Selective breeding for disease resistance and fast growth in Sydney rock oysters

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5.2. Nell, J.A., Hand, R.E, 2003. Evaluation of the progeny of second generation Sydney rock oyster Saccostrea glomerata (Gould, 1850) breeding lines for resistance to QX disease Marteilia sydneyi. Aquaculture. *24*

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I thank the Fisheries Research and Development Corporation (FRDC) for partial funding of the project, Ben Perkins for maintaining farming equipment and the oyster farmers in Port Stephens and Georges River for making their leases available for this study. Special thanks are due to Bob and Len Drake for handling oyster trays and looking after the oysters whilst out on the leases in Georges River.

NON-TECHNICAL SUMMARY

96/357	Selective breeding for disease resistance and fast growth in Sydney rock oysters

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OBJECTIVES:

1. Evaluation of resistance of the progeny of 2nd generation Georges River disease resistance lines to QX disease and winter mortality against controls.
2. Evaluation of the growth rates of the progeny of 3rd generation Port Stephens selection line diploids and triploids against non-selected diploid and triploid controls.

NON-TECHNICAL SUMMARY:

Outcomes achieved:

1. A 29% reduction in mortality from QX disease at Lime Kiln Bar, Georges River after two generations of selection for disease resistance.
2. Triploid progeny of a 3rd generation Port Stephens breeding line were 74% heavier than controls. These triploids reached market size (> 50 g whole weight), at 28 months of age versus 38 months past settlement for the non-selected diploid controls.

Both objectives of the project were met. After two generations of selection for QX disease resistance in Georges River, NSW mortality among progeny from the most improved breeding lines was 63% compared with 92% for progeny of non-selected controls. This is a reduction in mortality of 29% after only two generations of selection. These oysters were also 26% heavier on average than the control group (Fig. 1). QX survivors still host the QX parasite *Marteilia sydneyi*. Because of the overwhelming effect of mortality from QX disease any improvement in resistance to winter mortality *Mikrocytos roughleyi* was masked. However, it is expected that selection for resistance to winter mortality can be just as successful as that for QX disease.

Selection for fast growth has also been very successful. The triploid progeny of a 3rd generation breeding line reached market size (50 g whole weight) 10 months earlier than the diploid progeny of a non-selected control group of oysters (Fig. 2). At the end of the study in November 2002, when oysters were 3 years and 2 months old, the mean whole weight of diploid controls, triploid controls, diploid breeding line and triploid breeding line were 52, 71, 63, and 90 g respectively. The growth advantages of triploidy and selective breeding were synergistic.

KEYWORDS: Sydney rock oyster, triploidy, selective breeding, disease resistance, fast growth

Figure 1. Photograph of progeny of the most improved 2nd generation Georges River breeding line (line 3) (left) with control (right) at Lime Kiln Bar at the end of the experiment (July 2000 – February 2002). The breeding line and control oysters had a mean weight of 48 and 38 g and a cumulative mortality of 63 and 92% respectively.

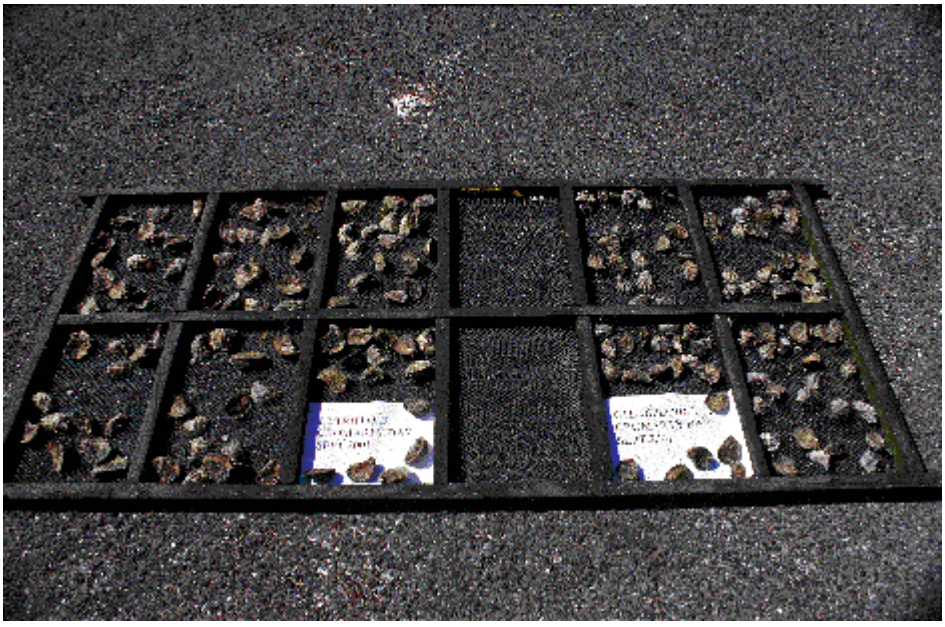

Figure 2. Photograph of triploid progeny of 3rd generation Port Stephen breeding line (38 g) with control (24 g) grown at Cromarty Bay, Port Stephens at an age of two years in September 2001.

1. BACKGROUND

A breeding program for the Sydney rock oyster, *Saccostrea glomerata*, was established by NSW Fisheries in 1990 (Nell et al., 2000). Four breeding lines selected for fast growth were maintained in Port Stephens, NSW, Australia (Nell et al., 1996; Nell et al., 1999). Four breeding lines were also maintained in the Georges River, NSW, Australia, to select for fast growth and resistance to winter mortality (Nell et al., 2000) caused by the protistan parasite *Mikrocytos roughleyi* (Farley et al., 1988). Winter mortality was first observed in Georges River in the early 1920's (Roughley, 1926). The taxonomy of *M. roughleyi* is under review and it is currently thought that it should be grouped in the *Bonamia* genus (Cochennec-Laureau et al., 2001).

Selection for weight gain in Port Stephens has been very successful. A mean increase of 18% in whole weight for the progeny of the four 2nd generation breeding lines, represents a reduction in time to market size (50 g whole weight) of 5 months out of the usual 3½ years (Nell et al., 1999). A 6 months reduction in time to market for triploid Sydney rock oysters was reported by Hand et al. (1998). It was thought to be of commercial interest to determine if the fast growth of selective breeding and triploidy were additive.

Progress in selection for fast growth and winter mortality resistance in Georges River was severely interrupted when QX disease occurred in 1994 (Adlard and Ernst, 1995). This disease is caused by a second protistan parasite *Marteilia sydneyi* (Perkins and Wolf, 1976) and it had such a devastating effect on the breeding lines that 85% of all oysters died at two out of three sites in Georges River in 1995 (Nell et al., 2000). In January 1997, the program was re-established and modified to incorporate one line selected for resistance against winter mortality, one for resistance against QX disease and one for resistance against both winter mortality and QX disease (Nell et al., 2000). The breeding lines were re-organised in this way as one site in the upper reaches of the estuary (Lime Kiln Bar) was severely affected by QX disease, the site in the middle reaches (Woolooware Bay) of the estuary was affected by both QX disease and winter mortality and the one at the lower reaches, near the mouth of the estuary (Quibray Bay) was affected by winter mortality only.

Selection for resistance to protistan parasites has been successful in other oyster species. In eastern oysters *Crassostrea virginica*, selection for resistance to *Minchinia nelsoni*, more commonly known as MSX, reduced mortality from 93% in controls to 56% after three generations (Haskin and Ford, 1979) and from 96% to 35% after six generations (Allen, 1998). A similar response was obtained with selection for *Bonamia* resistance in European flat oysters *Ostrea edulis* in France, with mortality reduced from 87% in controls to 41% after three generations of selection for disease resistance (Naciri-Graven et al., 1998).

Both QX disease and winter mortality occur seasonally, although the severity of these two diseases depends on temperature and salinity (Nell and Smith, 1988; Nell, 1993). In Georges River, NSW, infestation of oysters with QX disease parasites commences in February (summer) and most mortality occurs in April/May (autumn), whereas winter mortality infestations commence around April (autumn) and most mortality occurs in September/October (spring). In both diseases, weakened survivors may die from heat stress in late spring or early summer (November/December).

2. NEED

The NSW oyster industry has long suffered from two major diseases, namely winter mortality which occurs from Port Stephens south and QX disease which occurs mainly in southern Queensland, the northern rivers of NSW and Georges River NSW. Although, the effects of winter mortality may be severe, it tends to be restricted to the mouth of estuaries, and farmers can, at least to some extent, avoid the major impact from this disease by moving oysters upstream in autumn and holding them at a higher growing height over autumn and winter (Nell and Smith, 1988; Smith et al., 2000). This is in contrast with QX disease, first recorded in NSW in 1976 (Perkins and Wolf, 1976), where farm management practices offer no respite.

As a result of QX disease, production in the Tweed, Richmond and Clarence Rivers in northern NSW during the past 26 years, decreased from 379,200 dozen in 1974/75 to 168,504 dozen in 2000/01 - a drop of 56%, compared with a NSW statewide drop of 45% over the same period. In 1994, QX disease was first diagnosed in central NSW in the Georges River. The disease had a devastating effect on oyster production in the Georges River which declined from 1,111,171 dozen in 1993/94 to 62,000 dozen in 2000/01, a drop of 94%, as the disease in this river kills up to 90% of all Sydney rock oysters annually. As the Pacific oyster is not affected by QX disease, it has partially displaced Sydney rock oysters in Georges River and now makes up 80% of the oysters on the foreshore of the upper reaches of the river (Nell, 2001). As leases have been surrendered and shore depots converted to residential or industrial uses, production is unlikely to return to previous levels. It is therefore important that action is taken to avoid further collapses in the industry should QX spread to other estuaries.

In addition to problems with disease, the NSW industry is also competing with faster growing Pacific oysters, produced in Tasmania, South Australia and New Zealand, which on average take two years to reach market size versus 3½ years for Sydney rock oysters.

In order to make the Sydney rock oyster industry more competitive and protect it from further losses from QX disease, a fast growing disease resistant Sydney rock oyster is needed.

3. OBJECTIVES

3.1. Evaluation of resistance of the progeny of 2nd generation Georges River disease resistance lines to QX disease and winter mortality against control.

3.2. Evaluation of the growth rates of the progeny of 3rd generation Port Stephens selection line diploids and triploids against non-selected diploid and triploid controls.

4. METHODS

NSW Fisheries established the Sydney rock oyster breeding program in 1990. Initially there were four fast growth breeding lines in Georges River and another four fast growth breeding lines in Port Stephens. However, after the appearance of QX disease in Georges River in 1994, these breeding lines were reorganised into three disease resistance lines in 1997. The Georges River and Port Stephens breeding lines were bred in alternate years (Table 1) and progress evaluated (Table 2) and achievements noted (Table 3, Fig. 1).

Each breeding line comprised three trays (1.8 x 0.9 m) of oysters at each of three sites, 9 trays in all. Initial tray stocking rates are >1 000 spat/tray. Stocking densities are re-set at 50% tray coverage every 3-4months by sieving out the smallest oysters. The sieves are made from plastic trays with round holes drilled through the bottom. Sieving oysters retains the widest oysters and culls long thin or small oysters. For each generation, the 24 fastest growing oysters for each replicate tray were selected on a whole weight basis. Oysters were selected on a whole tray, of around 350 oysters, basis, i.e. approximately 7% of oysters were selected.

