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DISEASE PREVENTION STRATEGIES FOR QX DISEASE (*MARTEILIA SYDNEYI*) OF SYDNEY ROCK OYSTERS (*SACCOSTREA GLOMERATA*)

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ABSTRACT The Sydney rock oyster (*Saccostrea glomerata*) forms the basis of an important aquaculture industry on the east coast of Australia. During the 1970s, production of *S. glomerata* began to decline, in part as a result of mortalities arising from Queensland unknown (QX) disease. Histological studies implicated the paramyxean parasite *Marteilia sydneyi* in the disease outbreaks. Disease zoning was implemented to prevent the spread of *M. sydneyi*-infected oysters. This control measure hindered rock oyster farming, which historically has relied on transferring wild-caught spat between estuaries for on-growing to market size and has not prevented the subsequent occurrence of QX disease in the Georges and Hawkesbury rivers in central New South Wales. Management of QX disease has been hampered by the complicated life cycle of *M. sydneyi*, with outbreaks of QX disease likely to be regulated by a combination of the abundance of intermediate host of *M. sydneyi*, environmental stressors, and the immunocompetence of *S. glomerata*. The future of the Sydney rock oyster industry relies on understanding these factors and progressing the industry from relying on farming wild-caught seed to the successful commercialization of hatchery-produced QX-resistant *S. glomerata*.

KEY WORDS: disease management, *Marteilia*, oyster, *Saccostrea*, QX disease

INTRODUCTION

This article focuses on advances in our understanding and management of Queensland unknown (QX) disease (causative agent, *Marteilia sydneyi*) in Sydney rock oysters (*Saccostrea glomerata*). A detailed description of the history and challenges facing the Sydney rock oyster industry is provided in Nell (2001) and O'Connor and Dove (2009). The Sydney rock oyster, *S. glomerata* (formerly *Saccostrea commercialis*), is an important aquaculture industry on the eastern seaboard of Australia and is reported to be the 4th largest edible oyster industry in the world (Simonian et al. 2009b). Production of farmed *S. glomerata* peaked in Australia during the 1970s, following the development of “highway farming”—a system that optimizes production by transferring oysters between estuaries by road at different stages of their production cycle to take advantage of each individual estuary’s prime growing period (Nell 2001). With the benefit of hindsight, it is perhaps not surprising that outbreaks of disease started to coincide with the implementation of highway farming in southeast Queensland and northern New South Wales (NSW). The cause of mortality was at first unknown, and so was referred to as QX disease. Subsequent histological observation of diseased oysters led to the identification of the paramyxean protozoan parasite *M. sydneyi* as the causative agent of QX disease (Perkins & Wolf 1976). Since the first description of *M. sydneyi*, extensive research into the parasite’s life cycle, risk factors for disease outbreaks, the immune system of *S. glomerata*, and development of selectively bred QX-resistant oyster lines has been conducted in an attempt to develop management options for the Sydney rock oyster industry.

MARTEILIOSIS OF CULTURED BIVALVES AND QX DISEASE

Marteiliosis occurs in numerous species of farmed bivalves around the world (Berthe et al. 2004). It is caused by paramyxean protists belonging to the genera *Marteilia* and *Marteilioides* (Berthe et al. 2004). *M. sydneyi* is the causative agent of QX disease, which causes recurring mass mortalities of *S. glomerata* in some estuaries on the east coast of Australia (Nell 2001). Although the parasite is present in *S. glomerata* populations in the majority of estuaries tested to date, expression of the disease, for whatever reason, only occurs in a limited number of these estuaries. *M. sydneyi* usually infects oysters in estuaries endemic for QX disease during the southern hemisphere summer months of January to April (Wesche et al. 1999), with mortalities in those estuaries usually reported between April and June. Similarly, *Marteilia refringens* causes mass mortalities in a number of economically important bivalve species in Europe, such as the flat oyster, *Ostrea edulis*, and the blue and Mediterranean mussels, *Mytilus edulis* and *Mytilus galloprovincialis* (Carrasco et al. 2008). *Marteilioides chungmuensis* infects the gonad of the Pacific oyster, *Crassostrea gigas*, in Korea and Japan (Comps & Park 1987). Mortality is not associated with *M. chungmuensis* infections of *C. gigas*, but the infection does cause unattractive brown lesions on the gonad, making the oysters unmarketable (Park & Chun 1989). There is a single report of *Marteilioides branchialis* infecting the gills of *S. glomerata*, causing focal gill lesions similar to *M. sydneyi* (Anderson & Lester 1992). *M. branchialis* infection of *S. glomerata* can be differentiated from those of *M. sydneyi* because the sporangiosorus stage of *M. branchialis* is tricellular rather than bicellular, as seen in *M. sydneyi* (Kleeman et al. 2002a).

