Investigation and Management of a Major Oyster Mortality Event in Wonboyn Lake, Australia

DAMIAN OGBURN1, RICHARD CALLINAN[1], IMOJEN PEARCE[2], GUSTAAF HALLEGRAEFF[2] AND MATTHEW LANDOS[1]

[1] *NSW Fisheries, Private Bag 1, Nelson Bay, NSW 2315, Australia*

[2] *School of Plant Science, Private Bag 55, University of Tasmania, Hobart, Tasmania 7001, Australia*

ABSTRACT

In March 2002, an unprecedented mortality (15-100%) of Sydney rock oyster (*Saccostrea glomerata*) was reported on aquaculture leases in Wonboyn Lake (37°S) on the south coast of New South Wales, Australia. This paper describes the field and laboratory investigations including mortality mapping, a pollution survey, histopathological examination of oysters, and analyses for biotoxins and microalgae. Oyster mortalities, associated with severe necrosis and inflammation in the digestive gland were observed. The dinoflagellate *Prorocentrum minimum* has been implicated as a possible causative agent together with a changing ecology of the lake, partly resulting from a restricted entrance to the lake due to sand accretion. We evaluate the emergency response process and make recommendations for the management of similar aquatic animal disease emergencies. Options for ongoing farming practices and an algal-monitoring program for the oyster industry in Wonboyn Lake are discussed.

INTRODUCTION

The Sydney rock oyster (*Saccostrea glomerata*) aquaculture industry has operated for over 130 years on approximately 4000 hectares of inter-tidal leases in over 35 estuaries on the east coast of Australia between Moreton Bay (27°S) and Wonboyn Lake (37°S). Methods employed are off-bottom culture using a variety of infrastructure including oysters set on tarred hardwood sticks or individually placed on trays and baskets that are placed on timber racks or in floating culture (Nell, 1993). The industry has faced a number of significant oyster health-related issues including mud worm (principally *Polydora websteri*) infestation, winter mortality (*Mikrocytos roughleyi*) infection, QX disease (*Marteilia sydneyi*), flat worm (*Imogine mcgrathi*) infestation and heat kill (Nell, 2001). Reports on Sydney Rock oyster mortalities in estuaries have occurred through the history of the industry (eg Roughley, 1926). In this paper, we report on the investigation sequence of an unprecedented mass mortality of oysters in Wonboyn Lake that occurred in February-March 2002, with particular reference to the aquatic animal health incident management perspective. The cause for the mortality appears to be a combination of factors. The small (10-15 µm diameter)

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dinoflagellate, *Prorocentrum minimum*, has been implicated as a possible causative agent. We evaluate the emergency response process and make recommendations for the management of future events. Options for ongoing farming practices and an algal monitoring program for the oyster industry in Wonboyn Lake and elsewhere are discussed.

MATERIALS AND METHODS

Wonboyn Lake is considered a wave dominated, barrier estuary (after Roy *et al*., 2001). Wonboyn Lake oyster leases date back to at least 1930 with reported mean annual production increasing 10 fold during the last 15 years compared to the previous 50 years. More than 95% of the catchment is forest with minimal catchment development to date. It is considered near pristine and has recently been recommended for significant protection (Healthy Rivers Commission, 2002). It has a water surface area of approximately 358 hectares (Land Property Information Centre - cadastre layer) and contains approximately 45 hectares of oyster lease from which approximately one million *S. glomerata* are harvested annually.

Following the report by farmers of mass mortality of oysters on March 1, 2002, the following actions and investigations were undertaken.

A closure on shellfish harvest for both human consumption and translocation was implemented as precautionary measures.

NSW Fisheries conducted a field survey, linked with farmer interviews, within five days of the mortality event. Information collected included details and dates on farming activity, hydrological, climatic, water quality (visual and physical) and any other unusual signs or details to establish a diary of events. Mortalities on most of the 45 leases were estimated in conjunction with farmers with attention to variations in mortality within individual leases. Data was recorded using a Geographic Information lease mapping system to depict mortality variation on leases across the estuary.

A pollution survey was conducted on March 14 and 15, 2002. Sampling of waters and biota in the Wonboyn Lake area was undertaken as follows: Live oysters from various sites that were subject to the rise and fall of tides; oysters that had only been submerged or floating; oyster shells that farmers said had a pink coating on them following the heavy rainfall; live whelks from the shore of the Lake. Concentrated algae samples using 20-micron plankton net. Water profile and grab samples for algal assessment. Water grab samples for nutrient and faecal coliform testing; inorganic chemical analysis, metal and pesticide/herbicide screen.

