WASHINGTON SEA GRANT PROGRAM

BREEDING FOR RESISTANCE TO SUMMERTIME MORTALITY IN THE PACIFIC OYSTER (CRASSOSTREA GIGAS)

By J. Hal Beattie, William K. Hershberger, Kenneth K. Chew, Conrad Mahnken, Earl F. Prentice and Chris Jones

July 1978

WSG 78-3

DIVISION OF MARINE RESOURCES
UNIVERSITY OF WASHINGTON 98195

Prepared under the
National Sea Grant Program

WASHINGTON SEA GRANT PROGRAM

BREEDING FOR RESISTANCE TO SUMMERTIME MORTALITY IN THE PACIFIC OYSTER (CRASSOSTREA GIGAS)

By

J. Hal Beattie, William K. Hershberger, Kenneth K. Chew
Conrad Mahnken, Earl F. Prentice

and

Chris Jones

July 1978

WSG 78-3

Washington Sea Grant Program
Division of Marine Resources
University of Washington Seattle 98195

ABOUT THE AUTHORS

J. Hal Beattie, William K. Hershberger, and Kenneth K. Chew are all with the College of Fisheries at the University of Washington in Seattle. Conrad Mahnken and Earl F. Prentice are with the National Marine Fisheries Service in Seattle; and Chris Jones is with the Washington State Department of Fisheries, Point Whitney Shellfish Laboratory in Brinnon, Washington.

Support for publication of this manuscript was provided by Grant No. 04-7-158-44021 from the National Oceanic and Atmospheric Administration to the Washington Sea Grant Program.

Additional copies of this publication may be obtained from Washington Sea Grant Communications, Division of Marine Resources, University of Washington HG-30, Seattle, WA 98195 for $1.50 each. Bulk rates on request. Make checks payable to University of Washington. Washington residents please add sales tax.

CONTENTS

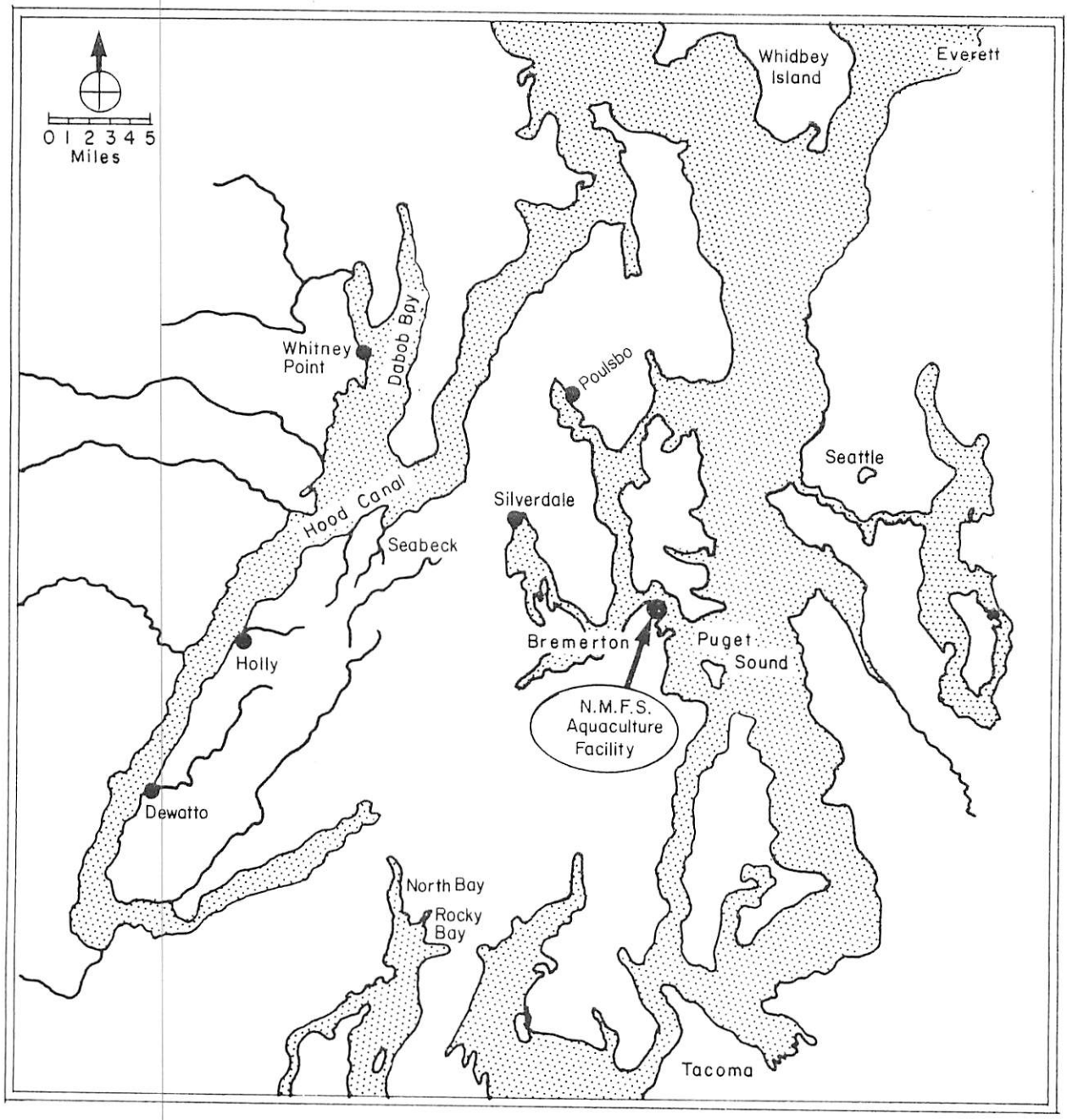

FIGURE 1. Map of Puget Sound indicating the sites used in this study.

