17 Parasitic Diseases of Shellfish

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Introduction

Numerous species of parasites have been described from various shellfish, especially representatives of the Mollusca and Crustacea (see Lauckner, 1983; Sparks, 1985; Sindermann and Lightner, 1988; Sindermann, 1990). Some parasites have had a serious impact on wild populations and shellfish aquaculture production. This chapter is confined to parasites that cause significant disease in economically important shellfish that are utilized for either aquaculture or commercial harvest. These pathogenic parasites are grouped taxonomically. However, the systematics of protozoa (protistans) is currently in the process of revision (Patterson, 2000; Cox, 2002; Cavalier-Smith and Chao, 2003). Because no widely accepted phylogeny has been established, parasitic protozoa will be grouped according to the hierarchy used in both volumes edited by Lee *et al.* (2000). In that publication, Perkins (2000b) tentatively included species in the genera *Bonamia* and *Mikrocytos* in the phylum Haplosporidia. As discussed below, subsequent analysis has verified that *Bonamia* spp. and *Mikrocytos roughleyi* are Haplosporidia but that *Mikrocytos mackini* is not and has unknown taxonomic affiliations. In addition, several other pathogenic protozoa (three species unofficially grouped as Paramyxea that parasitize oysters; and a recently encountered pathogen of pandalid shrimp) that were not mentioned in Lee *et al.* (2000) and have unknown taxonomic affiliations are discussed prior to presenting the metazoans that are problematic for shellfish.

Protozoa Related to Multicellular Groups

Microsporida

Introduction

Many diverse species of microsporidians (Fig. 17.1) (genera *Agmasoma*, *Ameson*, *Nadelspora*, *Nosema*, *Pleistophora*, *Thelohania* and *Microsporidium* – unofficial generic group), have been described from shrimps, crabs and freshwater crayfish worldwide (Sparks, 1985; Sindermann, 1990). The majority of these parasites are detected in low prevalences (< 1%) in wild populations. Although the economic impact of most species of Microsporida on crustacean fisheries is unknown, some species are perceived to have adverse economic impacts. Also, these parasites occasionally cause serious disease epizootics in penaeid aquaculture (Lightner, 1996).

Host range

Ameson (= *Nosema*) *nelsoni* infects at least six species of shrimp throughout the Gulf of

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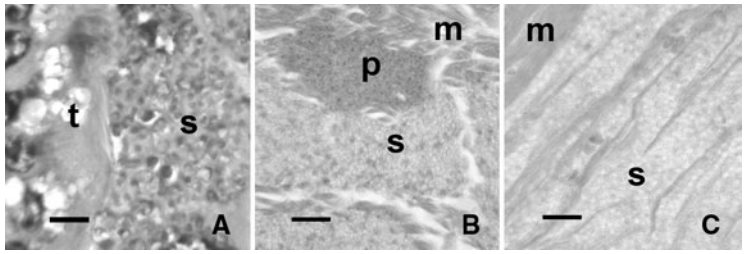

Fig. 17.1. Histological images of plasmodia (p) and developing spores (s) of unidentified Microsporida in crustaceans in British Columbia, Canada. A. Infection congesting the connective tissue between the hepatopancreas tubules (t) of a *Pandalus platyceros* in which the muscle tissue was not infected. B. Infection replacing the skeletal muscle (m) tissue of a *Pandalus jordani* in which the hepatopancreas was not infected. C. Similar infection to B in a *Cancer magister*. All bars = 10 μm.

Mexico and north along the Atlantic coast of the USA to Georgia (Sparks, 1985). It is a common pathogen and has caused significant financial losses to the bait and food shrimp industries (Sindermann, 1990). Microsporidosis in captive–wild *Penaeus* brood stock (infections not apparent at time of collection) resulted in losses of up to 20% (Lightner, 1988). Also, prevalences (16% and 15%, respectively) of *A. nelsoni*, in pond-reared brown shrimp from Texas and in white shrimp in a net-enclosed bay in Florida suggest a potential threat to shrimp reared in extensive culture (Lightner, 1975).

Ameson (= *Nosema*) *michaelis* is widely distributed at low prevalences in blue crab on the Gulf and Atlantic coasts of the USA (Sparks, 1985). Diseased blue crabs (*Callinectes sapidus*) often inhabit sheltered areas near the shore and experience high mortalities when stressed (Overstreet, 1988). However, unlike *Ameson* in shrimp, the transmission of *A. michaelis* is direct, i.e. by ingestion of infected tissue (Sparks, 1985; Overstreet, 1988). Some authors indicated that this parasite was a significant factor in blue crab mortality and thus a potential threat to the industry. However, more information is needed on pathogenicity, geographical distribution and prevalence in various populations before its economic significance can be established (Sparks, 1985).

Nadelspora canceri occurs in Dungeness crab (*Cancer magister*) along the US Pacific Coast from Bodega Bay, California, to Gray's Harbor, Washington, with prevalences lowest in open oceans (0.3%) and highest in estuaries (usually about 14% but up to 41.2% in one location) (Childers *et al.*, 1996). Like *A. michaelis*, *N. canceri* was also directly transmitted to juvenile and adult crabs in the laboratory by allowing them to ingest infected tissue and to megalope and early juvenile crabs by placing them in a suspension of 10^6 spores/ml (Olson *et al.*, 1994).

An unidentified microsporidian in the hepatopancreatocytes of tiger shrimp (*Penaeus monodon*) was associated with low production, slow growth rates and occasional mortalities in brackish-water pond culture in Malaysia (Anderson *et al.*, 1989). Also, unidentified Microsporida have been presumed to cause high mortalities in freshwater crayfish in Western Europe and England (Pixell Goodrich, 1956).

Morphology

Each species of Microsporida is characterized by the number of spores per sporont, the spore size, the tissues infected and, to some extent, the host species (Sparks, 1985; Lightner, 1996). Although the spores of most Microsporida are ovoid and relatively small (about 3 to 5 μm in length), the spores of *N. canceri* are unique in being exceptionally long (about 10 μm) and needle-shaped (0.2 to 0.3 μm in diameter), tapering to a posterior pointed end (Olson *et al.*, 1994).

Host–parasite relationships

Microsporidians replace host tissue with spores as they grow, without invoking host inflammatory responses. Infected individuals exhibit poor stress resistance and poor stamina and are thus prone to loss by predation and to poor survival following capture and handling. Infection of the gonad by some species renders infected individuals sterile and may cause feminization of infected male penaeids (Lightner, 1996).

Diagnosis of infection

Infected tissue, especially muscle, is eventually replaced by spores, giving it an opaque appearance. Due to this white discoloration, heavy infections are apparent and justify the common names of 'cotton', 'milk' or 'cooked' shrimp and crabs. In addition, the cuticle of some crustaceans may have blue-black discoloration due to expansion of cuticular melanophores (Lightner, 1996). The fluorescent technique described by Weir and Sullivan (1989) for screening for Microsporida in histological sections may be useful for detecting light infections. A molecular probe has been developed for the detection of *Agmasoma* sp. in *Penaeus* spp. (Pasharawipas *et al.*, 1994).

Prevention and control

The only known method of prevention is removal and destruction (freezing may not destroy spores) of infected individuals (Lightner, 1988; Overstreet, 1988). The intermediate hosts (fin fish) should be excluded from culture systems and water supplies (e.g. *Ameson penaei* became infective for pink shrimp following passage through the gut of a shrimp predator, the spotted sea trout (*Cynoscion nebulosus*) (Lightner, 1988)). A single treatment of buquinolate (used to treat coccidiosis in boiler chickens) prevented microsporidosis caused by *A. michaelis* in most exposed blue crab (Overstreet, 1975). Lightner (1988) suggested that Fumidil B (an antibiotic used to control microsporidosis in honeybees) and benomyl (a systemic fungicide used to control microsporidosis in the lucerne weevil) may be suitable treatments for this disease in penaeid shrimp. However, Overstreet (1975) found that Fumidil B seemed to exacerbate *A. michaelis* infection in blue crabs and benomyl was not as effective as buquinolate and apparently killed some crabs.

Alveolates

Apicomplexa

Introduction

Many species of gregarines and coccidians have been described from shellfish. Because information to date indicates that most of these species are relatively benign in pathogenicity, they will not be mentioned here. However, two species of coccidia have been associated with pathology.

Coccidians (family Eimeriidae) have been described from the kidneys and less frequently other organs of bivalves. Although they were all designated as species of *Pseudoklossia* (Upton, 2000), only the first (type) species and one other appear to be heteroxenous. Because the other species undergo monoxenous development in their molluscan host, Desser and Bower (1997) proposed the creation of a new genus, *Margolisiella*, to accommodate these parasites. Disease concerns were associated with *Margolisiella kabatai* (Fig. 17.2A) in Pacific littleneck clams (*Protothaca staminea*) that were found on the surface of the substrate in Washington State (Morado *et al.*, 1984) and *Margolisiella* (= *Pseudoklossia*) *haliotis*, which can occur in extremely heavy infections in the kidneys of abalone (*Haliotis* spp.) from California (Friedman *et al.*, 1995).

In addition to gregarines and coccidians, Levine (1978) proposed that *Perkinsus marinus* (= *Dermocystidium marinum* = *Labyrinthomyxa marina*), a pathogen of eastern (American) oyster (*Crassostrea virginica*), also be included within the phylum Apicomplexa. Subsequent taxonomic analysis based on nucleotide sequences indicated that this parasite may be more closely related to the Dinoflagellida (Perkins, 1996;

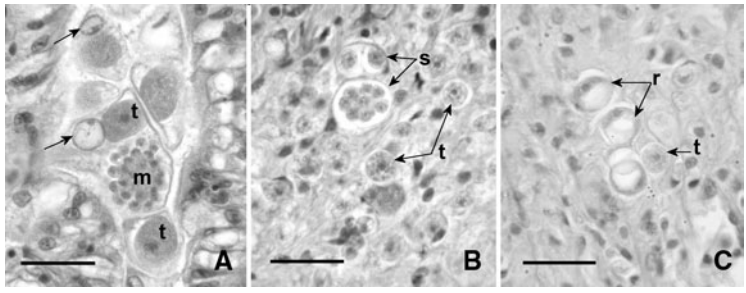

Fig. 17.2. Histological images of Apicomplexa in molluscs from British Columbia, Canada. A. Mature microgamont (m) with peripherally arranged microgametes and trophozoites (t) of *Margolisiella kabati* in the cytoplasm of renal epithelial cells with hypertrophied nuclei (arrows) in *Protothaca staminea*. B and C. Trophozoites (t), mature trophozoites (signet-ring stage, r) and two schizonts (s) consisting of two and eight trophozoites, respectively, of *Perkinsus qugwadi* in the connective tissue of the gonad of *Patinopecten yessoensis*. All bars = 20 μm.

Reece *et al.*, 1997b; Siddall *et al.*, 1997). Norén *et al.* (1999) proposed that perkinsids, which share features with both dinoflagellates and apicomplexans, be assigned to the phylum Perkinsozoa, equivalent to other alveolate phyla. However, Perkins (2000a) tentatively suggested maintaining the link with the Apicomplexa because molecular phylogeny assays have not yet been applied to parasitic dinoflagellates or to more primitive Apicomplexa, which seem to have morphological features akin to those of *Perkinsus* spp. Nevertheless, the genus *Perkinsus* (Fig. 17.2B, C) incorporates several species that are highly pathogenic to molluscs and are thus described in further detail.

Host range

Members of the genus *Perkinsus* (order Perkinsorida, family Perkinsidae) have been detected in over 67 species of molluscs (primarily bivalves) from temperate to tropical regions of the Atlantic and Pacific Oceans and the Mediterranean Sea (Perkins, 1996). Although several species have been named (see related pathogens below), the best known and first named species, *P. marinus*, is one of the prime challenges to the productivity of the eastern oyster, including the devastation of the once profitable oyster industry in Chesapeake Bay, USA, and has caused up to 50% mortality in areas of the Gulf of Mexico (Andrews, 1988a; Burreson and Ragone Calvo, 1996; Ford, 1996; Cook *et al.*, 1998). Also, mass mortalities of eastern oysters (30 to 34 million oysters or 90 to 99% of the stock) imported into Pearl Harbour, Hawaii, were attributed to this pathogen (Kern *et al.*, 1973). In addition to mortalities, meat yields are drastically reduced by high levels of infection, and infections may reach 100% in eastern oysters exposed to two consecutive summers of *P. marinus* activity (Andrews and Ray, 1988; Crosby and Roberts, 1990).

P. marinus occurs along the east coast of the USA from Massachusetts to Florida, along the Gulf of Mexico to Venezuela and in Puerto Rico, Cuba and Brazil. However, the development of *P. marinus* is correlated with salinity and temperature (Crosby and Roberts, 1990; Ford, 1992, 1996). The parasite is most virulent in eastern oysters at salinities above 15 ppt during periods of elevated water temperatures (above 20°C for at least 1 month) (Chu and Greene, 1989). Thus, the disease is prominent for about half the year in high-salinity areas of Chesapeake Bay and active for most of the year in the Gulf of Mexico (Lauckner, 1983). Also, Delaware Bay is periodically free of the disease, owing to: (i) poor propagation of the parasite due to cool temperatures; and (ii) an embargo placed on importation of eastern oysters from more southern areas (Andrews, 1988a).

Most described species of *Perkinsus* lack distinctive morphological features that

can be used for specific identification. As indicated below, at least one other species is known from within the geographical range of *P. marinus* and both species have been reported from the same bivalve hosts, including the eastern oyster and the sympatric clams *Macoma balthica*, *Macoma mitchelli* and *Mercenaria mercenaria* (Coss *et al.*, 2001). In addition, *P. marinus* was reported to develop in the ectoparasitic snail *Boonea impressa* (White *et al.*, 1987). However, the host range of *P. marinus* has probably not been fully identified. The recent development of molecular diagnostic tools for the detection and identification of *Perkinsus* spp. should facilitate the identification of host species susceptible to *P. marinus*.

Morphology and life cycle

The disease caused by *P. marinus* is popularly known as 'dermo', and fungal terminology, stemming from previous taxonomic interpretations, is still occasionally used for various stages in the life cycle (Fig. 17.3). In the eastern oyster, the trophozoite (aplanospore or meront) ranges in size from 2 to 20 μm, with the smaller forms often observed within the phagosome of a haemocyte. At maturity, the trophozoite (10 to 20 μm in diameter) has an eccentric vacuole (often containing a refringent vacuoplast), which may comprise 90% of the cell volume. The peripheral location of the nucleus produces the characteristic signet-ring configuration. The mature trophozoite undergoes schizogony by successive bipartitioning of the protoplast (alternating karyokinesis and cytokinesis) to form a schizont (sporangium, tomont or 'rosette') stage (ranging from 15 to 100 μm but usually less than 25 μm in diameter) containing from two to 64 (usually eight to 32) trophozoites (coccoid or cuneiform and 2 to 4 μm in the longest axis), which are released when the schizont ruptures (Perkins, 1996).

