Brackish Water Fish Culture Research Station

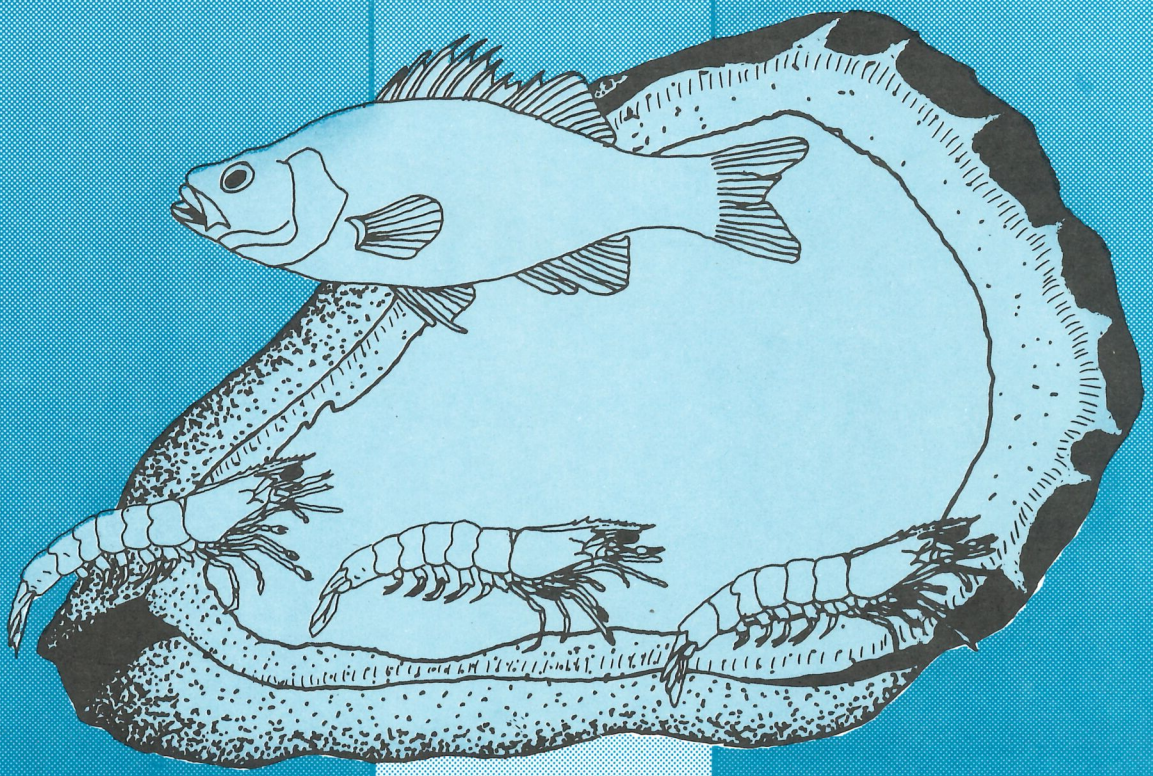
PACIFIC OYSTERS IN NEW SOUTH WALES

FINAL REPORT

TO

FRDC

JOHN A NELL AND CAROLINE J MASON



Department of Agriculture New South Wales

PACIFIC OYSTERS IN NEW SOUTH WALES

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FISHERIES RESEARCH AND DEVELOPMENT CORPORATION

FINAL REPORT (FIRTA 86/66; DAN11Z)

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BRACKISH WATER FISH CULTURE RESEARCH STATION
SALAMANDER BAY NSW 2301 (AUSTRALIA)

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SECTION 9 - APPENDICES

SECTION 1

INTRODUCTION

INTRODUCTION

Virtually all of the research in the orginal 'Pacific oysters in New South Wales' grant (FIRTA 86/66; DAN11Z) application (1986-1989) was carried out.

The major objectives of the grant were as follows:

a Determination of the optimum conditions for spawning, larval survival and spat settlement of the Pacific oyster currently present in Port Stephens.

b Assessment of the numbers and seasonal distribution of Pacific and Sydney rock oyster larvae in Port Stephens.

c Determination of the present distribution, abundance and age composition of Pacific oysters in the oyster growing areas of New South Wales.

d Establish if possible, by electrophoresis, the strain of the Pacific oyster now abundant in Port Stephens.

e Comparison of seasonal changes in gonad and meat condition of adult Pacific and Sydney rock oysters in Port Stephens.

f Monitoring of Sydney rock oyster spat put out on commercial leases to determine the potential for breeding QX and 'winter mortality' resistant oysters. These spat were produced at the Brackish Water Fish Culture Research Station by staff employed on the FIRT 81/2 grant.

The assessment of the numbers and seasonal distribution of Pacific and Sydney rock oyster larvae in Port Stephens, was carried out be Mr S McOrrie under his 'Oyster settlment and recruitment study' FIRDC grant (88/104). The proposed strain identification of the Pacific oyster in Port Stephens was not attempted because the Pacific oyster in Port Stephens was directly imported from Tasmania in 1983/84 (Ayres, 1990), making strain identification rather superfluous.

In addition to the major objectives some growth studies of Sydney rock oysters were carried out. All studies have been of great relevance to the NSW oyster industry and much of the information obtained will also benefit the oyster industries in other states.

The report is composed of separate manuscripts using the format required for 'Aquaculture', except if they were prepared for publication in other journals. Three manuscripts have been published and others are still being prepared for publication in refereed journals. Progress reports were presented to oyster farmers at the Brackish Water Fish Culture Research Station annual 'Open Days' in 1987, 88 and 89.

Reference

Ayres, P. A., 1990. Pacific oysters in New South Wales. Present and future implications for the NSW rock oyster industry. The Oyster Farmer's Association of Australia, Waitara, NSW, 59pp.

SECTION 2

COMPARATIVE BIOLOGY OF SYDNEY ROCK AND PACIFIC OYSTERS

COMPARATIVE GROWTH STUDY OF SYDNEY ROCK OYSTERS (SACCOSTREA COMMERCIALIS) AND PACIFIC OYSTERS (CRASSOSTREA GIGAS) IN PORT STEPHENS, NSW.

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ABSTRACT

In Port Stephens, NSW, Pacific oysters (Crassostrea gigas) grow at approximately twice the rate of Sydney rock oysters (Saccostrea commercialis) in both intertidal and deepwater culture. If not protected with a plastic shade mesh, small intertidally grown Pacific oyster spat may suffer a higher mortality rate from heat kill than Sydney rock oyster spat of the same size. Subtidally cultured Sydney rock oysters suffered a high incidence of mudworm (Polydora websteri) infestation.

INTRODUCTION

A heavy spatfall of Pacific oysters (Crassostrea gigas) in the inner harbour of Port Stephens occurred during the summer of 1984/85 (Holliday and Nell, 1985). Traditionally the Sydney rock oyster (Saccostrea commercialis) is grown intertidally in Port Stephens (Malcolm, 1987) and heavy spatfalls of the faster growing Pacific oyster may have a dramatic impact on this industry. Overcatch with Pacific oyster spat would increase the culling cost and make it less viable to grow Sydney rock oysters in Port Stephens. The objectives of the study were firstly to determine whether the Pacific oyster would survive and grow in Port Stephens and secondly to assess its growth rate and that of the indigenous species using deepwater and intertidal culture.

