Brackish Water

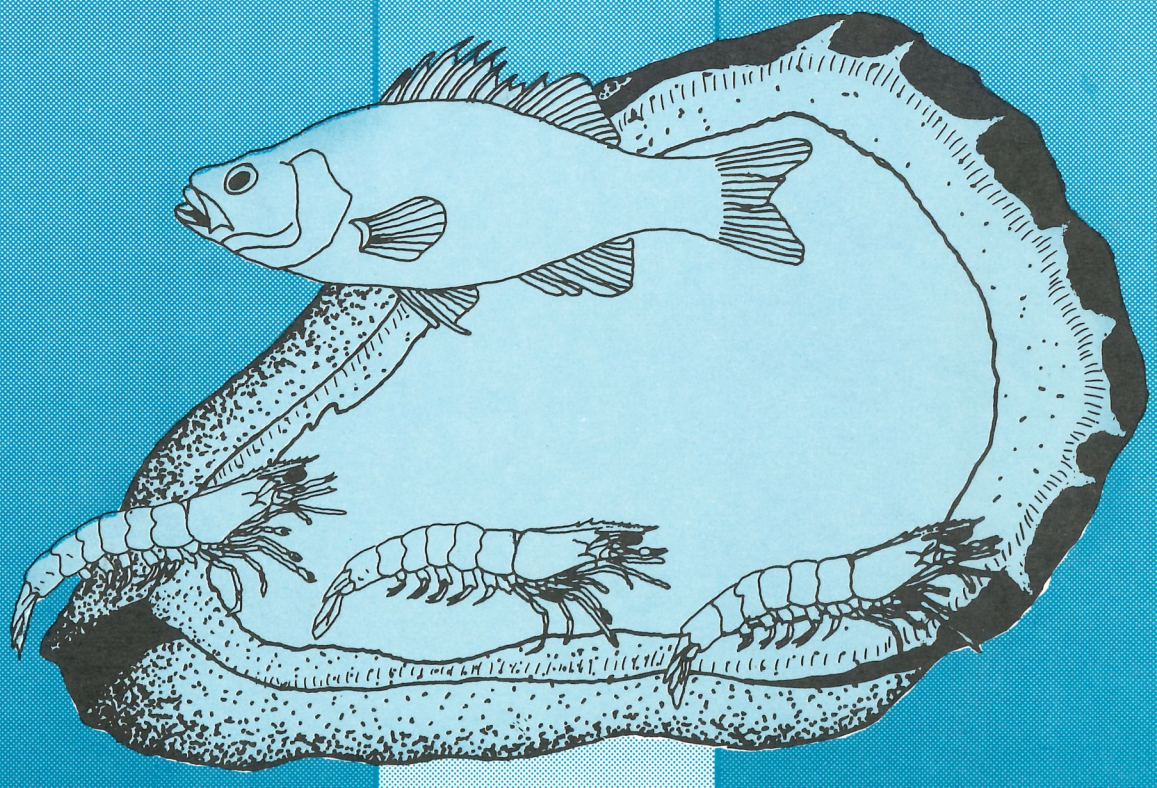
Fish Culture

Research Station

SYDNEY ROCK OYSTER GENETICS PROGRAMME

FINAL REPORT

Caroline Mason



Department of Agriculture New South Wales

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OVERVIEW

The aim of the genetics programme was to produce genetically improved Sydney rock oysters by a process of selective breeding. Because of unforseen problems in rearing the oyster larvae, this aim was not acheived. The substance of this report, then, is an account of the two batches of larvae that were partially successful, the attempt at rearing QX resistant larvae and of experiments and observations made in the course of the work. A discussion of the reasons for the failure of the programme is presented, with the hope that it might be of use to future work in similar programmes.

APPENDICES

I. Summary of the results of the larval rearing

II. Larvae batch 1. Regressions of size and mortality on age.

III. Spat batch 9. Mean sizes at 6 months.

IV. Post-spawning mortality of winter mortality broodstock.

V. Effect of delay in fertilisation on early larval development.

VI. Table of oyster farmers' observations of QX.

VII. 1984 FIRTA report.

VIII. 1985 FIRTA report.

IX. Article published in Australian Fisheries.

1) LARVAL REARING

BASKET SYSTEM - METHODS

A rearing system was devised to keep families of free-swimming, microscopic oyster larvae separate, while sharing the same water supply, to minimise the environmental component of phenotypic variance. This consisted of tall plankton mesh cylinders, (referred to as baskets) suspended in one of the 2000 L fibreglass tanks used in routine larval rearing (Plate 1). A similar system using plankton mesh containers has been used to keep fresh water prawns, *Macrobrachium rosenbergii*, families separate in the larval stage. (Malecha et al, 1982)

The rearing conditions were kept as close as possible to those of the routine larval rearing system, and the water in the tanks was changed every 2 days. During the water changes, the larvae were emptied from the baskets, rinsed off over plankton mesh seives of successivly larger mesh size to remove dead larvae, and a sample of larvae preserved in formalin for estimation of larval density and size. Larval densities were obtained by counting aliquots of the sample through a disecting microscope, and size by taking a photo-micrograph of the samples from each basket, and measuring the image on a print produced by a microfilm copier (Plates 2,3).

A number of different setting substrates were tested, including scallop shell chips, glass petri dishes and "Rens" (stacks of sheets separated by spacers, hung on a string) of mylar (which have been used successfully to settle hatchery reared Pacific oyster, *Crassostrea gigas*, larvae in New Zealand). These were placed or hung in each basket when the larvae reached the pediveliger stage.

RESULTS

The larvae used in the first batch were produced from one spawning and distributed amongst the baskets at stocking densities ranging from 2 to 10 per ml, to find the environmental component of variance in growth rates and survival in the baskets.

A few larvae from almost every basket survived to the setting stage, and about 4,000 spat were obtained on the petri dishes and scallop shell chips; the mylar rens were apparently not attractive setting substrates.

There were no significant differences in larval size between baskets at each age. The size distributions were normal (95% confidence limit), and the coefficient of variation of the distributions increased with age. There was no ($P>0.10$) correlation between growth rate of the larvae and the initial stocking density in the baskets. There were no significant differences in percentage mortality rates between baskets

stocked at different densities, although the higher density baskets appeared to have higher mortality rates (because more larvae died per day).

From this it was deduced that the effects of different larval densities and other environmental effects on the growth rate and survival of the larvae were negligible. The 4,000 spat were given to the routine hatchery team as it was considered that there were too few to be worth ongrowing at that time.

The second batch was spawned by survivors of winter mortality obtained from oyster farmers. (For details of observations in post-spawning mortality see appendix IV). This batch did not survive beyond 4 days. The fate of this and the next 6 unsuccessful batches is summarised as a table in Appendix I.

40 LITRE TANKS - METHODS

Because of the lack of further success using the baskets, twenty-four 40 L plastic tanks were set up and stocked with the progeny of twelve female and two male oysters. As with the baskets, rearing conditions were as close as possible to the conditions used in the routine hatchery at the time. No estimates of size or numbers were made other than checking samples of larvae under the dissecting microscope during the 48 hour water changes. The setting substrate used were flat, nesting, plastic cones with ridged upper and lower surfaces, that are used commercially in Spain as cultch for the

Plate 1. One of the larval rearing baskets. The black grid is a supporting frame made of plastic mesh to keep the plankton mesh in shape. The funnel and tube are used to introduce the larvae back into the basket after it has been washed in the containers in the foreground. The two 2000 L rearing tanks, and the frames from which the baskets were suspended in the tanks may be seen in the background.

Plate 2. The measurements of the images of the larvae on the photomicrographs were scaled by taking a photomicrogaph of a 2mm micrometer slide at the same magnification. The numbers refer to batch 1, day 15.

Plate 3. A copy of a photomicrograph of the larvae (sample from basket 34 on day 15). The measurements of the images are in mm, for converting to the actual size of the larvae in microns using the micrometer scale above. The difficulty of reproducing half-tones with the photocopier makes the print look rather rough. In fact, the technique is a very good way of recording large amounts of data about the larvae for processing at a later date.

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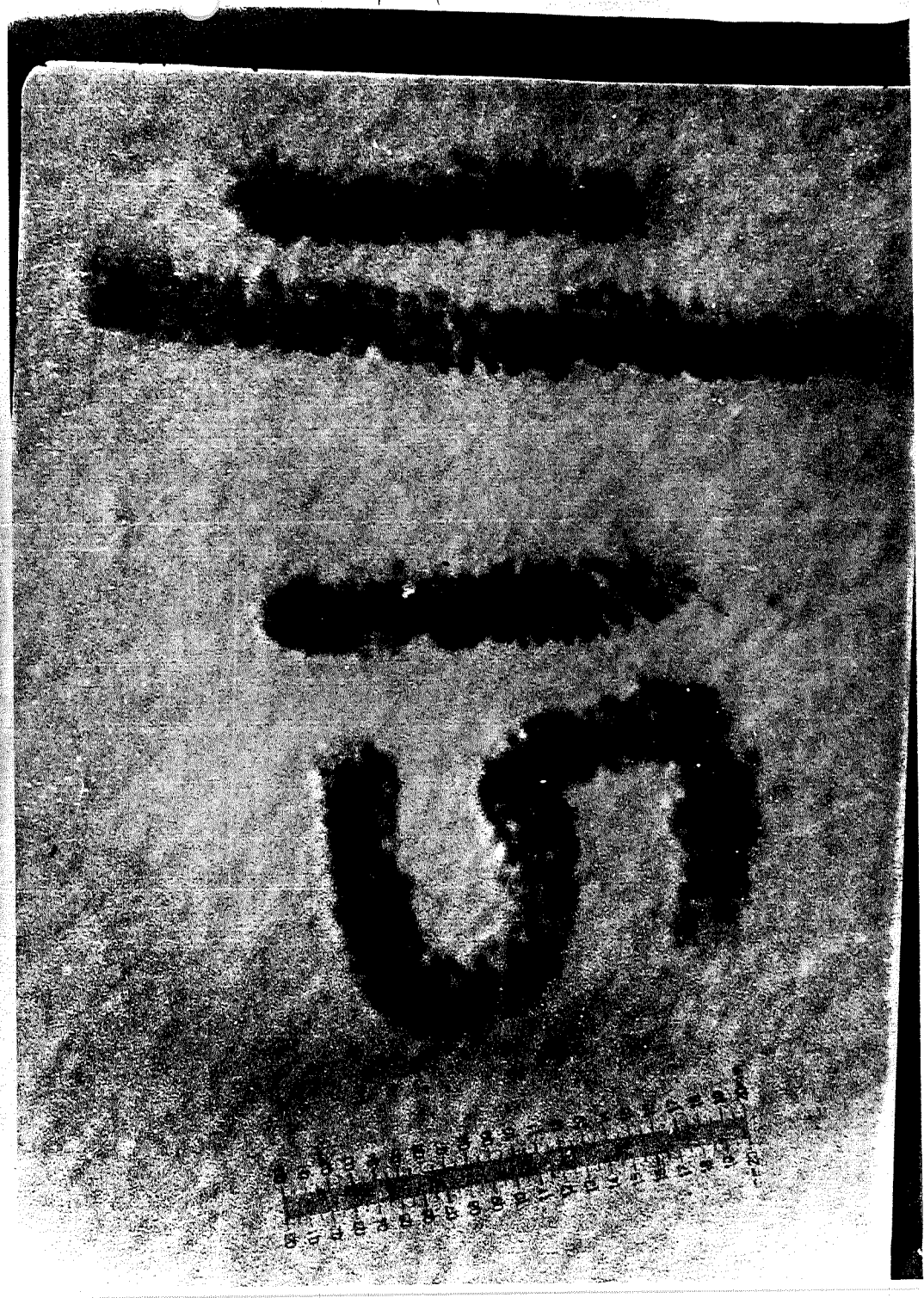

Plate 4. Spat in trays used in the trough. The tags show that both sets of spat are from the same family (13), and set on day 32 (left), and day 37 (right). Note the difference in size between spat from the two groups.


