Final report

to

Fisheries Research and Development Corporation (FRDC)

September 1994

Full project title:

Evaluation of triploid Sydney rock oysters (*Saccostrea commercialis*) on commercial leases in New South and Triploid Pacific oysters (*Crassostrea gigas*) on leases in Tasmania (FIRDC No 89/63).

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CONTENTS

b Gardner, N. C., 1993. A literature review of triploidy in commercial bivalves. 40 pp. From Gardner, N. C., 1993. Aspects of the Performance of Diploid and Triploid Pacific Oysters (*Crassostrea gigas*, Thunberg) in Southern Tasmania. Unpublished Thesis, University of Tasmania, Launceston, 183 pp. 220

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d Maguire, G. B., Gardner, N. C., Nell, J. A., Kent, G. and Kent, A. Studies on triploid oysters in Australia. 2. Growth, condition index, gonad area and glycogen content of triploid and diploid Pacific oysters, *Crassostrea gigas*, from oyster leases in Tasmania, Australia. In: Book of Abstracts, Fifth International Symposium on Genetics in Aquaculture, Halifax, NC, Canada, pp. 90 (Abstract). 256

e O'Meley, C., Maguire, G. B. and Lleonart, M., 1992. Molluscan Culture Techniques. Multi-Skilling in Aquaculture: A Hands-on Training Workshop. University of Tasmania, Launceston, Tas., pp. 1-23. 258

f Ryan, T., 1992. Aquaculture and remote data collection. UTAS, June 1992, p. 5. 282

10 OTHER SEMINAR PRESENTATIONS

Sydney rock oysters

In 1992 a series of seminars were given to oyster farmers along the NSW coast.

Pacific oysters

Presentations have been given to the Tasmanian Aquaculture Co-operative Society (Rutherglen, 1990), Australian Mariculture Association (Brisbane, 1991), Port Stephens Research Centre (NSW Fisheries, 1992) and the University of Tasmania at Launceston (1992) and Hobart (1993).

1 SUMMARY

1 SUMMARY

Sydney rock oysters

1 A two and a half year study was conducted to compare the performance of sibling diploid and triploid Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley) at three intertidal and one subtidal site in Port Stephens, New South Wales (NSW). The triploid Sydney rock oysters were on average 41% heavier than their diploid siblings after two and a half years of growth.

2 The triploid oysters maintained higher dry meat weight, higher condition index (CI) values and glycogen levels than their diploid siblings at all sites, during the final ten months' growth to market size (40 to 60 g whole weight). The triploid oysters at the subtidal site had the highest CI values recorded in this study.

3 In the farming experiment, the triploids suffered less mortality (12.1±1.1%; x±SE) than the diploids (21.5±1.9%), although this difference was not significant ($P>0.05$). When in a 'winter mortality experiment' oysters were exposed to the protistan parasite *Mikrocytos roughleyi* which causes winter mortality disease, no differences in mortality between diploid and triploid groups occurred.

4 Sensory evaluation of diploid and triploid Sydney rock oysters showed that there was no consumer preference for either type of oyster. However, consumers do prefer 'fat' oysters over 'poor' oysters.

5 Triploids developed smaller gonads than diploids (maximum as % of cross sectional area was 26% compared to 49% for diploids).

6 Most females triploids during the breeding season for diploids (summer - autumn) had a few mature oocytes but in contrast to overseas studies no triploid females had well developed gonads and no spermatozoa were found in triploid males.

7 There was no evidence of spawning in the triploids.

8 During sampling, only one hermaphrodite was found and only 2% of oysters were of an indeterminate sex. Four triploid hermaphrodites were found but the most notable difference between diploids and triploids was the large number of indeterminate triploids (57%), as opposed to 2% for diploids. The male to female ratio for triploids (1:1.8) did not differ significantly ($P>0.05$) from the diploid sex ratio (1:1.3).

These findings have major implications for the NSW oyster industry as triploid Sydney rock oysters have good consumer acceptability, reach market size 6-18 months faster and maintain better meat condition than diploid oysters without an increased risk of winter mortality.

Pacific oysters

Project goals have been met and the major findings have been as follows:

1 Triploid Pacific oysters maintained marketable meat condition during the summer-autumn period after reaching market size (>60 kg) whereas, at two of the three sites used, diploid oysters spawned and remained in much poorer condition than the triploids for at least three months. There was no evidence of spawning of triploid oysters. In the absence of a spawning among diploids at the third site, diploids developed higher condition index (CI) than triploids but both ploidy groups were very high quality oysters so triploidy conferred no major disadvantage at that site.

2 At the three sites triploids grew 20-23% faster but this was mostly shell growth after commercial size had already been reached. This shell growth is disadvantageous as it tends to reduce condition index.

3 At two sites mortality of diploid and triploid oysters was negligible from the time they were placed in 6 mm baskets in August 1990 through to mid 1992. At the third site runt oysters for both diploids and triploids generally perished during the study.

4 Triploids had acceptable shell shape (deep cup shape, not elongated).

5 Both the diploids and triploids were acceptable to taste panels over the three seasons examined (spring - autumn). However, in each taste test trial, the diploids were in quite good meat condition.

6 Triploids developed higher glycogen reserves and maintained these through the spawning season. However, glycogen content did not seem to be a reliable guide to taste panel appraisal of oysters.

7 Triploids developed much less gonad than diploids (maximum as % of cross sectional area was 12% compared to 73% for diploids). Triploid males had larger gonads than triploid females.

8 Most females triploids during the breeding season for diploids (summer - autumn) had a few mature oocytes but in contrast to overseas studies no triploid females had well developed gonads and no spermatozoa were found in triploid males.

9 Triploid samples had a greater proportion of males than diploids and in contrast to other studies overseas there were relatively few hermaphrodites.

10 In a separate experiment triploids and diploids in an age range of 1 - 1.5 years old exhibited similar growth and meat condition. However, the four combinations of tidal position (growing height) and stocking density, that the oysters were subjected to, affected growth and meat condition results and provided information on strategies for repressing shell growth in triploids.

11 This project provided a detailed description of the performance of diploid Pacific oysters in modern farming systems in Tasmania; no equivalent data have been published before. Growth results were consistent with the two year (two sites) and three year (one site) production economics models, developed in a concurrent FRDC Project, by ABARE and Maguire.

12 The modest changes in size variation that occurred over two years at the better sites suggests that there is potential for reducing the amount of grading oysters are subjected to.

13 A new technique for distinguishing triploids from diploids using histology was developed. The project also led to the development of better methods for glycogen analyses and the commissioning of a data logger system for water temperature and aerial exposure.

This project has been very successful and has generated considerable demand for triploid oysters from Tasmanian oyster farmers. From the batch of triploids, described above and produced by the FRDC research team and Shellfish Culture Pty Ltd, 800 000 triploid spat were made available to Tasmanian and South Australian farmers. Feedback was generally favourable. A new research grant application (Commercialisation of triploid Sydney rock and Pacific oysters) was made to FRDC to help overcome problems in hatcheries which previously had been unsuccessful with meeting this demand.

2 BACKGROUND

The New South Wales oyster industry which is worth in excess of $30 Million (124 million oysters) annually to the growers has an oyster condition problem in winter. Farmers are either forced to sell oysters in poor condition. (which lowers the price and the reputation of the Sydney rock oyster) or postpone oyster sales until spring and summer. This affects continuity of supply, and cash flow to the farmers.

The Tasmanian industry, which is worth $9 million (35 Million oysters) annually, has similar problems with meat condition.

A comparison of oyster meat and gonad condition between diploid and triploid oysters was necessary to determine whether triploids do have any benefits for the oyster industry in solving the problem of poor condition. Comparative taste tests were also required to determine if there would be any consumer preference between the two types of oysters for either species.

3 OBJECTIVES

a To compare growth and survival rates of diploid and triploid oysters on 3-4 sites in major oyster growing estuaries, ie. Sydney rock oysters in Port Stephens (NSW) and Pacific oysters in Tasmania.

b To compare gonad and meat condition (condition index) and chemical composition of meats in Sydney rock oysters grown at 3-4 sites in Port Stephens (NSW), and Pacific oysters grown at 3-4 sites in Tasmania.

c To compare consumer acceptability of triploid with diploid oysters using recognised taste panel studies.

4 DESCRIPTION OF INTELLECTUAL PROPERTY and TECHNICAL SUMMARY OF ALL INFORMATION DEVELOPED

All information obtained during the life of the project is contained in this report and will be published in the scientific literature.

5 COVERING LETTER TO FRDC

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30 September 1994

Dear Sir

I hereby submit the Final Report for Project "Evaluation of triploid Sydney rock oysters (*Saccostrea commercialis*) on commercial leases in New South and Triploid Pacific oysters (*Crassostrea gigas*) on leases in Tasmania; FIRDC No 89/63". All the objectives as set out in the original research grant application have been achieved. Triploid oysters of both species grew faster and held meat condition better than their diploid siblings. On the basis of these results FRDC approved a new three year (August 1993 - August 1996) research grant "Commercialisation of triploid Sydney rock and Pacific oysters" (FRDC No 93/151).

Summary of total funds and other contributions to this project:

	$
FRDC	263 996
NSW Fisheries	106 500
University of Tasmania	70 000
Marine Culture Developments Pty Ltd (Brooms Head Hatchery)	2 150
Shellfish Culture Pty Ltd (Bicheno Hatchery)	1 500
Tasmanian Development Authority	3 500
Agritechnology Pty Ltd (US $500)	720
Total	$448 366

Yours sincerely

Dr John A Nell

Yours sincerely

Dr Greg B Maguire

6 FARMING POTENTIAL OF TRIPLOID OYSTERS

a Nell, J. A., Cox, E. , Smith, I. R. and Maguire, G. B., 1994. Studies on triploid oysters in Australia. I. The farming potential of triploid Sydney Rock oysters *Saccostrea commercialis* (Iredale and Roughley). Aquaculture, (in press).

b Maguire, G. B., Gardner, N. C., Nell, J. A., Kent, G. and Kent, A. Studies on triploid oysters in Australia. II. Growth, condition index, glycogen content and gonad area of triploid and diploid Pacific oysters, *Crassostrea gigas* (Thunberg), in Tasmania.

c Maguire, G. B. and Kent, A., 1991. Performance of triploid and diploid Pacific oysters at four combinations of growing heights and stocking densities. In: G, Maguire (Editor), Oyster Research Workshop. Tasmanian Aquaculture Co-operative Society, Smithton, Tas., November, 1991, pp. 13-16.

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Studies on triploid oysters in Australia. I. The farming potential of triploid Sydney Rock oysters *Saccostrea commercialis* (Iredale and Roughley).

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ABSTRACT

A two and a half year study was conducted to compare the performance of sibling diploid and triploid Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley) at three intertidal and one subtidal site in Port Stephens, New South Wales (NSW). The triploid Sydney rock oysters were on average 41% heavier than their diploid siblings after two and a half years of growth. The triploid oysters also maintained higher dry meat weight and higher condition index (CI) values than their diploid siblings at all sites, during the final ten months' growth to market size (40 to 60 g whole weight). The triploid oysters at the subtidal site had the highest CI values recorded in this study. When exposed to the protistan parasite *Mikrocytos roughleyi* which causes winter mortality disease, no differences in mortality between diploid and triploid groups occurred. These findings have major implications for the NSW oyster industry as triploid Sydney rock oysters can reach market size 6-18 months faster and maintain better meat condition than diploid oysters without an increased risk of winter mortality.

Keywords: farming, triploid, oysters, growth, disease

INTRODUCTION

Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley) generally spawn in the summer and autumn period and as a result are in poor condition during winter and spring, making them difficult to market for up to six months of the year (Nell, 1993). It was thought that because triploid oysters have reduced gonad development (Beaumont and Fairbrother, 1991), triploid Sydney rock oysters would be a good winter crop for oyster farmers. Triploid Pacific oysters *Crassostrea gigas* (Akashige and Fushimi, 1992) and triploid American oysters *Crassostrea virginica* (Barber and Mann, 1991; Matthiessen and Davis, 1992) also grow faster than diploid oysters. In our study, the effects of triploidy on seasonal changes in meat condition in Sydney rock oysters were investigated to assess their marketability in winter. Growth rates of the oysters were also recorded for comparison with those reported for other species (Table 1).

In other oyster species, triploids may vary, compared with their normal diploid forms, in their response to challenge by protistan parasites. No differences in mortality were found between the diploid and triploid forms of the American oyster (Barber and Mann, 1991; Meyers et al., 1991) or the Pacific oyster (Meyers et al., 1991) exposed to the parasite *Perkinsus marinus*, although triploid American oysters survived better than diploids exposed to the parasite *Haplosporidium nelsoni* (MSX) (Matthiessen and Davis, 1992). The relative susceptibility of diploid and triploid oysters to the disease winter mortality is important as high losses of Sydney rock oysters occur in southern growing areas, particularly during cold, dry winters (Nell and Smith, 1988). An experiment to compare survival of triploid and diploid oysters was conducted in a commercial oyster growing area where this disease, which is caused by the protistan parasite *Mikrocytos roughleyi* (Farley et al., 1988), is prevalent.

Substantial differences in the size of the adductor muscle between diploid and triploid Pacific oysters have been reported (Akashige, 1990) and NSW oyster farmers were concerned that triploid Sydney rock oysters might have an excessive shell to whole weight ratio. Therefore three morphometric characteristics, adductor muscle diameter, cavity volume and shell weight were measured, relative to whole oyster size, in market size (40 to 60 g whole weight) diploid and triploid oysters at the end of the study.

MATERIALS AND METHODS

Induction of Triploidy

Ripe Sydney rock oysters were obtained from Port Stephens, New South Wales (NSW), in January 1990 (mid summer) and strip spawned (Allen et al., 1989; Allen and Bushek, 1992). Eggs from nine females were divided in two portions, half for the production of normal diploid oysters and the rest for the production of their triploid siblings. Sperm from two male oysters were mixed then added, within 30 min of stripping, to each group of eggs so that up to five sperm per egg could be seen at the periphery. Triploidy was induced in a single batch of fertilised eggs by blocking meiosis II with cytochalasin B (CB) at a concentration of 0.5 mg CB l^{-1} at 23 min after sperm addition for 20 min at a temperature of 25°C (Downing and Allen, 1987; Allen et al., 1989).

Larvae and spat rearing

Larvae were reared in 1 000 l tanks and at day 18 the "eyed" larvae were moved to settling tanks with finely ground scallop shell (200 to 350 μm in diameter) in downweller screens (Utting and Spencer, 1991). Larvae remained in the settling system for one week and the resulting spat were reared in the hatchery for four weeks. The spat were then transfered to upweller screens in an outdoor nursery (Bayes, 1981) supplied with estuarine water. These spat were ongrown until June 1990, when most were approximately 14 mm in height.

Oyster growth study - Experiment 1

Experimental design

Three intertidal sites (Tilligerry Creek, North Arm Cove and Pindimar; Holliday et al., 1991a) and one subtidal (Tilligerry Creek; about 3.2 km upstream from the intertidal site) site in Port Stephens, NSW (32° 45'S, 152° 10'E), were chosen for the grow-out study, which was carried out from June 1990 to November 1992. Oysters were held on trays (0.9 m x 1.8 m; divided in twelve sections) covered with plastic (polyethylene) mesh (Holliday et al., 1991b) and an additional tray was used as a lid to prevent accidental loss of oysters. Trays were held on intertidal, off-bottom racks or held continuously suspended from a raft (Nell, 1993).

Before the experiment commenced, the oysters had been carefully sieved through nylon mesh screens to minimise size variation between and within the two groups of oysters. The average shell height and whole weight of the diploid oysters at the start of the experiment were 13.8±0.06 mm (n = 800; x±SE) and 0.28±0.03 g respectively and for the triploid oysters were 14.3±0.06 mm and 0.30±0.03 g respectively. These differences in shell height and whole weight between diploid and triploid oysters were significant ($p<0.001$).

Four replicates of 500 diploids and 425 triploids (85% triploidy) each were placed at each site at the start of the experiment in June 1990. The slight differences in initial stocking densities were not expected to affect growth as oysters were redistributed on the trays periodically.

Oyster sampling

At three monthly intervals from June 1990 to November 1992, 50 randomly selected oysters per replicate were individually weighed and their shell heights measured, after which they were returned to the experiment. In addition, six oysters were removed monthly from each replicate from July 1991 onwards. These were shucked and the meats dried separately at 90°C for 48 h and weighed for individual oyster condition index calculations. From November 1991 to November 1992, the six dried meats for each replicate were pooled, ground finely and analysed for glycogen analyses, which involved a modified Keppler and Decker (1974) procedure (B. Day and G. B. Maguire, unpublished data, 1994). Condition index (CI) [(dry meat weight (g) x 1000)/cavity volume (g)] was measured as recommended by Crosby and Gale (1990).

At the end of the experiment in November 1992, 48 oysters of each type were sampled from each site to determine:
adductor muscle diameter (%) [(adductor muscle diameter (mm) x 100)/shell height (mm)],
cavity volume (%) [(cavity volume (g) x 100)/whole oyster volume (g)], where whole oyster volume (g) is (shell weight (g) in air - shell weight (g) in water) + cavity volume (g), and
shell weight (%) [(shell weight (g) x 100)/whole oyster weight (g)]. Note cavity volume is whole weight (g) - shell weight (g) in air (Lawrence and Scott, 1982).

Oyster management

At the start of the experiment, stocking densities for the intertidally cultured oysters were set at 70% tray coverage (Holliday et al., 1991b; ie the percentage of the tray covered with a single layer of oysters) respectively and stocking density was adjusted at each quarterly measurement. Oysters were spread out over additional tray sections or onto additional trays if necessary to maintain an appropriate stocking density. The stocking rate for the subtidally cultured oysters (50% stocking density) was lower than that for the intertidally cultured oysters to allow for their faster growth rate (Nell, 1993). Oysters were not graded during the course of the experiment.

Two mesh sizes 3 mm or 9 mm (diagonal measurement) were used for the plastic trays and covers. As soon as possible the oysters were placed on trays with the larger mesh size to maximise the water flow. However, in the case of the intertidally cultured oysters the small mesh was used for the lid throughout the first summer season to provide shade and protect the oysters from heat kill (Nell, 1993).

Oysters and trays were checked monthly and if necessary washed on the leases with a high pressure jet of estuarine water to remove silt. Once during both the first and the second summer, oysters and trays were left indoors out of the water, for up to a week to kill Pacific oyster and Sydney rock oyster spat that had settled on the oysters (up to 20 spat per oyster); any remaining larger spat were removed when the quarterly measurements were taken. Once during the second summer, the intertidally cultured oysters were submerged in a fresh water bath for 24 h to kill barnacles.

Winter mortality study - Experiment 2

Triploid and diploid oysters from the same batch produced for Experiment 1 were used in Experiment 2. Five trays of triploids (160 oysters/tray) and an identical set of trays of diploid oysters were placed on an intertidal rack in Woolooware Bay, Botany Bay, NSW (33° 59'S, 151° 12'E), in May 1992 and left in place till December 1992 for exposure to the parasite *M. roughleyi* (Farley et al., 1988). Woolooware Bay was chosen because oysters there regularly suffer winter mortality (Roughley, 1926). Care was taken to ensure that the whole live weight of the 27 month old triploid (23.7±0.3 g (x±SE) range 15.1-38.0 g) (n=125) and diploid (22.4±0.3 g; range 14.5-32.7 g) oysters chosen for the experiment were similar, because oyster farmers believe that large oysters are more susceptible to the disease than small oysters. However, because the triploid oysters had grown much faster than the diploid oysters, slow growing triploid oysters were matched with fast growing diploid oysters. At the start and end of the experiment 25 randomly selected oysters per tray were each weighed.

Statistical analysis

All meat weight, condition index, whole oyster weight and shell height data in the figures are presented as means ± 95% confidence intervals, except for the whole oyster weight and shell height data for which 95% confidence intervals were too small (≤1.5% of the mean) to be displayed in the figures.

Homogeneity of variance of the data was confirmed using the Cochran's test (Winer, 1971). The data were analysed by ANOVA (Sokal and Rohlf, 1981). The whole oyster weight data required a $\log_{10}$ transformation and oyster mortality and their morphometric characteristics were arcsin $x^{0.5}$ transformed.

RESULTS

The percentage triploidy was determined by flow cytometry (Chaiton and Allen, 1985) in larvae immediately before metamorphosis (81% in February 1990). However, four months later (June 1990), just before commencement of the oyster growth study, 85% of the spat were triploids.

Oyster growth study - Experiment 1

Whole oyster weight

For all treatments whole oyster weights increased with time, but there were very large differences between ploidy type and sites (Fig. 1). At all sites the triploid oysters were significantly heavier ($P<0.05$) than their diploid siblings at the end of the study (Table 2). The order, for highest to lowest whole oyster weight for both diploids and triploids, was Tilligerry Creek subtidal, Pindimar, North Arm Cove and Tilligerry Creek intertidal. The whole weights of the subtidally grown diploid and triploid oysters in Tilligerry Creek were double those of their counterparts at the nearby intertidal site in Tilligerry Creek.

Shell height

The seasonal trend, ploidy type and site differences in shell heights (Fig. 2) were less pronounced but similar to those in whole oyster weights (Fig. 1). At all sites the shell heights of the triploid oysters were significantly greater ($P<0.05$) than those of their diploid siblings (Table 2) and the order of greatest to smallest for shell height was the same as for whole weight.

Dry meat weight

Dry meat weights increased over summer and were relatively constant over winter (Fig. 3). Despite substantial seasonal differences (which varied among sites), there were large increases in dry meat weights for all treatments over time.

The triploid oysters maintained higher dry meat weights than their diploid siblings at all sites, from the age of two years until the end of the experiment and the subtidally cultured triploid oysters had particularly high dry meat weights over the last 11 months of the study.

Condition index

The seasonal differences in condition indices varied among the sites, however, at all four sites tested, condition index values increased over summer (Fig. 4). The triploid oysters maintained better meat condition than their diploid siblings at all four sites over the last 11 months of the study. The subtidally cultured triploid oysters had the highest average condition index of all oysters.

Oyster meat glycogen content

The triploid oysters had a much higher meat glycogen content than their diploid siblings (Fig. 5) at all sites, and for most months these differences were significant (based on 95% confidence limits). Meat glycogen content varied less with time in triploid oysters at all sites, than for diploids, which displayed lower levels of glycogen during the spawning season from January to May.

Oyster mortality

The percentage mortality data (Table 2) were still not homogenous ($P=0.02$) after the arcsin $x^{0.5}$ transformation and care should be taken in comparing them, however, overall the triploids suffered less mortality ($12.1\pm1.1\%$; $x\pm SE$) than the diploids ($21.5\pm1.9\%$). At least one third of this mortality occurred during summer, when oysters died from heat exposure (Nell, 1993) while being handled on shore.

Morphometric characteristics

There were small but significant differences ($P<0.01$) between the triploid and diploid oysters in adductor muscle diameter, cavity volume and shell weight (Table 3). However, the significant ($P<0.05$) site differences in adductor muscle diameter were larger. The subtidally cultured oysters had a significantly greater ($P<0.05$) adductor muscle diameter ($20.1\pm0.2\%$) than the intertidally cultured oysters, but that of the intertidally cultured oysters at Pindimar ($17.8\pm0.2\%$) was in turn significantly greater than that for the other intertidally cultured oysters ($17.1\pm0.2\%$). Although there were significant ($P<0.05$) site differences for all of the morphometric characteristics, there were no significant interactions ($P>0.05$) between ploidy level and site for any of the morphometric characteristics.

Winter mortality study - Experiment 2

Both the triploid and diploid oysters suffered from winter mortality caused by the parasite *M. roughleyi*. Death from winter mortality was confirmed by observation of superficial lesions on gills and palps and focal necroses in gonad tissue in some of the dead experimental oysters and from live oysters on nearby oyster leases. The average death from winter mortality for the triploid and diploid oysters was $45.9\pm1.7\%$ ($x\pm SE$) and $41.3\pm1.6\%$ respectively, but this difference was not significant ($P>0.05$). Nor was there a significant difference ($P>0.05$) between the surviving triploid (36.2 ± 0.5 g; $n=125$) and diploid (36.5 ± 0.6 g; $n=125$) oysters in whole weight.

DISCUSSION

In this study the triploid Sydney rock oysters grew much faster during the warmer months of the year (August to May) than their diploid siblings (Figs. 2 and 3) and the morphometric differences between diploid and triploid oysters were small (Table 3). Both whole oyster weight gain and seasonality of growth of diploid Sydney rock oysters were consistent with both the study by Holliday et al. (1991a) and commercial farming practice (Nell, 1993). The triploid Sydney rock oysters were on average 41% heavier (Table 1) than their diploid siblings after two and a half years of growth on commercial oyster leases in Port Stephens, NSW. This result is similar to that reported for triploid Pacific oysters (20 to 23% heavier; G. B. Maguire, personal communication, 1993) and American oysters (27% heavier; Barber and Mann, 1991) in medium to long term (15 to 31 months) farming trials but lower than that reported for triploid Pacific oysters (71% heavier; Akashige and Fushimi, 1992) and American oysters (64% heavier; Matthiessen and Davis, 1992) in short term (5 to 8 months) farming trials (Table 1). The faster growth of the triploid oysters in comparison to their diploid siblings was probably due to energy savings achieved by the reduced gonad development in triploid oysters (Beaumont and Fairbrother, 1991).

The similarity in weight gain of the diploid and triploid oysters used in the winter mortality study could have been partly due to a lack of gonad development during that time of the year (May to December; winter and spring) (Dinamani, 1974). In addition, this was a comparison between slow growing triploid oysters with fast growing diploid oysters as a result of matching the two types of oysters on a whole weight basis at the beginning of the experiment. Oyster farmers also claim that survivors of a severe winter mortality outbreak such as that experienced in Experiment 2 of this study, show slow growth for some time.

Subtidal culture of Sydney rock oysters greatly increased growth rates of both diploid and triploid Sydney rock oysters. However subtidal culture also greatly increases the risk of oyster mortality caused by the polychaete worm (*Polydora websteri*) and two diseases (Nell and Smith, 1988; Nell, 1993) winter mortality (*M. roughleyi*) (Farley et al., 1988) and QX (*Marteilia sydneyi*) (Perkins and Wolf, 1976). Subtidal culture of Sydney rock oysters requires extra care (Nell and Smith, 1988; Nell, 1993) and it only suits relatively few oyster growing areas as most leases are situated on shallow mudflats (Nell, 1993).

Although Port Stephens, NSW is the largest growing area in the state (it has about 30% of total area of oyster leases) and is an excellent spat catching and juvenile oyster growing area, it is not a good fattening estuary. A condition index of 100 or above is preferred for marketing the Sydney rock oyster and was reached consistently by the triploids in this study (Fig. 4). Once the oysters reached two years of age in February 1992 (their second summer), triploids maintained higher condition indices at all sites and in all sampling periods until the end of the study in November 1992 (Fig. 4). The better meat condition of triploid oysters may be due to their reduced gonadal development (Beaumont and Fairbrother, 1991). Similarly, the faster growth rate of triploids was more apparent during the second summer. The advantages of subtidal culture and farming triploid oysters were accentuated when the benefits of faster growth rate and higher condition index were jointly expressed in dry meat

weight (Fig. 3).

During the months November till January, which precede the spawning season (January till May; Roughley, 1933; Dinamani, 1974), the diploid oysters depleted their glycogen stores, whereas the triploid oysters maintained higher oyster meat glycogen levels than their diploid siblings throughout the year (Fig. 5). Seasonal variation in oyster meat glycogen level was much greater than that for the condition index, because of the use of glycogen stores in gamete production (Beaumont and Fairbrother, 1991).

No differences in tolerance to winter mortality was found between diploid and triploid Sydney rock oysters. This is consistent with the response to *P. marinus* challenge by triploid and diploid American oysters (Barber and Mann, 1991; Meyers et al., 1991) and Pacific oysters (Meyers et al., 1991). However, Matthiessen and Davis (1992), found lower mortalities in triploid American oysters than diploids from the parasite *Haplosporidium nelsoni* (MSX).

The Sydney rock oyster industry in New South Wales faces increased competition from the sale of faster growing Pacific oysters from Tasmania, Australia (Nell, 1993). Treadwell et al. (1991) showed that shortening the production cycle by one year greatly improves the profitability of oyster farming. Fast growing triploid Sydney rock oysters therefore provide the Sydney rock oyster industry with an opportunity to improve its economic viability.

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TABLE 1
Comparative performance of diploid and triploid oysters

Species	Country/ Region	Length of experiment (months)	Number of sites tested	Average whole weight of oysters (g)				Triploid: diploid growth index[1] (%)	Reference
				Start of exp		End of exp			
				Dip	Trip	Dip	Trip		
Pacific oyster (*Crassostrea gigas*)	Japan	8	4	4	6	41	70	71	Akashige and Fushimi, 1992
Pacific oyster (*Crassostrea gigas*)	Australia (Tasmania)	21 31	2 1	<1 <1	<1 <1	86 65	105 78	23 20	G. B. Maguire, pers comm, 1993
American oyster (*Crassostrea virginica*)	US East coast	15	1	1	3	41	52	27	Barber and Mann, 1991
American oyster (*Crassostrea virginica*)	US East coast	5	3	<1	<1	11	18	64	Matthiessen and Davis, 1992
Sydney rock oyster (*Saccostrea commercialis*)	Australia (New South Wales)	29	4	<1	<1	39	55	41	This study

[1] Mean of index results for each site. Index is: $\frac{\text{whole weight of triploids (g)}}{\text{whole weight of diploids (g)}} \times 100 - 100$

Table 2

Comparison[1] of whole oyster weight[2], shell height[2] and mortality of diploid and triploid Sydney rock oysters *(Saccostrea commercialis)* in Port Stephens, NSW, at the end of a 29 months study, completed in November 1992.

	Whole oyster Weight (g)		Shell height (mm)		Mortality (%)[3]	
Growing sites	Diploid	Triploid	Diploid	Triploid	Diploid	Triploid
Intertidal						
Tilligerry Creek	27.5 ± 0.3	36.3 ± 0.5	63.7 ± 0.3	68.8 ± 0.4	18.7±1.6	9.8±0.7
North Arm Cove	34.6 ± 0.4	47.6 ± 0.6	66.4 ± 0.4	70.5 ± 0.4	20.2±3.1	14.2±3.3
Pindimar	41.0 ± 0.5	55.9 ± 0.8	69.1 ± 0.4	73.8 ± 0.4	25.8±7.0	10.2±1.1
Subtidal						
Tilligerry Creek	53.7 ± 0.6	79.9 ± 1.0	68.0 ± 0.4	75.0 ± 0.4	21.3±2.1	14.2±2.2

1 Values are means ± SE.

2 At all sites tested the triploid oysters (n = 200) had significantly greater ($P<0.05$) whole oyster weights and shell heights than their diploid siblings (n = 200).

3 This data was still not homogenous ($P=0.02$) after an arcsin $x^{0.5}$ transformation and care should be taken in comparing them.

TABLE 3

Morphometric characteristics[1] in two years and nine months old diploid and triploid Sydney rock oysters *Saccostrea commercialis* (n=192; data from all four sites combined).

	Oysters[2]	
Morphometric characteristics	Diploid	Triploid
adductor muscle diameter[3] (%)	17.5±0.2	18.5±0.2***
cavity volume[4] (%)	41.1±0.3	39.9±0.2***
shell weight[5] (%)	71.2±0.2	72.1±0.2**

1 For statistical analyses an arcsin $x^{0.5}$ transformation was used.
2 Values are means ± SE; **P<0.01; ***P<0.001
3 Adductor muscle diameter (%) is (adductor muscle diameter (mm) x 100)/shell height (mm)
4 Cavity volume (%) is (cavity volume (g) x 100)/ whole oyster volume, where whole oyster volume (g) is (shell weight in air (g) - shell weight (g) in water) + cavity volume (g)
5 Shell weight (%) is (shell weight (g) x 100)/whole oyster weight (g)

Fig. 1. Whole oyster weight (g) of diploid and triploid Sydney rock oysters *Saccostrea commercialis* (n=200) at three intertidal and one subtidal site in Port Stephens, NSW, from June 1990 to November 1992. (a) Tilligerry Creek intertidal; (b) Tilligerry Creek subtidal; (c) North Arm Cove; (d) Pindimar. The 95% confidence intervals were too small (≤1.5% of the mean) to be displayed.

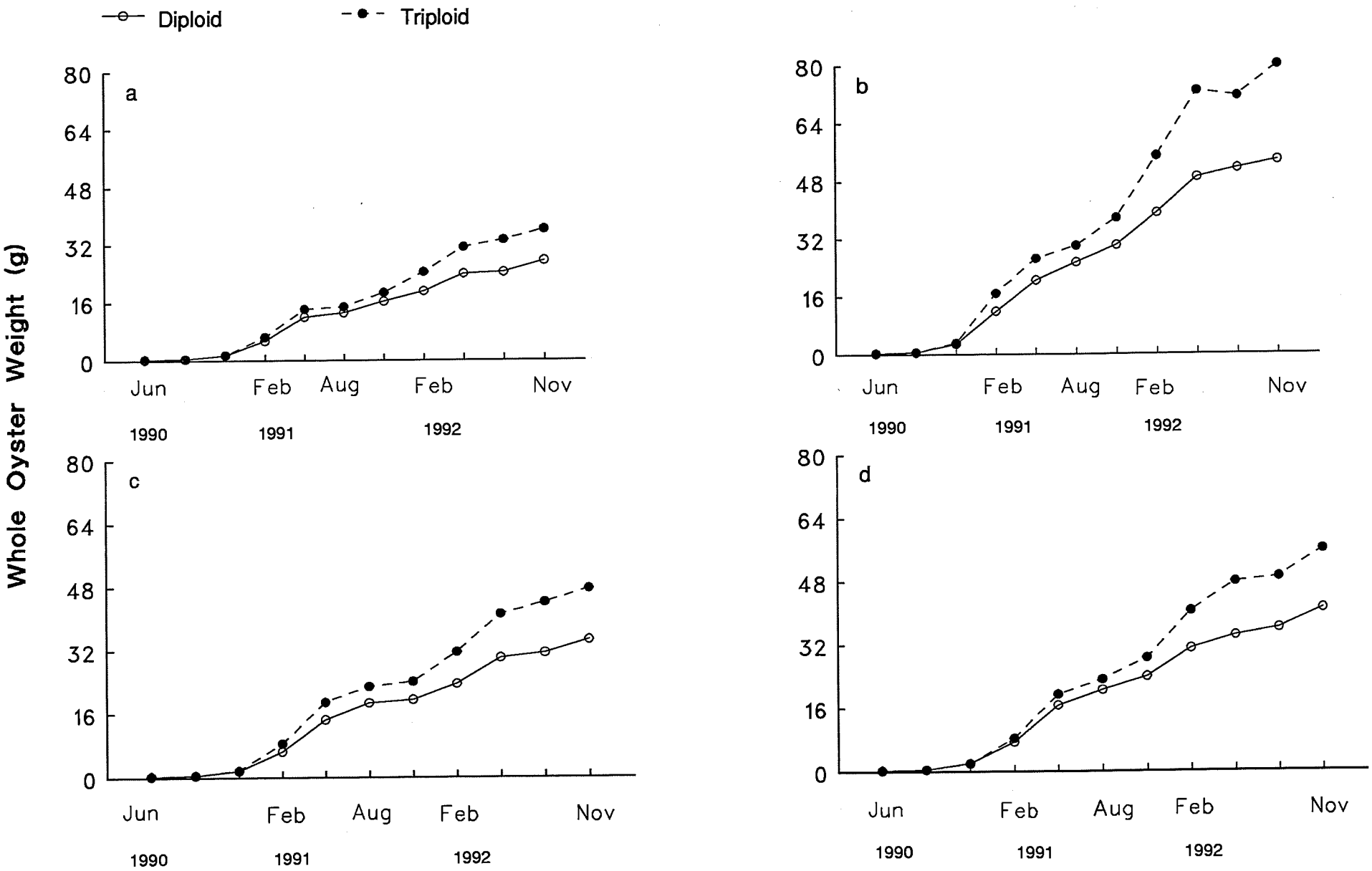

Diploid
Triploid
Whole Oyster Weight (g)
a
b
c
d
80
64
48
32
16
0
Jun
Feb
Aug
Feb
Nov
1990
1991
1992

Fig. 2. Shell height (mm) of diploid and triploid Sydney rock oysters *Saccostrea commercialis* (n=200) at three intertidal and one subtidal site (Tilligerry Creek) site in Port Stephens, NSW, from June 1990 to November 1992. (a) Tilligerry Creek intertidal; (b) Tilligerry Creek subtidal; (c) North Arm Cove; (d) Pindimar. The 95% confidence intervals were too small (≤1.5% of the mean) to be displayed.

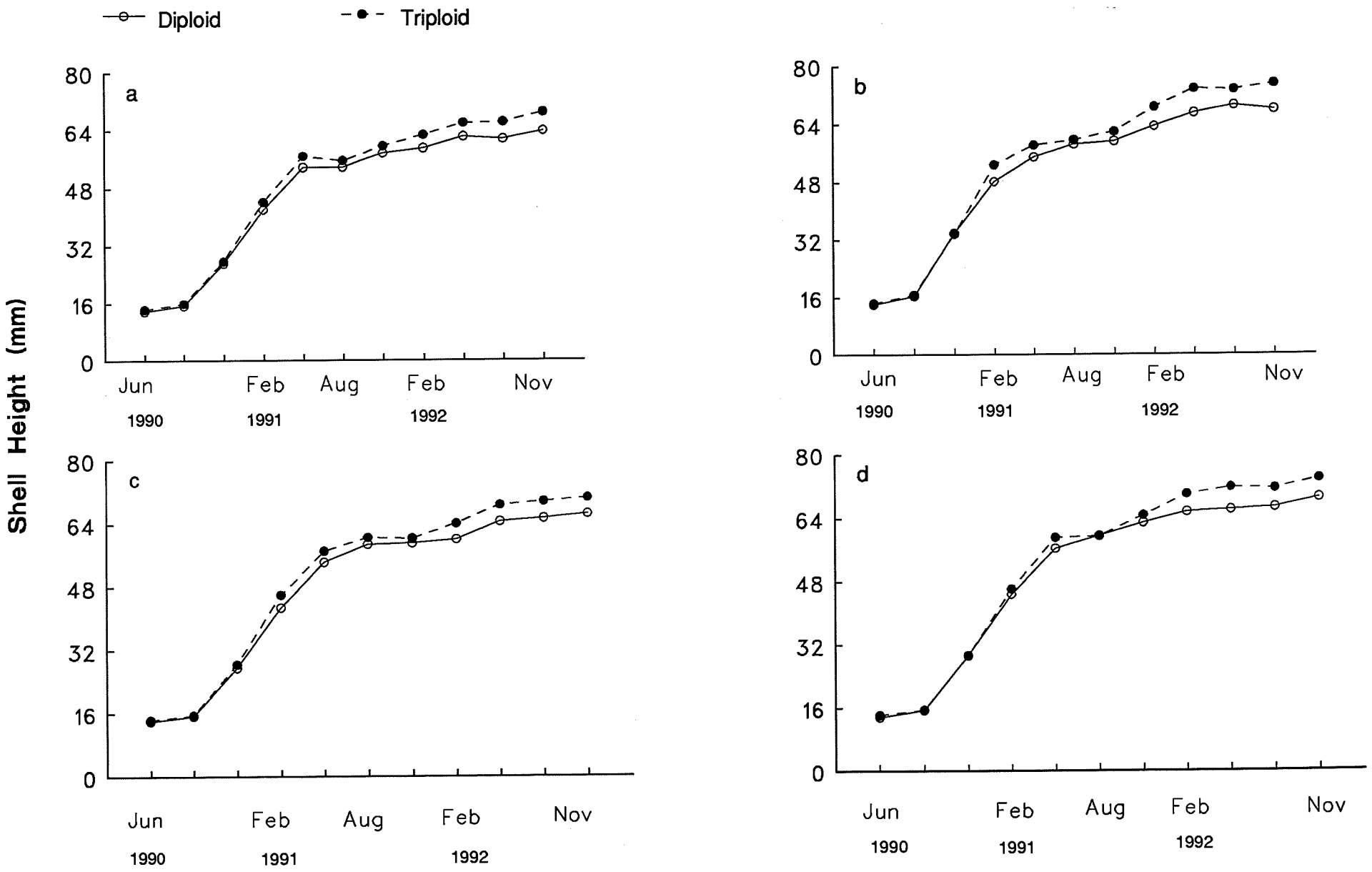

Diploid
Triploid
Shell Height (mm)
a
b
c
d
80
64
48
32
16
0
Jun
Feb
Aug
Feb
Nov
1990
1991
1992

Fig. 3. Dry meat weights (means ± 95% confidence intervals) of diploid and triploid Sydney rock oysters *Saccostrea commercialis* (n=24) at three intertidal and one subtidal site in Port Stephens, NSW, from June 1990 to November 1992. (a) Tilligerry Creek intertidal; (b) Tilligerry Creek subtidal; (c) North Arm Cove; (d) Pindimar.

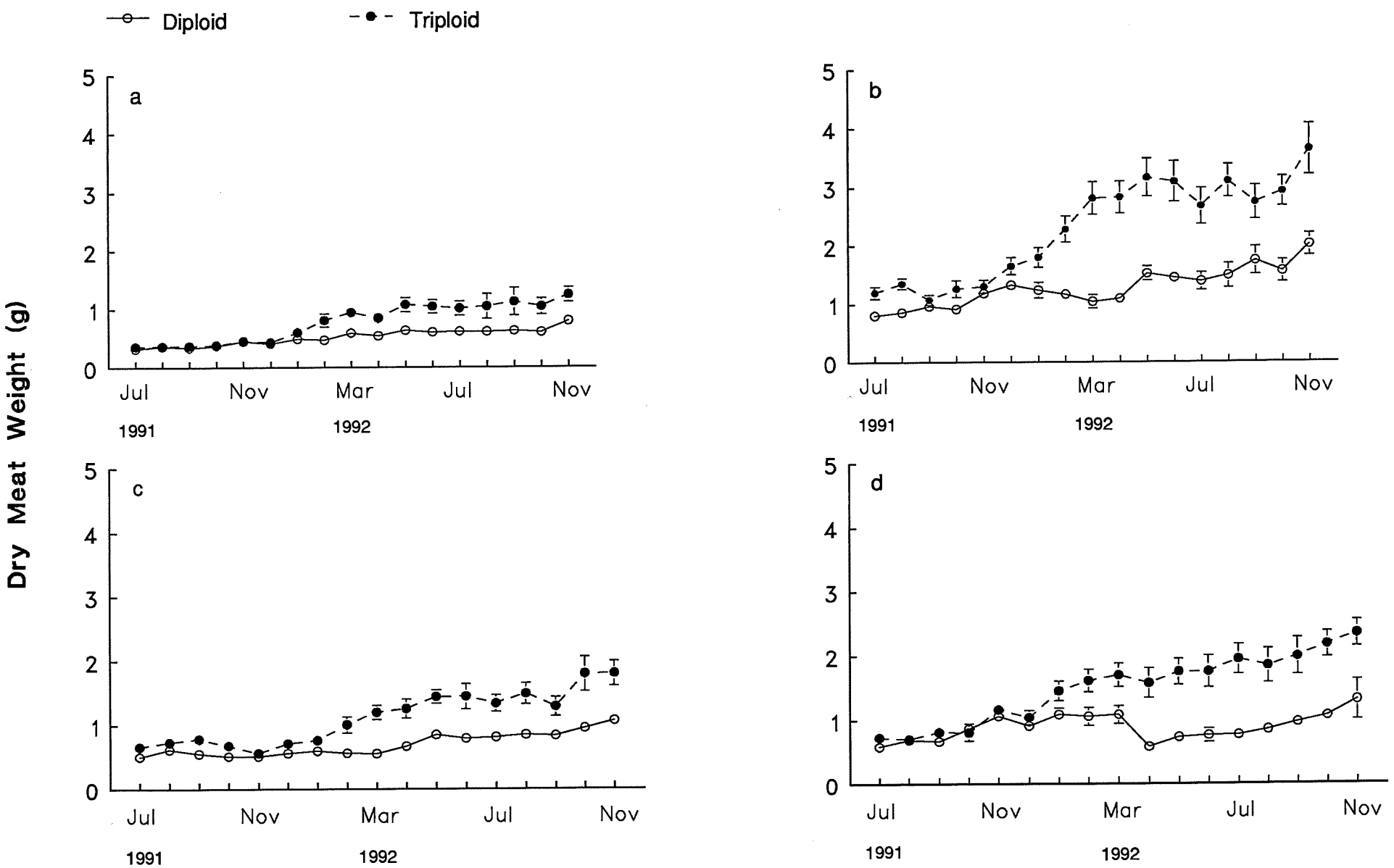

Diploid
Triploid
Dry Meat Weight (g)
a
b
c
d
0
1
2
3
4
5
Jul
Nov
Mar
Jul
Nov
1991
1992

Fig. 4. Condition index (means ± 95% confidence intervals) of diploid and triploid Sydney rock oysters *Saccostrea commercialis* (n=24) at three intertidal and one subtidal site in Port Stephens, NSW, from July 1991 to November 1992. (a) Tilligerry Creek intertidal; (b) Tilligerry Creek subtidal; (c) North Arm Cove; (d) Pindimar.

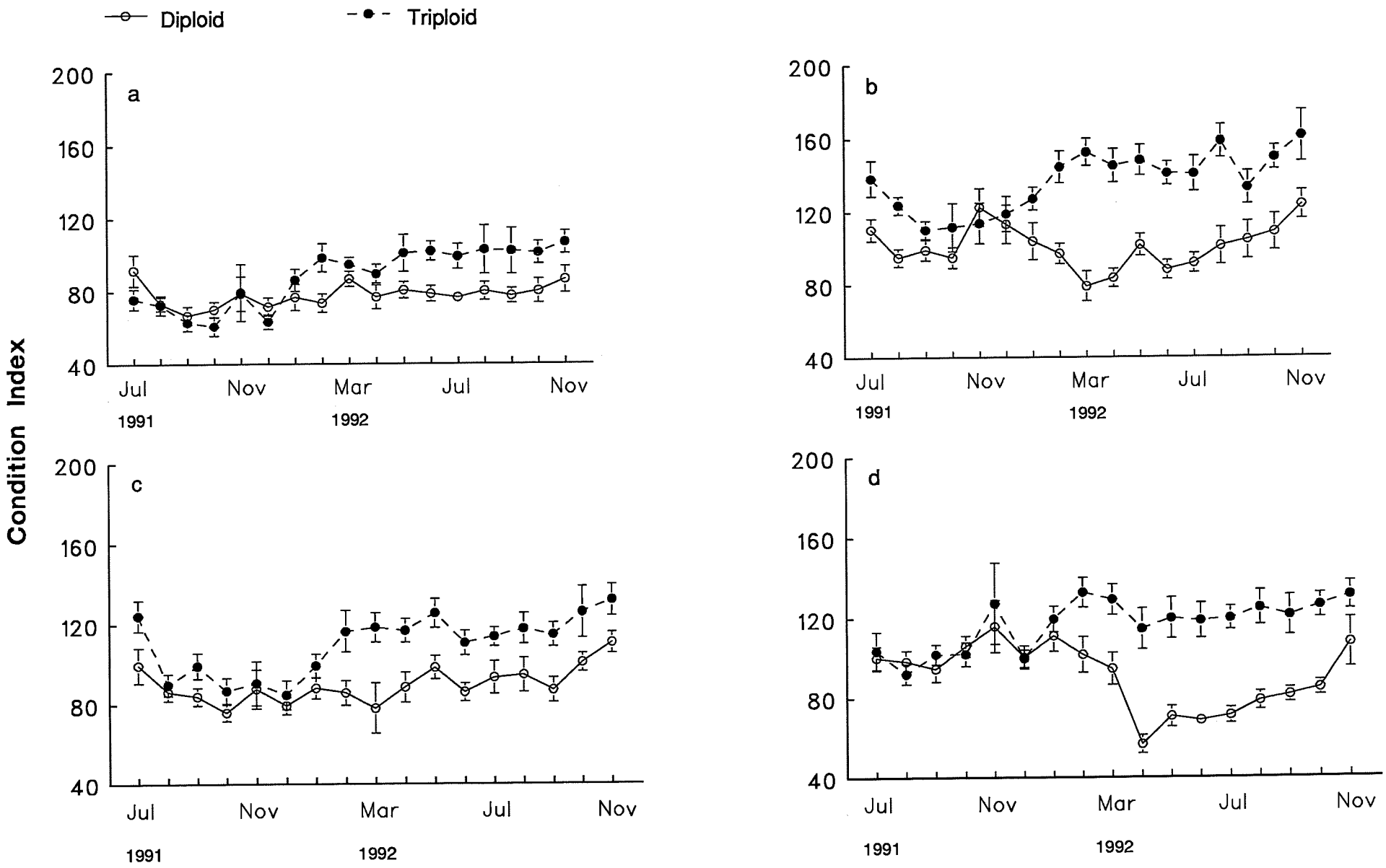
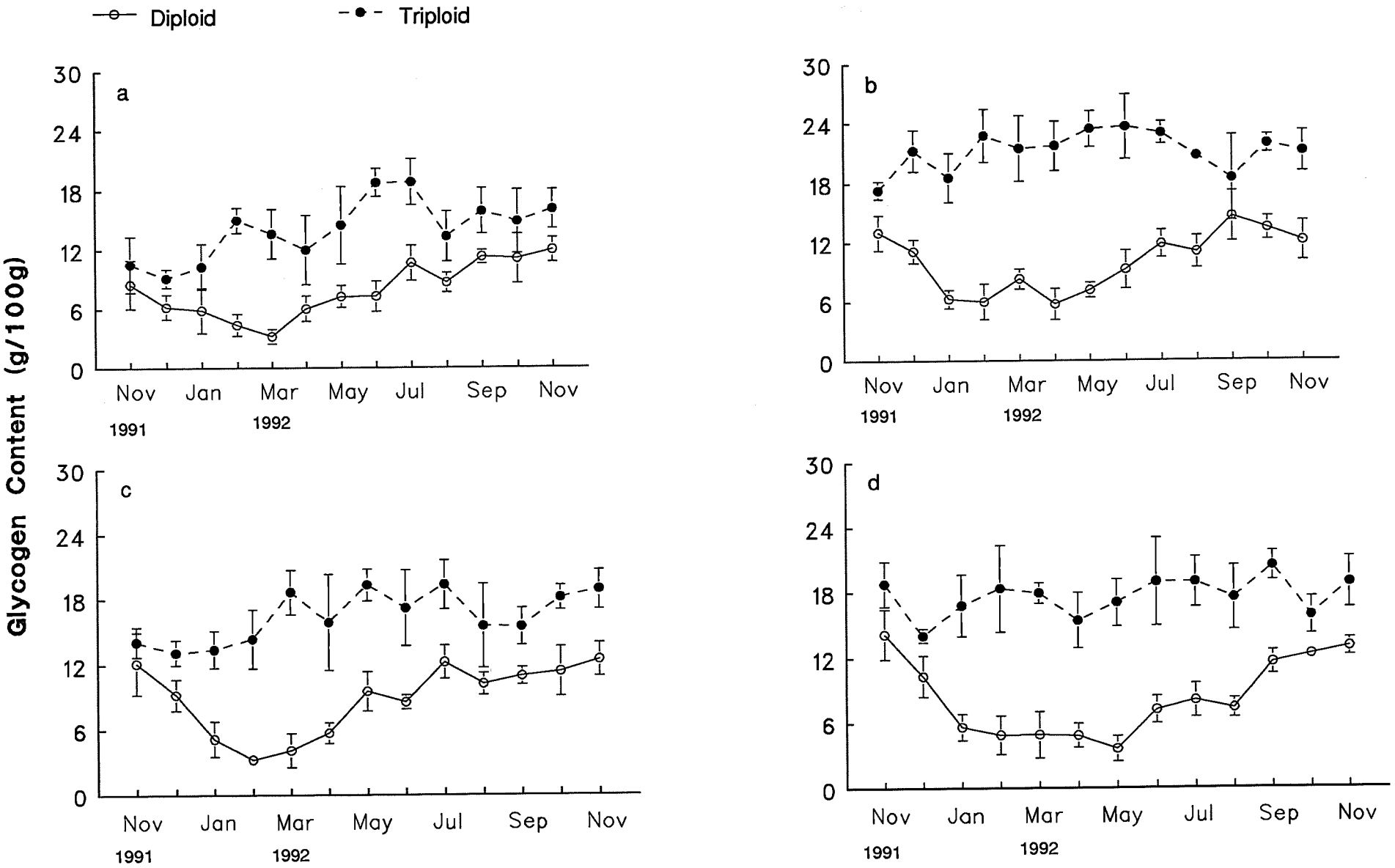

Diploid
Triploid
Condition Index
a
b
c
d
200
160
120
80
40
Jul
Nov
Mar
Jul
Nov
1991
1992

Fig. 5. Glycogen content of meats (means ± 95% confidence intervals) of diploid and triploid Sydney rock oysters *Saccostrea commercialis* (n=4) at three intertidal and one subtidal site in Port Stephens, NSW, from November 1991 to November 1992. (a) Tilligerry Creek intertidal; (b) Tilligerry Creek subtidal; (c) North Arm Cove; (d) Pindimar.


Diploid
Triploid
Glycogen Content (g/100g)
a
b
c
d
30
24
18
12
6
0
Nov
Jan
Mar
May
Jul
Sep
Nov
1991
1992

Studies on triploid oysters in Australia. II. Growth, condition index, glycogen content and gonad area of triploid and diploid Pacific oysters, *Crassostrea gigas* (Thunberg), in Tasmania

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Abstract

This research was undertaken to evaluate triploid Pacific oysters *Crassostrea gigas* (Thunberg) under Tasmanian commercial growout regimes. Sibling diploid and triploid Pacific oyster spat were produced through blocking of polar body 2 with cytochalasin B. From age 6.3 months, oysters were grown at three commercial leases in 6-12 mm plastic mesh, intertidal baskets and reached commercial size (about 60 g whole oyster weight) as they approached two or three years of age. Triploid oysters (76 % triploidy) grew faster than diploids after reached commercial size and were 23.4 % larger than diploids on a whole weight basis at the two better sites (age 27.1-27.8 months) and 19.6 % larger at the poorest site (age 38.7 months). At the two better sites, mortality of diploid and triploid oysters was negligible during growout but was 1.0 and 0.3 %/month for diploids and triploids respectively at the third site from 6.3-38.7 months. At all sites triploids maintained marketable meat condition during the summer - autumn spawning period after attaining commercial size. At the two sites where diploid oysters spawned, they remained in much poorer condition than triploids for at least four months post spawning. Triploids maintained much higher minimum glycogen content and much lower maximum gonad size than diploids. Triploid Pacific oysters show promise for overcoming marketing problems caused by poor meat condition of diploid oysters in summer and autumn in Tasmania. To exploit this marketing niche, most of the triploids would need to be marketed at about 2-2.5 years of age and shell growth rates may have to be repressed through use of elevated growing heights.

Keywords: triploidy, Pacific oyster, *Crassostrea gigas*, growth, gonad area, glycogen, condition index

1. Introduction

Triploid bivalves usually exhibit reduced gonad development, acceptable meat condition through inhibition of spawning, and faster growth (Beaumont and Fairbrother, 1991; Nell et al., 1994). Associated attributes are improved consumer acceptability, possibly associated with relatively high glycogen reserves, (Allen and Downing, 1991) and a reduction in unwanted spatfall particularly for exotic species (Beaumont and Fairbrother, 1991). Other research on triploid bivalves has focussed on their potential for reducing the impact of oyster diseases (Barber and Mann, 1991; Matthiessen and Davis, 1992; Nell et al., 1994), their response to high temperatures (Shpigel et al., 1992), and their genetic value through enhanced heterozygosity (Mason et al., 1988).

The Pacific oyster *Crassostrea gigas* industry in Tasmania is a small but innovative industry, farming hatchery reared spat as single (cultchless) oysters usually in intertidal, off-bottom systems. It was worth around A$ 10.5 million (farm gate) in 1991, based on production for the half shell trade (Stanley, 1993). Because of spawning activity, continuity of supply of oysters with adequate meat condition during summer and autumn is a major problem in Tasmania (Graham et al., 1993). While triploid oysters have been useful in addressing this problem in other countries, the performance of triploids is mediated by site characteristics (Davis, 1988). This study was undertaken to assess the performance of triploid Pacific oysters, in relation to growth, condition index, glycogen content and gonadogenesis, at three commercial farming sites in Tasmania.

For the Tasmanian Pacific oyster industry, some production techniques have been described (Dix, 1991; Graham et al., 1993), the market situation analysed (Graham et al., 1993), and profitability modelled (Treadwell et al., 1991). Growth data have been presented for oysters grown, from natural spatfall, on cultch (Sumner, 1980a,b) but there are no published growth data for cultchless oysters in the growout systems now used commercially in Tasmania. Hence, the performance of diploid oysters from spat to market size at these commercial farming sites is also documented in this study.

2. Materials and Methods

Induction of Triploidy

Seven female and three male Pacific oysters, originating from Tasmanian oyster farms and conditioned within the Shellfish Culture P/L bivalve hatchery at Bicheno Tasmania, were strip spawned in February 1990. Triploidy was induced by blocking the second polar body through exposure of eggs to 0.5 mg/l cytochalasin B (CB) for 20 minutes beginning 23 minutes after sperm addition, at a temperature of 25 °C (Downing and Allen, 1987; Allen et al., 1989). Untreated embryos, arising from the same pool of gametes, were used to produce diploid controls.

Spat production

Triploid and diploid larvae were reared in 7000 l fibreglass tanks using proprietary hatchery technology. After higher initial mortality among

triploids, larval densities and development rates were similar for the ploidy groups (larval shell height of about 290 μm , 14 d post spawning). Pediveliger larvae were set in downwellers on fine scallop shell chips before being transferred to a land-based upweller system. Subsequently, spat were transferred to 1.7 mm and 3.0 mm square plastic mesh and timber sectionalised trays (Holliday et al., 1991) within Little Swanport estuary (Fig. 1). Spat within these nursery systems were graded regularly and, where possible, mid size ranges were retained to avoid bias. In August 1990, oysters retained on 12 mm mesh but passing through 17 mm mesh were stocked at three growout sites (Fig. 2).

Experimental design

Three commercial leases in major oyster growing areas in the east (Little Swanport), south east (Pittwater) and south (Birch's Bay) of Tasmania were selected (Fig. 1). At each site, for each ploidy group, there were four randomly arranged replicates; each replicate consisted of four 6 mm spat bags within adjacent baskets (one bag/basket and 190 spat/bag; 3040 triploids and 3040 diploids per site). Average initial shell height and whole weight were 23.0±4.2 mm and 0.81±0.25 g/oyster for triploids and 22.8±4.1 mm and 0.79±0.24 g/oyster for diploids respectively (mean±s.d.; n=50 spat).

Triploidy assessment

Percentage triploidy was determined using flow cytometry (Chaiton and Allen, 1985). Several readings were taken; 6.3 months old spat (when transferred to growout systems, Fig. 2), 26.2 months old oysters at Pittwater, and 30.8 months old oysters at Birch's Bay (≥100 oysters/reading). Percentage triploidy for spat or adults was 76.0±1.7, mean±s.e., n=3 groups ≥100 oysters). For gonad area data only, diploids within triploid groups were identified, using image analysis of integrated optical density for haemocyte nuclei (Gérard et al., 1991; Gardner, 1993; Gardner et al., 1995).

Oyster management

Growout strategies including stocking densities, method of containment and average aerial exposure, based on data logger results for different intertidal growing heights (Kent and Maguire, 1992), are summarised in Fig. 2. Management followed commercial practices at each farm although the baskets used (6 mm or 12 mm plastic mesh, 380 mm x 550 mm x 130-150 mm depth) were standardised and initially oysters were contained in 6 and, later, 9 mm plastic mesh oyster bags within the baskets at all sites. Units (two baskets threaded lengthways by two 1650 mm x 25 mm x 25 mm treated pine sticks) were arranged across intertidal treated timber supporting frames (post and rail) for off-bottom culture (Nell, 1993). Throughout the experiment, biomass within baskets was regulated by redistributing oysters into additional or fewer baskets in response to growth, mortality or sacrificial sampling (Fig. 2).

After a period of very slow growth (from 15.6-21.4 months), oysters at Birch's Bay were moved to subtidal modules for three months before being moved back to intertidal baskets on a different intertidal section of the lease. The other major difference in farming strategies among the three sites was that

at Little Swanport oysters were exposed for longer, particularly from age 19.8 months (Fig. 2).

Sampling procedures

In December 1991 (age, 6.3 months) three monthly sampling, with replacement, of 50 oysters per replicate for whole weight and shell height began followed by monthly sacrificial sampling from 13.5 months. At Little Swanport and Pittwater dry meat weight, moisture and glycogen content and condition index (CI) were assessed monthly using 32 oysters per replicate. Wet meats from an additional 16 oysters per replicate were preserved for gonad histology. Whole oyster weight and shell weight and height were based on all 48 oysters. At age 16.9 months sampling intensity was reduced by 25% until oysters were 27.1-27.7 months old. At 28.2-29.1 months an extra sample of 12 oysters per replicate was taken to assess additional morphometric characteristics.

Prior to December 1991 storm damage caused the loss of about half of the bags at Birch's Bay. Consequently, the experimental design at this site only was modified to eleven replicate bags per ploidy group. Initially, sacrificial sampling (12 per replicate including four for histology) was every three monthly due to low growth rates. Monthly sampling began at 18.2 months when sampling intensity was reduced by 25%; this strategy continued until 27.7 months. Sampling was then three monthly until oysters approached commercial size (about 60 g/ oyster) prior to the third summer at Birch's Bay. Thus at age 33.8 months, the eleven replicates were pooled to form three replicates with monthly sampling, per replicate, based on 20 oysters for all performance indices except for moisture content and CI (13 oysters) and histology (seven oysters). Intensive monthly sampling continued until age 38.7 months and an extra sample of ten oysters per replicate was taken at 41.9 months to assess additional morphometric characteristics.

Oyster measurements

Whole weight was measured after oysters were immersed overnight in a recirculating system approximating oceanic conditions, scrubbed, and dried superficially with paper towel. Oyster height was measured as the difference between opposite extremities including the hinge section of the lower valve without removing new fragile shell growth (frill); in contrast commercial practice in Tasmania often involves measurement of the upper valve with frill removed. Condition index CI, (dry meat weight (g) x 1000/ cavity volume) , was assessed by drying individual meats at 80°C to constant weight in a forced draft oven (24-48 h); cavity volume was estimated as the difference between whole weight and shell weight (Crosby and Gale, 1990). Dry meats for each replicate group were subsequently ground and used to determine glycogen content as per a modified Keppler and Decker (1974) procedure (B. Day and G. B. Maguire, unpublished data, 1994).

A transverse slice was taken from oysters about 3 mm above the labial palps (Morales-Alamo and Mann, 1989) and a 5 μm section prepared and stained as in Gardner et al. (1995). Video images, magnified with microscopy or with expansion rings for larger specimens, were translated to an IBM™ compatible microcomputer using CUE-2™ software for image analysis to

quantify gonad area relative to whole cross sectional area, excluding gills. Spawning events were evident from gonad area data but were confirmed by in a parallel study of gametogenesis (Gardner et al., 1995).

The additional morphometric characteristics of adductor muscle diameter (% of upper valve length), cavity volume (% of whole oyster volume), and shell weight (% of whole oyster weight) were determined as in Nell et al. (1994). Shell density was also assessed as shell volume (g)/ shell weight (g) where shell volume is (shell weight in air (g)-shell weight in water (g)) and whole oyster volume is (shell volume (g)+cavity volume (g)) (Nell and Mason, 1991).

Temperature and salinity measurements

Surface readings (about 10 cm depth) were taken several times per week by farming staff at the Pittwater lease using a thermometer and hydrometer.

Statistical analysis

Except where indicated, data for the three sites were analysed separately and all statistical analyses for triploid groups were based on results prior to exclusion of data for diploids within triploid samples. Size, CI, soft tissue composition and gonad area data were subjected to two factor ANOVA (ploidy and age). Subsequent multiple comparisons of means were restricted a priori to differences between triploid and diploid oysters of the same age using Fisher's Least Significance Difference test. Data for the extra samples for morphometric analyses were assessed using two factor ANOVA (ploidy and site). All factors within all ANOVAs were considered to be fixed rather than random. Homogeneity of variance was assessed using Cochran's test (Sokal and Rohlf, 1981). An a priori alpha value of 0.05 was adopted throughout except where variances were heterogeneous; in those cases alpha = 0.01 was adopted to reduce the probability of a Type 1 error although this reduces power (Underwood, 1981).

3. Results

Comparison of sites

Both triploids and diploids grew faster at Little Swanport and Pittwater than at Birch's Bay in terms of whole oyster weight, shell height and weight, and dry meat weight (Fig. 3-4). Oyster size was more variable at Birch's Bay with stunted individuals often perishing during the study. Mortality was negligible at Little Swanport and Pittwater (<1% overall) while at Birch's Bay mortality of diploids and triploids averaged 0.3 % / month and 1.0 % / month respectively (excluding storm damage). At the two better sites, both triploids and diploids attained higher CI and lower meat moisture content while diploids also developed larger gonads than at Birch's Bay (Fig. 5-6). Maximum glycogen content was highest at Little Swanport (Fig. 6a). There was no evidence of spawning of diploid oysters at that site (Fig. 6b) but diploids spawned at Pittwater in summer between 22.5 and 23.4 months (Fig. 6d) and at Birch's Bay between 23.4 and 25.4 months and again in the following summer between 34.8 and 35.8 months of age (Fig. 6f).

Two factor ANOVAs (age and ploidy groups)

Results for the two factor ANOVAs for the eight performance indices above, excluding additional morphometric analyses, for each of three sites are given in Table 1. Results of subsequent comparisons of ploidy groups of the same age are given in Fig. 3-6; comparisons which were significantly different, using alpha=0.01 because of heterogeneity of variance, are indicated by the symbol #.

Whole oyster and shell weights

Triploids and diploids reached commercial size (>60 g/ oyster) at 19.9 months (Little Swanport), 22.5 months (Pittwater) and 34.8 months (Birch's Bay). They grew at similar rates until oysters approached their second summer at the Little Swanport and Pittwater sites (Fig. 3a,c). A trend towards a growth advantage of triploids over diploids first became apparent by 22.3-22.5 months at these two sites; by 27.1-27.7 months triploids were 23.4% heavier than diploids in terms of whole weight. In contrast, triploids and diploids grew at similar rates until oysters approached their third summer at Birch's Bay; differential growth rates became apparent by 35.8 months and after 38.7 months triploids were 19.6% heavier than diploids (Fig. 3e). Trends for shell weight were similar (Fig. 4a,c,e) and the equivalent size advantages were 25.5, 22.4 and 21.9 % respectively.

Shell height

At Little Swanport and Pittwater shell height tended to be greater for diploids until growth rates for triploids surpassed those of diploids in the second summer (Fig. 3b,d). At Birch's Bay few significant differences in shell height, between diploid and triploid groups of the same age, were recorded (Fig. 3f).

Dry meat weight

Apart from the last two samples (from 27.1 months), diploids had slightly higher dry meat weight than triploids at Little Swanport and meat size for both ploidy groups increased steadily at this site (Fig. 4b). At Pittwater, diploids had slightly higher meat weight than triploids until after spawning of diploids between 22.5 and 23.4 months; triploids then had much larger meats than diploids until the end of the study (Fig. 4d). However, meat growth for both ploidy groups was very slow for 5.7 months after 23.4 months. At Birch's Bay spawning during the second summer (23.4-25.4 months) did not result in a significant difference in meat size between ploidy groups and diploids tended to have larger meats after this event until spawning in the third summer (34.8-35.8 months) (Fig. 4f). Meat size for triploids and diploids increased steadily after the third summer.

The major spawning events at Pittwater and at Birch's Bay (34.8-35.8 months) caused reductions in diploid meat size of 28.7 and 48.9% respectively (Fig. 4d,f). Subsequently, triploids at Pittwater and Birch's Bay maintained significantly larger meats than diploids for 5.7 and 6.1 months respectively. Average meat size advantage of triploids over diploids, for the penultimate

samples at Little Swanport, Pittwater and Birch's Bay, was 6.1, 42.3 and 66.0% respectively.