MATERIALS AND METHODS

Sydney rock and Pacific oyster spat were produced in the Research Station's hatchery in March, 1986 using an established technique (Walne, 1974; Holliday, 1985). The experiment was established with 1000 Sydney rock oyster spat (0.2g) and 1500 Pacific oyster spat (0.2g) per intertidal site (Karuah River, Big Swan Bay and Tanilba Point and per intertidal and deepwater site (Tilligerry Creek) in Port Stephens, NSW in August 1986 (Fig 1). Oysters were restocked on the trays every weighing time (Table 1) at the density recommended for maximum biomass gain (per tray) for Sydney rock oysters of 70% tray coverage for sectionalised trays (Holliday, et al., 1990). The experiment was commenced with one sectionalised tray (3mm mesh) per species per site. For better water flow oysters were restocked in trays with larger mesh (9mm) as they grew and additional trays were used as required. The trays used in deepwater culture were thoroughly

cleaned at every weighing to remove barnacles and filamentous algae. Because of the opposition by NSW oyster farmers to the proliferation of Pacific oysters, they were harvested before they reached commercial plate size (40g) to remove them from the pool of potential broodstock.

Salinity measurements were taken weekly at each site with a salinity dip meter (inductive coupled cell sensor, model 605; Yeo-Kal Electronics Pty Ltd, Brookvale, NSW, 2100) at 0.3m below the surface. Water temperatures were taken at the same time with a thermometer.

RESULTS

The Pacific oysters grew much faster than the Sydney rock oysters (Tables 1 and 2; Fig 2) with deepwater and intertidally cultured Pacific oysters requiring 13 and 24 months respectively (Table 2) to reach a small plate size (>35g). Pacific oysters grew faster intertidally than Sydney rock oysters grown in deepwater culture (Table 2). The majority of the Sydney rock oysters grown in deepwater were infested with mudworm (Polydora websteri) between 24 and 28 months. Most of the Pacific oysters reached market size (Tables 1 and 2) before the mudworm struck but those remaining were also infested. After 33 months growth in intertidal culture (Table 2) the Sydney rock oysters (25±5g) were still approximately 12 months from reaching a small plate size (>35g) and the time taken to reach market size was not determined. Growth rates [$\log_{10}$ (10 x whole weight of oysters in grams)] of oysters of both species grown in either deepwater culture or at rack height began to level off after 6 months (February, 1987; Fig 2).

Mortality rates of the Pacific oysters were higher than those of the Sydney rock oysters (Table 1), but the differences were not significant ($P>0.05$). The between-site variations in mortality rates for Pacific oysters were very high, as indicated by the high standard deviations (Table 1).

There was little variation in water temperature (n=132) and salinity (n=132) between sites; 21±4°C, 28±8‰, ranges 13-28°C, 0-36‰ Karuah River; 21±4°C, 30±5‰, ranges 12-28°C, 7-39‰ Big Swan Bay; 21±4°C, 31±5‰, ranges 12-28°C, 9-39‰ Tanilba Point; 21±4°C, 29±5‰, ranges 11-30°C, 8-38‰ Tilligerry Creek (intertidal site); 21±4°C, 29±5‰, ranges 12-29°C, 0-38‰ Tilligerry Creek (deepwater site). At any site salinity levels <15‰ did not last longer than 2 weeks.

DISCUSSION

This study clearly demonstrated that Pacific oysters can be successfully grown on trays in Port Stephens and that they reach market size in approximately half the time of that of Sydney rock oysters. In Port Stehens, deepwater culture increased the growth rates of oysters of both species as compared to those grown intertidally. Pacific oyster grown in deepwater culture in the Tamar River, Tasmania (Sumner, 1981) also grew faster than intertidally grown oysters. The

mortality of small Pacific oysters during their first summer was probably caused by heat stress. The large variations in the mortality of Pacific oysters between sites may have been caused by differences in air temperature on very hot days. However, to avoid heat kill of small spat (<5g), 3mm mesh tray lids should be used for shade on intertidal racks. This study showed that Pacific oysters can be successfully grown in Port Stephens as single seed oysters on trays at the conventional rack height for Sydney rock oysters. Pacific oysters have also been successfully grown to harvest size on sticks in Port Stephens (J. A. Nell, personal observation, 1988) and in the Tamar River in Tasmania (Sumner, 1980).

Both Sydney rock and Pacific oysters in deepwater culture became infested with mudworm. This parasite has detrimentally affected the NSW oyster industry for a long time (Nell and Smith, 1988). Pacific oysters in New Zealand also suffer from mudworm infestation (Curtin, 1986). Oysters in deepwater culture should be regularly lifted out of water to be washed and left to dry for a few days to avoid mudworm infestation (Skeel, 1979), although this treatment should be applied with caution to Pacific oysters, as they do not survive for as long out of water as Sydney rock oysters (Mason and Nell, 1991).

Both Pacific and Sydney rock oysters tolerated a wide range of salinities (0-39‰) and water temperatures (11-30°C) and survived in freshwater (0‰) and low salinity water (<15‰) for short periods of time during the course of this study. Pacific oysters however, have a wider salinity tolerance range than Sydney rock oysters (Nell and Gibbs, 1986; Nell and Holliday, 1988).

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TABLE 1

Comparative study of growth[1] and mortality[1] of Sydney rock oysters (Saccostrea commercialis) and Pacific oysters (Crassostrea gigas) in Port Stephens NSW (August 1986 - May 1989).

Period		Cultivation[2]	Oyster weights			Cumulative oyster mortality		
			Pacific (g)	Sydney rock (g)		Pacific (%)	Sydney rock (%)	
August	1986	Intertidal (4)	0.2	0.2		no data	no data	
		Deepwater (1)	0.2	0.2				
November	1986	Intertidal (4)	2.2±1.1	1.3±0.4	NS	17.6±17.6	10.0±3.3	NS
		Deepwater (1)	7.8	1.8		2.0	0.0	
February	1987	Intertidal (4)	4.9±1.2	3.4±0.8	NS	23.5±24.0	11.4±4.1	NS
		Deepwater (1)	14.7	5.2		6.8	0.6	
May	1987	Intertidal (4)	9.3±2.3	5.1±1.1	*	26.8±25.4	12.4±4.5	NS
		Deepwater (1)	26.1	9.8		9.2	1.7	
September	1987	Intertidal (4)	15.5±2.3	7.4±1.3	*	27.0±25.6	12.6±4.6	NS
		Deepwater (1)	43.1	14.2		9.3	2.6	
December	1987	Intertidal (4)	19.7±3.9	10.7±2.3	*	27.3±25.5	13.2±4.7	NS
		Deepwater (1)	harvested	21.4		harvested	7.7	
April	1988	Intertidal (4)	26.0±2.5	13.2±2.6	*	27.8±25.4	14.1±4.1	NS
		Deepwater (1)	harvested	25.6		harvested	14.8	
August	1988	Intertidal (4)	38.0±5.6	17.5±4.5	*	29.0±24.7	16.3±5.6	NS
		Deepwater (1)	harvested	27.9		harvested	24.1	
December	1988	Intertidal (4)	harvested	22.0±2.8		harvested	17.6±6.5	
		Deepwater (1)	harvested	terminated		harvested	terminated	
May	1989	Intertidal (4)	harvested	25.1±5.0		harvested	19.6±4.9	
		Deepwater (1)	harvested	terminated		harvested	terminated	

1 Data presented as mean $\pm$ S.D.

2 Number of sites per cultivation type is shown in brackets.
The statistical comparison of oyster weights and cumulative mortalities between oyster species for the intertidal cultivation for each weighing time is indicated [NS = no significant difference ($P>0.05$); * = significant different ($P<0.05$)]. An arcsin $x^{0.5}$ transformation was applied to the mortality data prior to statistical analyses. There were 1500 Pacific and 1000 Sydney rock oyster spat stocked on trays at each location at the start of the experiment.

3 Treatment terminated because of mudworm infestation

TABLE 2

Growth rates[1] of Sydney rock oysters (Saccostrea commercialis) and Pacific oysters (Crassostrea gigas) in Port Stephens NSW, from August 1986 - May 1988.