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Portuguese oyster, *C. angulata*, (Spanish discs). The discs were replaced daily or at the water change, depending on how heavy the set was. Petri dishes were also placed in some tanks.

Spat were grown on the Spanish discs in a 10,000 L outdoor tank supplied with cultured algae. After three months, they were flexed off the discs and put into shallow, fly-screen bottomed trays in a trough (Plates 4,5). The water supply to the trough was from one of the 0.1 ha ponds and effluent was returned to the pond. After 6 months, samples were taken for weighing.

RESULTS

The larvae survived and set extremely well.

There was no more resemblance between the duplicates of the same family than between unrelated groups of larvae, for either survival or setting success, so as might be expected, the environmental effects were relatively more important than for the basket system.

The measurements of the spat (summarised in Appendix III) show that while there were significant differences in mean weight between the different groups of spat (Plate 6), there was no more resemblance between related groups than unrelated

groups of spat. There were differences in the numbers of spat remaining, with 5.7 times more spat with male 1 as the father than male 2 (however these were not statistically significant as the variance was high).

The mean weights of the spat were affected by two other factors, one mostly environmental, and the second, probably a combination of genetic and environmental factors; Some of the larvae settled on glass surfaces, and were scraped off within 48 hours, as "cultchless" spat. At six months, these were less than a third of the weight of spat from the same tank caught on Spanish discs, which were allowed to grow onto the discs for 6-8 weeks before being flexed off. Some groups set so prolifically that it was feasible to keep spat from the same group that set on different days separate by writing the day on which the disc was placed in the tank on the disc, and putting the spat into different trays when they were flexed off. The mean weights of spat settled on different days are significantly different, and there is a trend toward lower mean weights in spat that set at 32-42 days post-spawning than spat set between 25-30 days post-spawning (Appendix III, see also Plate 4).

Plate 5. The spat rearing trough. Water is pumped in (at the top end of the picture) through perforated pipes running along the bottom of the trough, and flows to waste through the stand pipe (at the bottom end of the picture).

Plate 6. Typical 6 month old spat taken from several different families, showing the differences in size and appearance between families.

QUARANTINE - RESULTS

The major genetic improvement aimed at in this project was to be production of oysters with improved resistance to QX disease. It was considered that oysters that had survived QX would probably be carrying the disease, so to prevent contamination of Port Stephens water with QX, a quarantine system was set up. Broodstock that had survived greater than 99% QX mortality were kept in a 1000 L polyethylene tank within a 10,000 L concrete tank. Approximately 4-500 L of cultured algae per day were given to the 200 broodstock, and the water was changed every two days. The effluent water was sterilised with 5-10 ppm hypochlorite for 24 hours (this was considered enough to kill QX spores; F.O. Perkins, personal communication, 1986) and pumped to waste in the middle of a sand bed measuring three metres deep, by approximately 0.4 ha (the adjacent new hatchery site).

The larval rearing system consisted of an insulated, portable site cabin with drainage connected to a 3000 L concrete tank. All waste water from the cabin was collected in the tank and chlorinated with >10ppm hypochlorite for at least 24 hours before being pumped to waste.

The larvae were kept in 40 L plastic tanks in the cabin (Plate 7). The larval rearing water was filtered to 1µm and

pre-heated to 23°C before being pumped into the tanks. Thereafter the temperature was maintained by keeping the air temperature in the cabin at 24°C. At day 31, potassium chloride was added to the tanks to a final concentration of 12mM KCl to induce settlement (Nell and Holliday, 1986), and maintained at this concentration until the termination of the experiment at day 38.

RESULTS

A spawning system was set up in a part of the research station where spilt water would be unable to flow to the surrounding swamps, and 5 unsuccessful attempts to induce the oysters to spawn were made. On the sixth attempt seven females and five males spawned. Twelve crosses were made and distributed among thirty-six 40 L tanks.

Overall survival was fair despite a drop in water temperature to 12°C on day 4, when the cabin door blew open in the night. As had been noted before when using the tank rearing system, larval mortality appeared to be contagious. During a water change, the tanks were washed sequentially in the same order. It was observed that tanks washed after a tank that had suffered heavy mortality often also suffered mortality over the next two days (which indicates a possible bacterial aetiology for the mortality.).

Plate 7. Plastic 40 L larval rearing tanks in the QX quarantine site cabin.

The larvae developed slowly and the first pediveligers were not seen until day 25 (a week overdue). Despite the provision of three sorts of cultch, the larvae did not set, so on day 31, potassium chloride was added to the water to stimulate setting. This did not seem to help, and by a process of attrition all the larvae were dead by day 38.

2) HORMONAL INDUCTION OF SPAWNING

To prevent contamination of the spawn of one oyster with the spawn of another oyster, which might lead to undesirable crosses, the oysters should be spawned in separate containers. This is not practical, as one of the major stimuli for spawning in oysters is the presence of oyster sperm in the water, and it is difficult to get many oysters to spawn in individual containers. In the USA many hatcheries producing Pacific oyster spat strip their broodstock, but this kills the oyster, which would not be appropriate for a genetics programme. Hormonal stimuli based on prostaglandins or serotonin (a molluscan neurotransmitter) have been used to induce molluscs to spawn (Morse *et al*, 1978; Gibbon and Castagna, 1984). Prostaglandins are expensive, so a cheap alternative is to add hydrogen peroxide to the water; the synthesis of prostaglandins from their fatty acid precursors is via tha addition of an oxygen atom by a dioxegenase, and this is stimulated by addition of hydrogen peroxide at concentrations in the order of 1 - 10mM (Morse *et al*, 1978).

Optimum conditions for inducing the Sydney rock oyster to spawn using hydrogen peroxide were examined in two experiments. The first was to find the optimum concentration of hydrogen peroxide, as conflicting reports of the optimum concentration were available (Morse *et al*, 1978; and P. Dinamani, *pers. comm.*). Six concentrations of hydrogen peroxide were used, and two controls with no hydrogen

peroxide. Oysters (eight lots of 20) were distributed among eight 20 L photographic developing trays, each containing 10 L of filtered sea water at 23°C. The trays were in a water bath maintained at 23°C. The pH of the water in seven of the trays had been raised to 9.1 (Morse *et al*, 1978), and one (control 1) was left at 8.1. The oysters were left in the trays for two hours to acclimate to the change in pH. hydrogen peroxide was then added slowly to all but one of the trays adjusted to pH 9.1 (control 2) to bring the concentration in the trays to 0.03 to 10mM (table 1). Morse *et al* (1978) report that spawning in the American oyster, *Crassostrea virginica*, starts from three to five hours after addition of hydrogen peroxide at 2 to 4mM, so the oysters were left in the trays for a further two hours before being transferred to individual containers. These contained filtered sea water kept at 23°C, and were kept in the thermostatically controlled water bath. The oysters were checked two and four hours later for signs of spawning, and the results are given in table 2. None of the control oyters in trays 1 or 2 spawned. One oyster spawned after exposure to 0.1mM H_2O_2, three after exposure to 1mM H_2O_2, eight after exposure to 3mM H_2O_2 and five spawned after exposure to 10mM H_2O_2. The lower response at 10mM H_2O_2 is probably because some of the oysters reacted to addition of the hydrogen peroxide by closing up, preventing exposure to the chemical. The optimum result at 3mM H_2O_2 agrees with the results obtained by Morse *et al* (1978).

Table 1.

pH and concentration of Hydrogen peroxide.

	Controls							
Tray	1	2	3	4	5	6	7	8
pH	8.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1
H_2O_2 (mM)	-	-	0.03	0.1	0.3	1.0	3.0	10.0

Table 2.

Number of oysters spawned (out of 20)

	Controls							
Tray	1	2	3	4	5	6	7	8
No. oysters spawned	0	0	0	1	0	3	8	5

Table 3.

Temperature, algae added and Hydrogen peroxide.

Tray	1	2	3	4	5	6	7	8	9
Temperature	23	23	23	25	25	25	28	28	28
1 L algae	+	-	+	+	-	+	+	-	+
3mM H_2O_2	-	+	+	-	+	+	-	+	+

Table 4.

Numbers of oysters spawning (out of 20)

Temperature	Algae	H_2O_2	Algae+H_2O_2
23	0	7	4
25	*	8	10
28	*	7	*

* Indicates that one or more oysters spawned in the tray before being put into separate containers.

The second experiment was to test the effect of temperature and the presence of food (algae) on induction of spawning with hydrogen peroxide. Nine trays, each with 20 oysters as before, were half filled with 10 L of filtered sea water adjusted to pH 9.1. Three each of the trays were put in water baths maintained at 23, 25 and 28°C. After two hours acclimatisation of the oysters, each tray in the temperature group received 1 L thick algal suspension (a mixture of *Pavlova lutherii* and Tahitian *Isochrysis* aff. *galbana* at 5 x 10^7 cells/ml), or 1 L algal suspension plus hydrogen peroxide to a final concentration of 3mM, or 1 L filtered sea water plus hydrogen peroxide to a final concentration of 3mM (table 3). The oysters were left in the trays for two hours, then transferred to individual containers containing filtered sea water. The containers were in the same water bath as the trays, so the temperature remained the same. The oysters were checked for spawning, and the results shown in table 4. It will be seen that at least one of the oysters in each of trays 4, 7 and 9 spawned spontaneously before being put into the individual containers, causing the other oysters in the tray to spawn as well. There seems to be little useful effect of increased temperature or added food on hydrogen peroxide induced spawning.

Serotonin has been used to induce spawning in a number of molluscs including the American oyster. Serotonin was suspended in membrane filtered sea water to a concentration of 2mM, and 0.4ml injected into the gonads of each of 20

oysters, all of which started to spawn within 15 minutes of injection (Gibbons and Castagna, 1984). The proceedure was tried in July, using oysters that had been kept in an outdoor tank since February. None of the 30 oysters injected spawned, and they were found to have no trace of mature ova or sperm in the gonads, so the experiment was put off until oysters in good spawning condition could be obtained.

(2) QX MORTALITY FIELD EXPERIMENT.

In order to be able to select for resistance to a disease (in this case, QX), the resistant oysters must be distinguishable from the susceptible oysters, so that the more resistant oysters may be used as broodstock. If there is resistance to QX disease in oysters, the only way to distinguish resistant from susceptible oysters will be to expose them to QX, in which case the resistant oysters will be more likely to survive. For optimum conditions to select broodstock, the following conditions should apply:

(1) The disease should be transmissible under controlled, laboratory conditions.

(2) A given "dose" (infective unit) of the disease should cause the same incidence of the disease in unselected hosts, (i.e. incidence should be predictable and repeatable.)

(3) The disease should not be too virulent, as the use of too few broodstock might lead to genetic "bottlenecks" and inbreeding.

