PROJECT COMPLETION REPORT

96-8 "Genetic Selection of Oysters, *Crassostrea virginica*, for Growth and Resistance to Juvenile Oyster Disease (JOD)"

Termination Report Period: July 1, 1996 though February 28, 1998

NRAC Total Funding: $41,049 (July 1, 1996 through December 31, 1997)
(No-Cost Ext'n through February 28, 1998)

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	Mook Sea Farms	Maine
	Cape Cod Oyster Co.	Massachusetts
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Project Objectives:

1. Perform a controlled selection (based on size) of an F2 generation of oysters produced in 1994 (previously exposed to JOD), and to produce F3 select and control sublines.

2. Evaluate both control and select sublines in the field in two locations (Damariscotta River, Maine and Cotuit Bay, Massachusetts) for growth and JOD resistance in 1996. Growth and survival of the F3 sublines will be compared with the F2 sublines at age 18 months and with a susceptible control (to account for year to year variability in JOD pressure).

3. Make JOD resistant seed commercially available to the oyster aquaculture industry of the northeastern U.S., where JOD is endemic.

Anticipated Benefits:

The product of this work will be a genetically improved line of oysters. The primary beneficiary of this product will be the oyster aquaculture industry of the Northeastern United States. This product will be made commercially available (as seed oysters) to growers throughout the country by Mook Sea Farms, Damariscotta, Maine. The economic benefits of this product will be measurable in terms of a decreased mortality of juvenile oysters (potential market oysters).

Principal Accomplishments:

Objective 1

Oysters (Flowers F2 select and control sublines) produced in Mar. 1994 were weighed prior to conditioning for spawning to produce F3 sublines. Broodstock for the control subline (n=35) was selected around the population (n=206) mean live weight of 46.2 g. Broodstock for the select subline (n=35) was based on the largest 20% of the parental population (n=168). Parents of the susceptible control oysters (not previously exposed to JOD) were taken from the Piscataqua River (provided by Spinney Creek Shellfish, Eliot, ME). After conditioning, all three groups of broodstock were spawned at Mook Sea Farms. Table 1 provides the spawning dates, and numbers of parents that contributed gametes to each cohort.

Table 1. Dates of spawning and number of males and females contributing gametes to each of the three cohorts of oysters produced for this project. *

Larvae were reared in 375 l conical tanks containing 10 µm filtered water from the Damariscotta River, which was replaced every other day. Temperature in the tanks was maintained at 22-24 ^{o}C and food (mixed diet of *Isochrysis galbana*, *Tetraselmis* sp., and *Thalassiosira pseudonana*) was added twice per day. Eyed larvae were collected and placed into a setting tray containing mini-cultch (crushed oyster shell) on a screen. After the settlement period (48 hr.), spat were held in upwellers (35 l tanks) and fed a diet similar to that given to the larvae.

Objective 2

Spat (2-3mm) were deployed at both sites (Damariscotta River, ME and North Bay, MA) on July 4-5, 1996. Five replicate trays each containing 500+ oysters were deployed for each of the 3 cohorts produced. On each sampling date, shell height measurements from a random subsample of 60 individuals per replicate were obtained from photographs (image analysis) for small (<5mm) oysters or with digital calipers for larger oysters (>5mm). All individuals were checked for general health and all dead oysters were counted and removed from trays. At the North Bay site, salinity was recorded on each sampling date. Temperature was recorded every 3 hr with a datalogger. At the Damariscotta River site, salinity was determined biweekly with a YSI salinometer and temperature was recorded every 6 hr. with a Ryan thermograph. All oysters were removed from their respective sites on the last sampling date. The Damariscotta River oysters were moved to the flowing seawater laboratory at the Darling Marine Center for overwintering.

In May 1997, oysters monitored in the Damariscotta River in 1996 were re-deployed. Growth (shell height) and mortality were assessed monthly over a second growing season (May through November). Temperature and salinity data were collected as in 1996.