Paramyxean parasites have complex life cycles spanning a wide range of hosts, including crustaceans, annelids, and molluscs. They also often utilize multiple hosts throughout their

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life cycle (Berthe et al. 2004, Carrasco et al. 2007). The complete life cycle of *M. sydneyi* is currently unknown, even though understanding the life cycle is of central importance to the development of effective management solutions for QX disease. Currently, laboratory studies of *M. sydneyi* are not possible, so researchers rely on field infections of QX disease, which are unpredictable, particularly with respect to intensity of infection. Despite this, the infective stages and life cycle of *M. sydneyi* within *S. glomerata* have been described. Infective stages of *M. sydneyi* initially enter the oyster via the gills or labial palps (Kleeman et al. 2002a), usually over a short period (sometimes less than 2 wk) during the summer (Adlard & Ernst 1995). These undergo extrasporogonic proliferation in the gills, releasing parasitic cells into the surrounding connective tissue and hemolymph spaces (Fig. 1). After systemic dissemination, the parasite infiltrates the digestive gland and becomes established as a nurse cell beneath the epithelial cells in a digestive tubule. Here, cell-within-cell proliferation results in the eventual liberation of daughter cells from the nurse cell into spaces between adjacent epithelial cells (Kleeman et al. 2002a). Daughter cells internally cleave secondary cells, after which degeneration of the nurse cell occurs. Sporulation proceeds, and mature sporonts containing 2 tricellular spores are shed into the digestive tubule lumen (Fig. 1) (Perkins & Wolf 1976). The majority of sporonts infecting the digestive gland are shed into the water column by the infected oysters before they die (Roubal et al. 1989). The main cause of mortality of *S. glomerata* infected with *M. sydneyi* is thought to be starvation resulting from massive disruption of digestive gland tissue (Roubal et al. 1989), and the oysters cease feeding, as evidenced by the reabsorption of gonad tissue (Roubal et al. 1989) and the lack of bacteria associated with filter feeding within the digestive gland (Green & Barnes 2010a). However, starvation does not explain why a proportion of *M. sydneyi*-infected *S. glomerata* die before they have exhausted their energy reserves, so that the role of secondary infections warrants investigation.

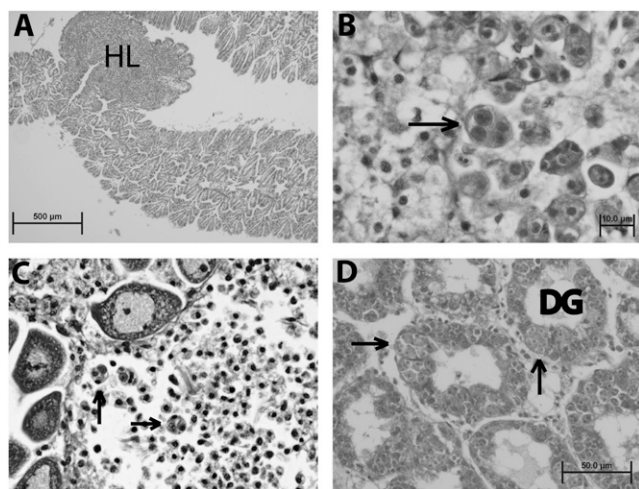


Figure 1. Histological pictures of QX disease. (A) Focal hemocytic inflammation (HL) in response to *M. sydneyi* infecting the gill filaments of *S. glomerata* (stage A). (B) The bicellular sporangium stage of *M. sydneyi* in the gills of *S. glomerata*. (C) The transient systemic stage of *M. sydneyi* (stage B) disseminating throughout oyster tissues. Note the strong hemocytic response of the host. (D) *M. sydneyi* (stage C) embedded in the digestive gland tubules (DG).

