2-2

4. One Way ANOVA

$$\frac{(\text{Mean Square}_{\text{between groups}} - \text{Mean Square}_{\text{within groups}})(1-1)}{\sum_i J_i - \frac{\sum_i J_i^2}{\sum_i J_i}}$$

$i = 1 \ldots l$
J_i is the count of non-missing non excluded values for the i^{th} group

5. Fisher's PLSD Test

$$t_a \cdot \sqrt{\frac{MS_x}{r}}$$

Where: $r = \frac{1}{N_a} + \frac{1}{N_b}$

N_a is the count of group a
N_b is the count of group b
t_a is the two tailed t value at the entered significance level for the within groups df
MS_x is the between groups mean square.

3. RESULTS

3.1
Conditioning Time

The major purpose of this study was to bring abalone to spawning condition in captivity. This was achieved and a sample of the experimental animals were induced to spawn on 21 August 1990, 112 days following the commencement of conditioning. A sample of wild abalone obtained from the same area as the experimental population on 5 September 1990 was found to be considerably less mature than conditioned specimens (Table 1 and Section 3.14). This demonstrates the conditioning process accelerated the rate of gonad maturity. The Franklin Sound source population of abalone apparently spawned between November 1990 and March 1991 (Section 3.14). Additionally, surplus abalone from the source population maintained at ambient temperature in a second conditioning tank at Abalone Hatcheries Pty Ltd were induced to spawn by hatchery staff in late November 1990.

The August spawning is believed to be the first successful out of season spawning of H.laevigata. The mean water temperature during conditioning was 16.0°C (s.d.=1.1) and the number of elapsed degree days was 1750. Following 150 days of conditioning the majority of female specimens displayed extensive areas of egg necrosis (Section 3.12).

Table 1

ABALONE CONDITIONING PERFORMANCE MEASURES

Gonad Indices				Feed Consumption (% Wt./d)
GBI (%)		MGBI (mm^3/g)		
Initial	Final	Initial	Final	
14.1 ± 4.4	72.3 ± 9.2	0.4 ± 0.2	7.0 ± 2.0	6.0 ± 1.1

Gonad Indices Field Specimens		Degree Days
GBI (%) 5/09/1990	MGBI (mm^3/g) 5/09/1990	
58.5 ± 14.4	3.3 ± 0.9	1750

Length (mm)		Weight (g)	
Initial	Final	Initial	Final
139.7 ± 9.6	142.6 ± 11.4	359.9 ± 83.4	431.0 ± 103.0

Mean Length Change ($\mu m/d$)	Mean Weight Change (mg/d)	Mean Specific Growth	
		Length (10^{-4})	Weight (10^{-3})
25.9	634.8	1.83	1.61

The growth period was 112 days from 27/04/90 (n=128) to 21/08/90 (n=51). Final gonad indice measures (n=10) were recorded following 105 days of conditioning. N=12 for field comparison gonad index data and n=14 for feed consumption data.

Male specimens required longer to become fully ripe and maximum maturity values were recorded after 24 weeks of conditioning (Section 3.11). Gonad index values initially and a week before spawning are shown in Table 1. These data are compared to available data from other conditioning studies in Appendix B and discussed in Section 4.1

3.2

Growth

Growth of broodstock abalone is not of primary importance in abalone culture since these animals would not normally be sent to market. However, growth is used as an indicator of conditioning tank suitability at Japanese culture facilities (Hahn, 1989) and for this reason was measured in the present study. Growth was also measured because as the Australian abalone culture industry is in its infancy there are few available data on the captive growth rates of species believed to have farming potential. Furthermore, such measurement allows for the examination of differential growth rates between the sexes as reported by Shepherd and Hearn (1983) at one site of a field study.

Pooled sex growth data for the first 112 days of conditioning are displayed in Table 1. The mean weight increase during this time was 71 g. Dissection of gonads from specimens taken five weeks later (week 21) when gonad volume had increased further yielded a mean gonad weight of 21.7 g (s.d.=7.5, n=12) indicating a large somatic tissue contribution to overall weight gain. To examine whether there was a difference in growth rate between the sexes, mean individual specific weight and length increases were measured using those animals with retained tags at day 112 of the conditioning period. The unpaired t-test method (two tailed) showed no significant difference between the sexes ($P>0.05$). Mean, sex pooled specific length and weight growth data are displayed in Table 1.

The length-weight relationship before and after conditioning are displayed in Figures 7a and 7b. Correlation coefficients for the two regression lines are R-squared = 0.823 ($p = 0.0001$) and R-squared = 0.771, ($p = 0.0001$) respectively.

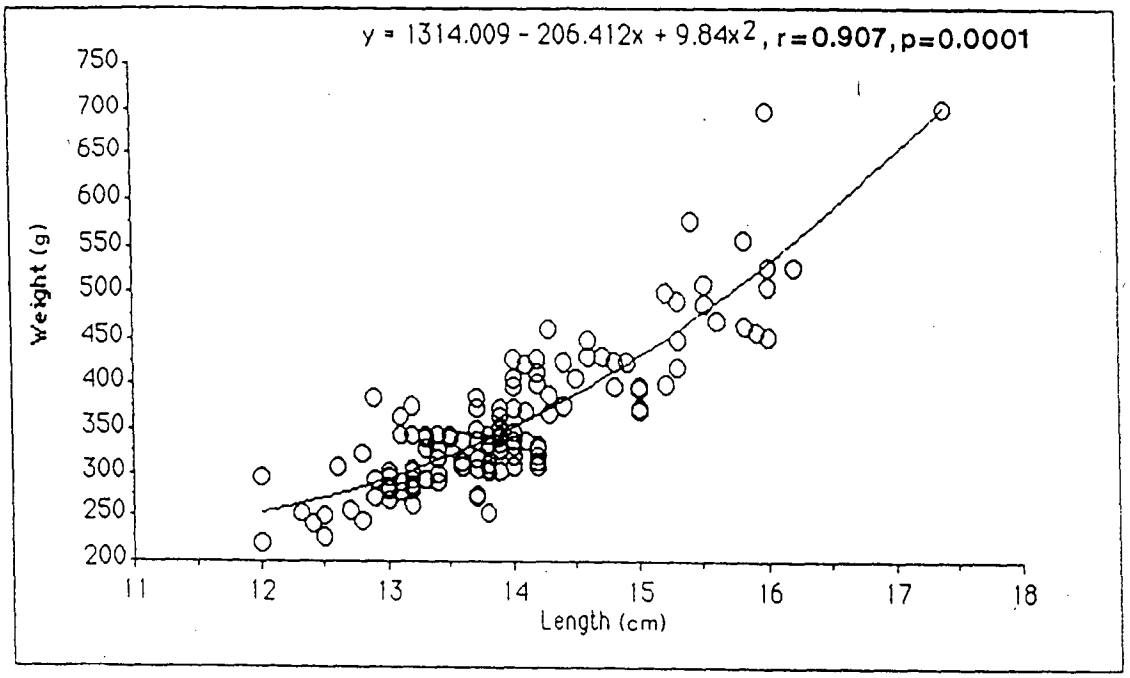


FIGURE 7a

Length-weight regression, before conditioning

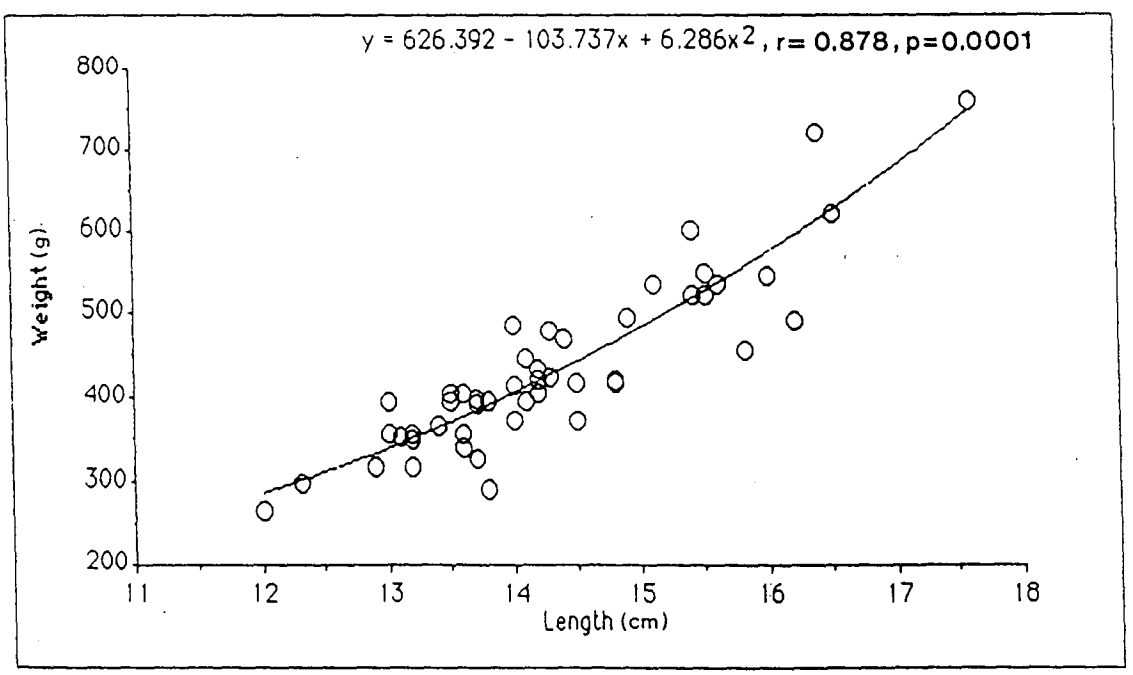


FIGURE 7b

Length-weight regression, following conditioning

3.3

Feed Consumption and Seaweed Preference

Abalone were fed to excess where possible and actual feed consumption was measured on a regular basis. Data on broodstock feed consumption is important for deciding hatchery resource allocation. Haliotids fed seaweed show poor feed conversion rates e.g. 4-7 % on a wet weight basis as cited by Hahn (1989). Thus the number of broodstock animals may be limited by the amount of feed that can be conveniently acquired. This is particularly true of species such as H.laevigata which exhibit strong feed preferences.

Mean feed consumption based on three day trials was 6.0 % body weight daily (s.d.=1.1, n=14) at a mean water temperature of 16.0 °C. Seaweed preference data are shown in Table 2. Except when feed preference trials were conducted more than one species of seaweed was present in a holding basket at a time. Observation showed abalone selectively consumed certain species of seaweeds present in mixtures of species. The very fine red seaweed R.coccinea consistently would be removed first from a mixture of other macroalgae, even if present in relatively small quantities. The locally abundant seaweeds, M.pyrifera and Codium sp. were not preferred by the abalone when present with red algae. Indeed, in a pre-experimental trial where only the kelp M.pyrifera was present, feed consumption was essentially zero after five days.

Table 2
SEAWEED PREFERENCE (% Body weight consumed /day).

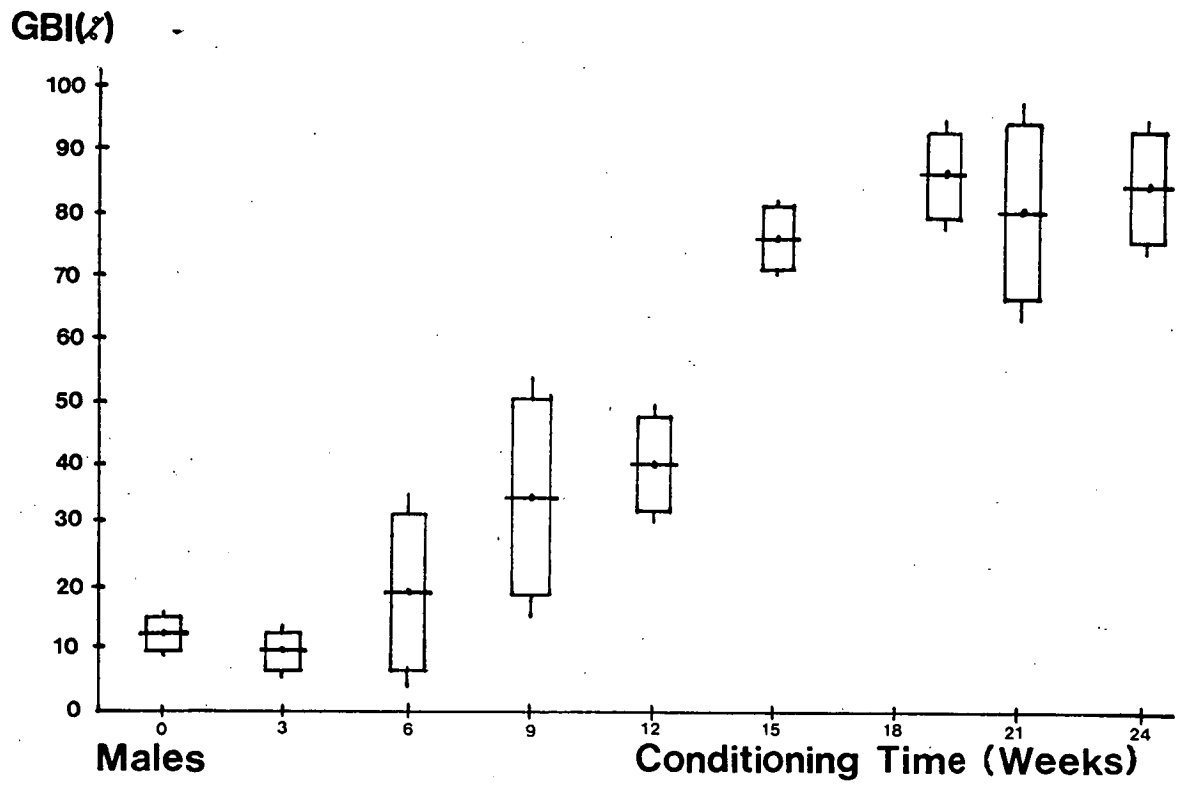
Species	Consumption Rate $\bar{x}$	s.d.	n
Rhabdonia coccinea (frozen)	7.1	2.4	2
Laurencia filiformis	6.0	1.8	11
Hypnea sp.	5.2	1.9	2
Chiracanthia arborea	4.5	2.1	3

3.4

Gonad Bulk Index Data

Temporal changes in gonad bulk index with the sexes separate are shown in Figure 8. One factor ANOVA using arcsine transformed data was highly significant for both sexes (P=0.0001). This signifies a genuine change in GBI values during the conditioning process. Comparison of sample means by Fisher PLSD Test are displayed in Table 3. The table shows the GBI was able to detect gonad growth in both sexes by week nine of the conditioning period. By week 15, one week before induced spawning the GBI values for males and females respectively were 76.1% and 68.5%. There was no further significant change in mean male GBI after week 15. A further significant increase in female GBI occurred by week 18, at which time histological examination showed widespread necrosis of eggs. Two factor ANOVA using sex and sample time as factors showed no significant difference for the time/sex interaction (P>0.05),indicating that there was no difference in the way the gonads of the two sexes developed. This differs from the data available from more accurate histological methods which indicates females reached maximum maturity before males (Table 3).

FIGURE 8
Temporal Change in the Gonad Bulk Index (x, s.d., 95% c.l.)


GBI(%)
100
90
80
70
60
50
40
30
20
10
0
0
3
6
9
12
15
18
21
24
Males
Conditioning Time (Weeks)

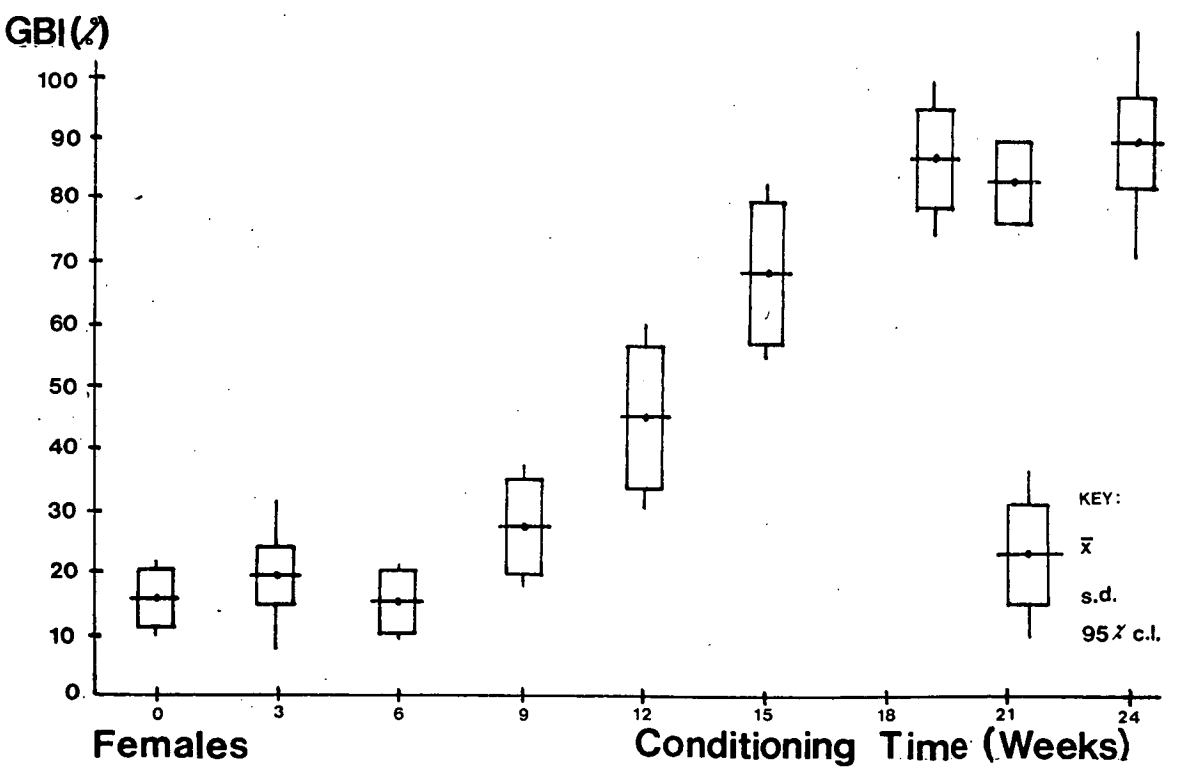

GBI(%)
100
90
80
70
60
50
40
30
20
10
0
0
3
6
9
12
15
18
21
24
KEY:
x̄
s.d.
95% c.l.
Females
Conditioning Time (Weeks)

Table 3
SUMMARY OF GONAD MATURITY MEASURE DATA FOR CONDITIONING OF CAPTIVE H.LAEVIGATA

Time (Wk)	Ovarian Maturity Phase Dominant phase:	Oocyte Size/Frequency Distribution +ve Std. Residuals:	Mean Oocyte Diameter (μm) $\bar{x}$	sd	Mature Sperm Cells (%) $\bar{x}$	sd	Gonad Bulk Index (%) MALES $\bar{x}$	sd	FEMALES $\bar{x}$	sd	Modified Gonad Bulk Index ($mm^3.g^{-1}$) MALES $\bar{x}$	sd	FEMALES $\bar{x}$	sd
0	100% P1	0-40 μm	30.7^{A}	2.0	0^{A}	0	11.9^{A}	2.8	16.4^{AB}	4.8	0.3^{A}	0.1	0.5^{A}	0.2
3	100% P1	0-40 μm	30.1^{A}	3.8	0^{A}	0	9.4^{A}	3.3	19.5^{AB}	4.8	0.3^{A}	0.2	0.8^{AB}	0.2
6	87% P1	20-60 μm	38.5^{B}	5.4	2.2^{AB}	4.8	19.3^{A}	12.7	14.9^{A}	5.0	1.5^{B}	1.0	1.0^{B}	0.3
9	63% P1	20-80 μm	43.4^{B}	3.6	6.7^{B}	5.6	34.4^{B}	15.8	27.3^{B}	7.8	1.4^{B}	0.6	1.3^{B}	0.8
12	55% P2	40-140 μm	65.5^{C}	10.7	22.2^{C}	5.7	40.0^{B}	7.8	45.1^{C}	11.8	2.6^{C}	0.7	2.3^{C}	1.1
15	73% P3	60-240 μm	109.7^{D}	6.0	32.2^{C}	9.9	76.1^{C}	5.0	68.5^{D}	11.4	7.7^{D}	1.2	6.4^{DE}	2.5
18	100% P5	-	-	-	51.3^{D}	17.2	86.5^{C}	7.2	87.2^{E}	8.0	15.8^{EF}	6.3	7.3^{DE}	2.1
21	48% P5	-	-	-	68.4^{D}	14.9	80.6^{C}	14.3	83.6^{E}	6.8	10.6^{DF}	3.2	9.1^{D}	4.1
24	93% P5	-	-	-	90.6^{E}	16.3	85.1^{C}	9.1	89.7^{E}	7.5	9.9^{DF}	6.1	4.1^{CE}	1.6

The sample size was 5 in each case except for maturity measures of females in weeks 3 and 24 where n=3 and for weeks 18 and 21 where n was 4 and 7 respectively.

Means sharing common superscripts are not significantly different ($P \geq 0.05$. Fisher PLSD Test). Significance tests were performed on arcsine transformed data for GBI and % spermatozoa analysis. Log transformation was used for MGBI and mean oocyte diameter data.