At the Damariscotta River location in 1996, oyster growth was continual until mid-September for both the Flowers select and control sublines (Table 2). In contrast, growth in the Piscataqua wild line ceased in mid-August. Flowers select and control sublines had a mean shell height of 33 mm on November 12 (not significantly different), while final mean shell height in the Piscataqua cohort (18.7 mm), was significantly less than the Flowers sublines. Mortality occurred primarily in a one month period, from late August to late September, for all cohorts, and was associated with the characteristic signs of JOD. For both Flowers sublines, cumulative mortality on November 21 was below 15%. Cumulative mortality in the Piscataqua cohort reached 92% on September 25 and 95% on November 21; this was significantly greater than the cumulative mortality of both Flowers sublines.

Table 2. Mean shell height (mm) and cumulative mortality (%) for each of the three cohorts at the Damariscotta River site in 1996. *

Growth of all oyster cohorts at the North Bay site in 1996 was continual throughout the study period and greater than that seen in the Damariscotta River (Table 3). On November 14, mean shell height of both Flowers sublines (38 mm) was significantly greater than that of the Piscataqua cohort (28 mm). Cumulative mortality in North Bay was quite low, reaching (on November 14) only 5% in the Piscataqua cohort and less than 2.5% in the Flowers sublines. There were no significant differences in cumulative mortality among cohorts at this location.

Table 3. Mean shell height (mm) and cumulative mortality (%) for each of the three cohorts at the North Bay, MA site in 1996. *

JOD was prevalent in the Damariscotta River but not in North Bay in 1996. At both sites, growth of both Flowers sublines exceeded that of the Piscataqua cohort. In the Damariscotta River, where JOD was prevalent in 1996, mortality of the Piscataqua cohort exceeded that of the Flowers sublines, indicating that the genetically selected sublines not only had superior growth rates, but also had greater survival than the JOD-susceptible Piscataqua cohort.

In 1997, only the Flowers control and select sublines were placed back in the Damariscotta River, as the few remaining Piscataqua oysters perished over the winter. Growth of both cohorts was steady from June through September (Table 4). Final mean shell height of the select group (70.4 mm) was significantly greater than that of the control group (63.0 mm). Mortality occurred in both groups during May and June, probably related to overwintering stress. After June, little further mortality occurred in either group (Table 4); final cumulative mortality was similar for select (25%) and control (23.4%) groups, respectively. JOD did not affect these oysters in 1997, even though it was present in the river.

Table 4. Mean shell height (mm) and cumulative mortality (%) for each of the three cohorts at the Damariscotta River, ME site in 1997. *

Based on the successful performance of the Flowers F3 sublines, commercial production of selected oysters is presently being undertaken by Mook Sea Farms, Damariscotta, Maine and Pemaquid Oyster Company, Waldoboro, Maine. Broodstock is similarly available for loan to any interested commercial hatchery.

Impacts:

- Oysters selected for fast growth also have a genetically based tolerance to JOD
- Use of selected broodstock will reduce impact of JOD in future
- Savings to industry from increased survival of oyster seed to market size

Recommended Follow-Up Activities:

This project has demonstrated the value of a selective breeding program to aquaculture. At age 18 months, the F3 select subline was significantly larger than the control subline. Thus additional gains could be anticipated with a continuation of the program, i.e., selecting the F3 generation and producing the F4. Since 1986, this effort has been supported by several funding agencies and the Agricultural and Forestry Experiment Station at the University of Maine. No funds are currently in hand for the continuation of this program. Therefore, the industry should carefully consider whether this breeding program is valuable enough to sustain and how it should be supported.

Publications, Manuscripts, or Papers Presented:

Publications in Print: None yet - see below

Manuscripts:

Barber, B. J., C. V. Davis, and M. A. Crosby. 1998. Cultured oysters, Crassostrea virginica, genetically selected for fast growth also exhibit increased tolerance of Juvenile Oyster Disease (JOD). Submitted to Journal of Shellfish Research.

Papers Presented:

C.V. Davis, M.A. Crosby, B.J. Barber and R.O. Hawes, Genetic selection in oysters for growth and resistance to juvenile oyster disease (JOD). National Shellfisheries Association, Fort Walton Beach, FL, Apr. 20-24, 1997.

Barber, B. J., C. V. Davis, and M. A. Crosby, Genetic selection of oysters for growth and resistance to JOD. World Aquaculture Society, Las Vegas, NV, February 15-19, 1998.

