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Fig.M.1.1.4g. Polydoriid tunnels underlying the nacre at the inner edge of an American oyster (*Crassostrea virginica*) shell, plus another free-living polychaete, *Nereis diversicolor* on the inner shell surface (**SE McGladdery and M Stephenson**)

SECTION M.2 BONAMIOSIS

Fig.M.2.2a. Haemocyte infiltration and diapedesis across intestinal wall of a European oyster (*Ostrea edulis*) infected by *Bonamia ostreae* (**SE McGladdery**)

Fig.M.2.2b. Oil immersion of *Bonamia ostreae* inside European oyster (*Ostrea edulis*) haemocytes (arrows). Scale bar 20µm (**SE McGladdery**)

Fig.M.2.2c. Systemic blood cell infiltration in Australian flat oyster (*Ostrea angasi*) infected by

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Bonamia sp. Note vacuolised appearance of base of intestinal loop and duct walls (H&E) (PM Hine)

Fig.M.2.2d. Oil immersion of *Bonamia* sp. infecting blood cells and lying free (arrows) in the haemolymph of an infected Australian flat oyster, *Ostrea angasi*. Scale bar 20µm (H&E) (PM Hine)

Fig.M.2.2e. Focal infiltration of haemocytes around gut wall (star) of *Tiostrea lutaria* (New Zealand flat oyster) typical of infection by *Bonamia* sp. (H&E) (PM Hine)

Fig.M.2.2f. Oil immersion of haemocytes packed with *Bonamia* sp. (arrows) in an infected *Tiostrea lutaria* (H&E) (PM Hine)

SECTION M.3. MARTEILIOSIS

Fig.M.3.2a. Digestive duct of a European oyster, *Ostrea edulis*, showing infection of distal portion of the epithelial cells by plasmodia (arrows) of *Marteilia refringens*. Scale bar 15µm (H&E) (SE McGladdery)

Fig.M.3.2b. Digestive tubule of a European oyster, *Ostrea edulis*, showing refringent spore stage of *Marteilia refringens* (star). Scale bar 50µm (H&E) (SE McGladdery)

Fig.M.3.4.1.1a. Tissue imprint from *Saccostrea commercialis* (Sydney rock oyster) heavily infected by *Marteilia sydneyi* (arrows) (QX disease). Scale bar 250µm (H&E) (RD Adlard)

Fig.M.3.4.1.1b. Oil immersion of tissue squash preparation of spore stages of *Marteilia sydneyi* from Sydney rock oyster (*Saccostrea commercialis*) with magnified inset showing two spores within the sporangium. Scale bar 50µm (H&E) (RD Adlard)

SECTION M.4 MIKROCYTOSIS

Fig.M.4.2a. Gross abscess lesions (arrows) in the mantle tissues of a Pacific oyster (*Crassostrea virginica*) severely infected by *Mikrocytos mackini* (Denman Island Disease) (SM Bower)

Fig.M.4.3.2.1a. Histological section through mantle tissue abscess corresponding to the gross lesions pictured in Fig.M.4.2a, in a Pacific oyster (*Crassostrea gigas*) infected by *Mikrocytos mackini* (H&E) (SM Bower)

Fig.M.4.3.2.1b. Oil immersion of *Mikrocytos mackini* (arrows) in the connective tissue surrounding the abscess lesion pictured in Fig.M.4.3.2.1a. Scale bar 20µm (H&E) (SM Bower)

SECTION M.5. PERKINSOSIS

Fig.M.5.1.2a. *Arca* clam showing a *Perkinsus*-like parasite within the connective tissue. Magnified insert shows details of an advanced 'schizont' like stage with trophozoites showing vacuole-like inclusions. Scale bar 100µm. (H&E) (PM Hine)

Fig.M.5.1.2b. *Pinctada albicans* pearl oyster showing a *Perkinsus*-like parasite. Magnified insert shows details of a 'schizont'-like stage containing 'trophozoites' with vacuole-like inclusions. Scale bar 250µm (H&E) (PM Hine)