Condition Index (CI)

In the absence of a spawning at Little Swanport, diploids maintained a CI advantage of up to 24.1 units, for 7.2 months, from 21.0 months (Fig. 5a). At Pittwater, differences were minor until diploids spawned after which triploids maintained a significant advantage (8.5-28.4 units) for 5.7 months despite a progressive decline in CI for triploids after 22.5 months (Fig. 5c). At Birch's Bay, diploids had a small advantage in CI until spawning in the third summer after which triploids maintained an advantage of 18.2-25.2 units for at least four months (Fig. 5e). The major spawning events at Pittwater and Birch's Bay (in the third summer) caused reductions in diploid CI of 48.5 and 38.1 units respectively.

Moisture content

At all three sites triploids and diploids had similar moisture content with values declining until the second and third summers when moisture content for both groups rose for several months (Fig. 5b,d,f). At Little Swanport, diploids then developed lower moisture content than triploids over the final four months (Fig. 5b). After spawning, diploids developed higher moisture than triploids over 5.2 months at Pittwater (Fig. 5d) and over 7.3 and 2.9 months in the second and third summers respectively at Birch's Bay (Fig. 5f). Overall the changes in moisture content through time were relatively large with the maximum differences between the monthly values ranging 8.6-9.5 g/100 g for diploids and 7.5-10.2 g/100 g triploids for the three sites.

Glycogen content

Oyster glycogen content increased in the early spring periods prior to the second or third summers and at each site ploidy groups reached similar maximum values (Fig. 6a,c,e). Glycogen content of diploids then declined by 10.1-16.3 g/100 g dry soft tissue (range for three sites); this occurred over a period of at least three months prior to the spawning events in the second summers at Pittwater (Fig. 6c) and Birch's Bay (Fig. 6e). The subsequent decline at Birch's Bay occurred at least 1.4 months prior to the spawning event in the third summer. Glycogen content in diploids reached a minimum just prior to the spawning event at Pittwater and soon after spawning events at Birch's Bay and then recovered steadily. Diploids at Little Swanport reached a similar minimum glycogen content to the other sites (3.2-4.4 g/100 g dry soft tissue) before recovering steadily (Fig. 6a). Triploids exhibited much smaller declines in glycogen content and maintained significantly higher values than diploids for periods of 6.0 months at Little Swanport (Fig. 6a), 8.9 months at Pittwater (Fig. 6c) and 2.9 and 4.9 months at Birch's Bay (Fig. 6e).

Gonad area

In the spring prior to the second summer at Little Swanport and Pittwater, the gonad area of diploid groups increased rapidly (Fig. 6). At Little Swanport, diploids reached a peak of 69.5-71.3% of cross sectional area at 24.1-25.9 months and this only declined, to 57.4%, in the final sample at 27.1 months

(Fig. 6b). Gonad area for diploids at Pittwater peaked at 22.5 months (72.6%) but declined to 46.9 % after spawning and continued to contract; by 27.7 months it was negligible (4.4%) (Fig. 6d). Triploid groups had much smaller gonads than diploid groups at Little Swanport and Pittwater from 21.0 and 20.1 months respectively and subsequent maximum values for monthly samples of triploid groups were 16.8% and 33.4% respectively. However, when diploids within the triploid groups were excluded, the maximum gonad area values for triploids were only 9.2% and 11.9% respectively (Fig. 6b,d).

At Birch's Bay, gonad area for diploids peaked in successive summers after expanding rapidly in late spring but maxima (47.7 and 43.6% respectively) were lower than at other sites; these values declined to 33.3 and 18.1% after spawning events (Fig 6f). In between these maxima, diploid gonad area was very contracted for about three months (minimum of 2.7% at 30.8 months). Triploid groups had much smaller gonads than diploid groups during the periods 21.5-26.2 months and 33.8-35.8 months. When diploids within the triploid groups were excluded, the maximum gonad area values for triploids in these periods were only 5.5 and 7.7 % respectively (Fig 6f).

Other morphometric characteristics

Two factor ANOVAs indicated that ploidy group affected all characteristics except cavity volume, site affected all characteristics except shell density and there were no significant ploidy group x site interactions (Table 2). Triploids had higher shell weight (%) and larger adductor muscle diameter (%) (Fig. 7b,c). Triploid shells (2.38±0.11 g/g, mean±s.d., n=121 oysters, sites pooled) were also 1.9 % more dense than diploids (2.33±0.12, mean±s.d., n=115). Oysters from Little Swanport had the highest average values for cavity volume (%) (Fig. 7a) and adductor muscle diameter (%) (Fig. 7c) but the lowest for shell weight (%) (Fig. 7b).

4. Discussion

Whole oyster and shell growth of diploid oysters in relation to site

The sites chosen are within important waterways for commercial oyster production in Tasmania (Graham et al., 1993) and can be considered as cool temperate (Fig. 8). Pittwater is a relatively enclosed high salinity lagoon (Fig. 8) while Little Swanport is a more open estuary that exhibits rapid recovery in salinity after moderate rainfall (C. O'Meley, unpublished data, 1990). Birch's Bay experiences the most wave action and, as part of the D'Entrecasteaux Channel, is a less estuarine site. The higher whole oyster and shell growth rates at Little Swanport and Pittwater than at Birch's Bay, for diploid oysters, reflect commercial results at these sites (G. Sumner, Coal River Oysters, personal communication, 1994). Many factors can cause differences, among sites, for Pacific oyster growth rates (Brown and Hartwick, 1988) and an explanation of growth differences among diploids is beyond the scope of this study. However, differences in food supply are a likely cause and these are being addressed in a separate FRDC study, of carrying capacity of major oyster farming areas, by the Tasmanian Department of Primary Industries & Fisheries.

There are no comparable published data for growth rates of cultchless Pacific oysters in Tasmania but our shell height data for Pittwater are similar to results for Pacific oysters grown intertidally on cultch in nearby Pipeclay Lagoon (Sumner, 1980a). However, this comparison is complicated by the difference in shell shape between single oysters and those on cultch (Smith et al., 1995), the effect of spat density on growth rates of attached oysters (Holliday et al., 1993) and differences in degree of aerial exposure (Spencer, 1990). Oysters at the two better sites reached marketable size at less than two years of age which is comparable with commercial results for Pacific oysters in Tasmania (Treadwell et al., 1991; Graham et al., 1993) and New Zealand (35-38 °S) (Dinamani, 1991). The apparent absence of seasonal growth patterns was in contrast to the non-linear growth results recorded by Sumner (1980a,b).

The remarkable improvement in shell growth rate during and after a three month phase of subtidal culture at Birch's Bay (Fig. 2, 3e, 4e) is probably indicative of the value of integrating intertidal and subtidal culture techniques at some sites (Holliday et al., 1988).

Relative performance of triploid oysters (whole oyster and shell growth)

The higher growth rate of triploid oysters has been seen as a major advantage (Beaumont and Fairbrother, 1991; Nell et al., 1994) and triploids in this study grew 19.6-23.4 % faster than diploids in terms of whole weight and 21.9-25.5 % faster for shell weight. Faster growth of triploid bivalves, on a whole weight basis, has often been attributed to enhanced heterozygosity of triploids (Stanley et al., 1984) or to the increase in energy available for somatic growth due to reduced gametogenesis (Beaumont and Fairbrother, 1991; Akashige and Fushimi, 1992). Gardner (1993) suggested that spawning and the associated costs of resorption and replacement of soft tissue may also help explain growth differences between ploidy groups. Heterozygosity was not assessed in our study but was not expressed as a growth advantage prior to periods of gonad development. A growth advantage for triploids was evident at Little Swanport in the absence of spawning of diploids (Fig. 3a, 4a) and at Pittwater prior to spawning of diploids (Fig. 3c, 4c) These findings support the explanation based on reduced energy expenditure on gametogenesis. At Birch's Bay a significant size difference between triploids and diploids was not evident until after spawning in the third summer and this finding could support an explanation based on energy costs of spawning and recovery.

A growth advantage for triploids, as whole weight, was evident from about 22 months at the better sites and 36 months at Birch's Bay. In contrast, this occurred at an age of about 12-13 months for Pacific oysters near Hiroshima, Japan (Akashige and Fushimi, 1992) and Sydney rock oysters in Port Stephens, New South Wales (NSW), Australia (Nell et al., 1994) and at 4-5 months for *Crassostrea virginica* in the United States (Barber and Mann, 1991; Matthiessen and Davis, 1992). It should be noted that the *C. virginica* studies are complicated by the fact that the ploidy groups did not arise from the same pool of gametes and also, deliberately, by the presence of pathogens. Similarly, the research by Akashige (1990) and Akashige and Fushimi (1992) involved comparisons between diploids from natural spatfall and hatchery reared triploids. The differences between our sites suggest that the onset of growth differences may be related to size rather than age, however, diploid growth

rates at our better sites were similar to those recorded by Akashige and Fushimi (1992). A probable factor is the onset of advanced gonad development. Akashige (1990) found that Pacific oysters from around Hiroshima can have cross sectional gonad area values exceeding 50%, when diploids are less than 12 months old, and maturation can occur in young Sydney rock oysters (<10 months) (Roughley, 1933).

As well as occurring later than in other studies, the growth advantage of triploids that we recorded for whole weight were also smaller; Akashige and Fushimi (1992) recorded an advantage for four sites of 40-90% for Pacific oysters near Hiroshima while for three studies on *C. virginica* and Sydney rock oysters the average advantage was 27-64% (Nell et al., 1994). Summer surface water temperatures were 5-6 °C warmer near Hiroshima (Akashige and Fushimi, 1992) than at Pittwater (Fig. 8) and this may explain the smaller growth advantage in our study. Davis (1989) attributed differences in relative growth rates of ploidy groups at two sites to the effects of temperature on reproduction of Pacific oysters. Shpigel et al. (1992) recorded larger differences in gametogenesis between diploid and triploid Pacific oysters in heated water compared to ambient conditions.

Dry weight and moisture content of meats for ploidy groups

Diploids meats lost 29-49% of their dry meat weight due to the two major spawnings at Pittwater and Birch's Bay (in the third summer) although gonad histology indicated that the Pittwater event was only a partial spawning (Gardner et al., 1995). The equivalent estimates for diploids by Akashige and Fushimi (1992) were 56-67 %.

Wet meat weight is used in commercial oyster quality assessment in Tasmania because it allows rapid processing of samples; however, dry matter content of wet meats for diploid and triploid oysters increased by about 55-65% of minimum levels at the three sites until an increase in moisture content for all groups towards the end of sampling. The maintenance of lower moisture content in triploids for several months after the spawning of diploid Pacific oysters is consistent with the results for Akashige and Fushimi (1992) but not Allen and Downing (1986) who recorded a difference between sibling diploids and triploids for only one month post spawning.

Relative performance of triploid oysters (CI)

The higher diploid CI values at Little Swanport and Pittwater compared to Birch's Bay are consistent with preliminary sampling of commercial stocks in the summer-autumn period in 1989-90 (Kent, 1990). Minimum acceptable CI values vary depending on site and markets but for Tasmanian Pacific oysters Maguire et al. (1995) used ≥70 as acceptable although Gardner (1993) noted that oysters from Birch's Bay with CI ≥65 could be marketed.

One of the major reasons for evaluating triploids was the possibility that they would maintain acceptable CI through the summer and autumn spawning season after diploids reached commercial size. Differences in CI between ploidy groups varied greatly among sites as was the case for Pacific oysters around Hiroshima (Akashige and Fushimi, 1992) but less so for Sydney rock oysters in Port Stephens, NSW (Nell et al., 1994). In the absence of a spawning at Little Swanport, both diploids and triploids retained very high CI values

through this period and the substantially lower CI for triploids would have been of little commercial significance. At Pittwater, spawning of diploids in summer resulted in a major advantage in CI for triploids which remained marketable through to the end of the study in the following late autumn. However, the decline in CI values for triploids at this site from 22.5-27.7 months is of concern (Fig. 5c). CI is a composite index that is influenced directly by cavity volume, and hence shell growth and shape, and by dry meat weight which is determined by wet meat growth and moisture content. During this decline in CI for triploids, conditions within Pittwater were unfavourable for meat growth in triploids or diploids (Fig. 4d) but more importantly shell growth for triploids was much faster than for diploids (Fig. 4c). We argue that the much shorter aerial exposure time, due to the lower growing height used at Pittwater (10.9 %) compared to Little Swanport (59.2 % from 19.8 months, Fig. 2), favoured rapid shell growth. Shell growth accelerated from about 20 months (late spring) at Pittwater after having been slower for both ploidy groups at this site than at Little Swanport up to this time. This assertion is supported by research on the effects of growing height on diploid and triploid Pacific oysters (Maguire and Kent, 1991). In Tasmania, the faster shell growth of triploids, which occurs around the breeding season, should be seen as a disadvantage as the oysters are already marketable and merely need to maintain acceptable CI without whole oyster size becoming excessive for marketing.

At Birch's Bay, triploids maintained a clear advantage in CI after diploids spawned in the third summer, however, subsequently shell growth of triploids at this low growing height (10.0% exposure, Fig. 2) was relatively fast. Hence CI of triploids was commercially marginal, after diploids spawned, until both diploids and triploids improved in CI in autumn. The decline in CI for triploids when the diploid groups spawned at Birch's Bay is probably due in part to spawning of diploids within triploid groups (Gardner, 1993). In general, having only about 76% triploidy reduces the value of triploidy for commercial operations and for this study probably caused underestimation of the differences between diploids and triploids.

Gonad area and glycogen content of ploidy groups

The variation among sites observed for spawning activity was not unusual for commercial diploid Pacific oyster stocks in Tasmania (G. Sumner, Coal River Oysters, personal communication, 1994). All individuals spawned in the third summer at Birch's Bay, some spawned in the second summer at that site and at Pittwater, and no individuals spawned at Little Swanport even though most commercial stocks on that lease did spawn that summer (Gardner et al., 1995; Maguire et al., 1995). In contrast, Pacific oysters at Port Stephens, NSW spawned in mid spring (Mason and Nell, 1995). Maximum gonad cross-sectional area for diploids at Little Swanport and Pittwater (70-71%) was similar to that recorded for Pacific oysters (70-79%) by Akashige (1990) and Allen and Downing (1986) while maximum values for the second and third summers at Birch's Bay (44-48%) were lower. The maximum gonad area values for triploids (diploids excluded) in our study (7.7-11.9%) were lower than those recorded for triploid Pacific oysters in other studies; (male area(%) + female area(%))/2 was 29 and 41% for Allen and Downing (1986) and Akashige (1990)

respectively. However, some of these differences could be due to position of cross section and sex ratio as well as the presence of diploids within triploids for Akashige (1990).

Triploids maintained much higher glycogen reserves than diploids in this and other studies (Allen and Downing, 1986; Akashige and Fushimi, 1992; Nell et al., 1994). Utilisation of glycogen reserves was first evident for diploids at about the same time as diploid gonad area began to expand rapidly (Fig. 6). This coincided with the majority of diploids being in Stage 3 of gonad development; a phase, prior to full prespawning development, characterised by expansion of secondary oocyte size or the occurrence of numerous spermatids although at Birch's Bay many of the diploids had more advanced development (Dinamani, 1974; Gardner et al., 1995). These trends are generally in accord with the review by Gabbott (1975) of glycogen metabolism in bivalves. The decline in glycogen content in diploids led to minimum values that were similar at all sites to the results recorded by Allen and Downing (1986), Akashige and Fushimi (1992) and Nell et al. (1994).

One surprising result in our study was the absence of a growth advantage for triploids in association with substantial gonad development and a subsequent partial spawning of diploids at Birch's Bay (23.4-25.4 months) in the second summer (Fig. 3e, 4e). Davis (1988) suggested that growth differences between ploidy groups may depend on differential utilisation of glycogen and hence glycogen analyses for the Birch's Bay were extended back to assess changes during that second spring-summer period. However, while results were variable (Fig. 6e), triploids maintained significantly higher glycogen reserves for much of that period. These oysters were grown subtidally for three months around this time (Fig. 2) and this may have affected relative growth rates although triploid Sydney rock oysters have grown faster than diploids at a subtidal site (Nell et al., 1994). The fact that it was only a partial spawning (Gardner et al., 1995) among relatively small oysters may have been more influential.

Other morphometric characteristics for ploidy groups

Triploids had greater shell weight (as % of whole weight) and larger adductor muscle diameter (as % of shell height) than diploids, both in this study and for Sydney rock oysters (Nell et al., 1994). In both studies site differences were quite large for adductor muscle diameter (Fig. 7). Akashige (1990) also reported that triploid Pacific oysters had large adductor muscle area, however, the functional significance is uncertain. Nell et al. (1994) also reported that diploid Sydney rock oysters had larger cavity volume (as % of whole volume) but differences were minor in our study. The small difference in shell density was not enough to account for the differences we observed in shell growth rate, on a weight basis, between diploids and triploids. When our results and those of Nell and Mason (1991) and Nell et al. (1994) were compared, Pacific oysters consistently had much larger cavity volume (%) and shell density (%), larger adductor muscle diameter (%) but much lower shell weight (%) than Sydney rock oysters. The difference in cavity volume indicates that for the same size oyster, on a whole weight basis, a larger Pacific oyster meat is required for Pacific and Sydney rock oysters to achieve a similar CI value.

Survival of ploidy groups

The negligible mortality of diploid and triploid oysters grown from 0.8 g spat to in excess of minimum market size over about two years at Little Swanport and Pittwater is consistent with the observation by Dix (1991) and is indicative of the quality of these growing areas and the absence of major pathogens to Pacific oysters in Tasmania (Wilson et al., 1993). The higher mortality of triploids relative to diploids at Birch's Bay should be interpreted cautiously as opposite trends were observed by Allen and Downing (1986), Akashige and Fushimi (1992), Matthiessen and Davis (1992), and Nell et al. (1994).

5. Conclusion

Diploid Pacific oysters at better sites in Tasmania can reach marketable size in terms of whole weight and CI within two years and mortality on growout leases can be negligible. Not all diploid oysters spawn during their second summer on leases but spawning does adversely affect marketability. The lower CI for triploids in summer, if diploids do not spawn, is unlikely to be important commercially unless the CI of the diploids is marginal. Triploid Pacific oysters, with their greatly reduced gonad development, show promise for overcoming marketing problems caused by poor meat condition of diploid oysters in summer and autumn in Tasmania. However, to replace diploids in this marketing niche, most of the triploids would need to be marketed at about 2-2.5 years of age and shell growth rates may have to be repressed through use of elevated growing heights. Based on the results of the present study we consider that the faster shell growth of triploids in Tasmania is a disadvantage although it could allow for selective exclusion of smaller diploids, within triploid groups, through size grading prior to marketing.

Other attributes, of triploid bivalves, considered to be advantageous in industries elsewhere, may not be important for Pacific oysters in Tasmania although they could be in smaller Pacific oyster industries in warmer Australian states. Triploids did not confer an advantage in consumer acceptability over diploid Pacific oysters with acceptable CI probably because Australian consumers do not discriminate against sexually mature oysters (Maguire et al., 1995). While Pacific oysters are an exotic species in Australia, heavy spatfall is not as common in Tasmania as in New South Wales (Nell, 1993) and hence sterility may not be an important justification for use of triploids. There is also little immediate need in Tasmania for Pacific oysters with greater disease resistance or better tolerance to high temperatures except when translocated to warmer sites interstate. Thus the major incentive for the Tasmanian industry to use triploids is to overcome marketing problems associated with spawning of diploid Pacific oysters although polyploidy could have a role in selection strategies that are being developed with this industry. Current research on triploid Pacific oysters in Australia is aimed at improving the percentage triploidy, examining heterozygosity in triploid and diploids and evaluating triploids at warmer sites interstate.

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Table 1
Results of two factor ANOVAs for size, Condition Index, soft tissue composition and gonad area of triploid and diploid Pacific oysters (*Crassostrea gigas*) from Little Swanport (LS), Pittwater (PW) and Birch's Bay (BB), Tasmania. All factors, at all sites, were significant *** unless indicated. Some Null Hypotheses are retained (approximate P>0.01=#) rather than (P>0.05) because of heterogeneity of variance.

Variable	Factor		
	Ploidy group (P)	Age (A)	P x A
Whole weight	***	***	***
Shell height	LS^{NS} ; BB**	***	BB#
Shell weight	***	***	***
Dry meat weight	***	***	LS**
Condition Index (CI)	BB#	***	***
Moisture content of meats	***	BB**	LS*; BB#
Glycogen content	***	***	***
Gonad area (%)	***	***	***

Condition index = (dry meat weight (g) x 1000/ cavity volume) as per Crosby and Gale (1990).
Glycogen content as g/ 100 g dry soft tissue.
Gonad area = % of cross sectional area excluding gills.
*** = P<0.001; ** = P<0.01; * = P<0.05; NS = P>0.05

Table 2
Results of two factor ANOVAs for additional morphometric characteristics of triploid and diploid Pacific oysters (*Crassostrea gigas*) from three sites in Tasmania.

Variable	Factor		
	Ploidy group (P)	Site (S)	P x S
Cavity volume (%)	NS	***	NS
Shell weight (%)	**	***	NS
Adductor muscle diameter (%)	***	***	NS
Shell density (g/ g)	**	NS	NS

*** = P<0.001; ** = P<0.01; NS = P>0.05

Fig. 1. Location of Tasmania experimental sites in relation to mainland Australia.

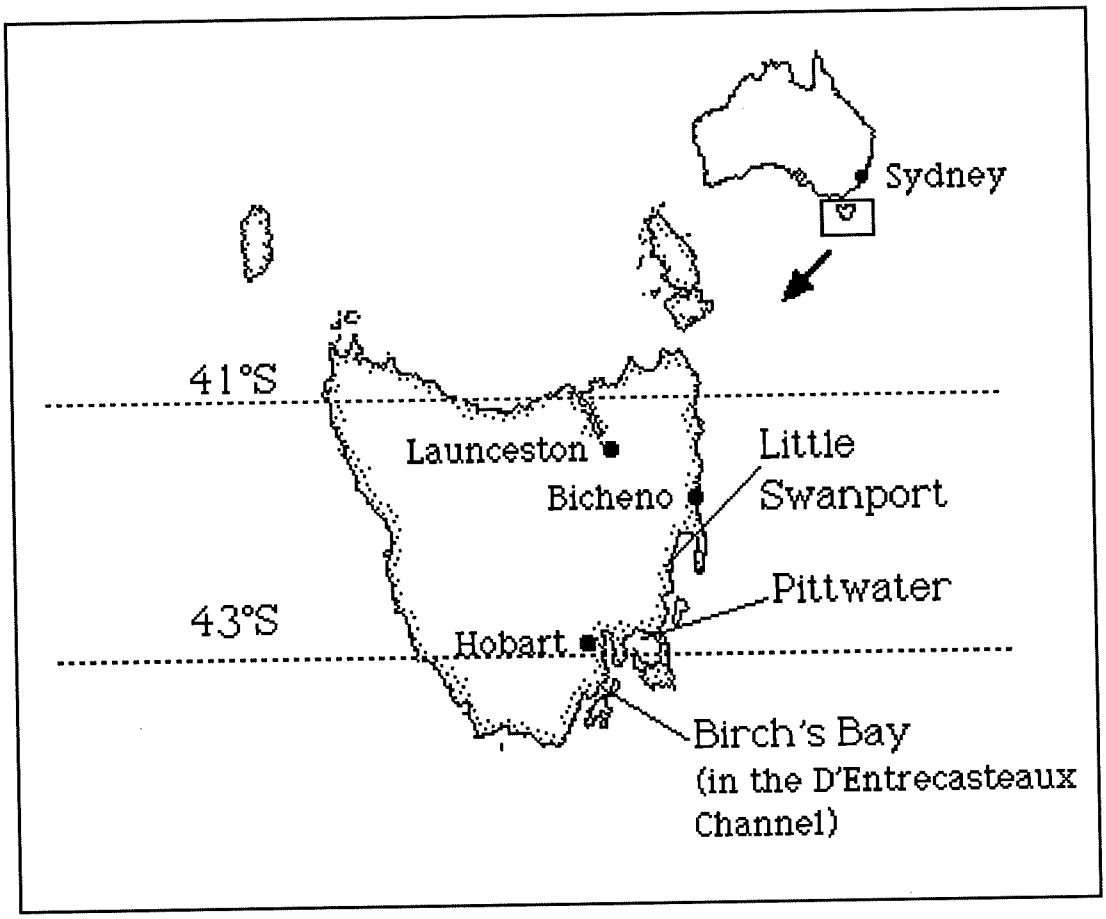

Sydney
41°S
Launceston
Bicheno
Little
Swanport
Pittwater
43°S
Hobart
Birch's Bay
(in the D'Entrecasteaux
Channel)

Fig. 2. Flow chart of management procedures at the three experimental sites. Each box for each site indicates a change in growout system, intertidal growing height (% exposure) or stocking density {number of oysters/ m^2 of floor area of growout container}. For each phase in the production cycle, age [months] is given.

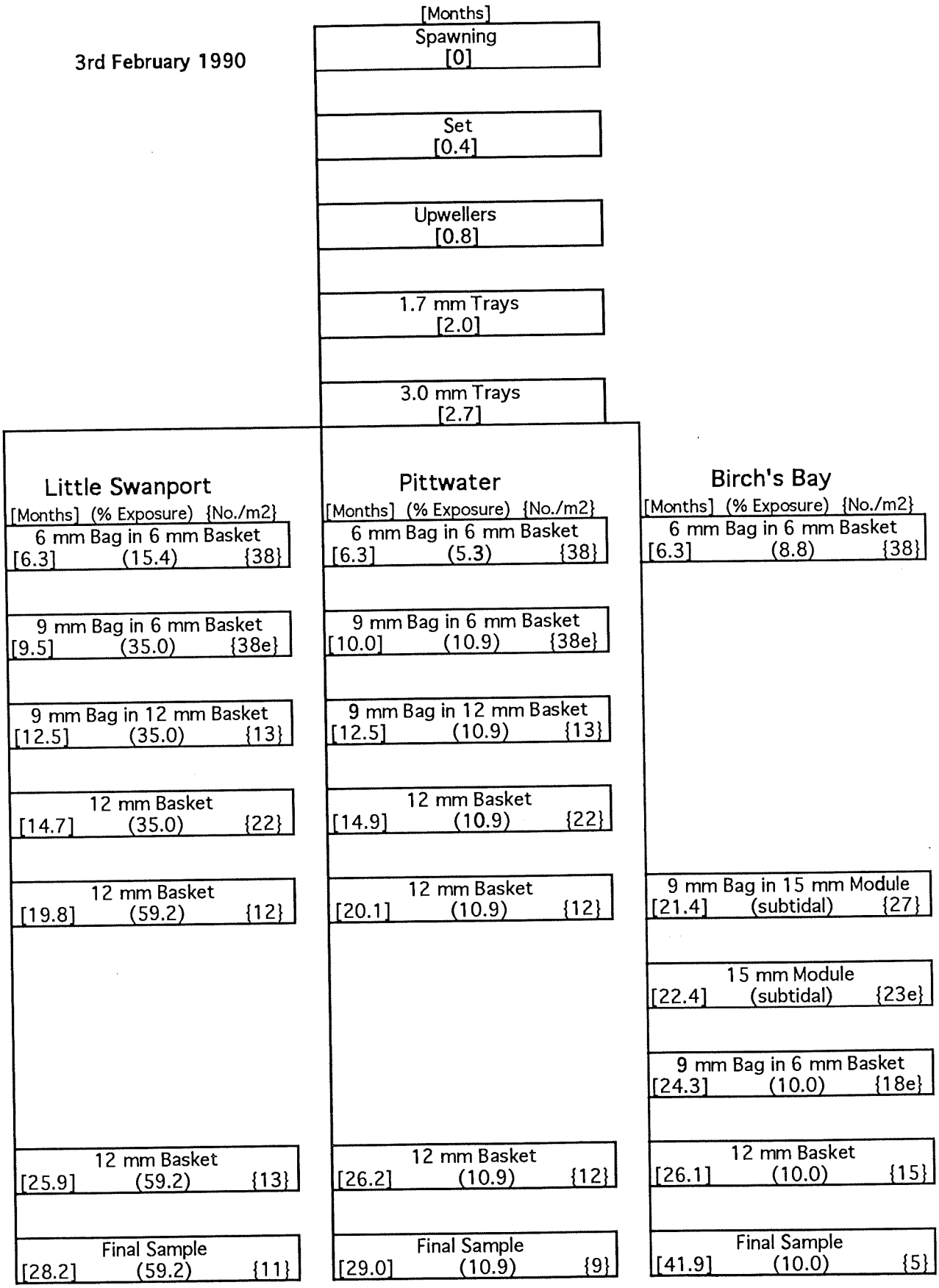

[Months]
3rd February 1990
Spawning
[0]
Set
[0.4]
Upwellers
[0.8]
1.7 mm Trays
[2.0]
3.0 mm Trays
[2.7]
Little Swanport
[Months] (% Exposure) {No./m2}
6 mm Bag in 6 mm Basket
[6.3] (15.4) {38}
9 mm Bag in 6 mm Basket
[9.5] (35.0) {38e}
9 mm Bag in 12 mm Basket
[12.5] (35.0) {13}
12 mm Basket
[14.7] (35.0) {22}
12 mm Basket
[19.8] (59.2) {12}
12 mm Basket
[25.9] (59.2) {13}
Final Sample
[28.2] (59.2) {11}
Pittwater
[Months] (% Exposure) {No./m2}
6 mm Bag in 6 mm Basket
[6.3] (5.3) {38}
9 mm Bag in 6 mm Basket
[10.0] (10.9) {38e}
9 mm Bag in 12 mm Basket
[12.5] (10.9) {13}
12 mm Basket
[14.9] (10.9) {22}
12 mm Basket
[20.1] (10.9) {12}
12 mm Basket
[26.2] (10.9) {12}
Final Sample
[29.0] (10.9) {9}
Birch's Bay
[Months] (% Exposure) {No./m2}
6 mm Bag in 6 mm Basket
[6.3] (8.8) {38}
9 mm Bag in 15 mm Module
[21.4] (subtidal) {27}
15 mm Module
[22.4] (subtidal) {23e}
9 mm Bag in 6 mm Basket
[24.3] (10.0) {18e}
12 mm Basket
[26.1] (10.0) {15}
Final Sample
[41.9] (10.0) {5}

Fig. 3. Whole weight and shell height of diploid and triploid Pacific oysters *Crassostrea gigas* at three sites in Tasmania; n=4 replicate groups except at Birch's Bay (n=11 up to age 32.4 months and n=3 from 33.8 months).
* ($P<0.05$)
#=($P<0.01$, approximate because of heterogeneity of variance).
Cross hatched sections on X axis indicate summer periods while S indicates a spawning event for diploids.
Ranges for standard error values are given.

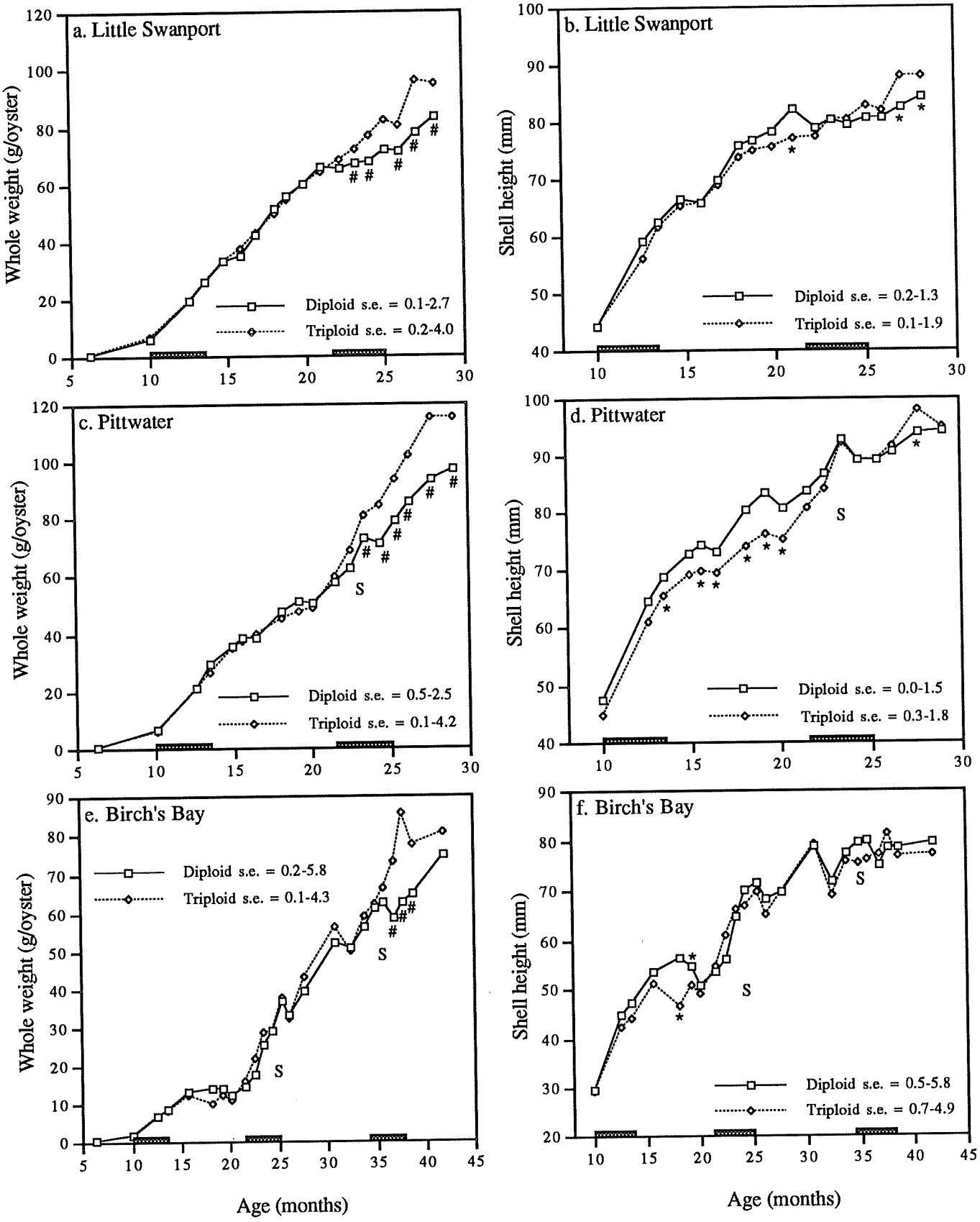

a. Little Swanport
Whole weight (g/oyster)
Diploid s.e. = 0.1-2.7
Triploid s.e. = 0.2-4.0
b. Little Swanport
Shell height (mm)
Diploid s.e. = 0.2-1.3
Triploid s.e. = 0.1-1.9
c. Pittwater
Whole weight (g/oyster)
Diploid s.e. = 0.5-2.5
Triploid s.e. = 0.1-4.2
d. Pittwater
Shell height (mm)
Diploid s.e. = 0.0-1.5
Triploid s.e. = 0.3-1.8
e. Birch's Bay
Whole weight (g/oyster)
Diploid s.e. = 0.2-5.8
Triploid s.e. = 0.1-4.3
f. Birch's Bay
Shell height (mm)
Diploid s.e. = 0.5-5.8
Triploid s.e. = 0.7-4.9
Age (months)
Age (months)

Fig. 4. Shell weight and dry meat weight of diploid and triploid Pacific oysters *Crassostrea gigas* at three sites in Tasmania; n=4 replicate groups except at Birch's Bay (n=11 up to age 32.4 months and n=3 from 33.8 months).
* ($P<0.05$)
#=($P<0.01$, approximate because of heterogeneity of variance).
Cross hatched sections on X axis indicate summer periods while S indicates a spawning event for diploids.
Ranges for standard error values are given.

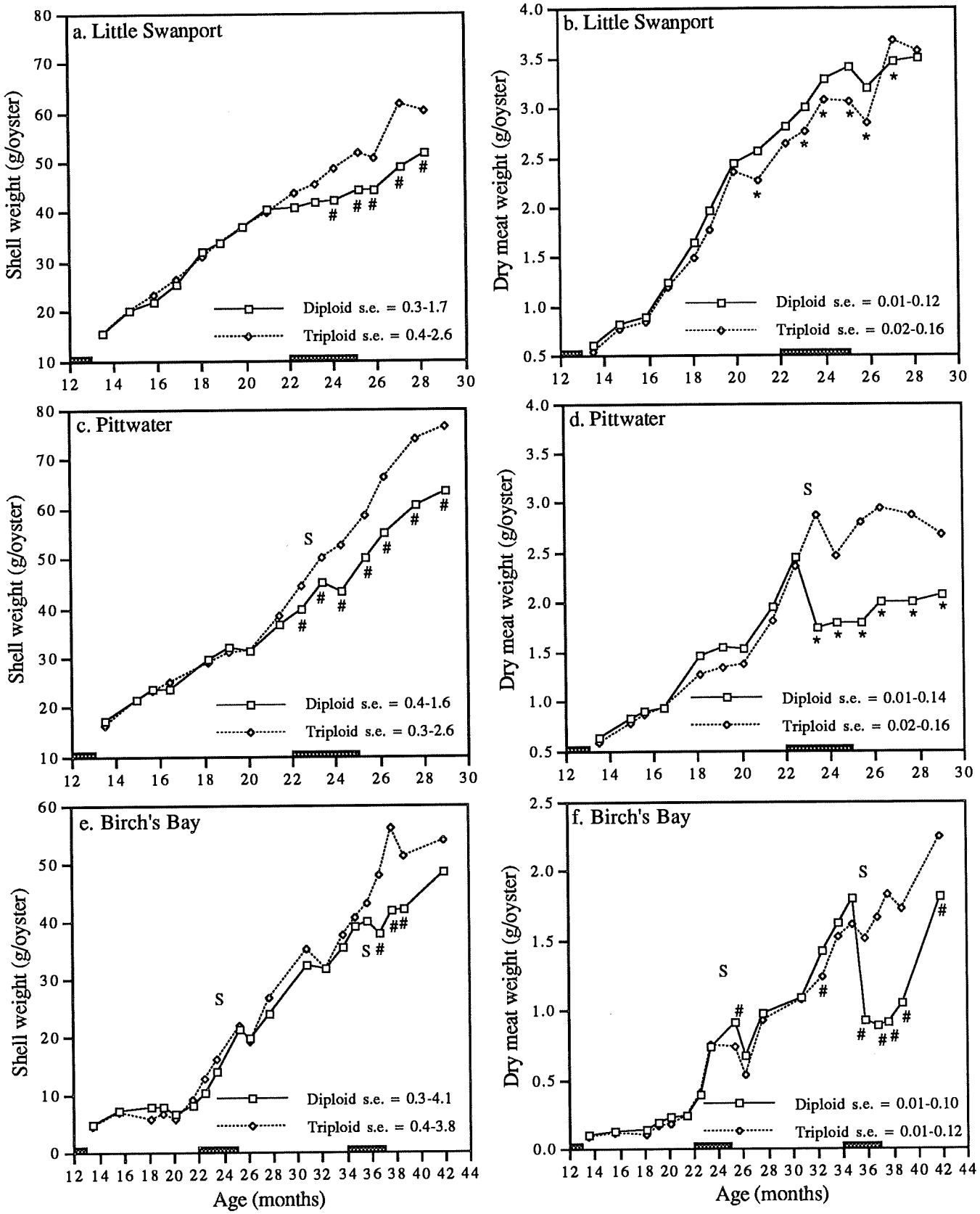

a. Little Swanport
Shell weight (g/oyster)
Diploid s.e. = 0.3-1.7
Triploid s.e. = 0.4-2.6
b. Little Swanport
Dry meat weight (g/oyster)
Diploid s.e. = 0.01-0.12
Triploid s.e. = 0.02-0.16
c. Pittwater
Shell weight (g/oyster)
Diploid s.e. = 0.4-1.6
Triploid s.e. = 0.3-2.6
d. Pittwater
Dry meat weight (g/oyster)
Diploid s.e. = 0.01-0.14
Triploid s.e. = 0.02-0.16
e. Birch's Bay
Shell weight (g/oyster)
Diploid s.e. = 0.3-4.1
Triploid s.e. = 0.4-3.8
Age (months)
f. Birch's Bay
Dry meat weight (g/oyster)
Diploid s.e. = 0.01-0.10
Triploid s.e. = 0.01-0.12
Age (months)

Fig. 5. Condition index (CI) and moisture content of meats of diploid and triploid Pacific oysters *Crassostrea gigas* at three sites in Tasmania; n=4 replicate groups except at Birch's Bay (n=11 up to age 32.4 months and n=3 from 33.8 months).
* ($P<0.05$)
#=($P<0.01$, approximate because of heterogeneity of variance).
Cross hatched sections on X axis indicate summer periods while S indicates a spawning event for diploids.
Ranges for standard error values are given.

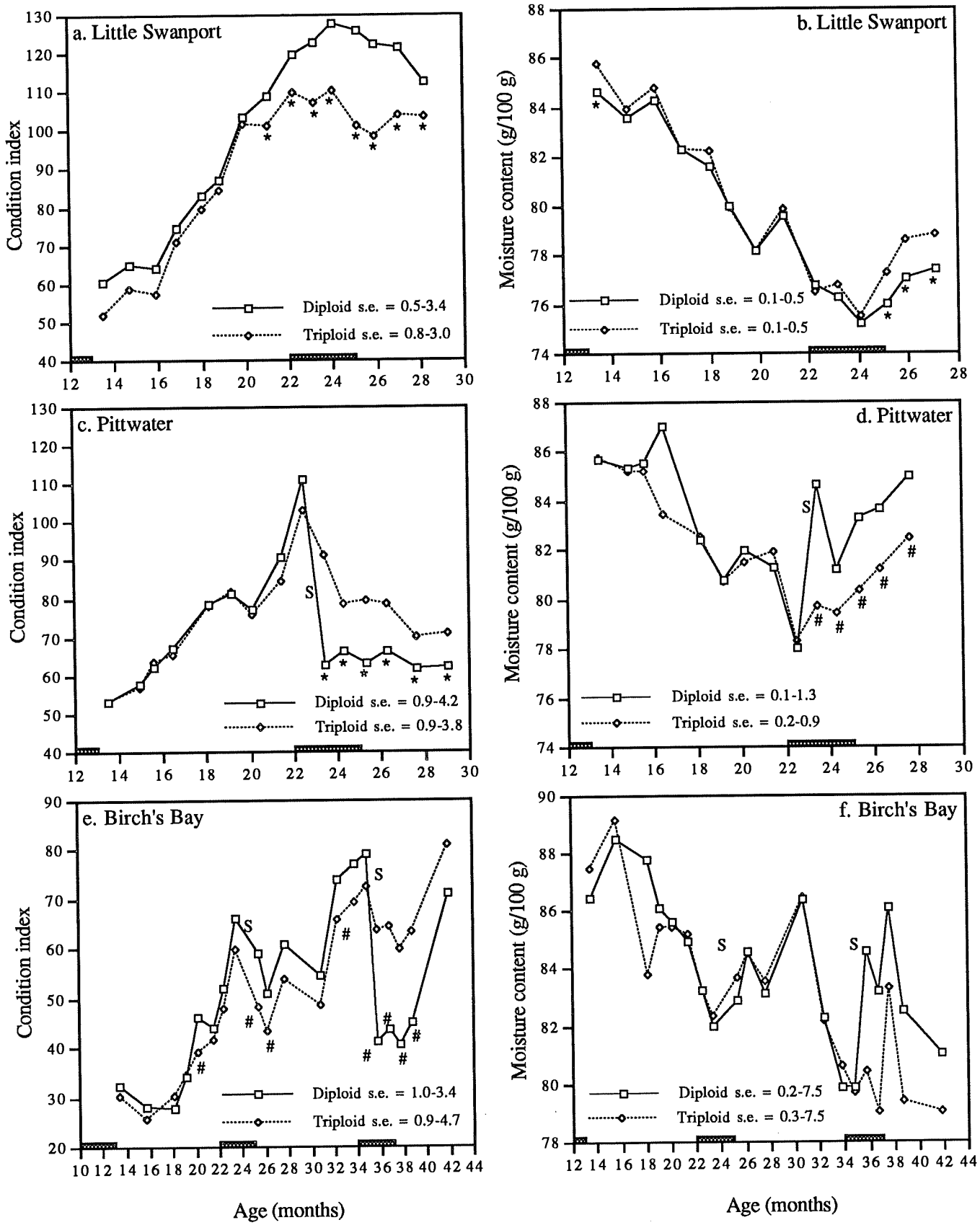

a. Little Swanport
Condition index
Diploid s.e. = 0.5-3.4
Triploid s.e. = 0.8-3.0
b. Little Swanport
Moisture content (g/100 g)
Diploid s.e. = 0.1-0.5
Triploid s.e. = 0.1-0.5
c. Pittwater
Condition index
Diploid s.e. = 0.9-4.2
Triploid s.e. = 0.9-3.8
d. Pittwater
Moisture content (g/100 g)
Diploid s.e. = 0.1-1.3
Triploid s.e. = 0.2-0.9
e. Birch's Bay
Condition index
Diploid s.e. = 1.0-3.4
Triploid s.e. = 0.9-4.7
f. Birch's Bay
Moisture content (g/100 g)
Diploid s.e. = 0.2-7.5
Triploid s.e. = 0.3-7.5
Age (months)
Age (months)

Fig. 6. Glycogen content and gonad area (% of cross sectional area excluding gills) of diploid and triploid Pacific oysters *Crassostrea gigas* at three sites in Tasmania; n=4 replicate groups except at Birch's Bay (n=11 up to age 32.4 months and n=3 from 33.8 months).
* ($P<0.05$)
#=($P<0.01$, approximate because of heterogeneity of variance).
Cross hatched sections on X axis indicate summer periods while S indicates a spawning event for diploids.
Ranges for standard error values are given.
For gonad area, values are for diploid groups, triploid groups and trip. (act.) i.e. triploid groups with diploid oysters, within triploid groups, excluded.

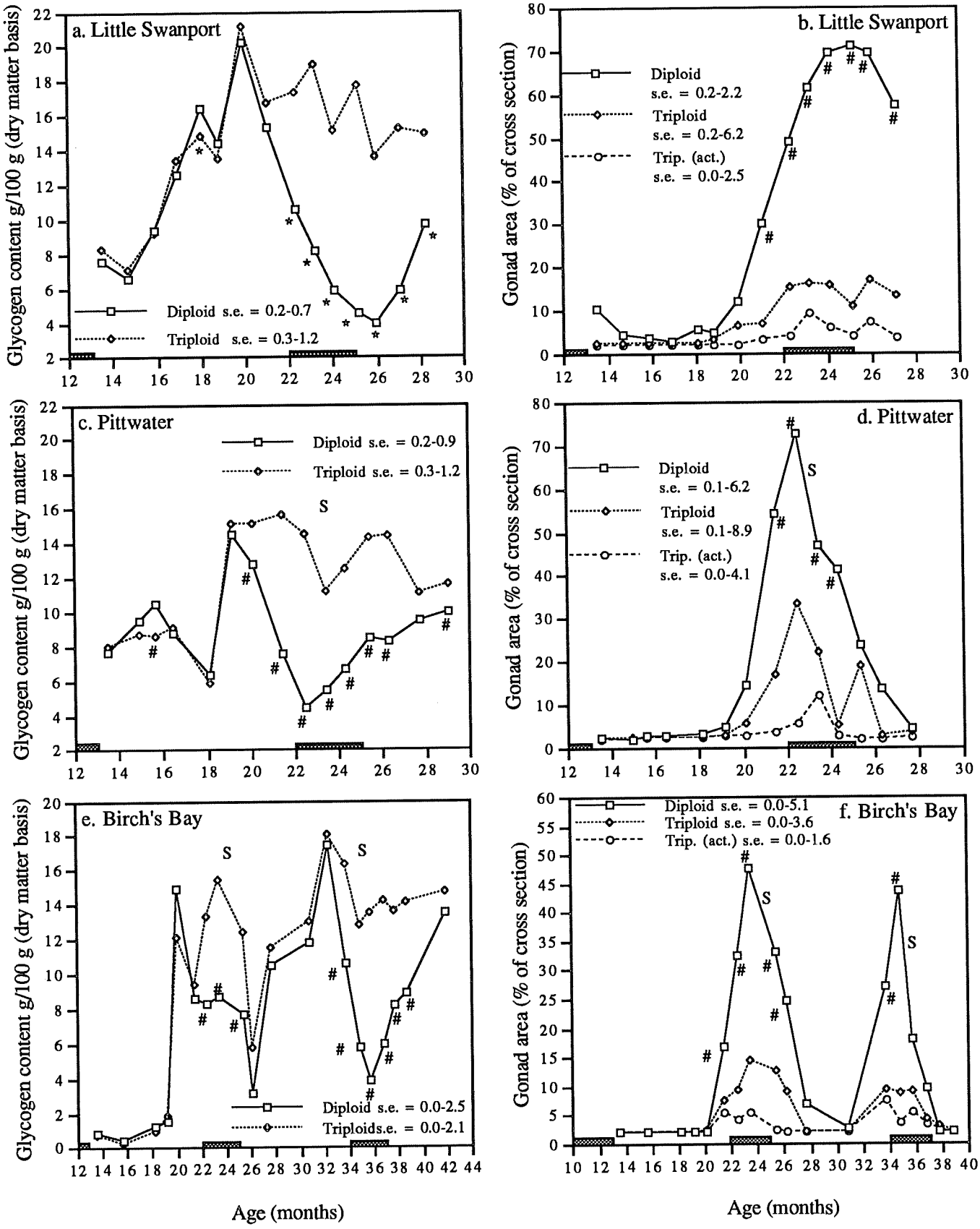

a. Little Swanport
Glycogen content g/100 g (dry matter basis)
Diploid s.e. = 0.2-0.7
Triploid s.e. = 0.3-1.2
b. Little Swanport
Gonad area (% of cross section)
Diploid
s.e. = 0.2-2.2
Triploid
s.e. = 0.2-6.2
Trip. (act.)
s.e. = 0.0-2.5
c. Pittwater
Glycogen content g/100 g (dry matter basis)
Diploid s.e. = 0.2-0.9
Triploid s.e. = 0.3-1.2
d. Pittwater
Gonad area (% of cross section)
Diploid
s.e. = 0.1-6.2
Triploid
s.e. = 0.1-8.9
Trip. (act.)
s.e. = 0.0-4.1
e. Birch's Bay
Glycogen content g/100 g (dry matter basis)
Diploid s.e. = 0.0-2.5
Triploids.e. = 0.0-2.1
f. Birch's Bay
Gonad area (% of cross section)
Diploid s.e. = 0.0-5.1
Triploid s.e. = 0.0-3.6
Trip. (act.) s.e. = 0.0-1.6
Age (months)
Age (months)

Fig. 7. Additional morphometric characteristics of diploid and triploid Pacific oysters Crassostrea gigas at three sites in Tasmania; n=4 replicate groups except for Birch's Bay where n=3.
Asterisks indicate significant differences between ploidy groups at individual sites; *** = P<0.001; ** = P<0.01; * = P<0.05.
a. Cavity volume (%) is (cavity volume (g) x 100)/whole oyster volume, where whole oyster volume (g) is (shell volume (g) + cavity volume (g)). Shell volume is (shell weight in air (g) - shell weight in water (g)) and cavity volume is (whole oyster weight in air (g) - shell weight in air (g)).
b. Shell weight (%) is (shell weight in air (g) x 100)/whole oyster weight in air (g)).
c. Adductor muscle diameter (%) is (adductor muscle diameter (mm) x 100)/shell height (mm)).

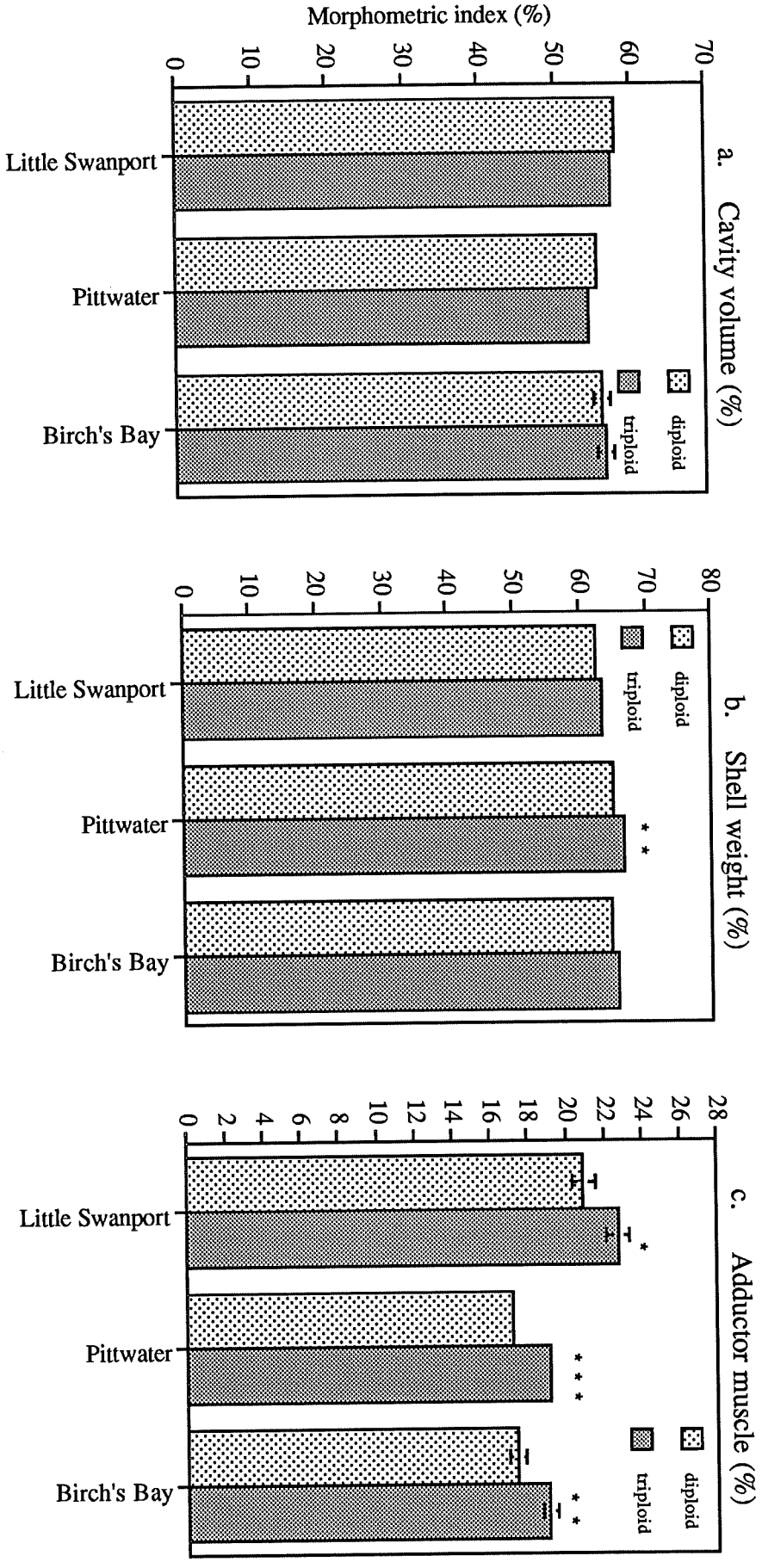
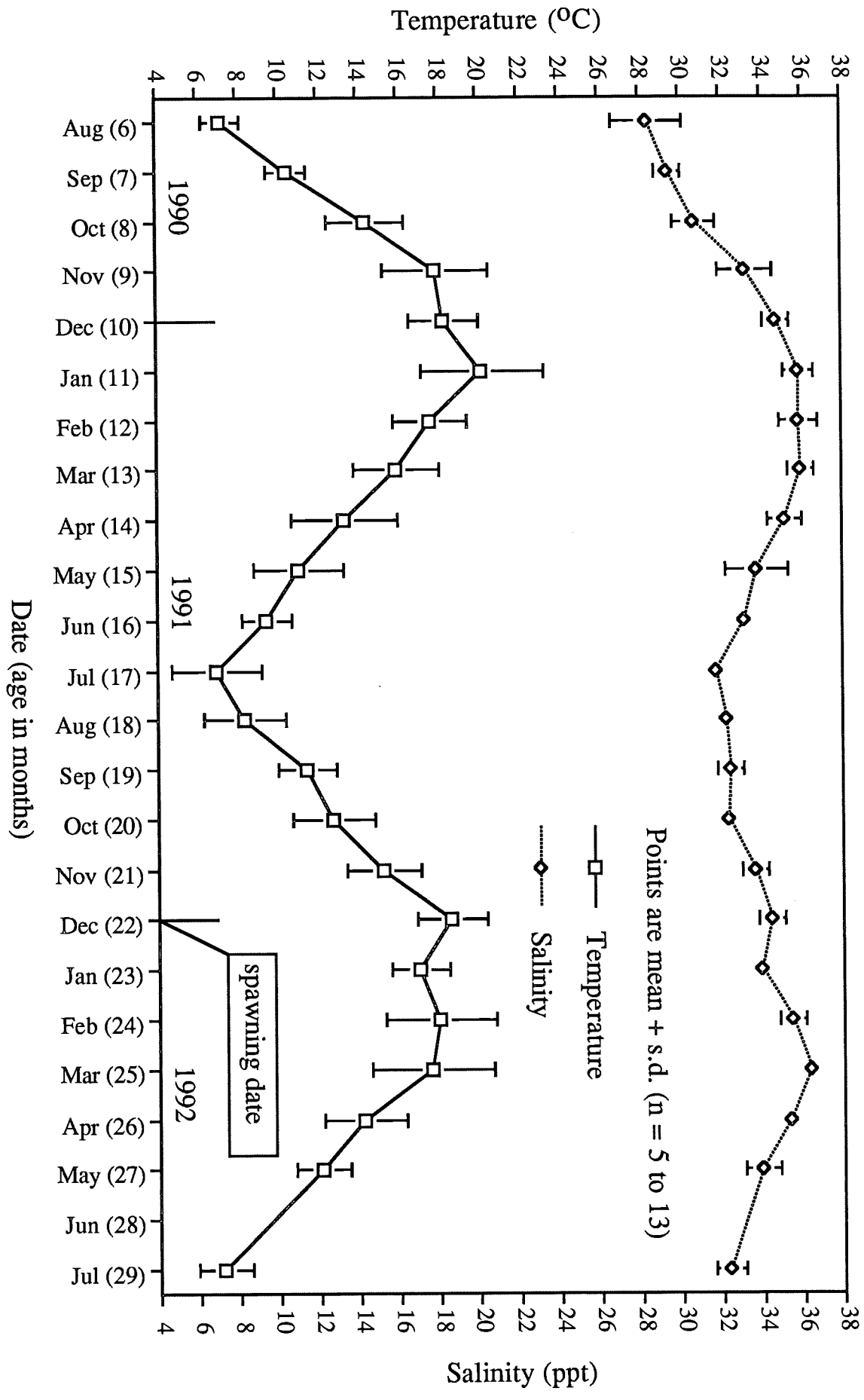

Morphometric index (%)
0
10
20
30
40
50
60
70
a. Cavity volume (%)
Little Swanport
Pittwater
Birch's Bay
diploid
triploid
b. Shell weight (%)
80
* *
c. Adductor muscle (%)
28
26
24
22
* *
* * *
* *

Fig. 8. Surface water temperature and salinity at Pittwater, Tasmania. The spawning event for diploid Pacific oysters *Crassostrea gigas* at this site and the age of oysters for each month are indicated.


Temperature (°C)
Salinity (ppt)
Date (age in months)
Aug (6)
Sep (7)
Oct (8)
Nov (9)
Dec (10)
Jan (11)
Feb (12)
Mar (13)
Apr (14)
May (15)
Jun (16)
Jul (17)
Aug (18)
Sep (19)
Oct (20)
Nov (21)
Dec (22)
Jan (23)
Feb (24)
Mar (25)
Apr (26)
May (27)
Jun (28)
Jul (29)
1990
1991
1992
spawning date
Temperature
Salinity
Points are mean + s.d. (n = 5 to 13)

PERFORMANCE OF TRIPLOID AND DIPLOID PACIFIC OYSTERS AT FOUR COMBINATIONS OF GROWING HEIGHTS AND STOCKING DENSITIES.

Dr. Greg B. Maguire and Mr. Greg Kent

National Key Centre for Teaching and Research in Aquaculture, University of Tasmania at Launceston.

As part of a larger study on evaluation of triploid Pacific oysters in Tasmania (Table 1), diploid and triploid oysters were grown for five months (December to May) at four combinations of two growing heights (a finishing rack and 30cm below this) and two stocking densities (130 oysters per basket and 80 per basket). The aim was to see if triploids (3 sets of chromosomes per cell) are appropriate under a variety of lease management strategies. For each combination there was a triploid group and a diploid group (these have two chromosomes per cell as have virtually all of the Pacific oysters in Tasmania). The experiment was intended to complement a much larger study of triploids and diploids over two years at three sites in Tasmania. The key results for the whole project will be obtained over this coming summer, if spawning occurs.

The key findings of the study on combinations of polyploidy (triploids and diploids), growing height and stocking density were as follows (all groups combined unless stated) :

WHOLE WEIGHT :

- On average triploids grew 7.6 % faster than diploids.
- At the lower height oysters grew 62 % faster than at the higher growing height.
- Oysters at 130 per basket grew 4 % faster than those at 80 per basket. However, at the low growing height, faster growth was recorded at 80 per basket while at the high growing height faster growth was recorded at 130 per basket.

SHELL WEIGHT :

- The patterns were similar to those for the whole weight data.

DRY MEAT DATA :

- Meat weights were similar for triploids and diploids.
- Meat weights were 11 % higher for the lower growing height.
- After 3 months the low density oysters (80 per basket) developed larger meats than the high density oysters (130 per basket). By the end of the trial the difference was 12.7%. However, for both triploids and diploids the combination of low growing height and low density gave much larger meats (25.7 % larger than the rest).

CONDITION INDEX :

- Most groups reached minimum marketable condition (index greater than 80) by February after being only about 55 in December. Subsequently, condition declined in all groups by the end of the experiment.
- Because of similar meat size but smaller shells, diploids had slightly better condition at the end of the trial (4.7 % better).
- From February to March the difference in relation to height for condition index values was about 35 units, thus the condition of oysters at the high growing height was **massively better** than at the low height.
- By the end of the trial, the high density groups (130 per basket) had much poorer condition than the low density group (80 per basket); the difference was about 10 condition index units.

"CUPPINESS", i.e. SHAPE :

- There were no consistent differences between triploids and diploids but low density oysters and high growing height oysters generally had better shape.

SHELF LIFE, GONAD DEVELOPMENT AND GLYCOGEN CONTENT :

These will be presented on another occasion.

CONCLUSION :

Triploids generally performed well under different combinations of lease management strategies. Data from the 1991 / 1992 Summer period for our other groups of triploids will be crucial to the assessment of their value. The present experiment, along with Ms. O'Meley's work, shed more light on the effects of growing height and stocking density. These two variables interact to affect growth but generally high growing height favours better condition, chiefly through repressing shell growth. A high stocking density will lead to poor meat growth if the oysters are not thinned out after a few months. A deep shell shape is favoured by low density and high growing height. Clearly, the choice of density and growing height depends on what the farmer gives highest priority to, i.e. shell growth, meat growth, condition or shell shape.

Additional data analyses are required on available data and samples for shelf life, gonad development and glycogen content.

TABLE 1 KEY ELEMENTS OF PACIFIC OYSTER TRIPLOIDY PROGRAM

INDUCE TRIPLOIDY IN OYSTER EMBRYOS

PRODUCE LARGE NUMBERS OF TRIPLOID AND DIPLOID SPAT

800,000 OF EACH GROUP MADE AVAILABLE TO FARMERS IN TASMANIA, S. A. AND VICTORIA

EVALUATE TRIPLOID AND DIPLOID OYSTERS IN TASTE PANEL TRIALS DURING DIFFERENT SEASONS

COMPARE GROWTH, MEAT CONDITION, AND GONAD DEVELOPMENTIN BOTH GROUPS AT THREE SITES IN TASMANIA

COMPARE BOTH GROUPS AT FOUR COMBINATIONS OF INTERTIDAL GROWING HEIGHT AND STOCKING DENSITY AT ONE SITE IN TASMANIA

NATIONAL KEY CENTRE FOR TEACHING & RESEARCH IN AQUACULTURE

TRIPLOIDY FOR PACIFIC OYSTERS

By Dr Greg Maguire (Senior Lecturer) and Mrs Adele Kent (Technical Officer)

Tasmanian State Institute of Technology, P.O. Box 1214, Launceston, Tasmania, Australia 7250.Tel: (003) 260 229, Fax: (003) 263 664

INTRODUCTION

A research grant from FIRDTF (Fishing Industry Research and Development Trust Fund) is being shared by the above Key Centre staff and NSW Agriculture and Fisheries staff from the Brackish Water Fish Culture Research Station, Port Stephens (Dr John Nell and Ms Caroline Mason).

The aim is to evaluate triploid Pacific (*Crassostrea gigas*) and Sydney rock oysters (*Saccostrea commercialis*) under commercial farming conditions. In this article triploidy and its possible benefits are explained and the research program for Pacific oysters outlined. Most of the oysters produced in Tasmania and South Australia are Pacific oysters.

TRIPLOIDY IN OYSTERS

Most animal cells contain two sets of chromosomes, one set of paternal origin and the other maternal. These are said to be diploid (2N). During the development of eggs a process called meiosis occurs whereby the chromosomes are duplicated (4N). However, excess chromosomes are also extruded as polar bodies in two stages. In the first stage a 2N polar body is ejected leaving a cell with 2N chromosomes. A second polar body (1N) is ejected leaving a 1N cell. Similarly, a sperm cell (spermatozoon) only contain one set of chromosomes (1N).

When unfertilised eggs are spawned by female oysters they are still in a 4N state. When the sperm penetrates the egg it stimulates the expulsion of the two polar bodies and the egg is left with its 1N set of chromosomes. These combine with the 1N set from the sperm cell to give the normal complement of 2N chromosomes. The fertilised egg will then develop into an oyster larva (2N).

In contrast triploid oysters contain three sets of chromosomes per cell (3N). Triploidy in oysters is usually induced by preventing the extrusion of the second polar body so that the egg retains 2N chromosomes which combine with the 1N set from the sperm cell leading to a 3N oyster larva.

There are a variety of methods for inducing triploidy (see Forteath, 1989) but a chemical method is preferred for Pacific oysters. A precise concentration is selected within the range 0.5 - 1.0 mg per litre for the fungal antibiotic cytochalasin B. This is added to a dense concentration of oyster eggs (20 - 25 million eggs per litre) and the timing depends on temperature e.g. at 25oC it is usually added 20 - 30 minutes after fertilisation (Allen et al., 1989). The success rate will depend on the broodstock chosen, the cytochalasin B concentration, timing, temperature, egg development rate and general hatchery procedures.

ADVANTAGES OF TRIPLOIDY

The major aim of inducing triploidy in oysters is to ensure continuity of supply of oysters in an acceptable condition for marketing. If an oyster spawns, it loses condition and may be too "lean" for marketing.

The oyster does of course progressively recover condition but particularly in some areas the slow rate of recovery can seriously delay marketing. In some areas of South Australia this recovery for Pacific oysters takes several months while in Tasmania spawning may not even occur annually in summer and recovery rates at some sites are reputed to be quite rapid. Triploid oysters are less likely to spawn and generally do not suffer as large changes in condition as diploid oysters.

For the United States market there is buyer resistance to sexually mature Pacific oysters and during periods of advanced sexual development, triploid oysters are preferred. The Sea Fisheries Division of the Tasmanian Department of Primary Industries has a growing area classification program underway in an attempt to meet standards for export of Pacific oysters to the United States. Clearly, it may be necessary to provide triploid oysters for the United States market during certain months. In addition, as oyster production in Australia expands the domestic market will become more competitive and it is possible that triploid oysters will be sought on a seasonal basis by more discriminating consumers.

Triploid Pacific oysters tend to maintain high glycogen levels (Allen and Downing, 1986) and this can enhance eating qualities as well as resistance to physiological stress and associated mortality (Chew, 1986).

Triploid bivalves can, however, exhibit characteristics which may be undesirable (e.g. lower larval survival, lower peak meat condition, larger adductor muscle and relatively poor growth in nutrient deficient waters).

HATCHERY PRODUCTION

Induction of triploidy in Pacific oysters appears to be simple on paper as a very helpful manual is available (Allen et al., 1989). However, failures have been common in initial trials overseas and in Australia. It is crucial to produce groups of oysters with a high percentage of triploid individuals, preferably more than 90%.