INTRODUCTION

Significant summer mortalities of Pacific oysters (*Crassostrea gigas*) are common in Matsushima Bay, Japan, and have also occurred in California and Washington. During the early 1960's, these mortalities reached 35 to 50%, prompting an investigation coordinated by the National Marine Fisheries Service in California, Oregon, and Washington (Glude 1975; Katkansky et al. 1972; Scholz et al. 1968, 1971, 1973). The epidemiology of the mortality in the U.S. was similar in character to that described for Matsushima Bay (Imai et al. 1968; Kanno et al. 1965; Koganezawa 1975). High temperatures (18° C to 21° C) and elevated nutrient levels were associated with these mortalities.

Beginning in 1972, the University of Washington College of Fisheries began a study of possible pathogenic organisms and their mode of infection by observing oysters in laboratory-simulated summertime conditions of elevated temperature and nutrient enrichment (Lipovsky and Chew 1972). Mortality was found to be associated with *Vibrio*-type bacteria. Later studies implicated *Vibrio alginolyticus* and *Vibrio anguillarum* as facultative pathogens (Grischkowsky and Liston 1974). Since treatment of this disease by use of antibiotics is economically impractical, it was concluded that a logical approach to reducing mortality would be to determine if stocks of oysters could be genetically selected for improved survival during summertime stresses. This study was initiated with the following two objectives: 1) To determine whether resistance to the stress of elevated temperature is a genetically modifiable trait; accomplishment of this would be indicated by significantly greater survival of experimental crosses over imported Japanese stocks at elevated temperatures; 2) To assess the genetic variability within and among experimental crosses using electrophoretic analysis. This would shed light on the nature of inheritance in oysters and might help identify a marker associated with improved survival.

METHODS AND MATERIALS

Figure 1 indicates the locations included in this study. Adult challenges and larval rearing were accomplished at Sea Farms, Inc., a private hatchery then located on Liberty Bay at Poulsbo, Washington. Post-larval oysters were reared at Whitney Point, Rocky Bay, and North Bay, Washington. Progeny were tested at the National Marine Fisheries Service Aquaculture Station on Clam Bay, Washington. The overall experimental approach is diagrammed in Figure 2.

Challenge of Brood Stock

Three- and four-year-old Japanese stock *Crassostrea gigas* were challenged to select those oysters that could survive elevated temperature stresses. The challenging was performed at 21° C with water changed every 2 to 3 days, and each water change was enriched with 1 ml per liter of Davis' nutrient solution. After 60 to 70% mortality, the challenge was terminated. The survivors were placed into ambient (12 to 14° C) seawater for a recovery period of 10 to 14