Cultivation[2]	Oyster Weights (g) 13 months	24 months	28 months	33 months
SYDNEY ROCK OYSTERS				
Rack height(4)	7±1 (13±5)	18±5 (16±6)	22±3 (18±7)	25±5 (20±5)
Deepwater(1)	14 (3)	28 (24)	terminated[3]	
PACIFIC OYSTERS				
Rack height(4)	16±2 (27±26)	38±6 (29±25)	harvested	
Deepwater(1)	43 (9)	harvested		

1 Data are expressed as mean ± S.D. The percentage cumulative mortality is shown in brackets. The experiment was commenced with 1000 Sydney rock and 1500 Pacific oyster spat per site. The average initial weight of spat of both species was 0.2g. The number of months indicate the length of the experimental period, not the age of the oysters.

2 Number of sites under cultivation is shown in brackets.

3 Treatment terminated because of mudworm infestation.

Fig 1. Location of experimental oyster cultivation sites (Karuah River, Big Swan Bay, Tanilba Point and Tilligerry Creek) in Port Stephens NSW, from August 1986 - May 1989.

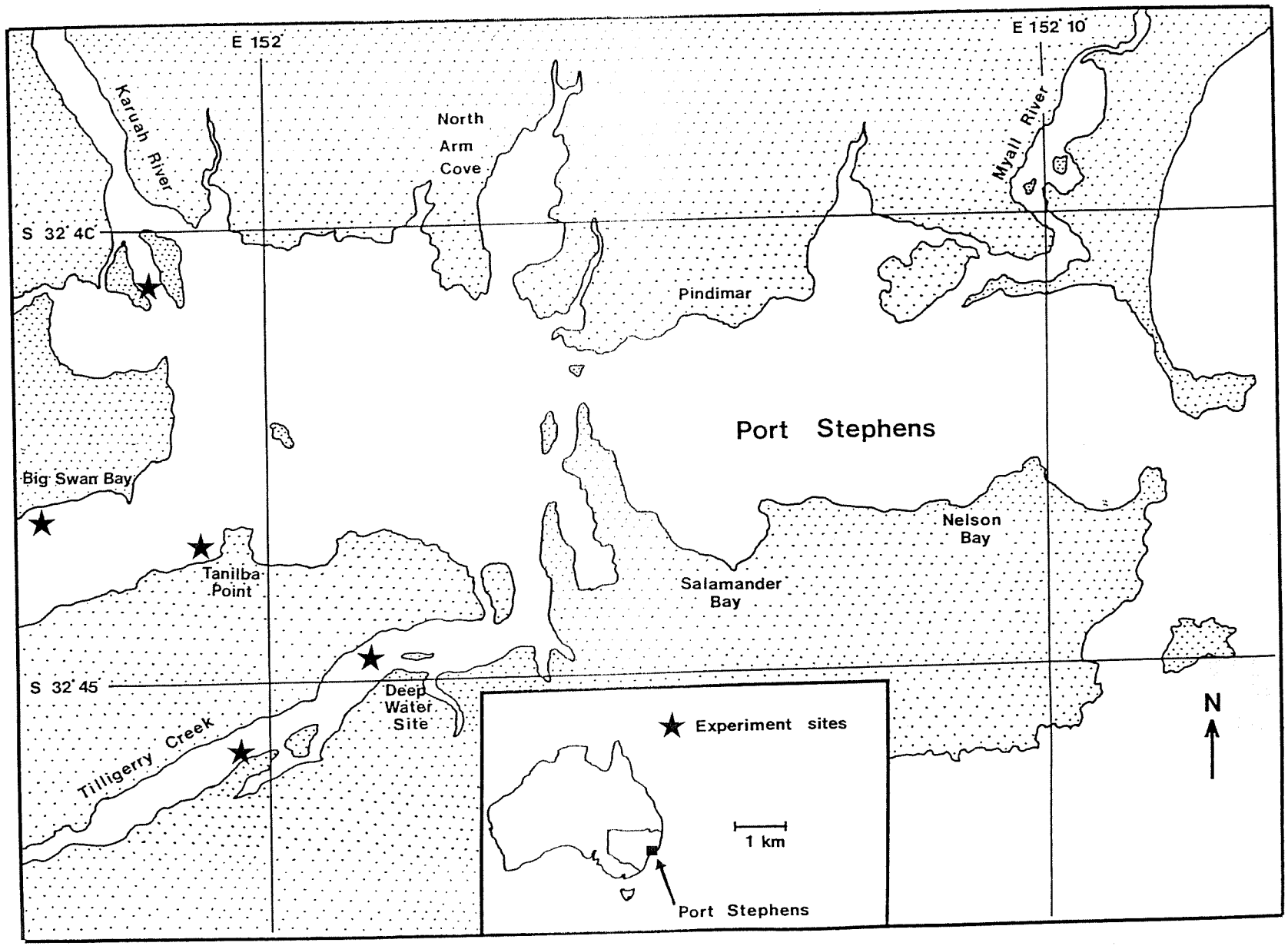
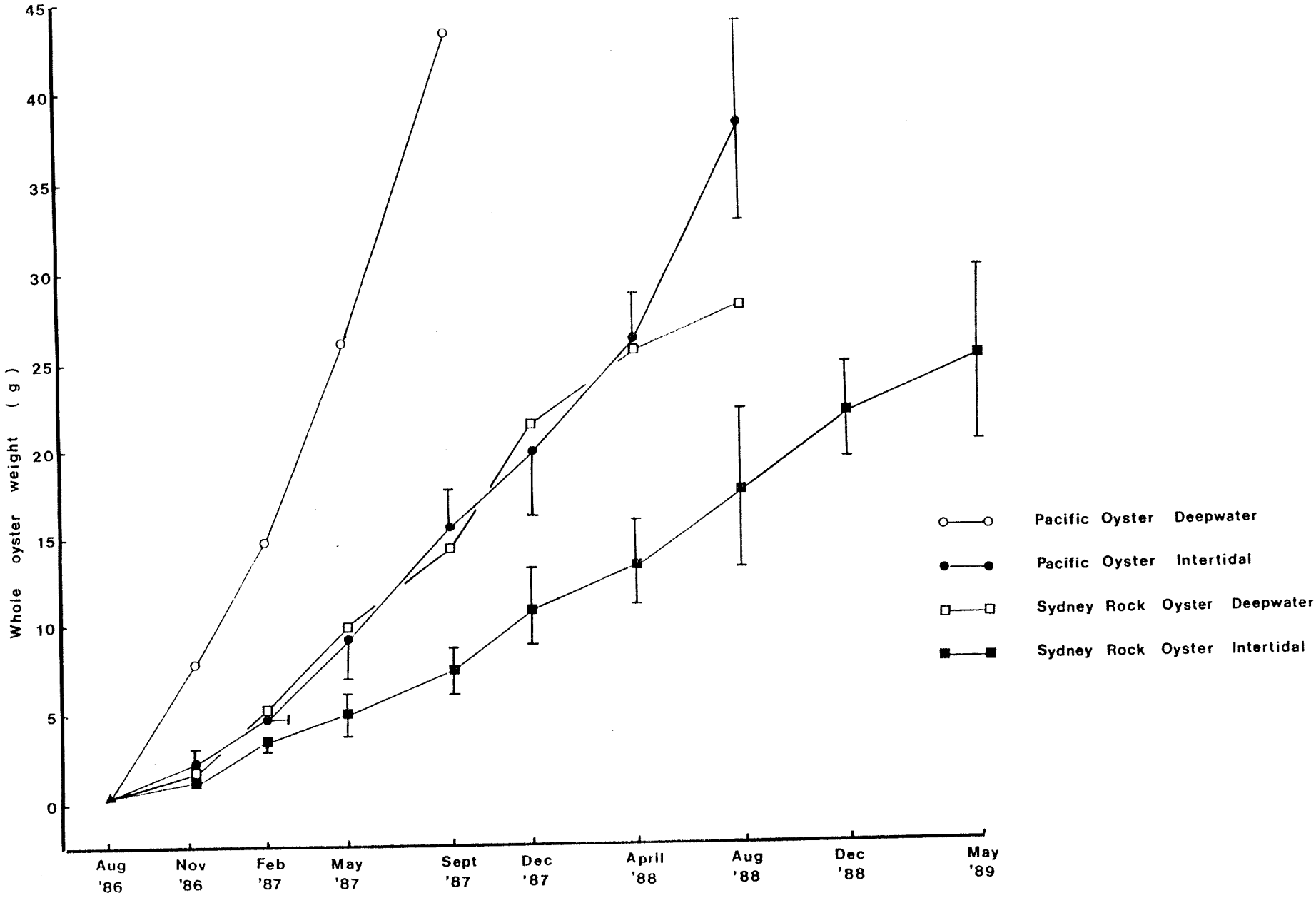
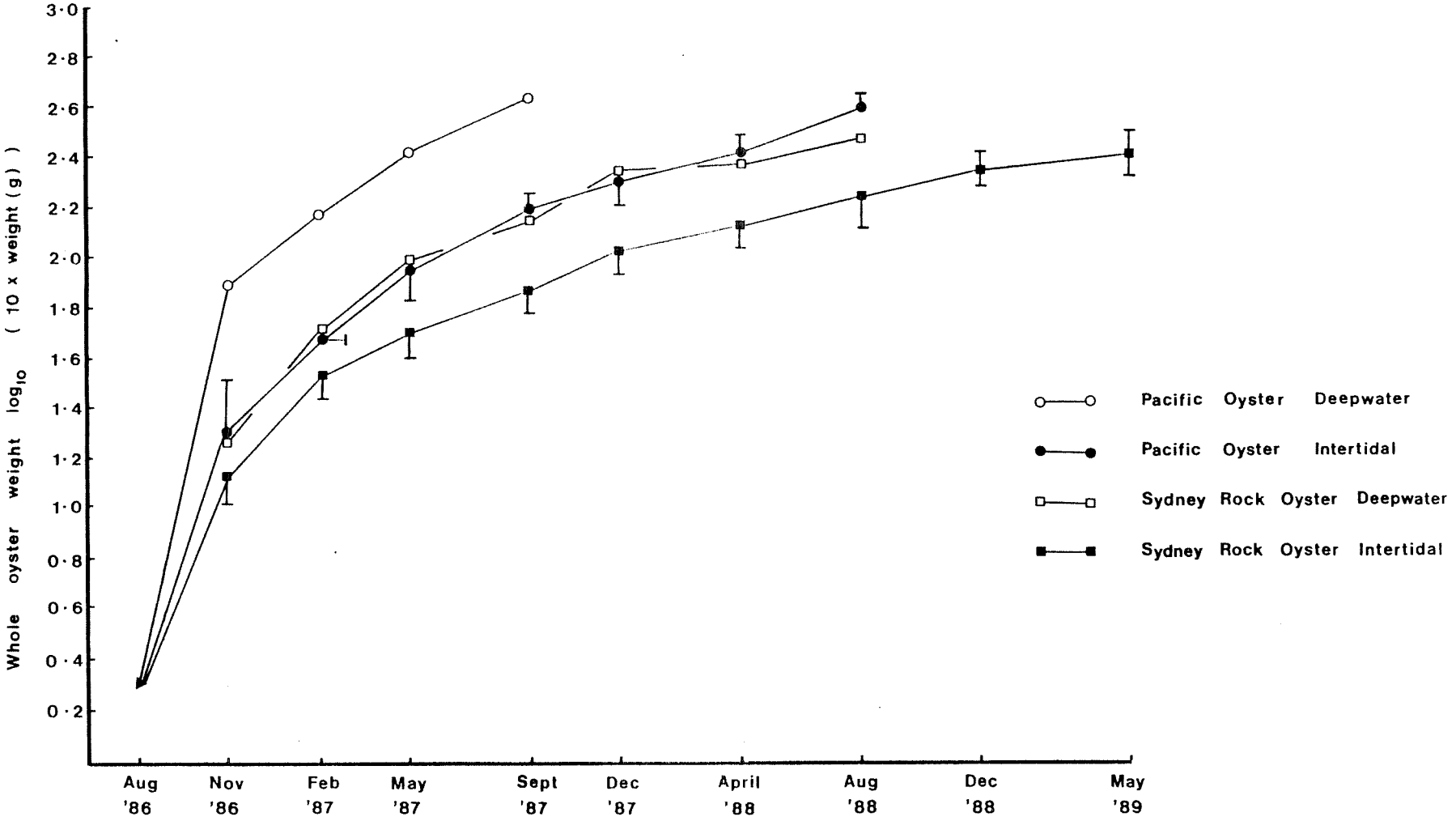