Samples of surviving oysters were collected on March 6 and 12 and submitted for histopathological examination to NSW Fisheries.

The following samples were submitted to the University of Tasmania for microalgal examination: A concentrated water sample on March 25; live samples of surviving oysters on April 4; seagrass (*Zostera capricorni*) samples on May 29.

Oyster samples were collected on March 20 and submitted for algal biotoxin examination using mouse bioassay for Paralytic Shellfish Poison (PSP) (conducted by MedVet, Adelaide). Oyster samples were collected on April 21 and submitted for Diarrhetic Shellfish Poison (DSP) and ether extract mouse bioassays for Neurotoxic Shellfish Poison (NSP) (conducted by AgriQuality, New Zealand).

Semi-continuous bulk cultures of the dinoflagellate *P. minimum* isolated from water samples in Wonboyn Lake were established and fed to juvenile Pacific Oyster (*Crassostrea gigas*) spat and brine shrimp (*Artemia salina*). Survival of both species was compared to a control fed the green flagellate *Tetraselmis* suecica. A subsequent experiment involving "aged" culture of *P. minimum* was fed to a second batch of *C. gigas* spat. Water and lipid soluble *P. minimum* extracts were tested for toxicity using intraperitoneal mouse assay (MedVet, Adelaide).

A sentinel *S. glomerata* monitoring program was undertaken between June and August 2002. Oysters were placed at four sites (one unaffected and three heavily affected sites in different wind prone sites) in the estuary. At each site 20 oysters were located at each of three heights above the substrate: on the tidal plane at normal rack height (NRH); at NRH+100mm; and NRH -100mm to determine. The variable heights were used to determine the impact of extended emersion, if any, on sentinel oysters. The sentinel stock were removed after one month and submitted for histopathological examination.

An ongoing plankton-monitoring program for Wonboyn Lake was developed as result of the mass mortality. A component tree for improved aquatic animal health incident management was developed as the basis for managing future incidents. This will provide the framework for a control centre database currently being developed for tracking the management process.

RESULTS

Oyster farmers advised that from about January 24-27, 2002 oysters on racks were out of the water. Salinity level in the Lake on February 4 was 33 ppt. No unusual discolouration in the waters of Wonboyn Lake at this time was observed. From February 4-12, approximately 270mm of rain fell in the catchment. The Lake flooded on February 10 and the level rose approximately 0.7 metres. The water was too turbid to note discolouration and the oysters remained submerged until February 16. During this time a strong on shore south-west wind was blowing. On February 17-20 the tide went out completely and oysters on rack cultivation were emerged for approximately 60 hours. Air temperatures were relatively mild (max. 27°C) and a westerly breeze. During this time oysters "appeared" to be normal. The tide returned on February 20 and oysters did not re-emerge until the tide went out on March 1. Peak water temperature was 25°C during this time. This sequence of events had occurred in the past without major effect on cultivated oysters in the lake. On March 1 the mass mortality was observed. Oysters had been dead for some days (many with only shell left). There were no reported fin-fish mortalities. Wild mussels and *C. gigas* on lease racks were also dead. A pinkish discolouration in valvular fluid of live oysters was observed for several weeks after the mortality. Sand appeared to be discoloured dark brown to dark red at the Lake entrance on March 3-5.

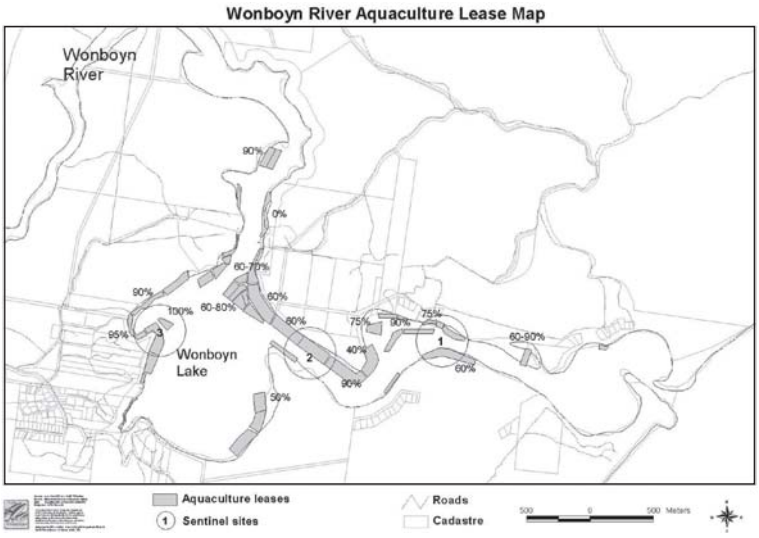

Figure 1. Mortality estimates on oyster leases in Wonboyn Lake and sentinel oysters site locations.