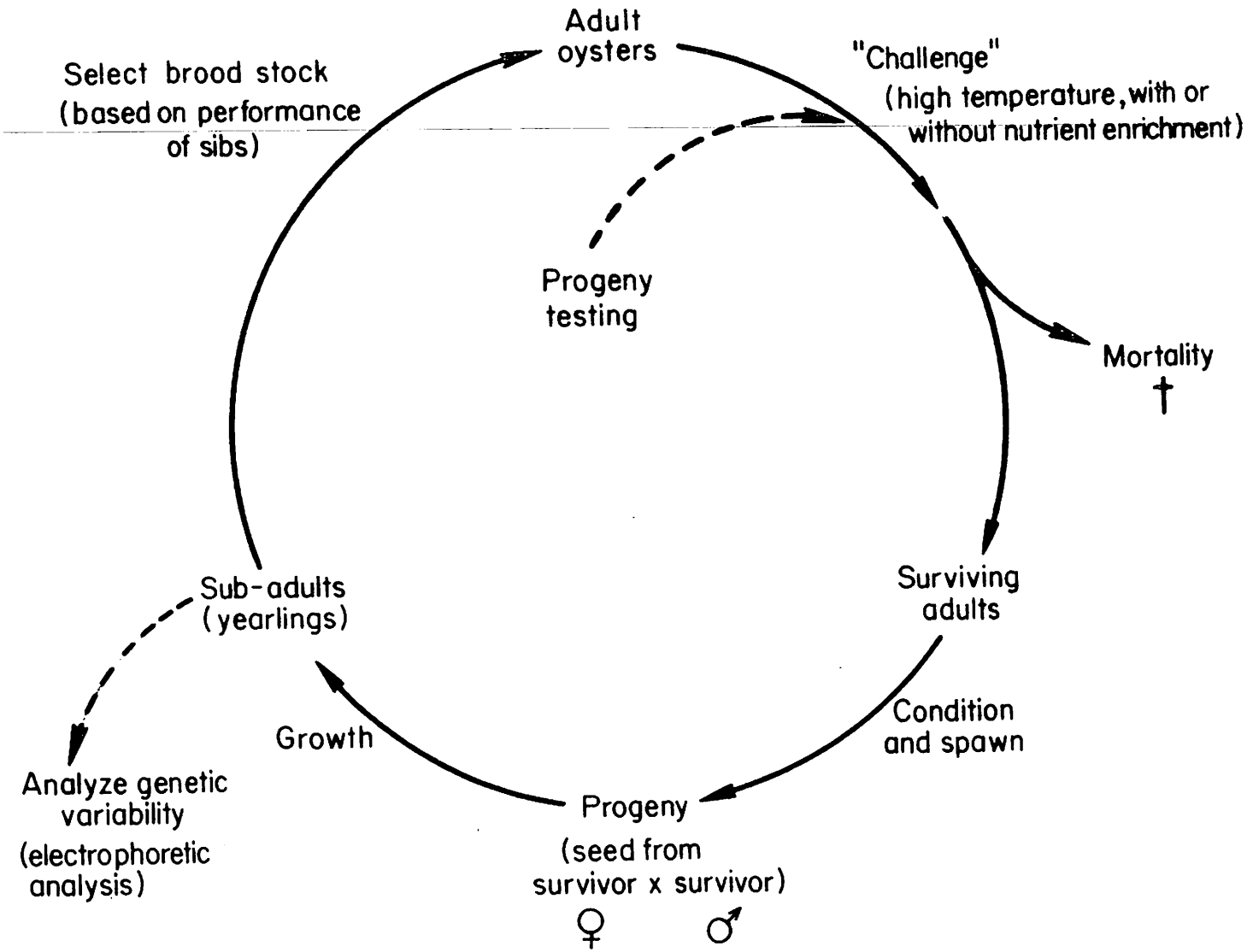

FIGURE 2. Diagram of the selection design and genetic analysis used in breeding oysters for resistance to mortality during simulated summertime stress.

days, then moved to trays of running seawater at 18° C for conditioning. There was usually a delayed mortality during the recovery and conditioning periods. Parent groups exhibited overall survival, varying from 3 to 35%.

Spawning

Oysters were placed in individual containers of seawater at 27° C for 4 hours to induce spawning. If spawning did not commence in this time period, water was added at 15-minute intervals. As a last resort, sperm from one stripped parent was used as a spawning stimulus.

Larval Rearing

Each family resulted from crossing one male with one female and was labeled by the date of spawning (year, month, day) and coded. The methods of rearing bivalve larvae described by Loosanoff and Davis (1963) and detailed by Chanley (1975) were followed. Food for the larvae consisted of *Monocrysis lutheri* and *Thallasiosira pseudonana*. Oyster shell was used as a setting substrate. During 1974, six crosses were made; three survived through setting. In 1975, four of eight crosses were set. Thus seven genetically separate families were produced.

Post-larval Rearing

Newly set families were held at 20° C and fed *T. pseudonana*. When the juveniles reached 2 mm, they were rafted in Liberty Bay, Washington. Because of water quality problems, Sea Farms ceased operations in August 1975. The experimental oysters were moved at that time to a Washington State Department of Fisheries (WDF) holding pond at Whitney Point on Hood Canal. During the spring of 1976, the seven experimental families, plus 1974 and 1975 Japanese stocks and 1974 Dabob Bay stocks, were moved to WDF tidal grounds on North Bay in Case Inlet. The oysters were placed at about the +1 tidal height in wooden trays with hardware cloth bottoms. The trays rested on concrete blocks, out of the sediment. Other Japanese stock oysters were grown in Rocky Bay and also in Case Inlet.

Challenging the F_1 Generation

The families and stocks were tested at 21° C. No nutrient enrichment was used. Japanese stocks were used as a standard of comparison. Dabob Bay stock (1974) was also tested to see if a local breeding population would have any acquired resistance. One test each was performed in October, November, and December of 1976. The duration of each test period was up to the time at which the cumulative mortality of the Japanese stock reached 50% (LT 50_{JS}).

All oysters in the first test were from the growing trays in North Bay. In the second and third tests, the 1975 Japanese stock was from Rocky Bay. Between 44 and 132 oysters of each family and stock were tested simultaneously in the same container. Control animals were held in a refrigerated container at 9° C to monitor background mortality. Survivors of the first test were taken to the College of Fisheries for electrophoretic analysis.

Table 1. The enzyme systems studied in each F_1 family and test population subjected to the mortality-inducing challenge

Enzyme/Protein System (Abbreviation)	74-10-14-X	75-6-24-X	74-9-15-Y	75-6-24-Y	74-10-14-Y	75-6-24-Z	74-Dabob Bay	Japanese test
Alanine Amino Transferase (AAT)	X	X	X	X	X	X		
Phospho-glucose Isomerase (PGI)	X	X	X	X	X	X	X	X
Phosphoglucomutase (PGM)	X	X	X	X	X	X	X	X
Isocitrate Dehydrogenase (IDH)	X	X	X	X	X	X	X	X
Leucine Amino Peptidase (LAP)		X			X	X	X	X
Esterase (Est)		X	X	X	X	X		X
Muscle Protein (MP)					X	X	X	X
Acid Phosphatase (APh)						X	X	
Peptidase (PEP)						X		

Electrophoretic Analysis

To assess the genetic variability in families and stocks, about 40 individuals from each were analyzed by electrophoretic separation of proteins and/or enzymes. For the analysis, gut and adductor muscle tissues were removed from each oyster, homogenized, and centrifuged. The supernatant from each sample was then analyzed electrophoretically according to the procedures described by Buroker (1975). The enzyme and protein systems analyzed in each family or stock are indicated in Table 1. Between five and ten separate loci were monitored.