Technical Analysis and Summary:

Growth and mortality of hatchery-produced juvenile oysters, *Crassostrea virginica*, selected for fast growth (select and control sublines) were compared with unselected, wild oysters at sites in Maine (Damariscotta River) and Massachusetts (North Bay) where Juvenile Oyster Disease (JOD) is enzootic. Over the course of the study, JOD occurred primarily in Maine, even though temperature and salinity at both sites were conducive for JOD development. From July to November 1996, mean shell height of select and control sublines was similar but significantly greater than that of wild oysters at both sites. Final mean shell heights of all groups were significantly greater in North Bay than in the Damariscotta River. In Maine, mean cumulative mortality of both select and control groups was similar and significantly less than that of wild oysters. Cumulative mortality of all groups was low in North Bay. Cumulative mortality was significantly greater in the Damariscotta River than in North Bay for all groups. Differences in growth and mortality of oysters between sites was most likely due to differences in JOD occurrence. The difference in survival between select (and control) and wild oysters in the Damariscotta River was not related to size differences between groups at the time of initial exposure to JOD, but rather to a genetically derived tolerance to the disease achieved through previous exposure.

Select and control sublines were re-deployed in the Damariscotta River in 1997 for a second year of study (May through November). Select oysters reached a significantly greater size (shell height and weight) than control oysters but cumulative mortality of both groups was similar.

Thus we conclude the following:

Outbreaks of JOD are site specific (not dependent on source of seed Under challenge from JOD, selected oysters not only grow faster than wild (unselected) oysters, but exhibit a genetically based tolerance to this disease At age 18 months, the F3 select subline was larger than the control subline, indicating that the selection program is viable and that further gains may be realized with further selection

Introduction:

The culture of oysters, *Crassostrea virginica* (Gmelin, 1791) in the northeastern United States has been severely impacted since 1988 by mortalities of hatchery-produced seed caused by Juvenile Oyster Disease (JOD). Signs of the JOD syndrome include reduced growth, shell deformation (cupping of left valve), tissue emaciation, mortality approaching 100%, and heavy conchiolin deposits on inner valve surfaces (Bricelj et al. 1992, Davis and Barber 1994, Lewis et al. 1996). Juvenile Oyster Disease is site and species specific. Certain locations in Maine, Massachusetts and New York have chronic disease

outbreaks while others do not. Only hatchery-produced *C. virginica* is affected by JOD (Bricelj et al. 1992); *Ostrea edulis* cultured in close proximity to *C. virginica* does not contract the disease (Crosby and Barber unpublished data). Although a specific causative agent has yet to be identified, Lewis et al. (1996) demonstrated that JOD is transmissible and that greatest transmission occurs at water temperatures of 22-26 ^{o}C and salinities of 18-30 ppt.

Mortality caused by JOD is inversely related to size, with cohorts having a mean shell height <25 mm at the time of initial JOD occurrence experiencing the greatest mortality (Bricelj et al. 1992, Davis and Barber 1994). Juvenile Oyster Disease occurs only during summer months, primarily at water temperatures >20 ^{o}C (Bricelj et al. 1992). Seasonal aspects of JOD were investigated by Barber et al. (1996) who produced eight cohorts of oysters at two week intervals throughout a growing season and found that mortality was dependent on the timing of placement in the field; cohorts deployed before the end of May and after mid-August had <20% mortality, but all other cohorts had mortalties >65%. The cohort that was deployed in May was exposed to JOD, but because it had reached a mean size >25 mm at the time of JOD occurrence, suffered minimal mortality; the cohort deployed in mid-August also experienced little effect from JOD. It was concluded that etiological agent(s) of JOD are only present or active seasonally, but that within the "window of infection", signs of JOD (reduction in growth, valve cupping, mortality) were not manifested until 3-4 weeks after placement in the field (Barber et al. 1996).