At death and decomposition of an infected host or when infected tissue is placed in anaerobic fluid thioglycollate medium supplemented with salt and antimicrobial agents (described by Ray (1966a) and now

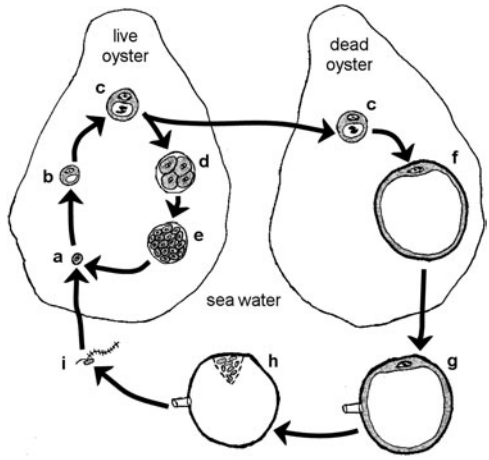

Fig. 17.3. Diagrammatic developmental cycle of *Perkinsus marinus* within living and dead *Crassostrea virginica* and free in the marine environment as adapted from Perkins (1996). Within the living oyster, immature trophozoites (a) grow and develop a cytoplasmic vacuole (b). Mature trophozoites (c) have a large eccentric vacuole containing a vacuoplast, thereby displacing the nucleus to the cell periphery (signet-ring stage). Palintomy (d, e) occurs and results in the formation of a schizont (e) from which the immature trophozoites (about four to 64 per schizont) escape through a tear in the wall. When the oyster dies and the tissue becomes anoxic, the mature trophozoite (c) develops into a large prezoosporangium (f). On liberation into the marine environment, the prezoosporangium develops a discharge tube (g) and undergoes palintomy (h), resulting in the formation of numerous biflagellated zoospores (i) capable of initiating a new infection.

called RFTM), the trophozoites transform into prezoosporangia (hypnospores), which enlarge to diameters usually between 30 and 80 μm, with extremes of 480 μm (Perkins, 1996). Upon release into sea water (aerobic conditions), the prezoosporangia (from either dead oysters or RFTM) differentiate into flask-shaped zoosporangia, within which numerous biflagellated zoospores develop in about 4 days. The biflagellated zoospore (ovoid body 4 to 6 μm by 2 to 3 μm) has a row of long filamentous mastigonemes (tinsels) along the length of the anterior flagellum and a naked posterior flagellum. It also has an apical complex, consisting of a conoid, polar ring, up to 39

subplasmalemma microtubules, rhoptries and micronemes (Perkins, 1996). Zoospores escape from the zoosporangium via the discharge tube and may initiate infection in the epithelium of gill, mantle or gut, where they become trophozoites (Perkins, 1996). Apparently, trophozoites contained within haemocytes that underwent diapedesis are infective and represent an alternate means by which the infection is transmitted.

Hoese (1964) speculated that dissemination of *P. marinus* might be achieved through scavengers because he detected prezoosporangia in the faeces of fishes, oyster drills and crabs that fed on dead or moribund infected oysters. He was able to infect eastern oysters with this material. In addition, White *et al.* (1987) speculated that *P. marinus* might also be transmitted by an ectoparasitic pyramidellid snail, *B. impressa*, which directly injected *P. marinus* into the oyster mantle during feeding or *P. marinus* was transferred through the water between the snail and oyster, perhaps entering the feeding wound made by the snail.

Extensive data on the influence of environmental conditions on the prevalence and intensity of infection and the disease process have been integrated into mathematical models of host–parasite–environmental interactions (Hofmann *et al.*, 1995; Powell *et al.*, 1996; Ragone Calvo *et al.*, 2001). The models simulate infection cycles within the oyster and in oyster populations under different environmental conditions and forecast conditions that can initiate and end epizootics in oyster populations.

Host–parasite relationships

Gross signs of 'dermo' are severe emaciation, gaping, pale appearance of the digestive gland, shrinkage of the mantle away from the outer edge of the shell, inhibition of gonadal development, retarded growth and occasionally presence of pus-like pockets (Lauckner, 1983; Sindermann, 1990). In the early stages of infection, many *P. marinus* trophozoites are engulfed by haemocytes and the infection spreads systemically throughout the oyster. Although the extent of the haemocytic response is variable, several layers of haemocytes can encapsulate trophozoites. Also, host cell destruction appeared limited to the immediate vicinity of the pathogen. Advanced infections were characterized by haemocyte activation and recruitment, with concomitant exuberant production of haemocyte-derived oxygen intermediates (oxyradicals), which may be associated with the pathogenesis of the disease (Anderson *et al.*, 1992).

Foci of infection or abscesses containing thousands of *P. marinus* and host debris may attain several hundred micrometres in diameter during later stages of infection. In addition, the pathogen often occludes haemolymph sinuses. Although the epithelium and adductor muscle are invaded, they do not appear to be damaged until late in the infection. By the time the eastern oyster becomes moribund, large numbers of *P. marinus* have accumulated in all tissues. Paynter and Burreson (1991) have indicated that, in Chesapeake Bay, groups of eastern oysters, which incurred high prevalences and intensities of infection, exhibited low mortalities during their first year but suffered high mortalities during the following year. Bushek and Allen (1996a,b) observed variations in the virulence of *P. marinus* to genotypically different stocks of eastern oysters and proposed that different strains of *P. marinus* may vary in virulence or different oyster stocks may vary in resistance to infection.

In vitro *propagation*

P. marinus is one of the few shellfish pathogens that can be maintained by continuous *in vitro* propagation of the trophozoite (La Peyre, 1996; Casas *et al.*, 2002b). In addition to having biological characteristics similar to the histozoic stages of *P. marinus* (i.e. morphology, antigenicity, biochemistry and development in thioglycollate medium, as described by Ray (1966a)), some cultured isolates were infective to eastern oysters.

The transformation of the trophozoites into prezoosporangia in RFTM (Ray, 1966a) is frequently referred to as a culture technique. However, prezoosporangia have poor survival in RFTM. The subsequent

transformation of the prezoosporangia into zoospore-producing zoosporangia can be achieved by transferring the prezoosporangia from RFTM to sea water. The use of RFTM is now considered to be part of a diagnostic technique, as described below.

Diagnosis of infection

In addition to routine histopathological examination of oyster tissues for the detection of *Perkinsus*, other diagnostic techniques have been developed. The RFTM procedure indicated above involves the incubation of test mollusc tissues in fluid thioglycollate medium as modified by Ray (1966a) for about 1 week at room temperature, which induces the development of prezoosporangia. When the sample is stained with dilute Lugol's iodine solution, the prezoosporangia readily stand out as dark brown to blue-black spheres. A semi-quantitative estimate of disease intensity was determined by the apparent percentage of squashed mantle or rectal tissue that contained *P. marinus* prezoosporangia (Andrews, 1988a; Choi *et al.*, 1989). Gauthier and Fisher (1990) demonstrated that haemolymph could be assayed by RFTM to produce a sensitive, reliable and completely quantitative method of estimating the intensity of infection. Although this method is inadequate for detecting light infections, it does not require that the oyster be sacrificed (Bushek *et al.*, 1994). Because RFTM is not species specific, it has been used to detect other species of *Perkinsus* in various Mollusca (see below).

Monoclonal and polyclonal antibodies produced against the prezoosporangia can be used in ELISA or immunofluorescent assays for identification and quantification of *P. marinus*. The various antibodies show differences in cross reactivity with other life stages of *P. marinus* and with other species of *Perkinsus* (Choi *et al.*, 1991; Dungan and Roberson, 1993).

Molecular techniques, including specific and sensitive semi-quantitative and competitive polymerase chain reaction (PCR) and multiplex PCR (simultaneous testing of two or more pathogens in a single test reaction), based on the sequence of the small subunit ribosomal RNA (SSU rRNA) gene, have been developed (De la Herrán *et al.*, 2000; Penna *et al.*, 2001). The use of PCR primers to amplify up to six polymorphic loci of genomic DNA from cultured *P. marinus* indicated that, *in vitro, P. marinus* is diploid and that oysters may be infected by multiple strains of this parasite (Reece *et al.*, 1997a, b). Allelic and genotypic frequencies differed significantly among isolates from regions of the north-east and south-east US Atlantic coast and the coast of the Gulf of Mexico. The inter- and intraspecific genetic variation among *Perkinsus* species has provided the opportunity to design genus- and species-specific molecular diagnostic assays (Casas *et al.*, 2002a; Dungan *et al.*, 2002; Murrell *et al.*, 2002). In addition, Yarnall *et al.* (2000) developed a quantitative competitive PCR that proved to be more sensitive than the RFTM tissue assay. However, before molecular analysis (e.g. PCR) can be recommended as the method of choice for disease diagnosis, more research is necessary to validate the various molecular diagnostic assays and compare them with standard diagnostic techniques (Burreson, 2000).

Prevention and control

Continuous bath treatment with low levels of cyclohexamide (1 µg/ml/week for 45 days) prolonged the life of laboratory stocks of eastern oysters infected with *P. marinus* (Ray, 1966b). However, chemical treatment is impractical in the field. Andrews (1988a), Andrews and Ray (1988) and Sindermann (1990) indicated that control of the disease depends on isolation and manipulation of seed stock and recommended the following procedures: (i) avoid use of infected seed stocks; (ii) plant oysters thinly on beds; (iii) isolate newly planted beds (0.4 km) from infected eastern oysters; (iv) continually monitor eastern oysters (especially oysters at 2 years of age or older in the late summer or early autumn) for the disease, using RFTM; (v) harvest early if beds become infected; and (vi) fallow beds after harvest to allow all infected oysters to die before

replanting. Goggin *et al.* (1990) further recommended that the spread of *Perkinsus* sp. from shellfish processing plants could be prevented by not returning untreated mollusc tissues to the sea.

Although *P. marinus* persisted in eastern oysters held at low salinities (6 ppt), it was less virulent at salinities below 9 ppt (Ragone and Burreson, 1993). The occurrence of disease only at higher salinities has been used in management practices (Paynter and Burreson, 1991). In Chesapeake Bay, uninfected eastern oyster seed are acquired from areas of low salinity, which are not suitable for oyster culture because oyster growth and condition are reduced by low salinity. In the Gulf of Mexico, where warmer temperatures allow the infection to remain active year-round, freshwater diversions into high-salinity bays have been proposed in order to revive or enhance areas that are marginally productive for eastern oysters (Andrews and Ray, 1988). The possibility of breeding eastern oysters that are resistant to *P. marinus* is under investigation (Gaffney and Bushek, 1996). Also, the introduction of a non-endemic species that is more tolerant of *P. marinus* (Meyers *et al.*, 1991) is being considered as a method for the recovery of stable oyster production in areas of Chesapeake Bay where native eastern oysters have been eliminated (Mann *et al.*, 1991).

Related pathogens

Prezoosporangia of *Perkinsus* sp. have been detected by RFTM in many species of Mollusca from temperate to tropical waters of the world. For example, in Australia, *Perkinsus* spp. were detected in at least 30 species of Mollusca (Lester *et al.*, 1990). Although *Perkinsus* sp. was associated with giant clam (*Tridacna gigas*) mortalities (Alder and Braley, 1989) and lesions in the tissues of pearl oysters (*Pinctada maxima*) (Norton *et al.*, 1993), many *Perkinsus* sp. infections seem to have no detectable adverse affects on their hosts (Goggin *et al.*, 1990).

In addition to *P. marinus*, six other species have been named. The most distinctive species is *Perkinsus qugwadi*, considered enzootic in British Columbia, Canada, but only known from Japanese scallops (*Patinopecten yessoensis*) that were introduced into Canada from Japan for culture purposes (Blackbourn *et al.*, 1998). Scallops native to enzootic areas (*Chlamys rubida* and *Chlamys hastata*) were resistant to infection, while mortalities among cultured Japanese scallops often exceeded 90% (Bower *et al.*, 1999). Unlike all other *Perkinsus* spp., *P. qugwadi*: (i) proliferated and was pathogenic at cool temperatures (8–15°C); (ii) developed zoospores within tissues of juvenile living hosts instead of outside the host; and (iii) did not produce prezoosporangia in RFTM or stain blue-black with Lugol's iodine (Bower *et al.*, 1998). In addition to these differences, phylogenetic analyses based on the internal transcribed spacer (ITS) regions of rRNA of *P. qugwadi* consistently place this species at the base of a clade containing the other *Perkinsus* spp. (Coss *et al.*, 2001; Casas *et al.*, 2002a,b; Dungan *et al.*, 2002).

The second named species was *Perkinsus olseni*, first reported as a pathogen of abalone (*Haliotis rubra*) in Australia (Lester and Davis, 1981). This species is now reported from three other species of abalone (*Haliotis laevigata*, *Haliotis cyclobates* and *Haliotis scalaris*) along the southern coast of Australia and is often associated with significant mortalities. It is also believed to occur in a wide variety of molluscan species from the Great Barrier Reef but was not detected in abalone from that area (Goggin and Lester, 1995). *Perkinsus olseni* was experimentally transmitted and highly infectious to a range of molluscs under laboratory conditions (Goggin *et al.*, 1989).

The third species to be named was *Perkinsus atlanticus*, a pathogen of native clams (*Ruditapes* (= *Tapes* = *Venerupis*) *decussatus*, *Ruditapes* (= *Tapes*) *semidecussatus*, *Ruditapes pullastra*, *Venerupis aurea*, *Venerupis pullastra*) and the introduced Manila clam (*Venerupis* (= *Tapes* = *Ruditapes*) *philippinarum*) along the coasts of Portugal, Spain (Galicia and Huelva areas) and the Mediterranean Sea (Azevedo, 1989; Rodríguez *et al.*, 1994; Ordás *et al.*, 2001; Casas *et al.*, 2002a).

In the late 1990s, a *Perkinsus* sp. was associated with significant mortalities of

native stocks of Manila clams in Korea, Japan and China (Choi *et al.*, 2002). Hamaguchi *et al.* (1998) found that the nucleotide sequence of two internal transcribed spacers (ITS1 and ITS2) and the 5.8 S region of the SSU rRNA of the *Perkinsus* sp. from Manila clams in Japan were almost identical to those of *P. atlanticus* and *P. olseni* and suggested that the parasite in Japan may be *P. atlanticus*. Several other investigations found similar results (Goggin, 1994; Robledo *et al.*, 2000; Casas *et al.*, 2002a). However, in all investigations, the gene sequences of the *P. atlanticus* and *P. olseni* isolates were distinct from those of *P. marinus* (from the oyster *C. virginica* from Virginia, USA). Based on similarity (98–99%) in the sequences of the non-transcribed spacer (NTS) region, Murrell *et al.* (2002) proposed that *P. olseni* and *P. atlanticus* be synonymized, with the name *P. olseni* having priority. If this synonymy is upheld, *P. olseni* will have a wide host range (infecting gastropods as well as bivalves) and a wide geographical range (including the coasts of Australia, New Zealand, Japan, Korea and Europe). The wide variability in the pathogenicity of this parasite may be attributed to either differences in strains of the parasite or differences in host responses.

The validity of another species, *Perkinsus karlssoni*, has been refuted. *P. karlssoni* was identified as a pathogen of cultured bay scallops, *Argopecten irradians*, being conditioned for spawning under hatchery conditions in Atlantic Canada (McGladdery *et al.*, 1991; Whyte *et al.*, 1994). This parasite was described because a Lugol-positive organism was detected in diseased scallop tissues incubated in RFTM. However, diagnosis by RFTM alone is controversial and Goggin *et al.* (1996) surmised that the description was based on a contaminant biflagellate organism. This species may be reinstated if further RFTM-positive prezoosporangia are obtained and phylogenetic analyses of sequence data place the species within the *Perkinsus* clade.

Another species, *Perkinsus chesapeaki*, has been isolated from the gills of soft-shell clams (*Mya arenaria*) from the same location (Chesapeake Bay, USA) as *P. marinus* (McLaughlin *et al.*, 2000). Although the life-cycle stages and zoosporulation process were similar to those described for other *Perkinsus* spp., *P. chesapeaki* was identified based on minor differences in the morphology of the zoospore. Also, the genetic sequence of the SSU rRNA of this isolate was found to be distinct from that of *P. marinus* (Casas *et al.*, 2002a; Dungan *et al.*, 2002). McLaughlin and Faisal (2001) reported a difference in the production of extracellular proteins by *P. chesapeaki* and *P. marinus*, which may help to explain the difference in pathology observed in infected soft-shell clams and the eastern oysters, respectively.