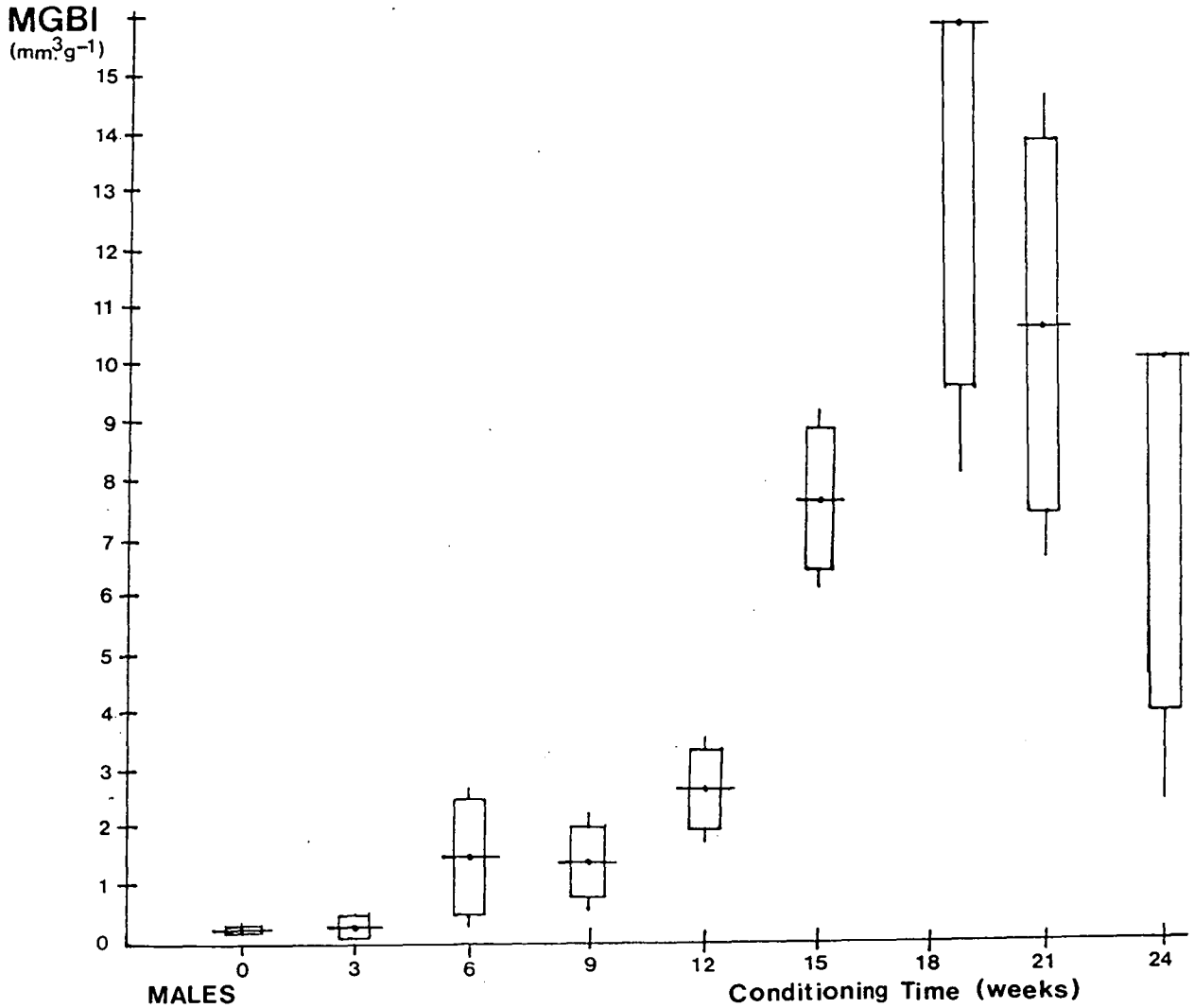
3.5

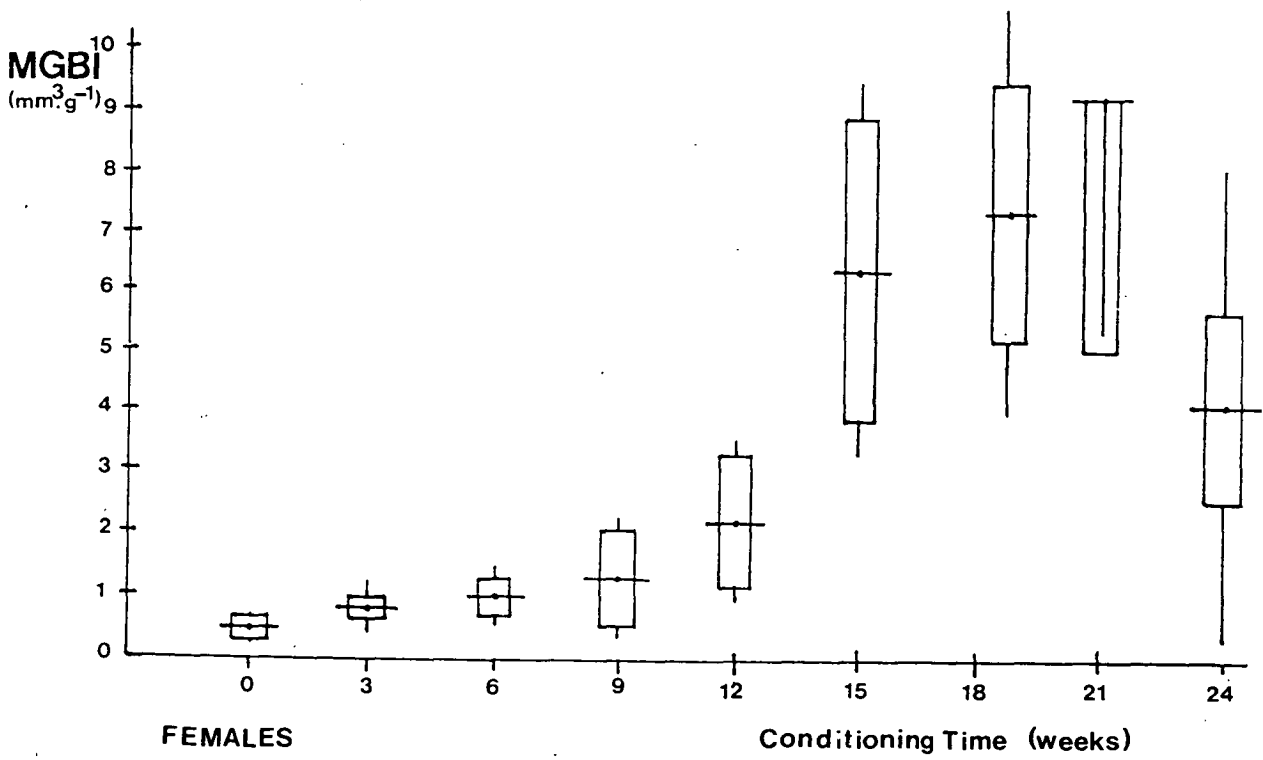
Modified Gonad Bulk Index Data

Temporal changes in modified gonad bulk index are shown in Figure 9. One factor ANOVA performed on log transformed data with the sexes treated separately was highly significant in both cases ($P=0.0001$). This indicates the actual change in gonad maturity which occurred and resulted in spawning was detected by the MGBI. Comparison of sample means by Fisher PLSD Test are displayed in Table 3. These data indicate the MGBI is more sensitive than the GBI as gonad growth was detected by week six of conditioning by the former index as compared to week nine for the latter (Table 3). In both sexes the MGBI indicates the gonad developed rapidly between weeks 12 and 15 prior to spawning. The decline in the week 24 MGBI value for females compared to the week 15 value is possibly explained by the necrosis observed (Section 3.12) resulting in a reduction of ovarian volume. Two factor ANOVA showed a significant difference ($P<0.05$) for the time/ sex interaction, indicating differences existed between the way the sexes matured over time.

The relationship between the GBI and MGBI was tested using the Spearmann rank correlation coefficient. For each abalone sampled over the course of the conditioning trial the GBI and MGBI were paired. The overall correlation was significant ($Rho=0.901$, $n=87$) at the 5 % probability level.

FIGURE 9
Temporal Change in the Modified Gonad Bulk Index (x, s.d.,c.l.)


MGBI
(mm³g⁻¹)
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
0
3
6
9
12
15
18
21
24
MALES
Conditioning Time (weeks)


MGBI
(mm³g⁻¹)
10
9
8
7
6
5
4
3
2
1
0
0
3
6
9
12
15
18
21
24
FEMALES
Conditioning Time (weeks)

The correlation between the two indices was highest when abalone gonads were actively growing, up until week 15 (Rho = 0.911, n = 58) but was poor between weeks 18 and 24 (Rho = 0.003, n =29). During this latter time period there was no further increase in maturity, as detected by the gonad indices (Table 3). The correlation was also significant for field specimens collected between September and November 1990 when gonad maturity was occurring (Rho = 0.709, n = 31).

Samples taken during week 21 when gonad size was near its maximum level were used to test the MGBI method assumption that most of the gonad tissue is contained in the conical appendage. Only 23.5% (s.d.=6.3, n=12) of the gonad was found in the conical appendage as defined by Figure 3b. Two tailed unpaired t-test found no significant difference between male and female specimens ($p>0.05$). A sample of field specimens collected 20 November 1990 yielded a mean gonad percentage in the conical appendage of 24.4% (s.d.=6.0, n=5). T-test analysis (2 tailed, unpaired) found no difference between the means of the conditioned and field samples ($p=0>0.05$).

3.6

Gonad as Percentage Body Weight

Reproductive development in abalone rarely has been monitored by calculating the percentage of animal weight contributed by the gonad. As noted previously (Section 2.13) this is because of the tedious nature of the dissection involved. The method was used on samples of ripe abalone in this study to allow comparison with the available data for other haliotid species. However, the method was not used for routine analysis.

Dissection of 12 specimens (7 female: 5 male) selected during week 21 of the experiment yielded a mean of 5.7% gonad by weight (s.d.=1.2) including the shell. The 2 tailed unpaired t- test method showed no significant difference between the sexes ($P>0.05$). The gonad as a percentage of soft body weight was 8.7% (s.d.=2.1). A sample of field specimens collected 20 November 1990 (the natural spawning season) yielded a mean gonad value of 7.4% (s.d.=4.3, n=5) with the shell intact. T-test analysis (2 tailed, unpaired) found no significant difference between data for field and conditioned specimens ($P>0.05$).

3.7

Visual Assessment of the Developing Gonad

It was possible to determine the sex of abalone with some certainty by week 12 of the conditioning period. Males were more easily identified than females at first. This was because the creamy yellow of the testis contrasted more obviously with the grey colour of the underlying digestive gland than did the green of the ovary. The conical appendage became progressively longer, bulkier and more concave in relation to the inside of the shell as the gonad developed. Eventually the tip of the conical appendage became blunt and rounded. This last feature was observed in some of the abalone which were induced to spawn during the sixteenth week of conditioning. The gonad did not become so large that it projected from the shell in any of the artificially conditioned abalone. Neither was this seen in any of approximately 100 wild abalone examined 20 November 1990.

3.8

Oocyte Size/Frequency Distribution Data

Temporal changes in oocyte size/ frequency distribution are displayed graphically in Figure 10. Contingency table analysis of the data was significant (The Chi-square calculated value of 380.96 being higher than the critical value of 73.31, d.f = 55). This indicates a genuine change in the frequency distribution of oocyte size classes occurred over the study period. Standardized residuals are displayed in Table 4. These indicate which samples and size classes contribute most to the Chi-square value. A positive value indicates the frequency of oocytes in that size class is greater than expected while negative values indicate lower than expected frequencies. Thus there is no significant change in oocyte size distribution during the first three weeks of conditioning. The distribution of oocyte sizes from the initial sample indicates that the population spawned synchronously before collection. Also, individual specimens had spawned completely as there were no large remnant oocytes. By week six the standardized residual for the 40-60 µm class had changed from a negative to a positive indicating an increase in oocytes of that size. Also the residual for the 0-20 µm size category has changed from a positive value to a negative, signifying a decline in frequency of very small oocytes. The greatest oocyte growth occurs between weeks 12 and 15 when six of the twelve size classes experience a change in the sign of the standardized residual.

FIGURE 10
Temporal Change in Oocyte Size/ Frequency Distribution
(x, s.d.)

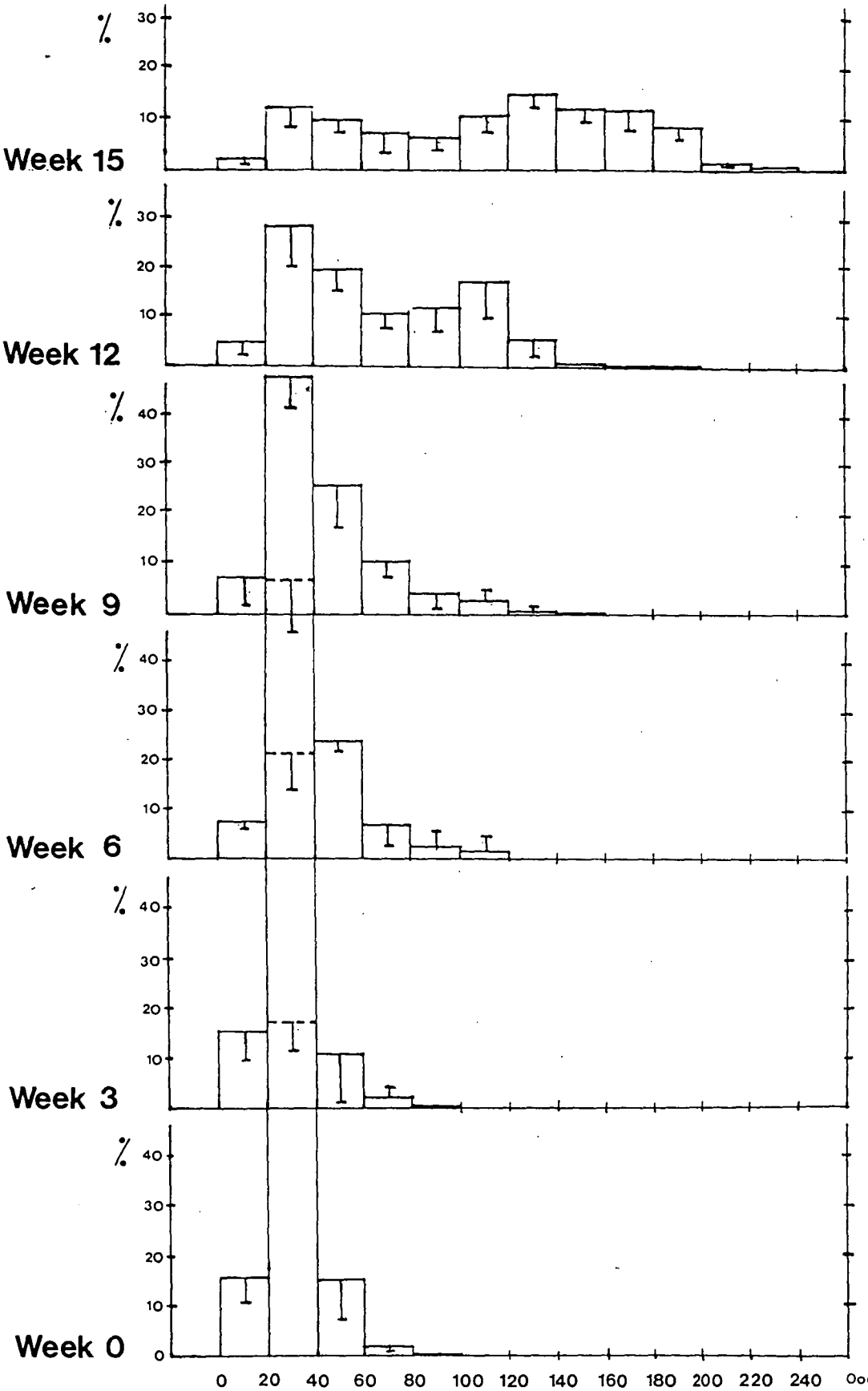

%
Week 15
Week 12
Week 9
Week 6
Week 3
Week 0
0 20 40 60 80 100 120 140 160 180 200 220 240
Oocyte Size (µm

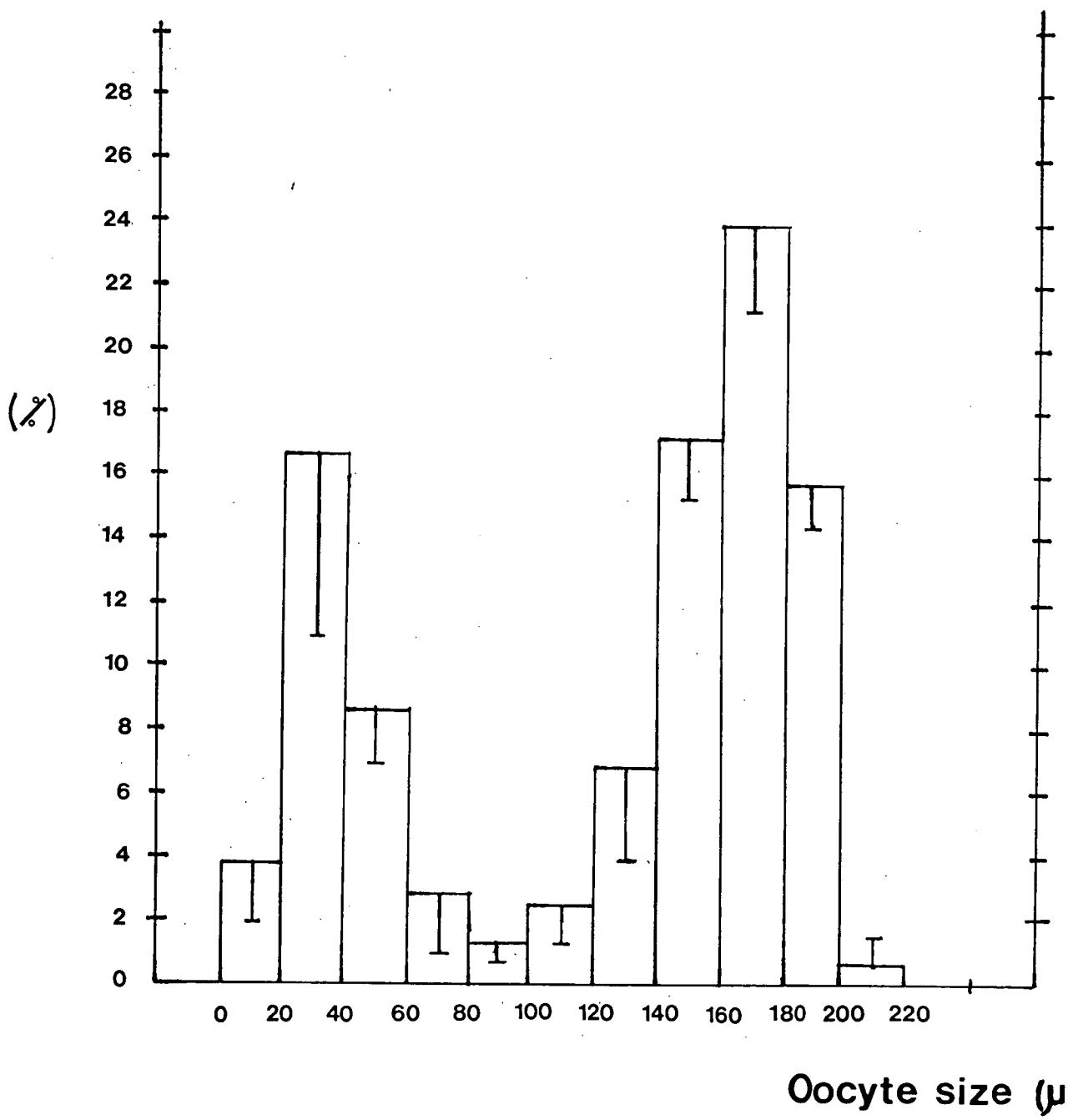
Table 4
STANDARDIZED RESIDUALS FOR CONDITIONING TIME / OOCYTE SIZE CLASS CONTINGENCY TABLE.

	Conditioning Time (Weeks)					
Size Class (μm)	0	3	6	9	12	15
0 - 20	3.11	2.82	-0.38	-0.64	-1.97	-2.93
21 - 40	6.34	7.96	3.23	0.13	-6.28	-11.36
41 - 60	-0.87	-2.45	2.18	2.99	0.80	-2.66
61 - 80	-2.31	-2.14	0.00	1.93	1.96	0.56
81 - 100	-2.56	-2.55	-1.10	-0.03	4.76	1.47
101 - 120	-2.96	-2.96	-2.19	-1.45	6.59	2.98
121 - 140	-2.32	-2.32	-2.32	-2.03	1.23	7.75
141 - 160	-1.80	-1.80	-1.80	-1.67	-1.09	8.14
161 - 180	-1.72	-1.72	-1.72	-1.73	-1.56	8.45
181 - 200	-1.45	-1.45	-1.45	-1.45	-1.39	7.19
201 - 220	-0.55	-0.55	-0.55	-0.55	-0.55	2.75
221 - 240	-0.29	-0.29	-0.29	-0.29	-0.29	1.47

The oocyte growth at this time indicates that vitellogenesis was occurring. By week 15 the percentage of ripe oocytes greater than 180 µm in diameter had reached 10.2%.

Monitoring of gonad development by the oocyte size/frequency distribution method was discontinued after week 15. Figure 10 does not display data subsequent to week 15 of the experiment because of the presence of necrotic oocytes. However, sampling of female abalone in week 21 of the experiment yielded some individuals that did not have extensive areas of necrotic oocytes. These have been used to construct an oocyte size/frequency distribution histogram showing the bimodal pattern more typical of fully ripe abalone (Figure 11).

FIGURE 11
Oocyte Size/ Frequency Distribution for Ripe Abalone (x, s.d.)

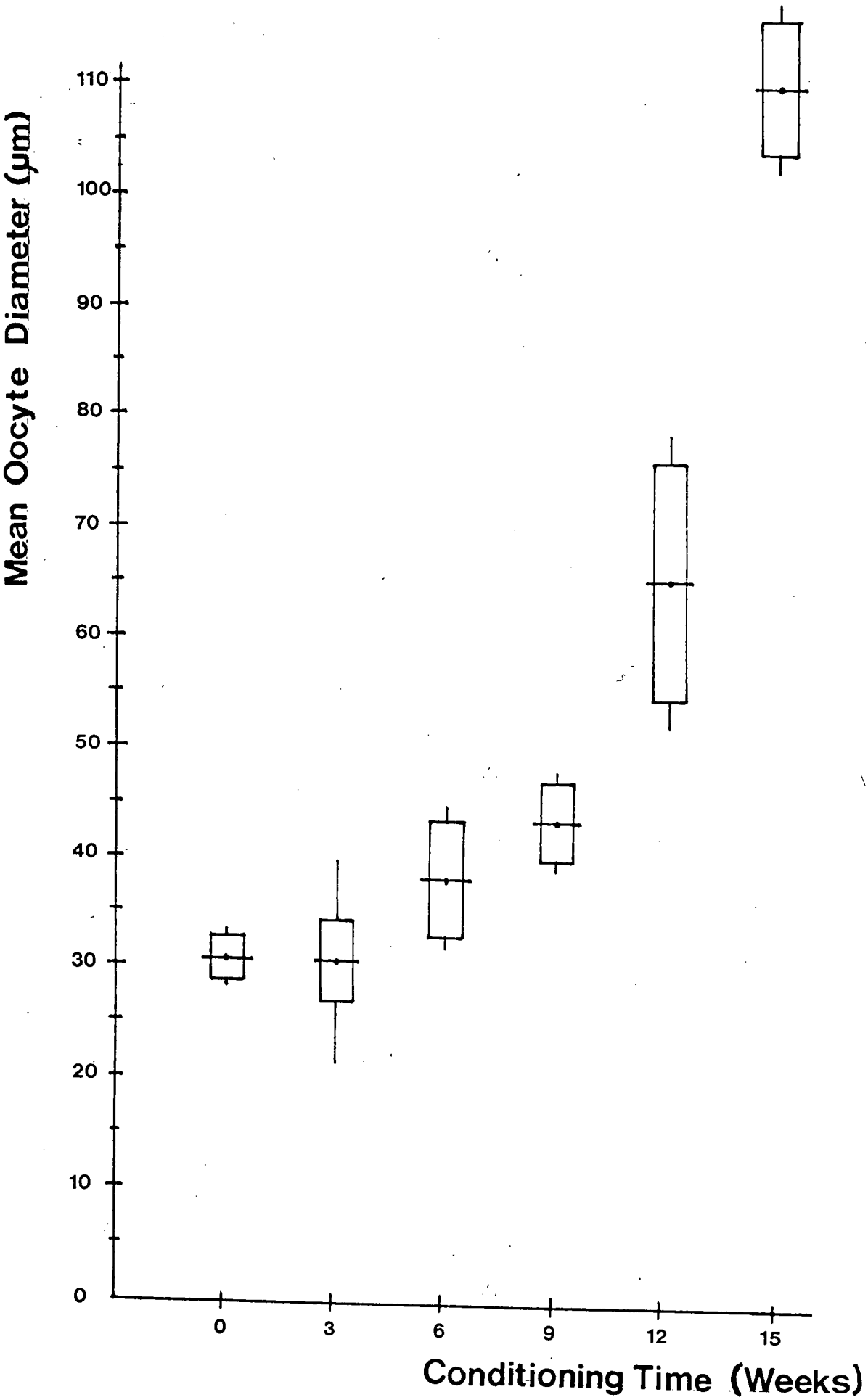

(%)
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0 20 40 60 80 100 120 140 160 180 200 220
Oocyte size (µm

3.9

Mean Oocyte Diameter Data

Changes with time in mean oocyte diameter are shown in Figure 11. The initial mean value of 30.7 µm increased to 109.7 µm after 15 weeks of conditioning. This maximum value is considerably less than the size of fully ripe eggs. This is because such eggs do not dominate the ovary numerically at maximum ripeness, therefore reducing the mean size. One factor ANOVA, performed on log transformed data was as expected highly significant (P=0.0001). Comparison of sample means by Fisher PLSD test are shown in Table 3. The data indicate the first significant increase in oocyte size had occurred by week six of the conditioning process. Oocyte size increase was slow until the ninth week of conditioning. Rapid development late in the reproductive cycle is shown by the large increase in oocyte diameter between weeks 12 and 15. The use of this method was discontinued after week 15 due to the presence of large numbers of necrotic oocytes.

FIGURE 12
Temporal Change in Mean Oocyte Diameter (x, s.d., 95% c.l.)