With this in mind Industry, government and research grant funding has been

used to bring out a triploidy expert, Ms. Sandra Downing from the University of Washington, Seattle. During January - February 1990 she assisted with the production of triploid Pacific oysters at the Shellfish Culture Pty. Ltd. hatchery at Bicheno, Tasmania and triploid Sydney rock oysters as well as diploid ones for selective breeding trials at Port Stephens.

To ensure that all eggs develop at a similar rate, broodstock are dissected and the gametes stripped at similar times. While larval survival is lower with triploid Pacific oysters, this is not necessarily a problem as this species has a remarkably high reproductive output. Larval rearing methods are similar for diploids and triploids.

GROW-OUT TRIALS

The aim is to produce both diploid and triploid groups from the same Pacific oyster broodstock and to hold these in nursery systems before comparing the groups on commercial leases for about 18 months. Performance indices will include survival, total weight, shell weight, meat weight, condition index, gonad development, glycogen content and acceptability in terms of taste, texture and appearance. Hopefully funds will be available to not only compare the groups at normal intertidal growing heights but at a range of heights and under other lease management conditions.

PRELIMINARY STUDIES

Diploid Pacific oysters are being sampled regularly at three leases in Tasmania to both establish appropriate sampling methods and to monitor spawning and recovery times for meat condition. Partial spawnings among these groups of oysters indicate considerable variation among sites in terms of timing. The production of triploid Pacific oyster larvae at Bicheno was very successful with initial samples indicating that more than 90% of the larvae were triploid. It was apparent that broodstock which have regressed in gonad condition within broodstock tanks could give poorer triploidy results.

INTEREST SHOWN BY INDUSTRY

Interest in triploidy varies depending on site but several farmers in Tasmania and South Australia (and New Zealand - Ed) are keen to evaluate triploid Pacific oysters. Some are making lease space available for this research and a few farmers have visited the northwest Coast of the U.S. to inspect triploid oysters first hand.

The aim of the project is to assess triploidy under local conditions and to give farmers more options for growing this species in the future rather than to try to convince farmers to adopt triploidy. It should be emphasized that the chemical cytochalasin B (CB) is only applied to oyster eggs and these are rinsed thoroughly to remove excess CB. Hence, there is no problem with chemical residues when the oysters are harvested two years later.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the support of their colleagues in Tasmania and at Port Stephens as well as the material and financial contributions from FIRDTF, Tasmania Development Authority, Shellfish Culture Pty. Ltd. and individual oyster farmers.

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OYSTER VIDEO & MANUAL FOR SALE

The University of Washington's laboratory manual and an instructional video are recommended for anyone wishing to undertake triploidy trials.

AAM can organise bulk purchase purchases of the manual at A$20 (within Australia) or A$30 elsewhere. Orders should be sent ASAP (any invoices which must be raised will be charged a A$5 book keeping fee). Otherwise purchase details can be obtained direct from the supplier : University of Washington Press, PO Box 50096, Seattle, WA 98145-5096, USA.

The video is in an U.S. format which is not compatible with Australian or New Zealand machines, so AAM is trying to organise for it to be dubbed onto VHS-PAL format for sale. The approximate cost will be A$200-A$400 depending on costs and numbers of orders. Initial advice of interest is requested ASAP so we have some idea of the demand.

PRELIMINARY RESULTS FROM THE PACIFIC OYSTER TRIPLOIDY PROGRAM

Dr Greg Maguire and Mr Greg Kent
National Key Centre for Teaching and Research in Aquaculture
University of Tasmania at Launceston

In late 1989 a joint project on triploid oysters was funded by the fishing Industry Research and Development Committee for three years. Dr John Nell, NSW Department of Fisheries at Port Stephens, began work on triploid Sydney rock oysters and our research team took on the task of producing and evaluating triploid Pacific oysters.

With the cooperation of Shellfish Culture P/L and the University of Washington, triploid and diploid Pacific oysters (three sets and the normal two sets of chromosomes per cell, respectively) were reared from the same pool of eggs at Bicheno in February 1990. Subsequently, the small juvenile oysters were maintained at the company's nursery at Little Swanport by university staff including Ms Adele Kent. Oysters which were not retained by the researchers were distributed to commercial oyster farmers, through Shellfish Culture P/L, in Tasmania, South Australia and Victoria. We of course cannot guarantee the percentage of the oysters, supplied by the company, that were actually triploids as most of these spat were looked after by company staff for extended periods.

The oysters retained by the researchers were placed on commercial leases in Tasmania in August 1990; two sets of groups were kept at Oyster Bay Oysters at Little Swanport, one set (triploid and diploid groups) were kept at Coal River Oysters at Pittwater, and one set were kept at Bailinga Oyster and Mussel Farm at Birch's Bay. The triploids were not all triploids and had a triploid: diploid ratio of about 7:3. The expectation was that the triploids would not spawn, and hence would not become unmarketable, in the crucial summer of 91/92 when the oysters were approaching market size.

While not all of the gonad samples have been processed yet, none of the triploid groups seemed to have spawned. However, there were major spawnings for two of the four groups of diploids. The data summarised below are for the set at Pittwater where the diploids spawned and for one set at Little Swanport in which the diploids did not spawn.

Key findings for these two sets

1. Based on the last comparable samples, the triploids grew about 23% faster than the diploids at both sites. This growth advantage, in average whole oyster weight, only became evident in the later part of the study.

2. When the Pittwater diploids spawned, the triploids maintained their meat condition and developed a 20% advantage in wet meat weight. Because the Little Swanport diploids did not spawn, no such advantage was evident in that set. Not all of the meats have been dried yet so we cannot present our preferred index of oyster meat condition, i.e. dry condition index. (See the notes from the November 15, 1991 TACS Workshop at Smithton).

3. For both the Pittwater set and the Little Swanport set, the triploids had a 10 - 15% advantage in shell shape, based on a "cuppiness index" which indicates the degree to which the oysters develop a desirable deep cup shape.

4. Final counts on one set at Little Swanport indicate that there has been virtually no mortality in either the diploids or the triploids since they were placed on the Little Swanport lease in August 1990.

5. While further data analysis is required, the scientifically designed taste testing trials we conducted with our colleague Mr Bob Boocock indicated that triploid oysters were well accepted by university staff who professed a liking for natural oysters.

6. Despite not being graded since August 1990, the above groups of oysters exhibited a quite acceptable amount of size variation within a group. This has created interest among some oyster farmers who see an opportunity to reduce labour costs.

We will provide more information as we progressively complete the sample and data analysis. Looking to the future, increasing the percentage of oysters that are actually triploid must be a priority if this work is to be used by farmers.

An inexpensive remote monitoring system trialed on Tasmanian oyster farms

By G. N. Kent
and G. B. Maguire

Monitoring and maintenance of important water quality variables are integral components of many aquaculture enterprises. Similarly, observing and logging critical environmental conditions, lends greater clarity and credibility to research conducted on aquatic animals. In many instances monitoring is done manually, and as such is too labour intensive to allow for multiple readings spaced evenly over each 24 hr cycle.

The advent of microprocessor technology in recent years has made electronic data logging and recording of environmental parameters an alternative solution. Remote data loggers are labour efficient, allow for regularly spaced multiple readings, and will automatically store the data in a form suitable for further processing.

Unfortunately, for many farmers and researchers, the majority of systems available for this work have proven to be too sophisticated and consequently too expensive for consideration. For example, a remote data logger monitoring dissolved oxygen, pH, conductivity, and temperature, may cost in excess of $20,000.

Two Key Centre research programs are aimed at assessing triploidy (Maguire and Kent, 1990) and other Pacific oyster (*Crassostrea gigas*) farm management options (O'Meley and Maguire, 1990). At several Tasmanian oyster farms Key Centre research staff have utilized a low cost, data recording system to monitor water temperature, and rack exposure resulting from tidal movement. This system was used first on Australian oyster farms by Mr Ian Smith from NSW Fisheries' Port Stephens Research Station.

The equipment being used, is a personal computer based data logging system designed and manufactured by the Perth based company, WESDATA, and is depicted in Figure 1. At each site the system may consist of two, model 390 data loggers, connected to a temperature probe and an exposure probe. The loggers have the ability to internally manipulate their approach to sampling data. This may include sample averaging, maximum plus minimum format, or data compression. For this work, however, the units were programmed simply to read the sensors at 15 minute intervals and store the resultant information. Programming of the loggers functions may be conducted on site using a field transfer unit, or alternatively the loggers may be removed from the sensors and programmed directly from an IBM compatible computer on land.

It is interesting to note that for a 15 minute sampling period (ie. 96 samples per day), the loggers each recorded over 3000 pieces of data per month of operation. This used approximately 5000 bytes (5K) of logger memory. Hence, given a new set of batteries, these 32K loggers are capable of sampling data at 15 minute intervals for six months without data having to be "down loaded", via the field transfer unit, to a computer in that period.

Results

Data recording, to monitor oyster exposure at two intertidal growing heights 15 cm apart, began in May 1991 on a farm near Hobart, and continued until early August of that year. Over this time period, oyster exposure on the high rack was calculated to be approximately 9.7% or 2 h 20 min per 24 h cycle. Similarly, exposure for oysters on the lower experimental rack was calculated to be approximately 3.5% or 45 min per 24 h cycle. Water temperature was shown to fluctuate daily by as much as 3-4°C, predominantly in response to tidal movement.

In late August 1991, the system was moved to a farm on the east coast where it is currently operating. At this site two experimental growing heights, differing by 30 cm, are being examined. Average exposure of oysters grown on the high rack was calculated to be approximately 28% or 6 h 40 min per 24 h while oysters held on the lower experimental rack received 0% exposure (ie continuously submerged).

Currently a 1.5 metre exposure probe has been placed on an oyster lease near Hobart airport and it is envisaged that a third exposure probe will be placed on a farm in the D'Entrecasteaux Channel.

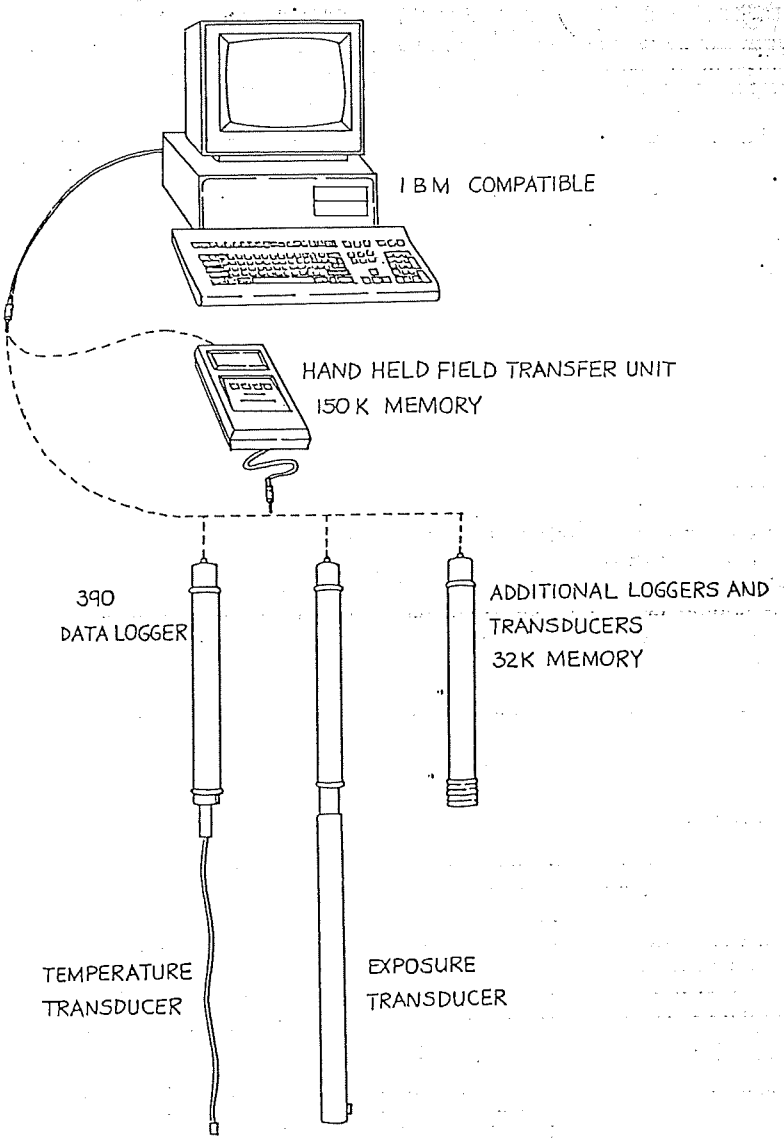
Other options

In addition to water temperature and exposure, it is anticipated that a salinity

measurement probe will also be included when it becomes available from WESDATA.

Other sensors available for use with the 390 data logger include humidity measurement, photosynthetic irradiance (which provides a measurement of light intensity or solar irradiance, within the wavelengths generally accepted to sustain photosynthesis), wind speed and direction, and water pressure (for monitoring water depths outside the range of the exposure probes). The system is extremely cost effective with the majority of sensors ranging in price from $50.00 to $150.00 and the 390 data loggers costing approximately $220.00 each. The Field Transfer Unit (FTU) is the most expensive item at approximately $660.00. (All prices are for October 1990 and do not include sales tax or freight). As previously mentioned, the FTU is not essential for operating the system and could be replaced by a portable IBM compatible computer, or alternatively the loggers could be brought in from the field to down load data.

Figure 1: The Wesdata data logging system



Technical considerations

Both the data loggers and the FTU employ CMOS technology (Complementary Metal Oxide Semiconductor) to reduce power consumption and thereby increase battery life. Unfortunately, however, unless properly shielded, CMOS devices are very susceptible to static electricity. During initial programming and testing of loggers at the Key Centre, logger failure did occur as a result of this deficiency. Other teething problems included a computer virus and a manufacturing defect in a connection socket which both had the effect of short circuiting the batteries in the data logger. However, since being placed out on the leases, no data or logging time has been lost as the equipment has shown itself to be extremely reliable.

Resolution and accuracy of the sensors has also met expectations. For the temperature sensor, resolution is stated as being 0.025 degrees Centigrade. Accuracy is dependent on calibration by the user, but generally accuracy better then +/- 0.5 °C can be expected. For the water level or exposure probes, resolution is quoted as being approximately 0.3 mm. Dependent on calibration, an accuracy better then +/- 1.0 mm is certainly achievable.

Summary

The WESDATA data logging system has proven to be a cost effective means of monitoring and recording information relating to temperature and oyster exposure on Tasmanian oyster farms. Whilst initially suffering from a number of teething problems, the WESDATA data logging system, has demonstrated itself to be both a reliable and accurate research tool under field conditions. With the future addition of a salinity sensor for the data loggers, and ongoing development of products already available, the WESDATA system represents a relatively inexpensive means for farmers and researchers to effectively monitor important environmental aspects of bivalve culture.

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The authors would like to thank our Key Centre colleague, C. O'Meley, and the following farmers and their staff; C. and S. Dyke, P. and J. Chew, C. and G. Sumner, and J. Baily.

Evaluation of Triploid Sydney Rock Oysters

John Nell

It is proposed to test a method of altering the genetic makeup of oysters that has been used successfully overseas so as to improve the quality of Pacific oysters.

Normal oysters inherit one set of genetic material (in the form of chromosomes) from each parent, so normal oysters have two sets of chromosomes. Triploid oysters have been altered so they have three sets of chromosomes. This does not affect their normal growth, but it does reduce the production of sperm and eggs and they don't spawn as much as normal oysters. We hope that they hold their condition into the winter months which would be a great marketing advantage.

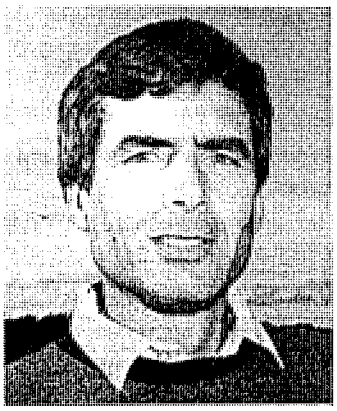
The study will be carried out over four intertidal sites in Port Stephens to expose the oysters to a wide range of conditions. Although triploid oysters have been shown to be successful overseas, it is still important to test triploid Sydney Rock oysters under New South Wales conditions, to see if they really do hold their condition in winter time and also to check that they don't suffer a higher mortality rate.

The problem with triploid oysters is that because they are largely sterile, farmers would have to keep buying spat from hatcheries. Unfortunately, the technique is not well enough developed to ensure 100% triploids, but chances are that with further refinements, high percentages are possible. For this study it is anticipated that we may have to settle for 60% triploids, although we would aim for a higher percentage.

The study would be conducted for three years and during this time oysters would be regularly checked and weighed to compare mortalities and growth rates. The meat and spawning conditions of thc two types of oysters would be compared for 13 months. A properly conducted taste comparison is planned to complete the study.

Fast Growing Triploid Sydney Rock Oysters for Oyster Farmers

by John Nell and Elizabeth Cox*



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The aim of this program was to test if triploid Sydney rock oysters (*Saccostrea commercialis*) would be suitable as a winter crop for NSW oyster growers. Triploid oysters have three sets of chromosomes or genetic material, while normal diploid oysters have two sets. Although there are no obvious external differences between the two oysters, triploid oysters are sterile and do not spawn, and it was thought that they may hold their condition in winter.

Funds to evaluate triploid Sydney rock oysters on commercial oyster leases in Port Stephens were obtained from the Fishing Industry Research and Development Council (FRIDC). To save a lot of time in trying to "re-invent the wheel", Ms Sandra Downing from the University of Washington in Seattle was brought over to assist with the triploid induction. She has been involved in much of the original research on triploid induction in oysters. Five batches of triploid oysters were produced by treating eggs with an antibiotic for 20 min in seawater at 25°C at approximately 20 min after fertilisation. The percentage in the best batch was 92% and in other batches in earlier attempts the percentage ranged from 35 to 51%.

The research on triploid Sydney rock oysters is in its final year and some interesting results are now apparent (Table 1). The main objective of providing "fat" winter oysters was not achievable, but it seems that on some leases triploid Sydney rock oysters keep their condition longer in autumn. As the triploids were on average 34% heavier after two years, it appears that these could be grown to a market size of 40 to 60 grams in 2 to 2.5 years compared with 3 to 3.5 years for normal diploid oysters. At all sites tested (both slow growing and fast growing) the triploids grew faster. The faster growth rate of the triploid oyster shows up during summer when the diploids use some of their energy on spawning activity. Although the triploids don't have this energy saving advantage in winter they are so far ahead at the end of summer, that the diploids can't catch up in winter.

The encouraging results with triploid oysters, raises the question of whether commercial production of triploid Sydney rock oysters is likely to benefit the industry, and if so, how to encourage its development. It should be stressed, however, that a pilot scale experiment is essential before any attempt at full scale commercialisation is made. This is simply to ensure that the results which were achieved experimentally, can be repeated under commercial conditions, in a variety of estuaries.

It should be stressed that because the aim of the program was to evaluate triploid oysters on leases, little time was spent on training Departmental staff in triploid induction. If the Brackish Water Fish Culture Research Station hatchery, or any other hatchery for that matter, were to produce more triploid Sydney rock oyster for experimental purposes, time and money would need to be set aside for staff training. ■

Table 1: Comparison of weight (g) of two year old diploid and triploid[1] Sydney rock oysters[2] (*Saccostrea commercialis*) in Port Stephens, NSW

Sites	February 1992	
	Diploid	Triploid
Tilligerry Creek	19.1±0.3	24.4±1.0
Tilligerry Creek Raft	39.1±0.9	54.9±1.9
Pindimar	31.0±1.3	40.5±0.7
North Arm Cove	23.5±0.4	31.6±1.2

[1] The percentage triploidy at the start of the experiment was 92%
[2] Spat were set in February, 1990

TECHNICAL NOTE

Hatchery production of diploid and triploid clams *Tapes dorsatus* (Lamarck 1818): a potential new species for aquaculture.

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Abstract

To assess the potential for hatchery production of the venerid clam *Tapes dorsatus*, diploid and triploid clams were produced and ongrown until the spat reached 5 - 8 mm in size. Triploidy percentages at metamorphosis that ranged from 56 to 85%, were induced using a 15 min exposure to 1 mg/l cytochalasin B. No differences were observed in the growth rate of sibling diploid and triploid *T. dorsatus* larvae. Survival of diploid clams to pediveliger stage was however higher. Post-metamorphic growth and survival of diploid and triploid clams remained the same until the clams were removed from the hatchery to outdoor nurseries. Throughout the larval and early spat phases of production, *T. dorsatus* growth was similar to that reported for the Manila clam *Tapes philippinarum*. Based upon its ease of culture, rapid growth and marketability, *T. dorsatus* are thought to have considerable aquaculture potential.

1. Introduction

Despite the development of clam mariculture in Asia, Europe and the USA, clam farming in Australia is in the experimental stage (Treadwell et al., 1992). Licences have been issued for temperate clam farming (Maguire, 1991), with interest shown in species of the genera *Venerupis*, *Katelysia* and *Anadara* (Maguire, 1991; Nell et al., 1994). To enhance the export prospects for clams and to cater to the tastes of those familiar with clams overseas, we believed that advantages may be gained from the selection of a clam similar to those farmed elsewhere. We therefore searched for an Australian alternative to the Manila clam *Tapes philippinarum*.

The venerid clam *Tapes dorsatus* (Lamarck 1818) are found in estuarine seagrass beds and sand flats where it grows to about 100 mm in length. The species occurs throughout the Indo-West Pacific, and in Australia is spread from Western Australia to Queensland (Wells and Bryce, 1988), with isolated populations as far south as Victoria (Robinson and Gibbs, 1982). Unfortunately, the small populations of *T. dorsatus* found in southern Australia are unlikely to sustain commercial harvesting or to provide sufficient natural spatfall for the development of mariculture ventures. Investigations were therefore undertaken into hatchery propagation of the species with two objectives: to determine if current hatchery production and triploidy induction techniques were suitable for producing *T. dorsatus* spat; and to compare growth characteristics of diploid and triploid *T. dorsatus* larvae and spat to determine their suitability for culture.

2. Materials and Methods

T. dorsatus broodstock was gathered from Sydney Harbour, New South Wales (35° 04'S, 150° 44'E) in November 1993 and held in an oyster conditioning system for five days. Twenty five clams were then spawned by raising and lowering water temperature by 5.5°C at 30 min intervals for 2 h, followed by the injection of 0.05 ml of a 10^{-3} M serotonin solution (creatinine sulphate complex, $C_{14}H_{21}N_5O_6S.H_2O$, Merck, Darmstadt, Germany). Spawning commenced within 5 min of the serotonin administration and had ceased within 2 h. Spawning clams were removed from the table, rinsed in fresh 1 μm filtered seawater and placed in individual 500 ml plastic containers of fresh filtered seawater. The eggs from the first six clams to spawn were pooled in 4 l of fresh filtered seawater and sperm solution was added.

The embryos were divided into two groups. One group was divided to stock four 90-l cylindro-conical tanks at 5 eggs/ml as controls. The second group was divided into four and polar body development at 23°C was monitored. When approximately 45% of eggs had developed the first polar body, each of the four batches was separately treated with cytochalasin B (1 mg CB/l in 1% dimethylsulfoxide, DMSO) for 15 min at 23°C (Dufy and Diter, 1990). The eggs were then rinsed and treated with 0.1% DMSO. After a further 15 min, each batch of eggs was rinsed in filtered seawater and stocked into four separate 90-l cylindro-conical tanks, similar to the controls.

All seawater used for spawning, larval rearing and for the settlement of postlarvae was treated with 1 mg/l disodium ethylenediaminetetraacetic acid (EDTA) before use (Utting and Helm, 1985) as a precaution against metals contamination. Seawater was changed every second day with the larvae being retained on polyester mesh netting cylindrical sieves. On Day 11, pediveligers were collected on a 150 μm mesh sieve and put to set on screens (without culch) in PVC downweller units (Utting and Spencer, 1991). Each 450 mm diameter screen was stocked with approximately 50 000 larvae and placed in a 1 300-l downwelling nursery system as used for oyster larvae (Bayes, 1981). Water in the downwelling system was changed thrice weekly and maintained at temperatures between 24-26°C.

T. dorsatus larvae were fed *Pavlova lutheri* (Droop) Green, Tahitian *Isochrysis* aff. *galbana* Green and *Chaetoceros calcitrans* (Paulsen) Takano on an equal dry weight basis (Nell and O'Connor, 1991). Feeding rates ranged from 5 000 cells/larvae/day at the first feed on Day 3 to 14 000 cells/larvae/day at the commencement of settlement on Day 12. During metamorphosis, feeding rates were increased to about 20 000 cells/larvae/day. Following metamorphosis, stocking densities and feed rates were based on those recommended by Utting and Spencer (1991).

When mean spat size had reached 2.0 mm shell length, spat were relocated to outdoor upwelling nursery systems. One system was located on an intertidal pond within the grounds of the research station and the other at the entrance to Port Stephens. Spat growth and water quality parameters i.e. salinity, temperature, pH and turbidity were monitored at each site. Growth throughout the study has been

reported in terms of shell width (distance across the longest axis, parallel to the hinge) to allow comparison with other studies.

Attempts to determine percentage triploidy early in the run using chromosome counts of embryo and larval spreads failed. Flow cytometry was then used at metamorphosis and again at the time of deployment to nursery systems to determine percentage triploidy (Chaiton and Allen, 1985).

3. Results

Of the 25 *T. dorsatus* used in the spawning attempt, nine male and eleven female clams spawned. Fecundity ranged from 0.55 - 1.51 x 10^6 eggs, with a mean (± s.d.) of 1.11 ± 0.27 x 10^6. These eggs were 67.7 ± 3.6 μm in diameter and appeared white in suspension. The first D veligers were observed approximately 40 h post fertilisation, with the remaining trochophores metamorphosing within 48 h. Approximately 80% of eggs developed to veligers of mean shell width 97.1 ± 3.7 μm and mean shell height 82.0 ± 2.0 μm. By Day 4, development of the umbo was clearly evident and by Day 11 the first pediveligers were observed at a shell width of approximately 195 μm.

At metamorphosis the percentage triploidy varied between replicates from 56 to 85%. Overall survival of triploid larvae was lower than that of their diploid siblings. In the triploid treatment, the replicate with the lowest percentage triploidy showed the highest survival. Survival of diploid and triploid clams during metamorphosis was 68 and 72% respectively. At the time of deployment to the nursery systems the triploid clams were combined and the percentage triploidy in duplicate samples were 83 and 87%.

The growth of diploid and triploid *T. dorsatus* larvae and spat, as indicated by increases in shell width, did not differ (Fig. 1). Comparisons of clam growth at Tomaree and the research station indicated sibling diploid and triploid clams were on average 4% larger at Tomaree within 3 weeks. Two bouts of severe mortality (50-60%) were experienced, one at each site, although temporally separated by four weeks. Environmental variables monitored did not give a clear indication of the cause.

4. Discussion

The need for more research into both the ecology and aquaculture of temperate clam species has been highlighted by Maguire (1992) and should involve the development of hatchery production techniques. Experience in the USA has shown that existing oyster hatchery facilities require little modification for clam production (Castagna and Manzi, 1989), and this appears to be the case with the venerids *Katelysia rhytiphora* (Nell et al., 1994) and *T. dorsatus*.

In comparison to the widely cultured Manila clam, larval and early spat growth of the venerid clam *T. dorsatus* has been promising. *T. dorsatus* larvae reached metamorphosis in approximately the same time as *T. philippinarum*, and although diploid survival from hatch to the onset of metamorphosis (32%) was lower than

the minimum of 40% suggested by Utting and Spencer (1991), survival through metamorphosis (68%) was good. Growth from metamorphosis until 2-3 mm and 5-8 mm in size at temperatures in the range 18 - 23°C was also achieved inside 6 weeks and 12 weeks respectively, as is expected for *T. philippinarum* (Utting and Spencer, 1991; Williams, 1980).

The mean percentage of triploid *T. dorsatus* larvae induced (68%) was higher than the results achieved with *T. philippinarum* by Gosling and Nolan (1989), similar to those by Utting and Child (1994) but lower than those of Beaumont and Contaris (1988) and Dufy and Diter (1990). In this study, concentrations of CB (1 mg/l) and DMSO (0.1%) were based on the method used by Dufy and Diter (1990) and were higher than those used by Gosling and Nolan (1989) and Beaumont and Contaris (1988) for *T. philippinarum*. With further development, the techniques may be improved to provide consistently higher triploidy percentages in *T. dorsatus*. It remains to be seen if the lower survival to metamorphosis of triploid larvae was a product of the chemical treatment or of the additional handling required or both. Similar reduced survival of CB induced triploids has been observed with other clam species, oysters and scallops (Stanley et al., 1981; Tabarini, 1984; Dufy and Diter, 1990). In any case, a mean larval survival to metamorphosis of 18.5 % may be acceptable if the possible benefits of higher meat yield exist with this species or if triploid stocks do prove to have greater disease resistance (Gosling and Nolan, 1989).

Based on the criteria described by Kraeuter and Castagna (1989) for assessing the aquaculture potential of a clam species, *T. dorsatus* would appear promising. *T. dorsatus* is an attractive clam with good market potential that may be enhanced by the similarities it has with *T. philippinarum*. The species is easily spawned and reared and has exhibited rapid growth to a stage at which it may be removed from the hatchery. They are not deep burrowers, usually exposing the distal anterior portion of the shell to feed. In addition, *T. dorsatus* inhabit shallow estuarine areas and therefore should be tolerant of changes in major environmental variables such as temperature and salinity.

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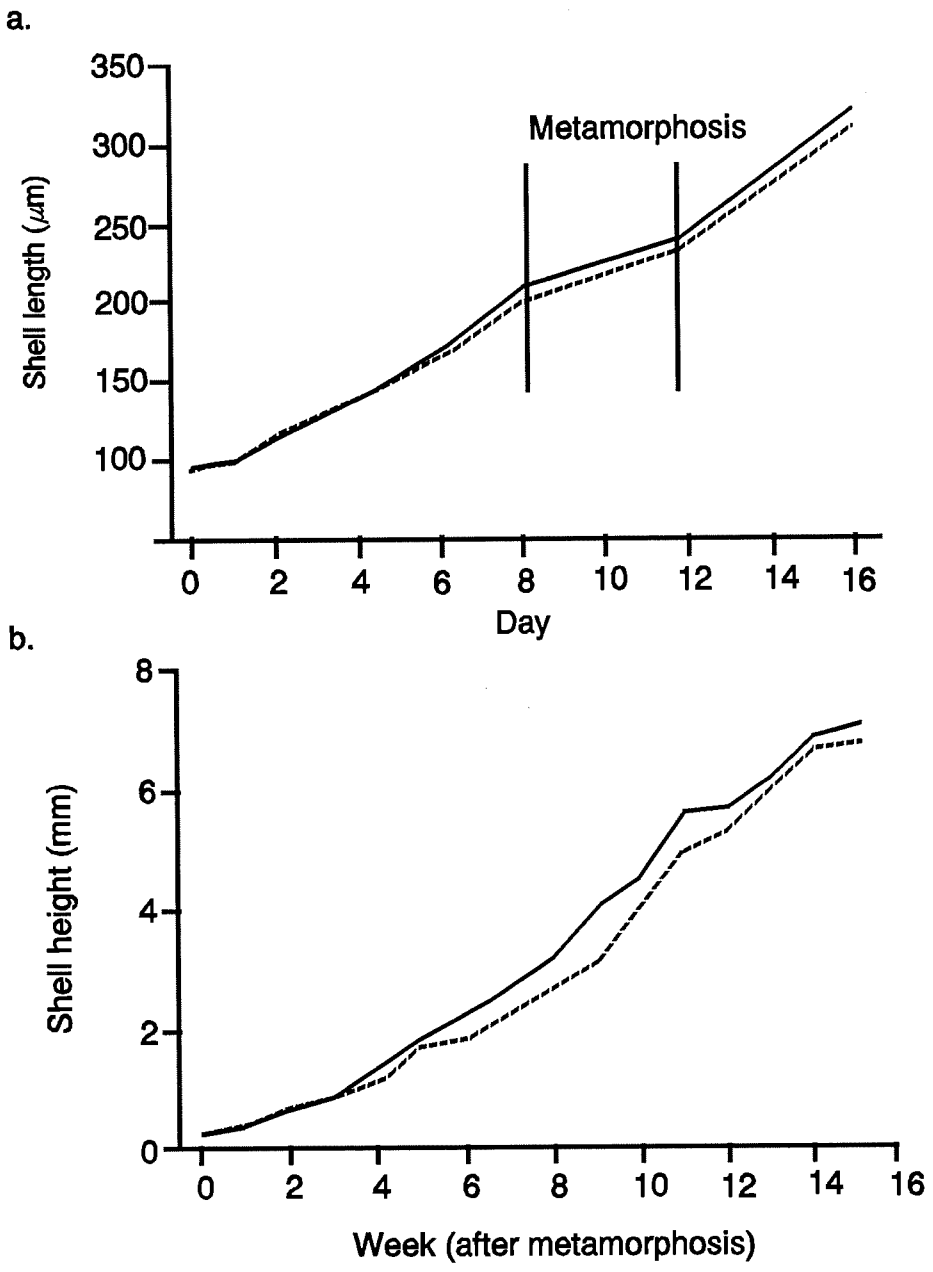
Fig.1 Growth of diploid (——) and triploid (------) *Tapes dorsatus* larvae (a.) and spat (b).



7 REPRODUCTIVE CYCLE AND GONADIAL CHANGES IN TRIPLOID OYSTERS

a Gardner, N. C., Smith, M. S. R., Maguire, G. B., Kent, G. and Nell, J. A. Studies on triploid oysters in Australia. III. Gametogenesis of diploid and triploid Pacific oysters, *Crassostrea gigas* (Thunberg), in Tasmania.

b Cox, E., Smith, M. S. R., Nell, J. A. and Maguire, G. B. Studies on triploid oysters in Australia. VI. Changes in gonad development in diploid and triploid Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley).

c Gardner, N. C., 1995. Verification of triploidy in Pacific oysters, *Crassostrea gigas* (Thunberg), with image analysis.

Studies on triploid oysters in Australia. III. Gametogenesis of diploid and triploid Pacific oysters, *Crassostrea gigas* (Thunberg), in Tasmania.

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Abstract

Gametogenesis of diploid and triploid Pacific oysters, *Crassostrea gigas*, was studied at three intertidal sites in Tasmania, Australia. Sampling began in late summer when oysters were 13 months old and continued over 14 months at two sites and 26 months at the third. Gametogenesis was suppressed in triploids relative to diploids with female triploids characteristically producing few mature oocytes while males did not produce spermatozoa. No evidence of spawning was observed in triploids despite spawning of diploids at two sites. Some triploids were not sexually differentiated during the summer periods; there was a significantly greater ($P<0.05$) proportion of these oysters at the site producing poorer growth (51%) than at the two better sites for growth (25% and 14%). Sex ratio of oysters was influenced by site, ploidy and age. At the poorer site, there were significantly more males than at the other sites ($P<0.05$), for both diploids and triploids. Diploid oysters were predominantly female (up to 89%) at all sites and for both summers (at the one site samped over 26 months). Triploid samples tended to have a greater proportion of males than diploid samples. The proportion of females in both diploid and triploid samples increased from the second to the third summer at the poorer site. Few oysters were hermaphrodites in either diploid or triploid groups. The reduction of gametogenesis in triploid Pacific oysters was greater than has been reported elsewhere although the extent of reduction was influenced by site and

sex.

Keywords: Oyster-Culture, *Crassostrea-gigas*, Triploidy, Gametogenesis

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1. Introduction

Recently, much research has been directed to the production of triploid fish and molluscs to improve performance for aquaculture. In bivalves, several factors contribute to the potential commercial benefits of triploidy including sensory characteristics (Alllen and Downing, 1991), faster growth (Nell et al., 1994) and heterozygosity (Stanley et al., 1984). However, the impact of triploidy on reproduction is of greatest commercial interest in oyster industries (Beaumont and Fairbrother, 1991).

Promising results with triploid oysters in the United States prompted investigation of triploid oysters in Tasmania with the aim of overcoming marketing problems associated with the spawning of diploids in Summer and autumn (Maguire and Kent, 1990; Maguire et al., 1995a). Water temperature has been shown to influence gametogenesis (Shpigel et al., 1992) in Pacific oysters and this implies that gametogenesis will vary with lattitude. This study examined the gametogenesis of triploid Pacific oysters in the most extreme lattitudes reported (42°20'S to 43°20'S); Allen and Downing (1990) examined the gametogenesis of triploid oysters from Humbolt Bay (41°N) and Akashige (1990) examined oysters from Hiroshima Bay (35°N).

In Tasmania, the production of market size Pacific oysters is concentrated in estuarine regions along the eastern and southern coastal areas. Regional variation in gametogenesis was assessed in this study by conducting trials at three distinctly different sites within these regions. Growth rates at these sites differ so that the time required to attain market size may vary from 2 to 3 years (Treadwell et al., 1991; Maguire et al., 1995a). Other studies of gametogenesis in triploid oysters have examined gametogenesis over short periods (3 months) in their first summer and at a single site (Akashige, 1990; Allen and Downing, 1990). In this study, gametogenesis and the sex ratio of oysters were examined over extended periods, including their second and third reproductive seasons, at different sites within Tasmanian waters.

2. Materials and Methods

Husbandry and Sampling

The oysters used in this study were derived from the same groups as those used to assess growth, glycogen content, survival, condition and gonad area (Maguire et al., 1995a). Triploids were produced in February 1990 by treatment with 0.5 mg/l cytochalasin B for 20 min. as described by Allen et al. (1989) and diploid controls were drawn from the same spawning. Single seed spat were transferred from the hatchery and on-shore upwellers to intertidal mesh trays at 2 months. At six months the oysters were relocated to three sites around Tasmania: Little Swanport (eastern), Pittwater (south-eastern) and Birch's Bay (southern; see map, Maguire et al., 1995a). Oysters were held in mesh baskets on intertidal racks and the mesh size was increased from 6 mm to 12 mm during the trials to parallel commercial management. Intertidal growing heights reflected site specific management practices, however, subtidal culture methods were used at one site (Birch's Bay) for three months to improve exceptionally poor growth trends (November 1991 to January 1992).

Sampling occurred at approximately monthly intervals and began in February 1991 when oysters were 13 months old. Sampling continued until May 1992 at Little Swanport and Pittwater. During this period, 351 and 354 diploids and 299 and 289 triploids, from Little Swanport and Pittwater respectively, were collected for histology. Oysters at Birch's Bay took longer to reach commercial size (approximately 60 g whole weight) than at the other two locations so sampling was extended until April 1993. This allowed the reproductive patterns of triploid oysters to be assessed in their third reproductive season. A total of 537 diploids and 479 triploids were sampled from Birch's Bay.

Determination of Triploidy

The ratio of triploids to diploids within the cytochalasin B treated group was estimated, in spat at 6 months and in adults at 26.2 and 30.8 months, with flow cytometry (Chaiton and Allen, 1985). The proportion of triploids within the cytochalasin B treated group remained stable throughout (73-79%) and no triploids were found in the diploid control group. Diploid individuals, within several samples of triploids used for histology, were identified by image analysis of the integrated optical density for haemocyte nuclei (Gérard et al., 1991; Gardner, 1993). Image analysis of haemocytes was done with 155 oysters, sampled from each of the three sites (throughout the trial) and allowed definitive characterisation of gonad development stages for triploids and diploids. The proportion of triploid oysters in the cytochalasin B treated group as estimated by image analysis was similar (81%) to that estimated with flow cytometry (73-79%). From the appearance of these stages, individuals in other monthly samples of triploids were identified as triploids or diploids. Data for diploids within the cytochalasin B treated group are excluded from data sets presented in this paper.

Histology

Transverse slices of tissue were taken approximately 3 mm above the labial palps as recommended by Morales-Alamo and Mann (1989). Tissue was fixed in formal calcium, processed by standard paraffin histology, sectioned at 5 μm and stained with Mayer's haematoxylin and eosin Y.

Measurements of the percentage of tissue occupied by gonad, excluding gills, were taken from histological sections using image analysis (CUE II™ software, IBM™ compatible). Gonad area measurement was performed for samples in which greater than 90% of the diploids sampled were in ripe condition as assessed by examination of histological sections (stages M4 and F4; Dinamani 1974). These samples were: for Little Swanport, February 1992-April 1992; for Pittwater, November 1991 to February 1992; and for Birch's Bay, November 1991 to January 1992, and November 1992 to February 1993.

Gonad tissue from 12 diploid and triploid oysters was processed for transmission electron microscopy (TEM) using 2% paraformaldehyde and 1% gluteraldehyde fixation and standard resin embedding. The ultra-thin sections were stained with lead citrate and uranyl acetate.

Stages of Gametogenesis

The development of gametes in diploid controls was quantified using the staging system described by Dinamani (1974) for diploid New Zealand rock oysters (*Crassostrea glomerata*). This system divided the reproductive cycle into four maturation phases (G1-4) (Fig. 5), a spawned phase (G5) and a fully regressive phase (GX). Some diploids were also classified as indeterminate sex (I), where no gamete development was apparent, and hermaphrodite (H), where both male and female gametes were present.

The system applied to diploids was found to be unsuitable for the staging of triploids due to the different pattern of gametogenesis observed in triploids. A qualitative staging scheme, based partially on that described by Dinamani (1974) for diploid oysters, was created to classify triploid reproductive development. This triploid staging scheme distinguished between males, females, hermaphrodites (H) and oysters of indeterminate sex (I, Fig. 1). Triploid males were further subdivided into two developmental phases (TM1-TM2) and one regressive phase (TMX) while triploid females were divided into four phases (TF1- TF4). The staging of triploid female gonad tissue as regressive was complicated by the presence of numerous phagocytes in most specimens, throughout the sampling period. In addition, many female sections contained relatively few ova. Consequently, the classification of individual females as regressive could not be done definitively and no regressive stage was used for triploid females.

Site and ploidy appeared to affect the period at which oysters matured. The periods for which sex ratios were compared were determined by the proportion of oysters that had developed past indeterminate stage and could be ascribed to a sex group. This proportion was set at 75% for diploids and 35%

for triploids.

Triploid Staging System

Male Gonad Development Phases

These stages generally equate to those of diploids in the staging system described by Dinamani (1974) although development does not continue to maturity. The male gonad development stages correspond to those used in a concurrent study by Cox et al. (1995) for triploid Sydney rock oysters *Saccostrea commercialis*.

TM1 - Follicles contain spermatogonia and spermatocytes but no spermatids are present. The gonad size at this stage can be highly variable in triploids (Fig. 2).

TM2 - Spermatids are seen as small dark stained cells approximately 1.5 μm in diameter. They are present in small numbers, usually in the centre of the follicle. Where there are few spermatocytes, the spermatids are more randomly distributed (Fig. 3).

TMX - This phase corresponds to the regressive MX phase of diploids described by Dinamani (1974). As in diploids, residual gametes are present and numerous phagocytes are observed within the follicle.

Female Gonad Development Phases

These stages are similar to those used by Cox et al. (1995) except that an additional stage was used to define female gonads where immature, developing oocytes are present. Three phases were used to describe the extent of proliferation of apparently mature oocytes. These three divisions permitted an investigation of the developmental synchrony of gametes in female triploids. The staging divisions used for female triploids do not relate to those used for female diploids.

TF1 - Follicles contain developing oogonia and oocytes up to 15 μm in diameter. All oocytes remain attached to the follicle wall by a stalk and cytoplasm is granular and basophilic (Fig. 4).

TF2 - Follicles are small and lined with male-like cells (Figs. 6). Oocytes are round to oval and approximately 35 μm in diameter. The cytoplasm of the oocytes is eosinophilic and generally clear. There are very few oocytes present and these tend to be isolated throughout the gonad (Fig. 7).

TF3 - Most follicles contain a few oocytes. As in TF2, the majority of oocytes appear mature and are round to oval. Some developing oocytes may be present and the follicles are lined with male-like cells and oogonia (Fig. 8).

TF4 - Oocytes appear mature and are round to oval and approximately 35 μm in diameter. All follicles contain oocytes (often numerous) although the follicles are still separated by large areas of connective tissue. Follicles are lined with a basophilic band of male-like cells and oogonia (Fig. 9).

Hermaphrodites

Two forms of hermaphrodites were recognised. The first appeared to result from the incomplete resorption of male gametes before gametogenesis, of female gametes, commenced the following season (Fig. 10). In the second form, both male and female gametes were seen to be developing simultaneously (Fig. 11).

Intertidal Exposure

The daily exposure duration of intertidal racks was recorded with Wesdata™ dataloggers (Kent and Maguire, 1992). Readings were taken from a level equivalent to the base of the oyster basket.

Analyses

The significance of differences in sex-ratio and incidence of sexually indeterminate animals between sites and gonad size between sexes was tested with chi-square (Dixon and Massey, 1983).

3. Results

Gametogenesis of Diploids

In 1991, most diploid oysters (>50%) at Little Swanport and Pittwater began gametogenesis in early winter (June-July, Fig. 12a and 12b). Gametogenesis was initiated later at Birch's Bay where most of the population began development in September 1991 (early spring; Fig 12c). Diploid oysters reached the peak of maturity in summer (December 1991-February 1992) at all three sites and gonads of female diploids at this time contained numerous mature oocytes free within the follicle lumen. In males, the gonad follicles were lined with a band of spermatocytes and spermatids and the centre of the follicle was occupied by spermatozoa. All diploids sampled during that summer had begun gametogenesis. The extent of gonad development, as a percentage of cross-section area, was not significantly different (P>0.05) between males and female diploids (Table 1).

Evidence of spawning was observed in diploid oysters collected in January 1992 at Pittwater and in December 1991 at Birch's Bay. The follicles of diploid oysters from these samples, both male and female, were collapsed and the

remnant gametes appeared disrupted. None of the diploids sampled from Little Swanport appeared to have spawned, and it appeared that not all the oysters sampled at Pittwater and Birch's Bay in the 1991/1992 summer had spawned. In the following summer (1992/1993), at the only remaining site (Birch's Bay), all diploids had begun gametogenesis by November 1992 and those sampled in January 1993 appeared to have spawned.

Resorption of gonads at Pittwater and Birch's Bay in 1991/1992 occurred in late summer (January-March 1992). The absence of a February 1992 sample from Birch's Bay (inadvertently left out of water) prevented the determination of the precise time at which resorption began. It appeared that many of the diploid oysters undergoing resorption at Pittwater and Birch's Bay in Autumn (March - May, 1992) did not spawn. This is supported by the disparity between the proportion of oysters observed to have spawned at these sites and those seen to have undergone resorption by May 1992 (Fig. 12b and 12c). Resorption of gonad tissue at Little Swanport was not observed until May 1992.

Gametogenesis of Triploids

The onset of gametogenesis began at approximately the same time in both female and male triploids (Figs. 13 and 14) and no difference was observed between the onset of gametogenesis in diploids and triploids at Little Swanport or Birch's Bay. However, at Pittwater, some of the diploids had begun gametogenesis in April 1991 yet no triploids were observed to begin gametogenesis until August 1991.

In triploids, the degree of maturity attained by gametes and the size of the gonad was extremely reduced, compared to diploids (Table 1). Many individuals did not develop either spermatogonia or oogonia and remained at "indeterminate" stage (I) through summer. A significantly greater proportion of the triploid oysters sampled from Birch's Bay, in either year, failed to develop spermatogonia or oogonia compared to those cultured at Little Swanport or Pittwater ($P<0.05$). The proportion of oysters at Birch's Bay, that failed to develop gametes, decreased from approximately 30% in the first summer of sampling (1991/1992) to approximately 15% in the second summer of sampling (1992/1993).

The gonad size of male triploids was significantly greater ($P<0.05$) than that of females at all sites (Table 1). The gametes of males were never observed to develop to maturity (spermatozoa) while the oocytes present in females, although generally few, appeared to be mature. No evidence of spawning was observed in triploids and the resorption of gonads in males occurred at the same time as in diploids.

Except at little Swanport, few female triploids developed past the TF2 stage, in which only a few isolated mature oocytes develop. Although numerous spermatocytes were produced by males, few gametes developed beyond this stage and only small numbers of spermatids were observed. Site or age (at Birch's Bay) did not appear to influence the extent of maturation of gametes in

male triploids.

Sex ratio and Hermaphroditism

Sex ratio was influenced by both site and ploidy (Table 2). Differences between sites were most noticeable between Little Swanport and Birch's Bay as both diploid and triploids from Little Swanport had a significantly larger proportion of females ($P<0.05$). A significantly higher ($P<0.05$) proportion of diploid oysters were female than in triploids at both Little Swanport and Birch's Bay (1991/1992 and 1992/1993) although not at Pittwater.

The proportion of hermaphrodites was relatively small at all sites for both diploids and triploids (Table 2) and this prevented meaningful statistical analysis. The highest proportion of hermaphrodites in samples was found in triploids from Little Swanport (6.2%). Most hermaphrodites appeared to have been produced by incomplete resorption of male gametes before the commencement of female gametogenesis (Fig. 10). However, the production of male and female gametes appeared to be occurring simultaneously in three triploid oysters (Fig. 11).

Environmental Variables

The degree of intertidal exposure at Pittwater and Birch's Bay was similar and averaged approximately 10% daily. The exposure of oysters at Little Swanport was far greater than that of the other two sites and ranged from approximately 15% to 60% during the growout phase (Maguire et al., 1995a).

4. Discussion

Gonad Development

A semi-quantitative staging scheme was devised to monitor gametogenesis in triploid Pacific oysters. Many schemes have been developed for staging development in diploid oysters (Kennedy and Battle, 1964; Dinamani, 1974; Mann, 1979) but the erratic and comparatively slight development of gametes in triploids impairs the direct application of these schemes to triploid development. The diploid staging scheme described by Kennedy and Battle (1964) was used by Allen and Downing (1990) to classify triploid development and consists of 4 developing phases, 1 ripe phase, a resorbing phase and 2 resting stages. The scheme presented in this paper simplified the classification of developmental stages to 1 (female) or 2 (male) developing phases so that staging became more consistent, especially where only a few gametes were present in a section. Resorption was difficult to define in female triploids, especially where only a few oocytes were present in a section, so no resorbing phase was used for female triploids.

Gonadogenesis of triploid Pacific oysters in this study was observed to be severely retarded, as has been reported in all of the published studies of gametogenesis in triploid bivalves. This was in respect of the overall size of

the gonad (Table 1) and also the extent of maturity of gametes. Maturation of diploids appeared to be normal when compared with other reports of diploid gametogenesis in Pacific oysters (Dinamani, 1987; Kent, 1990); proliferation of gametes was extensive and mature oocytes and spermatozoa were produced.

Gametogenesis was reduced to such an extent in triploids oysters that they appeared to be effectively sterile. In other research on triploid Pacific oysters, gametogenesis was reduced, but did occur in all oysters (Akashige, 1990; Allen and Downing, 1990). We found that there were many individuals that developed follicles but failed to develop any spermatogonia or oogonia during the reproductive season, which is a similar pattern of gametogenesis to that reported for triploid *Mya arenaria* and *C. virginica* (Allen et al., 1986; Barber and Mann, 1991). The complete suppression of gametogenesis observed in some triploid Pacific oysters in this study may have resulted from environmental factors as the proportion of triploids that remained of indeterminate sex was significantly lower at Little Swanport and Pittwater than at Birch's Bay ($P<0.05$). Although it is impossible to conclusively determine which environmental variable between the sites altered the proportion of oysters in which gametogenesis was blocked, some conditions were clearly different. The greatest proportion of triploids where gametogenesis did not occur was recorded at Birch's Bay. This site sustained a much lower growth rate (Maguire et al., 1995a) and appeared to be more exposed to wave action in comparison to the other two sites. This leverage of site on suppression of gametogenesis may influence the degree to which triploids are useful for culture in different regions.

Gametogenesis of both male and female triploids appeared reduced in comparison to that reported in other trials of triploid Pacific oysters (Akashige, 1990; Allen and Downing, 1990). In contrast to Pacific oysters sampled by Akashige (1990) and Allen and Downing (1990), no male triploid Pacific oysters were observed to have produced mature gametes. It is surprising that development of male gametes occasionally continued through meiosis 2 to the formation of spermatids (Fig. 3), yet the morphological changes associated with formation of spermatozoa did not occur. Interference of cellular processes resulting from an unequal division of chromosomes at meiosis 2 may have repressed the metamorphosis of spermatids to spermatozoa. Whilst apparently mature oocytes were produced by female triploids, these gametes were very sparse. The maximum extent of development of female triploids in this study (Fig. 9) appeared reduced compared to that photographed by Allen and Downing (1990) and was more in accordance with that described by Barber and Mann (1991) for triploid *C. virginica*.

Allen and Downing (1990) and Akashige (1990) reported that triploid Pacific oysters appeared to have spawned. In this study, despite extensive spawning of diploids from Birch's Bay (in January 1993), no triploids appeared to have spawned. This complete suppression of spawning from triploidy has also been reported in *Mercenaria mercenaria* and *C. virginica* (Eversole et al., 1992; Barber and Mann, 1991).

Thus, a combination of factors indicate that reproduction is blocked in triploid Pacific oysters in Tasmanian conditions: gametogenesis is totally blocked in some oysters so that no gametes are produced; where gametogenesis does occur in males, gametes do not mature to spermatozoa; gametes are produced in low numbers compared to diploids; and spawning does not appear to occur. Although the triploid oysters sampled in this research appeared to be sterile, Akashige (1990) found that the spermatozoa of triploid Pacific oysters were able to fertilise eggs produced by diploid females. Allen and Downing (1990) noted that spermatozoa from triploid Pacific oysters are viable and capable of producing diploid embryos. More recently, triploid Pacific oysters have been strip spawned to provide gametes for the production of tetraploids (Guo and Allen, 1994). The more extreme suppression of gametogenesis in triploid Pacific oysters observed in our study compared to that reported by Allen and Downing (1990) and Akashige (1990) may have resulted from different environmental conditions. Tasmanian water temperatures are cooler than in Hiroshima (35°N) where Akashige's (1990) research was conducted (Maguire et al., 1995a) but our sites were at latitudes (42° - 43°S) comparable to Humbolt Bay, California (approximately 41°N) used by Allen and Downing (1990).

Sexual Differentiation

The sex determination mechanism proposed by Haley (1977) implies that increasing the number of "male-type" alleles through triploidy will increase the likelihood of the production of male gametes in triploids. This may have contributed to the increased proportion of males observed in triploids compared to diploids in our study. Other researchers have found that the sex ratios of triploid and diploid Pacific oysters sampled after one year were equivalent (Akashige, 1990; Allen and Downing, 1990). Allen et al. (1986) found that two-year-old triploid *Mya arenaria* were predominantly female (93%). An increase in the proportion of males within triploid groups has only been reported elsewhere in hybrids of *C. rivularis* with *C. gigas* (Downing, 1988).

Dinamani (1987) assessed the sex ratio of one year old diploid Pacific oysters and found that there was a preponderance of males. In following seasons, the ratio tended toward an equal ratio between males and females. The diploid oysters at Birch's Bay did not follow this pattern, rather, the ratio of females to males increased from 1.4:1 to 4.3:1 from the second to the third summer. Site affected sex ratio in both diploid and triploid groups as a significantly greater ($P<0.05$) proportion of the sample was female at Little Swanport than at Birch's Bay. Many environmental factors have been shown to alter the sex ratio in oyster stocks and it has been suggested that stressful conditions cause an increase in the proportion of males (Kennedy, 1982). The oysters cultivated at the three sites sampled in this study were clearly subjected to different environmental conditions as growth rates (Maguire et al., 1995a) and the extent of intertidal exposure varied considerably. The poorer growth of oysters

at Birch's Bay (Maguire et al., 1995a) indicates that the proportion of males increased in the more stressful environment as suggested by Kennedy (1982).

The larger proportion of females in triploid oysters from Little Swanport and Pittwater compared to Birch's Bay may improve the feasibility of triploids for commercial culture in these estuaries. Allen (1988) reported female triploids mobilise less glycogen during gametogenesis than males, and increased concentration of glycogen has been associated with improved meat quality in Pacific oysters as assessed for North American markets (Allen and Downing, 1991). This may be of less relevance to Australian consumers who do not necessarily exhibit a preference for oysters with high glycogen content (Maguire et al., 1995b).

Oysters were classed as hermaphrodites where developing or mature oocytes were present in the same section as spermatocytes, spermatids or spermatozoa. The follicles of all triploid females were lined with cells that appeared to be spermatogonia based on transmission electron microscopy. These cells formed a dark staining band that lined the follicle and they were not present in diploid females. It was considered that the presence of these cells did not constitute a basis for classification of the oyster as a hermaphrodite until gametogenesis continued to the formation of spermatocytes. Hermaphrodites occurred infrequently in both triploid and diploid groups, which was also reported by Akashige (1990). Due to the low number of hermaphrodites found in this study (7 in 1242 diploids, 11 in 1067 triploids), differences between the groups could not be assessed statistically. The proportion of hermaphrodites in the samples tested by Allen and Downing (1990), which was as high as 60% of triploids in one sample period, was dramatically greater than that met with in this study. Haley (1977) suggested that sex in oysters is determined by the two alternative alleles, male and female, at a minimum of three-gene loci. This indicates that the incidence of hermaphroditism (and sex type) in triploids will be influenced by the selection of broodstock and can be expected to differ between experiments. The numbers of broodstock used by Allen and Downing (1990) and Akashige (1990) were not reported but in our study (10 broodstock oysters) and that of Barber and Mann (1991; 5 broodstock oysters) the number of broodstock may have been sufficiently low for such a difference to occur.

Acknowledgments

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Table 1.
Gonad area as a percentage of whole cross-section area (excluding gills) of two and three year old Pacific oysters (*Crassostrea gigas*) from three sites in Tasmania, Australia.

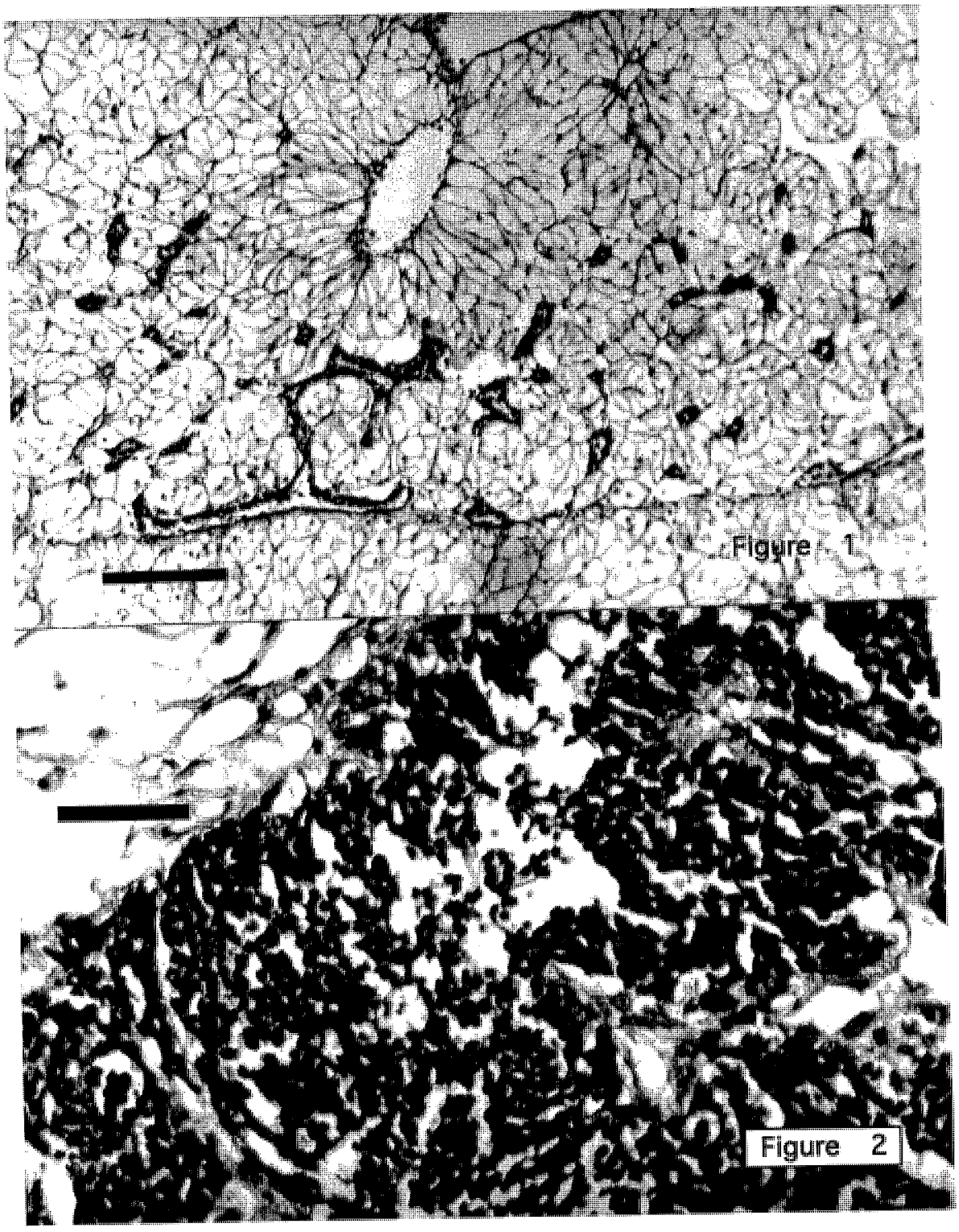
	Male[1]	Female[1]	Male vs Female
Diploid			
Little Swanport	71.5 ± 2.0 (8)	70.0 ± 6.7 (64)	N.S.
Pittwater	57.3 ± 15.5 (28)	50.7 ± 18.5 (65)	N.S.
Birch's Bay (1991/92)	36.6 ± 16.8 (32)	32.9 ± 20.1 (50)	N.S.
Birch's Bay (1992/93)	23.4 ± 14.2 (15)	26.1 ± 16.2 (65)	N.S.
Triploid			
Little Swanport	13.4 ± 8.4 (11)	4.4 ± 3.9 (34)	***
Pittwater	17.1 ± 17.1 (11)	3.9 ± 3.8 (35)	*
Birch's Bay (1991/92)	10.1 ± 8.5 (29)	2.7 ± 1.4 (9)	*
Birch's Bay (1992/93)	10.4 ± 7.5 (24)	2.6 ± 0.8 (17)	**

[1]Data was drawn from samples in summer where > 90% of diploid oysters sampled had gonads in ripe condition (mean ± standard deviation (n)).
*** = P<0.001; ** = P<0.01; * = P<0.05; NS = P>0.05

Table 2.
Sex of two and three year old Pacific oysters (*Crassostrea gigas*) from three sites in Tasmania, Australia.

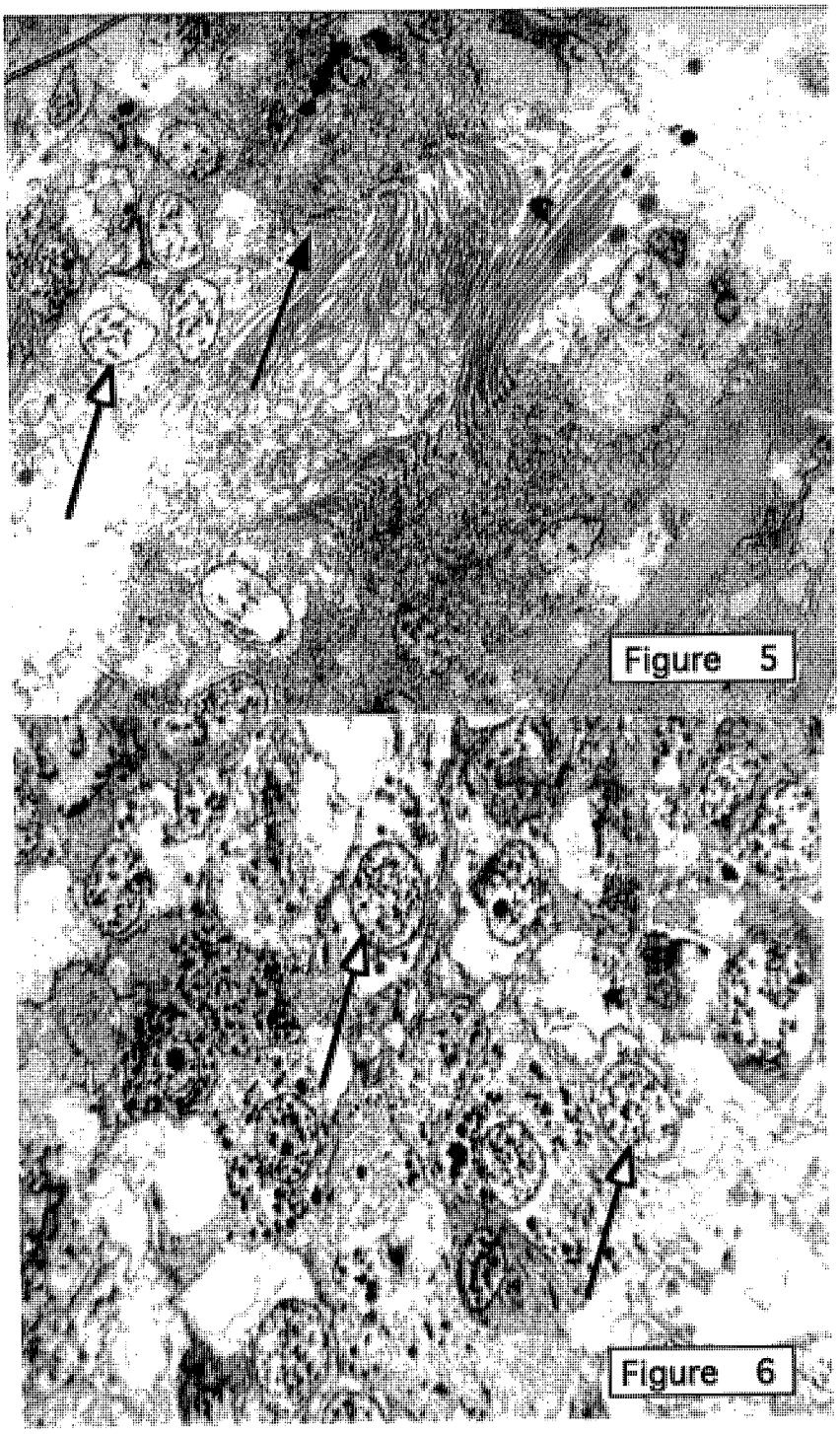
	Male (%)	Female (%)	Hermaphrodite (%)	Indeterminate (%)
Diploid[1]				
Little Swanport [a]	24 (9.2)	231 (88.8)	3 (1.2)	2 (0.8)
Pittwater [b]	50 (30.3)	111 (67.3)	1 (0.6)	3 (1.8)
Birch's Bay (1991/92) [b]	72 (37.1)	103 (53.1)	3 (1.6)	16 (8.2)
Birch's Bay (1992/93) [c]	15 (17.9)	65 (77.4)	0 (0)	4 (4.8)
Triploid[1]				
Little Swanport [a]	19 (17)	71 (63)	7 (6.2)	16 (14)
Pittwater [a]	11 (17)	35 (55)	2 (3.1)	16 (25)
Birch's Bay (1991/92) [b]	30 (39)	8 (10)	0 (0)	39 (51)
Birch's Bay (1992/93) [c]	24 (34)	19 (27)	2 (2.8)	26 (37)

Data was drawn from samples where > 75% of diploid oysters sampled had commenced gametogenesis or > 35% for triploids.
[1]Within a single ploidy group, sites sharing a common superscript do not have a significantly different ratio of males to females ($P>0.05$).