More recently, *Perkinsus andrewsi* was described from Baltic clams (*M. balthica*) from the east coast of the USA. The species identification was based on sequence data from the SSU rRNA locus, which differed from those of *P. marinus*, *P. atlanticus*, *P. olseni* and *P. qugwadi* (Coss *et al.*, 2001). DNA analysis (using PCR assays on regions of the SSU rRNA loci (mainly ITS1 and ITS2)) indicated that *P. andrewsi* can coexist with *P. marinus* in Baltic clams and other sympatric clams (*M. mitchelli* and *M. mercenaria*) and in the eastern oyster (Coss *et al.*, 2001). Subsequent analysis of the ITS regions of several species of *Perkinsus* (including several isolates of some species) consistently grouped *P. chesapeaki* and *P. andrewsi* (Casas *et al.*, 2002a; Murrell *et al.*, 2002). Analysis of the ITS sequence from cloned isolates of *Perkinsus* sp. from Baltic clams and another sympatric clam (*Tagelus plebeius*) from Chesapeake Bay suggested that the minor variations among ITS sequences of *P. chesapeaki* and *P. andrewsi* indicate true polymorphism within a single parasite species (Dungan *et al.*, 2002). If these species are synonymous, *P. chesapeaki* will have precedence over *P. andrewsi*.

The current major limitations to identifying the various species of *Perkinsus* are the absence of significant morphological differences among known species and the broad host range encountered for isolates tested in the laboratory and assayed from the field. A conservative perspective suggests that only four of the named species may be valid (i.e. *P. marinus*, *P. olseni*, *P. qugwadi* and *P. chesapeaki*). If this perspective is

correct, then the host and geographical range is very broad for at least one of these species (i.e. *P. olseni*) and at least two other species can infect the same hosts in the same geographical area (i.e. *P. marinus* and *P. chesapeaki*). These characteristics and the lack of distinctive morphological features render the identity of *Perkinsus* spp. encountered in the field and the specific identity of all new isolates open to question. Because of the significant negative economic impact caused by some of these parasites, it is important to be able to differentiate between pathogenic and supposedly non-pathogenic species or to determine which species are pathogenic for which hosts. Investigations into the genetic sequence and associated biology of various isolates and the continuing development of molecular assays will address this problem in the future.

Ciliophora

Two closely related genera of holotrich ciliates (class Oligohymenophorea, subclass Scuticociliatia, order Philasterida, family Orchitophryidae) periodically cause high mortalities among Crustacea in captivity. *Mesanophrys* (= *Paranophrys* = *Anophrys*) spp. have been observed in the haemolymph of Dungeness crabs (*C. magister*) and red rock crabs (*Cancer productus*) on the west coast of North America (Fig. 17.4A), in blue crabs (*C. sapidus*) on the east coast of the USA and in edible crabs (*Cancer pagurus*) and green crabs (*Carcinus maenas*) in Europe. *Mugardia* (= *Paranophrys* = *Anophrys*) sp. occurs in lobsters (*Homarus americanus*) on the east coast of North America (Sindermann, 1990; Morado and Small, 1995). Although the ciliates are rare in the haemolymph of most wild-caught Crustacea, *Mugardia* sp. was observed in stained hepatopancrease smears from all 89 lobsters from ten locations along the coast of Maine in November 1990 (Sherburne and Bean, 1991). The ciliate is infectious and lethal for animals held in artificial enclosures. Presumptive diagnosis of these ciliates is made by observing numerous ciliates of typical elongate form and reduced numbers of haemocytes in the

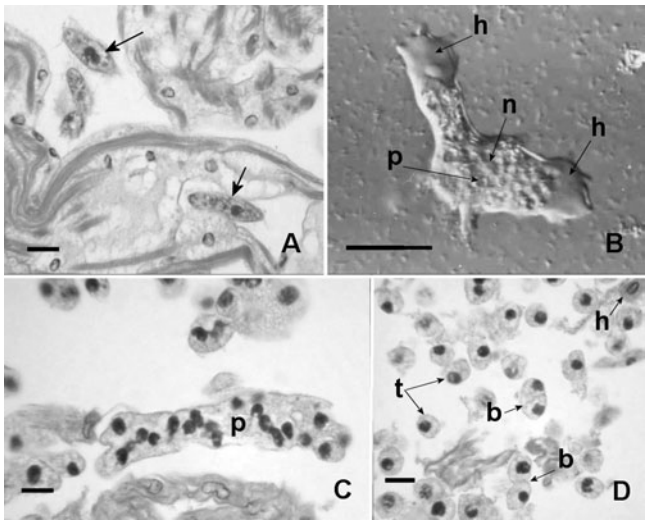

Fig. 17.4. A. Histological images through *Mesanophrys pugettensis* (arrows) in the haemal sinuses of the heart of *Cancer magister* from British Columbia, Canada. B. Wet-mount preparation (Nomarski optics) of *Paramoeba invadens* (hyaline region (h), nucleus (n) and parasome (p)) from *in vitro* culture isolated from *Strongylocentrotus droebachiensis* in Nova Scotia, Canada (courtesy of R.E. Scheibling). C and D. Histological section through *Hematodinium* sp. (plasmodium (p), trophozoites (t) and binary fission in trophozoites (b)) in the heart sinus (haemocyte (h)) of *Chionoecetes tanneri* from British Columbia, Canada. All bars = 10 μm.

haemolymph. Ciliates can also be observed in histological sections of the soft tissues, especially the heart (Fig. 17.4A) and gills and may be associated with tissue destruction, especially of the intestine (Sherburne and Bean, 1991). Protargol-stained preparations are required for specific identification (Armstrong *et al.*, 1981). Because most reports of infection pertained to injured crabs and lobsters being held in enclosures, lowering densities (i.e. less stress of crowding) and reducing mechanical damage during holding may be beneficial (Sindermann and Lightner, 1988).

Dinozoa (Dinoflagellida)

Introduction

The parasitic Dinoflagellida in the genus *Hematodinium* spp. (order Syndiniales) are significant pathogens of commercially harvested crabs and lobsters (Shields, 1994).

Host range

The first reported and type species, *Hematodinium perezi*, was originally described from the haemolymph of crabs (*C. maenas* and *Liocarcinus* (= *Portunus*) *depurator*) from European waters (Chatton and Poisson, 1930) and was more recently reported to cause high mortalities in *C. pagurus* and *Necora puber* in France (Wilhelm and Mialhe, 1996). On the western side of the North Atlantic Ocean, from New Jersey to the western coast of Florida and in the Gulf of Mexico to southern Texas, a *Hematodinium* sp. that is believed to be the same parasite was reported from other species of crabs, including the blue crab, *C. sapidus* (Couch, 1983). Based on results from epizootiology studies, Messick and Shields (2000) suggested that this parasite represented a significant threat to blue crab populations in high-salinity estuaries along the Atlantic and Gulf coast of the USA. The second species, *Hematodinium australis*, occurs in *Portunus pelagicus*, *Scylla serrata* and possibly *Trapezia* spp. from the coast of Queensland, Australia (Hudson and Shields, 1994).

Based on differences in nucleotide sequence, two additional unnamed species have been documented (Hudson and Adlard, 1996). *Hematodinium* sp. causes an astringent aftertaste (bitter crab syndrome) and mortalities in Tanner crabs (*Chionoecetes bairdi* and *Chionoecetes opilio*) along the coast of Alaska (Meyers *et al.*, 1996). Meyers *et al.* (1990) conservatively estimated that the total economic loss to fishermen due to rejected diseased crabs was about 5% of the catch for the 1988/89 season. In addition, data from the commercial Tanner crab fishery suggested that there was an increase in prevalence and spread of the disease to new areas. A similar parasite was found in *C. opilio* from the coast of Newfoundland (Pestal *et al.*, 2003) and in *Chionoecetes tanneri* from coastal British Columbia (Bower *et al.*, 2003). The other *Hematodinium* sp. occurs in the Norway lobster (*Nephrops norvegicus*) off the west coast of Scotland and in the Irish Sea (Field and Appleton, 1995). Severe infection has an adverse effect on meat quality, noted by fishermen and processors. Peak infections of 70% were found in some trawl samples, which seasonally coincided with the annual moult. The decrease in Norway lobster abundance in the last decade may in part reflect the higher level of infection by *Hematodinium* sp. during this time (Field *et al.*, 1998).

Parasite morphology

Superficially, *Hematodinium* spp. appear similar, with only slight differences in size for the two named species (Hudson and Shields, 1994). The most abundant form is a round trophozoite (about 6 to 18 μm in diameter) with a single dinokaryon nucleus, which is characteristic of the dinoflagellates (Fig. 17.4D). Binucleate cells and multinucleate ovoid to vermiform plasmodia (usually containing fewer than about 20 nuclei (Fig. 17.4C)) are occasionally observed in the haemal sinuses (Couch, 1983; Hudson and Shields, 1994). A flagellated dinospore occurs during the terminal stages of infection

and is rarely observed in some crustacean hosts. Two sizes of motile dinospores were observed in tissues of *C. bairdi* in Alaskan waters during August and September (Love *et al.*, 1993). A common ultrastructural characteristic is the presence of cytoplasmic organelles called trichocysts in mature trophozoites and dinospores (Hudson and Shields, 1994).

Host–parasite relationships

The haemolymph of heavily infected crustaceans is opalescent or milky, slow to clot, devoid of haemocytes and filled with non-motile trophozoites. Also, there may be total lysis of hepatopancreatic tubules and partial destruction of muscle fibres (Couch, 1983). These crustaceans are lethargic and have a short survival time in captivity. The main cause of death may be the disruption of gas transport and tissue anoxia caused by proliferation of large numbers of dinoflagellate cells in the haemolymph. Alternatively, Shields *et al.* (2003) indicated that infected blue crabs probably die from metabolic exhaustion, as indicated by reduced levels of haemolymph proteins, haemocyanin and glycogen. Meyers *et al.* (1987) suggested that the bitter flavour in cooked infected Tanner crabs is the result of either the dinoflagellate itself or its metabolite(s).

Infected blue crabs were found only in areas above 11 ppt salinity and in all seasons except late winter and early spring (Messick and Shields, 2000). A reduction in prevalence of the disease in the winter was also reported in Tanner crabs. Laboratory transmission by injection indicated that both types of dinospores from Tanner crabs (Eaton *et al.*, 1991) and trophozoites and plasmodia from blue crabs (Messick and Shields, 2000) are infectious to their respective host crabs. However, *Hematodinium* spp. have not been transmitted by feeding (predation or cannibalism) or cohabitation, but moulting may predispose crabs to invasion (Messick and Shields, 2000). The complete life cycle of all *Hematodinium* spp. in the field is poorly understood.

Diagnosis of infection

The non-motile trophozoites are evident as numerous spheres (6 to 18 μm in diameter) in wet mount preparations of haemolymph from discoloured crustacea examined microscopically (× 100 magnification). *Hematodinium* spp. are also apparent in histological sections (Figs. 17.4C, D). However, the paucity of morphological characteristics for species identification has resulted in the development of PCR techniques based on the nucleotide sequences of parts of the SSU rDNA gene (Hudson and Adlard, 1996). Gruebl *et al.* (2002) described an 18S rRNA gene-targeted PCR-based diagnostic technique capable of detecting one *Hematodinium* sp. in 300,000 blue crab haemocytes. The partial sequences of the 18S rDNA gene of *Hematodinium* sp. from blue crabs deposited in GenBank (accession numbers AF421184 and AF286023) are nearly identical to the equivalent sequences of the parasite from *C. tanneri* in British Columbia, which is believed to be a different species. Because of this close association, this region of the genome will not be useful in differentiating between species. The development of specific molecular tools to differentiate between species will probably necessitate the analysis of more divergent genes, such as the ITS regions of the SSU rDNA. Currently such gene sequences are not available for most *Hematodinium* spp. from various crustaceans around the world.

Field and Appleton (1996) developed an indirect fluorescent antibody test (IFAT) to detect *Hematodinium* sp. in the haemolymph and tissues of Norway lobsters. This technique was more sensitive than gross observations and wet-mount examinations and was capable of detecting low-level haemolymph infections as well as previously undiagnosable tissue infections. However, the species specificity of this assay has not been assessed.

Prevention and control

The management of the Tanner crab fishery to avoid product quality problems (bitter crab syndrome) with infections of *Hematodinium*

sp. may be possible by harvesting Tanner crabs in the winter, when fewer crabs are severely parasitized and meats are more marketable. Also, proper disposal of infected Tanner crabs is essential in controlling dissemination of the parasite (Meyers *et al.*, 1990).

Stramenopiles

Residual heterotrophic Stramenopiles – Labyrinthulida

Introduction

Only one named species of Labyrinthulida has been documented as a pathogen of economically important shellfish. *Labyrinthuloides haliotidis*, an achlorophyllous, eukaryotic protist, is pathogenic to small, juvenile, northern abalone (*Haliotis kamtschatkana*) and small, juvenile, red abalone (*Haliotis rufescens*) (Bower, 1987a).

Host range

To date, *L. haliotidis* has only been observed in small abalone (less than 1 cm in shell length) from an abalone culture facility in British Columbia. Within 2 weeks of first being detected in a raceway, over 90% of the small abalone succumbed to infection and the disease quickly spread between raceways. The high mortalities caused by *L. haliotidis* were one of the reasons why this particular abalone culture facility is no longer in operation. Small abalone that are susceptible to infection are rarely found in the field. Thus, the geographical distribution of this parasite and its effect on wild stocks are not known.

Parasite morphology and life cycle

The trophozoite of *L. haliotidis* was spheroid (5 to 9 μm in diameter) and had a unique organelle of this group called the sagenogenetosome (Fig. 17.5A). Several sagenogenetosomes were scattered on the surface of each organism, forming openings in the thin laminated cell wall, and each produced the ectoplasmic net upon which the parasite moved (0.3 ± 0.16 μm/min) and obtained nutrients. Following removal from sources of nutrients (i.e. placement in sterile sea water), the trophozoite underwent synchronous multiple fission to form a zoosporoblast (6 to 10 μm in diameter) containing about ten developing zoospores (Fig. 17.5B). The uninucleate, ovate, motile, biflagellated zoospores (Fig. 17.5C) escaped through a rupture in the zoosporoblast wall (Bower, 1987a). When the zoospore contacted a hard surface or after about 24 h of active swimming in sea water, both flagella were shed. The resulting cell was morphologically similar to the trophozoite and survived in sterile sea water for at least 2 years (Bower, 1987a).

Trophozoites that developed from zoospores were infective to small abalone.

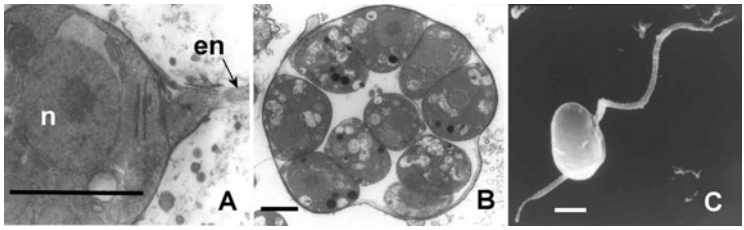

Fig. 17.5. Electron micrographs of *Labyrinthuloides haliotidis* from British Columbia, Canada. A. Trophozoite within the muscle tissue of a juvenile abalone (*Haliotis kamtschatkana*) showing the nucleus (n) and the ectoplasmic net (en) originating from the sagenogenetosome. B. Zoosporoblast from sea water containing well-developed zoospores. C. Zoospore illustrating the subapical attachment site of the two flagella, the coarse texture of the longer anterior flagellum, where debris has attached to the mastigonemes, and the thin tapered tip of the short posterior flagellum. All bars = 2 μm.

Within 4 h of contact, sagenogenetosomes were evident and host epithelial cells adjacent to the parasite were lysed. By 24 h post-exposure, the ectoplasmic net was well developed, the parasite had moved into the head and foot tissues of the abalone and dividing forms were observed (Bower *et al.*, 1989b). Within 10 days after exposure to about 10^4 parasites in 20 ml of seawater, about 90% of the abalone (less than 4.0 mm in shell length and 140 days of age) died with numerous parasites throughout the head and foot (Bower, 1987b). As dead abalone decomposed, trophozoites released from the tissues developed into zoosporoblasts, which produced zoospores within about 24 to 72 h. Parasites released from infected abalone were infective to other abalone on contact. Although alternative hosts have not been described, *L. haliotidis* can utilize diverse sources of nutrients but seems incapable of coexisting with bacteria.