Mean Oocyte Diameter (µm)
110
100
90
80
70
60
50
40
30
20
10
0
0
3
6
9
12
15
Conditioning Time (Weeks)

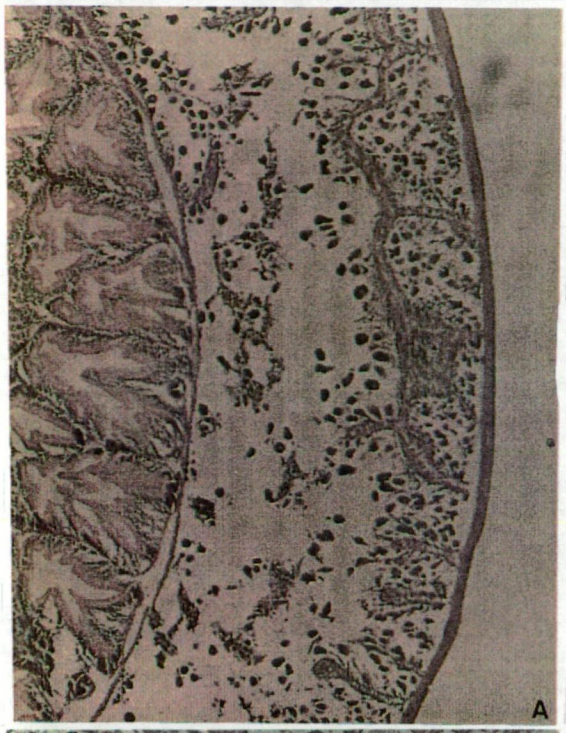
3.10

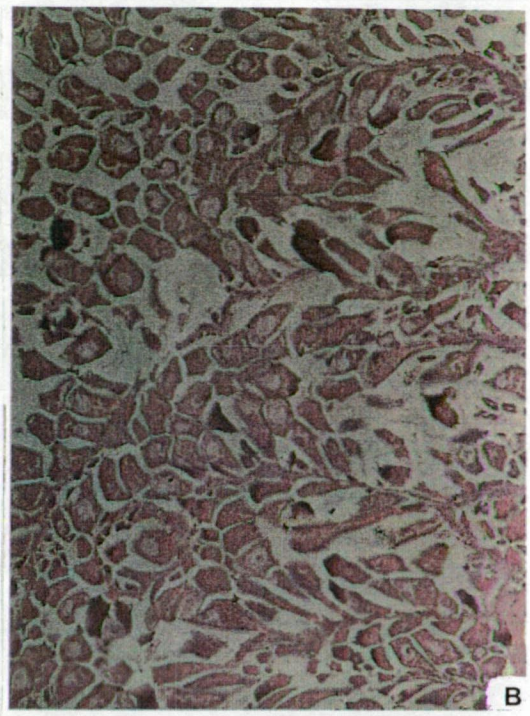
Ovarian Phase Data

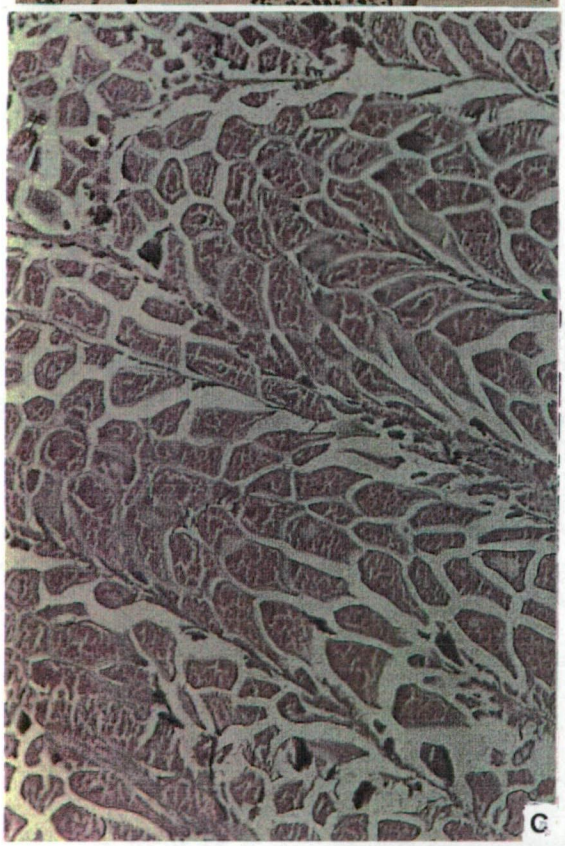
The ovarian phase method of assessing reproductive development allows rapid diagnosis of gonad condition. The method, though subjective is based on ovarian histology and therefore gives a more complete picture of reproductive development than other simple to use methods such as gonad indices. In this study all the ovarian phases present in each sample were quantified and expressed as percentages rather than merely recording the dominant phase. Based on examination of histological preparations five simply recognized ovarian phases were defined. Typical examples of each of the phases are shown in Figure 13.

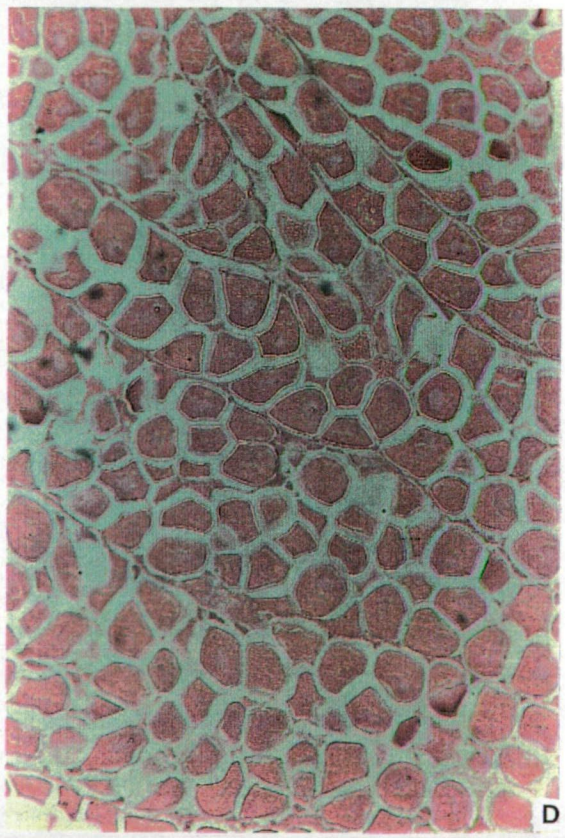
1. Recovery Phase - The gonad is immature with the vast majority of cells being smaller than 60 µm and closely associated with the germinal epithelium and trabeculae. Oocytes are strongly basophilic.

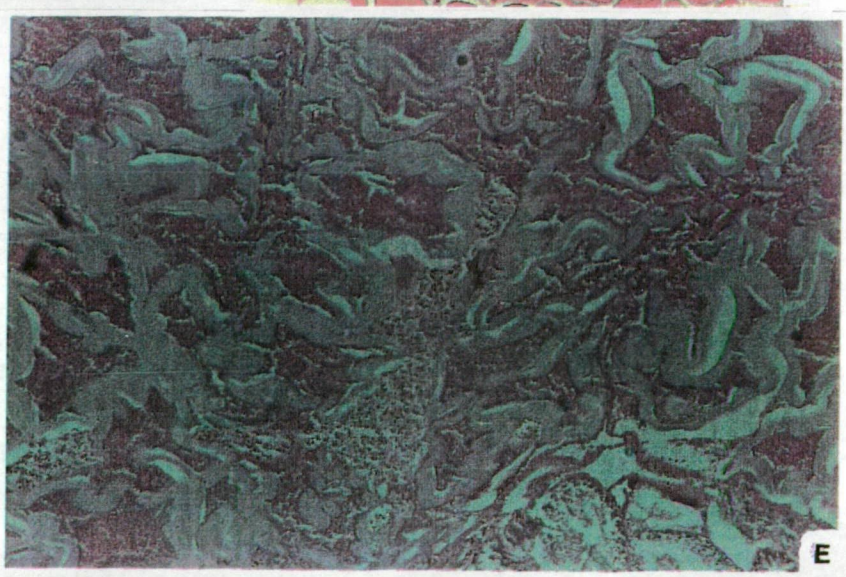
2. Growing Phase - This phase covers quite small oocytes which are noticeably stalked to large elongate (up to 300 µm) oocytes. Vitellogenesis occurs during this phase and the majority of cells are attached to trabeculae. Late in the phase the trabeculae extend well into the lumen of the ovary. There is still considerable empty space between individual cells and clusters of cells. Oocytes are devoid of the gelatinous coating.











Key to phases:

A– Recovery phase, ×50.

B– Growing phase, ×50

C– Mature phase, ×50

D– Spawning phase, ×50

E– Necrotic phase, ×125

FIGURE 13

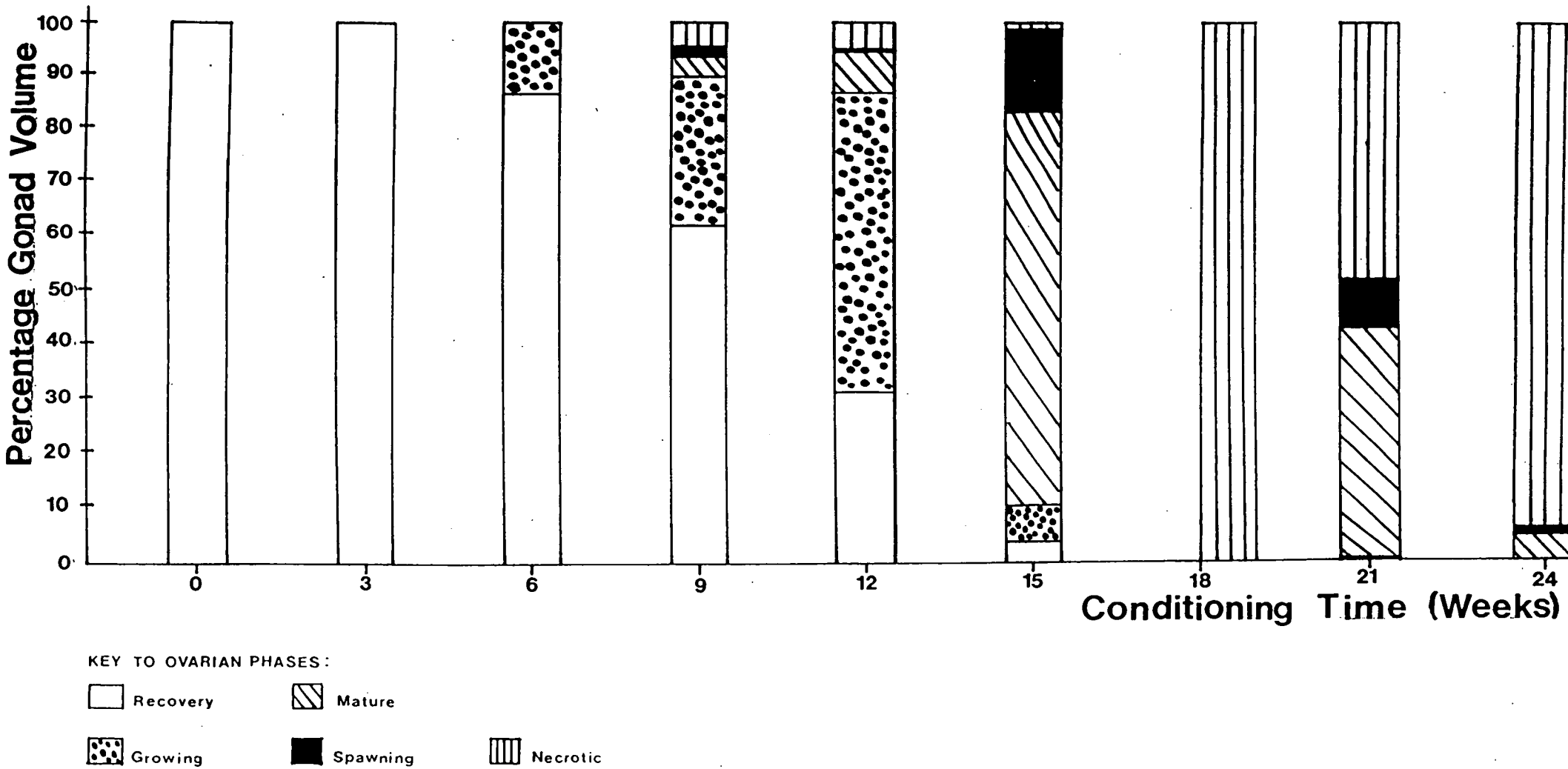
3. Mature phase - The ovaries of mature phase specimens are densely packed with large oocytes. The smooth teardrop shape characteristic of oocytes from the previous phase is now replaced with relatively shorter, broader cells of hexagonal appearance. The gelatinous coating is apparent.

4. Spawning Phase - In spawning phase the oocytes are at maximum size and rounded. They are densely packed and the gelatinous coating is thick.

5. Necrosis. - Cells in this condition are easily distinguished by their irregular shape, lack of a nucleus, and characteristic strong eosinophilic staining. Some oocytes appear to become necrotic before reaching maximum size.

Changes in ovarian phase over the gonad conditioning period are shown in Figure 14. Gonad development was underway by the sixth week of conditioning. Development was at first slow with the recovery phases dominating the ovary until week nine of the study. Ovarian maturity then increased more rapidly with the growing, mature, and necrotic phases successively dominating the next three samples. The proportion of the ovary considered to be in spawning phase reached a sampled maximum of 16% one week before induced spawning. For comparison purposes with other maturity measures these data are summarized by showing the dominant phase in each sample (Table 3).

FIGURE 14
Temporal Change in Ovarian Phase

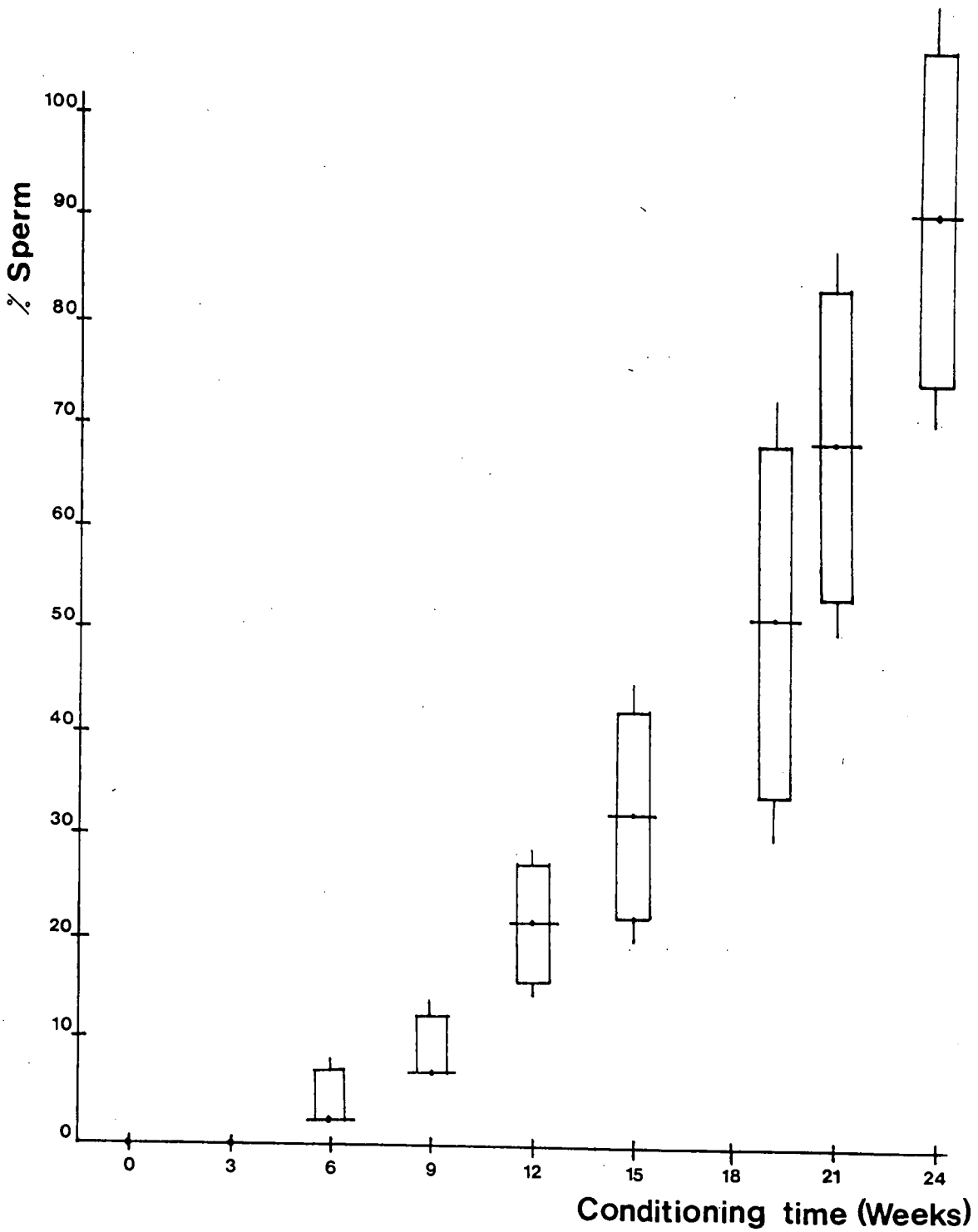

Percentage Gonad Volume
100
90
80
70
60
50
40
30
20
10
0
0
3
6
9
12
15
18
21
24
Conditioning Time (Weeks)
KEY TO OVARIAN PHASES:
Recovery
Mature
Growing
Spawning
Necrotic

3.11

Testis Maturation Data

Temporal changes in the percentage of male gametes present as mature sperm cells are displayed in Figure 15. One factor ANOVA performed on arcsine transformed data was highly significant (P=0.0001). Thus the actual increase in maturity noted by visual assessment and detected by the gonad indices was also detected by this method. Comparison of sample means by Fisher PLSD Test are shown in Table 3. The first traces of mature sperm in samples were detected after six weeks of conditioning (Figure 15), though there was no significant statistical change compared to the initial sample until week nine (Table 3). The percentage of the testis occupied by sperm cells continued to increase until the end of the study when the value peaked at 90.6% of cross sectional area. This differed from the pattern observed for female specimens where maximum gonad ripeness occurred between weeks 15 and 18. It should be noted that the continued increase in maturity displayed by male specimens after week 18 was not detected by either gonad index (Table 3).

FIGURE 15
Temporal Change in Percentage Spermatozoa (x, s.d., 95% c.l.)

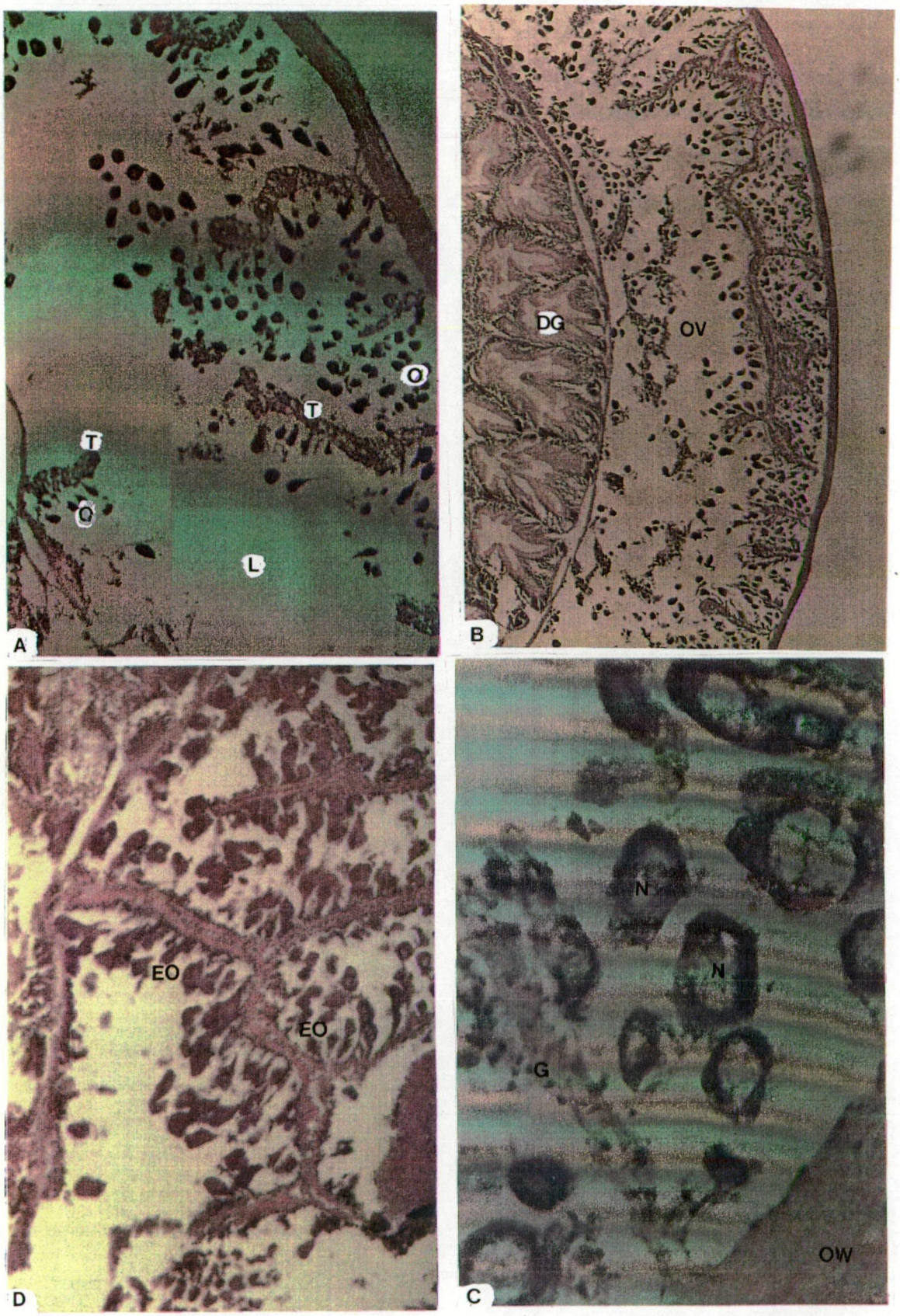

% Sperm
100
90
80
70
60
50
40
30
20
10
0
0
3
6
9
12
15
18
21
24
Conditioning time (Weeks)

3.12

Descriptive Histology of the Ovary

Examination of specimens taken prior to the commencement of gonad conditioning revealed the reproductive state to be highly synchronous. The ovary at this stage consisted of a very thin layer which could be barely seen macroscopically. Gametogenic activity was mainly confined to the germinal epithelium of the outer gonad wall, distal from the digestive gland. In addition oogonia and small primary oocytes could be seen in close association with the trabeculae which project from the inner ovarian walls (Figures 16a,b,c). Small detached oocytes were also present in spaces between adjacent trabeculae and trabeculae and the ovarian walls. Oocytes at this time were either rounded or becoming teardrop shaped, the latter type were especially common on the ends of trabeculae projecting into the lumen of the ovary. The cells were uniform in size with the majority being less than 40 µm in diameter. No large oocytes residual from the previous spawning were present. The cytoplasm of oocytes was strongly basophilic indicating protein synthesis.

No change was evident in samples taken after three weeks of gonad conditioning but after six weeks differences could be observed. Oocytes attached to trabeculae were beginning to elongate so that the stalks holding them were more obvious (Figure 16d). Also for the first time some distinctly larger cells, up to 120 µm were seen.



A– Overview of immature ovary. O = primary oocytes, T = trabecula, L = lumen of ovary. ×50.

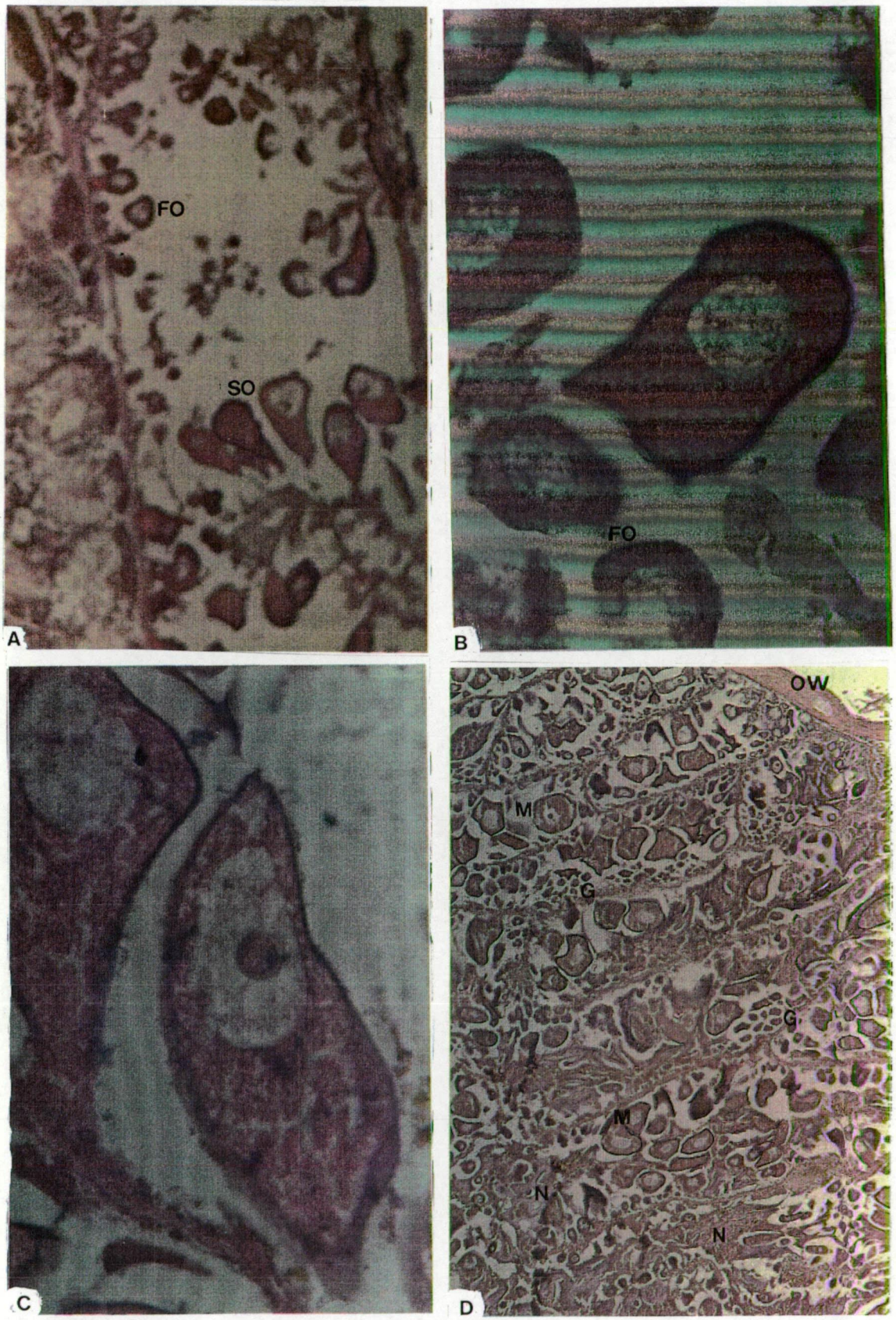
B– DG = digestive gland, OV = immature ovary. ×50. C – Small oocytes. G = germinal epithelium, OW = outer ovarian wall, N = cell nucleus. ×500. D – EO = elongating small oocytes. ×125

FIGURE 16

These oocytes were not recorded in all specimens from the week six sample. Some of the cells were teardrop shaped and in clusters (Figure 17a) while others were more rounded and free from the trabeculae and ovarian walls (Figure 17b). These oocytes appear to correspond to the oil drop stage described by Tomita (1967) and the late growing stage described by Lee (1974).