Fig.M.5.3.2.1a. Trophozoite ('signet-ring') stages of *Perkinsus marinus* (arrows), the cause of 'Dermo' disease in American oyster (*Crassostrea virginica*) connective tissue. Scale bar 20µm (H&E) (SM Bower)

Fig.M.5.3.2.1b. Schizont ('rosette') stages of *Perkinsus marinus* (arrows), the cause of 'Dermo' disease in American oyster (*Crassostrea virginica*) digestive gland connective tissue. Scale bar 30µm (H&E) (SE McGladdery)

Fig.5.3.2.2. Enlarged hyphospores of *Perkinsus marinus* stained blue-black with Lugol's iodine following incubation in fluid thioglycollate medium. Scale bar 200µm (SE McGladdery)

SECTION M.6 HAPLOSPORIDIOSIS

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Fig.M.6.1.3d. Oil immersion magnification of *Haplosporidium*-like spores (arrow) associated with heavy haemocyte infiltration in a Sydney rock oyster (*Saccostrea cucullata*). Scale bar 10µm. (H&E) **(PM Hine)**

Fig.M.6.3.1.2a. Plasmodia (black arrows) and spores (white arrows) of *Haplosporidium costale*, the cause of SSO disease, throughout the connective tissue of an American oyster (*Crassostrea virginica*). Scale bar 50µm **(SE McGladdery)**

Fig.M.6.3.1.2b. Plasmodia (black arrows) and spores (white arrows) of *Haplosporidium nelsoni*, the cause of MSX disease, throughout the connective tissue and digestive tubules of an American oyster (*Crassostrea virginica*). Scale bar 100µm **(SE McGladdery)**

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Fig.M.6.4.2.2b. Oil immersion magnification of MSX spores in the digestive tubule epithelium of an American oyster *Crassostrea virginica*. Scale bar 25µm. (H&E) **(SE McGladdery)**

SECTION M.7 MARTEILIODOSIS

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Fig.M.7.4.2.1. Histological section through the ovary of a Pacific oyster (*Crassostrea gigas*) with normal ova (white arrows) and ova severely infected by the protistan parasite *Marteiloides chungmuensis* (black arrows). Scale bar 100µm **(MS Park)**

SECTION 4 CRUSTACEAN DISEASES

SECTION 4.1 GENERAL TECHNIQUES

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Fig.C.1.1.1.3b. Light coloured shrimp with full guts from a pond with healthy phytoplankton **(P Chanratchakool)**

Fig.C.1.1.2.1a. Black discoloration of damaged appendages **(P Chanratchakool)**

Fig.C.1.1.2.1b. Swollen tail due to localized bacterial infection **(P Chanratchakool)**

Fig.C.1.1.2.2a,b. Shrimp with persistent soft shell **(P Chanratchakool/MG Bondad-Reantaso)**

Fig.C.1.1.2.3a. Abnormal blue and red discoloration **(P Chanratchakool)**

Fig.C.1.1.2.3b. Red discoloration of swollen appendage **(P Chanratchakool)**

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Fig.C.1.1.3b. Brown discoloration of the gills **(P Chanratchakool)**

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Fig.C.1.1.2d. Dead phytoplankton **(P Chanratchakool)**

Fig. C.1.3.6. Points of injection of fixative **(V Alday de Graindorge and TW Flegel)**

SECTION C.2 YELLOWHEAD DISEASE (YHD)

Fig.C.2.2. Gross sign of yellow head disease (YHD) are displayed by the three *Penaeus monodon* on the left **(TW Flegel)**

Fig.C.2.3.1.4a,b. Histological section of the lymphoid organ of a juvenile *P. monodon* with severe acute YHD at low and high magnification. A generalized, diffuse necrosis of LO cells is shown. Affected cells display pyknotic and karyorrhectic nuclei. Single or multiple perinuclear inclusion bodies, that range from pale to darkly basophilic, are apparent in some affected cells (arrows). This marked necrosis in acute YHD distinguishes YHD from infections due to Taura syndrome virus, which produces similar cytopathology in other target tissues but not in the LO. Mayer-Bennett H&E. 525x and 1700x magnifications, respectively **(DV Lightner)**

