The Pearl Oyster

Pinctada maxima (Jameson, 1901)

An Atlas of Functional Anatomy, Pathology and Histopathology

JD Humphrey and JH Norton

Australian Government
Fisheries Research and Development Corporation

Northern Territory Government
Department of Primary Industry, Fisheries and Mines

Queensland Government
Department of Primary Industries and Fisheries

THE PEARL OYSTER

Pinctada maxima (Jameson, 1901)

AN ATLAS OF FUNCTIONAL ANATOMY,

PATHOLOGY

AND

HISTOPATHOLOGY

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PREFACE

The commercial farming of the pearl oyster *Pinctada maxima* and the production of pearls from this species comprise a major industry in northern Australia. Although this industry is entirely dependent on the mollusc, the histology, pathology and physiology of *P. maxima* remain poorly described. As with all animal species, an understanding of the normal anatomic and microscopic structure is a pre-requisite for the recognition of disease or pathological states characterised by altered structural changes at the gross and cellular level. This is no less the case with *P. maxima*

The recognition of such pathological states often leads to a diagnosis of disease, or, importantly in many cases, the exclusion of serious diseases, including those that may be exotic. As well, the interpretation of altered physiological states, often manifest as clinical or subclinical disease, is also greatly assisted by an understanding of the anatomic and cellular basis of normal physiological function.

In producing this atlas on the functional anatomy, histology and histopathology of *P. maxima*, the normal structure and function of the oyster is described together with a range of inflammatory and degenerative processes associated with infectious and non-infectious causes of disease. The aim of the atlas is to provide a practical and "user-friendly" guide to assist in the day to day management of pearl oyster aquaculture by:

- Providing the comparative pathologist with a basis for the recognition and interpretation of the normal gross and microscopic structure of *P. maxima* and of abnormalities which may occur as a result of disease processes.
- Assisting biologists, aquaculturalists and farm technicians in understanding the structure and function of *P. maxima*, providing an aid to the recognition of disease processes and providing guidelines for the management, collection and sampling of oysters for disease or other investigations.

John D. Humphrey
John H. Norton
May 2005

ACKNOWLEDGEMENTS

This atlas is the evolutionary product of a number of years experience in examining large numbers of pearl oysters from northern Australia both as survey material and diagnostic specimens, and the assimilation of new and contemporary information on infectious and non-infectious conditions in the species. The atlas owes much to the persons and organisations who assisted and facilitated its production.

The authors thank the Northern Territory Department of Primary Industry, Fisheries and Mines, the Queensland Department of Primary Industries and Fisheries, and the Western Australian Department of Agriculture for their interest in, and support for, the work. Thanks are also due to the pearl farming companies and staff in Western Australia, the Northern Territory and Queensland who generously assisted in the collection of material for gross and histopathological examinations on the oysters, and to the Queensland Boating and Fisheries Patrol, Thursday Island, for transport to pearl farms in Queensland.

Thanks are due to Dr Bob Rose and his staff of the Darwin Hatchery Project for the supply of pearl oyster larvae and spat, the Cooperative Research Centre Aquaculture for their financial support for the collection and preparation of histological specimens and to Dr Judith Handlinger of the Tasmanian Department of Primary Industries, Water and Energy during this work with hatchery-reared molluscs.

We especially thank Dr Brian Jones, Western Australian Department of Agriculture, for providing a number of figures for the atlas and for valuable and informed discussions on the nature and significance of a range of parasites, diseases and conditions found in pearl oysters. Dr Dave Mills provided considerable insight into pearl farm management and the role that a work of this nature may play in assisting the pearl oyster aquaculture industry. His comments, advice and suggestions are gratefully acknowledged. Dr Richard Weir and Dr Alex Hyatt are thanked for their assistance with electron microscopy and ultrastructural interpretations.

The preparation and staining of the large number of high quality histological sections from which the illustrations were selected represented a major undertaking. Special thanks are due to Ms Cheryl Day, Ms Lisa Simington, Ms Linda McLean, Ms Nikki Elliot, Ms Natalie Cox and Ms Sue Aumann of the Berrimah Veterinary Laboratories, Department of Primary Industry, Fisheries and Mines, Darwin, and to Ms Michelle Hoogland of the Oonoonba Veterinary Laboratories, Department of Primary Industries and Fisheries, Townsville, who assisted in this regard over a number of years. The technical assistance of Ms Maria Connell in collating and indexing the large number of histological sections from which much of the photographic material was derived is also gratefully acknowledged.

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SECTION 1

GENERAL ANATOMY,

CLINICAL EXAMINATION

AND

GROSS PATHOLOGY

GENERAL ANATOMY

Pearl oysters of the genus *Pinctada* belong to the order Pteriidae, family Pinctidae and include the silver- or gold-lip pearl oyster, *Pinctada maxima*, the black-lip pearl oyster, *P. margaritifera* and the Japanese pearl oyster *P. fucata*. The taxonomic characteristics of the genus *Pinctada* are described in detail by Hynd (1955). A schematic representation of the distribution and location of organs and tissues in a mature *P. maxima* is shown in Figure 1.

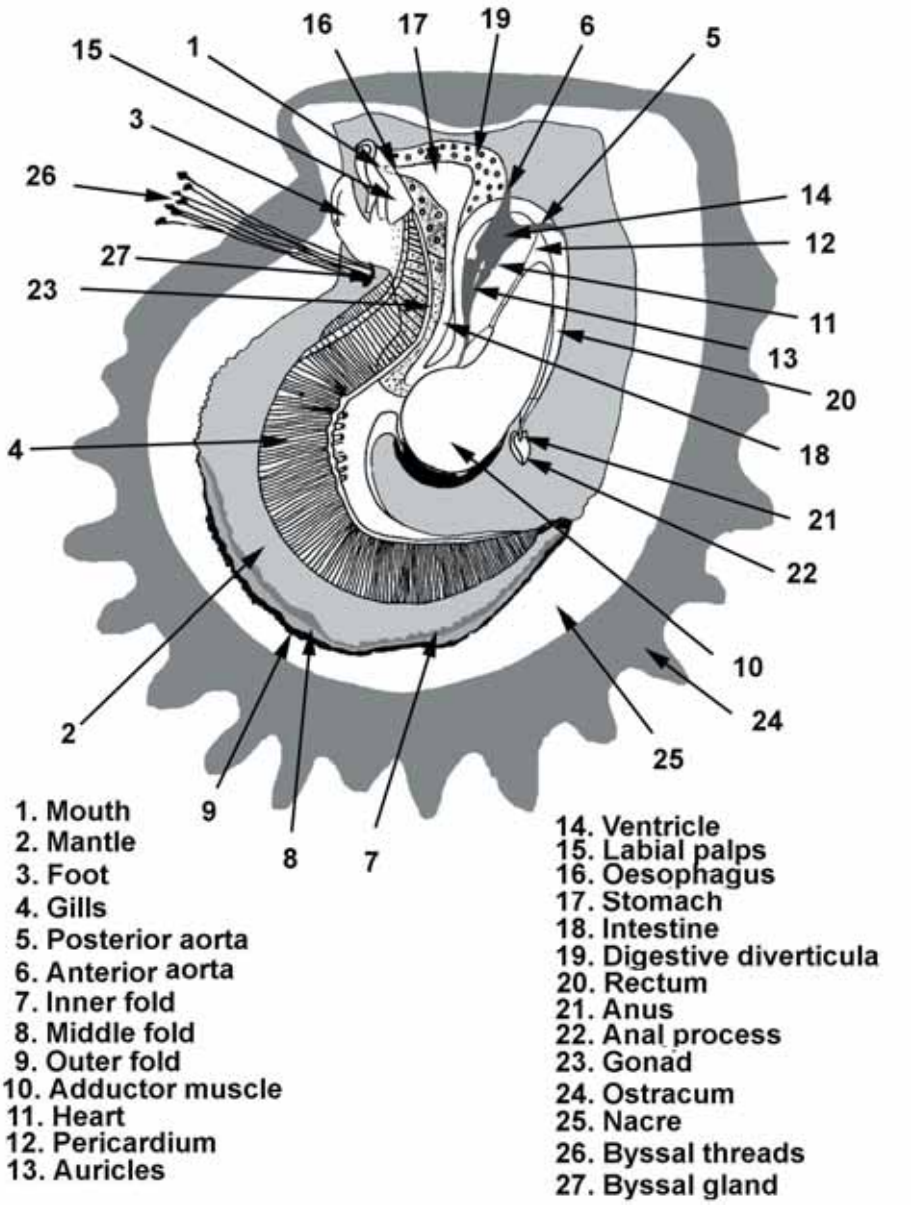

Figure 1. Schematic diagram showing location and relationships of organs and tissues of a mature *P. maxima*. Redrawn after Doumenge *et al.* 1991.

THE IMMATURE OYSTER: LARVAE

The larval development of the *Pinctada* species appears similar. The early life history of *P. margaritifera* is described by Shokita *et al.* (1991) and McCormack (1989) describes the early life stages of *P. maxima*. At spawning, eggs and sperm are released into the water column and fertilised eggs, approximately 50-60 um in diameter, begin to develop. The rate of development is in part dependent on temperature and feed availability. In about 12 hours, motile trochophore larvae swim from the eggs. These develop into D-shaped larvae or D-shaped veligers in about 8 hours. The planktonic D-shaped veligers are approximately 75-85 um shell length and feed on micro-phytoplankton. The umbo of the shell extends behind the hinge at approximately 7-12 days and by around 20 days, larvae form the umbonal veliger stage of approximately 200 um shell length with a well-developed umbo. The larvae subsequently develop a pair of eyespots, forming the eyed veliger. A foot develops in a further three days forming the fully developed larval stage, the pediveliger, possessing velum and foot, with swimming and crawling behaviour.

After about 30 days, the fully developed larvae lose their velum and continue development in the early post-larval phase or spat, at which stage attachment to the substrate occurs.

In comparison with their adult counterparts, early larval forms of *P. maxima* are morphologically different, with few internal features of the adult stage recognisable.

Larval Shell Development

The progressive development of the shell of early D-shaped larvae of *P. maxima* up to the formation of the umbo is shown in Figures 2 to 7.

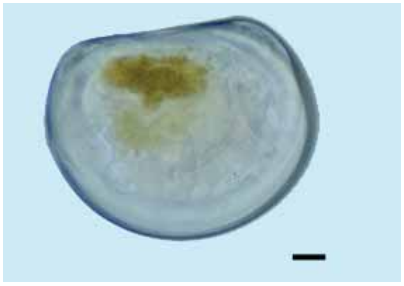

Figure 2. Shell of 3-day-old larval *P. maxima*. "D-larva". Bar = 6 microns.

Figure 3. Shell of 4-day-old larval *P. maxima*. Note progressive rounding of shell. Bar = 6 microns.

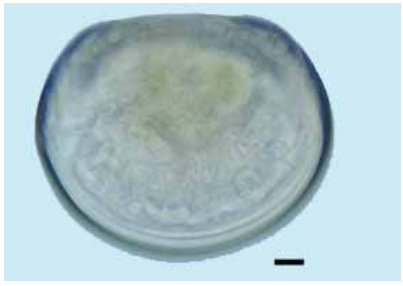

Figure 4. Shell of 6-day-old larval *P. maxima*. Bar = 8 microns.

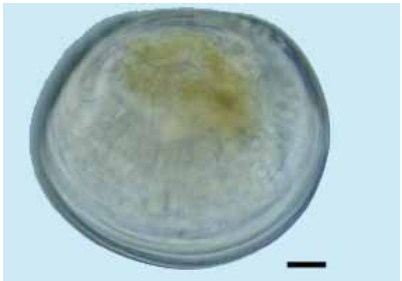

Figure 5. Shell of 8-day-old larval *P. maxima*. Bar = 12 microns.

Figure 6. Shell of 12-day-old larval *P. maxima*. Note early umbo formation. Bar = 10 microns.

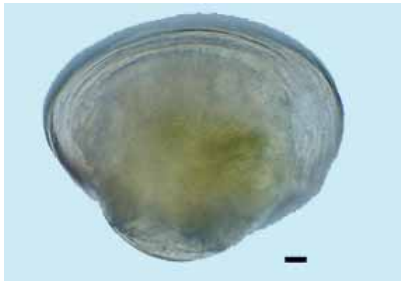

Figure 7. Shell of 13-day-old larval *P. maxima*. Note umbo formation of shell. Bar = 10 microns.

THE IMMATURE OYSTER: SPAT

Post-larval spat are approximately 240-260 um shell length when the oyster takes on a typical bivalve appearance. At about 5-7 mm shell length, scale-like projections arise from the shell. Juveniles, up to approximately 20 mm shell length, are normally attached but can move using their foot after detaching their byssus from the substrate. Over about 20 mm shell length, typical finger-like projections develop from the shell margin (Shokita *et al.* 1991). Spat develop by progressively taking on typical adult characteristics as they grow. There appears to be no strict definition of spat. Juvenile oysters are generally considered spat up to approximately 50 mm shell length, although industry often refers to oysters up to about 110 mm shell length as spat.

THE MATURE OYSTER

Mature oysters of the family Pinctidae are characterised by possessing a long and straight hinge. The left shell valve is usually deeper than the right. A byssal notch is present on each shell valve at the base of the anterior lobe. The colour of the periostracum varies, often being brown with radial markings. The silver- or gold-lipped pearl oyster, *Pinctada maxima* is differentiated from other species of *Pinctada* by the absence of denticles on the hinge. The shell valves are flatter and less hollow than in *P. margaritifera*.

Aspects of the anatomy of *Pinctada spp.* are discussed in detail by a number of authors including Herdman (1904), Shiino (1952), Takemura and Kafuku (1957), Velayudhan and Gandhi (1987) and Fougerouse-Tsing and Herbaut (1994).

Shell Structure, Orientation and Principal Axes

The general anatomy of the shell, the principal axes and orientation used to describe anatomic relationships for *P. maxima* are based on Hynd (1955), Takemura and Kafuku (1957) and Knuckey (1995) and are shown in Figure 8. Orientation described by Fougerouse-Tsing and Herbaut (1994) is helpful in this regard. When the shell is held in the hands, hinge uppermost and horizontal and the byssus is directed away from the observer, the left and the right shell valves of the oyster correspond to the left and right hands of the holder. The hinge corresponds to the dorsal side, the region at the opening of the shell valves is the ventral margin. The byssus marks the anterior region. It is noteworthy that the left shell valve is more convex or arched than the right.

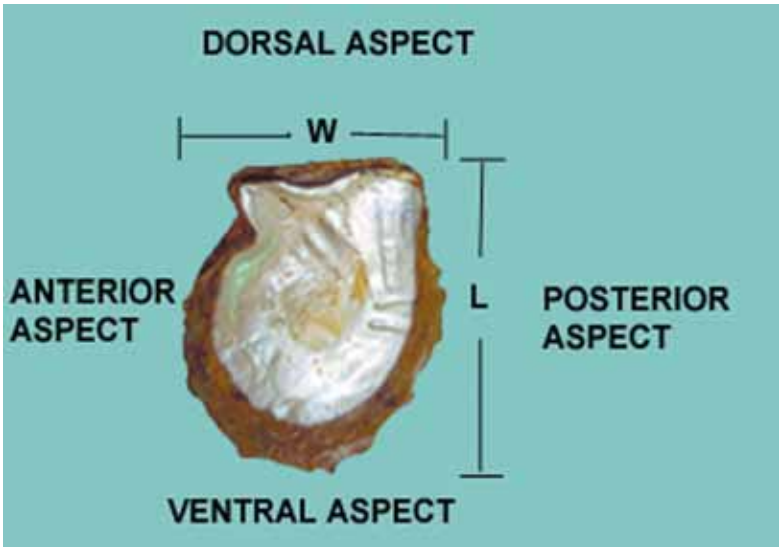

Figure 8. General view of internal surface of right shell valve of *P. maxima* showing major axes and orientation. W = shell width, L = shell length.

The major anatomic features and points of reference on the external shell surface of *P. maxima* are shown in Figure 9.

The internal surface of the shell is in direct contact with the external surface of the mantle and is generally covered by nacre giving the inner shell surface its silver iridescent appearance. At the margins of the shell, the nacreous layer merges sharply with a zone of dark, semi-soft proteinaceous conchiolin forming the periostracum. Finger-like projections of this material extend beyond the general periphery of the shell (Figure 10). In farmed oysters, these projections may be lost in cleaning, especially in older animals.

A number of features and points of reference are apparent on the internal shell surface (Figure 11). The attachment of the adductor muscle is seen as a comma-shaped depression in the centre of the shell. The depressions for the attachment of the retractor muscles of the foot lie immediately anterior-dorsal to the attachment of the adductor muscle. The pallial muscle attachments are seen as irregular linear depressions between the adductor depression and the pallial line. The pallial line is where the pallial muscles attach to the shell valves.

Principal Organs and Tissues

The general structure of the organs and tissues of the oyster is best viewed in an animal opened by cutting the adductor muscle from the upper shell valve (Figure 12) and then removing the upper mantle and gill structures as shown in Figure 13.

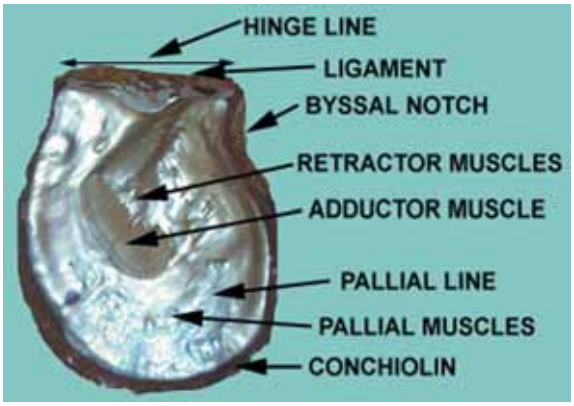

Figure 11. Internal shell surface of left shell valve of a mature *P. maxima* showing major anatomic and surface features, including roughened, depressed regions of muscle attachment. The pallial line includes the pallial muscles and line of mantle attachment to the shell.

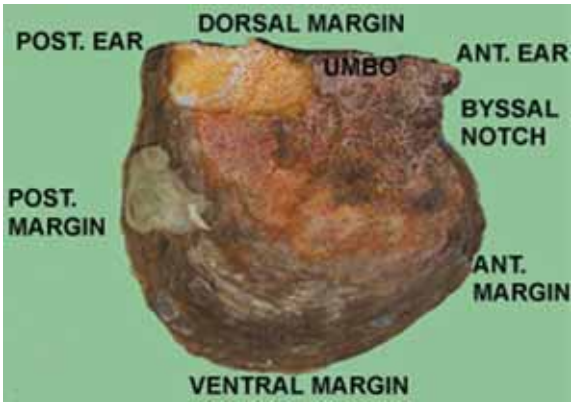

Figure 9. Major external anatomic features of the shell surface of a mature *P. maxima.* Note invasion by boring sponge causing condition known as "red bum".

Figure 10. Pronounced finger-like projections of conchiolin at periphery of shell in spat of *P. maxima.* These projections are generally lost during routine cleaning of farmed shell.

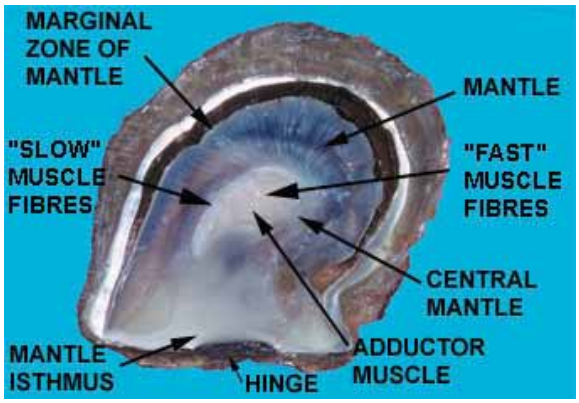

Figure 12. View of mature oyster with left shell valve removed to expose the underlying adductor muscle and mantle.

Mantle

The mantle is a thin, flattened expansive organ that, in the relaxed living animal, covers the internal surfaces of the shell valves. The expansive nature of the mantle is not generally appreciated in animals disturbed by handling, or in animals placed directly into fixative for histological examination without prior anaesthesia, as rapid contraction of this organ occurs. In the living animal, the margins of the mantle expand beyond the nacreous layer of the shell valves, over the finger-like projections of conchiolin at the margins of the shell.

In a well-nourished animal, the mantle is pale creamy-white and semi-opaque. Fine radial markings representing muscle striations and haemolymph vessels are visible. Along the extremities of the outer margin of the mantle, a distinct pigmented thickening is visible.

Gill

The gill is a crescent-shaped, brown organ that lies between the mantle lobes within the mantle cavity and generally surrounds the outer portion of the adductor muscle. The gills, normally suspended in the water within the shell cavity, collapse on opening of the shell.

Adductor Muscle

The adductor muscle is a large, pale, conspicuous comma-shaped mass that lies generally central to the shell valve, apposing the gills and the mantle. The adductor is firmly attached to the inner surface of each shell valve.

Visceral Mass

The visceral mass contains the digestive gland, gonad, alimentary tract and byssal gland. It lies anterior-dorsal to the adductor muscle. In well-nourished animals, the visceral mass is semi-soft in consistency. In male oysters it appears pale white whereas in female oysters it appears grey-brown. The stage of gonadal development influences the size and colour of the visceral mass.

The anatomical relationships in the region of the apposition of the gills, palps, foot and visceral mass are shown in Figure 14.

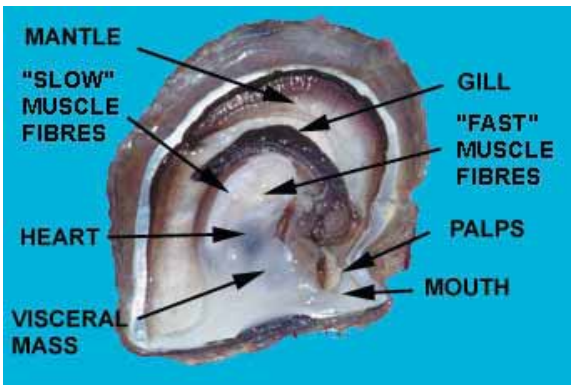

Figure 13. Mature oyster with overlying mantle and left shell valve removed exposing gill structure, underlying mantle, visceral mass and associated structures.

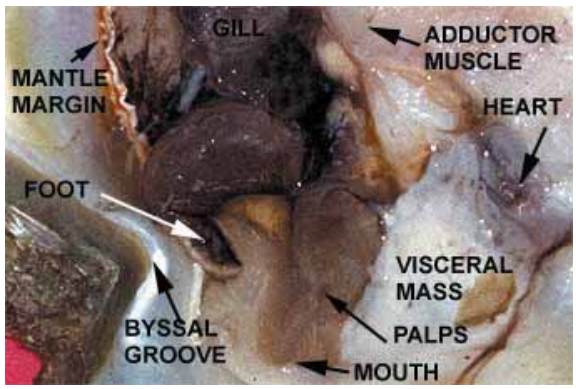

Figure 14. Region of apposition of gill, palps, foot and visceral mass in mature oyster showing major anatomic features.

Byssal Threads

The byssal threads, when present in the intact animal, form a conspicuous bundle of green, flattened fibres arising from the byssal gland on the anterior-dorsal aspect of the visceral mass, extending towards and through the byssal notch in the shell. In mature farmed shell, the byssal threads are often lost due to frequent removal of shell for cleaning.

Foot

The foot arises as a protrusive, tongue-like organ from the antero-dorsal aspect of the visceral mass, extending towards the byssal notch.

Palps

The palps extend from the proximal termination of the gills to the mouth as four flattened brownish sheets of tissue, arising lateral to the byssal threads and foot. Laterally, the palps merge with the anterior-dorsal mantle.

Mouth

The mouth, located deep in the terminal closure of the palps and mantle in the anterior-dorsal aspect of the visceral mass, leads to the oesophagus within the visceral mass.

Heart

The heart is seen as a dark mass within the fine, membranous pericardial cavity, adjacent to the visceral mass on the postero-dorsal aspect of the visceral mass, between the adductor muscle and the visceral mass. Two portions can be readily visualised, the ventricle and the auricles.

Gonad

The gonad generally occupies the posterior region of the visceral mass, but extends into and surrounds the digestive gland within the visceral mass. A prominent protrusion arises from the region of the gonad on the antero-ventral aspect of the visceral mass, which contains the loop of descending and ascending segments of the intestine.

Intestine

The intestine arises from the stomach in the dorsal aspect of the visceral mass. It extends as descending and ascending segments within the visceral mass before continuing ventrally as the rectum on the posterior surface of the adductor muscle. The intestine terminates as the anus. A conspicuous flap of tissue in the midline of the posterior-ventral aspect of the adductor muscle, the anal process, covers the anus.

Pearl Production

Farmed or cultured pearls are produced by the deposition of nacre over a seed pearl, nucleus or mould. Natural pearls form by deposition of nacre over a foreign body, for example, a parasite, within the tissues of the oyster.

Round Pearls

Round pearls (Figure 15) are formed by the insertion of a seed pearl or nucleus produced from the shell of freshwater mussel, together with a small section of living mantle tissue, both of which are implanted into the gonadal tissue of the oyster. The mantle tissue proliferates forming a sac around the pearl, the pearl sac. The cells of the pearl sac subsequently produce concentric layers of nacre around the nucleus to form the pearl. Following harvest of the pearl, a further nucleus maybe inserted into the now present pearl sac of the same oyster.

Half Pearls (Mabe Pearls)

Half pearl production occurs through the deposition of nacre by the mantle over a plastic nucleus or mould attached to the inner surface of the shell (Figures 15, 16). Half pearls may be produced in a variety of shapes, depending on the shape of the mould (Figure 17). These are subsequently removed by cutting the shell.

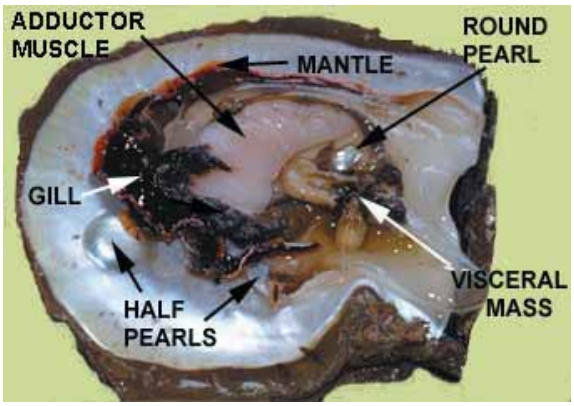

Figure 15. View of opened oyster showing half pearl formation on nacreous inner surface of shell valve and round pearl extruding from cut pearl sac in visceral mass. Note also the contraction of the mantle and collapse of gill structure which occurs when the oyster is removed from the water and the shell valves are separated by incising the adductor muscle. Immersion in fixative in this state results in artefactual distortion of histological structure.

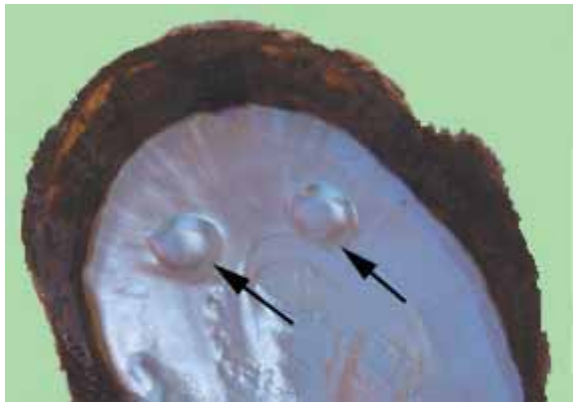

Figure 16. Half pearl production (arrows) with deposition of nacre over moulds attached to inner shell surface.

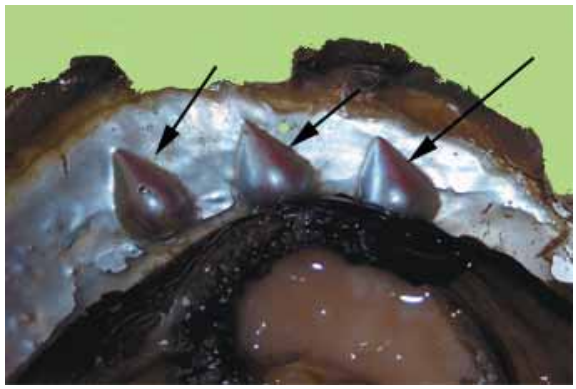

Figure 17. Internal shell surface showing tear-shaped half pearls (arrows) produced by nacre deposition over plastic moulds glued to shell surface.

CLINICAL EXAMINATION

The determination of the nature and cause of disease is primarily a function of careful and thorough consideration of all factors that interact in the ultimate expression of disease. Careful investigation of the background to the disease event (history), observations of unusual behaviour or abnormalities in the living oyster (clinical signs) and observations of abnormal tissue changes at the gross or histological level (pathological changes) are necessary to arrive at a logical conclusion regarding the cause of a disease event.

Clinical Examination

The diagnosis of disease in farmed pearl oysters and the differentiation of healthy from diseased oysters based on clinical examination alone are often difficult. A thorough post-mortem examination and further laboratory investigation are often warranted.

Disease may be *clinical*, i.e., there may be signs of disease in the individual animal or in the population as a whole. Such signs may include deaths, empty shells, gaping shells, poor growth, shell valve anomalies and mantle retraction. Disease may also be *sub-clinical*, i.e., not accompanied by obvious signs of disease. Sub-clinical disease is less readily appreciated and may be manifested as sub-optimal shell growth, decreased body weight gain, poor condition indices and poor quality and/or quantity of pearl production. Sub-clinical disease may be difficult to evaluate on a population basis and the detection of sub-clinical disease necessitates the maintenance of detailed farm production records. The importance of maintaining such records cannot be over-emphasised if optimal productivity is to be attained and the source of losses identified.

Clinical examination of living, farmed oysters may present difficulties. Restricted visual access inside the shell cavity limits direct observation of the living animal. In the living animal viewed undisturbed in water, the observer is generally dependent on relatively subjective and variable criteria to evaluate the health of individual animals. Adductor muscular tone, assessed on strength and speed of closure of the shell, and mantle retraction are two common criteria evaluated in response to external stimuli. Retraction of the mantle and failure to lay down new shell may suggest disease.

An assessment of the general nutritional condition of the body and mantle may be possible. Poorly nourished animals will appear wasted, with transparent mantle tissues. Prolonged retraction of the mantle is indicative of disease states. Mantle retraction may result in fouling of the nacreous surface of the shell normally covered by the expanded mantle, together with deposition of conchiolin then nacre by the retracted mantle. This process results in a growth check, with the deposition of new shell on previously normal shell and gives rise to the name "double backs" for these oysters (Figure 18). Shells with such lesions are good indicators of a pre-existing insult to the animal. In the absence of accurate farm records and production data, there are few other criteria on which to evaluate the earlier clinical status of pearl oysters.

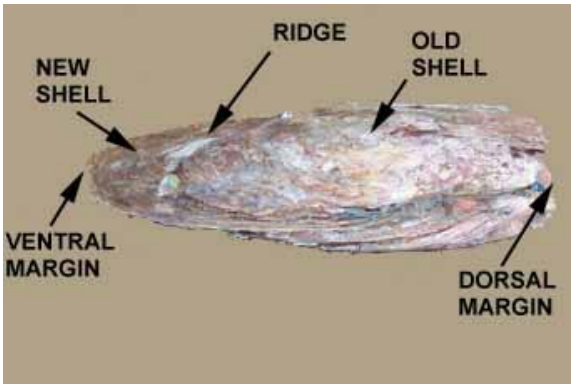

Figure 18. "Double-back" oyster. Cleaned shell showing distinct ridge between new growth and old growth resulting from earlier check in shell deposition and subsequent re-deposition of shell.

In undertaking a clinical examination of pearl oysters, a number of oysters should be randomly selected directly from their holding panels in the sea. The following features should be checked:

- Are the oysters open and feeding before collection?
- Are the mantles retracted?
- How many of the oysters are dead or the panel pockets empty of oysters?
- Are the oysters heavily fouled? Type or nature of fouling?
- Are the oyster shell valves badly distorted, i.e., are there double-backs?

Further investigation requires that a selection of typically affected oysters be taken and a post-mortem examination done. This is recommended because of the difficulty of observing the soft organs hidden inside the two shell valves and because most of the agents that affect the health of pearl oysters tend to be microscopic in nature, for example, bacteria and protozoa.

History

Investigation of a current disease outbreak should include consideration of the history of the event and an examination where possible of farm records. The following factors should be noted:

- Size and age of oysters affected
- Origin of oysters, for example, from another farm or hatchery
- Date of start of the problem
- Signs of disease
- Number of sick and dead oysters to date
- Number of oysters in group
- Recent management changes imposed on the affected group in the past 3-4 months
- Recent environmental changes, e.g. rain events, cyclones
- Recent introductions to farm
- Extent of problem on farm; are other groups affected?
- Identification of stresses that have occurred in past months prior to the onset of the disease problem

Farm Records

Farm records are an essential component of good management and are particularly important in identifying the possible causes of disease events and losses. Such records are also particularly important if future losses and drops in production are to be avoided.

Farm records may be generally divided into *Environmental Records* and *Management Records*.

Environmental Records

These include:

- ***Water temperature.*** Daily maximum and minimum, especially when extremes of temperature are anticipated

- ***Weather.*** Major changes in weather events, including cyclones, rainfall
- ***Water quality*****.** Silt, suspended solids, algal blooms, salinity
- ***Accidents.*** Nearby accidents, for example, oil spills, industrial discharges

Management Records

These include;

- ***Growth rate.*** Periodic measurement of shell length to establish growth performance
- ***Oyster introductions.*** All introduced oysters should be isolated so that any sickness or deaths are readily recorded and investigated
- ***Oyster cleaning.*** The degree of fouling, the number of oysters dead or sick on cleaning, the water pressure used in sprays and the operators of the cleaning machines
- ***Pearl seeding operations.*** Nuclei data, the number of oysters seeded, number of oysters dying within six weeks of seeding and the names of technicians undertaking seeding on particular groups of oysters
- ***Pearl harvest data.*** Number of pears harvested /number of pearls seeded, the number of quality pearls produced per batch seeded and the mortality rate of the original batch seeded

All records should be compared against previous production data and/or industry standards. If farm production is decreasing, the reason should be investigated.

Stress in Pearl Oysters

A sound understanding of the role of stress is essential if one is to understand the cause or causes of sickness, disease and deaths in pearl oysters.

Stress in pearl oysters may be caused by any variation from the normal range of conditions necessary for their optimal maintenance, growth and reproduction. Stresses may be categorised as ranging from slight to severe in intensity and may be either singular or multiple. Multiple stresses tend to be additive. Severe stress or the combined effect of multiple stresses usually leads to secondary bacterial infections. These in turn result in acute and/or chronic sickness, and death may follow.

Some examples of both environmental and management stresses in pearl oysters follow:

Environmental Stressors

Temperature. Temperatures less than approximately 21^{o}C and greater than approximately 31^{o}C may induce severe stress in pearl oysters.

Low dissolved oxygen. Low oxygen levels in the water may occur as a result of stratification or may be associated with decaying algae. Low oxygen levels in water may severely stress the oyster.

Silt and suspended solids. High silt levels may arise as a result of heavy rains or large tidal movements. Silt impedes feeding activity. Accompanying high bacterial loadings may favour secondary bacterial infection.

Blue-green algal blooms. Blooms of blue-green algae may deplete oxygen levels, may impede water flow and may be toxic.

Low salinity. Rapid large scale changes in salinity are detrimental to pearl oysters. Prolonged low salinity may also be detrimental, especially to spat and juvenile oysters, favouring bacterial colonisation.

Climatic events. Very strong currents may cause panels of oysters to rise parallel to the surface with possible reduction of optimal growth conditions. Cyclones may result in loss of panels and smothering by sand on the sea floor.

Pollutants. Numerous pollutants adversely impact on pearl oysters resulting in stress, secondary bacterial infection, debility and death. These include petroleum products, detergents, sewage, bilge water and anti-fouling boat paints.

Management Stressors

Excessive marine fouling. Fouling occurs on net panels and on oysters themselves. Fouling reduces water flow, oxygen, food availability and discharge of wastes.

Overcrowding. In small baskets or panels, excessive numbers of spat and juveniles may incur starvation and lowered oxygen levels. Similarly effects may be seen with overstocking of adults.