For each generation, 216 of the largest survivors per line are selected for breeding on a within tray basis. Four separate mass spawnings/fertilisations are used per line. Oysters are induced to spawn rather than 'strip' spawned because the quality of 'strip' spawned eggs is more variable. An additional problem of stripping eggs is that is may increase asynchrony of fertilisation, which impairs triploidy yields. As soon as an oyster commences spawning it is taken off the spawning table and placed in a separate beaker of clean seawater. For each spawning group, eggs and sperm are pooled separately before fertilisation.

The Georges River breeding lines were spawned in an oyster farmer's shed at Georges River and fertilised eggs (washed in 1 µm filtered seawater) were taken to the Cronulla Fisheries Centre at Port Hacking for rearing in one (4 500 L) tank for each breeding line. The Port Stephens breeding lines were reared in two (1 000 L) tanks for each line. Larvae were set on scallop shell chips and spat reared in upwellers, until they could be transferred to 1.7 mm plastic mesh nursery trays on leases. When spat reached a shell height of 8-10 mm the lines were re-established with 9 trays/line and experiments to evaluate performance of the breeding lines against controls on 3 mm mesh trays. When oysters were big enough they were transferred to 8 mm mesh trays to benefit from better water flow.

Table 1. Breeding times of the Sydney rock oyster breeding program

February 1990	Bred base population. Oysters were collected from Wallis Lake, Port Stephens, Hawkesbury River and Georges River.
Georges River lines	
January 1992	Bred first-generation Georges River fast growth lines to produce second-generation.
January 1994	Bred second-generation Georges River fast growth lines to produce third-generation.
February 1997	Bred first-generation (previously known as third generation) Georges River disease resistance lines to produce second generation.
January 1999 & January 2000	Bred second-generation Georges River disease resistance lines to produce third-generation.
January 2002	Bred third-generation Georges River disease resistance lines to produce fourth-generation.
Port Stephens lines	
February 1993	Bred first generation Port Stephens fast growth lines to produce second-generation.
December 1994	Bred second generation Port Stephens fast growth lines to produce third-generation.
March 1998	Bred third generation Port Stephens fast growth lines to produce fourth generation.
January 2001	Bred fourth generation Port Stephens fast growth lines to produce fifth-generation.
January 2003	Bred fifth-generation Port Stephens fast growth lines to produce sixth-generation.

Table 2. Sydney rock oyster breeding program evaluation experiments.

Georges River experiments	
February 2000 – February 2002	Evaluation of progeny of second-generation Georges River disease resistance breeding lines with controls (Nell and Hand, 2003).
Port Stephens experiments	
February 1993 – January 1995	Evaluation of progeny of first-generation Port Stephens fast growth breeding lines with controls (Nell et al., 1996).
February 1995 – May 1997	Evaluation of progeny of second-generation Port Stephens fast growth breeding lines with controls (Nell et al., 1999).
September 1999 – November 2002	Comparison of growth of diploid and triploid progeny of a third generation Port Stephens fast growth breeding line with controls (Hand et al., in press).

Table 3. Achievements of the Sydney rock oyster breeding program.

Georges River lines	
February 2002	Demonstrated a 29% per generation reduction in death from QX disease in progeny of second-generation QX disease resistance line at Lime Kiln Bar. Mortality for the control was 92% and that for the selectively bred line was 63% and selected oysters were also 26% heavier than controls (Nell and Hand, 2003).
Port Stephens lines	
January 1995	Demonstrated a 1 months reduction in time to market size (50 g whole weight) for progeny of first-generation Port Stephens fast growth breeding line as compared to non-selected controls (Nell et al., 1996).
May 1997	Demonstrated a 5 months reduction in time to market size (50 g whole weight) for progeny of second-generation Port Stephens fast growth breeding line as compared to non-selected controls (Nell et al., 1999).
November 2002	Demonstrated a 7 months reduction in time to market size (50 g whole weight) for progeny of third-generation Port Stephens fast growth breeding line as compared to non-selected controls (Hand et al., in press).
November 2002	Demonstrated that the faster growth of selective breeding and triploidy are fully additive. Triploid progeny of third-generation Port Stephens fast growth breeding line reached market size in 28 months versus 38 months for non-selected diploid controls (Hand et al., in press).

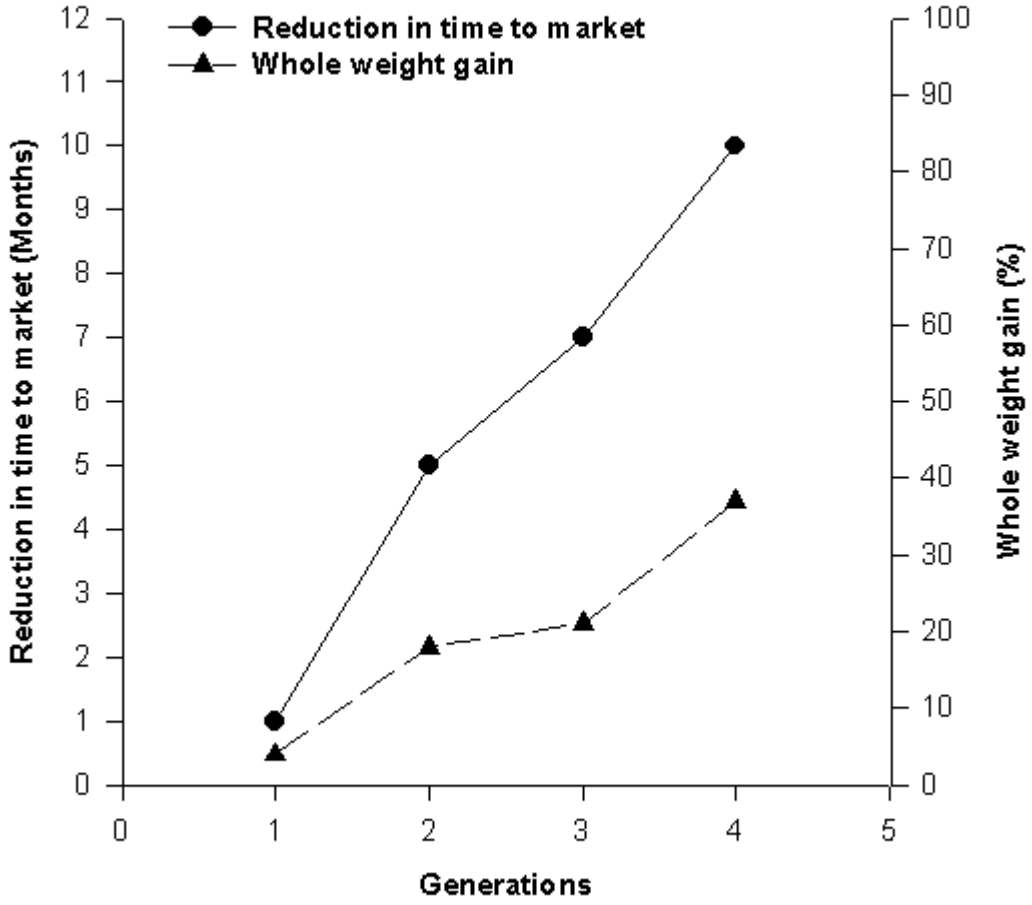

Figure 1. Gain per generation of selection for fast growth in Sydney rock oysters in Port Stephens.

5. RESULTS AND DISCUSSION

5.1 Hand, R.E., Nell, J.A., Thompson, P.A. Studies on triploid oysters in Australia. XIII. Performance of diploid and triploid Sydney rock oysters, Saccostrea glomerata (Gould, 1850) progeny from a third generation breeding line. Aquaculture.

Studies on triploid oysters in Australia. XIII. Performance of diploid and triploid Sydney rock oysters, *Saccostrea glomerata* (Gould, 1850) progeny from a third generation breeding line

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Abstract

The performance of diploid and triploid selected and control oysters was compared at three sites to determine if the improvements in growth of selected oyster line (L2) Sydney rock oysters were additive to the faster growth of triploids. After a grow-out period of 32 months, both mean whole weights and shell heights were in the order: L2 triploids > control triploids > L2 diploids > control diploids. Mean whole weights of different oyster lines were all significantly different ($p < 0.05$). A significant ($p = 0.02$) site*line interaction effect on oyster weights was also detected. On average, L2 triploids were 74% heavier than control diploids, indicating that growth improvements from selective breeding and triploidy were at least additive and could reduce the time to market by at least ten months. Oyster type had no effect on the meat condition index, percent cavity volume, percent shell weight or cumulative mortality but did affect whole weight:shell height ratios after 21 months grow-out.

Keywords: Oysters; Triploidy; Selection; Growth; Breeding

1. Introduction

Progeny from second generation Sydney rock oyster selection lines produced by NSW Fisheries at Port Stephens, NSW, Australia had previously shown improvements of 14 - 23% in mean whole weight of oysters after 18 months grow-out (Nell et al.,1999; Nell et al., 2000). With an average time to reach market size of 3½ years for unselected oysters, this improvement equates to a three-month reduction in time taken to reach market size. Oyster breeding programs have shown growth rate to be a heritable trait (Toro and Newkirk, 1990; Jarayabhand and Thavornyutikarn, 1995; Sheridan, 1997) and heritable traits that influence the physiology of oysters are known to influence growth. Bayne et al. (1999) confirmed that the "L2" Sydney rock oyster selection line produced by NSW Fisheries (23% heavier than controls), had higher ingestion rates as well as a lower cost of growth than control oysters. Selection for improved survival has also been successful in several bivalve species (Baud et al., 1997; Naciri-Graven et al., 1998). In a separate breeding program, Sydney rock oyster mortality was reduced from 86% for the controls to 64% after two generations of selection for resistance to QX disease, caused by the protistan parasite *Martelia sydneyi* (Nell and Hand, In Press).

Meiosis II triploid Sydney rock oysters have been evaluated under a broad range of conditions (Hand et al., 1998a, b; Hand and Nell, 1999). Triploids were 30-40% heavier than same-parent diploids after 2-2½ years grow-out. This more rapid growth corresponds to a reduction of approximately six months to market size. In addition, overall mortality rates of triploids were frequently less than that of diploids (Hand et al., 1998a, b). Enhanced performance of triploid bivalves is generally attributed to increased energy diversion to somatic growth due to limited gametogenesis (Tabarini, 1984; Allen and Downing, 1986; Ruiz-Verdugo et al., 2000), increased cell size and/or increased heterozygosity (Guo et al., 2001). Hawkins et al. (1994) found that the

faster growth of meiosis I triploids is due to both a reduction in gametogenesis as well as an increase in heterozygosity and its associated effects on metabolism such as a reduction in rates of whole-body protein turnover and differences in oxygen consumption. Meiosis II triploids however, are not more heterozygous than diploids (Stanley et al., 1984; Hawkins et al., 1994). Their superior growth rates are generally attributed to limited gametogenesis (Li et al., 1992) and/or increased cell size (Guo et al., 2001).

Kesarcodi-Watson et al. (2001) attributed faster growth of triploid Sydney rock oysters to reduced gametogenesis and different energy partitioning between tissue types of diploids and triploids. In contrast to lines selected for fast growth, feeding and physiological energetics were shown not to be responsible for faster growth rates of triploid Sydney rock oysters. As the pathways for improved growth of selected and meiosis II triploid Sydney rock oysters are therefore separate, the hypothesis that the improvement in growth of the selected line would be additive to that of triploids was examined. An additive response would equate to a minimum growth improvement from triploidy of 30% over the 23% improvement from the best (L2) selection line. Assuming an unselected diploid weight of "x", then:

L2 oyster weight = $x + 23\%x$
L2 triploid oyster weight = L2 weight + 30%(L2 weight) =
$x + 23\%x + 30\%(x + 23\%x) = x(1 + 0.23 + 0.30 + 0.07) = x1.60$

That is, if the growth benefits were additive, triploid L2 oysters should be approximately 60% heavier than unselected diploid oysters. In the experiment described in this paper, the growth and mortality of selected diploid, selected triploid and control (unselected) diploid and triploid oysters were compared.