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Fig.C.2.3.1.4c. Histological section of the gills from a juvenile *P. monodon* with YHD. A generalized diffuse necrosis of cells in the gill lamellae is shown, and affected cells display pyknotic and karyorrhectic nuclei (arrows). A few large conspicuous, generally spherical cells with basophilic cytoplasm are present in the section. These cells may be immature hemocytes, released prematurely in response to a YHV-induced hemocytopenia. Mayer-Bennett H&E. 1000x magnification **(DV Lightner)**

SECTION C.3 INFECTIOUS HYPODERMAL AND HAEMATOPOIETIC NECROSIS (IHHN)

Fig.C.3.2a. A small juvenile *Penaeus stylirostris* showing gross signs of acute IHHN disease. Visible through the cuticle, especially on the abdomen, are multifocal white to buff colored lesions in the cuticular epithelium or subcutis (arrows). While such lesions are common in *P. stylirostris* with acute terminal IHHN disease, they are not pathognomonic for IHHN disease **(DV Lightner)**

Fig.C.3.2b. Dorsal view of juvenile *P. vannamei* (preserved in Davidson's AFA) showing gross signs of IHHNV-caused RDS. Cuticular abnormalities of the sixth abdominal segment and tail fan are illustrated **(DV Lightner)**

Fig.C.3.2c. Lateral view of juvenile *P. vannamei* (preserved in Davidson's AFA) showing gross signs of IHHNV-caused RDS. Cuticular abnormalities of the sixth abdominal segment and tail fan are illustrated **(DV Lightner)**

Fig.C.3.4.1.2a. A low magnification photomicrograph (LM) of an H&E stained section of a juvenile *P. stylirostris* with severe acute IHHN disease. This section is through the cuticular epithelium and subcuticular connective tissues just dorsal and posterior to the heart. Numerous necrotic cells with pyknotic nuclei or with pathognomonic eosinophilic intranuclear inclusion bodies (Cowdry type A) are present (arrows). Mayer-Bennett H&E. 830x magnification **(DV Lightner)**

Fig.C.3.4.1.2b. A high magnification of gills showing eosinophilic intranuclear inclusions (Cowdry type A inclusions or CAIs) that are pathognomonic for IHHNV infections. Mayer-Bennett H&E. 1800x magnification **(DV Lightner)**

SECTION C.4 WHITE SPOT DISEASE (WSD)

Fig.C.4.2a. A juvenile *P. monodon* with distinctive white spots of WSD **(DV Lightner)**

Fig.C.4.2b. Carapace from a juvenile *P. monodon* with WSD. Calcareous deposits on the underside of the shell account for the white spots **(DV Lightner/P. Saibaba)**

Fig.C.4.3.3.1.2a. Histological section from the stomach of a juvenile *P. chinensis* infected with WSD. Prominent intranuclear inclusion bodies are abundant in the cuticular epithelium and subcuticular connective tissue of the organ (arrows) **(DV Lightner)**

Fig.C.4.3.3.1.2b. Section of the gills from a juvenile *P. chinensis* with WSBV. Infected cells show developing and fully developed intranuclear inclusion bodies of WSBV (arrows). Mayer-Bennett H&E. 900x magnification **(DV Lightner)**

SECTION C.4a BACTERIAL WHITE SPOT SYNDROME (BWSS)

Fig. C.4a.2. *Penaeus monodon* dense white spots on the carapace induced by WSD (M. Shariff)

Fig. C.4a.4.2.2a, b. Bacterial white spots (BWS), which are less dense than virus-induced white spots. Note some BWS have a distinct whitish marginal ring and maybe with or without a pinpoint whitish dot in the center **(M. Shariff/ Wang et al. 2000 (DAO 41:9-18))**

Fig. C.4a.4.2.2c. Presence of large number of bacteria attached to exposed fibrillar laminae of the endocuticle **(M. Shariff/ Wang et al. 2000 (DAO 41:9-18))**

SECTION C.5 BACULOVIRAL MIDGUT GLAND NECROSIS (BMGN)

Fig.C.5.1.2a. Section of the hepatopancreas of *P. plebejus* displaying several hepatopancreas cells containing BMN-type intranuclear inclusion bodies. Mayer-Bennett H&E. 1700 x magnification **(DV Lightner)**