Statistical Analysis

Cumulative mortalities were analyzed using the Kolmogorof-Smirnof test, a nonparametric analysis for comparison of distributions (Siegel 1956). The populations in each test were compared up to $LT50_{JS}$ within each year-class and experiment.

RESULTS AND DISCUSSION

Thermal Challenging

Cold-water controls had few mortalities. Cumulative percent mortality graphs of the F_1 tests are arranged to show comparison by year set: Figures 3, 4, 5 (1974), and 6, 7 and 8 (1975).

Statistical Results

During the first test (Figure 3), all 1974 families and the 1974 Dabob Bay stock showed significantly greater survival ($P < .05$) than the 1974 Japanese stock, but none were significantly different during any later test (Figures 4, 5). Families 75-6-24-Y and 75-6-24-Z had significantly higher survival than the 1975 Japanese stock in all tests (Figures 6-8). Family 75-6-24-W survived significantly better in its only test (Figure 6). Survival of 75-6-24-X was not significantly different from that of the Japanese stock in any test.

There were differences between tests. The time required for $LT50_{JS}$ was longer for each succeeding test: 6 to 9 days in October, 12 to 14 days in November and 19 to 20 days in December. This is suspected to be related to the changing gonadal condition of the oyster from mid- to late-fall. Gonadal condition has been implicated with oyster mortalities in Japan (Koganezawa 1975).

The challenge results showed variations in survival between families. However, the demonstration of a significant survival advantage of some families over imported stocks indicated that in these cases resistance to the stress of elevated temperature had been selected for. Hopefully, future selection based on the results of electrophoretic analysis and more sophisticated breeding techniques will provide families uniformly resistant to this laboratory-induced mortality.

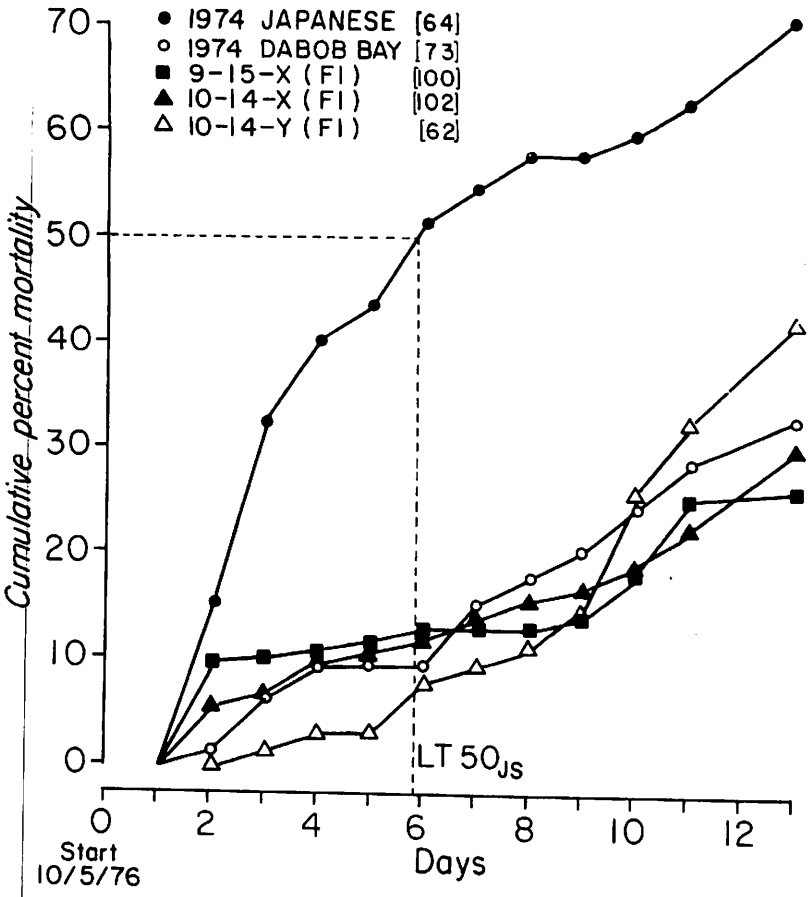

FIGURE 3. Cumulative percent mortality of 3 experimental families, Dabob Bay stock and Japanese stock. Total number of oysters tested for each are shown in brackets.