All individuals appeared to have at least some oocytes in the oil drop stage by week nine of the conditioning process. The majority of cells present were still less than 60 µm long. However, the small fraction of more mature oocytes had begun to fill some of the empty space in the ovarian lumen. The most mature cells present appear to correspond to phase 4 of the development cycle described by Takashima et al. (1978) and the primary yolk globule stage described by Tomita (1967). The cytoplasm of more mature cells stained eosinophilic. One of the specimens sampled at this time was considerable more advanced than the others. Some very large elongate cells, still lacking the gelatinous layer were seen in this specimen (Figure 17c). In other areas of the ovary patches of cells in an advanced stage of necrosis were observed, including the remains of gelatinous membranes (Figure 17d).

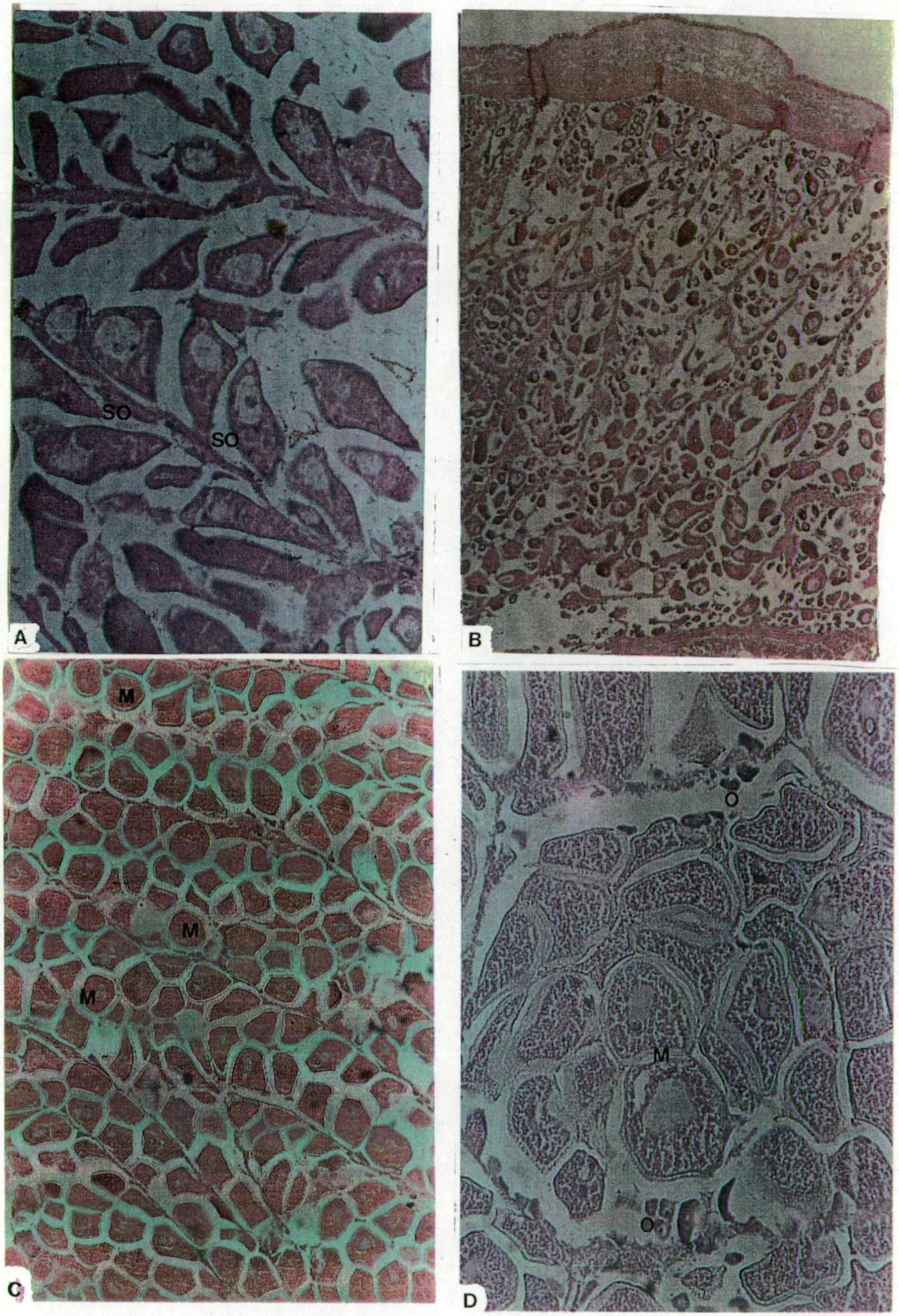
Samples taken after twelve weeks in the conditioning tank showed ovaries dominated for the first time by large stalked oocytes up to 300 µm in length (Figures 18a and 18b).



A – Overview of growing ovary. SO = stalked growing oocytes, FO = free growing oocyte. ×125.

B – FO = free oocytes. × 500. C – Deposition of oil droplets and yolk granules in large elongate oocytes. × 500 D – OW = outer ovarian wall, M = mature oocytes, N = necrotic areas, G = growing oocytes ×50.

FIGURE 17



A– SO= large stalked oocyte, ×125. B– overview of growing ovary, ×50.

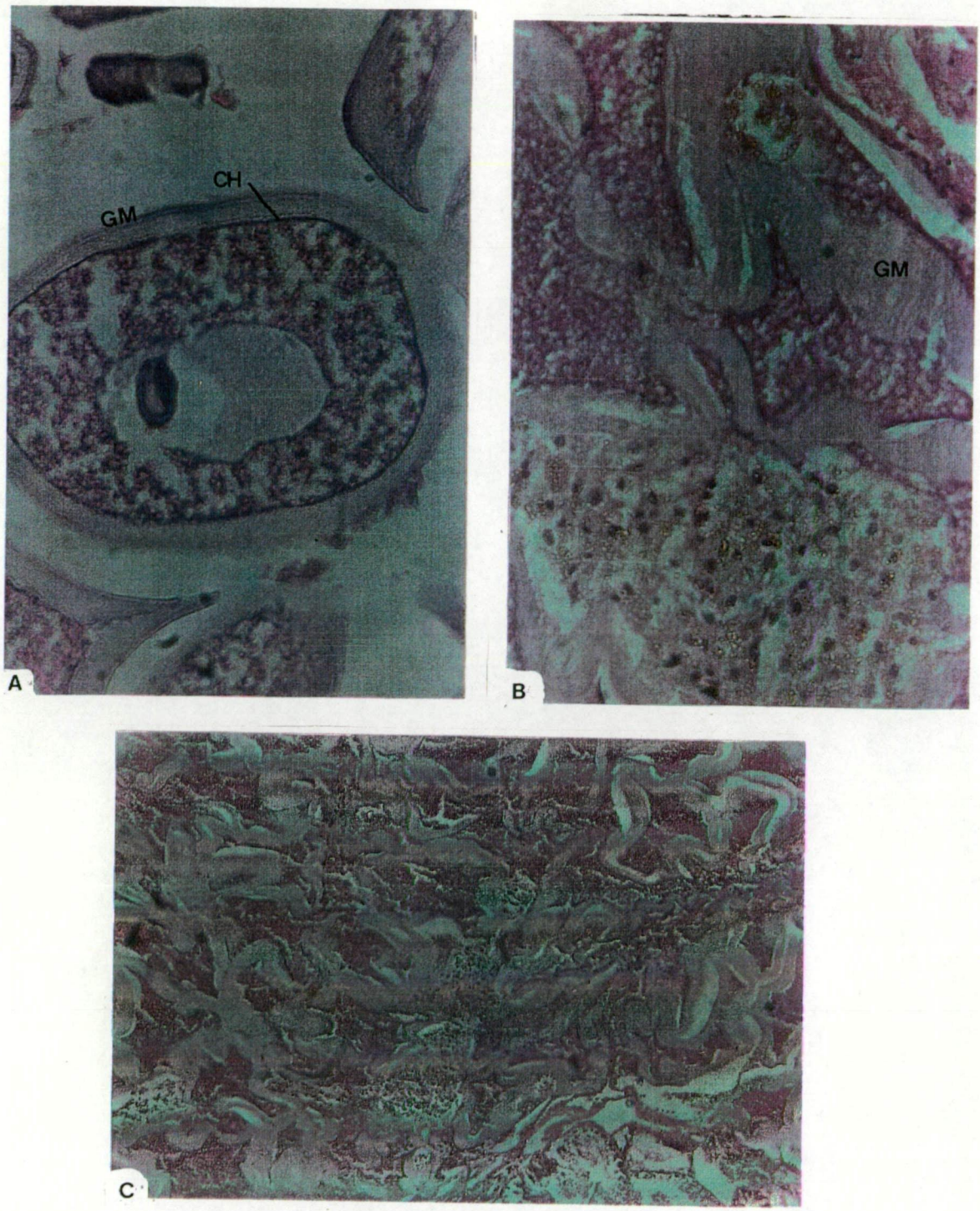
C– M= mature eggs, ×50. D– M=mature eggs, O= small oocytes, ×125

FIGURE 18

These large cells were found mainly in the central part of the ovary, attached to the trabeculae but distant from where trabeculae join the ovarian walls. Generally gelatinous membranes had not yet formed. Numerically, small oocytes 20-40 µm in diameter were still dominant and tended to cluster near the inner ovarian walls and bases of the trabeculae.

By week 15 the ovary was densely packed and patches of fully developed eggs were seen (Figures 18c and 18d). More of the large stalked oocytes seen previously had become detached from the trabeculae and rounded. The gelatinous membrane was now common on large oocytes (Figure 19a).

All seven samples taken during week 18 of the conditioning process showed extensive necrosis. The only oocytes not affected were those smaller than 40 µm. Necrotic cells were strongly eosinophilic (including the gelatinous coat) ,irregular in shape and in many cases the nucleus had disappeared (Figures 19b,c). Abalone sampled during weeks 21 and 24 were almost entirely necrotic with the exception of some individual specimens from the week 21 sample. Small areas of a yellow staining granular substance (possibly lipo fuchsin) were associated with some necrotic sites. This substance has been previously reported by Giorgi and DeMartini (1977) in areas of advanced necrosis. Young and DeMartini (1970) reported extensive proliferation of oogonia during advanced necrosis. This was not seen by week 24 of the study when the remaining animals were sacrificed.



A– Mature egg. CH= chorion, GM= gelatinous membrane, × 500. B– Necrotic area. GM= remains of gelatinous membrane, ×500. C– Necrotic eggs, ×125.

FIGURE 19

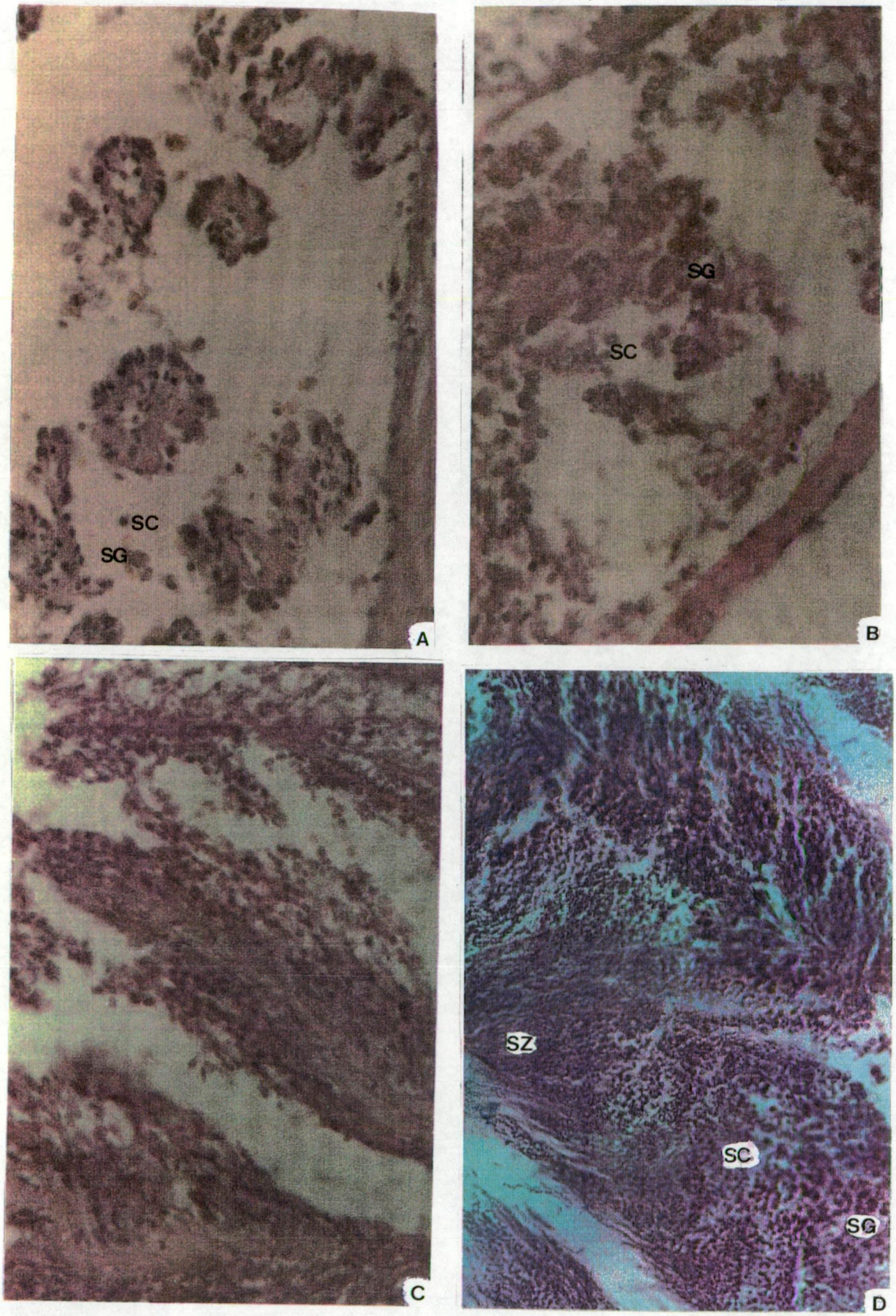
3.13

Descriptive Histology of the Testis

The testes of animals sacrificed for sampling when first collected were very small, consisting of a thin layer just visible macroscopically. Examination of stained sections revealed the testes to be largely devoid of gametes. What appeared to be degenerating cells left over from the previous spawning were observed. Spermatocytes were seen (Figure 20a) and appeared to be the most common cell type present, spermatogonia also were observed (Figure 20b).

By the sixth week of conditioning empty spaces in the testes had begun to reduce as the number of spermatogonia and spermatocytes associated with the germinal epithelium and trabeculae increased in number (Figure 20c). Necrotic cells appeared to be no longer present. One of the sample individuals was considerably more mature than the other specimens and some areas occupied by mature sperm cells were seen (Figure 20d). Traces of spermatozoa could be seen in all individuals from the following sample three weeks later.

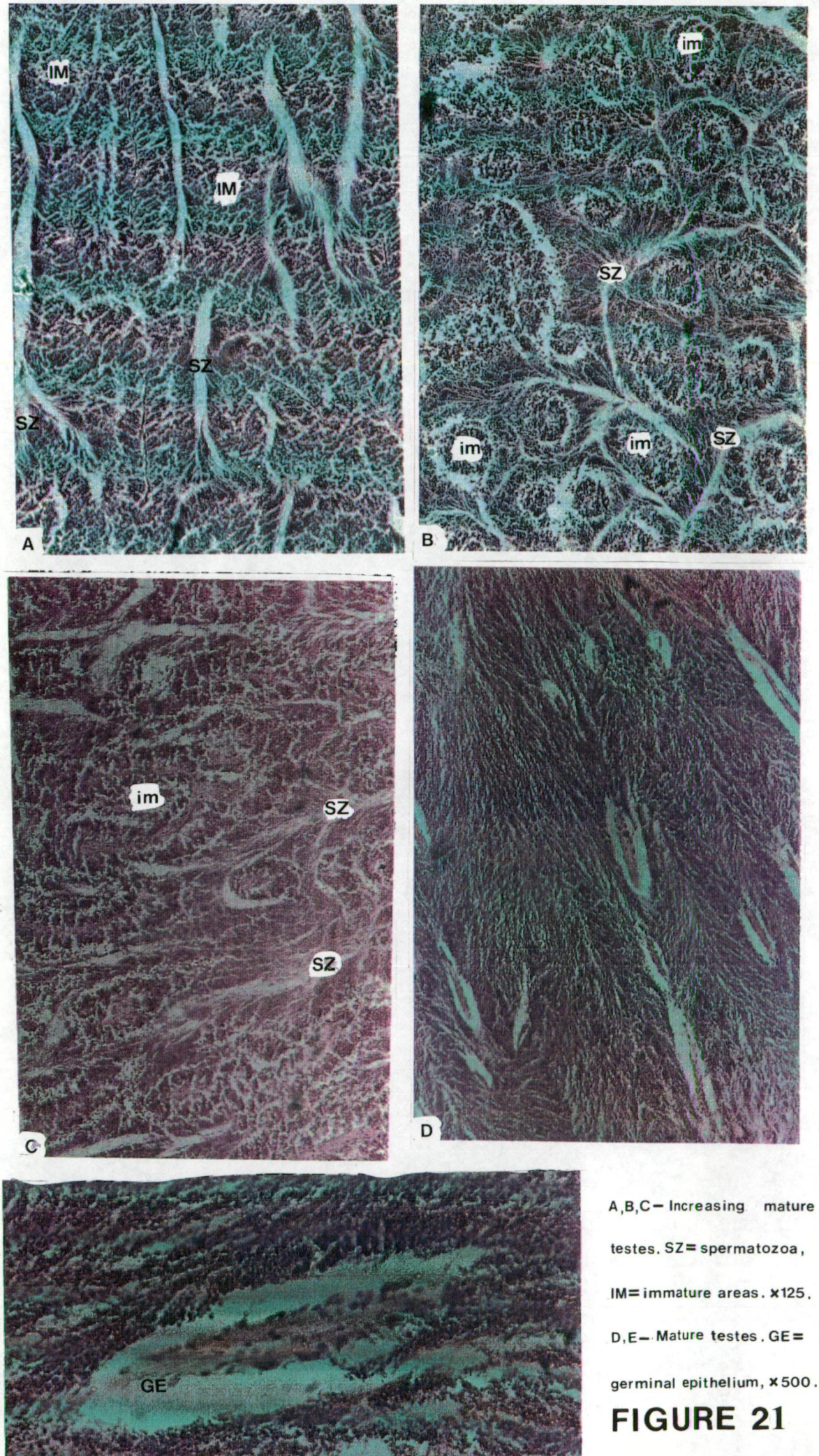
After week 12 the volume of the testis occupied by mature sperm steadily increased (Figures 21a,b,c). The volume of gonad occupied by spermatozoa was at its maximum in the final samples taken during week 24 of the experiment (Figure 21d). By then the only immature cells present were found clustered around small islands of germinal epithelium (Figure 21e).



A+B—Overview of immature testis. SG= spermatogonia, SC= spermatocytes, ×500.

C—Increasing cell area, ×500. D—SG= spermatogonia, SC= spermatocytes, spermatozoa, ×125.

FIGURE 20



A,B,C – Increasing mature testes. SZ = spermatozoa, IM = immature areas. ×125.
D,E – Mature testes. GE = germinal epithelium, ×500.

FIGURE 21

Using the testis maturation stages developed by Tomita (1968) samples from weeks zero to nine were considered to be in the recovery stage, weeks 12 to 15 in the premature stage, weeks 15 to 18 in the mature stage and samples from weeks 21 and 24 were considered to have reached the spawning stage.

3.14

The Reproductive Cycle of the Source Population

Field studies by Shepherd and Laws (1974) and McShane (1988) in South Australia and Victoria respectively have shown H.laevigata to be a summer spawning species. This was also found to be the case for the source population of abalone used in the present study (Figures 22 and 23). Spawning appears to have occurred between November 1990 and March 1991. This was consistent with the spawning pattern observed the following summer. Between mid November 1991 and late February 1992 spontaneous spawning of animals occurred in holding tanks used for live shipment at Furneaux Aquaculture, Flinders Island.

In the present study, a proportion of the conditioned abalone were induced to spawn on 21 August 1990 ie. late winter. Comparisons between conditioned samples taken on 14 August 1990 (week 15) and field specimens collected on 5 September 1990 were made using the T-test method (2 tailed, unpaired). Data for both gonad indices were significantly different ($P<0.05$). Conditioned abalone had higher GBI values (72.3 ± 9.2 vs 58.5 ± 14.4, n=10 and 12 respectively) and higher MGBI values (7.0 ± 2.0 vs 3.3 ± 0.9, n=10 and 12 respectively) than field specimens.

FIGURE 22
Seasonal Change in GBI of Source Population (x, s.d.)