In addition to oyster size, the meat condition, proportion of shell by weight and shell cavity size are all factors that influence the marketability of oysters. Production of an oyster with greater cavity volume and lighter shell could allow for greater meat volume (Nell et al., 1999). The ratio of whole oyster weight to shell height also affects the marketability of an oyster. That is, an increase in weight to height ratio will increase oyster value, as it is often associated with a more deeply cupped shell. Triploid Sydney rock oysters have been shown to differ from diploids in terms of condition index (Hand and Nell, 1999), shell cavity volume and shell weight (Nell et al., 1994). By measuring oysters in several dimensions, Landau and Guo (1999) found that triploid Pacific oysters had a more deeply cupped shell (with a presumably greater cavity volume) than diploids due to their larger shell width. After two generations of selection, results were inconclusive as to whether selected Sydney rock oysters were better in terms of meat condition, cavity volume and shell weight (Nell et al., 1999). In the present study, selected diploid, selected triploid and control diploid and triploid oysters were compared in terms of weight to height ratios, meat condition, shell weight (%) and cavity volume (%).

2. Materials and methods

2.1. Production of larvae and spat

Diploid and triploid oyster larvae and spat were produced in August 1999 in Port Stephens, NSW, Australia (32°45′S, 152°10′E). Selection line diploids and triploids were produced from third generation selection line (mass selection) oysters from the "Loose 2" line (referred to as L2) described in Nell et al. (1999). Spawned eggs from 52 L2 females were pooled and then randomly divided into two groups, one for diploids and one for triploids. Both groups were fertilized with pooled sperm from the same three L2 males. Triploid zygotes were stocked into a single 20 000 l tank and diploid zygotes were split equally into duplicate 1000 l tanks.

Control diploids and triploids were produced from oysters from Port Stephens. Eggs from 11 females were pooled and then randomly divided into two groups, one for diploids and one for triploids. Both groups were fertilized with pooled sperm from the same three non-selection line

males. Zygotes of control diploids and triploids were each split equally and stocked into duplicate 1000 l tanks.

Triploidy was induced in both control and selection line oysters using similar methods to those described in Nell et al. (1996). Fertilised eggs were treated with 1 mg/l cytochalasin B (CB) in 0.1% dimethylsulphoxide (DMSO) in 1µm (nominal) filtered sea water for 15 minutes when approximately 50% of the eggs had extruded the first polar body. Eggs were then rinsed in filtered sea water and resuspended in 0.1% DMSO for 15 minutes to remove residual CB.

Larvae were reared using standard hatchery techniques for Sydney rock oysters (Frankish et al., 1991). Following settlement, spat were transferred to outdoor upwelling units where the four lines were on-grown under the same conditions until March 2000 when they were a suitable size for establishing the field experiment.

2.2. Ploidy determination

Ploidy was determined in shelled larvae (day 5) (Nell et al., 1996), spat and adult oysters (after 21 months grow-out) by flow cytometry (Allen et al., 1994). Ploidy of spat was measured immediately prior to stocking the experiment using spat from the same size grade (see section 2.3). Three replicate groups of 30 randomly selected oysters were sampled from both the control and L2 triploids with diploid reference peaks for flow cytometry established using a mixture of control and L2 diploids. After 21 months grow-out, ploidy of the triploid lines was determined for the oysters from Cromarty Bay, one of three experimental field grow-out sites described below. The ploidy of 30 oysters from each replicate half tray (section 2.3) of control and L2 triploids was determined using flow cytometry of oyster gill tissue. A diploid reference peak for flow cytometry was confirmed after every 20 samples using a mixture of control and L2 diploids.

2.3. Experimental design and data collection for grow-out experiments

In March 2000, prior to stocking the experiment, the four lines (control diploid, control triploid, L2 diploid and L2 triploid) of spat were graded several times between 7 mm and 10 mm mesh screens to achieve a similar initial size for all lines. A random sample of 100 oysters from each line was measured for initial shell height and whole weight. Oysters were placed on mesh trays for grow-out experiments. Trays measured 0.9 m x 1.8 m and were divided into 12 sections to enable better control of oyster density which affects growth rates (Holliday et al. 1991). The experiment comprised four replicates (200 oysters per replicate) of each of the four lines of oysters randomly allocated to half of an oyster tray (two replicates per tray) placed at each of three sites. That is, there were eight complete trays at each of the three sites: Cromarty Bay, Tea Gardens and Tilligerry Creek in Port Stephens (Fig. 1). By using sectionalised trays, stocking rates of trays could be maintained at approximately 50% tray coverage throughout the experiment to reduce the effect of oyster density on growth rate (Holliday et al. 1991). At the start of the experiment and then every three months until November 2002, a random subsample of 50 oysters from each replicate was measured for whole oyster weight. The total number of live and dead oysters was counted every three months for each replicate and dead oysters removed from the trays. Due to the large size attained by triploid selection line oysters by June 2001, densities could not be maintained at 50% tray coverage without removing some of the oysters. To maintain the standard stocking density, the total number of oysters was counted for each replicate and 50% randomly removed from all treatments.

In December 2001, shell heights and whole weights of 50 oysters from each replicate were measured. Weight:height ratios were calculated for oysters reared at the Tilligerry Creek site. As weight:height ratios change with oyster size, ratios were compared for oysters of an equivalent size. Data was analysed for oysters in the initial whole weight range of 0.12 – 0.16 g and final weight range of 35 – 40 g. These weight ranges were selected as they approximated the overall mean weight taken across all four lines whilst maximising the number of samples for each line. In addition, 10 oysters randomly selected from each replicate were measured for percent shell weight,

percent cavity volume and condition indices according to the gravimetric methods of Crosby and Gale (1990):

1. Percent cavity volume = (cavity volume/whole oyster volume) x 100, where: whole oyster volume = shell weight in air (g) – shell weight in water (g) + cavity volume (g) and cavity volume = whole weight (g) – shell weight (g)
2. Percent shell weight = [shell weight (g)/whole weight (g)] x 100
3. Condition Index (CI) = dry meat weight (g) x 1000/cavity volume (g)

2.4. Statistical analyses

Ploidy data were analysed by Students t-test. Initial and final weight data and shell heights, cavity volumes and condition indices were analyzed for significant differences using a two factor (site and line) ANOVA after homogeneity of variances was confirmed using Cochran's C test. Where significant differences were detected, pairwise comparisons of means were conducted using the Student-Newman-Keuls (SNK) procedure (Underwood 1997). ANOVA was conducted using the GMAV5 for Windows software package (Institute of Marine Ecology, University of Sydney, NSW). Weight data are shown in figures as means ± 95% confidence intervals.

Data (whole weight, shell height, weight:height ratio) for oysters in the ranges selected for comparisons of weight:height ratios were analysed by a single factor (line) ANOVA. These data were analysed using SPSS for Windows Release 9.0.0 (SPSS Inc. 1998). Where significant differences were detected, pairwise comparisons of means were conducted using the SNK procedure. Homogeneity of variances was confirmed using Levene's test.

Cumulative mortality data at the end of the experiment were compared by a two factor (site and line) ANOVA. A ln (X) transformation was required to satisfy Cochran's C test for homogeneity of variances.

3. Results

3.1. Ploidy

The ploidy readings of day 5 larvae were 79.2 and 75.2% triploid for triploid control and triploid L2 lines respectively. The ploidy reading of spat used for the experiment was higher than initial larval readings with 87.8 ± 10.7% and 87.6 ± 2.1% triploid respectively (mean ± sd; n=3 groups of 30 oysters). There was no significant difference between the percent triploid in control and L2 triploid groups ($p = 1.0$) prior to stocking the experiment. After 21 months grow-out, adult oysters were 91.7 ± 4.3% and 89.2 ± 8.8% for triploid control and triploid L2 lines respectively (mean ± sd; n=4 groups of 30 oysters) with no significant difference between the percent triploid in control and L2 triploid groups ($p = 0.6$). The percent triploid did not change significantly for either control ($p = 0.5$) or L2 triploid ($p = 0.8$) lines. A small number of aneuploid oysters with around 23-26 chromosomes was detected in triploid control (1.7%) and L2 (0.8%) adult oysters but no mosaics were detected.

3.2. Size

ANOVA showed no significant effect ($p > 0.05$) of site or site*line interaction on initial whole weights of oysters. There was however, a significant effect of line on initial mean whole weights ($p = 0.004$) and on shell heights ($p = 0.000$) although variances of height data were heterogeneous ($C = 0.38$; $p < 0.01$). Post-hoc comparisons of initial weight data showed that only control diploids were significantly heavier ($p < 0.05$) than the other lines (Table 2).

Selection line, growing site and line*site interaction had a significant effect ($p < 0.05$) on final whole weight (Table 1). Final whole weights were heaviest at Tilligerry Creek, then Cromarty Bay with slowest growth at Tea Gardens (Fig. 2). At each site, final mean whole weights were all significantly different ($p < 0.05$) except for control triploids and L2 diploids at

Tea Gardens and Tilligerry Creek. Mean whole weights (in grams) across the three sites were greatest for L2 triploids (90.1 ± 1.9), then control triploids (70.5 ± 1.8), L2 diploids (62.7 ± 2.4) and control diploids (51.7 ± 1.5) (means ± se; n = 12 replicates of 50 oysters) with a weight advantage of 74% for L2 triploids over control diploids (Table 2).

3.3. Condition index and morphometric measurements

After 21 months grow-out, condition indices of the four lines were significantly affected by the site at which they were grown ($p < 0.001$) with best meat condition of oysters at Tilligerry Creek (Fig. 3). Triploid control and triploid L2 oysters had slightly higher condition indices than their diploid counterparts (averaged over three sites) but ANOVA showed there was no significant effect of oyster line or line*site interaction on condition indices ($p > 0.05$).

Similar to condition indices, both percent cavity volume and percent shell weight were unaffected by oyster line ($p > 0.05$) but were affected significantly by growing site ($p < 0.001$ for both). Greatest percent shell weights of oysters were at Cromarty Bay whilst greatest percent cavity volumes were recorded in oysters from Tilligerry Creek (Fig. 3).

Analysis of weight data within the ranges selected for comparisons of weight:height ratios confirmed that there was no significant difference between initial weights or final weights of the four lines (Table 3; $p > 0.05$). Initial weight:height ratios were similar across the four lines (Table 3) with no significant difference ($p < 0.05$) between any of the lines. Final weight:height ratios, however were significantly affected by oyster line (Table 3; $p < 0.001$) with significantly greater weight:height ratios for both the L2 diploids and triploids when compared to both control diploids and triploids ($p < 0.05$). Final weight:height ratios were in the order control diploid < control triploid < L2 diploid < L2 triploid. Final mean shell heights in the selected range were significantly affected by line ($p < 0.001$); post hoc comparisons of means showed control diploids had significantly greater ($p < 0.05$) shell heights than selected diploids and triploids.