E 152
E 152 10
Karuah River
North
Arm
Cove
Myall River
S 32 4C
Pindimar
Port Stephens
Big Swan Bay
Tanilba
Point
Nelson
Bay
Salamander
Bay
S 32 45
Deep
Water
Site
Tilligerry Creek
Experiment sites
1 km
Port Stephens
N

Fig 2. Comparative growth of Sydney rock oysters (Saccostrea commercialis) and Pacific oysters (Crassostrea gigas) at intertidal rack height (4 sites) or in deepwater culture (1 site) in Port Stephens NSW, from August 1986 - May 1989. Mean ± SD.

a. whole oyster weight
b. whole oyster weight $\log_{10}$ (10 x weight (g))


Whole oyster weight (g)
45
40
35
30
25
20
15
10
5
0
Aug '86
Nov '86
Feb '87
May '87
Sept '87
Dec '87
April '88
Aug '88
Dec '88
May '89
Pacific Oyster Deepwater
Pacific Oyster Intertidal
Sydney Rock Oyster Deepwater
Sydney Rock Oyster Intertidal


Whole oyster weight log10 (10 x weight (g))
3·0
2·8
2·6
2·4
2·2
2·0
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
Aug '86
Nov '86
Feb '87
May '87
Sept '87
Dec '87
April '88
Aug '88
Dec '88
May '89
Pacific Oyster Deepwater
Pacific Oyster Intertidal
Sydney Rock Oyster Deepwater
Sydney Rock Oyster Intertidal

COMPARISON OF SHELL DENSITY, PERCENTAGE CAVITY VOLUME AND SHELL WEIGHT OF SYDNEY ROCK (Saccostrea commercialis) AND PACIFIC OYSTERS (Crassostrea gigas)

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ABSTRACT

Adult Pacific oysters (Crassostrea gigas) had a larger shell cavity volume expressed as a percentage of whole oyster volume (56 and 49%) than adult Sydney rock oysters (Saccostrea commercialis; 40 and 36% for single seed and stick oysters respectively). Pacific oysters contained less shell expressed as a percentage of whole oyster weight (63 and 65%) than Sydney rock oysters (73 and 77% for single seed and stick oysters respectively).

INTRODUCTION

Pacific oysters (Crassostrea gigas) have become well established in Port Stephens, NSW since they were first found in large numbers in November 1984 (Holliday and Nell, 1985). As a result of the sudden population explosion of Pacific oysters in Port Stephens, growth studies were conducted. Weight measurements of oysters of both species cultivated either as single seed (Holliday et al., 1988) or stick (Malcolm, 1987) oysters were taken to make physiological comparisons of oysters.