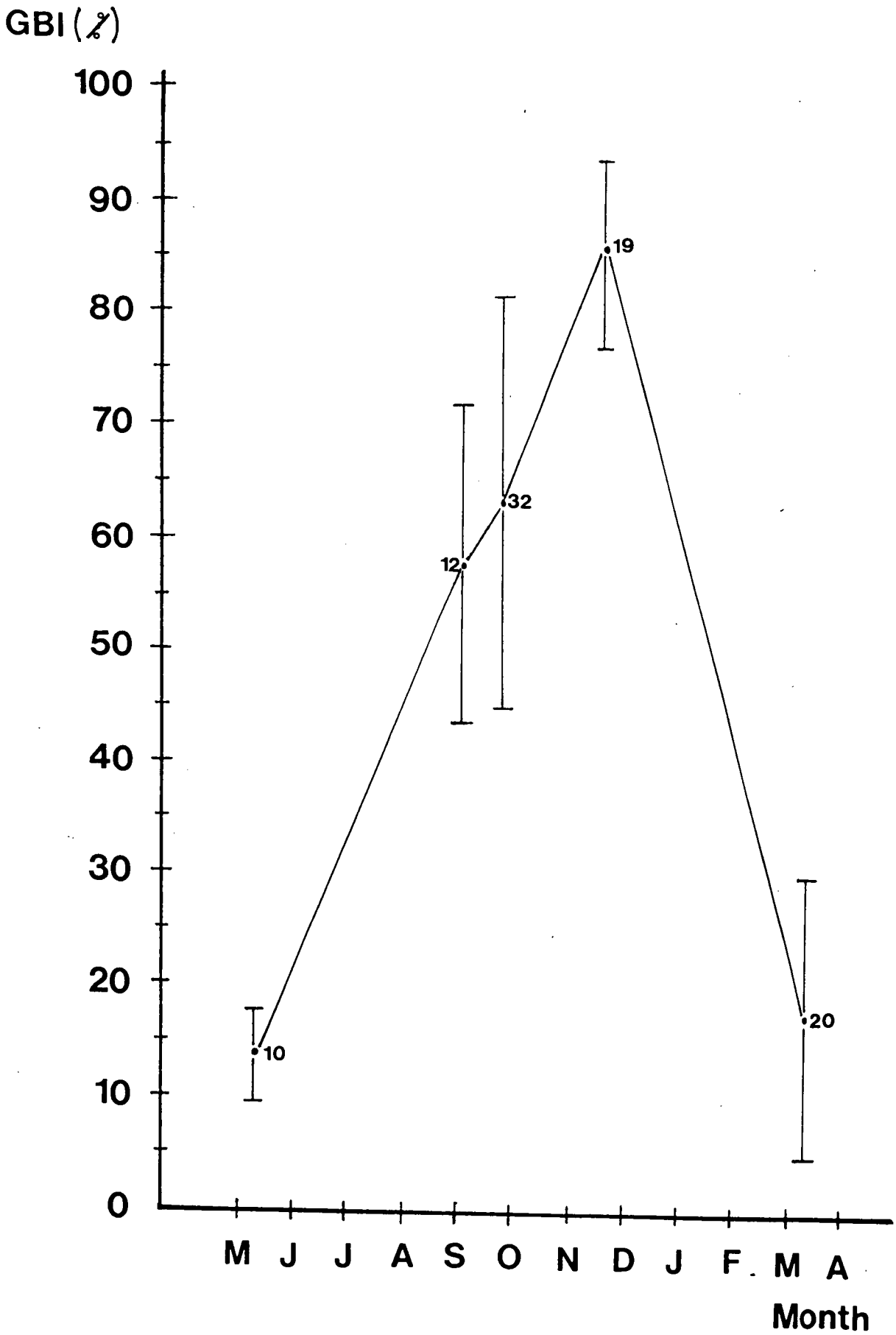
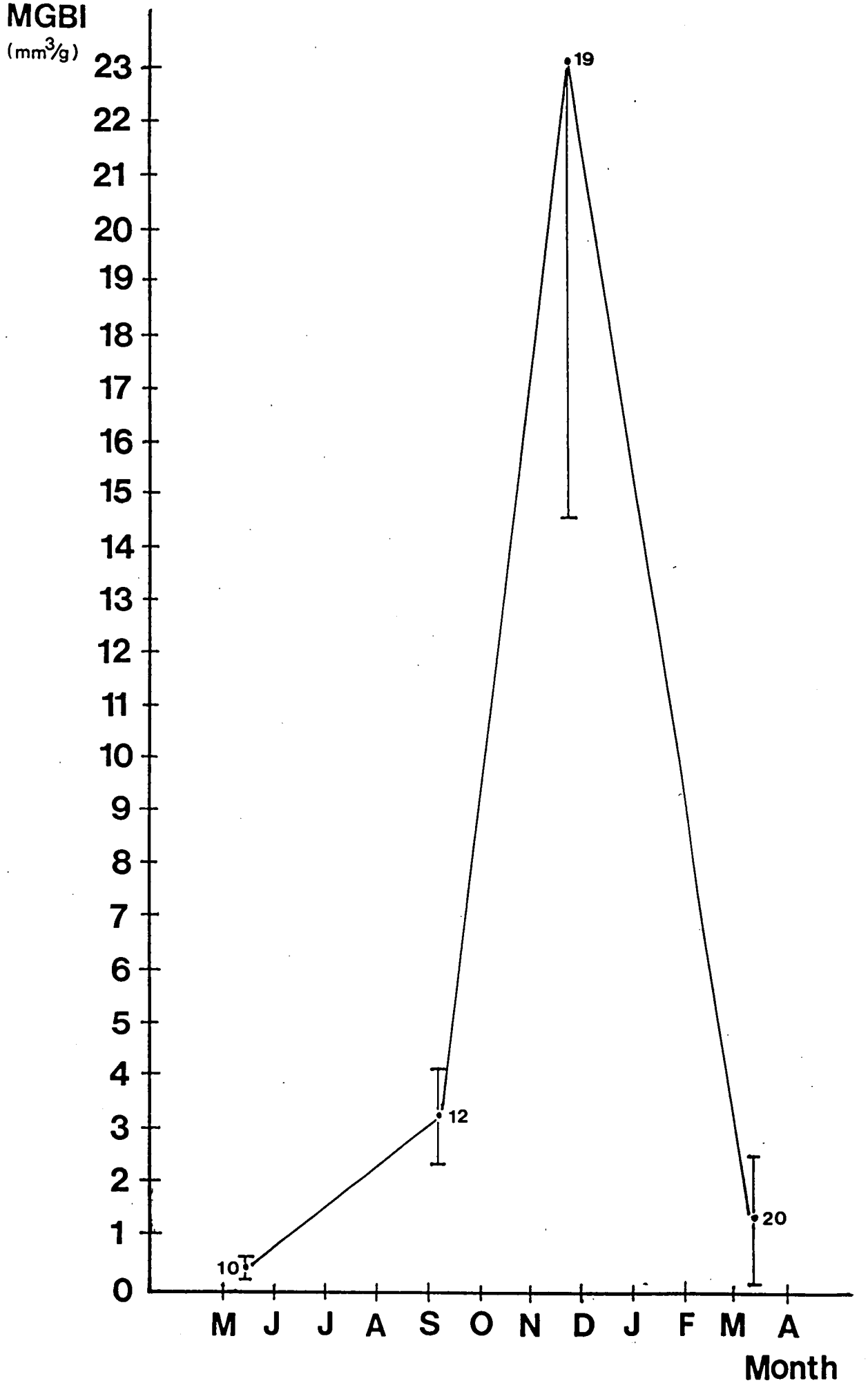

GBI (♂)
100
90
80
70
60
50
40
30
20
10
0
10
12
32
19
20
M
J
J
A
S
O
N
D
J
F
M
A
Month

FIGURE 23
Seasonal Change in MGBI of Source Population (x, s.d.)


MGBI
(mm³/g)
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
19
12
10
20
M J J A S O N D J F M A
Month

3.15

Fecundity Data

Following conditioning for 112 days 6 of 16 female and 1 of 6 male abalone were induced to spawn. One of the female specimens spawned approximately one million viable eggs, the remainder produced one to three hundred thousand eggs each. Such fecundity is relatively low for haliotids (refer to Appendices C and D) and it was considered likely the abalone were not sufficiently mature to release the majority of their eggs. Further attempts to induce spawning were made three weeks later and were unsuccessful. Subsequent histological analysis showed egg necrosis in the specimens. Additional fecundity estimates were made on the necrotic ovaries by the weight and volume subsample techniques (sections 2.23 and 2.24). These methods yielded mean values of 4.42 million eggs/animal (s.d.=2.03 million) and 4.00 million eggs/animal (s.d.=2.47 million) respectively. Seven female abalone were sampled in week 21 to provide the data, which are displayed as a polynomial regression in Figure 24. Alternatively, if the data are fitted to a linear regression $r=0.797$, $p=0.03$. In addition, for comparative purposes five wild abalone were sampled in mid November and fecundity estimated by the known weight subsample method (Tables 5a and 5b).

In January 1992 further fecundity estimates of conditioned abalone were made at Furneaux Aquaculture Pty Ltd. Cultured abalone in the size range 6-9 cm were induced to spawn (Section 2.25) and fecundity estimated as described in section 2.22. These data are shown in Table 6, mean fecundity was 315,000 eggs/animal (s.d.=111,000, n=13).

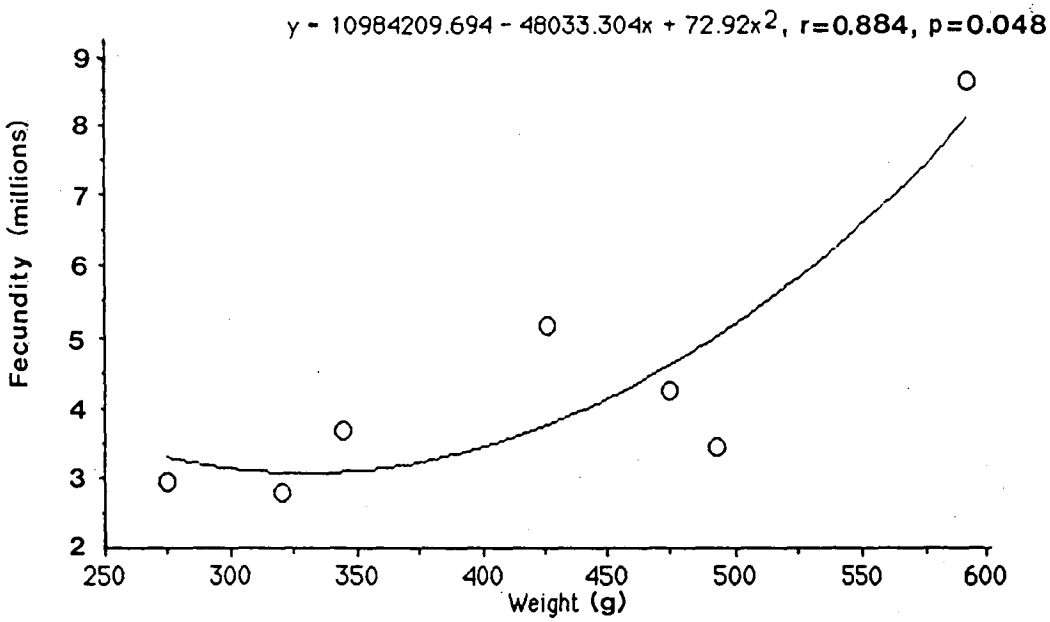


FIGURE 24

Weight, fecundity regression for conditioned abalone

Table 5a
FECUNDITY ESTIMATES FOR CONDITIONED ABALONE SAMPLED WEEK 21.

Weight (g)	Length (cm)	Fecundity (Weight Estimate)	Fecundity (Volume Estimate)	EGV (mm^3)	MGBI (mm^3/g)	GBI (%)
274.6	13.8	2 940 000	1 640 000	2752	10.0	81.1
320.2	14.3	2 820 000	1 210 000	1160	3.6	89.6
344.4	14.8	3 690 000	2 010 000	2048	6.0	85.0
426.3	14.4	5 160 000	4 290 000	5779	13.6	75.9
474.5	15.6	4 260 000	3 690 000	3275	6.9	75.7
492.4	15.1	3 440 000	2 480 000	4236	8.6	84.2
592.0	15.4	8 640 000	8 390 000	8915	15.1	94.1

Table 5b
FECUNDITY ESTIMATES FOR FIELD ABALONE SAMPLED 20/11/1990

Weight (g)	Length (cm)	Fecundity (Weight Estimate)	EGV (mm^3)	MGBI (mm^3/g)	GBI (%)
445	15.0	5 420 000	15 359	34.5	84.5
563	16.8	5 550 000	16 098	28.6	92.9
581	16.2	6 400 000	9 527	16.4	91.3
634	17.7	1 830 000	13 336	21.0	75.4
669	17.9	1 920 000	10 714	16.0	75.7

Table 6

FECUNDITY ESTIMATES FOR CULTURED ABALONE

Length (mm)	Weight (g)	Fecundity
60	23.9	162,000
61	24.1	189,000
69	36.9	214,000
69	37.5	277,000
70	38.1	216,000
74	44.9	374,000
77	51.6	369,000
78	53.0	373,000
79	52.9	209,000
81	57.5	458,000
84	65.0	455,000
86	65.8	508,000
88	76.0	290,000

3.16

Correlations Between Fecundity and Gonad Indices

The Spearmann rank correlation was used to examine the relationship between fecundity of ripe abalone and the two gonad indices used in the study. Data used were obtained from conditioned abalone sampled during week 21 (Table 5a). The correlation between gonad size as measured by the EGV (the numerator in the MGBI formula) and fecundity (weight estimate) was significant (Rho=0.821, n =7, $P<0.05$). The correlations between fecundity and the two gonad indices were not significant (Rho=0.679 and Rho= - 0.071) for the MGBI and GBI respectively. Correlation between fecundity of field samples taken 20 November 1990 (Table 5b) and EGV, MGBI, and GBI were not significant (Rho=0.400, 0.600, and 0.900 < 1.00 {Rho critical}, n =5).

3.17

Sex Ratio

Since the sex ratio of H.laevigata in South Australia has been reported to be unequal (Shepherd and Laws ,1974) data on Tasmanian stocks was collected in this study for the purposes of comparison. The sex of abalone could not be determined at the time of capture. However, histological study and subsequent gonad development allowed the sex ratio to be determined. Chi-square analysis indicated the sex ratio of experimental animals used in the present study (70 males : 48 females) differed significantly from 1 : 1 ($P<0.05$).

4. DISCUSSION

4.1

Comparison of Conditioning Tank Results

The major purpose of this study was to induce out of season spawning in H.laevigata. This goal was achieved and ripe individuals were induced to spawn in August 1990, some months before the natural spawning season (Section 3.1). The process of gonad conditioning is used routinely in the commercial culture of H.discus hannai and H.rufescens which are the two most extensively farmed haliotid species. The advantages offered by conditioning (Section 1) are such that the commercial culture of many abalone species may not be viable without this level of control over reproduction.

Appendix B summarises the available gonad conditioning data for seven abalone species in addition to H.laevigata. The major species cultured in Japan H.discus hannai is probably the most studied haliotid species. Work by Uki and Kikuchi (1984) showed that gonad maturity is proportional to the effective accumulative temperature (EAT). The EAT is calculated as the sum of the differences between daily temperature and an experimentally derived biological minimum temperature required for gonad growth. In the case of H.discus hannai, the biological minimum is 7.6 °C and spawning may be induced after an EAT of 1000 degree days, with maximum maturity seen once the EAT reaches 1500. Male specimens become mature before females. The quantification of gonad development has also been achieved for H.discus (Kikuchi and Uki, 1974). For this species the biological minimum is 5.3°C and full

maturity occurs when the EAT equals 3500. Spawning may be achieved once the EAT reaches approximately 2600.

It was beyond the scope of the present study to determine the EAT and biological minimum temperature for H.laevigata. However, comparison between H.laevigata and the two Japanese species is possible by substitution of mean water temperature for the present study (16°C) into EAT formulae for H.discus hannai and H.discus. This yields a conditioning time to achieve spawning of 119-179 days for H.discus hannai and 248-327 days for H.discus at 16°C. It should be noted that specimens of H.laevigata could not be regarded as fully mature when spawned after 112 days of conditioning. Rather, this may be close to the minimum time required for spawning, with a range of 112 to 150 days for females and yet a further five weeks required for maximum maturity of males.

Interestingly, a study of H.gigantea found no relationship between water temperature and rate of reproductive development (Kikuchi and Uki, 1975). Instead, the sexes could be gradually distinguished after 120 days of conditioning and spawning could be induced following 220 days of conditioning independent of temperature.

In various studies of North American abalone (Appendix B), the question of EAT and biological minimum temperature has not been examined. The North American red abalone H.rufescens can be conditioned to spawn in less than three months, with some individuals going through three reproductive cycles in a year (Ault, 1985). Similarly, the green abalone H.fulgens can be conditioned to spawn in only 90 days (Leighton et al.,1981).

Two further species H.corrugata Wood and H.cracherodii Leach may require only one or two months of gonad conditioning to reach spawning readiness (Morse,1984). In comparison with published data H.laevigata requires a conditioning period in the mid range of conditioning times for haliotid species. More than one spawning a year is almost certainly possible and further work could determine whether two or three reproductive cycles can be completed in a year.

In addition to the time taken for the various haliotid species to complete a captive reproductive cycle, the growth rate in conditioning tanks may be compared. Such data are useful since the growth rate of a species is very important in assessing its culture potential. Somatic growth in abalone continues to some extent in abalone during gonad development (Appendix B) and has been used as an indicator of conditioning tank suitability. The specific growth rate (Section 2.5) gives a measure of growth independent of animal size. Appendix B shows that broodstock animals used in the present study were in all cases larger than abalones used in other conditioning studies where growth has been recorded.

Specific weight increase for H.laevigata in this study was very similar to that for H.discus and H.gigantea and less than that for H.discus hannai. The difference in specific shell growth data between this and other studies is more marked. As seen in Appendix B the specific shell growth of H.laevigata was less than that for other species in conditioning studies at similar temperatures. The reason why shell growth is relatively less than weight gain in this study is unclear. Possibly, the larger size of animals used and the

tagging method may be factors. Respiratory pore tagging was found to inhibit growth of H.rubra less than 10 cm in length in field and in aquarium experiments (Harrison and Grant, 1971) and also in a field study by McShane et al. (1988). In these studies the growth referred to is shell growth; the effect on weight is unknown.

Animals were tagged as described previously (Section 2.5) so that individual growth could be measured. During the first 112 days in the conditioning tank 20 out of 128 animals lost their tags. Further tags were lost or damaged beyond use following this time until the completion of the experiment. The numbers drawn on the plastic tags with a water proof marking pen soon erased under the experimental conditions. This was not the case in pre-experimental tests and it is assumed that the activities of the animals, especially crawling over exposed tags, contributed to the failure of the ink. Alternative methods of tagging abalone include the use of fast setting epoxy glue (McShane et al.,1988) and bolting tags directly to the shell (Hahn,1989).

Studies on H.discus and H.gigantea have established that a relatively long conditioning time is necessary to spawn these species. This may be in part linked to the feed consumption of the abalones. Examination of Appendix B reveals that H.laevigata in the present study had a higher feed consumption than either of these Japanese species, though the latter were smaller animals and reared at higher temperatures in most trials.

4.2

Comparison of Conditioning Tank Design

It is important to design conditioning tanks to meet the requirements of individual abalone species. As described in Section 2.2.1, the conditioning tank at Abalone Hatcheries Pty Ltd simulated conditions of water movement typical of the natural habitat of H.laevigata. Shepherd (1973) noted that water movement elicits a characteristic feeding response in the species and that feeding is poor when the water is too calm or too rough. Shepherd also found that H.laevigata occupies positions on reefs where drifting seaweed is likely to settle or be carried past. In the conditioning tank the preferred station was high up on the sides of the mesh baskets often within 10 cm of the water surface. This position offered maximum feeding opportunities as seaweed placed in the tank would be forced to the surface by the aeration where it came into contact with the sides of the baskets. In field studies Shepherd noted that H.laevigata is a sedentary species. The majority of animals he studied remained on the same rock for months at a time. This was the case in the conditioning experiment where animals were observed only to move when the water level was lowered exposing part of the shell during tank cleaning.

The conditioning tank on Campus to which the abalone in this experiment were transferred after they became ripe (week 16) was not suitable for long term conditioning of the species. Efforts to aerate the water sufficiently to force seaweed to the surface resulted in the blockage of drainage pipes. The seaweed settled out on the tank bottom since

aeration had to be reduced to avoid such occurrences. As a result some abalone were found on the bottom, rather than the sides of the tank. This was doubly undesirable because animals preferring to occupy vertical surfaces had reduced feeding opportunities while animals on the tank bottom were exposed to their own faecal matter. Feed consumption appeared to be less in this conditioning tank than the original one. However, it was not possible to quantify this difference since seaweed in the second tank could become unavailable to abalone when tangled in mesh screening the outlets. A further factor that makes comparison between the tanks difficult is that feed consumption decreases after the gonad is fully developed in volume according to Uki and Kikuchi (1984). This was the case after week 18 of the experiment (Section 3.5) when the animals had been moved to the second tank. The circular water movement in the second conditioning tank was capable of moving seaweed along the tank bottom but feeding opportunities did not appear to be as frequent as in the original tank. Accordingly, abalone were hand fed every second or third day to ensure appetites were satiated. The extra maintenance required to ensure adequate living conditions in this system renders it impractical for commercial situations.

A conditioning tank at Furneaux Aquaculture Pty Ltd based on the same principles of water movement to elicit a feeding response and distribute feed was used to condition 9-10 cm specimens of H.laevigata in 1991. The animals were observed to feed in the adult fashion, trapping moving seaweed. Conditioning was begun in September before sex could be distinguished and by the use of elevated temperature gonad maturity was observed in November.

In this case artificial photoperiod was not used and abalone instead were reared in almost total darkness.

Further specimens of H.laevigata in the size range 5-8 cm also were also observed to come into spawning condition over summer 1991/92 at Furneaux Aquaculture. These abalone were in a growout tank of a quite different design to that used for conditioning larger animals. The small abalone were observed to be active feeders at night, moving about the tank base and walls in search of food. This is consistent with sub adult behaviour in the wild as reported by Shepherd (1973). Spontaneous spawning of approximately 500 of these abalones was observed in early February 1992. This tank was at ambient temperature (approximate seasonal range 11-17°C) and there was little illumination.

The behaviour of abalones in the experimental tank at Bicheno and in the other three systems, demonstrates the importance of conditioning tank design. Small abalone which actively forage for food appear suited to any culture vessel that allows seaweed to settle out on the bottom and provides a degree of water movement. As specimens of H.laevigata grow they become less active and begin to favour vertical areas of tanks. The change in behaviour begins to occur at approximately 8 cm necessitating a system which moves seaweed past sedentary animals. Ironically, because captive rearing may bring specimens of H.laevigata as small as 6 cm into spawning condition (Section 3.15) the need for specialized conditioning tanks as distinct from growout tanks may be avoided.

The typical conditioning tank used in Japanese abalone culture facilities (Hahn, 1989) and for the gonad conditioning experiments performed by Uki and Kikuchi which are summarised in Appendix B is as follows. Tanks have a volume of 1000 L (1.5 m by 0.9 m by 0.8 m) and consist of a frame lined with PVC coated canvas. Water enters the tank through a perforated pipe along one side of the bottom and air is supplied through tubing on the opposite side. This system creates water movement capable of removing smaller faecal matter and detritus via an overflow. Shelters are provide at the bottom of tanks which are used by abalone during the day when they are inactive. The animals emerge from shelter at night to feed. An artificial photoperiod to match that found during the natural spawning season is used. Alternative tank designs for the conditioning of H.discus hannai have been tested by Uki and Kikuchi (1981) as summarised in Appendix B. Three systems were used: a conventional tank as described previously; rearing of abalone in screened pipes suspended in tanks, and rearing of abalone in baskets with open mesh sides and a solid base suspended in tanks. There was considerable variation in feed consumption and growth of abalone as a result of the rearing conditions (Appendix B). Thus like H.laevigata there appears to be an important relationship between feeding behaviour and conditioning tank design for H.discus hannai.

A broodstock management system for H.rufescens has been described by Ebert and Houk (1984). Abalone to be conditioned are housed in 15 litre plastic containers with perforated lids. A central hole in the lid allows entry of the water inlet pipe which extends to the container bottom. Twenty micron filtered, ultra-violet irradiated water at ambient

temperature is supplied to each container. A flow rate of three to four litres a minute is used. Photoperiod does not appear important since abalone would be subjected to little light with the lids in place. Morse (1984) describes a conditioning tank suitable for various species of North American abalone. Tanks hold 1200 L of filtered water at a density of less than 0.25 animals per litre. A water replacement rate of 10 L/min is used and strong centrifugal flow is used to stimulate feeding and distribute food. Indirect natural illumination is used and abalone are fed M.pyrifera or other brown seaweeds.

4.3
Comparison of Feed Consumption and Feed Preference Data

An important aspect of all abalone broodstock conditioning systems is the provision of adequate quantities of seaweeds which are acceptable to the cultured species. Where species are selective feeders such as H.laevigata data on preferred algae species and consumption rates are important. This is particulary true where abalone are cultured outside their natural range, and the provision of suitable seaweed species is difficult.

There are some difficulties in comparing mean feed consumption data obtained in this study (6.0% body weight/day) with data from other conditioning experiments (Appendix B). This is because of differences in abalone size, macroalgal diets and rearing temperatures. Relative feed consumption in ectothermic animals tends to decrease with increasing age and

size and increase with temperature. Also, data provided by Hahn (1989) show daily feeding rates vary considerably depending on the seaweed species provided. None the less it is striking that H.laevigata in this experiment consumed more food daily than either H.gigantea or H.discus although the latter species were smaller and conditioned at higher temperatures in most trials. The importance of feed consumption as a factor regulating the reproduction of haliotids is discussed in Section 4.18.

The major purpose of the present study was to induce gonad maturity in the abalone. Seaweed preference trials were performed only where it was thought that the species of seaweed would not compromise this aim. The feeding response of abalone when presented with the various seaweeds in the present experiment indicated that R.coccinea and L.filiformis were the preferred species. Abalone were observed to detect the presence of R.coccinea in the water even when it was held up to 10 cm away from individual animals. The animals would characteristically react by lifting the anterior part of the body off the substrate and holding position. It appears possible that this seaweed species contains a strong chemical attractant. The abalone also responded in this way to L.filiformis and Ulva sp. Other species of seaweed generally needed to come into direct contact with abalone before the feeding response was observed. The animals were capable of selectively removing preferred algae such as R.coccinea and Ulva sp. from a mixture of seaweed species. This is consistent with the results of Hone (1989) who found that Ulva australis was highly palatable to H.laevigata. By contrast, in the present study another green algae, Codium sp. was generally

the last algae to be consumed in a mixture and constituted the majority species where excess food remained. As noted by Hone (1989) the palatability of different seaweeds within genera may vary. For instance, in this conditioning study Hypnea sp. was not greatly favoured by the abalone, whereas the study by Hone (1989) identified algae of this genus as highly palatable to H.laevigata. In addition, L.filiformis was well received by animals in the present study but less favoured by abalone in the study by Hone (1989).

In general, abalone prefer to feed on brown seaweeds such as kelps according to Hahn (1989). However, H.laevigata is not alone in its preference for red seaweeds. Shepherd (1973) states that four other species of Australian haliotids : H.roei, H.rubra, H.cyclobates Peron and H.scalaris Leach also show a preference for red algae. Two species from New Zealand : H.iris Martyn and H.australis Gmelin also showed a preference for red algae in laboratory trials. According to Poore, (1972) H.iris may feed almost exclusively on the brown alga M.pyrifera in the wild if red seaweeds are not abundant, but given a choice will consume species of the latter group. There is a great abundance of Rhodophyta present in the waters of Australasia (Christianson et al., 1981). Therefore, in an evolutionary sense the feed preferences of Australasian haliotids may be a response to the availability of this feed type. The red seaweed Gracilaria sp. routinely is fed to abalone in the Taiwanese culture of H.diversicolor. Interestingly, Chen (1984) notes that abalone prefer to consume Ulva sp. though the growth rate is superior when fed the former.