Host–parasite relationships

The tissues of heavily infected abalone were slightly swollen, with a loss of integrity. Prevalence and intensity decreased and time to death increased as the abalone increased in age and size. Abalone greater that 15 mm in shell length could not be infected even when injected intramuscularly with about 1.5×10^4 *L. haliotidis*. The mechanism of defence against this parasite is not known. There was no indication of a haemocytic response in young susceptible abalone. Possibly the resistance of older abalone corresponded to the development of cellular or humoral defence mechanisms as the abalone matured (Bower, 1987b).

Small juvenile Japanese scallops (*P. yessoensis*) and juvenile Pacific oysters (*Crassostrea gigas*), both less than 8 months of age, were resistant to infection. However, two oysters with badly cracked shells became infected, suggesting that *L. haliotidis* was capable of utilizing oyster tissue for growth and multiplication if it was able to gain access to the soft tissues of the oyster (Bower, 1987b).

In vitro *culture and propagation*

Aseptic cultures of *L. haliotidis* grew well on several different liquid media and agar-based solid media. An *in vitro* life cycle could be produced by placing the parasite alternately in minimum essential medium with 10% fetal calf serum (where rapid production of trophozoites occurred through binary fission) and in sterile sea water (where the trophozoites transformed into zoosporoblasts and zoospores were produced) (Bower, 1987c). Cultured *L. haliotidis* were infective to small abalone (Bower, 1987b; Bower *et al.*, 1989b). The trophozoite also grew on pine pollen (*Pinus contorta*) in sea water but failed to produce zoosporoblasts and zoospores (Bower, 1987c).

Diagnosis of infection

The spheroid parasite is readily observed with light microscopy ($\times$ 100 magnification) in the head and foot of small infected abalone squashed in sea water between a glass slide and a cover slip. *L. haliotidis* is also evident in histological sections prepared using routine procedures. However, due to the morphological similarities of *L. haliotidis* to other thraustochytrids, identification of the parasite outside its host is impossible by microscopy. A direct fluorescent antibody technique showed promise in facilitating the detection of this parasite (Bower *et al.*, 1989a). However, this technique has not been fully tested to verify its specificity. A partial sequence of the SSU rRNA has been identified and used to assess phyletic relationships (Leipe *et al.*, 1996). However, this information has not been utilized in the development of a diagnostic tool.

Prevention and control

The source of infection in the abalone hatchery was not established. Transmission of the disease between raceways could be prevented by employing sanitary techniques. The parasite was destroyed in 20 min when exposed to 25 mg/l of chlorine in sea water. The fungicide cyclohexamide at 1–2 mg/l for 23 h per day on 5 consecutive days

cured infected abalone. However, this treatment had the disadvantages of: (i) being detrimental to diatoms upon which the abalone fed; (ii) being ineffective against non-growing but infective zoospores such that reinfection occurred within 2 to 3 weeks following treatment; and (iii) inducing resistant forms (as few as three successive treatments resulted in the production of forms twice as resistant to cyclohexamide) (Bower, 1989). Ozone treatment of incoming water may only be efficacious if ozone exposure is greater than 0.97 mg ozone/l for 25 min (Bower *et al.*, 1989c).

Amoeboid protists

Introduction

Two species in the order Euamoebida and family Paramoebidae are significant pathogens of shellfish. *Paramoeba perniciosa* is the cause of 'grey crab disease' or paramoebiasis in the blue crab (*C. sapidus*) and is infectious to other crustaceans. *Paramoeba invadens* is pathogenic to sea urchins (*Strongylocentrotus droebachiensis*).

Host range

P. perniciosa has been reported in blue crabs along the east coast of the USA from Connecticut to Florida, including the high-salinity areas of Chincoteague Bay and Chesapeake Bay, where it periodically causes mass mortalities and has caused ongoing low-level mortalities since 1967 (Couch, 1983; Sparks, 1985). Epizootics with high mortalities (about 17%) were reported from Chincoteague Bay in early summer and mortalities (20–30%) were observed in shedding tanks (for production of newly moulted softshell crab) (Johnson, 1988). It has also been reported from the rock crab *Cancer irroratus*, the exotic European green crab *C. maenas* and the American lobster *H. americanus* (Couch, 1983).

P. invadens was associated with mass mortalities of the sea urchin along the Atlantic coast of Nova Scotia in the early 1980s (Jones, 1985; Jones *et al.*, 1985). From 1980 to 1983 sea urchin mortalities were estimated to be at least 245,000 t (Miller, 1985). No mortalities were observed in other echinoderms, including other echinoids, asteroids and ophiuroids from the same area (Scheibling and Stephenson, 1984). However, the transformation of echinoid-dominated 'barren grounds' into kelp beds provided increased areas for American lobster (*H. americanus*) recruitment and thus increased lobster productivity (Wharton and Mann, 1981).

Morphology and life cycle

P. perniciosa is round to elongate, with linguiform lobopodia, and can be differentiated into small (3 to 12 μm) and large (15 to 35 μm) forms (Couch, 1983). Each amoeba contains a vesicular nucleus with a large central endosome and a 'second nucleus', 'Nebenkörper' or elongate parasome (1 to 4 μm) with a Feulgen-positive middle bar and two opposing basophilic polar caps. *P. invadens* is similar in size (20–35 μm in length and 8–15 μm in width) but is more elongated in shape, with a length/width ratio of about 2, and has digitiform pseudopodia (Fig. 17.4B). Also, the parasome (2 to 3 μm in size) has Feulgen-positive poles but no Feulgen-positive central band (Jones, 1985). Due to the unusual ultrastructure of the parasome, which is characteristic for *Paramoeba* spp., Perkins and Castagna (1971) proposed that the parasome may be a discrete organism of unknown taxonomic affinities and not an organelle of the amoeba.

The mode of transmission in the field has not been fully elucidated for either amoeba. Some attempts to infect blue crabs by injecting *P. perniciosa* or feeding infected crab tissues failed (Couch, 1983). However, Johnson (1977) observed the disease in two blue crabs 34 and 39 days post-inoculation with infected haemolymph and Sparks (1985) claimed that the disease was transmitted by consumption of moribund or dead infected blue crab. The transmission of *P. invadens* was direct and the infection was waterborne (Jones, 1985).

Host–parasite relationships

P. perniciosa, a parasite of the connective tissues and haemal spaces, occurs along the

midgut, antennal gland and Y organ in light infections. Haemal spaces in gills are usually invaded in medium and heavy infections and, in the terminal phase, the infection becomes systemic (Johnson, 1977; Sparks, 1985). In heavy infections, pathological changes caused by large numbers of amoeba include: tissue displacement; probable lysis of some types of tissue, including haemocytes; and significant decreases in protein, haemocyanin (the oxygen-binding and transport molecule of crustaceans) and glucose (Pauley *et al.*, 1975; Johnson, 1977). Sparks (1985) suggested that the probable cause of death was a combination of anoxia and nutrient deficiency. Terminal infections are usually observed during the late spring to early autumn, but infected blue crabs are found throughout the year (Johnson, 1988).

Most infected blue crabs demonstrate a defence response, which is manifested usually as phagocytosis of amoeba by hyaline haemocytes and infrequently as encapsulation of amoeba by haemocytes, but destruction of amoeba by humoral factors also occurred (Johnson, 1977). Occasionally a blue crab would overcome the infection.

In vitro *propagation*

P. perniciosa could not be cultured in various media, including a medium that supported continuous growth of *Paramoeba eilhardi*, a free-living species originally isolated from algal material. However, *P. perniciosa* survived for about 2 weeks in 10% calf serum agar overlaid with sterile sea water and incubated at 18°C (Sprague *et al.*, 1969). Unlike *P. perniciosa, P. invadens* was easily cultured on malt–yeast–seawater agar and on non-nutrient agar with marine bacteria as a food source. However, there was some loss of virulence after 15 weeks in monoxenic culture and 58 weeks in polyxenic culture (Jellett and Scheibling, 1988).

Clinical signs and diagnosis of infection

In blue crabs, signs of infection include a greyish discoloration of the ventral exoskeleton, general sluggishness, reduced or absence of clotting of the haemolymph and poor survival subsequent to handling or holding in tanks (Sparks, 1985). Infection is easily diagnosed only in the terminal phase, when numerous *P. perniciosa* and virtually no haemocytes are present in circulating haemolymph. Characteristic signs of paramoebiasis in sea urchins included muscle necrosis, general infiltration of coelomocytes, reddish-brown discoloration, poor attachment to substrate and high mortalities (Jones, 1985; Jellett *et al.*, 1988). Amoeba of both species can be observed with phase contrast either live or fixed in 5% formalin sea water and stained with dilute methylene blue. Smears can be stored following fixation in Bouin's, Davidson's, Hollande's or 10% formalin solutions and staining with iron haematoxylin or Giemsa's stain. Before amoebae appear in the circulation, they may be observed in squashes of subepithelial connective tissue examined with phase contrast if the infection is sufficiently advanced (Johnson, 1988). Infection can also be diagnosed by histological examination.

Haplosporidia (Haplospora)

Introduction

Included within the Haplosporidia are several species that are significant pathogens of oysters. Species of Haplosporidia were first described from oysters in the early 1960s and since that time the group and its affiliations have undergone several changes in classification. Initially these parasites were included in an order that was changed from Haplosporida to Balanosporida in the phylum Ascetospora (Sprague, 1979; Levine *et al.*, 1980), Perkins (1990) indicating that they were sufficiently distinct to warrant the status of phylum with the name Haplosporidia. Subsequently, Siddall *et al.* (1995) indicated that the haplosporidians are more closely related to alveolates (ciliates, dinoflagellates and apicomplexans) than to other spore-forming protozoans based on sequence comparisons of the 16S SSU rDNA gene. More recently, Cavalier-Smith and Chao (2003) indicated that the order status should be reinstated under the recently

created phylum Cercozoa. The proposal by Cavalier-Smith and Chao (2003) has not yet received wide acceptance and thus the phylum status of this group as indicated by Lee *et al*. (2000) is used here.

In addition to challenges in determining higher taxonomic affiliations, the sorting of species at the genus level has also undergone modifications. The initial grouping only included spore-forming endoparasites but differentiation between genera proved problematic, with species being transferred between *Haplosporidium* and *Minchinia* (Lauckner, 1983). Although the visibility and morphology of spore ornamentations (e.g. the presence of prominent extensions (tails) on the spore wall, visible by light microscopy) were proposed as features for differentiating between these two genera (Perkins, 2000b), recent phylogenetic analysis by Reece *et al*. (2003) supported an earlier proposal that composition of the spore ornamentation is a key characteristic (not the morphology of spore ornamentation). Specifically, species with spore ornamentation composed of epispore cytoplasm are *Minchinia* and those within it composed of spore wall material are *Haplosporidium* (Azevedo, 2001).

Recently, oyster pathogens in the genus *Bonamia*, which are not known to produce spores, were incorporated into the phylum Haplosporidia based on molecular phylogenetic analysis (Carnegie *et al.*, 2000). The following review focuses on the most significant representatives of the spore-forming and non-spore-forming members of this phylum. Specifically, *Haplosporidium nelsoni* (commonly known as MSX from the original assignation of multinucleate sphere unknown) and then *Bonamia ostreae* will be discussed. In addition, other significant species in each genus are mentioned under the headings 'Related pathogens'.

HAPLOSPORIDIUM NELSONI

Host range and economic significance

Catastrophic epizootics caused by *H. nelsoni* were first encountered in the late 1950s in eastern oysters (*C. virginica*) from Delaware Bay (Haskin *et al.*, 1966). This parasite (Fig. 17.6A, B, C) currently occurs along the

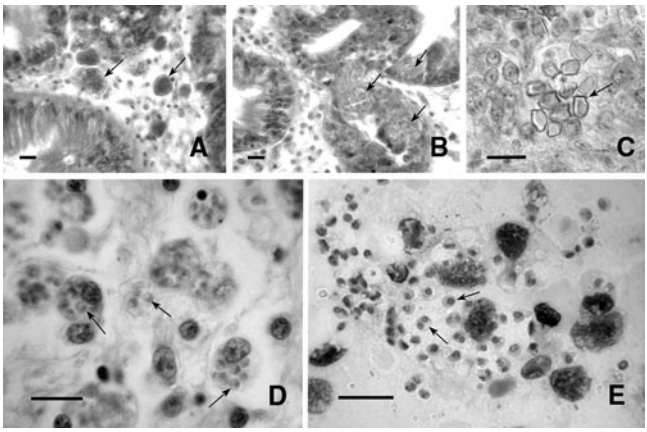

Fig. 17.6. Histological images (A to D) and tissue imprint (E) of Haplosporidia in oysters. A to C. Developmental stages of *Haplosporidium nelsoni* in *Crassostrea virginica* from Nova Scotia, Canada. A. Plasmodia (arrows) in the connective tissue of the digestive gland between the tubules. B. Sporocysts (arrows) within the epithelium of the digestive-gland tubules. C. Spores with a prominent operculum (arrow) in the disrupted tissue of a digestive-gland tubule (courtesy of M. Maillet). D and E. *Bonamia ostreae* from heavily infected *Ostrea edulis* from France. D. Numerous *B. ostreae* (arrows) located within haemocytes in the connective tissue between the tubules of the digestive gland. E. Imprint of connective tissue containing many *B. ostreae* (arrows), some with two nuclei, freed from ruptured haemocytes. All bars = 10 μm.

eastern coast of the USA from Florida to the Piscataqua River estuary in Maine/New Hampshire. A recent (autumn of 2002) epizootic, with localized high mortalities (about 80%), occurred in Bras d'Or Lakes, Nova Scotia, Canada (Stephenson *et al.*, 2003). However, the parasite has not yet been detected in oyster stocks between the southern end of Maine and Bras d'Or Lakes.

Sporogonic and/or plasmodial stages of haplosporidians that resemble *H. nelsoni* were observed in the Pacific oyster (*C. gigas*) from California and Washington State, USA, Taiwan, Korea and Japan (Friedman, 1996). With the development of molecular diagnostic techniques, reports of *H. nelsoni* in Pacific oysters have been confirmed from California, USA, France, Korea and Japan (Burreson *et al.*, 2000; Renault *et al.*, 2000; Kamaishi and Yoshinaga, 2002). Effects of *H. nelsoni* on Pacific oysters have not been described, but some authors speculate that it may be pathogenic, especially for juvenile oysters. However, haplosporidosis has not been associated with mortality of this oyster species.

In 1957, 85% mortality (with 50% dead within 6 weeks) occurred among eastern oysters planted in Delaware Bay. The high mortality represented a loss in production from 7.5 million lbs of shucked meats prior to the enzootic to about 100,000 lbs of production in 1960, and production has not significantly recovered (Lauckner, 1983; Sindermann, 1990). Average mortalities in eastern oysters have been estimated at 50 to 60% in the first year with a 50% further loss in the second year of oyster grow-out. Also, eastern oyster culture in the lower Chesapeake Bay was abandoned for at least 25 years due to this disease (Andrews, 1988b).

Morphology and life cycle

Despite more than 40 years of intensive research, the complete life cycle, the mode of infection and several aspects of the general biology remain obscure (Ford, 1992). In eastern oysters, spheroid plasmodia (4 to 30 μm in diameter; Fig. 17.6A) are usually multinucleate (up to about 60 nuclei per plasmodium, with each nucleus about 2 μm in diameter). Smaller plasmodia are formed by cytoplasmic cleavage of the larger ones. They are first observed in the gills, palps and suprabranchial chambers but subsequently occur in the vesicular connective tissues adjacent to the digestive tract, and eventually become systemic (Lauckner, 1983). Plasmodia and prespore stages (Fig. 17.6B) are most frequently observed, while sporocysts containing mature spores (Fig. 17.6C) are rare in adult oysters (< 0.01%). However, sporulation occurs in at least 75–85% of the infected young oysters (< 1 year) in Delaware Bay (Burreson, 1994). Sporulation, when present, occurs in the epithelial cells of the digestive gland tubules (Fig. 17.6B). Spores are operculate and measure 7.5 μm by 5.4 μm unfixed (Couch *et al.*, 1966; Rosenfield *et al.*, 1969). Ford and Haskin (1982) and Perkins (1993) noted that the parasite could not be transmitted in the laboratory with either infected tissues or spore suspensions and foci of infection persisted in areas where eastern oysters were sparse. Although no hosts other than oysters have been found (Ford, 1992), there is speculation that an intermediate host is required for the completion of the life cycle of *H. nelsoni* (Ford and Tripp, 1996).