Knowledge of the timing of JOD outbreaks and the relationship between mortality and oyster size has led to disease management techniques which include producing seed early in the spring or in the fall as well as using alternate nursery sites where JOD does not occur (Barber et al. 1996). Additional benefit can be achieved by utilizing oysters selected for fast growth. A program of genetic selection for fast growth of C. virginica was initiated at the University of Maine in 1986 in conjunction with the state's expanding oyster culture industry (Hawes et al. 1989). In general, "selected" sublines (parents from largest 20% (total weight) of previous generation of selected oysters) have outperformed "control" sublines (parents from entire weight distribution of previous generation of control oysters), both in terms of growth and survival in the Damariscotta River, Maine, where JOD is endemic (Davis et al. 1990, 1991, Davis and Barber, unpublished data). Thus while these oysters have been consciously selected for fast growth, they have also, by default, been selected for tolerance to JOD in recent years. To assess the effectiveness of this selection process to date, we compared growth and survival of a selected cohort with that of an unselected, JOD-susceptible cohort at two locations where JOD is enzootic.

Methods:

Broodstock oysters used to produce the "selected" subline were the largest 20% (total weight) of surviving Flowers F2 selected oysters produced in 1994. Similarly, the "control" subline was produced by parents randomly chosen (entire size distribution) from surviving F2 control oysters produced in 1994. Parents of the unselected "wild" oysters (not previously exposed to JOD) came from natural beds in the Piscataqua River, Maine. The three groups of broodstock were conditioned separately beginning in February 1996. Spawning was induced using thermal shock on 23 May, 1996. The number of parents contributing gametes was 13 males and 5 females for the selected group; 8 males and 2 females for the control group; and 8 males and 12 females for the wild group.

Larvae were reared in 2000 l conical tanks containing filtered (10 µm) water from the Damariscotta River, which was replaced every other day. Temperature in the tanks was maintained at 22-24 ^{o}C and food (mixed diet of *Isochrysis galbana*, *Tetraselmis* sp., and *Thalassiosira pseudonana*) was added twice per day. Eyed larvae were collected and placed into a setting tray containing mini-cultch (crushed oyster shell) on a screen. After the settlement period (48 hr), spat were placed in downwellers and fed a diet similar to that given to the larvae.

Spat (2-3mm shell height) were placed at two sites: Damariscotta River, Newcastle, Maine (lat 44^{o}01'N; long 69^{o}32'W) and North Bay, Osterville, Massachusetts (lat 41^{o}37'N; long 70^{o}26'W) on 4 and 5 July 1996, respectively. Five replicate trays each containing 500+ oysters (stocking density = 2000 oysters/m2) were deployed for select, control, and wild groups. Oysters at both locations were sampled regularly between July and November 1996. On each sampling date, shell height measurements were obtained from a random subsample of 50 individuals per replicate using either image analysis (from photographs) for small (<5mm) oysters or digital calipers for larger oysters (>5mm). All individuals were checked for general health and all dead oysters were counted and removed from trays.

On the final sampling date in 1996 (14 November for North Bay and 21 November for the Damariscotta River), 30 oysters from each group were weighed (whole) prior to being shucked. Meats and shells of each individual were dried to a constant weight at 45 ^{o}C prior to final weighing. Thus mean whole weights, tissue dry weights, and shell dry weights were obtained for each group at each site. All remaining Damariscotta River oysters were placed into tanks in the Flowing Seawater Laboratory of the Darling Marine Center for holding.

In May 1997, remaining Damariscotta River oysters were re-deployed and monitored for growth (shell height) and mortality as in 1996. On the final sampling date (2 December 1997) oysters were weighed, shucked, and dried as above for determination of mean whole weight, dry tissue weight, and dry shell weight.

At the North Bay site, salinity was recorded on each sampling date with a refractometer, and temperature was recorded every 3 hr with a datalogger (Onset StowAway). At the Damariscotta River site, salinity was determined biweekly with a YSI salinometer and temperature was recorded every 6 hr with a thermograph (Ryan RTM 2000).

Mean shell heights and cumulative mortalities (arcsin transformed) of select, control, and wild groups were statistically compared between and within sites using analysis of variance and Duncan's Multi-range post hoc test (1996) and between groups (1997) with t-tests (DataMost, 1995).