Bacterial fouling of water. Bacterial proliferation may occur in water in transport tanks on ships and in land-based holding or rearing tanks. This is generally due to poor hygiene and to low water exchange rates. Exposing oysters on long lines to biofouling wastes following cleaning may also result in high bacterial loadings.

Rough handling and trauma. Excessive physical force may severely stress oysters and may result in damage to the fragile tissues inside the shell. Dropping oysters on a hard surface, constant pounding of oysters out of water in small boats in rough seas, forcing oysters open and breaking the shell edge and excessive water pressure on cleaning, especially in small oysters, are examples of excessive physical force, causing trauma and stress.

POST-MORTEM EXAMINATION AND GROSS PATHOLOGY

Routine Examination of Oysters

Unless circumstances dictate otherwise, oysters are only seen during recurrent husbandry procedures such as cleaning of shells and pearl seeding and harvest operations. Examinations at this time may not be critical and diseased oysters may go unnoticed. Oysters from populations reported to be “sick”, or with a history of mortalities may show subtle clinical signs, especially in the recovery phase. Commonly, mortalities are detected well after the initial event. In such cases, dead oysters characterised by the presence of empty shells, or shells containing putrefying remnants of tissue may be the sole evidence of a disease event. Such samples are generally of no value for diagnostic purposes.

External Shell Valves

The examination of oysters should also include a detailed evaluation of the external shell valves, noting shell growth and development and the occurrence and impact of fouling and boring organisms on the shell structure. Aspects of shell abnormalities are covered under External Skeleton (Shell Valves).

Gross examination of intact and opened oysters combined with critical examination of the organs and tissues provides the best clinical and gross pathological information. This approach also allows for the collection of fresh, appropriate samples for laboratory examination.

Post-mortem Procedure

- Randomly select a minimum of six sick, but not dead, oysters from the affected group
- Perform a post-mortem examination as soon as possible after these oysters are collected

Organs and Tissues

With a long bladed, sharp knife inserted between the shell valves, cut through the adductor muscle. The two shell valves will open to display the soft organs and tissues. Examine each organ methodically and note abnormalities, for example.

- Shell nacre for retracted mantle and fouling
- Mantle for retraction
- Soft organs for wasting
- Stomach and intestine for presence of ingesta
- Digestive gland; gills; adductor muscle; heart; foot/byssus; gonad

The colour, size, texture, consistency and turgidity of organs and tissues, including the cut surfaces, should be assessed as part of the gross examination. In the freshly killed animal, mantle tissue should retain contractility and be translucent. The developed gonad should be conspicuous as a pale white or yellow-white, turgid structure in the body of the animal. The intestinal tract should contain food material. In diseased oysters, mantle tissues may be pale, gonadal development may be suppressed and visceral organs may be atrophied or smaller than normal.

Internal Shell Surface

The gross examination should carefully examine the internal shell surface, especially the margins of the mantle to determine the extent, if any, of mantle retraction and the deposition of ostracum and fouling material on the nacreous surface. Brownish discolouration of exposed nacre associated with the retracted mantle may be a feature of such oysters (Figures 19, 20, 21).

Wedge-shaped regions of discolouration may be seen commonly in diseased oysters. These regions are associated with local or regional lesions in the mantle resulting in local retraction and dysfunction, with deposition of ostracum on the nacre, colonisation by epiphytic organisms and accumulations of detritus. Evidence for prolonged mantle retraction may be seen as a distinct line on the shell surface (Figure 22). Subsequent laying down of shell by the mantle over these regions may result in a distinct ridge in the shell, giving rise to the term “double back” as described previously (Figure 18).

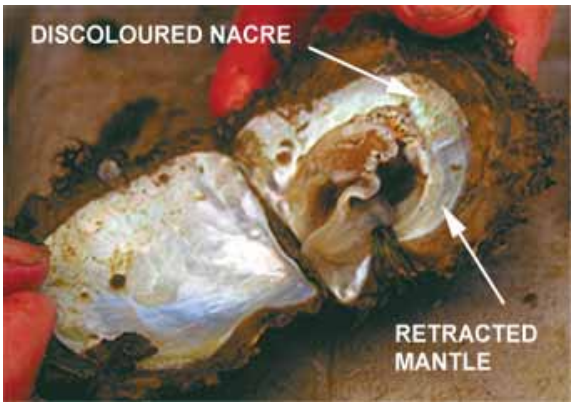

Figure 19. Diseased oyster showing marked mantle retraction and brown discolouration of nacre caused in part by colonisation by marine epiphytes in marginal zone of mantle normally covered by healthy, expanded mantle tissue and in part by deposition of conchiolin on nacre.

Figure 20. Severe discolouration of nacre (arrows) by recently deposited conchiolin associated with prolonged retraction of mantle.

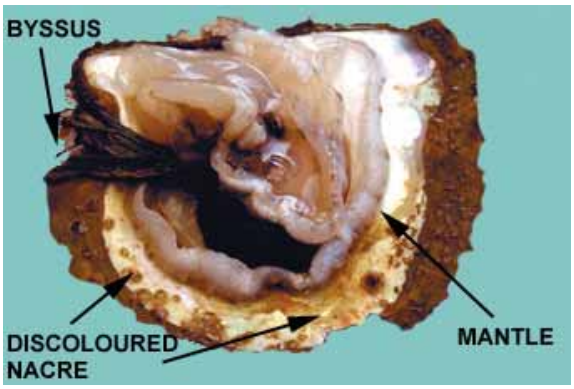

Figure 21. Prolonged retraction of mantle in chronically diseased oyster, with nacre discoloured by recently deposited conchiolin. Note poor development of visceral mass.

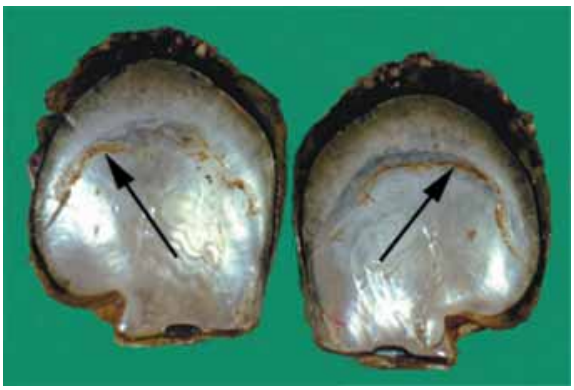

Figure 22. Internal surfaces of shell valves of mature *P. maxima* showing distinct lines of discoloured nacre (arrows) associated with prolonged mantle retraction and deposition of conchiolin which will ultimately result in a "double back" oyster.

Histopathology

As noted, the histological or microscopic examination of organs and tissues at a diagnostic laboratory forms a critical component of the diagnostic examination on diseased oysters. Successful interpretation of histological changes necessitates the rapid collection and thorough preservation of organs and tissues.

For histopathology, place a small piece of each organ in 10% seawater formalin, or place the whole oyster in a bucket of 10% seawater formalin as described under "Techniques and Stains" (Section 4). Efforts must be made to ensure adequate penetration of fixative into the tissues of the oyster. In small shells, this can be achieved in the whole oyster by breaking a piece of the shell at the margin to allow entry of fixative. Larger oysters are best opened prior to fixation and several deep cuts made into the tissues, especially the digestive gland, stomach and gonad, to ensure entry of the fixative into the tissues.

EXTERNAL SKELETON (SHELL VALVES)

Functional Anatomy and Normal Shell Formation

The shell structure of *P. maxima*, in common with other bivalve molluscs, consists of three principal layers; the outer periostracum, a prismatic layer and the inner calcite-ostracum or nacreous layer. Each layer is produced by specialised cells in the marginal zone of the mantle, with apposition of calcite on the periostracum resulting in radial growth and enlargement of the shells.

The periostracum consists of a firm sheet of the scleroprotein conchiolin which, in undamaged shells forms brown, semi-translucent finger-like projections around the edge of the shell (Figure 10) and extends inside the shell margin as a shiny brown zone.

The prismatic layer consists of aggregates of calcite crystals laid in a matrix of conchiolin, while the inner nacreous layer consists of sheets of calcite laid down between thin membranes of conchiolin.

Specialised cells in the mantle, the hinge gland, produce the flexible hinge that unites each shell valve.

Pathology

Pathological examination of mature pearl oysters should include a critical examination of the external and the internal surfaces of each shell. Disease or stress may result in checks to shell growth, manifest as "double backs" (Figure 18). The shell surface and matrix provide habitats for a diversity of fouling and boring organisms. These may be significant causes of disease. Ideally, from a farming perspective, inner surfaces of each shell valve should be free of blemishes and the external surface of the shell should be free of organisms that may invade the shell matrix, for example, boring molluscs, mudworms and boring sponges.

Fouling Organisms

A diverse range of algae and invertebrates are capable of colonising the shell surface of the pearl oyster (Figure 23). Algal growth on the nacre of the inner shell surface is indicative of earlier disease and prolonged mantle retraction. Heavy external epiphytic fouling alone can restrict water flow with consequential decreased availability of feed, oxygen and restricted expulsion of waste products. Fouling can restrict full closure of the shell valves. Fouling necessitates frequent cleaning in farmed oysters.

Boring Organisms

Numerous marine organisms representing a diverse range of taxa including molluscs, mudworms and sponges, may invade the shell matrix or penetrate beneath the conchiolin layer around the lip of the shell to produce blemishes. Full thickness penetration of the shell valves by such

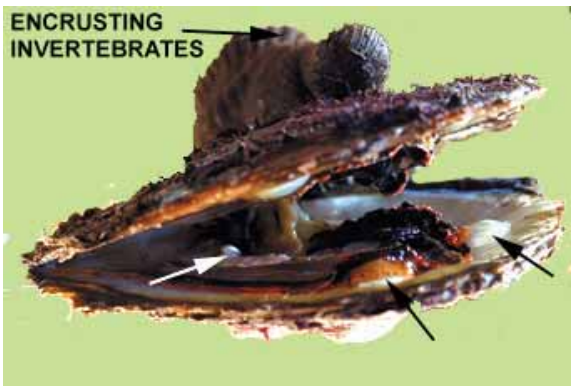

Figure 23. Growth of invertebrates on outer surface of shell valve. Note shell has been opened to expose round pearl (white arrow) and half pearls (black arrows) for harvesting.

organisms may result in local or systemic infestation of the oyster and may be accompanied by secondary invasion by microbial agents, especially bacteria.

Boring sponges cause a condition known as "red bum" (Figures 24, 25) and may render the shell so fragile such that it may collapse during handling and cleaning.

Boring bivalves may create numerous holes and tracts within the shell matrix, substantially weakening the shell and often penetrating into the nacreous layer (Figures 26, 27). Full thickness penetration of such organisms to the nacre may result in expansive, irregular areas of dark discolouration (Figures 28, 29, 30, 31).

A dynamic interaction exists between boring organisms and the deposition of nacre by the oyster. Damage resulting to the shell matrix and erosion of the nacreous surface is subsequently covered by deposition of nacre. This process is frequently manifest as irregular raised, nodular dark regions on the inner surface of the shell valve, overlaid by a thin layer of nacre indicating recent damage, to heavy deposits of nacre with a more normal appearance, indicating deposition over a prolonged period of time.

Figure 24. External surface of shell valve of mature *P. maxima* showing extensive invasion of the shell matrix by boring sponge. The orange-red coloration gives rise to the common name of "red bum". Invasion results in marked thinning and fragility of the shell.

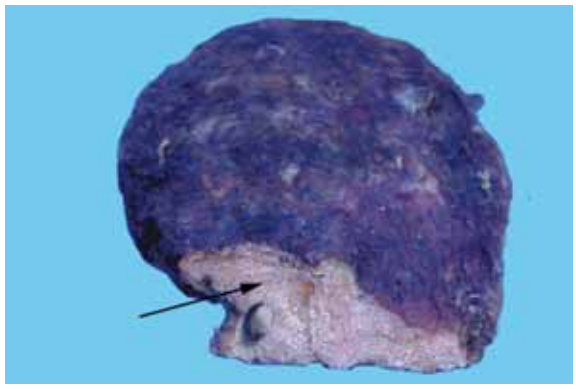

Figure 25. External surface of mature *P. maxima* showing a further example of invasion of shell matrix by boring sponge (arrow).

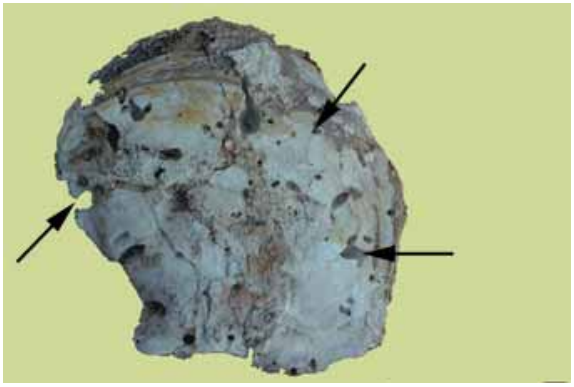

Figure 26. Tracts (arrows) left by invading bivalve molluscs following penetration of shell matrix.

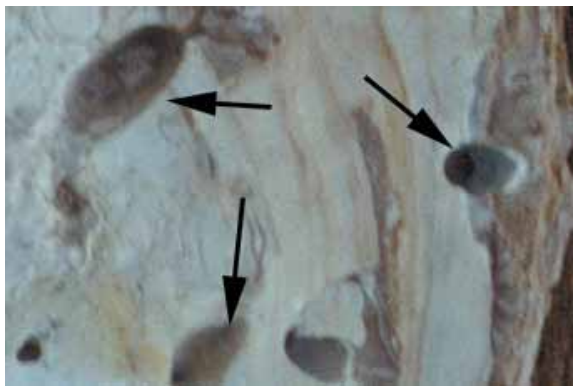

Figure 27. Detail of tracts in shell matrix (arrows) caused by invading bivalve molluscs.

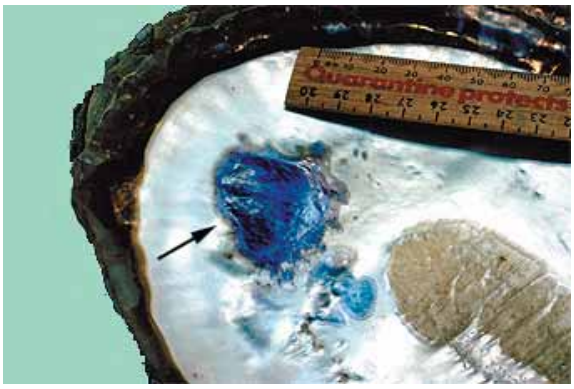

Figure 28. Internal surface of shell with large blemish (arrow) resulting from invasion of shell matrix by boring bivalve mollusc.

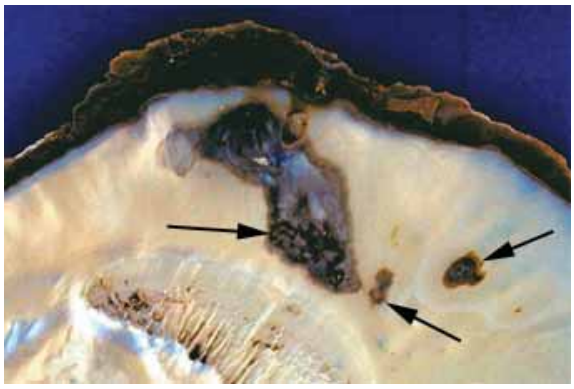

Figure 29. Internal surface of shell with irregular region of discolouration and erosion of nacre (arrows) associated with invasion of shell matrix by multiple boring bivalve molluscs.

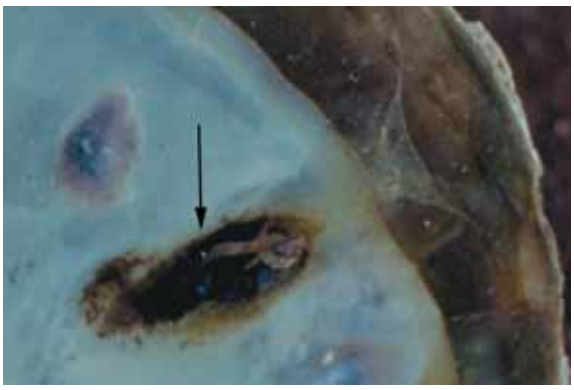

Figure 30. Dark, detritus filled "blister" (arrow) under nacre caused by invasion of polychaete "mudworm" in mature oyster.

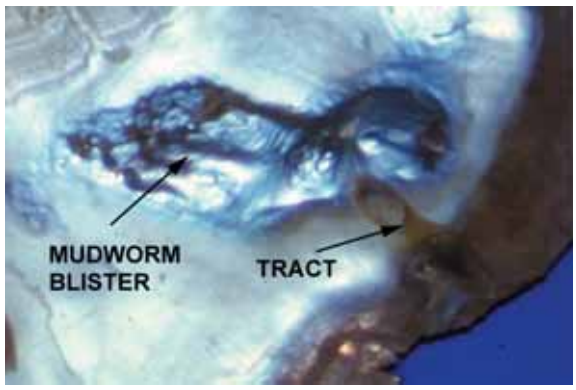

Figure 31. Detail of mudworm blister showing irregular accumulation of detritus under nacre and tract leading from conchiolin layer to blister.

SECTION 2

FUNCTIONAL ANATOMY, HISTOLOGY AND HISTOPATHOLOGY

THE IMMATURE OYSTER: LARVAE

Functional Anatomy and Histology

The progressive histological development of the larval stages of *P. maxima* is shown in Figures 32 to 45. With increasing age, cellular differentiation into histologically recognisable organs and tissues occurs. The simple velar stage at two days of age comprises shell, velum and stomach (Figure 32), with the development of oesophagus, mantle and digestive gland by day five; adductor muscle by days six-seven and foot and alimentary tract by day 14 (Figure 41). By day 60, all the morphological features of the juvenile oyster are present (Figures 44, 45).

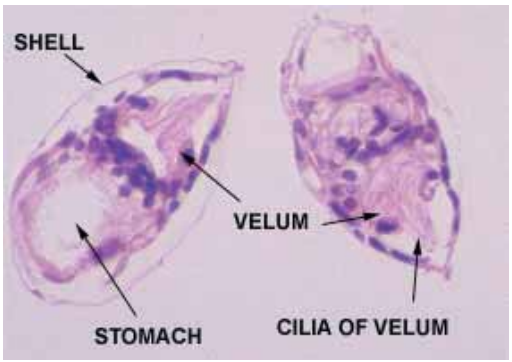

Figure 32. Section of two-day-old larvae.

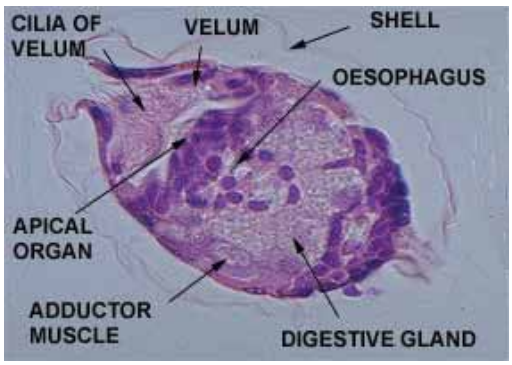

Figure 33. Section of four-day-old larva.

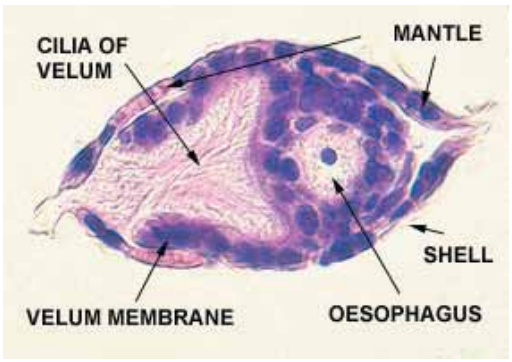

Figure 34. Section of five-day-old larva.

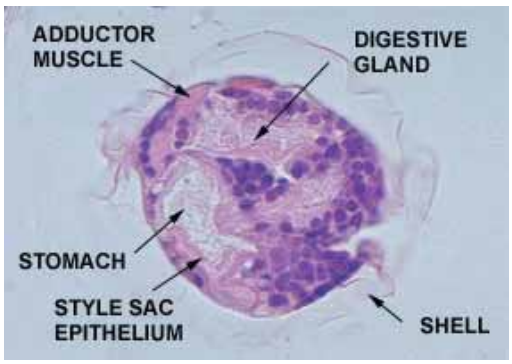

Figure 35. Section of six-day-old larva.

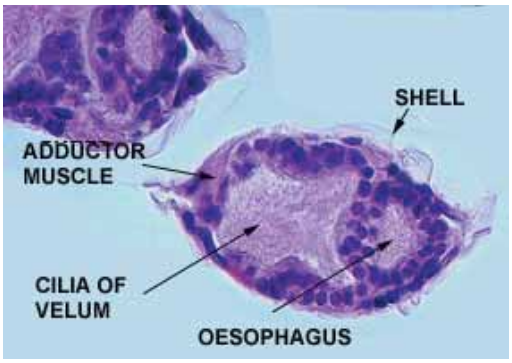

Figure 36. Section of seven-day-old larvae.

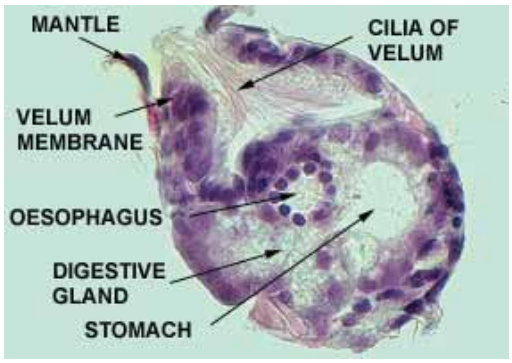

Figure 37. Section of nine-day-old larva.

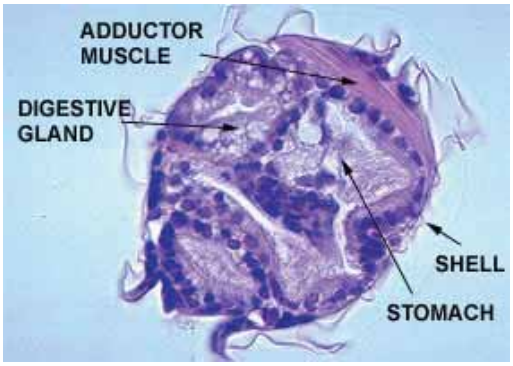

Figure 38. Section of 10-day-old larva.

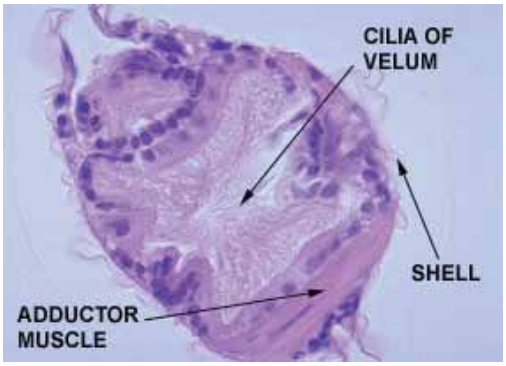

Figure 39. Section of 11-day-old larva.

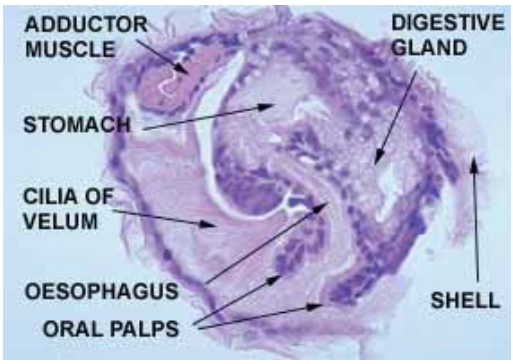

Figure 40. Section of 13-day-old larva.

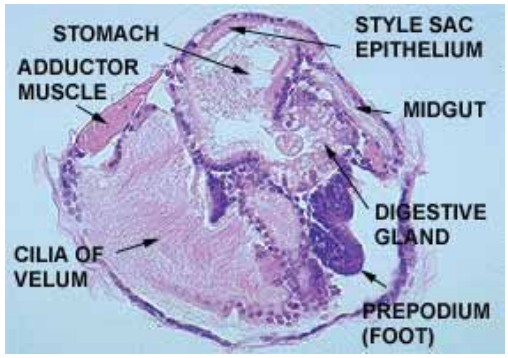

Figure 41. Section of 14-day-old larva.

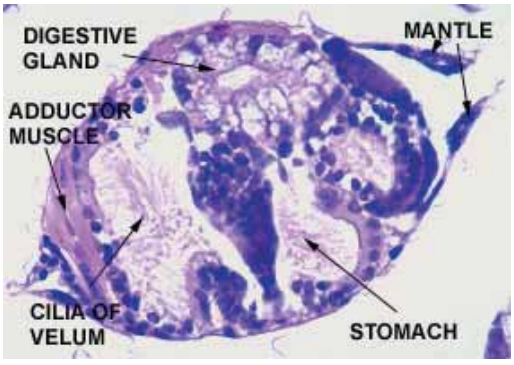

Figure 42. Section of 15-day-old larva.

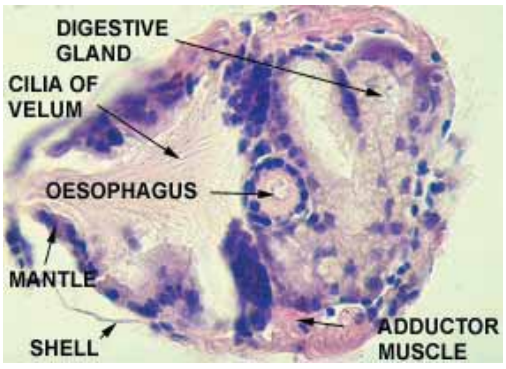

Figure 43. Section of 25-day-old larva.

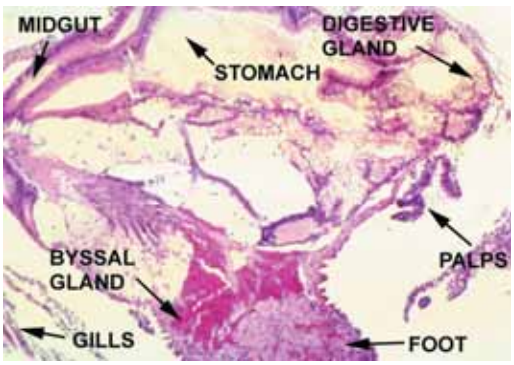

Figure 44. Section of 62-day-old larva.

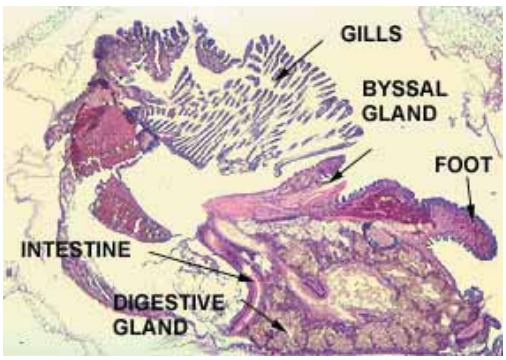

Figure 45. Section of 64-day-old larva.

Pathology and Histopathology

Larval *P. maxima* are particularly susceptible to bacterial infectious and/or adverse environmental conditions which may predispose the larvae to bacterial infections. Successful histological interpretation depends on rapid fixation of larvae in the early stages of disease.

Degenerative Lesions

Hydropic Degeneration

Hydropic degeneration or the accumulation of water in cells of the developing digestive gland may occur in larval oysters (Figure 46).

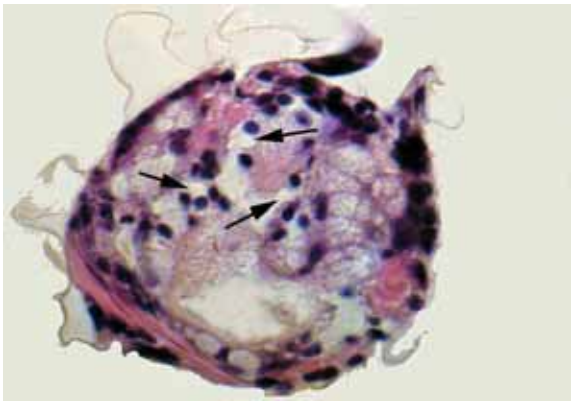

Figure 46. Hydropic degeneration, seen as clear, fluid-filled cytoplasm in the digestive gland of a 12-day-old larval *P. maxima* (arrows).

Infectious and Commensal Organisms

Bacterial Infection and Inflammation

Bacterial invasion of larval oysters, especially associated with marine vibrionic organisms, may occur rapidly in populations and is usually accompanied by necrosis of tissues (Figures 47, 48). Advanced bacterial necrosis results on dissolution and fragmentation of the larvae, and may be associated with colonisation by saprophytic ciliated protozoa (Figure 49). Typically, numerous bacteria may be seen proliferating within the body cavity or intestine of infected larvae (Figure 50). Infection may result in high mortalities.

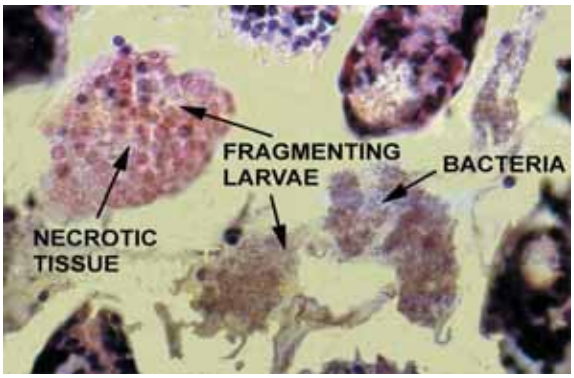

Figure 47. Bacterial necrosis in 2-day-old larval *P. maxima* showing advanced necrosis and fragmentation of larvae (arrows).

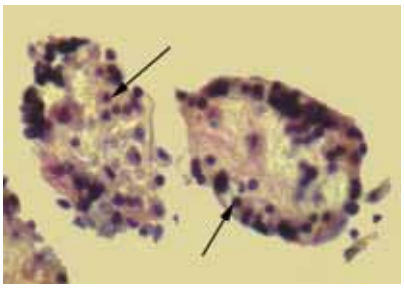

Figure 48. Low magnification view of 5-day-old larval *P. maxima* showing dissolution of tissue structure, and pyknosis or condensation of nuclei (arrows) resulting from bacterial infection.

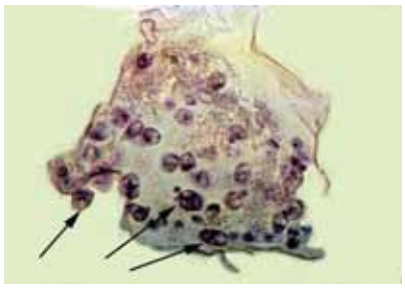

Figure 49. Advanced bacterial necrosis and fragmentation of 10-day-old larval *P. maxima*, with invasion of larval tissue by saprophytic ciliated protozoa (arrows).

Figure 50. Bacterial necrosis in 11-day-old larval *P. maxima*. Note large numbers of bacteria (arrow) proliferating within tissues.

THE IMMATURE OYSTER: SPAT

Functional Anatomy and Histology

Spat are generally considered to be post-settlement animals up to approximately 50 mm. The histological structure of spat of *P. maxima* 55 days and 5 months post-settlement is shown in Figures 51, 52 and 53 and Figures 54, 55 and 56, respectively. Histologically, the structure of the organs and tissues of the spat become progressively analogous to those of the mature oyster as the spat grow.

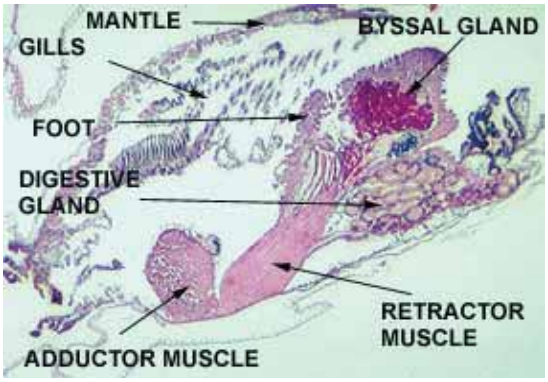

Figure 51. Histological structure of spat of *P. maxima* 55 days post settlement.

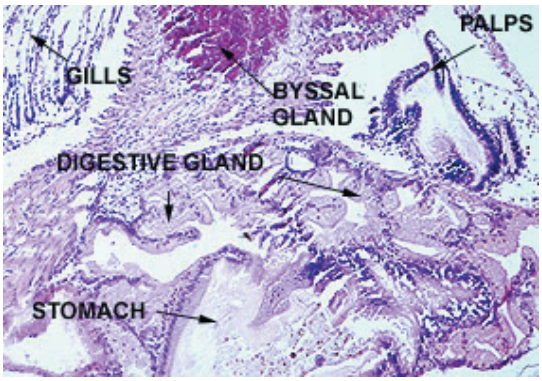

Figure 52. Histological structure of spat of *P. maxima* 55 days post-settlement.

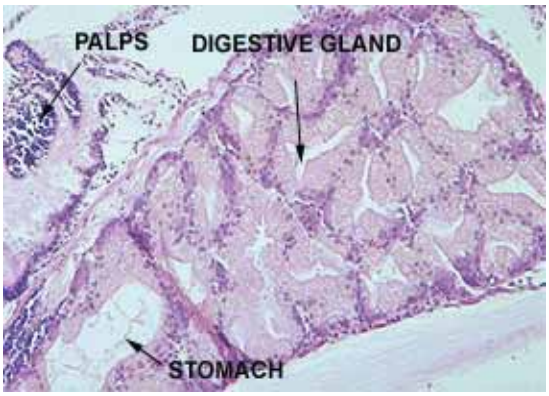

Figure 53. Histological structure of spat 55 days post settlement.

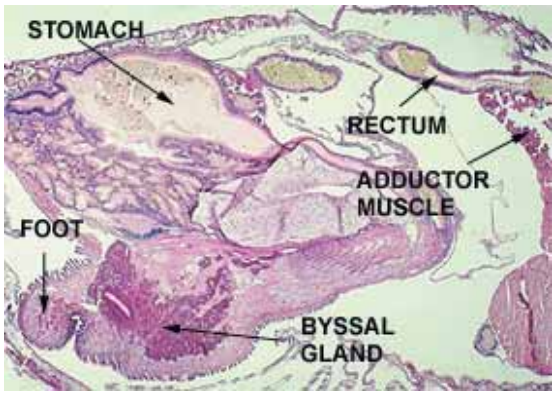

Figure 54. Histological structure of spat at 6 months of age.

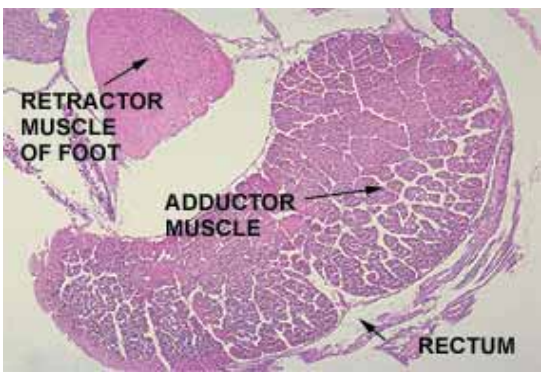

Figure 55. Histological structure of spat at 6 months of age.

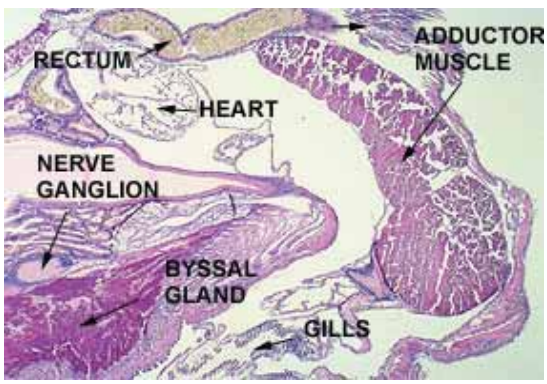

Figure 56. Histological structure of spat at 6 months of age.

Pathology and Histopathology

Spat generally exhibit a range of lesions and histological changes similar to their mature counterpart which are described under the respective organs and tissues of the mature oyster. Examples of conditions commonly encountered in spat are described here.