Extensive epizootiological data indicate that infections acquired in the early summer become patent in July and mortalities begin in early August and peak in September, with a subsequent decline to low levels by November (Andrews, 1982). A few mortalities occur in late winter, followed by increased mortalities in June and July of the second year resulting from infections acquired during the late summer and autumn of the previous year. Essentially the disease is regulated by temperature, with both parasite and host being inactive below 5°C. Between 5 and 20°C, the parasite multiplies faster than the host can contain it. Above 20°C, resistant oysters can inhibit parasite multiplication or undergo remission (Sindermann, 1990). Levels of *H. nelsoni* have fluctuated in a cyclic pattern, with peaks in prevalence every 6 to 8 years and reduced parasite activity following very cold winters (Ford and Haskin, 1982). In addition to temperature, salinity is also known to affect

the pathogenicity of *H. nelsoni*. The disease is restricted to salinities over 15 ppt (*H. nelsoni* cannot survive below 10 ppt), rapid and high mortalities occur at about 20 ppt and the disease may be limited by salinities between 30 and 35 ppt (Andrews, 1988b). Extensive data on the influence of environmental conditions on the prevalence and intensity of infection and the disease process have been integrated into a mathematical model of host–parasite–environmental interactions (Ford *et al.*, 1999; Paraso *et al.*, 1999; Powell *et al.*, 1999).

Host–parasite relationships

H. nelsoni is highly virulent for eastern oysters and the occurrence of moribund oysters with relatively light infections suggests a toxic effect (Andrews, 1988b). The course of the infection seemed dependent on the history of exposure in eastern oyster stocks (Farley, 1975). In susceptible populations, the prevalence of infection can reach 100%, with mortalities ranging between 40 and 80% (Andrews, 1988b). However, in enzootic areas such as Delaware Bay, natural selection has increased the proportion of disease-resistant eastern oysters and mortalities were about half those of naïve stocks (Ford and Haskin, 1982). Further development of high disease resistance in wild oyster populations was attributed to drought conditions in the mid-1980s, which caused a salinity increase in the usually lower-salinity areas of the upper Delaware Bay, thereby allowing incursion of *H. nelsoni*, with resulting high mortalities and thus selection for disease resistance in the brood stock (Ford, 2002).

During their second year, eastern oysters that survived the infection were able to suppress or rid themselves of the parasite in the late spring as temperatures approached 20°C (Ford and Haskin, 1982). Remission was characterized by diminution of infection and localization of parasites to external epithelium, with diapedesis resulting in the deposition of moribund parasites and necrotic tissues against the shell, followed by external conchiolinous encapsulation (Farley, 1968). Ford and Haskin (1982) indicated that resistance to mortalities was not correlated with an ability to prevent infection but with restriction of parasites to localized non-lethal lesions. Chintala and Fisher (1991) proposed that lectins in the haemolymph could be related to disease resistance or affected by *H. nelsoni* infection.

Parasitism was associated with reduced meat yield, impaired gonadal development and lower fecundity (Barber *et al.*, 1988). The greatest effect on reproduction occurred when gametes were in the formative stage rather than after they matured (Ford *et al.*, 1990a). There was also a threefold increase in the proportion of females among infected oysters, which Ford *et al.* (1990a) suggested was due to inhibition of the development of male more than female gametes. However, infected oysters that underwent temperature-associated remission during the summer developed mature gonads and spawned before new or recurrent infections proliferated in the autumn (Ford and Figueras, 1988).

Propagation

H. nelsoni has not been cultured *in vitro* and controlled transmission has not been achieved. Even transplantation of infected tissues was unsuccessful (Lauckner, 1983). Enriched suspensions of *H. nelsoni* plasmodial stages can be obtained using the 'panning' technique described by Ford *et al.* (1990b).

Clinical signs and diagnosis of infection

The only specific but rare sign of this disease is a whitish discoloration of the digestive gland tubules due to the presence of mature spores. Other non-specific signs are: emaciation, mantle recession, failure of shell growth, retracted mantle and, rarely, brown patches of periostracum opposite lesions on the mantle surface (Lauckner, 1983; Andrews, 1988b). Histological examination is used to confirm the presence of infection, and heavy infections can be detected by microscopic examination of stained haemolymph smears (Andrews, 1988b; Burreson *et al.*, 1988; Ford and Kanaley, 1988). When mature spores are present, the sporoplasm specifically stains

bright red with a modified Ziehl–Neelsen carbol fuchsin technique (Farley, 1965). The location of the spore stages in the epithelium of the digestive gland tubules can be used to differentiate *H. nelsoni* from a closely related and potentially cohabiting species *Haplosporidium costale* with spores that occur in the connective tissue between the tubules. If spores are not present (usually the case for adult oysters infected with *H. nelsoni* and during early developmental stages of *H. costale*), the plasmodial stages of the two species cannot be differentiated by histological examination. Although Barrow and Taylor (1966) and Burreson (1988) illustrated the potential use of immunoassays for detecting infection and possibly for identifying alternative hosts, molecular diagnostic techniques have proven to be more widely used.

An SSU rRNA gene of *H. nelsoni* was sequenced (Fong *et al.*, 1993) and DNA probes that targeted this region were tested for sensitivity and specificity (Stokes and Burreson 1995; Stokes *et al.*, 1995). In addition to being used by various researchers to identify *H. nelsoni* in eastern oysters, DNA sequence equivalency (tested by PCR amplification of genomic DNA and *in situ* hybridization) provided conclusive evidence that the haplosporidian in Pacific oysters from other areas of the world was *H. nelsoni* (Burreson *et al.*, 2000; Renault *et al.*, 2000; Kamaishi and Yoshinaga, 2002). The primer sequence was also used to develop a competitive, quantitative PCR assay for this parasite (Day *et al.*, 2000). The subsequent identification of the SSU rRNA gene for *H. costale* (Ko *et al.*, 1995) and the development of specific PCR assays confirmed by *in situ* hybridization have led to the differential diagnosis of mixed *H. costale* and *H. nelsoni* infections in eastern oysters (Stokes and Burreson, 2001). Also, multiplex PCR (simultaneous testing of two or more pathogens in a single test reaction) was developed for *H. nelsoni* and two other cohabiting parasites, *H. costale* and *P. marinus* (Penna *et al.*, 2001). Unfortunately, attempts to use PCR technology to identify the complete life cycle of *H. nelsoni* were unsuccessful (Stokes *et al.*, 1999).

Prevention and control

Reduced salinities (< 10 ppt) adversely affected the pathogenicity and survival of the parasite in oysters (Haskin and Ford, 1982). Thus, management strategies depend, in large measure, on avoiding the disease by culturing oysters in areas of low salinity and/or altering the time at which oysters are moved to enzootic areas of high salinity to take advantage of better growth. Continuous monitoring and early diagnosis of infections are important because they allow mortality to be predicted so that growers and managers can make informed decisions on when or whether to plant and harvest (Ford and Haskin, 1988). The recently developed mathematical model of host–parasite–environmental interactions has been used to simulate infection cycles within the oyster and in oyster populations under different environmental conditions to forecast conditions that can initiate and end epizootics in oyster populations (Ford *et al.*, 1999; Paraso *et al.*, 1999; Powell *et al.*, 1999).

Excellent survival has been achieved in enzootic areas using eastern oysters that were experimentally selected for disease resistance (Ford *et al.*, 1990a). Barber *et al.* (1991) indicated that resistance in the selected strain may be the result of physiological responses that inhibit parasite development and basic metabolic adjustments to parasitism. Much of the physiological response may be derived from an increased number of haemocytes, which plug lesions, remove debris and repair tissue, thereby helping resistant oysters to survive infection (Ford *et al.*, 1993). Because of economic limitations, resistant oysters have not yet been produced in commercial quantities (Andrews, 1988b). However, triploid eastern oysters seem more resistant to the disease than diploid cohorts (Matthiessen and Davis, 1992). The increased resistance in triploids may provide a viable alternative for the eastern oyster culture industry in areas where the disease occurs. Also, natural selection in Delaware Bay seems to have resulted in wild oysters that are more resistant to disease than oysters from populations that have not undergone such long-term, intensive, selection processes (Ford, 2002).

The possibility of growing non-native oyster species that appear to be more resistant to *H. nelsoni*, such as the Pacific oyster and Suminoe oyster (*Crassostrea ariakensis* = *Crassostrea rivularis*) are being assessed.

Related pathogens

In addition to numerous reports of unidentified species of *Haplosporidium* or *Minchinia* in marine invertebrates (Burreson and Ford, 2004), three named species occur in bivalves of economic importance.

H. costale (commonly referred to as SSO, an acronym for seaside organism) has been detected in eastern oysters along the east coast of North America but has caused significant disease only in high-salinity (> 25 ppt) areas from Delaware to Virginia (Andrews, 1988c). It can be differentiated from *H. nelsoni* by: (i) a smaller spore size (3.1 μm by 2.6 μm); (ii) occurrence of sporulation throughout all connective tissue and not in the epithelium of the digestive gland; (iii) antigenic differences; and (iv) species-specific molecular diagnosis, as indicated above. Initially thought to have a regular and clearly defined life cycle (a 4- to 6-week period of disease, sporulation and concurrent mortalities in May and June, followed by an 8- to 10-month prepatent period in newly exposed oysters), the application of molecular diagnostic tools has revealed unseasonably advanced infections in the autumn (Stokes and Burreson, 2001). Also, mixed infections with *H. nelsoni* are more frequent than originally thought. *H. costale* is not as serious a pathogen as *H. nelsoni* and losses can be minimized by harvesting oysters at 18 to 24 months of age (Andrews, 1988c).

Haplosporidium (= *Minchinia*) *armoricana* causes brown meat disease in flat oysters (*Ostrea edulis*) in Brittany (France) to Spain and in the Netherlands among flat oysters imported from Brittany (van Banning, 1985a; Azevedo *et al.*, 1999). Numerous operculate spores (5.0 to 5.5 μm by 4.0 to 4.5 μm) with two long projections (70 to 100 μm) in sporocysts (35 to 50 μm in diameter) throughout the connective tissue result in brownish discoloration of heavily infected flat oysters. Although the disease is fatal, the prevalence of infection to date has been low (< 1%) with an insignificant impact on the flat oyster culture industry in Europe (van Banning, 1979; Lauckner, 1983).

Minchinia (= *Haplosporidium*) *tapetis*, was described from European littleneck clams (*R.* (= *T.*) *decussatus*) in Portugal and France (Lauckner, 1983; Chagot *et al.*, 1987; Azevedo, 2001). Slightly ovoid spores (4 to 6 μm in diameter) were observed in the connective tissue of the gills, mantle and ventral to the digestive gland tubules. Reported prevalences of infection were low (4%) and pathogenicity was minimal.

BONAMIA OSTREAE

Host range and economic significance

B. ostreae (commonly called a microcell because of its small size; Fig. 17.6D, E) is a lethal pathogen of flat oysters (*O. edulis*), in which it causes a disease called bonamiasis (Pichot *et al.*, 1980). However, other oyster species, including Australian flat oysters (*Ostrea angasi*), New Zealand dredge oysters (*Ostrea chilensis* (= *Tiostrea chilensis* = *Tiostrea lutaria*)), *Ostrea puelchana* and Suminoe oysters (*C. rivularis*) were experimentally infected (Cochennec *et al.*, 1998). The Pacific oyster (*C. gigas*), mussels (*Mytilus edulis* and *Mytilus galloprovincialis*) and clams (*R. decussatus* and *V.* (= *R.*) *philippinarum*) could not be naturally or experimentally infected and these bivalves did not appear to act as vectors or intermediate hosts for *B. ostreae* (Culloty *et al.*, 1999). This parasite occurs along the Atlantic coast of Europe from Spain to Denmark, Great Britain (excluding Scotland), Ireland and Italy (OIE, 2003a). *B. ostreae* also occurs in some introduced flat oyster populations on the west (California and Washington) and east (Maine) coasts of the USA (Zabaleta and Barber, 1996). Evidence suggests that *B. ostreae* was inadvertently introduced into Europe, Maine and Washington from California by the translocation of infected flat oysters in the late 1970s (Elston *et al.*, 1986; Friedman and Perkins,

1994; Cigarría and Elston, 1997). The source of infection for the introduced flat oysters in California is not known.

This parasite was first associated with mortalities in Brittany, France, in 1979 and the disease quickly spread throughout the major flat oyster culture areas in Europe. Average losses were about 80% or higher (Grizel, 1986; Hudson and Hill, 1991; van Banning, 1991). In conjunction with the protist *Marteilia refringens* (see below), *B. ostreae* reduced flat oyster production in France from 20,000 t per year in the 1970s to 1800 t in 1995 (Boudry *et al.*, 1996). Production of flat oyster has not recovered, and the Pacific oyster (*C. gigas*) is now the main species of oyster cultured in Europe.

Morphology and life cycle

Two morphological forms of *B. ostreae* have been identified (Lauckner, 1983; Grizel *et al.*, 1988). The most frequently observed 'dense forms' (2 to 3 μm in diameter) have basophilic, dense cytoplasm with a pale halo around the nucleus. This form is usually free (extracellular) in tissues altered by the disease and may represent the transmission stage. The slightly larger and less dense 'clear forms' (2.5 to 5 μm in diameter) may be the trophozoite, schizogonic stage and typically occur within the cytoplasm of haemocytes and in branchial epithelial cells (Montes *et al.*, 1994). The usual mode of multiplication in the oyster is by simple binary fission. However, Brehélin *et al.* (1982) described a true plasmodial multinucleate stage with three to five nuclei and about 6 μm in diameter.

Unlike the spore-forming haplosporidians, *B. ostreae* can be directly transmitted between flat oysters, and lethal infections usually develop within 3 to 6 months after exposure (Grizel *et al.*, 1988; Sindermann, 1990). Transmission occurs year-round, with the highest prevalence of infection found during the summer. Van Banning (1990) suggested that an infectious phase may occur in the ovarian tissue of flat oysters.

Host–parasite relationships

Bonamiasis is usually systemic because *B. ostreae* normally resides within haemocytes and has not been reported in other host cells. Infections are often accompanied by dense, focal haemocyte infiltration into the connective tissue of the gill and mantle and around the gut, and may result in tissue lesions (Cochennec-Laureau *et al.*, 2003a). Many of the infiltrating haemocytes contain several microcells (Fig. 17.6D), which are often in cytoplasmic vacuoles. As the infection progresses, infected haemocytes occur in the vascular sinuses, and microcells may be released by lysis of haemocytes and found free in necrotic tissues (Balouet *et al.*, 1983). Two years of age appeared to be critical for disease development in oysters, and infection level was statistically independent of oyster gonadal development and sex (Culloty and Mulcahy, 1996).

In vitro tests were used to determine that haemocytes of Pacific oysters were able to bind more *B. ostreae* than were haemocytes of flat oysters (Fisher, 1988), but haemocyte infection rates were similar for both species (Mourton *et al.*, 1992). The apparent inability of flat oyster haemocytes to inactivate the parasites once they are ingested may explain differences in susceptibility and disease development in oysters (Chagot *et al.*, 1992; Xue and Renault, 2000).