Results - 1996:

In the Damariscotta River, Maine, growth of all groups was rapid between 4 July and 16 August 1996, but slower thereafter (Figure 1). Mean shell height in the wild group on 16 August (16.5 mm) increased only slightly to a final mean shell height of 18.7 (± 2.8 SD) mm on 21 November. In contrast, the select and control groups continued to grow until 12 September, reaching mean shell heights of 33.5 (± 0.5 SD) mm and 33.2 (± 1.4 SD) mm, respectively, on 21 November. Over the entire study period in 1996, mean shell height of both select and control oysters was significantly greater than that of wild oysters (P=0.0002).

Select and control oysters also grew faster than wild oysters in North Bay, Massachusetts in 1996 (Fig. 2). Compared to the Damariscotta River site, however, growth of all groups was more continual and occurred over a longer period of time (5 July to 17 Oct.). On 14 November, mean shell height of select, control, and wild oysters was 38.9 (± 0.5 SD) mm, 38.8 (±1.0 SD), and 27.7 (± 1.3 SD) mm, respectively. Mean shell height of both select and control groups was significant greater than that of wild oysters throughout 1996 (P=0.0034).

Differences in oyster growth occurred between sites (compare Figures 1 and 2). Final mean shell height of all three groups was significantly greater in North Bay than the respective groups in the Damariscotta River (P=0.0002). However, the difference in final mean shell height between sites was greater (9.0 mm) for wild oysters than select (5.4 mm) and control (5.5 mm) oysters.

Final whole weights of select and control oysters were significantly greater ($P<0.05$) than that of wild oysters at both locations (Figures 3 and 4). Because there were so few wild oysters remaining at the end of the 1996 study period from the Damariscotta River, animals were not sacrificed for determination of mean tissue dry weight and mean shell dry weight. Whole weight, shell dry weight and tissue dry weight were greater for all groups at the North Bay site than their respective groups at the Damariscotta River site.

Juvenile Oyster Disease was highly prevalent in the Damariscotta River in 1996. Growth of wild oysters ceased after 16 August and mortality, with characteristic valve cupping and conchiolin deposits on inner valve surfaces, was initially observed on 30 August. Mortality in all groups occurred primarily during a one month period (Figure 5). For the wild group, mortality was greatest between 30 August and 25 September. Most mortality in the select and control groups occurred later, between the 12 September and 25 September sampling dates. Little mortality occurred in any group after 25 September. Final mean cumulative mortality on 21 November was 95.7 (± 2.5 SD) % in the wild group compared to 11.2 (± 5.0 SD) % in the select group and 14.7 (±12.1 SD) % in the control group; the difference between select and control and wild groups was statistically significant (P=0.0001).

In North Bay, JOD was not seen until late in the growing season, and then its impact was minimal. As a result, growth of both groups was continual over the study period, and valve cupping and conchiolin deposits on inner valve surfaces were rare. Cumulative mortalities in both selected and wild groups were low (Figure 6). Final mean cumulative

mortality on 14 November was 2.5 (± 3.9 SD) % in the select group, 0.8 (± 0.8 SD) % in the control group, and 4.6 (± 3.2 SD) % in the wild group. Differences among groups were not statistically significant (P=0.2187).

Differences in the impact of JOD between the two sites was further illustrated by differences in cumulative mortality (compare Figures 5 and 6). Final mean cumulative mortality was significantly greater in the Damariscotta River than in North Bay for all three groups of oysters (P=0.0079).

Water temperature was generally greater in North Bay than in the Damariscotta River, particularly in July and August (Figure 7). The maximum daily temperature recorded in North Bay was 25.5 ^{o}C while that in the Damariscotta River was 24.7 ^{o}C. Water temperature at both locations began to decline in September. Overall, mean daily temperature in North Bay (18.5 ^{o}C) was significantly greater (P=0.0004) than in the Damariscotta River (16.0 ^{o}C).

Salinity at both sites was between 21 and 30 ppt throughout the entire study period (Figure 8). In July, salinity was greater in North Bay than in the Damariscotta River, but from early August onward, salinity was greater in the Damariscotta River than in North Bay.

Results - 1997:

No wild oysters survived the winter, so only select and control groups were followed from May through November 1997 in the Damariscotta River. Growth of both groups occurred primarily from June through August (Figure 9). Mean shell height of the select group on 2 December 1997 was 70.4 mm, which was significantly greater (t-test, P=0.0001) than the mean shell height of the control group (63.1 mm).

