

# QX RESISTANT OYSTER CHALLENGE TRIAL 2005 - 2007

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Cover picture: QX Resistant Sydney rock oysters at age 20 months from Kimmerikong Bay, Hawkesbury River, NSW.

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The authors wish to acknowledge and thank the following Hawkesbury River oyster farmers, Robbie Moxham, John Stubbs, Bruce Alford, Les Wadham and Dale Witchard, for the use of their lease areas, work boats and equipment and assistance during the course of this project.

## NON-TECHNICAL SUMMARY

205/076	QX Resistant Oyster Challenge Trial 2005-07
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### OBJECTIVES:

1. To assess under normal commercial oyster farming practice the commercial viability of selectively bred fourth-generation QX resistant Sydney rock oysters on QX disease affected oyster leases in the Hawkesbury River NSW.
2. To assist the uptake by Hawkesbury River oyster farmers of handling and growing technology necessary for the commercial scale cultivation of small hatchery produced oyster spat.

### NON TECHNICAL SUMMARY:

The Hawkesbury River (33° 35'S; 151° 20'E) is located approximately 50 km north of Sydney NSW. In 2003, the Hawkesbury River was the third largest Sydney rock oyster *Saccostrea glomerata* (SRO) producing estuary in NSW, with an annual farm gate production of \$3.6M (10.1 million oysters).

The commercial production of SRO in the Hawkesbury River was based exclusively on the traditional intertidal stick and tray method of oyster farming. This method of oyster production is reliant on the annual settlement of SRO larvae that occurs towards the mouths of estuaries during the summer and autumn months. To take advantage of this natural oyster settlement, oyster farmers placed tar and cement coated oyster sticks on intertidal timber racks in early summer in areas known for reliable SRO settlement. The following spring the oyster sticks caught with wild spat (juvenile oysters) are moved upstream to be on-grown on intertidal racks in areas away from further competing SRO settlement. Once the SRO have reached a suitable size they are removed from the oyster sticks by hand and placed on timber, mesh bottom, trays and returned to the growing areas where they remain until they reach a marketable size of around 50g whole weight (approximately 3.5 years of age).

Due to the high productivity of the waters of the Hawkesbury River and the historical reliability of the stick and tray method of production, Hawkesbury River oyster farmers had not seen any need to move away from their traditional farming methods towards single seed oyster farming technology that would enable them to take advantage of oyster seed stock produced by commercial oyster hatcheries. Before 2003, hatchery production of commercial quantities of SRO was unreliable. Hatchery production of SROs commenced in the early 1980s but has been plagued by recurrent mass mortality (>80%) of larvae and spat. A Fisheries Research & Development Corporation (FRDC) project (Number 2003/209) commenced in 2003 with the aim of overcoming constraints to

commercial scale hatchery production of SRO and developed techniques that have resulted in commercial hatcheries supplying the SRO industry with spat that are selectively bred for faster growth and disease resistance.

In mid 2004, QX disease was detected in the key major upstream SRO commercial oyster harvest areas in the Hawkesbury River. QX disease, which is specific to the SRO, is caused by the protozoan parasite *Marteilia sydneyi* (Wolf, 1979); highly virulent; and, often results in oyster mortalities in excess of 90%. QX has led to the collapse of commercial oyster cultivation in a number of areas in NSW and southern Queensland. Due to the poor visual meat condition of QX affected Hawkesbury River oysters and adverse publicity surrounding the disease outbreak, commercial sales of Hawkesbury River oysters quickly ceased. By mid 2005, QX disease surveillance conducted by the NSW Department of Primary Industries (NSW DPI), in cooperation with Dr Rob Adlard of the Queensland Museum, indicated that the disease causing QX parasite had spread throughout the majority of the commercial farming areas in the Hawkesbury River system. By this time most farmers were in serious financial difficulties and were retrenching staff. Given the history of QX outbreaks in other estuaries, the Hawkesbury River oyster industry quickly recognised that oyster production based on the wild QX susceptible SRO was no longer viable.

Since the mid 1990's, NSW DPI has been developing a breeding line of SRO that has been selected for fast growth and has demonstrated good resistance (>75% survival) to outbreaks of QX disease on commercial oyster leases under experimental conditions in the Georges River NSW (Nell and Perkins, 2006). However, the commercial viability of this breeding line of QX resistant (QXR) SRO under Hawkesbury River commercial farming conditions was unknown. Hawkesbury River farmers also expressed concern regarding the unknown marketability (i.e. possible poor visual meat condition) of the QXR SRO stock should it be grown in the heavily QX infested areas in the Hawkesbury River. They also had concerns as to their individual financial ability to invest in single seed technology which they believed was unproven under Hawkesbury River oyster farming conditions.

During 2005 a rescue package for the Hawkesbury River oyster industry was developed by NSW DPI that involved; the provision of \$2,700,000 available to Hawkesbury River oyster farmers to assist them to remove and dispose of dead and dying stock and collapsing oyster encrusted infrastructure from their leases; and in cooperation with FRDC the provision of \$10,000 to conduct a commercial farm proof of concept trial of QXR SRO developed under FRDC Project (Number 96/357). Following a favourable environmental impact assessment, approval was also granted to Hawkesbury River oyster farmers to import and cultivate triploid Pacific oysters directly from the controlled environment of a Tasmanian commercial oyster hatchery. Under the proof of concept trial, NSW DPI has made available approximately 200,000 QXR SRO spat to seven Hawkesbury River oyster farmers who expressed interest in an evaluation of the suitability of QXR SRO as an alternative oyster crop under normal farming conditions. The spat were supplied to participating farmers in specialised single seed oyster trays developed and supplied by NSW DPI. NSW DPI also provided ongoing technical advice and field assistance regarding the maintenance of the spat on commercial leases until the oysters could be transferred to traditional oyster growing tray systems used by oyster farmers. Concurrently, NSW DPI maintained QXR SRO at three key QX infection sites with the cooperation of farmers.