Degenerative Lesions

Dilation and Epithelial Attenuation of the Digestive Gland

Dilation of the digestive gland diverticula and flattening or attenuation of the epithelium, sometimes accompanied by irregular cystic degeneration resulting from breakdown of the glandular architecture and coalescence of adjoining diverticula occurs in spat (Figures 57, 58). This change may occur in mature oysters where it is generally focal or regional. The cause and the pathogenic significance are unclear and the changes are generally seen in the absence of clinical signs. Further, affected spat appear to grow and develop normally. In edible oysters, dilation and epithelial attenuation may be associated with tidal movements and feeding patterns in the intertidal zone (Elston 1999). Flattening of the epithelial cells may also occur as a result of reduced feeding associated with periods of cold water temperatures. Similar changes have been associated with extremes of high water temperature. Attenuation of the epithelium in *P. maxima* may also occur during periods of abundant phytoplankton, suggesting that toxic elements in certain plankton species may result in reduced feeding activity and a decrease in epithelial height, or they may exert a direct toxic effect. Negri *et al.* (2004) associated mortalities and digestive gland pathology in *P. maxima* with exposure to the bloom-forming alga *Trichodesmium erythraeum.*

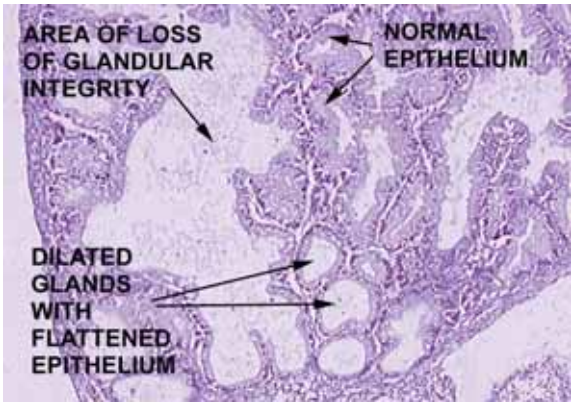

Figure 57. Lower magnification view of spat showing breakdown of normal glandular architecture, dilation of diverticulae and severe flattening of epithelium.

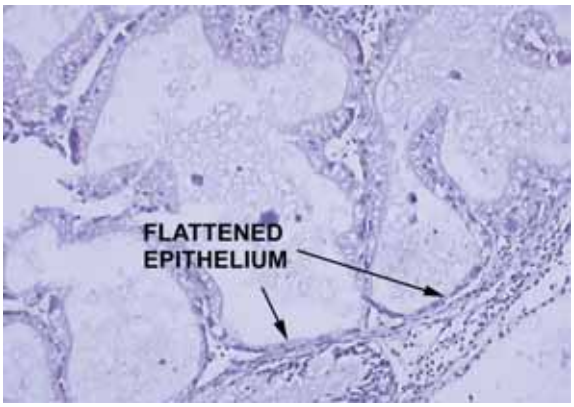

Figure 58. Higher magnification view of flattened, attenuated epithelium of digestive gland of *P. maxima* spat, accompanied by collapse of normal architecture of glands.

Infectious and Commensal Organisms

Viruses and Viral Infections

Spat of *P. maxima* are susceptible to papova-like viral infections, characterised histopathologically by hypertrophy of infected cells and the presence of large intra-nuclear inclusion bodies, especially in the epithelium of the palp (Figure 59), but also in the cells of the gill. The histopathology of these infections are further discussed under palp in the mature oyster.

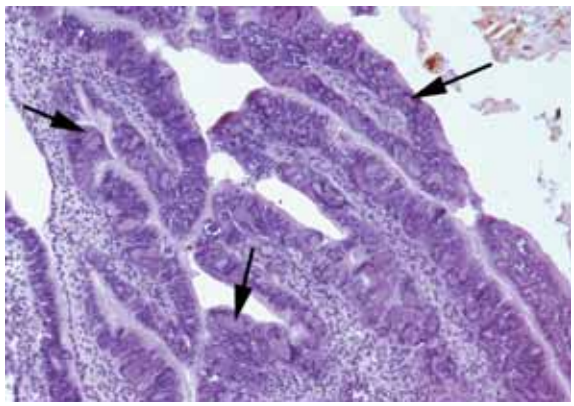

Figure 59. Lower magnification view of palp of spat with numerous large basophilic papovavirus-like inclusion bodies in the epithelium (arrows).

Bacteria and Bacterial Infections

Bacterial infection of spat may result in an intense haemocytic infiltrations in or adjacent to organs and tissues including palps (Figure 60), intestinal tract (Figures 61, 62, 63) and myocardium (Figure 64).

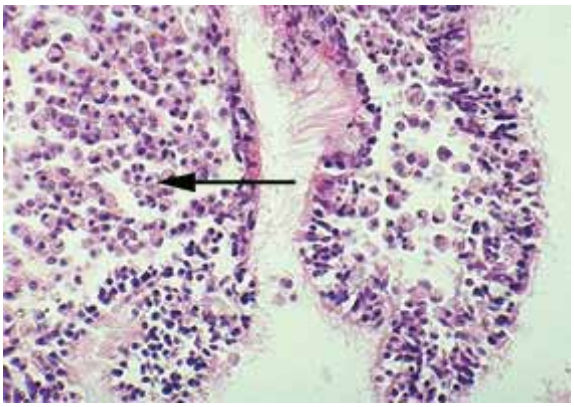

Figure 60. Palp of larval *P. maxima* showing intense haemocytic accumulation in stromal tissues beneath the epithelium (arrow), resulting from infection by marine vibrionic bacteria.

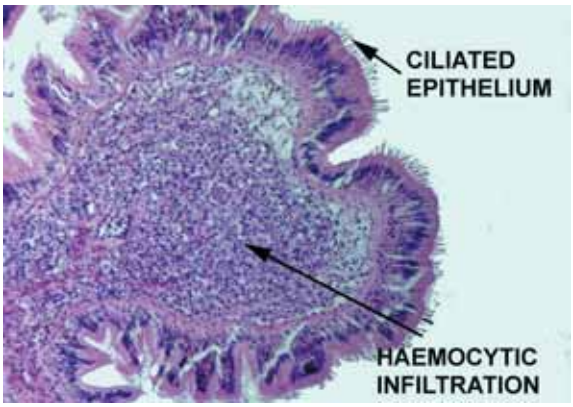

Figure 61. Intense accumulation of haemocytes in internal fold of distal intestine of spat resulting from infection by marine vibrionic bacteria.

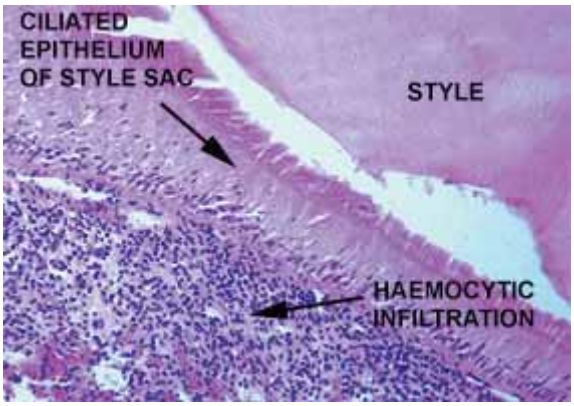

Figure 62. Intense haemocytic infiltration of sub-epithelial tissue adjacent to the style sac.

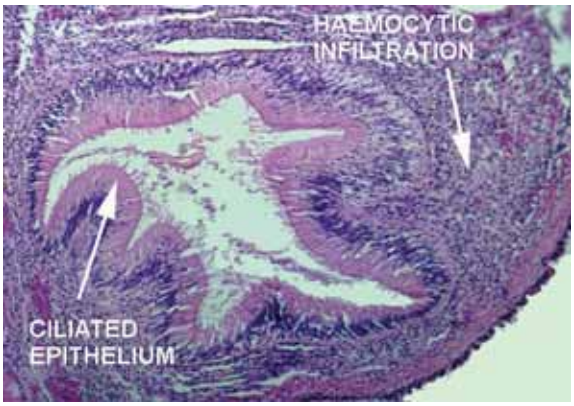

Figure 63. Intense haemocytic infiltration around the intestine of *P. maxima* spat, a typical in response to bacterial invasion.

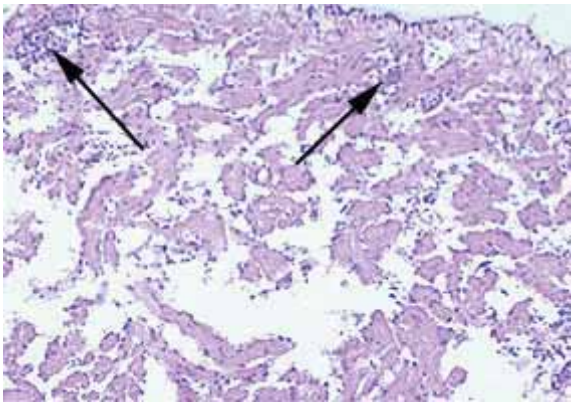

Figure 64. *P. maxima* spat showing occasional focal haemocytic accumulation (arrows) in myocardial tissues.

Focal Parasitic (Larval Cestode) Granulomas

Focal parasitic granulomas occur occasionally in mature oysters and are generally considered an incidental finding of little consequence to the health of the oyster. Infections of spat are recorded infrequently, with larval metazoa morphologically consistent with metacestodes associated with single or multiple focal granulomas in the stroma beneath the epithelium of the oesophagus, stomach, palps or mantle (Figures 65, 66). A high prevalence of infection may occur in the population, especially if spat are reared on the sea floor and exposed to faecal contamination from sharks or rays. Infection may be accompanied by bacterial invasion of tissues and an associated cellular inflammatory response.

Parasitic granulomas caused by larval cestodes are well recognised in bivalve and gastropod molluscs. The parasites are generally considered to be lecanicephalid metacestodes of the genera *Tylocephalum* or *Polypocephalus*, although other species may be involved. The adult stages of these tapeworms reside in the intestine of sharks or rays. Mature eggs pass from the shark or ray host in its faeces and hatch to form a motile coracidium. The coracidium invades the tissues of the intermediate molluscan host. In suitable intermediate hosts, the coracidium develops into a metacestode which, if eaten by the shark or ray definitive host, develops into a mature tapeworm.

In spat, the intense inflammatory reaction to the parasites suggests that *P. maxima* may not be a particularly suitable intermediate host for the cestode parasite. In high numbers, the peri-oesophageal parastic granulomas may impede passage of ingesta.

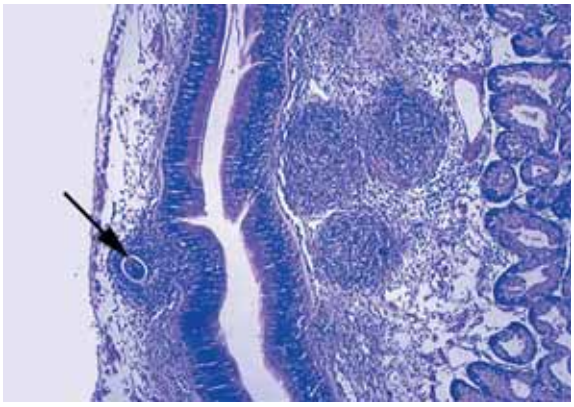

Figure 65. Lower magnification view of oesophagus of spat of *P. maxima* showing multiple focal granulomas associated with metacestodes (arrow) of the lecanicephalid cestodes *Tylocephalum sp*. or *Polypocephalus sp.*

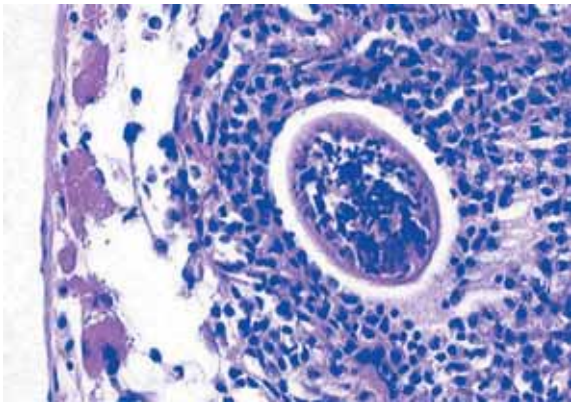

Figure 66. Higher magnification view of a metacestode typical of the lecanicephalid cestodes *Tylocephalum sp.* or *Polypocephalus sp.,* in the centre of a focal granuloma adjacent to the oesophagus in a spat of *P. maxima.*

THE MATURE OYSTER

MANTLE

Functional Anatomy and Histology

The ***mantle*** or ***pallial lobes*** of *P. maxima* comprise two symmetrical, thin, flap-like expansions of the outer body wall of the oyster. Externally, each pallial lobe lies adjacent to the internal surface of its respective shell valve.

Internally, the lobes form and enclose the pallial or mantle cavity. With the lobes extended, the mantle tissue is semi-opaque and numerous fine radiating muscles arise from the pallial line extending to the margin. The margin is thickened and pigmented, giving the characteristic colourings to the oyster in life.

Within the pallial cavity, the gill arch is weakly adherent to the medial margin of the mantle via the cuticular zone, histologically recognisable as a "zipper"-like structure which extends in a crescenteric manner from the postero-ventral aspect of the oyster, terminating near the palps (Figures 67, 68).

Central to the crescent shaped attachment of the gill to the mantle, the mantle continues as a flattened sheet of tissue to terminate on the posterior, ventral and anterior aspects of the adductor muscle. At the anterior-dorsal aspect of the visceral mass, the pallial lobes unite with the base of the outermost labial palps. In this anterior-dorsal segment of the oyster, the pallial lobes enclose the palps, foot, byssus and oral cavity.

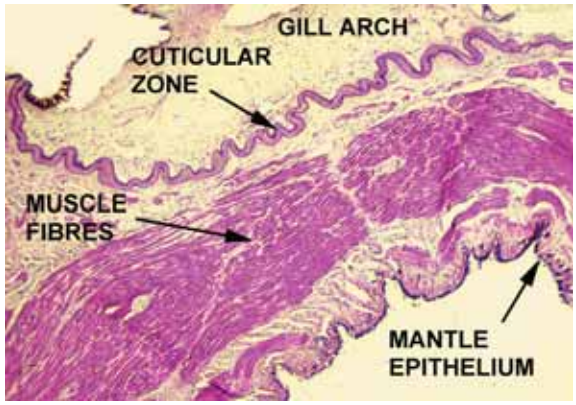

Figure 67. Lower magnification view of base of medial mantle showing cuticular zone and the junctional region between the gill arch and mantle.

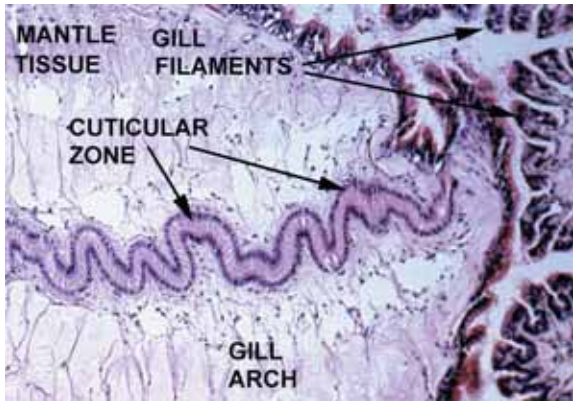

Figure 68. Cuticular zone at base of gill.

The pallial lobes are separated anteriorly, ventrally and posteriorly but unite dorsally underneath the hinge-line of the shell. The mantle cavity is divided at the termination of the gills and the inner mantle folds into the larger, central inhalant chamber and a smaller exhalant chamber near the anus.

The mantle is a sensory and neuromuscular organ, richly supplied with haemolymph sinuses and capable of considerable contraction and expansion. The mantle performs a number of functions including secretion of the shell and formation of inhalant and exhalant currents via the inhalant and exhalant chambers.

Externally, each pallial lobe terminates centrally at the pallial line, the line of attachment of the mantle with the shell. Internally, within the pallial cavity, on the extreme postero-ventral face of the gills, each pallial lobe arises as an indentation or fold of tissue of the mantle, the pallial fold, from the extreme postero-ventral face of the gills (Figure 13). The mantle covers the visceral organs within the pallial cavity.

Grossly, each pallial lobe shows regional differences in anatomy and physiological function and is generally divided into the peripheral ***marginal zone*****,** the muscular ***central zone*** and the ***isthmus*** where the lobes are joined (Figure 12).

The mantle is composed mainly of connective tissue traversed by muscle bundles, nerve fibres and numerous haemolymph sinuses and is covered on both inner and outer surfaces by epithelium. Considerable regional diversity in histological structure exists in the mantle of *Pinctada* species and is described in detail by Herdman (1904), Dix (1973a), Jabbour-Zahab *et al.* 1994 and Garcia-Gasca *et al.* (1994).

The Mantle and Pearl Production

The mantle is of prime importance in pearl aquaculture. Half pearls (Figures 16, 17) are produced by the deposition of nacre over a nucleus attached to the shell surface. For round pearl production, mantle grafts are inserted, together with a nucleus, into the visceral tissue of the oyster to form the cultured pearl. In this operation, the mantle cells proliferate to form the pearl sac around the nucleus, with subsequent deposition of nacre on the nucleus to form the pearl (See The Pearl Sac).

Marginal Zone

The free pallial margin or free edge of each pallial lobe is thickened and in most cases deeply pigmented and fringed with branched tentacles. The mantle edge terminates in three folds, the outer, middle and inner folds (Figure 69). The outer and inner folds are thin, membranous and have pigmented papillae. The outer fold is smaller, while the middle fold is quite conspicuous.

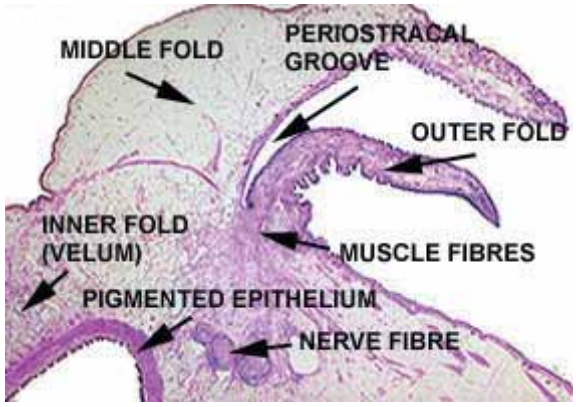

Figure 69. View of marginal zone of mantle showing inner, middle and outer folds.

The outer fold is the smaller of the three folds and bears papillae which inter-digitate with the inner surface of the shell and forms the true pallial edge. The inner fold bears flattened palmate papillae, the pallial veil or velum, and projects inwards at right angles from the mantle edge, so that the veil of one pallial lobe stretches toward and meets that of the other, forming the mantle cavity. Along the posterior margins of the body, from the pallial fold to the posterior edge of the hinge, the papillae of the velum become reduced in size and simpler in form.

In the living animal, the edges of the pallial veil or velum, formed by the inner fold of the marginal zone of the mantle, are generally in contact along the median plane of the body except at two places where they gape forming the inhalant and exhalant apertures. These apertures are not readily apparent in the dead oyster or the oyster removed from water.

The inhalant aperture is variable around the middle of the ventral surface and forms the inhalant gap through which the main in-flowing current passes. The exhalant aperture is ovoid or round and localised near the posterior end, opposite the opening of the anal funnel and the supra-branchial chamber. The exhalant current serves to direct wastes and particulate matter from the animal.

Histologically, the three folds of the marginal zone show marked regional differentiation and structural differentiation related to function (Dix 1973a). Each fold extends along the distal edge of the mantle and is morphologically similar along its length. Marked differences in morphology occur, however, between folds. The middle and outer folds are separated by the periostracal groove (Figure 69). As with other bivalves the inner, middle and outer folds of *P. maxima* are primarily concerned with muscular, sensory and secretory functions, respectively.

Inner Fold

The inner fold, which forms the velum, is covered by a single layer of ciliated, pigmented columnar epithelium, with ciliation conspicuous at the tip of the fold. The inner surface contains few secretory or mucous cells, whereas goblet-shaped granular acidophilic cells and basophilic mucous cells are common in the outer epithelial surface (Figures 69, 70).

Conspicuous muscle fibres traverse the stromal connective tissues, and large numbers of acidophilic cells, ovoid in shape containing a finely granular eosinophilic cytoplasm and small, conspicuous nuclei, may be present in the inner fold.

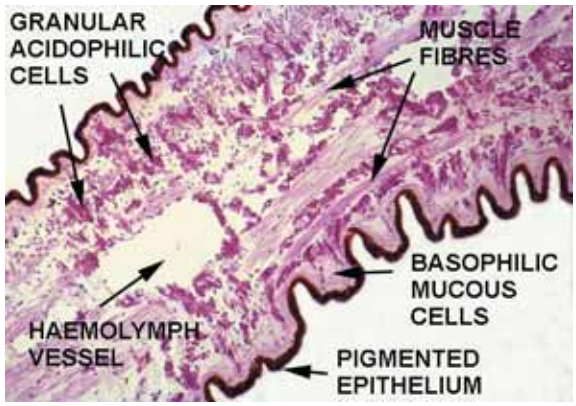

Figure 70. Inner fold of marginal zone of mantle showing heavily pigmented epithelium.

Middle Fold

Regional differentiation is present in the epithelium of the middle fold (Figures 69, 71). A strongly ciliated columnar epithelium generally lines the outer surface of the middle fold, i.e., the side forming the periostracal groove, with cells near the base appearing cuboidal, with a central nucleus and brush border. Cells lining the inner side of the middle fold appear similar to those of the inner fold, but are non-pigmented and with lightly basophilic cytoplasm. Non-pigmented cells of the outer surface are ciliated and similar in size to the pigmented cells.

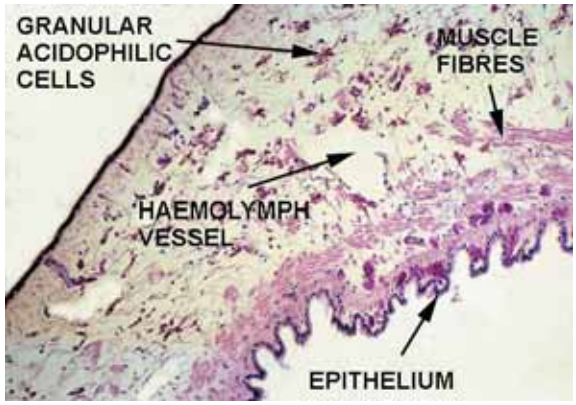

Figure 71. Middle fold of marginal zone of the mantle.

Wandering acidophilic secretory cells are reported by Dix (1973a) to be less common in the stromal tissue of the middle fold compared with the inner fold. Basophilic mucous cells are extremely abundant in the sub-epithelial areas, especially near the tip of the fold, where clear connections with the fold exterior are evident. Dix (1973a) reported that a few acidophilic cells with large granules are found scattered among the outer epithelial cells, with clusters forming in the sub-epithelium towards the bottom of the periostracal groove.

Outer (Shell) Fold

The epithelium of the outer fold (Figures 69, 72) is columnar in nature and shows marked regional differentiation with regard to height. The epithelium is not ciliated and shows variable pigmentation. Basophilic mucous cells occur below the epithelium at the

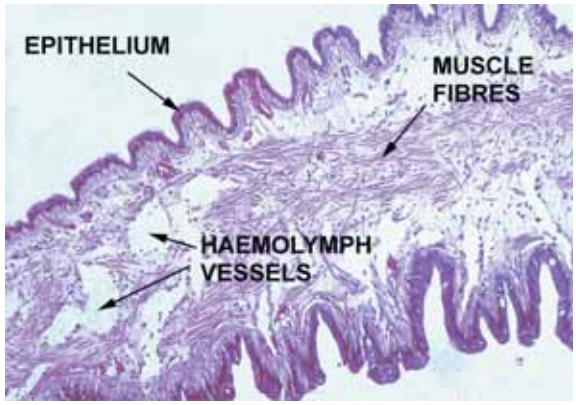

Figure 72. Outer fold of the marginal zone of the mantle showing variably pigmented, infolded epithelium and conspicuous muscle fibres.

periostracal groove and goblet mucous cells are common on the inner and outer fold surfaces. Acidophilic secretory cells with large granules are present on the outer fold surface near the tip. It is considered that the tall columnar cells at the base of the periostracal groove secrete at least part of the periostracum and part of the outer fold probably secretes the inner calcareous (prismatic) layer of the shell.

Central (Pallial Or Muscular) Mantle

The central mantle is muscular, translucent and capable of considerable contraction and expansion by the influx of haemolymph into the large vascular sinuses. Histologically, the pallial mantle consists of a thick layer of loose connective tissue traversed by nerves, haemolymph spaces and by radially orientated pallial muscles. The pallial retractor muscles together constitute a series of fan shaped muscles that radiate towards the mantle edge from a number of points of insertion on the shell, the semi-circular pallial line (Figure 11).

The central mantle is covered by low columnar epithelium on both inner and outer surfaces. The epithelium encloses muscular tissue. Differences between the epithelium on the inner and outer surfaces occur. The inner epithelium has dense cilia and melanin pigment in the cytoplasm and has deeply staining ovoid basal nuclei. In contrast, cilia and cytoplasmic pigmentation is absent in the outer epithelium, with the nuclei basal to central.

Dix (1973a) reported basophilic mucous and coarsely granular acidophilic secretory cells on both sides of the central mantle. The larger secretory cells of the outer surface occurred in the sub-epithelium as well as the epithelium.

Proximally, the central mantle extends from the pallial line where the shell is marked with muscle attachment scars. The insertions of the adductor muscle, the retractor and the levator of the foot and the pallial muscles penetrate this part of the pallial lobe. Here also, the epithelial of central mantle extends and overlies the visceral mass.

The epithelium of the outer side of the central mantle is simple low columnar. A concentrated layer of secretory cells, with basophilic mucous goblet cells overlying course granular acidophilic cells occurs beneath the epithelium (Figure 73). The connective tissue in this region is continuous with the stromal tissues of the gonad and digestive gland, and is interspersed with muscle fibres.

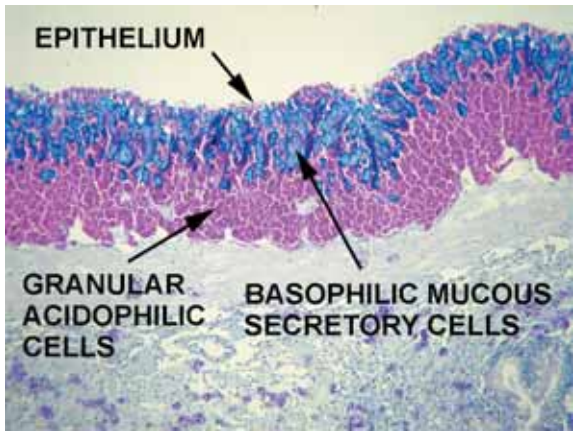

Figure 73. View of central zone of the mantle overlying gonad showing layer of basophilic mucous secretory cells below surface epithelium and underlying layer of granular eosinophilic cells.

Mantle Isthmus

The isthmus is formed by fusion of the dorsal marginal mantle immediately inside the shell hinge line (Figure 12).

The dorsal marginal mantle forms the mantle isthmus immediately inside the shell hinge line. The mantle isthmus consists of a tall, non-ciliated columnar epithelium resting on connective tissue containing sparse muscle fibres. Epithelial and sub-epithelial secretory cells are absent. The epithelium lining the dorsal isthmus is sharply demarcated from that of the inner isthmus, which resembles and is continuous with the epithelium of the central mantle.

Hinge Gland

A specialised projection of the epithelium of the dorsal margin of the mantle isthmus forms the hinge gland, a protrusion of the mantle stroma covered by a specialised secretory epithelium (Figures 74, 75). The function of this epithelium is to produce the tough, flexible proteinaceous material that forms the joint or hinge between the two shell valves.

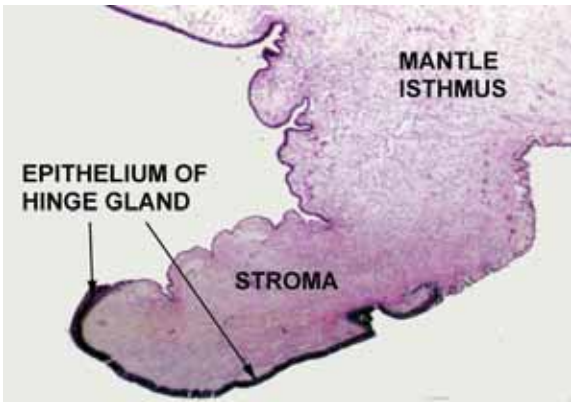

Figure 74. Lower magnification of hinge gland arising from mantle isthmus.

Pathology and Histopathology

Degenerative Lesions

Mantle Oedema

Oedema of mantle may occur commonly in populations of *P. maxima*. Local oedema beneath crater-like depressions in the mantle adjacent to the palps containing pea crabs (*Pinnotheres sp*.) occurs frequently (Figure 76) and has been described by Dix (1973b). Apart from the local oedema in the mantle caused by the pea crabs, no other pathology is associated with these organisms.

Generalised oedema may be associated with prolonged withdrawal from water and associated stress and is characterised by marked dilation of haemolymph sinusoids and irregular spongiform change in stromal tissues.

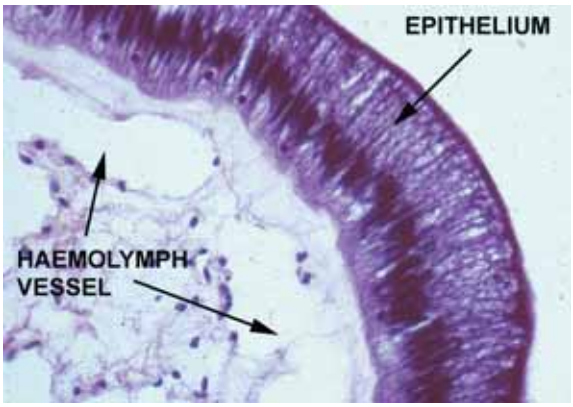

Figure 75. Higher magnification view of epithelium of hinge gland showing tall, non-ciliated columnar secretory epithelium.

Non-specific Inflammatory Lesions

Non-specific Inflammation

Non-specific inflammatory changes characterised by focal or focally extensive infiltrations of haemocytic cells in the stroma of the mantle, in some cases extending through the epithelium, occur occasionally in mature *P. maxima* but are seen more commonly in spat. Where they occur, they appear generally as a response to bacterial infection, especially marine vibrionic bacteria. Often, however, bacterial organisms are not evident histologically.

Focal or multifocal, sub-epithelial accumulations of haemocytic cells in the stroma of the marginal and central mantle, unassociated with an obvious aetiological agent,

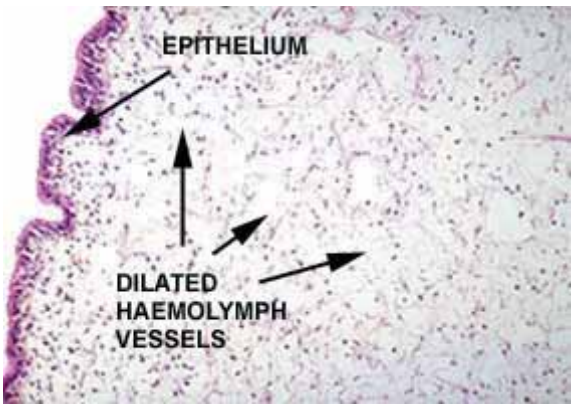

Figure 76. Oedema of mantle stroma in depression in mantle colonised by pea crab *Pinnotheres sp*. Note dilation of haemolymph vessels and spongy appearance of tissue.

may be related to mantle retraction and wedge-shaped deposition of conchiolin on the nacreous surface (Figures 19, 20, 21). This change is generally considered an indication of earlier or pre-existing localised infection by marine vibrionic bacteria.

Discrete, focal, granulomatous accumulations of inflammatory cells are seen occasionally in mantle tissues. These resemble the focal granulomas seen with metacestode infections of the mantle (Figure 77), but may show no evidence of a parasite, even following serial sectioning of the tissue. They may represent earlier parasitic invasion.

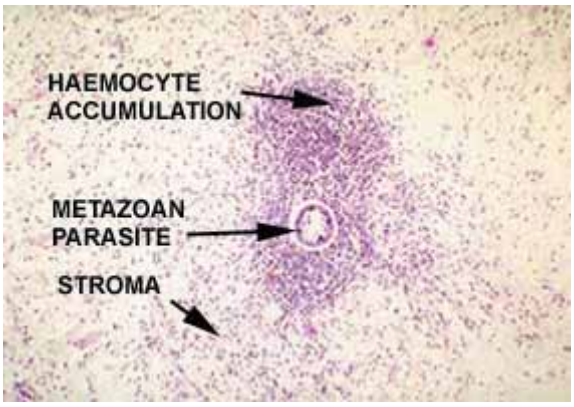

Figure 77. Focal granuloma in stroma of mantle associated with invasion of a metacestode typical of the lecanicephalid cestodes *Tylocephalum sp*. or *Polypocephalus sp.*

Infectious and Commensal Organisms

Commensal Shrimps and Peacrabs

Populations of *P. maxima* may have a high prevalence of commensal shrimps *Conchodytes sp.* (Figures 78) and peacrabs *Pinnotheres sp*. (Figure 79). These are generally found residing between the gill and mantle and do not appear to compromise the health of the oyster. The peacrabs are usually found in an irregular, crater-like depression in the mantle.

Figure 78. Commensal shrimp *Conchodytes maculatus* (arrow) inhabiting mantle region in *P. maxima*.

Internal Metazoa

Focal granulomas with peripheral fibrous encapsulation associated with metazoan parasite infestations occur infrequently (Figure 77). These are often found in the same animals in which focal non-specific granulomas are present, representing a section through the inflammatory cells but avoiding the parasite.

Parasitic granulomas caused by larval cestodes are relatively well recognised in a range of bivalve and gastropod molluscs, as well as *P. maxima*. The parasites are considered to be larval lecanicephalid cestodes of the genera *Tylocephalum* or *Polypocephalus*. The adult stage of these tapeworms resides in the intestine of sharks or rays. Mature eggs pass from the ray in faeces and hatch to form a motile coracidium, which invades the tissues of the intermediate molluscan host. In suitable intermediate hosts, the coracidium develops into a metacestode. Although the larval cestode granulomas occur infrequently in mantle tissues, they are relatively common in the stroma of the palp and oesophagus.

Figure 79. Pea crab *Pinnotheres sp*. adjacent to crateriform depression in mantle (arrow) of *P. maxima.*

GILLS (CTENIDIA)

Functional Anatomy and Histology

The branchial apparatus, ctenidium or gills perform water transport, feeding and metabolic exchange functions. A major role of the gills is food collection prior to assimilation by the oyster. The gills are comprised of two longitudinal crescent-shaped structures, the branchial arches that arise from the anterior-dorsal aspect of the visceral mass and lie anterior and ventral to the visceral mass and adductor muscle in the pallial cavity.

Each branchial arch is adherent to the mantle via the cuticular zone on its outer margin for approximately 75% of its length. The free ends of each arch extend posteriorly into the pallial cavity, to a point immediately posterior to the anus where they attach briefly to the inner mantle folds. The inner margins of each arch are loosely adherent for the length of the arch, except near the termination of the gills near the mantle and near the foot.

Along the entire length of each branchial arch, two V-shaped, ***branchial lamellae*** arise at right angles from supporting ladder-like extensions of the arch, forming the outer and inner branchial lamellae. Each branchial lamellum consists of two sets of radial, perpendicular branchial or gill filaments, which arise independently at their base, supported by the ladder-like expansions of the branchial arch, and which unite at their apex to form a triangular space.

Two types of branchial filaments occur: the ***principal filaments*** and the ***ordinary filaments***. The principal filaments are larger and thicker, occur in the hollows or depressions of each fold or pleat in the lamellae and provide structural support for the ordinary filaments. The ordinary filaments are most numerous and constitute the folds of the lamellum. Striations at regular intervals along the length of the ordinary filaments parallel to the branchial axis consist of the ***ciliary discs*** that interlock with neighbouring filaments by way of their stiff cilia.