On 2 December, mean whole weight, dry shell weight, and dry tissue weight of select oysters were significantly greater (P=0.0001) than those of control oysters (Figure 10). Whole weight of select oysters was 53.8 (± 1.7 SD) g compared to 39.3 g (± 3.4 SD) g for control oysters. Dry shell weight of the select group was 39.2 (± 1.6 SD) g compared to 24.6 (± 2.3 SD) g for the control group. Tissue dry weight of select oysters was 2.1 (± 0.1 SD) g while that of control oysters was 1.3 (± 0.1 SD) g.

Most mortality in both select and control groups occurred between 21 May and the end of June (Figure 11). During this period, the select group had a slightly higher mortality rate. From July through November, little additional mortality was noted in either group. Final mortality on 2 December was 24.9 (± 5.4 SD) % in the select group and 23.4 (± 5.4 SD) % in the control group; these values were not significantly different (t-test, P>0.05).

Temperature in the Damariscotta River increased steadily through May and June, exceeding 20 ^{o}C in late June. Weekly mean temperature remained above 21 ^{o}C until mid-August, before declining (Figure 12). Salinity in the river remained between 31 and 33 ppt from July through September (Figure 12).

Discussion:

The fact that JOD did not manifest itself in North Bay at the same time and to the extent that it did in the Damariscotta River in 1996 indicates that either the causative agent was not present at the time that seed was deployed or the environmental conditions were not suitable for JOD development. Temperature (22-26 ^{o}C) and salinity (22-30 ppt) in North Bay, however, were well within the range of those preferred by JOD (Bricelj et al. 1992, Lewis et al. 1996). If anything, given the generally greater water temperature in North Bay, it might be expected that the disease would have been evident sooner in North Bay than in the Damariscotta River, had the causative agent been present. Thus it is likely that the causative agent of JOD was not initially present and therefore not transported with the oysters from their production site in Maine to the commercial lease in Massachusetts. This supports previous findings that occurrences of JOD are site specific and cannot be related to a single hatchery or common broodstock (Bricelj et al. 1992).

The select and control groups grew faster than the wild group at both locations. This is explained primarily by the fact that the Flowers line itself originated from a selected stock. Thus both select and control groups used here had undergone previous selection for fast growth. The selection process undertaken in Maine since 1986 defined the select subline as the top 20% by weight at age 18 months. Thus is was not until the end of the 1997 growing season that the select subline reached a size that was statistically greater than the control subline. These findings support previous studies which reported improved growth as a result of selective breeding (Newkirk and Haley 1983, Davis et al. 1990, 1991, Hadley et al. 1991). It would be reasonably expected that continuation of the selection process would lead to further improvements in growth rate.

The finding that all groups grew to a greater final size in North Bay than in the Damariscotta River may have been related to the greater water temperature at the North Bay site. More importantly, however, differences in JOD pressure also undoubtedly played a role in suppressing growth of oysters in the Damariscotta River. The fact that the difference in growth exhibited by select and control oysters between the two sites was about half that of wild oysters, suggests that the select and control groups had an increased tolerance to JOD as well as a genetically derived growth advantage. The evidence for a genetically based disease resistance is further supported by the fact that in the Damariscotta River, select and control oysters continued to grow for a month after growth in wild oysters had ceased (due to JOD infection).