The QXR SRO were exposed to two consecutive QX infection events. The QXR SRO demonstrated excellent survival and growth and maintained good marketable condition under both experimental and normal Hawkesbury River farming conditions. The observed QXR oysters' resilience to QX infection was reinforced by the fact that the QX parasite was not detected in the digestive gland of any of the oysters examined following visual meat condition assessments undertaken by Hawkesbury River oyster farmers following the QX infection events. The marketability of the QXR SRO was demonstrated by the fact that the majority of QXR SRO held

on prime Hawkesbury River fattening leases by participating farmers were sold by 24 months of age. Participating farmers also had no difficulty in adopting small single seed management practices prior to transferring stock to the traditional tray growing technology used in the Hawkesbury River. Hawkesbury River oyster farmers were also able to apply the principals of small single seed oyster management to the development of a flexible and cost effective floating bag single seed system for use on their leases.

## OUTCOMES ACHIEVED

The proof of concept trial has demonstrated that:

- survival of QXR SRO on QX affected Hawkesbury River oyster leases is in line with that reported by Nell and Perkins (2006) for QXR SRO in the Georges River and is also in line with normal historical background mortality rates observed by Hawkesbury River oyster farmers prior to the outbreak of QX in the River in 2004;
- QXR SRO grown on QX affected Hawkesbury River oyster leases reached the premium market “Plate Grade” threshold of 50 g whole weight in approximately 24 months which is similar to the growth rates reported by Nell and Perkins (2006) for QXR SRO grown in the QX affected areas of the Georges River. This is quicker than would normally be expected for wild caught SRO in the Hawkesbury River;
- exposure to the QX parasite does not detrimentally affect the visual meat condition and therefore commercial marketability of the QXR SRO grown on QX affected leases in the Hawkesbury River; and
- Hawkesbury River oyster farmers were able to quickly grasp the principals and techniques required to handle small hatchery produced oyster spat and adapt these principals to suit Hawkesbury River growing conditions.

### KEYWORDS:

*Saccostrea glomerata*, *Marteilia sydneyi*, QX, disease resistance, oyster, commercial production

## 1. BACKGROUND

The Hawkesbury River (33° 35'S; 151° 20'E) is located approximately 50 km north of Sydney NSW. In 2003, the Hawkesbury River was the third largest Sydney rock oyster *Saccostrea glomerata* (SRO) producing estuary in NSW with an annual farm gate production of \$3.6M (10.1 million oysters).

The commercial production of the SRO in the Hawkesbury River was based exclusively on the traditional intertidal stick and tray method of oyster farming. This method of production is reliant on the annual settlement SRO larvae that occurs towards the mouths of estuaries during the summer and autumn months. To take advantage of this natural oyster settlement, oyster farmers placed tar and cement coated oyster sticks on intertidal timber racks in early summer in areas known for reliable SRO settlement. The following spring the oyster sticks caught with wild spat (juvenile oysters) are moved upstream to be on-grown on intertidal racks in areas away from further competing SRO settlement. Once the SRO have reached a suitable size they are removed from the oyster sticks by hand and placed on timber, mesh bottom, trays and returned to the growing areas until they reach a marketable size of around 50g whole weight (approximately 3.5 years of age).

Due to the high productivity of the waters of the Hawkesbury River and the historical reliability of the stick and tray method of production, Hawkesbury River oyster farmers had not seen any need to move away from their traditional farming methods towards single seed oyster farming technology that would enable them to take advantage of the small oyster seed stock produced by commercial oyster hatcheries. Before 2003, hatchery production of commercial quantities of SRO was unreliable. Hatchery production of SROs commenced in the early 1980s but has been plagued by recurrent mass mortality (>80%) of larvae and spat. A Fisheries Research & Development Corporation (FRDC) project (Number 2003/209) commenced in 2003 with the aim of overcoming constraints to commercial scale hatchery production of SRO and developed techniques that have resulted in commercial hatcheries supplying the SRO industry with spat that are selectively bred for faster growth and disease resistance.

In mid 2004, QX disease was detected by NSW Department of Primary Industries (NSW DPI) in the key major upstream SRO harvest areas in the Hawkesbury River. QX disease which is specific to the SRO is caused by the protozoan parasite *Marteilia sydneyi* (Wolf, 1979) is a highly virulent and often results in oyster mortalities in excess of 90%. QX has led to the collapse of commercial oyster cultivation in a number of areas in NSW and southern Queensland. Due to the poor visual meat condition of QX affected Hawkesbury River oysters and adverse publicity surrounding the disease outbreak, commercial sales of Hawkesbury River oysters quickly ceased. By mid 2005, QX disease surveillance (PCR and imprint cytology) conducted by the NSW DPI, in cooperation with Dr Rob Adlard of the Queensland Museum, indicated that the QX parasite had spread throughout the majority of the commercial farming areas in the Hawkesbury River system. By this time most farmers were in serious financial difficulties and were retrenching staff. Given the history of QX outbreaks in other estuaries, the Hawkesbury River oyster industry quickly recognised that oyster production based on the wild QX susceptible SRO was no longer viable.

Since the mid 1990's, NSW DPI with funding assistance from FRDC has been developing a breeding line of SRO that has been selected for fast growth and QX disease resistance. This breeding line, referred to as Lime Kiln Bar Line, has demonstrated good resistance (>75% survival) to outbreaks of QX disease on commercial oyster leases under experimental conditions in the Georges River NSW (Nell and Perkins, 2006). However, the commercial viability of the QX resistant (QXR) Georges River Lime Kiln Bar line (Nell & Perkins 2006) under Hawkesbury River

commercial farming conditions was unknown. Hawkesbury River farmers also expressed concern regarding the unknown marketability (ie. possible poor visual meat condition) of the QXR SRO stock should it be grown in the heavily QX infested areas in the Hawkesbury River. They also had concerns as to their individual financial ability to invest in single seed technology which they believed was unproven under Hawkesbury River oyster farming conditions.

During 2005 a rescue package for the Hawkesbury River oyster industry was also developed by NSW DPI that involved; provision of \$2.7M available to Hawkesbury River oyster farmers to assist them to remove and dispose of dead and dying SRO stock and collapsing timber oyster encrusted infrastructure from their leases, thus reducing the potential QX disease infective load within the estuary. Under this proof of concept trial, NSW DPI with financial assistance from FRDC, also provided approximately 200,000 QXR SRO spat to Hawkesbury River farmers to enable a collaborative farm based commercial viability assessment of the stock to be undertaken on QX affected Hawkesbury River oyster leases.