Propagation

B. ostreae is readily propagated *in vivo* by injection of infected haemocytes or purified parasite suspensions and by cohabitation of diseased and uninfected oysters (Hervio *et al.*, 1995). Comps (1983) reported *in vitro* proliferation of *B. ostreae* in the presence of flat oyster cells after 48 h of incubation but the viability of the cultures over longer periods was not indicated.

Diagnosis of infection

Microcells are detected by histological examination (Grizel *et al.*, 1988). Although many infected oysters appear normal, others may have yellow discoloration and/or extensive lesions (i.e. perforated ulcers) on the

gills and mantle. The isolation and purification of *B. ostreae* from infected flat oysters (Mialhe *et al.*, 1988a) have led to the production of monoclonal antibodies (Rogier *et al.*, 1991) and the development of an IFAT (Boulo *et al.*, 1989) and of an ELISA diagnostic technique with 90% reliability in comparison with standard histopathological light microscopic examinations (Cochennec *et al.*, 1992). Because classical histological (Fig. 17.6D) and heart smear (Fig. 17.6E) techniques are unreliable for detecting light infections (Culloty *et al.*, 2003) and immunoassays (ELISA kits) are no longer commercially available, molecular diagnostic techniques were developed.

A PCR reaction specific for an rDNA amplicon (528 base pairs (bp) spanning 341 bp of 18S rDNA and 187 bp of ITS1) with a gene sequence resembling that belonging to members of the phylum Haplosporidia was identified and found to detect the parasite in naturally infected *O. edulis* in Maine, USA (Carnegie *et al.*, 2000). This PCR assay proved to be more sensitive, more specific and less ambiguous than standard histological and cytological (tissue imprint) techniques. Another DNA probe identified from the same area of the genome also detected another species of *Bonamia* (see *B. exitiosus* below) and *H. nelsoni* (Cochennec *et al.*, 2000).

Prevention and control

Following the recognition of bonamiasis in Europe, measures such as the destruction of infected stocks and restricting movement of flat oysters were implemented (van Banning, 1985b; Grizel *et al.*, 1986; Hudson and Hill, 1991). In many instances, these measures were employed too late to prevent the spread of the pathogen. Studies in the Netherlands indicated that *B. ostreae* persisted in low levels for at least 6 years in areas where flat oysters were virtually eradicated (van Banning, 1987). Mortalities due to bonamiasis were reduced by using suspension culture and lower stocking densities and marketing the oysters at a relatively young age (after 15 to 18 months of culture) (Lama and Montes, 1993; Montes *et al.*, 2003).

Alternative resistant species, such as Pacific oysters, are now being cultured in areas where flat oyster populations were devastated by bonamiasis. However, flat oyster production has marginally persisted in a few areas of France in which the seeding of young oysters was reduced from 5 to between 1 and 2 t/ha, and by the use of 'deep water', where the parasite is apparently not transmitted (Grizel *et al.*, 1986). Also, the absence of infection in juveniles has allowed the use of oyster seed produced in areas where *B. ostreae* occurs (Grizel *et al.*, 1988). Selecting for disease-resistant flat oysters is showing some success (Culloty *et al.*, 2001). However, there is evidence from DNA microsatellite loci analysis that a population bottleneck has occurred during the selection process in some stocks of bonamiasis-resistant *O. edulis*. The small effective number of breeders is expected to lead to increasing inbreeding and have important consequences for the future management of at least three selected bonamiasis-resistant populations (Launey *et al.*, 2001).

Related pathogens

Bonamia exitiosus has devastated dredge oysters (*O.* (= *T.*) *chilensis* (= *lutaria*)) populations in the Foveaux Strait south of South Island, New Zealand (Hine *et al.*, 2001b). Stocks of dredge oysters were reduced by 67% in 1990 and by 91% in 1992 from levels recorded in 1975. The commercial dredge oyster fishery was closed in 1993, with severe economic impacts on South Island coastal communities (Doonan *et al.*, 1994). Like *B. ostreae*, *B. exitiosus* resides in haemocytes, is small in size (2 to 7 μm) and has light and dense forms, which vary in prevalence seasonally (Hine, 1991a,b). However, *B. exitiosus* can de differentiated from *B. ostreae* by antigenic features (Mialhe *et al.*, 1988b), divergent regions in the SSU rDNA sequence and ultrastructural differences in

the dense forms. Dense forms of *B. exitiosus* are less dense and slightly larger in size (3.0 ± 0.3 μm mean diameter in comparison with 2.4 ± 0.5 μm for *B. ostreae*), have more haplosporosomes, mitochondrial profiles and lipoid bodies per ultrastructure section, have smaller tubulovesicular mitochondria and have nuclear membrane-bound Golgi/nuclear cup complexes and a vacuolated stage that are lacking in *B. ostreae* (Hine *et al.*, 2001b). Ultrastructural examination of cytoplasmic structures in *B. exitiosus* led Hine and Wesney (1992) to suggest that the haplosporosome-like bodies may be a sign of disease within the microcells. In part, this suggestion was derived because of the similarities: (i) between haplosporosomes and virus-like particles; and (ii) between haplosporogenesis and virus production within host cells. As with *B. ostreae*, molecular diagnostic assays (*in situ* hybridization and PCR amplification) were more sensitive in the detection of *B. exitiosus* than traditional techniques (microscopic examination of heart imprints and histology) (Diggles *et al.*, 2003).

The microcell that causes winter mortalities in Sydney rock oysters (*Saccostrea glomerata* (= *commercialis*)) in New South Wales, Australia, initially described as *Mikrocytos roughleyi* (Farley *et al.*, 1988), is now believed to be a species of *Bonamia* (Cochennec-Laureau *et al.*, 2003b). The morphological features that separate this parasite from the other *Bonamia* spp. have not been identified. Like other *Bonamia*, this microcell also occurs within haemocytes and is associated with focal abscess-type lesions in the gill, connective and gonadal tissues and the alimentary tract. Disease is associated with low temperatures and high salinities (30–35 ppt). It can kill up to 70% of mature Sydney rock oysters in their third winter before marketing and mortalities seem to be highest in autumns and winters with low rainfall (Wolf, 1979). The high mortalities can be reduced by harvesting large oysters before the winter and by overwintering smaller oysters on up-river leases where lower salinities and higher racks protect them from the disease (Anderson, 1990).

Unknown Taxonomic Affiliations

Mikrocytos mackini

Like *Bonamia* spp. described above, *M. mackini* (Fig. 17.7A, B, C) is commonly referred to as a microcell (2 to 4 μm in diameter) and is infective to at least four species of oysters (Pacific oysters, *C. gigas*; eastern oysters, *C. virginica*; flat oysters, *O. edulis*; and Olympia oysters, *Ostrea conchaphila*). However, *M. mackini* is not related to *Bonamia* spp. and its taxonomic affiliations remain unknown. This parasite has been reported from oysters in the southern part of British Columbia, Canada and adjacent areas of Washington, USA. Disease caused by *M. mackini* appears to be restricted to older oysters (over 2 years) in some locations in British Columbia and mortalities (recorded as high as 30% in older oysters at low tide levels) occur in the spring (April and May) after a 3–4-month period when temperatures are less than 10°C. The requirement for cool temperatures and the long prepatent period may explain why the disease only occurs during the spring and seems to be confined to oysters cultured in more northerly locations. *M. mackini* can de differentiated from *Bonamia* spp. by its location in vesicular connective tissue cells, in adductor muscle myocytes and, less frequently, in haemocytes, and by the apparent lack of mitochondria and haplosporosomes. Also, *M. mackini* seems to have a very unique way of obtaining energy from its host cell. Hine *et al.* (2001a) depicted tube-like structures extending into the cytoplasm of *M. mackini* from the mitochondria of its host cell (Fig. 17.7A, B). *C. gigas* seems to be more resistant to the disease than the other species of oysters challenged experimentally under laboratory and field conditions (Bower *et al.*, 1997). Hybridomas that produce monoclonal antibodies specific for *M. mackini* were produced (Hervio *et al.*, 1996), but this product has not been developed into an immunodiagnostic assay. Carnegie *et al.* (2003) described PCR and fluorescent *in situ* hybridization assays for *M. mackini*, based on the SSU rDNA, which detected three to four times more infections than standard histopathology.

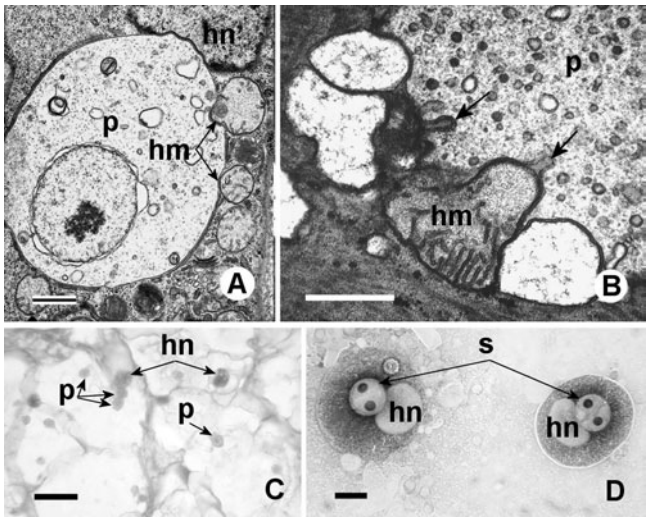

Fig. 17.7. Transmission electron micrographs (A and B) and a histological image (C) of *Mikrocytos mackini* in the cells of *Crassostrea gigas* and *Ostrea edulis,* respectively, from British Columbia, Canada. A. Protist (p) against the host cell nucleus (hn) and two closely associated host mitochondria (hm). B. Higher magnification of a host mitochondrium (hm) with tube-like structures (arrows) extending into the cytoplasm of *M. mackini* (p). C. Several *M. mackini* (p) in the cytoplasm of vesicular connective-tissue cells (hn, nuclei of host cells) of the labial palps of *O. edulis*. D. Tissue imprint of the gonad of *Crassostrea gigas* from Japan with a sporangiosorus (s) of *Marteilioides chungmuensis* in each ovum against the host cell nucleus (hn). Each sporangiosorus contains two sporonts and each sporont contains one basophilic developing spore. A and B bars = 0.5 µm, C and D bars = 10 µm.

Mortalities caused by *M. mackini* can be circumvented by well-timed plantings and harvests of Pacific oysters in relation to season and tide levels (Bower, 1988).

Paramyxea

Introduction

These spore-forming bivalve pathogens were initially assigned to the phylum Ascetospora in the same class (Stellatosporea) as the haplosporidians (Levine *et al*., 1980). Because of significant morphological and developmental differences, Desportes (1984) moved them to the class Paramyxea and order Marteiliida, and Desportes and Perkins (1990) suggested that the class Paramyxea be raised to the rank of phylum. Based on an SSU rDNA gene sequence that was very different from all known sequences of eukaryotic organisms, including myxosporeans and haplosporeans, Berthe *et al*. (2000) supported this phylum designation. These parasites are characterized by the presence of several cells enclosed inside one another, which arose by a process of internal cleavage ('endogenous budding') within a stem cell. Included in this group are pathogens in two genera, *Marteilia* (several species) and *Marteilioides* (two species), that have had a significant impact on bivalve production in different areas of the world. Each genus will be presented separately.

MARTEILIA SPP.

Host range and economic significance

Species of *Marteilia* produced disease of economic concern on the coast of Europe, eastern Australia and Florida, USA. In Europe, especially along the Atlantic coast of France, *M. refringens*, commonly known as Aber disease or digestive-gland disease, caused recurrent serious mortalities (from 1967 to about 1977) in flat oysters (Grizel

et al., 1974; Sindermann, 1990). Alderman (1979) indicated that the decline (to about 47%) of flat oyster production in France over 6 years (from 18,000 t in 1969 to 8400 t in 1975) was a direct result of the spread of this parasite. To date, *M. refringens* has been detected in flat oysters from the coasts of France, Portugal, Spain, Italy, Greece and Morocco (OIE, 2003b). In addition to flat oysters, *M. refringens* was reported from blue mussels (*M. edulis*), European cockles (*Cardium edule*) and imported Pacific oysters (*C. gigas*), and was experimentally infective to New Zealand dredge oysters (*O.* (= *T.*) *chilensis* (= *lutaria*)) and Australian flat oysters (*O. angasi*). Interestingly, negligible pathology was found in most of these other species (Cahour, 1979; Grizel *et al.*, 1983; Bougrier *et al.*, 1986). As the impact of Aber disease seemed to be subsiding in the late 1970s, the flat oyster industry in Europe was struck by another devastating disease caused by the microcell *B. ostreae* (see above), from which it has not yet recovered.

In addition to *M. refringens*, two other species, *Marteilia maurini* in 'gallo' mussels (*M. galloprovincialis*) and blue mussels from France, Spain and the Persian Gulf (Comps *et al.*, 1981), and *Marteilia lengehi* from oysters (*Saccostrea* (= *Crassostrea*) *cucullata*) from the Persian Gulf (Comps, 1976), have been described. Due to the morphological similarity between these marteilias, the validity of the latter two species is questioned. Nevertheless, in some areas, mortalities of mussels attributed to *M. maurini* are significant, especially for 'gallo' mussels from rías in Galicia, northwest Spain (Villalba *et al.*, 1997). High prevalences (37 to 70%) have also been reported in blue mussels from the north coast of Brittany (Auffret and Poder, 1983).

On the east coast of Australia, in subtropical and tropical regions of southern Queensland and northern New South Wales, another species, *Marteilia sydneyi*, the cause of QX disease, was associated with high mortalities (often exceeding 80%) in Sydney rock oysters (*S.* (= *Crassostrea*) *glomerata* (= *commercialis*)) (Lester, 1986). The same, or a very similar, species of marteilid has been reported in the black-lip oyster (*Saccostrea* (= *Crassostrea*) *echinata*) in Australia but has not been associated with overt pathology (Hine and Thorne, 2000).

On the coast of Florida, another *Marteilia* sp. has been implicated in mass mortalities approaching 100% of the calico scallop (*Argopecten gibbus*). The fishery, which produced 11–40 million lbs of adductor muscle meats annually prior to December 1988, was destroyed within 1 month, with mortalities spread over a 2500 square mile area. As of the spring of 1992, the scallops had not returned to commercially harvestable quantities (Moyer *et al.*, 1993).

Parasite morphology and life cycle

Infections by all *Marteilia* spp. are presumably initiated by a primary cell or stem cell (5 to 8 μm in diameter) in the epithelial cells of the gut or gills (Grizel *et al.*, 1974). The early development of *M. sydneyi* in the gill and palp epithelium of the Sydney rock oyster was revealed by Kleeman *et al.* (2002a) using molecular *in situ* hybridization techniques. The primary uninucleate cell contained a secondary uninucleate daughter cell in a vacuole within its cytoplasm. The daughter cell divided by binary fission to produce four daughter cells within the enlarged primary (stem) cell and within each daughter cell a uninucleate cell developed by internal cleavage. The primary cell degenerated to release the daughter cells, which became new primary cells. For *M. sydneyi*, initial proliferation in the epithelial cells of the gills and palps was followed by systemic dissemination to the digestive-gland tubules, where the basal membrane of the tubules was penetrated and the parasites became established as nurse cells at the base of the epithelial cells. Nurse cells containing daughter cells proliferated and eventually degraded. Daughter cells of *M. sydneyi* and *M. refringens* in the digestive gland tubules become sporangiosori, described as 'primary cells' by Perkins and Wolf (1976). Sporulation occurred within the sporangiosorus via a unique process of internal cleavages (endosporulation) to produce cells within cells (Fig. 17.8).