Much of the life histories of the haplosporidan oyster diseases are still poorly understood, and so far it has not been possible to infect healthy oysters with haplosporidan parasites in the laboratory. This was a major obstacle in the QX selective breeding programme, because in NSW the occurence of QX is sporadic, and mortality

varies from a few percent to complete loss of the oysters on several leases. To test the QX resistance of progeny of "resistant" parents, enough spat must be produced to be placed in several different rivers, in the hope that at least one area will suffer a heavy enough QX mortality to kill off an appropriate number of the spat. In an attempt to circumvent the problem of lack of predictability of QX incidence, an experiment to try to get graduations in the severity of QX mortality was set up in south Queensland.

Oyster leases at the southern end of the South Stradbroke Island channel suffer 100% mortality of any oysters (including wild spat) left in the water between January and April. After that time, the oysters could safely be relaid on the leases to fatten for the next season. It was proposed that oysters transplanted to the area between the period of 100% mortality and the "safe period" would experience a reduction in the infective dose, and hopefully, successive reduction of mortality. If this was the case, then spat could be exposed to a pre-determined QX infective dose simply by choosing the right time to put them on the lease.

Five lots of 120 oysters of 20-30g were sent up to South Stradbroke, South Queensland, at weekly intervals between mid-April and the end of May, and relaid on the lease of a local farmer who was helping with the experiment. The oysters were relaid about a month later than would have

been ideal, because of the need to seek cooperation from the Queensland DPI and because the first lot of oysters was sent off late (a misunderstanding on the part of the supplier). The oysters were collected in mid-June, and mortality and QX incidence estimated. There was no difference in mortality between the lots of oysters sent up at different times, and no QX spores were found, although some oysters had inflamatory reactions in the digestive diverticulae.

It would appear from the lack of heavy mortality even in the oysters relaid early on that the safe period started earlier than mid-April. A similar experiment will be run from the begining of March 1987 by the biologist working on oyster diseases at BWFCRS to try to answer some of the questions left unanswered:

(1) Is there a gradual reduction in mortality at the end of the 100% mortality period?

(2) If it is found that the mortality rate drops from 100% to 0% very quickly, what might be the reason?

(3) Is the 100% mortality period the same from year to year?

Answers to these questions could help in the reducing losses caused by QX as well providing a valuable tool for testing QX resistance in oysters.

4) DISCUSSION OF LARVAL REARING

Introduction

The aim of the project was to produce genetically improved Sydney rock oysters. The species seemed to be an ideal candidate for such a programme because of the great fecundity and high level of genetic variability found in the genus. Unfortunately, it was not appreciated at the time that substantial problems with hatchery and nursery techniques still remained to be solved, and that those problems would be execerbated by scaling down the size of the rearing containers (so that large numbers of family groups could be reared at once). Genetic improvement of the Sydney rock oyster remains a desirable goal, but progress will not be made until small-scale hatchery production of spat becomes reliable.

Hatchery techniques current when the genetics programme was first proposed.

When the genetics programme was set up in 1984, hatchery techniques had improved to the point that about one in two batches survived to the setting stage, and the setting rate was up to 1% of the larvae stocked initially. It was considered that because reasonably large numbers of larvae

did actually survive to set, the techniques were basically correct, and that when the factors causing mortality were identified and corrected the survival and setting rates would improve. The basket rearing system for the selective breeding programme was designed to use the advantages of large scale larval rearing. The environmental variance would also be kept to a minimum because all families would share the same tank of water. The first batch of larvae to be reared in this system survived to the setting stage. This was perhaps unfortunate, as it generated a false sense of optimism, and the system was kept on well after it should have been abandoned. The first batch of the second rearing system to be tried also survived and set much better than any subsequent batches (the other factor in common with the two successful batches was that both were spawned at the end of the year).

Possible reasons for poor larval survival.

It seems that the larvae of the Sydney rock oyster are simply difficult to rear in a hatchery, as experimental batches of Pacific oysters and Mud oysters, *Ostrea angasi*, survived and set well. Physical, and lately, bacteriological data have been recorded for batches of larvae in both the routine hatchery and the genetics programme, but no one factor stands out as a cause of larval mortality.

It has been observed that heavy mortality often follows heavy rain, and poor "water quality" is blamed. Possible explanations include low salinity (Sydney rock oyster larvae survive best at salinities between 27 and 36 ppt., J. Nell, unpublished observations, 1986); the presence of agricultural chemicals in run-off from adjacent farm land; the leaching of organic acids and tannins from tea-tree and mangrove stands around BWFCRS; and the presence of harmful *Vibrios* adhering to silt particles stirred up from the sediment.

During recent attempts to cross Sydney rock with Pacific oysters, it was found that it is extremely difficult to get unfertilised eggs from females spawning on the communal spawning table. It seems that enough of the the sperm that acts as a stimulus to start the female spawning is retained in her mantle cavity to fertilise virtually all her eggs. If one male starts to spawn some time before the others, it is quite probable that his sperm will fertilise most of the eggs subsequently spawned, no matter how many males contribute to the sperm suspension to be added to the eggs later. It is apparent from factorial crosses of different male and female oysters that the male parent has a large effect on larval survival. It is therefore possible that the early loss of some batches of larvae occur because many of the eggs have been fertilised by poor quality sperm. The opposite case, the exceptional survival of larvae fertilised by a single male of good

quality, might lead to inbreeding because of a founder effect unless care is taken to ensure that enough males really do contibute to the fertilisation.

Changes in larval rearing conditions.

Over the last three years, improvements in larval survival and setting rate have been made, so that a million set spat from 30 million larvae originally stocked is now the rule rather than the exception. This improvement has been mostly the result of two changes in rearing techniques. The first change was that rather than trying to keep the rearing water virtually sterile (by filtration, UV irradiation and treatment with Neomycin), the water should be filtered to remove excessive numbers of bacteria, while leaving an ecologically balanced population of bacteria in the water. This practice reduced larval mortality, but many batches still failed. Routine bacteriological monitoring of the algal cultures supplying the hatchery was also introduced (algae with greater than $5x10^5$ bacteria per ml are automatically dicarded). The algal cultures used to feed the larvae were the major source of bacterial contamination in a Tasmanian hatchery. On the assumption that the same was probably true at BWFCRS, the amount of algae fed to the larvae was greatly reduced (from an average of 10^5 cells per ml to around 15,000 cells per ml). Since then, the rearing of the larvae has

become much more reliable, but only in the later months of the year. The cause of the lack of success in the first half of the year is not known, but it is suspected that it may be poor spawning condition of the broodstock. It has been demonstrated with Pacific oysters (Lannan et al, 1980) that broodstock not in the peak of spawning condition produce inferior larvae with poor survival and setting success.

The valuable advice on hatchery hygeine, water treatment, and the importance of reducing the numbers of bacteria in algal cultures, given by Dr. Christian Garland (who visisted BWFCRS while on a FIRTA funded programme examining the effects of bacteria on larval rearing in a Tasmanian hatchery) is greatly appreciated.

Improvements in hatchery technique applied to the genetics programme.

That the condition of the broodstock may be important is supported by the fact that the only two batches (numbers 1 and 9) to survive to the setting stage were spawned in November and December, respectively, when the oysters were fat. Oysters may be kept fat (whether "fat" is exactly the same as "in good spawning condition" is not known) by feeding them with very large volumes of cultured algae, as was done to keep the QX broodstock in condition, but it is

expensive in terms of the labour required to produce the algae. A conditioning system to heat algae-rich pond water has been designed, but it will be too expensive to run until a heat-exchanger to recover some of the heat from the effluent water is obtained.

Once it became clear that changes in rearing techniques had resulted in improved larval survival in the routine hatchery production, similar changes were adopted for the genetics programme; unfortunately, the larval survival did not seem to improve as a result. It seems likely that the reduction in numbers of bacteria that occurred in the large routine rearing tanks was cancelled out by the much larger surface area to volume ratio of small tanks or the basket system (aquatic bacteria require surfaces on which to grow). Apparently, the larger the volume of water, the better the larval survival.

Conclusions

In hindsight, many of the problems besetting the genetics programme were caused by the small scale of the venture.

For a programme aimed at genetic improvement of Sydney rock oysters to make progress, the progeny of selected oysters should be the spat reared for production, rather than a small scale side-line maintained for provision of broodstock only: Facilities to keep spat of different

genetic origin separate would be an integral part of the nursery system, and potential broodstock would be taken from the spat to be offered to the farmers. The broodstock would then be evaluated for the desired traits. It would be important to prevent deleterious genetic traits from becoming prevalent, as these would be felt immediately in production of poor quality spat, or in the loss of several years' selection.

Correct spawning condition of the broodstock is also vitally important, and as much effort should be devoted to bringing the broodstock into condition as to the hatchery and nursery operations.

Finally, to minimise inbreeding because the sperm of one male can fertilise most of the eggs in one spawning, the broodstock should be induced to spawn (using hormones, or possibly a cell-free sperm extract) in isolation, or should be spawned in small isolated groups.

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APPENDIX I

NOTES ON LARVAL REARING ATTEMPTS

BATCH	DATE SPAWNED	INITIAL STOCKING DENSITY (larvae/ml)	CON-TAINER	MEAN TEMP (°C)	MIN/MAX SALINITY (‰)	MIN/MAX pH	REMARKS	NO: DAYS SURVIVED	NO: SET	EXPERIMENTAL DETAILS
1	8/11/84	2 to 10 WITHIN BAGS	30L MESH BAGS	24°	27 - 32	7.8-8.2	WATER STERILSED WITH UV & NEOMYCIN COAST SEAWATER USED WHEN SALINITY DROPPED	FINISHED SETTING AT D 33	4.10^3	SEE DETAILS IN MAIN TEXT
2	7/2/85	20 WITHIN BAGS	30L MESH BAGS	24°	35	7.8-7.8	WINTER MORTALITY SURVIVORS AS BROODSTOCK UV & NEOMYCIN	4	0	
*	13/3/85	40 (FERT EGGS)	40L POLY-THENE BAGS IN BASKETS	23° → 28°	-	-	½ BAGS AERATED ½ BAGS STILL	48h	0	* TO TEST LAUNDRY BASKETS AS AN INITIAL REARING SYSTEM
**	20/3/85	50 (UNFERT EGGS)	20L AQUARIA	21° → 24°	-	-	STERM ADDED TO EGGS AT HOURLY INTERVALS	48h	0	** FERT SUCCESS WITH TIME. SEE APPENDIX FIVE
3	18/3/85	11	2000L TANK	24°	35	7.5-8.0	UV & NEOMYCIN LARVAE DISAPPEARED	3	0	TO TEST THE EFFECT OF STOCK-ING DENSITY IN BAGS
4	25/3/85	20 WITHIN BAGS	30L MESH BAGS	23°	34 - 35	7.5-8.0 (MOSTLY VERY LOW)	WINTER MORTALITY BROODSTOCK UV & NEOMYCIN	10	0	
5	11/4/85	2 to 50	30L MESH BAGS	22°	34	7.5-8.0	UV & NEOMYCIN WINTER MORTALITY BROODSTOCK	6	0	STOCKING DENSITY IN BAGS
6	18/4/85	2 to 50	30L MESH BAGS	23°	32 - 35	7.5-7.8	UV & NEOMYCIN HATCHERY BROODSTOCK	8	0	STOCKING DENSITY FERT. TIME & CHLORINE RINSE
7	8/10/85	19	30L MESH BAGS	23°	32 - 35	7.9-8.0	BROODSTOCK FROM NAMBUCCA WATER FILTERED TO 1µm NO UV OR NEOMYCIN	10	0	AN ANTIBIOTIC OR UV STER'N
8	21/10/85	9.5 - 10	30L MESH BAGS	24°	27 - 30	7.8-8.0	MANY ROTIFERS. WATER TO 33°C AT D 10	14	0	
9	6/12/85	15	40L AQUARIA	26°	33 - 36	-		FINISHED SETTING AT D 30	50.10^3	SEE DETAILS IN MAIN TEXT
10	20/±1/86	15	40L AQUARIA	25°	34 - 36	-	COAST WATER			
11	11/6/86	-	40L AQUARIA	22°	36	-	COAST WATER WINTER MORTALITY BROODSTOCK MANY COPEPODS PRESENT	FINISHED SETTING AT D 30	5.10^3	TO TEST EFFECTS OF FEEDING & LIGHT INTENSITY
12	20/6/86	15	40L AQUARIA	23°	36	-	QX SURVIVORS AS BROODSTOCK LARVAE KEPT IN QUARANTINE SHED COASTAL WATER	38	0	

APPENDIX II

APPENDIX II

Larvae from batch 1 (basket system).