The cilia on the branchial filaments create a current of water, which enters the mantle cavity and passes over and through the branchial lamellae. Inhaled water enters the shell anteriorly through the inhalant opening in the mantle, flows into the large inhalant chamber in the pallial cavity and reaches the gill. Ciliary movements redistribute the water by channeling it along the filaments, then they direct water to the smaller exhalant chamber and then to the exhalant aperture in the mantle. The gills represent a series of ciliated sieves with a large surface area exposed to the water in the mantle cavity whereby water is moved over the surface by ciliary action to facilitate feeding and metabolic exchange.

Two haemolymph veins follow the branchial axis. The afferent vein brings haemolymph from the kidneys and the efferent vein channels blood towards the auricle. A network of capillaries distributed throughout the filaments links the two veins. Hollow interlamellar junctions contain branches from the afferent vessels which convey haemolymph from the axial trunk to the base of the lamellae. The haemolymph enters individual filaments, flows outward to the free margin and passes to the filaments returning inwards to the branchial axis where it joins the efferent vessel.

The orderly gross and sub-gross structure of the gill is not readily apparent in histological section. On removal from water, the branchial lamellae and gill filaments collapse and distort, presenting a randomly interwoven meshwork of principal and ordinary filaments and their stromal supporting elements (Figures 80, 81, 82).

Close histological examination reveals four major elements in the gill which assist in histological orientation. These elements comprise: the space or external environment normally occupied by water, the epithelial elements of the ordinary and principal filaments in direct contact with the external environment, supportive stromal elements of the branchial lamellae and the internal haemolymph spaces.

At the respiratory surface, the principle and ordinary gill filaments comprise finger-like projections lined externally by ciliated epithelium. The filaments appear as multiple, conjoined and folded epithelial projections, which vary in size and shape depending on the plane of section (Figures 81, 83). The epithelium in the protruding portion of the fold is cuboidal and heavily ciliated, with a tendency for loss of ciliation deep in the folds, where the epithelium becomes more squamous. A fine fibrous stroma supports the overlying epithelium of the gill filaments and encloses the haemolymph sinuses, seen as clear, endothelial-lined spaces extending into the filaments.

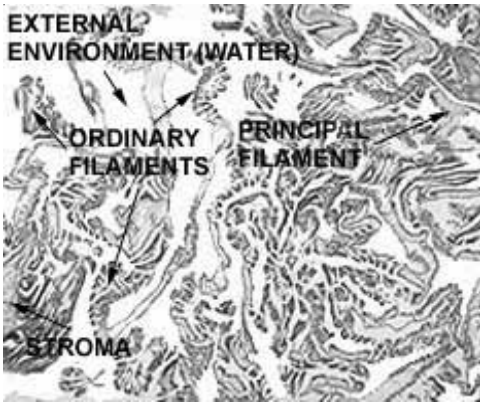

Figure 80. General structure of gill at low magnification showing typical collapsed and disorganised architecture of the principal and ordinary filaments with their stromal tissues. Different architectural aspects of the gill are dependent on plane of section.

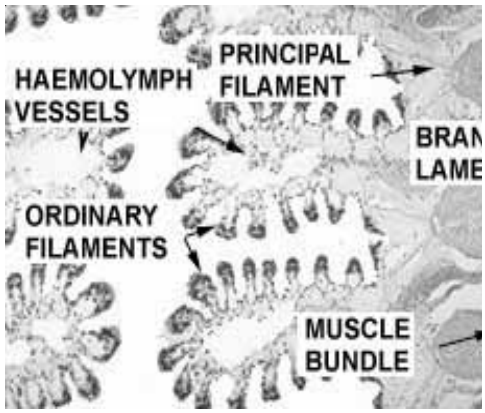

Figure 81. Fine structure of branchial filament showing principal and ordinary filaments.

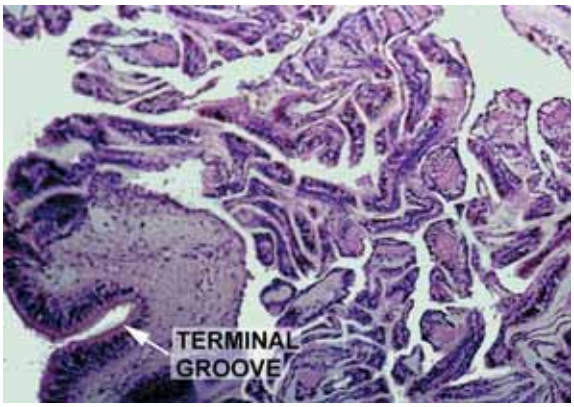

Figure 82. Lower magnification view of gill showing the terminal groove for channeling of food particles.

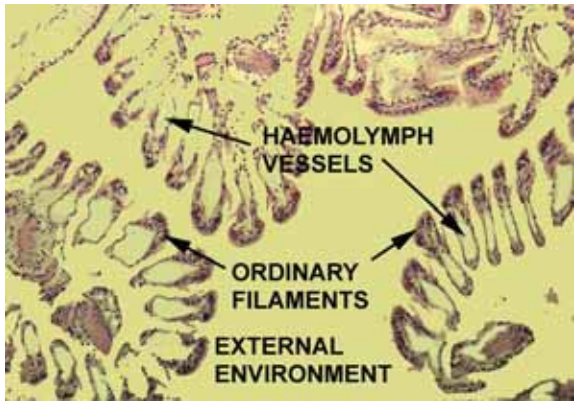

Figure 83. Higher magnification view of section through gill filaments showing multiple, epithelial-lined finger-like projections forming ordinary filaments, with haemolymph vessels within each projection.

An area of specialised infolding of the gill structure, the terminal groove, is present at the base of the lamellae (Figure 82). The terminal groove is covered by a ciliated columnar epithelium and acts to channel feed particles towards the palps and ultimately the mouth.

The stroma of the filaments is continuous with the branchial lamellum and branchial arches and contains the ***branchial muscles***. These muscle fibres run within each ctenidial axis from end to end, and also run longitudinally down each side of the principal filaments. The branchial muscles cause slanting of the gills and withdrawal of their posterior extremities.

Pathology and Histopathology

Degenerative Lesions

Cysts and Mineralisation

Rarely, cysts or cystic structures of unknown origin appear in gill tissues and focal areas of mineralisation may be occasionally observed in the gill.

Non-specific Inflammatory Lesions

Non-specific Inflammation

Focal or regional areas of non-specific inflammation characterised by haemocytic infiltrations or accumulations are seen occasionally in clinically normal oysters.

Infectious and Commensal Organisms

Papova-like Virus

Infections of spat with a papova-like virus, with large intranuclear inclusions in the epithelial cells of the gills, have been reported from Queensland, Australia, usually accompanying similar inclusions in the palps (Figures 84, 85).

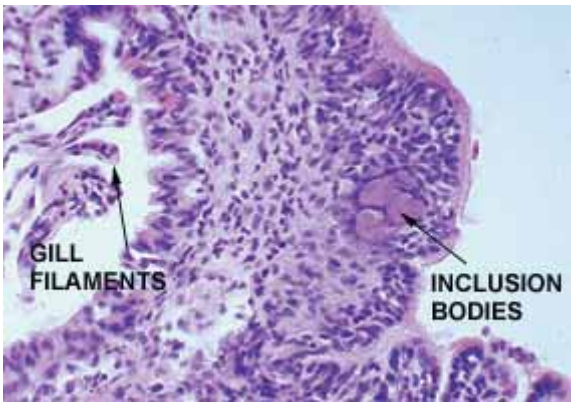

Figure 84. Papova-like viral inclusions in the superficial epithelium of the gills. Note focal epithelial hypertrophy associated with inclusions and diffuse inflammation.

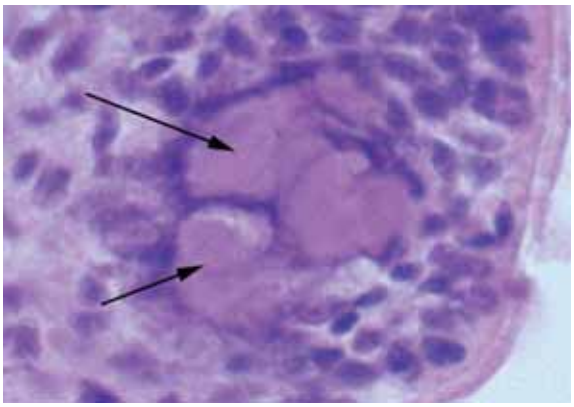

Figure 85. Higher magnification view of papova-like viral inclusions in enlarged cells (arrows). Note that inclusions substantially replace the cell.

Rickettsiales-like Infection

Rickettsiales-like organisms, characterised by granular, basophilic bodies approximately 20-30 um diameter, occupying epithelial cells or intimately associated with epithelial cells, occur infrequently in the gill of clinically normal mature *P. maxima*. The cysts contain numerous small, fine, Gram negative bodies. There is no apparent tissue damage or inflammatory response associated with these bodies (Figure 86).

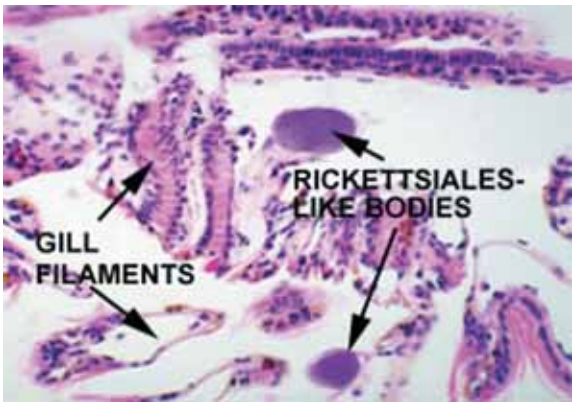

Figure 86. Rickettsiales-like cysts intimately associated with epithelium of gill lamellae. Note absence of cellular response and absence of clear tissue damage.

Unidentified Protozoan-like Bodies

Ovoid, eosinophilic organisms approximately 7-10 um diameter resembling the protozoan *Haplosporidium* have infrequently been visualised in the gill tissues of adult pearl oysters in Western Australia. The organisms were associated with epithelial degeneration and an intense focal inflammatory cell response.

Ancistrocoma-like Ciliates

Ancistrocoma-like ciliates occasionally observed in the intestine have also been reported in the gills of spat of *P. maxima* from Western Australian waters (Figure 146, 147).

External Metazoa

A variety of metazoan agents including pea-crabs and shrimp may be found associated with the external surfaces or epithelium of the gills, generally in the absence of inflammatory or degenerative responses. Histological sections of gill may inadvertently include a section through one of these symbionts (Figure 87).

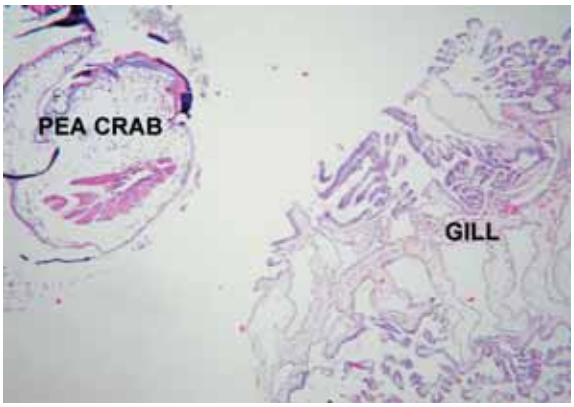

Figure 87. Section of metazoan commensal peacrab between gill and adductor muscle.

Occasionally, metazoa may invade and localise within the stromal tissues of the gill, often without inciting an inflammatory or other host reaction (Figure 88).

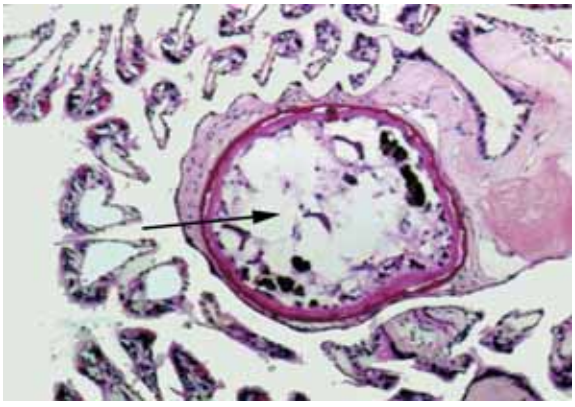

Figure 88. Unidentified metazoan parasite (arrow) localised in the stromal tissues of the gill of mature *P. maxima*. Note absence of host inflammatory cell response.

ALIMENTARY SYSTEM

The alimentary system includes the labial palps, mouth, oesophagus, stomach, digestive gland (digestive diverticula), intestine or midgut, rectum and anus. The oesophagus, stomach, digestive gland and greater portion of the intestine lie within the visceral mass and are generally surrounded by the gonad.

LABIAL PALPS

Functional Anatomy and Histology

The labial palps are formed by the lateral extension of two pairs of projecting lips, one pair on either side of the oral cavity, that conceal the oral cavity and aperture of the mouth. Grossly, the palps are smooth on the surface turned away from the mouth but closely grooved on the apposed faces enclosing the mouth aperture. The role of the palps is to sort and convey food particles to the mouth.

Histologically, the external surfaces of the labial palps are smooth whereas the internal surfaces are heavily folded. The epithelium on the external surface tends to be cuboidal while on the internal surface it comprises tall, ciliated columnar cells and numerous goblet cells, arranged on a fibrous stroma (Figures 89, 90).

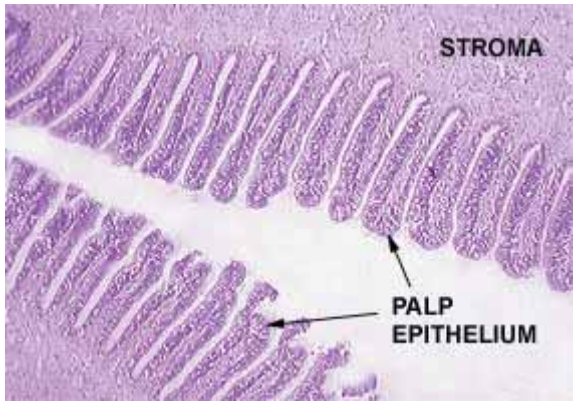

Figure 89. Lower magnification view of normal palp structure showing infolding of the labial palp epithelium on a fibrous stroma.

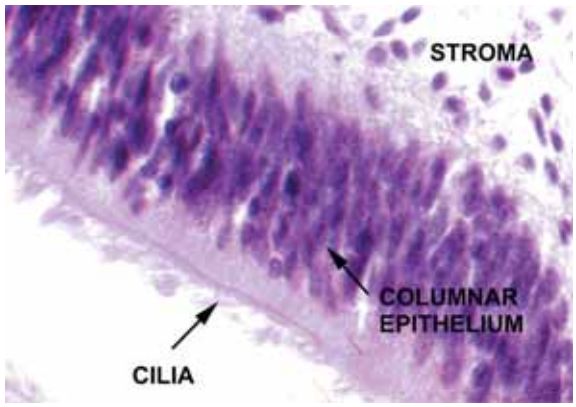

Figure 90. Higher magnification of labial palp showing tall ciliated columnar epithelium resting on fibrous stroma.

Pathology and Histopathology

Degenerative Lesions

Oedema

Oedematous changes characterised by marked dilation of haemocytic sinusoids and irregular spongiform change in stromal tissues may be observed in the stroma of the palp.

Non-specific Inflammatory Lesions

Non-specific Inflammation

Non-specific inflammatory cellular responses in the stroma of the palps occur infrequently in mature *P. maxima* in the absence of observable aetiological agents. Focal or focally extensive haemocytic infiltrations involving both the stroma and epithelium likely represent a response to bacterial infection (Figure 91). Discrete, focal or multifocal granulomas also occur in the sub-epithelial stroma (Figure 92) and are similar in character to those initiated by larval cestode invasion. These granulomas may be in the stroma of the fold of the palp and may significantly expand the fold (Figure 93). In many cases, no agent is evident and serial sections may not reveal any such agents. These granulomas may represent earlier invasion by metazoan larvae.

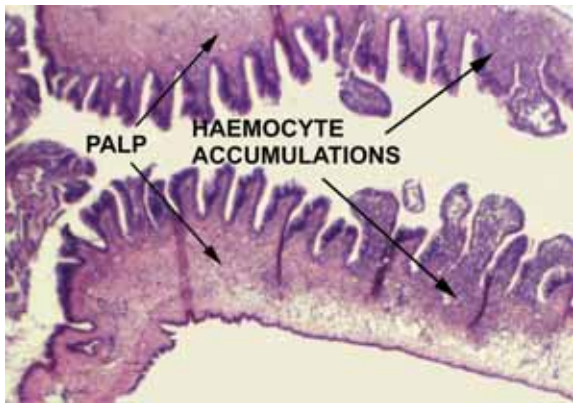

Figure 91. Low magnification of projections of palp with multiple focal haemocytic accumulations in stroma below the epithelium, typical of local bacterial invasion in juvenile oysters or spat.

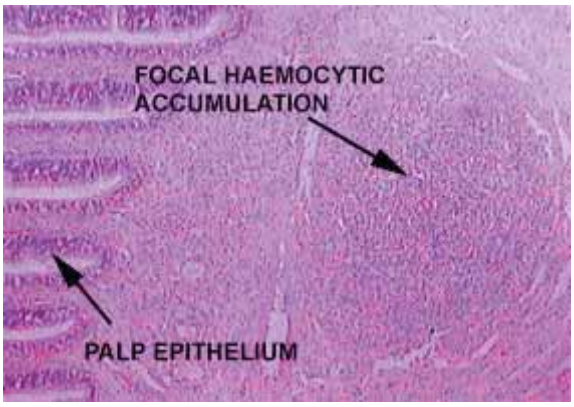

Figure 92. Focal granuloma in stroma of palp. These are similar to those seen with invading cestode larvae, but larvae may not be demonstrable, even with serial sectioning of the lesion.

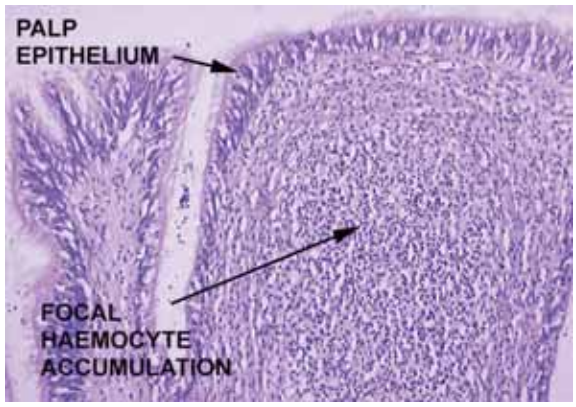

Figure 93. Intense focal haemocytic infiltration in stroma of fold of palp epithelium. Note expansion of the fold.

Infectious and Commensal Organisms

Papova-like Virus

Large intranuclear inclusion bodies consistent with papovavirus, and associated with epithelial hyperplasia and hypertrophy occur commonly in some circumstances in the epithelium of the palps (Figure 94) and have been described in detail by Norton *et al.* 1993a. Expansive regions of the palp may be involved and the extent of involvement, together with the marked epithelial hypertrophy and loss of cilia in affected cells, suggests the agent may be pathogenic, especially in younger animals. The inclusion bodies and the epithelial lesions have been recorded only from Queensland waters and have occurred at a prevalence of up to 50 percent (Humphrey *et al.* 1998). In spat, the large intranuclear inclusions may also occur in cells within the underlying stromal tissues. An intense haemocytic infiltration of the palp tissues and loss of epithelial ciliation may occur (Figures 59, 95). Similar viral inclusions may also occur in the gills of spat (Figure 84).

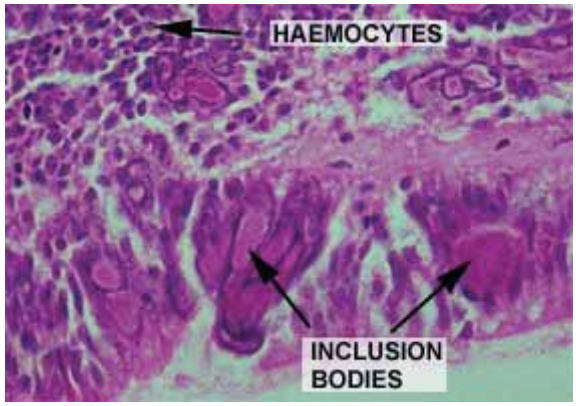

Figure 94. Palp epithelium with papovavirus-like intranuclear inclusion bodies. There is marked hypertrophy of affected epithelial cells, with loss of ciliation on surface.

Viral-like Inclusions

A viral-like inclusion has been described in the palp epithelium of one spat examined from Western Australia. The inclusion appears morphologically different to the papovavirus-like inclusions observed in Queensland *P. maxima* (Norton *et al.* 1993a).

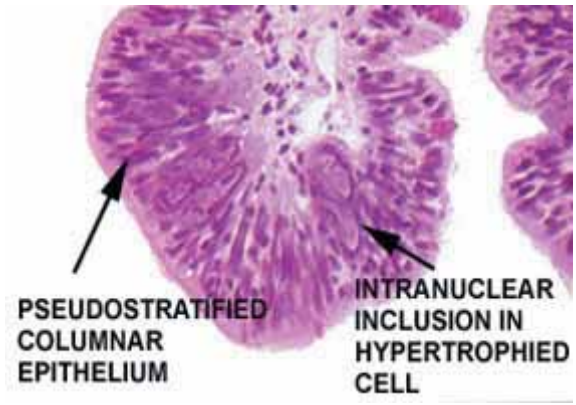

Figure 95. Palp tissue of spat infected with a papovavirus-like virus. There are numerous large, basophilic, intranuclear inclusion bodies in the epithelial cells and a moderate, diffuse infiltration of haemocytes in the stromal tissues. Enlargement of the infected cell occurs, with loss of cilia and loss of normal epithelial architecture.

Rickettsiales-like Infections

Rickettsiales-like bodies occur in the palp epithelium or immediately below the palp epithelium of mature *P. maxima* at a prevalence up to 13 percent (Figures 96, 97, 98). While occurring in other tissues, these rickettsiales-like bodies have not been recognised in the palps of *P. maxima* from Western Australian or Northern Territory waters (Humphrey *et al.* 1998). Although clinical disease has not been associated with the presence of these agents, an inflammatory cell infiltration may occur, associated with the presence of the bodies in the sub-epithelial tissues (Figure 98).

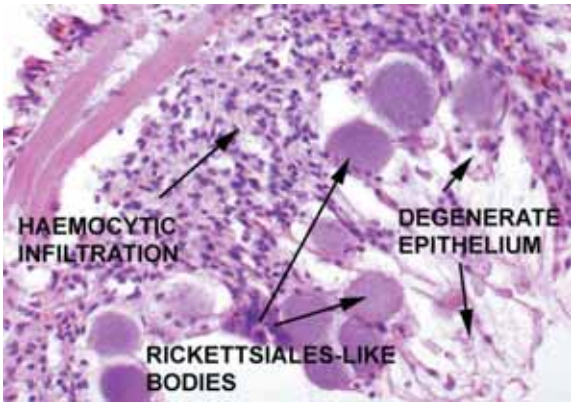

Figure 97. Multiple rickettsiales-like bodies in epithelium of palp of spat with associated degeneration and loss of epithelium and an underlying haemocytic inflammatory cell infiltration.

Turbellarian-like Agents

Turbellarian-like ciliated metazoan agents approximately 300-400 um in length colonise the surface of the palp epithelium of wild-harvested oysters and have been found at a prevalence of up to 12 percent (Humphrey *et al.* 1998). No tissue damage is apparent with these agents (Figure 99).

External Metazoa

A variety of metazoan agents may be occasionally found external to the palp epithelium. Generally, there are no associated inflammatory or degenerative responses associated with these agents and they are considered to be incidental occurrences of free-living or commensal organisms.

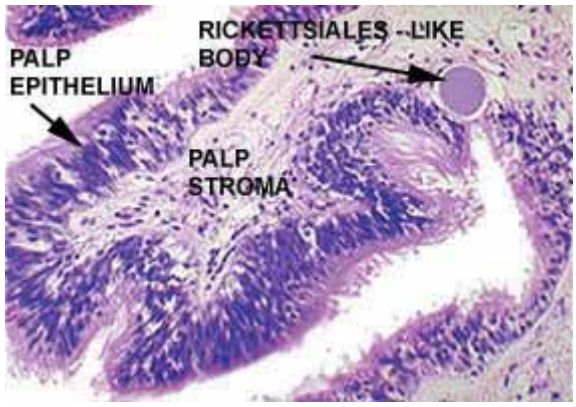

Figure 96. Ciliated epithelium of palp with single rickettsiales-like body in base of fold. Apart from local expansion of the colonised epithelium, there is no apparent tissue damage or inflammatory response.

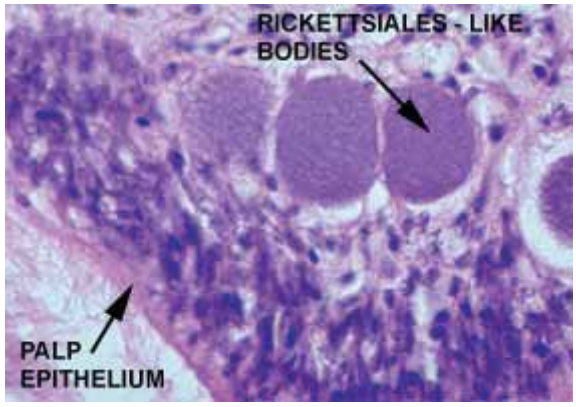

Figure 98. Higher magnification showing fine detail of rickettsiales-like bodies associated with the palp epithelium. These appear beneath the epithelium and have an associated low-grade haemocytic cell accumulation.

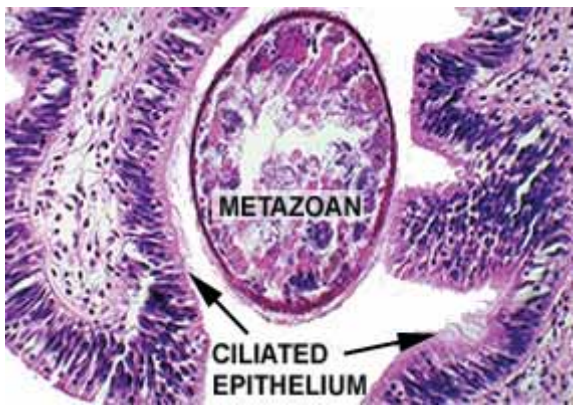

Figure 99. Section showing turbellarian-like metazoan inhabiting the zone above the epithelial surface of the palp.

Internal Metazoa

Single or multiple focal concentric haemocytic accumulations or granulomas peripheral to metazoan parasites in the stroma underlying the palp epithelium may be prominent in populations of mature oysters (Figures 100, 101). Older lesions show well-developed fibrous fibrous encapsulation. As noted in mantle tissues (Figure 79), parasitic granulomas caused by larval lecanicephalid cestodes of the genera *Tylocephalum* or *Polypocephalus* are well recognised in *P. maxima*. The adult stage of these tapeworms resides in the intestine of sharks or rays.

Unidentified metazoa may occasionally localise in the stroma of the palp, forming small granulomas (Figure 102).

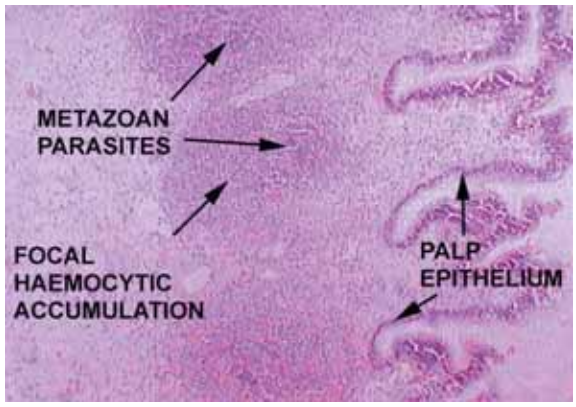

Figure 100. Lower magnification view showing multiple focal parasitic granulomas in the stroma below the palp epithelium. The parasites are morphologically consistent with metacestodes of the lecanicephalid genera *Tylocephalum* or *Polypocephalus.*

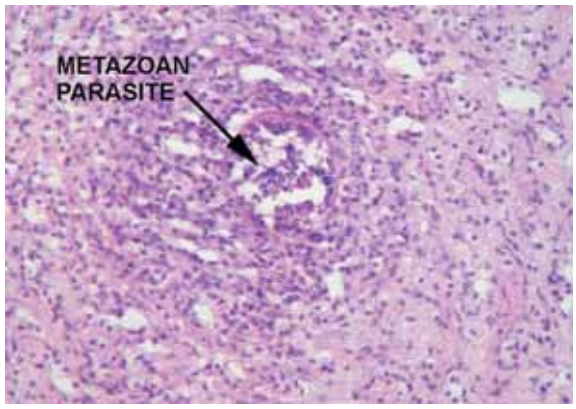

Figure 101. Higher magnification view of metazoan parasite in stroma of palp.

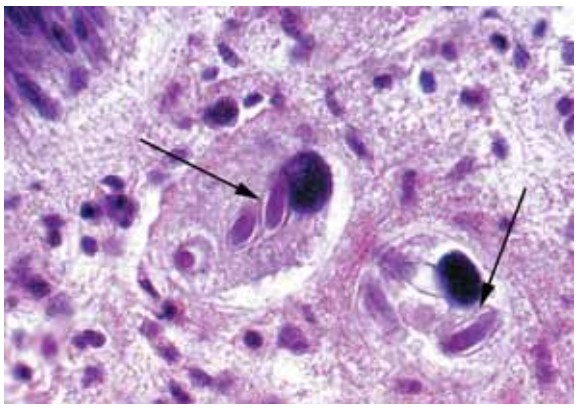

Figure 102. High magnification view of unidentified parasitic bodies forming discrete masses in microgranulomas in the stroma of the palp (arrows).

MOUTH

Functional Anatomy and Histology

The mouth or oral cavity lies at the end of the terminal grooves of the gills in a deep cleft formed by the approximation of the two pairs of labial palps. It is a slit-like orifice located between the anterior levator muscles of the foot. The corners of the oral cavity merge laterally with the depression that marks the junction of the palps of each side. The mouth continues as a depression that narrows inwards towards the oesophagus.

Pathology and Histopathology

No specific lesions are reported in the mouth. The copepod *Anthessius pinctadae* may on occasions be found in the region of the mouth.

OESOPHAGUS

Functional Anatomy and Histology

The oesophagus is a short, straight, dorso-ventrally compressed tube that opens into the stomach. The mucosa shows considerable infolding and the epithelium is ciliated, (Figures 103, 104).

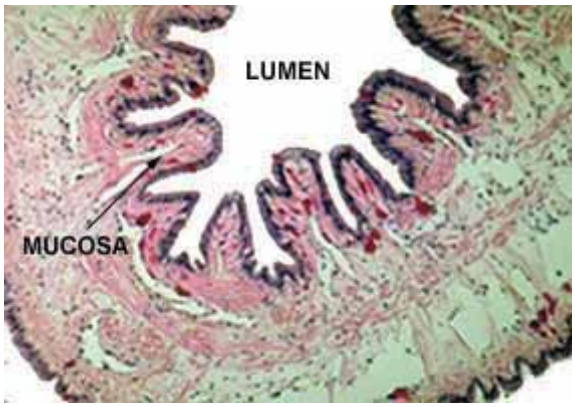

Figure 103. Low magnifiation view showing general structure of oesophagus.

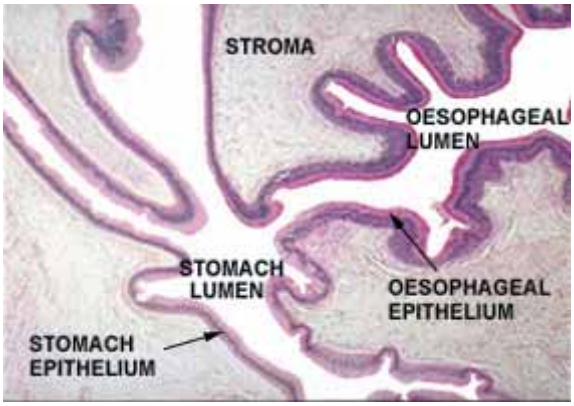

Figure 104. Section through junction of oesophagus and stomach.

Pathology and Histopathology

Infectious and Commensal Organisms

Internal Metazoa

Metazoa associated with discrete, focal or multifocal granulomas in stromal tissues below the epidermis appear regularly in certain populations of mature *P. maxima*. These lesions are characterised by concentric haemocytic accumulations peripheral to the metazoan. Peripheral fibrosis of the lesion is evident in more chronic infections (Figure 105).

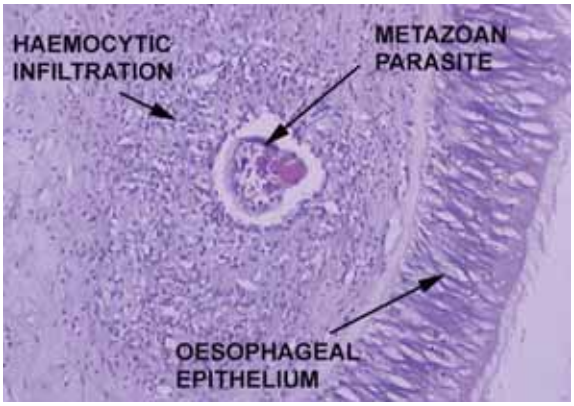

Figure 105. Higher magnification view of an encysted lecanicephalid metacestode adjacent to the oesophageal epithelium. Note the focal haemocytic response around the parasite.

As in mantle and palp tissues, these parasitic granulomas are typical of lecanicephalid metacestodes of the genera *Tylocephalum* or *Polypocephalus*. The adult stage of these tapeworms resides in the intestine of sharks or rays.

While focal metacestode granulomas appear particularly prevalent in tissues peripheral to the oesophagus and palps, they also occur in the stroma of the stomach. High numbers of parasitic granulomas in populations and in individual oysters may be particularly evident in areas frequented by sharks and rays. Adult *Tylocephalum sp.* have been identified in the spiral valve of rays near farms having a high prevalence of metacestode infections in the oysters.

The pathogenic significance of metacestode infections is uncertain. High numbers of parasitic granulomas may cause dysfunction in affected tissues.

Anthessius pinctadae

The copepod *Anthessius pinctadae* has been reported at high prevalence and intensity in the mouth and oesophageal lumen in certain populations of mature *P. maxima* from the Northern Territory (Figure 106). Occlusion of the lumen by large numbers of parasites and epithelial erosion and ulceration associated with feeding of the parasite has been observed in some animals.

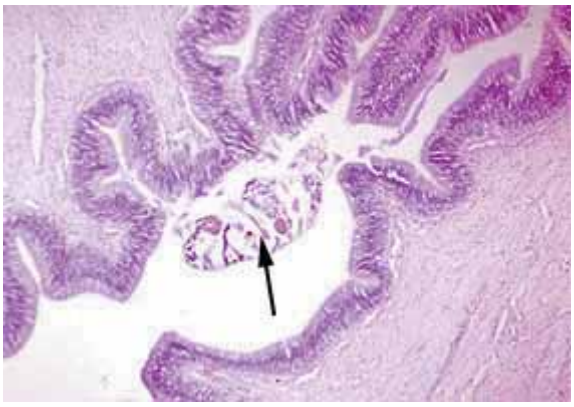

Figure 106. The copepod *Anthessius pinctadae* (arrow) inhabiting the oesophageal lumen.

STOMACH

Functional Anatomy and Histology

The stomach is a folded multilocular organ that lies within the visceral mass and is enclosed by the glands of the digestive diverticula. The organ is divided by large indentations of the mucosa forming well-defined chambers or pits. The mucosa is generally rugose or folded. The terminal ducts of the surrounding digestive gland ramify into the chambers of the stomach. Herdsman (1904) described eleven collecting ducts entering the stomach of *P. fucata*. Conspicuous projecting folds subdivide the stomach into cardiac and pyloric regions. A gelatinous rod, the crystalline style, is located in the sub-central position and projects from the sac where it is formed. The epithelium of the stomach is typically tall columnar and ciliated (Figure 107). The crystalline style abuts on the gastric shield (Figure 108), a region of non-ciliated epithelium opposing the opening of the style sac.