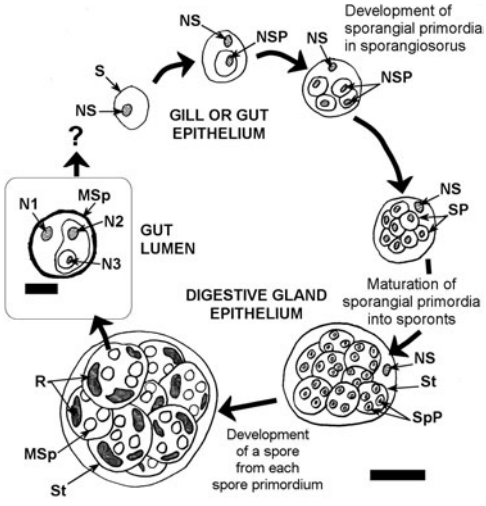

Fig. 17.8. Schematic drawing to scale of the sporulation process of *Marteilia* spp. based on descriptions by Grizel *et al*. (1974), Perkins (1976) and Kleeman *et al*. (2002a). S, sporangiosorus (or primary cell); NS, nucleus of sporangiosorus; SP, sporangial primordium (or secondary cell); NSP, nucleus of sporangial primordium; St, sporont; SpP, spore primordium (or tertiary cell); MSp, mature spore; R, refringent bodies; N1, nucleus of outermost sporoplasm; N2, nucleus of middle sporoplasm; N3, nucleus of innermost sporoplasm. Bar = 10 μm and 2 μm for inset of spore.

At the initiation of sporulation, uninucleate segments become delimited within the cytoplasm of the sporangiosorus to form the sporangial primordia (secondary cells). Eventually, eight to 16 sporangial primordia (each about 12 μm in diameter at maturity) form within the sporangiosorus, which retains its nucleus and enlarges to about 30 μm in diameter. Each sporangial primordium matures into a sporont containing two to four spore primordia (tertiary cells), which mature into spores (Fig. 17.8). Each spore contains three uninucleate sporoplasms of graded sizes, with each of the smaller sporoplasms being enclosed within the cytoplasm of the next largest one (i.e. consecutive internal cleavage of two sporoplasms within the spore primordium) (Perkins, 1976). A continuous spore wall with no operculum occurs around each spheroid mature spore, which measures 3.5 to 4.5 μm in diameter. As the spore matures, light-refractile inclusion bodies appear in the sporont cytoplasm surrounding the spores. The specific name of *M. refringens* was derived from these refringent inclusion bodies. Mature spores are shed into the tubule lumen for evacuation from the oyster and infected oysters may shed large numbers of spores before oyster death.

Marteilia sydneyi can be differentiated from *M. refringens* by: (i) the formation of eight to 16 sporangial primordia in each sporangiosorus instead of eight for *M. refringens*; (ii) each sporont contains two or infrequently three, rather than four, spores; and (iii) the heavy layer of concentric membranes surrounding mature spores of *M. sydneyi* is lacking around *M. refringens* spores (Perkins and Wolf, 1976).

The development of *M. refringens* and *M. sydneyi* was directly related to water temperature, with the disease being most severe towards the end of summer, which coincides with the sporulation process. Transplantation experiments in France suggested that new infections of *M. refringens* were acquired from early May to early September (Grizel, 1979). However, similar experiments in Australia indicated that oysters may be exposed to infection over a very short interval (possibly only a few weeks per year) during the summer (Lester, 1986; Wesche, 1995). Warm temperatures favoured parasite development and, at lower temperatures, host mortality was retarded and parasite development suppressed. Young plasmodia may persist throughout the winter and reinitiate clinical infections the following spring (Lauckner, 1983).

The mechanism of infection has not been determined for any species of *Marteilia*. Experimental attempts to transmit the disease to oysters in the laboratory met with failure, although field exposures were successful (Roubal *et al*., 1989; Berthe *et al*., 1998). As with *Haplosporidium* spp., an intermediate host is suspected (Perkins, 1993). Audemard *et al*. (2002) detected *M. refringens* with molecular tools in the gonad of the copepod *Paracartia grani* and speculated on its involvement in the life cycle of this parasite.

Host–parasite relationships

Signs of disease in oysters include a poor condition index, with glycogen loss (emaciation), discoloration of the digestive gland, cessation of growth, tissue necrosis and mortalities (Sindermann, 1990). The pathogenesis of *M. refringens* remains obscure due to the lack of consistent correlation between the degree of infection and mortality (Lauckner, 1983). Some flat oysters kept in high-prevalence areas for extended periods showed characteristic signs of disease without notable numbers of parasites, while other flat oysters heavily infected with young sporangiosori and mature spores exhibited virtually no histological alterations. To explain these inconsistencies, Balouet (1979) and van Banning (1979) suggested that either: (i) the parasite produced toxins inconsistently; (ii) the parasite required the synergistic effect of another, as yet unidentified, pathogen; (iii) an intermediate host was required to amplify parasite abundance; and/or (iv) unfavourable environmental conditions (e.g. physicochemical factors in sea water) played prominent roles in determining the apparent pathogenicity of *M. refringens*. Anderson *et al.* (1994a) determined that fluctuations in pH, salinity and water temperature in close proximity to the Sydney rock oysters did not correlate with epizootics of *M. sydneyi*.

Diagnosis of infection

Because there are no specific clinical signs, infection can best be confirmed by histological examination (Grizel, 1979; Kleeman *et al.*, 2002a). A diagnostic feature is the presence of *Marteilia* spp. in histological sections of the digestive gland tubule epithelium and occasionally in the gills and palps (Sindermann, 1990). Gutiérrez (1977) described a modified staining technique for enhancing the detection of the parasite in paraffin-embedded histological sections. An IFAT, based on the polyclonal antibodies that were specific for sporulating stages of *M. sydneyi*, failed to detect presporulation stages of *M. sydneyi* in the connective tissue of recently infected oysters (Anderson *et al.*, 1994b). Subsequent identity of segment sequences within the SSU rDNA of both *M. sydneyi* (Anderson *et al.*, 1995; Kleeman and Adlard, 2000) and *M. refringens* (Le Roux *et al.*, 1999; Pernas *et al.*, 2001) was used in the development of PCR and *in situ* hybridization assays. Assays developed from the ITS1 by Kleeman and Adlard (2000) proved specific to *M. sydneyi* when tested for their potential to cross react with related species of Paramyxea (Kleeman *et al.*, 2002b). Although the 'Smart 2 probe' identified by Le Roux *et al.* (1999) cross-reacted with various species of Paramyxea (Kleeman *et al.*, 2002b), this probe provided a stronger signal in the detection of sporont stages and was more reliable in the detection of mature spores of *M. sydneyi* than the ITS1 probe. Thus, Kleeman *et al.* (2002b) indicated that the Smart 2 probe was preferred for use in the screening or surveillance of oyster populations and that the ITS1 probe should be used as one means of confirming the specific identity of the pathogen as *M. sydneyi*.

Prevention and control

Similar farm management practices were employed to reduce the risk of infecting oysters with *M. sydneyi* in Australia and *M. refringens* in Europe (Lester, 1986; Sindermann, 1990). During the summer, oysters were not planted in areas of risk and young oysters were held in high-salinity water, where they grow more slowly but remain free of infection until after the risk of infection has passed. Also, large oysters were harvested prior to the onset of the transmission period. In Europe, Pacific oysters, which seem to be resistant to the disease, are cultured in most areas affected by *M. refringens*.

MARTEILIOIDES SPP.

Protozoa in the genus *Marteilioides* can be differentiated from *Marteilia* spp. in that two sporonts usually develop in each sporangiosorus, each sporont produces a single pluricellular spore and the mature spore contains two concentric cells rather than three. The *Marteilioides* species of greatest

concern is *Marteilioides chungmuensis*, which causes a nodular appearance (like multiple tumours) in the gonad of Pacific oysters in western Japan and southern Korea. Surveys in Gokasho Bay, Japan, in 1996 and 1997 revealed prevalences in cultured female oysters between 18 and 20% from autumn to spring, with up to 52% infected in July (Imanaka *et al.*, 2001). In Gosung Bay, Korea, surveys conducted in 2000 did not detect *M. chungmuensis* between February and May and prevalence peaked at about 16% in December (Ngo *et al.*, 2003). A similar-looking parasite was reported from the ova of black-lip oysters (*Saccostrea echinata*) from Western Australia and Northern Territory, Australia (Hine and Thorne, 2000).

M. chungmuensis infects the cytoplasm of oocytes (Fig. 17.7D) and can affect large areas of the reproductive follicles, causing irregular enlargement of the infected gonadal tissues (Itoh *et al.*, 2002). This parasite may cause a significant reduction in the reproductive output of an infected female oyster. Also, *M. chungmuensis* can have a serious economic impact because infected oysters lose their marketability due to the unaesthetic appearance caused by the disease. Basic biological information pertaining to the complete life cycle of this parasite, including the route of infection, early infective and multiplication stages in the oyster and method of transmission, remains unknown (Imanaka *et al.*, 2001). The recent identification of a partial sequence of the SSU rDNA and development of specific molecular diagnostic assays will be used to elucidate the life cycle of *M. chungmuensis* and to determine the phylogenetic position of this parasite (Itoh *et al.*, 2003).

A related species, *Marteilioides branchialis*, in conjunction with *M. sydneyi*, was associated with significant economic losses among Sydney rock oysters (Anderson and Lester, 1992). *M. branchialis* was differentiated from *M. chungmuensis* by sporulation in the cytoplasm of epithelial cells, connective tissue cells and occasionally haemocyte accumulations within lesions on gill lamellae and not in ova.

Spot prawn parasite

A parasite in pandalid shrimp (*Pandalus platyceros* and *Pandalus borealis*) reported from the west coast of northern North America was initially identified as *Hematodinium*-like (Meyers *et al.*, 1994). However, a few inconsistent morphological features (massive plasmodia and binary fission of the trophozoites, with mitosis unlike that of parasitic dinoflagellates; Fig. 17.9), antigenic characteristics that were inconsistent with those of other parasitic dinoflagellates and genetic analysis that consistently grouped this parasite with members of the phylum Haplosporidia clearly indicated that this lookalike pathogen was not related to the Dinoflagellida (Bower and Meyer, 2002). The identity of this shrimp parasite remains unknown because none of the morphological features found to date can be used to affiliate it with the Haplosporidia.

Phylum Annelida

The cosmopolitan spionid polychaetes include several species (most in the genera *Polydora* and *Boccardia*) that burrow into the shells of living molluscs. Spionid polychaetes are filter feeders and do not derive nutrients from their host; however, the burrows that they create in mollusc shells can be problematic. Due to the overall low economic significance of this group of parasites, the taxonomic problems, as indicated by Lauckner (1983), will not be reiterated here. Instead, instances where these polychaetes have had an impact on commercial stocks of molluscs in various parts of the world will be mentioned.

In European waters, mortalities and loss of market quality of blue mussels were caused by *Polydora ciliata* (Lauckner, 1983). The burrows excavated by *P. ciliata* in blue mussel shells not only caused unsightly blisters containing compacted mud but also resulted in significant reductions in shell strength, thereby increasing susceptibility to predation by birds and shore crabs (Kent, 1981). Nacreous blisters

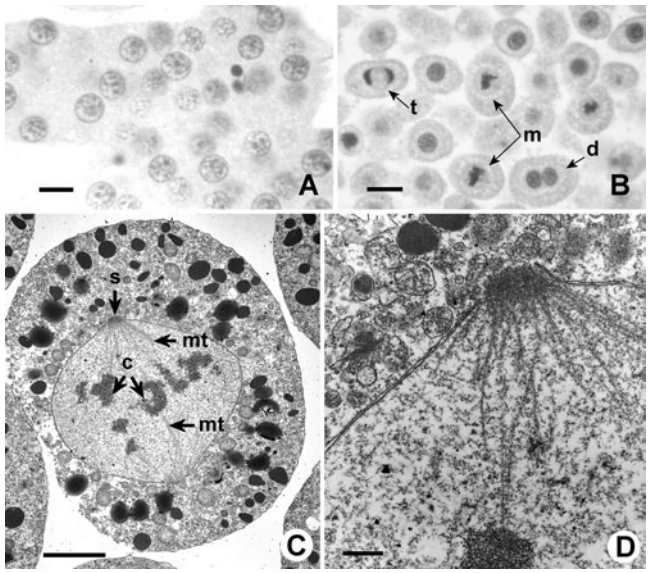

Fig. 17.9. Histological images (A and B) and electron micrographs (C and D) of an unnamed protist with unknown taxonomic affiliations from *Pandalus platyceros* in British Columbia, Canada. A. Plasmodium with numerous nuclei. B. Trophozoites in the process of binary fission showing metaphase (m), late telophase (t) and one cell in which the nucleus has recently divided (d). C. Trophozoite in late metaphase with an intact nuclear membrane surrounding condensed chromosomes (c), which are connected by microtubules (mt) to spindle-pole bodies (s) emerging through the nuclear envelope. D. A higher magnification of C illustrating the microtubules connecting to the spindle-pole body at a gap in the nuclear membrane. A and B bars = 10 µ m, C bar = 2.5 µm and D bar = 0.5 µm.

produced by blue mussels in response to *P. ciliata* may result in atrophy and detachment of the adductor muscle and possibly interference with gamete production when the calcareous ridges occur adjacent to these organs (Lauckner, 1983).

On the east and south coasts of North America, *Polydora websteri* may cause unsightly mud blisters in the shell and yellowish abscesses in the adductor muscle (when the burrow comes in contact with the muscle tissue) of eastern oysters (Lauckner, 1983). Prevalence and intensity vary considerably with local ecological conditions, but there is a general tendency for infection to be more severe on the south and south-east coasts. Infection rarely causes mortalities and infected oysters can be marketed. However, mud blisters may interfere with shucking and this reduces the commercial value of oysters to be served on the half-shell. Similar conditions caused by unidentified species of *Polydora* were observed in Pacific oysters cultured on the south coast of Brazil and in Baja California, Mexico (Caceres-Martinez *et al*., 1998).

In British Columbia, Canada, stunting and high mortalities caused by high intensities of *P. websteri* (burrows too numerous and interwoven to count in shells of dead scallops) have precluded the culture of introduced Japanese scallops in a few localities (Bower, 1990). However, *P. websteri* only occurred in low intensities (fewer than ten per shell) and had no apparent effect on Pacific oysters and giant rock scallops (*Crassedoma giganteum*) cultured in the same localities (S.M. Bower, unpublished data).

In southern Australia, five species of polydorid polychaetes (*Polydora haswelli*, *Polydora hoplura*, *P. websteri*, *Boccardia chilensis* and *Boccardia polybranchia*) were observed in up to 95% of blue mussels. Although the intensity of infection was generally low, about 15% of the blue mussels from two localities had serious shell damage

attributed to polydora. The most heavily infested blue mussels were from bottom samples (Pregenzer, 1983). Also, spionid polychaete infestations along the east coast of Australia caused Sydney rock oyster aquaculture to change from bottom culture to an intertidal stick-and-tray culture system (Anderson, 1990; Handley, 1997).

Phylum Trematoda

Numerous species of digenean trematodes have been described from various shellfish worldwide. In general, the trematodes that cause the greatest economic impact are species in the families Bucephalidae and Fellodistomidae that utilize bivalves as primary hosts. In such instances, miracidia are infective to bivalves and the larval trematode life stages of sporocyst and development of cercariae occur within the tissues of the bivalve. Four cases in which trematodes from other families were reported to cause pathology are noted.

Family Bucephalidae

Introduction

Numerous species of Bucephalidae (suborder Gasterostomata) have been described from marine and freshwater fishes and the larval forms have been reported from bivalves worldwide. However, few experimental life cycle studies have been conducted. Thus, the taxonomy of many larval Bucephalidae in bivalves remains obscure (Lauckner, 1983). For simplicity, the Bucephalidae will be considered as a group, with examples of certain species presented where appropriate.