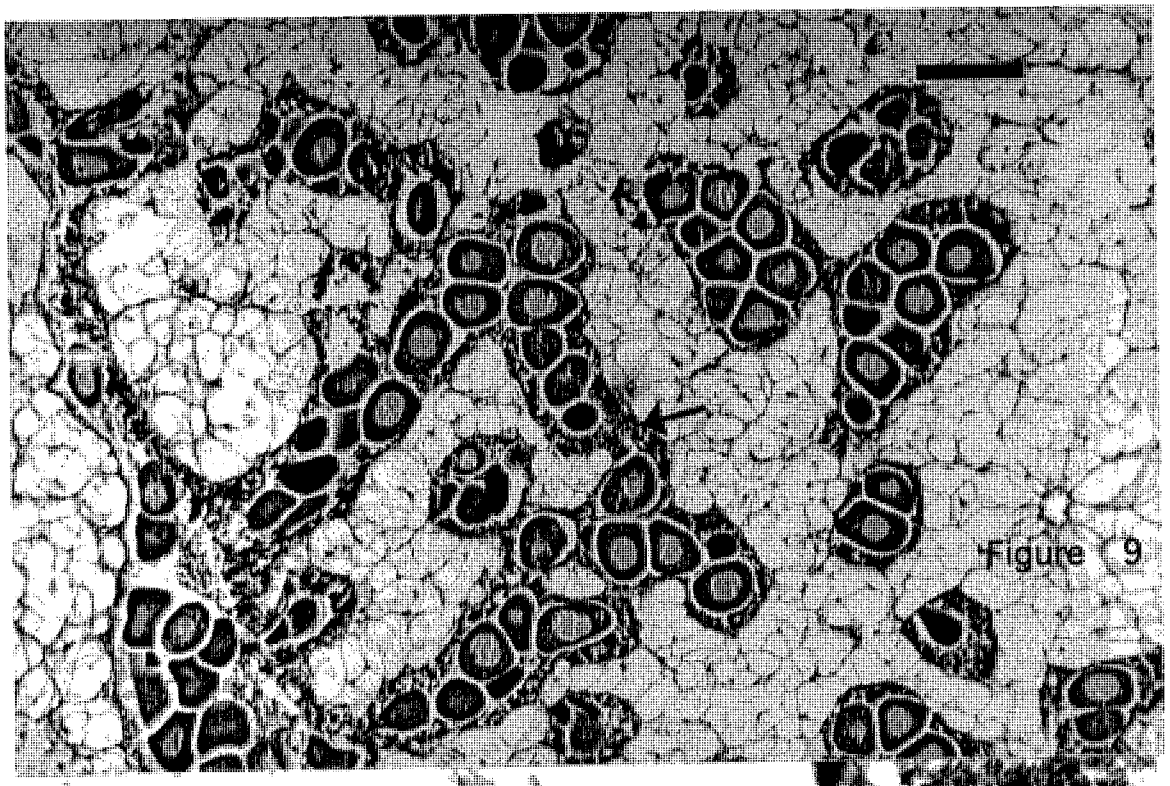
Figures 1 and 2: Stages in the development of gametes in triploid Pacific oysters (*Crassostrea gigas*). **1**, Indeterminate stage (I), follicular development is slight and no spermatogonia or oogonia are seen. Bar = 110 µm. **2**, TM1 stage, spermatogonia and spermatocytes (often numerous) are present. Bar = 25 µm.

Figures 3 and 4: Stages in the development of gametes in triploid Pacific oysters (*Crassostrea gigas*). 3, TM2 stage, spermatids are visable (arrow), usually within the centre of the follicle. This is the maximum extent of development observed in triploid males. Bar = 25 µm. 4, TF1 stage, developing oocytes with basophilic cytoplasm (arrow) are observed attached to the follicle wall. Bar = 20 µm.



Figures 5 and 6: Transmission electron micrographs of gonad tissue from Pacific oysters. **5**, male diploid with normal spermiogenesis. Both spermatozoa (solid arrow) and spermatogonia (hollow arrow) are present. **6**, lining of follicle from a female triploid showing the presence of cells (hollow arrows) similar to the spermatogonia seen in diploid males.

Figures 7 and 8: Stages in the development of gametes in triploid Pacific oysters (*Crassostrea gigas*). **7**, TF2 stage, single, large oocytes are observed infrequently within the lumen of follicles. Bar = 20 μm. **8**, TF3 stage, most follicles contain large oocytes. Bar = 60 μm.



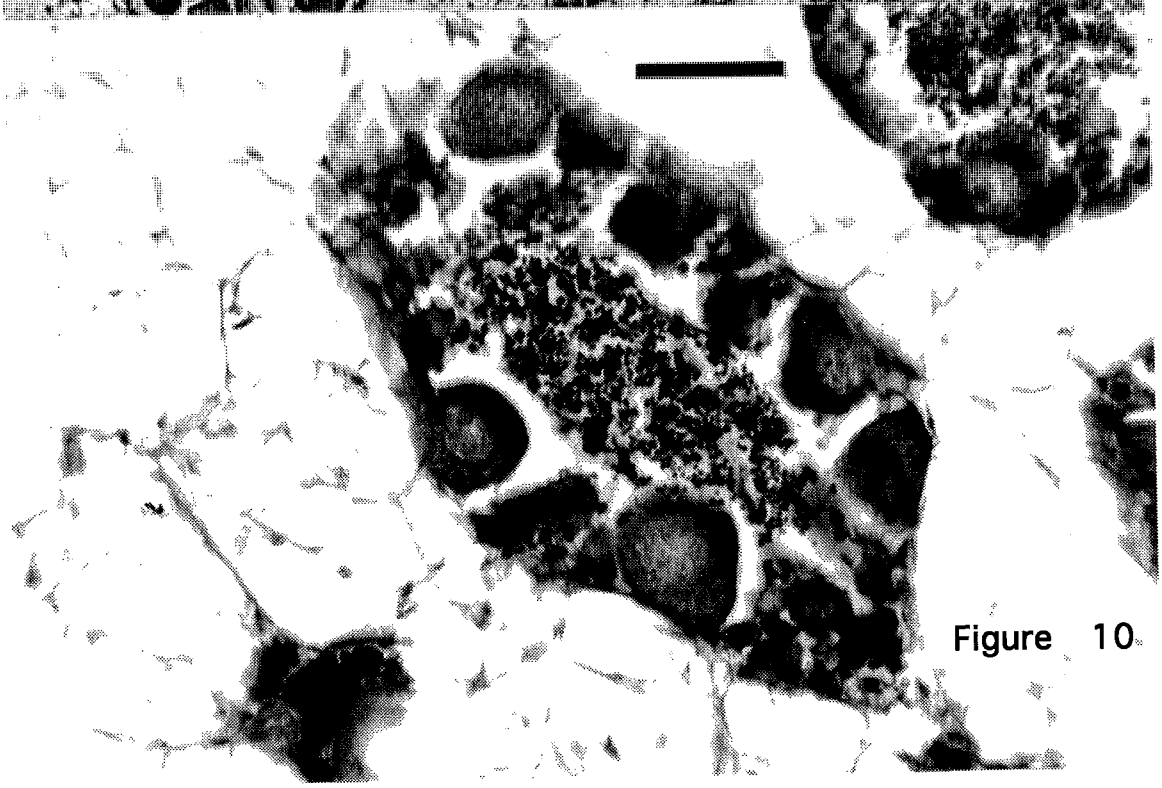


Figure 9. Stages in the development of gametes in triploid Pacific oysters (*Crassostrea gigas*). TF4 stage, large, apparently mature oocytes are plentiful within follicles. This is the most developed female gonad seen in triploids. Characteristically, follicles of females are lined with male-like cells (arrow). Bar = 55 µm.

Figure 10. Example of one of the two forms of hermaphrodite observed in Pacific oysters. Spermatozoa are present in the centre of the follicle which is lined with developing oocytes. There is no evidence of the concurrent development of male and female gametes. This form was seen in both diploids and triploids. Bar = 20 µm.

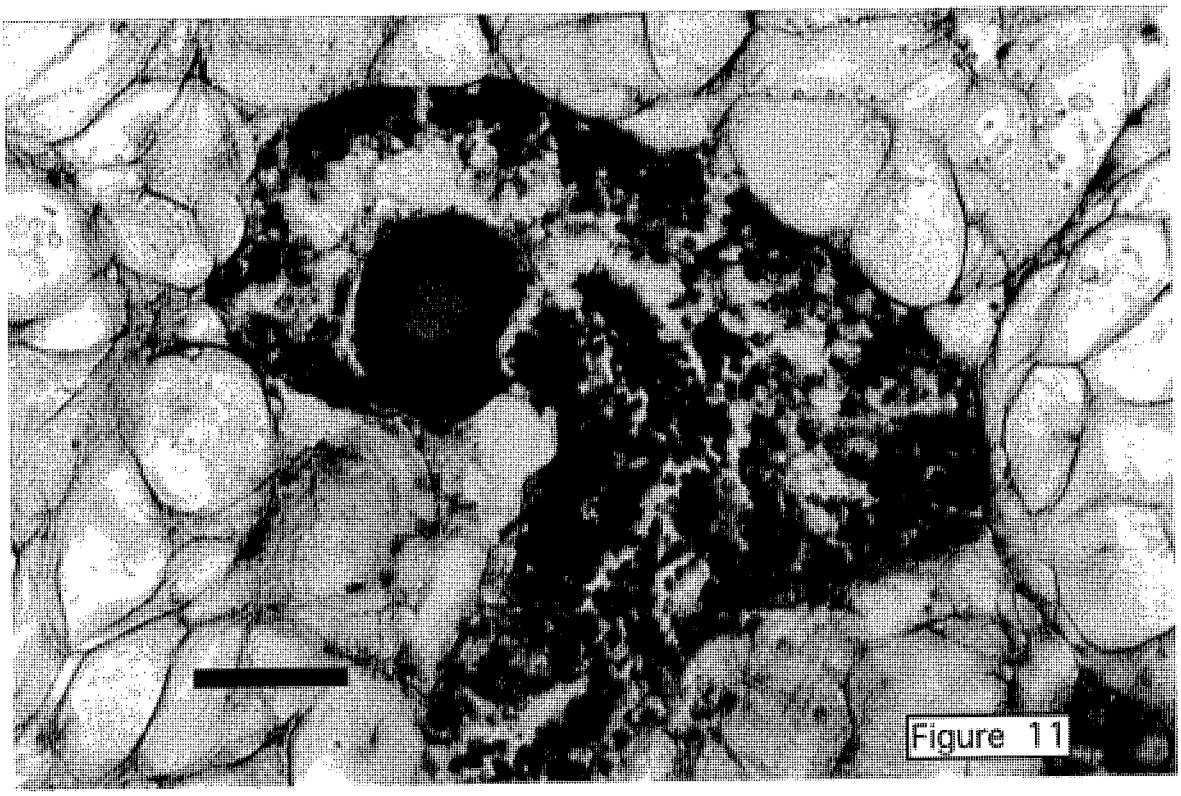


Figure 11: example of one of the two forms of hermaphrodite observed in Pacific oysters (*Crassostrea gigas*). Concurrent development of male (spermatocytes) and female (oocytes) gametes occurs. This form of development was observed in three triploids. Bar = 20 µm.

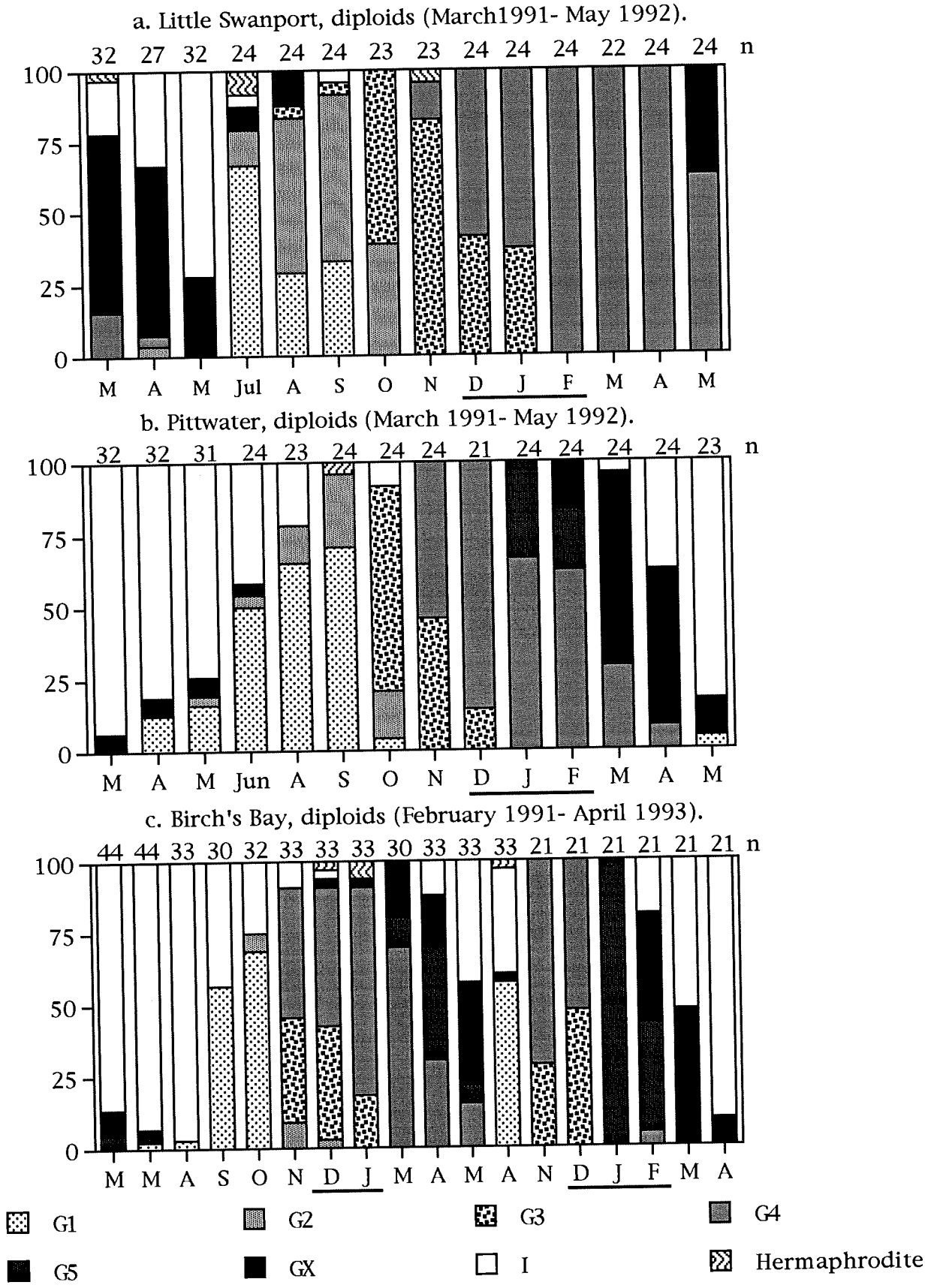


Fig.12: a-c. Gonadial stages (sex groups combined) of diploid Pacific oysters (*Crassostrea gigas*) from three Tasmanian sites expressed as a percentage of the sample. Gonadial phases classified as described by Dinamani (1974). Summer months are underlined.

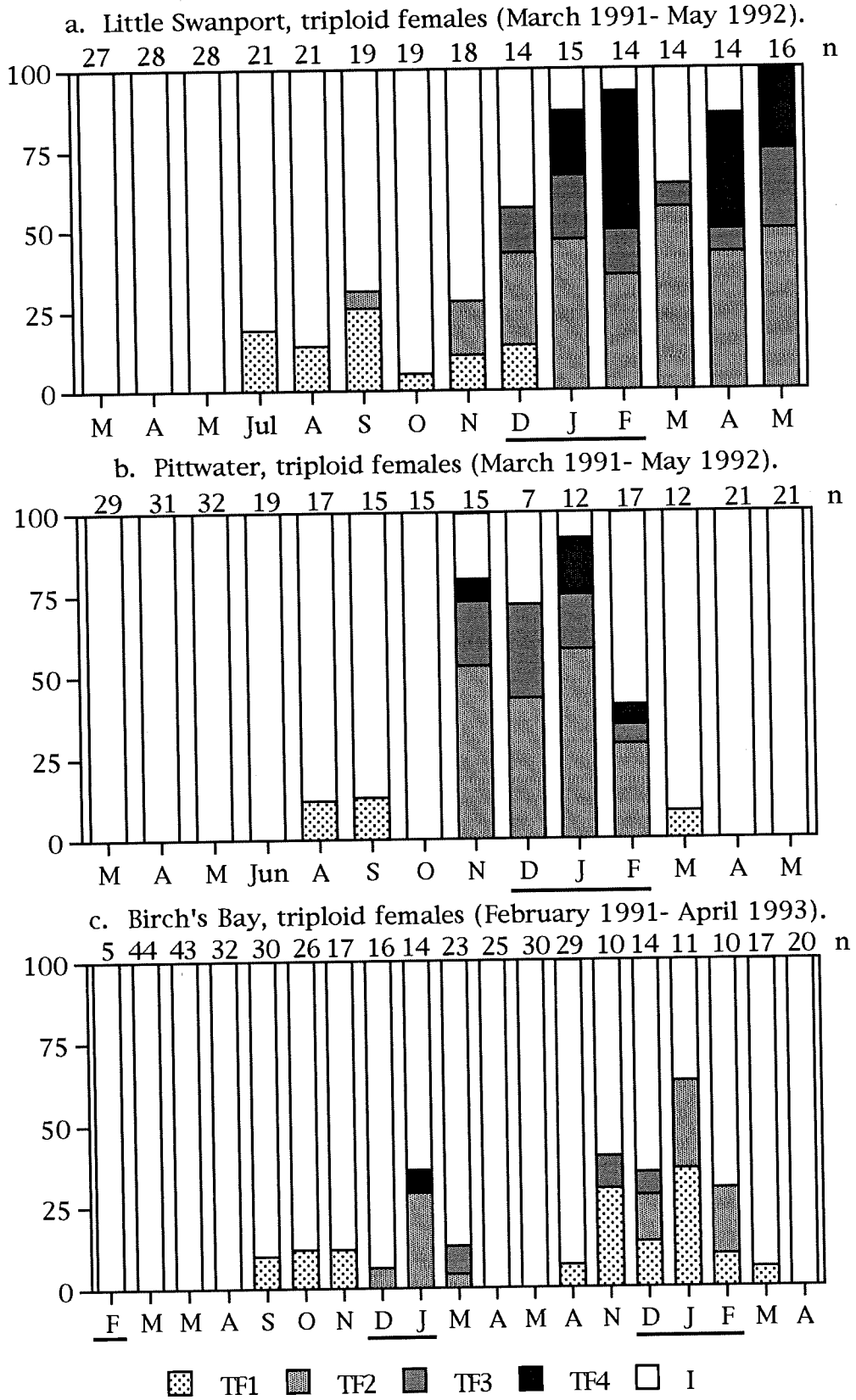


Fig.13, a-c. Gonadial stages of female triploid Pacific oysters (*Crassostrea gigas*) from three Tasmanian sites expressed as a percentage of the sample. Gonadial phase classification is described in text. Summer months are underlined.

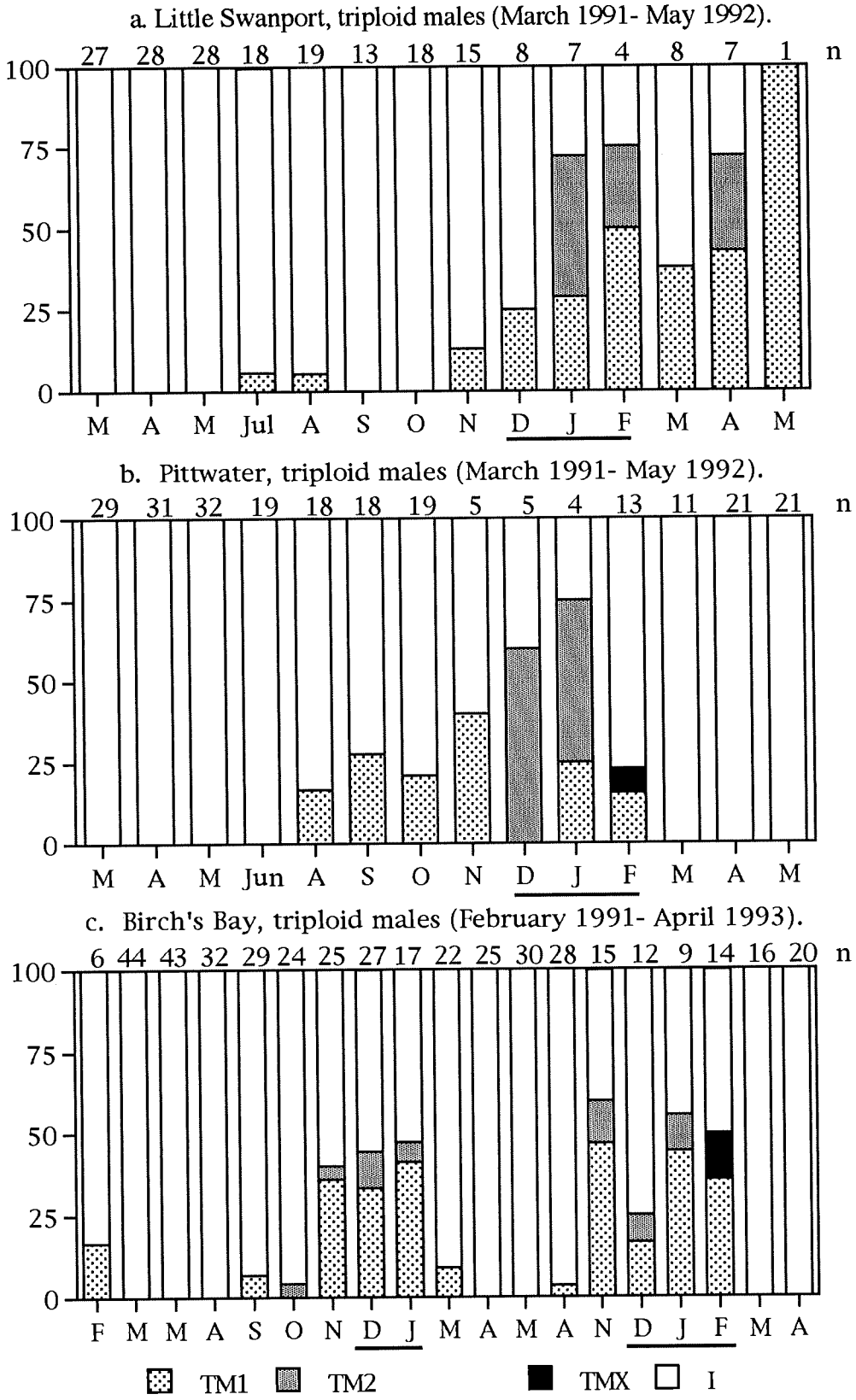


Fig.14: a-c. Gonadial stages of male triploid Pacific oysters (*Crassostrea gigas*) from three Tasmanian sites expressed as a percentage of the sample. Gonadial phase classification is described in text. Summer months are underlined.

Studies on triploid oysters in Australia. VI. Gonad development in diploid and triploid Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley)

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Abbreviated running title: Gonadial changes in diploid and triploid Sydney rock oysters.

Abstract: Triploid Sydney rock oysters, *Saccostrea commercialis*, (produced using cytochalasin B to treat newly fertilised eggs) were sampled from 18-30 months of age to encompass a full reproductive season. A comparative assessment of the extent of gametogenesis was made for both diploid and triploid Sydney rock oysters. It was necessary to develop separate staging criteria for the triploids. Triploid gametogenesis was highly retarded compared to the diploids, in particular for triploid females which developed abnormally; follicle branching was severely retarded with few mature ova present. Development in the male appeared to halt at spermatocyte formation; on the few occasions that spermatids were present they were sparsely distributed throughout the gonad. In the thirteen months of sampling, there was no evidence of spawning in the triploids.

Key words: gametogenesis, oyster, *Saccostrea commercialis*, triploid.

Introduction

Triploidy in oysters provides commercial benefits including faster growth (Beaumont & Fairbrother, 1991) increased heterozygosity (Allen & Downing, 1986; Stanley *et al.*, 1984; Nell *et al.*, 1994) and reduced sexual maturation. In the US, triploid Pacific oysters *Crassostrea gigas* are used primarily to produce a marketable, less gravid oyster over the summer months when diploids mature sexually. Triploids also have higher glycogen levels which increases consumer acceptability (Allen & Downing, 1991). The Sydney rock oyster (*Saccostrea commercialis*: Iredale and Roughley), however, is accepted by consumers when it is ripe, and so the main benefit of triploidy lies in the increased growth (Nell, 1993). Importantly, reduced maturation of triploids allows glycogen reserves to be utilised for somatic growth rather than being channelled into the production of gametes.

Although the process of gametogenesis has been reasonably well described for diploid oysters there have been very few studies of the annual cycle of triploid oysters and their relationship to diploids. In the few studies which do provide detailed histological descriptions of triploid bivalves (Allen & Downing, 1990; Komaru & Wada, 1989; Akashige, 1990) the development of gametogenesis has been shown to be abnormal with sexual maturation being considerably retarded. Triploid oyster histology has been documented for both the American oyster, *Crassostrea virginica,* (Barber & Mann, 1991) and the Pacific oyster (Allen & Downing, 1986, 1990). However, for the Sydney rock oyster, *Saccostrea commercialis*, only the gonadal histology of diploids of the closely related New Zealand rock oyster *Crassostrea glomerata* has been described (Dinamani, 1974). *S. glomerata* is considered to be a sub-species of *S. commercialis* (Buroker *et al.*, 1979). This present histological study was initiated to compare changes in gametogenesis and the seasonal gonadal cycle (particularly during the diploid spawning seasons) of both diploid and triploid Sydney rock oysters from two different sites within Port Stephens, NSW; and to develop a staging procedure for triploid Sydney rock oysters.

Materials and Methods

Triploids (85% triploidy) used in this study were induced using cytochalasin B as described by Downing and Allen (1987) and Allen *et al.* (1989) for the Pacific oyster. Untreated eggs from the same spawning were used as the control diploid oysters. Details of larval and spat rearing are described fully in an associated study by Nell *et al.*, 1994. Spat were deployed on trays to two sites (one subtidal and one intertidal) in Port Stephens, NSW (32°45'S, 152°10'E) where they were grown for eighteen months before collection of samples (November 1991 to November 1992) commenced (Nell *et al.*,1994). Samples consisting of 24 diploid and 24 triploid oysters were randomly selected from both sites on a monthly basis. There were no histological differences between sites for either male or female triploids or diploids and consequently data were combined for analysis.

Staging for the diploid oysters was based on stages of oogenesis and spermatogenesis previously described by Dinamani (1974) for *C. glomerata.*

Histology

A cross section was taken from each oyster by cutting a standard transverse (anterior) section level with the join of the labial palps and the gills and a second cut was taken approximately 3 mm below this section. The tissue was placed in Davidson's solution (Shaw & Battle, 1957) for 24 h before routine histological processing. Davidson's solution was the preferred fixative for marine invertebrates and produced minimal shrinking of the reproductive tissues. Standard paraffin embedded sections cut at 5-7 μm were stained with haematoxylin and counterstained with eosin. For electron microscopy, samples (N = 12) of the reproductive organs were fixed in a solution of 2% paraformaldehyde and 1% glutaraldehyde. Following staining with osmium tetroxide, ultramicroscopic sections of diploid and triploid specimens were examined.

Gonad Area Index (GAI)

The layer or thickness of gonad in oysters increases as gametogenesis progresses, whereas spawning causes a reversal of this process. Gonad area indices may therefore assist in estimates of the extent of gametogenesis. GAI values for this study were determined using image analysis with a Graphics Tablet (Apple Computer Company, CA) and Camera Lucida (Wild, Heerbrugg, Switzerland) as described by Morales-Alamo & Mann (1989) with final GAI being expressed as a percentage, i.e. the part of total body area occupied by the gonad (x100).

GAI data for male, female and base triploids were combined for graphical presentation as there were insufficient numbers to graph the sexes individually.

Statistical analysis

The male to female sex ratios were analysed for significant differences (P=0.05) by use of a Chi-square contingency table (Winer et al., 1991).

Results

Diploid Gametogenesis

Diploid males (M1-MX) and females (F1-FX) were assessed in six stages of development, following Dinamani (1974) and are detailed as follows:

Diploid males

M1: During the experimental period (Nov. 1991 to Nov. 1992) this stage was rarely observed. It is the period when the follicles contained mostly spermatogonia and very few spermatocytes. **M2**: Spermatogonia 5 - 6 μm in diameter were attached to the follicle wall and were associated with a band of primary and secondary spermatocytes on the luminal side. A few spermatids with dark round profiles were observed but no tails of spermatozoa were present.
M3: This stage was identified by rapid development in the spermatogenic cycle. There were many spermatids and a few spermatozoa present in the centre of the follicle, with the interstitial connective tissue still prominent. **M4**: The follicular tissue occupied most of the gonad and a small amount of interstitial connective tissue was present. The most notable feature of this stage was the streaming of the spermatozoa into the lumen (Figs. 1 & 2). **M5**: A distinct period of spawning was difficult to observe as it was rarely a complete process, but an indicative feature was the reappearance of prominent spermatocytes close to the basement membrane of the follicle. **MX**: At the end of the period the follicles had reduced in size to that approximating the M2 stage and there was commonly an increase in cells with small round nuclei, haemocytes, which have been classified as a phagocytic cell.

Diploid females

F1: Indeterminate gonia began to differentiate at this stage and follicles were small and formed a small proportion of the gonad. Primary oocytes 9-19 μm in diameter and secondary oocytes were attached to the follicular wall. **F2**: Follicles were more developed than in F1 but there were still large areas of interstitial tissue

present. Secondary oocytes ≤25 μm in diameter were attached to the follicle wall. Most oocytes were elongated, irregular in shape and attached by a peduncle and the secondary oocytes had a granular and strongly basophilic cytoplasm (Fig. 3).
F3: During this period the extent of the follicles increased and most of the secondary oocytes were still ≤ 25 μm in diameter. Late in this stage, oocyte peduncles became more elongated and some oocytes were observed free in the lumen (Fig. 4).
F4: At this stage follicles occupied the majority of the ovary and there were many mature, ripe ova observed free in the lumen; these were oval in shape and ≥35 μm in diameter. **F5**: This was assessed as the most advanced stage of spawning when all or part of the gonad had started to discharge ova from the follicles. At the end of this stage phagocytes (haemocytes) increased in the follicles and were present with residual oocytes and ova. There were marked similarities between this stage and stages found in some triploid specimens. **FX**: In this period the follicles were contracted and a few relict ova were observed and the proportion of interstitial tissue increased markedly (Fig. 5).

R: (Base diploid in Table 1). This is an indeterminate period when it is difficult to determine the sex of the oyster. There was an abundance of interstitial tissue and the follicles are greatly contracted. The base triploids resemble this stage (Fig. 6).

The monthly frequency of the gonadial cycle of diploid male Sydney rock oysters (Fig. 7) demonstrates that although a large portion of male diploids spawned between March and July 1992 there did not appear to be a specific spawning period as there were males in spawning condition throughout most of the year. By Aug., many males had spawned and were regressing as evidenced by increasing numbers of haemocytes invading the tissues. In Nov. 1991 there was an increase in spawning which was not observed in Nov. 1992, and did not correspond to an equivalent pattern in the females.

In contrast to the prolonged spawning period of the males, the monthly frequency of the reproductive cycle of the female diploids (Fig. 8) indicated that most females spawned in the period from Jan. to May 1992 with large numbers of regressive females present in the winter months. Both male and female samples from Nov. 1992 indicated more advanced development than in Nov. 1991 probably as a result of this being the second year of spawning.

Triploid Histology

Gametogenesis in the triploid Sydney rock oyster was abnormal and differed from the diploids to such a degree that separate staging criteria had to be developed. The most notable difference from studies on triploid Pacific oysters was that few males were observed with spermatids present and there was no evidence of spawning in contrast to findings by Allen and Downing (1990), who observed extensive spermatid differentiation and also spawning of both male and female triploids. In this study, triploid oysters have been classified as; base triploids, those with minimal gonadal development; male triploids, those with a predominance of male-like gonadal development and female triploids, those with a predominance of female - like development.

Indeterminate Triploid

The indeterminate triploid can be loosely compared with the indeterminate stage of the diploid oyster. While the indeterminate diploid stage had neither male or female cells, the indeterminate triploid appeared to have only male cells, in this case spermatogonia and primary spermatocytes. Follicles were greatly contracted and the cells were found in a tight band around the basement membrane with all specimens having aggregations of dark staining cells which were classified as aberrant spermatogonia (Fig. 9). Four main cell types were present in the follicular wall at this stage: (i) oval cells about 7 - 8 μm in diameter usually near the basement membrane of the follicle. These could be equated with the spermatogonia (Fig. 9); (ii) larger dark staining cells about 8 - 10 μm in diameter and often in aggregations, probably aberrant spermatogonia (Fig. 6); (iii) spermatocytes which were spherical cells with obvious chromatin in strands in the nucleus (Fig. 9), and (iv) the last group were smaller cells being 4-5 μm in diameter, oval and darkly stained. These cells (haemocytes) were present in large numbers in the follicles and can be phagocytic.

With ultrastructural examination of the indeterminate triploid gonad, the spermatogonia and spermatocytes were obvious and there appeared to be considerable vacuolation of the cytoplasm in a majority of the cells (Fig. 10). The interstitial tissue formed the main component of the indeterminate triploid gonad.

Male Triploids

Male triploids in the Port Stephens population have been classified into two stages based on the presence or absence of spermatids. The cells lining the follicular wall were similar to those observed in the base triploid. The majority of male triploids attained spermatocyte differentiation and then development appeared to be arrested. In a few triploid males spermatids were sparsely distributed in the follicles. In over 300 samples of male triploids, there was no evidence of spawning.

TM1: Spermatogonia had differentiated into spermatocytes as observed in the base triploid but nests of dark staining spermatids were present (Fig. 11). **TM2**: This stage (Fig. 12) was rare and many spermatids were observed in the follicle lumen and the follicular basement membrane wall was not obvious. The spermatids had not developed into spermatozoa and spermatozoa were not observed streaming into the lumen.

Female Triploids

Female triploids demonstrated various stages of oogenesis but only development up to secondary oocytes, as described in the diploid staging, was observed. The nucleus often had chromatin strands at the periphery and commonly development appeared to be abnormal. Many oocytes had light staining nuclei or were anucleate, which is associated with cell death. The follicles were mostly contracted with the tight packing of the base triploid male like cells at the basement membrane. Female triploids have been assigned three stages which were based on the numbers of immature oocytes present and not maturity, which is difficult to determine in the triploid oyster.

TF1: This stage is demonstrated in Fig. 13 where the spermatogonia and spermatocytes were observed in the follicular wall and there were randomly scattered immature oocytes present. Most female triploids appeared to be in this category (Fig. 14). The numbers of haemocytes (cell type 4 in base triploids), appeared to decrease at this stage. **TF2**: At this stage most follicles had few immature oocytes present and the maturity of the cells varied within the gonad and between oysters assessed at the same stage. Some had mature ova and others a combination of mature and developing oocytes. This stage is demonstrated in Fig. 15 where the increase in oocytes is obvious and the follicular wall is similar to TF1. The ultrastructure of the oocyte and the surrounding spermatocytes is demonstrated in Fig. 16. **TF3**: Very few triploids were assessed as TF3 (Fig. 17) which resembled the wasted diploid female although the follicular wall is more complex in the triploids.

The most common stage present in the triploid Sydney rock oysters was the indeterminate triploid which was most abundant from July to September (Fig. 14). The triploid females were at a peak from March to May which mirrored the spawning period of the diploid female, however, the male triploids did not demonstrate a distinct pattern which could correlate to diploid males spawning. To add to the evidence indicating that the triploid Sydney rock oyster is sterile, a comparison of the GAI of diploid males and females and triploid males/females combined was undertaken (Fig 18). Only in November 1992 (when diploid indices averaged 40) did the highest triploid GAI of 26 reach the lowest recorded diploid index of 25 (recorded for post-spawning females in September, 1992) suggesting that triploid gonads did not mature sufficiently to allow spawning to occur.

Sex Ratio

The sex ratio (Table 1) for male and female diploids over the entire sampling period was 1:1.3. During sampling, only one hermaphrodite was found and only 2% of oysters were of an indeterminate sex. The male to female ratio for triploids (1:1.8) did not differ significantly ($\chi^2 = 2.339$ (df 1); $P>0.05$) from the diploid sex ratio. Four triploid hermaphrodites were found but the most notable difference between diploids and triploids was the large number of indeterminate triploids (57%), as opposed to 2% for diploids.

Discussion

Triploid Sydney rock oysters exhibited abnormal and severely retarded gametogenesis which was similar to previous observations for Pacific oysters (Allen & Downing, 1990); scallops, *Chlamys nobilis* (Komaru & Wada, 1989) and *Argopecten irradians* (Tabarini, 1984), and the soft-shell clam *Mya arenaria* (Allen *et al.*, 1986). Triploid male Sydney rock oysters advanced to the stage of spermatocyte differentiation and females developed to secondary oocytes before development appeared to be arrested. Few spermatids and no spermatozoa were observed in male triploids. Male and female diploids appeared to develop normally with gametogenesis being similar in the closely related species, *C. glomerata* (Dinamani, 1974) and also the Pacific oyster (Allen & Downing, 1990). Full differentiation was achieved for both sexes with large numbers of mature ova and

spermatozoa present in the gravid gonads.

In earlier studies of gonadogenesis in triploid marine bivalves it was observed that the GAI for triploids was smaller than for diploids and the extent of gonadal cellular development was significantly reduced although the triploids tended to follow the gonadal development of the diploids (Allen & Downing, 1986; Komaru & Wada, 1989; Akashige, 1990; Allen & Downing, 1990; Barber & Mann, 1991).

In this study the highest GAI for the triploids, 26 in Nov. 1992 (Fig. 18) only just reached the lowest GAI for the diploid males (26 in Nov. 1991) and females (24 in Sept. 1992) which is the period when most gonads were in the regressive stage, suggesting that spawning of the triploids could not occur. This suggestion is reinforced by the histological studies that demonstrated no triploid males or females reached the advanced stage of gonad development observed in diploids. However, since the percentage triploidy was 85%, it is likely that some diploids were sampled as triploids. Although this might have had an effect on the GAI, depending on the number of diploids sampled as triploids, it is likely to increase, not decrease the GAI figures and so it is possible that the triploid GAI were actually less than recorded.

The results of this gonadal study of the triploid Sydney rock oyster demonstrate that the triploids from Port Stephens are functionally sterile. The absence of any evidence of spawning within the triploid samples of the Sydney rock oyster in this study differs from findings by Allen & Downing (1990) who have demonstrated spawning in triploid Pacific oysters on the west coast of the US. Whether this absence of spawning of the triploids is species or site related is uncertain as a study of triploid Pacific oysters in Tasmania, Australia (Gardner *et al.*, in review) also reported no evidence of spawning, which is in contrast to studies of Pacific oysters in the US.

Another major difference between the Sydney rock oyster and the Pacific oyster is the frequency of reported hermaphrodites. In our study less than 1.2% of the triploids (less than 1% of diploids) showed any signs of hermaphroditism whereas Allen & Downing (1990) observed a much higher proportion ($\leq$60%) of triploid hermaphrodites which could be positively identified as such histologically.

In the absence of a comprehensive range of gonadal stages through to fully mature gonads, as described by Allen & Downing (1990), an appropriate staging system for triploid Sydney rock oysters was not obvious. Therefore a new staging criteria was developed. The most frequently occurring triploid stage has been classified as an indeterminate triploid which resembles the indeterminate or early male stage of the diploid Sydney rock oyster. The walls of all the triploid follicles are lined with spermatogonia and primary spermatocytes, and in the majority of triploids, maturation of the male gametes appears to be inhibited at the second meiotic division, i.e. between secondary spermatocyte and spermatid. Allen & Downing (1990) observed that the first meiotic division was rarely affected in Pacific oysters and that inhibition is at cytokinesis rather than during pairing of the chromosomes during spindle formation at meiosis 1. Barber & Mann (1991) found only one confirmed triploid *Crassostrea virginica* with spermatozoa in their study of

the American oyster and few if any with advanced oocytes, a pattern similar to the Sydney rock oyster. The reason triploid Pacific oysters can produce and spawn mature gametes, unlike other species of bivalves examined to date may be related to the great fecundity displayed by this species (Allen & Downing, 1986).

Sex Ratio

A higher proportion of females to males was observed in both diploids (55% to 43%) and triploids (27% to 15%). There did not appear to be a seasonal pattern to the occurrence of male and female triploids. Small numbers of indeterminate diploids were present from September to November as spawning decreased although fewer occurred than observed by Dinamani (1974) for the New Zealand rock oyster. The most notable difference between diploid and triploid sex ratios (Table 1) was the occurrence of large numbers of indeterminate triploids (57%) compared to indeterminate diploids (2%). Development of indeterminate triploids was aberrant and the fact that maturation was arrested early at either the first or second meiotic division probably resulted in the higher incidence of indeterminate triploids.

Oogenesis and the pattern of sexual maturation in the triploid female was abnormal. Developing oocytes were rarely observed in follicles containing mature (usually isolated) ova suggesting that gametogenesis was arrested early on. The few mature ova that were present appeared to have developed abnormally and were frequently in the process of being resorbed.

This study has examined in detail the development of the Sydney rock oyster and has proposed a staging procedure for both triploid males and females. Triploids from Port Stephens were functionally sterile with no evidence of spawning in any samples. Any differences between the triploid samples of the Sydney rock oyster and the Pacific oyster require further study with reference to the incidence of hermaphrodites and the possibility of spawning.

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TABLE 1

Distribution of sexes in diploid and triploid Sydney rock oysters *Saccostrea commercialis* (27-39 months of age) in Port Stephens, NSW

Month	Diploid				Triploid			
	Male	Female	Base Diploid	Hermaphrodite	Male	Female	Base Triploid	Hermaphrodite
	No(%)	No(%)	No(%)	No(%)	No(%)	No(%)	No(%)	No(%)
Nov 1991	14(58)	9(38)	0	1(4)	11(46)	2(8)	10(42)	1(4)
Dec	10(42)	14(58)	0	0	7(29)	4(17)	12(52)	0
Jan 1992	10(42)	14(58)	0	0	4(17)	11(46)	7(29)	2(8)
Feb	13(54)	11(46)	0	0	1(4)	14(58)	9(38)	0
Mar	11(46)	13(54)	0	0	3(13)	10(42)	11(46)	0
Apr	8(33)	16(67)	0	0	1(4)	11(48)	11(48)	0
May	12(50)	12(50)	0	0	4(17)	9(38)	10(42)	1(4)
June	8(33)	15(63)	1(4)	0	1(4)	6(25)	17(71)	0
July	5(21)	19(79)	0	0	2(8)	5(21)	17(71)	0
Aug	11(46)	13(54)	0	0	1(4)	0	23(96)	0
Sept	11(46)	10(42)	3(13)	0	1(4)	1(4)	22(92)	0
Oct	12(50)	10(42)	2(8)	0	8(33)	4(17)	12(50)	0
Nov	8(33)	15(63)	1(4)	0	3(13)	7(29)	14(58)	0
Total	133(43)	171(55)	7(2)	1(0.4)	47(15)	84(27)	175(57)	4(1.2)

Fig. 1. A male diploid (M4) of *S. commercialis* showing the streaming pattern of spermatozoa in the centre of the lumen. The follicular area is enlarged and occupies most of the gonad area (Bar: 100 μm).

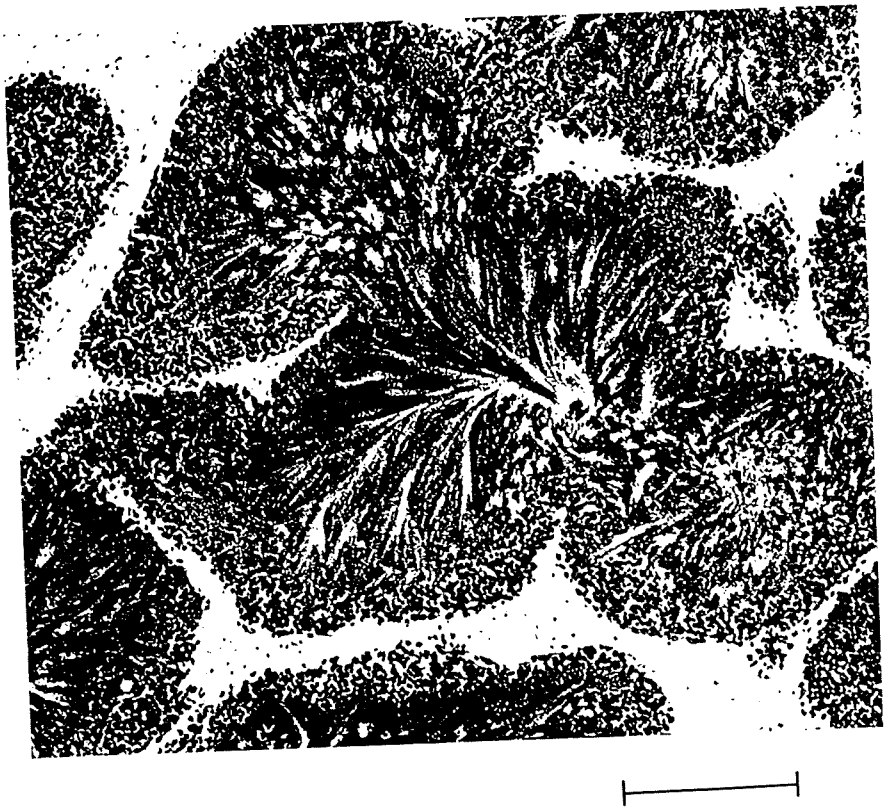


Fig. 2. An electron micrograph of the male diploid (M4) demonstrated in Fig. 1, showing primary spermatocytes (S) at the periphery of the follicle and streams of spermatozoa (S3) in the lumen (Bar: 10 μm).

Fig. 3. An example of an F2 diploid female of *S. commercialis* showing many secondary oocytes, mostly irregular in shape with many demonstrating elongated peduncles (Bar: 100 μm).

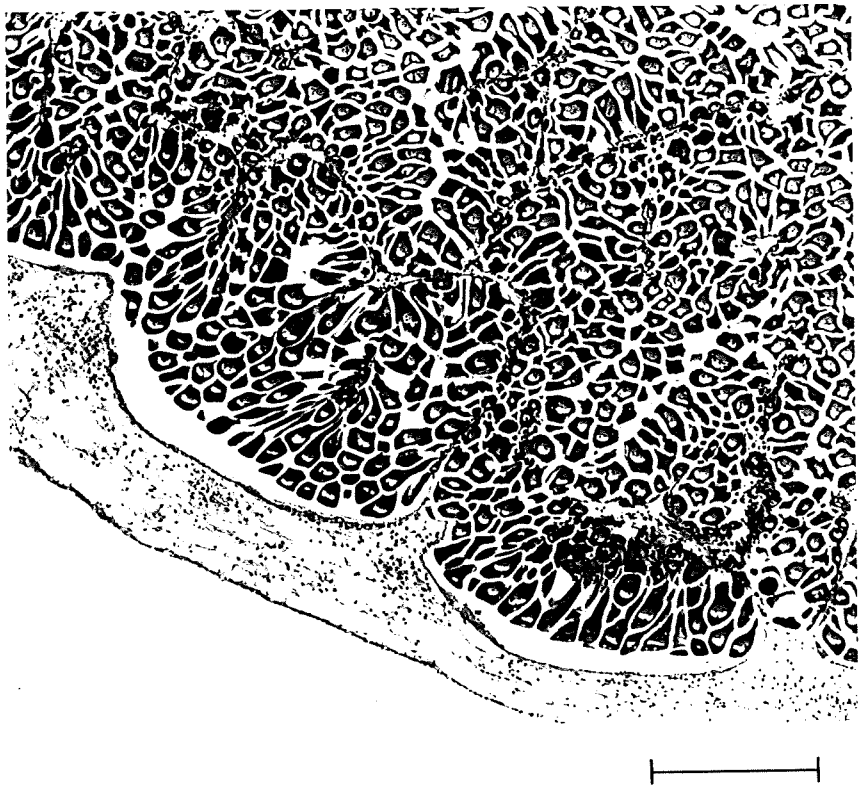


Fig. 4. An example of an F3 diploid female of *S. commercialis* showing a few oocytes free in the lumen (Bar: 50 μm).

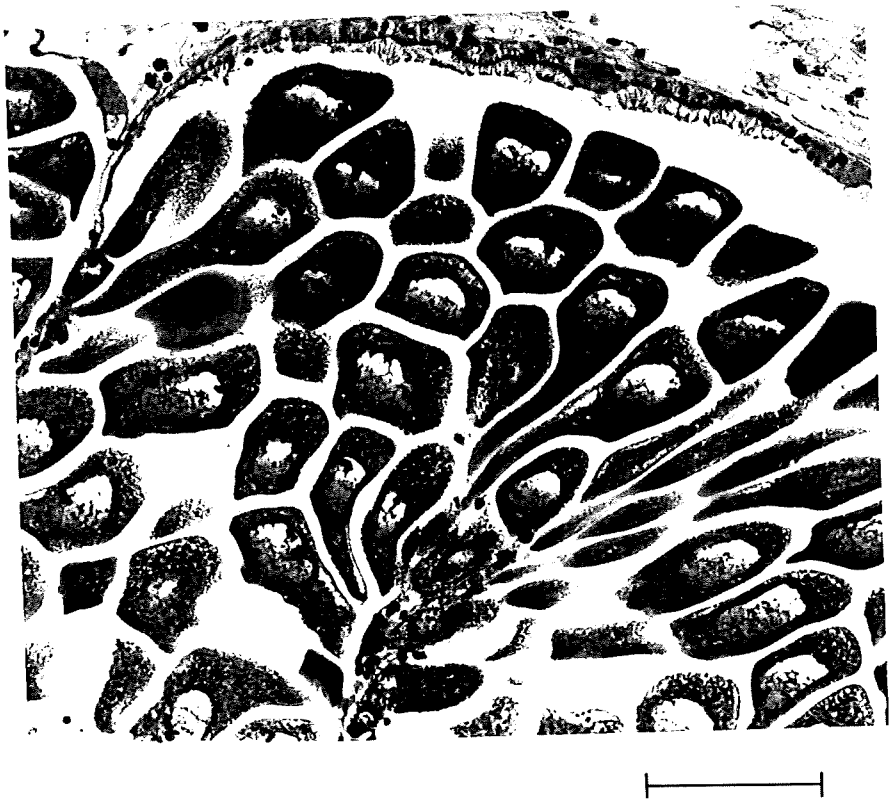


Fig. 5. An example of a post-spawned diploid female (FX) of *S. commercialis* with markedly contracting follicles and an increased area of connective tissue. Relict ova are found in the lumina and many phagocytes are present. This stage was similar to the most developed female triploid stage (Bar: 100 μm).

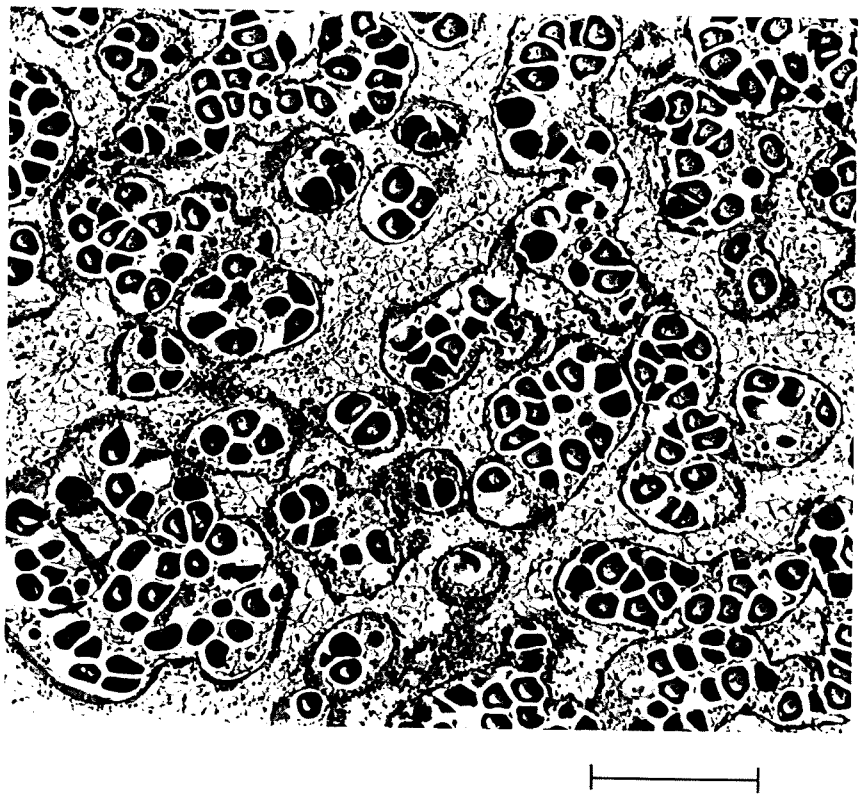


Fig. 6. An example of a base triploid of *S. commercialis* which formed between 30-95% of the total triploids sampled each month. The dark staining follicles are contracted and the bulk of the gonad is interstitial tissue. Aberrant spermatogonia form a band at the periphery of the follicle (Bar: 100 μm).

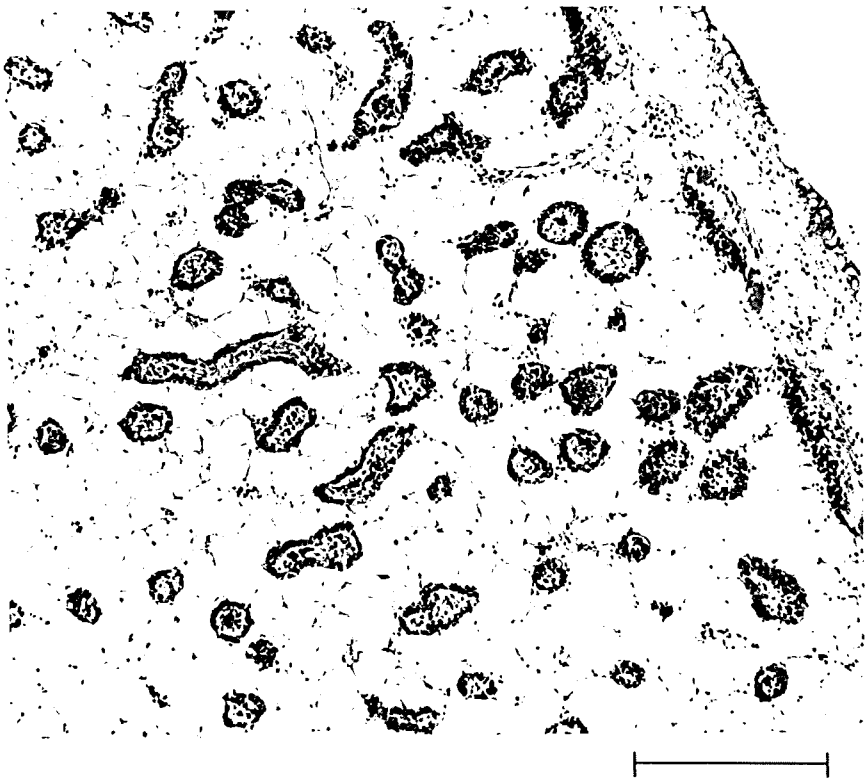


Fig. 7. Monthly frequency of gonadial phases in diploid male Sydney rock oysters *Saccostrea commercialis*.

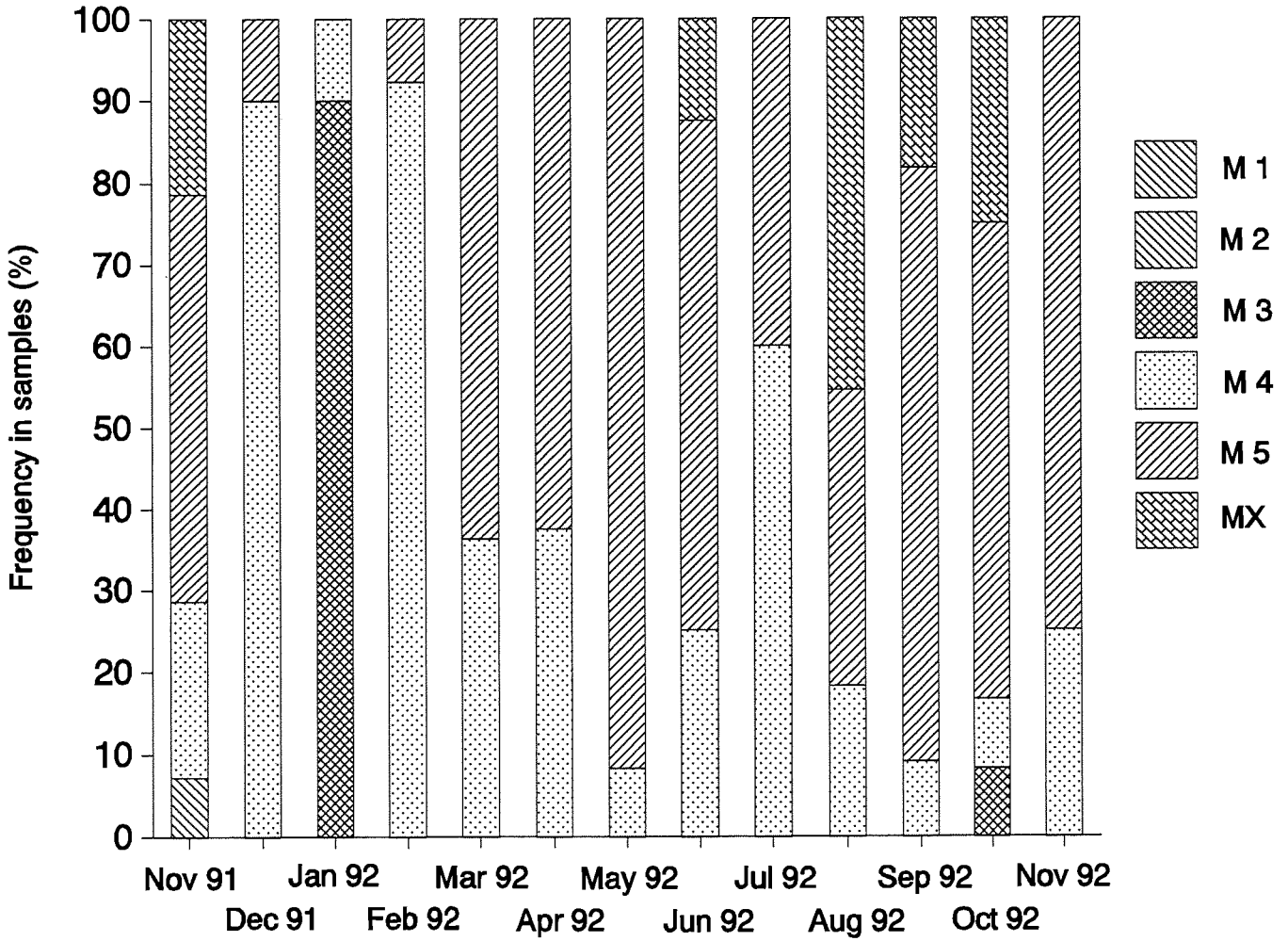


Fig. 8. Monthly frequency of gonadial phases in diploid female Sydney rock oysters *Saccostrea commercialis*.

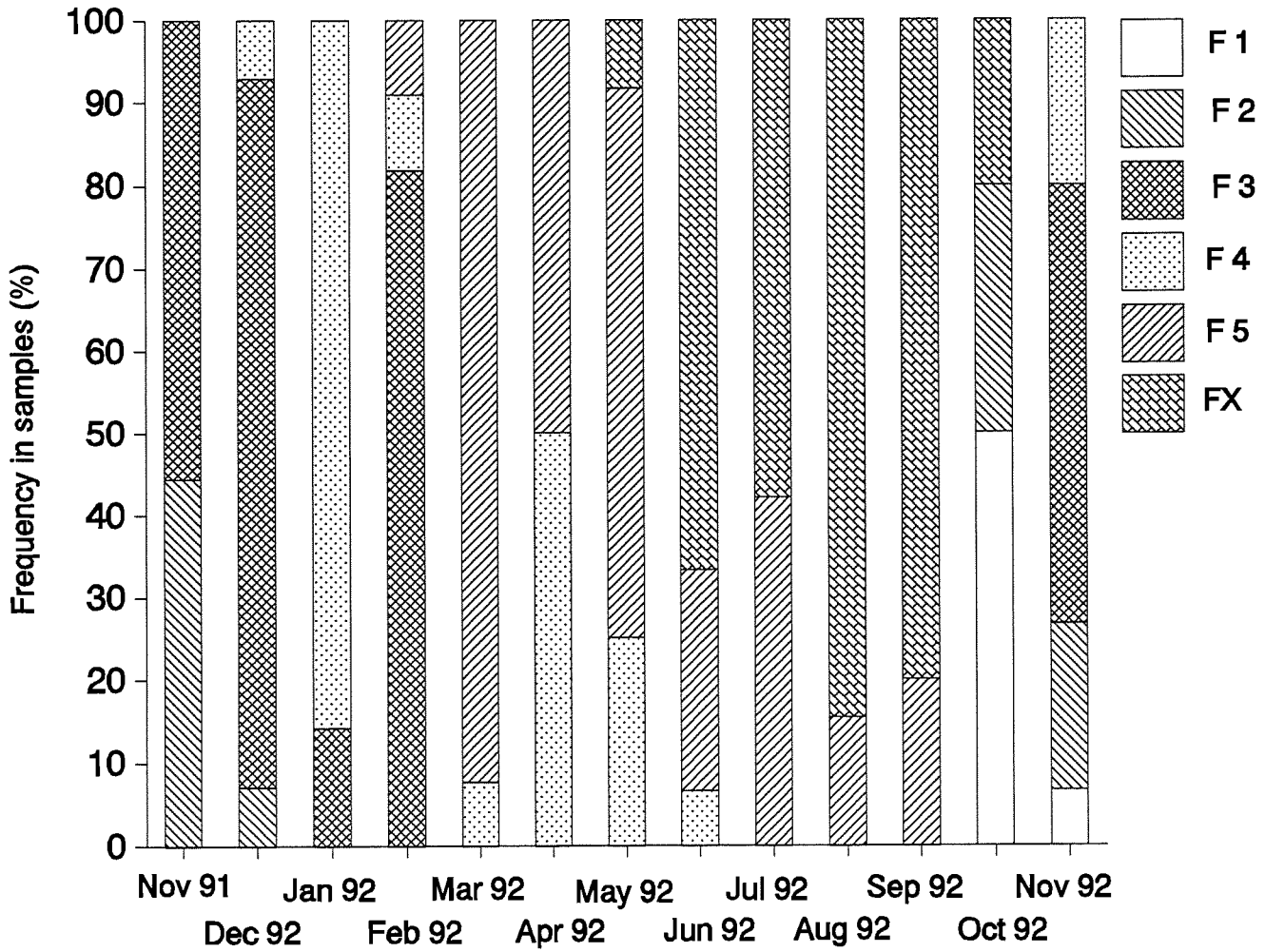


Fig. 9. A base triploid of *S. commercialis* showing a follicle lined with spermatogonia (Sg) and primary spermatocytes (S) lining the lumen (Bar: 50 μm).

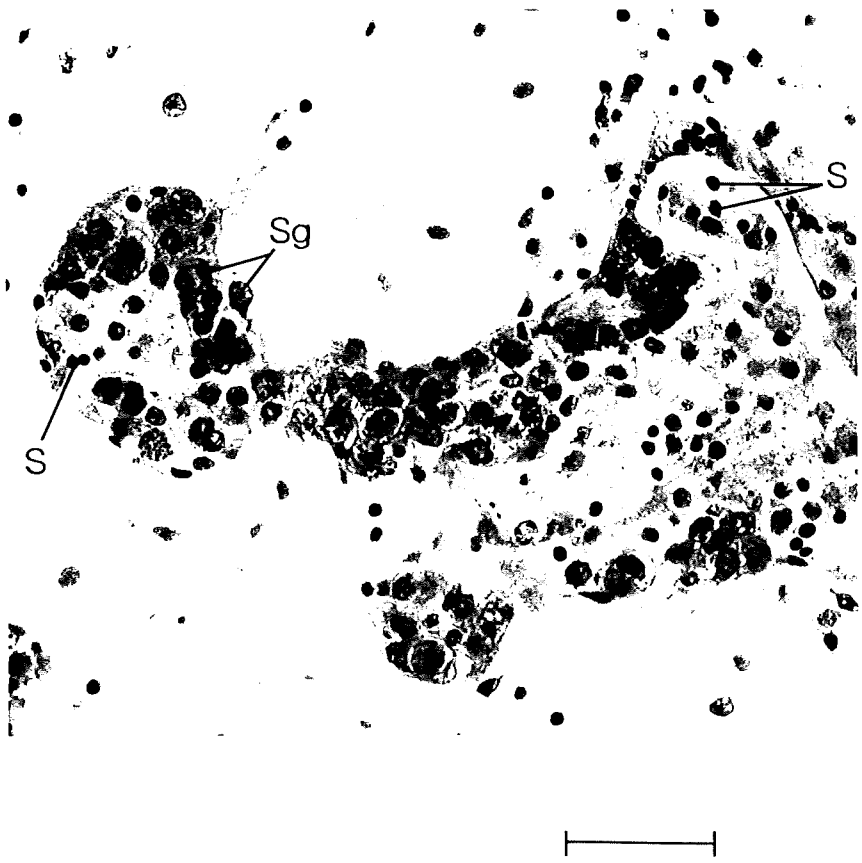


Fig. 10. Ultrastructure of the base triploid of *S. commercialis showing* vacuolation of cytoplasm from most cells. Spermatogonia (SG), spermatocytes (S) and haemocytes (H) characteristics of base diploids are clearly visible (Bar: 10 μm).

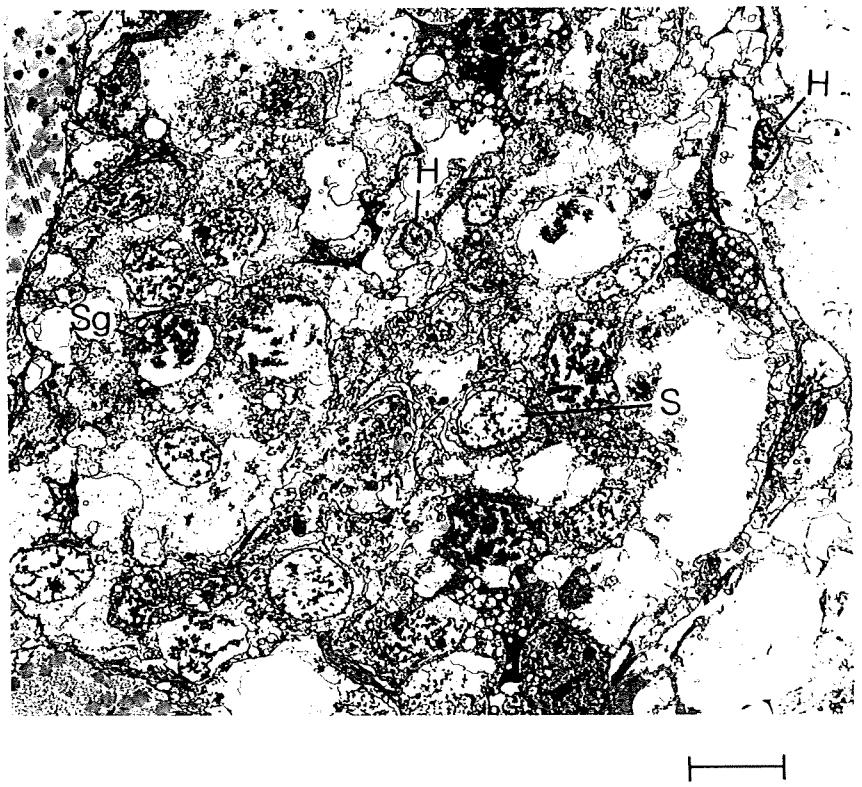


Fig. 11. An example of a triploid male (TM1) of *S. commercialis* with development through to a few spermatids seen as dark nuclei (Bar: 100 μm).