There were no significant differences in diameter between larvae from the different baskets at each age.

Mean diameter, larvae from all baskets:

Age (days)	Diam. (um)	SD (um)	Coef. var (%)	N.
7	96.0	8.6	8.9	1341
9	109.5	12.3	11.3	966
11	128.1	16.0	12.5	1341
13	144.7	20.8	14.4	1708
15	172.6	28.0	16.2	1371
17	199.2	31.4	15.7	1081

Up to day 13, the size distributions of the larvae were normal (95% confidence limit). At days 15 and 17, there was pronounced skew to the right, as more larvae reached the eyed (pre-setting) stage.

Regressions of percent survival on age, with the data grouped according to the initial stocking density (day 6).

These data have been arcsin transformed (Sokal and Rohlf, 1969) to remove the bias introduced by expressing the larvae remaining as percent of the stocking density. The intercept is greater than 100%, because it is extrapolated back to day 0.

Group	Initial Stocking Density/ml	Intercept (%)	Slope	r^2.
Very low	<5.5	130.8	-4.430	0.352
Low	5.6 - 6.5	133.0	-4.770	0.595
Medium.	6.6 - 7.5	138.5	-5.122	0.690
High	7.6 - 8.5	136.9	-5.162	0.617
Very high	>8.6	134.1	-5.361	0.785

Note that the slope increases (daily % mortality increases) with increasing initial stocking density, This is not statistically significant, however, as the variances are very high.

Regressions of numbers of larvae remaining, against time.

Basket	Intercept Larvae/ml(Day 0)	Slope (Larvae/ml)	r^2
1	7.9	-0.277	0.570
2	11.2	-0.500	0.786
3	13.2	-0.714	0.691
4	9.2	-0.348	0.825
5	8.9	-0.393	0.627
6	6.5	-0.221	0.380
7	10.6	-0.441	0.725
8	11.2	-0.546	0.799
9	9.2	-0.407	0.548
10	7.6	-0.283	0.341
11	6.3	-0.216	0.329
12	13.0	-0.591	0.847
13	8.8	-0.370	0.650
14	12.3	-0.568	0.892
15	7.2	-0.248	0.507
16	9.2	-0.377	0.666
17	11.4	-0.527	0.709
18	10.1	-0.438	0.773
19	11.9	-0.571	0.783
20	7.4	-0.391	0.669
21	14.3	-0.702	0.821
22	9.9	-0.432	0.961
23	4.3	-0.246	0.795
24	2.9	-0.152	0.692

Regressions of numbers of larvae remaining, against time (cont).

Basket	Intercept Larvae/ml(Day 0)	Slope (Larvae/ml)	r^2
25	11.0	-0.433	0.383
26	4.4	-0.168	0.448
27	10.6	-0.554	0.927
28	15.1	-0.779	0.811
29	12.2	-0.607	0.783
30	11.4	-0.538	0.907
31	11.0	-0.475	0.792
32	7.3	-0.314	0.734
33	10.0	-0.425	0.526
34	9.0	-0.330	0.440
35	14.8	-0.714	0.977
36	9.2	-0.432	0.858

APPENDIX III

APPENDIX III

Spat from Batch 9 (tank system).

Thse spat were the product of crosses between two male and twelve female oysters. The crosses and tank numbers are as follows:

Group 1 (Male 1 as father):

Female number:	1	2	3	4	5	6
Tank numbers:	1,13	3,15	5,17	7,19	9,21	11,23

Group 2 (Male 2 as father):

Female number:	7	8	9	10	11	12
Tank numbers:	2,14	4,16	6,18	8,20	10,22	12,24

There was no significant difference in weight between spat of groups 1 and 2 ($p<0.25$).

The difference between groups 1 and 2 in numbers of spat surviving to 6 months is nearly significant ($p=0.947$):

Mean number of spat per tank surviving to 6 months:

Group 1:	830
Group 2:	145

Mean weights and numbers of spat surviving to 6 months.

Tank number	Mean weight (g)	Number
1	4.21	21
2	3.06	147
3	2.43	2456
5	2.83	176
6	3.42	866
7	3.51	1044
8	4.64	110
11	0.58*	660
13	3.10	2043
14	2.99	262
15	2.38	297
19	2.13	1269
22	4.67	40
23	3.21	323
24	3.36	66

*All the spat from tank 11 were cultchless spat, scraped off the glass petri dish 24 hours after setting.

The three highest mean weights are amongst the four lowest total spat numbers, which is a crowding effect in the spat trays.

Mean weights of spat setting at different ages.

	TANK					
Age (days)	3	6	7	13	19	MEAN
24	-	-	-	1.02*	-	1.02
25	-	2.43	-	-	-	2.43
26	3.07	4.07	3.85	2.65	-	3.41
28	3.37	-	3.50	3.55	-	3.47
30	2.03	4.03	2.80	3.36	4.63	3.37
32	2.15	-	4.20	-	3.69	3.35
37	1.61	-	-	-	1.07	1.34
42	-	-	-	-	1.41	1.41

* These spat set (before cultch had been provided for them) on the glass aquarium heater in the tank, and were scraped off about a week later, and so are also cultchless spat.

APPENDIX IV

APPENDIX IV.

SURVIVAL OF WINTER MORTALITY BROODSTOCK AFTER SPAWNING

The Winter mortality broodstock had each been marked by drilling a hole through the horn (the projection of shell behind the hinge) of the oyster and tying a tag with a code identifying the lease of origin and the individual oyster. 555 oysters from 22 leases in seven areas were induced to spawn on the 7th of February. The sex and tag code of each spawner was recorded, and the broodstock returned to trays in one of the 0.1 ha ponds at BWFCRS.

The broodstock were inspected on 12th February, and again on the 25th February, 5th March, thereafter at approximately monthly intervals. Dead oysters were removed, and the tag codes and the cause of death recorded. There was 11% mortality by the 25th February, the causes of death being: mud crab predation (the only remains, fragments of shell and the tag) or penetration of the shell while it was being drilled for the tag (both refered to as "accidentally dead"); or death for no obvious reason (refered to as "dead").

The tags were compared with those that had spawned 3 weeks previously. There were no significant differences between the ratios of males to females (that had spawned; the sex of the non spawners was unknown) among the live, accidentally dead or dead oysters. There was no significant difference in the ratio of spawners to non-spawners between the live and

accidentally dead oysters.

Oysters that had spawned were less likely to die than the oysters that had been exposed to the same conditions, but had failed to spawn:

Mortality of oysters immediately after spawning.

	Total no. oysters	Oysters that had spawned	% spawned
Dead oysters	41	3	7%
Live oysters remaining	490	71	15%

Five days post-spawning, 7% (3 out of 41) of the dead oysters had spawned, compared to 15% (71 out of 490) of the live oysters, and by eighteen days post-spawning, 7% (5/73) of the dead oysters had spawned compared to 15% (65/409) of the remaining live oysters (the accidentally dead oysters were not included in the statistical analyses). These differences are not significant ($0.5>p>0.1$).

The oysters were then grouped into their leases of origin, and the percentage of oysters that had spawned and died compared to the percentage that had spawned and survived were calculated for each lease. The data were arcsin transformed (Sokal and Rohlf, 1969) to remove the bias caused by expressing the data as percentages, and mortality regressed on spawning. The regression equation is:

$$\text{mort \%} = (0.451 \times \text{spawning \%}) + 28.4\%,$$

and is significant ($0.05 > p > 0.01$). This bears out the general finding that the fattest oysters suffer worse from stress than thin (spawned out) oysters.

Oysters continued to die after 25th February, but the mortality was lower, and the difference between oysters that had and had not spawned was no longer significant.

APPENDIX V

APPENDIX V

VIABILITY OF LARVAE WITH DELAY IN FERTILISATION

The use of many broodstock to reduce the degree of inbreeding in the hatchery population has its own problems, in that the man-power available cannot cope with making the desired crosses of about 20 oysters in each of 36 family groups. A delay of at least two hours between spawning of the gametes and fertilisation was normal for the batches run in the first part of the year.

An experiment was run to find the effect of delay in fertilisation on the viability of larvae up to 48 hours old. Six female oysters were divided into 4 groups, groups 1, 2 and 3 being females 1, 2 and 3 respectively, and group 4 being females 4, 5 and 6 (which had each produced fewer eggs than females 1, 2 or 3). The four groups of eggs were mixed thoroughly, and a 50 ml aliquot (about 800,000 eggs) mixed with 1 ml of sperm suspension from a single male at approximately ten minutes post-spawning, thereafter at hourly intervals to six hours. The fertilised eggs were put into a 20 L PVC bucket with 1 L of UV sterilised water at 25°C, and topped up to 5 L after an hour. The buckets were kept at 23$\pm$1°C overnight in a thermostatically controlled water bath.

The next day, 22 hours post-spawning, the buckets were emptied gently through a 15 um plankton mesh seive to rinse

out sperm and unfertilised eggs, and the larvae replaced in the buckets with 15 L of UV sterilied water at 23°C. At 30 hours post-spawning 1 L algae (Tahitian *Isochrysis* aff. *galbana*, 2.7 x 10^6 cells/ml) was added to each bucket to feed the larvae.

At 24 hours and 48 hours after adding sperm suspension to each set of eggs the larvae were sampled by mixing the contents of each bucket (using a mixer shaped like a mushroom with large holes drilled in it, which mixes without forming vortices in which the larvae accumulate) and taking eight 0.5 ml samples with a micropipette. The larvae were counted in the cells of a haemagglutinin plate, under a dissecting microscope at 12x magnification.

The eggs from the three oysters making up Group 4 are significantly ($0.05>p>0.01$) more affected by delay in fertilisation than Groups 1 and 3, and Group 2 at 24 hours ($p<0.01$). This is even more pronounced at 48 hours post-fertilisation, as the counts are less variable; Group 4 is different to Group 3 ($0.05>p>0.01$) and to Groups 1 and 2 ($p<0.01$). Group 2 appears to be less affected by delay in fertilisation than the other groups, but the differences are not significant at the 95% level.