Fig.C.5.4.2.1a. High magnification of hepatopancreas from a PL of *P. monodon* with a severe infection by a BMN-type baculovirus. Most of the hepatopancreas cells display infected nuclei. Mayer-Bennett H&E. 1700x magnification **(DV Lightner)**

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Fig. C.5.4.2.1b, c. Sections of the hepatopancreas of a PL of *P. japonicus* with severe BMN. Hepatopancreas tubules are mostly destroyed and the remaining tubule epithelial cells contain markedly hypertrophied nuclei that contain a single eosinophilic to pale basophilic, irregularly shaped inclusion body that fills the nucleus. BMNV infected nuclei also display diminished nuclear chromatin, marginated chromatin and absence of occlusion bodies that characterize infections by the occluded baculoviruses. Mayer-Bennett H&E. Magnifications: (a) 1300x; (b) 1700x **(DV Lightner)**

Fig.C.5.4.2.1d. MBV occlusion bodies which appear as eosinophilic, generally multiple, spherical inclusion bodies in enormously hypertrophied nuclei (arrows). Mayer-Bennett H&E. 1700x magnification **(DV Lightner)**

SECTION C.6 GILL-ASSOCIATED VIRUS (GAV)

Fig. C.6.4.2.1. Transmission electron microscopy of GAV **(P Walker)**

SECTION C.8 TAURA SYNDROME (TS)

Fig. C.8.4.1.1a,b. a. Moribund, juvenile, pond-reared *Penaeus vannamei* from Ecuador in the peracute phase of Taura Syndrome (TS). Shrimp are lethargic, have soft shells and a distinct red tail fan; b. Higher magnification of tail fan showing reddish discoloration and rough edges of the cuticular epithelium in the uropods suggestive of focal necrosis at the epithelium of those sites (arrows) **(DV Lightner)**

Fig. C.8.4.1.1c,d,e. Juvenile, pond-reared *P. vannamei* (c – from Ecuador; d – from Texas; e – from Mexico) showing melanized foci mark sites of resolving cuticular epithelium necrosis due to TSV infection **(DV Lightner/F Jimenez)**

Fig. C.8.4.1.2a. Focal TSV lesions in the gills (arrow). Nuclear pyknotosis and karyorrhexis, increased cytoplasmic eosinophilia, and an abundance of variably staining generally spherical cytoplasmic inclusions are distinguishing characteristics of the lesions. 900x magnification **(DV Lightner)**

Fig. C.8.4.1.2b. Histological section through stomach of juvenile *P. vannamei* showing prominent areas of necrosis in the cuticular epithelium (large arrow). Adjacent to focal lesions are normal appearing epithelial cells (small arrows). Mayer-Bennett H&E. 300x magnification **(DV Lightner)**

Fig. C.8.4.1.2c. Higher magnification of Fig. C.8.4.1.2b showing the cytoplasmic inclusions with pyknotic and karyorrhectic nuclei giving a ‘peppered’ appearance. Mayer-Bennett H&E. 900x magnification **(DV Lightner)**

Fig. C.8.4.1.2d. Mid-sagittal section of the lymphoid organ (LO) of an experimentally infected juvenile *P. vannamei*. Interspersed among normal appearing lymphoid organ (LO) cords or tissue, which is characterized by multiple layers of sheath cells around a central hemolymph vessel (small arrow), are accumulations of disorganized LO cells that form LO ‘spheroids’. Lymphoid organs spheres (LOS) lack a central vessel and consists of cells which show karyomegaly and large prominent cytoplasmic vacuoles and other cytoplasmic inclusions (large arrow). Mayer-Bennett H&E. 300x magnification **(DV Lightner)**

SECTION C.9 NUCLEAR POLYHEDROSIS BACULOVIROSES (NPB)

Fig. C.9.3.2.1a. Wet mount of feces from a *P. vannamei* infected with BP showing tetrahedral occlusion bodies (arrows) which are diagnostic for infection of shrimp’s hepatopancreas or midgut epithelial cells. Phase contrast, no stain. 700x magnification **(DV Lightner)**