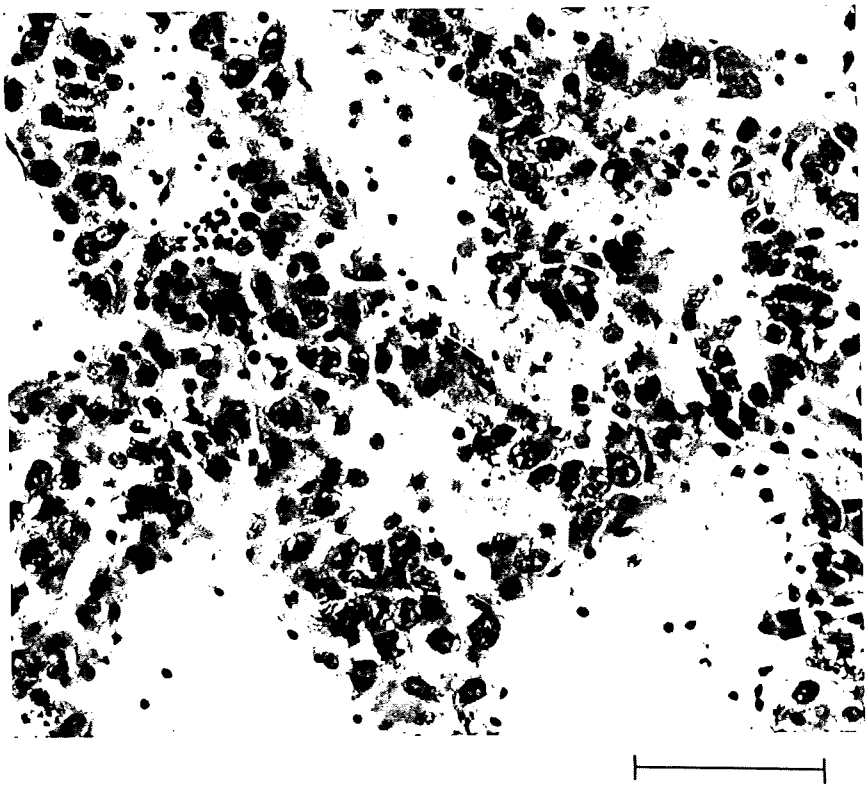


Fig. 12. An example of a TM2 triploid male of *S. commercialis* which was only rarely observed. The density of spermatids at the luminal edge of the follicle has markedly increased and there was reduced interstitial tissue. Unlike the diploids, no spermatozoa were observed (Bar: 100 μm).

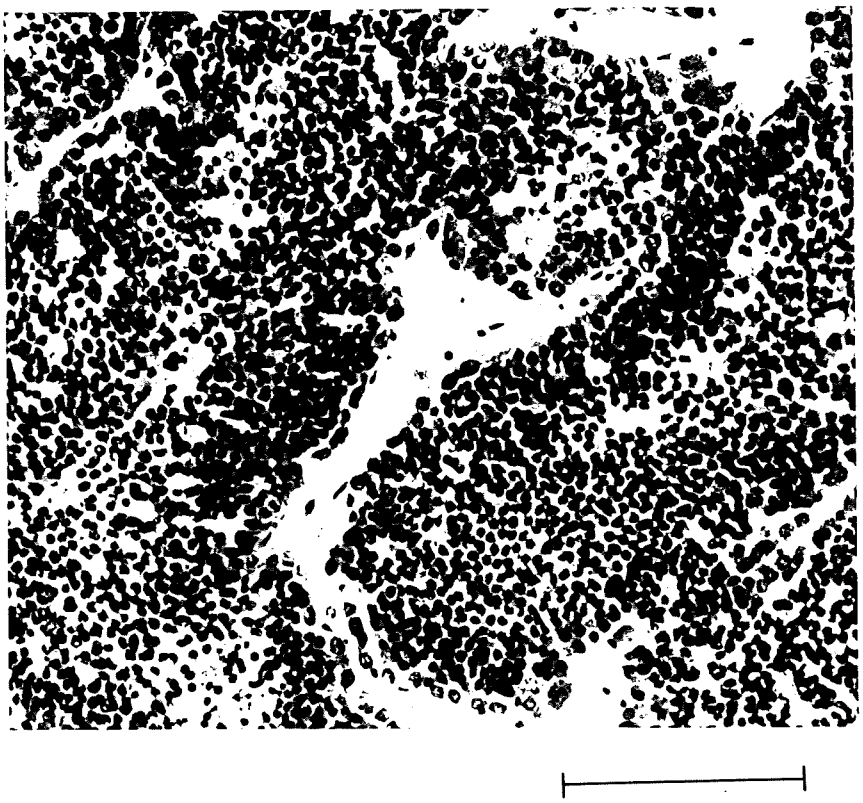


Fig. 13. An example of a TF1 female triploid *S. commercialis* demonstrating isolated oocytes (O) in the follicle lumen. The follicular wall has many spermatogonia (Sg) and primary spermatocytes (S) (Bar: 50 μm).

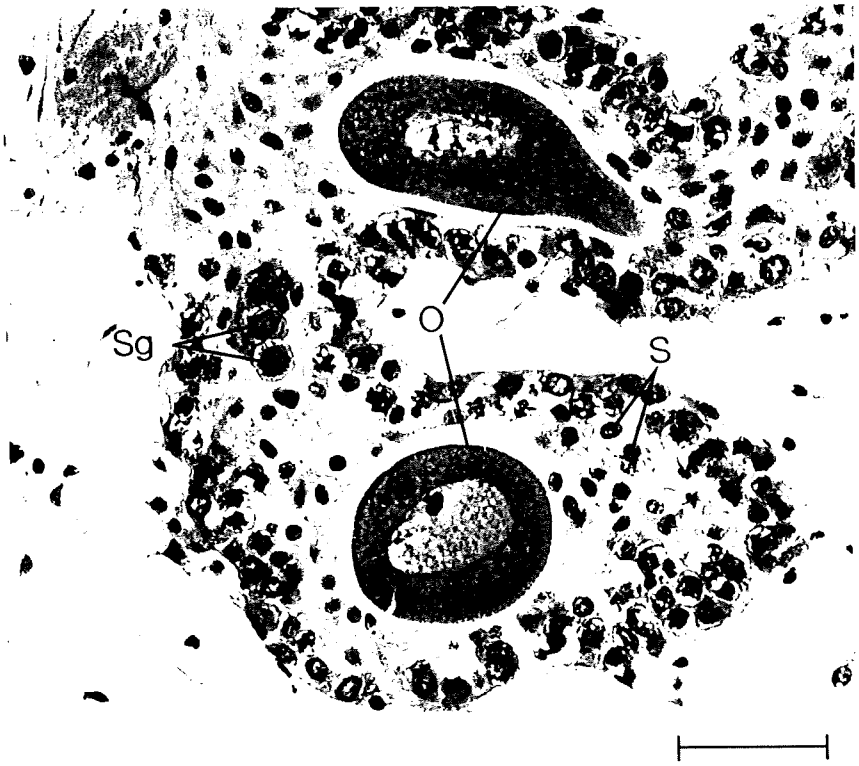


Fig. 14. Monthly frequency of gonadial phases in triploid Sydney rock oysters *Saccostrea commercialis.*

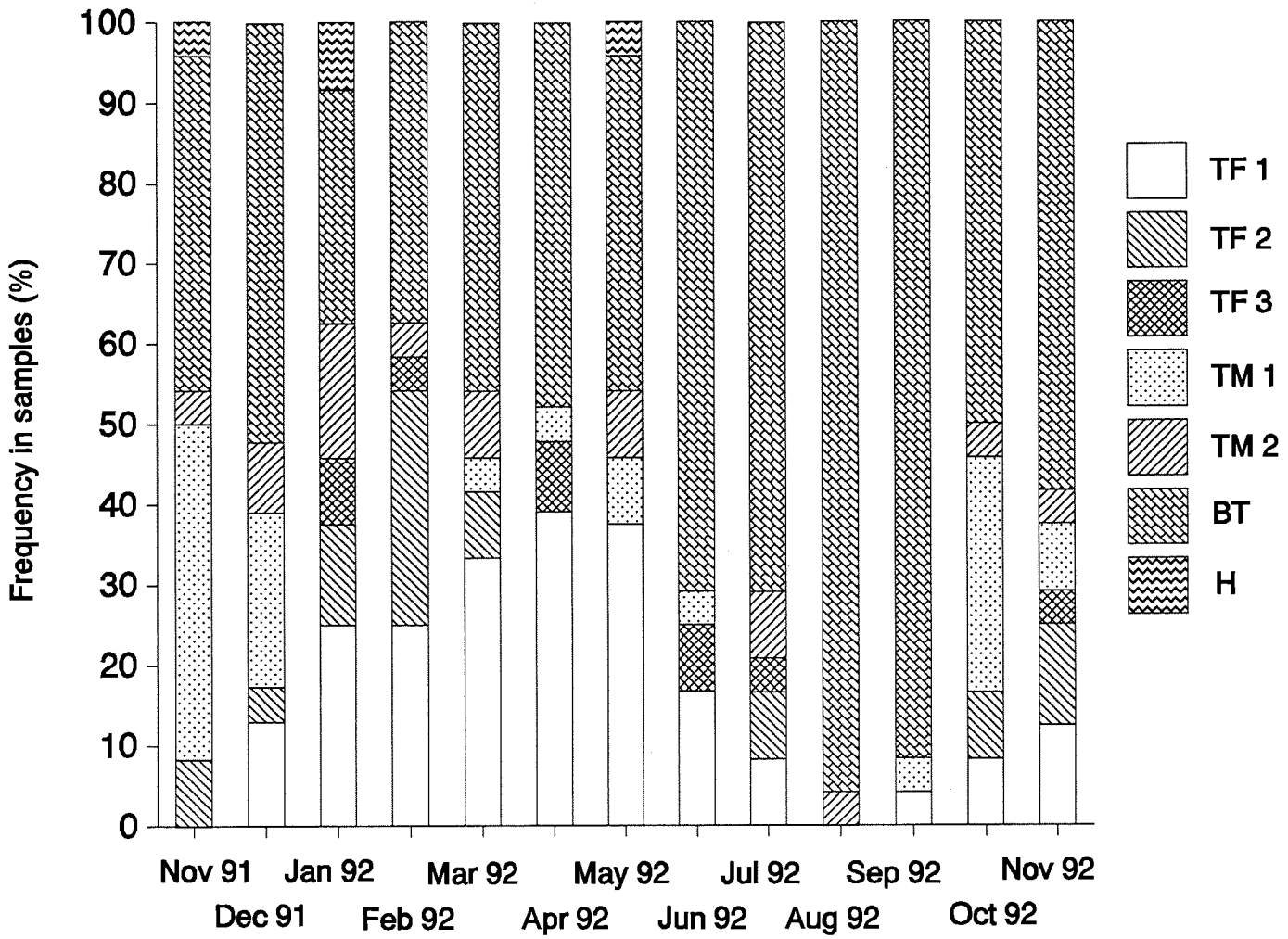


Fig. 15. A TF2 female triploid *S. commercialis* demonstrating an increase in the numbers of oocytes (O) in the follicular lumina with the follicular wall similar to the TF1 example in Fig. 13 (Bar: 50 μm).

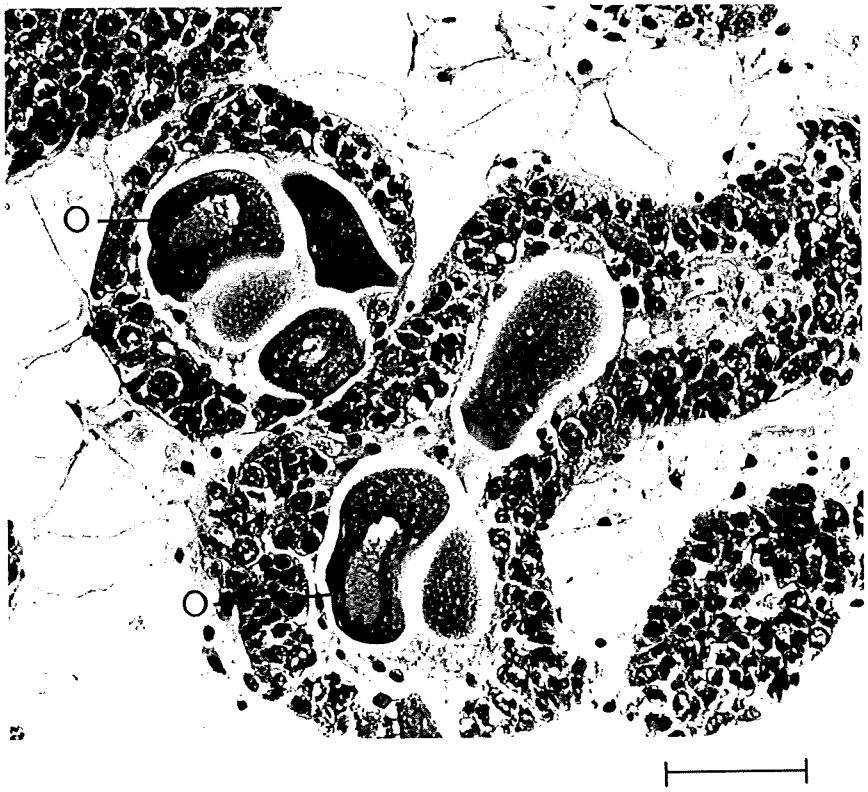


Fig. 16. Ultrastructure of TF1 triploid *S. commercialis* with an oocyte (O) and oocyte nucleus (ON) distinguishable in the lumen. Primary spermatocytes (S) characteristic of the triploid females were visible on the follicle wall. (Bar 10 μm).

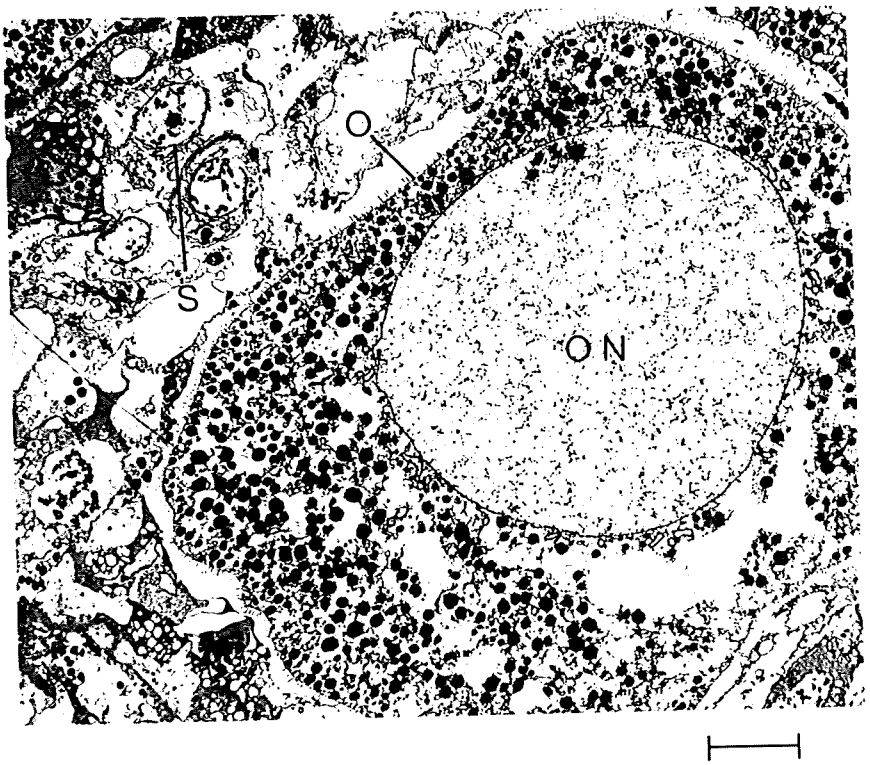


Fig. 17. The most developed gonad, TF3 of triploid *S. commercialis* was similar in appearance to the post-spawned diploid female, FX. Follicles contained few ova (O) and male sex cells still in line the follicle wall. (Bar: 50 μm).

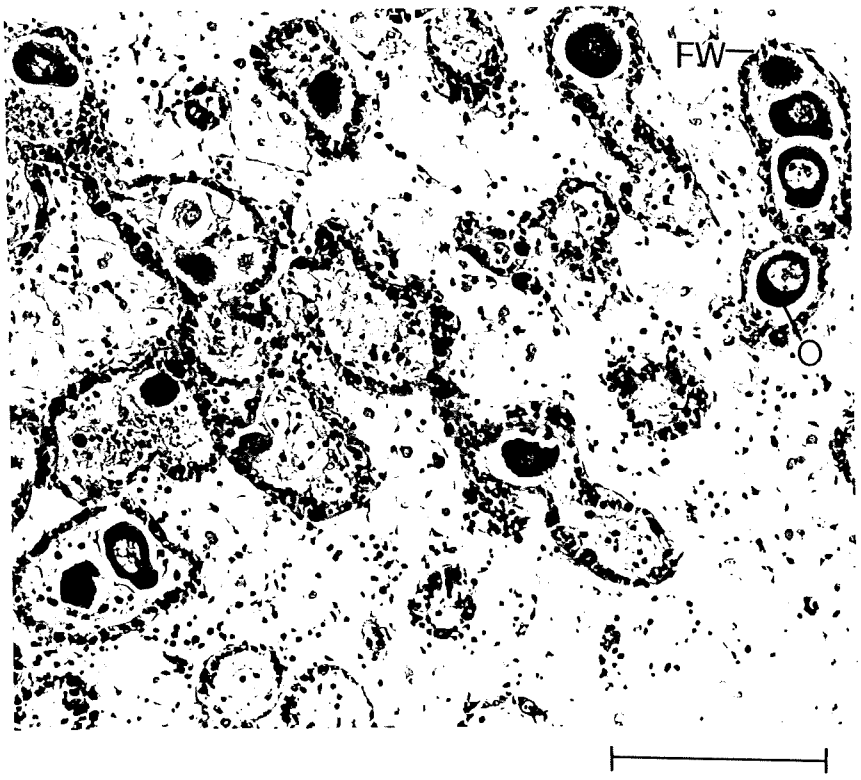
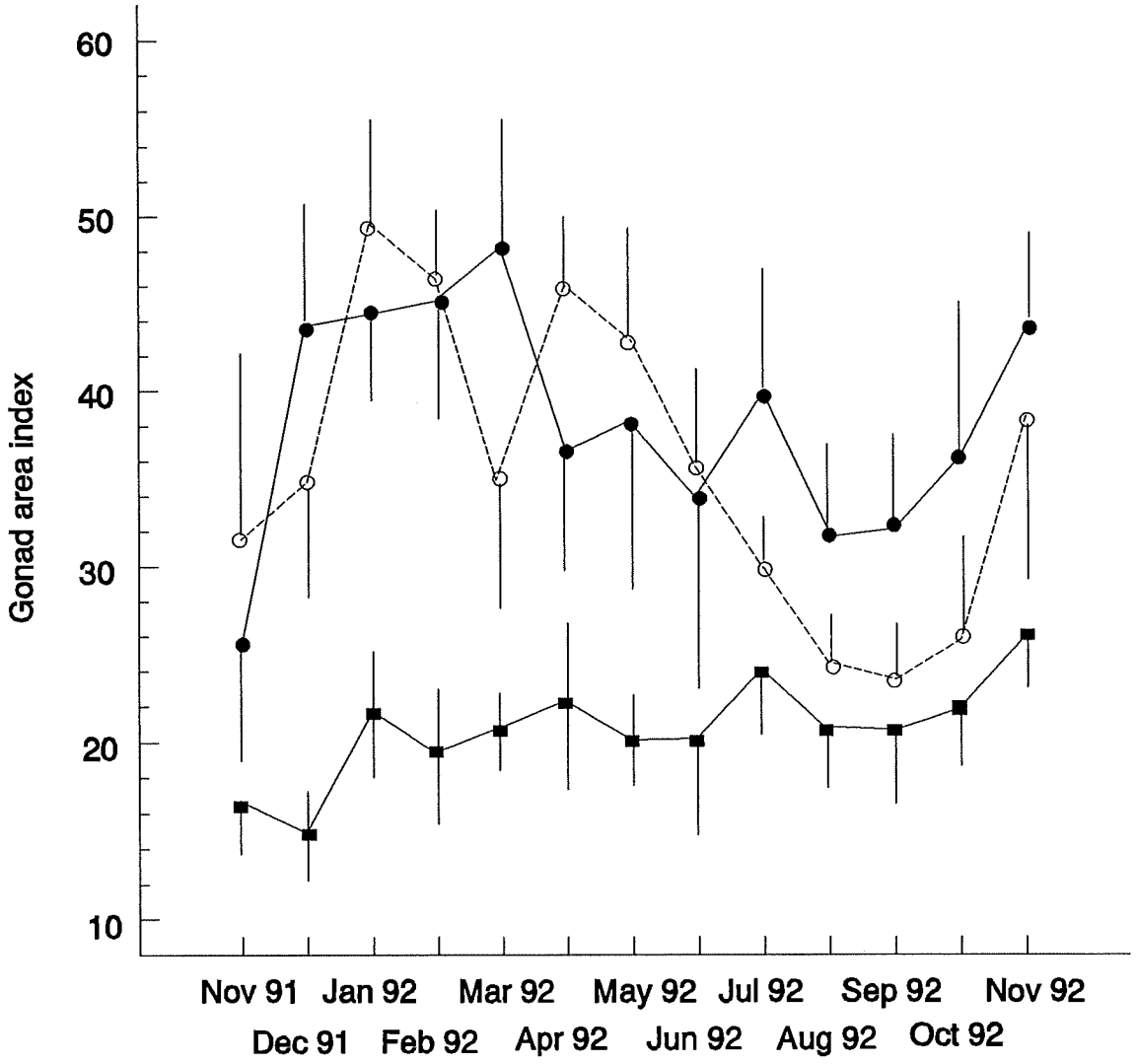


Fig. 18. Gonad area index of Sydney rock oysters *Saccostrea commercialis* (means ± 95% confidence intervals). Diploid males (•——•), diploid females (○------○) and male, female and base triploids combined (▪——▪).



Verification of triploidy in Pacific oysters, *Crassostrea gigas* (Thunberg), with Image Analysis

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Abstract

A method for the separation of diploid and triploid Pacific oysters (*Crassostrea gigas*) is described which involves image analysis of histological sections. The method utilises differences between diploids and triploids of nuclear size of haemocytes and the intensity of staining for haemocyte nuclei. Histological sections, prepared using standard paraffin histology, were stained for nuclear histones with Gill's haematoxylin. Integrated nuclear optical density and nuclear area were recorded with image analysis. When histological specimens are required, this method is less expensive or time consuming than other techniques used to determine triploidy.

Keywords: triploid, oysters, adductor muscle, nuclei, image analysis

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1. Introduction

In any attempt to alter the ploidy of bivalves it is necessary to test to what degree this has been achieved. Generally, diploid individuals are also produced and the ratio of these to triploids needs to be established. In the hatchery this can save wasted labour rearing batches of larvae when the proportion of triploids is low (Beaumont and Fairbrother, 1991).

Usually, verification of triploidy in the hatchery is undertaken to obtain a ratio of diploids to triploids or a measure of the percentage success achieved in inducing triploids. In an evaluation of the performance of triploid oysters in Tasmania (Gardner et al., 1995; Maguire et al., 1995), it was useful to establish the ploidy of individual adult oysters rather than obtaining a ratio of triploids within the population. Samples of triploid oysters used for the study of gametogenesis described by Gardner et al. (1995) were found, using flow cytometry, to contain approximately 25% diploids. Individuals within the triploid group that developed large gonads may have been diploids, alternatively, they may have been triploids where gametogenesis was less inhibited. By distinguishing diploids from triploids it was possible to accurately describe the gametogenesis of triploid oysters. Differences due to sex in the extent of suppression of gametogenesis could also be assessed once

triploidy was verified.

Several techniques have been described for the determination of the ploidy of bivalves; the two approaches most frequently used are karyotypic analysis and flow cytometry. Karyotypic analysis involves the preparation, staining and counting of the chromosomes of separate nuclei (Kilgerman and Bloom, 1977; Allen, 1983). Whilst karyotypic analysis is a very accurate measure of ploidy, it is also very time consuming (Komaru et al., 1988). Flow cytometry is capable of recording the DNA content of cells at a much greater rate, in the order of 10,000-100,000 nuclei per sample. This technique is particularly useful for the estimation of the percentage of triploids in a population, as is required in hatcheries (Chaiton and Allen, 1985). Flow cytometry is an efficient technique for the evaluation of triploidy but expensive where numerous ploidy assessments of individuals are required.

This experiment aimed to find an alternative method of verification of ploidy that would be both time- and cost-efficient for the assessment of individual oysters. This was approached in two ways: (1) the evaluation of body structure, specifically adductor muscle size, with the aim of distinguishing ploidy on a morphological basis, and (2) examining the nuclear size and staining intensity in histological sections.

The first approach, measurement of body structure, was assessed by measuring the size of the adductor muscle in relation to the size of the whole organism. It was hypothesised that the increased DNA content of muscle fibre nuclei may be reflected in the diameter of muscle cells. This in turn may affect the diameter of the entire adductor muscle.

The additional set of chromosomes within the nucleus of triploid cells can be expected to alter either the size of the nucleus or the density of the DNA contained therein. In flow cytometry, this is measured by staining DNA with a fluorescent dye. The degree of fluorescence of the nuclei after staining is proportional to the DNA content of the nucleus (Coon and Weinstein, 1992). Image analysis uses a similar technique to quantify the DNA content of nuclei. Instead of measuring the light emitted by stained nuclei (as in flow cytometry), image analysis can be used to measure the light absorbed by the stained nuclei (Jarvis, 1992a) or simply the size of the nuclei. By measuring the light absorbed by Feulgen stained nuclei as a function of their area (as integrated optical density), Gérard et al. (1991) independently used image analysis to distinguish diploid oysters from triploids.

Nuclear size and integrated optical densities were calculated for commercial size oysters (approximately 60 g) sampled in the months on either side of a spawning recorded at Birches' Bay (southern Tasmania, Australia), December 1992 and January 1993. This allowed evaluation of individuals within the triploid group that behaved like diploids. Were these individuals triploids where gametogenesis was not reduced or were they simply diploids?

2. Materials and Methods

The diploid and triploid Pacific oysters used in these experiments were grown at Birch's Bay, Tasmania and were from the same groups as those described by Maguire et al. (1995). Triploids were induced by suppression of polar body 2 with cytochalasin B in February 1990 (Allen et al., 1989).

Indices for adductor muscle were formulated by dividing adductor diameter (mm) with valve height (mm) and whole oyster volume (ml), obtained by allometric analysis (Gardner, 1993). Oysters for adductor muscle measurements were cultured in a replicated trial (3 replicates per ploidy group) and sampled at commercial size (40 months of age). Significance between diploids (n = 30) and triploids (n = 30) was tested with a one way ANOVA (Walpole and Myers, 1989).

Standard paraffin (7 μm) histological sections were prepared as described in Gardner et al. (1995). Staining of tissue for image analysis must be specific and of high quality (Jarvis, 1992b). Jarvis (1992b) suggests that the Feulgen technique is the most appropriate stain for image analysis of DNA. This was not found to be the case in this study as Feulgen staining produced a pale stain that provided poor contrast against the surrounding tissue. Variation in staining intensity between slides was also encountered with the Feulgen technique and this masked variation due to differences of ploidy. The source of variation in Feulgen staining was considered to arise from a critical step of acid hydrolysis. Consequently, an alternative stain was sought which produced strong and specific staining of DNA and was also very simple so that variation was diminished. Gill's haematoxylin (Gill et al., 1974) was found to be a suitable stain. Sections were stained for precisely two minutes then rinsed with tap water for five minutes. Although Feulgen staining was inconsistent in this study, Gérard et al. (1991) used Feulgen staining to accurately distinguish triploids with image analysis.

Nuclear area and densiometric (integrated optical density) measurements were taken using a BH2, Olympus™ compound microscope. Slides were viewed with a 100x planachromat objective with oil immersion to produce magnification of x1000. Nuclear area and integrated optical density measurements were recorded for 20 haemocyte cells from each specimen. Measurements were taken with a CUE II™, IBM™-compatible, image analysis system. Haemocyte cells were chosen as they were widely distributed through the tissue and there appeared to be little variation of haemocyte nuclear size within specimens. Also, the nuclei of haemocytes are round which assists in defining the shape to be measured with image analysis. The proportion of somatic tissue (including gills) occupied by gonad was also determined with image analysis as described by Maguire et al. (1995).

Illumination between sections was standardised by adjusting the light intensity of the microscope to a standard intensity. This was done when viewing a section of each slide without tissue. The purpose of this step was to remove variation in light intensity due to differences in the slide or cover slip thickness, and variation in the optical density of the mounting medium. Densiometric measurements were made with microscope adjustment and illumination filtration and illumination adjustment as advised by Jarvis (1992b). Basophilic components within the cytoplasm produced pale staining which compounded nuclear optical density

measurements. To correct for this, an optical density reading (from an area the exact size of the nucleus) was taken from the cytoplasm of each haemocyte sampled. By subtracting the integrated optical density of cytoplasm from the integrated optical density of the nucleus, this component of light absorption was removed.

Significant difference between triploids and diploids was tested with a Wilcoxon non-parametric test (Walpole and Myers, 1989).

3. Results and Discussion

Adductor muscle

There appeared to be a trend of greater adductor muscle diameter in relation to shell height of triploid oysters compared to diploids (Table 1). However, it was found that no significant difference ($P>0.05$) existed between ploidy groups for both indices of adductor diameter. In contrast, Maguire et al., (1995), using larger sample sizes, found that the adductor muscle diameter relative to shell height, was 10.4% larger for triploids than diploids. However, this difference may have resulted from differences in the shell shape of triploids relative to diploids rather than changes in the size of the adductor muscle relative to the size of the whole oyster.

Table 1.
Means of adductor muscle indices for diploid and triploid Pacific oysters (±SE, n=3)

Ploidy	Adductor Diameter / Whole Volume	Adductor Diameter / Valve Height
Diploid	0.3269 (0.0127)	0.1741 (0.00576)
Triploid	0.3308 (0.0098)	0.1914 (0.00299)

Image Analysis

A highly significant difference ($P<0.001$) was found between diploids and triploids for both parameters assessed by image analysis. This was the case for both December and January analyses. This demonstrates the potential for haemocyte nuclear area and integrated optical density to be effective tools in the determination of ploidy. However, for the purpose of this study, it was necessary to distinguish diploid and triploid oysters on an individual basis rather than simply separating populations. The degree to which this was achieved was assessed by comparing values obtained by image analysis against the gonad area of individual oysters (Fig. 1-4).

Both nuclear area and integrated optical density values appeared to distinguish triploid oysters from diploid oysters in the December 1992 sample. Neither measurement conclusively separated all individuals but a clear indication of ploidy

was apparent when both measures were compared for each oyster. The relationship of each of the nuclear parameters (integrated optical density and nuclear area) to ploidy was based on both the gonad area measurements of each oyster and the ratio of diploids within the triploid group. The nuclear area and integrated optical density values indicated that eight oysters within the triploid group, from both months (a total of 40 triploids), were diploid which equates to 20 % of the sample (specimens 22, 28 and 32 from the December sample, specimens 21, 32, 36, 37 and 38 from the January sample). Although the sample size is very small, this is in the order of what would be expected based on the original ratio obtained by flow cytometry (approximately 25% diploids within triploid samples). Additional samples from two other sites indicated a similar incidence of triploidy (Gardner et al., 1995).

Although there was an influence of ploidy on both integrated optical density and nuclear area, there tended to be more variation in integrated optical density measurements (Table 2). The nuclear staining capacity of haematoxylins has been attributed to binding of the dye molecule to nuclear histones (Stevens, 1982) but other proteins will also bind. The variation in integrated optical density measurements observed in this study may have been caused by non-specific staining of proteins associated with the nucleus, rather than the staining of histones alone. Staining intensity can be expected to vary from day to day and there appeared to be some difference in intensity between the December and January samples which were stained separately (Figs. 2 and 4). To eliminate error caused by changes in staining of the haematoxylin, diploid controls are necessary for each batch of analyses. Although uniform and intense Feulgen staining could not be achieved in this study, it has been used elsewhere for the determination of ploidy (Gérard et al., 1991; Jarvis et al., 1992b) and it is highly specific to DNA. Variations on the Feulgen technique, used for determining integrated optical density in human pathology (Schieck et al., 1987; Schulte et al., 1988), may improve the staining intensity in oyster sections and assist in determining ploidy.

Table 2.
Comparison of the variation (by coefficient of variation)[1] between nuclear area and integrated optical density measures for diploid and triploid oysters[2]

Ploidy	Integrated Optical Density		Nuclear Area	
	December	January	December	January
diploid	7.97	17.01	7.20	6.32
triploid	8.69	11.64	9.96	8.40

1. Coefficient of variation = (standard deviation / mean) x 100
2. Oysters within the triploid group which appeared to be diploids were excluded from analyses

The nuclear area and integrated optical density values of triploids from the December 1992 sample confirmed the suppression of gametogenesis from triploidy. Those individuals that did develop extensive gonad were shown to be diploids. In the following sample, January 1993, there were three individuals within the triploid group with large gonad area that appeared to be legitimate triploids based on image analysis (specimens 22, 24 and 33 of the January sample). All of these triploids were male and gonad size is less retarded in male triploid Pacific oysters than in female triploids (Allen and Downing, 1990). Numerous spermatocytes were present in the follicles of these individuals yet no spermatozoa were observed which is a pattern of gametogenesis consistent with that reported for triploid *Crassostrea virginica* (Barber and Mann, 1991).

Compared to other techniques for the determination of ploidy, the cost of consumables for image analysis is low. However, the preparation of sections and measurement of nuclear parameters with image analysis is time consuming compared to flow cytometry. This may undermine the feasibility of this technique except for applications where histological sections are also required. In these situations, the technique of image analysis provides a cost efficient and rapid means of determining ploidy and may also serve as a useful adjunct to flow cytometry.

Acknowledgments

Financial assistance from the Key Centre for Teaching and Research in Aquaculture and the Fishing Research and Development Corporation is gratefully acknowledged. Thanks are also due to Dr Greg Maguire for assistance in the preparation of the manuscript.

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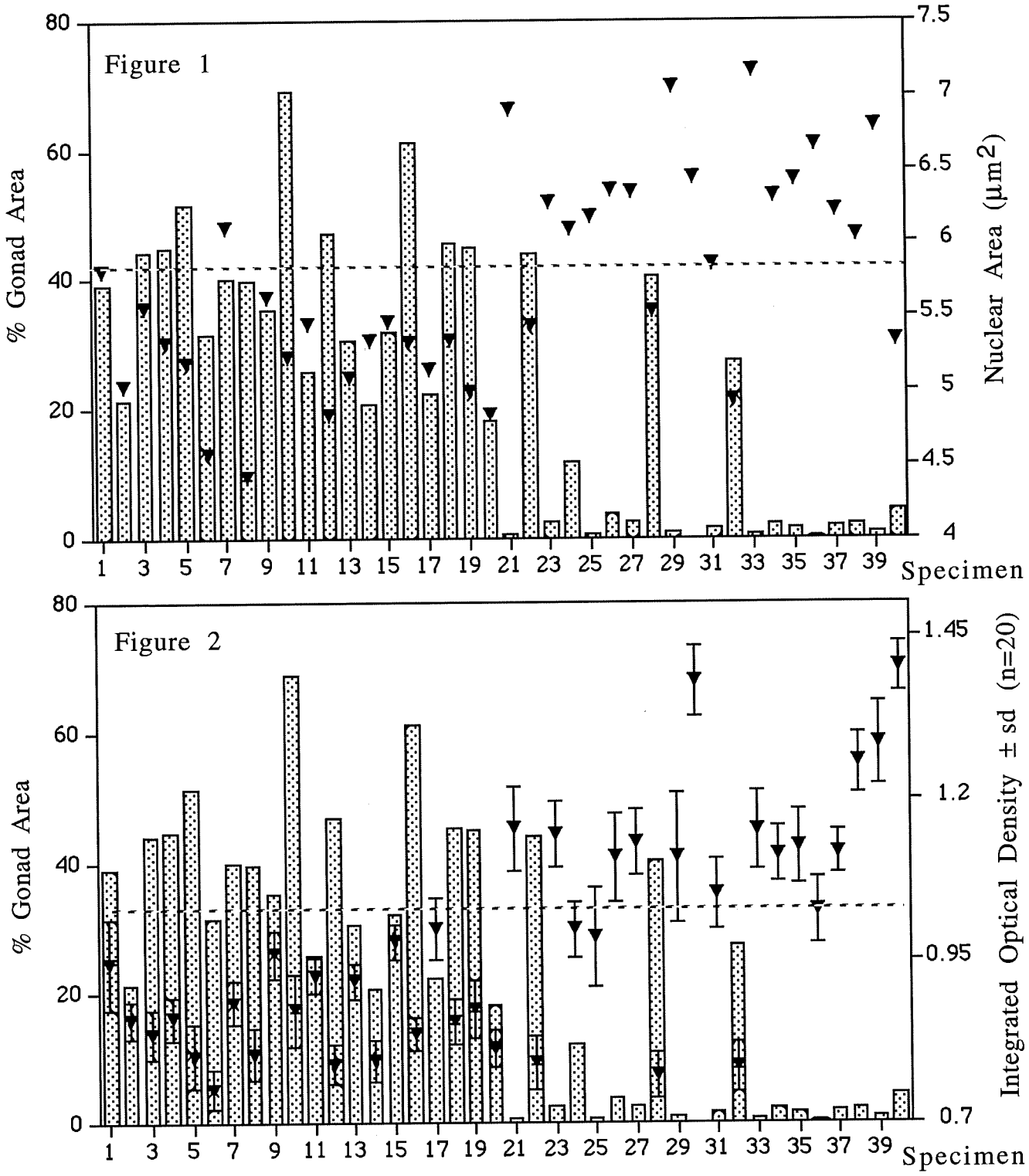
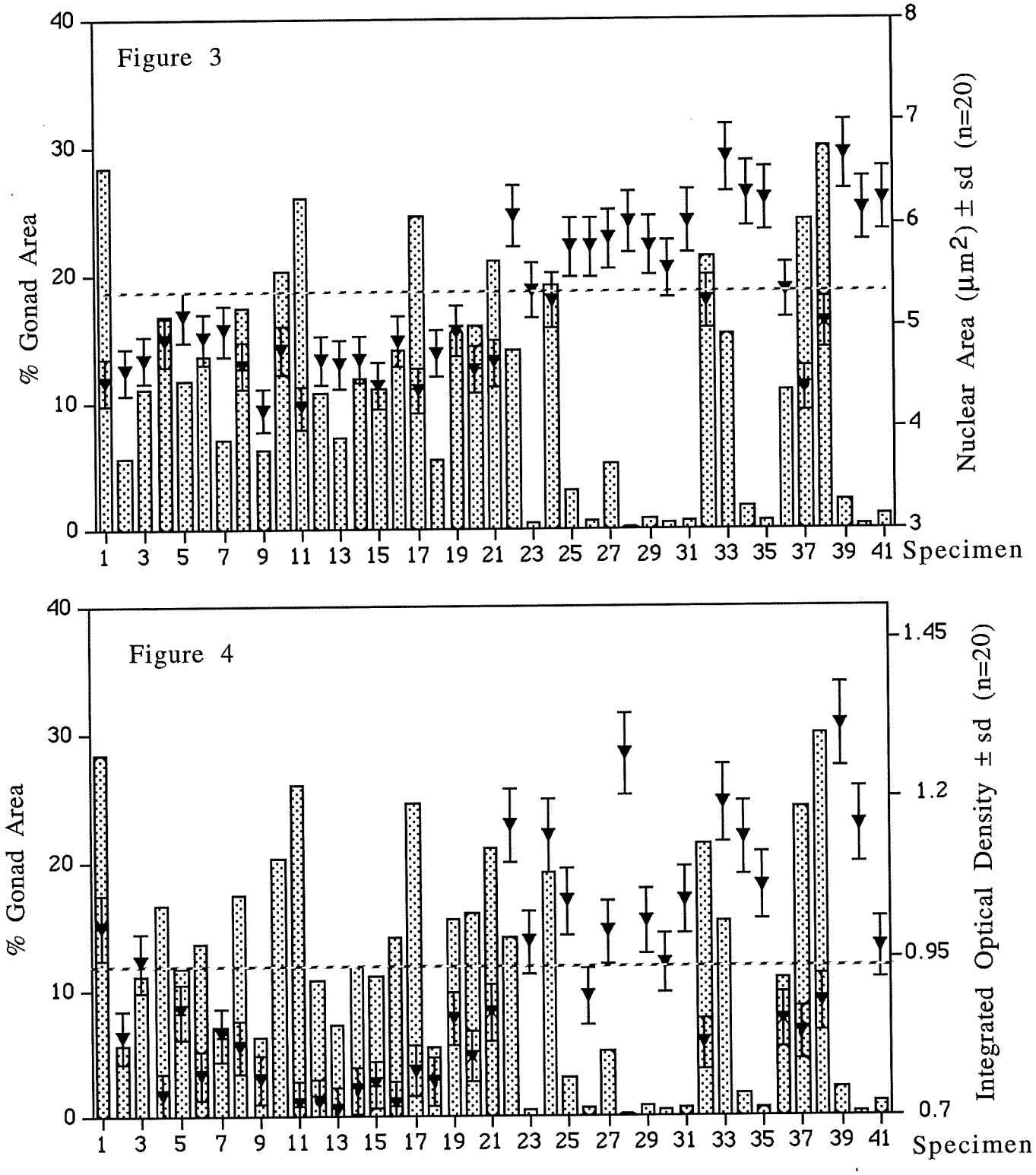


Figures 1 and 2. Mean haemocyte nuclear area (Fig. 1) and integrated optical density (Fig. 2) in relation to proportion of somatic tissue occupied by gonad for December 1992 sample. Gonad area values are represented by columns (⬚) and nuclear parameters by solid triangles (▼). Specimens 1 to 20 from the diploid group, specimens 21 to 40 from the triploid group. No standard deviations were recorded for nuclear area measurements from this sample.



Figures 3 and 4. Mean haemocyte nuclear area (Fig. 3) and integrated optical density (Fig. 4) in relation to proportion of somatic tissue occupied by gonad for the January 1992 sample. Gonad area values are represented by columns (⊡) and nuclear parameters by solid triangles (▼). Specimens 1 to 20 from the diploid group, specimens 21 to 41 from the triploid group.

8 CONSUMER ACCEPTABILITY OF TRIPLOID OYSTERS

a Allen, S. and Prescott, J., 1993. Sensory evaluation of Sydney rock oysters. Report No. 91, Commonwealth Scientific and Industrial Research Organisation, Division of Food Science & Technology, Food Research Laboratory, Sensory Research Centre, North Ryde, NSW, 13 pp.

b Maguire, G. B., Boocock, B., Kent, G. N. and Gardner, N. C. Studies on triploid oysters in Australia. IV. Sensory evaluation of triploid and diploid Pacific oysters, *Crassostrea gigas* (Thunberg), in Tasmania.

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IN CONFIDENCE

REPORT NO. 91

SENSORY EVALUATION OF SYDNEY ROCK OYSTERS

COMMISSIONED BY:

NSW Agriculture and Fisheries

Salamander Bay
Australia 2301

SUMMARY

Samples of diploid and triploid Sydney rock oysters were evaluated for appearance, flavour, texture and overall acceptability in October 1991, January 1992 and November 1992.

Samples were presented in a counterbalanced order, coded to prevent identification, and evaluated by a panel of 42 adult males and females. Oysters were presented raw, one at a time at a temperature of approximately ten degrees Celsius.

The analysis of variance carried out for each harvesting time separately, found no significant differences for any of the attributes at January 1992 or November 1992. The only significant differences were texture and overall acceptability at October 1991, with the diploid oyster being rated as more acceptable in texture and overall acceptability than the triploid oyster. There were no significant differences between the diploid and triploid oysters over time for any of the attributes evaluated.

These results suggest that the triploid Sydney rock oyster may be a useful alternative to the diploid Sydney rock oyster.

1. AIM OF THE SENSORY EVALUATION PANEL

To evaluate and compare samples of diploid and triploid Sydney rock oysters on a variety of flavour and texture characteristics over three discrete testing periods.

2. PROCEDURE AND TESTING METHOD

Oysters were evaluated over three testing periods, October 1991 (time 1), January 1992 (time 2), and November 1992 (time 3).

Three oysters from each sample (diploid = oyster 1; triploid = oyster 2), were presented one group at a time, on a white plastic plate with a white plastic fork.

Oysters were shucked on the morning of the tasting session for each testing period. Samples were served raw at a temperature of approximately ten degrees Celsius.

Samples were presented in a counterbalanced order to compensate for positional bias. To further reduce bias samples were coded with three digit random numbers which were changed after each set of ten panellists.

The taste panel comprised of 42 adults. The panelists ranged in age from 18-53 years, with a mean age of 27 years. 24 panellists were male (57%) and 18 panellists (43%) were female.

The oysters were evaluated on appearance (before tasting), flavour, texture/mouthfeel and overall acceptability. The score sheet is included in Appendix 1. For each tasting session panellists were instructed to rate all four attributes for each sample and return the scoresheet before the next sample was presented. Panellists rated their opinions of each sample on 150mm graphic rating scales. Panellists were instructed to rinse their mouths with water to cleanse the palate between samples, hence avoiding cross-sample contamination. Standard sensory evaluation conditions prevailed.

Following each tasting session, the ratings given by the panellists were converted to scores out of 100. The data for each attribute were then subject to a one-way Analysis of Variance (ANOVA) to determine if there were any significant differences between the two products. The tests were carried out using a 5% ($p<0.05$) level of significance. The ANNOVA summary is included in Appendix 2. Dot plots of the individual data for each oyster type are included in Appendix 3. These plots show the distribution of the data across the range of 0 to 100 displaying the spread of the individual scores.

3. RESULTS

The results given below are from the statistical analysis of the following scales (with their end point descriptors) as rated by the panellists during each tasting period for each oyster type.

Appearance: (before tasting) (0 = extremely poor, 100 = extremely good)

Flavour: (0 = extremely poor, 100 = extremely good)

Texture/ Mouthfeel: (0 = extremely poor, 100 = extremely good)

Overall Acceptability: (0 = not at all acceptable, 100 = extremely acceptable)

The mean scores for each attribute of each oyster sample, for each tasting session are shown below.

Results for Time 1. have been analysed in two lots. The first analysis includes all panellists and the second analysis removes one panellist whose results were considered as an outlier.

Means With Outlier Present.

(statistically significant differences are indicated by *)

TIME 1.

Appearance

Oyster 1.	49.136
Oyster 2.	50.364

Flavour

Oyster 1.	60.273
Oyster 2.	56.500

Texture *

Oyster 1.	64.682
Oyster 2.	57.091

Overall Acceptability

Oyster 1.	59.364
Oyster 2.	49.318

Means With Outlier Removed

TIME 1.

Appearance

Oyster 1.	49.952
Oyster 2.	49.810

Flavour

Oyster 1.	63.143
Oyster 2.	57.762

Texture *

Oyster 1.	65.476
Oyster 2.	56.524

Overall Acceptability *

Oyster 1.	62.190
Oyster 2.	51.143

TIME 2.

Appearance

Oyster 1.	53.280
Oyster 2.	51.840

Flavour

Oyster 1.	56.000
Oyster 2.	59.000

Texture

Oyster 1.	60.120
Oyster 2.	60.600

Overall Acceptability

Oyster 1.	54.480
Oyster 2.	57.560

TIME 3.

Appearance

Oyster 1.	51.680
Oyster 2.	57.760

Flavour

Oyster 1.	67.800
Oyster 2.	57.880

Texture

Oyster 1.	63.800
Oyster 2.	64.320

Overall Acceptability

Oyster 1.	64.800
Oyster 2.	61.880

All Testing Sessions Combined With Outlier Present

Appearance		Flavour	
Oyster 1.	51.37	Oyster 1.	61.36
Oyster 2.	53.32	Oyster 2.	57.79
Texture		**Overall Acceptability**	
Oyster 1.	62.87	Oyster 1.	59.55
Oyster 2.	60.67	Oyster 2.	56.25

All Testing Sessions Combined With Outlier Removed

Appearance		Flavour	
Oyster 1.	51.64	Oyster 1.	62.31
Oyster 2.	53.14	Oyster 2.	58.21
Texture		**Overall Acceptability**	
Oyster 1.	63.13	Oyster 1.	60.49
Oyster 2.	60.48	Oyster 2.	56.86

All figures and future discussion is based on data with the outlier removed.

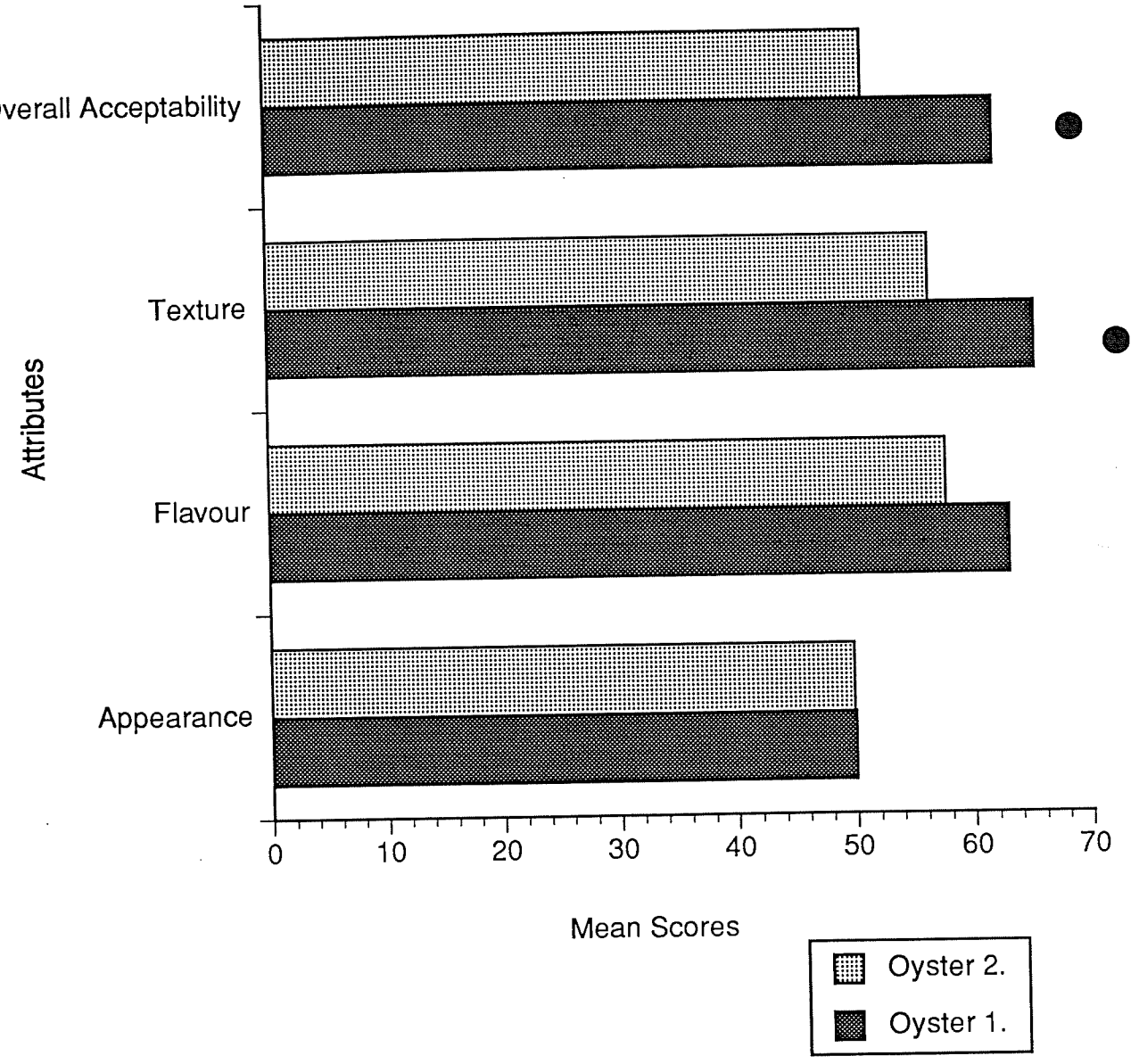
Figure 1. shows the mean scores for Oyster 1. and Oyster 2. at October 1991.

Figure 2. shows the mean scores for Oyster 1. and Oyster 2. at January 1992.

Figure 3. shows the mean scores for Oyster 1. and Oyster 2. at November 1992.

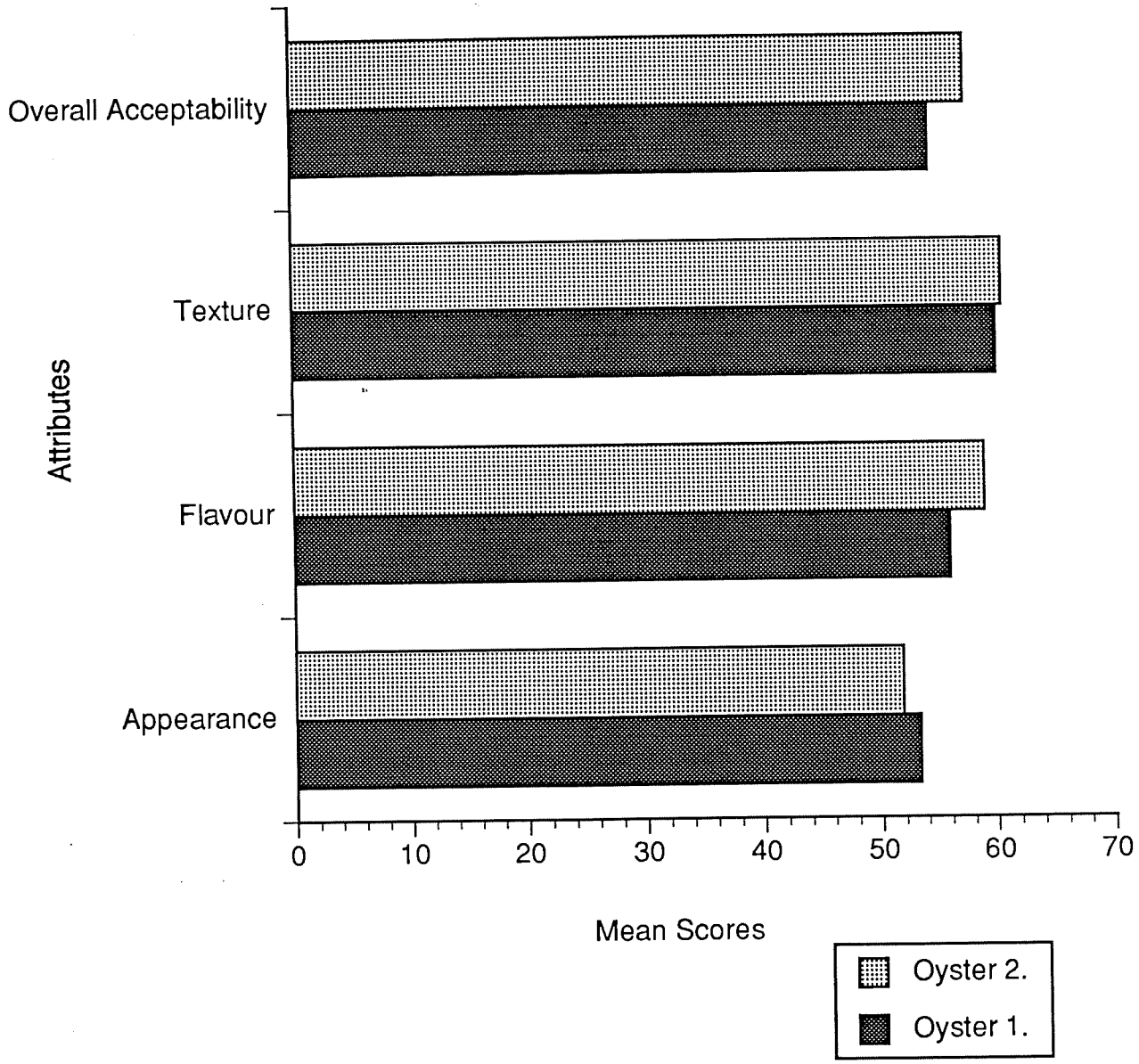
Figure 4. shows the mean scores for Oyster 1. over time compared with Oyster 2. over time.

Figure 1. Evaluation of Oyster 1. and Oyster 2. October 1991



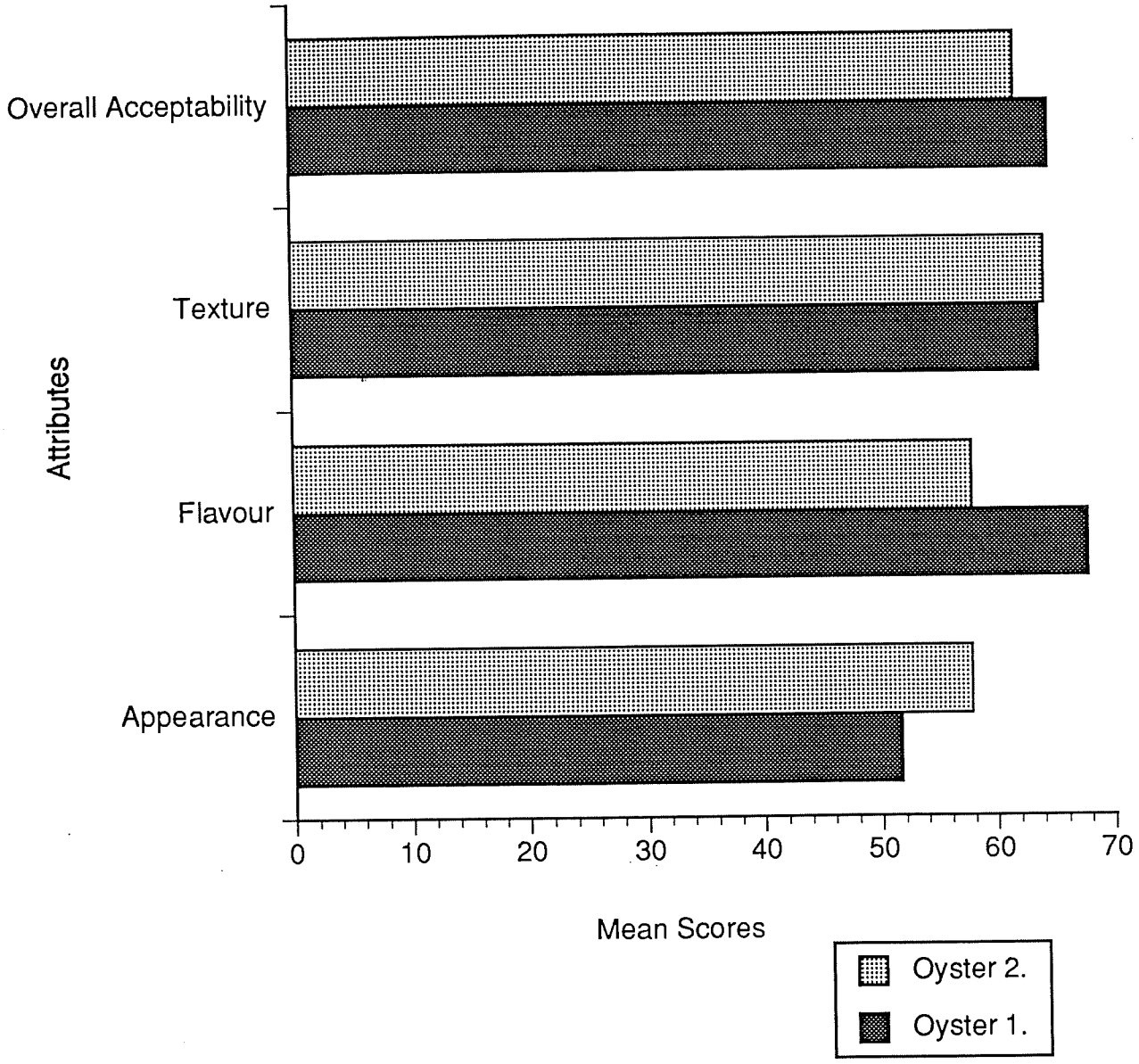
Attributes with a dot have statistically significant differences between oysters.

Figure 2. Evaluation of Oyster 1. and 2. January 1992



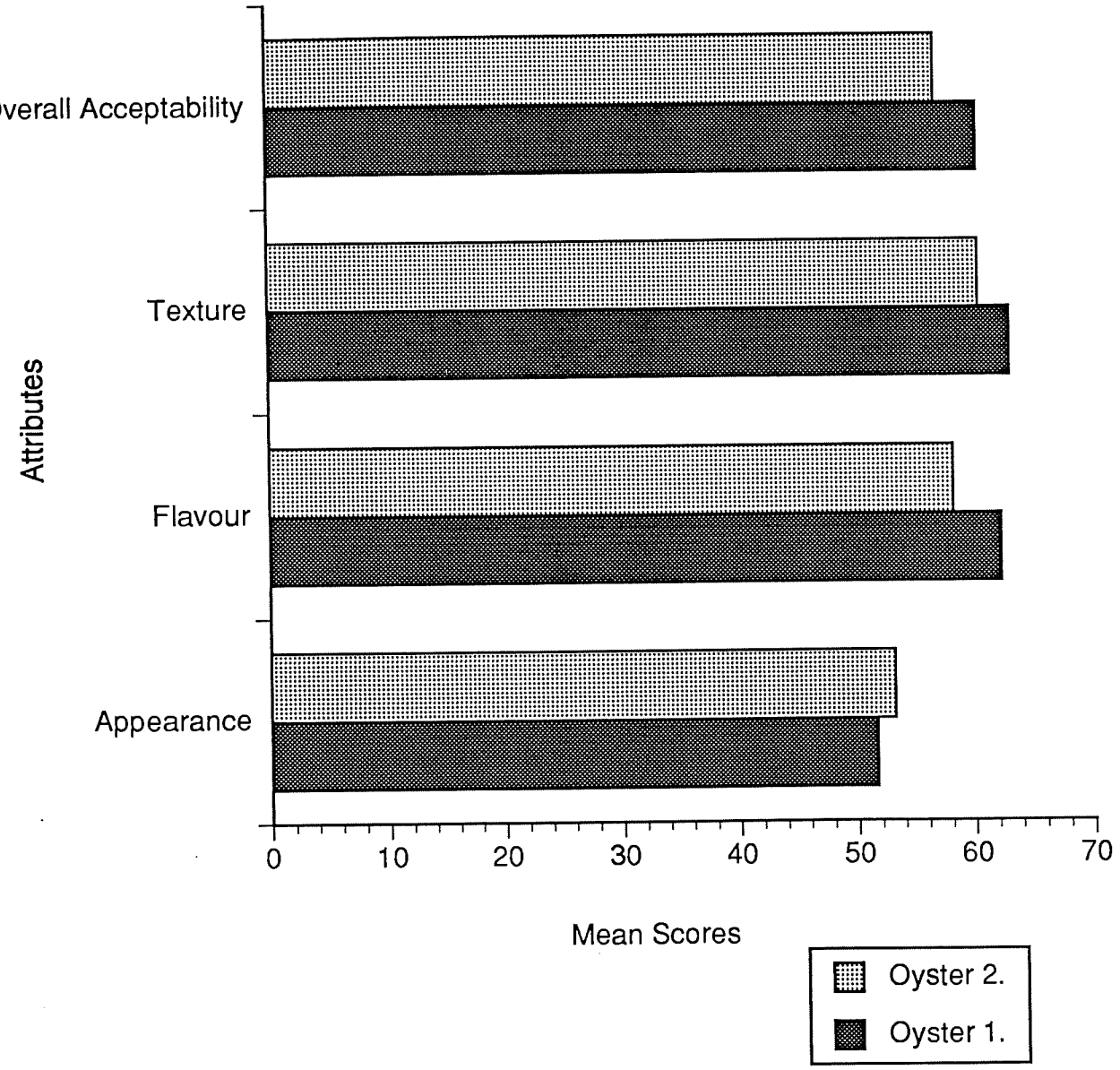
Attributes with a dot have statistically significant differences between oysters.

Figure 3. Evaluation of Oyster 1. and 2. November 1992



Attributes with a dot have statistically significant differences between oysters.

Figure 4. Evaluation of Oyster 1. and 2. Over Time



Attributes with a dot have statistically significant differences between oysters.

SENSORY EVALUATION OCTOBER 1991

Appearance

There were no significant differences in appearance between Oyster 1. and 2. at Time 1.

Flavour

There were no significant differences in flavour between Oyster 1. and 2. at Time 1.

Texture

There was a significant difference in texture between Oyster 1. and 2. at Time 1. ($p=0.015$), with Oyster 1. having a mean rating of 65.48 and Oyster 2. a mean rating of 56.52. Oyster 1. being rated further towards the "extremely good" end of the rating scale than Oyster 2.

Overall Acceptability

There was a significant difference in overall acceptability between Oyster 1. and 2. at Time 1. ($p=0.045$) with Oyster 1. having a mean rating of 62.19 and Oyster 2. a mean rating of 51.14. Oyster 1. being rated as more acceptable overall than Oyster 2.

SENSORY EVALUATION JANUARY 1992

There were no significant differences in appearance, flavour, texture or overall acceptability between Oyster 1. and 2. at time 2.

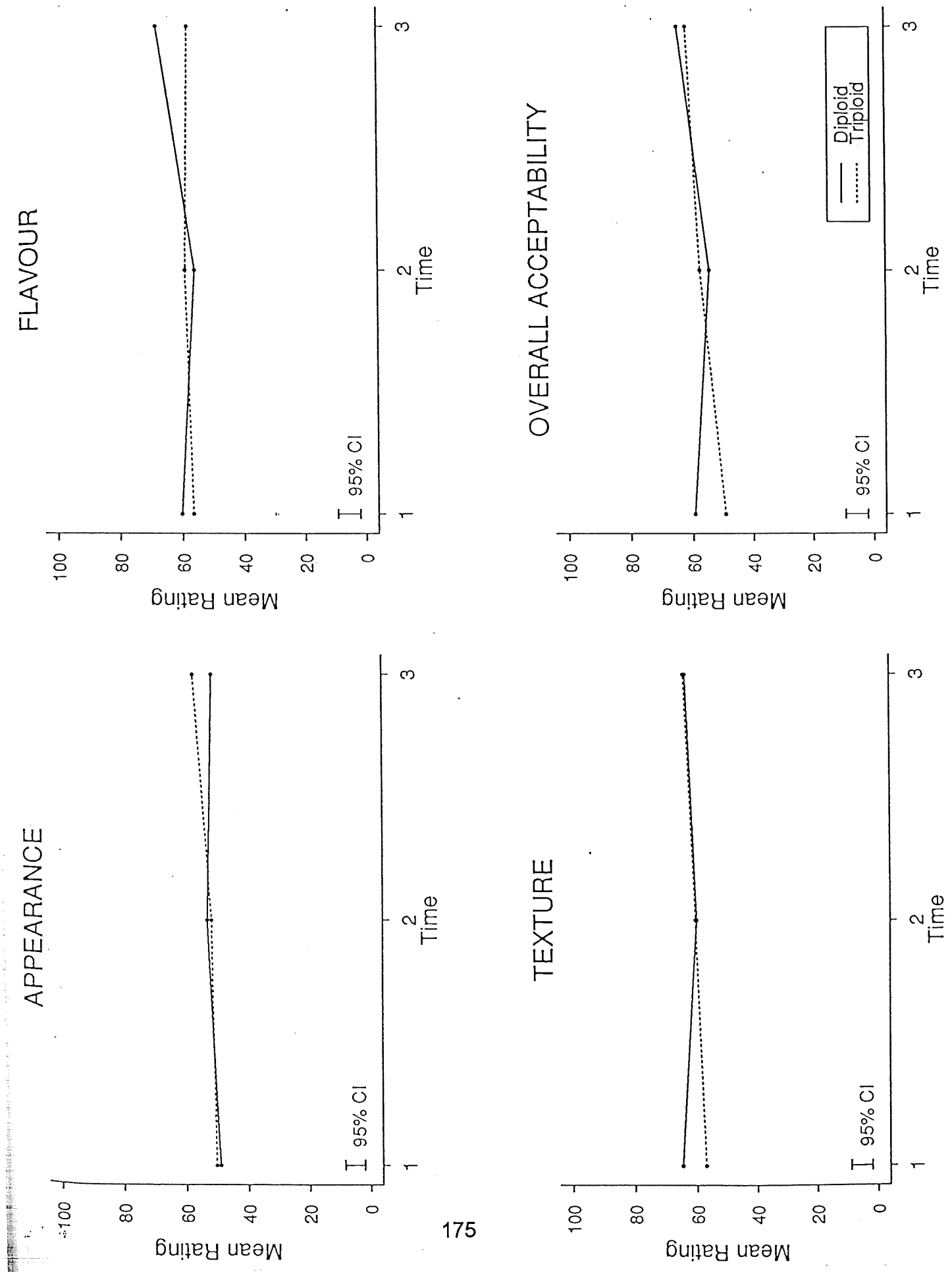
SENSORY EVALUATION NOVEMBER 1992

There were no significant differences in appearance, flavour, texture or overall acceptability between Oyster 1. and 2. at time 3.