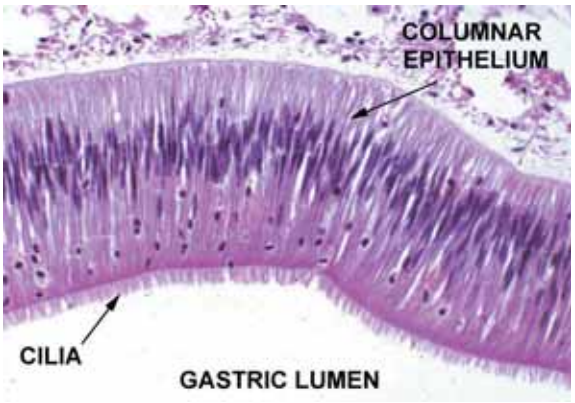

Figure 107. High magnification of gastric epithelium showing tall ciliated columnar epithelium overlying stromal tissues.

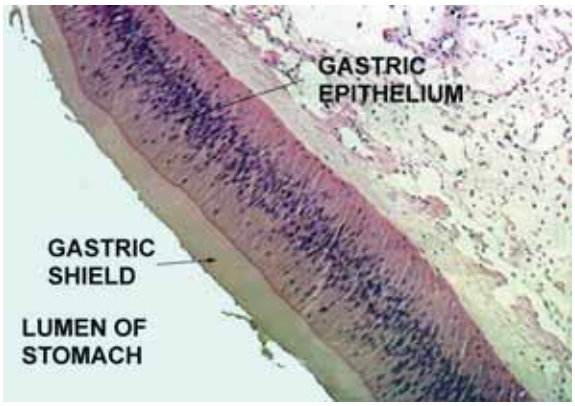

Figure 108. Tall non-ciliated epithelium beneath the gastric shield.

Pathology and Histopathology

Non-specific Inflammatory Lesions

Non-specific Inflammation

The stromal tissues surrounding the gastric epithelium in mature *P. maxima* normally contain low numbers of haemocytes. Focal or regional haemocytic inflammatory cell infiltrations occur infrequently in the stroma of the stomach, associated with bacterial infections. Erosion or ulceration of stomach epithelium of unknown cause, with an underlying stromal cellular inflammatory response is rarely observed (Figure 109).

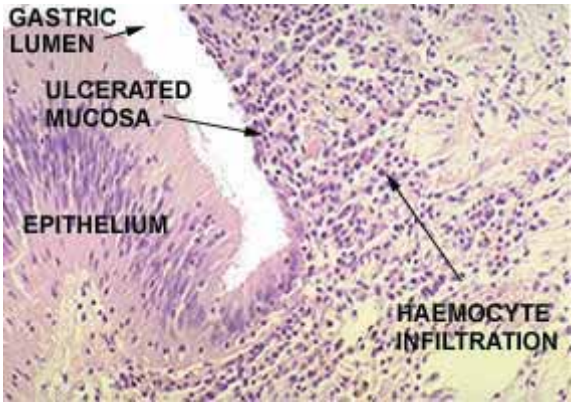

Figure 109. Section of gastric mucosa showing normal tall columnar ciliated epithelium. Adjacent epithelium is ulcerated, with loss of epithelial cells and an intense inflammatory cell infiltration of the stromal tissues by haemocytes. Cause unknown.

Infectious and Commensal Organisms

Gregarine Protozoa

Non-ciliated, indented, ovoid Gregarine protozoa are recorded as colonising the epithelium of the stomach in mature *P. maxima* in Queensland waters (Figure 110). These appear innocuous, causing no apparent damage or host response and have been characterised by Cui (1997).

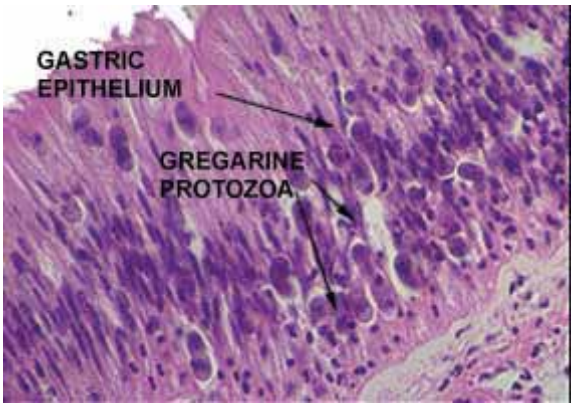

Figure 110. View of gastric epithelium showing numerous ovoid gregarine protozoa colonising the epithelium. There is an apparent lack of tissue damage and absence of a significant inflammatory response. These organisms appear to be harmless, even in large numbers.

Internal Metazoa

Focal haemocytic accumulations peripheral to metazoan agents, often with fibrous encapsulation, are occasionally located in the stroma of the stomach (Figure 111). In most cases, the metazoa are typical of larval lecanicephalid cestodes. Occasionally, crustacea are found which may elicit an intense focal haemocytic inflammatory response (Figure 112).

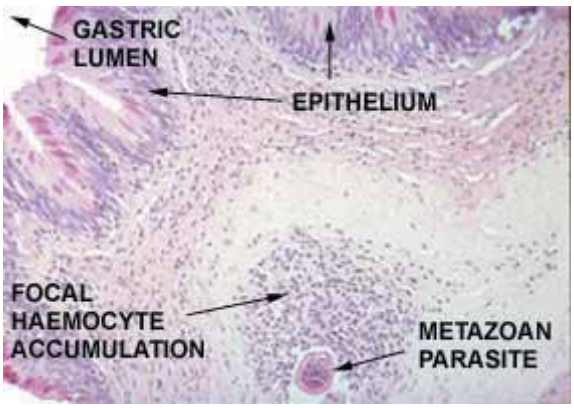

Figure 111. Section of gastric mucosa showing a focal parasitic granuloma with a central metazoan typical of a lecanicephalid metacestode.

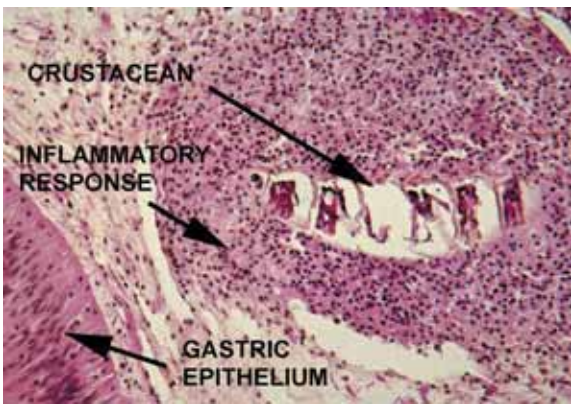

Figure 112. View of crustacean parasite in wall of stomach with associated intense focal haemocytic inflammatory response.

DIGESTIVE GLAND

Functional Anatomy and Histology

The digestive gland or digestive organ comprises adjacent clusters of secretory glandular tissue, the digestive tubules or diverticula, uniting as ductules, leading into the stomach as distinct ducts (Figures 113, 114). As with edible oysters (Galtsoff 1964), the digestive gland of *P. maxima* is considered to be the primary organ of digestion.

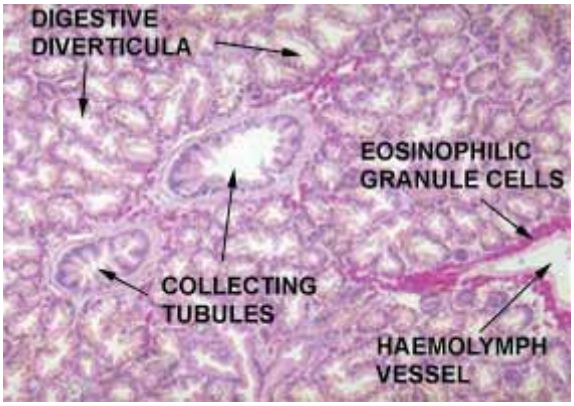

Figure 113. Lower magnification view of digestive gland showing expansive regions of digestive diverticula, collecting tubules and fibrous stromal tissue.

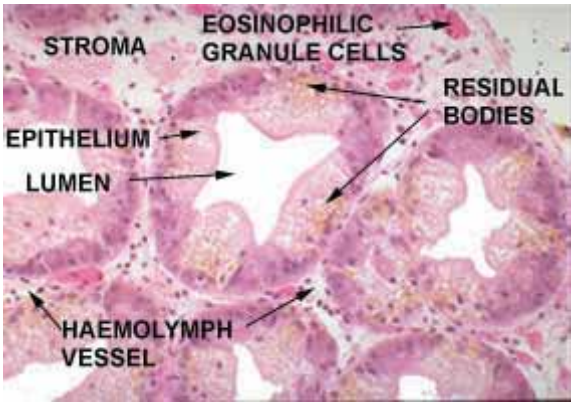

Figure 114. Higher magnification of digestive gland showing detail of digestive diverticula. Note golden-brown residual bodies and differential staining of cells forming glands.

The overlying integument is an extension of the epithelium of the external surface of the mantle and consists of a complex mucous epithelium. A superficial layer of columnar epithelial cells, interspersed with eosinophilic granule cells, is present resting on a thin fibrous stroma. Below this layer, a sub-epithelial layer of mucous cells and eosinophilic granule cells is present, supported by the stromal tissues of the visceral mass (Figure 73).

The digestive diverticula are complex multilocular structures having alternating zones of tall, deeply staining, non-ciliated columnar epithelial and shorter, poorly staining regions of cells. At least three different cell types are recognised. ***Absorptive cells*** are tall, columnar with a basal nucleus and abundant, foamy eosinophilic cytoplasm. ***Secretory cells*** are dark basophilic cells with an apical nucleus arranged in aggregates between the absorptive cells. ***Undifferentiated cells*** are low cuboidal and line the basement epithelium (Figures 114, 115). Collecting ductules arise at the proximal ends of the tubules and have a ciliated low columnar epithelium. These ductules progressively unite to form ducts leading into the stomach.

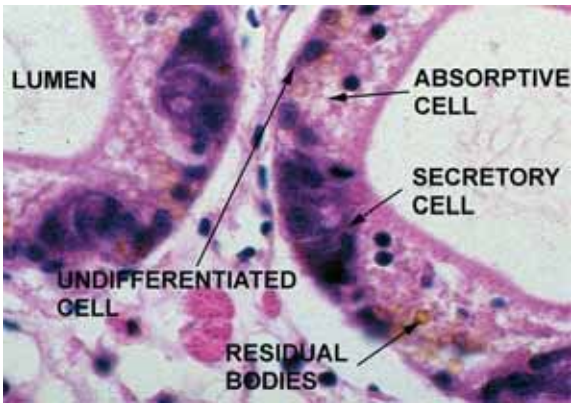

Figure 115. Higher magnification of digestive diverticular epithelium showing alternating zones of tall, deeply staining, non-ciliated tall secretory cells, foamy adsorptive cells and undifferentiated germinal cells.

Residual Bodies

Residual bodies, small spherical bodies up to approximately 3 um diameter and containing golden pigment are commonly encountered in the epithelium of the digestive diverticula (Figure 115). These bodies have been described as "protistan parasites" by Wolf and Sprague (1978) who considered them to be parasitic and associated their presence with degenerative changes and mortalities. The opinion of Pass and Perkins (1985), who considered these bodies to be "residual bodies", normal constituents of digestive cells comprising storage or secretory products bound within lysosomes is, however, widely accepted.

Pathology and Histopathology

Degenerative Lesions

Dilation and Degeneration of Diverticular Glands

In mature oysters, dilation of digestive gland diverticula, with flattening or attenuation of the epithelium may be commonly observed in individual populations (Figures 116, 117). The dilation may be regional, often occurring in the more peripheral regions of the gland. In some cases, dilation may be irregular or generalised.

Figure 116. Digestive gland showing dilation of digestive diverticula with flattening and thinning of epithelium.

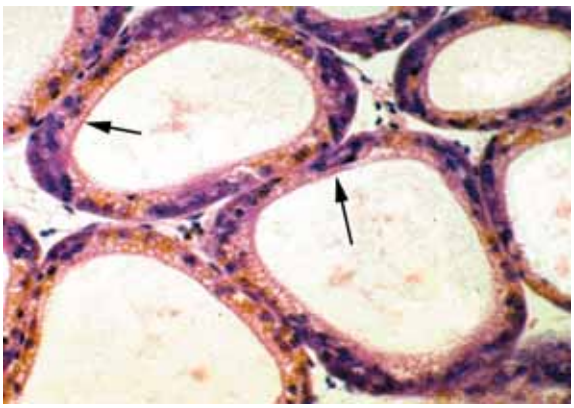

Figure 117. High magnification view of digestive diverticula showing flattening of the epithelium (arrows) and dilation of the lumen of the gland.

In edible oysters, changes in epithelial height are associated with tidal movements and/or feeding patterns in the inter-tidal zone but not in subtidal oyters (Wilson and La Toucha 1978, Winstead 1998). Flattening of the diverticular epithelium in *P. maxima* may occur during periods of abundant phytoplankton, suggesting that toxic elements in certain plankton species may result in reduced feeding and a decrease in epithelial height, or they may have a direct toxic effect. Atrophy and necrosis of the digestive gland absorptive cells are recorded in scallops *Argopectan irradians* exposed to the dinoflagellate *Prorocentrum minimum* (Wikfors and Smolowitz 1995).

Dilation of the digestive gland diverticula and flattening or attenuation of the epithelium, sometimes accompanied by breakdown of glandular architecture and fusion of adjacent glands forming cystic, multilocular cavities occurs in spat of *P. maxima* (Figures 57, 58). The cause and significance are unclear, but heavy blooms of the algae *Trichodesmium* have been associated with the condition (Negri *et al.* 2004).

Inclusion Bodies of Uncertain Aetiology

Occasional large amphiphilic inclusion bodies showing internal granular structure rarely occur in the digestive diverticular epithelium (Figure 118). Smaller eosinophilic inclusions with granular internal structure are also seen rarely (Figure 119). The cause and pathogenic significance of these bodies are uncertain.

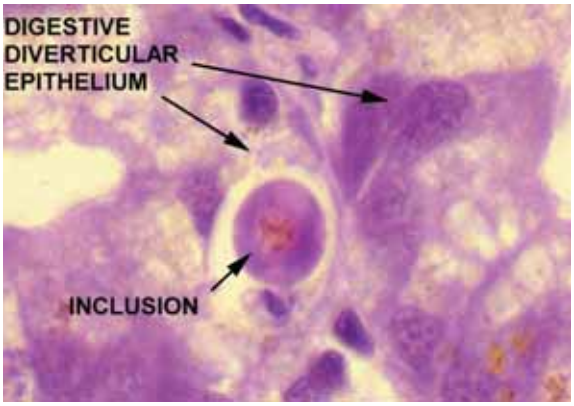

Figure 118. Higher magnification view of inclusion body of uncertain cause and significance in the epithelium of a digestive diverticulum (Courtesy of Dr B. Jones).

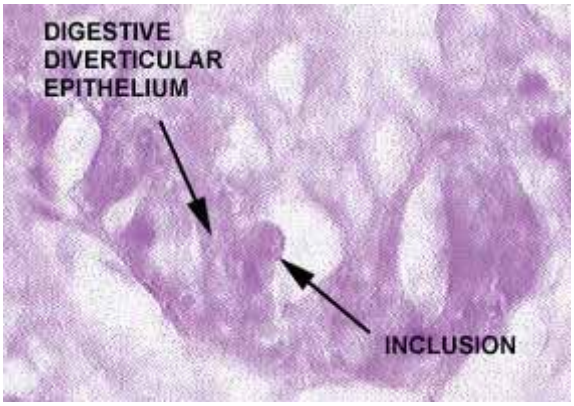

Figure 119. Eosinophilic inclusion in the epithelium of a digestive gland diverticulum. Fine internal structure is present. The cause and significance of these bodies are unknown (Courtesy of Dr B. Jones).

Non-specific Inflammatory Lesions

Non-specific Inflammation

Regional or focal infiltrations of haemocytes are occasionally found in the stroma of the digestive gland of apparently normal oysters in the absence of an obvious aetiological agent (Figure 120). Intense, focally extensive haemocytic infiltrations associated with degeneration of digestive gland epithelium have also been seen in apparently clinically healthy oysters. No causal agent, however, has been found (Figure 121). Negri *et al.* (2004) reported haemocytic accumulations and epithelial degeneration in the digestive gland of oysters exposed to the alga *Trichodesmium erythraeum*. This response also appears commonly in bacterial infections involving the digestive gland.

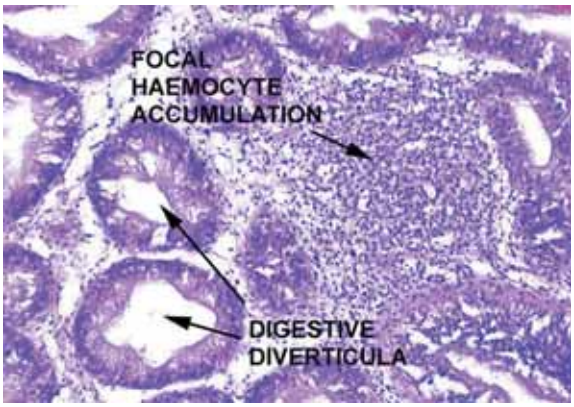

Figure 120. Focal haemocytic accumulation in stroma of digestive gland of clinically normal mature *P. maxima.*

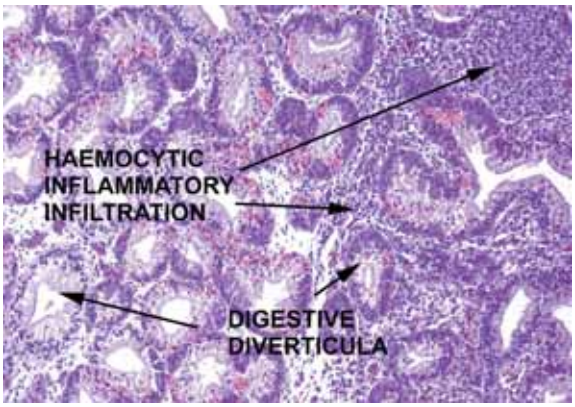

Figure 121. Lower magnification view of digestive gland with severe regional cellular inflammation characterised by an intense haemocytic infiltration of the stroma suggestive of a response to bacterial infection.

Infectious and Commensal Organisms

Intranuclear Viral Inclusions

Intranuclear viral inclusion bodies in the digestive gland epithelium (Figures 122, 123, 124), morphologically identical with the viral inclusions described by Pass *et al.* (1988), are common in populations of mature *P. maxima*. A prevalence of infection up to 53 % has been recorded (Humphrey *et al.* 1998). Individual oysters may have high numbers, in excess of 20 inclusions bodies per high power field, in some areas. Inclusions generally appear randomly distributed in the digestive diverticula and are usually unassociated with any inflammatory, degenerative or proliferative changes. Localised hyperplasia and degeneration of digestive gland epithelium are occasionally associated with the presence of these bodies, especially where individual glands are heavily infected. These viral inclusion bodies are also recorded in the digestive gland epithelium of spat. Electron microscopic examination of these inclusions shows arrays of regular icosahedral viral particles resembling herpesvirus or adenovirus (Figure 125). No clinical disease has been associated with the inclusions.

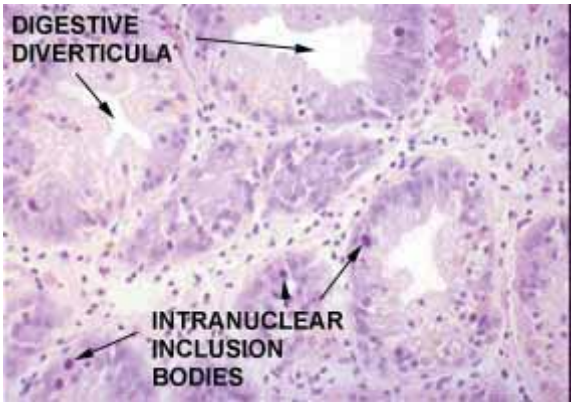

Figure 122. Lower magnification general view of digestive gland showing conspicuous amphiphilic intranuclear inclusion bodies within epithelial cells of digestive diverticula.

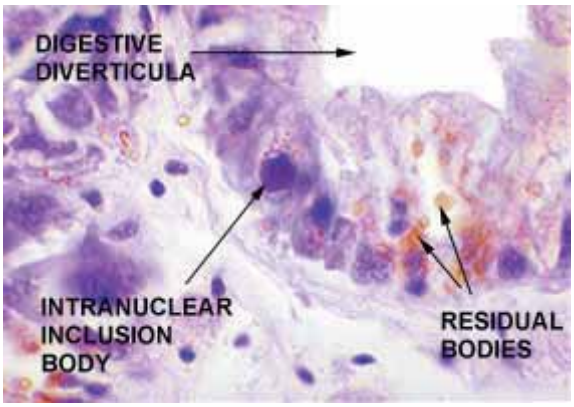

Figure 123. Higher magnification view of well developed viral intranuclear inclusion body in epithelial cell of digestive diverticulum. Note also conspicuous golden-brown residual bodies within epithelium cells.

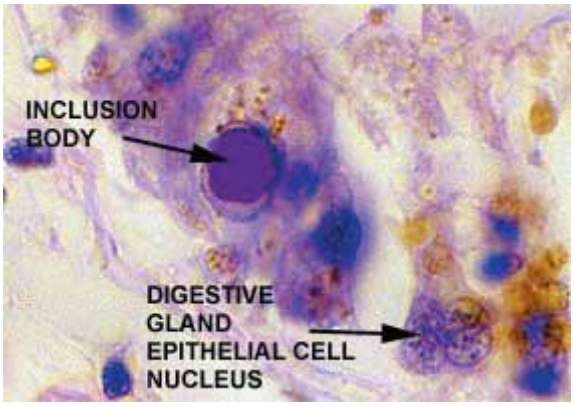

Figure 124. Higher magnification view of well developed basophilic viral intranuclear inclusion body in digestive gland diverticulum epithelium. Note margination of chromatin in the nucleus containing the inclusion.

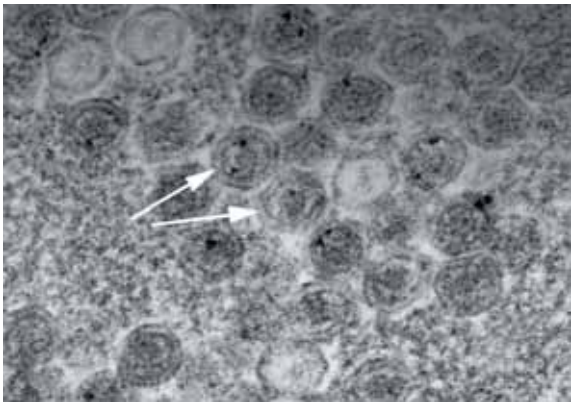

Figure 125. Electron micrograph showing array of large icosahedral viral particles (arrows) in intranuclear inclusion in a digestive diverticular epithelial cell (Courtesy of Dr R. Weir).

Rickettsiales-like Infections

Large rickettsiales-like granular basophilic bodies approximately 20-30 um diameter containing numerous fine organisms and occupying epithelial cells or intimately associated with epithelial cells of the digestive diverticula, are relatively common in mature *P. maxima* in the absence of disease (Figures 126, 127). Generally there is no apparent tissue damage or inflammatory response associated with these organisms but rarely an intense inflammatory cell infiltration and necrosis of epithelium accompanies infection. Smaller rickettsiales-like cysts are also recorded in the digestive gland in spat of *P. maxima* (Figure 128). Sporadically, these organisms appear to initiate an intense inflammatory response.

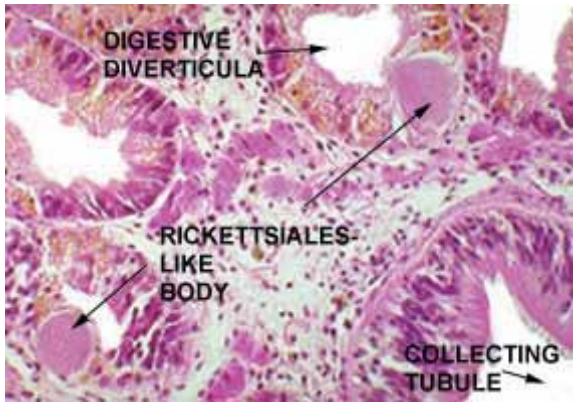

Figure 126. Section of digestive gland showing rickettsiales-like bodies in epithelial cells of digestive diverticula. Apart from local expansion, there is no apparent damage to the epithelium and no associated inflammatory response.

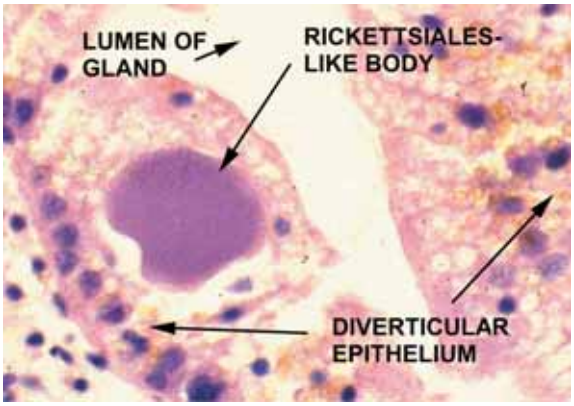

Figure 127. Higher magnification view of rickettsiales-like cyst in the epithelium of a digestive gland diverticulum.

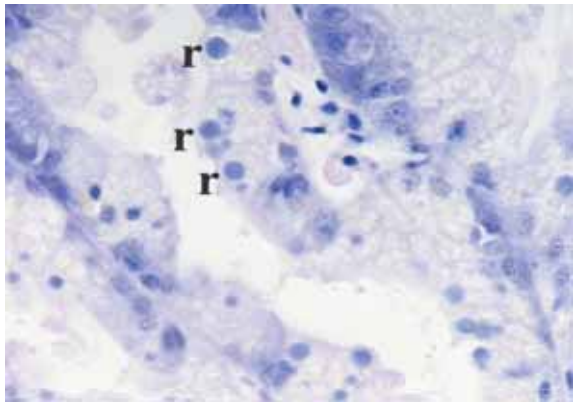

Figure 128. Small form of rickettsiales-like cyst "r" in the digestive gland diverticular epithelium (Courtesy of Dr B. Jones).

Gregarine Protozoa

Gregarine protozoa commonly colonise the epithelium of the digestive diverticula in some populations of mature *P. maxima* (Figures 129, 130, 131). These non-ciliated, indented, ovoid bodies approximately 10-15 um in length appear to cause no tissue damage to the host.

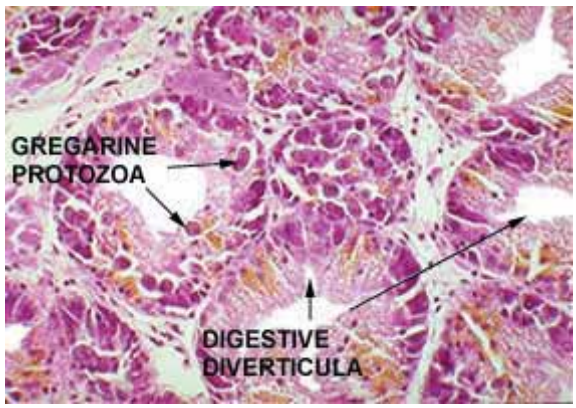

Figure 129. Digestive gland diverticula demonstrating heavy colonisation by gregarine protozoa. Note conspicuous residual bodies in epithelium.

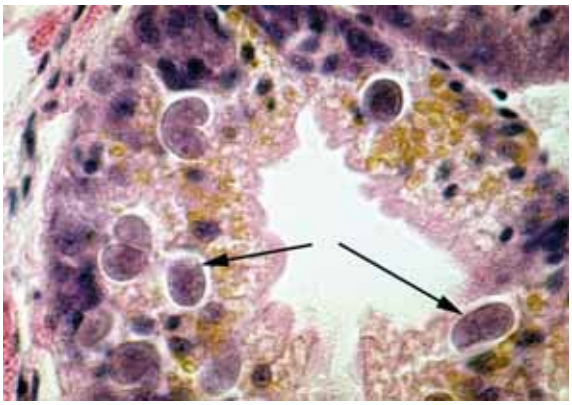

Figure 130. View of digestive gland diverticula showing colonisation by gregarine protozoa (arrows).

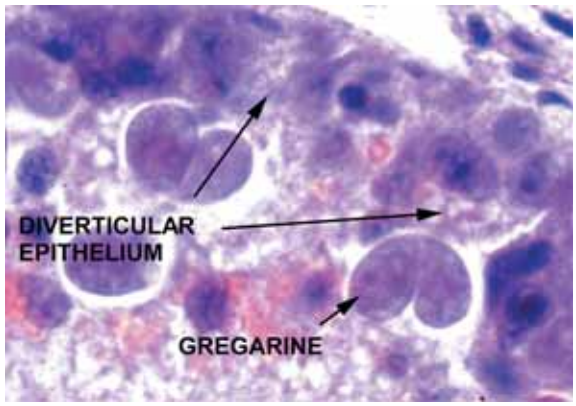

Figure 131. High magnification view of epithelium of digestive diverticulum showing detail of gregarine protozoa.

Haplosporidium sp.

Isolated cases of haplosporidiosis have been recorded at a high prevalence in populations of spat of *P. maxima* from Western Australia, in which *Haplosporidium sp*. subtend the epithelial cells of the digestive gland diverticula (Figure 132). On detection, infected populations have been destroyed thus the natural course of infection is unknown. The high numbers of organisms and the extent of infection suggest that clinical disease and death are probable sequelae (Dr B. Jones, personal communication). Infection with the agent has been described by Hine (1996) and Hine and Thorne (1998). The organism is considered to represent a serious potential threat to the pearling industry in Australia.

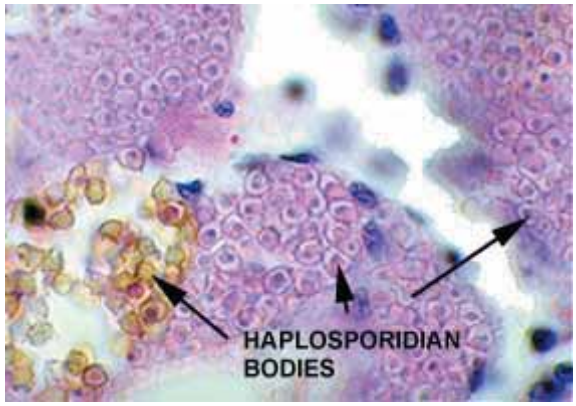

Figure 132. Higher magnification view of digestive gland of spat of *P. maxima* showing heavy infection by *Haplosporidium*. The haplosporidians are in the stromal tissues adjacent to the digestive diverticula. The golden bodies appear to be degenerate haplosporidians (Courtesy of Dr B. Jones).

Rhynchodid-like Ciliates

Rhynchodid-like ciliated protozoa have been reported in juvenile *P. maxima* from Western Australian in oysters residing near the southern limits of their distribution. On occasions, infection is associated with an intense haemocytic response (Figures 133, 134), however, they have not been associated with clinical disease (Dr B. Jones, personal communication).

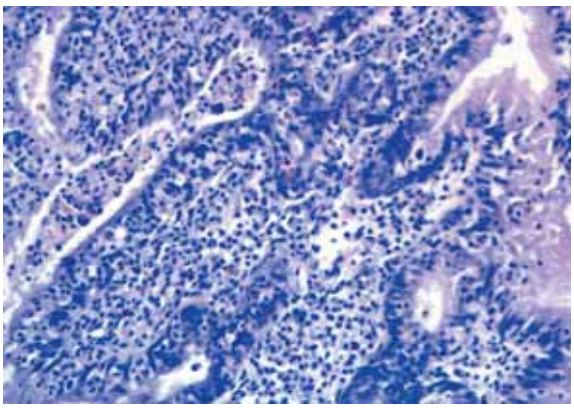

Figure 133. Lower magnification view of digestive gland of a juvenile *P. maxima* showing intense haemocytic infiltration associated with *Rhynchodid*-like ciliated protozoa (Courtesy of Dr B. Jones).

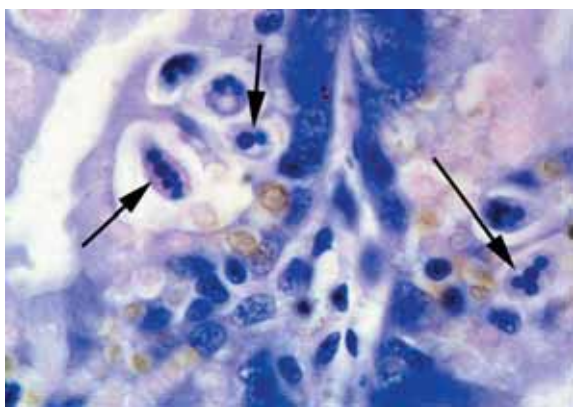

Figure 134. Higher magnification view of epithelium of digestive gland diverticula harbouring *Rhynchodid*-like ciliated protozoa (arrows) (Courtesy of Dr B. Jones).

Cryptosporidium-like Organisms

Multiple, small basophilic bodies approximately 2-3 um in size and resembling *Cryptosporidium*, have been recorded on one occasion in or closely associated with the epithelium of the digestive gland diverticula of a single mature oyster from the Northern Territory in the absence of disease (Humphrey *et al.* 1998). Cytoplasmic enlargement and foamy degeneration of the epithelium were associated with the bodies (Figure 135).

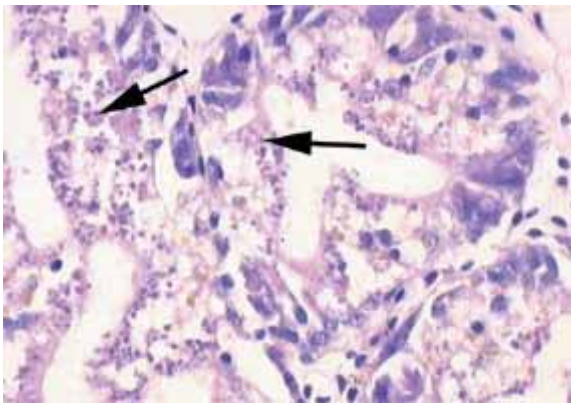

Figure 135. Multiple, small unidentified bodies resembling *Cryptosporidium* (arrows) in and on the digestive gland diverticular epithelium. Note epithelial degeneration and fragmentation.

Enigmatic Bodies

Eosinophilic, ovoid granular bodies approximately 10-15 um diameter, in or closely associated with the digestive diverticular gland epithelium have been observed in populations of mature oysters from the Northern Territory at a prevalence of up to 3.3%. The bodies were associated with a moderately intense localised granulomatous inflammatory response and epithelial degeneration (Figures 136, 137). Some of the bodies showed fine basophilic granular internal structure. No definite association with clinical disease could be established and the possibility that they represent degenerate cellular components has not been excluded.

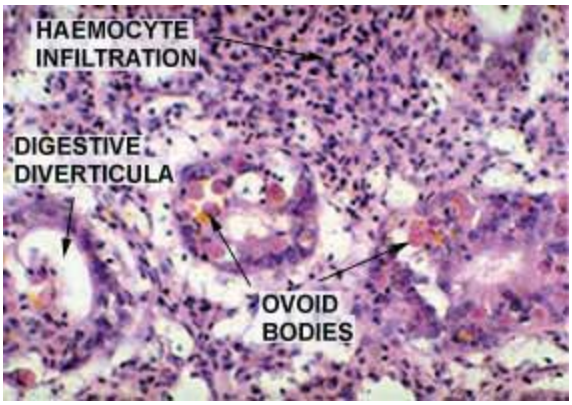

Figure 136. Digestive gland showing unidentified ovoid bodies associated with digestive diverticular degeneration and an intense haemocytic infiltration. There is loss of epithelium associated with the bodies. The inflammatory response is focussed on areas undergoing epithelial damage.

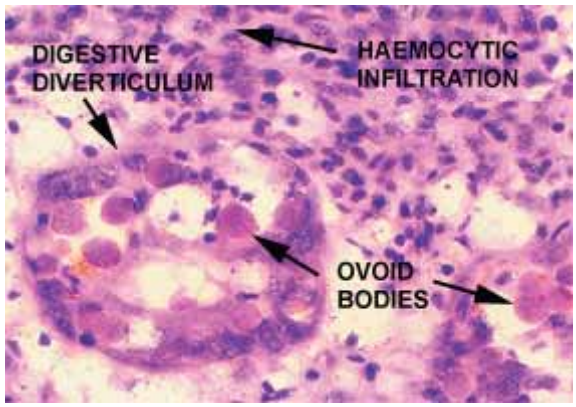

Figure 137. Higher magnification of a digestive gland diverticulum containing ovoid bodies. Note the intense haemocytic inflammatory infiltration adjacent to affected diverticulum.