Until recently, the life cycle of *M. sydneyi* after shedding from its oyster host was unknown. Early experiments suggested that the parasite requires multiple hosts to complete its life cycle (Lester 1986, Roubal et al. 1989) because numerous attempts to horizontally transfer *M. sydneyi* infections in the field and laboratory have been unsuccessful (Lester, 1986). Other experiments also revealed that *M. sydneyi* sporonts are negatively buoyant and short lived when isolated from the host, with the majority dying within 7–9 days (Wesche et al. 1999). Moreover, spores do not survive for more than 2 h when ingested by fish and birds (Roubal et al. 1989, Wesche et al. 1999), implicating either a benthic filter feeder or benthic detritivorous invertebrate rather than scavenging invertebrates or fish in the life cycle of *M. sydneyi* (Roubal et al. 1989).

Identifying alternative hosts in the marine environment is particularly difficult because of the high diversity and abundance of organisms (Audemard et al. 2002). However, the development of highly sensitive molecular diagnostic techniques, such as polymerase chain reaction (PCR) and *in situ* hybridization, have allowed further insights into the life cycle of *M. sydneyi* (Anderson et al. 1995, Le Roux et al. 1999, Kleeman & Adlard 2000, Kleeman et al. 2002b). Recently, the DNA of *M. sydneyi* has been detected in benthic polychaetes by PCR, and confirmed in tissues by *in situ* hybridization (Robert Adlard, Queensland Museum, pers. comm.). In contrast, the copepod *Paracartia grani* has been shown to be a host for *M. refringens* in Europe. Field and laboratory studies have revealed that *M. refringens* can be transmitted from *O. edulis* to *P. grani*, but not vice versa (Audemard et al. 2002, Audemard et al. 2004). These studies suggest that there is at least a third host in the life cycle of *M. refringens*. Further examination of zooplankton has found that 6 different zooplankton taxa appear to be parasitized by *M. refringens*, including 5 copepod species (*Acartia discaudata*, *Acartia clausi*, *Acartia italica*, *Oithona* sp., and *Euterpina acutifrons*) and 1 decapod crustacean zoea (*Portunus* sp.) (Carrasco et al. 2007). The role of benthic polychaetes and the strong possibility of other hosts in the life cycle of *M. sydneyi* warrant further investigation to improve management options for controlling QX disease. Understanding the life cycle may lead to a consistent laboratory challenge model for QX disease, which is crucial for epidemiological and immunological studies, as well as for benefiting a selective breeding program.

Population genetic analyses of *M. sydneyi* has revealed two distinct variants of the parasite common to the Great Sandy Strait, Richmond River and Georges River, as distinct from the variants in the Pimpama and Clarence rivers (Kleeman et al. 2004). These findings, together with historical data on outbreaks of QX disease, support the contention that the parasite originated in the Great Sandy Strait and/or the Richmond River, and then extended southward along the east coast of Australia as a result of the transfer of oysters during routine “highway” farming practices (Kleeman et al. 2004). Differences in the pathogenesis between the two variants of *M. sydneyi* are currently unknown. In Europe, two species of *Marteilia* sp. exist. *M. refringens* (type O) and *Marteilia maurini* (type M) are thought to be responsible for marteiliosis in oysters and mussels, respectively (Le Roux et al. 2001). However, doubt persists about the existence of these distinct species of *Marteilia* in Europe based on molecular characterization of the rDNA intergenic spacer. Alternatively, type O and type M of *Marteilia* may represent two different strains of the same species (Lopez-Flores et al. 2004).

Some data also suggest that type O and type M lack precise host specificity for their respective oysters or mussels (Novoa et al. 2005), even though differences in specificity for intermediate hosts between type O and type M strains have been observed (Carrasco et al. 2008).