3.4. Mortality

The data required a ln (X) transformation to satisfy Cochran's C test for homogeneity of variances ($C = 0.31$; $p > 0.05$), subsequently ANOVA showed growing site had a significant ($p < 0.001$) effect on the cumulative mortality of oysters at the end of the field trials in November 2002. However, there was no significant effect of oyster line or site*line interaction on cumulative mortality ($p > 0.05$). Mean cumulative mortalities in November 2002 (data combined from the three sites) were 18.4 ± 1.4%, 18.6 ± 2.6%, 17.4 ± 2.1% and 20.2 ± 1.9% (means ± se, n = 12 replicates) for control diploid, control triploid, selected diploid and selected triploid lines respectively.

4. Discussion

Similar to earlier experiments with triploid Sydney rock oysters (Hand et al., 1998a; 1999), initial ploidy readings of oyster larvae were lower than those for spat and adult oysters. For flow cytometry, larvae are analysed in groups of thousands of larvae rather than individually making it difficult to identify mosaics and aneuploids with a chromosome number that is close to those of diploids or triploids. This might have influenced the triploid reading, although final flow cytometry readings showed a lack of mosaics in adult oysters, indicating that mosaics are unlikely to have been responsible for the change in ploidy level. Nell et al. (1996) detected aneuploidy levels of 1-11% in day 0 larvae in triploidy induction experiments. The majority of these aneuploids fell within the chromosome number range of 22-24 (R.E. Hand, unpublished data), which is close to the diploid number of 20 chromosomes. Although aneuploids can be viable (He et al., 2000; section 3.1), their generally poor survival as larvae (pers. obs.) could account for the increase in the percent triploid in spat and adult oysters.

L2 diploids were 21% heavier than control diploid oysters at the end of the experiment. The weight advantage of triploid oysters observed in this experiment (36%) was similar to that

achieved in commercial scale farming experiments (Hand et al., 1998a). If the weight advantage in this experiment, from selection (21%) is additive to that from triploidy (36%) and assuming a diploid control weight of "x", then:

L2 oyster weight = $x + 21\%x$
L2 triploid oyster weight = L2 weight + 36%(L2 weight) =
$x + 21\%x + 36\%(x + 21\%x) = x(1 + 0.21 + 0.36 + 0.08) = x1.65$

That is, using the results from the experiment if the growth benefits were additive, triploid L2 oysters should be approximately 65% heavier than diploid control oysters. In this experiment, after a grow-out period of 32 months, L2 triploids were on average 74% heavier than control diploids. Kesarcodi-Watson et al. (2001) suggest that the growth advantage of triploid Sydney rock oysters is unlikely to be due to physiological energetics. In contrast, Bayne et al. (1999) showed that L2 selected oysters grow faster than controls, due to greater ingestion rates and a lower cost of growth. The separate pathways responsible for growth advantages of selected and triploid oysters appear to allow for at least an additive growth response when combining selection with triploidy. In fact, there seemed to be some sort of positive feedback or synergism which allowed for a greater than additive growth response when combining triploidy with selection. Under experimental conditions, L2 triploids had achieved market size (≥50 g) after only 22 months grow-out, whilst control diploids took 32 months (Table 2).

The effects of line, site and line*site interaction were significant for weights of oysters. The effect of the line*site interaction is most likely due to the environment-dependent performance of triploids relative to diploids (Shpigel et al., 1992; Allen 1995). Nell et al. (1999) found no line*site interaction effect on selected oysters; however, it would be expected that with further selection, the metabolic differences in L2 selected oysters will enable even better performance of L2 triploids at more productive sites.

Diploid Sydney rock oysters are usually marketable during summer as condition index increases in late spring/early summer with increasing water temperatures (Nell et al., 1994). Thus, with the high meat condition of diploids there is often little difference between diploids and triploids in terms of condition indices prior to spawning (Maguire et al., 1994). Oysters in this study were measured for condition indices in December (summer) prior to diploids spawning (Sydney rock oysters usually spawn between February and May (Nell, 1993)) so large differences in condition indices of diploids and triploids were not expected. In addition to this, L2 oysters were selected for weight and not for meat condition (Nell et al., 1999). Although, condition index can be affected by size of oysters (Nascimento and Pereira, 1980), condition indices were unaffected in this case, by oyster line (diploid control, triploid control, diploid L2 and triploid L2) in December. This is partially due to the good meat condition of the diploid oysters at this time of year and would be expected to differ during cooler months after spawning.

Triploid Pacific oysters had a more deeply cupped shell (with a presumably greater cavity volume) than diploids in the study by Landau and Guo (1999) due to their greater shell width. Shell weight is also frequently heavier for triploid oysters than diploids (Scarpa et al., 1996). However, when cavity volume and shell weight are expressed as a percentage of whole volume and whole weight respectively, the trends between diploids and triploids are sometimes less clear. For example, triploid Sydney rock oysters had a slightly lower cavity volume (%) than diploids in the previous study of Nell et al. (1994) and in the present study there were no apparent patterns in cavity volume (%) or shell weight (%) in different oyster lines. It may be misleading to express trends in morphometric measurements such as cavity volume and shell weight without accounting for the total size of the oyster.

After two generations of selection, results from the previous study of Nell et al. (1999) were inconclusive as to whether L2 oysters selected for whole weight were better in terms of cavity volume and shell weight. In the present study there were no apparent trends in cavity volume (%) or shell weight (%) in response to oyster line (diploid control, triploid control, diploid L2 and

triploid L2) in December 2001. L2 oysters were selected for fast growth (whole weight), not for shell weight or cavity volume (Nell et al., 1999). Nevertheless, faster growing Pacific oysters have been shown to have greater meat:shell ratios (so greater cavity volumes and smaller shell weights) than slower growing oysters (Cigarria, 1999), so it is possible that with further selection for fast growth L2 oysters will improve in meat:shell ratios. If this is so, meat:shell ratios may be improved upon more by combining further selection with triploidy as triploid Sydney rock oysters were shown by Kesarcodi-Watson et al. (2001) to allocate/store more energy in soft tissue than diploids of an equivalent size.

Despite the similar cavity volumes (%) and shell weights (%) between the four oyster lines, weight: height ratios in December 2001 appeared to have been improved by both selection and triploidy. Although larger, the weight:height ratios of triploids were not significantly different from diploids for both control and selected oysters. An improvement in weight:height ratio within a set size range without a corresponding increase in overall percent shell weight or percent cavity volume suggests that both shell weight and meat weight may be increasing by selection giving a wider or longer oyster without an increase in percent cavity volume. Selected diploids and triploids had significantly smaller shell heights than control diploids and triploids supporting the suggestion that the increase in weight:height is due to an increase in width or length of selected oysters.

During the course of this experiment, there were no outbreaks of the disease winter mortality that sporadically affects oysters in parts of Port Stephens. Having been exposed to winter mortality during the first and second generations of selection (Nell et al., 2000), there may have been some degree of selection of L2 oysters for resistance to this disease. Triploid Sydney rock oysters frequently survive the winter mortality disease better than diploids (Hand et al., 1998b), consequently, it is likely that survival would be relatively high for L2 triploids through outbreaks of the disease.

The greater than additive response in this trial suggests a degree of interaction in the mechanisms by which triploidy and selection improve growth. As stated earlier, a 74% heavier whole weight of L2 triploids compared to normal diploid oysters equates to a reduction in grow-out time of at least ten months. This growth advantage will presumably be improved upon with each subsequent generation of selection for size. In addition to a faster crop turnover, growing L2 triploids, could allow farmers to avoid keeping their oysters for a third winter on leases when oysters are more prone to the disease winter mortality (Wolf, 1977). Oyster type (control diploids, control triploids, selected diploids and selected triploids) had no effect on final meat condition index, percent cavity volume, percent shell weight or cumulative mortality.

Further research is required to investigate the performance of L2 triploids relative to diploids over the cooler months, in terms of meat condition and resistance to winter mortality. With the drop in meat condition of diploids following spawning during late summer to autumn (Nell, 1993) triploid oysters often have better meat condition during autumn and winter (Maguire et al., 1994; Hand and Nell, 1999). The design of the current experiment did not allow for sacrificial sampling during winter. Nevertheless, it would be interesting to measure the meat condition of diploids versus triploid L2 oysters throughout the year in any future experiments/trials with these oyster lines to test the hypothesis that selected triploids have seasonally superior meat condition to control diploids, control triploids and selected diploids, especially during winter. The performance in terms of resistance to winter mortality of L2 triploids relative to unselected diploids should also be examined in view of 1) the better survival of triploids over diploids through outbreaks of winter mortality (Hand et al., 1998b) and 2) the previous exposure of selection line oysters to the disease allowing for possible inadvertent selection for resistance to it.

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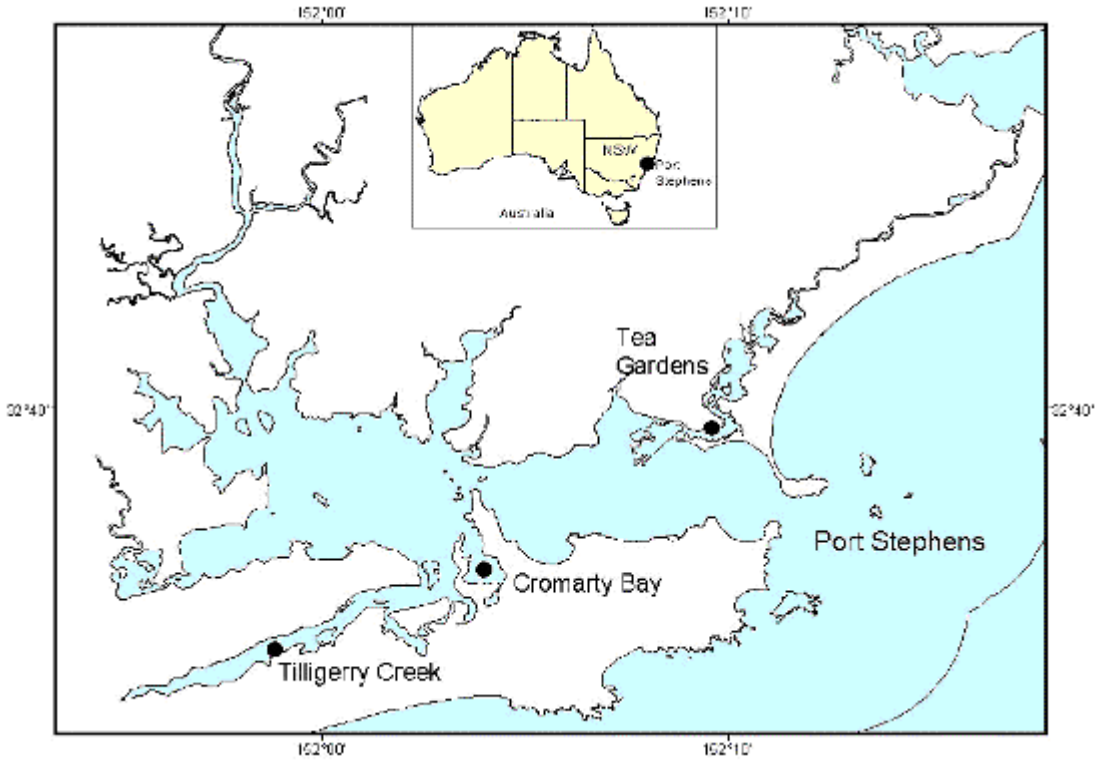

Figure 1. Location of experimental oyster leases in Port Stephens, NSW (by F. Dorman, NSW Fisheries).