MATERIALS AND METHODS

Both Sydney rock (Saccostrea commercialis) and Pacific oysters were cultivated under intertidal conditions in Port Stephens, NSW as either single seed or stick oysters from August 1986 to May 1989. All oysters (>35g) were removed from seawater immediately before measurements were taken to ensure that all shell cavities were filled with seawater. Oysters and shells were wiped dry before weighing. All weight measurements of whole oysters and shells were taken in air unless stated otherwise. The percentage cavity volume and shell weight were calculated as shown below.

$$\text{Cavity Volume (\%)} = \frac{\text{Cavity Volume (mL)} \times 100}{\text{Whole oyster volume (ml)}}$$

$$\text{Shell Weight (\%)} = \frac{\text{Shell weight (g)} \times 100}{\text{Whole oyster weight (g)}}$$

$$\text{Cavity Volume (ml)} = \text{Whole oyster weight (g)} - \text{Weight of shell (g)}$$

The cavity volume calculation of oysters (Lawrence and Scott, 1982) is based on the principle that the density of the cavity contents (meat and mantle fluid) is approximately 1g/mL and therefore can be measured as whole weight of oysters (g) minus shell weight (g). The shell volume calculation is based on the principle that the shell volume (mL) is equal to the volume of water displaced when shells are weighed suspended in water (the density of water is 1g/mL).

Shell weight (g) in water = shell weight (g) in air - weight (g) of volume of water displaced

Shell volume (mL) = shell weight (g) in air - shell weight (g) in water

Oyster volume (mL) = shell volume (mL) + cavity volume (mL)

Shell density was calculated as shown below:

$$\text{Shell density (g/mL)} = \frac{\text{shell weight (g)}}{\text{shell volume (mL)}}$$

RESULTS

Pacific oysters had a larger shell cavity volume (Table 1) expressed as a percentage of whole oyster volume (56 and 49%) than Sydney rock oysters (40 and 36% for single seed and stick oysters respectively). Pacific oysters contained less shell (Table 1) expressed as a percentage of whole oyster weight (63 and 65%) than Sydney rock oysters (73 and 77% for single seed and stick oysters respectively). The average shell densities (Table 1) of Pacific oysters (2.2 and 2.1g/mL) was higher than that of the Sydney rock oysters (1.7 and 1.8g/mL for single seed and stick oysters respectively).

DISCUSSION

Single seed Pacific oysters have a 40% larger cavity volume expressed as a percentage of whole oyster volume than single seed Sydney rock oysters. This means that a bag of single seed Pacific oysters (plate size) should contain 40% more cavity volume than a bag of single seed Sydney rock oysters (plate size). If oyster of both species are in a similar condition, the bag of Pacific oysters should contain 40% more meat than the bag of Sydney rock oysters. Similarly a bag of single seed Sydney rock oysters (plate size) should contain 10% more meat than a bag of stick oysters of the same species in the same condition. If oysters are sold by weight instead of volume similar principles apply, because of the differences in the percentage shell weight of oysters of the two species and methods of cultivation.

The differences in the shell density between oysters from the different cultivation methods could not be explained and may be an artefact of the measurement procedure.

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TABLE 1

Comparison[1] of shell density, percentage cavity volume and shell weight of Sydney rock oysters[2] (*Saccostrea commercialis*) and Pacific oysters[2] (*Crassostrea gigas*)

Species	Cultivation	Number of oysters measured	Cavity Volume[3] (%)	Shell weight[4] (%)	Shell density (g/mL)
Sydney rock oyster	single seed	185	40 ± 5^{b}	73 ± 4^{c}	1.7 ± 0.1^{a}
Sydney rock oyster	stick	179	36 ± 4^{a}	77 ± 3^{d}	1.8 ± 0.2^{b}
Pacific oyster	single seed	220	56 ± 6^{d}	63 ± 6^{a}	2.2 ± 0.2^{d}
Pacific oyster	stick	201	49 ± 9^{c}	65 ± 5^{b}	2.1 ± 0.4^{c}

1 Mean $\pm$ S.D. within columns, mean with a common superscript do not differ significantly ($P>0.01$).

2 All oysters (weight range 25-76g) were grown intertidally in Port Stephens, NSW from August, 1986 - May, 1989.

3 Expressed as a percentage of whole oyster volume.

4 Expressed as a percentage of whole oyster weight.

SEASONAL VARIATION IN MEAT AND GONAD CONDITION OF SYDNEY ROCK OYSTERS (*Saccostrea commercialis*) AND PACIFIC OYSTERS (*Crassostrea gigas*) IN PORT STEPHENS, NSW

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ABSTRACT

Adult Sydney rock oysters (*Saccostrea commercialis*) and Pacific oysters (*Crassostrea gigas*) were kept on commercial oyster leases at three sites in Port Stephens, NSW, and on an experimental raft at a fourth site between July 1988 and September 1989. Oysters were sampled from each site at approximately monthly intervals for chemical and histological (discussed elsewhere) analysis.

The dry condition index and percentage glycogen of the Pacific oysters was higher than that of the Sydney rock oysters during the winter and spring, but tended to be lower during summer and autumn. The gonads of the Pacific oysters matured two months earlier than the Sydney rock oysters, and they apparently spawned in October 1988 at all sites. The Sydney rock oysters spawned later, during December 1988 - January 1989, and did not lose as much condition as the Pacific oysters in the period immediately after spawning. The amount of glycogen in the meats of both species dropped at the expense of protein and lipid as the oysters became fully ripe.

It is considered likely that the residue of the dry meat weight that cannot be accounted for as glycogen, protein or lipid represents refractory gut contents.

For both species, the overall condition of the oysters was best at the raft site, although both spcies were badly affected by mudworm, and poorest at the Karuah site, which is strongly affected by fresh water runoff after rain. The highest dry condition indices were found in Sydney rock oysters at the Corrie Island site, which was the site most affected by oceanic water from the heads of Port Stephens.

INTRODUCTION

As has been described elsewhere (Mason and Nell, 1991, this volume) Pacific oysters (*Crassostrea gigas*) first appeared in large numbers in Port Stephens, NSW, in the summer of

1984-85. This fast-growing introduced species threatened the cultivation of the native Sydney rock oyster (*Saccostrea commercialis*), and was declared a noxious fish by the then NSW Department of Agriculture. The experiment described herein was carried out to provide a baseline study of the gonad maturation and fattening of each species in four different growing environments within Port Stephens, over 13 months from late July 1988 to mid September 1989.

Roughley (1933) in his treatise on the life history of the Sydney rock oyster (called *Ostrea commercialis* at the time), described the stages of development of male and female gonads of Sydney rock oysters from histological sections. His specimens were derived from estuaries from the whole length of the NSW coastline, however, and apart from generalisations about summer and winter condition, he did not link the gonad condition of the oysters to the time of the year in any more detail. He did remark that the oysters often spawned on the spring (full moon and new moon) tides of the summer months, which has been confirmed in Port Stephens (C. J. Mason, unpublished data, 1992). Dinamani (1973) described seasonal changes in the gonads of the New Zealand rock oyster, *Saccostrea glomerata* (which is considered to be a sibling species to the Sydney rock oyster, if not conspecific; Buroker et al, 1979).