GEOGRAPHICAL DISTRIBUTION OF *M. SYDNEYI* AND RISK FACTORS FOR DISEASE OUTBREAKS

The presence of *M. sydneyi* has been confirmed by PCR in all but one of the major Sydney rock oyster growing areas on the east coast of Australia (Adlard & Wesche 2005). However, mass mortality of *S. glomerata* resulting from *M. sydneyi* occurs in only a small proportion of these estuaries. This suggests that there is potential for outbreaks of QX disease in all the major commercial growing areas in the Australian states of NSW and Queensland. The intensity of *M. sydneyi* infections has also been found to vary annually within individual estuaries. For example, histological examination of 176 oysters in the Pimpama River, Queensland, between December 2006 and April 2007 revealed only two oysters infected with *M. sydneyi*. During the following year, maximum prevalence of the parasite was observed at the end of March 2008, with 83.3% of oysters found to be infected with *M. sydneyi* at the exact same location (Green 2009). Similar temporal differences in the prevalence of *M. sydneyi* has been reported previously (Adlard & Wesche 2005). This suggests that outbreaks of QX disease are regulated by a combination of the dynamics of the parasite's life cycle, the immunocompetence of *S. glomerata*, and interactions with the environment. Anecdotal evidence suggests infection with *M. sydneyi* occurs during periods of warmer water temperatures and after heavy summer rainfall (Lester 1986). According to the Australian Bureau of Meteorology (www.bom.gov.au), rainfall was substantially higher in the Pimpama River catchment during the 2007/2008 QX disease risk period when compared with 2006/2007, and could possibly explain the higher prevalence of *M. sydneyi* infection observed (Green 2009). However, statistical analyses have found no correlation between changes in salinity and/or pH (from acid sulfate soil leachate resulting from a rising water table) and the timing of QX disease outbreaks (Anderson et al. 1994, Wesche 1995), even though these studies did not consider the effect of rapid changes in salinity on host fitness.

Scientific studies have revealed that *S. glomerata* are immunocompromised during the QX infection risk period as a result of an environmental stress (Peters & Raftos 2003, Butt & Raftos 2007). A sudden decrease in salinity causes a sustained inhibition of the activity and transcription of immune enzymes that are known to be important to the immunological defense of *S. glomerata* against *M. sydneyi* (Butt et al. 2006, Green & Barnes 2010b). Higher mortality rates may occur when *S. glomerata* are osmotically stressed and are subsequently challenged with *M. sydneyi*. Hence, further investigation of environmental stressors that could cause *S. glomerata* to become immunocompromised during the QX disease risk period is warranted.

PREVENTION AND MANAGEMENT OF QX DISEASE

QX disease is a reportable disease in Australia, and farmers are required to notify their local state fisheries office if they suspect mortality of their oysters is occurring on their leases as

a result of *M. sydneyi*. Fisheries officers from both NSW and Queensland will then investigate the cause of mortality on oyster leases free of charge to farmers to prevent the spread of *M. sydneyi*-infected oysters, cultivation equipment, and farm infrastructure from diseased to nondiseased estuaries. In August 2008, NSW DPI (now Industry and Investment NSW; I&I NSW) introduced a single closure or movement control that manages the movement of *S. glomerata* within the state of NSW. This single closure manages the risk of transferring QX disease between estuaries within NSW by ranking oyster farming locations according to their likelihood of having an outbreak of QX disease (high, medium, or low risk). Under this closure, oysters and cultivation equipment can be moved freely between estuaries with the same risk ranking, and estuaries with lower risk can move oysters to higher risk estuaries. However, higher risk estuaries cannot move oysters to lower risk estuaries. This single closure within NSW, and risk-based approach to the movement of oysters within the state, has several advantages. First, it allows farmers to continue the practice of transferring oysters between estuaries to take advantage of each estuary's individual characteristics (e.g., high spatfall or proximity to markets) throughout the production cycle. Second, it simplifies the complex series of closures and quarantines that had been applied to NSW estuaries prior to 2008. However, because *M. sydneyi* can be detected in the majority of estuaries by PCR (Adlard & Wesche 2005), the rationale for the use of disease zoning to stop the spread of *M. sydneyi* could be questioned. This point highlights the emphasis for increased understanding of the risk factors that contribute to outbreaks of QX disease so that effective management solutions can be applied to the industry to prevent further outbreaks of QX disease.

In this light, the future of the Queensland and NSW oyster industry relies on either the development of QX-resistant *S. glomerata* or converting to farming the naturally resistant triploid Pacific oyster *C. gigas*. The farming of *C. gigas* is only sanctioned in NSW if permission has been granted by I&I NSW (O'Connor & Dove 2009). Farming the nonnative and invasive *C. gigas* in NSW and Queensland has met with resistance from conservation and indigenous groups, and the long-term resistance of this species to parasites and diseases endemic to NSW and Queensland is unknown. Several introductions of nonnative oyster species have occurred in France to sustain commercial oyster production when local species were decimated by disease. These introduced oysters initially flourished, but eventually succumbed to new and emerging diseases (Roch 1999, Hegaret & Mazurie 2005, Garnier et al. 2007). Thus, the future of the Queensland and NSW oyster industry still relies on improving management of QX disease and production of QX-resistant *S. glomerata*.