The normal timetable of deveopment at 23°C for Sydney rock oyster larvae is trochophore (ciliated swimming stage) by 6 hours post-fertilisation; and "D" stage, so called because the newly developed prodissoconch (larval shell) is

distinctly D-shaped, by 22 hours post-fertilisation. Qualitative observations of larvae at 48 hours show larvae that apparently failed to progress beyond the trochophore stage (but were still swimming) start to appear in samples from Groups 3 and 4 after 2 hours or more delay in fertilisation. Larvae that had not progressed beyond the intermediate stage between trochophore and D stage were present in all groups after 2 or more hours delay in fertilisation, but there seemed to be fewer in Group 2 than in the other groups. All groups had increasing numbers of D stage larvae with a saddle-shaped deformation of the prodissoconch with the longer delays in fertilisation.

Some time after this experiment was run, two facts came to light which could affect the interpretation of the results:

Firstly, Brown (1973) published photomicrographs of American oyster larvae with the saddle-shaped deformations observed in larvae fertilised after a long delay. These deformations were caused by exposing the larvae to large numbers of bacteria (mostly *Vibrios* and *Pseudomonas spp.*). It seems quite likely that the deformations in the larvae fertilised with longer delays were of similar aetiology, possibly because of bacterial proliferation in the sperm suspension.

Secondly, although the eggs were examined microscopically after they were spawned, and did not appear to have been fertilised (soon after fertilisation, the club-shaped eggs "round-up"), no unfertilised control was kept for checking at

24 hours and 48 hours. In light of the difficulty of obtaining unfertilised eggs from females spawned on the communal table (see the discussion, section 3), it is possible that there was sperm present with the eggs, and that they were fertilised fairly soon after spawning. However, the development of eggs "fertilised" with longer delays did appear to lag behind those that were fertilised earlier.

APPENDIX VI

APPENDIX VI Summary of oyster farmers' observations about QX

FARMER	N. Henry	C. Lucas	F. Kirkham/ R. Murray	F.Kirkham	L. Perandis	F. Knudson
RIVER/LEASE	Bribie Island QLD.	Opp. the north arm of the Coomera River, QLD.	Tweed River/ Terranora Inlet	Brunswick (QX free)	Lower Tweed River	Richmond River
CURRENT/ EXPOSURE	Some choppiness in mid-channel, not much fetch.	Some choppiness, lease protected by conveyor belting.	Little tide in lake.	High tidal range at catching leases at the bridge.	High tide-fall, strong current.	
SALINITY/ LOCATION	Gets freshes in the wet season.	Complex hydrography caused by north and south arms of the Coomera. North end of the Island much higher salinity.	Lower salinity at the lake. Fresh or cyclone causes immediate kill.	High salinity, small cachment area.	High salinity, gets big freshes.	
MUDDINESS/ MUDWORM	No mudworm. Fresh brings down black, silty, smelly mud.	Mudworm in oysters brought up from south. Sand/mud substrate.	Mudworm prevalent if trays put low and shadecloth used.	Little mud at catching or growing leases, except during floods.	Very muddy.	
OYSTER CONDITION	Oysters with QX have no frill.	QX infected oysters look emaciated and watery. Generally, recover condition within two weeks of spawning.	Fatten very well.	Much less fat than at Terranora. Good shell growth.	Look very fat and shiny [flesh] during and just after a fresh.	
FISH: PRESENCE/ CONDITION.			Whiting and mullet are ulcerated in bad QX years. The "little crabs" [on trays] die when oysters get QX.			Bream get ulcers.
SCAVENGING OF QX-DEAD OYSTERS		Mud crabs kill many oysters, not known if weakened by QX.	Fish and crabs do not touch oysters dead ofa QX kill.	Nothing eats dead QX oysters.		
AGRICULTURAL CHEMICALS AND LAND USE	Cachment conifer forestry, swamps and cattle grazing.	Cachment area cattle grazing and swamps. Fertilise in April.	Firmly believes the pesticides and herbicides used on cane fields to blame.	No cane. Bananas only.		Cachment area tea-tree swamps and grassland.
OTHER REMARKS	Very heavy over-spatting. He takes wild oysters from mid-channel rock lease for on-growing on trays.	Says Prior's leases (stick) to the north get a maximum of 20% mortality compared to 100% at the southern end. Oysters with QX die within four weeks.	No Mud oysters left on previously well-populated bank opposite the sheds. QX kill occurs two weeks earlier in main river (Perandis' leases) than up-river.	Knudson says some mudworm occurs on his Brunswick stick leases if shadecloth used.	Oysters die within three weeks after starting to die.	

APPENDIX VII

APPENDIX VII. 1984 FIRTA report.

A larval rearing system has been devised and set up that will enable us to rear enough oyster families (with the resources available) to give valid results; up to 36 families of larvae may be reared at one time with the minimum of environmental variation, as each family is contained by a plankton mesh "basket" suspended in the same tank, and each family sees the same water quality and food concentration. The larvae settle on glass collectors within the baskets, and the spat transferred to small individual upwellers which share the same water supply. At 5-8 mm the spat are glued to strips of plastic mesh, and when the spat have grown onto the mesh it is trimmed to leave single oysters with a loop of mesh firmly embedded in the shell. An identifying tag may be attached to the loop and the spat put out in trays without fear of mixing up families or individuals.

Baseline experiments have been carried out with larvae from a single spawning to establish the variation inherent in the rearing system and the effects of larval density within the baskets on larval growth and survival. Larval growth is measured by photographing preserved larvae taken at intervals throughout the experiment and measuring the enlarged image. Daily water quality measurements have indicated that larval mortality experienced recently may be associated with low pH, so an experiment to determine the optimum pH for larval rearing is in progress.

A pilot experiment on the effect of neomycin (routinely used in the rearing tanks) on bacterial (Vibrio sp.) growth has been run with the assistance of the Microbiologist at Brackish Water Fish

Culture Research Station and the results warrant further investigation into the stability of the neomycin in seawater and concentration.

In collaboration with Agriculture Department geneticists, a rotational line breeding scheme has been chosen as it allows maximal genetic improvements while minimising inbreeding for the small (30) number of families that may be reared at one time; for a given trait such as fast growth rate, or disease resistance, 5 lines plus a control line (each of 6 families) are maintained. There is no crossing between lines; within lines, female oysters from family 4 are mated with males from family 5 - the progeny are called family 4; females (5) are mated with males (6) to produce the next generation of family 5; females (6) are mated with males (1) etc. etc. This minimises inbreeding to which oysters (a closely related species, C. virginica) are very susceptible.

Survivors of winter mortality kills ranging from 30% to 90% + were collected as broodstock. A half dozen oyster farmers in N.S.W. have offered further broodstock and space on their leases for growout and evaluation of the hatchery produced spat. The broodstock were kept for weeks in a closed conditioning tank with high food levels, with water changes every few days. This suggests that quarantining of QX broodstock and sterilisation of the waste water at Brackish Water Fish Culture Research Station will be perfectly feasible.

APPENDIX VIII

APPENDIX VIII. 1985 FIRTA report.

Introduction

In 1980, following representations by oyster farmers in northern New South Wales to the Deputy Prime Minister, New South Wales State Fisheries was approached by the Department of Primary Industry Research Committee to investigate the heavy mortality of Sydney rock oysters in northern New South Wales and southern Queensland. An application was prepared and submitted to the Committee in September, 1980, and the project was subsequently approved for commencement in July, 1981.

Because previous research by New South Wales State Fisheries had shown that a parasite, Marteilia sydneyi, then classified as a haplosporidian but now known as an ascetosporan parasite, was responsible for heavy mortality in the Sydney rock oyster in northern New South Wales and southern Queensland, the objectives of the proposed programme were as follows:-

(i) Investigation of the parasite, Marteilia sydneyi, to obtain a clearer understanding of its life history including the time and nature of infestation of the oysters, to identify the secondary host and to determine incidence of infestation and mortality in relation to selected environmental parameters.

(ii) Development of cultivation techniques that will reduce mortality.

(iii) Examination of the possibility of establishment of a disease resistant strain of oyster by selective breeding.

RESULTS

(a) The Parasite, Marteilia sydneyi

In order to obtain a clearer understanding of the life history of the parasite and the incidence of infestation and mortality in relation to selected environmental parameters, Sydney rock oysters were sampled from July 1975 to June 1983 at the rate of 10 per site per month from the major oyster producing areas of northern New South Wales and southern Queensland, i.e. the known range of the ascetosporan parasite M. sydneyi in commercially grown oysters. During the observed period the parasite extended its range southward to the Clarence River in 1978 and to the Macleay River in 1982. In response the sampling range was extended to include these estuaries and also to the uninfected growing areas within this range. Port Stephens was included in the sampling programme at this time because oysters were normally relayed there from the Macleay River and it was essential to detect any spread of the parasite to this major centre of production.

The sampled oysters were routinely fixed (Davidson's fluid), and 6µm tissue sections of the digestive diverticula were prepared and stained (haematoxylin/eosin) for histological examination under the light microscope.

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The prevalence of developmental stages of M. sydneyi in the samples was recorded and the results then forwarded to the oyster farmers concerned. Where new infections for the season were detected the oyster farmers were telephoned directly with the information.

Oyster farmers assisted with the collection of temperature and salinity data as near as practicable to sampling sites, using thermometers and hydrometers checked and supplied by the biologist.

Where data were sufficient (Tweed, Richmond, Evans and Clarence Rivers) the prevalence of the QX parasite in monthly samples was graphed against salinity (figs. 1-4). M. sydneyi epizootics can be seen to vary in intensity between estuaries and between years. Some estuaries have a very low prevalence in some years, for example, the Tweed in 1979 and the Clarence River in 1980. These graphs show that a sharp drop in salinity often, but not always, precedes or accompanies a M. sydneyi epizootic. This does not provide evidence of a very close relationship between low salinity and prevalence of M. sydneyi in oysters. It is of relevance because of the belief held by some oyster farmers that run-off containing agricultural chemicals is likely to occur with the first "fresh" after a long dry period, and that these chemicals may precondition the oysters to the onset of parasitic infection. Considerably more data, and more detailed data than could be obtained here, would be required to test this hypothesis.

The frequency of occurrence of months showing M. sydneyi for all samples for each estuary (Table 1) shows a tendency for the parasite to occur longer and later in the southern parts of its range, but more data would be required, especially from Queensland to validate this possibility. A histogram of the sum of positive monthly samples for all estuaries (fig. 5) shows December to be the only month when the parasite was never detectable and that the period covering the bulk of infections was from February to August with the highest occurrence in April.

MONTH	J	F	M	A	M	J	J	A	S	O	N	D
Pumistone		1		1		1						
Redland Bay		1	1	2								
Southport	1	1	1	2	1	2						
Tweed		2	6	6	5	3	2	1				
Brunswick												
Richmond		1	3	5	5	4	4	3	2			
Evans	2	2	2	1	2	2	3	1	1	2	1	
Clarence		1	4	6	5	4	5	3	2			
Sandon		1	1					1				
Wooli												
Belligen												
Nambucca												
Macleay			1	2	1					1	1	
Pt.Stephens												
TOTALS	3	10	18	25	19	16	15	9	5	3	2	0

.../3

TABLE 1. Marteilia sydneyi positive monthly samples, totalled for estuaries sampled from July, 1975 to June 1983.