The simplest measure of meat condition is based on the assumption that the the cavity enclosed by the oysters shell represents the largest possible size that that oyster could attain. The ratio of the meat volume (in practice, meat weight) to cavity volume is the wet meat condition index (Wet CI). Since the proportion of water in the meat varies widely according to season and condition, and even between individual oysters, the dry conditon index (Dry CI), which is the ratio of the dry meat weight to cavity volume (multiplied by a constant) is used more often to describe the condition of oysters; the constant may be 100 (Haven, 1960; Soniat and Ray, 1985) or 1000 (Gabbott, 1974; Walne, 1970; Whyte and Englar, 1982). An alternative measure of dry meat condition, the ratio between the dry weight of the meat and the dry weight of the shell valves (Shell condition index), has been used by some authors (Gabbott and Walker, 1971; Littlewood and Gordon, 1988; Walne and Mann, 1975). The two methods for determining dry meat condition were compared by Brown and Hartwick (1988), who concluded that the first method (Dry CI) was "primarily correlated to whole weight and secondarily to water temperature, ... and chlorophyll *a* concentration" whereas the Shell condition index was "correlated to the same environmental factors, but not whole weight".

A number of authors (for instance: Littlewood and Gordon, 1988; Walne, 1970; Whyte and Englar, 1982) have looked at the change in chemical composition of oysters with time. Glycogen is the major storage material in bivalve molluscs (Gabbott, 1975), and the proportion of glycogen in oyster

tissues varies strikingly according to season; it is at its greatest in early summer, before the oysters start to develop their gonads, then drops as gonads develop. Once spawning has been completed, the oysters start to build up their glycogen reserves before the winter (Gabbott, 1975; Gabbott and Walker, 1971; Humphrey, 1941; Mann, 1979 Perdue and Erikson. 1984).

Apart from the importance of glycogen as a storage material in oysters, a high level of glycogen is also responsible for the qualities of creamy white mantle, smooth texture and sweet taste of "fat" oysters (Stanley et al, 1981). In the USA and Europe, Pacific oysters and Flat oysters (*Ostrea edulis*) are considered to be in the best condition for eating when they have not yet started to develop their gametes, and are still glycogen-rich; mature Pacific oysters are considered to be unpalatable (K. K. Chew, pers. comm. 1989). In Australia however, the local taste for the Sydney rock oysters is an oyster with mature gonads (Wisely et al, 1979), thus in Australia, oysters are considered to be "fat" in all stages of sexual maturity from early gamete development to just prior to spawning.

Materials and Methods

Location of experimental sites

Four sites in Port Stephens, NSW, Australia (32° 45'S, 152° 04'E) (Fig. 1) were selected as being representative of a particular type of growing environment.

Site 1 was an experimental raft anchored in Tilligerry Creek (Fig. 1). The oysters were kept on trays suspended under the raft 0.4m below the surface of the water. Tilligerry Creek is affected by run-off from the cachement area, which is a mixture of farmland and low-density residential areas, and the salinity may drop below 15 gL^{-1} for several weeks after heavy rain. It is considered by local oyster farmers to be a good fattening area.

Site 2 was an intertidal growing lease (Malcolm, 1988) in Tilligerry Creek (Fig. 1). The oyster trays were submerged by the tide for approximately 70% of the time (I. R. Smith, unpublished data, 1989).

Site 3 was a catching lease (Malcolm, 1988) at Corrie Island, Port Stephens (Fig. 1). This site is strongly influenced by oceanic water from the heads of Port Stephens, except after heavy rain, when fresh water from the Myall lake system dominates (S. McOrrie pers. comm., 1990).

Site 4 was a depot lease (Malcolm, 1988) in the Karuah River, Port Stephens (Fig. 1). Silt levels are high, and the

oysters quickly became covered with a thick layer of fine, sticky mud.

Experimental procedures

Sydney rock oysters *Saccostrea commercialis*, and Pacific oysters *Crassostrea gigas* (96 dozen of each) were scrubbed free of fouling and put out on standard 1.8 x 0.9m oyster trays, fitted with hinged lids, at each of the four sites. Eight dozen oysters of each species were put on each tray and four trays were put out at each site. Oysters on one tray at each site (the 'spare' tray) were air-dried and sprayed with yellow enamel paint before being put out on the lease. These yellow-painted oyters were used to replace dead oysters and oysters sampled from the other trays (the 'experimental' trays) to maintain the stocking densities within the trays. The trays were brought back to the reseach station every four months to clean off fouling and respray any spare oysters which had lost some of their original yellow paint.

Sampling procedures

The oysters were sampled at approximately monthly intervals between the end of July 1988 (southern hemisphere winter) and mid-September 1989. Twelve oysters of each species were sampled from each site, with care taken that no yellow-painted oysters were included in the sample. Any dead oysters and the sampled oysters were replaced with yellow-painted oysters. The oysters were taken from the trays on the falling tide, before they had been exposed to the air, and put into a 20L bucket of seawater from the site; upon return to the research station, the buckets were aerated with an airstone until the oysters were processed.

Sample procssing

The oysters were processed as quickly as possible after being brought to the research station. Each oyster was blotted dry with paper towel, and the length (hinge to lip), the width (the greatest distance between the shell margins perpendicular to the length axis) and the depth (the greatest thickness of the oyster perpendicular to both length and width) were measured with calipers. The whole weight, weight of the oyster in water, meat weight (blotted dry for 5 seconds on paper towel), shell weight (blotted dry on paper towel) and shell weight in water were recorded. Six oyster meats of each species from each site were each weighed on disposable plastic weighing tray marked with a unique identification code. The tray and meat was then put into a sealable polythene bag, and frozen at -20°C The remaining six meats of each species from each site were fixed for histological analysis (C. J. Mason, unpublished data, 1991).

The frozen meats were dried to constant weight at 80°C and weighed, before being ground to a fine powder. The powdered meats were stored in sealable plastic bags in a dessicator at room temperature, until they could be analysed.

Chemical analysis

Individual oyster meats were analysed for glycogen by the enzymatic method of Keppler and Decker (1974), modified to account for the small dry weights of the individual oyster meats. Protein was measured spectrophotometically (Layne, 1957). Lipid was measured using a modification of the method due to Bligh and Dyer (1959). The modification was necessary because of the yolky nature of many of the meats, which made proper separation of the aqueous and chloroform phases of the lipid extraction solvent nearly impossible. The modification was based on a procedure used by the analytical laboratories of the NSW Egg Board (Lidcombe, NSW, Australia), and is as follows: 1g of dried oyster meat was homogenised with 100ml of 1 : 2 : 0.8 parts chloroform : methanol : distilled water (the single phase solvent of Bligh and Dyer, 1959), and filtered through a medium-fast paper filter. 50ml of chloroform and 50ml of distilled water was added to the filtrate, and the mixture evaporated to dryness on a steam bath. The lipid fraction was rinsed out of the dried residue with 100ml chloroform into a tared beaker, and the solvent evaporated off. This residue was total lipid from the dried meat.

Percent ash was obtained for individual oysters for which more than 200mg of dry meat remained after the above analyses were carried out. The dry meat was put into a tared crucible, weighed, and heated in a muffle furnace at 550°C for 48h.

Data processing

The following calculations were carried out on the raw data for each oyster:

Cavity Volume = whole weight - shell weight.

This is based on the assumption that the meat and mantle fluid of the oyster fills the shell cavity, and has a density of 1.0 (Lawrence and Scott, 1982).

Condtion Index (CI) = (dry meat weight/Cavity Volume)x100

This definition of Condition Index has been used by Haven (1960), Nell and Wisely (1982) and Soniat and Ray (1985). The same definition, but using a constant of 1000 rather than 100 was used by Gabbot and Stephenson (1974), Brown and Hartwick (1988) and Walne (1970). An alternative definition of

Condition Index:

Condition Index (SCI) = dry meat weight / shell weight

has been used by Gabbott and Walker (1971), Walne and Mann (1975), Brown and Hartwick (1988) and Littlewood and Gordon (1988). Lucas and Beninger (1985) recomended its use over the cavity volume based definition of Condition Index. Estimates of Condition Index based on the shell weight (SCI) have been made for comparison with the publications of authors using this definition of Condition Index.