Following a favourable environmental impact assessment, approval was also granted by NSW DPI for Hawkesbury River oyster farmers to import and grow triploid Pacific oysters on their oyster leases.

This is the first instance that SRO selected for QX disease resistance have been formally assessed under commercial cultivation.

## 2. NEED

In 2004 a virulent outbreak of QX disease in the Hawkesbury River led to oyster mortalities in excess of 90% at commercial oyster harvest areas. As oyster farming at the Hawkesbury River was totally reliant on the traditional stick cultivation of QX susceptible wild caught SRO seed stock, ongoing commercial oyster cultivation in the river was deemed by the Hawkesbury River oyster industry to be commercially non-viable. By 2005 individual farms were in serious financial difficulties and were retrenching staff. The commercial viability of an existing line of QXR SRO developed under experimental conditions in the QX affected Georges River by NSW DPI was unknown and farmers were reluctant or unable to invest in unproven technology. The QXR SRO had not been formally assessed in an estuary other than the Georges River. No data were available for the Hawkesbury River farmers regarding the condition, and hence the marketability, of QXR SRO that had been exposed to the QX disease agent. Mortality and growth performance of the QXR SRO in the Hawkesbury River was not known. Hawkesbury River farmers needed this information on the performance of the QXR SRO in a commercial context to enable cultivation of SRO to continue in this estuary beyond 2005. Prior to the outbreak of QX disease the Hawkesbury River in 2004 the estuary was the third largest oyster producing estuary in NSW with an annual farm gate production of \$3.6M (10.1 million oysters) and 23 farming businesses directly employing around 50 people.

### **3. OBJECTIVES**

1. To assess under normal commercial farming practice the commercial viability of selectively bred fourth-generation Georges River (Lime Kiln Bar) breeding line of QX resistant Sydney rock oysters (QXR SRO) on QX disease affected oyster leases in the Hawkesbury River NSW.
2. To assist the uptake by Hawkesbury River oyster farmers of handling and growing technology necessary for the commercial scale cultivation of small hatchery produced oyster spat.

## 4. METHODS

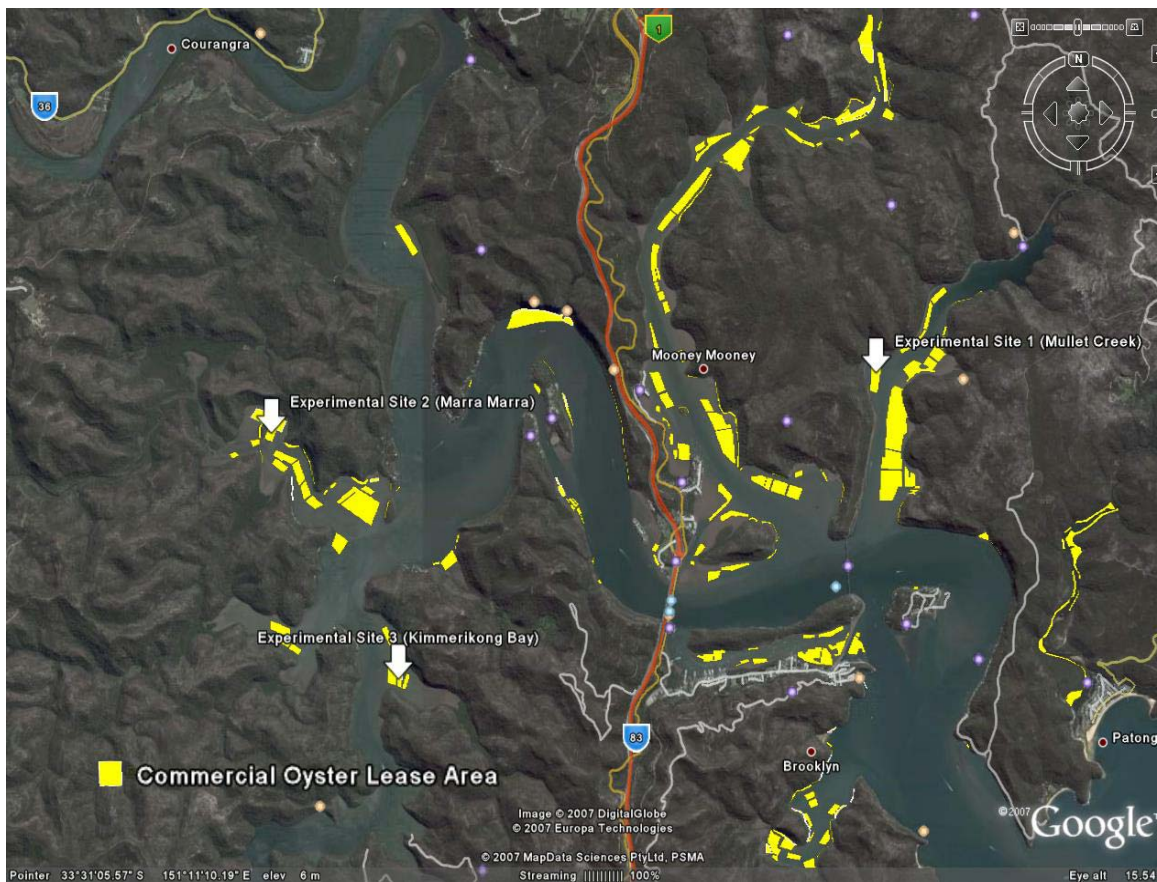
### 4.1 Survival of QXR SRO on QX Affected Hawkesbury River Oyster Leases

To assess the survival of QXR SRO in the Hawkesbury River, QXR and Control oysters were placed at three QX affected Hawkesbury River oyster leases. The parents of QXR oysters were Line 1 (Nell & Perkins 2006) oysters that had been selected for QX disease resistance at Lime Kiln Bar in the Georges River (34° 00'S; 151° 10'E) over four generations. Control oysters were bred from non-selected parents collected from Wallis Lake (32° 11'S; 152° 30'E) and Port Stephens (32° 42'S; 152° 10'E) in December 2005. Ripe broodstock for both lines were spawned synchronously in January 2005 at Never Fail Bay, Georges River. Larvae were transferred from Georges River to the Port Stephens Fisheries Centre (PSFC) bivalve hatchery in accordance with quarantine protocols to minimise QX disease transfer. Polymerase Chain Reaction (PCR) testing for the presence of *Marteilia sydneyi* DNA was conducted on larval samples transferred from Georges River and results of this testing were negative. The larvae and spat were reared at the PSFC bivalve hatchery until April 2005 and spat were approximately 3 mm in size, at which time they were transferred to fine mesh (1.6 mm) hardwood seed trays (1.8 m × 0.9 m) for nursery rearing in Port Stephens.