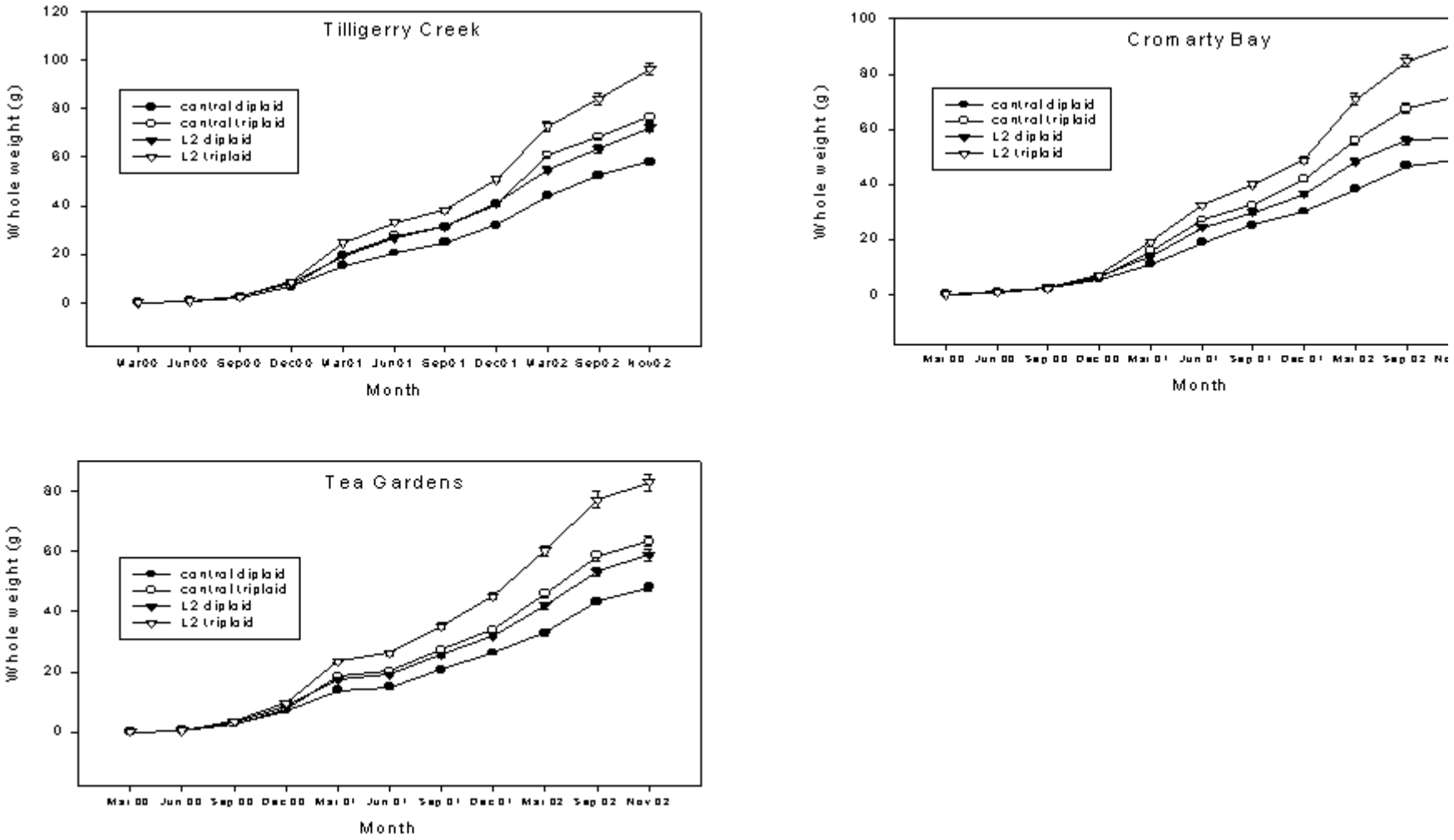

Figure 2. Whole weights of diploid and triploid control and selection line Sydney rock oysters, *Saccostrea glomerata,* at three sites in Port Stephens, NSW from March 2000 - November 2002. Mean values ± 95% c.i.

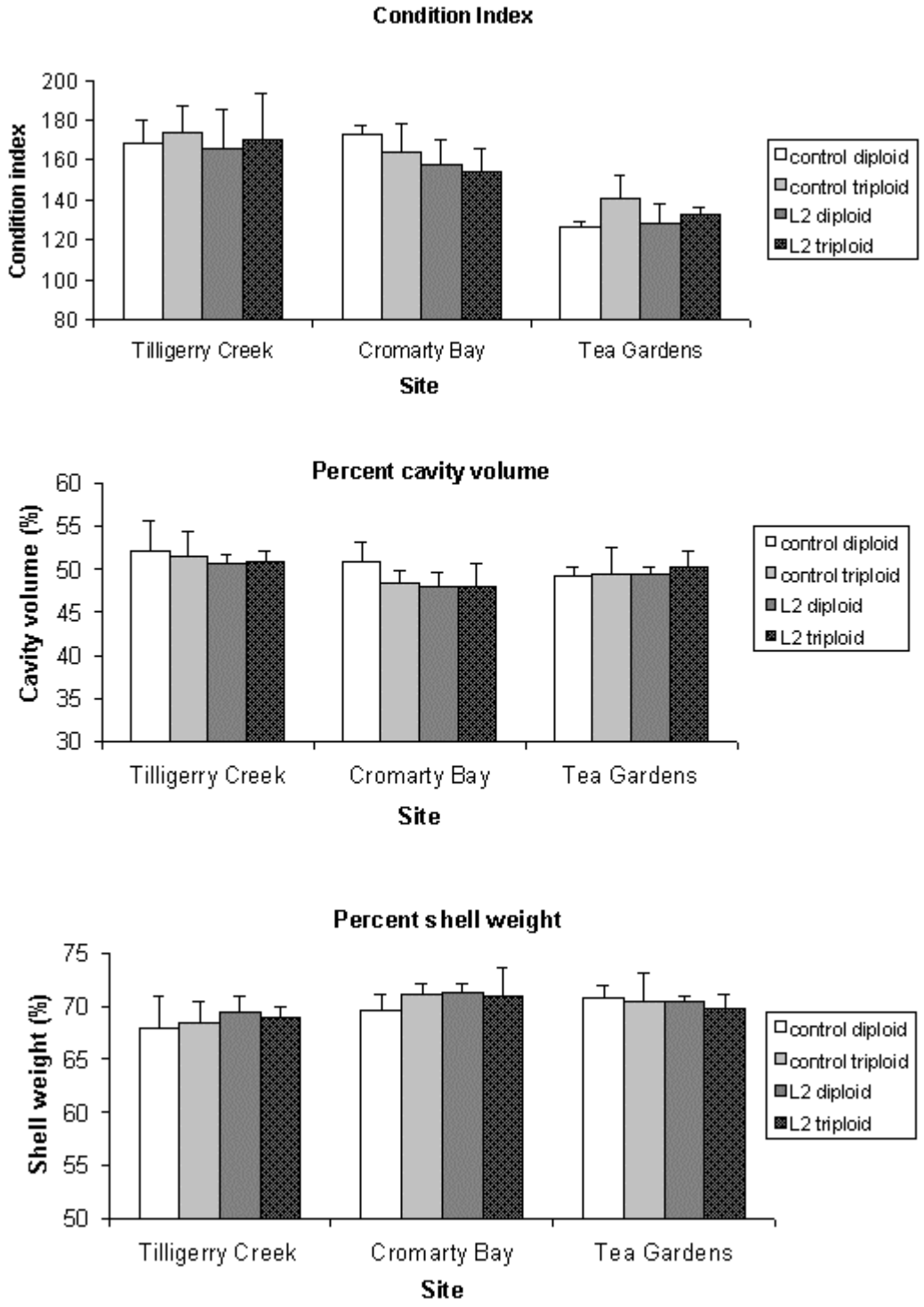

Figure 3. Condition index, cavity volume (%) and shell weight (%) of diploid and triploid, control and selected Sydney rock oysters, *Saccostrea glomerata*, after 21 months at three sites in Port Stephens, NSW. Mean values ± 95% c.i.

Table 1. Results of ANOVA[1] of whole oyster weights[2] at the end of a 32-month growth comparison of diploid and triploid, control and selected Sydney rock oysters grown in Port Stephens, New South Wales.

Source of variation	Fixed or random	Sum of squares	df	MS	F	p
Site (A)	R	1281.92	2	640.96	51.35	0.00
Line (B)	F	9453.32	3	3151.11	87.38	0.00
AxB	R	216.37	6	36.06	2.89	0.02
Error		449.36	36	12.48		
Total		11400.97	47			

[1]Cochrans C = 0.19; p > 0.05.
[2]50 oysters weighed per replicate

Table 2. Initial and final[1] whole weights of diploid and triploid, control and selected Sydney rock oysters, *Saccostrea glomerata* grown from March 2000 - November 2002.

Line	Initial whole weight[2] (g)	Final whole weight (g)	Final weight advantage (%)[3]	Reduction in time to reach market size (months)
Control diploid	0.143 ± 0.002[a]	51.7 ± 1.5[a]	-	-
Control triploid	0.136 ± 0.002[b]	70.5 ± 1.8[b]	36.4	9
L2 diploid	0.137 ± 0.001[b]	62.7 ± 2.4[c]	21.3	7
L2 triploid	0.134 ± 0.002[b]	90.1 ± 1.9[d]	74.3	10

[1]Means ± se (n = 12 replicates of 50 oysters). Means from 3 sites combined.

[3] Weight advantage of $X = \frac{\text{Weight X (g) - Weight control diploid (g)}}{\text{Weight control diploid (g)}} * 100$

[2]Within columns, means with different letters differ significantly ($p < 0.05$).

[3] Weight advantage of $X = \frac{\text{Weight X (g) - Weight control diploid (g)}}{\text{Weight control diploid (g)}} * 100$

[4]Market size ≥ 50g

Table 3. Initial and final[1] weight:height ratios of diploid and triploid, control and selected Sydney rock oysters, *Saccostrea glomerata* of equivalent whole weights[2], grown from March 2000 - December 2001.

Line	Initial[3]			Final		
	whole weight (g)	weight:height (g/mm)	N	whole weight (g)	weight:height (g/mm)	n
Control diploid	0.141 ± 0.002[a]	0.013 ± 0.000[a]	33	37.4 ± 0.2[a]	0.537 ± 0.004[a]	43
Control triploid	0.135 ± 0.002[a]	0.013 ± 0.000[a]	30	37.4 ± 0.2[a]	0.549 ± 0.005[a]	59
L2 diploid	0.139 ± 0.002[a]	0.013 ± 0.000[a]	34	37.8 ± 0.2[a]	0.571 ± 0.004[b]	62
L2 triploid	0.143 ± 0.003[a]	0.014 ± 0.000[a]	24	38.0 ± 0.4[a]	0.575 ± 0.011[b]	15

[1]Means ± se.
[2]Oysters analysed were within the range 0.12 - 0.16 g for initial weights and 35 – 40 g for final weights.
[3]Within columns, means with different letters differ significantly ($p < 0.05$).

5.2. Nell, J.A., Hand, R.E, 2003. Evaluation of the progeny of second generation Sydney rock oyster Saccostrea glomerata (Gould, 1850) breeding lines for resistance to QX disease Marteilia sydneyi. Aquaculture.