Mortality estimates for oyster leases are shown in Figure 1. Nearly all 45 leases in Wonboyn Lake suffered mortalities of between 15-100% in late February 2002. Oysters that did not emerge (ie. were held in floating baskets or as bottom cultivation) during the large tide-out on February 17-20 did not suffer mortality. Temperatures were not extreme and heat kill was not apparent. One lease in an upwind section of the lake and wild oysters at the mouth of the lake did not suffer mortality. Kills of shellfish were heaviest on the western side (down wind) of the estuary. Bioassay for DSP, NSP and PSP biotoxins were negative. Nutrient, metals and pesticide analyses of water and oyster samples did not reveal any unusual results. There was no evidence to implicate pollution and the food safety closure was lifted in the middle of May, 3.5 months after the mortality. Restriction on translocation of oysters remained in place.

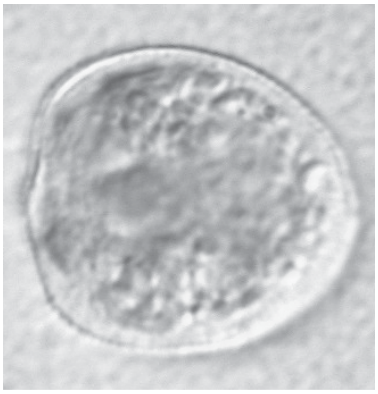

Figure 2. *Prorocentrum minimum,* size = 15 mm diameter.

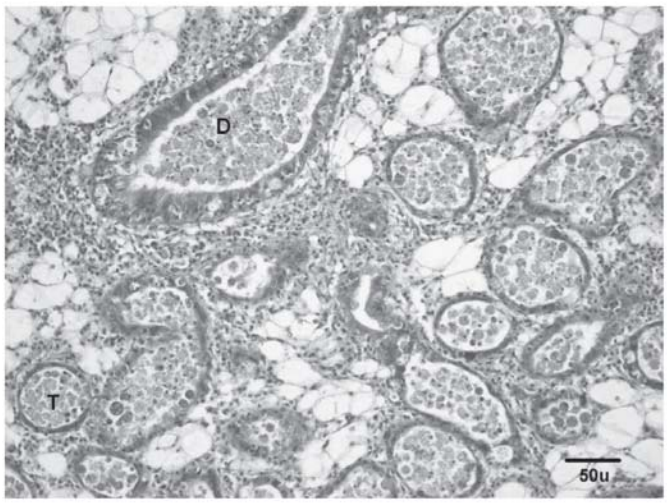

Figure 3. Digestive gland - dilated lumens of digestive gland ducts (D) and tubules (T). T epithelium markedly attenuated. Intense infiltration of inflammatory cells in adjacent connective tissues. Haematoxylin and eosin.

Live oysters removed from affected leases shortly after the event "leaked" badly and would desiccate and die within 36 hours; normal *S. glomerata* can survive for at least 7 days out of water. The dominant phytoplankton, *P. minimum* (Figure 2), was identified from selected samples (March 25 for water and May 29 from Z. capricorni) as a potential causal agent. An additional observation made during the examination of oyster stomachs (April 24 samples) was the presence in some (but not all) shellfish of *Prorocentrum rhathymum*.

On histopathological examination, surviving oysters showed severe lesions in digestive gland ducts, tubules and adjacent connective tissues (Fig. 3). Typically, large quantities of cells (including sloughed epithelial cells and haemocytes), cell debris and amorphous material were present in dilated lumens of digestive gland ducts and tubules. Digestive gland tubule epithelium was often markedly attenuated and dense accumulations of haemocytes were present in adjacent connective tissues. In some cases, massively dilated digestive gland ducts had ruptured.

P. minimum cultures from Wonboyn Lake were non-toxic to *A. salina*. Mouse bioassays (intraperitoneal injection) of both water and lipid soluble cell extracts were also negative. There were no mortalities in *C. gigas* spat fed dense *P. minimum* cultures for two weeks at 10-16 °C. However, histopathological examination showed differences in gut morphology in spat subjected to *P. minimum* compared to controls. A second feeding experiment conducted at 20 °C with "aged" *P. minimum* culture resulted in deaths in 100% of test spat in four days. All control spat fed equal densities of non-toxic *T. suecica* survived.

Table 1. Histopathology results for sentinel oysters on selected leases in Wonboyn Lake following the oyster kill expressed as means over the three-month sentinel-monitoring program.