In July 2005, both oyster types were graded to obtain oysters with a shell length greater than 8 mm but less than 10 mm. The whole weight (mean ± SD) of QXR and Control oysters at this stage was 0.09 ± 0.005 g and 0.11 ± 0.006 g, respectively. QXR and Control oysters were then transported to the Hawkesbury River to commence the experiment.

To provide a notional range of increasing QX infection and mortality, sites were chosen at Mullet Creek (approximately 25% QX disease mortality in 2004), Kimmerikong Bay (approximately 50% QX disease mortality in 2004) and Marra Marra Creek (100% QX disease mortality in 2004), based on previous QX surveys conducted by NSW DPI in cooperation with Dr Rob Adlard from the Queensland Museum (Unpublished data). Experimental oysters were stocked onto plastic mesh trays (XL6 Aquatray, Tooltech Pty Ltd, Carole Park QLD, Australia). At each site there were four replicate trays of QXR oysters and four replicate trays of Control oysters and each replicate tray contained 400 oysters. Additional QXR SRO oysters were taken to the Hawkesbury River at this time for assessment of meat condition at Kimmerikong Bay oyster leases (Section 4.3) and for commercial assessment of QXR oysters on QX affected Hawkesbury River oyster leases (Section 4.4).

Survival and growth measurements were done in: October 2005; January 2006; April 2006; July 2006; November 2006; February 2007; and May 2007. On each sampling date, the whole weight and shell height of 40 oysters were measured from each tray. The number of dead oysters and live oysters were counted in each tray to calculate the cumulative mortality percentage. All trays were washed clean of mud using a high pressure water spray and visible overcatch and fouling organisms were chipped from the experimental oysters with a metal file on each sampling date. Oysters were exposed to two consecutive QX infection periods in the late summer to early autumn period of 2006 and the late summer to early autumn period of 2007.



**Figure 1** Map of the Hawkesbury River Oyster lease areas indicating the location of QX resistance assessment sites, (Site 1) Mullet Creek, (Site 2) Marra Marra Creek and (Site 3) Kimmerikong Bay.

#### 4.2 Infection by the QX Parasite of SRO Held on Kimmerikong Bay Oyster Leases

The confirmation of QX infection was restricted to one site due to financial limitations associated with the high cost of QX parasite detection. The Kimmerikong Bay site was chosen due to the high prevalence of QX infection and mortality at this site during 2004–2005. Additionally, Kimmerikong Bay is a commercially important SRO harvest area.

To confirm that both oyster types maintained at this site were exposed to QX disease, the timing and level of QX infection was determined by the sacrificial assessment of QX “free” SRO maintained at the site over the previously observed infective period, December to April (NSW DPI, unpublished data). The sacrificial oysters were sourced from a commercial grower in the Crookhaven River NSW, where previous QX surveys (PCR and imprint cytology) have not detected the QX parasite in commercial oyster crops. To confirm QX “free” status of the sacrificial stock, the stock was also sub-sampled for the presence of the QX parasite prior to placing the stock at Kimmerikong Bay.

From December 2005 to June 2006, 50 sacrificial SRO were placed at Kimmerikong Bay approximately every 2 weeks where they were maintained for approximately four weeks. This provided a two week overlap between samples to allow for sufficient progression of QX infection prior to the sample batch being sacrificed and examined microscopically for the presence of the QX

parasite by imprint cytology (Kleeman et al., 2003). The assessment of the level of QX infection in the sacrificial oysters was determined by veterinary staff from NSW DPI Elizabeth Macarthur Agriculture Institute (EMAI), Camden NSW, Australia.

From December 2006 to June 2007, 50 sacrificial SRO were placed at Kimmerikong Bay approximately every three weeks where they were maintained for approximately six weeks. The assessment of the level of QX infection in the sacrificial oysters was determined by Mr John Stubbs (Hawkesbury River oyster farmer) in cooperation with Ms Kristy Guise and Mr Peter Coad (Environmental Section, Hornsby Shire Council). Mr Stubbs, Ms Guise and Mr Coad were trained in sample preparation and QX parasite identification by NSW DPI EMAI veterinary staff with further technical and diagnostic verification assistance provided by Dr Rob Adlard of the Queensland Museum.

#### **4.3 Assessment of Visual Meat Condition of QXR SRO Held at Kimmerikong Bay**

At Kimmerikong Bay, sacrificial QXR SRO stocks were also maintained to enable farmers to undertake an assessment of commercial marketability of the stock exposed to the QX parasite. To determine commercial marketability, 100 randomly selected QXR SRO survivors were opened and visually examined by participating oyster farmers on 10 May 2006 and 24 April 2007. To investigate any relationship between QX infection and marketable condition, imprint cytology was performed on 50 of the randomly selected QX Resistant SRO.

#### **4.4 Farmer Assessment of the Performance of QXR SRO Grown on QX Affected Hawkesbury River Oyster Leases**

Approximately 200,000 QXR SRO spat were also allocated to Hawkesbury River oyster farmers to enable them to self-assess the performance of the stock under routine culture methods used on their leases.