The relationship between these granular bodies and 7-10 um diameter protozoan-like bodies associated with microgranulomas seen in the digestive gland (Figure 138 and gill tissues in mature *P. maxima* in Western Australia and the Northern Territory is unclear. The ovoid bodies appear to have internal structure and do not resemble host tissue.

Similarly, the relationship between these bodies and other single or multiple, eosinophilic or amphiphilic, ovoid bodies approximately 5-7um diameter seen occasionally in the digestive gland diverticular epithelium in the absence of inflammatory changes is also unclear (Figures 118, 119). These bodies may represent effete epithelial cells or eukaryotic parasitic cells.

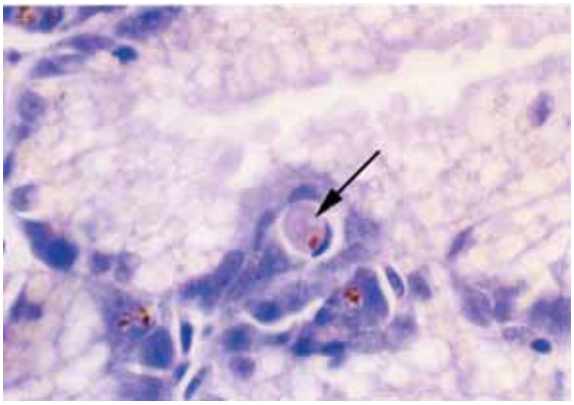

Figure 138. Microgranuloma associated with unidentified ovoid body (arrow) in stroma of the digestive gland (Courtesy of Dr B. Jones).

Internal Metazoa

Morphologically and taxonomically diverse metazoan agents may occasionally be observed in the lumen of the digestive gland tubules or diverticula or in the interstitial tissues (Figure 139). In general, these agents appear to incite little if any tissue damage or inflammatory response. Occasionally, metazoa in the digestive gland are associated with atrophy of the epithelium, haemocytic response and mineralisation. A low proportion of *P. maxima* spat examined from Western Australia are recorded with the copepod *Anthessius pinctadae* encysted in the digestive gland (Dr. B. Jones, personal communication).

On occasions, metazoa, including lecanicephalid metacestodes may invade the interstitial tissues peripheral to the digestive gland, inciting an intense haemocytic inflammatory response (Figure 140).

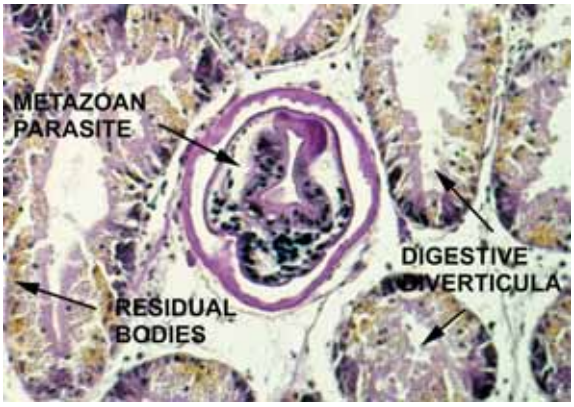

Figure 139. Unidentified metazoan in the lumen of the digestive gland diverticula. Note post mortem autolysis of digestive gland, with loss of normal structure.

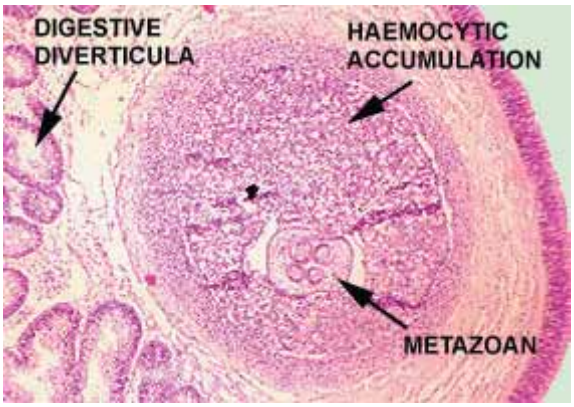

Figure 140. Encapsulated haemocytic inflammatory accumulation associated with invasion of the tissues adjacent to the digestive gland by a lecanicephalid metacestode typical of *Tylocephalum sp*. or *Polypocephalus sp.*

INTESTINE

Functional Anatomy and Histology

The intestine of *P. maxima* comprises three sections of approximately equal length.

- Descending segment
- Ascending segment
- Rectum

The descending segment arises from the stomach and passes ventrally through the posterior part of the visceral mass, behind the base of the byssal gland and between the two retractor muscles of the foot. The intestine then curves backwards and dorsally to the visceral mass as the ascending segment.

A longitudinal fold projects from the wall of the descending intestine at its origin near the pyloric region of the stomach, forming a distinct tubular cavity continuous with the descending segment. This cavity forms the sheath of the crystalline style, from which this clear gelatinous cylinder extrudes (Figure 141). The distal style projects into the stomach, which is protected by the gastric shield (Figure 108). The style has a digestive role.

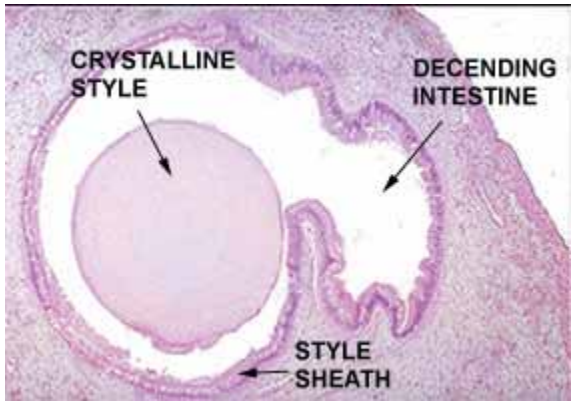

Figure 141. Section through upper descending intestine showing crystalline style, sheath of style and descending segment of intestine.

The epithelium of the intestine is columnar and possesses long cilia (Figure 142). The epithelium of the sheath of the style is similar. Distally, the lumen of the rectum is compressed by an infolding of stroma (Figure 143).

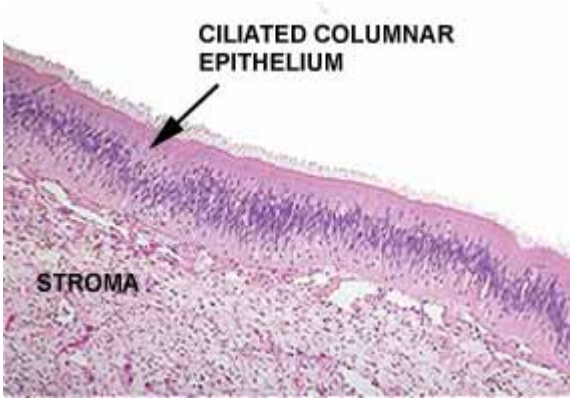

Figure 142. View of tall ciliated columnar epithelium of intestine.

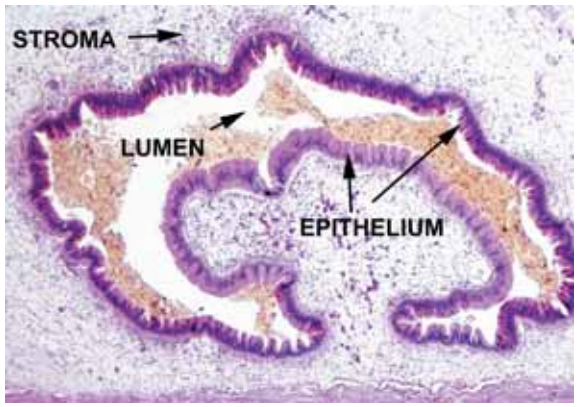

Figure 143. Lower magnification view of rectum. Note typical infolding of central stroma covered by low ciliated columnar epithelium.

Pathology and Histopathology

Degenerative Lesions

Pigmented Macrophages

Occasionally, large numbers of pigmented macrophages or "brown cells" may be present in the stroma adjacent to the alimentary tract. These cells are generally considered to contain metabolites of cell degeneration.

Siliceous Obstipation

Ingestion of fine silica spicules may cause impaction and erosion of cilia in the small intestine (Figure 144) resulting in secondary bacterial infection and death. This condition has occurred when oysters have been placed on seabeds containing degenerating siliceous sponges.

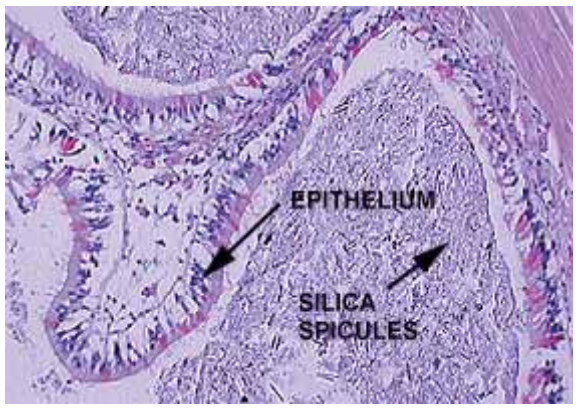

Figure 144. Impaction of the intestine by silica spicules from sponge, with loss of ciliation.

Non-specific Inflammatory Lesions

Non-specific Inflammation

Diffuse infiltrations of haemocytes of light to moderate intensity commonly occur adjacent to the intestinal epithelium, especially in bacterial infections. Erosion and ulceration of the intestinal epithelium may occur, with intense inflammatory cell infiltrations extending into and partially occluding the lumen of the intestine (Figures 63, 145).

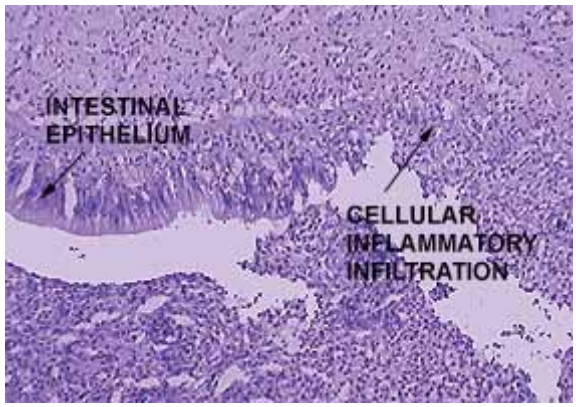

Figure 145. Intense haemocytic inflammatory cell infiltration of the intestine with epithelial erosion, ulceration and cellular exudation into the intestinal lumen associated with marine vibrionic bacteria.

Infectious and Commensal Organisms

Ancistracomid Protozoa

Elongate, *Ancistrocoma*-like ciliated protozoa, approximately 25-30 um in length, may be common in the intestine of mature *P. maxima* (Figures 146, 147). *Ancistrocoma*-like ciliates have also been recognised in the intestine of spat of *P. maxima.*These organisms appear to be non-pathogenic.

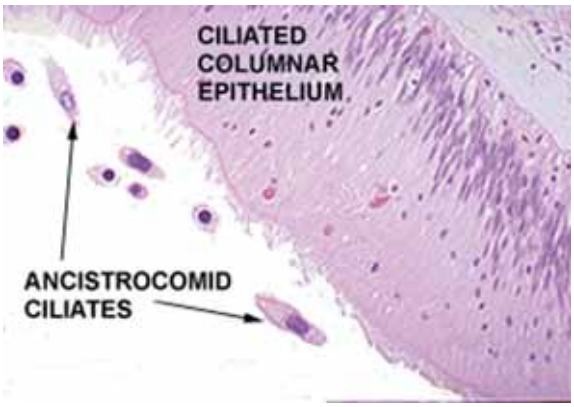

Figure 146. Intestine showing normal epithelium and ancistrocomid ciliates in lumen. Note absence of any apparent tissue response to these protozoa.

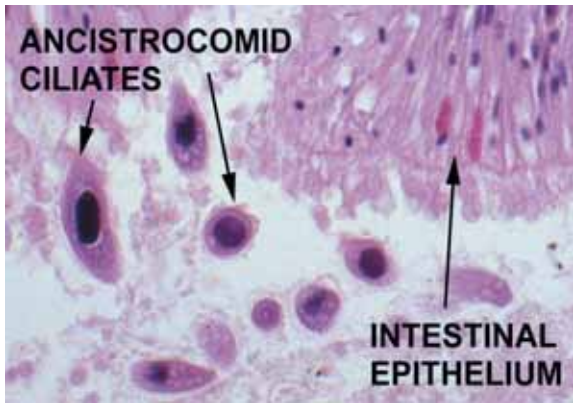

Figure 147. Higher magnification view of ancistracomid ciliates in lumen of intestine.

Gregarine Protozoa

Gregarine protozoa morphologically identical to those in the stomach and digestive gland (Figure 131) occur occasionally in the epithelium of the intestine in the absence of tissue damage. These agents have only been recorded in *P. maxima* from Queensland (Humphrey *et al.* 1998).

Internal Metazoa.

A range of morphologically diverse metazoan agents may be observed in the lumen of the intestine and rectum. In general, these appeared to incite no tissue damage and no inflammatory response. Occasionally mollusc larvae are present in the stomach and the intestine (Figure 148).

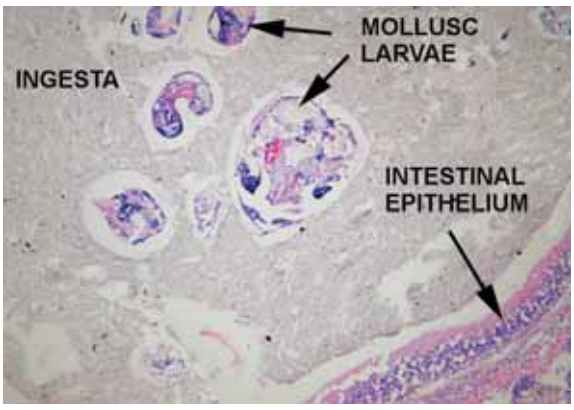

Figure 148. Mollusc larvae in lumen of intestine.

CIRCULATORY SYSTEM

Functional Anatomy and Histology

The circulatory system includes the heart, blood vessels and a system of vascular sinuses that allow for the circulation of haemolymph throughout the animal.

The Heart

The heart is situated in the pericardial cavity within the posterior region of the visceral mass and is covered by a fine membrane, the pericardium. The heart is bordered posteriorly by the thin end of the adductor muscle and ventro-laterally by the retractor muscles of the foot and byssus. Aspects of the anatomy of the heart of Pterid bivalves including *Pinctada* are described by Suzuki (1985).

The heart of *P. maxima,* consists of a single ventricle and a pair of contractile, thin walled auricles which together form a triangular structure. The pale ventricle lies medially and dorsally to the brown auricles. At their dorsal extremity, the auricles each communicate individually with the ventricle. The auricles are united ventrally at their base adjacent to the kidney. Ventricular contraction impels haemolymph into the anterior and posterior aortae. One way flaps or valves (auriculo-ventricular or A-V valves) between the ventricle and auricles (Figure 149) prevent backward flow of haemolymph.

Histologically, the ventricle consists of a meshwork of muscle fibres that create the interconnected cavernous sinuses of the ventricular lumen and which are lined by endothelium (Figure 150). Occasional or numerous accumulations of granular acidophilic cells may be associated with the muscle fibres. The auricles (Figure 149) have a similar structure comprising muscle fibres and numerous pigmented cells but the vascular spaces are considerably reduced. Pigmentation of the heart, notably the auricles, is a normal feature of *P. maxima* (Figures 151, 152). A fine endothelium lines the muscle tissues of auricle and ventricle.

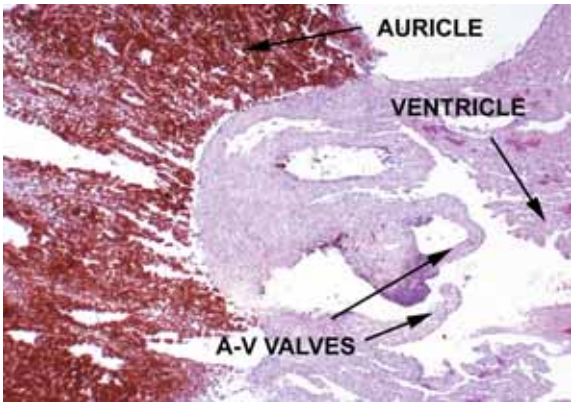

Figure 149. Lower magnification view of junction of auricle and ventricle in heart of mature *P. maxima*. Note dark region on valve indicating focal inflammation.

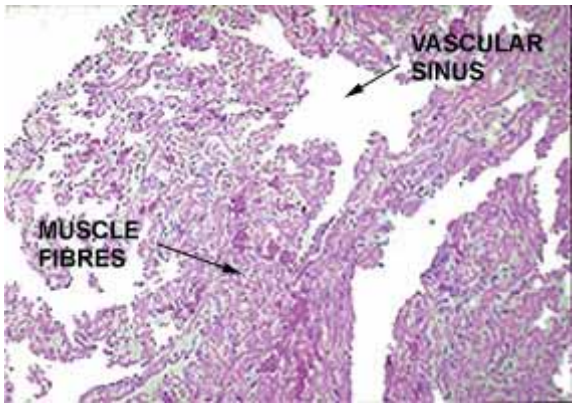

Figure 150. Lower magnification view of ventricle showing muscle fibres and vascular sinuses lined by endothelium.

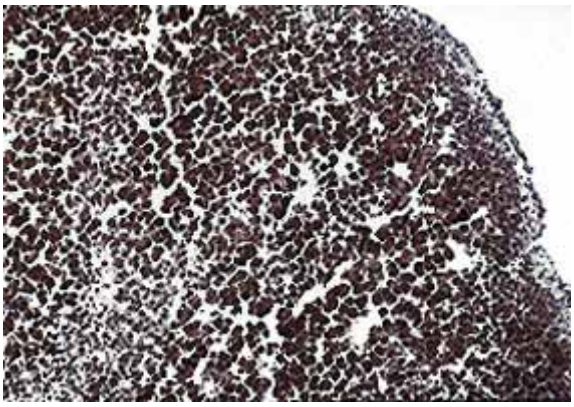

Figure 151. Lower magnification view of auricle showing heavily pigmented muscle fibres.

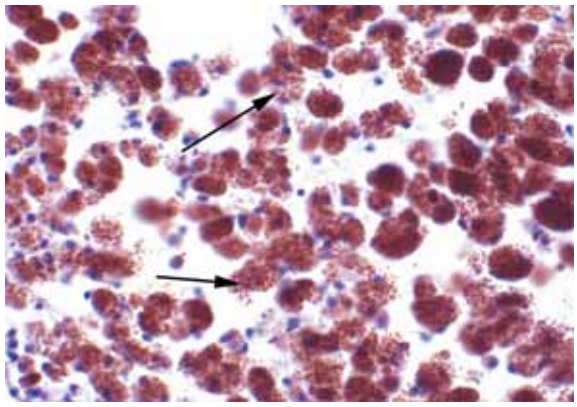

Figure 152. Higher magnification view of auricle showing fine granular melanin-like pigment (arrows) within endothelial cells lining haemolymph sinuses.

Vasculature

Two macroscopically visible major vessels arise from the dorsal extremity of the ventricle, the anterior and posterior aortae. The anterior aorta passes through the anterior visceral mass and gives rise to a series of minor arteries which open into vascular sinuses and which ultimately communicate with larger vessels in the margins of each mantle lobe. The posterior aorta, a shorter vessel, runs posteriorly along the rectum and serves the adductor muscle, rectum and anus.

Deoxygenated haemolymph collects in veins where it is transported to the gills for re-oxygenation and to the kidneys for elimination of metabolic wastes or osmoregulation. Haemolymph from the mantle and the gills returns to the heart through the efferent branchial vein to the auricles.

Major and minor haemolymph vessels are readily seen in the stroma of the digestive gland (Figures 153, 154). Major vessels have a clearly defined endothelium and fibrous media (Figure 155). Vessels often have a conspicuous outer cellular component of eosinophilic granule cells, which surround major haemolymph vessels.

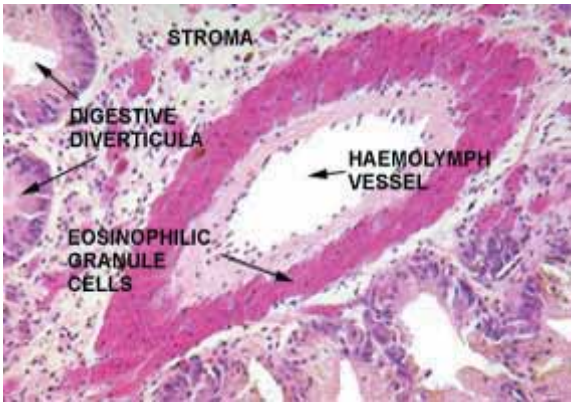

Figure 153. Major haemolymph vessel in digestive gland. Note conspicuous granular acidophilic cell layer.

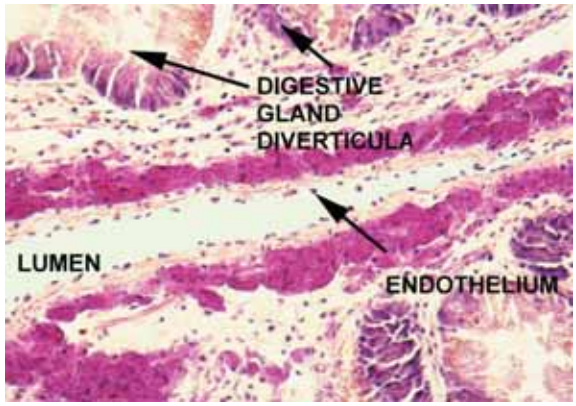

Figure 154. Longitudinal section of major haemolymph vessel in digestive gland.

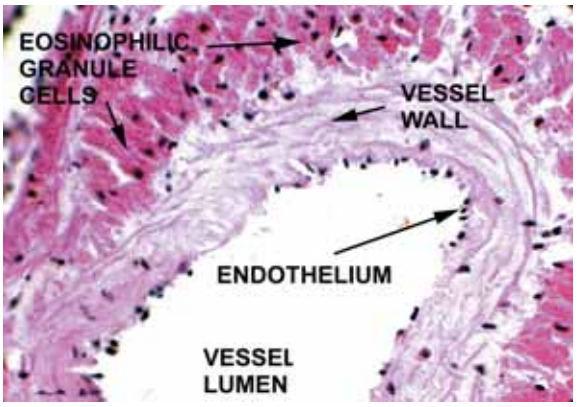

Figure 155. High magnification of major vessel showing endothelial lining, fibrous media and surrounding granular acidophilic cells.

Pathology and Histopathology

Non-specific Inflammatory Lesions

Non-specific Inflammation

Non-specific inflammatory changes in the heart, characterised by mild to intense, focal or regional infiltrations of haemocytes in the stroma, sometimes accompanied by degeneration and necrosis may be observed occasionally in apparently normal oysters in the absence of obvious causative agents. Haemocytic accumulations on the valves may occur occasionally (Figures 149, 156, 157). Such foci may be granulomatous in nature and show peripheral fibrous encapsulation. Vasculitis or inflammation of the haemolymph vessel (Figure 158) may accompany inflammatory responses in any tissue or organ.

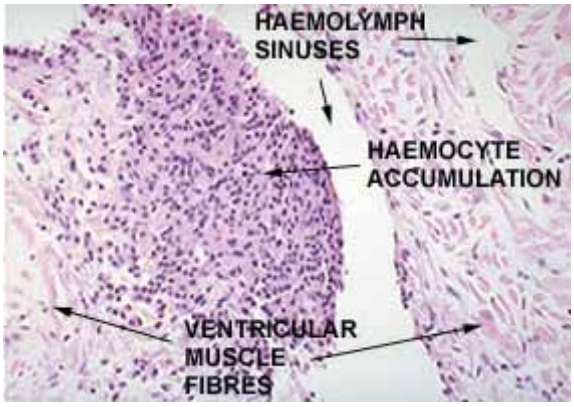

Figure 156. Lower magnification view of section of heart valve showing intense focal haemocytic infiltration.

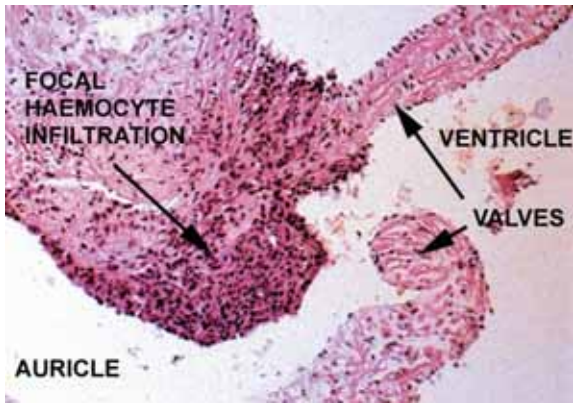

Figure 157. Higher magnification of heart valve showing intense local inflammatory reaction, typically caused by localisation of vibrionic bacteria following septicaemia.

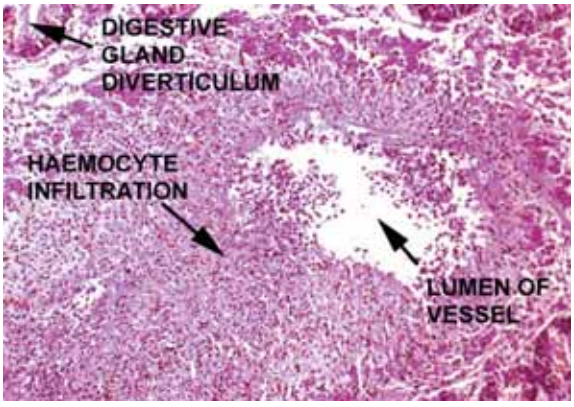

Figure 158. Lower magnification view of intense vasculitis in the digestive gland. The vasculitis is characterised by an extensive infiltration of the vessel and surrounding tissue by haemocytes, loss of the endothelium of the vessel and infiltration of haemocytes into the vessel lumen.

Infectious and Commensal Organisms

Bacterial Colonisation and Micro-abscessation

Focal or regional infiltrations of haemocytes in the heart may be observed in diseased animals with bacterial septicaemia, in which bacilliform bacteria typical of marine vibrios may often be seen in the lesion. The heart appears to be a primary site for bacterial localisation in oysters with bacterial septicaemia, where abscess or granuloma formation with central necrosis is a frequent finding. The presence of focal haemocytic inflammation in the myocardium (Figures 64, 159, 160), in the absence of obvious aetiological agents, is typical in bacterial septicaemia, especially associated with *Vibrio* spp. Valvular inflammatory lesions are not uncommon, especially in oyster spat with vibriosis (Figures 149, 156, 157).

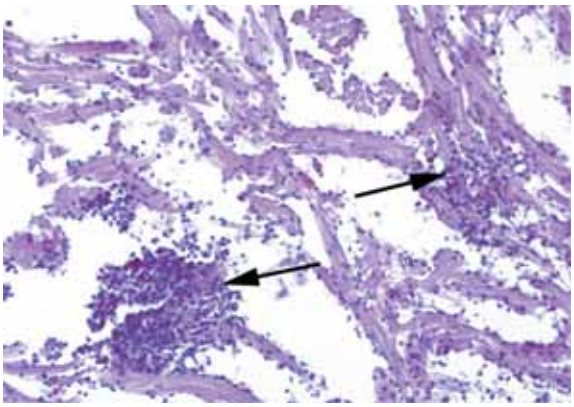

Figure 159. Acute multifocal myocarditis characterised by focal haemocytic accumulations in the myofibres of the ventricle (arrows).

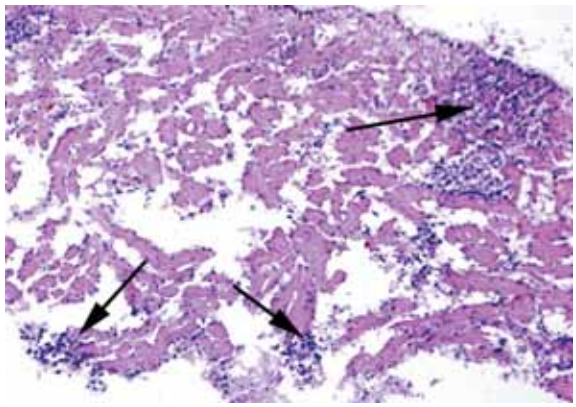

Figure 160. A further example of focal myocarditis, with a large and multiple small discrete accumulations of haemocytes (arrows) in the ventricular muscle fibres of the heart.

Protozoa

An apicomplexan has been reported in the heart of spat of *P. maxima* from Western Australia.

Internal Metazoa

Uncharacterised metazoan agents may occasionally occur in the sinuses of the heart and in haemolymph sinusoids (Figure 161). There are no reports of clinical disease associated with these agents.

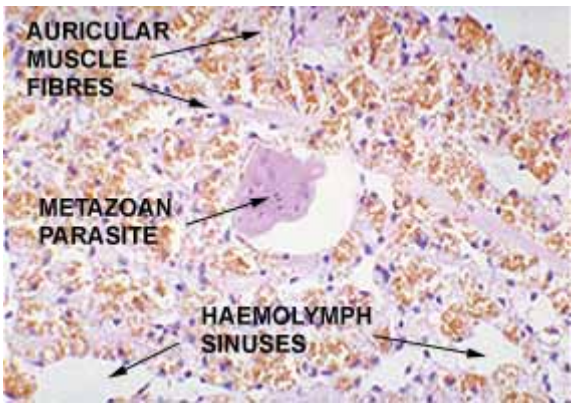

Figure 161. Lower magnification view of auricle of heart showing uncharacterised metazoan in haemolymph sinus. Note absence of inflammatory response.

MUSCULAR SYSTEM

Functional Anatomy and Histology

The muscles of the pearl oyster are well-defined structures as follows;

- Adductor muscle
- Retractors of the foot
- Levators of the foot
- Branchial muscles
- Pallial muscles
- Heart

Muscle fibres may also be found interspersed in the interstitial tissues, associated with the stomach, intestine and digestive gland.

Adductor Muscle

The pearl oyster is a monomyarian mollusc, i.e., a mollusc that only has one adductor, which corresponds to the posterior adductor in other bivalves. The anterior adductor, present in the larvae, disappears during metamorphosis shortly after attachment of the larvae (Galtsoff 1964). The adductor muscle, comma shaped in lateral view and wedge shaped when viewed in the vertical plane, is the largest muscle in the oyster, extending transversely across the body from shell valve to shell valve. The adductor muscle is attached to the shell valves by a specialised adhesive epithelium. The function of the adductor muscle is to close the shell valves.

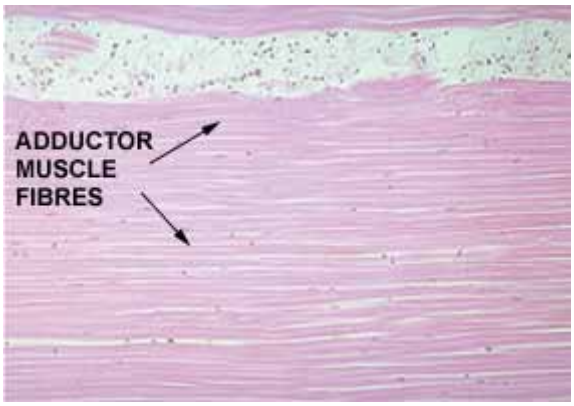

Figure 162. Adductor muscle showing lamellar muscle fibres.

Two distinct regions in the adductor occur: A narrow tendinous strip of white glistening non-striated fibres, the "slow" muscle fibres forming the posterior border and a broad region of soft, striated, translucent fibres, the "fast" muscle fibres which form the bulk of the muscle mass (Figures 12, 13, 162). Striations are difficult to visualise in muscle stained with haematoxylin and eosin, however, they may be demonstrable using special staining techniques, for example, phosphotungstic acid haematoxylin.

The adductor muscle is strongly adherent to the internal surface of the shell valves by way of a layer of simple cuboidal cells subtended by secretory cells, the adhesive epithelium (Figure 163).

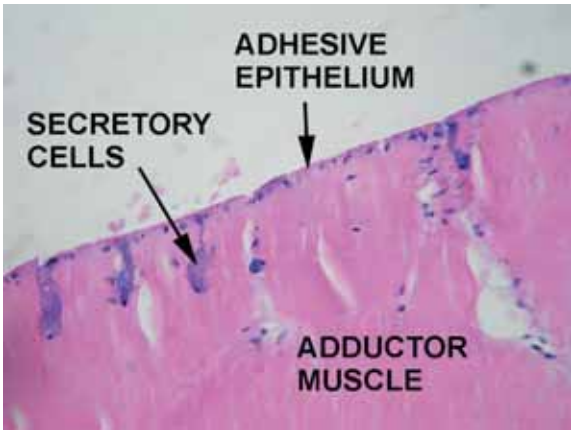

Figure 163. Adductor muscle showing adhesive epithelium which bonds the muscle fibres to the overlying shell valve.

Retractor Muscles of the Foot

The retractors of the foot form a pair of symmetrical V-shaped muscles lying in the horizontal plane of the body. Each muscle arises from the region of the foot, attaching to the right and left shell valves adjacent to the adductor muscle, without making a separate scar on the nacre (Figure 11).

Levator Muscles of the Foot

The levator muscles of the foot comprise two anterior and two posterior muscle bundles. Each of the anterior levator muscles arise in the foot, pass vertically on either side of the mouth, spreading laterally in a fan-shape and insert at the apex of the umbonal recess of the shell valve. The zone of insertion is visible on the internal face of the shells. The posterior levator muscles are two short muscle bundles that originate in the foot and pass through the visceral mass, attaching to the shell valves behind the anterior levator scar. The posterior levator muscles are reduced in size and are difficult to observe grossly. Contraction of the levator muscles causes the foot to be retracted and raised dorsally.

The **branchial, pallial** and **heart muscles** are described under gill, mantle and circulatory systems, respectively. Pallial muscle fibres of the mantle are shown in Figures 67, 70 and 72.

Pathology and Histopathology

Degenerative Lesions

Oedema

Oedema of the adductor muscle is occasionally recorded. This may occur in oysters removed from the water and subject to high air temperatures.

Mineralisation

Focal areas of concentric lamelliform mineralisation may be seen occasionally in adductor muscle.

Non-specific Inflammatory Lesions

Non-specific Inflammation

Non-specific foci of inflammation are common, likely associated with bacterial infections, especially in younger oysters. Focally extensive or diffuse infiltrations of the muscle fibres of the adductor by haemocytes may occur (Figure 164), associated with tissue damage caused by excessive force when opening the shell. More intense, focal haemocytic accumulations in the muscle may occur in bacterial septicaemia.

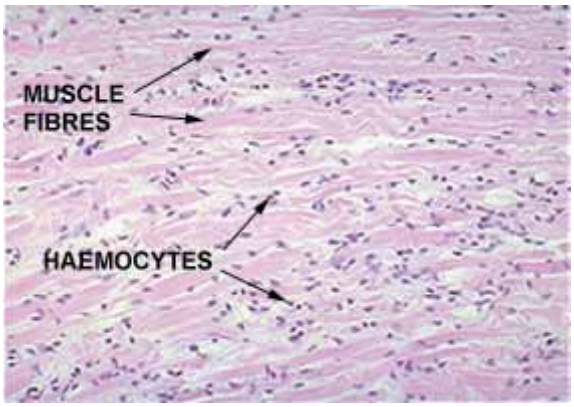

Figure 164. Adductor muscle showing mild, generalised haemocytic infiltration. This may be associated with stretching and tearing of muscle fibres which occurs with forceful seperation of the shell valves.

Infectious and Commensal Organisms

Internal Metazoa

On rare occasions, metazoa may invade the muscle tissues, probably as incidental infections. Figure 165 shows invasion of the levator of the foot by an unidentified metazoan, likely a crustacean parasite.