DEVELOPMENT OF DISEASE-RESISTANT SYDNEY ROCK OYSTERS

A breeding program to develop fast-growing *S. glomerata* was established by I&I NSW in 1990 (Nell et al. 2000) and is now in its 7th generation. Progress in selection for fast growth and disease resistance to winter mortality (*Bonamia roughleyi*) in the Georges River was severely interrupted when QX disease occurred in that estuary in 1994 (Adlard & Ernst 1995). Since then, QX disease has had a devastating effect in the Georges River, with annual mortality levels as high as 94% (Nell 2001).

In January 1997, the breeding program was reestablished in the Georges River and modified to incorporate selection for resistance to QX disease using mass selection from the survivors of the Georges River fast growth lines (Nell et al. 2000). Continuous improvement in QX disease resistance has been observed for each generation of *S. glomerata* selected for QX resistance (Nell & Perkins 2006), suggesting to date that QX resistance is controlled by multiple genes and is heritable in *S. glomerata*.

The I&I NSW breeding program for QX-resistant oysters has been hailed a success with commercially viable batches of disease-resistant *S. glomerata* produced in the Hawkesbury and Georges rivers, NSW (Nell & Perkins 2006). This has returned farming of *S. glomerata* to estuaries from which it had been previously lost as a result of QX disease outbreaks. However, the breeding program has a number of challenges and limitations to overcome. These include: (1) the risk of inbreeding depression; (2) its reliance on field-based selection, which differs in the intensity of QX disease between years; (3) the fact that mortality of oysters in the field were not always tested to confirm that mortality was caused by *M. sydneyi*; and (4) the selection of oysters for broodstock that may have survived infection by chance.

The risk of inbreeding depression occurring in the QX-resistant line is of concern because similar selection programs based on mass selection have encountered difficulties in the United States when the eastern oyster, *Crassostrea virginica*, was bred for resistance to the protozoan parasite *Haplosporidium nelsoni* (MSX) (Allen 1998). Attempts to minimize these risks to the *S. glomerata* selective breeding program have included the use of large numbers of contributing parents per line (a minimum of 216 parent per generation and, when possible, equal number of each sex) (O'Connor & Dove 2009), periodic microsatellite diversity assessment, assessment of single-paired matings to determine the genetic contribution of individual parents, and investigation of allozyme and DNA markers for QX resistance (Newton et al. 2004, Bezemer et al. 2006, Green et al. 2009) so that marker-assisted selection can occur independently of exposing broodstock to field infections of QX disease.

The *S. glomerata* breeding program is also undergoing fundamental change. Although mass selected lines are still being maintained, the QX-resistant lines in the Georges River are no longer fully nested within a single site, but are rotated across sites to allow exposure to QX disease and winter mortality (*B. roughleyi*) in summer and winter, respectively. This rotation is intended to increase the severity of exposure to both diseases and hopefully to improve response to selection across both disease traits. Furthermore, selected lines are now routinely maintained for more than 2 y in QX disease-affected sites to ensure multiple exposures to the disease. The selective breeding program has also been expanded to incorporate pedigreed families, with more than 90 pair-mated families having been created from a mixture of QX- and winter mortality-resistant parents and nonselected oysters obtained from estuaries along the NSW coastline. Performance evaluation of these families is underway across four estuaries to determine the genetic contribution of individual parents to increased performance (O'Connor & Dove 2009).