OYSTER 1. COMPARED WITH OYSTER 2. OVER TIME

There were no significant differences in appearance, flavour, texture or overall acceptability between Oyster 1. and 2. over time. Figure 5. is a plot of means for interaction (oyster over time) and illustrates the conclusion of no significant differences over time.

Figure 5. Plot of Means for Interaction (oyster over time)



DISCUSSION

The analysis of variance carried out for each harvesting time separately, found no significant differences for any of the attributes at Time 2. or at Time 3. The only significant differences were texture at Time 1. and overall acceptability at Time 1., with the diploid oyster being rated as more acceptable in texture and overall acceptability than the triploid oyster. All attributes evaluated, with the exception of appearance, were rated above a score of 50. Appearance at Time 1. may have been marked down as the size of the oysters for both groups was smaller than average.

There were no significant differences between the diploid and triploid oysters over time for any of the attributes evaluated.

CONCLUSION

The sensory evaluation of diploid and triploid Sydney rock oysters suggests that there is little difference between the two genetic strains over time, allowing perhaps the use of triploid oysters as a winter crop in New South Wales.

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Principal

Studies on triploid oysters in Australia. IV. Sensory evaluation of triploid and diploid Pacific oysters, *Crassostrea gigas* (Thunberg), in Tasmania.

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Abstract

Triploid and diploid Pacific oysters *Crassostrea gigas* (Thunberg), of marketable size, were compared using taste panels and biochemical analyses. Sibling triploids (percentage triploidy = 76.0%) and diploids were grown as cultchless oysters in mesh baskets on intertidal, off-bottom racks. Freshly shucked, raw oysters (21.5-27.5 months old) were provided as half shell product to untrained members of taste panels on three occasions (spring, summer and autumn) to compare sensory attributes (overall acceptability, appearance, flavour, and texture/mouthfeel). Of the twelve possible comparisons between triploids and diploids (four sensory attributes for each of three seasons), only two were significantly different with triploids eliciting lower scores for appearance (spring) and texture (autumn) than diploids ($P<0.05$). Both groups of oysters exhibited commercially acceptable condition index values although these declined through time.

Glycogen content of triploids was higher than for diploids ($P<0.01$). Glycogen extracted from Pacific oysters was largely tasteless and changes in glycogen content only paralleled seasonal trends for sensory attributes of diploids but not triploids. Analyses, based on results for individual oysters (ploidy groups and sensory trials combined), indicated significant positive correlations between all sensory attributes ($P<0.001$, n = 611 oysters). However, overall acceptability was more strongly correlated with flavour (correlation coefficient = 0.85) and texture/mouthfeel (0.78) than with appearance (0.50) ($P<0.001$).

Triploid Pacific oysters are acceptable, in terms of sensory attributes, to potential consumers in Australia. Evaluation of taste and texture/mouthfeel could be useful in commercial oyster quality inspection programs. However, glycogen content is not necessarily a good predictor of Australian consumer response to Pacific oysters with acceptable condition index.

Keywords: triploidy, Pacific oysters, *Crassostrea gigas*, taste, texture, appearance, glycogen, glucose

1. Introduction

Triploidy has proved to be a useful technique for reducing gonad development, maintaining acceptable meat condition through inhibition of spawning, and increasing growth rates of bivalves (Beaumont and Fairbrother, 1991). In the U.S.A. there is consumer resistance in summer months to diploid Pacific oysters *Crassostrea gigas* with advanced gonad development but the use of triploid oysters overcomes this problem (Allen and Downing, 1991). Sydney rock oysters *Saccostrea commercialis*, the major edible oyster produced in Australia, and Pacific oysters are acceptable for domestic markets during periods of advanced gonad development provided they retain a high condition index (Graham et al., 1993; Nell, 1993). Triploid Pacific oysters are being assessed in Tasmania (Gardner et al., 1995; Maguire et al., 1995) to evaluate their suitability for overcoming marketing problems following spawning of diploids in summer or autumn (Graham et al., 1993).

Taste panels have often been used for assessing the quality of aquacultural products including molluscs (Naidu and Botta, 1978; McBride et al., 1988). Comparisons have been made between sensory attributes and biochemical composition of shellfish products (Stroud and Dalgarno, 1982). Specifically, Allen and Downing (1991) suggested that the preference of U.S. taste panels for triploid Pacific oysters was due to their higher glycogen content relative to diploids.

In this study the acceptability of triploids and diploids to taste panels was compared as sensory attributes, along with issues associated with a "clean natural image", will largely determine marketability. Glycogen, glucose and moisture content and the relationships among sensory attributes were also investigated to help explain any differences in overall acceptability of two year old triploid and diploid oysters sampled in spring, summer and autumn.

2. Materials and methods

Oysters and husbandry

Triploid Pacific oysters were produced using strip spawning, cytochalasin B stress (Allen et al., 1989), and proprietary commercial hatchery technology in February 1990 as summarised in Maguire et al. (1995). Sibling triploid and diploid oysters were grown in the same hatchery, nursery (land based upwellers then intertidal, off-bottom, sectionalised trays), and growout systems (intertidal, off-bottom, mesh baskets) (Maguire et al., 1995). The percentage triploidy for spat or adults in growout systems was determined on three occasions for oysters from this single batch of triploids (76.0±1.7 %, mean±s.e., n=3 groups ≥ 100 oysters). The oysters used for taste tests were from a husbandry experiment (Maguire and Kent, 1991) and were maintained in elevated 12 mm mesh baskets from December 1990; on average, oysters were submerged 58.6 % of the time (Kent and Maguire, 1992). For the taste tests in November 1991 (spring, age 21.5 months), February 1992 (summer, 24.8 months), and May 1992 (autumn, 27.5 months), oysters were collected from these baskets within a commercial oyster lease in Little Swanport estuary,

Tasmania (42° 20'S, 148°E). They were transported by road, out of water, and submerged overnight in a recirculating seawater holding tank at the University of Tasmania, Launceston before the taste tests.

Measurement of size, condition index and composition of oysters

Oyster size (whole weight, shell height and dry meat weight) and moisture content of meats were measured as in Maguire et al. (1995) using 8-12 oysters per ploidy group for each trial. The condition index (CI) [(dry meat weight (g) x 1000)/ cavity volume (g)] of each oyster was measured as recommended by Crosby and Gale (1990). The dried meats were then separated into two groups for each treatment, ground, and analysed for glucose and glycogen using a modified Keppler and Decker (1974) procedure (B. Day and G.B. Maguire, unpublished data, 1994).

Taste tests

For each of the three taste tests at the University of Tasmania, 31 - 36 untrained volunteers from a range of age groups and cultural and socio-economic backgrounds were used. The selection of these volunteers was based only on a liking for natural (raw) oysters. Three triploid and three diploid oysters (scrubbed and freshly shucked with meats turned over and served in the half shell) were arranged randomly on a white disposable plate for each panel member with alphabetical oyster positions marked in pen. The oysters were not deliberately matched for size. Panel members used a taste test laboratory, at the Launceston campus, designed to meet the requirements of the Standards Association of Australia (Anon., 1984). They were provided with squares of crustless white bread and a glass of water. The trials were hedonic; on the result sheet provided panel members wrote the letter associated with each oyster on a non graduated 145 mm scale the beginning of which was denoted as "extremely poor" and the end as "extremely good". Panel members entered results for four sensory attributes (appearance, flavour, texture/ mouthfeel, and overall acceptability) on separate scales on the same data sheet along with any specific comments. Subsequently, letter positions ware measured in mm from the origin and divided by 1.45 to give a potential range of 0-100.

Glycogen from Pacific oysters was extracted, with trichloroacetic acid, ethanol, water, and diethyl ether (Clark, 1964, pp. 23-24), so that the authors could assess its taste. A commercial source of oyster glycogen (Sigma™ catalogue number G-8751) was also tasted.

Statistical analyses

An a priori alpha value of 0.05 was adopted throughout. Size, CI and composition data were subjected to two factor ANOVA (ploidy and season as fixed factors) followed by comparisons of means for triploid and diploid oysters in each trial using Fisher's Least Significance Difference test. Homogeneity of variance was assessed using Cochran's test. Sensory data for each group of three oysters, of the same ploidy group and evaluated by the same panel member, were averaged and the panel members considered as replicates for paired t-tests between triploid and diploid oysters. As each taste test involved many of the same panel members used in the other tests, the three

taste tests were not considered to be independent. Hence separate t-tests were done and no statistical comparisons were made between tests (Sokal and Rohlf, 1981).

Relationships among sensory variables were examined using correlation analyses with data from 611 individual oysters (results for both ploidy groups and three trials combined). Correlation coefficients for pairs of sensory attributes were compared to see which attributes correlated most strongly with overall acceptability (Snedecor and Cochran, 1989, pp. 186-190).

3. Results

Size, condition index and composition of oysters

Two factor ANOVAs (Table 1) indicated that size, CI and composition of oysters changed over time, that triploids and diploids differed in whole weight, dry meat weight and glycogen content, and that significant season x ploidy group interactions were only evident for glycogen and glucose content. Both ploidy groups grew consistently in whole weight (Fig. 1a) and, while being similar in size at 21.5 months, by 27.5 months triploids had a whole weight advantage of 31.1 % ($P<0.001$). Average shell height increased steadily from 78.0 mm for both groups at age 21.5 months to 101 and 95 mm, for triploids and diploids respectively, at 27.5 months. Dry meat weight increased over time except for 27.5 month old diploids which had declined in meat size to be 37.4 % smaller than triploids of the same age ($P<0.01$) (Fig. 1b). CI declined through time from high initial values (92.1 - 102.0) to more commercially marginal values (69.4 - 74.7). Triploids maintained condition between 24.8 and 27.5 months whereas diploids deteriorated rapidly during that period (Fig. 1c). While moisture content of oyster meats was similar for ploidy groups, the trends over time were the inverse of those for CI (Fig. 1d). Glycogen content was 3.2-8.6 g/100 g (dry soft tissue) higher in triploids than diploids ($P<0.01$); by 24.8 months the average value for diploids had declined to 4.2 g/100 g but had recovering by 27.5 months (Fig. 1e). The significant season x ploidy group interaction for glycogen content was evident in the small difference between triploids and diploids after 27.5 months. Oysters contained much less glucose than glycogen and seasonal changes exhibited a similar pattern except for the elevated glucose content at 24.8 months for triploids (Fig. 1f).

Taste tests

Of the twelve possible comparisons between triploids and diploids (four sensory attributes for each of three seasons), only two were significantly different with triploids scoring lower for appearance (spring, 21.5 months) and texture (autumn, 27.5 months) than diploids ($P<0.05$) (Fig. 2a,c). All mean values for different attributes and ploidy groups in separate trials were in the range 55.0 - 65.0 (n=22 means) except for appearance of triploids at 21.5 and 24.8 months (53.1-53.2). Seasonal trends were complex with opposite trends for diploids and triploids evident between summer and autumn (24.8-27.5 months). Mean values for diploids improved while triploids deteriorated for all attributes except appearance (Fig. 2).

In general, variation among average results for different panel members, as measured by standard error values for each sensory attribute, were similar for triploid and diploid oysters (Fig. 2). Variability among triploids and diploids was also assessed using the range of scores, for a single sensory attribute, recorded for the three oysters of the same ploidy group provided to a single panel member. The mean values for these ranges were determined, with the result for each panel member providing replication (Fig. 3). Of the twelve possible comparisons between triploids and diploids for the same age and sensory attribute, only two of these means were significantly different with triploids exhibiting higher and lower variability for texture (autumn, 27.5 months) ($P<0.01$) and overall acceptability (spring, 21.5 months) ($P<0.05$) respectively (Fig. 3c,d).

Analyses, based on results for individual oysters (ploidy groups and sensory trials combined), indicated significant positive correlations among all four sensory attributes ($P<0.001$, n=611 oysters). However, overall acceptability was more strongly correlated with flavour (correlation coefficient = 0.85) and texture/mouthfeel (0.78) than with appearance (0.50) ($P<0.001$). Flavour and overall acceptability were more strongly correlated than were texture/mouthfeel and overall acceptability ($P<0.01$).

Oyster glycogen from a commercial source or extracted from Pacific oysters was largely tasteless and had a mild starch-like texture.

4. Discussion

Size, condition index and composition of oysters

Seasonal trends and differences between diploids and triploids for whole oyster weight (Fig. 1a) were consistent with results for sibling oysters held on a different part of this Little Swanport oyster lease at the same time (Maguire et al., 1995). Those oysters reached an advanced stage of gonad development in November - December 1991, as indicated by the occurrence of spermatozoa and mature oocytes and a rapidly increasing gonad area, but did not spawn in the summer - autumn of 1991-92 (Gardner et al., 1995; Maguire et al., 1995). However, the decline in meat weight and condition index for diploids between 24.8 and 27.5 months in oysters in the present study was consistent with spawning activity noted in adjacent commercial stocks of non sibling diploids. However, while CI did decline through time, all groups in the present study maintained CI values that were at least acceptable (≥ 70).

Taste test methodology

Untrained taste panels were used in our study and this may account for the lower average scores compared to those for diploid Pacific oysters from New South Wales in spring in the study by McBride et al. (1988) using trained panels. We considered that most groups of diploid and triploid oysters offered to the Tasmanian taste panels were of high quality. Cardello et al. (1982) found that there can be "clear differences between consumer and trained panel judgements".

For side-by-side comparisons McBride et al., (1988) provided sets of Pacific and Sydney rock oysters in sequence and this significantly favoured the

set evaluated first. We used several randomly arranged oysters per experimental group rather than single oysters; this should have increased the power of the trial, as well as reducing sequence problems, although Allen and Downing (1991) were able to demonstrate significant differences using single oysters after eliminating diploid individuals within triploid treatments.

In contrast to the studies by McBride et al. (1988) and Allen and Downing, (1991), we presented the same type of product (raw oysters) over several seasons. Given that there can be seasonal differences in CI between ploidy groups (Maguire et al., 1995) and species of oysters (Mason and Nell, 1995), this seems to be a worthwhile approach.

Taste tests

As few significant differences in sensory attributes between triploid and diploid Pacific oysters were detected by Tasmanian taste panels from diverse geographic and cultural backgrounds, the sensory attributes of triploids are likely to be acceptable to Australian consumers. In contrast, the taste panels used by Allen and Downing (1991) had a clear preference for triploid Pacific oysters in summer for all attributes examined (overall acceptability, flavour and texture). This is probably due to the traditional dislike of the sensory attributes of sexually mature diploid Pacific oysters in the U.S.A. (Allen and Downing, 1991). The two significant differences for sensory attributes that were recorded in the present study, a preference for diploids for appearance (spring, 21.5 months) and texture (autumn, 27.5 months), are not readily explained by differences in CI, or glycogen, glucose or moisture content. The only obvious difference in appearance occurred in summer (24.8 months) when 5.9% of the triploids but none of the diploids had brown patches on the meats. Histological examination revealed only surface pigmentation and a similar phenomenon has been observed in triploid and diploid Sydney rock oysters (J. Nell, personal communication, 1992). The average dry meat weight for triploids in autumn was much higher than for diploids ($P<0.01$) and a few panel members objected to the large size of some meats in that taste test. Scores for triploids declined between summer (24.8 months) and autumn (27.5 months) for flavour, texture and acceptability; meat size could have influenced the perceptions of some panel members in the autumn taste test. Meat size for diploids and triploids was similar in both spring and summer tests ($P>0.05$).

Allen and Downing (1991) suggested that the better sensory attribute scores for triploids could be due to their higher glycogen content. However, changes in glycogen or glucose content only followed seasonal trends in sensory attributes for diploids but not triploids in the present study. As glycogen is a macromolecule, it is not surprising that oyster glycogen from both a commercial source or extracted from Pacific oysters was tasteless. No studies which definitively related glycogen content to oyster taste were found and flavour seems to be determined by a complex mixture of compounds in oysters (Soliman et al., 1985). Glucose is the major blood sugar in bivalves (Whyte and Englard, 1982) but the concentration of glucose in wet meats would have been below the taste threshold for humans (Aurard et al., 1987, pp. 156-157). However, an influence of glycogen content on the texture of oysters seems plausible.

Another contrasting result was the finding by Allen and Downing (1991) that diploids elicited more variable responses from panel members. In the present study, variability for each sensory attribute was similar both in terms of standard errors, associated with average scores (Fig. 2), and range values (Fig. 3) for the groups of three oysters of each ploidy treatment provided to each panel member. This was despite the fact that triploids tended to be more variable in terms of whole weight, dry meat weight, and glycogen content presumably because the triploid group was a mixture of triploids and diploids (Fig. 1a,b,e).

The correlation analyses indicated overall acceptability was correlated with flavour, texture/mouthfeel and appearance (in descending order of correlation coefficients). This is consistent with the results of regression analyses in an unpublished undergraduate report on the sensory evaluation of Sydney rock oysters (Longley, 1982). Both appearance and odour were considered to be poor predictors of general acceptability (Longley, 1982) while in the present study appearance was not as closely linked, as other attributes, to general acceptability; aroma was not assessed. Our results suggest that sensory evaluation of taste and texture/mouthfeel could be useful in commercial oyster quality inspection programs; one such program operates within the Tasmanian Pacific oyster industry. However, appearance should not be overlooked as it is usually the only criterion available to consumers prior to purchasing oysters.

In conclusion, triploid Pacific oysters are acceptable, in terms of sensory attributes, to potential consumers in Australia. However, other perceptual issues such as a "clean healthy image" can influence marketability. Classification and monitoring of growing areas, in terms of microbiology, pollutants and algal toxins, in Tasmania and increasingly in other Australian oyster industries have addressed health issues (Nell, 1993). However, chemical induction of triploidy has the potential to adversely affect the marketing image of triploids, regardless of the absence of health risks. Currently, relevant oyster research in Australia is aimed at replacing cytochalasin B as a stressor for induction of triploidy and at producing tetraploid broodstock that may eliminate the need to stress eggs used to produce triploid offspring.

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Table 1
Results of two factor ANOVAs for size, condition and composition of 21.5-27.5 month old triploid and diploid Pacific oysters (*Crassostrea gigas*) from Little Swanport, Tasmania used in taste tests over three seasons (spring to autumn, 1991-92).

Variable	Factor		
	Ploidy group (P)	Season (S)	P x S
Whole weight	***	***	NS
Shell height	NS	***	NS
Dry meat weight	*	*	NS
Condition index	NS	**	NS
Moisture content (meats)	NS	**	NS
Glycogen content (meats)	***	***	**
Glucose content (meats)	**	NS	*

*** = $P<0.001$; ** = $P<0.01$; * = $P<0.05$; NS = $P>0.05$

Fig. 1. Seasonal changes (mean±s.e.) in size, condition and composition of 21.5-27.5 month old triploid and diploid Pacific oysters (*Crassostrea gigas*) from Little Swanport, Tas. used in taste tests over three seasons (spring to autumn, 1991-92).
(Condition index = dry meat weight (g) x 1000/ cavity volume (g))
*** = $P<0.001$; ** = $P<0.01$

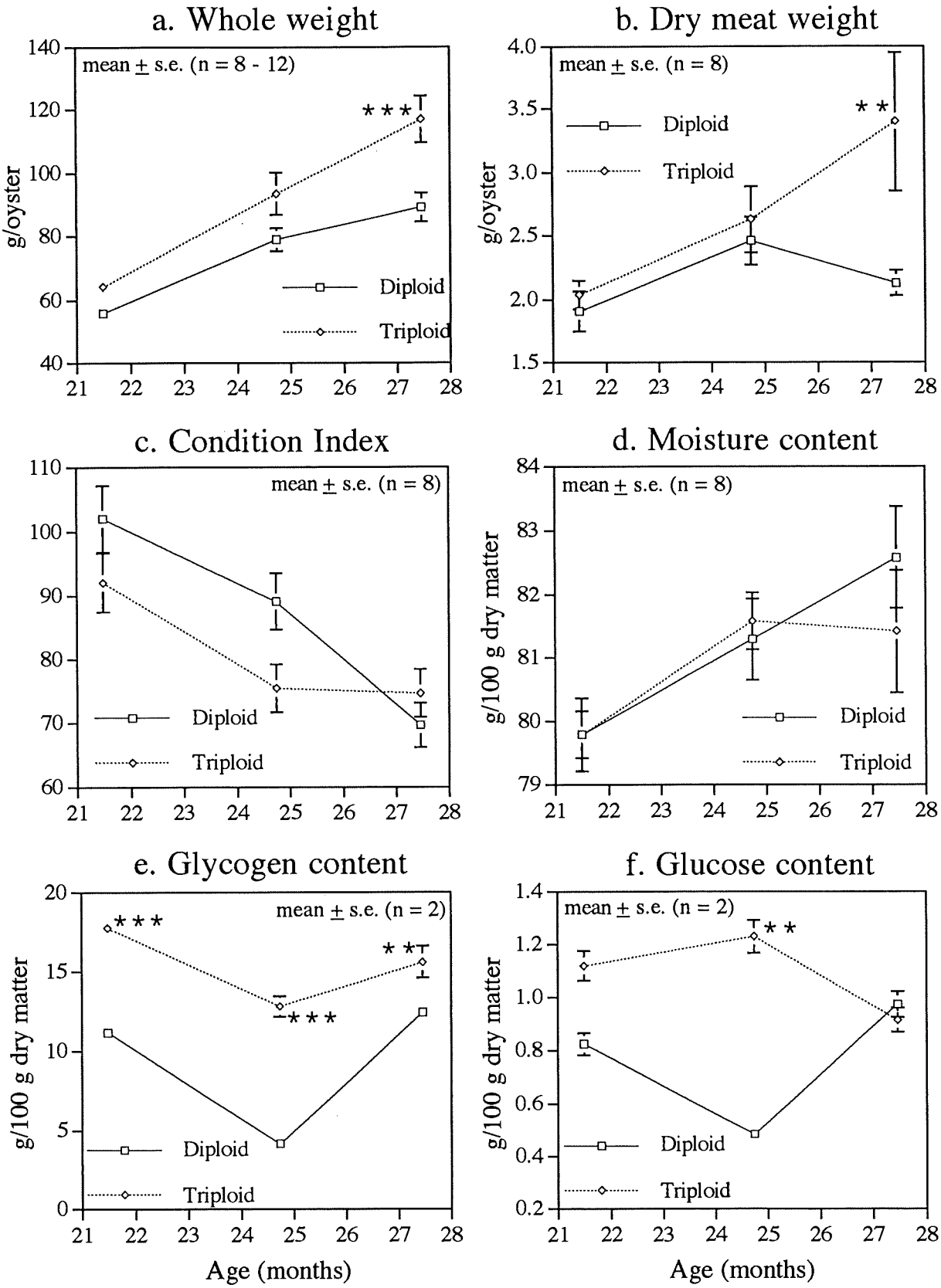
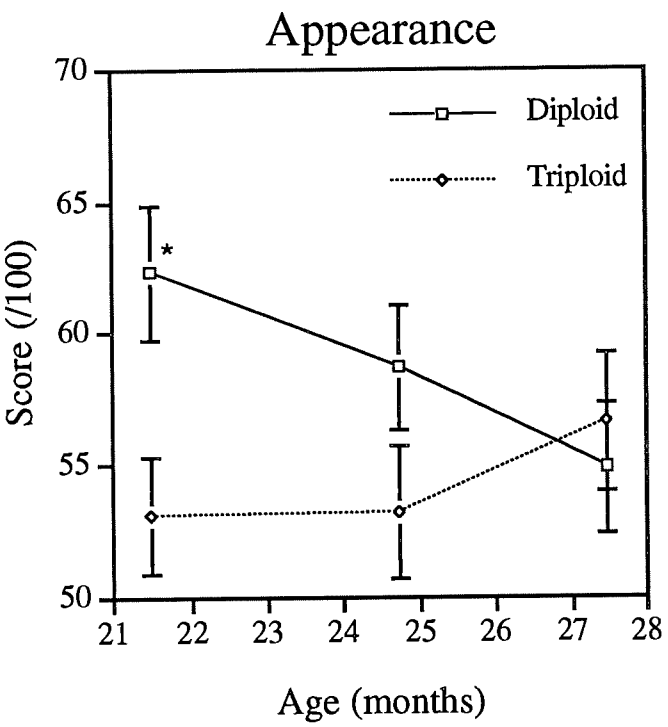

a. Whole weight
mean ± s.e. (n = 8 - 12)
g/oyster
Diploid
Triploid
b. Dry meat weight
mean ± s.e. (n = 8)
g/oyster
Diploid
Triploid
c. Condition Index
mean ± s.e. (n = 8)
Diploid
Triploid
d. Moisture content
mean ± s.e. (n = 8)
g/100 g dry matter
Diploid
Triploid
e. Glycogen content
mean ± s.e. (n = 2)
g/100 g dry matter
Diploid
Triploid
Age (months)
f. Glucose content
mean ± s.e. (n = 2)
g/100 g dry matter
Diploid
Triploid
Age (months)

Fig. 2. Seasonal changes in sensory attributes of 21.5-27.5 month old triploid and diploid Pacific oysters (*Crassostrea gigas*) from Little Swanport, Tasmania used in taste tests over three seasons (spring to autumn, 1991-92). Values are mean±s.e. (n=31-36 panel members).
* = $P<0.05$


Appearance
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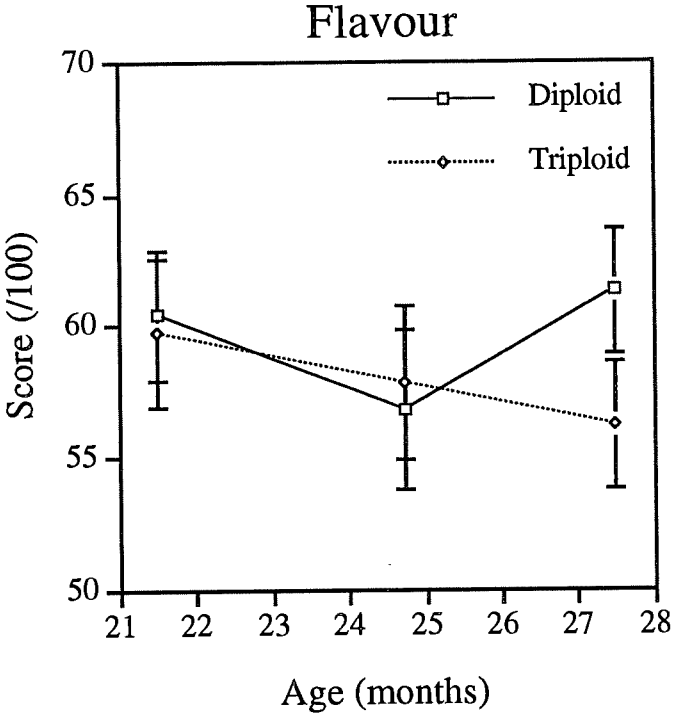
Flavour
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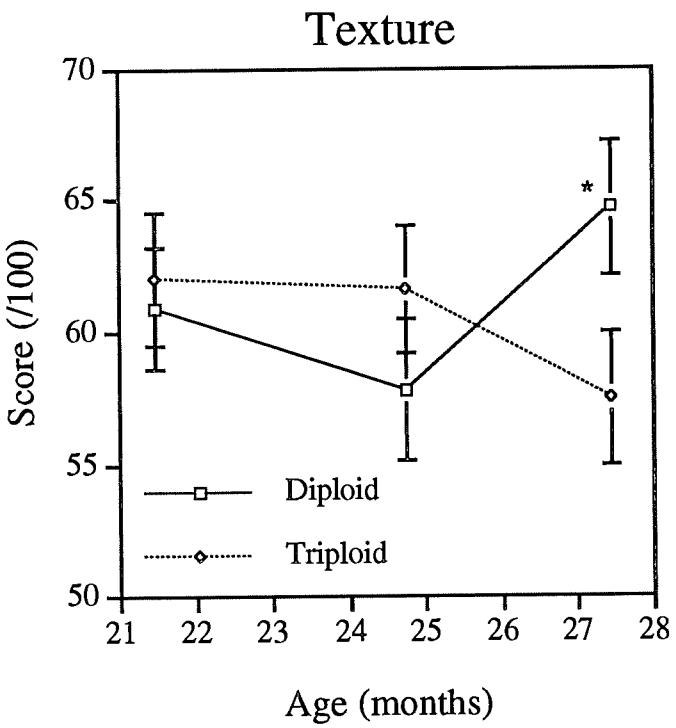
Texture
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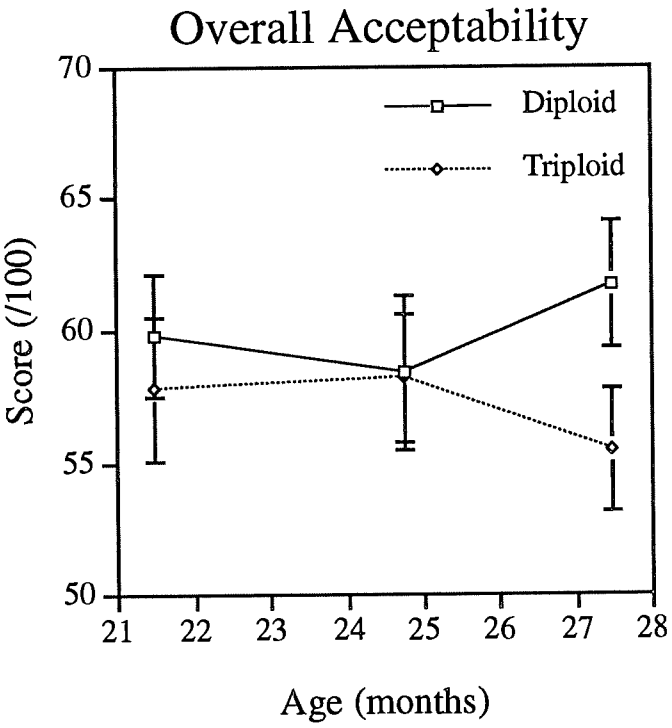
Overall Acceptability
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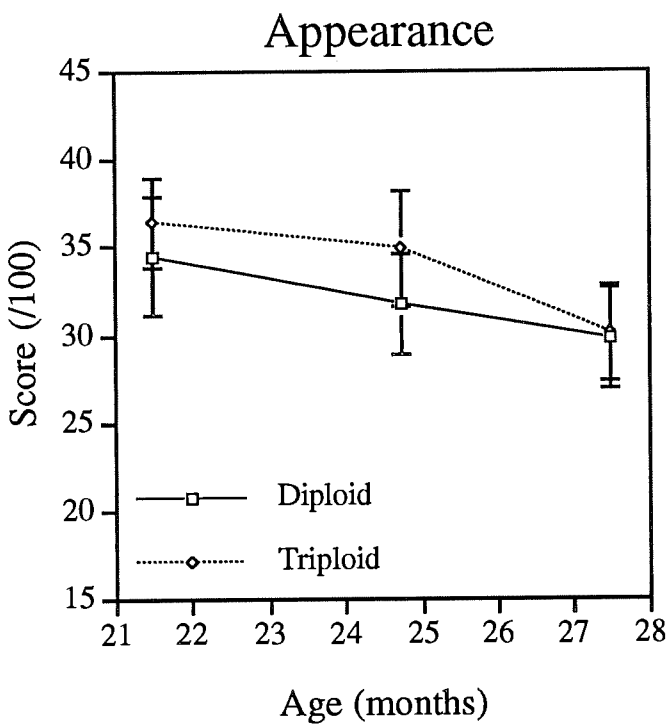
Age (months)

Fig. 3. Seasonal changes in range values, among three oysters supplied to an individual taste panel member, for each sensory attribute of 21.5-27.5 month old triploid and diploid Pacific oysters (*Crassostrea gigas*) from Little Swanport, Tasmania used in taste tests over three seasons (spring to autumn, 1991-92). Values are mean±s.e. (n=31-36 panel members).
** = $P<0.01$; * = $P<0.05$


Appearance
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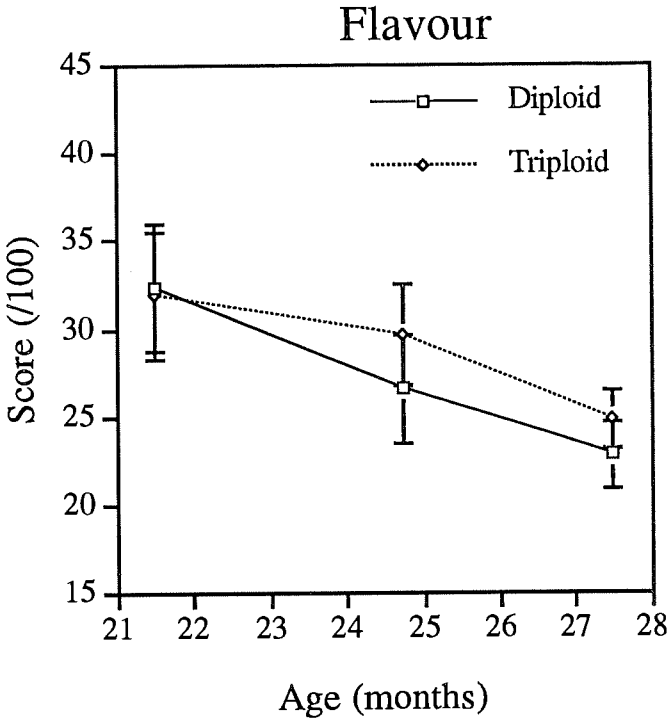
Triploid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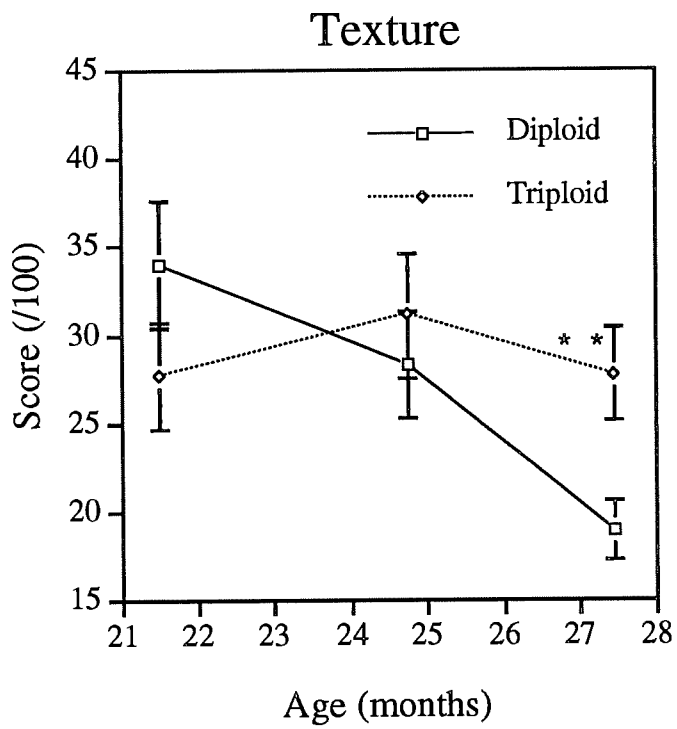
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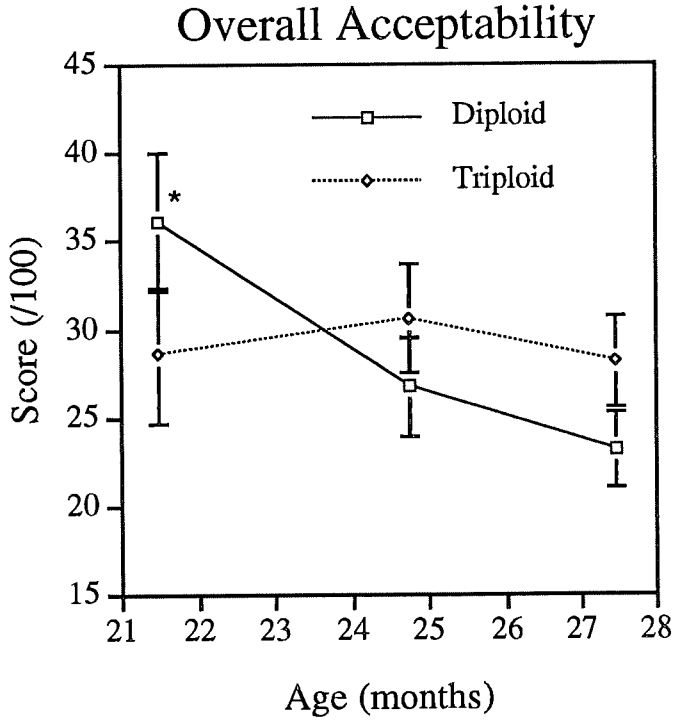
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Score (/100)
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Age (months)
Diploid
Triploid
*

9 APPENDICES

a Nell, J. A., 1993. Farming the Sydney rock oyster (*Saccostrea commercialis*) in Australia. Reviews in Fisheries Science, 1: 97-120.

b Gardner, N. C., 1993. A literature review of triploidy in commercial bivalves. 40 pp. From Gardner, N. C., 1993. Aspects of the Performance of Diploid and Triploid Pacific Oysters (*Crassostrea gigas*, Thunberg) in Southern Tasmania. Unpublished Thesis, University of Tasmania, Launceston, 183 pp.

c Nell, J. A., Cox, E. , Smith, I. R. and Maguire, G. B., 1994. Studies on triploid oysters in Australia. I. The farming potential of triploid Sydney Rock oysters *Saccostrea commercialis* (Iredale and Roughley). In: Book of Abstracts, Fifth International Symposium on Genetics in Aquaculture, Halifax, NC, Canada, pp. 105 (Abstract).

d Maguire, G. B., Gardner, N. C., Nell, J. A., Kent, G. and Kent, A. Studies on triploid oysters in Australia. II. Growth, condition index, gonad area and glycogen content of triploid and diploid Pacific oysters, *Crassostrea gigas*, from oyster leases in Tasmania, Australia. In: Book of Abstracts, Fifth International Symposium on Genetics in Aquaculture, Halifax, NC, Canada, pp. 90 (Abstract).

e O'Meley, C., Maguire, G. B. and Lleonart, M., 1992. Molluscan Culture Techniques. Multi-Skilling in Aquaculture: A Hands-on Training Workshop. University of Tasmania, Launceston, Tas., pp. 1-23.

f Ryan, T., 1992. Aquaculture and remote data collection. UTAS, June 1992, p. 5.

Reviews in Fisheries Science, 1(2):97–120 (1993)

Farming the Sydney Rock Oyster (Saccostrea commercialis) *in Australia*

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ABSTRACT: Commercial production of Sydney rock oysters (*Saccostrea commercialis*) in Australia began simultaneously in New South Wales (NSW) and southern Queensland around 1870. It began with the exploitation of dredge beds, intertidal oyster beds, and with the placement of a range of catching and growing substrates such as sticks, slabs of rocks, and shell placed on intertidal mud flats. As dredge beds were depleted and problems with accumulation of silt and mudworm (*Polydora* sp.) increased, the industry progressively adopted the stick and tray culture on intertidal racks. However, the use of sticks for growing is now rapidly diminishing as farmers are learning to scrape small (4 to 8 mm) spat off sticks and grow them in specially adapted trays or other growing containers (single-seed culture). At its peak in 1976–1977, the combined NSW and southern Queensland industry produced 9267 t (wet weight including shell) or 154,454 bags (1200 oysters per bag), but it has declined over the last decade to a production of 5306 t or 88,429 bags in 1990–1991, with a total value of A$30.1 million.

This review discusses current farming methods such as stick and tray, single-seed and subtidal (raft, pontoon, dredge bed) culture, processing and marketing techniques, problems with diseases and environmental hazards (heat kill, floods, and pollution), competitors (other bivalves and barnacles), as well as government regulations, industry trends, and triploid oysters.

KEY WORDS: History, geographic distribution, biology, farming techniques, processing, depuration, marketing, production trends, diseases, environmental hazards, competitors, predators, government regulations, industry trends, triploid oysters.

I. INTRODUCTION

The Sydney rock oyster (*Saccostrea commercialis*), which belongs in the subfamily Crassostreinae (cupped oyster) in the family Ostreidae (Arakawa, 1990a), has been farmed (Figure 1) in New South Wales (NSW) (Roughley, 1922) and southern Queensland (Smith, 1981/82) since the 1870s. A related species (P. Dixon, personal communication, 1992) has been farmed in Western Australia since the early 1980s. In 1989–1990, about 72% of the edible oysters produced in Australia were Sydney rock oysters and about 27% Pacific oysters (*Crassostrea gigas*), which were produced mainly in Tasmania and South Australia. Other species of edible oysters farmed in Australia are the native flat oyster (*Ostrea angasi*), which is farmed in NSW, Tasmania, Victoria, and South Australia, the milky oyster (*S. amasa*), and the blacklip oyster (*S. echinata*), which are farmed in northern Queensland (Nell et al., 1990).

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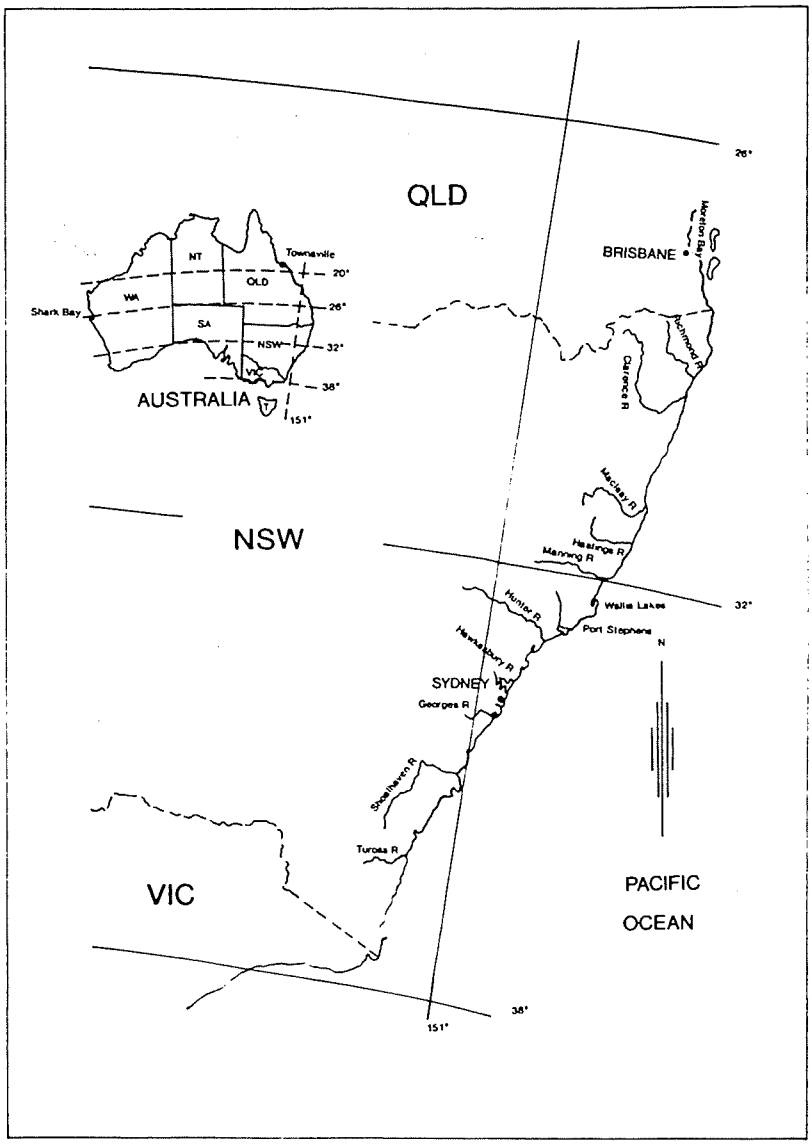

FIGURE 1. A map of Australia and New South Wales (not to scale) showing the major oyster producing estuaries for NSW.

Although other species of the genus *Saccostrea* are farmed throughout Asia (FAO/UNDP, 1990, 1991) and to a limited extent in northern Queensland (Nell et al., 1990), the genus and its geographic distribution are so diverse that this review is restricted to the Sydney rock oyster. Several different aspects of Sydney rock oyster farming have been reviewed, including a brief industry overview (Nell et al., 1990), management and disease interactions (Nell and Smith, 1988), alternative cultivation methods (Holliday et al., 1988) and production trends (Maguire et al., 1988); however, these were all limited in their scope, and the excellent review of oyster farming by Korringa (1976) has now become dated. The following is a review of the

major issues concerning the farming of the Sydney rock oyster, including the development of the industry, farming methods, disease, processing and marketing techniques, government regulations, triploid oysters, and industry trends.

II. HISTORY

Before Europeans settled in Australia, Sydney rock oysters were collected for consumption by aborigines along the coastal regions of eastern Australia and some of the shell deposits in aboriginal kitchen middens have been carbon-dated to around 6000 B.C. (Malcolm, 1987). The early European settlers gathered oysters not only for food, but also for their shells, which were collected in large numbers and burnt to provide lime for building mortar (Malcolm, 1987). As the European population increased, natural oyster stocks were depleted. This resulted in the introduction of legislation prohibiting the burning of live oysters for lime and led to the development of farming practices (Roughley, 1925a; Smith, 1985).

Systematic oyster cultivation first began in NSW around 1870 with attempts to establish the French system of growing oysters in canals ("claires") on the banks of the Georges River, NSW (Roughley, 1922). The project was eventually abandoned due to high mortalities caused by high summer temperatures and the build-up of silt on the oysters (Roughley, 1922). The failure of this project deterred further attempts at cultivation for a short period. Oyster farmers then began to set out stick, stone, and shell to catch and grow oysters in the intertidal zone until they could be harvested (Roughley, 1922). Around 1888, New Zealand rock oyster spat (*S. glomerata*, considered to be a subspecies of *S. commercialis* [Buroker et al., 1979]) were imported into NSW to replenish the depleted oyster stocks (Roughley, 1922). The appearance of mudworm (*Polydora* sp.) in NSW, which happened concurrently with the introduction of New Zealand oysters, forced the development of intertidal farming methods (Roughley, 1922) because this type of culture provides some protection against mudworm infestation. The remains of the early farming methods (Roughley, 1925b), such as wooden pegs driven into the mud bottom, rock culture (slabs of rocks standing upright in the mud), and bottom culture (shell spread out on a prepared base) can still be seen in some estuaries today (Figure 2). A few dredge bed areas are still operational.

The southern Queensland oyster industry flourished from the 1870s to 1910 but has since declined (Smith, 1981/82). Although largely a dredge bed industry, it also involved the exploitation of oysters occurring naturally in the intertidal zone. This was called "bank oystering" (Smith, 1981/82), referred to as "bottom culture" in NSW. Some farmers also experimented with rocks, shells, and sticks as alternative substrates on which to grow oysters (Smith, 1981/82).

Disaster struck the industry in 1895 when mudworm (*Polydora* sp.) killed large numbers of oysters (Smith, 1981/82). The industry also suffered from flood damage, predation by fish, oyster theft, mismanagement, and the lack of secure tenure of oyster banks. Although mudworm was the greatest single contributor to the decline in production (Smith, 1981/82), in the 1970s QX disease was responsible for a further decline (Wolf, 1979; Potter, 1983; Moxon, 1986). Since 1936, Queensland oyster farmers have begun to import half-grown oysters from NSW to grow them to market size (Smith, 1981/82).

FIGURE 2. Raised bed culture, Lime Kiln Bar, Georges River, NSW, April 1992. Raised, well-drained growing beds were built early this century in intertidal areas where current flow is sufficient and mud deposition can be controlled. The growing beds are stocked with natural spat, and with small oysters produced by the culling process.

The 1978 outbreak of viral gastroenteritis was a severe setback for the industry. Although no fatalities occurred, many consumers were affected (Murphy et al., 1979), and the press coverage that ensued resulted in a severe setback to sales, which did not fully recover for almost 2 years (Figure 3; Maguire et al., 1988).

III. GEOGRAPHIC DISTRIBUTION AND BIOLOGY

The distribution of the Sydney rock oyster ranges from the temperate climate of the Victorian/NSW border (37°S), the subtropical conditions in northern NSW and southern Queensland, and the tropical conditions of Townsville in Queensland (19°S; Figure 1). A related species is found along the north of Australia as far south as Shark Bay in Western Australia (25°S, 114°E; Figure 1) (P. Dixon, personal communication, 1992). The Sydney rock oyster occurs subtidally on natural dredge beds (Smith,1981/82) and intertidally on rocks, mangroves, and man-made structures (Malcolm, 1987).

Sydney rock oysters have the ability to change their sex, and it is likely that they spawn for the first time as males or at least develop initially as males (Roughley, 1933; Dinamani, 1974) and in those aspects they are similar to other cupped oysters

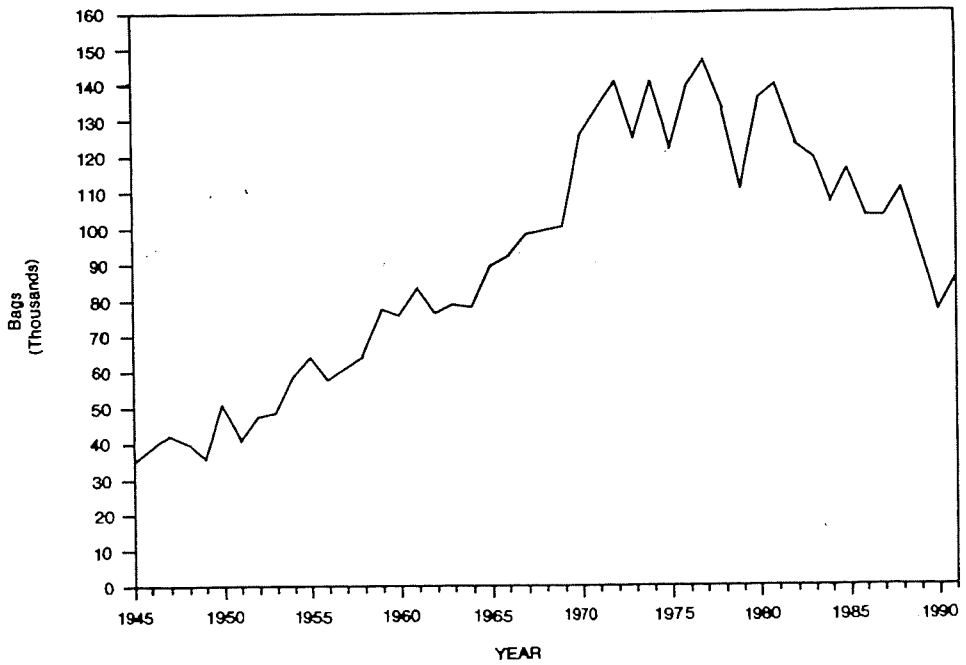

FIGURE 3. Oyster production in New South Wales from financial year 1944–1945 to 1989–1990 (based on annual returns provided by oyster farmers). One bag contains about 100 dozen "plate" oysters (40 to 60 g).

(Galtsoff, 1964; Arakawa, 1990b). During the active breeding season, there is a consistently higher percentage of females among older oysters when compared with younger oysters (of 1 year or less in age) (Roughley, 1933; Dinamani, 1974). The percentage of females drops off after the breeding season in autumn, with a rise in the number of sexually indeterminate oysters with regressed gonads. Examples of hermaphroditism have been observed only rarely in Sydney rock oysters (Roughley, 1933; Dinamani, 1974).

The main spawning and spat settling season (Wisely et al., 1979a; Holliday, 1985a; Holliday and Goard, 1986) for the Sydney rock oyster in Port Stephens, NSW (32°S, 152°E; Figure 1) ranges from February (last month of summer) until May (last month of autumn) when water temperatures are generally above 20°C (Korringa, 1976; Maguire and Bell, 1982). Sydney rock oysters are serial spawners; they spawn several times during a season and have a high fecundity, with females producing up to 25 million eggs per spawning (Holliday, 1985b). Spawning may be triggered on the ebb tide following a high spring (new moon) tide (Roughley, 1933). Larvae are transported passively by river-, tidal-, and wind-driven currents and aggregated by hydrological factors toward the mouths of estuaries, which are important as commercial spat catching areas.

When exposed, intertidally grown oysters may survive high air temperatures of up to 36°C, but may survive even higher temperatures if they are shaded (Potter and Hill, 1982). The susceptibility of oysters to heat kill is highly variable, with subtidally grown oysters being most at risk. Under laboratory conditions of unlimited food supply, Sydney rock oyster larvae show the best growth and survival rates at salinities between 27 and 39‰, whereas adult oysters grow best

at salinities of between 25 and 35‰ (Nell and Holliday, 1988), although they can survive a salinity range of 15 to 55‰ (Nell and Gibbs, 1986). Under natural conditions, growth may be limited by a lack of food, particularly near the mouths of estuaries where coastal conditions of low productivity may prevail. At salinities <15‰, adult Sydney rock oysters can survive by keeping their valves closed for up to 10 days (Nell and Dunkley, 1984). They may be able to survive fresh water flooding for longer periods if a tidal salt wedge (15‰ or higher) reaches their level during high tides.

IV. FARMING TECHNIQUES

A few remnants of the older farming methods, such as dredging for subtidal oysters or culture on raised shell beds or on stones, still may be found but do not contribute much production to the industry. Spat caught on natural shell beds or stones may, however, be used to stock growing trays, especially when shortages have occurred as a result of poor spatfall or losses of stock to disease. The "stick and tray method" of production (Korringa, 1976), which was developed to avoid the overwhelming losses from mudworm (*Polydora* sp.) around the turn of the 19th century (Roughley, 1922), is still widely used today. Other techniques have arisen recently as a result of the availability of new materials and economic pressures. Suspended culture, single-seed culture, and the use of plastic mesh baskets are becoming increasingly important in an industry that is changing rapidly.

A. STICK AND TRAY CULTURE

For the last 30 to 40 years, the most common method for culture has been the stick and tray method (Malcolm, 1987). Oyster spat are collected following natural settlement on tarred hardwood sticks (Figure 4) and then, following several years of growth attached to these sticks, knocked off and sorted. "Plate" oysters (40 to 60 g whole weight) are marketed and smaller ones are transferred to trays for further growth. "Blocks" of sticks, comprising five to six "frames" of sticks stacked on top of each other, are put out on intertidal racks in areas renowned for reliable spatfall. A "frame" consists of about 22 hardwood sticks (25 mm × 25 mm × 1.8 m) nailed together so that 16 to 18 of them lie parallel at 100-mm intervals (Thomson, 1954; Malcolm, 1987). Within each block, the space between the sticks is sufficient to allow good water flow, but not enough space to allow predation by fish. These blocks of about 100 sticks are then dipped into cold liquid tar to protect the timber from marine borers and to provide a surface on which the oyster larvae can attach and grow (Korringa, 1976; Malcolm, 1987). The intertidal racks, which are made of tarred hardwood posts and rails (Korringa, 1976; Malcolm, 1987), are set to a height so that the trays or sticks that they support spend approximately 70% of the time under water (I. R. Smith, personal communication, 1992).

About 6 months after settlement, the blocks of sticks with the newly settled oysters attached are relocated upstream to depot leases, where better growth can be expected and overcatch of spat is avoided. The sticks are left in blocks on the depot lease until the following winter to reduce the predation of spat by fish. The blocks

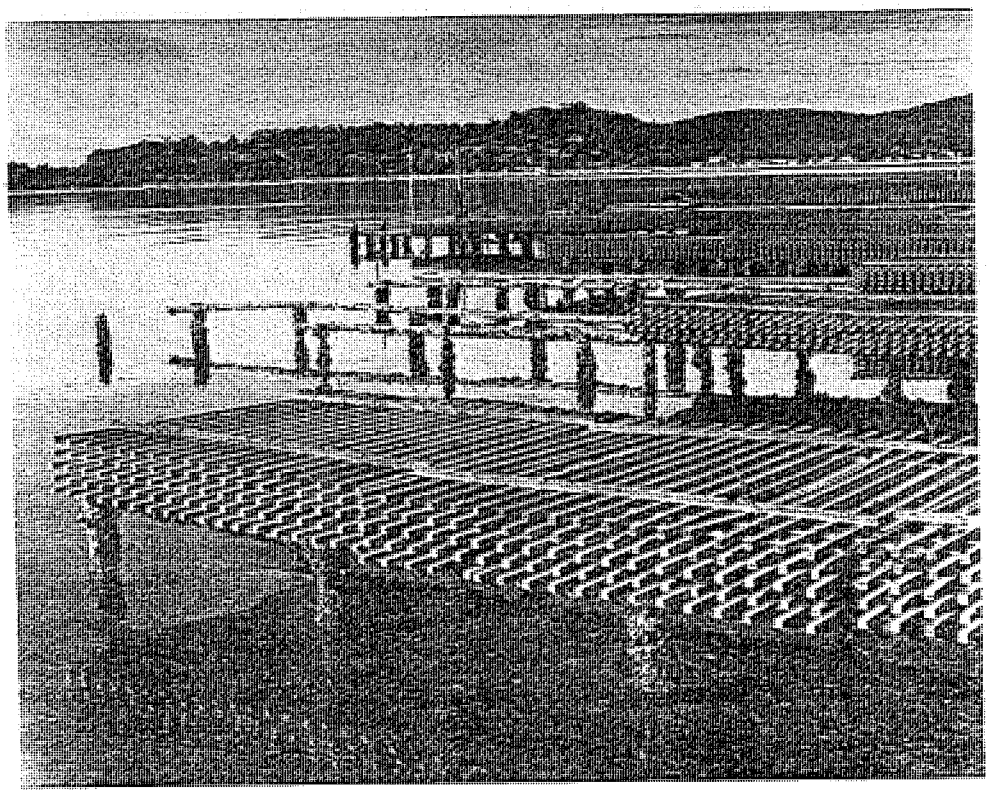

FIGURE 4. Catching sticks laid out on Salamander Bay, Port Stephens, NSW, April 1992. Hardwood sticks are nailed together and tarred in blocks before laying out in catching areas. The tar reduces marine borer attack for the 3 years until the oysters are marketable. Close spacing denies predatory fish access to the oysters when they are young and vulnerable.

are then separated into single frames and nailed horizontally onto post and rail racks in the growing areas (Figure 5). Clumps of oysters are separated by hand (Figure 6) and the individual oysters are either marketed or on-grown on tarred wooden trays (Figure 7; 0.9 m × 1.8 or 2.7 m) to "plate" size. Although individual sticks can yield up to 100 "plate" oysters per stick, average yields are in the range of 30 to 50 oysters per stick (Holliday et al., 1988).

Although the intertidal stick and tray culture technique does not maximize growth and does not produce cup-shaped oysters, it requires less capital and labor than other growing techniques. It is sometimes referred to as a "set and forget system" because prior to the problem of overcatch with Pacific oysters (Holliday and Nell, 1985), Sydney rock oysters on nailed-out single frames required little attention for approximately 2 years until they were harvested (at about 3 years of age). The cost and scarcity of good quality hardwood and tar also are causing some problems for this type of culture and forcing farmers to look for alternative culch materials such as plastic collectors, as well as new production methods such as single-seed and subtidal culture (Holliday et al.,1988).

Mechanization has had a major impact on oyster farming methods in the past 2 decades. Faster boats, lifting gear (Figure 8), and road transport allowed "highway

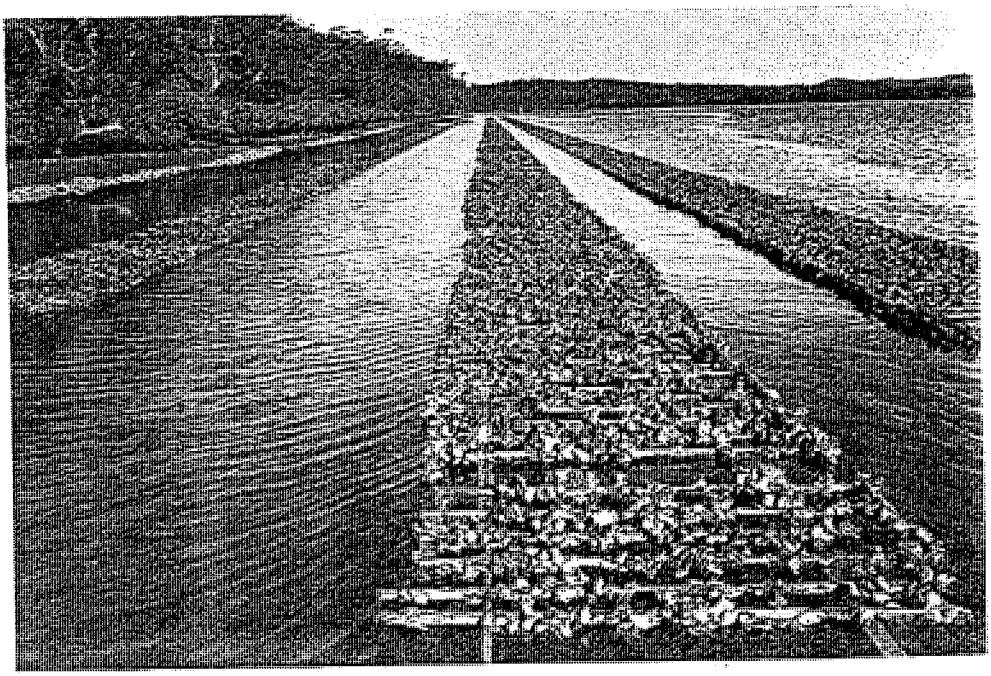

FIGURE 5. Nailed-out sticks in the Karuah River, Port Stephens, NSW, June 1992. Blocks of sticks are moved away from catching areas before the next year's spatfall, then later separated into single frames on these racks to grow to market size in 3 to 4 years after catching.

oyster farmers" to move oysters from one estuary to another to use the best characteristics of each. Half-grown oysters (3 to 3.5 years old) on trays are moved to another estuary to fatten for market for 6 to 8 months. Quarantine measures to prevent the spread of the introduced Pacific oyster have limited movements of Sydney rock oysters since 1985.

B. SINGLE-SEED CULTURE

Some NSW oyster farmers specialize in one section of the industry such as the production of sticks with newly settled oyster spat attached ("caught sticks"), single-seed spat scraped from sticks or PVC culch ("scrape-offs"), or ungraded half-grown oysters knocked off sticks ("all-ins") (Holliday et al., 1988). Since 1985, farmers have adopted techniques of scraping small spat (4 to 8 mm) from hardwood sticks or plastic collectors (Figure 9; Holliday and Goard, 1986), which are then grown as single oysters on trays (0.9 m × 1.8 or 2.7 m; Holliday et al., 1991b), in cylinders (Figure 10; 0.84 m long × 0.27 m diameter or 48.1 l volume; Holliday et al., 1993a), or in baskets (0.32 × 0.55 m). The trays, cylinders, or baskets are placed on intertidal racks, which are made of tarred hardwood posts and rails (Korringa, 1976; Malcolm, 1987). The removal of spat from tarred hardwood sticks may yield about 400 small spat per stick, although this may be increased by using a lime and cement coating. An even higher yield can be obtained by using a variety of plastic collectors (Holliday et al., 1993b). The best surfaces for catching and retaining Sydney rock oyster larvae and spat in order of numbers of spat harvested (per unit surface area)

FIGURE 6. Oyster culling, Port Stephens, NSW, February 1992. Manually culling clumps of oysters into individuals is a major labor cost in the stick and tray method of oyster production. Single-seed culture avoids this but requires more frequent handling.

by farmers are lime and cement-coated plastic collectors, plastic collectors, lime and cement-coated tarred hardwood sticks, and tarred hardwood sticks (Holliday et al., 1993b). Although growth of single oysters on trays or in cylinders may be somewhat slower than those attached to sticks, the losses that may occur from oysters dropping from the sticks are avoided.

Single-seed culture also has the advantage of producing uniform cup-shaped oysters, which are ideal for the important half-shell trade in Australia. The major disadvantage of single-seed culture is the greatly increased labor cost, resulting from increased handling and the greater investment required, compared to stick culture. Single-seed culture may be carried out both intertidally and subtidally.

C. SUBTIDAL CULTURE

Subtidally grown Sydney rock oysters in NSW (Wisely et al., 1979b,c) and Pacific oysters in Tasmania (Sumner, 1981) generally grow faster than those grown intertidally because the immersed oysters have more time to feed. Subtidally cultured oysters improve their meat condition faster than intertidally cultured oysters, which is important for marketing. This type of culture also enables the use of deep water areas that are not suitable for intertidal culture, it protects oysters from heat kill

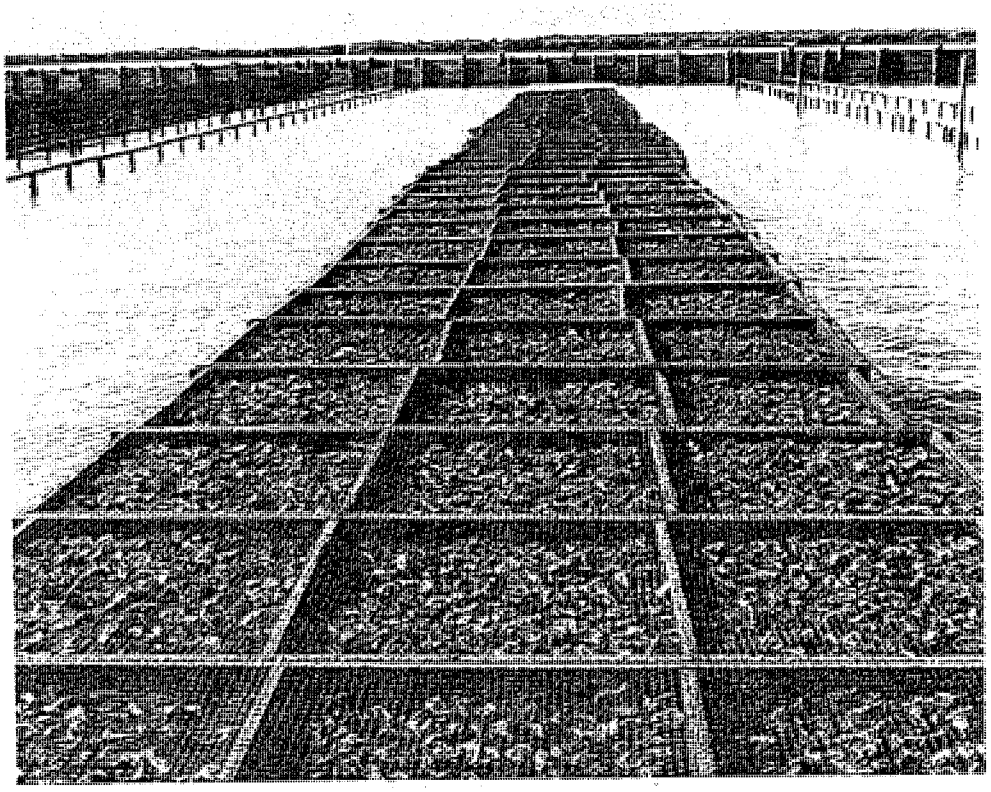

FIGURE 7. Timber breakwall and trays (0.9 × 1.8 m), Woolooware Bay, Georges River, NSW, April 1992. Hardwood and wire mesh trays protected with a tar coating have been used for decades to grow or fatten oysters to market size after they have been culled from sticks. Breakwalls and mesh covers reduce losses caused by wave action and boat wash.

(Potter and Hill, 1982), and allows farmers to work their oysters regardless of tides (Wisely et al., 1979b). Fouling from barnacles, tunicates, sponges, and hydroids is a major disadvantage with this farming technique (Wisely et al., 1979c); however, fouling organisms may be killed by periodically leaving oysters and equipment out of water for a few days. Although intertidally grown Sydney rock oysters may be left out of water for up to 2 weeks, subtidally grown oysters are less tolerant and when exposed should be checked daily for signs of mantle fluid loss, which may be an early warning sign of impending death. Other problems of subtidal culture include the effects of flooding (Wisely et al., 1983), an increased risk of mudworm (*Polydora* sp.) infestation (Wisely et al., 1979c), and winter mortality (Wisely et al., 1979b).

1. Rafts

Raft culture, which is the preferred method for the cultivation of Pacific oysters in Japan (Ventilla, 1984), is used in British Columbia (Quayle, 1988) and also has been adapted for use in Australia (Wisely et al., 1983). Rafts with stacks of trays suspended beneath them are used commercially in eight rivers in NSW and in Moreton Bay in southern Queensland. The rafts vary in design but are similar to the large rafts (which consist of six bays each holding five trays) described by Wisely et al. (1983).