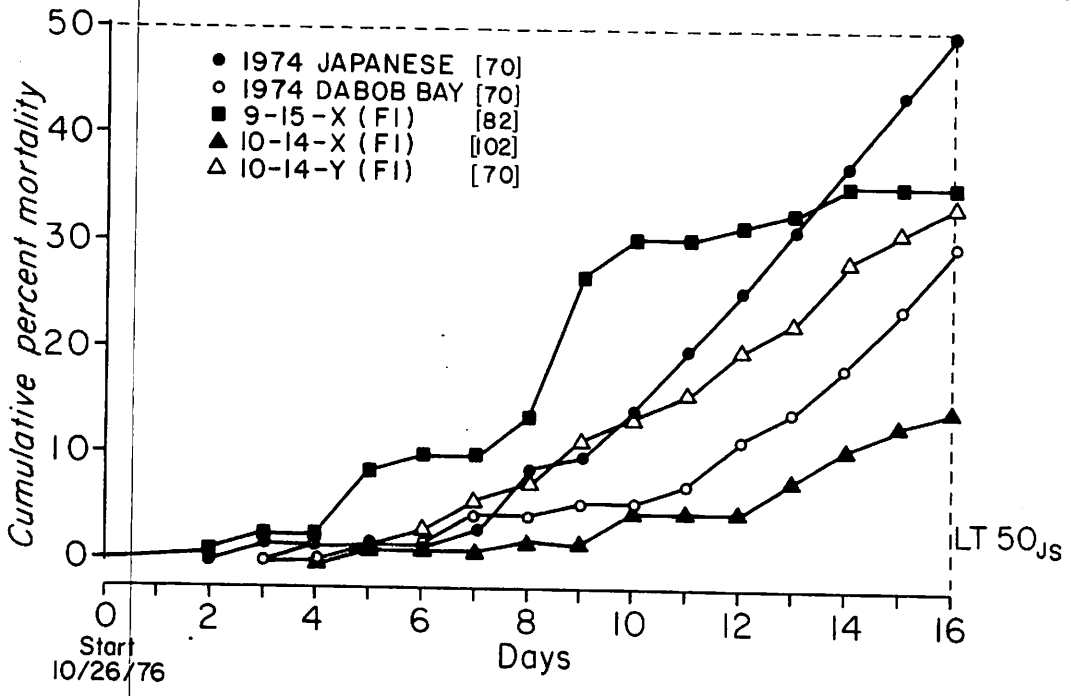

FIGURE 4. Cumulative percent mortality of 3 experimental families, Dabob Bay stock and Japanese stock. Total number of oysters tested for each are shown in brackets.

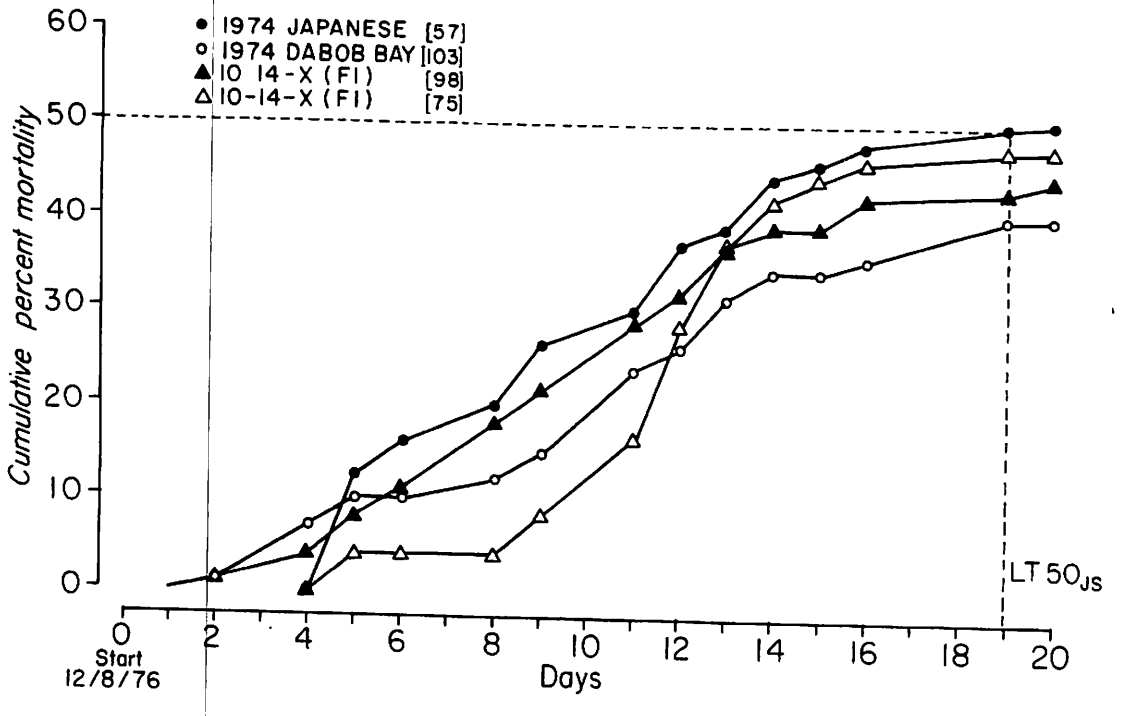

FIGURE 5. Cumulative percent mortality of 3 experimental families and Japanese stock. Total number of oysters tested for each are shown in brackets.