IMMUNOLOGICAL STUDIES ON SYDNEY ROCK OYSTERS

Histological examination has revealed that *S. glomerata* have a strong immune response to the early stages of *M. sydneyi* infection (stages A and B; Fig. 1). Proliferation of the parasite

within the gills and dissemination of *M. sydneyi* throughout the tissues is associated with hemocytic infiltration of the connective tissue, diapedesis across tubule epithelia (Kleeman et al. 2002a), and phagocytosis of *M. sydneyi* (Fig. 1). It appears that *S. glomerata* resistant to QX disease eliminate *M. sydneyi* before it establishes itself in the digestive gland. *S. glomerata* bred for QX resistance have been shown to have higher phagocytic indexes for ingesting *M. sydneyi* sporonts than nonselected oysters (Butt & Raftos 2008, Kuchel et al. 2010). However, the relevance of this observation is questionable, as histological observations suggest that hemocytic immune response of *S. glomerata* ceases when sporogenesis of *M. sydneyi* begins in the digestive gland tubules (Kleeman et al. 2002a). This coincides with the observation of four novel proteins that are similar in molecular weight and isoelectric point appearing in the hemolymph of *S. glomerata* infected with sporulating *M. sydneyi* (Simonian et al. 2009b). The nature of these proteins remains unclear, because mass spectrometric analysis failed to identify any homologues in the available databases, and it is unknown whether these proteins are produced by *S. glomerata* or *M. sydneyi* (Simonian et al. 2009b). If these novel proteins are derived from sporulating *M. sydneyi*, it may explain the lack of immune response of *S. glomerata* to sporulating *M. sydneyi*, because protozoan parasites are known to produce extracellular proteins that inhibit the immune response of their bivalve hosts (Garreis et al. 1996, Schott et al. 2003).

In terms of immune responses, *S. glomerata* that are resistant to *M. sydneyi* have higher titers of the defensive enzyme phenoloxidase and increased expression of the gene encoding extracellular superoxide dismutase, but reduced expression of peroxiredoxin 6 (Butt & Raftos 2008, Green et al. 2009, Simonian et al. 2009a). Melanization of pathogens by phenoloxidase is a major innate defense system in invertebrates (Cerenius & Soderhall 2004). This enzyme is normally present in the hemolymph as an inactive form (prophenoloxidase), avoiding unnecessary production of highly toxic and reactive compounds (Cerenius & Soderhall 2004). The prophenoloxidase cascade is triggered in *S. glomerata* in response to microbial invasion (Aladaileh et al. 2007), and results in the production of melanin, which is deposited on the surface of *M. sydneyi* (Butt & Raftos 2008). Other components of the phenoloxidase cascade are also involved in opsonizing pathogens for phagocytosis, or appear to be cytotoxic (Hellio et al. 2007). Early studies revealed that the activity of the phenoloxidase enzyme was inhibited in estuaries where QX disease is endemic (Peters & Raftos 2003) as a result of transient environmental stressors (Butt & Raftos 2007). Field and laboratory studies have subsequently shown the activity of the phenoloxidase enzyme in *S. glomerata* is inhibited by low salinity (Butt et al. 2006) and starvation (Butt et al. 2007), which have both been suggested as risk factors for QX disease outbreaks (Lester 1986). The activity of the phenoloxidase enzyme in *S. glomerata* selectively bred for QX disease resistance is higher than nonselected control oysters (Newton et al. 2004, Butt & Raftos 2008), and novel isoforms of the phenoloxidase enzyme exist in *S. glomerata* selectively bred for QX resistance (Newton et al. 2004, Bezemer et al. 2006). However, the genetic polymorphisms that control these different isoforms and the biochemical advantage conveyed by their expression are currently unknown. Further research is required to identify the polymorphisms within the genes controlling these different phenoloxidase isoforms.

Comparison of the transcriptome of hemocytes isolated from either *S. glomerata* bred for QX resistance with those from control oysters over the QX disease risk period revealed differences in the constitutive expression of immune genes (Green et al. 2009). Changes in the constitutive expression of immune genes, or increased general vigor, have also been observed for other species of shellfish selected for disease resistance (Lorgeril et al. 2008, Taris et al. 2009). This may explain why *S. glomerata* selected for resistance to *M. sydneyi* are also resistant to disseminating neoplasia, potentially of viral etiology (Green et al. 2008). In QX-resistant *S. glomerata*, the basal expression of an extracellular superoxide dismutase (EcSOD) is higher, and the basal expression of peroxiredoxin 6 (Prx6) is lower when compared with nonselected oysters (Green et al. 2009). This observation is supported by complementary proteomic and mass spectrometry analysis of the hemolymph proteins from QX-resistant and nonselected oysters (Simonian et al. 2009a). The expression of these 2 antioxidant genes could not be induced by microbial invasion (Green & Barnes 2009), leading to the hypothesis that another mechanism used by *S. glomerata* selectively bred for QX resistance is their ability to generate the antiparasitic compound hydrogen peroxide (H_2O_2) at a faster rate and higher concentration when challenged with disease agents. This is likely a result of the elevated titer of EcSOD, which produces H_2O_2 , and the reduced titer of peroxiredoxin 6, which detoxifies H_2O_2 (Nikapitiya et al. 2009) (Fig. 2). The elevated phenoloxidase activity of QX-resistant *S. glomerata* may be beneficial in the melanization of *M. sydneyi* (stage B). This might prevent the spread of the parasite from the gills to the digestive gland and/or may support encapsulation to stop the host's immune response (such as H_2O_2) from escaping the site of infection and damaging host tissue (Holmblad & Soderhall 1999) (Fig. 2).