SAMPLE	Digestive atrophy	Digestive necrosis	Haemocyte infiltration
Control	0.76	0.08	0.66
Site 1 +100mm	1.11	0.37	0.73
Site 1 RH	0.94	0.44	0.65
Site 1-100mm	1.03	0.24	0.82
Site 2 +100mm	1.17	0.52	1.04
Site 2 RH	0.96	0.38	0.81
Site 2-100mm	0.89	0.36	0.71
Site 3 +100mm	1.17	0.45	0.93
Site 3 RH	0.93	0.20	0.64
Site 3-100mm	1.07	0.17	0.66

Average lesion severity: nil (0), mild (1), moderate (2), severe (3), very severe (4)

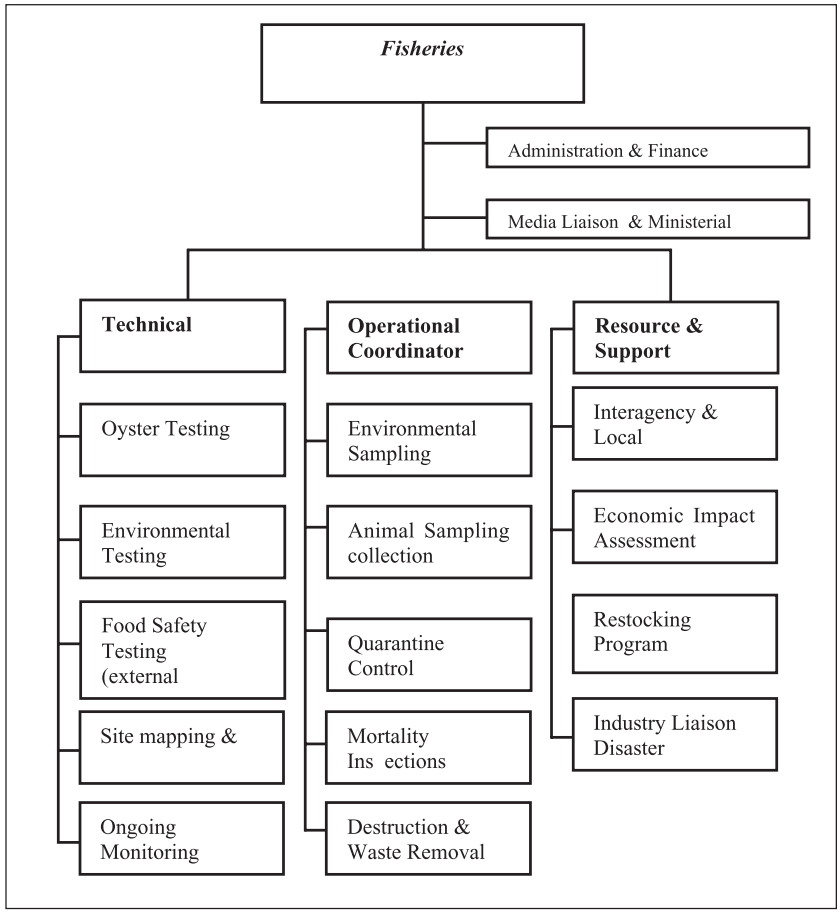

Figure 4. Component tree for aquatic animal health incident management.

Results of histopathological examinations of sentinel oysters conducted during June-August are presented in Table 1. No significant lesions were observed. Results suggested the incident had abated and that restocking of Wonboyn oyster leases with spat from other estuaries could be undertaken.

The investigation of this incident spanned several months and involved a range of technical, management and socio-economic responses across various government agencies, laboratories and academic institutions. The mass mortality in an otherwise pristine lake attracted considerable media coverage and has had severe impact on the livelihood of several oyster-farming families at Wonboyn Lake. The need for an incident management program under this scenario was apparent. A model component tree for aquatic animal health incident management is outlined in Figure 4. This will form the framework for a database for a proposed state Control Centre Manual. This will be established following a simulation exercise under the auspices of the Australian national animal health program known as Aquaplan (Commonwealth of Australia, 1999).