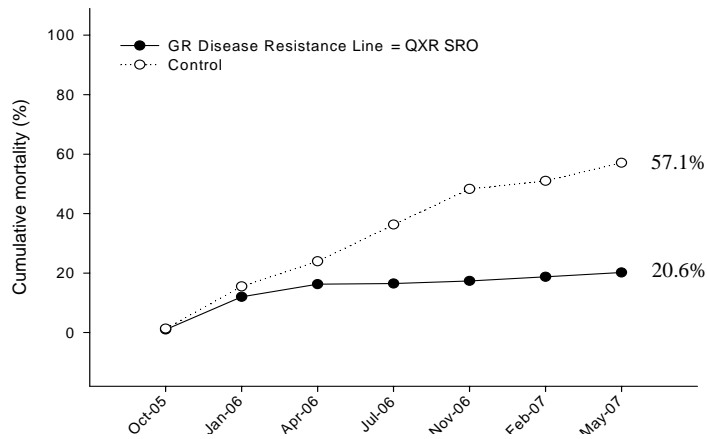
The spat were produced at the NSW DPI PSFC bivalve hatchery and supplied to participating farmers at range of sizes (3-15 mm) in specialised 1.5 mm, 3.0 mm and 6.0 mm mesh single seed oyster trays supplied by NSW DPI. NSW DPI also provided ongoing technical advice and field assistance regarding the maintenance of the spat until they reached a size that enabled them to be transferred to commercial oyster trays in normal use in the Hawkesbury River prior to the QX outbreak.

During the course of the assessment, DPI staff have demonstrated grading and spat handling procedures to Hawkesbury River oysters farmers and have worked closely with the farmers in the maintenance of the small hatchery reared QXR SRO on commercial leases.

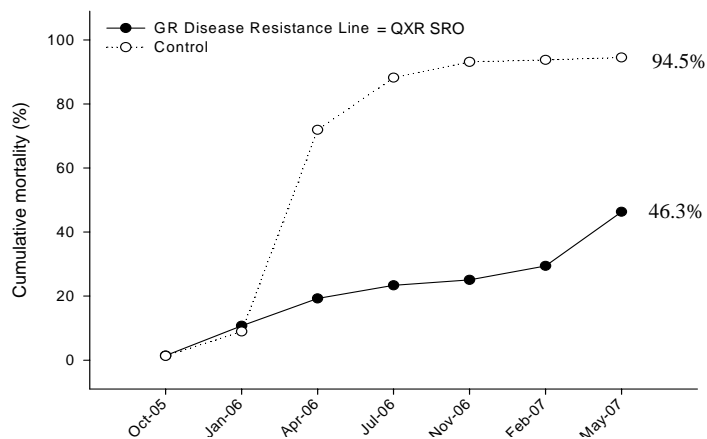
## 5. RESULTS

### 5.1 Survival of QXR SRO on QX affected Hawkesbury River Oyster Leases

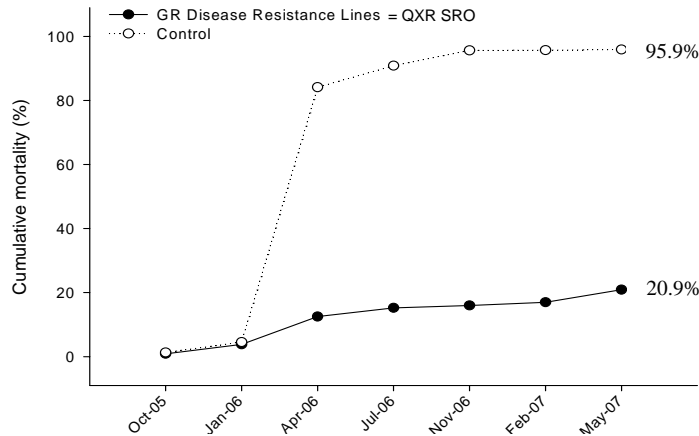
The cumulative mortality rates of QXR and Control oysters stocked at Mullet Creek, Marra Marra and Kimmerikong Bay are summarised graphically in Figures 2, 3 and 4 respectively. Between January and April 2006, major mortality of Control oysters occurred at Marra Marra (69%) and Kimerikong Bay (83%) compared to the mortality of QXR oysters which only suffered 10% and 9%, respectively. Over the same period at Mullet Creek the mortality of Control oysters was 9% which was slightly higher than the 3% measured for the QXR oysters.



**Figure 2** Mortality of oysters at Mullet Creek, Hawkesbury River, New South Wales, October 2005 to May 2007.

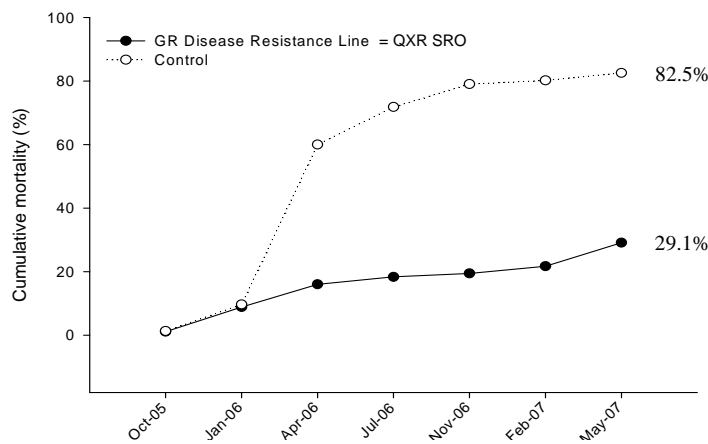


**Figure 3** Mortality of oysters at Marra Marra Creek, Hawkesbury River, New South Wales, October 2005 to May 2007.



**Figure 4** Mortality of oysters at Kimmerikong Bay, Hawkesbury River, New South Wales, October 2005 to May 2007.

The cumulative mortality rates of QXR and Control oysters stocked at all sites are shown in Figure 5. After exposure to two consecutive QX infection periods at QX affected Hawkesbury River leases the cumulative mortality was 82.5% for Control oysters and 29.1% for QXR oysters. This cumulative mortality measurement only takes into account oysters that died quickly from the second QX disease exposure (February to April 2007, Figure 6). The experiment was not continued beyond May 2007 for two reasons: firstly, QXR oysters were of a marketable size; and secondly, due to the low numbers of Control oysters still alive in the experimental trays.