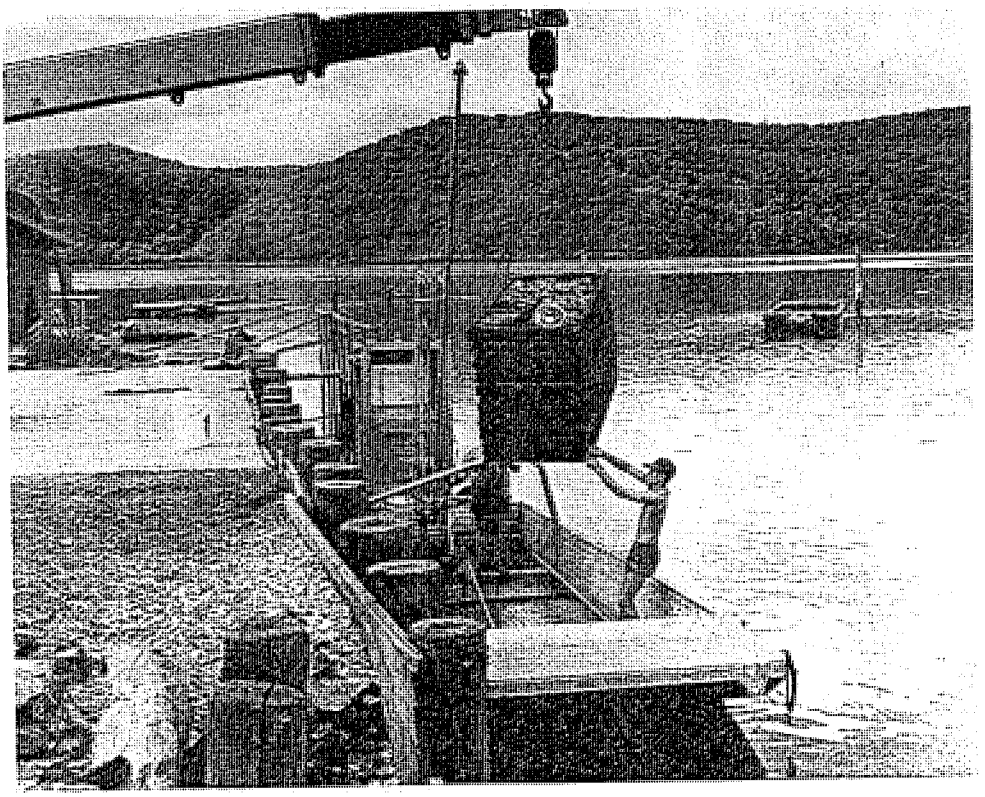

FIGURE 8. Shore depot, Mooney Mooney Creek, Hawkesbury River, NSW, April 1992. Shore depots are used for oyster handling, to store materials, and to make and repair farming equipment.

Some farmers use rafts for short-term "fattening" of "plate" oysters and finishing shell growth of "all-ins," whereas other farmers use rafts to promote shell growth in single-seed spat during their nursery stage, or to grow small spat to "plate" size oysters (Holliday et al., 1988).

In many estuaries, farmers avoid using their rafts at times when heavy fouling occurs, i.e., summer, and often stock them with grades of oysters that will reach market size before fouling problems become excessive (Holliday et al., 1988). Other farmers take their oysters out of water for a few days every 6 to 8 weeks when necessary to kill fouling that has occurred (Holliday et al., 1988). Subtidal oysters also must be examined regularly for the build-up of silt, which may increase the incidence of mudworm (Wisely et al., 1979a,c; Holliday et al., 1988).

2. Pontoons

Commercial pontoons are constructed using plastic pipes (6 m × 0.1 m diameter) glued together and capped at the ends to form long floats of 18 to 24 m in length (Wisely et al., 1979b; Holliday et al., 1988). They are used in pairs to support single layers of sticks or trays. Sticks may be continuously submerged under water for periods of 8 to 12 months, after which the oysters are knocked off the sticks and grown on trays (Holliday et al., 1988). Oysters on sticks suspended beneath pontoons cannot be taken out of water to kill off marine fouling organisms because

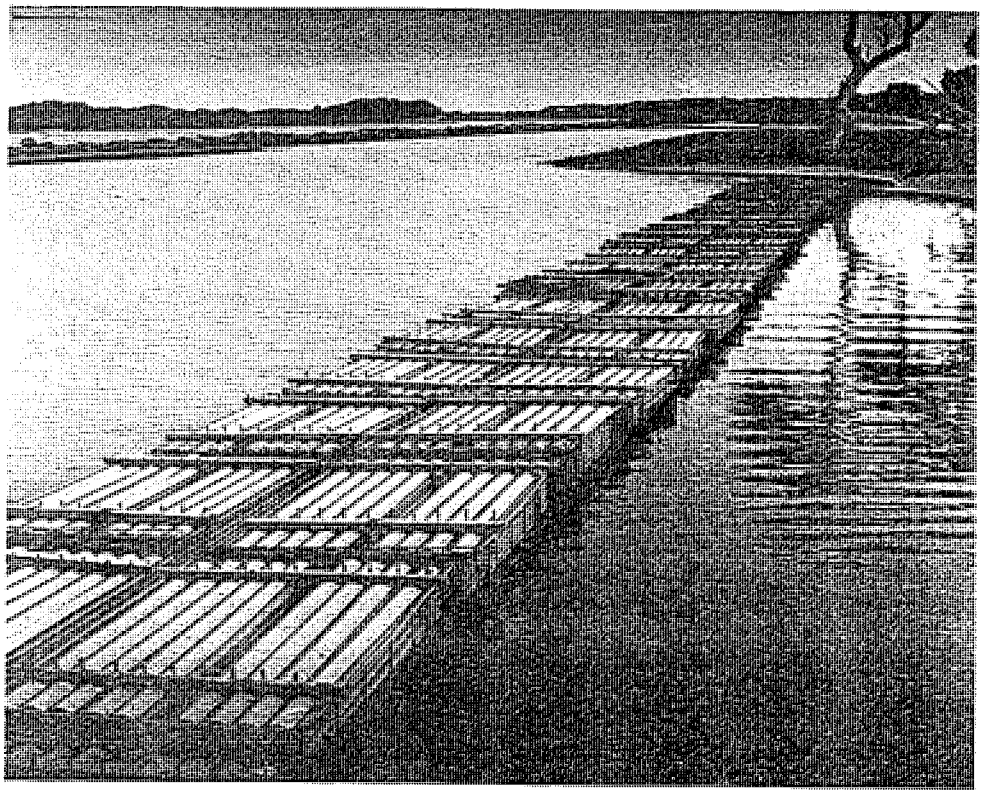

FIGURE 9. PVC catching sticks, Bellinger River, NSW, May 1992. PVC pipe sections coated with a cement and lime slurry are used as culch for single-seed oyster culture. The oysters are removed when a few months old by flexing the plastic and then are reared in specialized nursery trays or cylinders.

too many oysters fall off when sticks are handled; therefore great care must be taken in site selection.

3. Bottom Culture

With bottom culture, stacks of trays are placed on a small platform on the floor of the estuary (Wisely, 1980) to increase shell growth, fatten oysters, and avoid damage from floods (Holliday et al., 1988). This type of culture is only suitable for areas with a firm bottom substrate and where mudworm is not a serious problem (Holliday et al., 1988). Even so, care must be taken to keep oysters clear of the bottom to avoid siltation (Holliday et al., 1988). Oysters can be taken out of water to kill off fouling organisms, and management for this type of culture is similar to that for raft culture.

4. Dredge Bed Culture

This very simple and economic way of farming oysters is still used extensively for the culture of the American oyster *C. virginica* on the east coast, and for Pacific oysters on the west coast of the U.S. (Korringa, 1976). Because of siltation and mudworm (Roughley, 1922), and the increased risk of losses from winter mortality and QX disease (Nell and Smith, 1988), there are only a few small dredge beds left in commercial operation in isolated areas of NSW. This is unfortunate because it is

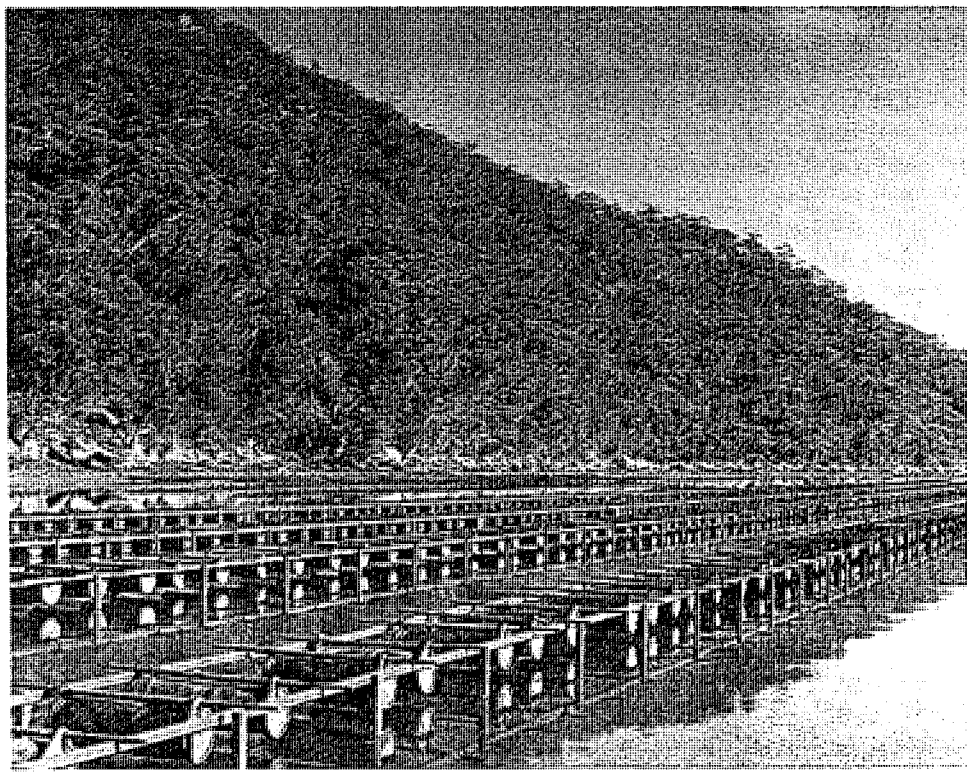

FIGURE 10. Cylinders on Mooney Mooney Creek, Hawkesbury, NSW, May 1992. The cylinders are recently patented devices comprising a plastic mesh growing cylinder with eccentric flotation resulting in a complete rotation with each tidal cycle. They can be effectively used in sheltered areas for nursery or juvenile-stage oysters.

a very simple and effective way of growing oysters. In dredge bed culture, oyster shell is deposited on hard clean bottoms of estuaries to catch spat. The spat attached to the oyster shell are left to grow on the bottom (Thomson, 1954). The crop from a dredge bed is harvested by systematically working the area with a small dredge (about 1 m wide with a wire mesh net attached) (Roughley,. 1925b).

V. PROCESSING AND MARKETING

Since 1968, microbiological analyses have shown that oysters harvested from one of NSW largest producing areas, the Georges River, have been subject to unpredictable and unacceptable levels of sewage pollution associated with periods of heavy rainfall (Souness et al., 1979). In 1978, a serious outbreak of viral gastroenteritis occurred in which the Norwalk virus was implicated as the causative organism (Murphy et al., 1979; Grohmann et al., 1980,1981). The outbreak was believed to have occurred as a result of overflows from a sewage system overloaded during heavy rain. These problems led to the introduction of depuration procedures for oysters, first for Georges River oysters and subsequently to oysters from the rest of the State.

It has been mandatory since 1983 that all oysters sold for consumption in NSW are depurated for 36 h (Ayres, 1991; Holliday et al., 1991a). It also is recommended that farmers avoid harvesting their oysters after heavy rainfall if the salinity has dropped significantly. The aim of this depuration process is to improve the microbiological quality of the oysters. Most farmers operate their own depuration plants, which are based on shallow (240 to 440 mm deep) rectangular tanks that use ultraviolet light (wavelength of around 254 nm) to sterilize estuarine water. The tanks use either a recirculation or a flow-through system and operate at salinities >18‰, with temperatures ranging from 14 to 27°C. These conditions are based on laboratory scale trials as well as research conducted in commercial depuration tanks (Souness and Fleet, 1991). Fortunately, the sewage systems in most of the oyster-growing estuaries have improved greatly since the original outbreaks occurred.

Farmers in the Georges River, NSW, also have been regularly testing their depurated oysters for bacteriological purity since 1990 under a voluntary quality assurance program (QAP). It was announced by the NSW State government in March, 1992, that the voluntary QAP for Georges River, NSW, will be replaced by a compulsory program and that similar programs will be developed for all oyster-producing estuaries over the next 3 years.

Whole oysters are usually sold in jute bags, which contain about 100 dozen "plate" oysters (40 to 60 g), 120 dozen "bistro," oysters (30 to 40 g), or 140 dozen spat, or in waxed cardboard boxes of 50 dozen "plate" oysters. Whole oysters may be stored out of water at temperatures between 8 and 10°C for up to 2 weeks (Holliday et al., 1991a). At temperatures <8°C or >10°C, mortalities are likely to increase and storage times will be much shorter. Opened oysters on the half-shell are sold in boxes of 10 dozen each. The meats of smaller ("bottle" grade) oysters (20 to 30 g) immersed in fresh water are sold by the count in glass jars. Oysters on the half-shell should be stored at refrigeration temperatures of 3 to 5°C and consumed within a few days of opening. Bottled oysters may be stored refrigerated for up to 2 weeks (Korringa, 1976).

In 1992, the relative prices for a bag of "plate," "bistro," and "bottle" grade oysters were approximately A$340, A$240, and A$160, respectively, whereas "plate" and "bistro" oysters on the half-shell sold for A$5.60 and A$3.70 per dozen, respectively, and two dozen meats from "bottle" grade oysters in 250 ml glass jars sold for A$4.20 per jar.

Farmers usually sell some depurated unopened oysters directly to the public, but the majority of oysters are sold opened on the half-shell to restaurants. Oysters may be opened by the farmers themselves or more commonly by processors. About half of the Sydney rock oysters sold are eaten raw from the half-shell; the rest are cooked on the half-shell with a variety of toppings, sauces, and spices.

VI. PRODUCTION TRENDS

The NSW and Queensland industries are characterized by a large number of small producers (Korringa, 1976; Marshall and Espinas, 1987; Nell et al., 1990). In 1983–1984, there were 787 farmers in NSW, although 92% of all oysters were produced by only 208 of the largest producers (Marshall and Espinas, 1987). In NSW, there are 41 oyster-growing estuaries with a total lease area of about 4700 ha. Further lease

allocations are unlikely as leases tend to conflict with the increasing water area demanded by recreational water users.

During the 1950s and 1960s, the NSW Sydney rock oyster industry exhibited consistent growth as production methods improved and total lease area increased (Figure 3; Maguire et al., 1988). However, around 1970, production rapidly increased from about 100,000 to 140,000 bags (with each bag containing about 1200 oysters) or 6000 to 8400 t (wet weight including shell) (Maguire et al., 1988). Much of this increase was attributed to the increase in the number of farmers who had decided to transport oysters from estuary to estuary to take advantage of differences between estuaries in the timing of prime growing or fattening conditions (Maguire et al., 1988). This practice is known as "highway oyster farming." Production rates stabilized during the 1970s at around 130,000 bags (7800 t) per year and the industry was constrained by problems encountered in obtaining new leases due, in part, to commercial fishing and changes in foreshore zoning in estuaries (Maguire et al., 1988). From the late 1960s until quite recently, estuaries were closed to the granting of new oyster leases (Maguire et al., 1988).

The development of Pacific oyster industries in New Zealand during the 1970s (Dinamani, 1987) and in Tasmania during the 1970s and 1980s (Sumner, 1980) generated competition for the Sydney rock oyster industry and resulted in the loss of large parts of interstate markets and competition on the NSW domestic market. Consumers readily accept both species of oyster (McBride et al., 1988) and this is reflected in market pricing. The cost of production of Pacific oysters is lower as they require only half the time (2 years) to reach "plate" size compared with the Sydney rock oyster. Economic recession during 1990–1991 also has reduced the restaurant trade where the majority of oysters are marketed, and competition from a wider range of entrees that have become available on menus also may have contributed to the reduction in demand for oysters. The downturn has resulted in a 38% decrease in Sydney rock oyster sales in NSW, from 123,521 bags (7411 t) in 1981–1982 to 85,429 bags (5126 t) in 1990–1991 (Figure 3). At its peak in 1976–77, the combined NSW and southern Queensland industry produced 9267 t or 154,454 bags, but it has declined over the last decade to a production of 5306 t or 88,429 bags in 1990–91, with a total value of A$30.1 million. Even at the height of production, however, the edible oyster industry in Australia was small (approximately 1% of world production) compared with 256,313 t in Japan, 242,956 t in South Korea, 158,425 t in the U.S., and 140,038 t of oysters in France in 1989 (FAO, 1991).

VII. DISEASES

A. MUDWORM

The mudworm (*Polydora websteri*) is thought to be the most damaging of four spionid polychaete worms that infest and kill large numbers of oysters (Skeel, 1979). It also infests the Pacific oyster, the edible mussel (*Mytilus edulis*), and the native flat oyster. The adult mudworm is up to 25 mm long, 1 mm wide, and red in color (Skeel, 1979). It lives on the inside of the oyster shell, where it gives rise to the formation of a mud blister, but maintains a tube across the lip of the shell to the outside (Nell and Smith, 1988). Healthy, rapidly growing oysters may quickly be able

to cover the worm and its mud patch with shell and recover from the attack, whereas weak oysters may succumb (Nell and Smith, 1988). Mudworm losses are often very high, and the remaining infested oysters become unsaleable because of their poor condition and unsightly and foul-smelling mud blisters, which rupture easily when the oysters are opened (Nell and Smith, 1988). Infested oysters also can be left out of water in the shade for up to 10 days to kill mudworm, and boom-sprays on punts are used by some oyster farmers to wash mud from oysters on their leases, thus lowering the risk of infestation (Nell and Smith, 1988).

B. WINTER MORTALITY

Winter mortality is believed to be caused by *Mikrocytos roughleyi* (Farley et al., 1988), which is a protoctistan parasite (*insertae sedis*). It occurs in NSW between Port Stephens and the Victorian border, i.e., in the southern or cooler half of the range in which the Sydney rock oyster is farmed, but it has a patchy distribution within its range (Nell and Smith, 1988). Mortality can occur in winter, but usually most of the oysters do not die until the warmer spring weather of September or October (Nell and Smith, 1988). The severity of the kill can vary markedly between years, between and within estuaries, and even between adjacent leases or within leases. Oysters on sticks are less susceptible to the disease than oysters on trays (Nell and Smith, 1988). Dry autumns (high salinities) and early winters increase the likelihood of a severe kill (Wolf, 1967). To a large extent, farmers can avoid this disease by increasing the growing height of the oysters to 150 mm above the normal growing height, or moving their oysters to leases further upstream before the end of autumn (May), where lower salinities and higher growing heights offer some protection. Alternatively, farmers may sell their oysters for consumption before the onset of winter (Nell and Smith, 1988).

C. QX DISEASE

QX disease is believed to be caused by *Marteilia sydneyi* (Perkins and Wolf, 1976), which also is a protoctistan parasite (*insertae sedis*). The disease, whose route of infection remains incompletely known (Roubal et al., 1989), may kill large numbers of oysters in the infested estuaries. It was responsible for a decline of the industry in southern Queensland and northern NSW during the 1970s (Wolf, 1979; Potter, 1983; Moxon, 1986). The range of the disease has extended as far south as the Macleay River (31°S). The parasite appears to require low salinities and high water temperatures and is more likely to occur after heavy rain in early autumn (March and April; Lester, 1986; Nell and Smith, 1988; Witney et al., 1988), although mortalities may occur during most of the year (Wolf, 1979). On the east coast of the U.S., where American oysters (*C. virginica*) suffer from MSX, a disease similar to QX and caused by *Haplosporidium nelsoni*, wild oysters acquired natural resistance and this has been further enhanced by selective breeding (Haskin and Ford, 1979). The northern NSW and southern Queensland oyster farmers, however, resorted to importing half-grown oysters from noninfested southern estuaries for short-term oyster growing by selling them for consumption during summer before the main risk

period in autumn (March and April). Although this effectively avoids problems with QX disease, and it actually increased the number of oysters sold for consumption from these estuaries, it does not allow the development of resistance in farmed oysters. Quarantine regulations for QX, which prohibit the movement of oysters from affected estuaries into disease-free estuaries, were first put into place in 1986 (Nell and Smith, 1988).

VIII. ENVIRONMENTAL HAZARDS

A. HEAT KILL

Severe losses through death of oysters may result from the effect of summer heat, when air temperatures on exposed oyster leases reach 30 to 40°C. The problem is most acute when a low tide coincides with the warmest period on a hot summers day, exposing intertidally grown oysters to strong sunlight and high air temperatures (Potter and Hill, 1982). Many oysters farmers of the Georges River, NSW, have installed irrigation sprinkler systems on their leases and salt water is sprayed over the oysters to keep them cool (Nell and Smith, 1988). Other farmers use shade cloth over trays to keep oysters cool and predators out, but unfortunately this practice restricts water flow over the trays and increases the risk of mudworm infestation (Nell and Smith, 1988). Spraying oysters with seawater results in far lower oyster meat temperatures than the use of shade cloth (Potter and Hill, 1982).

B. FLOODS

Because oyster rafts may suffer severe physical damage or be lost during floods in rivers, care is taken that they are not placed in the main channel and are properly anchored. Floating trees and other debris may damage rafts as well as intertidal racks. If flooding reduces salinities to 10‰ or less for 2 weeks or more, mortalities are likely to occur (Nell and Gibbs, 1986). Some oyster leases in the Georges River, NSW, silt up after flooding and oyster farmers may use high-pressure water jets at low tide to remove silt from the lease. Damage to racks by erosion or burial in sediment also can occur during floods.

C. POLLUTION

The use of antifouling paints containing tributyltin oxide (TBTO), which had a severe impact on the culture of Pacific oysters in France (Alzieu, 1986), England (Thain, 1986), Scotland (Davies, et al., 1988), and the U.S. (Stephenson et al., 1986; Wolniakowski et al., 1986), also affected the NSW Sydney rock oyster industry during the 1980s (Batley et al., 1989). High concentrations of bis-tributyltin oxide (17.5 to 170.0 ng TBTO l^{-1}) occurred in the Hawkesbury and Georges Rivers (Figure 1), two of the main oyster-growing estuaries in NSW, during the 1980s (Batley et al., 1989). These high TBTO concentrations caused shell deformities and reduced growth rates of oysters. It was subsequently shown by Nell and Chvojka (1992) that

growth of both the Sydney rock and Pacific oysters was reduced by up to 50% by as little as 5 ng TBTO l^{-1} in laboratory studies. Fortunately, since the government banned the use of antifouling paints containing TBTO on boats smaller than 25 m in 1989 in NSW, the prevalence of shell deformities in oysters has declined and growth has improved.

IX. COMPETITORS

A. PACIFIC OYSTERS

A heavy spatfall of the exotic Pacific oyster occurred in the inner harbor of Port Stephens, NSW, during the summer of 1984–1985 (Holliday and Nell, 1985; Chew, 1990), and by 1988 the population of Pacific oysters in Port Stephens was estimated to have reached 26 million. Before 1984–1985, farmers in Port Stephens who caught their Sydney rock oyster spat near the mouth of the estuary, could grow their oysters relatively free of overcatch in the upper half of the estuary. This is no longer the case because Pacific oyster spat settle in both the lower and upper halves of the estuary, periodically forcing farmers to take their Sydney rock oysters out of water for up to 2 weeks to kill off the overcatch of Pacific oysters. In an attempt to control the number and spread of Pacific oysters in NSW, regulations were first introduced in 1985. In estuaries other than Port Stephens, oyster farmers are legally required to remove Pacific oysters from their leases if they are found. As a result of the overwhelming numbers of Pacific oysters in Port Stephens, farmers there were given permission by the NSW State government to grow and market Pacific oysters in 1991. The species remains prohibited in other estuaries. The proliferation of Pacific oysters in Port Stephens has boosted the overcatch of spat and increased the handling costs of Sydney rock oysters.

Pacific oysters were deliberately introduced into the southern states of Australia in the late 1940s and early 1950s (Medcof and Wolf, 1975) but they were refused entry into NSW because that State already had a valuable Sydney rock oyster industry. However, by the 1970s, small numbers of Pacific oysters were found in southern NSW estuaries (Medcof and Wolf, 1975). The risks to the industry were again pointed out to oyster farmers and they were encouraged to destroy Pacific oysters. The sudden appearance of large numbers in Port Stephens in 1984–1985 (Holliday and Nell, 1985) suggests deliberate introduction. Control measures were introduced, but because Port Stephens was a major source of Sydney rock oyster spat and juvenile oysters for growing or fattening in other NSW estuaries (Chew, 1990), large numbers of Pacific oysters were dispersed to other estuaries. This resulted in Pacific oysters being found in 18 of the 41 oyster-producing estuaries in NSW by 1990 (D. Reid, unpublished data, 1990).

B. MUSSELS AND BARNACLES

The small brown mussel *Xenostrobus securis* sometimes settles in large numbers on oyster sticks and trays in areas of lower salinity (15 to 25‰). These mussels can dislodge oysters from sticks and, if they catch on oysters in trays, the clumps of

oysters and mussels holding them together may silt up and increase the risk of mudworm infestation.

A number of species of barnacles are often found on oysters, sticks, and trays. In some spat-catching areas, barnacles may settle during the early part of the spat-catching season, reducing oyster spat settlement (Holliday and Goard, 1986; Holliday et al., 1993b). Farmers try to avoid this problem by putting their catching materials out later in the season to minimize the barnacle catch and leave clean sticks for oyster spat to settle on. Subtidal culture may suffer seasonally from heavy fouling from barnacles to such an extent that they may interfere with the feeding behavior of the oysters and reduce growth rates. Barnacles may be killed by leaving oysters and equipment out of water for a few days (Wisely et al., 1979c).

X. PREDATORS

Many species of fish, including bream (*Acanthopagrus australis*) and juvenile Australian snapper (*Pagrus auratus*), feed on small oysters. As a means of protection, young oysters on sticks are left in blocks for 18 months after catching (Korringa, 1976). Sometimes blocks of caught sticks also are covered in netting to keep these predators out. If sticks are nailed out in single layers when they are still small, they also are covered with netting for protection. Other predators such as the mudcrab (*Scylla serrata*) and various species of stingrays may feed on oysters on trays, and trays are therefore usually covered with plastic or wire mesh. These predators are attracted to oysters damaged during handling or culling. A predatory flatworm (*Stylochus* sp.) also is believed to kill oysters (Korringa, 1976).

XI. GOVERNMENT REGULATIONS

In NSW, oyster-growing areas are leased from the government for a yearly rental fee of A$37/ha for a period of 15 years, with the option of renewal in the last year of the lease. The conditions require that leases are kept tidy. The NSW State government announced in March 1992, that it will introduce a research levy (to be 0.25% of value of industry, which will be subsidized by the Federal government on a dollar for dollar basis) to fund oyster research priorities determined by the industry. The introduction of a separate levy of A$50/ha to fund oyster marketing and promotion, which will be phased in over 3 years, was announced at the same time. Both levies will be based on the area leased. The government also is considering the introduction of an aquaculture license that stipulates what activities can be carried out on a lease. This license will allow farmers to seek approval to diversify into the culture of other species of bivalves, gastropods, or fish that may have potential for aquaculture.

In NSW, strict regulations prohibit the transfer of oysters from estuaries with a history of QX disease to other estuaries, and other, frequently reviewed, regulations exist in both NSW and Queensland to control the spread of Pacific oysters. State governments also regulate depuration, processing, storage, and marketing conditions for oysters and the marking of oyster leases as an aid to navigation. In NSW, council rates (local government tax) also are paid on oyster leases. In NSW, liaison

with industry and government is maintained through joint consultative committees known as the Oyster Management and Advisory Committee (OMAC) and the Oyster Industry Research Committee (OIRC).

XII. INDUSTRY TRENDS

The Sydney rock oyster industry is undergoing a period of rapid change after many decades of stability. A restructured industry consisting of a reduced number of more efficient oyster farmers producing oysters through intensive cultivation of the more productive leases is emerging. Although Sydney rock oyster spat have been produced by the NSW Fisheries hatchery at the Brackish Water Fish Culture Research Station for experimental purposes since 1981 and a commercial hatchery has produced small quantities of spat since 1986, the industry will continue to use cheaper natural spat until triploid or other genetically superior oysters are made available in commercial quantities. However, unlike the Pacific oyster industry on the west coast of the U.S. (Matthiessen, 1991), the Sydney rock oyster industry relies almost entirely on natural spatfall and it is therefore likely that any changeover from naturally caught spat to hatchery-produced spat will be gradual.

Triploid oysters can be produced by a physical or chemical shock after egg fertilization (Beaumont and Fairbrother, 1991; Allen and Bushek, 1992). Triploid Pacific oysters are grown on the west coast of the U.S. (Allen et al., 1989; Barber and Mann, 1991) to produce marketable oysters for the 4-month period in summer (May to August) when standard Pacific oysters cannot be sold because they are in a spawning or immediate post-condition (Allen and Downing, 1991). The market for Sydney rock oysters prefers plump sexually mature animals so triploid oysters have no advantage for this purpose. However, the energy that triploid oysters would otherwise use for sexual development is diverted to additional growth (Beaumont and Fairbrother, 1991). A study of triploid Sydney rock oysters has recently demonstrated an average 40% whole weight advantage after 2 years compared with normal diploid oysters, indicating that they could be marketed after 2 to 2.5 years growth, compared with 3 to 3.5 years for diploid oysters. Once the diploid oysters reached an age of 2 years or older and had reached full sexual maturity, the meat condition of the triploid oysters was better most of the time and at no time worse than that of the diploid oysters. Therefore, the farming of triploid Sydney rock oysters would assist the NSW oyster industry in its drive to ensure a consistent high-quality product.

Marketing and promotion strategies will need to be developed and implemented for the industry to compete with increased competition from Pacific oysters cultivated in New Zealand, Tasmania, South Australia, and Port Stephens, NSW (Treadwell et al., 1992). It is essential that high microbiological standards be maintained, that minimum meat condition standards be set, and that a range of fairly tight size grades be adopted for marketed oysters. To regain consumer confidence, the NSW industry has, in principle, adopted the QAP (the regular testing of oysters for bacteriological purity) developed and tested in the Georges River, NSW.

The industry is reducing labor and production costs to regain profitability and is trying to regain market share with quality control, attractive presentation, and vigorous promotion. The changeover to single-seed culture, which produces a

uniform cup-shaped oyster for the half-shell trade, is assisting the industry in its modernization, but oyster farmers also could consider diversification into the cultivation of other species on other leases such as venerid clams, gastropods, and fish.

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A Literature Review of Triploidy in Commercial Bivalves

by
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Contents

1. Introduction

Bivalves devote an exceptionally high proportion of their energy reserves and tissue to gonad and gamete development (referred to as gonadogenesis and gametogenesis respectively). Indeed, gonad tissue of *Crassostrea gigas* can represent 70% of total tissue in cross section (Akashige, 1990). Consequently, an understanding of the minimal size at sexual differentiation, gonadogenic cycles, the extent of gonado/gametogenic development, and related sexual topics is of major importance in research on bivalves. From a commercial perspective it is deemed desirable to limit the development of sexual tissue and spawning (Chaiton and Allen, 1985). Perdue and Erickson (1984) proposed four possible ways of achieving this: hybridisation with another species to produce sterile offspring; selective breeding; utilising two species with different (temporal) gonadic cycles and polyploidy.

This review deals with the use of polyploidy, particularly triploidy, to retard gametogenesis. The effectiveness of this strategy is discussed as are the methods of induction of polyploidy and several of the implications of decreased sexual development. These are changes in growth, condition index, glycogen content and disease resistance.

2. Induction of Triploidy

Usually, triploid molluscs are produced by the blocking polar body formation in spawned eggs. Consequently an extra chromosome set is retained (Figure 1). Alternative methods for producing polyploid individuals include the suppression of mitotic division, cell fusion, polyspermy (Gould and Stephano, 1990) and gynogenetic activation (Guo *et al.*, 1991b).

Critical factors involved in the induction of triploidy by suppression of polar body formation are the means of polar body suppression, duration of the treatment, intensity of the treatment, temperature of treatment, time after fertilisation that the treatment is administered and the species of bivalve (Table 1).

Treatments that have successfully induced triploidy in molluscs include pressure, the antibiotic cytochalasin B (CB), 6-dimethylaminopurine (6-DMAP), thermal (hot and cold), caffeine, electric field and calcium ion (Table 1). The most widely used of these, cytochalasin B, prevents polymerisation of actin filaments at the barbed end (Pollard and Mooseker, 1981) thus indirectly affecting segregation of chromosomes (Guo *et al.*, 1989). The ability of cytochalasin B to suppress polar body formation has been known since Longo (1972) treated zygotes of the clam *Spisula solidissima* with cytochalasin B. Polar body formation was prevented and the zygotes retained four sets of maternal chromosomes.

Factors to be considered in the selection of a treatment for commercial application include; percentage of triploidy achieved, extent of abnormality caused by treatment,

impact of treatment on survival, operator safety and consumer acceptance. Cytochalasin B is classified as a carcinogen and is a potential hazard in bivalve hatcheries (Desrosiers *et al.*,1993). Safer alternatives such as non-chemical methods or 6-DMAP are considered desirable.

Polar body formation can be blocked at meiosis one or two. The first meiotic division (metaphase 1) occurs during and shortly after spawning in *Crassostrea gigas* and the second after fertilisation (Gould and Stephano, 1990). There is some indication that the resultant progeny are different depending on which stage is blocked. Stanley *et al.* (1984) observed no difference in growth rates between diploid and meiosis II triploids while those created in meiosis I exhibited increased growth. The observed differences were attributed to increased heterozygosity (Stanley *et al.*, 1984). Enhanced heterozygosity is known to increase growth (Beattie *et al.*, 1987; Hu, 1991). It should be noted that Stanley *et al.* (1984) used *Crassostrea virginica* which has limited suppression of gametogenesis from triploidy (Hidu *et al.*, 1982; Hidu *et al.*, 1983). In other species, such as *Crassostrea gigas*, the blockage of gametogenesis is extensive (Akashige, 1990; Allen and Downing, 1990). In this situation the extensive physiological effects of triploidy on gametogenesis may mask differences of heterozygosity. Consequently, we would expect less advantage to be observed in the induction of meiosis I as opposed to meiosis II triploids.

Generally, shocking of the zygotes results in higher mortality (Yamamoto *et al.*, 1988; Allen *et al.*, 1989, Desrosiers *et al.*, 1993). However, thermal induction of triploidy performed by Quillet and Panelay (1986) did not increase larval mortality.

Polyspermy provides a mechanism for the incorporation of an additional chromosome set into the zygote and hence the induction of triploidy. *Crassostrea gigas* eggs are highly susceptible to polyspermy although an electrical block develops during spawning or after incubation in sea water (Gould and Stephano, 1990).

Table 1. Induction of Triploidy

AUTHOR (S)	TREATMENT	INTENSITY	DURATION (minutes)	TIME AFTER FERT. (minutes)	MEIOSIS STAGE	SPECIES	TEMP. (°C)	% POLYPLOID
Longo, 1972	Cytochalasin B	1.0 & 10 mg/l		3	1 & 2	*Spisula solidissima*	21	*
Stanley et al., 1981	Cytochalasin B	0.1 mg/l	20	50-70	2	*Crassostrea virginica*	26-29	13 in 22
	Cytochalasin B	0.5 mg/l	15	0-15	2	*Crassostrea virginica*	26-29	10 in 20
	Cytochalasin B	0.5 mg/l	15	15-30	2	*Crassostrea virginica*	26-29	34 in 46
	Cytochalasin B	1.0 mg/l	20	50-70	2	*Crassostrea virginica*	26-29	3 in 4
Allen et al., 1982a	Cytochalasin B	0.5 mg/l	15	0-15	1	*Crassostrea gigas*		0.54
Hidu et al., 1982	Cytochalasin B	0.5 mg/l	15	0	1	*Crassostrea virginica*		0.6
	Cytochalasin B	0.5 mg/l	15	15	2	*Crassostrea virginica*		0.73
Allen et al.,1982b	Cytochalasin B	1.0 mg/l	15	0		*Mya arenaria*	22	0.84
	Cytochalasin B	1.0 mg/l	15	15		*Mya arenaria*	22	0.8
Tabarini, 1984	Cytochalasin B	0.05 mg/l	10	10		*Argopecten irradians*		0.66
	Cytochalasin B	0.1 mg/l	10	10		*Argopecten irradians*		0.94
Stanley et al., 1984	Cytochalasin B	0.5 mg/l	15	0	1	*Crassostrea virginica*	28-29	0.61
	Cytochalasin B	0.5 mg/l	15	15	2	*Crassostrea virginica*	28-29	0.72
Allen and Downing, 1985	Cytochalasin B & Pressure					*Crassostrea gigas*		*
Chaiton and Allen, 1985	Pressure	6000-8000 PSI	10	10		*Crassostrea gigas*	25-29	0.57
Allen et al., 1986	Cytochalasin B		15	0		*Mya arenaria*		0.895
	Cytochalasin B		15	15		*Mya arenaria*		0.785
Allen and Downing, 1986	Cytochalasin B	1.0 mg/l	15	30	2	*Crassostrea gigas*	20	*
Quillet and Panelay,1986	Heat Shock	35°C	10	10 to 15	1	*Crassostrea gigas*		0.25
		38°C	10	35 to 40	2	*Crassostrea gigas*		0.45
Downing and Allen, 1987	Cytochalasin B	1.0 mg/l	15	0-135	1 & 2	*Crassostrea gigas*	18	0.52
		1.0 mg/l	15	0-135	1 & 2	*Crassostrea gigas*	20	0.76
		1.0 mg/l	15	0-135	1 & 2	*Crassostrea gigas*	25	0.9
Komaru et al., 1988	Cytochalasin B	0.5 mg/l	15	15		*Chlamys nobilis*		*
Hidu et al., 1988	Cytochalasin B	0.5 mg/l				*Mercenaria mercenaria*		*
Guo et al.,1988	Polyethylene glycol (tetraploid-blastomere fusion)	50% in seawater	1,2 and 5	2 Cell	N/A	*Crassostrea gigas*		*
Downing,1988	Cytochalasin B		15	20	2	*Crassostrea gigas/rivularis* hybrid	25	0.5
Yamamoto et al., 1988	Cytochalasin B	0.5 mg/l	20	10	2	*Crassostrea gigas*	19	0.672
	Heat	35°C	10	20	2	*Crassostrea gigas*	19	0.683
	Cold	0°C	10	15	2	*Crassostrea gigas*	19	0.667
Beaumont and Kelly,1989	Heat	20°C	10	10	1	*Mytilus edulis*	15	*
	Heat	25°C	10	10	1	*Mytilus edulis*	15	0.25
	Heat	30°C	10	10	1	*Mytilus edulis*	15	*
	Cytochalasin B	0.1 mg/l	15	5	1	*Mytilus edulis*	15	0.6
	Cytochalasin B	0.1 mg/l	15	5	2	*Mytilus edulis*	15	0.67
	Cytochalasin B	0.5 mg/l	15	5	1	*Mytilus edulis*	15	0.32

* No information presented in paper

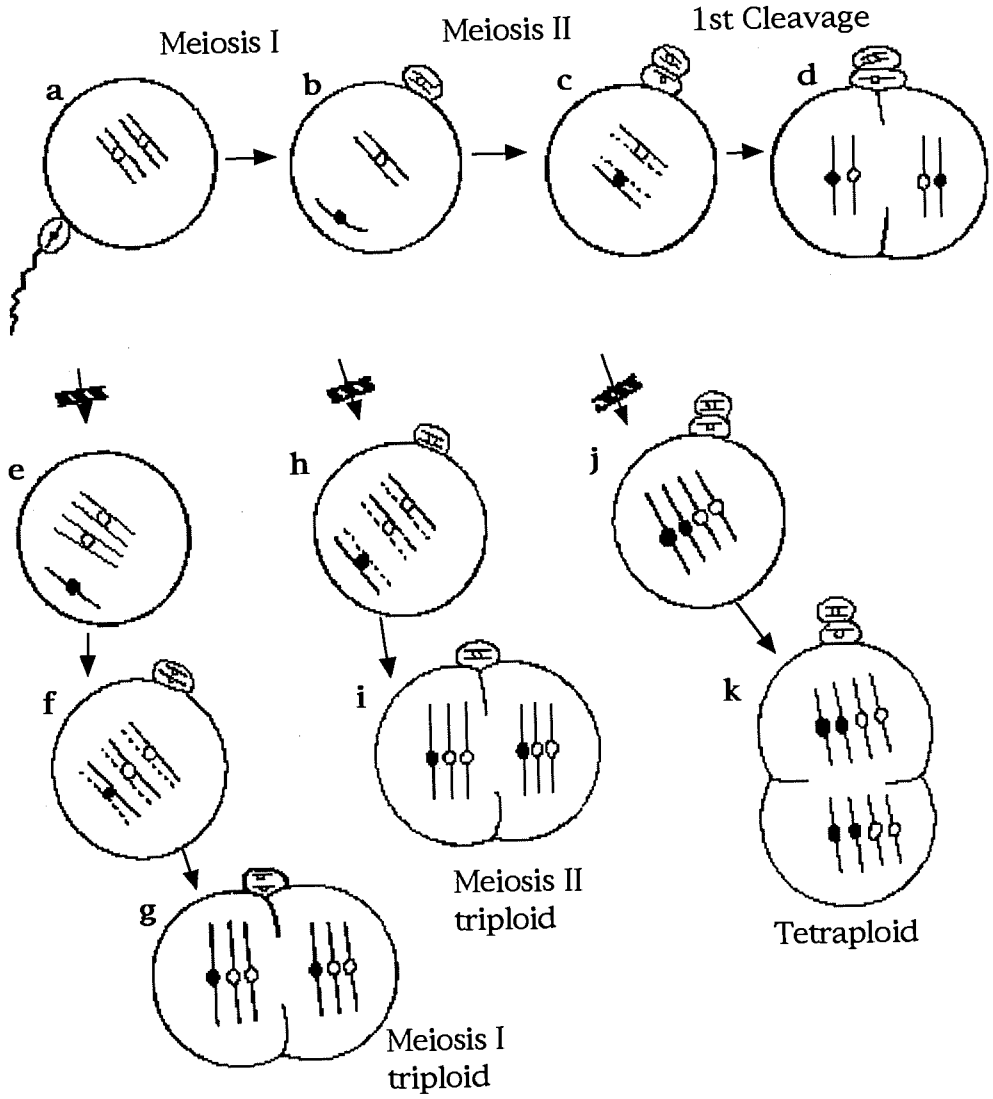
	Cytochalasin B	0.5 mg/l	15	5	2	*Mytilus edulis*	15	0.46
	Cytochalasin B	1 mg/l	15	5	1	*Mytilus edulis*	15	0.5
AUTHOR (S)	TREATMENT	INTENSITY	DURATION (minutes)	TIME AFTER FERT. (minutes)	MEIOSIS STAGE	SPECIES	TEMP. (°C)	% POLYPLOID
Beaumont and Kelly,1989	Cytochalasin B	1 mg/l	15	5	2	*Mytilus edulis*	15	0.38
Gendreau et al., 1989	Cytochalasin B	1mg/l	20	40	1	*Ostrea edulis*	20	0.7
	Cytochalasin B	1mg/l	20	100	2	*Ostrea edulis*	20	0.68
Uchimura et al., 1989	Cytochalasin B	0.5 mg/l	15	5		*Pinctada fucata martensii*	24	0.654
Stephens and Downing, 1989	Cytochalasin B			15-30	2	*Crassostrea gigas*		0.85
Komaru and Wada, 1989	Cytochalasin B or Hydropressure	0.5 mg/l 200kg/sq. cm				*Chlamys nobilis* *Chlamys nobilis*		0.714 *
Shatkin and Allen, 1989	Cytochalasin B	0.5 mg/l	15	25	2	*Crassostrea virginica*	25	0.96
Guo et al., 1989	Cytochalasin B				1	*Crassostrea gigas*	25	0.6
Downing, 1989	Cytochalasin B		15	20	2	*Crassostrea gigas/virginica* hybrid	25	84-100%
Durand et al., 1990	Caffeine/Heat shock	13mM/31.5°C	12	12		*Pinctada fucata martensii*		*
Komaru and Wada, 1990	Cytochalasin B & cold shock					*Pinctada fucata martensii*		0.6
Allen and Downing, 1990	Cytochalasin B	1.0 mg/l	15	30	2	*Crassostrea gigas*	20	*
Akashige, 1990	Cytochalasin B	1.0 mg/l	10	20	2	*Crassostrea gigas*	26.5	*
Yamamoto et al.,1990	Heat Shock	37°C	15	40	2	*Crassostrea gigas*		0.833
	Heat Shock	32°C	10	10		*Mytilus edulis*		0.975
	Cold Shock	0°C	10	15		*Crassostrea gigas*		0.667
	Cold Shock	1°C	10	35		*Mytilus edulis*		0.853
	Heat/Caffine	32°C/10mM	10	10		*Crassostrea gigas*		40.3% more than heat alone
	Heat/Caffine	29°C/15mM	10	10		*Mytilus edulis*	20	42.0% more than heat alone
	Caffine	10mM	10			*Crassostrea gigas*		0.4
	Calcium ion	0.01, 0.05, 0.1 M	12	8		*Crassostrea gigas*	23	0.28
	Ca/Caffine	0.1M/10mM				*Crassostrea gigas*		0.53
	Ca/Heat	0.1M/30°C				*Mytilus edulis*		0.88
Guo et al., 1991a	Blastomere fusion				N/A	*Crassostrea gigas*		8-44%
	heat shock				1	*Crassostrea gigas*		8-44%
	gynogenesis/ polar body block				1&2	*Crassostrea gigas*		80-100%
Guo et al., 1991b	gynogenesis (toluidine blue & UV)					*Crassostrea gigas*		0.84
Komaru and Wada, 1991	Cytochalasin B	0.5 mg/l	15	15	2	*Chlamys nobilis*	24.8	*
	Hydrostatic Pressure	300 kg/sq. cm	10	20	2	*Chlamys nobilis*	24.8	*
Barber and Mann, 1991	Cytochalasin B	1.0 mg/l	15	15	2	*Crassostrea virginica*		0.96
Allen and Bushek, 1992	Cytochalasin B	0.5 mg/l	20		2	*Crassostrea virginica*	27	58-93%
Barber et al., 1992	Cytochalasin B	0.1 mg/l	10	25	2	*Crassostrea virginica*	29	*
	Cytochalasin B	0.1 mg/l	22	20	2	*Crassostrea virginica*	29	*
	Cytochalasin B	0.5 mg/l	10	25	2	*Crassostrea virginica*	29	*

* No information presented in paper

Barber et al., 1992	Cytochalasin B	0.5 mg/l	22	20	2	*Crassostrea virginica*	29	*
	Cytochalasin B	1.0 mg/l	10	25	2	*Crassostrea virginica*	29	*
	Cytochalasin B	1.0 mg/l	22	20	2	*Crassostrea virginica*	29	*
	Cytochalasin B	0.25 mg/l	15	13	2	*Crassostrea virginica*	27	*
AUTHOR (S)	TREATMENT	INTENSITY	DURATION (minutes)	TIME AFTER FERT. (minutes)	MEIOSIS STAGE	SPECIES	TEMP. (°C)	% POLYPLOID
Cadoret, 1992	Electric field	600 V/cm	(50-200 μs) x 1-6 pulses			oysters and mussels		55 & 36%
Utting and Doyou, 1992	Cytochalasin B	0.5 mg/l	15	15		*Tapes philippinarum*		67 & 73%
Akashige and Fushimi, 1992	Cytochalasin B					*Crassostrea gigas*		*
Guo et al.,1992	Cytochalasin B	1.0 mg/l	15	5	1	*Crassostrea gigas*	25	15.6% triploid
	Cytochalasin B	1.0 mg/l	15	5	1	*Crassostrea gigas*	25	57.6% aneuploid
Shen et al., 1993	Hydroststic Pressure	200-250 kg/sq. cm	17-19	5-7	1	*Pinctada fucata martensii*	*	24.6 - 64.7 %
	Hydroststic Pressure	200-250 kg/sq. cm	17-19	10	2	*Pinctada fucata martensii*	*	76.00%
Desrosiers et al., 1993	6-dimethylaminopurine	300 μm	20	15	1 & 2	*Crassostrea gigas*	25	90.00%

* No information presented in paper

Figure 1:
Karyological Processes Involved in Ploidy Manipulation of Mollusc Eggs



Normal development in the bivalve mollusc egg (a-d). For simplicity only one pair of chromosomes is shown. (a) egg at release at metaphase of meiosis I, activation by sperm; (b) meiosis II complete, first polar body extruded, sperm nucleus has entered egg; (c) meiosis II completed, second polar extruded, male and female pronucleus unite; (d) first cleavage perpendicular to point of polar body extrusion.
Ploidy manipulation (e-k). (e) shock administered during meiosis I, both chromosomes of the pair retained in the egg, first polar body not extruded; (f) normal meiosis II allowed, second polar body pair extruded, 2N female pronucleus and N male pronucleus unite; (g) triploid first cleavage; (h) shock administered during first cleavage; (k) tetraploid chromosome complement in second cleavage (at right angles to first cleavage)(Beaumont and Fairbrother, 1991).

3. Determination of Ploidy

Determining the ploidy level of induced triploids is required to verify the degree of success achieved in any attempt to alter ploidy. Specifically, verification of triploidy is required in all research into the biological impact of triploidy.

Several techniques have been described for use in ploidy determination. These are: flow cytometry, karyotypic analysis, starch gel electrophoresis, image analysis, scanning electron microscopy, comparative cell nuclei cytology and microfluorometry. Techniques used in research on triploid bivalves are summarised in Table 2.

3.1 Flow Cytometry

Flow cytometry can be used to measure DNA content of nuclei by recording the intensity of staining of the nuclei (Allen, 1983; Oud *et al.*, 1987; Coon and Weinstein, 1992). Fluorescent stains are used which are specific for DNA (Oud *et al.*, 1987; Coon and Weinstein, 1992). Flow cytometry essentially measures the intensity of fluorescence of the stained DNA within the nuclei (Allen, 1983; *Oud et al.*, 1987; Coon and Weinstein, 1992). Nuclei are passed over a sensor in a suspension allowing many readings to be recorded rapidly (Oud *et al.*, 1987; Coon and Weinstein, 1992). Consequently it is possible to record the DNA content of many cells, in the order of 10,000-100,000 per sample (Oud *et al.*, 1987).

Preparation of nuclei for flow cytometry involves three steps; A) disassociation and suspension of cells, B) lysis of cells to leave nuclei, and C) staining of the DNA within the nuclei with an appropriate fluorochrome dye (Taylor and Milthorpe, 1980; Allen, 1983; Oud *et al.*, 1987; Coon and Weinstein, 1992).

A) Adductor muscle is selected for flow cytometry where possible. However in small individuals such as spat or larvae, this is not possible and the whole animal is used (Chaiton and Allen, 1985).

The suspension of cells is a mechanical process. Larvae can be prepared by first cracking their shell with mechanical shock (Chaiton and Allen, 1985). Spat cells are separated by first mincing with a scalpel then repeatedly drawing a suspension through a syringe needle before finally filtering (Chaiton and Allen, 1985). Centrifugation and resuspension with a vortex mixer assists to disassociate cells (Chaiton and Allen, 1985).

B) Lysing of the cellular membrane is achieved with a non-ionic detergent (Chaiton and Allen, 1985). Taylor and Milthorpe (1980) used Triton-X-100 for this purpose. The lysing of the cellular membrane is carried out in the same solution as staining (Chaiton and Allen, 1985).

C) Staining can utilise several fluorochromes. The most suitable stain varies with the

application (Coon and Weinstein, 1992). The most commonly used in bivalve triploidy determination is 4,6-diamidino-2-phenylindole (DAPI). This dye has a specificity for moieties (T-A (Coon and Weinstein, 1992)) in DNA (Taylor and Milthorpe, 1980). Other dyes used for DNA analysis include propindium iodide, ethidium bromide, acridine orange, Hoechst™ 33342 and mithramycin (Taylor and Milthorpe, 1980; Coon and Weinstein, 1992).

3.2 Karyotypic Analysis

Chromosome spreads require the preparation of stained, condensed and separated chromosomes for visualisation by light microscopy. Preparation often involves the halting of cell division at mitosis (karyokinesis) to ensure clumping of DNA with colchicine (Kilgerman and Bloom, 1977; Allen *et al.*, 1982b). The nuclear envelope and cell membrane are then split and the chromosomes spread (Allen *et al.*, 1982b). Chromosomes are stained, viewed and counted (Kilgerman and Bloom, 1977; Allen *et al.*, 1982b). Whilst this is the most accurate method of ploidy determination, it is very time consuming (Komaru *et al.*, 1988). A detailed methodology is given by Kilgerman and Bloom (1977). The Kilgerman and Bloom (1977) method was devised for teleost studies and was adapted by Allen *et al.* (1982b) for use with bivalves. The major change in method involved the storage of tissue prior to processing in methanol (Allen *et al.*, 1982b). This both increased the fragility of the cells and allowed batch processing of samples.

3.3 Electrophoresis

Electrophoresis of enzymes (coded by the biochemical loci, PGM, PRI, EST-1, EST-3 and 6PG) has been used by Allen *et al.* (1982a & b) to provide accurate confirmation of triploidy. However, this technique requires genetic diversity to be heightened in triploids and this is not always the case. Consequently, often this method is not useful (Komaru *et al.*, 1988).

3.4 Image Analysis

Nuclear DNA content is measured by the intensity of staining (densiometric) with image analysis (Jarvis, 1992a). This has become a popular technique in human ploidy studies for diagnosis of dysplastic of malignant cell characteristics and estimation of their prognostic significance (Jarvis, 1992a).

Comparative studies between flow cytometry and image cytometry by Oud *et al.* (1987) found that the techniques displayed a similar ability to determine ploidy in human pathology. Oud *et al.* (1987) stated that the main advantage of flow cytometry over image analysis is the number nuclear DNA contents it is possible to measure from a sample. This is in the order of 10,000-100,000 with flow cytometry whilst only fifty to a few hundred in image cytometry. Image cytometry possesses the advantage of being able to more selectively sample specific tissue prior to recording the DNA content (Oud *et al.*, 1987). This is achieved by selection of cells prior to measurement using histological examination. In bivalve triploidy analysis this is less relevant as ploidy determination is conducted to determine the ploidy of a whole organism. Conversely, in the study by Oud *et al.* (1987), ploidy was evaluated in cancer cells. This cancer tissue had anomalous ploidy compared to the surrounding tissue which assisted in diagnosis.

As with flow cytometry, staining type and technique is crucial for evaluation of ploidy with image analysis (Jarvis, 1992a). Traditionally, histology has utilised the natural cationic dye, haematoxylin to stain DNA (Schulte *et al.*, 1988). This dye is unsuitable for image analysis due to wide batch-to-batch variation (Schulte *et al.*, 1988). The most accepted staining technique for image segmentation of DNA is the Feulgen procedure (Jarvis, 1992a & b). This technique is described in Kiernan (1990). A variation on this technique, Feulgen Napthol Yellow-S, was used by Schieck *et al.* (1987) to assist computer visualisation of staining. Schulte *et al.* (1988) used the synthetic DNA specific cationic dye, Victoria Blue, to improve the clarity of staining for image analysis.

Image analysis determination of DNA content can be performed on resuspended nuclei from paraffin embedded tissue (Oud *et al.*, 1987), tissue sections (Jarvis, 1992) and smears (Schulte *et al.*, 1988). Whilst image analysis of DNA content is possible on paraffin sections, whole cell squashes are preferred (Jarvis, 1992b). This is due to the problem of measurement encountered with partially sectioned nuclei (Jarvis, 1992b).

3.5 Scanning Electron Microscopy

Scanning electron microscopy (SEM) is ideally suited to the examination of fine surface detail of cells. Stephens and Downing (1989) intended to examine differences between the gamete sizes and the micropyles of diploid and triploid *Crassostrea gigas* eggs although no results are given.

3.6 Visual Cytology

Visual confirmation of triploidy in fish is possible by examination of blood smears using standard giemsa staining (Kavumpurath and Pandian, 1992).

To date, only one research project used cytology of whole cells to determine ploidy in bivalves. Yamamoto *et al.* (1990) used fluorochrome stains (DAPI) to stain DNA which was then examined microscopically with UV illumination.

3.7 Microfluorometry

Confirmation of triploidy using microfluorometry in *Pinctada fucata martensii* larvae and *Chlamys nobilis* was achieved by Uchimura *et al.* (1989) and Komaru *et al.* (1988) respectively. Microfluorometry utilises the same principles as flow cytometry, that is, measurement of the intensity of fluorescence at a specific excitatory wavelength of fluorochrome stained nuclei. Fluorescence is measured by a microphotometer. In both studies DAPI was used to stain DNA for microfluorometry. The advantage of this technique in relation to flow cytometry is the use of simpler equipment. Sample preparation is similar for both techniques.

Table 2: Techniques Used For The Determination Of Ploidy

Author	Method	Species
Longo, 1972	Karyological (direct count)	*Spisula solidissima*
Stanley *et al.*, 1981	Karyological (direct count)	*Crassostrea virginica*
Allen *et al.*, 1982a	Electrophoresis	*Crassostrea virginica, Mya arenaria*
Allen *et al.*, 1982b	Karyological, Electrophoresis	*Mya arenaria*
Hidu *et al.*, 1982	Flow Cytometry, Karyological	*Crassostrea virginica*
Stanley *et al.*, 1984	DNA microfluorometry	*Crassostrea virginica*
Chaiton and Allen, 1985	Flow Cytometry	*Crassostrea gigas*
Allen and Downing, 1986	Flow Cytometry	*Crassostrea gigas*
Allen *et al.*, 1986	Flow Cytometry, Electrophoresis	*Mya arenaria*
Quillet and Panelay, 1986	Karyological (direct count)	*Crassostrea gigas*
Downing and Allen., 1987	Flow Cytometry	*Crassostrea gigas*
Komaru *et al.*, 1988	DNA microfluorometry	*Chlamys nobilis*
Downing, 1988	Flow Cytometry	*Crassostrea gigas/rivularis* hybrid
Beaumont and Kelly, 1989	Karyological (direct count)	*Mytilus edulis*
Stephens and Downing, 1989	Scanning Electron Microscopy	*Crassostrea gigas*
Uchimura *et al.*, 1989	DNA microfluorometry	*Pinctada fucata martensii*
Guo *et al.*, 1989	Flow Cytometry, Karyological (direct count)	*Crassostrea gigas*
Gendreau *et al.*, 1989	Karyological (direct count)	*Ostrea edulis*
Durand *et al.*, 1990	DNA microfluorometry	*Pinctada fucata martensii*
Akashige, 1990	DNA microfluorometry	*Crassostrea gigas*
Komaru and Wada, 1990	DNA microfluorometry	*Pinctada fucata martensii*
Allen and Downing, 1990	Flow Cytometry	*Crassostrea gigas*
Yamamoto *et al.*, 1990	Fluorescence microscopy	*Crassostrea gigas*
Guo *et al.*, 1991a	Karyological (direct count)	*Crassostrea gigas*
Barber and Mann, 1991	Flow Cytometry	*Crassostrea virginica*
Allen and Bushek, 1992	Flow Cytometry	*Crassostrea virginica*
Barber *et al.*, 1992	Flow Cytometry	*Crassostrea virginica*
Utting and Doyou, 1992	Karyological (direct count)	*Tapes philippinarum*
Desrosiers *et al.*, 1993	Image Analysis, Karyological & Nuclear size Cytology.	*Crassostrea gigas* *Placopecten magellanicus* & *Mytilus edulis*

4. Reproductive Biology

Reduction in the extent of sexual development is considered to be the major basis of any difference observed between triploids and diploids. While other factors undoubtedly also are involved, for example heterozygosity (Allendorf and Leary, 1984; Stanley *et al.*, 1984), the extent of the impact triploidy makes on reproduction is of primary importance.

4.1 Methods of Study

Traditionally, measurement of changes in the extent of development of bivalve gonad and gametes has involved qualitative histological staging or quantitative weight comparison methods (between gonad and somatic tissue). Gonado/somatic indices based on weight values require the separation of gonad tissue from the remaining soma (Peterson and Fegley, 1986). In oysters this is comparatively difficult as the gonad tissue infiltrates the mantle and digestive tissue (Dinamani, 1974). Consequently, histological staging has been used to determine the extent of development of the gonad and gametes in oysters. Such staging systems utilise changes in gonad follicles and gamete cell stage to categorise development.

The resultant quantitative scores have been used by Dinamani (1987) to provide a "gonad maturity index" (defined as the arithmetic mean of the developmental staging score). Many separate staging criteria utilising histological sections have been used to define reproductive development qualitatively. Relevant studies include: Brousseau (1982; cited by Heffernan and Walker, 1989) for *Geukensia demissa*; Kennedy and Battle (1964) for *Crassostrea virginica*; Dinamani (1974) for *Crassostrea glomerata*; Dinamani (1987) and Mann (1979) for *Crassostrea gigas*; Wilson and Simons (1985) and Mann (1979) for *Ostrea edulis* and Allen *et al.* (1986) for *Mya arenaria.*

Staging schemes are subject to variation in development within a gonad. Where this occurs the average developmental stage is assigned to the gonad (Dinamani, 1987).

Morphological observations from histological preparations also are needed to determine the sex and differentiation of oysters (Dinamani, 1974; Wilson and Simons, 1985; Allen *et al.*, 1986; Dinamani, 1987; Morales-Alamo and Mann, 1989; Allen and Downing, 1990).

For scientific analyses it is desirable to record change more quantitatively. Quantification of gonadogenesis from histological samples has been achieved by measuring gonad width relative to body width from several sites around a transverse section (to the anterio/posterior axis) (Kennedy and Battle, 1964). Similar measurements were used by Dinamani (1987) on longitudinal sections to provide a measure of percentage gonad tissue relative to somatic tissue.

Measurements taken with planometry allow greater quantification of the relative size of gonad tissue (Wilson and Simons 1985; Dinamani 1987). A combined index, termed a follicle index, was used by Dinamani (1987) to gauge the extent of gonad development. The follicle index was defined as:

> **percentage gonad area** (to somatic tissue) X
> **gonad density** (defined as the ratio of area occupied by gametes in follicles to the area of the gonad).

Wilson and Simons (1985) also quantified gonadogenesis in terms of follicle area within the gonad (percentage follicle area of gonad). Gametogenesis was quantified by Wilson and Simons (1985) with a method similar to Dinamani (1987). That is, planometry area calculation of the percentage gonad area occupied by gametes.

More recently computer analysis of light microscopy has enabled more detailed analysis and quantification of tissue types and area from histological sections (Jarvis, 1992a). This system is termed image analysis or photo-planometry. It is used to quantify using both shape (morphometry) and staining intensity (densiometry) (Jarvis, 1992a).

Image analysis has been used to record the percentage gonad area (Heffernan and Walker, 1989; Heffernan *et al.*, 1989; Morales-Alamo and Mann, 1989), percentage spermatozoa area (Heffernan and Walker, 1989), percentage oocyte area (Heffernan and Walker, 1989), mean oocyte diameter (Heffernan and Walker, 1989) and oocyte number (Heffernan and Walker, 1989).

The proportion of tissue occupied by gonad in transverse sections varies greatly along the anterio/posterior axis (Morales-Alamo and Mann, 1989). This is a potential source of experimental error in measurement of percentage gonad area. Morales-Alamo and Mann (1989) suggest that an anatomical structure, visible macroscopically, be used to standardise the site of gonad area sampling. It is suggested that the paired "H" shaped cavities of the appendix of the stomach caecum be utilised for this purpose.

4.2 Gonadogenesis

Many authors refer to gonadogenesis and gametogenesis separately but the distinction between the two terms is often superficial. Generally, gametogenesis is used to define the development of gametes on a cellular level. Gonadogenesis has been used to refer to the development of the whole sexual organ (including the gametes contained therein) and in this context is a measure of gametogenesis on a whole organism basis rather than of individual cells. This is the interpretation used by Allen *et al.* (1986), Akashige (1990), Allen and Downing (1990) and Corni and Cattani (1990).

Gonad development has been reported to occur in triploids but to a lesser extent

than diploids (Allen *et al.*, 1986; Akashige, 1990; Allen and Downing, 1990). Akashige (1990) measured whole gonad area in cross section and found triploids developed gonad in approximately 40% of total somatic area compared to approximately 60% in diploids *(Crassostrea gigas)*. This value of gonad area was derived by weighing the cut-out photocopies of histological photographs of ripe gonads.

Gonad development by triploids is variable with some individuals exhibiting virtually no development while others develop through to spawning condition (Allen *et al.*, 1986 *(Mya arenaria)*; Akashige, 1990 *(Crassostrea gigas)*; Allen and Downing, 1990 *(Crassostrea gigas))*. In diploid *Scapharca inaequivalvis* populations, differential gonad maturation amongst adult individuals of the same population over time can vary considerably (Corni and Cattani, 1990). This is a common reproductive strategy amongst bivalves (Corni and Cattani, 1990) and could account for observed sporadic or uneven gonad maturation within a group.

Both Akashige (1990) and Allen and Downing (1990) noted that retardation of gonadogenesis is more pronounced in female triploids.

Gonadogenesis incorporates development of the gonad follicular tissue. Allen and Downing (1990) recognised gonad follicle development of triploid *Crassostrea gigas* by two features: the extent of follicular branching and the extent of overall follicle growth. By these features Allen and Downing (1990) concluded that follicular development was severely retarded in triploids. Lee (1988) examined follicular development in triploid *Crassostrea virginica* and concluded that this was not affected. Theoretically this would seem to be the expected outcome of triploid impact on gonadogenesis as follicular tissue formation involves only mitosis which is not thought to be uninhibited by triploidy.

Following spawning in male *Crassostrea gigas*, gonadogenesis recommences more rapidly in triploids than diploids (Allen and Downing, 1990).

4.3 Gametogenesis

Generally, in triploid bivalves the extent of gametogenesis is reduced although this varies with species. Hidu *et al.* (1982) found that gametogenesis may not be blocked in *Crassostrea virginica*. Later work by these researchers also found gametogenesis was not blocked in *Mya arenaria* (Hidu *et al.*, 1983). The same research group reported the opposite in 1986, that is, gametogenesis is blocked in triploid *Mya arenaria* (Allen *et al.*, 1986). Male triploid *Mya arenaria* showed higher proportions of undifferentiated spermatogonia and fewer spermatocytes than diploids. No formation of spermatozoa was observed (Allen *et al.*, 1986).

Both Allen and Downing (1990) and Akashige (1990) found gametogenesis was markedly reduced in one year old triploid *Crassostrea gigas* as compared to sibling diploids. Although retarded, gametogenesis was not blocked by triploidy and both

oocytes and motile spermatozoa are formed (Akashige, 1990).

Gamete development in triploid *Argopecten irradians* was reduced relative to diploids (Tabarini, 1984). Another scallop, *Chlamys nobilis*, showed dramatic blockage of gametogenesis with male gametes not developing past spermatocytes in response to triploidy (Komaru and Wada, 1989). Oogenesis was similarly halted and no mature gametes were seen (Komaru and Wada, 1989).

Blockage of gamete formation is incomplete in triploid *Pinctada fucata martensii* and active spermatozoa and mature ova are formed (Komaru and Wada, 1990).

Retardation of gametogenesis in female triploid *Crassostrea gigas* is greater than in males (Allen and Downing, 1990). The opposite, that is, more severe retardation in the males, has been observed in *Mercenaria arenaria* (Allen *et al.*, 1986). Allen and Downing (1990) found that developing oogonia in *Crassostrea gigas* were often abnormal with pycnotic nuclei and appeared to be arrested in the zygotene stage. Development of ova appeared to occur earlier in triploid females although fewer oocytes developed to this stage. Lee (1988) also reported greater gametogenic retardation in female *Crassostrea virginica* triploids. These had limited oogonial proliferation with only slight development of primary oocytes while males exhibited proliferation of primary spermatocytes. No spermatids or spermatozoa were formed.

Inclusions within the gonad (which serve as a nutritive reserve for gametogenesis) have been shown to be significantly more abundant in triploid *Mya arenaria* than diploids (Allen *et al.*, 1986).