Constituent Indices = (% Constituent x CI) / 100

Where the Constituents are glycogen (GI), protein (PI), lipid (LI) or unidentified constituents (UI). The UI are caclulated as

UI = 100% - (% gycogen + % protein + % lipid)

and include the ash tissue components and refractory gut contents such as cellulose and silica (Hawkins and Rowell, 1987).

Statistical analyses

The percentages of the meat constituents were arcsin-transformed (Sokal and Rohlf, 1969). The means and standard deviations of the arcsin-transformed data were calculated, and the transformation reversed, to yield means expressed in the original units (percentage composition); since the standard deviation is not symmetrical about the mean when the data are de-transformed, the variance has been expressed as the 95% confidence interval about the mean. The data were analysed by multi-factor analysis of variance.

RESULTS

Temperature and Salinity

Variations in temperature and salinity at each site over the duration of the experiment are summarised in Fig. 2. The temperature in Tilligerry Creek ranged from a maximum of 26.5°C in December 1988 to January 1989, to a minimum of 14.5°C in late August 1988. The temperature at Corrie Island ranged from 27°C in March 1989 to a minimum 13.2°C in August 1989. The temperature in the Karuah River ranged from a maximum of 26.5°C in late December 1988 to a minimum of 13°C in August 1988. The salinity in Tilligerry Creek remained at about 30 - 33 gL^{-1} for most of the experiment, but fell to the middle 20's in April and May 1989. Therafter, the salinity in Tilligerry Creek did not recover fully until August 1989. The salinity at Corrie Island

fluctuated, starting low at 23 gL^{-1} during August 1988, then remaining at 31 - 34 gL^{-1} until February 1989, when rain between February and July caused the salinity at the surface to fluctuate between 20 and 30 gL^{-1} over this period. The salinity then remained around 30 - 32 gL^{-1} until September 1989. The salinity of the water measured 5m below the surface at Corrie Island remained much more steady at about 30 - 32 gL^{-1}, and did not drop below 28 gL^{-1} for the duration of the experiment. The salinity at Karuah fluctuated more than the other sites. The salinity increased from 27 gL^{-1} in August 1988 to a maximum of 38 gL^{-1} during November 1989. Rain caused the salinity to fall to 17 gL^{-1} in mid-January, and after recovering to 30 gL^{-1}, to a minimum of 10 gL^{-1} in April 1989. The salinity recovered again to around 30 gL^{-1}, dropping to 24 gL^{-1} during rain in August 1989.

Estimates of cavity volume

Estimates of weight cavity volume (WCV) based on the (whole weight - shell weight) were 104 $\pm$ 3% of the volume cavity volume (VCV) based on (whole volume - shell volume) for Sydney rock oysters at all sites, and 104 $\pm$ 7% for Pacific oysters at all sites. The regressions of WCV on VCV were:

$$\text{SRO WCV} = 0.50 + (1.0005 \times \text{VCV}) \quad (r^2 = 0.98)$$

$$\text{PO WCV} = 0.80 + (1.0012 \times \text{VCV}) \quad (r^2 = 0.98)$$

(SRO is Sydney rock oysters at all sites, PO is Pacific oysters at all sites).

Percent water in meats

The percent of water in the meats varied highly significantly throughout the year ($p < 0.001$). There was a small, but highly significant negative correlation between the water content of the meats and the CI of the meats:

$$\text{SRO \% water} = 95.8 - (1.48 \times \text{CI})$$
$$(F = 755: 1, 310 \text{ df}; \ r^2 = 0.71)$$

$$\text{PO \% water} = 95.9 - (1.56 \times \text{CI})$$
$$(F = 847: 1, 310 \text{ df}; \ r^2 = 0.73)$$

The regression of % water on CI - UI (ie protein + glycogen + lipid) had more unexplained variation, and was the same for both species:

$$\text{(both) \% water} = 91.1 - (1.14 \times (\text{CI} - \text{UI}))$$
$$(F = 923: 1, 597 \text{ df}; \ r^2 = 0.61)$$

Composition of dry meats

The mean percentages and 95% confidence intervals of glycogen, lipid, protein and unidentified constituents of the dried meats throughout the year for oysters of each species at each site are given in Table 1.

The percent glycogen varied significantly throughout the year, according to the state of gonad maturity of the oysters. The maximum glycogen for Sydney rock oysters was 21.9% (95% conf: 18.0 - 26.2%) for raft oysters in September 1988; for Pacific oysters the maximum was 19.2% (95% conf: 13.2 - 25.9%) on the raft in June 1989 (Table 1). Minimum glycogen percentages were 3.4% (95% conf: 1.8 - 5.4%) for Tilligerrry Creek Sydney rock oysters in March 1989 and 1.1% (95% conf: 0.2 - 3.0%) for raft pacific oysters in February 1989 (Table 1). The Pacific oysters at all sites spawned in November, and the percent glycogen started to fall in late September 1988 as their gonads matured, to reach a minimum between December and February 1989. The percent glycogen in Sydney rock oyster meats never dropped as low as that in the Pacific oysters, and declined later in the year (Table 1).

The percent lipid in oysters also varied significantly throughout the year (Table 1). Since lipid constitutes only a small proportion of the dry meat, changes in the percent lipid only reflects changes in the proportions of the major constituents, particularly glycogen, which ranged from less than 2% to more than 20% of the dry meats according to species, site and time of year (Table 1).

The percent protein varied throughout the year according to the gonad condition of the oysters, reaching a maximum in fully ripe oysters. There is a sudden increase in percent protein for both species at Tilligerry Creek, Corrie Is. and Karuah in August 1989.

Condition and constituent indices.

The condition Index (CI), glycogen index (GI), lipid index (LI), protein index (PI) and unidentified constituent index (UI) are summarised for raft oysters in Fig. 3, Tilligerry Ck. oysters (Fig. 4), Corrie Is. Oysters (Fig. 5) and Karuah oysters (Fig. 6).

Condition Index (CI)

The mean CI during the year was ranked raft (greatest), Tilligerry, Corrie Is., Karuah (least), with the overall mean CI of the Pacific oysters at each site lower than that of the Sydney rock oysters.

There was a trend for the CI of the Sydney rock oysters at each site (Figs. 3, 4, 5, 6) to be greater than that of the Pacific oysters, except just before the period in which the Pacific oysters spawned. The post-spawning drop in CI was less abrupt for the Sydney rock oysters than the Pacific oysters.

With the exception of the raft oysters (Fig. 3), the CI of the oysters in 1989 was lower than that for the same season in 1988, particularly at Karuah (Fig. 6). The CI of the raft Pacific oysters dropped as they spawned between August and December 1988, recovering to pre-spawning levels by June 1989. The CI of Sydney rock oysters held on the raft did not vary significantly ($p > 0.05$) throughout the year, although the CI of individuals dropped as low as 4.06 during February and March 1989, when many of the raft Sydney rock oysters spawned (C. J. Mason, unpublished data 1991).

The timing of the peak CI of the Pacific oysters is the same for Corrie Is. (Fig. 5) and Karuah (Fig. 6) (late September 1988), slightly later for Tilligerry Ck. (Fig. 4) (early October), and earlier for the raft (Fig. 3) (late August).

The timing of the peak CI of the Sydney rock oysters at Tilligerry Ck. (Fig. 3), Corrie Is. (Fig. 4) and Karuah (Fig. 6) was mid-December. The variance of the CI at Tilligerry Ck. and Karuah at this time was very high, with the CI of individual oysters ranging from 2.3 - 12.3 and 7.3 - 17.3 at each site respectively. The variance of CI of Karuah Sydney rock oysters was low throughout the year.