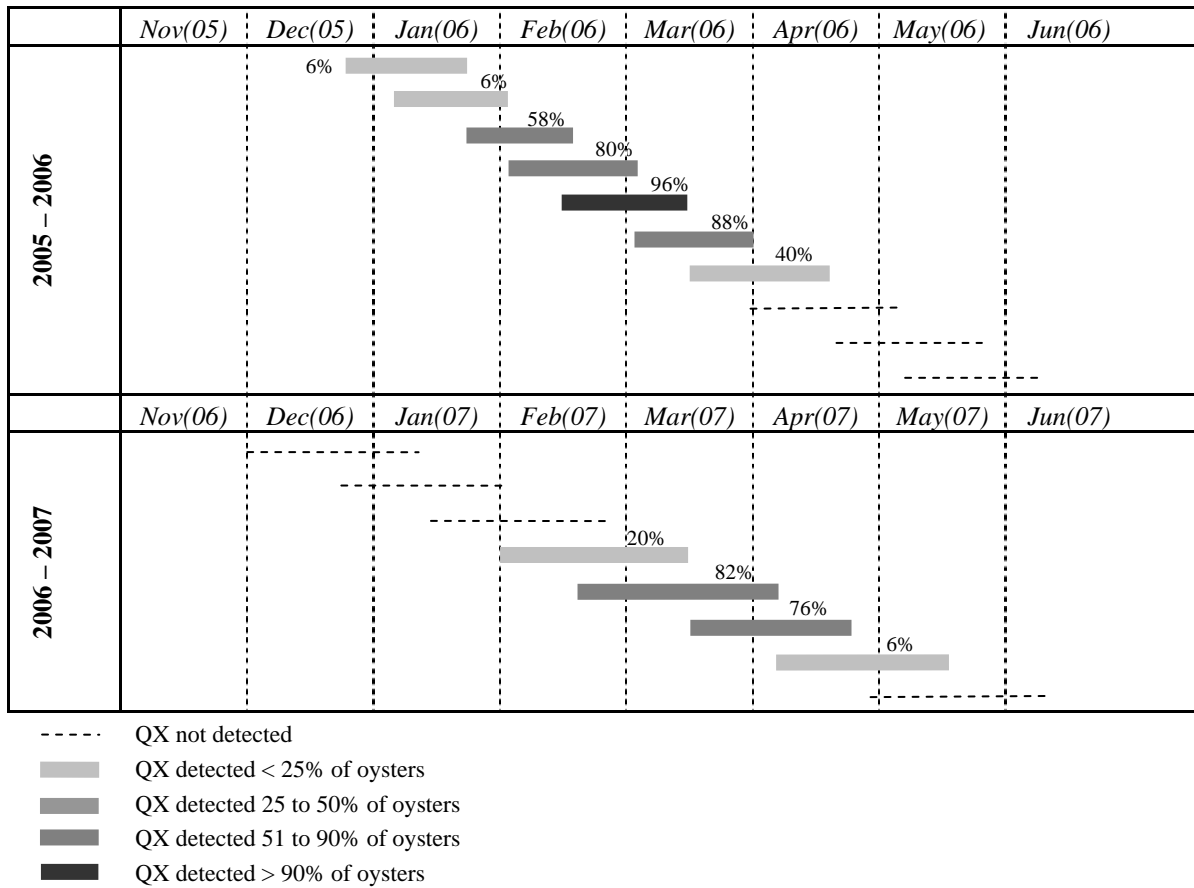


**Figure 5** Mortality of oysters (at all sites) in the Hawkesbury River, New South Wales, October 2005 to May 2007.

**5.2 Infection by QX Parasite of SRO Held on Kimmerikong Bay Oyster Leases**

Infection of QX naïve Crookhaven River stock held at Kimmerikong Bay was observed between late December 2005 and late March 2006, with peak QX infection (96%) observed in samples

deployed between mid February and mid March 2006. In 2006/07 QX infection was observed between early February and mid March 2007, with peak QX infection (82%) observed in samples deployed between mid February and early April 2007 (Figure 6).



**Figure 6** Temporal infection rate of QX naïve Sydney rock oysters held at Kimmerikong Bay during the 2005-2006 and 2006-2007 infective periods. QX naïve SRO were sourced from Crookhaven River (Crookhaven River has no previous history of the presence of the QX parasite *Marteilia sydneyi*).

**5.3 Assessment of Visual Meat Condition of QXR SRO Held at Kimmerikong Bay**

The visual assessment of meat condition of QXR oysters was conducted at the Mooney Mooney oyster depot on 10 May 2006 and 24 April 2007 by Hawkesbury River oyster farmers. The results of the assessment are summarised in Table 1 below.

The QX parasite, *Marteilia sydneyi*, was not detected by imprint cytology in the digestive gland of any of the QXR survivors assessed by oyster farmers on 10 May 2006 and on 24 April 2007.

Assessment Date	Average Whole Weight (g ± SD)	Age (months)	% Acceptable Visual Condition	% Poor Visual Condition	QX Infection
10 May 2006	34.4±5.6	15 months	84.0	16.0	Not detected
24 April 2007	68.9 ± 11.9	26 months	87.0	13.0	Not detected

**Table 1** Visual meat condition, mean whole weight (g ± SD) and QX infection rate of QXR SRO survivors maintained on a commercial oyster lease at Kimmerikong Bay between May 2005 and May 2007. QX infection was determined by digestive gland imprint cytology of a random sample of 50 of the 100 oysters assessed.

#### 5.4 Farmer Assessment of the Performance of QXR SRO Grown on QX Affected Hawkesbury River Oyster Leases

Prior to the assessment of Kimmerikong stock on 24 April 2007 (Section 4.3) the farm trial stock had been pooled among three remaining participating farmers. These farmers reported excellent growth and survival of the QXR SRO under normal Hawkesbury River farming conditions. The majority of QXR SRO held on prime Hawkesbury River fattening leases had been sold by 24 months of age (J. Stubbs and L. Wadham, pers. com.). Oysters held in Patonga Creek had also been sold for human consumption by 24 months but had been slower to reach a marketable size (B. Alford, D. Witchard, pers. com.). Participating farmers had no difficulty in adopting small single seed management practices prior to transferring stock to the traditional tray growing technology used in the Hawkesbury River (J. Stubbs, L. Wadham, R. Richards, B. Alford, D. Witchard, pers. com.). Hawkesbury River oyster farmers were able to apply the principals of small single seed oyster management to the development of a flexible and cost effective floating bag single seed system for use on their leases. This system was developed to accommodate small (3 mm shell height) hatchery reared triploid Pacific oyster spat that due to NSW DPI Biosecurity requirements had to be imported into NSW directly from the controlled environment of a Tasmanian hatchery.