According to Shepherd (1973) H.laevigata eats the leaves of the seagrass Amphibolis sp. and shows a preference for this plant over many non red species of seaweeds. The red algae L.filiformis which was a major feed species in this conditioning experiment grew as an epiphyte on the seagrass Amphibolis antarctica Ascherson. However, there was no evidence that abalone consumed any part of the seagrass as they stripped the epiphytes.

4.4

Comparison and Evaluation of Gonad Bulk Index (GBI) Data

The gonad bulk index is the most commonly used of the gonad indices for the monitoring of abalone reproductive cycles. It has been used to study abalone species by the following authors: Young and DeMartini (1970), Hayashi (1980), Ault (1985), Wells and Keesing (1989). In these studies the authors sectioned the conical appendage at the mid point. Minor variations on the method have been used by Poore (1973) who cut sections one third of the way from the base to the tip. McShane et al. (1986), sectioned the base of the conical appendage, while Shepherd and Laws (1974) took a mean value from base and mid point sections to measure the index in H.rubra and H.laevigata. For a smaller species, H.cyclobates, examined in the same study the index was calculated only from the mid point section. In a further study by Takashima et al., (1978) samples were taken one cm back from the tip of the conical appendage for calculation of the index. The proportion of gonad in a cross section of conical appendage increases towards the tip, with the final portion being entirely

composed of gonad tissue. Sectioning the base of the conical appendage gives lower index values throughout the reproductive cycle (Hayashi, 1980). However this is unimportant since the variations in the index still indicate the important features of the cycle.

Ault (1985) found that the gonad bulk index was not capable of detecting change in reproductive state in a conditioning experiment involving H.rufescens. Other methods, including the modified gonad bulk index (MGBI) were able to detect the gonad maturation. In a field study of H.rufescens (Young and DeMartini, 1970) a lack of seasonal variation in the GBI was found. In this case the authors concluded that the species was a potential spawner throughout the year. Ripe eggs were found in specimens at all samplings, thus supporting the GBI data.

Appendix E gives the maximum and minimum GBI values obtained in the reproductive studies referred to. Only the abalone populations that were found to have a well defined spawning season are included. Where data for the sexes were presented separately they have been combined. When making comparisons the modifications in the method referred to previously should be considered. For instance, the method used by McShane et al, (1986) for calculating the index in H.rubra would tend to lower values compared to taking the cross section only at the mid point, whereas the method of Takashima et al. (1978) would tend to raise them. For comparison the GBI values obtained in the present study are included.

Reproductive cycles monitored by the use of the GBI typically reach a maximum value of at least 70% per cent. In the study of H.iris (Poore, 1973) the maximum value recorded in one of the survey years was considerably less than this and the abalone population was believed not to have spawned. A decline in the GBI from its peak is assumed to represent the spawning period of a species. The extent of the decline in the GBI is probably an indicator of the intensity of spawning in the population. Histological evidence shows that the abalone used in the present study had previously undergone a complete synchronous spawning (Section 4.12). At the time of collection the GBI was 14% which indicates the populations of H.laevigata studied by Shepherd and Laws (1974) also spawned completely and synchronously (minimum values 2-7%). This was confirmed in one of the three populations studied by these authors where histological examination in the form of mean oocyte size data also was performed (Section 4.9). The large reduction in the GBI value for H.cyclobates in the same study is indicative of complete spawning which was also confirmed by the histological analysis. Data presented by McShane et al., (1986) for a population of H.rubra at Portsea, Victoria, in 1980/81 similarly displays a large difference in minimum and maximum GBI values indicative of complete spawning. Again, this assumption is confirmed by examination of mean oocyte diameter data provided by the authors.

Lesser decreases in the GBI from its peak value imply only partial spawning. A population of H.tuberculata Linnaeus studied by Hayashi (1980) could be considered partial spawners as the GBI increased to its peak from a low of 40% and following spawning decreased again to approximately 50%.

Comparison of GBI and oocyte size/frequency distribution data showed large residual oocytes to be present after spawning. In fact traces of large oocytes (>200 μm) were present in all but one of 12 monthly samples.

The gonad bulk index of males and females in the present gonad conditioning study had reached 76.1% and 68.5% respectively by week 15 of the study. One week later a proportion of the animals were successfully induced to spawn (6/15 females, 1/6 males). The GBI values of males in the study did not increase significantly after week 15 (Table 3) which means the continuing maturity increase shown by the histological methods (Section 3.11) was not detected by this gonad index. The mean GBI values for the female abalone did show a further significant increase (Table 3) between weeks 15 and 18, coincidental with the final stages of ovarian maturity.

The GBI appears to be the least sensitive of the measures of abalone reproduction used in this study. The index did not detect first gonad growth until three weeks after the other methods. Neither was it able to detect further maturity in male abalone after week 15. In addition the Spearmann rank correlation coefficient calculated between GBI and mean oocyte diameter data was not significant (Section 4.14). However, it is still a useful method and in this study was able to detect gonad growth over the length of the conditioning period.

The GBI is the simplest to use of the gonad measures examined, requiring neither microscopic examination or histological preservation. The measurements required to

calculate the index can be made quickly and may be taken from a fresh frozen gonad. Comparison of field and conditioning studies for a variety of species (Appendix E) demonstrates that a minimum value of approximately 70% as measured by the GBI is required before spawning may occur. This may provide a useful guide when attempting to predict the time of spawning in abalone populations.

4.5 Comparison and Evaluation of Modified Gonad Bulk Index (MGBI) Data

The MGBI was originally developed by Tutschulte and Connell (1981) for a field study of the reproductive biology of H.fulgens, H.corrugata and H.sorenseni Bartsch commonly called the green, pink and white abalone respectively. The index has also been used by Ault (1985) for a gonad conditioning study on red abalone H.rufescens. The index gives an estimate of gonad volume (EGV) relative to abalone weight. The EGV is calculated by treating the conical appendage of abalone as two concentric right circular cones (Appendix A). In the present study the original formula used by Tutschulte and Connell (1981) to calculate the EGV has been modified to allow calculation from measurements of tissue area rather than linear dimensions (2.12). This is believed to be more accurate and allows calculation of the MGBI from the same data used to obtain the GBI.

The mean minimum and maximum values for the MGBI in the reproductive cycles studied by the authors referred to above are given in Appendix F. For comparison the values of the

index obtained in the present study are included. No typical upper limit of the MGBI can be used to identify individual ripe abalone of different species as it can for the GBI (>70%, section 4.4). Examination of Appendix F shows considerable variation in maximum and minimum MGBI values between species and populations of the same species. Individual specimens of H.laevigata used in the present conditioning experiment spawned one week after sample mean MGBI values of only 7.7 and 6.4 were recorded for males and females respectively (Section 3.5). The maximum MGBI value of 12.0 (pooled sex data) recorded for abalone in this experiment during week 18 is less than the minimum values obtained during some studies of other species (Appendix F). A higher mean value of 23.0 (s.d.=8.60, n=19) was recorded for field specimens sampled during late November 1990 (Section 3.14). This was during the natural spawning season but may not represent the maximum MGBI value reached since frequent sampling of the population was not possible at this time. The significance of the difference in maximum MGBI values between field and conditioned specimens is discussed in Section 4.15.

Tutschulte and Connell (1981) made the assumption that most of the gonad of abalone is found within the conical appendage. Gonad dissection of ripe H.laevigata revealed that less than a quarter of gonad mass was located in the conical appendage (Section 3.5). Thus the total MGBI has been underestimated for the species. This does not invalidate the method for monitoring of the reproductive cycle but prevents accurate comparison of the MGBI between H.laevigata and other species. This is because the actual percentage of gonad located in the conical appendage for other species is unknown.

The MGBI was found to be a sensitive indicator of gonad maturity in this study of H.laevigata. Gonad growth was detected after six weeks of conditioning, as it was by the histological measures (Table 3). The increase in maturity shown by histological techniques between weeks 15 and 18 was detected by the MGBI in males but not in females (Table 3). The significant decrease in MGBI of female specimens between weeks 21 and 24 possibly indicates reduction in volume of the necrotic ovary. Thus as shown by Ault (1985) the MGBI provides a more accurate assessment of reproductive state than does the GBI. The use of the MGBI is recommended and the formula presented in this study simplifies the measurement of the index.

4.6

Comparison of Gonad as Percentage Body Weight Data

Abalone reproductive cycles rarely have been studied by calculating the percentage the gonad contributes to the body weight of the animal. This is because of the difficulty involved in removing the gonad from the underlying tissue. However, the method has been used by a few authors and allows some comparisons to be made. Webber and Giese (1969) used the method to study the reproductive cycle of H.cracherodii. They found the gonad made up approximately 20% of the weight (shell removed) prior to spawning and fell to 5% following spawning. A similar study of H.discus hannai by Rho and Park (1975) found the maximum gonad size was 14.3% of total weight (shell intact) declining to a minimum figure of 5.0% following spawning. By comparison the maximum gonad size of conditioned abalone in the present study was only 5.7% of total weight or

8.7% with the shell removed (Section 3.6). No attempt was made to quantify the minimum percentage of animal weight contributed by the gonad. Judging by the appearance of abalone gonads when the experimental animals were collected the gonad weight percentage would have been negligible. Field specimens collected during the natural spawning season did not have gonads of significantly different relative weight to conditioned animals (Section 3.6). The available gonad as percentage body weight data suggests that H.laevigata devotes less reserves to reproduction than other species.

4.7

Comparison of Subjective Gonad Indices (Visual Assessment)

The advantage of subjective gonad indices is that the level of maturation can be judged without killing the animal. Such indices are used in commercial culture.

The index used in gonad conditioning experiments by the Japanese researchers Nagahisa Uki and Shogo Kikuchi (Appendix B) is a subjective method of assessment. The abalone are turned ventral side up and the gonad is viewed by holding the shell edge at eye level. The gonad stage is rated "0" when the sex can not be distinguished, "1" when the gonad is concave in the shell, "2" when the gonad is level with the shell edge, and "3" when the gonad bulges above the shell edge (Hahn, 1989). Individuals of H.laevigata may be considered potential spawners when the sex can be clearly distinguished and the visible gonad is well rounded all the way to the tip of the conical appendage. However, no specimen of H.laevigata

examined, including the conditioned specimens, and large numbers of wild ripe abalone studied had sufficient gonad bulk that the animal could be assigned to stage 2 or 3 of the system used by the Japanese researchers. The relative lack of gonad bulk exhibited by the species makes selection of ripe broodstock for induced spawning difficult. The other large, local abalone species of commercial value (H.rubra) may develop mature gonads of a larger size. Anecdotal evidence from local divers suggests that the gonad of H.rubra can develop such bulk that it protrudes from the shell. A further species of abalone H.emmae Reeve also found in Tasmania has been observed by this author to develop gonad bulk comparable to a rating "3" in the assessment system described previously.

Ebert and Houk (1984) described a further subjective gonad index system for determination of maturity in H.rufescens. The system has four phases as follows: the gonad is rated "0" when the sex cannot be determined, "1" when the gonad colour has developed sufficiently to easily distinguish males, "2" when gonad is visible over the entire conical appendage and both sexes are easily distinguished, and "3" when the gonad has developed bulk which extends to the gonad tip. This system also could be used for H.laevigata and it appears these two species are more similar to each other in gonad development than to H.discus hannai.

Individual abalone in the present study developed sufficiently after nine weeks of conditioning for sex differentiation. It was not until week twelve however that the sex of all individuals in a sample could be distinguished with certainty. In species where it is some time before gonad

maturity can be detected simply by visual assessment, there may be doubt that the conditioning process is effective. This is likely to be the case in commercial culture of H.laevigata where sacrificial gonad indices are not employed. To monitor reproductive maturity in this situation awareness of the number of elapsed degree days will be important. In addition feed consumption should be measured on occasion as an indication of the suitability of the diet and rearing conditions.

In the present study, it was observed that as abalone matured their ability to attach to the substrate declined. Ripe abalone lose ability to cling to a vertical surface out of water quickly in comparison to immature animals. Abalone feebly attached to rocks when in spawning condition has been observed by Breen and Adkins (1980). This feature of abalone biology constitutes a further useful tool for selecting ripe broodstock.

4.8 Comparison and Evaluation of Oocyte Size/Frequency Distribution Data

Grant and Tyler (1983a,1983b) recommend the measurement of oocyte size for examining the reproductive cycles of molluscs. The method of examining standardized residuals in contingency tables constructed from oocyte size/frequency distribution data permits detailed study of oocyte growth and of differences in development within and between samples. The technique has been used to study reproduction of abalone by

Webber and Giese (1969), Lee (1974), Pearse (1978), Hayashi (1980) and McShane et al. (1986). Data from these studies are summarised in Appendix G.

In the present study the pattern of oocyte development is similar to that recorded in other studies. The numerically dominant size class for most of the reproductive cycle is the 20 to 40 µm size class. This can be seen by examination of Figure 10 (Section 3.8) which shows the dominance of this small size class until week 15 of the experiment. For most species examined these small oocytes make up 50 to 80% of all such cells early in the reproductive cycle. This proportion declines to a typical minimum value of approximately 20-30% when the ovary is fully mature (Appendix G). Generally abalone can be considered in spawning condition when the proportion of oocytes of the largest size class reaches 15 to 30%. At maturity these large oocytes dominate the ovary in terms of volume occupied. The largest size class present varies between species, with small species such as H.pustulata Reeve (Pearse, 1978) and H.coccinea canariensis Nordsieck,(Pena, 1975) having fully developed eggs of 150 and 103 µm in diameter, respectively.

When abalone with a well defined breeding season are in spawning condition the distribution of oocyte sizes is characteristically bimodal in all previous studies examined. The spawning period is thus easily determined from graphical displays as the distribution of oocytes changes to that of a unimodal distribution centred around oocytes of the smaller size classes. The sensitivity of the oocyte size/frequency distribution analysis also allows other important aspects of

the reproductive cycle such as gametogenesis and vitellogenesis to be identified. For these reasons the use of the method is recommended if a quantitative histological measure is to be employed. The availability of automated computer linked systems for the measurement of oocyte diameter or area in cross section would simplify the use of the method.

4.9
Comparison and Evaluation of Mean Oocyte Diameter Data

The mean oocyte diameter method like the O.S/F.D technique is very sensitive to changes in reproductive state. The chief advantage of the method is the simple interpretation of graphical data. In the present study the mean diameter increased from its initial minimum (30.1 µm) to 109.8 µm by week 15 (Figure 12, Section 3.9). The mean and small standard deviation of the first sample indicates, that at the time of collection a previous complete synchronous spawning had occurred.

Harrison and Grant (1971) studied the reproductive cycle of H.rubra in Tasmania for two years, using the mean oocyte diameter method in the first year. The minimum value of 145 µm and the maximum value of 235 µm were recorded in March and May 1969, respectively. The authors considered that spawning took place from June to October and possibly in February. The high value (compared to the present study) for the minimum mean oocyte diameter indicates that a sizable proportion of large oocytes were maintained in the ovary all year round.

The nature of the spawning event in the study by Harrison and Grant (1971) is difficult to interpret from the mean oocyte data alone. It is unclear whether the population of abalone spawned relatively synchronously with individual animals releasing only a portion of their eggs, or whether animals may spawn at any time with something of a peak occurring during the spawning season identified by the authors. The data of Harrison and Grant illustrate a difficulty with the mean oocyte diameter method as compared to the oocyte size/frequency distribution analysis. The minimum value obtained in the study when considered in isolation could well be assumed to indicate a growing or even ripe gonad. A given mean value may represent either oocyte populations of similar size or a bimodal population of oocytes considerably smaller or larger than the mean. Obviously, the measure of variation around each mean will give a clue to the real nature of the data and hence the reproductive state of the sampled population. However, the detail provided by the oocyte size/ frequency distribution method avoids such problems.

A field study of reproduction in several species of Australian abalone by Shepherd and Laws (1974) included data on mean oocyte diameter for H.laevigata and H.cyclobates. The seasonal mean size range was 25 to 205 µm for the former and 40 to 200 µm for the latter species. The authors considered the two species to have a well defined summer spawning season. The data also indicate both species were complete spawners in the year surveyed.This complete spawning makes interpretation of mean oocyte diameter data simpler than that for the partially spawning H.rubra population discussed previously.

There is a difference in the maximum mean oocyte size values between this study and that of Shepherd and Laws (1974) for H.laevigata. It should be noted that in the present study the time of maximum ovarian ripeness (between weeks 15 and 18) was not sampled. Subsequent to this time oocytes became necrotic and sampling by mean oocyte diameter analysis was discontinued. Also, the method used by Shepherd and Laws involved measuring oocytes teased from the ovary and placed in seawater on a slide. The authors note that this approach may underestimate the frequency of small oocytes which are attached to the ovarian walls and trabeculae.

4.10

Comparison and Evaluation of Ovarian Maturity Phase Data

Ovarian maturity phases are a useful relatively quick method of determining the state of female reproductive development. Because they are a histological method they provide a more accurate assessment of development than gonad indices but are more convenient to use than the tedious oocyte measuring techniques. For rapid use the major phase present in a sample need only be scored rather than quantifying each phase present as was done in this study.

Five phases or stages were identified for use in this study: recovery, growing, mature, spawning and necrotic. The definitions of these phases are given in section 3.10.
Maturity phases used by other authors include : recovery,pre mature, mature, spawning and spent (Tomita, 1967); recovery, multiplication, growing, mature and spent (Lee, 1974) and pre-

proliferative, proliferative, new stalk, old stalk and free (Ault, 1985). In addition Wells and Keesing (1989) used a staging system which included two spawning and recovery phases and a ripe phase.

In broad terms, the recovery phase in this study corresponds to the recovery and multiplication phases of Lee (1974); the pre- proliferative and proliferative stages of Ault (1985) and the first recovery stage of Wells and Keesing (1989). The recovery stage of Tomita (1967) appears more advanced corresponding to the growing phase used in this study.

The growing phase defined here is similar to the recovery and premature stages of Tomita (1967); the growing stage of Lee (1974); the new and old stalk stages of Ault (1985) and the second recovery stage described by Wells and Keesing (1989).

The phase described as mature is most similar to the mature stages of Tomita (1967) and Lee (1974). The free stage defined by Ault (1985) includes both the mature and spawning phases used here as does the ripe stage used by Wells and Keesing (1989). Only Tomita (1967) used a separate developmental stage to define eggs that appear to be ready to spawn as was done here. Since fully mature abalone did not spawn in this study a necrotic phase was described instead of the spent stages used by Tomita (1967), Lee (1974) and Wells and Keesing (1989).

As can be seen from Figure 14 (section 3.10) the recovery phase dominated the ovary until at least week nine of the conditioning process. Development was then accelerated with the growing, mature, and necrotic phases dominating in weeks 12, 15, and 18, respectively. This is similar to the pattern observed in a 13 month field study of H.discus hannai by Tomita (1967). This author found that the following stages could be observed within the ovaries of samples for the stated times (with overlaps) : recovery stage for seven months, premature stage for three months, mature stage for two and a half months, spawning stage for less than a month and spent stage for two months. The stages used in the field study by Tomita do not correspond exactly to those used in this study. However, in both cases it is obvious that ovarian development progresses slowly for a considerable proportion of the reproductive cycle with rapid development to spawning condition occurring late in the cycle. The total length of time for ovarian development was compressed by the conditioning process in this experiment but the characteristic long build up is still evident. Thus ovarian phase analysis showed the same pattern of abalone reproductive development as all other maturity measures employed in the study.

Ovarian phase analysis performed without quantifying each phase present, but instead scoring only the dominant phase in each sample, would undoubtedly have advantages in simplicity and rapidity of use compared to histological methods used in this study. The extra effort required to quantify all phases present in samples as done in this study is probably not justified in terms of extra information obtained.

However, where histological analysis of ovaries is to be performed and labour saving data analyzing devices are available measures such as the oocyte size/ frequency distribution are to be preferred.

4.11

Comparison and Evaluation of Testis Maturation Data

Attempts to quantify the percentage of mature sperm cells present in the testis have previously been made by Webber and Giese (1969) and Takashima et al.(1978). In addition, Tomita (1968) classified the maturation of the testis into five stages based on histology.

Webber and Giese (1969) examined the reproductive cycle of the North American black abalone H.cracherodii. They measured the percentage of spermatozoa in cross section and found the mean minimum value to be 65%. Two peaks were reported, one in summer prior to spawning with a sample range of 90 to 97 percent and further similar peaks in the winter months. The gonad index (expressed as the gonad percentage of the soft body weight) and oocyte size/frequency distribution data indicated a synchronized spawning in late summer. The combination of these data lead to the conclusion that males in the study, unlike females retained a high proportion of mature gametes following spawning. Partial spawning has been reported by Giorgi and DeMartini (1977) who found that male red abalone H.rufescens within the same population could be considered complete, incomplete or non spawners.

A field study of H.diversicolor by Takashima et al.(1978) found the spawning season to be in summer. Six months before spawning the proportion of spermatids and sperm in the testes was approximately 9%. This rapidly increased to 75% a month later and to nearly 100% just prior to spawning. Following spawning the percentage of the two most mature germ cell types fell to nearly zero, contrasting with the study by Webber and Giese (1969). The complete spawning shown by males in the study by Takashima et al. (1978) also appears to have occurred in the population of H.laevigata used in this conditioning experiment prior to collection.

It is interesting to note the high percentage of mature germ cell types before spawning in the two previously cited studies. In the present study only one of six selected males could be induced to spawn during week 16. Subsequent analysis showed the mean percentage spermatozoa to be 32 and 51% in weeks 15 and 18 respectively. Further attempts to induce spawning of individuals by the ultra-violet irradiation method were unsuccessful in weeks 18 and 19. Subsequent analysis found egg necrosis of females. Males however were still not fully ripe and did not reach their peak value of 91% until week 24. No attempt was made to induce spawning of remaining males in week 24 as unfortunately it was not known until afterwards that the percentage of spermatozoa had continued to increase. Spontaneous spawning was not noted in the conditioning tank which may not be surprising given the lack of temperature variation. Possibly male abalone must develop a very high percentage of mature sperm in the testis before spawning may take place.

Tomita (1968) defined five stages in the maturation of the testis : recovery, premature, mature, spawning and spent. Judging from the published photographs mature spermatozoa are found only in the mature and spawning stages. These stages occupied one and two and a half months respectively (with some overlaps) of the 14 month study. The longest time that a given stage was seen in the testes was nine months for the recovery stage. This is in contrast to the study on H.cracheroidii (Webber and Giese, 1969) where spermatozoa were seen all year round. In the present conditioning study of H.laeviqata the majority of individuals could be considered in the recovery or premature stages used by Tomita (1968) until at least week nine of the study.

The present study illustrates the importance of histological techniques when studying reproductive cycles. A two way ANOVA applied on arcsine transformed gonad bulk index data showed no difference in the pattern of development between the sexes (Section 3.4). However, as noted previously histological analysis found that females became fully ripe well before males. All female abalone sampled late in week 18 showed widespread egg necrosis (Section 3.12), while male specimens were at their most mature at the end of the study five weeks later. Given similar treatment of the sexes in this experiment the difference in time taken to reach maturity appears surprising. Histological analysis (section 3.13) revealed the presence of a small number of degenerating sperm cells in the initial (Week 0) sample. These were not apparent by the third week of conditioning. Female abalone showed no evidence of residual oocytes from a previous spawning. Thus the reproductive cycles of the sexes were not exactly

synchronized and the males lagged slightly behind. Whether this initial difference in reproductive state can fully explain the large time differential to reach maturity between the sexes is unclear.

4.12

Comparison of Ovarian Histology

Oogonia were seen in all samples in this study just as they were reported in all samples of year long field studies by Young and DeMartini (1970) working on H.rufescens and Takashima et al.(1978) working on H.discus hannai. The necrotic oocytes seen in most individual samples from weeks 18, 21, and 24 have been previously recorded by Young and DeMartini (1970), Giorgi and DeMartini (1977) (also in H.rufescens) and Takashima et al. (1978). In this study however necrotic oocytes were not seen early in the development cycle (to week nine) in any specimens and were recorded in few specimens after this until the week 18 sample. This contrasts with the field studies by Young and DeMartini (1970) and Giorgi and DeMartini (1977) who found necrosis in all samples taken during year long field studies. In the latter study it was concluded that populations of abalone spawned in spring and early summer and that necrotic oocytes were the result of autolysing residual gametes. The authors of this study recognized three types of spawning pattern : complete, incomplete and non spawning. They suggest that the number of necrotic oocytes varies with the previous spawning pattern so that in non-spawning abalone virtually all large oocytes are necrotic after the spawning season. In incomplete

or partially spawning abalone the number of necrotic oocytes varies while in fully spawned abalone the ovaries contain no such oocytes in the post spawning recovery phase.