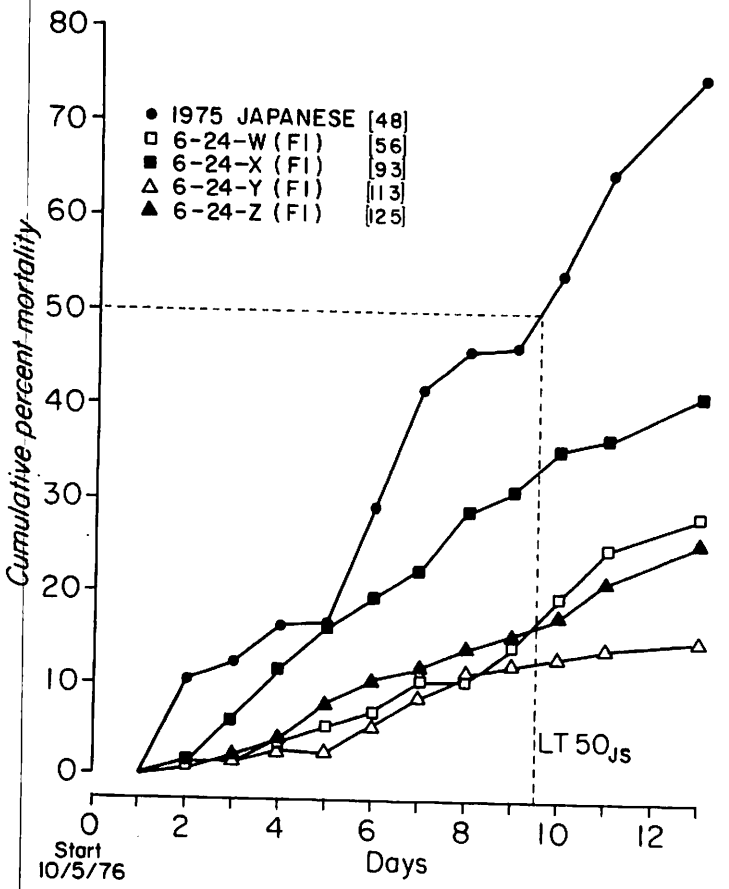

FIGURE 6. Cumulative percent mortality of 4 experimental families and Japanese stock. Total number of oysters tested for each are shown in brackets.

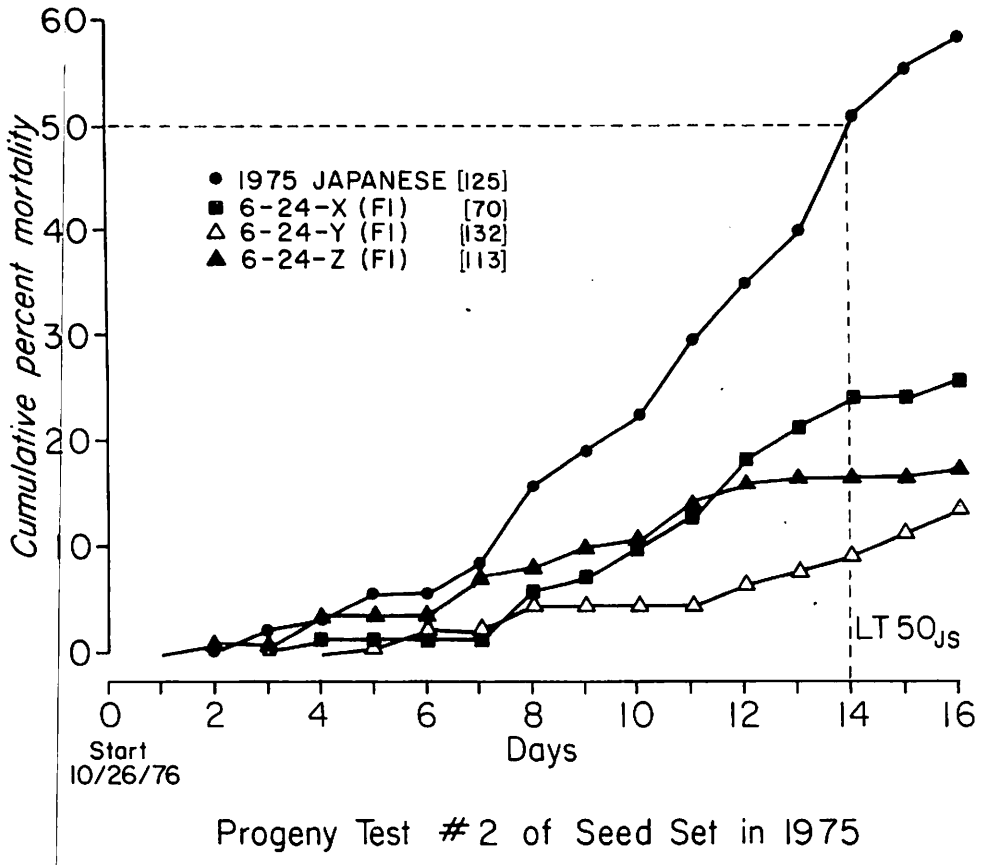

FIGURE 7. Cumulative percent mortality of 3 experimental families and Japanese stock. Total number of oysters tested for each are shown in brackets.