## 6. DISCUSSION

During the course of the assessment, QXR and Control SRO maintained continuously at Kimmerikong Bay were exposed to two consecutive QX infection events. The first of these events occurred between late January 2006 to late March 2006; and, the second between late February to late April 2007 (Figure 6). These events resulted in peak QX infection rates in the sacrificial QX naïve SRO of 96% and 82% respectively (Figure 6). This provided an excellent opportunity to assess the performance of QXR and Control oysters under conditions of heavy QX infection.

Following the peak infection period observed at Kimmerikong Bay in 2006, cumulative mortality of QX naïve Control oysters at this site had reached 84.1% by April 2006 compared to 12.5% observed for QXR oysters (Figure 4) and by May 2007 cumulative mortality of the QX naïve Control oysters had progressed to 95.9%, compared to 20.9% in the QXR oysters. A significant rate of annual unexplained background mortality is to be expected under normal oyster farming conditions. Unexplained background mortality rates of 10% over 17 months have been reported by Nell et al. (1996) and 18% over 21 months by Hand et al. (2004) in SRO grown under experimental conditions on commercial oyster leases in other estuaries. Annual unexplained mortality rates of around 10% in commercially grown SRO in the Hawkesbury River prior to the QX outbreak in 2004 were considered to be normal if not conservative by Hawkesbury River oyster farmers (J. Stubbs and R. Moxham, pers. com.). The cumulative mortality of 20.9% for QXR oysters over the 22 months deployment period at Kimmerikong Bay is therefore in line with normal commercial expectations of Hawkesbury River farmers prior to the outbreak of QX and compares favourably with that observed under experimental conditions in other estuaries.

The resilience of the QXR oysters to QX infection is further demonstrated by the fact that there were high levels of mortality in both QXR and Control oysters at all sites prior to the first QX infection period in late January 2006. The QXR oysters' resilience to QX infection is also reinforced by the fact that the QX parasite was not detected in the digestive gland of any of the 50 oysters randomly sampled from the 100 QXR oysters from Kimmerikong Bay assessed by farmers on 10 May 2006 and 24 April 2007.

The higher than normal mortality of QXR oysters measured at Marra Marra Creek between February and May 2007 was attributed to heat kill and an infestation of a predatory flatworm observed at that site. The lower level of mortality in Control oysters measured at Mullet Creek is in line with the lower incidence of QX that was observed in the more marine dominated areas of the Hawkesbury River, downstream of the Hawkesbury River Bridge, during 2004 and 2005 (NSW DPI, unpublished data).

In May 2006 Hawkesbury River oyster farmers undertook the first visual assessment of 100 randomly selected QXR SRO from Kimmerikong Bay. At this stage oysters had been exposed to QX disease, cumulative mortality in Control oysters had reached 84.1% and QX infection rates as high as 96% had been confirmed in the overlapping monthly batches of QX naïve SRO that were deployed at the site (Figure 6). The farmers assessed 84% of these oysters to be in good visual meat condition, while 16% were assessed as being in poor condition. At the time of assessment the sample of oysters had reached a whole weight (mean  $\pm$  SD) of  $34.4 \pm 5.6$  g and gonad development was observed in all oysters. As the QX parasite was not detected in the digestive gland of any of the oysters assessed to be in poor condition by oyster farmers it is thought that the poor condition observed was linked to patchy spawning activity that may have occurred prior to the assessment. The SRO is known to spawn serially over the summer and autumn months.

By the time of the second farmer assessment on 24 April 2007 approximately 95% of Control oysters and 20% of the QXR oysters had perished at Kimmerikong Bay. At this stage the oysters were 26 months of age and had undergone the second QX infection period in which QX infection rates as high as 82% had been observed in the overlapping six weekly batches of QX naïve SRO that were deployed at the site (Figure 6). When assessed the sample of oysters had reached a whole weight (mean  $\pm$  SD) of  $68.9 \pm 11.9$  g and well advanced gonad development was observed in all oysters. The farmers assessed 87% of these oysters to be in good visual meat condition, while 13% were assessed as being in poor condition. As in the previous year the QX parasite was not detected in the digestive gland in any of the oysters assessed to be in poor condition and it is thought that the poor condition observed was again linked to patchy spawning activity that may have occurred in the QXR SRO prior to the assessment.

The premium market “Plate Grade” threshold for SRO is considered to be 50 g whole weight (Nell and Perkins, 2006). At the second assessment the random sample of Kimmerikong Bay QXR oysters had reached the premium market “Plate Grade” threshold in under 26 months which was in line with growth rates reported by Nell and Perkins (2006) for third-generation QXR Lime Kiln Bar oysters grown in QX affected areas of the Georges River, NSW. Prior to the QX outbreak in the Hawkesbury River in 2004, wild caught SRO could take between three to four years to reach the “Plate Grade” threshold (J. Stubbs and R. Moxham, pers. com.). The commercial marketability of the QXR oysters grown on QX affected Hawkesbury River oyster leases is demonstrated by the fact that the majority of the QXR oysters supplied to Hawkesbury River farmers by NSW DPI had been sold for human consumption prior to the assessment that was conducted in April 2007.

During the course of the trial Hawkesbury River oyster farmers quickly grasped the principals and techniques required to handle small hatchery produced oyster spat. By late 2005 they had developed a floating bag system specifically to accommodate small (approximately 3.0 mm) triploid Pacific oyster spat imported from Tasmanian oyster hatcheries for on-growing on QX affected leases in the Hawkesbury River. This system has the potential to compliment the fine mesh tray system commonly used by SRO farmers in other NSW estuaries. Most Hawkesbury River farmers now have demonstrated proficiency in handling small hatchery reared oyster spat and have a substantial knowledge base that can be passed on to new entrants to the oyster industry in the Hawkesbury River.