In the present study the lack of residual large oocytes (necrotic or not) at the time of collection would appear to be explained by a previous complete spawning. This differs from the study by Giorgi and DeMartini (1977) where all three previously noted spawning patterns were found in the same population of abalone. Histological evidence showed that the conditioned abalone were becoming ripe by the fifteenth week of the experiment. Approximately a third of female abalone were induced to spawn one week later. Development then continued quickly with all females in the next sample showing necrosis of large oocytes. There was no evidence of spontaneous spawning in the conditioning tank. Therefore, it would appear that having previously been a population of complete spawners the animals were now demonstrating the non-spawning pattern. This possibly was caused by the high degree of temperature stability in the conditioning tank at the University. Temperature variation may be used to trigger spawning for abalone culture (Hahn,1989; Chen,1990) and may have the same effect naturally in the field. Giorgi and DeMartini (1977) refer to Newman (1967) who noted that low intensity spawning of a South African abalone H.midae Linnaeus occurs in areas of low water temperature fluctuation. Non-spawning of abalone has also been recorded by Poore (1973) who found spawning of field populations of H.iris and H.australis in 1967-68 but not in 1968-69.

In the present study gametogenesis had already commenced when the experimental animals were collected and had noticeably accelerated after six weeks of conditioning. Lee (1974) found gametogenesis initiates within approximately two months of spawning for H.gigantea, H.discus, H.discus hannai and H.sieboldii Reeve. Webber and Giese (1969) working on the reproductive cycle of H.cracheroidii found gametogenesis to be initiated immediately following spawning with no resting phase. There was little new gametogenic activity in the ovaries of H.laevigata in this study which had become necrotic. This was also noted in H.rufescens by Giorgi and DeMartini (1977) in animals classified as non-spawners. By contrast, full and partial spawners of the species showed gametogenesis in summer following spawning. These authors believed that the residual oocytes may have inhibited gametogenesis in non spawners. It seems likely that breakdown and recycling of unspent eggs must occur to provide reserves and/or space for gametogenesis to initiate. There appeared to be little progression in the break down and reabsorption of necrotic cells between weeks 18 and 24 of this study. Further research could determine the time period required for breakdown of residual oocytes and initiation of gametogenesis.

4.13
Comparison of Testicular Histology

The histology of the abalone testis has previously been described by Tomita (1968), Young and DeMartini (1970), Lee (1974), Giorgi and DeMartini (1977), and Takashima et al. (1978). When the animals used in this experiment were obtained

they were in the recovery phase described by Tomita (1968). Some spermatozoa residual from a previous spawning were evident and breakdown of these cells appeared well advanced. Such cells do not appear to have been recorded previously in the literature. Cells residual from the previous spawning were not seen in females at this time thus it appears the male reproductive cycle was lagging slightly behind the female. The testis was largely devoid of reproductive cells and the patches of immature cells present were clustered near the trabecula. The proportion of germ cells present as spermatozoa increased steadily after week 12 of the experiment to the maximum value recorded in week 24. At this time the only immature stages were seen near the trabeculae, as also recorded in ripe male abalone by Giorgi and DeMartini (1977). Spawning of male abalone did not occur spontaneously in the conditioning tank and no evidence of spawning was seen in samples taken after 24 weeks of conditioning.

4.14

Comparison and Evaluation Of Gonad Measures

The variety of measures used in this study to monitor changes in reproductive state ensured that when such changes occurred they would be detected. Also, by the use of such a number of measures the utility of the different methods could be examined.

Table 3 shows the temporal changes in each measure of reproductive maturity. Comparison of sample means is by Fisher PLSD Test unless otherwise stated. The sensitivity of the

gonad assessment methods can be compared by examining when the first significant increases in gonad maturity occur. Thus by week six the majority of the methods used had recorded a significant increase in gonad maturity indicating the conditioning process was effective. The gonad bulk index was less sensitive to the increasing gonad maturity, not recording a value significantly different to the week 0 result until week 9. Also, the histological method used to measure reproductive change in males did not record a statistically significant change until the ninth week of conditioning. However a small percentage of mature sperm cells were first recorded three weeks previously, signifying the process of gonad maturity was beginning. The histological measures all show that female specimens became fully mature before males. The difference in developmental pattern between the sexes was detected by the MGBI but not by the GBI (refer to 2 way ANOVA data, sections 3.4 and 3.5). Neither gonad index detected the increasing maturity of male specimens after week 18. This is because the increasing proportion of mature sperm cells has little effect on the size of the gonad since spermatozoa and spermatocytes are similar in size. This of course contrasts with the situation in developing ovaries where oocytes increase considerably in size as they mature.

All measures of gonad development demonstrate that the process of gonad maturity commenced slowly, accelerating between weeks 12 and 15 prior to induced spawning of a proportion of the population. This is consistent with Palmer (1907), as quoted by Poore (1973) who wrote, "the reproductive system of Haliotis is interesting ... because of the lateness of its development and the rapidity of the same once begun".

This developmental pattern makes it difficult to determine whether conditioning is actually occurring early in the process, especially when visual assessment is the only method used. Spearmann rank correlation coefficients calculated between sample means for all possible combinations of gonad maturity measure data are shown in Appendix H. The oocyte size/frequency distribution and ovarian phase methods are not included since these measures do not yield single mean sample values permitting correlation. All correlations between the remaining measures of maturity were significant at the five per cent probability level, except that between GBI (female samples) and mean oocyte diameter. There was a significant correlation between the sample means for GBI and MGBI data (Appendix H). The GBI though not perhaps as sensitive as the other techniques was capable of detecting gonad growth. This contrasts with the finding of Ault (1985) who attempted to use the index in a conditioning study of H.rufescens.

4.15
Discussion of Fecundity Data

Fecundity estimates performed on seven conditioned and five field specimens from the present study are given in Appendices C and D along with available fecundity data from studies of other haliotid species. Also included is fecundity data obtained from small cultured broodstock held at Furneaux Aquaculture Pty Ltd.

The appendices show that the fecundity of H.laevigata is somewhat similar to that of H.rufescens over a range of size

classes for both laboratory and field conditioned abalone. The maximum recorded MGBI values for these two species are relatively low and more similar to each other than to the MGBI data for other species (Appendix F). Through the estimate of gonad volume (EGV) in the numerator the MGBI provides a measure of gonad size and thus fecundity. However, it must be noted that comparison of MGBI data between different haliotid species requires knowledge of the proportion of the gonad found in the conical appendage (section 4.5). With the exception of H.rufescens it appears that adult H.laevigata are less fecund than other species of comparable size e.g., H.iris and H.midae (Appendix D). In addition, Appendices C and D demonstrate that smaller species such as H.australis, H.tuberculata, H.roei and H.discus are more fecund for their size than is H.laevigata.

The link between relatively low fecundity and relatively small gonad size as indicated by MGBI data for H.laevigata and H.rufescens is also suggested by descriptions of subjective gonad indices (section 4.7). Both species have relatively small gonads compared to certain Japanese species in which the ripe gonad may project from the shell. Comparison of percentage gonad by weight data (section 3.6) also support the view that specimens of H.laevigata have relatively small reproductive organs. If H.laevigata does in fact put less metabolic effort into reproduction than most other abalone species a relatively fast growth rate for adults may be expected. High growth rates for juveniles have been reported by Shepherd and Hearns (1983), Hone (1989) and would appear to make the species a prime candidate for culture. Of three abalone species maintained at Furneaux Aquaculture Pty Ltd the growth rate of H.laevigata has proven superior to that of H.rubra and H.emmae produced at the same time.

This provides more support for an inverse relationship between growth rate and reproductive effort. When all species came into spawning condition at the same time simple visual assessment showed H.laevigata to have a smaller gonad than the other species. The ripe gonad of the slowest growing species, H.emmae protruded from the shell, a condition not seen in specimens of other species at the time.

As seen in Section 3.15 conditioned specimens of H.laevigata in the size range 60 to 90 mm may be induced to spawn. Data presented by Shepherd and Laws (1974) shows the species first becomes sexually mature at between 75 and 120 mm depending on study site. Further, the authors suggest that spawning does not occur at first maturity but requires at least a further years growth. By way of example Shepherd (1990) notes that where H.laevigata attains maturity at 100 mm full spawning potential is not seen until animals are 120 mm in length. These observations are in agreement with the finding of Ault (1985) that conditioned specimens of H.rufescens may be spawned at a smaller size than wild abalone. Laboratory conditioned animals also were found to have higher fecundity than field specimens of the same size. Conditioning then, has obvious advantages to the culturist since larger egg numbers can be produced per individual and smaller abalone can be maintained as broodstock, requiring less feed, space and water exchange.

For abalone culture purposes animals with apparently large gonads are chosen as broodstock for induced spawning techniques. In this study an attempt was made to quantify the relationship between fecundity and gonad size as measured by the two gonad indices. For the conditioned animals gonad volume (EGV) correlated

significantly with fecundity but for field specimens this was not the case (Section 3.16). Interestingly, the gonad volume and MGBI values for the field specimens in this study are considerably higher than those for the conditioned animals (Section 3.16, Table 5). Ault (1985) notes that oocyte proliferation by the germinal epithelium is a surface phenomenon, the amount of proliferation is proportional to the available surface area. The author further states that folding allows for increased surface area of germinal epithelium and may account for significant increases in fecundity. It is suggested that as folding increases egg production eventually becomes a function of gonad volume. Thus extra folding of the germinal epithelium in laboratory conditioned abalone may explain the greater fecundity of such specimens and also why fecundity correlates with EGV for conditioned but not for field animals in the present study. In addition the phenomenon explains the apparent anomaly that conditioned abalone with gonads of smaller volume than those of field specimens were similarly fecund. Extra folding of the germinal epithelium, and therefore greater oocyte density may be the reason why there was no significant difference in percentage weight of gonads between field and conditioned specimens (Section 3.6), though field specimens had greater gonad volume.

There was no apparent relationship between size and fecundity of field specimens sampled in the present study. Poore (1973) found no relationship between shell length and fecundity for H.australis, while Giorgi and DeMartini (1977) reported considerable fecundity variation in specimens of H.rufescens of the same length. Hahn (1989) also notes that there is considerable variation in fecundity of larger animals. There is evidence for a relationship between size and fecundity of conditioned abalone in

the present study (Section 3.15). However, more work is required to determine the nature of the size/fecundity relationship.

The relationship between fecundity, length and weight has been examined by a number of authors. Egg production has been found to increase in a non-linear fashion with shell length by Poore (1973), Pena (1975), Hayashi (1980), McShane et al. (1986) and Wells and Keesing (1989). The relationship between weight and fecundity has been reported as linear by Newman (1967) as cited by Poore (1973), Poore (1973), Pena (1975), and McShane et al. (1986). Ault (1985) provides data that shows fecundity increases in a non-linear fashion with both weight and shell length for field and laboratory specimens.

The majority of the fecundity estimates displayed in Appendices C and D were derived by counting the number of mature eggs in a small gonad sample of known weight or volume. Both methods were used in the study and correlated closely ($P<0.05$, $Rho=0.964$, $n=7$). Because of the difficulty involved in measuring the volume of a small sample of gonad tissue as compared to weighing it, the latter method is preferred. An alternative fecundity estimation method is to induce spawning of animals and estimate the number of eggs by taking subsamples of the total. This technique was used by Kikuchi and Uki (1974,75) and Ault (1985) in the studies presented in Appendices C and D. The method is useful because it gives the number of eggs available for use by the culturist and does not involve the sacrifice of broodstock. However underestimation of total fecundity will occur if not all eggs present in the ovary are spawned.

4.16

Abalone Spawning Seasons

The Franklin Sound population of H.laevigata appeared to spawn between November 1990 and March 1991 (Section 3.14). Thus like populations of the species surveyed in South Australia (Shepherd and Laws, 1974) and Victoria (McShane, 1988) the abalone may be considered summer spawners.

Boolootian et al. (1962) summarising the literature available at the time concluded that 85% of molluscs are summer breeders, irrespective of their geographic distribution. Furthermore, seven out of eight abalone species surveyed were considered by the authors to be summer breeders. Further summaries of abalone spawning seasons have been presented by Poore (1973), Shepherd and Laws (1974) and Hahn (1989) since this early study. Appendices I and J have been compiled from these and other sources and present the spawning seasons for 23 species of abalone. For the majority of species included at least two studies are cited. This large body of information allows the spawning seasons of haliotids to be categorized with more certainty than previously.

The majority of species have fairly well defined spawning seasons generally limited to a maximum of four or five months. However, four species (H.roei, H.scalaris, H.kamtschatkana, and H.rufescens) have been shown to be capable of spawning during most months of the year. Of those haliotids with defined spawning seasons eight species can be considered summer spawners and these constitute the largest single group. These species are H.laevigata, H.iris, H.cyclobates, H.lamellosa Lamarck, H.fulgens, H.tuberculata, H.cracherodii, and H.discus hannai.

The spawning season of these last two species extends well into autumn. It should be noted that the proportion of abalone (8/23) showing a reproductive cycle which terminates with a summer spawning is well less than that given by Boolootian et al. (1962). Several species have spawning seasons which show considerable variation at different geographic locations. These species are H.rubra, H.midae, H.pustulata, H.corrugata, H.roei and H.rufescens. The variation in the latter two species extends to year round reproduction at some sites. The reproductive cycles of a further four species constitute a group which spawn in autumn and winter. These haliotids are H.diversicolor, H.discus, H.gigantea, and H.sieboldii. A further three species, namely H.australis, H.sorenseni and Haliotis walallensis have reproductive cycles that can not be easily categorized into any of the four groups described.

4.17

Comparison of Sex Ratio Data

Shepherd and Laws (1974) found that when total populations of H.laevigata were considered male abalone significantly outnumbered female abalone. Homogeneity of length classes was not found however and males were more numerous in the smaller size ranges and females more numerous in the larger. The population of abalone selected for the present study excluded very large animals that were available in the original catch (Section 2.1). This may have contributed to the observed sex ratio imbalance (Section 3.17). The findings have important implications for stock recruitment in the abalone fishery. Given that there are fewer females in populations of H.laevigata, but larger animals, which will be

taken first by divers tend to be female, then it would appear that the reproductive potential of abalone populations is very vulnerable to fishing pressure.

The question of sex balance in haliotids has been examined by a number of authors. In addition to H.laeviqata male abalone were found to be significantly more common in the following species : H.pustulata (Pearse,1978), H.roei (Wells and Keesing,1989), H.lamellosa (Bolognari,1954, as cited by Poore,1973) and H.rubra (Shepherd and Laws,1974). In the latter study however the authors noted that apparent behavioural differences between the sexes may lave led to biased sampling. Also, examination of data provided by Quayle (1971) for H.kamtschatkana showed the probability of the observed result (53% males out of a sample of 2914 animals) to be 0.0013 given equal sex ratios.

Equal sex ratios have been described in the following species: H.midae (Newman,1967 as cited by Poore,1973), H.cracherodii (Webber and Giese,1969), H.rufescens (Young and DeMartini,1970 and Giorgi and DeMartini,1977), H.iris, H.australis (Poore,1973), H.scalaris, H.roei, H.cyclobates (Shepherd and Laws,1974), H.fulgens, H.corruqata, and H.sorenseni (Tutschulte and Connell,1981). Giorgi and DeMartini (1977) also reported that in one population of H.rufescens studied female animals out numbered males.

4.18

The Importance of Exogenous Factors in Relation to Gonad Conditioning of Abalone.

The relationship between gonad maturity and cumulative temperature has been quantified for the abalone species H.discus Reeve (Kikuchi and Uki, 1974) and H.discus hannai (Uki and Kikuchi, 1984). Experiments conducted at a variety of different temperatures (Appendix B) demonstrate that temperature influences the rate of gonad development. This is to be expected since temperature drives the metabolic rate in ectothermic animals. Interestingly no apparent relationship between rate of gonad development and temperature was found for H.gigantea (Kikuchi and Uki, 1975). Instead the abalone gradually came to spawning condition after 220 days independent of temperature. Possible reasons for this are discussed later.

Maintaining controlled temperature regimes has not been considered of major importance for conditioning American abalones. Ebert and Houk (1984) and Ault (1985) conditioned H.rufescens to maturity throughout the year at ambient temperatures of 9-15 and 10-21°C respectively. Morse (1984) states that temperatures of between 18 and 25°C can be used to condition the thermophilic species H.fulgens, H.corrugata, and H.cracherodii. It is likely that in all these species there is a biological minimum below which reproduction is not initiated. However this temperature may not be encountered in ambient temperature conditioning tanks.

Year round production of H.rufescens (Ebert and Houk, 1984, 1989), H.corrugata (Morse, 1984, Ebert and Houk, 1988), H.fulgens and H.cracherodii (Morse, 1984) has been achieved using natural or ambient photoperiod. This was also probably the case for the species (H.rufescens) conditioned by Ault (1985) since photoperiod was not mentioned in the report.

Gonad conditioning of H.discus hannai (Uki and Kikuchi, 1984), H.discus Reeve (Kikuchi and Uki, 1974), H.gigantea Kikuchi and Uki, 1985) and H.fulgens (Leighton et al., 1981) has been achieved using a 12 hour light, 12 hour dark photoperiod. Hahn (1989) states that the photoperiod used to condition H.discus hannai (12 Light : 12 Dark) at the Oyster Research Institute in Japan is chosen to match the photoperiod during the natural spawning season. This choice of artificial photoperiod appears anomalous since the species is considered a summer spawner, though the season generally continues well into autumn (Appendix I). In the same way the choice of the same 12L:12D photoperiod for the conditioning of H.discus and H.gigantea appears odd given that these species may be considered winter spawners (Appendix I). Beinssen (1982) in a report written after a tour of Japanese abalone culture facilities noted that some species in Southern Japan were said to respond better to conditioning with use of 8L:16D photoperiod conditions. Such a daylight regime would be more similar to that experienced during the natural spawning season of H.discus and H.gigantea both of which are southern species. These two haliotids take a relatively long time to be conditioned to spawning potential (Appendix B). The authors of the original papers cited previously suggested that other factors, including photoperiod, may need to be explored to reduce the conditioning time. As noted previously H.fulgens has also been

conditioned to spawn out of season using 12L:12D artificial photoperiod. This is interesting because the natural spawning period is summer (Appendix I). Therefore the species can reproduce out of season when reared under artificial photoperiod conditions that do not mimic the natural spawning season. In addition, as referred to previously, Morse (1984) states H.fulgens can be conditioned using natural photoperiod. Further, as described in section 4.2 specimens of H.laevigata were conditioned to a state of reproductive maturity in November 1991 at Furneaux Aquaculture Pty Ltd. These animals were reared in darkness except for periods of indirect artificial illumination which did not simulate the photoperiod in the natural spawning season.

The fact that so many species of abalone can be conditioned out of season, using ambient photoperiod or artificial regimes that do not properly simulate conditions of photoperiod in the natural spawning season, casts serious doubts on the importance of this exogenous factor in controlling reproduction. In any case it is difficult to see how simulating natural spawning season day length could initiate reproduction. This is because in nature gametogenesis is initiated months before spawning when the prevailing photoperiod conditions are entirely different. Further evidence against the importance of photoperiod in influencing reproduction in abalone is provided by Ebert and Houk (1984). These workers conditioned H.rufescens to gonad maturity using both natural illumination and also by rearing the animals in total darkness. This example indicates that photoperiod does not act as a timer responsible for synchronization of reproductive development. Also a minimum amount of daylight e.g. 12 hours as suggested by Webber and Giese (1969) is not required to initiate reproduction.

Feed is of crucial importance for the successful gonad conditioning of abalone. Generally seaweeds of preferred species are supplied fresh and in quantities in excess of the daily requirement. Uki and Kikuchi (1984) showed that gonad development was proportional to feed intake at constant temperature for H.discus hannai. Also feed consumption levels of greater than 5% body weight daily were required for the relationship between gonad development and cumulative temperature to hold true.

As stated previously the Japanese species H.discus Reeve and H.gigantea require a relatively large number of degree days to achieve spawning condition. The maximum feed intake levels recorded in conditioning experiments (Appendix B) on the two haliotids were only 4.6 and 3.3% body weight daily, respectively. In may be that rearing conditions were in some way substandard. Feed preference could be an important factor in this case, as could, tank design or the number of hours of dark available for these nocturnally feeding species to consume food. As noted previously no relationship between gonad development and cumulative temperature was found for H.gigantea (Kikuchi and Uki,1974). This phenomenon, may also occur in H.discus hannai if feed intake levels are sufficiently low. Kim (1983) showed that the accumulative temperature concept could be replaced with an accumulative ingestion relationship, which the author quantified for H.discus hannai. This worker believed that in maturation of abalone temperature determines the satiation level and feed ingestion determines the gonad maturity rate. The seasonal availability of feed has been examined in studies of haliotid spawning seasons by Boolootian et al., (1962), Shepherd and Laws (1974), Giorgi and DeMartini (1977), and Wells and Keesing (1989). In these studies the availability of feed appears important in determining the timing of the reproductive cycle.

In conclusion it appears photoperiod has little effect in initiating and controlling the reproductive cycles of many, if not all haliotid species. Undoubtedly temperature influences the rate at which gonad development proceeds, but only if feed intake is adequate.

5. SUMMARY AND CONCLUSIONS

(i) Specimens of H.laevigata were collected from Franklin Sound in the Furneaux Group on 27 April 1990. The abalone were held in conditioning tanks for 24 weeks with the intention of accelerating gonad development.

(ii) Experimental animals came into spawning condition after 15 weeks of gonad conditioning. Induced spawning was achieved following 112 days of conditioning or 1750 degree days. Female abalone appeared to be fully ripe between 16 and 21 weeks following commencement of conditioning while male gonad maturity peaked during week 24.

(iii) Sampled abalone were induced to spawn on 21 August 1990, some months before apparent spawning of the wild source population. Allowing for minor yearly variations, natural spawning of the Franklin Sound population of H.laevigata takes place between November and March.

(iv) Conditioned abalone were sampled at 21 day intervals for gonad analysis. The methods used to assess gonad maturity were: GBI, MGBI, oocyte size/frequency distribution, mean oocyte diameter, an ovarian phase method and percentage ripe spermatozoa.

(v) ANOVA was used to analyse GBI, MGBI, mean oocyte diameter and percentage ripe spermatozoa data. Chi-square analysis was used for oocyte size/frequency distribution data.

(vi) Of the gonad indices used the MGBI was considered superior to the GBI. The MGBI detected first significant gonad growth 3 weeks before the GBI and was more consistent with the histological analyses.

(vii) An alternative version of the MGBI formula is presented. Use of the new formula simplifies the measurement required for calculation of the index.

(viii) Of the histological methods used for measuring the reproductive development of female abalone the oocyte size/frequency distribution method was considered the most informative.

(ix) Cultured abalone in the size range 60-90 mm were found to be capable of spawning. This is of potential importance in a hatchery since such broodstock require a smaller share of hatchery resources.

(x) From examination of regimes used for conditioning other abalone species, and comparison with natural spawning season conditions for these species, it appears that feed intake and water temperature are of greater importance than photoperiod in gonad conditioning of haliotids.

(xi) Adult abalone were observed to be inactive animals. The provision of water movement within broodstock tanks is considered essential to stimulate feeding and distribute algae to abalone. A tank design suitable for conditioning of H.laevigata is described.

(xii) By the use of gonad conditioning H.laevigata may be induced to spawn outside of the natural spawning season. This allows better use of hatchery resources since egg production can take place over a greater part of the year.