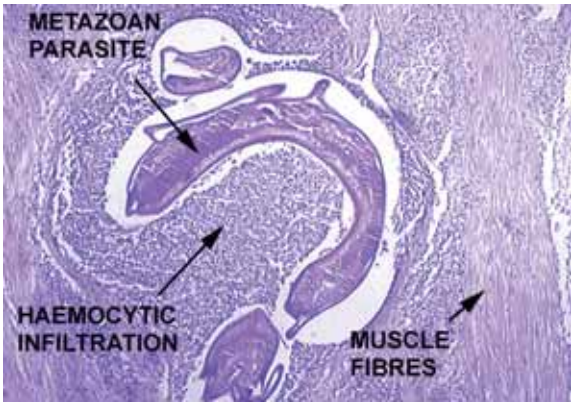

Figure 165. Unidentified metazoan parasite, probably a crustacean, invading the muscle of the levator of the foot. Note the intense haemocytic inflammatory response adjacent to the parasite

NERVOUS SYSTEM

Functional Anatomy and Histology

The structure of the nervous system of *Pinctada sp.* is described in detail by Velayudhan and Gandhi (1987). A bilaterally symmetrical network of ganglia and sensory and motor nerve fibres form the nervous system. Three pair of laterally and radially connected ganglia, the *cerebral, pedal and visceral ganglia*, are present. The cerebral ganglia are located on each side of the oesophagus, the pedal ganglia are located at the base of the foot and the visceral ganglia are located on the antero-ventral face of the adductor muscle ventral to the visceral mass (Figure 56). Typical ganglion and nerve trunk structures are shown in Figures 166 and 167.

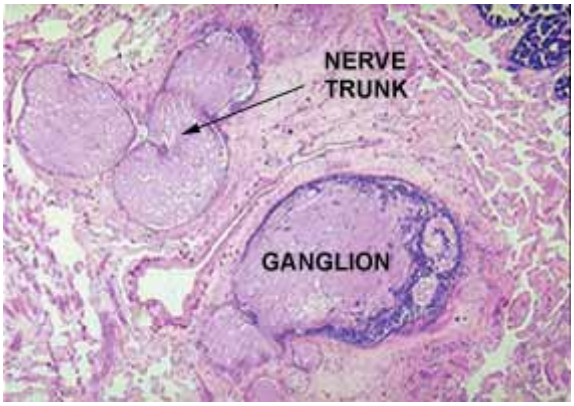

Figure 166. Lower magnification view of nerve. ganglion and associated nerve trunk.

Major nerves arise from each of the ganglia and these ramify into organs and tissues, providing sensory and motor innervation. Transversely, the cerebral ganglia are united by the supra-oesophageal nerve, the visceral ganglia appear united by a single visceral commissure and the pedal ganglia are joined to form a single ganglion, the pedal nerve mass. Major neural connections traverse the visceral mass between ganglia on each side of the animal, connecting the cerebral, visceral and pedal ganglia.

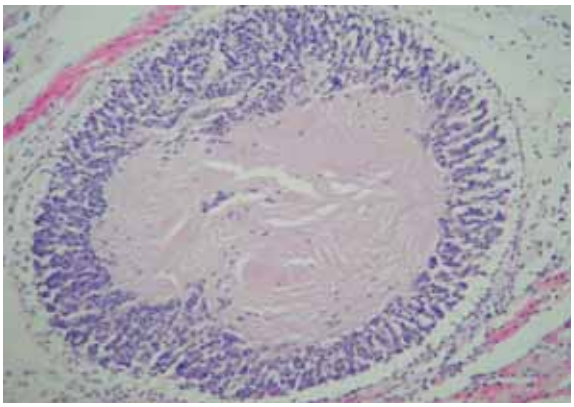

Figure 167. Higher magnification showing peripheral arrangement of neurones and glial cells within ganglion and central nerve trunk.

The cerebral ganglia give rise to nerves that innervate the pallial margins and palps, the pedal ganglia give rise to nerves that innervate the foot and byssal gland and the visceral ganglia give rise to nerves that innervate the gills. Within the muscular margin of the mantle, a network of nerves derived from the cerebral ganglia form the pallial plexus.

Pathology and Histopathology

The neural tissues of *P. maxima* appear relatively free from pathological processes.

Proliferative Lesions

In a comprehensive histopathological survey of mature *P. maxima* (Humphrey *et al.* 1998), only two proliferative lesions morphologically consistent with neurofibroma were recorded (Figure 168).

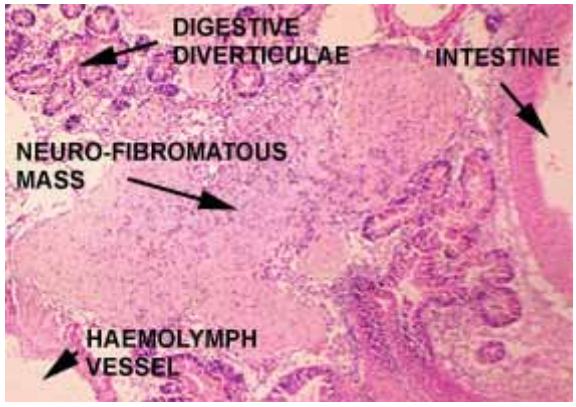

Figure 168. Irregular, multinodular neurofibromatous mass extending into digestive gland.

REPRODUCTIVE SYSTEM

Functional Anatomy and Histology

The sexes of *P. maxima* are generally separate, with approximately 0.003% of animals being hermaphroditic (Humphrey *et al.* 1998). Gonads are typically asymmetric, enveloping the stomach, digestive gland and sections of the intestine and occupying a major portion of the visceral mass. Grossly, the male gonads appear creamy-white whereas the female gonads appears more orange in colour.

Histologically, the testes and ovaries consist of branched tubules, the lining of which supports the development and maturation of spermatozoa or ova. With maturation, the mature gametes fill the tubules and pass into excretory ducts that lead to the external genital aperture.

The histology of the reproductive cycle of *P. maxima* has been described in detail by Rose *et al.* (1990), who characterised the seasonal gonadal development in the species. *P. maxima* is a protandrous hermaphrodite, reaching maturity as a male in the first year of its life, with the incidence of female animals increasing with age thereafter. Initial gonadal development is initiated with the formation and proliferation of reproductive follicles near the urogenital papilla, proximal to the retractor muscles, in the interstitial tissues between the visceral mass and the external integument (Figure 169). Male and female gonads undergo progressive gametogenesis in five stages (Rose *et al.* 1990).

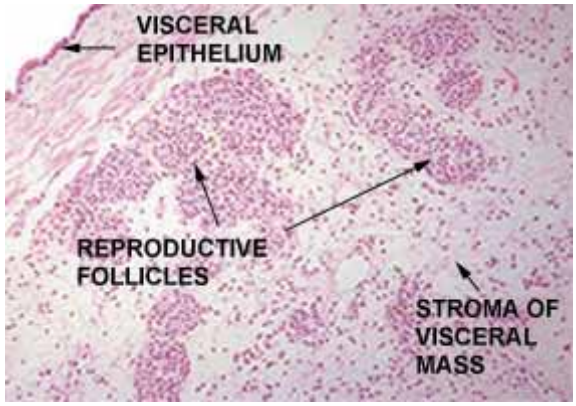

Figure 169. Low magnification of developing gonad in juvenile oyster showing follicular development in stromal tissues.

Stage 0

Sex indeterminate, early gametogenesis and follicles devoid of sperm or ova (Figure 170).

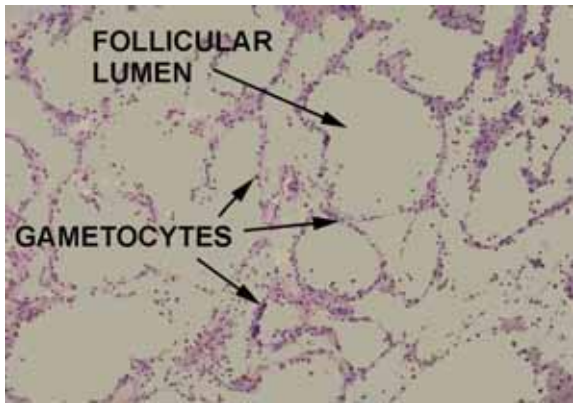

Figure 170. Early gametogenesis, mature oyster with gonad of indeterminate sex. Empty follicles and stromal tissue with granulocytes and phagocytes.

Testis

Stage 1

Follicles initially small and lined with stem cells and spermatogonia, with proliferation of primary and secondary spermatocytes filling the follicular lumen.

Stage 2

Follicles enlarge with spermatogonia and spermatocytes proliferating along the periphery of the lumen. Spermatids and some spermatozoa fill the centre of the lumen. At the end of this stage, the follicular lumen is packed with mature spermatozoa (Figures 171, 172).

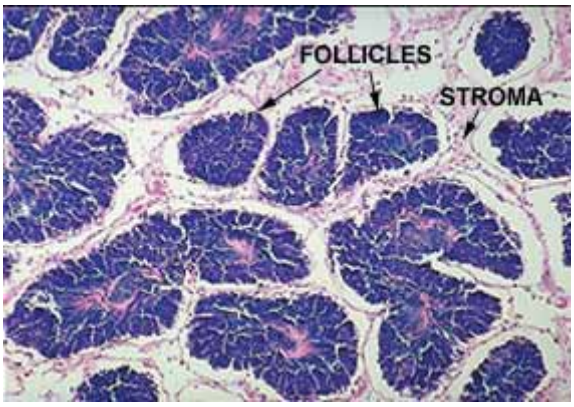

Figure 171. Testis showing follicles filled with spermatids and spermatozoa (stage 2).

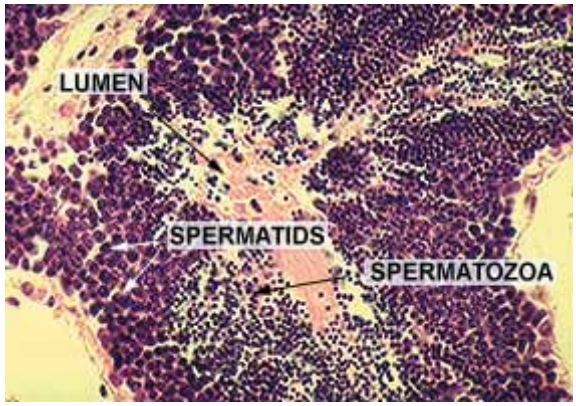

Figure 172. Higher magnification view of stage 2 testis with follicle with mature spermatozoa extending into lumen and a thick layer of spermatids.

Stage 3 (Spawning Ripe)

Follicles are distended, confluent and almost filled entirely with spermatozoa, remaining spermatids and spermatocytes restricted to the lining of the follicular wall.

Stage 4 (Partially Spawned to Spent)

Follicles have partially empty lumena, with a space between the wall and mass of spermatozoa in other areas. Spent follicles are empty except for residual pockets of sperm and phagocytes, with some areas of redevelopment along the walls of some follicles.

Ovary

Stage 1

Follicles initially small and lined with stem cells and developing oocytes. Oogonia and early or primary oocytes have little or no yolk and have a large basophilic nucleus, and frequently adhere to the follicular wall.

Stage 2

Oocytes connected to the follicular wall accumulate yolk material and expand into the lumen. Near mature follicles are packed with elongated oocytes, generally connected to the follicular wall by a narrow stem of yolk material (Figure 173).

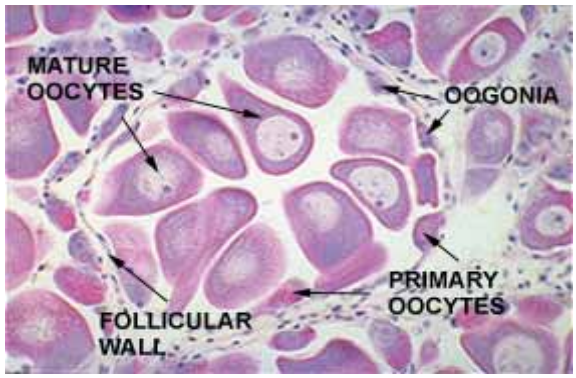

Figure 173. Higher magnification view of ovarian follicle showing developing oocytes connected to follicular wall.

Stage 3 (Spawning Ripe)

Follicles are distended, confluent and filled almost entirely with free, polygon-shaped oocytes displaying both nucleus and nucleolus.

Stage 4 (Partially Spawned to Spent)

Follicles partially empty, oocytes rounded to piriform, resorptive material surrounding free oocytes undergoing cytolysis. Spent follicles almost entirely devoid of oocytes with no sign of gametogenesis (Figure 174).

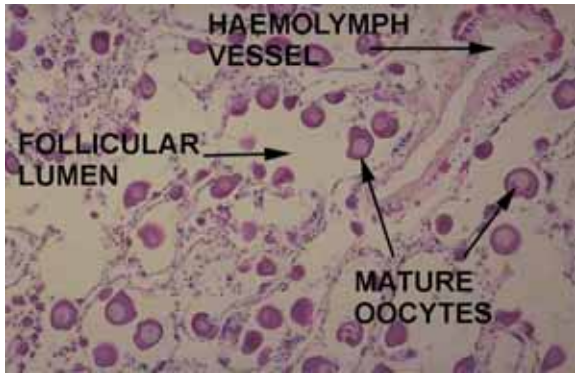

Figure 174. Lower magnification view of a stage 4 ovary, partially spawned to spent, with follicles partially empty and oocytes rounded to piriform.

Bisexual Animals; Hermaphrodites

Rose *et al* (1990) described two forms of bisexuality. One in which both sexes develop concomitantly in the same follicle (Figure 175) and one in which one sexual phase overlaps with another within the same gonad.

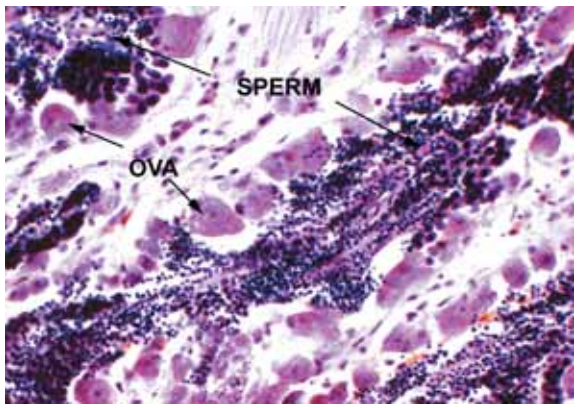

Figure 175. Mature hermaphrodite showing well developed sperm and ova within the same follicle.

Pathology and Histopathology

Degenerative Lesions

Mineralisation

Concentric, lamelliform, mineralised foci may be occasionally observed in the gonad.

Non-specific Inflammatory Lesions

Non-specific Inflammation

Regional haemocytic infiltrations are associated with regressing gonads as part of the normal cycle of regression (Figure 176). Occasional non-specific haemocytic accumulations may be seen in the gonadal stroma of mature *P. maxima*.

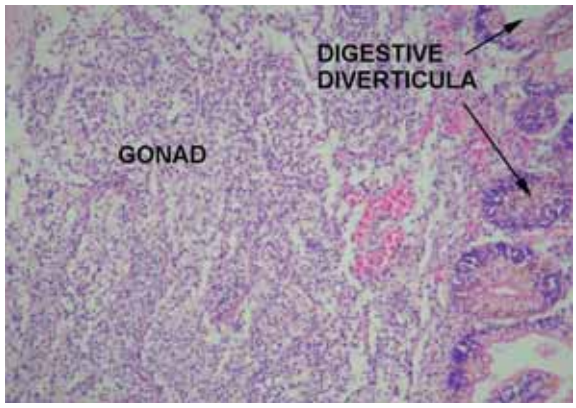

Figure 176. Low magnification view of resorbing gonad of *P. maxima* showing moderately intense infiltration of gonadal tissue by phagocytic haemocytes.

Infectious Organisms and Diseases

Rickettsiales-like Infections

Granular Rickettsiales-like bodies approximately 20-30 um diameter and containing numerous fine, basophilic bodies which occupy epithelial cells or which are intimately associated with epithelial cells, may occasionally be seen in the gonad, in the absence of tissue damage.

EXCRETORY SYSTEM

Functional Anatomy and Histology

The excretory system of *P. maxima* consists of paired, clear brown symmetrical sacs, the nephridia or kidneys which lie immediately adjacent to the walls of the auricles. Some confusion in nomenclature exists due to the use of a variety of names for the molluscan nephridium described variously as the organ of Bojanus, kidney, nephridium, renopericardial passage and nephropericardial passage (Galtsoff 1964). Aspects of the anatomy of the excretory organs in Pterid bivalves including *Pinctada* are described by Suzuki (1985).

The nephridia are located laterally in the visceral mass adjacent to the heart. Each nephridium is roughly triangular, the apex passing into the tissue under the auricle, while the elongate base coincides with the base of the anterior gill (Figure 177). The outer wall of the nephridium is thin and membranous. The inner wall is in intimate association with the pericardium. Each nephridium communicates with the pericardial area by a wide duct or sinus and a renal duct communicates to the exterior.

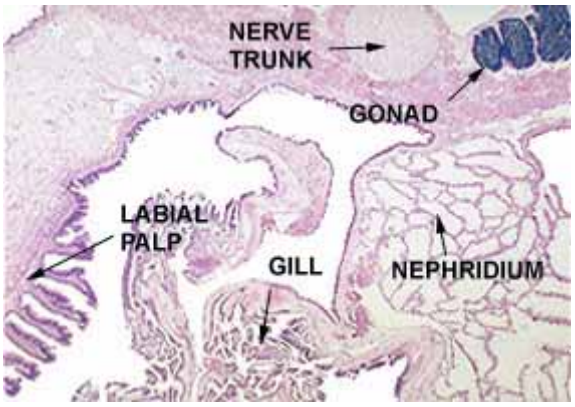

Figure 177. Lower magnification view showing structure and relationships of nephridia, gill and labial palp.

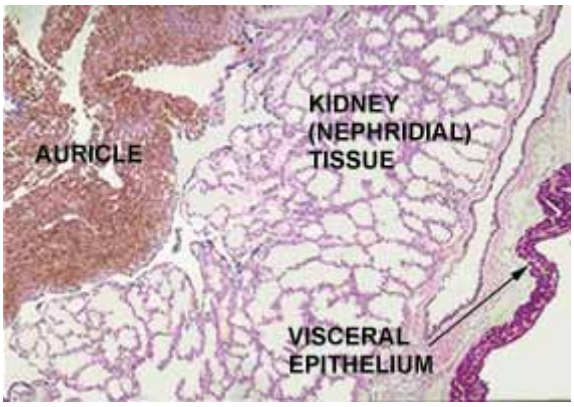

Figure 178. Histological relationship between nephridium and auricle.

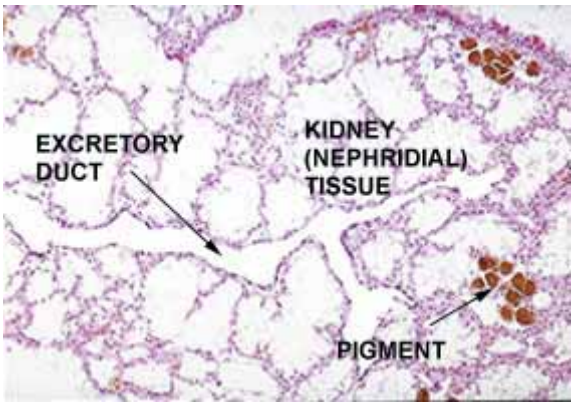

Figure 179. View of glandular tissue of nephridium.

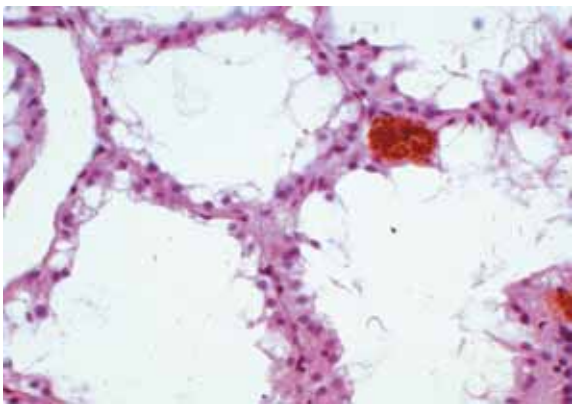

Figure 180. Higher magnification view of nephridium showing epithelial-lined glands.

The principle function of the paired nephridia is thought to be excretion of nitrogenous wastes. Studies on other bivalve species have demonstrated the excretion of amino acids, ammonia, purine, urea and uric acid (Galtsoff 1964). There is little information on the osmo-regulatory role of the nephridia. In keeping with nephridial function in other bivalves, urine formation appears to be a process involving filtration of haemolymph through the wall of the heart into the pericardium, which, together with possible secretions from pericardial cells, passes into the nephridial tubules. Here, secretion from the glandular nephridial tubules is added to the pericardial secretion, and is eliminated via an excretory duct.

Histologically, each nephridium consists of epithelial-lined glandular nephridial tubules with an irregular lumen. The majority of cells forming the glandular tissue are large and clear. Excretory products are assumed to pass from the haemolymph through the glandular region into the spacious cavity of the lumen (Figures 177, 178, 179, 180).

Pathology and Histopathology

Degenerative Lesions

Pigmentation

Increased pigmentation is reported in the nephridial glands, but this may reflect plane of section through pigmented glandular tissue or represent normal pigment deposits (Figure 179).

Non-specific Inflammatory Lesions

Non-specific Inflammation

Non-specific inflammatory changes, characterised by focal or regional haemocyte infiltrations occur infrequently in the nephridia.

Infectious and Commensal Organisms

Internal Metazoa

Occasionally, uncharacterised metazoa may be observed in the lumen of the kidney tubules in the absence of tissue damage.

Proliferative Lesions

Hyperplasia

Hyperplasia of the nephridial tubular epithelium, characterised by thickening and proliferation of the nephridial epithelium in the absence of gland formation, is occasionally observed (Figure 181). The cause is unknown.

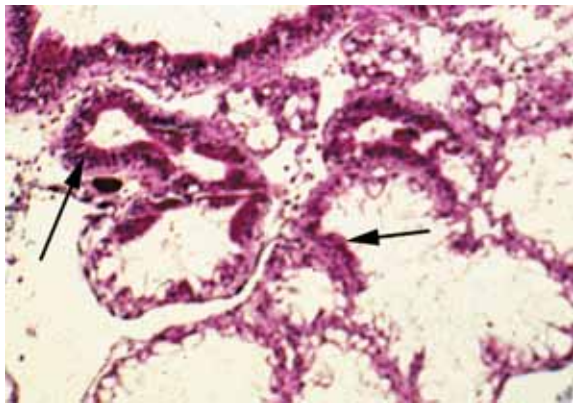

Figure 181. Hyperplasia of nephridial epithelium characterised by proliferation of the epithelium (arrows).

BYSSAL ORGAN

Functional Anatomy and Histology

The byssal organ or byssal gland comprises a compact bulbous mass in the central region of the retractor muscles of the foot adjacent to the proximal end of the foot on the dorsal aspect of the oyster (Figures 51, 52, 54, 56, 182). The organ comprises glandular tissue, crypts and ducts from which the secretion emerges as fine, laterally compressed green threads, the byssal threads. The byssal threads may grow up to several centimetres in length and emerge from the organ by way of epithelial lined ducts. The byssal threads coalesce to form a thick common byssal trunk or byssus. The byssus anchors the oyster to the substrate by means of discoid attachments approximately 150 um in diameter at the distal extremity of each thread. The rate of growth and attachment is considered an important indicator of body condition.

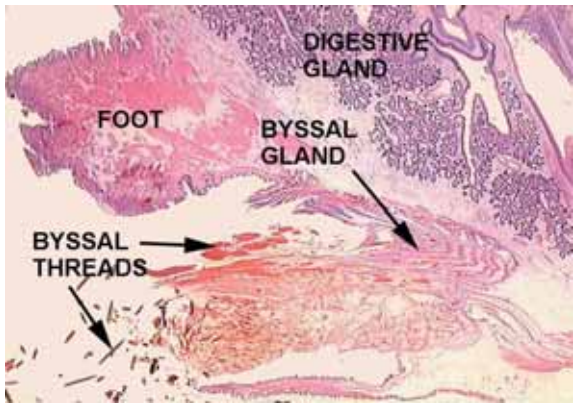

Figure 182. Low magnification view of byssal gland adjacent to the foot.

Histologically, the byssal crypts and ducts are surrounded by byssal glandular tissue. The glands are seen as regions of highly eosinophilic cells surrounding the crypts, extending to and encompassing the pedal groove of the foot. The ducts extends into the mantle cavity adjacent to the foot as a series of finger-like protrusions lined by a flattened, cuboidal epithelium with eosinophilic cytoplasm, supported on a fibrous stroma containing haemolymph vessels and basophilic mucous cells. The threads ultimately unite in the byssal canal. Byssal threads occur as elongate, thickened eosinophilic, proteinaceous threads extending from within the body of the byssal gland. (Figure 183). Other glandular secretions contributing to byssus formation appear to arise from sub-epithelial glands of the foot and glands within the byssal groove.

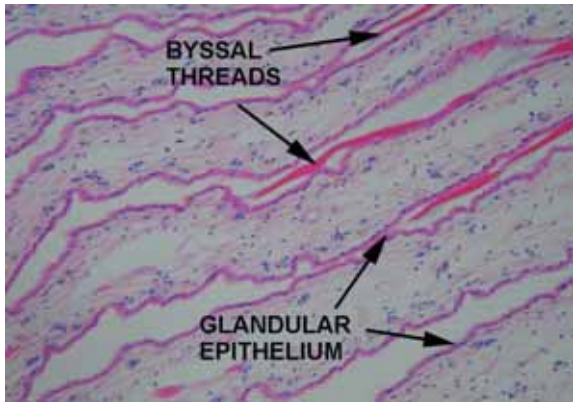

Figure 183. Higher magnification view of byssal gland showing epithelial lined glands and byssal threads.

Pathology and Histopathology

Non-specific Inflammatory Lesions

Non-specific Inflammation

Diffuse haemocytic cell infiltrations of the byssal organ may occur as part of a systemic reaction or in response to local bacterial colonisation. Focal or focally extensive haemocytic infiltration of the byssal gland (Figure 184), and in some cases focal abscessation, may occur. These lesions are considered to be the result of trauma and bacterial infection associated with tearing or pulling on the byssal threads during harvesting or removal from

culture panels as part of management procedures. In many cases, the byssal threads are removed entirely leaving a cavernous space within the gland susceptible to infection by marine vibrionic bacteria.

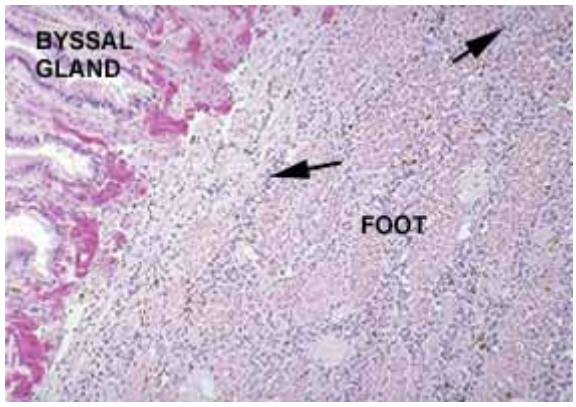

Figure 184. Lower magnification view of byssal gland and adjacent foot showing an intense haemocytic infiltration of the sub-epiothelial tissue (arrows), possibly associated with forceful removal of byssus.

FOOT

Functional Anatomy and Histology

The foot is a mobile, pigmented, elongate, tongue-shaped distensible organ which arises from the anterior region of the visceral mass adjacent to the mouth and byssal organ (Figure 14), approximately midway between the mouth and the intestinal lobe. The foot confers limited ability on the oyster to move in or on the substrate. The foot is of major size and importance in juvenile oysters. Before a byssal attachment to the substrate occurs, the foot acts as the major means of finding suitable substrates and anchoring the oyster. The relative size of the foot decreases with the age of the oyster.

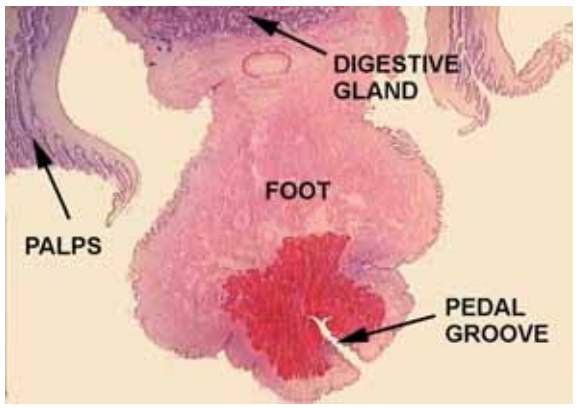

Figure 185. Lower magnification transverse view of foot showing general structure and anatomical association with digestive gland.

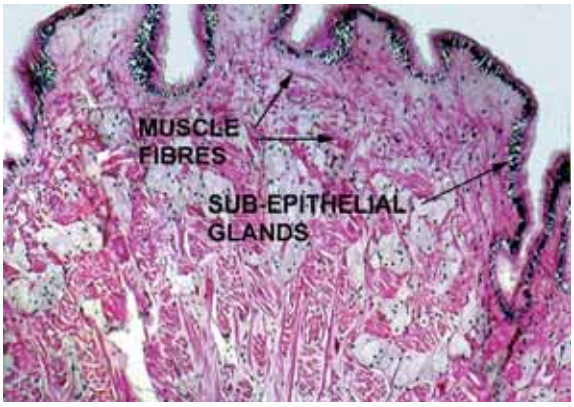

Figure 186. Lower magnification view of foot showing irregularly arranged muscle bundles below the pigmented epithelium.

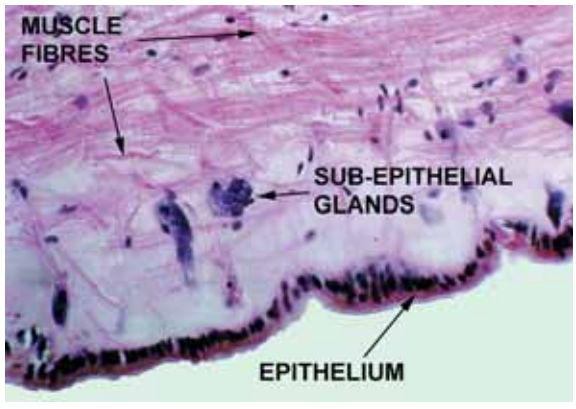

Figure 187. Higher magnification view of epithelium and sub-epithelial tissues of the foot. Note non-ciliated columnar epithelium and fine meshwork of interconnecting muscle fibres.

Pathology and Histopathology

Degenerative Lesions

Oedema

Commonly, oedematous dilation and swelling of the foot is seen in oysters taken straight from the water and may represent a physiological escape response mechanism. Gross oedema of the foot is seen in individual populations of mature *P. maxima*, possibly associated with prolonged time out of water between collection and examination or fixation.

Mineralisation

Foci of concentric, lamelliform, mineralisation occur occasionally in the foot of mature *P. maxima*.

Non-specific Inflammatory Lesions

Non-specific Inflammation

Focal or regional inflammation of the foot characterised by haemocytic infiltrations occurs uncommonly in clinically normal mature *P. maxima*.

Infectious and Commensal Organisms

Internal Metazoa

Occasional metazoan agents may be encountered internally in the haemolymph sinuses of the foot and externally in the pedal groove. Commonly, no tissue damage or inflammation is apparent with these agents.

INTERSTITIAL TISSUES

Functional Anatomy and Histology

The stromal tissues of the oyster provide the structural support for the organs and epithelial tissues of the animal and provide support for haemolymph vessels and nerves. The stromal matrix consists primarily of fibrous tissues through which inflammatory and phagocytic cells may migrate. Wandering phagocytes are considered to play an important role in mollusc excretory function and may be found throughout the visceral mass and gills. The cells accumulate on the surface of the body by diapedesis and are discarded (Galtsoff 1964).

Pathology and Histopathology

Degenerative Lesions

Brown Cells

On occasions, large numbers of phagocytic cells containing brown pigment may be found in the interstitial tissues (Figure 188). These are "brown cells" generally considered to be phagocytes of degenerate tissue and cellular debris. Their presence in numbers possibly reflects previous tissue damage.

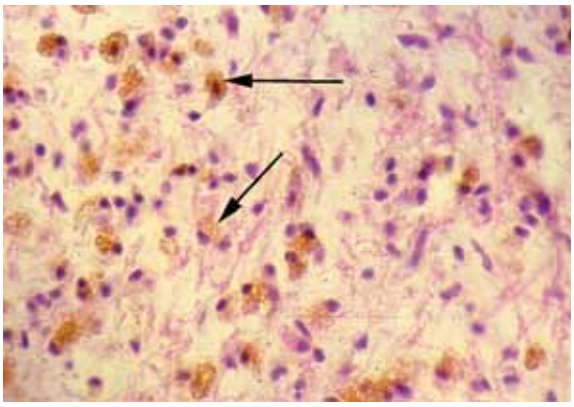

Figure 188. Interstitial tissue showing large numbers of "brown cells", phagocytic cells containing brown pigment (arrows).

Non-specific Inflammatory Lesions

Non-specific Inflammation

Focal or regional inflammation of the interstitium may involve adjacent organs or tissues and is characterised by focal, regional or generalised haemocytic infiltrations. These occur relatively commonly in clinically normal mature *P. maxima*, and may be associated with stress and bacterial invasion.

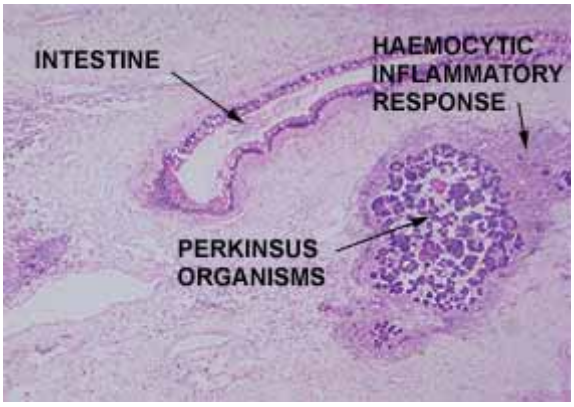

Figure 189. Lower magnification view of interstitial tissue of *P. maxima* infected with large focus of *Perkinsus*-like organisms, with associated peripheral inflammatory response.

Infectious and Commensal Organisms

Perkinsus-like Protozoa

Perkinsus-like protozoa were described by Norton *et al.* (1993b) in focal granulomatous lesions in the tissues of adult *P. maxima* from a population undergoing a high mortality in Torres Strait, Australia (Figures 189, 190). Affected oysters showed multifocal granulomatous systemic lesions that contained the protozoan.

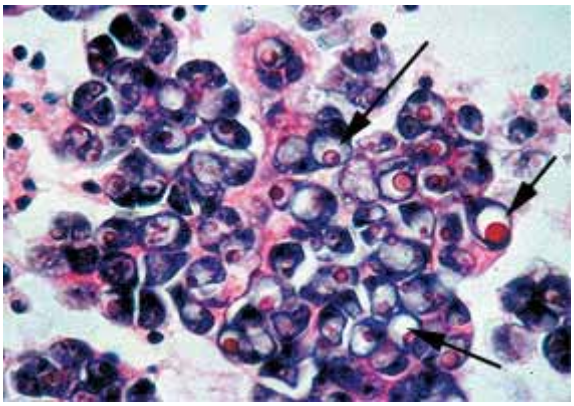

Figure 190. View of nests of *Perkinsus*-like organisms (arrows) in interstitium of *P. maxima*. Note typical "signet ring" forms with eccentric nucleus.

THE PEARL SAC AND PEARL FORMATION

Functional Anatomy and Histology

Pearls are mineralised or partially mineralised accretions formed by living bivalve molluscs. Pearls may be *natural*, occurring as a result of natural processes, or *cultured*, occurring as a result of human intervention or manipulation.

The elucidation of mechanisms of pearl formation are reviewed by Simkiss and Wada (1980). Initial theories of pearl formation included the deposition of nacre around grains of sand, at sites of injury and around parasites. Natural pearls appear to primarily result from invasion of the tissues of the oyster by larval cestodes (Sparks 1985) or larval trematodes, the latter considered a more important cause by Sindermann (1990).

Simkiss and Wada (1980) described initial evidence for pearl formation based on host secretory factors, whereby 13th century Chinese "Budda pearls" were produced by inserting a small leaden image of Budda between the mantle and shell of the mussel *Cristaria plicata*. Later in Europe a commercial process to produce pearls by inserting a limestone ball through the shell was developed, and in Japan the production of hemispherical or blister pearls was achieved by inserting moulds under the mantle of the oyster *Pinctada fucata*.