CONCLUDING REMARKS

Management solutions to prevent the spread of QX disease are evolving, and it remains to be seen whether they will halt the southward expression of the disease (Adlard & Ernst 1995). Moreover, disease zoning is, to some extent, constraining the industry, which benefits from transferring oysters between estuaries. Future advances in our understanding of the *M. sydneyi* life cycle will hopefully allow us to refine a disease zoning policy to prevent outbreaks of the disease from occurring, while limiting the effects on farming practices, such as highway farming. Understanding the complete life cycle of *M. sydneyi* will also facilitate the development of a laboratory infection model, which will aid the future success of the Sydney rock oyster breeding program, and will provide new tools for immunological

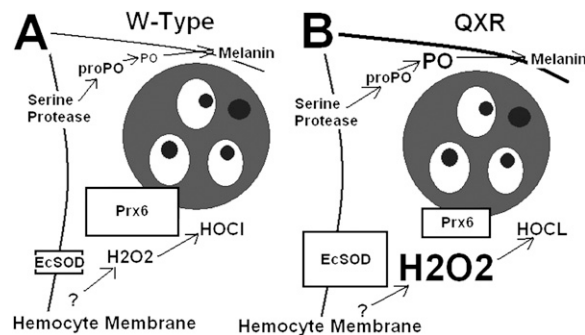


Figure 2. (A, B) Schematic representation of the immune system of *Saccostrea glomerata* (W-type) (A) and *S. glomerata* bred for QX resistance (QXR) (B). QXR oysters have a higher titer of phenoloxidase (PO) and extracellular superoxide dismutase (EcSOD), and a reduced titer of peroxiredoxin 6 (Prx6) (Butt & Raftos 2008, Green et al. 2009, Simonian et al. 2009). It is hypothesized that QXR oysters are resistant to *Marteilia sydneyi* because they can generate hydrogen peroxide (H_2O_2) at a faster rate and higher concentrations during respiratory bursts. The phenoloxidase activity of QXR oysters is higher compared with W-type oysters, which is important in preventing the spread of the pathogen into surrounding tissues and stops the host's immune response (such as H_2O_2) from escaping the site of infection and damaging host tissue.

and epidemiological studies. Epidemiological studies have shown that *M. sydneyi* is present in the majority of estuaries where *S. glomerata* are farmed. However, QX disease occurs only in a selected number of estuaries. Future research should focus on why QX disease occurs only in selected estuaries and whether these disease outbreaks are dependent on environmental stressors, the abundance of the parasite's intermediate hosts, or a combination of these factors. Determining the risk factors that result in QX disease outbreaks will allow farmers to position their farms in locations where QX disease outbreaks are unlikely to occur. Immunological studies have revealed the importance of heavy summer rainfall as a risk factor for QX disease outbreaks (Butt et al. 2006, Green & Barnes 2009) and the importance of antioxidant enzymes (PO, EcSOD, and Prx6) in the resistance of *S. glomerata* to *M. sydneyi* (Butt & Raftos 2008, Green et al. 2009, Simonian et al. 2009a). The Sydney rock oyster breeding program is now investigating whether oysters can be selected for disease resistance based on their phenoloxidase phenotype (O'Connor & Dove 2009), but selection of oysters for low-salinity tolerance may be an alternative selection strategy for QX resistance. The future prevention of QX disease is likely to comprise a combination of management solutions and the use of QX-resistant lines of *S. glomerata*. Overall, the future is bright for a sustainable Sydney rock oyster industry.

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