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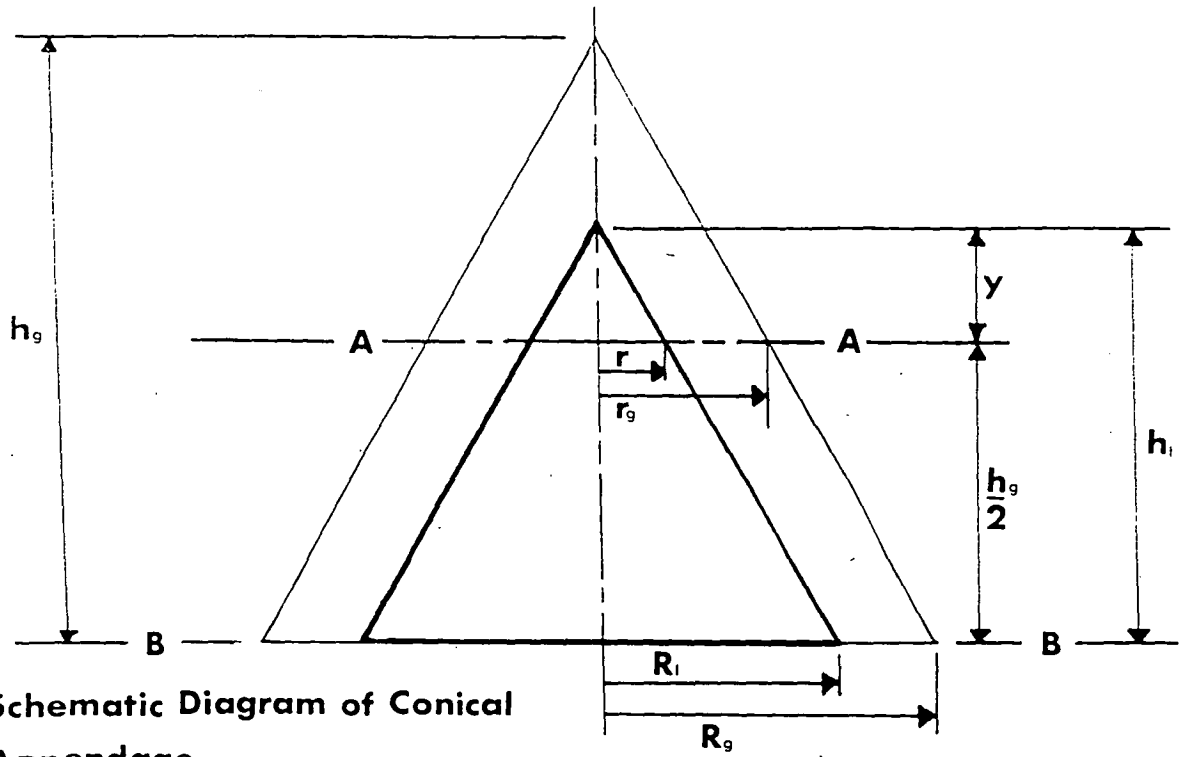
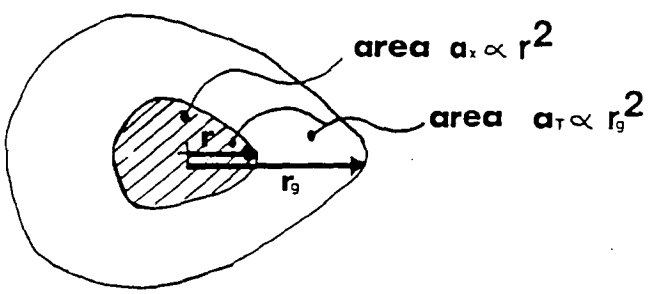
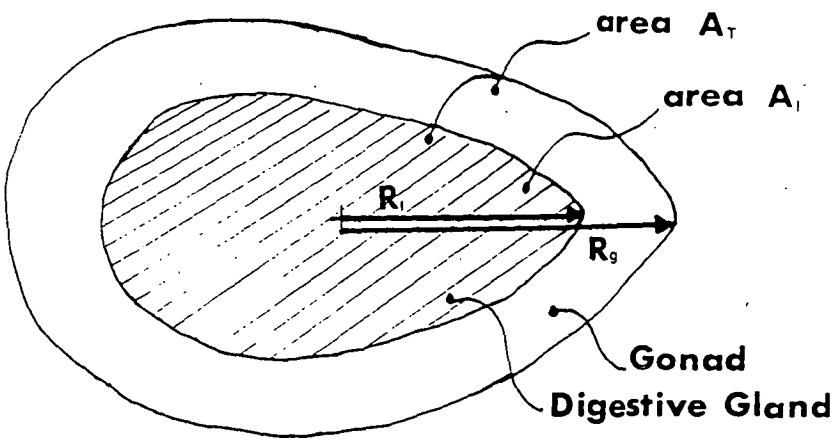
Appendix A. Derivation of EGV Formula



Schematic Diagram of Conical Appendage



Section AA



Section BB

Volume of right circular cone = $\frac{1}{3}$ area of base × height. Thus, gonad volume = (total volume − digestive gland volume).

$$V_g = \frac{1}{3}\left[A_T h_g - A_1 h_1\right] \qquad (1)$$

since $r_g = \frac{R_g}{2}$ then $a_T = \frac{A_T}{4}$

and $\frac{r}{R_1} = k$, thus $a_x = k^2 A_1$

Using similar triangles:

$$\frac{R_g}{h_g} = \frac{r_g}{h_g/2} \quad \text{and} \quad \frac{r}{r_g} = \frac{y}{h_g/2}$$

thus $y = \frac{r h_g}{2 r_g}$, also $h_1 = y + \frac{h_g}{2}$

substituting in equation (1)

$$V_g = \frac{1}{3}\left[4 a_T h_g - \frac{a_x}{k^2}\left(y + \frac{h_g}{2}\right)\right] \qquad (2)$$

next find k^2, (note $a_x \propto r^2$, $A_1 \propto R_1^2$)

$$k^2 = \frac{a_x}{A_1} = \frac{r^2}{R_1^2}$$

note that $\frac{r}{R_1} = \frac{y}{h_1}$ by similar triangles

$$\therefore \frac{r}{R_1} = y \Big/ \left(y + \frac{h_g}{2}\right)$$

$$\therefore V_g = \frac{1}{3}\left[4 a_T h_g - \frac{a_x}{y^2}\left(y + \frac{h_g}{2}\right)^3\right] \qquad (3)$$

now $y = \frac{r h_g}{2 r_g}$ and $a_T \propto r_g^2$, $a_x \propto r^2$

$\therefore y = \frac{h_g}{2}\sqrt{\frac{a_x}{a_T}}$ substituting in (3)

$$V_g = \frac{1}{3}\left[4 a_T h_g - \frac{4 a_x a_T}{h_g^2 a_x}\left(\frac{h_g}{2}\sqrt{\frac{a_x}{a_T}} + \frac{h_g}{2}\right)^3\right]$$

$$= \frac{4 a_T}{3}\left[h_g - \frac{1}{h_g^2} \cdot \left(\frac{h_g}{2}\right)^3 \cdot \left(\sqrt{\frac{a_x}{a_T}} + 1\right)^3\right]$$

$$= \frac{4 a_T h_g}{3}\left[1 - \frac{\left(\sqrt{\frac{a_x}{a_T}} + 1\right)^3}{8}\right]$$

$$V_g = \frac{a_T h_g}{6}\left[8 - \left(\sqrt{\frac{a_x}{a_T}} + 1\right)^3\right] \qquad (4)$$

[NOTE: EQUATION (4) IS ONLY VALID WHEN $h \geq h_1/2$]

Appendix B
A Comparison of Gonad Conditioning Time and Growth Data for Eight Haliotid Species

Species	Tank Design	Temp. (°C)	Length (mm) Initial	Final	Weight (g) Initial	Final	Length change (μm/d)	Weight change (mg/d)	Specific length change (10^{-4})	Specific weight change (10^{-3})	Feed Rate (%/d)	EA?	Time to Spawn (days)	Reference
H.laevigata	Basket	16.0	139.7	142.6	359.9	431.0	25.9	635	1.8	1.6	6.0	-	112	This study
H.discus	Con.	14.3	105.1	112.2	133.2	187.5	32.9	251	3.0*	1.6*	3.2		-	Kikuchi
	Jap.	18.9	105.2	114.9	134.0	197.9	44.9	296	4.1	1.8	4.2	3500	-	and Uki
	Tank	22.3	104.0	113.0	131.3	198.4	41.7	311	3.8	1.9	4.6		-	(1974)
H.gigantea	Con.	14.2	126.3	134.7	258.9	336.1	38.4	353	2.9*	1.2*	2.4	-	220	Kikuchi
	Jap.	18.4	121.3	134.4	224.4	330.1	59.8	483	4.7	1.8	3.6		220	and Uki
	Tank	22.2	123.5	134.6	235.4	324.7	50.7	403	3.9	1.5	3.3		220	(1975)
H.fulgens	-	20-24	-	-	-	-	-	-	-	-	-		90	Leighton et al (1981)
H.discus hannai	C.J.T	16.4	85.7	95.5	84.9	140.5	119.5	678	13.2*	6.1*	11.5	-	-	Uki and
	Pipe	19.9	78.2	88.6	62.4	93.4	83.9	250	10.1	3.3	8.2	-	-	Kikuchi
	Basket	17.5	81.4	82.6	69.9	86.9	17.6	250	2.2	3.2	6.7	-	-	(1981)
H.discus hannai		8.2	77.5	79.2	66.5	72.3	18.3	62	2.3*	0.9*	3.2		-	
	Con.	11.5	78.1	83.0	68.6	79.2	38.3	83	4.8	1.1	4.7		-	Uki
	Jap.	14.3	73.5	81.2	56.5	78.1	60.2	169	7.8	2.5	6.5	1500	-	and
	Tank	17.1	76.9	88.0	62.7	100.7	86.7	297	10.5	3.7	8.3		-	Kikuchi
		20.1	79.5	88.1	66.7	105.3	67.2	302	8.0	3.6	7.1		-	(1984)
		22.4	79.7	86.7	72.6	95.3	54.7	177	6.6	2.1	6.3		-	

Appendix B.(Continued)

Species	Tank Design	Temp. (°C)	Length (mm) Initial	Length (mm) Final	Weight (g) Initial	Weight (g) Final	Length change (μm/d)	Weight change (mg/d)	Specific Length change (10^{-4})	Specific Weight change (10^{-3})	Feed Rate (%.d)	RAT	Time to Spawn (days)	Reference
H.fulgens	-	18-	-	-	-	-	-	-	-	-	-	-	30-	Morse
H.corrugata	-	25	-	-	-	-	-	-	-	-	-	-	60	(1984)
H.cracheroidii	-		-	-	-	-	-	-	-	-	-	-		
H.rufescens	Recirc.	15	-	-	-	-	-	-	-	-	-	- -	75- 90	Ault (1985)

* = Values calculated from the available data.

Appendix C.
COMPARISON OF FECUNDITY ESTIMATES FOR HALIOTID SPECIES IN CONDITIONING STUDIES (Millions).

1. Comparison by Length

Species	Length (cm)														Reference
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
H.laevigata	[0.16 - 0.50]							[2.8 - 5.2]		[3.4-8.6]					This study
H.discus							* 3.6 - 7.3								Kikuchi and Uki (1974).
H.gigantea									* 2.1						Kikuchi and Uki (1975).
H.rufescens	[0.09 - 0.6]	[0.2 - 0.3]					[0.3 - 3.2]				[8.5 - 10.0]			* 11.1	Ault (1985).

2.Comparison by Weight.

Species	Weight (g)												Reference
	0	100	200	300	400	500	600	700	800	900	1000	1100	
H.laevigata	[.16-.5]			[2.8-3.7]		[3.4 - 8.6]							This study
H.discus			* 3.6 - 7.3										Kikuchi and Uki (1974)
H.gigantea					* 2.1								Kikuchi and Uki (1975)
H.rufescens	[.1-.6]	[0.2-0.3]			[0.3-3.2]							[8.5 - 11.1]	Ault (1985)

* = A mean weight or length value rather than a range of values.

Appendix D.
COMPARISON OF FECUNDITY ESTIMATES FOR HALIOTID SPECIES IN FIELD STUDIES (Millions).

1. Comparison by Length

Species	Length (cm)																	Reference
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
H.laevigata													[1.8 - 6.4]						This study
H.rufescens										[0.6 - 1.0][0.8-1.1][2.6 - 8.5][4.0 - 12.6]									Giorgi and DeMartini (1977)
H.rufescens										[0.1 - 1.7][0.2-2.0][0.6 - 5.4][1.4 - 4.2]									Ault (1985)
H.iris								[0.6-3.4][2.6-4.3][4.9-11.3]											Poore (1973)
H.australis				[0.1-1.1][1.0-2.9]															Poore (1973)
H.tuberculata	[0.1-0.3][0.4-1.8][0.9-4.4][2.9-5.1]																		Hayashi (1980)
H.roei		[0.3-0.4][0.6-1.0][1.6-2.5]																	Wells and Keesing (1989)
H.midae									[		2.6 - 15.5			]					Newman (1967) as cited by Ault (1985)

Appendix E

COMPARISON OF ABALONE REPRODUCTIVE CYCLES MONITORED BY USE OF THE GONAD BULK INDEX (GBI).

Species	Maximum and minimum GBI values recorded for species at various locations and/or years.												Reference
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	
H.laeviqata	87	15											This study
H.laeviqata	75	7	70	2	75	2 *							Shepherd
H.cyclobates	75	1											and Laws,
H.rubra	75	25 *											(1974)
H.rubra	52	13	41	17	49	16	86	35	>79	16			McShane et al. (1986)
H.rubra	73	9	86	6	*								Harrison and Grant (1971)
H.iris	68	36	46	12	* ‖								Poore (1973)
H.australis	>86	58	87	53	*								
H.diversicolor	70	8			*								Takashima et al. (1978)
H.tuberculata	88	40											Hayashi (1980)
H.roei	93	46	94	42	94	40	93	42	94	31	94	51	Wells and Keesing (1989)

‖ = spawning not observed

* = cross section not taken at midpoint of conical appendage

Appendix F

COMPARISON OF ABALONE REPRODUCTIVE CYCLES MEASURED BY USE OF THE MODIFIED GONAD BULK INDEX (MGBI).

Species	Minimum and maximum MGBI values for abalone populations at various locations and/or years								Reference
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	
H.laevigata	<1	12							This study (Conditioned)
H.laevigata	<1	23							This study (Field)
H.rufescens	17	35	3	31					Ault (1985)
H.sorenseni	12	105	15	130	12	69	6	100	Tutschulte
H.fulgens	24	60	32	76	14	68	10	65	and Connell
H.corrugata	25	73	31	61	20	40	21	67	(1981)
H.corrugata	32	61	30	57	23	52	12	56	

Appendix G
Comparison of Oocyte Size/ Frequency Distribution Data.

Species	Largest Size Class* (μm)	Max. % Largest Class (μm)	Min. % Largest Class (μm)	Commonest Size Class (μm)	Max. % Commonest Class (μm)	Min. % Commonest Class (μm)	Reference
H.laevigata	180-200	9	0	20-40	71	12	This study (Fig.9)
H.cracheroidii	141-160	15	0	20-40	70	30	Webber and Giese (1969)
H.rubra	175-200	25	0	25-50	70	22	McShane et al.,(1986)
H.gigantea	160-180	15	0	20-40	52	33	Lee (1974)
H.sieboldii	160-180	15	0	20-40	52	32	Lee (1974)
H.discus	160-180	16	0	20-40	51	31	Lee (1974)
H.discus hannai	160-180	15	0	20-40	64	30	Lee (1974)
H.pustulata	125-150	30	0	0-25	70	20	Pearse (1978)
H.tuberculata	200-220	30	0	40-60	26	8	Hayashi (1980)

* Data in this column refers to the size class of the most common large oocytes seen in the studies.

Appendix H
CORRELATION BETWEEN MEASURES OF GONAD MATURITY

Measure 1	Measure 2	Correlation coefficient	Significance and critical value	n
GBI	MGBI	0.954	significant, 0.954 > 0.700	9
GBI ♀	MGBI ♀	0.833	significant, 0.833 > 0.700	9
GBI ♀	M.O.D	0.771	not significant, 0.771 < 0.886	6
MGBI ♀	M.O.D	0.943	significant, 0.943 > 0.886	6
GBI ♂	MGBI ♂	0.962	significant, 0.962 > 0.700	9
GBI ♂	% sperm	0.946	significant, 0.946 > 0.700	9
MGBI ♂	% sperm	0.917	significant, 0.917 > 0.700	9

The correlation used is the Spearmann rank correlation. Signficance tests were performed at the 5% probability level.Correlations are between sample means (Table 3).

Appendix I
SPAWNING SEASONS OF ABALONE (NORTHERN HEMISPHERE)

Species	Winter		Spring			Summer			Autumn				Location	Reference
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		
H.discus hannai							+	♦	♦	♦	♦	♦	Korea	Lee (1974)
H.discus hannai								♦	♦	♦			Japan	Uki and Kikuchi (1984)
H.discus hannai								♦	♦				Rebun Is. Japan	Tomita (1967)
H.discus hannai										♦	♦	♦	Chiba, Japan	Ino,1952. Ref. Poore (1973)
H.discus hannai								♦	♦	♦	♦		Hokkaido,Japan	Ino,1952. Ref. Poore (1973)
H.discus hannai	♦			♦	♦					♦	♦	♦	Ibaragi, Japan	Ino and Harada,1961.Ref. " "
H.discus hannai									♦	♦			Matsushima Bay	Kanno & Kikuchi,1962.Ref.**
H.discus hannai							♦	♦	♦				Miyagi,Japan	Sakai,1962. Ref.Poore (1973)
H.discus hannai					♦	♦	♦		♦	♦	♦		Yeosu, Korea	Rho and Park (1975)
H.discus hannai								♦	♦	+			Hokkaido,Japan	Ono,1932.Ref. As above.
H.discus hannai								♦	♦	+			Hokkaido,Japan	Ono,1932.Ref. As above.
H.discus hannai								♦	♦	+			Hokkaido,Japan	Ono,1932.Ref. " "
H.discus hannai							+	♦	♦	+			Hokkaido,Japan	Ono,1932.Ref. " "
H.discus hannai									♦	+	+		Hokkaido,Japan	Ono,1932.Ref. " "
H.discus hannai									♦	+			Hokkaido,Japan	Ono,1932.Ref. " "
H.discus hannai								♦	♦	+			Hokkaido,Japan	Ono,1932.Ref. " "
H.discus hannai								♦	♦	♦			Aomori,Japan	Takahasi et al.1965.Ref.As above
H.discus hannai							+	+	♦	♦			Iwade,Japan	Auonuma,1953.Ref. As above
H.discus hannai								♦	♦	♦			Iwade,Japan	Auonuma,1953.Ref. " "

♦ = major spawning period + = less intense spawning period

Appendix I. Continued... SPAWNING SEASONS OF ABALONE (NORTHERN HEMISPHERE)

Species	Winter		Spring			Summer			Autumn				Location	Reference
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		
H.discus hannai									♦	♦			Miyagi,Japan	Onodera,1957.Ref.Rho & Park(1975)
H.discus hannai									♦	♦			Miyagi,Japan	Onodera,1957.Ref. " " "
H.discus hannai								♦	♦	♦			Miyagi,Japan	Sakai,1960.Ref. " " "
H.discus hannai								♦	♦				Dalian, China	Yongfeng et al. (1985)
H.rufescens	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	Calif. USA	Boolootian et al. (1962)
H.rufescens	♦	♦	♦	♦									Calif. USA	Bonnot,1930.Ref.Boolootian (1962)
H.rufescens		♦	♦										Calif. USA	Scofield,1930.Ref. " " " "
H.rufescens	♦	♦	♦										Calif. USA	Croker,1931.Ref. " " " "
H.rufescens	♦	♦	♦										Calif. USA	Bonnot,1940.Ref. " " " "
H.rufescens			♦	♦	♦	♦	♦	♦	♦				Calif. USA	Bonnot,1948.Ref. " " " "
H.rufescens	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	Calif. USA	Young and DeMartini (1970)
H.rufescens		♦	♦										Calif. USA	Heath,1925.Ref. As above
H.rufescens					♦	♦							Calif. USA	Cox,1962.Ref. " "
H.rufescens			♦	♦	♦	♦							Calif.USA,site 1	Giorgi and DeMartini (1977)
H.rufescens			♦	♦	♦	♦							Calif.USA,site 2	Giorgi and DeMartini (1977)
H.rufescens	♦			♦					♦				Calif. USA	Price,1974.Ref. As above
H.rufescens		♦	♦										USA	Curtner,1917. Ref. Ault (1985)
H.rufescens	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	Calif. USA	Leighton,1974. Ref.Ault (1985)
H.rufescens							♦	♦	♦				Calif. USA	Carlisle,1962. Ref. Poore (1973)
H.discus										♦	♦	♦	Korea	Lee (1974)
H.discus										♦	♦	♦	Japan, Warmer	Uki and Kikuchi (1984)

♦ = Major spawning period + = Less intense spawning period

Appendix I Continued... SPAWNING SEASONS OF ABALONE (NORTHERN HEMISPHERE)

Species	Winter		Spring			Summer			Autumn				Location	Reference
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		
H.corrugata			*	*	*	*							Calif. USA	Tutschulte and Connell (1981)
H.corrugata					*	*	*	*	*				Mexico,Baja Calif.	Quintanella,1966.Ref.Hahn (1988)
H.gigantea	*										*	*	Korea	Lee (1974)
H.gigantea									*	*			Japan	Tago,1931.Ref.Boolootian et al.(1962
H.gigantea									*	*	*	*	Japan	Kishinouye,1894.Ref. * * * *
H.gigantea									*	*	*	*	Japan	Higurashi,1934.Ref. * * * *
H.gigantea										*	*	*	Chiba,Japan	Ino,1952. Ref. Poore (1973)
H.gigantea											*	*	Japan,Warmer	Ino,1952. Ref. Poore (1973)
H.gigantea										*	*	*	Japan,Warmer	Uki and Kikuchi (1984)
H.sieboldii	*										*	*	Korea	Lee (1974)
H.sieboldii									*	*	*	*	Japan	Higurashi,1934.Ref.Boolootian,1962
H.sieboldii									*	*	*	*	Japan	Kishinouye,1894.Ref. * * * *
H.sieboldii											*		Chiba,Japan	Ino,1952. Ref. Poore (1973)
H.sieboldii										*	*	*	Japan, Warmer	Uki and Kikuchi (1984)
H.cracherodii							*	*	*	*			Calif. USA	Boolootian et al.(1962)
H.cracherodii					*	*	*	*	*	*			Calif. USA	Leighton&Boolootian.1961.Ref.Above
H.cracherodii									*				Calif.USA,site 1	Webber and Giese (1969)
H.cracherodii										*	*		Calif.USA,site 2	Webber and Giese (1969)
H.fulgens						*	*	*	*				Calif. USA	Tutschulte and Connell (1981)
H.fulgens					*	*	*		*	*			Calif. USA	Leighton et al. (1981)
H.fulgens				*	*	*	*	*					Mexico,Baja Calif.	Quintanella,1966.Ref.Hahn (1989)

Appendix 1. Continued...SPAWNING SEASONS OF ABALONE (NORTHERN HEMISPHERE)

	Winter		Spring			Summer			Autumn					
Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Location	Reference
H.lamellosa					*	*	*	*	*	*			Italy	Bolognari,1954. Ref. As below.
H.tuberculata							*	*	*				England	Crofts,1929.Ref.Boolootian (1962)
H.tuberculata						*	*	*					France	Wegmann.1884.Ref. " " " "
H.tuberculata					*	*	*	*					France	Crofts,1937.Ref. " " " "
H.tuberculata								*	*				Guernsey	Hayashi (1980)
H.tuberculata						*	*	*	*				Guernsey	Forster,1962.Ref.Hahn (1989)
H.kamtschatkana	+	+	+	+	+	+	+	+	+	+	+	+	B.C. Canada	Quayle (1971)
H.kamtschatkana				*									Calif. USA	Hahn,1981.Ref.Hahn (1988)
H.diversicolor										*	*		Chiba, Japan	Oba,1964. Ref. Poore (1973)
H.diversicolor	*	*								*	*	*	Taiwan	Chen (1984)
H.diversicolor									*	*			Japan, Warmer	Takashima et al. (1978)
H.pustulata						+	+	+	+	+	+	+	Gulf of Suez	Pearse (1978)
H.pustulata	*	*										*	Red Sea	Pearse (1978)
H.walallensis				*	*	*							Calif. USA	Hahn,1981.Ref. Hahn (1989)
H.sorenseni		*	*	*									Calif. USA	Tutschulte and Connell (1981)

* = Major spawning season + = Less intense spawning

Appendix J.
ABALONE SPAWNING SEASONS (SOUTHERN HEMISPHERE)

	Summer		Autumn			Winter			Spring					
Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Location	Reference
H.laevigata	♦	♦	+	+						+	+	+	S.A West Is.	Shepherd and Laws (1974)
H.laevigata	♦	♦	+	+						+	+	+	S.A Tipara Reef	Shepherd and Laws (1974)
H.laevigata	♦	♦	♦								+	+	Flinders Is. Tas.	This study
H.rubra	+	+	♦	♦						+	+	+	S.A West Is.	Shepherd and Laws (1974)
H.rubra			♦	♦	♦	♦	♦	♦					S.A. Tipara Reef	Shepherd and Laws (1974)
H.rubra		+				♦	♦	♦	♦	♦			Tasmania, Aust.	Harrison and Grant (1971)
H.rubra								♦	♦	♦	♦		N.S.W, Aust.	Shepherd and Laws (1974)
H.rubra	♦	♦		+								♦	Vic. Portsea	McShane et al.,(1986)
H.rubra	♦	♦								♦	♦	♦	Vic. Pt.Lonsdale	As above
H.rubra	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	Vic. Flinders	As above
H.rubra	♦	♦										♦	Vic. Lorne	As above
H.roei							♦	♦	♦	♦	♦	♦	W.A. Perth	Wells and Keesing (1989)
H.roei		♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	S.A. West Is.	Shepherd and Laws (1974)
H.iris		♦	♦	+									N.Zealand, Kaikoura	Poore (1973)
H.iris		♦											N.Zealand, Taylors mistake	Poore (1973)

♦ = major spawning period + = less intense spawning period ♦ = probable spawning period