The underlying cellular basis of natural pearl formation became evident from several early investigations reviewed by Galtsoff (1964) and Simkiss and Wada (1980). Such mechanisms included descriptions of amoeboid cells migrating through the mantle epithelium producing calcareous deposits with subsequent secretion of conchiolin; the recognition of a fundamental role of an epithelial sac, the "pearl sac", in pearl formation; and the formation of the pearl sac through proliferation of mantle epithelial cells under the stimulus of a foreign body. This later observation was quickly exploited through insertion of a nucleus of a pearl into the tissues of an oyster, together with a piece of mantle tissue from another oyster. The result was proliferation of mantle cells around the nucleus forming the pearl sac and production of a market quality, round pearl.

Subsequent to the demonstration that the basis of pearl formation is the secretory pearl sac derived from mantle epithelial cells, attention has focussed on the nature and morphology of the cells of the pearl sac.

The Pearl Sac and Mechanisms of Pearl Formation

The production of a gem quality pearl is dependent on a complex of cellular and biochemical responses which are poorly understood, but are believed to involve both extracellular and intracellular factors, together with selection for specific cell types.

Morphologically, the pearl sac is the epithelial lined cavity embedded in gonadal or connective tissue, derived from mantle tissue implants which grows and surrounds the nucleus introduced into the oyster for pearl production (Figure 191). The histology of the pearl sac in *Pinctada spp*. has been described by Aoki (1966) and Dix (1973a), who associated different types of pearl formations with differing histological structure of the pearl sac epithelium. Depending on the nature and cellular structure of the pearl sac epithelium, mineral deposition of the correct chemical structure to form a gem quality pearl may occur. It is not uncommon to find considerable regional variation in the morphology of the epithelium of the pearl sac.

The morphology of the epithelium is believed to influence the nature of the accretion. The epithelium and the accretions are generally divided into three types; Nacreous, prismatic and periostracal.

The Nacreous Pearl and Pearl Sac

The nacreous pearl is the desired gem quality pearl in which the mineral covering the outer nucleus forms horizontal layers of aragonite crystals. This layer gives the pearl its desirable physical qualities of lustre and colour. The epithelium is generally a single uniform layer of non-ciliated cuboidal or flattened epithelial cells, supported on a fine fibrous stroma and closely attended by gonadal or connective tissues (Figure 192).

The Prismatic Pearl and Pearl Sac

The prismatic pearl is a nucleus that is covered by vertical arrays of calcite crystals. Such pearls lack lustre and are not commercially desirable. The epithelium forming the prismatic pearl sac is generally a single layer of cuboidal cells being generally taller than those cells forming the nacreous pearl sac.

The Periostracal Pearl and Pearl Sac

Periostracal pearls are composed primarily of an organic matrix. This form of pearl sac comprises a single layer of tall cylindrical ciliated epithelial cells (Figure 193), often thrown into ridges, with accumulations of haemocytes in sub-epithelial areas.

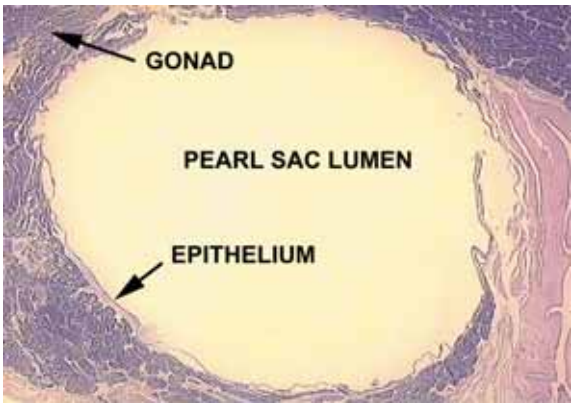

Figure 191. Pearl sac embedded in gonad. Note thin, simple squamous epithelium surrounding lumen of the pearl sac.

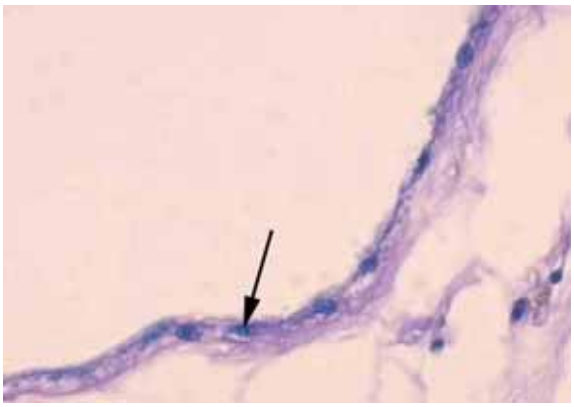

Figure 192 (above). Flattened, non-ciliated epithelium (arrow) of nacreous pearl sac.

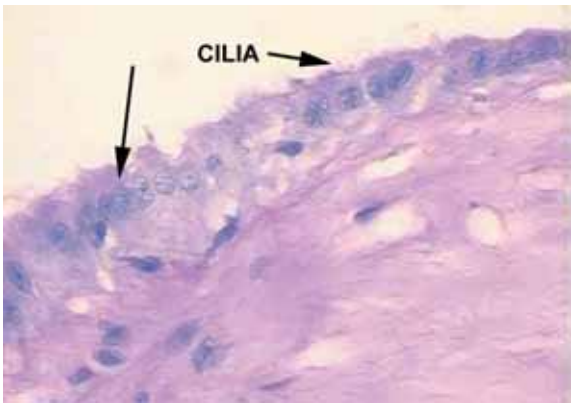

Figure 193 (right). Tall columnar to cuboidal, ciliated epithelium (arrow) typical of periostracal pearl sac.

SECTION 3

ARTEFACTS

ARTEFACTS

Artefacts are alterations of the natural appearance of organs or tissues. These alterations may occur following death, may be caused by a variety of chemical and physical insults to the tissue, or may be associated with the presence of confounding structures or objects. Artefacts may cause confusion in the interpretation of the appearance of oysters at both gross and histological levels and may lead to erroneous conclusions regarding the nature of possible disease states. As such, it is important that artefacts be recognised and not confused with true pathological alterations of tissues.

ANATOMICAL ARTEFACTS

Fixation Contraction

Immersion of the living oyster in fixative results in immediate contraction of the radial muscles of the mantle with distortion of the superficial architecture of the mantle and gills, hence histological interpretation of tissues may be difficult for the pathologist (Figure 15). Fixation contraction may mask lesions of the mantle and may cause difficulties in selecting representative tissues for histological examination.

Relaxation of the oyster and excellent fixation can be readily achieved by anaesthesia (Mills *et al.* 1997) followed by immersion in fixative (Figures 12, 13). This technique is recommended for critical gross and histopathological examinations.

HISTOLOGICAL ARTEFACTS

Post-mortem Changes

Post-mortem Autolysis

Post-mortem autolysis, i.e., enzymatic digestion of tissues following death, is a common artefact which particularly involves the digestive gland and results from:

- Prolonged time between death and fixation
- Poor or inadequate fixation due to tissues being too thick to allow penetration of fixative or insufficient volumes of fixative being used
- Immersion of unopened oysters into fixative. The shells prevent the fixative reaching the soft tissues of the oyster and as a consequence the oysters die and undergo autolysis. Fixative must penetrate the soft tissues to allow meaningful histological examination. See Section 4 Techniques and Stains

In stressed or diseased pearl oysters it is not uncommon for a region of the animal to become non-viable while adjacent tissues remain viable. Difficulties may be encountered in differentiating these degenerative changes from the artefacts produced by post-mortem autolysis.

For optimal fixation and to avoid post-mortem autolysis, tissues should be collected as soon as possible after death, be no thicker than approximately 5 mm and be fixed in 10 volumes of fixative for each volume of tissue.

Post-mortem autolysis is characterised by nuclear dissolution, loss of cytoplasmic detail and loss of cell boundaries. Autolytic change renders critical examination of histological changes difficult if not impossible.

The digestive gland of *P. maxima* undergoes rapid post-mortem autolysis, initially characterised by shrinkage and condensation of nuclear material, accompanied by loss of cellular detail, (Figures 194), followed by dissolution of nuclear chromatin and a "washed out" staining appearance (Figures 195). Ultimately, all nuclear material is lost through dissolution and the tissue stains generally eosinophilic (Figure 196).

Putrefaction

Post-mortem autolysis may be, and frequently is accompanied by putrefactive changes associated with bacterial colonisation of the dead tissues. These must be differentiated from ante-mortem bacterial invasion.

Vacuolation

Vacuolation under and within epithelial surfaces, especially of the palp and mantle, but also of the collecting ducts of the digestive gland and intestine is a common artefactual change associated with prolonged time between death and fixation (Figure 197).

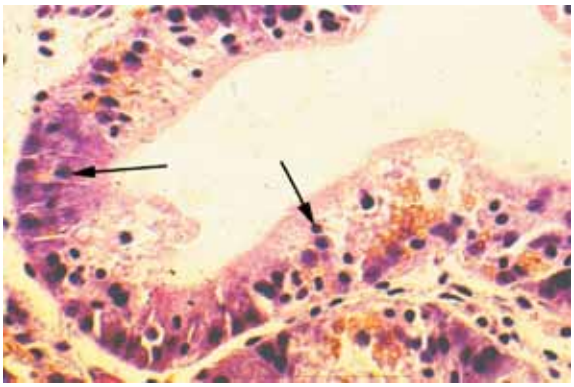

Figure 194. Early post-mortem autolysis in digestive gland. Note loss of nuclear and cytoplasmic detail, condensation of nuclei and granular cytoplasm (arrows).

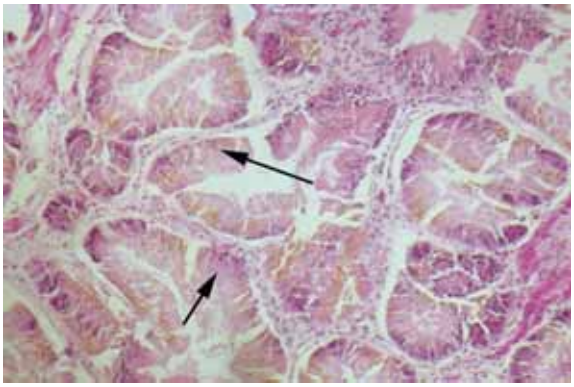

Figure 195. Advancing autolytic change showing loss of nuclear and cellular detail in digestive gland (arrows).

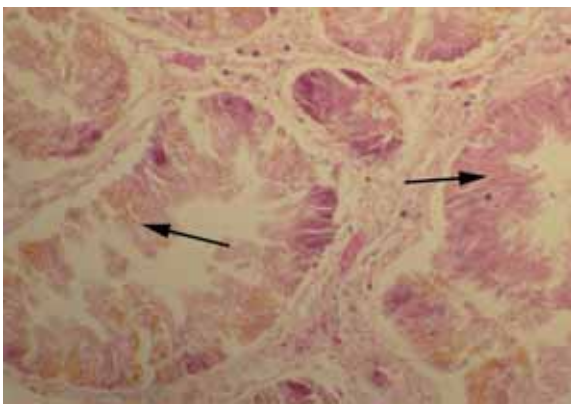

Figure 196. Advanced autolytic change showing almost total loss of nuclear and cellular detail in digestive gland (arrows).

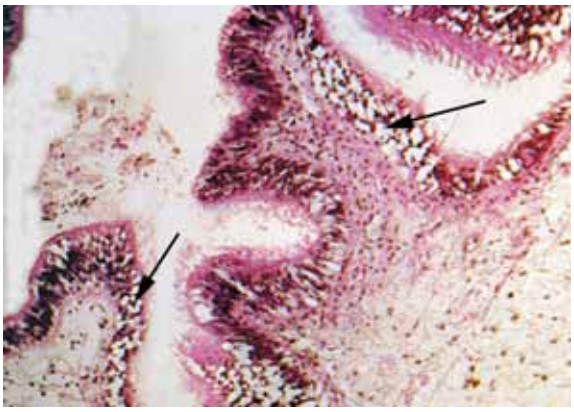

Figure 197. Vacuolation below and within palp epithelium indicating prolonged time between death and fixation or poor fixation.

Ciliate Invasion

Saprophytic ciliated protozoa may invade the external or internal tissues of the oyster following death (Figure 198), or while the oyster is in the process of dying.

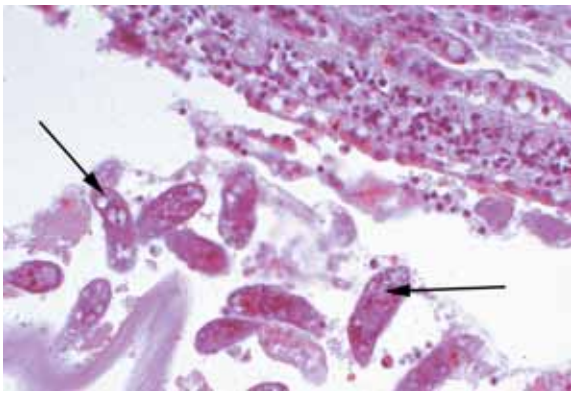

Figure 198. Post- mortem colonisation of the mantle by saprophytic ciliated protozoa (arrows).

Husbandry Induced Artefacts

Anomalous Pearl Sac Development

Anomalous placement and development of mantle epithelium may be seen occasionally as a round, epithelial-lined cyst-like cavity within organs or tissues other than the gonad. Figure 199 shows anomalous pearl sac development in the mantle tissue of *P. maxima*.

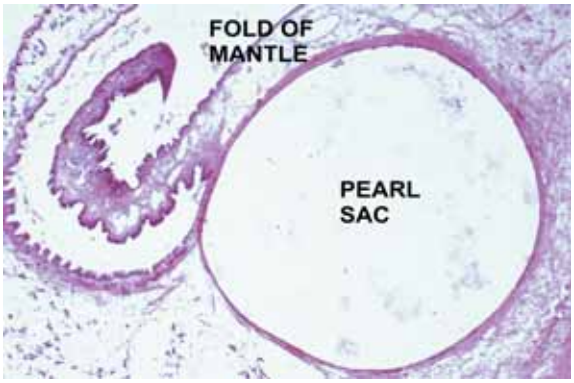

Figure 199. Anomalous location of pearl sac in the tissues of the mantle.

Other Artefacts

Spermatozoa

Spermatozoa from mature testes often contaminate other areas of the histological section or even other tissues being processed simultaneously. In addition, spermatozoa may gain entry to the digestive tract through normal filter feeding mechanisms (Figure 200). These small, basophilic bodies may be mistaken for bacteria.

Ova

Ova from mature ovaries may also contaminate other areas of the histological section or may be ingested and be seen in the lumen of the oesophagus and stomach (Figure 201). These large, nucleated, pyriform bodies may be mistaken for protozoa.

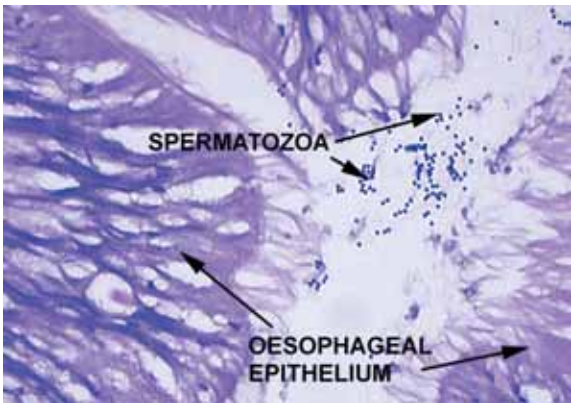

Figure 200. Spermatozoa resembling coccoid bacteria within the oesophagus of a mature *P. maxima.*

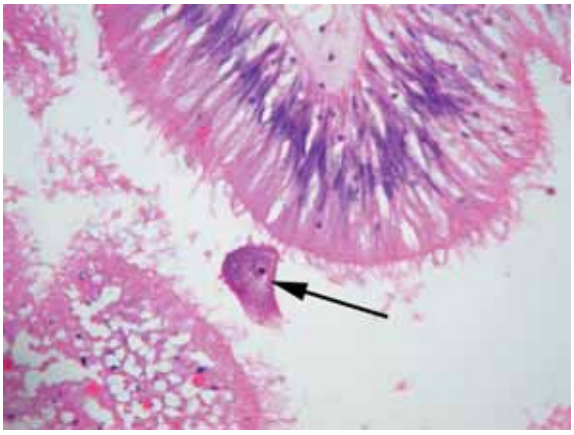

Figure 201. Ovum (arrow) resembling a protozoan in stomach of mature *P. maxima.*

SECTION 4

TECHNIQUES AND STAINS

TECHNIQUES AND STAINS

POST-MORTEM EXAMINATION

A systematic approach to the gross pathological examination should be followed (see Section 1).

SAMPLING AND DISPATCH OF OYSTERS FOR LABORATORY EXAMINATION

Laboratory examination to detect disease plays a central role in protecting and maintaining the health of farmed oysters. The likelihood of obtaining a positive laboratory finding is greatly enhanced if the oysters are sampled and forwarded such that they arrive in a state suitable for examination. The following provide easy-to-follow guidelines for collection and submission of live or preserved oysters for laboratory examination.

Sampling of Oysters

Mature Oysters

Select at least six oysters showing signs typical of the problem from each affected population or cohort. It is helpful to also select apparently normal animals for comparative purposes.

Larvae or Spat

Select at lease 50 larvae or spat from the batch showing the problem, together with a sample of normal or clinically healthy spat for comparison.

Submission of Live Pearl Oysters to the Laboratory

Packing

- Place **larvae or spat** in plastic bags containing moistened paper or rag towelling, fill the bags preferably with oxygen and seal. Place this bag in a second bag and seal
- Wrap **adult pearl oysters** in damp paper or cloth and place in a coolite container or similar protective box

Details to Accompany Oysters

Complete a laboratory Specimen Advice Sheet, or provide full details, including:

- Date
- Name of sender
- Address of sender
- Telephone and Fax
- Species involved

- Shell height
- Location
- Detailed history of disease occurrence; (movements, operations, water quality)
- Signs of disease
- No. oysters in group
- No. dead
- No. sick
- Date when sickness or deaths first observed
- Suspected cause of sickness or deaths
- Number and type of specimens submitted
- Advise the laboratory how and when the oysters are coming.

Submission of Preserved Pearl Oysters

If it is difficult to send live oysters, the alternative is to send fixed oysters. This may be preferable when long distances or travel times will result in a delay in getting oysters to the laboratory:

- Select only live, affected oysters for preservation. The selection of oysters which have been dead for any length of time severely limits the ability to obtain good results
- For larvae and spat, place 50 or more directly into the preservative (10% seawater formalin). For spat larger that approx 1cm, ensure penetration of the fixative into the shell cavity by removing a section of the shell.

For **juvenile oysters** up to 10 cm (dorso-ventral shell size), remove the soft tissues from each shell, make an incision into the middle of the soft tissues and place in 10% seawater formalin.

For **adult oysters**, process the tissues in one of two ways:

- Remove one shell valve by cutting the tissues close to the inside of that valve. Make one or two deep cuts into the tissues to allow preservative (10% seawater formalin) to enter and fix the tissues more rapidly. Remove all the tissues from the second valve and place tissues in 10% seawater formalin. If the containers are not large enough to hold the whole oyster, cut the oyster in half and send each half in a container
- Alternatively, wedge the oyster open and, using a large knife, cut the oyster tissues in half by bringing the knife down between the two open shell valves, eg, as for harvesting half pearls. Remove the tissues from each shell valve and place tissues in 10% seawater formalin. If there is insufficient room in the container send only one half of the oyster
- Generally, for good preservation, tissues should not be thicker than 5mm.

Following selection of tissues and initial preservation:

- Ideally, tissues should be immersed in 10 times their volume of 10% seawater formalin for a minimum of two days and preferably for 5-7 days: Shorter preservation times are acceptable for urgent cases
- Remove tissues from the 10% seawater formalin. Place specimens into a plastic bag, remove excess air and seal with heavy packing tape. Ensure there is no free fluid in the bag. Place a piece of paper towel or cloth in the bag to keep the specimens moist and to absorb any free seawater formalin

- Place the bag and specimens into a second plastic bag, remove excess air and seal with heavy packing tape. There should be no smell of formalin from the package
- Label each bag if more one than one lot of specimens is to be sent in the same container
- Place labelled bags containing the specimens into a strong container
- Add packing to protect the specimens being damaged during transport and seal container
- Complete a laboratory Specimen Advice Sheet available from the diagnostic laboratory or include all details described above relating to the samples
- Send to the appropriate State or Territory diagnostic laboratory.

Note: It is highly advisable to contact the Aquatic Animal Pathologist at the laboratory to discuss the case prior to sending the samples (see below).

Preparation of 10% Seawater Formalin

10% seawater formalin is prepared by adding one (1) volume of 40 % formaldehyde solution to nine (9) volumes of seawater.

Caution! Formaldehyde and formalin are toxic, corrosive and irritant. Contact with skin and eyes must be avoided. Inhalation and ingestion must be avoided.

HISTOLOGICAL STAINING

Histological stains can be used to identify different tissue components and can be found in most histology texts, for example. Luna (1968), Drury and Wallington (1980) and Bancroft and Stevens (1982). The following table gives the common stains used for histological sections of oysters and their applications.

Stain	Description
H & E	Haematoxylin & Eosin. Used routinely for most histological sections
PAS	Periodic acid Schiff reaction. Used for glycogen, mucoid substances & mucopolysaccharides
PAS/diastase	Used to confirm the presence of glycogen, as diastase extracts the glycogen rendering the PAS stain negative
Tri-chrome	Used to differentiate connective tissue from muscle tissue
Gram	Demonstration of Gram positive and Gram negative bacteria in tissue section

Stain	Description
Alcian blue	Alcian blue stain, pH 2.5, is used to demonstrate mucosubstances & mucopolysaccharides
Toluidine blue	Used to demonstrate the presence of sulphated acidic mucopolysaccharides
PTAH	Mallories phosphotungstic acid – haematoxylin stain. Used to detect striations in muscle
Von Kossa	Von Kossa stain is used to identify calcium
Giemsa	Useful for demonstration of protozoa in tissue section

CONTACT ADDRESSES FOR DIAGNOSTIC LABORATORIES

Northern Territory

Berrimah Veterinary Laboratories
Makagon Rd, Berrimah, NT
P.O. Box 3000
Darwin, NT, 0810

Tel: (08) 89992249
Fax: (08) 89992024

Queensland

Oonoonba Veterinary Laboratory
Abbott Street, Oonoonba
PO Box 1085
Townsville, Qld, 4810

Tel: (07) 4722 2624
Fax : (07) 4778 4307

Yeerongpilly Veterinary Laboratory
Animal Research Institute
665 Fairfield Road
Yeerongpilly, Brisbane, Qld, 4105

Tel: (07) 3892 9471
Fax: (07) 3362 9440

Western Australia

Animal Health Laboratories
3 Baron-Hay Court
South Perth, WA. 6151

Tel: (08) 9368 3351
Fax : (08) 9474 1881

SECTION 5

PREVALENCE OF HISTOPATHOLOGICAL CHANGES

Prevalence of Histopathological Changes in Pearl Oysters *Pinctada maxima* from Northern Territory, Queensland and Western Australia; 1994-1996 (from Humphrey *et al.* 1998)

Organ or Tissue	Histopathological Change	NT (n=1280) No. (%)	QLD (n=1068) No. (%)	WA (n=2154) No. (%)	TOTAL (n=4510) No. (%)
Mantle	Cellular inflammation	36 (2.8)	31 (2.9)	73 (3.4)	140 (3.1)
	Oedema	4 (0.4)		1 (0.1)	6 (0.1)
	Metazoa, stromal	8 (0.6)		40 (1.9)	48 (1.1)
	Macrophages, pigmented, numerous			6 (0.3)	6 (0.1)
Gill	Cellular inflammation	1 (0.1)	19 (1.8)	4 (0.2)	24 (0.5)
	Unidentified cysts		6 (0.6)		6 (0.1)
	Rickettsiales-like bodies; epithelial	5 (0.4)	7 (0.7)	6 (0.3)	18 (0.4)
	Metazoa, stromal &/or external	9 (0.7)	17 (1.6)	3 (0.1)	29 (0.6)
	Fibroma/Neurofibroma			1 (0.1)	1 (<0.1)
Palp	Cellular inflammation	40 (3.1)	36 (3.4)	54 (2.5)	120 (2.7)
	Papovavirus-like inclusions, epithelial		123 (11.5)		123 (2.7)
	Rickettsiales-like bodies; epithelial		17 (1.6)		17 (0.4)
	Metazoa, stromal &/or external	16 (1.2)	14 (1.3)	39 (1.8)	70 (1.6)
	Turbellarian-like ciliates; epithelial		18 (1.7)		18 (0.4)
	Oedema	1 (0.1)			1 (<0.1)
	Microgranulomata; protozoan-like			1 (0.1)	1 (<0.1)
Oesophagus	Cellular inflammation	8 (0.6)	12 (1.1)		20 (0.4)
	Metazoa, stromal &/or lumenal	41 (3.2)	7 (0.7)		48 (1.1)
Stomach	Cellular inflammation	16 (1.3)	9 (0.8)	1 (0.1)	26 (0.6)
	Gregarine protozoa, epithelial/lumenal		66 (6.2)		66 (1.5)
	Metazoa, stromal &/or lumenal	8 (0.6)	1 (0.1)	1 (0.1)	10 (0.2)
Digestive Gland	Cellular inflammation	42 (3.3)	67 (6.3)	57 (2.7)	166 (3.7)
	Viral-like intranuclear inclusions	165 (12.9)	143 (13.4)	223 (10.4)	531 (11.8)
	Intracytoplasmic inclusions, eosinophilic			5 (0.2)	5 (0.1)
	Eosinophilic ovoid bodies; epithelial	11 (1.1)			
	Rickettsiales-like bodies; epithelial	29 (2.3)	31 (2.9)	6 (0.3)	66 (1.5)
	Cryptosporidia-like bodies	1 (0.1)			
	Gregarine protozoa, epithelial/lumenal		426 (39.9)	1 (0.1)	427 (9.5)
	Metazoa, stromal &/or lumenal	15 (1.2)	11 (1.0)	11 (0.5)	37 (0.8)
	Microgranulomata; protozoan-like	1 (1.0)		4 (0.2)	5 (0.1)
	Glandular dilation	43 (3.4)			43 (1.0)
	Multinucleate cellular accumulation			1 (0.1)	1 (<0.1)
	Oedema			1 (0.1)	1 (<0.1)
	Macrophages, pigmented, numerous			2 (0.1)	2 (<0.1)
Intestine	Cellular inflammation	33 (2.6)	5 (0.5)	14 (0.7)	52 (1.2)
	Gregarine protozoa in lumen		2 (0.2)		2 (<0.1)
	Ancistrocomid-like ciliates in lumen	1 (0.1)	199 (18.6)		200 (4.4)
	Metazoa, stromal &/or lumenal	17 (1.3)		5 (0.2)	22 (0.5)
Gonad	Cellular inflammation	30 (2.4)	3 (0.3)	28 (1.3)	61 (1.4)
	Metazoa, stromal	1 (0.1)		1 (0.1)	2 (<0.1)
	Macrophages, pigmented, numerous			1 (0.1)	1 (<0.1)
Heart	Cellular inflammation	5 (0.4)	61 (5.7)		64 (1.4)
	Metazoa		1 (0.1)		1 (<0.1)
Kidney	Cellular inflammation	4 (0.3)	9 (0.8)		13 (0.3)
	Pigmentation	2 (0.2)			2 (<0.1)
	Metazoa, tubular	1 (0.1)			1 (<0.1)
Interstitial tissues	Cellular inflammation	49 (3.8)	15 (1.4)	24 (1.1)	88 (2.0)
	Metazoa, stromal / haemolymph sinuses	4 (0.3)	7 (0.7)	6 (0.3)	17 (0.4)
	Multinucleate cellular accumulations	12 (0.9)			12 (0.3)
	Oedema	12 (0.9)			12 (0.3)
	Fibroma/neurofibroma	1 (0.1)			1 (<0.1)
	Macrophages, pigmented, numerous	8 (0.6)		2 (0.1)	10 (0.2)
	Microgranuloma, protozoan-like			1 (0.1)	1 (<0.1)
Foot	Inflammation, focal or regional	1 (0.1)	10 (0.9)	3 (0.1)	13 (0.3)
	Metazoa, stromal &/or external	3 (0.2)		1 (0.1)	4 (0.1)
Adductor Muscle	Cellular inflammation	3 (0.2)	4 (0.4)		7 (0.2)
	Oedema	2 (0.2)			2 (<0.1)

SECTION 6

GLOSSARY

GLOSSARY

Acidophilic. Having the property of taking up acidic stains. Red staining with eosin.
Adductor. A muscle which brings one part towards another.
Afferent. Conveying towards a centre.
Agar. A gelatinous or jelly-like compound extracted from red algae.
Algae. Primitive plants which may be unicellular or multicellular.
Amphiphilic. Taking up both acidic and basic dyes: A purplish colour.
Anaesthesia. Induction of a state of reduced response to, or absence of pain or sensory stimuli.
Anal. Relating to the anus, the terminal orifice of the gastrointestinal or alimentary tract.
Anatomy. The normal structure of the animal.
Anterior. Nearer the head end.
Aorta. The major artery which carries blood or haemolymph from the heart.
Artery. A vessel carrying blood or haemolymph from the heart to the rest of the body.
Atrophy. To waste away.
Bacteria. Microscopic organisms which may be capable of causing disease.
Basophilic. Having the property of taking up basic stains. Blue staining with haematoxylin.
Bivalve. A mollusc with two shell valves joined by a hinge.
Branchial. Pertaining to the pteridia or gills.
Byssal. Pertaining to the byssus.
Byssus. A tuft of strong filaments which are secreted by the byssal gland and by which the mollusc is attached to the substrate.
Chromatin. The genetic material in the nucleus of a cell; DNA.
Cilia. Motile, hair-like outgrowths from the surface of a cell.
Columnar. Column-shaped, applied to cells which are longer than broad.
Commensal. An organism that lives in or on another organism but does not cause any harm.
Conchiolin. The organic, protein matrix secreted by the mantle on which mineral is deposited forming the shell valves.
Connective tissue. The supporting tissues of the animal body, usually applied to fibrous tissue.
Crescent. Shaped like a new moon.
Crypt. A simple blind tube or cavity.
Crystalline style. A translucent proteinaceous rod containing enzymes and involved in digestion in the alimentary canal.
Crystalline style sac. A tubular cavity containing the crystalline style.
Ctenidia. A comb-like structure, applied to the gills.
Cuboidal. Shaped like a cube.
Cuticular. Made of epithelial cells.
Cytolysis. Cell lysis or disintegration.
Cytoplasm. All the living parts of a cell outside of the nucleus.
Degenerating. Loosing its normal functions and structures.
Demibranch. Half of a gill.
Denticle. A tooth-like projection.
Detritus. Wastes or broken down tissues: Rubbish.
Digestive gland. Sac-like portion of the intestine which produces digestive enzymes and in which food is digested.
Dissection. A cutting into pieces to show its parts.
Dorsal. Upper aspect.
Duct. A tube which conveys fluid or other material.
Efferent. Conveying away from a centre.

Endothelium. Cell layer lining the internal surface of fluid filled cavities, especially haemolymph vessels.
Eosinophilic. Staining readily with eosin, a rose-red dye.
Epiphyte. A plant or algal organism growing on the external surface.
Epithelium. Sheets of cells tightly bound together lining any external or internal surface of an organ.
Eucaryotic. Organism having a nucleus, ie, higher organisms (c/f procaryotic).
Excretory. Pertaining to the discharge of waste products.
Excurrent. Outgoing current.
Filament. A slender thread like structure.
Follicle. A bag or sac-like structure.
Fouling. Covering of the shell by external growths of algae or other organisms.
Ganglion. Structure within the nervous system formed of a mass of nerve cell bodies.
Gape. To open wide; the distance between the edges of the shell valves when open.
Gastric shield. A horny structure in the stomach against which the crystalline style rubs and is worn away, releasing amylase, a digestive enzyme.
Genital. Relating to the reproductive system.
Gill. The organ of respiration.
Gland. A single cell or mass of cells specialised to secrete substances either into the body or to the exterior of the body.
Gonad. Reproductive organ.
Gonadal. Relating to the gonad.
Graft. Tissue taken from one animal and inserted into another.
Granulocytes. Cells containing granular structures.
Granuloma. A collection of inflammatory cells and macrophages.
Habitat. The environment in which an organism lives.
Haemolymph. Blood-like fluid.
Hermaphrodite. Having both male and female reproductive organs.
Hinge. A moveable joint.
Histological. Related to the microscopic study of the detailed structure of cells and tissues.
Histopathology. Pathological changes visible at the microscopic or cellular level.
Incurrent. Incoming current.
Isthmus. A narrow structure connecting two larger parts.
Juvenile. Immature or young.
Kidney. Organ associated with the excretion of wastes.
Labia. Lips.
Labial palp. Lobe-like structure near the mouth.
Lamellae. Leaf-like structures.
Larval. Immature or pre-adult stage.
Ligament. A strong fibrous band of tissue connecting two or more moveable structures.
Lobe. A round projecting part of an organ.
Lobular. Arranged in lobes.
Lumen. The internal space of any tubular or sac-like organ.
Macrophage. Large cell which ingests foreign material.
Mantle. Outer covering or layer of tissue enclosing the body of the oyster.
Marginal. Near or pertaining to the edge.
Matrix. A medium in which a substance is embedded.
Maturation. The process of becoming fully differentiated and fully functional.
Medial. Situated in the middle.
Membrane. A sheet-like tissue.

Metazoa. Multi-celled animals, compare with protozoa – single celled animals – usually refers to helminths (worms) and arthropod parasites.
Mollusc. A soft bodied, usually unsegmented coelomate animal, many of which are enclosed in a hard shell.
Mucous. Secretion of mucus.
Mucus. Slimy material rich in glycoproteins.
Nacre. The smooth, shiny calcite deposits forming the bulk of the shell.
Nephridium. Excretory organ or kidney.
Neurones. Nerve cells; the basic unit of the nervous system.
Nucleus. A large dense cellular organelle bounded by a double membrane which contains the chromatin.
Oedema. Accumulation of fluid in tissues.
Oesophagus. That part of the alimentary system between the mouth and the stomach.
Oral. Pertaining to the mouth.
Orifice. Opening of mouth or other cavity.
Ovary. The female reproductive or sex organ in which ova are produced.
Ovum. Egg.
Pallial. Pertaining to the mantle.
Papilla. Nipple like projection.
Parenchyme. The functional or essential elements of an organ.
Pathology. Abnormal changes caused by disease in organs or tissues.
Pedal. Pertaining to the foot.
Pericardial. Pertaining to the sac or membrane around the heart and enclosing the pericardial cavity.
Periostracal. Associated with the periostracum.
Periostracum. The external, non-calcareous layer of shell.
Perpendicular. At right angles to a given line or plane.
Posterior. Nearer the tail or rear end.
Protozoa. Single celled animals.
Pseudostratified. Having the appearance of several layers.
Rectum. The posterior part of the intestine or midgut extending to the anus.
Renal. Pertaining to the kidney.
Reproductive. Pertaining to the production or generation of sex cells, including ova and sperm.
Retractor muscle. A muscle which, when contracting withdraws the parts attached to it towards the body.
Saprophytic. Feeding on non-living material.
Sheath. An outer protective covering.
Spat. Early juvenile stage which attaches to substrate.
Spawning. The act of discharging sperm and ova.
Sperm. The male reproductive or sex cells produced in the testis.
Spermatozoa. Mature sperm or male gamete.
Stomach. The large pouch of the alimentary tract between the oesophagus and the small intestine or midgut.
Subepithelial. Beneath the covering epithelium of an organ.
Testis. The male reproductive or sex organ in which sperm or spermatozoa are produced.
Transverse section. A section cut through the length of the organ or oyster.
Umbo. A protuberance like a boss on a shield; beak or older part of the shell valve of a bivalve mollusc.
Vein. Vessel carrying haemolymph to the heart from the body.
Velum. A veil or curtain of tissue.
Ventral. Lower aspect; nearer the underside or under-structure.
Ventricle. A small cavity or chamber: one of the heart chambers.
Virus. Microscopic organism which may cause disease by entering and replicating within cells.
Visceral. Belonging to the internal organs.

SECTION 7

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BIBLIOGRAPHY

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