## **7. BENEFITS**

The benefits of the results of this proof of concept trial will flow directly to Hawkesbury River oyster farmers as well as SRO farmers in other estuaries affected by recurrent outbreaks of QX disease. These estuaries include, Georges River and Botany Bay, Macleay River, Clarence River, Richmond River and Tweed River in NSW and Morton Bay in QLD.

The benefits of the results of the proof of concept trial will also flow to oyster farmers in other estuaries in NSW where the QX parasite is present and where disease outbreaks have not been observed or reported by providing a proven oyster farming option should QX disease outbreaks occur in the future. The results of the proof of concept trial will also enable oyster farmers in QX at risk estuaries to confidently take proactive steps to minimise the affects of any future QX outbreaks by stocking their leases with QXR SRO.

The results of the proof of concept trial will be of benefit to local communities at QX affected estuaries by providing confidence to existing oyster farmers to invest in QXR SRO to improve the long term security and financial viability of their oyster farming businesses in these areas. This will also have flow on affects to local economies and employment. Improved long term security and financial viability has the potential to also reduce the call on public funds to undertake remediation of oyster lease areas where oyster farming businesses were likely to fail in the future due to the impact of QX disease outbreaks.

## **8. FURTHER DEVELOPMENT**

The results of the proof of concept trial will be made known to existing NSW oyster farmers and new entrants to the industry via NSW DPI aquaculture extension activities and the NSW Aquaculture Newsletter. The results will also be made available to the Select Oyster Company who manage the intellectual property associated with the QXR SRO for their dissemination.

## 9. PLANNED OUTCOMES

The proof of concept trial has demonstrated that;

- survival of QXR SRO on QX affected Hawkesbury River oyster leases is in line with that reported by Nell and Perkins (2006) for QXR SRO in the Georges River and is also in line with normal historical background mortality rates observed by Hawkesbury River oyster farmers prior to the outbreak of QX in the River in 2004;
- QXR SRO grown on QX affected Hawkesbury River oyster leases reached the premium market “Plate Grade” threshold of 50 g whole weight in approximately 24 months which is in line with growth rates reported by Nell and Perkins (2006) for QXR SRO grown in the QX affected areas of the Georges River. This is quicker than would normally be expected for wild caught SRO in the Hawkesbury River;
- exposure to the QX parasite does not detrimentally affect the visual meat condition and therefore commercial marketability of the QXR SRO grown on QX affected leases in the Hawkesbury River; and
- Hawkesbury River oyster farmers were able to quickly grasp the principals and techniques required to handle small hatchery produced oyster spat and adapt these principals to suite Hawkesbury River growing conditions.

## 10. CONCLUSIONS

The proof of concept trial has demonstrated that the survival of QXR SRO on QX affected Hawkesbury River oyster leases is in line with that reported by Nell and Perkins (2006) for third-generation QXR SRO grown on QX affected oyster lease in the Georges River. As unexplained background mortality of SRO on commercial oyster leases has been reported to be around 10% per annum (Nell et al., 1996; Hand et al., 2004) and as mortality of around 10% was regarded as normal if not conservative by Hawkesbury River oyster farmers (J. Stubbs and R. Moxham, pers. com.) prior to the QX outbreak in 2004, the performance of the QXR SRO on heavily QX affected Hawkesbury River oyster leases is considered to be commercially viable. The growth rates observed in the QXR SRO which reached “Plate Grade” in a around 24 months, albeit on oyster leases without competing SRO stock, is considerably quicker than the three to four years required by wild caught SRO to reach the premium market “Plate Grade” threshold prior to the QX outbreak in 2004. The quicker turn around of faster growing QXR SRO on Hawkesbury River oyster leases offers an opportunity for significant savings and rates of return to be made by oyster farmers from Hawkesbury River oyster lease areas. Further, the proof of concept trial has demonstrated that the exposure of QXR SRO to high levels of the QX parasite does not detrimentally affect the visual meat condition and therefore commercial marketability of the QXR SRO. This has alleviated the concern expressed by Hawkesbury River oyster farmers that surviving QXR SRO may not have been commercially marketable due to the possibility of poor visual meat condition associated with a non-lethal QX infection. These results further reinforce the work of Dr John Nell in developing the QXR SRO strain (FRDC Project Number 96/357) and will assist in promoting the advantage of QXR SRO and the uptake of QXR SRO by the NSW and QLD oyster industries.

The proof of concept trial has enabled NSW DPI staff to work closely and regularly on farm with Hawkesbury River oyster farmers in demonstration of single seed oyster production technology. This initially involved the handling of small hatchery reared seed and progressed to the maintenance of QXR and Control SRO stocks monitored by NSW DPI, as well as the maintenance of their allocated QXR SRO. This enabled farmers to quickly grasp the principals and techniques required to handle small hatchery produced oyster spat and adapt these principals to suite Hawkesbury River growing conditions. This was demonstrated by the ability of the Hawkesbury River oyster farmers to quickly adapt this technology in the development of a floating basket system for the nursery cultivation and management of small (3.0 mm) single seed triploid Pacific oysters which, due to NSW DPI Biosecurity requirements, had to be imported directly from the controlled environment of a Tasmanian shellfish hatchery. Further, the close contact and cooperation between NSW DPI staff, Hawkesbury River oyster farmers and the environmental section of Hawkesbury Shire Council that occurred during the proof of concept trial has lead to QX disease identification and surveillance methodology that can be done by the Hawkesbury River oyster industry. This will assist in the future management of QX disease in the Hawkesbury River.

The positive, cooperative and open approach taken by Hawkesbury River oyster farmers in dealing with the QX disease outbreak and the knowledge and skills they have developed since the detection of QX disease in 2004 will provide a valuable and accessible resource available to other oyster farmers in NSW and Queensland.

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## **11. APPENDICES**

### **Appendix 1 - Intellectual Property**

There is no intellectual property arising directly from this proof of concept trial. Intellectual property regarding QX resistant Sydney rock oysters used in this proof of concept trial is covered under FRDC Project (Number 2003/209).

### **Appendix 2 – Staff**

NSW DPI

Dr John Nell, Principal Research Scientist

Mr Steve McOrrie, Aquaculture Extension Officer

Dr Michael Dove, Scientific Officer - Molluscs

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