Fig. C.9.3.2.1b,c. Mid and high magnification views of tissue squash preparations of the hepatopancreas (HP) from PL of *P. monodon* with MBV infections. Most HP cells in both PLs usually display multiple, generally spherical, intranuclear occlusion bodies (arrow) that are diagnostic for MBV. 0.1% malachite green. 700x (b) and 1 700x (c) magnifications **(DV Lightner)**

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Fig. C.9.3.2.3a,b. a. Mid-magnification view of mid-sagittal sections of PL of *P. vannamei* with severe BP infections of the hepatopancreas showing multiple eosinophilic BP tetrahedral occlusion bodies within markedly hypertrophied hepatopancreas (HP) cell nuclei (arrows). Mayer-Bennett H&E. 700x magnification; b. High magnification of an HP tubule showing several BP-infected cells that illustrate well the intranuclear, eosinophilic, tetrahedral occlusion bodies of BP (arrows). Mayer-Bennett H&E. 1800x magnification (**DV Lightner**)

SECTION C.10 NECROTISING HEPATOPANCREATITIS (NH)

Fig. C.10.4.1.1. Juvenile *P. vannamei* with NHP showing markedly atrophied hepatopancreas, reduced to about 50% of its normal volume (**DV Lightner**)

Fig. C.10.4.1.2. Wet- mount of the HP of infected shrimp with inflamed hemocyte, melanized HP tubules and absence of lipid droplets. No stain. 150x magnification (**DV Lightner**)

Fig. C.10.4.1.3a,b. Low and mid-magnification of photographs of the HP of a severely NHP infected juvenile *P. vannamei*. Severe hemocytic inflammation of the intratubular spaces (small arrow) in response to necrosis, cytolysis and sloughing of HP tubule epithelial cells (large arrow), are among the principal histopathological changes due to NHP. Mayer-Bennett H&E. 150x (a) and 300x (b) magnifications (**DV Lightner**)

Fig. C.10.4.1.3c. Low magnification view of the HP of a juvenile *P. vannamei* with severe, chronic NHP. The HP tubule epithelium is markedly atrophied, resulting in the formation of large edematous (fluid filled or “watery” areas in the HP. Mayer-Bennett H & E. 100x magnification (**DV Lightner**)

Fig. C.10.4.1.3d. The HP tubule epithelial cells show no cytoplasmic lipid droplets, but instead contain masses of the tiny, non-membrane bound, intracytoplasmic NHP bacteria (arrow). Mayer-Bennett H&E. 1700x magnification (**DV Lightner**)

Fig. C.10.4.2.1. Low magnification TEM of a hepatopancreatocyte from a juvenile *P. vannamei* with NHP. Profiles of intracellular rod-shaped forms (large arrow) and helical forms (small arrow) of the NHP bacterium are abundant in the cytoplasm. 10 000x magnification (**DV Lightner**)

SECTION C.11 CRAYFISH PLAGUE

Fig. C.11.3.2.1a. Fresh microscopic mount of a piece of infected exoskeleton showing fungal spores (**EAFP/DJ Alderman**)

Fig. C.11.4.1.1a,b. Clinical signs of infected crayfish showing whitened necrotic musculature in the tail, and often accompanied in chronic infections by melanisation (blackening) of affected exoskeleton (**EAFP/DJ Alderman**)

The Asia Diagnostic Guide to Aquatic Animal Diseases or '*Asia Diagnostic Guide*' is an up- datable diagnostic guide for the pathogens and diseases listed in the NACA/FAO/ OIE Quarterly Aquatic Animal Disease Reporting System. It was developed from a large amount of technical contribution from aquatic animal health scientists in the Asia-Pacific region who supported the regional programme. The *Asia Diagnostic Guide*, which could be effectively used for both farm and laboratory level diagnosis in the region, not only complements the *Manual of Procedures* for the implementation of the Asia Regional *Technical Guidelines* on health management for the responsible movement of live aquatic animals, but also assists in expanding national and regional aquatic animal health diagnostic capabilities that will assist countries in upgrading technical capacities to meet the requirements in the OIE International Aquatic Animal Code and the OIE Diagnostic Manual for Aquatic Animal Diseases.

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