Evaluation of the progeny of second-generation Sydney rock oyster *Saccostrea glomerata* (Gould, 1850) breeding lines for resistance to QX disease *Marteilia sydneyi*

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Abstract

The progeny of second-generation Sydney rock oyster *Saccostrea glomerata* (Gould, 1850) breeding lines were tested for resistance to QX disease *Marteilia sydneyi* against a non-selected control. Mortality was reduced from 85.7±1.5% for the controls to 63.5±1.2% for the most improved breeding line. This is a reduction in mortality of 22% after only two generations of selection. These partially QX disease-resistant oysters in which *M. sydneyi* was found were also 21% heavier than controls. Selection for resistance to *M. sydneyi* is feasible and may be improved through further selection.

Keywords: Oysters; Breeding; Selection; Disease; Resistance; QX

1. Introduction

A breeding program for the Sydney rock oyster, *Saccostrea glomerata* (Gould, 1850) (formerly *S. commercialis*; Buroker et al., 1979; Anderson and Adlard, 1994), was established by the NSW Fisheries in 1990 (Nell et al., 2000). Four breeding lines selected for fast growth were established in Port Stephens, NSW, Australia (32°45'S, 152°10'E) (Nell et al., 1996; Nell et al., 1999). Four breeding lines were also established in Georges River, NSW, Australia (34°00'S, 151°10'E) to select for fast growth and resistance to the disease winter mortality (Nell et al., 2000). Winter mortality is caused by a protistan parasite, previously classified as *Mikrocytos roughleyi* (Farley et al., 1988); but is now thought to belong to the *Bonamia* genus instead (Cochennec-Laureau et al., 2001). Selection for weight gain in Port Stephens has been successful. The mean increase of 18% in whole weight achieved for the progeny of the four second-generation breeding lines, represents a reduction in time to market size (50 g whole weight) of 3 months out of the usual 3 ½ years (Nell et al., 1999). Progress in selection for fast growth and winter mortality resistance in Georges River was severely interrupted when QX disease occurred in 1994 (Adlard and Ernst, 1995). This disease is caused by a second protistan parasite *Marteilia sydneyi* (Perkins and Wolf, 1976); it had a devastating effect on the breeding lines in 1995 when 85% of all oysters died at two out of three sites in Georges River (Nell et al., 2000). The life cycle of the parasite is thought to include an intermediate host, the identity of which remains unknown. In January 1997, the breeding program was re-established and modified to incorporate one line selected for resistance to winter mortality, one for resistance to QX disease and one for resistance to both winter mortality and QX disease (Nell et al., 2000).

M. sydneyi, the aetilogical agent of QX disease, invades the gills and digestive glands of oysters and causes death through starvation. QX disease is more commonly found in northern, warmer estuaries and was responsible for a decline in the oyster industry in southern Queensland and in northern NSW during the 1970s. As a result of this disease, production in the Tweed, Richmond and Clarence Rivers in northern NSW during the past 26 years decreased from 379,200 dozens in 1974/1975 to 168,504 dozens in 2000/20001 - a drop of 56%. The disease had a devastating effect on oyster production in the Georges River which declined from 1,111,171

dozens in 1993/1994 to 62,000 dozens in 2000/20001, a drop of 94%, as the disease in this river kills up to 90% of all Sydney rock oyster annually. As the Pacific oyster is not affected by QX disease, it has partially displaced Sydney rock oysters in Georges River and now makes up 80% of the oysters on the foreshore of the upper reaches of the river.

Selection for resistance to protistan parasites has been successful in other oyster species. Selection for resistance to *Minchinia nelsoni*, more commonly known as MSX, has been successful in eastern oysters, *Crassostrea virginica* (Gmelin, 1791) (Haskin and Ford, 1979; Allen, 1998). Similarly, selection for resistance to *Bonamia* has been successful in European flat oysters *Ostrea edulis* (Linnaeus, 1750) (Naciri-Graven et al., 1998).

Both QX disease and winter mortality occur annually, although the severity of these two diseases depends on temperature and salinity (Nell and Smith, 1988; Nell, 1993). In Georges River, NSW, infestation of oysters with QX disease parasites commences in February (summer) and most mortality occurs in April/May (autumn), whereas winter mortality infestations commence around April (autumn) and most mortalities occur in September/October (spring) (Nell and Smith, 1988). For both diseases, weakened survivors may die from heat stress in late spring or early summer (November/December).

The aim of this experiment was to test oysters bred for resistance to QX disease after two generations of selection.

2. Materials and methods

2.1. Selection lines

In 1997, the four fast growth breeding lines in Georges River, NSW, Australia (34^{o}00'S, 151^{o}10'E), were reorganised to produce three disease-resistant lines (Table 1), namely, Line 1 at Lime Kiln Bar, Line 2 at Woolooware Bay and Line 3 at Quibray Bay (Fig. 1) (Nell et al., 2000). Resulting first-generation disease-resistant oysters were exposed to disease and selected on a whole weight basis at each of the three sites above (Nell et al., 2000). In 1998, the oysters at Lime Kiln Bar suffered around 85% mortality from QX disease only; i.e., after they had been selected for disease resistance for two generations (Nell et al., 2000). Broodstock at Woolooware Bay were exposed to both QX disease and winter mortality and those at Quibray Bay to winter mortality only. Mortality of oysters at Woolooware Bay and Quibray Bay was much less than that at Lime Kiln Bar. For all three selection lines, 216 of the heaviest survivors were selected on a within-tray basis for spawning. Non-selected oysters used as controls were taken in equal numbers from Wallis Lake, Port Stephens, and the Hawkesbury River, three of the four estuaries used in the establishment of the original base population for selection (Nell et al., 1996).

2.2. Second-generation selection lines

The second-generation Georges River disease-resistant breeding lines were spawned at the Georges River in January 2000. There were four separate mass spawnings/fertilisations for each breeding line or control group as described by Nell et al. (1996). Washed fertilised eggs were taken for rearing, to the Cronulla Fisheries Centre at Port Hacking, an estuary to the south of Georges River (Nell et al., 2000). There is no commercial oyster farming in Port Hacking and, by taking only washed fertilised eggs for rearing it was presumed that the wild oysters there would be protected from the possible introduction of the QX parasite. After settlement, spat were reared in upwellers at Georges River until the start of the experiment. In July 2000, spat were graded using nylon mesh screens into four size grades and those for the experiment were taken from the second largest size grade (7 – 10 mm), whereas those from the largest size grade were used to establish the next generation on leases.

2.3. Growth and mortality comparison

The experimental sites were the same three sites (Woolooware Bay, Quibray Bay and Lime Kiln Bar) as those used for the selection of the broodstock (Fig. 1). The experiment began in July 2000 and ended in March 2002. There were six replicates per line per site, each replicate comprising one-half-tray of 400 oysters. At approximately quarterly intervals, the individual whole weights of 50 randomly selected oysters were taken for each replicate and all live and dead oysters counted. However, at the end of the experiment, some replicates of some treatments no longer had 50 live oysters, and in those instances, all remaining live oysters were weighed.

2.4. Disease diagnosis

Infestation and mortality of oysters by the QX parasite *M. sydneyi* was established using the description of Wolf (1972) and Perkins and Wolf (1976). Twelve oysters were sampled monthly from November 2000 to May 2001. Infection in the digestive gland was first detected on February 2, 2001. At this time, plasmodia were observed undergoing sporulation to form 8 - 16 sporonts. The intensity of infection in both hemolymph and tissue increased continuously until the end of March 2001.

Infestation and mortality of oysters by the winter mortality parasite *M. roughleyi* was established on the basis of the presence of yellow to brown spots on palps, gills, mantle and surface of gonad and ulceration of palps and adductor muscle (Roughley, 1926).

2.5. Statistical analyses

Homogeneity of variance of data was checked using Cochran's test. The whole weight and growth data sets were homogeneous ($P>0.05$), whereas the mortality data was not ($P<0.05$). However, the Shapiro-Wilks test (Statgraphics 4.1, Manugistics, Rockville, MD) showed that none of the data sets were normally distributed ($P<0.05$). Therefore, the following transformations were used: log x for whole weight, arcsin $x^{0.5}$ for mortality and growth data. Whole oyster weight, growth and mortality data were analysed (on a half-tray basis) by a two-factor (site and line/control) ANOVA (WinGMAV5 software, Institute of Marine Ecology, University of Sydney, NSW). Data are expressed as means ± S.E. throughout the text, except for Fig. 2, where the mortality data is expressed as means ± 95% confidence intervals. The Student-Newman-Keuls (SNK) test was used as a post hoc comparison of response to selection and to compare mean values. Throughout the text, mean values with common superscripts do not differ significantly ($P>0.05$).

Oysters from line 3 selected at Quibray Bay had a significant lower initial weight ($P<0.05$) than any of the other breeding lines or control. This difference arose because there were more of them and they consequently had been held at a higher density in the upweller nursery system. Analysis of covariance showed a significant effect ($P<0.05$) of initial weight on final weight (Statgraphics 4.1, Manugistics, Rockville, MD). Therefore, growth rates were calculated, as direct comparison of final weights of line 3 with the other two lines or control would not have been valid due to initial differences in whole weights. To overcome this problem, instantaneous growth rate calculated as follows: $G = (\ln W_t - \ln W_0) /$ Duration (days), where W_t = final mean weight of oysters and W_0 = initial mean weight of oysters, was used (Askew, 1978).

3. Results

During the course of the experiment from July 2000 to February 2002, two major mortality events were observed (Fig. 2). The first was caused by winter mortality and was observed at Quibray Bay from July to February 2001. The second arose from QX disease at all three sites in May 2001, but was most pronounced at Lime Kiln Bar. Mortality from QX disease at Woolooware Bay and Quibray Bay was more prolonged than that at Lime Kiln Bar, but after weakened oysters died, mortality in late summer (February 2002), for oysters at Woolooware Bay was almost equal to that at Lime Kiln Bar. The QX parasite was also found in fast growing QX

survivors from line 1, at Lime Kiln Bar. Wild Sydney rock oysters on the foreshore at all three sites also died at the same time as our experimental oysters.

3.1. Comparison of breeding lines

The average initial and final weights, growth and mortality for the selection lines and control are shown in Table 2. The average initial weight of line 3 was significantly less ($P<0.05$) than that of any of the other lines or control. The average final weights of lines 1 and 2 were significantly greater ($P<0.05$) than that of either line 3 or control. However, line 3 oysters had the highest growth rate, followed by line 1 and 2 and the control had the lowest growth rate. Mortality of line 1 oysters was the lowest (63.5%), followed by line 2 (72.7%) and line 3 (80.4%). Not surprisingly the control had the highest mortality (85.7%).

3.2. Comparison of growing sites

There was no significant line x site interaction ($P>0.05$) for any of the parameters measured. There were however, significant site differences ($P<0.05$) for final weight, growth and mortality. The mean weights of all oysters at Lime Kiln Bar, Woolooware Bay and Quibray Bay were 42.7 ±1.0, 48.0±0.9 and 29.8±0.6 g, respectively, and they were all significantly different from one another. The mean instantaneous growth rates of all oysters for the above sites were 0.0130^{a}, 0.0131^{a} and 0.119^{b}, respectively. Instantaneous growth rate (0.0119) of all oysters at Quibray Bay was significantly lower ($P<0.05$) than that for those at the other two sites. The mean mortality for the above sites was 78.7 ± 2.6^{a}, $75.8\pm1.9\%^{ab}$ and $72.5\pm2.0\%^{b}$, respectively.