The M. sydneyi surveillance system operated under this grant recorded the prevalence of the parasite, monitored growing areas for new outbreaks so that quarantine measures could be rapidly taken, and provided growers with the knowledge that allowed them to more successfully relay their crops to take advantage of the "safe" growing period.

In spite of an enormous amount of study over the past twenty years the complete life cycle is not known for any of the ascetosporan parasites, several of which cause similar mortalities to M. sydneyi in oysters in other countries.

In a laboratory trial (Wolf, unpublished data) the disease could not be transferred directly from M. sydneyi infected oysters to uninfected ones. It is therefore possible that an intermediate or secondary host is responsible for the transmission of the parasite from oyster to oyster.

In an attempt to determine whether a secondary host existed a selection of estuarine animals closely associated with oysters and oyster beds was collected, fixed and sectioned. The specimens included Polychaete worms (3), Teredo sp. (18), jingleshell oyster (15), limpets (1), and blennies (16). No sign of any structures which could be related to M.sydneyi was detected within the tissues of any of these animals. The collection of specimens for examination had taken place in August of 1982 in the Tweed and Richmond Rivers after a relatively mild outbreak of M.sydneyi. There may have been few spores around at the time, so the task would be worth repeating. Species other than those examined may of course be the intermediate hosts, if such exist.

A workshop on haplosporidian parasites in oysters was conducted at the Brackish Water Fish Culture Research Station, Port Stephens in October 1982. Participants included scientists from France, New Zealand, United States of America, Victoria, Queensland, Australian Capital Territory and New South Wales. Talks were presented and discussions held on the parasites and related diseases and it was generally accepted that development of a disease resistant strain of oyster should be considered as a priority.

(b) The Effect of Oyster Growing Height on Marteilia sydneyi Induced Mortalities

It has been found that the incidence of winter mortality in the southern estuaries of New South Wales can be reduced by varying the height at which oysters are grown whilst it is considered that the growing level of oysters in relation to tidal range influences mortality rates attributable to M.sydneyi infections in the Sydney rock oyster (Armstrong,pers. comm. 1975). Potter and Rodgers (workshop paper not yet published) reported an experiment which examined the effect of height on M.sydneyi mortalities at Southport and Ningi Creek in southern Queensland. After a heavy infection of M.sydneyi at Southport, oysters died at all growing heights tested, but with the lower infection rate experienced at Ningi Creek the oysters grown 20cm above the usual commercial growing height lived through one summer season and on until termination of the experiment during the following December. In both situations the lowest oysters were the first to die, followed successively by those at increasing heights.

...4/

An experiment was designed to determine the effects of growing height on mortality in more detail in the Terranora Lagoon on the north coast of New South Wales. Oyster racks were laid out with growing heights ranging in 9 steps of 5 cm each from 20 cm below the normal commercial growing height for the area, to 20 cm above. Three replicate racks were used each with a set of tray heights randomly arranged. The standard oyster trays were each stocked with 100 local oysters and covered with woven plastic shade cloth to lower the risk of heat-kill and predation. During the first year of the experiment (1982) the shade cloth was too loose and oyster losses occurred. In 1983 this was rectified and losses were reduced to one whole tray, probably stolen.

Counts of oyster mortality were made in October, 1982 and early August, 1983 and the results tabulated. No final count was possible as the oyster farmer hosting the experiment removed it shortly after the retirement of the biologist. The oyster farmer had been informed that the remaining experimental oysters carried a very high M.sydneyi infection, and was understandably concerned that this may act as a focus of infection of his own and others stock.

Tables 2 and 3 show that growing height below that normally used by farmers is associated with increased mortality. Mean mortalities were lower for the trays grown higher but not enough for significance to be apparent in either 1982 or 1983. Mortalities were still unacceptably high in raised trays, and with the added risks of heat kill and the problem of low growth rates at the higher levels, raising trays could not be recommended as a management practice based on this experiment. The differences in mortality between lower and higher trays are clear and may prove worth further investigation in conjunction with, for example, studies on the method of infection of the parasite.

TABLE 2. The effect of oyster growing height on mortality rates in a Marteilia sydneyi infected area in the Tweed River, 1982*

Height mm	Mortality %	Arcsin /x Transformation
+200	31.3 ± 6.9	33.9 + 4.4^{a}
+150	40.6 ± 9.2	33.5 + 5.3ab
+100	34.5 ± 6.6	35.9 + 4.0^{a}
+ 50	28.8 ± 12.8	32.0 + 8.3^{a}
0**	47.0 ± 2.8	43.3 + 1.6^{ab}
- 50	44.4 ± 14.5	41.8 + 8.4^{ab}
-100	59.5 ± 8.5	50.5 + 4.9^{bc}
-150	61.7 ± 9.8	51.9 + 5.9^{bc}
-200	73.9 ± 10.1	59.2 + 7.1^{c}

* Mean S.D. Means which do not share a common superscript differ significantly ($p<0.01$)

** Normal commercial growing level, which is close to mid-tide. Three trays of 100 oysters each were used at each height.

...5/

TABLE 3. The effect of oyster growing height on mortality rates in a Marteilia sydneyi infected area in the Tweed River, 1983*

Height mm	Mortality %	Arcsin /x Transformation
+200	48.5 ± 6.5	44.1 + 3.7[a]
+150	53.0 ± 36.3	46.2 + 23.6[a]
+100	53.3 ± 6.1	46.9 + 3.5[a]
+ 50	60.7 ± 5.0	51.2 + 3.0[a]
0**	64.3 ± 3.2	53.3 + 1.9[abc]
- 50	72.7 ± 2.1	58.5 + 1.3[ab]
-100	78.7 ± 5.5	62.6 + 3.9[bcd]
-150	85.0 ± 2.6	67.3 + 2.1[cd]
-200	89.7 ± 6.4	72.0 + 7.0[d]

* Mean S.D. Means which do not share a common superscript differ significantly ($p<0.01$)

** Normal commercial growing level, which is close to mid-tide. Three trays of 100 oysters each were used at each height.

(c) Selective Breeding

Because it is possible that the only real means or reducing or eliminating the mortality may be by development of a disease resistant strain of oyster, one of the objectives of the programme was to examine the possibility of establishing such a strain. However, early conduct of this line of research was precluded by difficulties associated with the breeding of the Sydney rock oyster and the rearing of its larvae under laboratory conditions.

The development of techniques at Port Stephens for laboratory culture of the Sydney rock oyster subsequently allowed initiation of the programme.

With expert advice from four geneticists from the Department of Agriculture's Division of Animal Production, a simple rotational breeding scheme, based on four selection lines and two control lines (each consisting of 6 families) for each trait, was proposed. This was intended to optimise the rate of genetic information about the Sydney rock oyster, whilst remaining a manageable workload for the two people employed on the programme and preventing deleterious inbreeding of the selection lines.

To minimise the inevitable differences between the rearing environments of the families at the critical larval stage, a rearing system was designed to keep all 36 families of larvae in one 2000 l tank, each family contained in a long cylindrical cage of fine plankton mesh suspended in the tank. This system was tested in November 1984 using genetically identical groups of larvae in each plankton mesh cage to find out how much of the variability of the larvae would be caused by the system. The larvae survived through to the setting stage, and a number of different materials were tested for suitability as setting substrates. From counts of the numbers of larvae remaining and length measurements of about 30 larvae from each family taken every two days, it was shown that there were no significant effects of initial stocking density in the cage on larval survival or growth. Overall survival to the setting stage was not very high, but some of the larvae did settle on the substrates provided, and it was hoped that the next batch of larvae would show improved survival and setting success as the "bugs" had been ironed out of the rearing techniques.

...6/

Because there had been little QX mortality in New South Wales rivers in the past few years, it was decided to wait until higher QX mortalities occurred to provide a greater selection differential for the QX resistance selection lines. In the meantime, oysters that had survived one or more seasons of winter mortality of varying degrees of severity were collected from 11 locations in 5 southern rivers for laboratory breeding.

The initial promise of the first batch was not fulfilled; the 36 families of larvae produced from the winter mortality broodstock did not survive beyond 4 days. Subsequent batches spawned by the winter mortality broodstock or by fat oysters from Nambucca did not survive beyond 10 days, although a number of improved measures were taken. The delay between spawning and fertilisation was reduced, the larval rearing densities were reduced, and the plankton mesh cages were washed and sterilised every two days.

A visiting microbiologist, Dr. Christian Garland of the University of Tasmania, suggested that from his experience of the rearing of Pacific oyster larvae in the hatchery at Bicheno, that the practice of UV-sterilising the larval rearing water and addition of antibiotics to the rearing tanks be discontinued (as it provided a condition where an ecologically unbalanced population of bacteria could flourish), and that hygiene should be improved. This approach gave better survival of larvae in the routine hatchery tanks and will be used for future batches of larvae in the genetics programme. In addition a much simpler breeding scheme is to be used in order to achieve a higher success rate in the breeding experiments.

Although major problems associated with laboratory culture of Sydney rock oysters have been overcome at Port Stephens, it is not possible to actively proceed with the programme of selective breeding of a QX resistant strain because the low incidence of the disease in northern New South Wales in recent years has precluded the occurrence of suitable brood stock. In addition, because the programme will not be labour intensive it was decided not to seek additional funds from FIRTA but to use staff to be engaged on a programme on Pacific oysters to carry out the selective breeding work.

CONCLUSIONS

The heavy mortality occurring in the Sydney rock oyster in northern New South Wales and southern Queensland is due to an ascetosporan parasite, Marteilia sydneyi, but the complete life history of the parasite is not clearly understood. Laboratory experiments are taken to indicate that the parasite is not transferred directly from oyster to oyster but a study of a selection of estuarine animals closely associated with oysters and oyster beds failed to establish the presence of an intermediate host.

A clear understanding of life history of similar parasites causing heavy mortality in oysters overseas is also yet to be established in spite of intensive study over many years.

Previously prevalent in southern Queensland and the northern rivers of New South Wales such as the Tweed, Richmond and Evans, evidence is provided to indicate a southerly spread of the disease to the Clarence River in 1978 and the Macleay River in 1982. In order to prevent any further spread of the disease, action has been taken to prevent the movement of oysters from infected to non-infected areas.

...7/

The incidence of the disease varies from year to year and from area to area. Infestation is normally between February and August with the highest occurrence in April. An outbreak of disease often follows a fall in salinity but there is no strong relationship between low salinity and the incidence of disease.

Increasing the cultivation height can result in a decrease in QX mortality but adoption of this practice is unlikely to achieve any overall improvement in productivity because of slower growth and losses through heat kills of the oyster grown at higher levels.

To avoid heavy losses through QX mortality, oyster growers in the northern river areas of New South Wales tend to obtain half grown oysters from the more southern areas for fattening in the north. Where possible these oysters are then marketed before the onset of disease. Monitoring the growing areas and providing growers with information in relation to the occurrence of parasites has allowed more productive use of the northern rivers by more timely relaying or marketing of oysters.

Because it is possible that the only real means of reducing or eliminating the QX mortality will be by use of a disease resistant strain of the Sydney rock oyster, development of such a strain has now been afforded a high priority. However, because of the long-term nature of such research no early solution to the disease problem can be anticipated.