Mortality of all groups was minimal in North Bay because JOD did not manifest itself until late in the growing season, after which oysters had exceeded 25 mm in mean shell height. In the Damariscotta River, however, heavy challenge by JOD resulted in differential mortality between select and control and wild groups. Greater survival of the select and control groups can be attributed to either faster growth or a genetic component derived from previous selection from JOD, or a combination of both. Previous research has demonstrated that once oysters attain a shell height of 25-30 mm, mortality caused by JOD is 20% or less (Bricelj et al. 1992, Davis and Barber 1994). More important than size at the time of mortality, however, is size at the time of initial exposure to JOD infectious agent(s). Barber et al. (1996), in a study of the seasonal impacts of JOD, found that a cohort having a mean shell height of 27.1 mm at the end of July, 3-4 weeks prior to the onset of major mortality in smaller cohorts, had a final cumulative mortality of 19%; cohorts having a mean shell height of 22 mm or less experienced mortality ranging from 65 to 95%. In all cases, signs of JOD did not appear until 3-4 weeks after initial deployment; it was concluded that this period of time represented an incubation period for the JOD etiological agent (Barber et al. 1996). In this study, mortality of wild oysters in the Damariscotta River began in late August. Three weeks before the onset of mortality (2 August) thus represents the time of first exposure to JOD etiological agent(s). On this date, mean shell height of the select group was 13.2 mm, that of the control group was 12.3 mm, and that of the wild group was 10.6 mm, all well below the 25 mm refuge size. Thus on the basis of size alone, it would be expected that all groups of oysters would have experienced similar mortality. The fact that cumulative mortality in the select and control groups was only 11.2% and 14.7%, respectively (compared to 95.7% in the wild group), suggests strongly, however, that both select and control sublines have gained a genetically based tolerance to JOD as the result of previous exposure to and selection by the disease. Indeed, the select and control sublines used in this study (Flowers F3) were produced from previous generations that have been reared in the Damariscotta River, where JOD has been endemic since 1988 (Davis and Barber 1994, Barber et al. 1996, Davis and Barber, unpublished data).

In the Damariscotta River in 1997, select and control groups experienced the same level of cumulative mortality (about 30%), most of which occurred early in the growing season. Most likely this was related to the conditions under which they were held over the winter. There was no evidence that these oysters were affected by JOD, even though it was prevalent in the river in 1997 (Crosby and Barber, unpublished data).

Genetic selection of oysters for disease resistance has been previously demonstrated. In cases where diseases have caused massive mortality in host populations, natural selection has resulted in increased disease tolerance in offspring of surviving organisms. This has been reported for populations of *C. virginica* affected by Malpeque disease (Needler and Logie 1947) and diseases caused by protozoan parasites *Perkinsus marinus* (Andrews and Hewatt 1957), *Haplosporidium costale* (Andrews and Castagna 1978), and *H. nelsoni* (Haskin and Ford 1979). Evidence of naturally acquired disease resistance to *H. nelsoni* in populations of C. virginica in Delaware Bay led to a program of selective breeding to ascertain whether improved survival was heritable and could be enhanced relative to natural populations (Ford 1988). Ford and Haskin (1987) reported improved survival of oysters through six generations of selection by *H. nelsoni*. There is also evidence that JOD-induced mortality is lower in stocks of oysters previously exposed to JOD than in susceptible (unselected) stocks (Farley et al. 1997).

In spite of success at selecting for disease resistance, little is known of the defense mechanisms that lead to improved survival in selected strains. The absence of a clear defense mechanism has led to the conclusion that resistance to *H. nelsoni* mortality is under the control of multiple genes and results in selected oysters being generally physiologically superior (Ford and Haskin 1987, Barber et al. 1991).

Similarly, Baud et al. (1997) found that strains of oysters, *Ostrea edulis*, selected for resistance to the parasitic protozoan, *Bonamia ostreae*, grew faster and had lower mortality than control groups in the absence of disease pressure, but under less than optimal nutritional conditions. It was suggested that in this case, selection for disease resistance had resulted in an increased tolerance of stress in general. Identification of the mechanism of resistance to JOD mortality awaits determination of an etiological agent.

The fact that selected oysters produced in Maine have increased resistance to JOD mortality provides another approach for improving oyster production in areas where JOD is endemic. As previously reported, the effects of JOD can be minimized by obtaining seed that is greater than 25 mm in shell height at the time of exposure to JOD (Davis and Barber, 1994; Barber et al., 1996). This size can be achieved by conditioning broodstock out of season so as to obtain seed early (May) or late (August) in the year. Producing seed early in the year will ensure that a size >25 mm is attained before the onset of JOD. Placing seed in nursery trays after mid-August will avoid exposure to JOD that summer but requires holding the seed over the winter; subsequent deployment the early following spring will ensure that size-based tolerance to JOD is attained. Continued selection for fast growth will decrease the time required for oysters to attain the critical size refuge. The finding in this study that oysters selected for fast growth in the Damariscotta River also have increased tolerance to JOD, gained either directly via disease selection or indirectly via selection for fast growth, will ensure an even greater survival in areas where JOD is endemic.

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