4. Discussion

Line 1 from Lime Kiln Bar, which had the greatest exposure to QX disease over the previous two generations, had the lowest mortality of 63.5% versus 85.7% for the control. This is a reduction in mortality of 22% after only two generations of selection. Line 2 from Woolooware Bay, which had less exposure to QX disease over the previous two generations, had an intermediate mortality of 72.7%, whereas mortality in line 3, which had not had any known exposure to QX disease prior to this experiment, but had been exposed to winter mortality instead, had only a slightly reduced mortality of 80.4%, compared to the control.

The faster growth of line 3 from Quibray Bay may have been because of the greater selection pressure for growth as this line had suffered only around 50% mortality over the previous two generations versus 85% for line 1 from Lime Kiln Bar (Nell et al., 2000). Nevertheless, line 1 was still 21% heavier than the control, although there had only been minimal selection for fast growth because of the high mortality from QX disease over the previous two generations. As *M. sydneyi* was found in the partial QX disease-resistant oysters, it is expected that fully resistant Sydney oysters could still be hosts for this parasite.

There was a 22% reduction in mortality from 86% for the controls to 64% for the QX breeding line 1 (Table 2) after only two generations of selection for disease resistance. This is a similar response to that reported for selection for resistance to protistan disease in other oysters. Haskin and Ford (1979) found a reduction in mortality from 93% in controls down to 56% after three generations of selection for resistance to MSX in eastern oyster breeding lines. Allen (1998) found a reduction in mortality from 96% in controls down to 35% after six generations of selection in the same breeding lines. A better response was obtained with selection for *Bonamia* resistance in European flat oysters in France, with mortality reduced from 87% in controls to 41% after three generations of selection for disease resistance (Naciri-Graven et al., 1998).

The reduction in mortality of Sydney rock oysters after two generations of selection for resistance to QX disease during this study clearly demonstrates the effectiveness of selection for disease resistance in oysters. The success in selection for resistance to MSX in eastern oysters (Allen, 1998), and resistance to Bonamia in European flat oysters (Naciri-Graven et. al., 1998) support this. On the basis of these results, it seems quite feasible to breed a QX disease-resistant

Sydney rock oyster, although it is still able to host the *M. sydneyi* parasite. The life cycle of *M. sydneyi* would have to be fully understood to rule out the possibility of vertical transfer of QX disease from adults to larvae.

Care should be taken when comparing the results of different selection studies as the response to selection depends on both the selection intensity, which is often difficult to assess in oyster studies and the resistance mechanism involved. Fortunately, however, selection for fast growth and disease resistance has been successful in several commercially important oyster species.

As the industry in Georges River has collapsed, and production in 2000/20001 had dropped by 94% since QX disease was first diagnosed in this river in 1994, the breeding lines are now extremely vulnerable to theft and vandalism. Therefore, it may be necessary to relocate the QX disease resistance lines to another estuary in future. The possible association of the enzyme, prophenoloxidase, with resistance to *M. sydneyi* (Peters and Raftos, 2002), may facilitate the development of genetic markers needed to maintain disease-resistant breeding lines without continuing exposure to *M. sydneyi*.

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Table 1. Georges River breeding lines.

1990	Base population collected and spawned to produce Generation 1, which was established with four lines at each of the three sites: Lime Kiln Bar, Woolooware Bay and Quibray Bay (see Fig. 1).
1992	Generation 1 spawned to produce Generation 2, which was established on leases in Georges River as above.
1994	Generation 2 spawned to produce generation 3, which was established on leases in Georges River as above. QX disease first observed in Georges River.
1997	The four breeding lines selected at each of the three sites in Georges River were re-organised and spawned to produce three new Generation 2 disease-resistant lines (Nell et al., 2000), which were established on leases in Georges River as shown below ▪ Line 1 at Lime Kiln Bar to be selected for resistance against QX disease ▪ Line 2 at Woolooware Bay to be selected for resistance against both winter mortality and QX disease ▪ Line 3 at Quibray Bay to be selected for resistance against winter mortality These new disease-resistant lines were established on leases in Georges River.
2000	The three Generation 2 disease-resistant lines were spawned to produce the progeny of the second generation used in this study. Sibling of these oysters were used to establish Generation 3 disease resistant lines on leases in Georges River as above.

Table 2. Comparison of growth[a] and mortality[a] of progeny of second generation Sydney rock oyster breeding lines with a control in Georges River, NSW, from July 2000 to February 2002.

Line/Control	Site of selection of breeding line	Diseases found at sites over the two generations of selection	Initial weight (g)	Final weight (g)	Instantaneous growth rate[b]	Mortality (%)
1	Lime Kiln Bar	QX disease	0.23±0.007[a]	43.8±2.2[a]	0.0126[b]	63.5±1.2[d]
2	Woolooware Bay	QX disease and Winter mortality	0.24±0.011[a]	42.3±2.2[a]	0.0125[b]	72.7±1.1[c]
3	Quibray Bay	Winter mortality	0.15±0.003[b]	38.3±1.7[b]	0.0134[a]	80.4±2.4[b]
Control	-	-	0.24±0.005[a]	36.3±1.8[c]	0.0121[c]	85.7± 1.5[a]

[a]Data are mean ± S.E., (n = 6). Means with different letters differ significantly ($P<0.05$).
[b]Instantaneous growth rate: $G = (\ln W_t - \ln W_0) / \text{Duration (days)}$, where W_t = final mean weight of oysters and W_0 = initial mean weight of oysters.

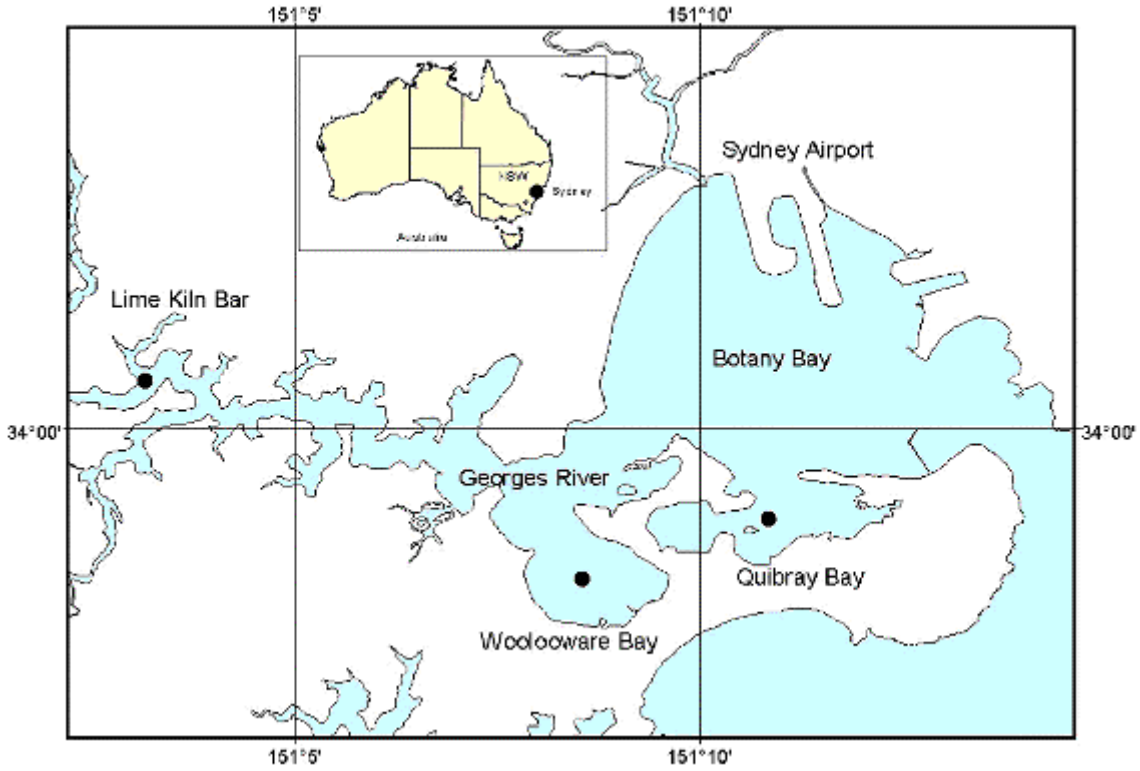

Figure 1. Location of experimental sites in Georges River, NSW, Australia by F. Dorman, NSW Fisheries.

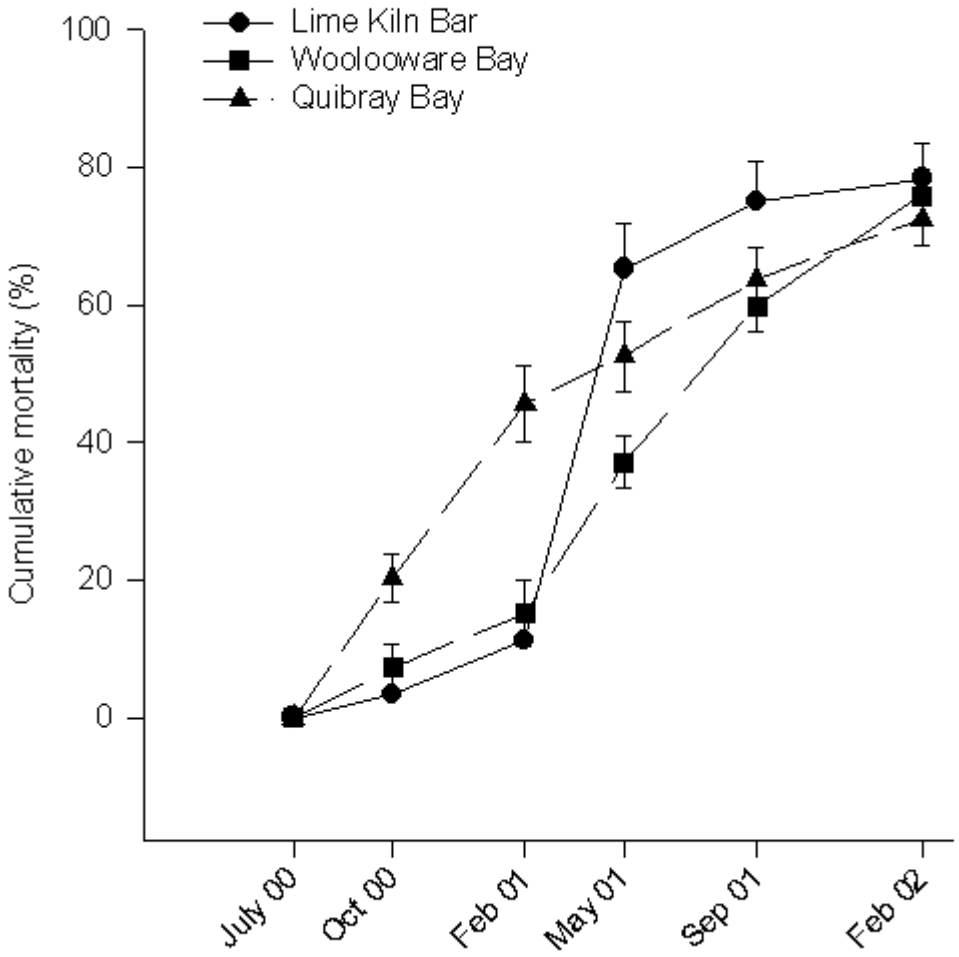

Figure 2. Cumulative mortality of Sydney rock oysters held in Georges River, NSW, from July 2000 to February 2002. Data are means ± 95% confidence intervals.