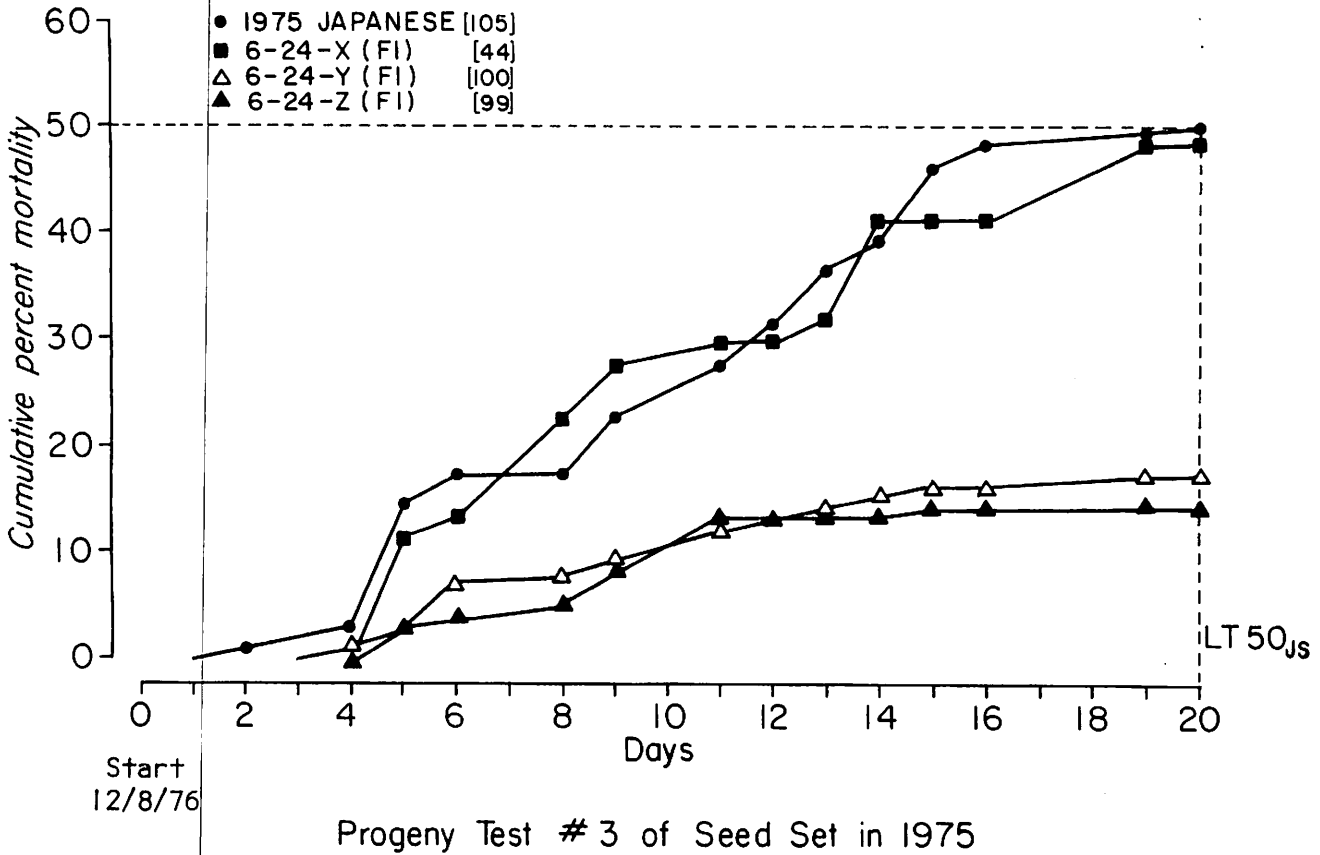

FIGURE 8. Cumulative percent mortality of 2 experimental families, Dabob Bay and Japanese stock. Total number of oysters tested for each are shown in brackets.

Electrophoretic Analysis

The results of the electrophoretic analysis of the families and stocks are presented in Table 2. These data show that there was considerable genetic variability among the families produced. For the loci tested, the stocks (Japanese and Dabob) contained 100% heteromorphic alleles whereas the percentage of heteromorphic alleles from the families varied from 100% to 60%. The loci that were monomorphic were primarily from two enzyme systems, isocitrate dehydrogenase (IDH) and leucine aminopeptidase (LAP).

Among the families that showed greater survival over the control populations, those that were monomorphic for the alleles determining LAP variability were somewhat more susceptible during testing. In addition, a high percentage of heteromorphic alleles does not appear to be associated with survival; that is, while the Japanese and Dabob Bay stock both contained 100% heteromorphic alleles for the loci tested, there was a difference in their survival.

CONCLUSIONS AND SUMMARY

The results to date indicate a good potential for the development of a strain of oysters resistant to thermal stress. First-generation progeny were produced from Japanese stock oysters that were survivors of thermal stress (21° C). Out of seven families, two consistently survived thermal stress significantly better than Japanese stocks of the same age, whereas no family showed poorer survival. Change in gonadal maturity is a suspected variable affecting the late autumn test results described in this report. Future tests will be conducted during the period of ripe gonadal development from mid- to late-summer.

The genetic analysis by electrophoresis showed that genetic change was being induced by the breeding techniques used. This change does not seem to be consistent in that varying percentages of loci were found to be monomorphic. However, there was some consistency in that the monomorphic loci were primarily of two enzymes: isocitrate dehydrogenase and leucine aminopeptidase. The question of whether any of these loci are associated with survival during thermal stress requires further investigation.