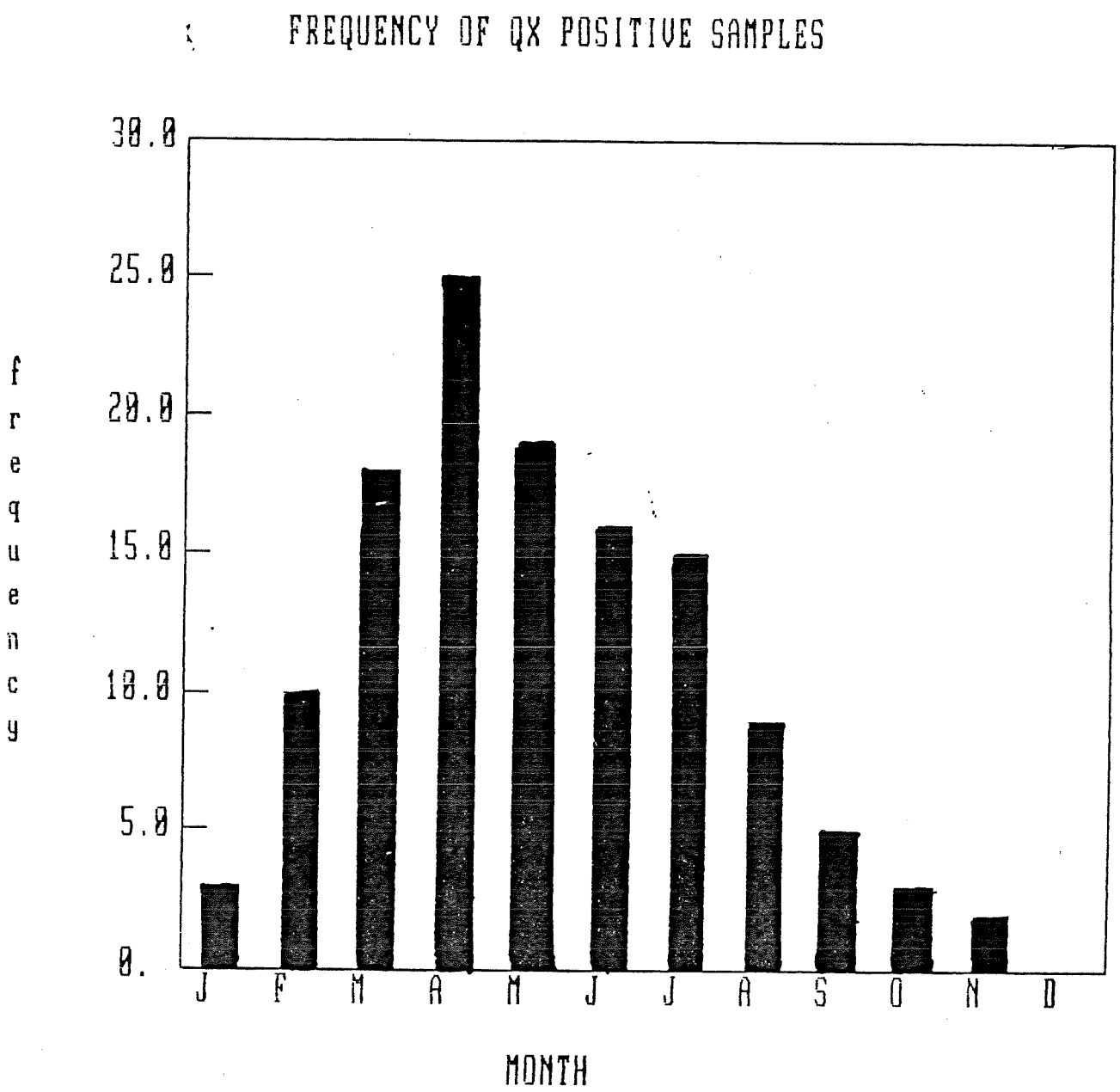
FREQUENCY OF QX POSITIVE SAMPLES
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FIGURE 5.

TWEED RIVER / Terranora

Figure 1

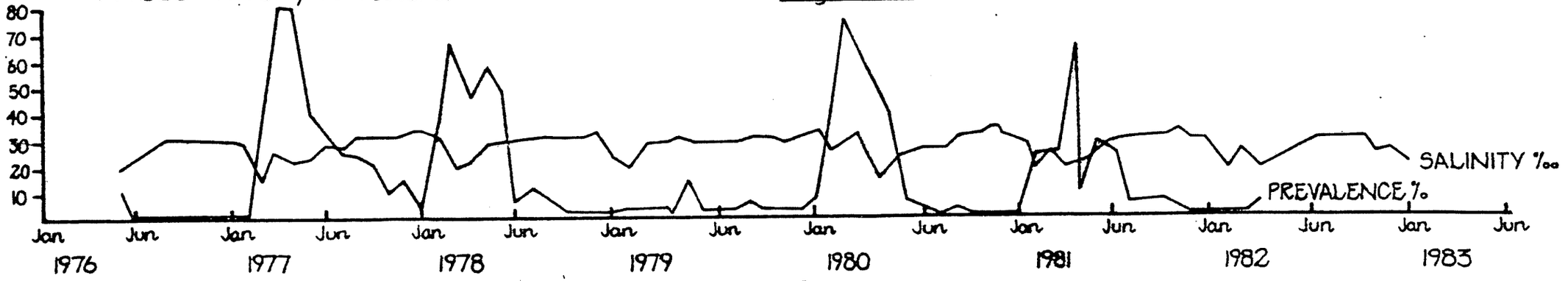


Figure 2

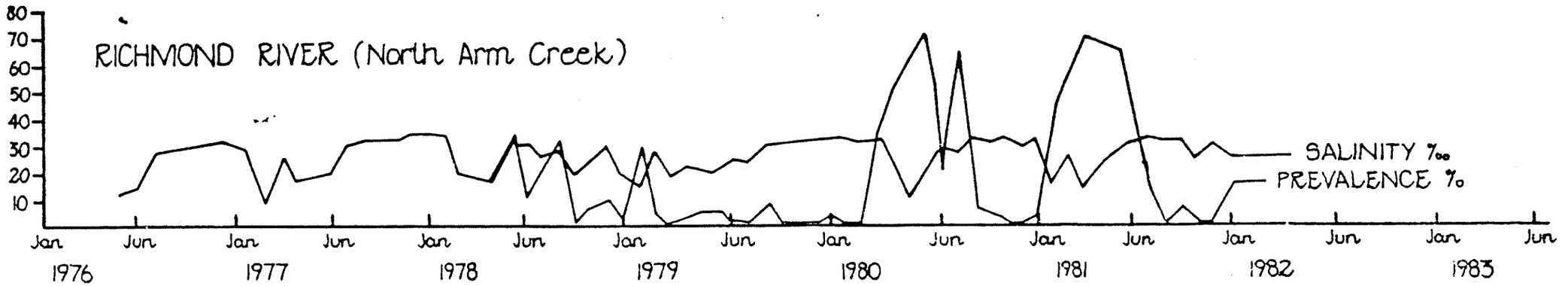


Figure 3

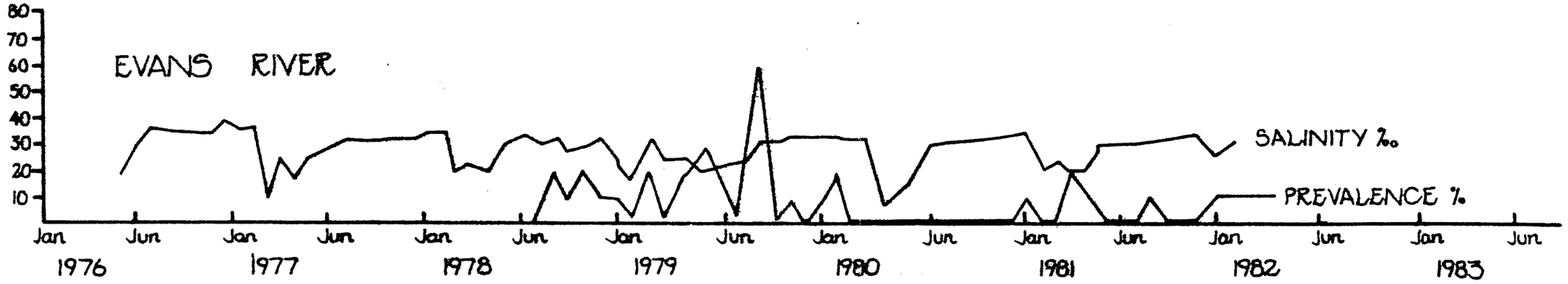
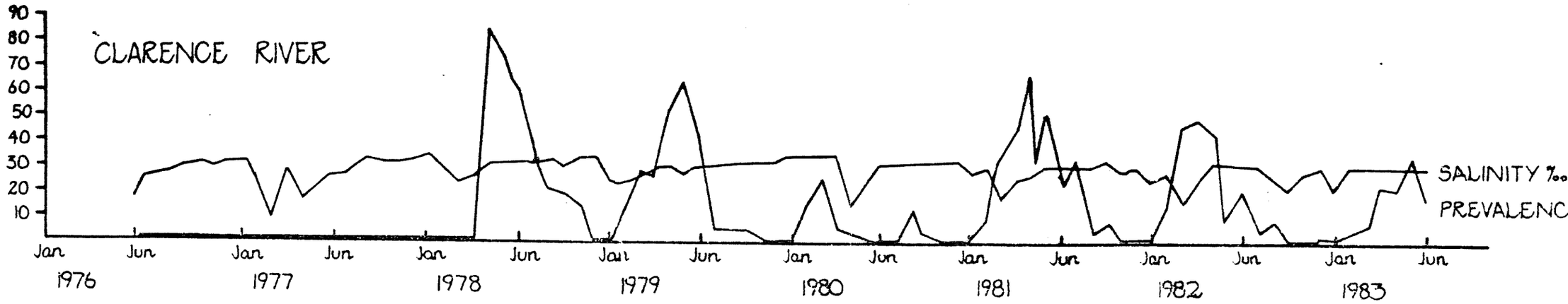


Figure 4



APPENDIX IX

The future of oyster breeding

by C. J. Mason
Caroline Mason is a technical officer at the NSW Brackish Water Fish Culture Research Station.*

THE COST of producing and marketing oysters has risen dramatically in the last decade, in everything from wages through materials to transport costs. To stay in business, an oyster farmer has two alternatives: to increase the price of oysters to cover costs, or try to reduce the cost of producing oysters. With the first alternative he risks losing customers to competitors, or even losing the market altogether if customers will not pay the asked price. To achieve the second alternative, one avenue may be to improve the oyster genetically by selective breeding.

And that is where the potential lies. Probably the three most important areas for genetic improvement in the Sydney rock oyster are increases in growth rate, and resistance to the two diseases QX and winter mortality.

The advantages of faster-growing strains of oysters are obvious: rapid turnover of stock; less time for oysters to be exposed to over-spatting, natural attrition or disease losses; and fewer seasonal movements of stock during the growing period.

The oysters now growing on sticks are essentially wild oysters, whose ancestors started to look like oysters about 190 million years ago. For oysters to have remained unchanged for 190 million years implies that they have hit on a pretty successful formula for survival, even though individual oysters are still very variable. The reason oysters look similar is because any that are grossly different from the others usually do not survive to reproduce themselves. For example, it is widely believed that faster-growing oysters are pushed off the stick, or very slow-growing, small oysters are eaten by bream or crabs. This is natural selection. When I pick the largest, fast-growing oysters to use as broodstock, I am changing the conditions. They produce fast-growing progeny, which instead of being at a disadvantage as they are in the wild, are now at an advantage because I am positively looking after them, and most important, taking the best of them to breed from. In each succeeding generation there will be more of the fast-growing oysters compared with normal growth rate oysters, which are now being selected against. This is artificial selection and it works on exactly the same principles as natural selection.