6. BENEFITS

This study has clearly demonstrated that Sydney rock oysters can be successfully selected for resistance against QX disease caused by *Marteilia sydneyi* and fast growth. Benefits of selective breeding can only be realised for the Sydney rock oyster industry in NSW and southern Queensland if the hatchery production problems of this species are overcome. A Sydney rock oyster hatchery health workshop was held in Port Stephens in August 2002, with the aim of giving direction to future hatchery production R&D.

7. FURTHER DEVELOPMENT

The 'Review of hatchery production technology and breeding program for Sydney rock oysters – FRDC Project Report 2001/213' recommended that:

1. Work on genetic improvement be halted until effective hatchery procedures are developed that will allow cost effective production of single pair matings, and commercial scale production of spat. It was recommended that the breeding lines be maintained during this period.
2. While the hatchery research is undertaken, a full evaluation of a range of genetic improvement plans is made to assess the benefits and their speed of delivery to industry.

8. PLANNED OUTCOMES

This project had three planned outcomes that would enable downstream benefits to the NSW oyster industry based on the following:

1. Demonstration that oysters can be effectively selected for resistance against QX disease *Marteilia sydneyi*.
2. Demonstration that the fast growth of triploidy and selective breeding are additive.
3. Demonstration that selective breeding for fast growth reduces time to market without any change in meat yield and percentage shell weight.

All planned outcomes were achieved and this has demonstrated the validity of using mass selection as the tool for the genetic improvement of the Sydney rock oyster. However, the benefits of the Sydney rock oyster breeding program cannot be transferred to the NSW oyster industry until hatchery production problems for this species have been overcome.

9. CONCLUSION

Both objectives in section 3 were met. After two generations of selection for QX disease resistance in Georges River, NSW mortality was reduced from 92% for the control to 63% for the most improved breeding line. These oysters were also 26% heavier than the controls. QX survivors still host the QX parasite *Marteilia sydneyi*, but they don't suffer the catastrophic mortality that is common in non-selected oysters. Because of the overwhelming effect of mortality from QX disease, any improvement in resistance to winter mortality *Mikrocytos roughleyi* was masked. However, it is expected that selection for resistance to winter mortality can be just as successful as that for QX disease.

The possible association of the enzyme, prophenoloxidase (Peters and Raftos, 2003), with resistance to *M. sydneyi* (Peters and Raftos, 2002), may facilitate the development of genetic markers needed to maintain disease-resistant breeding lines without continuing exposure to *M. sydneyi*.

If the breeding program is maintained, the annual mortality from QX disease remains high and the Georges River disease resistance breeding lines are bred every two years, it is expected that the evaluation of the progeny of the sixth generation of the most improved line will have demonstrated full resistance to QX disease in February 2010. This means 90% mortality for controls versus <20% mortality for the most improved selected oysters at Lime Kiln Bar, as the background mortality for Sydney rock oysters ranges from 10 – 20 %.

Selection for fast growth had also been very successful. The results showed that both 'wild' triploid oysters and the progeny of a 3rd generation breeding line reach market size (50-g whole weight) 6 months earlier than hatchery produced non-selected diploids. The triploid progeny of 3rd generation breeding lines reached market size 10 months earlier than diploid controls. At the end of the study in December 2002, when oysters were 3 years and 2 months old, the mean whole weight of the diploid controls, triploid controls, diploid breeding line and triploid breeding line were 52, 71, 63 and 90 g respectively. The growth advantages of triploidy and selective breeding were synergistic.

If the breeding program is maintained and the Port Stephens fast growth breeding lines are bred every two years, it is expected that the evaluation of the diploid progeny of the fifth generation of the most improved line will have demonstrated a 12 months or greater reduction in time to marker size (50 g whole weight) in February 2006. This means a 38 months time to market for controls versus 26 months for the most improved selected oysters in Port Stephens.

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Smith, I.R., Nell, J.A., Adlard, R.D., 2000. The effect of growing height and growing method on winter mortality in diploid and triploid Sydney rock oysters *Saccostrea glomerata* (Gould, 1850). Aquaculture 185, 197-205.

11. APPENDICES

Appendix 1: Intellectual Property

Georges River

In July 03, there were three (4th generation) disease resistance breeding lines in Georges River:

Line 1 at Lime Kiln Bar

Line 2 at Woolooware Bay

Line 3 at Quibray Bay

There were 9 trays of oysters for each breeding line held at one of the three sites above. Oysters are due to be bred to produce the 5th generation disease resistance lines in January 04.

Port Stephens

In July 03, there were four (6th generation) fast growth breeding lines in Port Stephens. There were 3 trays of oysters for each breeding line held at each of three sites in Port Stephens, ie a total of 9 trays for each breeding line. Oysters are due to be bred to produce the 7th generation fast growth lines in January 05.

NSW Fisheries retains the full ownership of both the Georges River and Port Stephens breeding lines and will maintain the breeding lines by breeding them every second or third year. The NSW Oyster Management and Advisory Group (OMAG), Oyster Research and Advisory Committee (ORAC) and Fisheries Research and Development Corporation (FRDC) will be consulted before any major changes are made to the breeding lines.

Appendix 2: Staff

NSW Fisheries

Name	Position	Qualification	Time (%)
Dr John Nell	Principal Research Scientist	Dip Appl Sc, Ph D	80
Dr Rosalind Hand	Scientist	B Sc Hons, Ph D	100
Mr Ben Perkins	Fisheries Technician	Master Class V	80

Appendix 3: Relevant Publications

Sydney rock oyster breeding papers

1. Smith, I.R., Sheridan, A.K., Nell, J.A., 1995. Evaluation of growing methods for use in a Sydney rock oyster *Saccostrea commercialis* (Iredale and Roughley) selective breeding program. Aquaculture 131, 189-195.
2. Nell, J.A., Sheridan, A.K., Smith, I.R., 1996. Progress in a Sydney rock oyster *Saccostrea commercialis* (Iredale and Roughley) breeding program. Aquaculture 144, 295-302.
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4. Nell, J.A., Smith, I.R., Sheridan, A.K., 1998. Third generation evaluation of Sydney rock oyster *Saccostrea commercialis* (Iredale and Roughley) breeding lines. Aquaculture 170, 195-203.
5. Bayne, B.L., Svensson, S., Nell, J.A., 1999. The physiological basis for faster growth in the Sydney rock oyster, *Saccostrea glomerata*. Biological Bulletin 197, 377-387.
6. Bayne, B.L., 2000. Relations between variable rates of growth, metabolic costs and growth efficiencies in individual Sydney rock oysters (*Saccostrea commercialis*). Journal of Experimental Marine Biology and Ecology 251, 185-203.
7. Nell, J.A., Smith, I.R., McPhee, C.C., 2000. The Sydney rock oyster *Saccostrea glomerata* (Gould 1850) breeding programme: progress and goals. Aquaculture Research 31, 45-49.
8. English, L.J., Nell, J.A., Maguire, G.B., Ward, R.D., 2001. Allozyme variation in three generations of selection for whole weight in Sydney rock oysters (*Saccostrea glomerata*). Aquaculture 187, 283-298.
9. Nell, J.A., Hand, R.E., 2003. Evaluation of the progeny of second generation Sydney rock oyster *Saccostrea glomerata* (Gould, 1850) breeding lines for resistance to QX disease *Marteilia sydneyi*. Aquaculture
10. Hand, R.E., Nell, J.A., Thompson, P.A., in press. Studies on triploid oysters in Australia. XIII. Performance of diploid and triploid Sydney rock oysters, *Saccostrea glomerata* (Gould, 1850) progeny from a third generation breeding line. Aquaculture

Sydney rock oyster ploidy manipulation papers

1. Nell, J.A., Cox, E., Smith, I.R., Maguire, G.B., 1994. Studies on triploid oysters in Australia. I. The farming potential of triploid Sydney Rock oysters *Saccostrea commercialis* (Iredale and Roughley). Aquaculture 126, 243-255.
2. Nell, J.A., Hand, R.E., Goard, L.J., McAdam, S.P., Maguire, G.B. 1996. Studies on triploid oysters in Australia: Evaluation of cytochalasin B and 6-dimethylaminopurine for triploidy induction in Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley). Aquaculture Research 27, 689-698.
3. Cox, E., Smith, M.S.R., Nell, J.A., Maguire, G.B., 1996. Studies on triploid oysters in Australia. VI. Gonad development in diploid and triploid Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley). Journal of Experimental Marine Biology and Ecology 197, 101-120.
4. Korac, S., Nell, J.A., Prescott, J., 1996. Studies on triploid oysters in Australia. VIII. Sensory evaluation of Sydney rock oysters *Saccostrea commercialis*. Asian Fisheries Science 9, 61-68.
5. Hand, R.E., Nell, J.A., Reid, D.D., Smith, I.R., Maguire, G.B., 1999. Studies on triploid oysters in Australia: effect of initial size on growth of diploid and triploid Sydney rock oysters *Saccostrea commercialis* (Iredale & Roughley). Aquaculture Research 30, 35-42.

6. Hand, R.E., Nell, J.A., Maguire, G.B., 1998. Studies on triploid oysters in Australia. X. Growth and mortality of diploid and triploid Sydney rock oysters, *Saccostrea commercialis* (Iredale and Roughley). Journal of Shellfish Research 17, 1115-1127.
7. Hand, R.E., Nell, J.A., Smith, I.R., Maguire, G.B., 1998. Studies on triploid oysters in Australia. XI. Survival of diploid and triploid Sydney rock oysters, *Saccostrea commercialis* (Iredale and Roughley) through outbreaks of winter mortality caused by *Mikrocytos roughleyi* infestation. Journal of Shellfish Research 17, 1129-1135.
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9. Nell, J.A., McMahon, G.E., Hand, R.E., 1998. Tetraploidy induction in Sydney rock oysters. Aquaculture CRC Project D.4.2, NSW Fisheries Final Report Series, Report No. 9, NSW Fisheries, Port Stephens Research Centre, Taylors Beach, NSW, 25 pp.
10. Hand, R.E., Nell, J.A., 1999. Studies on triploid oysters in Australia. XII. Gonad discolouration and meat condition of diploid and triploid Sydney rock oysters (*Saccostrea commercialis*) in five estuaries in New South Wales, Australia. Aquaculture 171, 181-194.
11. Kesarcodi-Watson, A., Lucas, J.S., Klumpp, D.W., 2001a. Comparative feeding and physiological energetics of diploid and triploid Sydney rock oysters, *Saccostrea commercialis*. I. Effect of oyster size. Aquaculture 303, 177-193.
12. Kesarcodi-Watson, A., Lucas, J.S., Klumpp, D.W., 2001b. Comparative feeding and physiological energetics of diploid and triploid Sydney rock oysters, (*Saccostrea commercialis*). II. Influences of food concentration and tissue energy distribution. Aquaculture 303, 195-216.
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2. Nell, J.A., 2001. The history of oyster farming in Australia. Marine Fisheries Review 63(3), 14-25.
3. Nell, J.A., 2002. Farming triploid oysters. Aquaculture 210, 69-88.

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No. 1 Andrew, N.L., Graham, K.J., Hodgson, K.E. and Gordon, G.N.G., 1998. Changes after 20 years in relative abundance and size composition of commercial fishes caught during fishery independent surveys on SEF trawl grounds. Final Report to Fisheries Research and Development Corporation. Project No. 96/139.

No. 2 Virgona, J.L., Deguara, K.L., Sullings, D.J., Halliday, I. and Kelly, K., 1998. Assessment of the stocks of sea mullet in New South Wales and Queensland waters. Final Report to Fisheries Research and Development Corporation. Project No. 94/024.

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