DISCUSSION

Field and laboratory investigations have implicated the dinoflagellate, *P. minimum* as a possible causative organism for the unprecedented Wonboyn oyster mortalities. However, evidence also suggested that a unique combination of geographical, climatic and biological factors stressed the oysters and may have allowed proliferation of *P. minimum.*

The restricted tidal entrance, due to sand accretion by natural processes, restricts tidal flushing within the Lake. Any discussion of the combination of causal factors, which led to the oyster mortalities, must account for the observation that those oysters, which remained submerged, either in bottom cultivation or in floating baskets, during the event, survived. *P. minimum* was known to be present in the lake prior to the flood in early February but is likely to have proliferated in response to the flood event (Feb 12-15) resulting in major ingestion of this organism while oysters were submerged. Subsequent emergence as flood waters receded, (Feb 16-18) of those oysters on racks/trays may have caused these oysters, already weakened by the effects of ingested *P. minimum*, to dry out and begin to die in spite of being re-submerged (Feb 19-28). These suggestions accord with the observation by farmers that oysters from affected leases 'leaked' and would desiccate and die within 36 hours if held out of water. It is likely therefore that most oysters that emerged died after being re-submerged but were only noted as dead when tide went out again on March 1.

P. minimum blooms have been linked to two factors: sudden freshwater influx and high loading of nutrients and dissolved organic matter (Grzebyk and Berland, 1996). Therefore, torrential rain reported from Wonboyn Lakes during early February may have created conditions suitable for P. minimum to bloom and thus oysters to ingest high cell numbers.

An additional observation made during the examination of the Wonboyn oyster stomachs was the presence in some (but not all) shellfish of *Prorocentrum rhathymum*. This is a benthic species, also sometimes implicated in mouse toxicity (Pearce *et al*., 2001) or poor shellfish feeding, suggesting that seagrass mats need to be sampled in addition to the water column. One interesting observation of farmers is the increasing prevalence of seagrass (*Zostera capricorni* and *Ruppia spp.*) in oyster lease areas of Wonboyn Lake during the last few years (J. Henry, pers. comm.). It may be possible that increased oyster farming in

recent years has assisted the proliferation of seagrass through phytoplankton filtering and enrichment of benthic sediments. The greater abundance of seagrass may lead to proliferation of blooms of *P. rathymum* known to be associated with seagrass (Pearce *et al*., 2001).

In Chesapeake Bay, USA, shellfish mortalities have been linked with *P. minimum* blooms (Luckenbach *et al*., 1993) and an unidentified Prorocentrum species has been implicated in mass mortalities of flat oysters (*Ostrea rivularis*) in South China (Yongjia *et al*., 1995). Laboratory feeding experiments have also demonstrated mortality in shellfish exposed to cultured *P. minimum* (Luckenback *et al*., 1993; Wikfors and Smolowitz, 1993; 1995). For example, *P. minimum* has been shown to kill scallops (Argopecten) with the following histopathology: "Poorly developed digestive diverticula, attenuation of the digestive tubule epithelium with abnormal vacuolation and necrosis. Residual cells were more numerous" (Wickfors and Smolowitz, 1993). These findings were attributed to the action of a toxin, rather than poor nutrition. The toxin/s produced by *P. minimum* are un-characterised, some (but not all) strains cause mouse death when injected intraperitoneally, with symptoms suggestive of neurotoxic activity (Grzebyk *et al*., 1997). The age of *P. minimum* culture is thought to influence toxicity; Grzebyk *et al*. (1997) found toxin production occurred only when *P. minimum* cultures were senescing and this was also suggested by the laboratory feeding experiments documented here.

The question remains as to whether the 2002 mass mortality is a "one off" event or likely to recur. Options for "farming around" the problem in future incidents include the development of an early warning plankton monitoring program which would enable farmers to respond by relocating to areas where blooms may be absent or by placing stock at a level on leases that avoids long emergence during bloom periods.

CONCLUSION

The sequence of events that lead to the mass mortality of oysters in Wonboyn Lake appears to have been exceptional. A combination of floods and low tidal flushing rates during late summer may have stimulated the development of toxicogenic *P. minimum* that severely weakened oysters. Subsequent emergence of stock on inter-tidal leases for approximately 60 hours during a big tide-out may have then led to desiccation and death of oysters on leases.

Further feeding experiments are to be conducted to determine the precise nature of the toxicity of *P. minimum* to oysters. An algal-monitoring program in Wonboyn Lake to be established by early 2003 will track phytoplankton densities in the Lake in subsequent months. The influence of oyster carrying capacity may need to be considered for the Lake. The translocation closure will need to be reviewed in mid-2003. In addition, a simulation exercise for emergency response to aquatic animal health incidents will be conducted with industry and government in mid-2003. The preparation of a Control Centre Manual and database will then form the basis for improved management of emergency responses.

The impact of phytoplankton such as *P. minimum* on shellfish health is clearly an area that needs more investigation. The establishment of plankton monitoring programs to determine trends in potentially harmful plankton blooms within Sydney rock oyster producing estuaries and possible links to previously undiagnosed oyster mortalities and poor production appears to be an area worth investigating as a result of this incident in Wonboyn Lake.

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Size 7.25 x 10 inches