In the case of resistance to disease, natural and artificial selection will be working in the same direction — that is, increase in numbers of the progeny of disease-resistant oysters at the expense of the susceptible oysters (that do not produce progeny because they are dead).

Artificial v natural selection

With natural selection working in our favour, it might seem unnecessary to select for disease-resistant strains, and so it would be if we were prepared to wait a hundred years. There are three reasons why artificial selection could make significant improvements in less than 1/10th of the time for natural selection.

First, hatchery spat may be produced from parents with known qualities and histories and oysters of known resistance may be mated, whereas in the wild, resistant and susceptible oysters may cross, producing fewer resistant progeny.

Second, the incidence of disease varies within a river or estuary. For example, oysters caught in one area and taken to three different leases in the same river might suffer 90, 50 and 0 per cent mortality respectively. When the oysters next spawned, the next generation would be derived from either resistant oysters, a mixture of resistant and susceptible oysters, or mostly susceptible oysters, depending on whether their parents were on the lease that was worst affected, partly affected or missed the disease altogether. (From this it follows that the only way you can distinguish between resistant and susceptible oysters is in the presence of disease when the susceptible oysters will probably die.) The susceptible oysters also spawn, so the resistant oysters will be diluted because only a limited number of oysters can survive to maturity. In genetic terms, the selection intensity has been reduced because some of the oysters were never subjected to selection pressure — in this case, the disease.

The third reason is related to the second — good growing areas are generally not suitable for spat catching, so spat is imported. As the spat is usually from disease-free areas, the parents will not have been subjected to selection. Most of the spat will be susceptible and will again dilute the local population that had been selected by disease mortality.

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At present we cannot transmit QX or winter mortality to a healthy oyster in the laboratory, so in order to find which oysters are resistant we will have to rely on natural disease outbreaks. Oysters to be used as broodstock will be tested on leases in areas that are likely to suffer disease outbreaks.

Genetic improvement

Virtually all genetic research on oysters has been carried out in North America on three species — the European flat oyster *(Ostrea edulis)*, the native American oyster *(Crassostrea virginica)* and the Pacific oyster *(C. gigas)*. I will summarise the results of a couple of experiments performed on growth rate and resistance to disease among the three species. First, however, I must define three terms that measure genetic improvements and the potential for genetic improvement:

$$\text{Heritability } (h^2) = \frac{V_A}{V_T}$$

Where V_A is the additive genetic variance and V_T is the phenotypic variance, which is the differences you actually see between individuals. Heritability is a measure of how much of a trait is determined by genetic factors compared with environmental factors, and is manifest as the resemblance between close relatives.

The Selection Intensity (S) has already been described; it is a measure of the distance between the mean (for a trait) of the population and the mean of the selected individuals.

The Response to Selection (R) is the difference between the means of the parental and the filial generations.

The three teams are related by:

$$H^2 = R/S$$

but it is not very useful in practice to try to estimate heritability from the ratio of the response to the selection differential.

In addition, I must mention that the above terms and formulae are used only for traits that are additive in effect. There are some genes with effects that are multiplicative in action, and rely on specific combinations to have their effect, rather as a royal flush is worth far more than the same value cards of different suits. The effects of these genes do not follow the same rules as those of additive genes, because the gene groups are split up when eggs and sperm are produced for the next generation.

Heritability

The heritability of various characters has been estimated for the Pacific oyster (Table 1, Lannan, 1972). It may be seen that the heritability of linear dimensions for 12 month oysters is very high. A heritability of .2 is conventionally considered high enough to get rapid gains through selection. By 18 months, however, the heritabilities for the same characters, measured from the same oysters, have dropped to between 0-.19. This almost certainly represents crowding with stunting of oysters in the disadvantaged positions, but it does emphasise the importance of knowing what is going on in the environment of the oyster in order to get meaningful information. The 18-month estimate of the heritability of total weight (or growth rate) is .33 and the heritability of the ratio of meat to shell is even higher at .46. Setting success is a trait with low estimated heritability, so the rate of improvement is likely to be very slow. In this case, it is best to concentrate on improving rearing techniques to improve the setting rate.

Using a slightly different approach, European oysters were selected for size at differing selection intensities and the offspring scored for response to selection (Table 2, Newkirk and Haley, 1982). Realised heritabilities calculated from the selection intensity and the response are similar in range and magnitude to those calculated in a different way for the Pacific oyster (Lannan, 1972, above). A second generation was produced (Newkirk and Haley, 1983). The offspring were on average, consistently heavier than the controls but the only statistically significant difference was that survival was improved. The improvement in growth rate does not equal that of the first generation. The authors conclude that the oysters were suffering from inbreeding depression, with only two or three individuals contributing to the different groups.

Table 1. Heritability estimates and predicted response to selection using the upper 20 per cent of the population as parents — Pacific oyster.

Attribute	*Heritability (estimated from full sibs.)*	*Parental Mean*	*Predicted Response*	*% Gain*
12 month oysters				
Length	.81 ± .07	34 mm	11 mm	33%
Width	1.17 ± .05	11 mm	9 mm	81%
Height	.81 ± .27	52 mm	19 mm	36%
18 month oysters				
Length	0	47 mm	0	0%
Width	.10 ± .12	17 mm	.7 mm	4%
Height	.19 ± .003	72 mm	5 mm	7%
Total weight	.33 ± .19	30 g	8 g	27%
Meat/total weight	.46 ± .22	.21	.02	10%
Setting success	.09 ± .08	4.5%	.02%	5%

From Lannan, 1972.

Table 2. Observed mean weights (g) and selection intensity of selected and control parents, and mean weights (g) and standardised response of offspring — *Ostrea edulis*.

		Parents		*Offspring*	
Year	*Line*	*Mean Weight*	*Selection Intensity (S)*	*Mean Weight*	*Response (R)*
1977	1	69.9	1.85	36.2	0.72
	2	56.6	1.05	32.7	0.49
	3	57.0	1.09	33.8	0.51
	4	53.3	0.86	33.4	0.62
Control	P1	—	0.00	26.2	0.00
1978	5	66.7	1.65	29.1	0.15
	6	59.8	1.25	32.6	0.44
	7	42.5	0.22	31.0	0.26
Control	P2	—	0.00	26.9	0.00

From Newkirk and Haley, 1982.
Weights for controls not comparable as they are 9-year-old oysters.

Inbreeding

The effects of inbreeding on oysters are under debate. Most authors (e.g. Longwell and Stiles, 1973; Newkirk, 1980; Imai and Sakai, 1961) agree that inbreeding has detrimental effects on growth and survival but some (Lannan, 1980; Mallet and Haley, 1983) have found no ill-effects. The rotational breeding scheme to be used in this genetics program is designed to minimise inbreeding when only a limted number of broodstock is used. At the same time, experiments will be carried out to determine the extent of inbreeding depression on growth and survival of Sydney rock oyster larvae and spat.

Disease resistance

Research on selection for disease resistance has been done in North America, principally on two oyster diseases that show similarities to the diseases that affect the Sydney rock oyster industry. MSX affects the American oyster on the east coast and is caused by a parasite closely related to the QX parasite, *Marteilia sydneyii*. On the west coast, and also in Japan, the Pacific oyster suffers losses caused by summer mortality, a disease which selectively kills females, older oysters and oysters resorbing their gonads. Summer mortality is thought to be stress mediated and shows some similarities to the winter mortality of the Sydney rock oyster.

In the late 50s and early 60s, the dredge oyster industry of the east coast was virtually wiped out by MSX (Haskin, 1964). Over the next 10 years it became apparent that stocks of oysters remaining in the worst affected areas survived the disease much better than oysters imported from other areas (Haskin and Canzonier, 1969) and the 'resistant' stocks showed a lower incidence of infection (Myhre and Haskin, 1969). Throughout the 70s improvements continued and a correlation between MSX resistance and resistance to *Labyrinthomyxa marina* (a fungus-like parasite) was found (Valiulis and Haskin, 1973).

Table 3. Survival of American oysters bred from parents selected for resistance to MSX.

	Total survival over three years
Susceptible	8%
Natives	22%
Generation F1 (laboratory-bred resistants)	31%
Generation F2	44%
Generation F3	50% (not complete at time of publication)

From Haskin, 1974.

Laboratory-bred resistants	Survival ratio (Res:Sus)
Generation F1	4.4
Generation F2	5.0
Generation F3	6.6
Generation F4	8.9
Natives	2.9
Susceptibles	1.0

From Haskin and Ford, 1978.

A selective breeding program to produce MSX resistant oysters (Table 3) produced oysters three times as resistant to MSX as native Delaware Bay oysters and nine times as resistant as susceptibles in four generations (Haskin and Ford, 1978). There were fears initially that improvements in resistance would level off (Haskin, 1974) but no plateau in resistance had been found in 1977.

Pre-stressing

Research in Washington State showed that there was a correlation between mortality caused by stressing Pacific oysters in the laboratory by holding them at elevated temperatures and summer mortality (Lipovsky and Chew, 1972 and Beattie *et al.*, 1980). This correlation meant that rather than having to rely on natural conditions causing an outbreak of summer mortality among the oysters, they could kill oysters in the laboratory with heat stress. Many of the oysters that died would also have died from summer mortality. In 1977, thirteen families of oysters produced by mating oysters that had survived heat stress were evaluated in the field and in the laboratory (Beattie *et al.*, 1980, Table 4). In 1978, summer mortality occurred in the test sites. Unselected controls suffered 47.5 per cent mortality and the mortality among selected families ranged from 5 per cent to 85 per cent. Most of the selected families did better than the controls however. Laboratory testing of oysters from the same families gave roughly the same results. Families with high survival of

summer mortality also survived the heat stress well. The authors also noted that on the whole, the dead oysters were larger than the survivors but the differences were only statistically significant in a couple of cases.

Table 4. Summer mortality of first generation Pacific oysters, bred from laboratory-selected parents. The L.D. 50 oysters were held at an elevated temperature until 50 per cent of the control oysters had died.

	% mortality in Rocky Bay		
Family	*Upper plot*	*Lower plot*	*Lab. L.D. 50 % mortality*
A	16.3		8
B	5.1		
C	20.0		
D	10.3		53
H	17.3	27.5	13.5
K	47.3	85.5	45
M	7.5	7.4	20.5
N		42.2	35
P	22.6	41.9	89
R		28.6	35
S	18.7	38.0	48
T	24.0	27.5	
Z	24.1		40
Control	33.0	48.0	50

From Beattie *et al.*, 1980.

Unfortunately, the experimental design makes it difficult to get any idea of the heritability or response to selection for summer mortality resistance.

To conclude, a word of caution — it might seem that given the potential for genetic improvement in the Sydney rock oyster I have just described, that within five years we will have bred a super oyster, resistant to disease and acts of God. However the minimum feasible generation time for the oysters is one year if we are lucky, and we will be hoping for improvements of a few per cent per generation. Compared with say electronics or biotechnology, genetic progress is slow and steady, but the gradual improvements we can pass on will be essential to the continuing success of the oyster industry.

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