Host range and economic significance

Larval bucephalids infecting commercially important scallops, oysters and mussels are possibly the most deleterious metazoan parasites of marine bivalves (Lauckner, 1983). Examples have been reported from various locations: (i) scallops (*Pecten alba*) from Bass Strait, Australia, parasitized by *Bucephalus* sp. (prevalence of 31%) were castrated and had significant adductor muscle (only part of this scallop that is marketed) atrophy (Sanders and Lester, 1981); (ii) *Bucephalus longicornutus* caused castration and significant mortalities of infected dredge oyster (*O. chilensis* (= *lutaria*)) under laboratory conditions with suspected impact on wild stocks in New Zealand (Millar, 1963; Howell, 1967); and (iii) weakness and gaping caused by *Prosorhynchus squamatus* (Fig. 17.10) in blue mussels from northwestern Europe, Britain, Iceland and the White Sea, Russia, reduced product value during shipping and marketing (Coustau *et al.*, 1990). In 1997, *P. squamatus* was encountered for the first time in mussels from Atlantic Canada and a similar-looking parasite was detected in a few mussels from the Pacific coast of Canada. Surprisingly, parasitic castration of blue mussels caused by *P. squamatus* (Coustau *et al.*, 1993) was

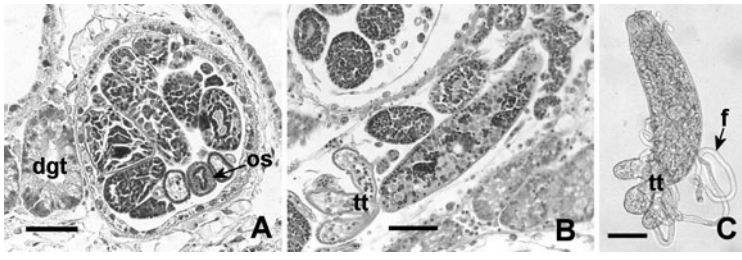

Fig. 17.10. Histological images (A and B) and a wet mount (C) of *Prosorhynchus squamatus* from *Mytilus edulis* in Nova Scotia, Canada (courtesy of S.E. McGladdery). A. Anterior end of sporocyst sectioned through oral sucker (os) adjacent to digestive-gland tubule (dgt). B. Sporocyst containing cercaria sectioned through the trilobate tail (tt). C. Cercaria with trilobate tail (tt) and curled furcae (f). All bars = 50 µm.

once thought to be beneficial for blue mussel culture, because parasitized blue mussels do not spawn and appear to remain in good condition during the summer spawning season. However, consumption of trematode-infested molluscs may be hazardous to humans, due to accumulation of toxic metabolites (butyric and other short-chain fatty acids) resulting from degeneration of the host's neutral fats by parasite-secreted enzymes (Cheng, 1967; Lauckner, 1983).

Morphology and life cycle

Bucephalids have fairly uniform life-cycle patterns. Sporocysts (Fig. 17.10A) and cercariae (Fig. 17.10B, C) occur in bivalves. Metacercariae occur in various parts of the central and peripheral nervous systems or in internal organs and musculature of teleost fish, and adults inhabit the alimentary tract of piscivorous fish (Lauckner, 1983). In bivalves, the large, dichotomously branching sporocyst forms a dense interwoven network, which infiltrates practically every organ, especially the gonad. Infection is terminal following growth into and occlusion of the haemal sinuses and the gradual destruction and replacement of molluscan tissue by the sporocyst. Prevalence of infection usually increases with bivalve age (Matthews, 1974). Cercariae (Fig. 17.10C), often several hundred at a time, are forcibly discharged through the bivalve's exhalant siphon. Although they are not active swimmers, transmission to the intermediate host is aided by the long, extendable and retractile furcae (Matthews, 1974; Lauckner, 1983).

Host–parasite relationships

Bucephalid sporocysts and cercariae cause castration of infected bivalves, tissue necrosis and debilitation, expressed as a significant reduction in tolerance of environmental stress (Lauckner, 1983). Despite the severe pathology associated with *Bucephalus* sp. infection in eastern oysters, there is usually little host response to the parasite, but massive biochemical alterations have been observed (Lauckner, 1983).

Prevention and control

Haplosporidian hyperparasites have been described from *Bucephalus* sp. parasitic in eastern oysters (Lauckner, 1983) and from *B. longicornutus* parasitic in dredge oysters (Howell, 1967). Although both hyperparasites are pathogenic for the bucephalids, Howell (1967) concluded that ecological conditions as well as the difficulty of collecting large numbers of infective spores precluded the effective use of the hyperparasites as biological controls.

Coustau *et al.* (1990) showed that blue mussels are more susceptible to *P. squamatus* than hybrids of blue mussels and 'gallo' mussels and suggested that it may be possible to select for a mussel stock that is resistant to this parasite.

Family Fellodistomidae

Although numerous species of this family parasitize many marine pelecypods as primary hosts and secondary hosts worldwide (Lauckner, 1983; Wolf *et al.*, 1987), *Proctoeces maculatus*, which infects blue mussels as well as other mollusca, has the greatest economic impact. Thus, this section presents information only on *P. maculatus*.

P. maculatus from shellfish and fin fish appear in the literature under a variety of synonyms, and life stages have been described from a wide variety of bivalves and gastropods (Bray, 1983). Metacercariae occur in various mollusca (including species of Amphineura, Gastropoda, Cephalopoda and Lamellibranchiata), Polychaeta (Annelida) and Echinoidea (Echinodermata). Adults have been reported in mollusc-eating fishes (mainly labrids and sparids) in tropical and subtropical areas, as well as in some Gastropoda, Lamellibranchiata and Polychaeta. However, sporocysts have only been reported from blue mussels, 'gallo' mussels, and hooked mussels (*Ischadium recurvum*). The wide host tolerance, global distribution in tropical and temperate marine waters and morphometric variability led Lauckner (1983) to speculate that more than one species of trematode may have been included

in *P. maculatus*. Thus, this species (group) requires further study, using biochemical and DNA analysis as well as life cycle studies in the laboratory.

P. maculatus was reported in up to 46% of blue mussels and 'gallo' mussels on both sides of the North Atlantic Ocean and in the Mediterranean and Black Seas (Lauckner, 1983). In 'gallo' mussels from the Black Sea, up to 28,000 sporocysts per mussel, comprising 20% of the biomass of the soft tissues, were observed (Machkevski, 1985). In Italy, extensive mortalities in cultured mussels were attributed to this parasite, which was thought to have been introduced via a depuration plant located nearby (Munford *et al.*, 1981).

In mussels, sporocysts of *P. maculatus* usually occur in the vascular system of the mantle (Lauckner, 1983). Infection causes an alteration in haemolymph components, a sharp decrease in energy stores, a reduction in growth rate and weakness in respect of valve closure and attachment to the substrate (Mulvey and Feng, 1981; Machkevski, 1985, 1988). In heavily infected mussels, sporocysts developing in the mantle can seriously reduce the glycogen content of the tissues and efficiency of the circulatory system. This results in disturbances to gametogenesis and possibly castration and death (Mulvey and Feng, 1981; Machkevski and Shchepkina, 1985; Feng, 1988). Mussels may also serve as a final host for *P. maculatus* (Lauckner, 1983). Progenetic development (Fig. 17.11) represents a mechanism by which *P. maculatus* could become established in new localities as a result of moving infected stocks. *P. maculatus* probably represents a threat to mussel culture worldwide. However, Lauckner (1983) indicated that the hazard would be minimal due to the supposed narrow range of ecological conditions under which *P. maculatus* is capable of disseminating.

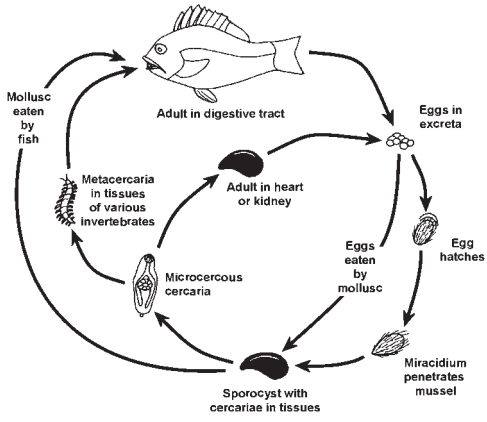

Fig. 17.11. Life-cycle alternatives of the Trematoda *Proctoeces maculatus*. The occurrence of a progenetic cycle (adult stages of *P. maculatus* in the heart or kidney of the blue mussel (*Mytilus edulis*)) eliminates the requirement of a final (definitive) fish host for the completion of the life cycle.

Other pathogenic Trematoda

In addition to the Bucephalidae and *P. maculatus*, several other digenetic trematodes have been reported as pathogens of shellfish. Some of the more prominent examples are as follows:

1. In the North Sea and adjacent areas, reduced byssal production and impaired shell cleaning were reported in young blue mussels infected with metacercariae of the bird trematode *Himasthla elongata* (family Echinostomatidae) (Lauckner, 1984). Also, *H. elongata* and *Renicola roscovita* were thought to have an impact on European cockle populations on the German North Sea coast (Lauckner, 1983).

2. On the west coast of Sweden, high intensities of *Cercaria cerastodermae* (family Monorchiidae) in about 20% of the cockles (*C.* (= *Cerastoderma*) *edule*) led to severe tissue damage, impairment of burrowing and eventual mortalities (Jonsson and André, 1992).

3. Several species of gymnophallid metacercariae, which occur between the mantle and shell of various lamellibranchs on both sides of the North Atlantic Ocean, are reported to cause soft-tissue pathology, induction of pearl formation and shell deformities (Lauckner, 1983).

As aquaculture operations expand and diversify, diseases caused by various

trematodes will probably be encountered. However, the requirement of at least two different hosts for completing the life cycle in most species renders these parasites vulnerable to control once the life cycles have been identified. Aquaculture practices alone may be sufficient to create an unfavourable environment for the completion of a trematode's life cycle, as illustrated by the reduced prevalence of *R. roscovita* in farmed (4 to 12%) as opposed to natural populations (96 to 100%) of blue mussels from the west coast of Sweden (Svärdh and Thulin, 1985).

Phylum Cestoda

Metacestodes (larval cestodes) have been reported from a wide variety of aquatic invertebrates. Among marketed shellfish, metacestode infections are economically insignificant. Nevertheless, there are a few isolated instances of high prevalences and intensities of metacestodes in bivalves and crustacea from various subtropical and tropical areas of the world (Lauckner, 1983; Sparks, 1985; Sindermann, 1990). Metacestodes of *Echeneibothrium* spp. were associated with unusual behaviour of Pacific littleneck clams (*P.* (= *Venerupis*) *staminea*) and fringed littleneck clams (*Protothaca laciniata*) in California (Warner and Katkansky, 1969) and caused histopathology and gonad atrophy in Atlantic calico scallops (*A. gibbus*) in North Carolina (Singhas *et al.*, 1993). In most cases, the final hosts of the cestodes are fishes, mainly elasmobranchs.

Phylum Nematoda

Nematodes are uncommon as parasites of shellfish (Lauckner, 1983; Sindermann, 1990). However, the exceptions are all larval stages and include the following:

1. Various species of the gnathostomid genus *Echinocephalus* from oysters, scallops and abalone from tropical and subtropical marine waters. Although the pathology in the bivalve hosts is minimal, there is concern that at least some species may have public health significance as potential invaders of the human digestive tract. The species (*Echinocephalus pseudouncinatus*) in pink abalone (*Haliotis corrugata*) from California causes blisters and weakens the foot as a holdfast organ in heavily infected specimens (Sindermann, 1990).

2. An ascaridoid *Sulcascaris sulcata* is widespread in warm seas and has a considerable host range, including scallops and clams (Lauckner, 1983; Sindermann, 1990). Although *S. sulcata* is a minor pathogen for its hosts, significant economic impact occurred on the east coast of North America where a haplosporidian hyperparasite (*Urosporidium spisuli*) caused the usually white to yellowish coloured worm to become dark brown. The epizootic spread of the hyperparasite in *S. sulcata* parasitizing Atlantic surf clams (*Spisula solidissima*) in the mid-1970s caused considerable economic concern for aesthetic reasons (Payne *et al.*, 1980).

3. *Angiostrongylus cantonensis*, the rat lungworm that causes human eosinophilic meningoencephalitis in parts of Asia, can utilize eastern oysters and quahogs (*M. mercenaria*) as aberrant intermediate hosts under experimental conditions (Sparks, 1985). These findings could be significant for some of the Pacific Islands where the rat lungworm occurs and oysters and clams may be eaten raw or poorly cooked (Lauckner, 1983).

4. The 'codworm' *Phocanema decipiens* in the North Atlantic has been observed in blue mussels and softshell clams (*M. arenaria*), which may serve as paratenic hosts for this parasite (Lauckner, 1983).

Phylum Arthropoda

The pathogenic arthropods all belong to the class Crustacea (subclass Copepoda, mainly in the order Cyclopoida and subclass Malacostraca, order Isopoda). Because the economic significance of all species is either disputable or confined to small local areas, these pathogens are only briefly mentioned.

Subclass Copepoda

The cycloid copepods presumed to cause the most significant mortalities among shellfish belong to the genus *Mytilicola*. These copepods have a direct life cycle and reside in the intestinal tract of a wide variety of bivalves (Dare, 1982; Gee and Davey, 1986). Prevalence and intensity of *Mytilicola intestinalis* in mussels in Europe can be high. For example, in Cornwall, UK, the prevalence in mussels from some localities only fell below 90% during the early summer months and intensity of infection often exceeded 30 copepods per mussel (Davey, 1989). Several workers concluded that some of the periodic mass mortalities in cultured mussels in Europe were attributable to *M. intestinalis* (Sparks, 1985; Blateau *et al.*, 1990). However, these conclusions: (i) were not substantiated by statistical analysis; (ii) were not supported by experimental evidence; and (iii) did not rule out the possibility that microscopic pathogens were responsible for the mortalities (Lauckner, 1983). From the results of a 10-year study conducted in Cornwall, England, Davey (1989) concluded that *M. intestinalis* is not a harmful parasite. Nevertheless, more work is required before the pest status of *M. intestinalis* can be fully appreciated, especially in respect of its synergistic relations with other pathogens and/or pollutants (Davey and Gee, 1988).

A parasitic copepod, *Pectenophilus ornatus*, of unknown taxonomic affinity and originally thought to be a species of rhizocephalan in the subclass Cirripedia, is considered a serious pest of commercial scallop production in Japan (Nagasawa *et al.*, 1991). The bright yellowish or orange female (up to 8 mm wide, consisting mainly of a brood pouch with no appendages) attaches to the gills and feeds on the haemolymph of commercially valuable scallops (*P. yessoensis* and *Chlamys* spp.). Heavy intensities of infection (greater than 20 *P. ornatus* per scallop) have detrimental effects on the condition of cultured scallops and the parasite also reduces market acceptability (Nagasawa and Nagata, 1992).

Subclass Malacostraca

Members of the family Bopyridae within the order Isopoda are common parasites of the branchial chamber of many species of shrimp worldwide. Infected shrimp are conspicuous due to the protruding lump on the lateral aspect of the carapace of the cephalothorax caused by the presence of the bopyrid (Sparks, 1985). Although the prevalence of bopyrids is usually low (< 5%), a few instances of high prevalences and associated pathology have been noted. Japanese red prawns (*Penaeopsis akayebi*) were frequently infected (up to 70%) with *Epipenaeon japonicus*, with associated gonad reduction or castration in some male prawns (Sindermann, 1990). In the Gulf of Carpentaria, Australia, the bopyrid *Epipenaeon ingens* infects up to 25% of the grooved tiger prawns (*Penaeus semisulcatus*), which it castrates and whose growth and geographical distribution it alters in comparison with those of uninfected prawns (Somers and Kirkwood, 1991).

Conclusions

A wide variety of parasites have been identified as causing significant economic losses in shellfish production worldwide. Many of these pathogens have the potential of causing significant losses either in endemic areas or if they inadvertently become established in other areas. In the past, transplants of commercial shellfish have been notorious for the accidental introduction of associated parasites (Sindermann, 1990, 1993). In order to avoid future disasters, all movements of shellfish must be conducted with caution. Equally essential is the acquisition of information on agents of disease, including parasites, such that risks associated with impending movements and aquaculture practices can be accurately assessed. This information should also prove useful for treating or controlling a disease in the event that an accidental introduction occurs.

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