The benefit of these investigations to oyster growers will be substantiated when selected hatchery crosses are shown to perform on the growing grounds as well as they do in the laboratory. In the spring of 1977, experimental families produced during 1976 at the College of Fisheries experimental shellfish hatchery at the National Marine Fisheries Service Aquaculture Station near Manchester, Washington, were planted in various oyster growing areas in the state; these areas include some with a history of oyster summer mortality. Several of the 1977 families were sent to Kahuku Farms, Inc., Waimanalo, Hawaii, where survival of Japanese stock oysters has been a problem. The performance of the families is being monitored in these areas. In the event that the results from this planting support the tentative conclusions presented here, oysters from the most promising families will be made available to commercial shellfish hatcheries for large-scale testing. In addition, work is now in progress producing second-generation families from the oysters discussed in this report.

Table 2. The genetic heterozygosity in each F_1 family and test population subjected to the mortality-inducing challenge as reflected by number heteromorphic or monomorphic gene loci

F_1 family or test population	No. of loci analyzed	No. of loci monomorphic	No. of loci heteromorphic	Specific loci monomorphic
74-10-14-X	3	2	3	IDH_1 and IDH_2
75-6-24-X	7	2	5	LAD_1 and LAP_2
74-9-15-Y	6	2	4	IDH_1 and IDH_2
75-6-24-Y	6	1	5	IDH_2
74-10-14-Y	9	4	5	LAP_1 and LAP_2 IDH and IDH_2
75-6-24-Z	10	0	10	---
Dabob Bay	7	0	7	---
Japanese Test	7	0	7	---

ACKNOWLEDGEMENTS

The Washington Sea Grant study in selective breeding for resistance to mortality during summertime stresses is a cooperative effort between the University of Washington College of Fisheries, the Environmental Protection Agency, the National Marine Fisheries Service, and the Washington State Department of Fisheries.

Sea Farms, Inc., a private oyster hatchery, provided the space and help necessary to produce the experimental genetic crosses. The Japanese stocks were provided by Dungeness Oyster Farms and Minterbrook Oyster Company.

Special thanks are due to Marsha Landolt of the College of Fisheries for constructive criticism of the manuscript and to Richard Bohn for performing the electrophoretic laboratory work.

LITERATURE CITED

Buroker, Norman E. 1975. Genetic variation in the Pacific oyster, *Crassostrea gigas*. J. Fish. Res. Board Can. 32(12): 2471-2477.

Chanley, Paul. 1975. Laboratory cultivation of assorted bivalve mollusks. Pages 297-318 in W.L. Smith and M.H. Chanley, eds., Culture of marine invertebrate animals, Plenum Press, New York.

Glude, John B. 1975. A summary report of Pacific Coast oyster mortality investigations 1965-1972. Proc. Third U.S.-Japan Meeting on Aquaculture at Tokyo, Japan, October 15-16, 1974. 1-28.

Grischkowsky, Roger S., and John Liston. 1974. Bacterial pathogenicity in laboratory-induced mortality of the Pacific oyster (*Crassostrea gigas*, Thunberg). Proc. National Shellfish. Assoc. 64: 82-91.

Imai, Takeo, Katsuyoshi Mori, Yoshio Sugawara, Hideo Tamate, Juichi Oizumi, and Osamu Itikawa. 1968. Studies on the mass mortality of oysters in Matsushima Bay VII pathogenic investigation. Tohoku Journal of Agricultural Research 19(4): 250-265.

Kanno, Hisashi, M. Sasaki, Y. Sakupai, T. Watanabe, and K. Susuki. 1965. General aspects of the mass mortality of oysters in Matsushima Bay and its environmental conditions. Bulletin of Tohoku Regional Fisheries Research Laboratory 25: 1-26.

Katkansky, Stanley O., Ronald W. Warner, Richard T. Burge, and Donald R. Manzer. 1972. Pacific oyster disease and mortality studies in California, May 1966 through June 1972. California Dep. Fish and Game, July 1972. Unpublished. National Marine Fisheries Service. 68 pp.

Koganezawa, Akimitsu. 1975. Present status of studies on the mass mortality of cultural oysters in Japan and its prevention. Proc. Third U.S.-Japan Meeting on Aquaculture at Tokyo, Japan, October 15-16, 1974. 29-34.

Lipovsky, Vance P., and Kenneth K. Chew. 1972. Mortality of Pacific oysters (*Crassostrea gigas*): the influence of temperature and enriched seawater on survival. Proc. National Shellfish. Assoc. (62): 72-82.

Loosanoff, Victor L., and Harry C. Davis. 1963. Rearing of bivalve mollusks. Pages 1-36 in F.S. Russell, ed., Adv. marine biology, Academic Press, Inc., London.

Scholz, Albert J., Ronald E. Westley, and Marvin A. Tarr. 1968. Pacific oyster mass mortality studies; summary report No. 2. Washington State Department of Fisheries. Unpublished report. 39 pp.

Scholz, Albert, J., Ronald E. Westley, and Marvin A. Tarr. 1971. Pacific oyster mass mortality studies; seasonal summary report No. 3. Washington State Department of Fisheries. Unpublished report. 26 pp.