Where gametes are formed by triploid bivalves the ploidy of gametes has seldom been investigated. Komaru and Wada (1990) found the spermatozoa of triploid *Pinctada fucata martensii* contained approximately 1.3 times the mean DNA content of diploid spermatozoa. This value was variable among triploid derived spermatozoa.

Attempts have been made to incorporate interspecific hybrids with triploidy to more profoundly retard gametogenesis (Downing, 1988a & b; Downing, 1989). These hybrids were between *Crassostrea gigas* / *Crassostrea rivularis* (Downing, 1988a & b) and *Crassostrea gigas* / *Crassostrea virginica* (Downing, 1989). The diploid hybrid between *Crassostrea gigas* / *Crassostrea rivularis* proved to be sexually viable with normal gametogenesis occurring. The effect on the triploid hybrid was not reported (Downing, 1988b).

4.4 Sex Type and Incidence of Hermaphroditism

Allen and Downing (1990) interpreted the multiple allele theory of Haley (1977) to imply that triploid sex ratios could be expected to differ from those in diploids with an increase in the number of males expected. This proposal of Allen and Downing (1990)

incorporated the findings of Allendorf and Leary (1984) on the increased heterozygosity of gynogenetic triploids. Haley (1977) explained sex determination in *Crassostrea virginica* by suggesting a model in which male individuals are produced by at least three heterozygous alleles. In conjunction with the proposal of Allendorf and Leary (1984) on heterozygosity it was concluded that triploids could be expected to be predominantly male. This conclusion assumed sex determination in *Crassostrea gigas* would be equivalent to that proposed by Haley (1977) for *Crassostrea virginica.* However, it would appear that sex determination in *Crassostrea gigas* is not genetically predetermined and varies with age and environment (Dinamani, 1987). Allen and Downing (1990) found an increase in males did not occur in practice with triploid *Crassostrea gigas* where the sex ratio was equivalent to diploids. Research by Allen *et al.* (1986) encountered the opposite to the proposed sex ratio of Allen and Downing (1990) when triploid clams (*Mya arenaria*) were found to be predominantly female (93%).

Triploid hybrids between *Crassostrea gigas* and *Crassostrea rivularis* yielded 85% males while 78% of diploid hybrids were male (Downing, 1988b).

Variation in the incidence of hermaphrodites has been reported to vary in response to triploidy. Allen and Downing (1990) found that hermaphrodites represented an average 29% of their triploid sample compared to only 1% of the diploids. This proportion increased with the sexual maturity of the oysters and peaked at 50% of the triploid sample. Allen and Downing (1990) found it possible to assign hermaphrodites a predominant sex. Based on this it was found that there was a preponderance of female types. Diploid populations of *Crassostrea gigas* sampled by Mann (1979) and Akashige (1990) contained no hermaphrodites.

Of 60 triploid clams *(Mya arenaria)* sampled by Allen *et al.* (1986) it was not possible to determine the sex of 4. These represent either undeveloped sexed (male or female) clams or sexually undifferentiated (effectively hermaphroditic) clams.

Sex ratio of *Crassostrea gigas* can also be expected to change with the age of the sampled population (Dinamani, 1987). Samples taken by Dinamani (1987) of oysters less than one year old had more males but this changed to a preponderance of females by the end of the second year.

4.5 Spawning

Triploid *Crassostrea gigas* examined by Allen and Downing (1990) exhibited spawning during the same period as the diploids. In the male triploids there was reduced involvement in spawning compared to diploids with only approximately 50% of males spawning. Most female triploids spawned during the same period.

It should be noted that gametes spawned by the triploids were less developed and fewer in number than diploids (Allen and Downing, 1990). Male triploids were found to have spawned while follicles contained large numbers of spermatocytes indicating

that these were evacuated in addition (or exclusively) to spermatids and spermatozoa (Allen and Downing, 1990).

Triploids exhibit similar post spawning histology of gonads to that of diploids (Allen and Downing, 1990). This is seen as infiltration of leucocytes and the phagocytosis of residual gametes (Dinamani, 1974; Allen and Downing, 1990).

Spawning of northern hemisphere populations of *Crassostrea gigas* occurs in summer. Allen and Downing (1990) reported a spawning season of some 4 weeks from late July to early August in California. Southern hemisphere populations (New Zealand) sampled by Dinamani (1987) exhibited spawning over several months beginning with light spawning in mid spring. Spawning extended to early Autumn and peaked in December.

4.6 Offspring of Triploid Spawning

Akashige (1990) fertilised eggs from diploid *Crassostrea gigas* with sperm from triploid males by stripping. It was found that the resultant offspring showed high levels of deformity at the D-veliger stage. Cellular DNA content was intermediate between triploids and diploids although there was a high variation amongst individuals.

5. Glycogen Utilisation

Research by Davis (1988a) and *Mason et al.* (1988) suggest that observed difference in growth between diploids and triploids is a function of difference in energy allocation. In a proposed mechanism energy expenditure varies primarily due to blockage of gametogenesis (Mason *et al.*, 1988). Gametogenesis and sexual differentiation has been shown to occur in 6 month old diploid *Crassostrea gigas* (Dinamani, 1987). Consequently no energetic advantage would be predicted for oysters less than 6 months old. Changes in the energetics of bivalves are reflected in their glycogen content as this is a major form of energy storage (Gabbott, 1975).

In *Crassostrea gigas* the reproductive cycle is correlated with cyclic changes in the carbohydrate content, specifically glycogen (Gabbott, 1975; Mann, 1979; Perdue and Erickson, 1984). Prior to gametogenesis is a period of storage metabolism which causes accumulation of glycogen. These reserves are depleted with the onset of gametogenesis (Gabbott, 1975; Mann, 1979; Perdue and Erickson, 1984). It is thought that depletion of glycogen results from the conversion of glycogen into the lipid reserves of the eggs (Soniat and Ray, 1985; Robinson, 1992).

If energetic differences between triploids and diploids are attributable to gametogenesis (Mason *et al.*, 1987), then it would be expected that differences will be reflected in glycogen utilisation of both sexes. Despite the metabolism of glycogen for

egg production, no significant difference of glycogen content is seen between male and female *Crassostrea rhizophorae* during the gametogenic period (Littlewood and Gordon, 1988). It would appear that gametogenesis is equally taxing for both sexes.

Gametogenesis is cyclic in temperate bivalves and glycogen levels usually fluctuate in response to this (Walne, 1970; Ansell, 1974a & b; Walne and Mann, 1975; Mann, 1979; Zandee *et al.*, 1980; Whyte and Englar, 1982; Muniz *et al.*, 1986). An exception is *Ostrea edulis* where glycogen levels were found to be unaffected despite marked gonadogenic activity (Mann, 1979). Glycogen stores are also affected by other environmental factors including temperature, culture system, food availability and stress from immersion (Seaman, 1991). These contribute to changes in glycogen content and must be considered when comparing differences between diploids and triploids.

5.1 Temperature

Gametogenesis occurs faster at higher temperatures in *Crassostrea gigas* (Mann, 1979). Mann (1979) found that glycogen content in this species decreased more rapidly with the onset of gametogenesis at higher temperatures. Gabbott and Walker (1971) simply considered temperature to be a stress which resulted in the catabolism of glycogen.

5.2 Culture System

The culture system employed to grow oysters can affect their growth (Chew, 1986) and has also been found to affect glycogen content (Whyte and Englar, 1982).

5.3 Nutrition

Glycogen levels fall in response to starvation or poor nutrition (Gabbott and Walker, 1971; Gabbott and Bayne, 1973; Riley, 1976; Davis, 1988c). Diploids require adequate levels of food for gametogenesis and starvation results in incomplete development (Riley, 1976). This is caused by insufficient glycogen reserves to supply lipid to developing gametes (Soniat and Ray, 1985). Effectively, the diploid individual is partially neutered by nutrition and this reduced gametogenesis narrows the dissimilarity of glycogen content between diploid and triploid (Davis, 1988c). When starvation occurs triploids utilise glycogen more rapidly than diploids and this eventually leads to higher mortality (Davis, 1988c). This could well have implications for the shelf life of triploids.

Glycogen reserves have been shown to be utilised extensively for gametogenesis by diploids even in high productivity areas (Robinson, 1992). However, utilisation is reduced and Davis (1989) found that the disparity in glycogen content during gametogenesis in triploid and diploid oysters is diminished in productive environments.

5.4 Glycogen Utilisation in Triploids

Crassostrea gigas has extensive gonad development and correspondingly, glycogen depletion is large during gametogenesis (Perdue and Erickson, 1984). Retardation of gametogenesis in triploid bivalves results in decreased metabolism of glycogen reserves. This is seen in higher glycogen levels in triploids in relation to diploids, especially during spring and summer (Allen and Downing, 1986; Akashige, 1990; Akashige and Fushimi, 1992). Triploid glycogen levels tend to fluctuate in the same pattern as diploids while depletion of reserves is reduced (Allen and Downing, 1986; Akashige, 1990; Akashige and Fushimi, 1992). Akashige and Fushimi (1992) recorded glycogen contents of triploid and diploid *Crassostrea gigas* . The range of glycogen contents within their sample decreased to 8.5-16.9% in triploids and 2.7-5.4% in diploids (dry weight).

Glycogen levels do not serve only as a guide to energy utilisation but can reflect the marketability of an oyster (Chew, 1986). Taste trials conducted by Allen and Downing (1991) concluded that triploid oysters were preferred which was attributed to their higher glycogen content. This is surprising as oyster glycogen is flavourless. Thus immature or partially sterile oysters, that is, those with high glycogen content, have high marketability in the U.S.A.(Chew, 1986; Allen and Downing, 1991).

High glycogen levels are positively correlated with condition index (Soniat and Ray, 1985). Both increase during the pre-gonadogenic fattening period of oysters (Soniat and Ray, 1985).

5.5 Method of Analysis

There are several methods available for the determination of glycogen content. Content is usually expressed as mg/ g dry weight of oyster. Most researchers evaluate glycogen contents of samples as a concentration of glycogen in the tissue. Gabbott and Stephenson (1974) suggest the use of a glycogen condition index which is defined as;

$$[\text{Glycogen (g)} / \text{Internal Shell Volume (ml)}] \times 1000$$

By incorporating internal shell volume, this formula aims to remove misleadingly high glycogen values due to oysters with a high water content.

Littlewood and Gordon (1988) precipitated glycogen from samples with ethanol. Phenol and sulphuric acid were added to the extracted glycogen. The products of the resultant reaction were measured with spectrophotometry (420 nm).

Keppler and Decker (1974) determined glycogen content by measuring the products produced by enzyme digestion. The procedure involved breakdown of glycogen to glucose with amyloglucosidase. The glucose produced was further reduced with

hexokinase to produce $NADP^+$. This product was converted to NADPH with a dehydrogenase which was then measured with spectrophotometry.

6. Condition Indices

Blockage of gametogenesis by triploidy leads to decreased spawning behaviour (Allen and Downing, 1990). This potentially produces one of the major benefits of triploidy in oysters, high condition during the spawning period (Maguire and Kent, 1990). Changes in condition of this nature are well suited to analysis by means of a condition index. Surprisingly, the only published research which measures condition in triploid bivalves is that of Davis (1988c). This paper measures decline in condition with starvation. The actual method of assessment of condition is not given.

Condition indices have been used to record physiological changes in response to temperature, starvation, spawning and disease in bivalves. Methods used vary widely, as can be seen in Table 3, and this presents difficulties in the comparison of studies.

Many works utilise condition indices based on the formula described by Baird (1958) which compares the dried meat weight with shell cavity measured in millilitres. The major difficulty with this system is the measurement of internal cavity volume. Baird (1958) uses displacement of the whole oyster and of the shell, thereby deriving cavity volume. Rodhouse (1977) suggested the use of Archimedes' principle to avoid errors associated with measuring displacement. This system measures the change in weight of a volume of water with the addition of the whole oyster and the shell. The oyster and shell were suspended so only their displaced volume was weighed.

Lawrence and Scott (1982) refined the measurement of cavity volume by utilising the assumption that oyster meat is of roughly the same density as water. They derived cavity volume by subtraction of the shell weight from the whole weight. While this method and the underlying assumptions would not hold true for all bivalves, it is a suitable method for analysis of oysters (Lucas and Beninger, 1985).

The Lawrence and Scott (1982) formula for the calculation of condition index is:

$$\text{C.I.} = \frac{\text{Dry Soft Tissue Weight (g)} \times 1000}{\text{Internal Shell Cavity Capacity (g)}}$$

Internal shell cavity capacity is determined by the gravimetric method outlined above.

Two recent reviews of condition indices cite this method as being the most useful for oyster research and recommend its application to assist standardisation between research (Lucas and Beninger, 1985; Crosby and Gale, 1990).

Table 3: Condition Indices Used in Bivalve Research

Author	Condition Index Used	Species
Baird, 1958	DMWT(g)/ ISV(ml) x 1000	Mussels and Oysters
Gabbott and Stephenson, 1974	DMWT(g)/ ISV(ml) x 1000	*Ostrea edulis*
Walne and Mann, 1975	DMWT(g)/SW (g) x 1000	*Crassostrea virginica*
Walne and Millican, 1978	DAshWT(g)/TDWT(g) x 100	Oyster spat
Muniz *et al.*, 1986	DMWT(g)/ ISV(ml) x 1000	*Crassostrea gigas*
	DMWT(g)/ ISV(ml) x 1000	*Crassostrea brasiliana*
Littlewood and Gordon, 1988	DMWT(g)/SW (g) x 1000	*Crassostrea rhizophorae*
Brown and Hartwick, 1988	DMWT(g)/WW(g)-SW(g) x 1000	*Crassostrea gigas*
	DMWT(g)/SW(g) x 1000	*Crassostrea gigas*
Barber *et al.*, 1988	DMWT(g)/ ISV(ml) x 1000	*Crassostrea virginica*
Marcus *et al.*, 1989	DMWT(g)/ ISV(ml) x 1000	*Crassostrea virginica*
Whyte *et al.*, 1990	DMWT(g)/ ISV(ml) x 1000	*Crassostrea gigas*
Lowell, 1991	MWT(g)/SW(g) X 100	*Artica islandica*
	MWT(g)/ ISV(ml) X 100	*Artica islandica*
	VWT(g)/ MWT(g) x 100	*Artica islandica*
Rainer and Mann, 1992	DMWT(g)/SW (g) x 100	*Crassostrea virginica*
	DMWT(g)/ ISV(ml) x 100	*Crassostrea virginica*

DMWT= dry meat weight; ISV= internal shell volume; SW= shell weight; WW= whole weight; MWT= dry meat or wet meat weight; VWT= visceral dry or wet weight; DashWT= dry ash weight; TDWT= total dry weight.

7. Growth

Ultimately, triploidy research is directed at producing a better quality or more profitable product (Chew, 1986). Of major interest is the possible growth advantage triploids have due to their decreased development of gametes and gonad (Barber and Mann, 1991).

Blockage of meiosis II in *Crassostrea virginica* produced triploids which showed little retardation of gametogenesis (Hidu *et al.*, 1982; Stanley *et al.*, 1984). Growth in this group was comparable to that of diploids. Siblings which had meiosis I blocked exhibited increased growth rate which was attributed to heterozygosity by Hidu *et al.* (1982) and Stanley *et al.* (1984). These growth results were similar to those found for

growth of meiosis I and meiosis II triploid *Mya arenaria* (Hidu *et al.*, 1983). Again, gametogenesis was not affected. The results of Matthiessen and Davis (1991) were contrary to that of Hidu *et al.* (1982) and Stanley *et al.* (1984). Growth of meiosis II triploid *Crassostrea virginica* were shown to be greater than that of diploids. This was evident at both six months and fifteen months post-set.

In other species gametogenesis is more severely affected. The energetic implications of this imply growth rate of triploids should be enhanced (Mason *et al.*, 1987). Barber and Mann (1991) found that the growth of triploid *Crassostrea virginica* was significantly more than that of diploid siblings. The triploids used by Barber and Mann (1991) were produced by suppression of formation of the second polar body.

Age of the triploid would be expected to influence difference of growth compared to sibling diploids. If the energy advantage afforded by reduced gametogenesis accounts for increased growth in triploids (Mason *et al.*, 1987), it would be expected that no growth advantage would be gained until after sexual maturity. This was observed by Komaru and Wada (1989) in *Chlamys nobilis.* Triploid shell width and meat weights were significantly greater than diploids at 14 months but not at 9 months (Komaru and Wada, 1989). Contrary to these results, Yamamoto *et al.* (1988) found that growth rates between diploid and triploid *Crassostrea gigas* were significantly different at 11 and 25 days. Triploids produced at meiosis II had slower growth (measured by change in shell length) than diploids while those produced at meiosis I had greater growth. Desrosiers *et al.* (1993) reported that no significant difference existed between diploid and triploid larvae after 20 days.

Triploid *Crassostrea virginica* studied by Matthiessen and Davis (1991) exhibited increased growth rate early in their development. Greater growth in triploids than diploids was found at 6 months. The onset of gametogenesis at this age would be unlikely (Kennedy and Battle, 1964). However, no histological description of the extent of gametogenesis was presented by Matthiessen and Davis (1991).

Davis (1988b) concluded that the growth of diploid Pacific oysters was not limited by gametogenesis in high energy environments. It would appear that growth is only improved by triploidy in those environments where there is limited food and gametogenesis taxes the total energy available to the organism (Davis, 1988b). In extremely low energy environments where starvation occurs, triploid *Crassostrea gigas* were reported to be less able to cope than diploids. Consequently higher mortality resulted (Davis, 1988c).

When triploidy is induced using cytochalasin B there are increased proportions of aneuploid and tetraploid larvae produced (Guo *et al.*, 1992). Aneuploidy in *Crassostrea gigas* is known to decrease growth rate (Thiriot-Quievreux *et al.*, 1988).

Growth is measured by either weight or length gain. Weight gain is measured as change in dry or wet meat weight or whole weight (Barber and Mann, 1991). Length is

taken as the biological height of the bivalve in oysters (Hidu *et al.*, 1982, Barber and Mann, 1991) or width in scallops (Komaru and Wada, 1989). Suppression of growth from intraspecific competition can affect growth trials in oysters where subjects are held communally (Jarajabhand and Newkirk, 1989).

8. Disease

Barber and Mann (1991) examined the incidence of *Perkinsus marinus* in triploid and diploid *Crassostrea virginica*. No difference in disease tolerance was observed between the two groups. The more resistant *Crassostrea gigas* was also found to exhibit no increased resistance to *Perkinsus marinus* through triploidy (Meyers *et al.*, 1991a & b).

Heightened disease resistance of triploid Crassostrea *virginica* against MSX was reported by Matthiessen and Davis (1991). Although infection was higher in triploids, mortality was lower than in diploids (Matthiessen and Davis, 1991).

Gendreau *et al.* (1989) researched induction of triploidy in the European flat oyster, *Ostrea edulis*. This was intended to increase resistance to *Bonamia*. The effectiveness of triploidy at reducing *Bonamia* infection and subsequent mortality was not assessed.

9. Ingestion of Food

Yamamoto *et al.* (1988) observed the digestion of food particles (*Isochrysis galbana*) by larval *Crassostrea gigas*. No significant differences were observed between diploids and triploids.

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STUDIES ON TRIPLOID OYSTERS IN AUSTRALIA. I. THE FARMING POTENTIAL OF TRIPLOID SYDNEY ROCK OYSTERS *Saccostrea commercialis* (Iredale and Roughley).

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Sydney rock oysters *Saccostrea commercialis* generally spawn in the summer and autumn period and as a result are in poor condition during winter and spring, making them difficult to market for up to six months of the year. Triploid *S. commercialis* were produced to determine whether the reduced gonad development, which is a characteristic of triploid oysters, would allow them to maintain sufficient condition to extend their marketable period.

Triploidy was induced in a single batch of fertilised *S. commercialis* eggs by blocking meiosis II with cytochalasin B (CB) at a concentration of 0.5 mg CB l^{-1} at 23 min after sperm addition for 20 min at a temperature of 25°C. Flow cytometry indicated 85% triploidy in the experimental spat. Larvae and spat were cultured under commercial hatchery conditions then reared on three intertidal commercial oyster leases and one subtidal (raft) site on Port Stephens, NSW.

Seasonal variation in growth rates and meat condition of triploid *S. commercialis* were compared with those of their diploid siblings for a period of 2.5 years. The average whole weight of triploid *S. commercialis* was 41% greater than that of the diploid controls after 2.5 years growth. They also maintained higher dry meat weight and higher condition index (CI) than diploids at all sites during the final ten months of the study. A condition index of 100 or above is preferred for marketing the Sydney rock and this was reached only by the triploids in this study. The highest CI values in the study were produced by the triploid oysters from the subtidal site.

In other oyster species, triploids may vary, compared with their normal diploid forms, in their response to challenge by protistan parasites. Winter mortality, caused by such a parasite, *Mikrocytos roughleyi*, kills a large proportion of *S. commercialis* in the southern part of its range, particularly in cold, dry winters.

Triploid and diploid *S. commercialis* were held in an area known for high incidence of winter mortality for eight months to determine their relative susceptibility to this disease. The difference in mortality (means ± SE) experienced by the triploids and diploids (45.9±1.7% and 41.3±1.6% respectively) was not significant ($P>0.05$).

The results indicate that triploid Sydney rock oysters can reach market size (40 to 60 g) 6-18 months faster than usual and maintain better meat condition without increased risk of winter mortality. This offers the NSW oyster industry an opportunity to improve its economic viability. Commercial trials by oyster farmers are in progress.

STUDIES ON TRIPLOID OYSTERS IN AUSTRALIA. 2. GROWTH, CONDITION INDEX, GONAD AREA, AND GLYCOGEN CONTENT OF TRIPLOID AND DIPLOID PACIFIC OYSTERS, *Crassostrea gigas*, FROM OYSTER LEASES IN TASMANIA, AUSTRALIA.

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This research was undertaken to evaluate triploid Pacific oysters under Tasmanian environmental conditions using local commercial growout techniques. Diploid Pacific oysters were strip spawned in February 1990, and diploid and Meiosis 2 triploid larvae reared from the same pool of gametes. Cytochalasin B (0.5 mg l^{-1} for 20 min) was the triploidy induction stress used. After setting, the oyster spat were held onshore in upwellers and then offshore in intertidal sectionalised trays. From August 1990 they were grown intertidally at three commercial leases in oyster bags within 6 mm mesh baskets and then free in 12 mm baskets.

At all sites triploids (76 % triploidy) maintained marketable meat condition during the summer - autumn spawning period after attaining commercial size (about 60 g whole oyster weight). At the two sites where diploid oysters spawned they remained in much poorer condition than triploids for at least four months post spawning. At two sites oysters reached commercial size, as they approached two years of age and at the other site at three years of age. Triploid oysters grew faster than diploids as they approached commercial size and were 23.4 % larger than diploids on a whole weight basis at the two better sites (age 27 months) and 19.6 % larger at the poorest site (age 38 months). At the two better sites mortality of diploid and triploid oysters was negligible during growout. At the third site mortality of triploid and diploid oysters (excluding sampling and whole bags lost through storm damage) averaged 1.0 and 0.3 % $month^{-1}$ respectively during growout through to April 1993. A greater proportion of the cross sectional area of oyster meats was occupied by gonad in diploids (maximum of 47.7 - 72.6 %, depending on site) than in triploids (maximum of 7.7 - 11.9 %). Similar maximum glycogen reserves were developed by triploids (15.6 - 21.2 g $100g^{-1}$ dry meat, depending on site) and diploids (14.5 - 20.2 g $100g^{-1}$ dry meat). Triploids maintained these reserves whereas diploids sacrificed most of their glycogen as they approached the spawning season.

Triploid Pacific oysters show great promise for overcoming marketing problems caused by poor meat condition of diploid oysters in summer and autumn in Tasmania. However, to exploit this marketing niche, most of the triploids would need to be marketed at about 2 - 2.5 years of age and shell growth rates may have to be repressed through use of elevated growing heights. This should ensure that the oysters do not become too large or that rapid shell growth during the maturation and spawning season does not reduce their condition index advantage over diploids.

MULTI-SKILLING IN AQUACULTURE
A HANDS-ON TRAINING WORKSHOP

June 28-July 2 1992
Key Centre for Teaching and Research in Aquaculture
University of Tasmania at Launceston

SESSION 4 B

MOLLUSCAN CULTURE TECHNIQUES

By Colleen O'Meley, Dr Greg Maguire and Mark Lleonart

HUSBANDRY AND MARKETABILITY OF OYSTERS

BY COLLEEN O'MELEY

1. INTRODUCTION

1.1 COMMERCIAL PRODUCTION IN AUSTRALIA

Edible oysters belong to a commercially important group of molluscs known as the bivalves which also includes scallops, mussels, clams and pearl oysters. The sale of cultured, edible oysters in Australia was worth $34.6 million in 1989-1990 and represented 18% of total aquaculture sales (Treadwell et al., 1992), while in terms of world-wide sales it represented about 1% (Graham, 1991). The main industries are based in New South Wales and Tasmania.

1.2 HISTORY OF OYSTER CULTURE IN AUSTRALIA

Aboriginals living in coastal areas, traditionally gathered oysters attached to stones, empty mollusc shells and sticks in sheltered, inshore waters. Over many centuries large middens were established comprising the waste shells of these oysters and other edible molluscs and these can still be seen along the shores of some estuaries and bays.

In the 1800's the culture of native Sydney rock oysters (*Saccostrea commercialis*) began in New South Wales. The culture methods simply involved placing rocks and stones in areas known to be favourable for natural spatfall collection and in this way natural stocks were enhanced. This method of collecting juvenile oysters or spat was further refined so that tea-tree branches and later tarred, hardwood sticks were used for spat collection. Today the majority of spat for New South Wales farms are still collected and on-grown on tarred, hardwood sticks and although there are two hatcheries in New South Wales, one of these is privately owned and mainly supplies its own farm operations while the government hatchery no longer sells commercial quantities of spat. In Queensland a small industry uses tarred sticks to collect and on-grow both Sydney rock and black-lipped oysters (*Saccostrea echinata*).

Dredge fisheries for native flat oysters (*Ostrea angasi*) began in the late 1800's in Victoria, but by the early 20th century, these had closed due to the threat of extinction of natural beds. In 1986, a government-run hatchery was established to provide spat to Victorian farmers, which helped to establish a small industry. The flat oyster is also cultured in Tasmania, Western

Australia and South Australia. This species has good export potential but the discovery of a parasitic disease *Bonamia* sp. in flat oysters in Victoria and Tasmania is of great concern. This parasite infects the oyster's blood cells and causes high mortalities, especially during the summer season, but is harmless to man.

Pacific oysters (*Crassostrea gigas*) from Japan were introduced into Australia by the Division of Fisheries, CSIRO. Survival of the initial sea shipments in 1947 and 1948 was low, possibly due to the lengthy travel period, and these were followed by air consignments in 1951 and 1952. Natural beds of breeding populations were established in the north of Tasmania and spat were collected from the Tamar river for several years using sticks. In the 1970's spatfalls in the Tamar river became irregular and were no longer commercially viable. A farmers cooperative hatchery was established in 1980 and further commercial hatcheries began operating in 1985, 1990 and 1991 (Dix, 1990). Together these hatcheries provide spat to both the Tasmanian and the rapidly expanding South Australian industries. Some hatcheries are also providing Sydney rock spat to farmers in New South Wales.

How Pacific oysters were introduced into New South Wales is not known but low numbers were present in the 1970's. Populations of this oyster dramatically increased in the 1980's so that it is now present in most bays and estuaries of New South Wales and southern Queensland. Although it is regarded as a noxious species, several farmers have government permission to culture the oyster in Port Stephens, New South Wales.

1.3 BIOLOGY OF OYSTERS

1.3.1 FEEDING AND RESPIRATION

Oysters are filter-feeding animals which usually live in estuarine or marine environments. The water surrounding the oysters contains many species of phytoplankton, bacteria and suspended organic and inorganic particles. The oysters filter the water with their gills (Figure 1) so that potential food particles which come into contact with the cilia of the gills are sorted usually by size. Organic particles of a suitable size (optimum 5 -15 μm) are moved by the cilia to the labial palps next to the mouth where further sorting occurs. Rejected particles are bound in mucus and ejected from the oyster as pseudofaeces.

Food particles enter the mouth and are carried to the stomach by cilia. In the stomach a crystalline style rotates against a gastric shield and enzymes are secreted at the same time so that both mechanical and chemical breakdown of food particles are achieved. The particles which are

now very small are pushed by cilia through ducts into the digestive tissue where more enzymes help to break down the particles before they are absorbed. Waste materials are ejected as faeces through the anus and urine through the gills.

Besides filter-feeding, the gills of the oyster are responsible for respiration where approximately 5% of the oxygen in seawater is transferred across the gill face and into the oyster's tissues.

1.3.2 SHELL GROWTH

The shell consists of a cupped valve which contains the oyster meat and another valve which is flatter. The valves are joined together by a ligament at one end of the oyster and by an adductor muscle which forms part of the oyster meat (Figure 1). Their function is to open and close the oyster, such that when the ligament is relaxed the two shells open but when the adductor contracts they close together.

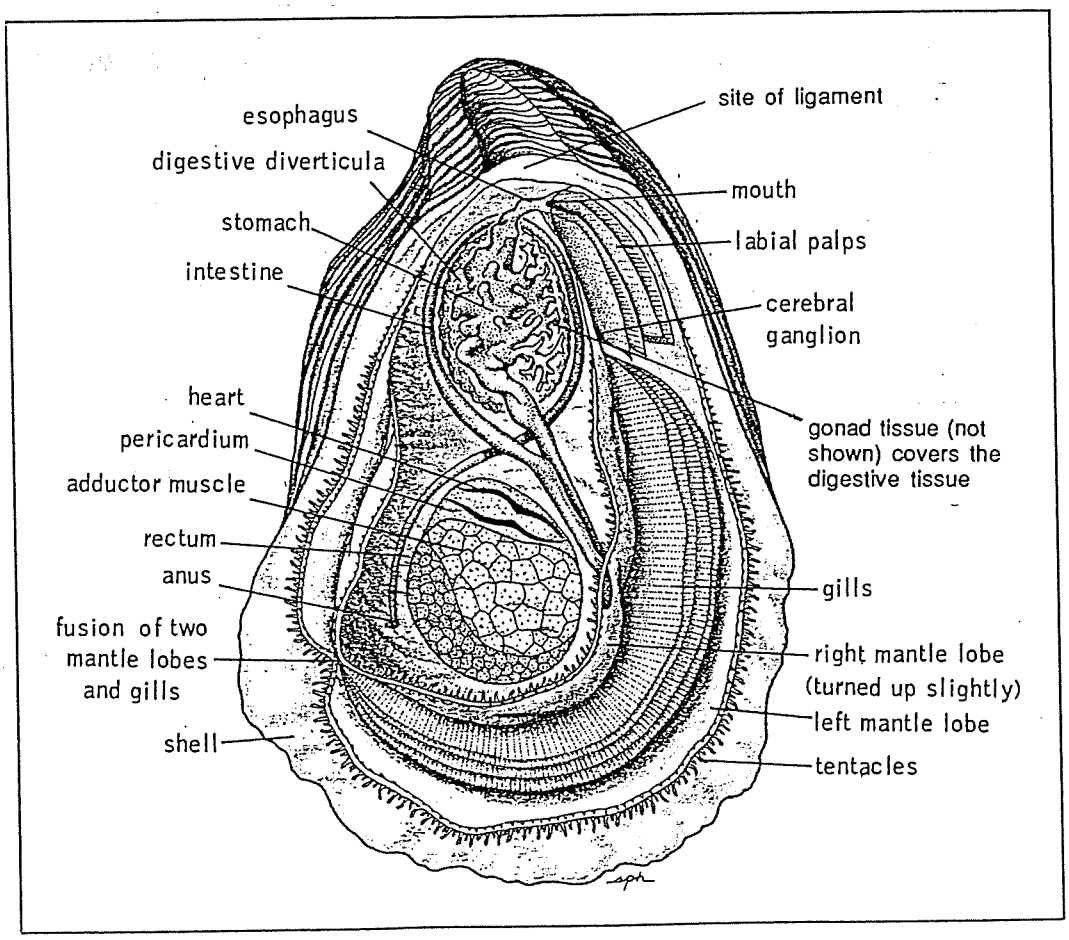

Figure 1. Anatomy of an oyster (taken from Barnes, 1980).

The mantle consists of two folds of tissue which encloses the rest of the oyster meat and its primary function is to secrete shell. The oyster shell consists of large amounts of calcium carbonate crystals held together by a matrix of protein. During growth periods the mantle extends beyond the shell border and the outer edge of the mantle lobe secretes the shell. The resulting shell growth is called shell-frill, which is at first fragile, but later becomes strengthened by further secretions from the mantle edge.

Generally, Pacific and flat oysters reach a market size of 65-75 mm in shell length in 2 years at favourable sites. Sydney rock oysters can take between 2-5 years to reach a size of 55-65 mm in length mainly because different husbandry methods are employed.

1.3.3 REPRODUCTION

The gonads or reproductive follicles lie over the digestive tissue and just under the surface epithelium (Figure 1). During the reproductive season, eggs and sperm are produced from glycogen stores surrounding the follicles and when these are ripe they are discharged through the gonoducts. Sydney rock and Pacifics oysters release eggs and sperm into the surrounding water (spawning) where fertilisation occurs. Native flat oysters, functioning as males, discharge sperm but the female oysters retain the eggs on their gills. Fertilisation of the eggs occurs within the female flat oyster and the larvae are kept on the gills for a period of about 10 days prior to their release (swarming).

The larval cycle begins with the fertilised egg which develops through a succession of phases to the final stage called a pediveliger (Figure 2). The larval cycle can last from 2-4 weeks, and during this time, an organ called a velum allows the larva to both swim and feed on phytoplankton in the water. The pediveliger is the final stage before metamorphosis or transformation of the free-swimming stage to a sedentary stage. When the larva is ready to metamorphose it sinks to the bottom and searches for a suitable substrate e.g. shell or rock. If it cannot find a suitable substrate it returns to the water column and tries again later. At metamorphosis the larva attaches itself to a substrate, the foot disappears and it becomes a juvenile oyster or spat.

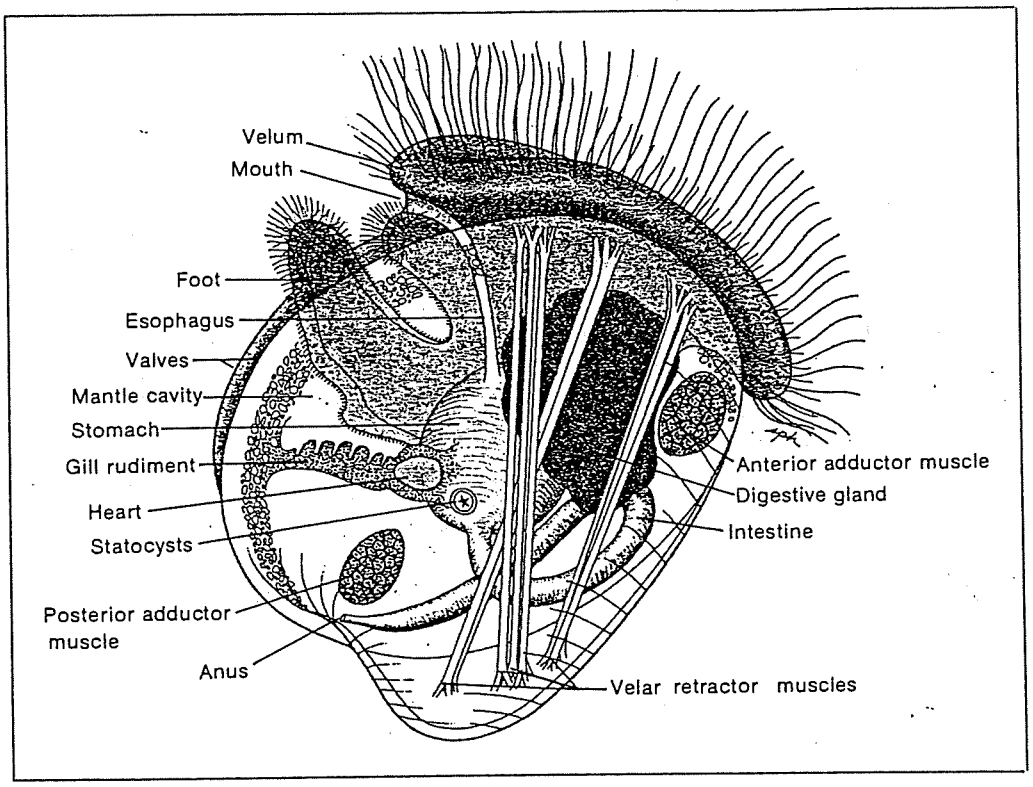

Figure 2. Anatomy of a pediveliger oyster larva (taken from Barnes, 1980)

2. MARKET REQUIREMENTS

Oysters are sold in Australia to retail fish shops, restaurants and hotels (Graham, 1991). The oysters are presented 'opened in the half shell' and therefore the appearance as well as the taste are important. The characteristics of a marketable oyster include:

- a marketable size e.g. 65-75 mm,
- a shell which is cupped and rounded in shape rather than being shallow and narrow,
- the meat fills the shell and is creamy and full in appearance,
- the meat should be flavour-some and without grit, and
- the live oyster should have a shelf-life out of water of at least one week.

The meat to shell ratio is one of the most important factors affecting the marketability of oysters and can be measured by the condition index:

$$\text{Condition index} = \frac{\text{Dry meat weight}}{\text{Internal shell volume}} \times 1000$$

The condition index varies throughout the year and is strongly correlated with the reproductive seasons. Oysters with condition index values greater than 80 are usually marketable. These oysters have full, creamy meats, prior to and during most of the reproductive season. Alternatively, oysters which have spawned have a grey and transparent appearance and are not marketable (condition index of much less than 80).

The condition index also varies widely between oyster species and sites and can be influenced by the husbandry methods employed (refer to Section 4). In Tasmania, Pacific oysters can be marketed for most of the year since the oysters generally do not spawn to completion, and may recover quite quickly after spawning. Alternatively in Victoria, flat oysters can be sold for only 6-8 months of the year, partly because the recovery period after spawning is quite long.

Most oysters sold to distributors and restaurants are sold live, which is why the shelf life of oysters is important. The oysters must survive out of water at temperatures and humidities experienced during transport and storage, usually for at least one week. The farmer can influence shelf-life of live oysters but if the distributor or marketer mistreats the oysters then they will open (gape) before they are sold.

3. SEED STOCK

Seedstock are obtained either from natural spat collection or from a hatchery. An advantage of buying hatchery/nursery stock is that the farmer has more control, over continuity of supply to the market, since spat can be bought throughout the year.

3.1 NATURAL SPATFALL

To catch natural spatfall farmers use materials called 'cultch' (also known as 'collectors'). The cultch may be oyster shells, sticks, plastic, bamboo or other materials which oyster larvae find attractive for settlement. The timing of collector placement in the water is crucial; if it is too early the collectors become fouled leaving few places for the larvae to attach to and if it is too late the farmer will have missed the spatfall season. Spatfalls can be irregular in timing and abundance although the Sydney rock industry has used this method of seed stock collection, mainly in Port Stephens (NSW), for several years.

Spat which are attached to large substrates such as tarred, hardwood sticks are called cultched (or attached) spat, since they are permanently attached to the substrate and cannot outgrow it. Alternatively, if seed caught by natural spatfall can be removed from the substrate then they are called cultchless (or unattached) spat. In New South Wales "oyster catchers" are used which are PVC guideposts ("mods"). The seed are easily removed by scraping the guideposts so that cultchless spat are obtained.

3.2 HATCHERIES

Hatcheries improve the condition index of broodstock oysters to the point that spawning can be achieved. The eggs are fertilised and the larvae are raised for 2-3 weeks, in large tanks, containing seawater and microalgae (cultured phytoplankton). Larvae approaching metamorphosis are provided with very small pieces of cultch such as ground up scallop or oyster shells which are the same size as the larvae (320-360 mm). After metamorphosis the young spat quickly outgrow the cultch (within a week) and become cultchless spat or single-seed oysters.

The spat are transferred to indoor upwelling nursery systems and fed microalgae. Upwellers are constructed from plastic cylinders e.g. PVC pipe and the spat are held in the vertical tubes by a nylon mesh base. Recirculating water, flows up through the spat and then through an out-flow pipe (mesh-covered to prevent loss of small spat) into a reservoir that contains the upwellers. The flow rates are adjusted so that the spat are partially suspended in the water-column; this allows large numbers to be held per upweller unit (O'Meley, 1992).

In Tasmania, Pacific oyster spat are held in these nursery systems until they have reached 3-4 mm in size. They are then sold to farmers in Tasmania and South Australia. The current price for spat with a shell length of 3-4 mm is $18.50/1000 spat. Added to this is a $1.00 research levy which goes to the Tasmanian Oyster Research Council (TORC) which is part of the Tasmanian Aquaculture Co-op Soc. Ltd. Contacts for suppliers of Pacific oyster spat in Tasmania are:

Shellfish Culture Ltd., P.O. Box 866, Bicheno, 7215. Ph. (003) 751188. FAX. (003) 751502.

Cameron of Tasmania Pty. Ltd., Main Road Dunalley, 7177. Ph. (002) 535111. FAX (002) 535278.

Marine Shellfish Hatcheries Pty. Ltd., P.O. Box 45, Bicheno, 7215. Ph. (003) 751412. FAX (003) 751488.

IGA Research Pty. Ltd., Binalong Bay Road, St Helens, 7216. Ph. (003) 761761. FAX (003) 761834.

Up to 50 % of the spat sold in Tasmania, are advanced spat that are produced by farmers who specialise in growing juvenile oysters for sale (Graham, 1991). Approximate prices for 10-15 mm spat are $48-50/1000 spat and for 40-50 mm spat $138-140/1000 spat. Major suppliers of these larger spat are:

Duck Bay Shellfish Pty. Ltd., Esplanade, West Smithton, 7330. Ph. (004) 522237.

Boulduans Bay Oysters Pty. Ltd., P.O. Box 84, Smithton, 7330. Ph. (004) 522262

The selling price, at the farm gate, for plate grade (suitable for serving in the half shell) Pacific oysters of 65-75 mm in length is $3.40-3.50/dozen.

4. SITE SELECTION

The best criterion of a suitable site is whether the oysters will grow and survive well at that site. For untried sites, small-scale, experimental trials should give the preliminary information within a year, so that the farmer will know whether both shell and meat growth are comparable to other successful areas. More conclusive trials could take up to two or three years which, for a potential farmer, may be too long. The environmental characteristics of the site in relation to the requirements of the species farmed will provide an initial basis for site selection.

4.1 TEMPERATURE

Seawater temperatures affect the growth and spawning activity of oysters although the extent to which these processes are affected, is largely dependent on the oyster species and the temperature range. Seasonal effects are most pronounced on the growth of flat oysters, native to the temperate regions of Australia where seawater temperatures range from 10-21^{0}C. During the spring and summer seasons, the shell growth rates are high whereas in winter, shell growth almost comes to a stand-still. Similarly the growth rates of Sydney rock oysters also exhibit a marked seasonal pattern. Alternatively the shell growth of Pacific oysters greater then 30 mm in length, in temperate regions, can increase relatively linearly throughout the year (Pers. comm. G.B. Maguire and C. Dyke, 1991).

Pacific oysters cultured in subtropical regions, where water temperature ranges of 10-27^{0}C are common, have high shell growth rates. This growth advantage, however, is offset by the fact that the oysters have an extended spawning season and 'spawn to completion', due to the high temperatures, so that the marketable period is shorter than for farms in the southern regions of Australia. For example, oysters that do not spawn completely, usually because temperatures do not exceed 20-21^{0}C, are able to recover quickly by resorbing unused eggs and sperm which are then converted into energy stores such as glycogen, so that market condition is quickly regained. A problem that many farmers in New South Wales face, because the water temperatures regularly exceed 21^{0}C, is that the larval numbers of Pacific oysters are huge and these compete with the Sydney rock oysters for settlement space on tarred sticks.

Intertidally grown oysters are exposed to the air for some period of the day, most days of the year. Air temperatures may be of concern, since for example, Sydney rock oysters suffer from heat stress which may cause mortality during summer. This problem can partly be overcome, by spraying the oysters with seawater or by selecting a lower growing height.

4.2 SALINITY

Pacific and Sydney rock oysters are mostly cultured in estuarine bays and inlets where the salinities can range from 25 parts per thousand (p.p.t.) to normal seawater salinity of 35 p.p.t. and higher. Occasional freshwater influxes from land run-off during rainy seasons can reduce the salinities down to 10-15 p.p.t. or lower. Since the oysters are osmoconformers they can tolerate these salinity fluctuations but in extreme situations e.g., below 5 p.p.t., they may resort to keeping their shells closed for several days until conditions improve. Native flat oysters, however, require salinity levels close to normal seawater salinity.

4.3 OTHER CONSIDERATIONS

Oysters have a huge filtering capacity of approximately 10 L per hour (i.e., for large oysters, 80-100 mm in length) and quickly strip the surrounding water of available food. Therefore, current flow is important as it provides a continuous supply of food particles to the oysters. The amount of wave action is another important consideration for intertidal, shore-based areas as too much wave action can slow the shell growth of the oysters or else damage the shell to such an extent that the meats are exposed to predators such as shell-boring molluscs.

There has been concern within the Tasmanian industry about the carrying capacity of estuaries, in which farms are already established. The carrying capacity of a water-body relates to the amount of food within the water. The concern is that, if established leases are expanded or new leases granted within an estuary, there may not be enough natural phytoplankton or other usable organic matter to support growth. Recent economic models indicate the profits for Tasmanian farmers could drop by two-thirds if Pacific oysters took up to three years rather than up to two years to reach marketable size (Treadwell, 1991).

The majority of oysters in Tasmania are grown in areas classified as clean-water areas and the farm lease sites are monitored by the Shellfish Quality Assurance Program (Department of Primary Industry). Where the water quality is doubtful on some farms, due to heavy rainfall and hence land run-off, the oysters are sold to farms in clean areas for on-growing. This ensures that the oysters are of the highest quality and safe for human consumption. In addition, this program has made possible export of Tasmanian shellfish to the United States of America, via a Memorandum of Understanding which is also recognised by many other countries. Similar programs have been initiated in Victoria and South Australia and more recently Western Australia.

In New South Wales, oysters by law, are depurated or purified prior to sale whether the lease site is clean or otherwise. The oysters are placed in a tank containing purified seawater for a period of 24 hours or more and, while the oysters are filtering the water, many of the bacteria and other microorganisms leave the digestive tract. This process ensures that the oysters are safe for human consumption, although it does not remove heavy metal contamination or a microorganism called *Vibrio vulnificus* which was implicated in poisoning some susceptible people in 1990. Unless the oysters are depurated other states cannot send live oysters to New South Wales markets (Graham, 1991).

5. HUSBANDRY METHODS FOR ATTACHED SPAT

Most of the world's oyster production is based on growing attached spat, on a variety of cultch materials, directly on the sea bed, or suspended below flotation units, or on horizontal, off-bottom, intertidal racks. However the husbandry methods that can be used for attached spat are fewer than for unattached spat. Below is a short description of the methods used for Sydney rock oysters in New South Wales by way of comparison to husbandry methods used for unattached spat.

"Crates" are constructed from tarred, hardwood sticks (1.8 m long x 25 mm^2) arranged in five to six vertical layers approximately 10 cm apart (the sticks are dipped in cold liquid tar to protect them from shipworm, *Teredo* spp.). During the Sydney rock spatfall season these crates are placed on intertidal wooden racks in spat collecting areas ("catching leases") located in the seaward end of estuaries. The spat caught are reared in this nursery system, designed to protect them from predators by the close-spacing of the sticks, for six to eight months. Large mortalities occur however on the outside of bundles exposed to predators such as fish and while the spat grow due to competition for space on the sticks.

After this period the bundles are separated in half and moved to "depot leases". These are areas upstream within the estuary and due to the lower salinity of the water "overcatch" is reduced (additional settlement of spat onto the sticks and juvenile oysters). The grow-out phase occurs on single-layer sticks arising from the further separation of the bundles. These are placed on racks, higher in the intertidal zone (refer to Section 6.2), into areas known as "growing" leases well up the estuary. After two or more years the oysters reach marketable size and condition. The sticks are loaded manually or by crane onto a punt and transported to shore-based facilities.

Clumps of oysters from the sticks are "culled" (broken apart) and sorted into three or more grades for the market. Labour costs for culling are high; it is hard work and large mortalities occur. The three grades include: "plate" grade (>55 mm) which sell for $3.60/dozen (farm gate); "bottle" grade (35-55 mm) which sell for $1.60/dozen (farm gate) and "spat" grade (20-40 mm). The plate grade oysters are depurated and then sold to restaurants to be served 'opened in the half-shell'. The bottle grade were traditionally used for bottling and sold to hotels and fish shops. Now these oysters are used as a plate grade oyster (in smorgasbords and bistros) and may encompass as much as 40-50% of the total supply of the plate-grade market (Pers. comm. B. Browne and G. Diemar, 1992). The spat grade which are too small to be sold, are returned to the lease and on-grown in tarred timber and wire or plastic mesh trays, for a futher 12-18 months in "finishing areas".

The major advantage of growing attached spat is that, during the grow-out phase, labour input can be very low until the oysters have to be culled ("the set and forget method"). One innovation which is attracting interest is the use of plastic sticks which are able to retain more oysters by the end of the grow-out phase. Hatcheries can also be used to provide pediveliger larvae which settle on the substrates to be used for growing attached spat.

6. HUSBANDRY METHODS FOR UNATTACHED SPAT

Although industries based on growing unattached oysters ("single-seed culture") already existed overseas, Tasmanian farmers developed many of their own techniques used for single-seed culture in Australia. Overseas farmers and experts have described the techniques as being extremely innovative and as a result are introducing these into their own countries. Some of the husbandry methods used include the type of grow-out unit, growing height or depth in the water column, grading and density manipulations.

6.1 NURSERY AND GROW-OUT UNITS

Nursery and grow-out units for unattached or single-seed culture include trays, cylinders, bags, and baskets (the last of these being constructed from folded, plastic mesh) and supported by treated wooden racks or else they are suspended below buoys. The mesh of single-seed units protect the oysters from predators whilst allowing water, containing food e.g., phytoplankton, to flow through the unit to the oysters.

The mesh, however, is prone to fouling by flora and fauna which reduce the amount of current flow to the oysters. Mesh enclosures used intertidally rather than subtidally, however, present fewer problems in terms of fouling due to a "drying-off" period for some time during the day. Other fouling control procedures are labour intensive and include cleaning the unit at sea using high-pressure water jets and/or transferral of the oysters to a clean unit. In most cases the oysters are transferred to units of a larger mesh size which not only increases the water flow but has less surface area on which the fouling organisms can grow. Note however, that it is important to keep the mesh size small, relative to that of the oysters, or else the oysters can grow into the mesh (or be lost through the mesh) and end up beak shaped rather then the rounded shape required for the market. Some units have been designed to help keep fouling to a minimum:

Stanway® cylinders: These are mesh cylinders suspended on racks in the intertidal zone, which rotate about 180° at each tidal change so that part of the cylinder is exposed to 'dry off' the fouling organisms.

Cylindrical units: These were designed in Victoria for the deep-water culture of flat oyster spat; they have brushes attached to the cylinder which remove fouling organisms during each tidal flow.

The units are designed for the particular lease site, whether it be intertidal or subtidal, and often it is the farmers that design and construct the grow-out units themselves. Since one of the major

capital costs involved in oyster farming is the grow-out units, they must be made with relatively cheap materials and be easy to construct so that labour costs are minimised. Well designed units are also durable so that they can withstand rough weather conditions and should be easy to move in and out of the water either manually or by machine.

In Tasmania, the types of single-seed units used for intertidal culture include, sectionalised trays to grow nursery spat and mesh baskets for intermediate and final grow-out of oysters:

Sectionalised trays: These are constructed from creosote-treated wooden frames (creosote treatment inhibits marine borers) to which 1.7 mm or 3 mm mesh is attached to the top and bottom of the frames. The mesh prevents predation of the small spat (3-20 mm) and also stops the spat from being washed out of the unit by wave action, while the internal sections help to prevent crowding into the corners of the tray which can reduce overall growth.

Basket units: These are constructed from rigid plastic mesh (6, 8, 12 or 20 mm mesh sizes) and are used for older stock. They are relatively inexpensive and easy to construct and consist of two mesh baskets through which two creosote-treated sticks are inserted. The units containing the oysters are placed on racks in the intertidal zone close to the foreshore.

Small oysters (20-40 mm) contained in open 6 mm mesh baskets are covered with bird mesh (light-weight 25 mm mesh) to help protect the spat from birds such as mollyhawks. Oysters greater than 30-40 mm are less prone to predation and are cultured in 12 mm baskets without bird mesh while oysters reaching marketable size (60-75 mm) are cultured in 20 mm baskets held high in the intertidal zone (Figure 3).

Harvesting of basket units is made easy by the fact that they can be handled by one person, many units can be stacked on a punt and the oysters can be emptied, simply by overturning the unit. Modifications of the design include baskets with high sides which can either fully or partially enclose the oysters in high wave-exposure sites and/or more baskets per unit in areas where the traditional rack supports are further apart.

Screen size (mm)	Spat size (mm)	Unit type (size - m^2)	Unit mesh size (mm)	Density /unit	Relative aerial exposure[a]	Time in unit (range)[b] (months)
2.8-3.5	3-4	Sectionalised tray (1.30)	1.7	80-100,000	Low	4 (2-7)
>4	6-15	"	3	20,000	Low	4 (2-7)[c]
>12	20-40	2 x Baskets (0.45)	6	530	Intermediate	3-4
>45[d]	50-60[e]	"	12	220	High	3
<45	30-40	"	12	220	Intermediate	3
>22.5	20-30	"	6	530	Low	3
>45[f] &	65-75[g]	"	20	130	High	2 (1-3)

a Rack height determines aerial exposure.
b Time in unit is seasonally dependent.
c Spat are graded 2-3 times while in 3 mm trays. Larger spat are moved to 6 mm mesh size.
d Hexagonal (chicken) wire mesh. Other screens are plastic or steel with square mesh.
e >70 % stay on 45 mm mesh. Sizes (30-60 mm) can be placed at varying levels in the intertidal zone to help ensure continuity of supply to the market.
f Hand-graded also.
g Finishing-off period for market-sized oysters.

Figure 3. Flow diagram showing culture operations at a Tasmanian Pacific oyster farm at a good site.

Other farms culture juvenile spat through to market-sized oysters in soft-mesh bags supported on rigid, plastic mesh which is nailed to wooden frames and supported by intertidal racks:

Soft-mesh bags: These are cheaper than mesh baskets and disposable. An advantage of bags, held in wooden frames, is that many of these units can be stacked on a punt, without 'squashing' the oysters. In Smithton (Tas.) these units can either be lifted by two people or by a forklift.

Or plastic trays may be used in subtidal leases:

Bread trays: These trays were designed to distribute bakery goods to milkbars and supermarkets but are now also used to on-grow 40-50 mm spat. The trays are suspended in groups below longlines, consisting of buoys held together by rope in subtidal areas (e.g., 2-10 m). They are sturdy but can quickly become fouled due to their large surface area.

In South Australia a novel method has been developed for sites exposed to large wave action:

PVC pipe: The pipe is open to the water via 2-3 mm slits in the sides and by mesh at each end. These are suspended in the intertidal zone on steel wire supports rather than timber racking.

The seabed?

The seabed: Oysters can be grown, unprotected on the seabed but these have a slower growth rate and higher mortality than oysters held in units, since they are exposed to predators (e.g., starfish and stingrays), fouling and can be covered by silt.

Another point that requires consideration, in terms of husbandry, is the effect these units have on the growth of the spat. For example, oysters grown in trays in calm-water areas, and left undisturbed, tend to grow long and fragile shells whereas those grown in cylinders or pipes have a more rounded shape and harder shell. The reason the cylinders produce a more rounded oyster is because the oysters are rumbled around inside the cylinder during each tidal cycle. This has the effect of removing shell-frill and hence overall length. Since part of the shell-frill is removed the oysters repair the damage but in so doing the edge of the shell becomes thicker and harder. This effect can be simulated for tray-grown oysters by handling them often so that some of the frill is removed (refer to Section 6.3).

6.2 GROWING-HEIGHT OR DEPTH

An advantage of intertidal sites is that the farming operations are usually located close to the shore and can easily be accessed and, if the substrate is firm, a tractor can be used to tow the punt out onto the lease during low tide. A disadvantage is that the tides dictate the times in the day or night that the farmer can work on the oysters. For example, it is not unusual for a farmer to be working in the early hours of the morning during winter in Tasmania. In contrast, subtidal farms can be worked at any time, except during poor or stormy weather but more substantial boats and equipment, to lift subtidal systems out of the water, are needed to work these farms particularly in exposed sites.

Oysters cultured in the intertidal zone are grown on racks and are exposed to the air for varying amounts of time each day. The time of exposure is dependent on the tidal cycle and weather patterns, for example, high pressure systems usually mean that the tide stays out for a longer period then predicted from the tide charts and in this case the farmer can work for a longer time on the lease.

Advantages of culturing oysters in the intertidal zone include an improved shelf life since the adductor muscle is strengthened and the shell growth rates can be regulated by culturing them at different intertidal heights. For example, in one study with Pacific oysters on racks differing in elevation by 30 cm, oysters which were continually submerged grew 62% faster than those exposed 28% of the time. However, the meat growth rate of the low-height group was only 11% better then for the high group (Pers. comm. G.B. Maguire, 1992). In the case of Pacific oysters, which are prolific growers, the ability to regulate their shell growth rates during all stages from spat to market is important, since the shell can 'outgrow' the meat. The height used is varied according to the size of the oyster (see Figure 3) and:

Small spat: of 3-15 mm shell length are grown on racks low in the intertidal zone so that aerial exposure is limited and shell growth rates are enhanced.

Intermediate spat: of 20-50 mm are cultured on racks which receive more aerial exposure so that the shell does not out-grow the meat; the shell is also more likely to become cup-shaped. Continuity of supply to the market can also be ensured by raising or lowering the oysters in the water and hence altering growth rates.

Large oysters: or close to market size (60-75 mm) are placed on racks with the highest amount of exposure in areas known to have high phytoplankton levels. This is done so that meat growth is encouraged, shell growth is slowed and to further improve the shelf life.

Some farmers 'finish off' Pacific oysters in subtidal culture sites where phytoplankton abundance may be higher, while other farms grow Pacific and Sydney rocks subtidally from spat through to market. The oysters have a faster growth rate so that time to market is shorter, however, there is a tendency for the shell of the oysters to become long and narrow compared to the market requirements of a rounded and cupped shell. In addition the shelf-life can be much shorter unless other measures are taken (Graham, 1991), such as grading and handling the oysters often so that the oysters 'learn' to stay closed while out of the water and so that some of the shell-frill is removed regularly. Alternatively subtidally grown oysters can be finished off on intertidal racks to improve their shelf life.

6.3 GRADING AND HANDLING

An advantage in growing single-seed oysters is that they can be graded into size groups as they grow. Small spat less than 15-20 mm in length are graded underwater, by hand-held or mechanised plastic-mesh sieves, to help limit shell damage. Larger oysters are graded using grading machines which operate by vibrating, steel or plastic, mesh sieves of varying sizes. Some farms grade their oysters by hand during grow-out while virtually all Tasmanian farms grade and check their oysters prior to market.

Grading is useful for farm management, since the farmer knows, for example, when baskets or trays of larger stock are likely to be ready for market. Oysters in Tasmania are graded 5-7 times during farm grow-out and it costs approximately 1 cent per juvenile or larger oyster per grading (Pers. comm. P. Chew, 1991).

When the oysters are graded and/or handled some of the shell-frill is removed. Some Tasmanian farmers deliberately use grading to break the frill of their oysters which has been shown by research (O'Meley and Maguire, unpubl., 1991) to improve the condition index and the shell-shape of the oysters.

Another management tool used by Tasmanian Pacific oyster farmers is "basket-shaking", where the baskets are shaken for less than a minute whilst out on the lease. This technique, like grading, improves the cupped shape by removing shell-frill. On two occasions, measurements were taken as part of (the above) research project, of oysters before and after basket shaking. The average reduction in length and width was 2-3 mm and 4-6 mm respectively after oysters had been growing undisturbed for 6 weeks.

6.4 DENSITY

The biomass (total weight) of oysters within a grow-out unit, will increase over time, as the oysters grow. Especially during seasons when high growth rates are typical, such as the summer season, the units quickly become overcrowded. Overcrowding leads to competition among the oysters for available food and this can limit growth; the incidence of poorly-shaped oysters also increases.

Density reduction is usually carried out in combination with routine grading of the oysters. As the oysters grow many more units are needed for the extra oysters. It is this near exponential increase, in the number of units required, which increases the costs of farming due to the materials and labour required to construct and manage them. The farmer must work out densities, for each size range of oysters, that will give good growth rates but still be profitable. Clearly if the density per unit is too low the venture will not be profitable. Typical densities used by Tasmanian farmers for Pacific oyster culture are shown in Figure 3.

6.5 SPATIAL VARIATION WITHIN LEASES

Farmers move oysters within a lease in relation to growing height. However a lease can exhibit other forms of internal variation, for example, in depth, current flow and likelihood that the seawater has already been filtered by a rack(s) of oysters before it reaches a specific rack. Herein lies two keys to successful oyster farming, i.e., observation and simple 'trial and error' movements within a lease to assess responses. This presumes that the farmer has a logical system of numbering racks, records oyster movements and regularly opens oysters to assess meat growth. While few Tasmanian farmers use computerised data bases, they do tend to have effective manual recording systems and do develop a good knowledge of the internal diversity and characteristics of their leases.

7. FUTURE DIRECTIONS

7.1 TRIPLOIDY AND SELECTIVE BREEDING

Triploidy is induced to ensure that animals or plants have three set of chromosomes per cell rather then the usual two. Triploid oysters, have reduced gonad formation during the reproductive season compared to diploid oysters, and the energy usually directed towards gonad development may be used instead, for tissue and shell growth, which should mean faster-growing oysters. A problem that hatcheries face however is that not all the oysters become triploid. This means that farmers will be wary of buying oysters unless the triploid success rate is close to 100%.

The advantage of triploid oysters is that they are much less likely to spawn to completion during the reproductive season, compared to diploid oysters. When diploids spawn the meat appears watery and the oysters may not be marketable for several months, until the oysters have recovered, whereas triploids may not develop outstanding meat condition but they generally maintain their meat condition throughout the reproductive season. Another advantage is that during their second summer on the oyster lease, triploids may grow 15-25% faster than diploids.

In the United States, oysters are considered unmarketable when their gonads are maturing prior to spawning. In contrast, consumers in Australia do not seem to discriminate against oysters which are in spawning condition. For export to the United States, triploid oysters may be desirable since these oysters have reduced gonadal development.

It is likely that triploid oysters will be produced commercially in Tasmania in the near future, following the completion of a successful research program. In the longer term selective breeding for growth and sensory characteristics of oysters is likely to be emphasised.

7.2 REMOTE SETTING

A technique called remote setting, used in America, may gain acceptance in Australia. The hatchery supplies the farmer with eyed-larvae (ready to set) and a concentrated algal paste (*Thalassiosira pseudonana*). The larvae are wrapped in a nylon cloth and damp paper towels, to prevent dehydration, and are transported in an insulated container at 5°C to the farmer (Jones and Jones, 1988). The farmers set the spat themselves by placing the larvae and algae into a tank (e.g. concrete) containing raw or filtered seawater and cultch material such as oyster, scallop or clam shells or plastic.

The larvae attach to the cultch within a short time (usually less than 2 days). Although large mortalities can result, the larvae are a lot cheaper than spat produced in hatcheries, since the hatchery/nursery does not have to pay for the costs of holding and feeding large numbers (and/or volumes of spat) in nursery systems. The attached spat usually are cultured directly on the seabed. Naturally, they are exposed to predators and get covered by silt, and large numbers are lost at this stage as well, but the method is economically viable, because only minimal maintenance is required.

8. ACKNOWLEDGEMENTS

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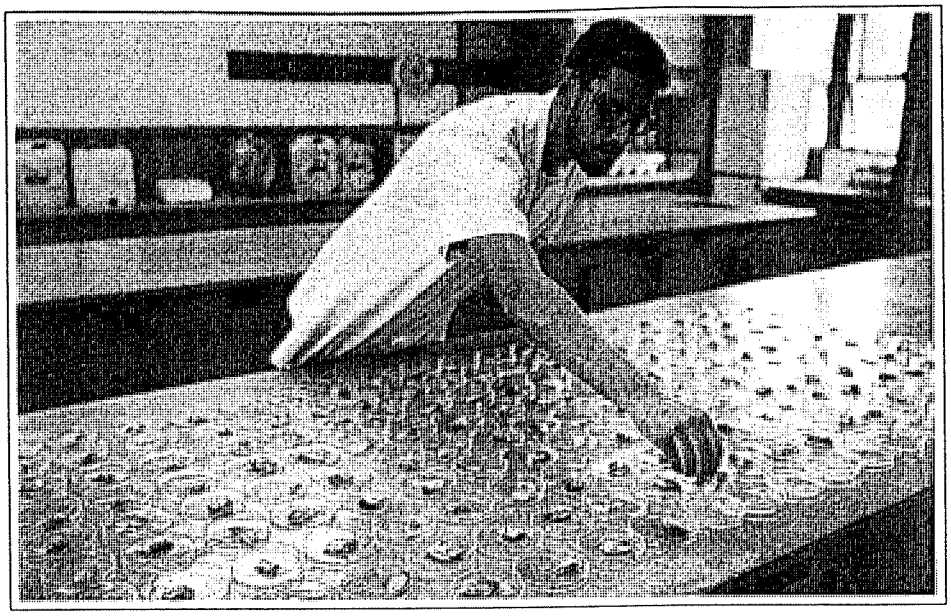
Aquaculture and Remote Data Collection



FROZEN triploid oysters being prepared for examination, Aquaculture Centre, Launceston. Remarkably simple but efficient techniques are used to gather data on conditions affecting their growth.

by Tony Ryan

UNDER the umbrella of the Department of Applied Science at Launceston, the National Key Centre for Teaching and Research in Aquaculture is flourishing.

One of its many areas of responsibility is the politically sensitive task of testing the effluent from pulp mills. Another is known as the Pacific Oyster Triploidy Program.

This research program, under Senior Lecturer Dr. Greg Maguire, is being funded by the Fishing Industry Research and Development Trust Fund to determine whether oysters grown under commercial farming conditions can benefit from an extra set of chromosomes – that is, from becoming 'triploid'.

Oysters lose condition when they spawn. Triploid oysters are less likely to spawn and therefore retain their condition and market appeal. They should be meatier and tastier. Of course all this, while simple in principle, can be quite complex in practice and the full consequences of triploidy are yet to be determined by sampling from three leases in Tasmania.

Results so far have indicated considerable variation amongst these groups of oysters and a system of remote monitoring has been set up on two of the leases, at Little Swanport and Pipeclay Lagoon, to measure water temperature and variations in the tide. Another sensor for salinity is expected to be added in the near future. Equipment for the two sites, from Wesdata, cost less than a thousand dollars.

Another person at the coalface, so to speak, is Technical Officer Greg Kent who collects and processes the data from the two sites. He confesses to have thoroughly gone off oysters – having opened 21,000 samples in the last year!

The remote monitoring system consists of two data loggers and two sensors (transducers) positioned, at different times, on the leases. The system allows the recording and storage of data for depth of overlying water and water temperature every 15 minutes. Data is down-loaded onto a small, hand-held field transfer unit and subsequently displayed on an IBM compatible PC.

Greg collects the data every month or so and stores it in an ancient IBM PC. Because so much data is collected each time he is having some trouble manipulating it into a spreadsheet. He has opted to use Lotus rather than Excel because that enables him to take work home and use his own PC which at least has a hard disc! The data for all other research in the Centre is processed in Excel on Macintoshes.

Because the sites are some distance off shore, collecting the data via a modem is not really an option. There has been some discussion about using a transmitter because, although the present collection rate suits the researchers, having the data in real time would be advantageous to the farmers. Greg is currently taking a course that he hopes will enable him to design the required transmitter.

Time will tell if the Centre's oysters are extra tasty (and if Greg Kent can ever face them again), but if the project is a success it will be remarkable – not least because of the simple and inexpensive computer technology it employed.

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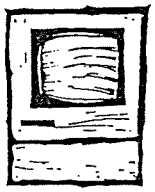

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