Shell Condition Index (SCI)

The mean SCI's and CI's of oysters of both species at each site with time are given in Table 2. The SCI's varied with time in the same way as CI's for oysters at each site with a coefficient of covariation of 0.38. The coefficients of variation of the estimates of SCI were greater than those of CI.

Glycogen index (GI)

The GI's of oysters of each species at the raft (Fig. 3), Tilligerry Ck. (Fig. 4) and Karuah (Fig. 6) were similar during the cold half of the year (April to August). Between August and September, when the Pacific oysters were aproaching spawning condition and the Sydney rock oysters were starting to develop their gonads, the GI of each species at all sites was not significantly different. The mean GI of Sydney rock oysters was higher than that of the Pacific oysters at the above sites between late September 1988 and late March 1989, although because the variance of a number of those means was very high, the differences were not always

significant (Figs. 3, 4, 6).

The GI of Sydney rock oysters at Corrie Is. were significantly lower ($p < 0.005$) than those of the Pacific oysters between April and August 1989 (Fig. 5). Unlike the other sites, where the GI of the Sydney rock oysters peaked in September, the GI of the Sydney rock oysters at Corrie Is. continued to increase until mid-December 1988, before decreasing.

The GI of raft oysters of both species reached higer levels than at other sites, which was also the case for percent glycogen in the dry meats.

Protein index (PI)

Pacific oysters at all sites (Figs. 3, 4, 5, 6) had PI's greater than those of the Sydney rock oysters at the site, until after they had spawned in October 1988. Thereafter, the Pacific oyster PI's dropped below those of the Sydney rock oysters at all sites until April 1989, when the PI's of both species at all sites became very similar. The highest PI's occurred after the peak GI levels, and just before the oysters spawned.

Lipid index (LI)

The LI of both species at all sites (Figs. 3, 4, 5, 6) followed the CI closely, since there was little variation in the percentage of lipid in the dry meats. As for the PI's, the highest LI levels occured as the GI levels dropped, before the oysters spawned.

Unidentified constituent index (UI)

The mean UI of each species over the year was ranked Tilligerry (greatest), Corrie Is., raft, Karuah (least), and the UI of Sydney rock oysters was greater than that of Pacific oysters at each site.

While the difference between the UI of oysters collected at different times of the year was highly significant ($p < 0.0001$), the differences between species and sites were not significant ($p > 0.5$) at each collection date.

DISCUSSION

There are a few problems with interpreting chemical composition data in oysters, caused by the physiology and anatomy of the oysters. Firstly, like many other

invertebrates, oysters devote an enormous amount of their resources to the production of gametes: over a period of two days, during which time the oysters spawned, Pacific oysters went from a mean wet CI (wet meat / cavity volume x 100) of 45.7 ± 5.1 to 37.3 ± 5.7, a loss of 18%. Sydney rock oysters went from a wet CI of 85.9 ± 5.1 to 60.6 ± 3.1 in the two days, and had fallen to 53.4 ± 3.9 two days later (n = 12 in each case). This is a loss of 30% of the wet meat weight in two days, and a loss of 38% over the four days (C. Mason, unpublished data, 1990). The loss of nearly 40% of the wet body weight of the oyster will influence the proportions of the constituents of the oyster meats. Secondly, the chemical composition of separate tissues of the oysters is relatively difficult to determine, since the tissues of the digestive system and the gonad are intimately connected. In scallops (*Chlamys septemradiata*), in which the gonad, adductor muscle and the mantle tissue are easily separated, the three groups of tissue are found to have chemical compositions which vary according to the sex of the scallop and season (Ansell, 1974b; Gabbott, 1975). Thus, the large change in the proportion of gonad to non-gonad tissue during gonad development and spawning makes it difficult to determine whether a change in the amount of a particular chemical entity, for instance lipid, has been caused by a change in the amount of tissues high in lipid, or a change in the amount of lipid in the rest of the oyster.

Two approaches to the presentation of chemical composition data have been used in the literature; firstly, the percentage composition of the dry meats. The second approach avoids relative measures by expressing the amount as an absolute quantity, normalised to permit comparison between animals of different sizes (see also Lucas and Beninger (1985) and Crosby and Gale (1990) for comprehensive reviews of bivalve condition indices).

The use of proportions or percentages of the chemical composition has the advantage of requiring only a simple calculation to transform the concentration of the constituent found in the analysis to a proportion of the dry meat from which it was derived. Thereafter no further transformations are required unless the range of the data is large, in which case an arcsin transformation will make the distribution of the data normal (Sokal and Rohlf, 1969). Any changes in the relative quantities of the constituents are very obvious, and indeed, may be exagerated. The major drawback with representing the quantities as proportions is that changes in one component will affect all the other components to some extent, for instance the large increase in the proportion of glycogen in spring causes a fall in the proportions of lipid and protein; it is not possible to determine whether there is an absolute increase or decrease in lipid or protein in the oysters at the same time.

With the second approach, the percentage of the constituent

is converted to an absolute amount by multiplying the percentage by the dry meat weight. The weight is then normalised to that of a 'standard animal' so that animals of different sizes may be compared (Ansell, 1974; Gabbott and Bayne, 1973). Alternatively, the CI may be multiplied by the percentage of the constituent to obtain an index for the constituent (Walne, 1970; Whyte and Englar, 1982). The extra calculations increase the error component, and care should be taken to avoid confounding errors such as a correlation between size and condition index. Changes in the relative proportions of the constituents are less noticeable with the indices than percentages, since they are independant, but absolute increases in the constituents are readily seen.

It may be seen from Table 1 and Figs. 3, 4, 5 and 6 that there was a wide range in both the proportion of unidentified material and the UI of the oysters. In the present study, the oysters were not purged in filtered water to void their guts (Pieters et al., 1980; Hawkins and Rowell, 1987). The proportion of unidentified material (including silt, cellulose, diatom frustules and biochemicals not identified as glycogen, protein or lipid) varied highly significantly between collection dates, but differences between species at each site and between sites for each species were not significant. This suggests that the major factor affecting the proportion of unidentified constituents was some environmental variable such as suspended solids in the water, and that the location and species were much less important. The assumption that the unidentified material was largely gut contents is supported by the highly significant correlation between UI and cavity volume ($P < 0.0001$); the cavity volume is a measure of the size of the animal, and it is to be expected that gut contents will be proportional to size. Quayle (1988) attributes the balance of chemical constituents of Pacific oysters in British Columbia unaccounted for to carbohydrates destroyed in the analytical procedure. The enymatic and spectroscopic procedures used to determine glycogen and protein in this experiment are much less harsh than the wet combustion and Kjeldahl methods, however, so loss of carbohydrate in this case is less likely. Since the proportion of unidentified material was so variable in this experiment, the percentages of protein, glycogen and lipid have a large error component. If proportions of protein, glycogen or lipid are to be compared, then they should be expressed as a proportion of the meat less unidentified material, not of the whole meat.

Glycogen is accumulated before the oysters were observed to have spawned, as has been reported for other oyster species and bivalves elsewhere in the world (Gabbott and Stephenson, 1974; Gabbott, 1975; Walne, 1970; Ansell, 1974a and 1974b; Perdue and Erickson, 1984; Soniat and Ray, 1985; Mann, 1979). The Pacific oysters at all sites developed their gonads and spawned earlier than the Sydney rock oysters (unpublished data), and this was reflected in the displacement of the rise

and fall of the GI in the Sydney rock oysters about 45 days later than the Pacific oysters. The protein and lipid indices also varied with the gonad condition of the oysters, although not in as striking a fashion as the GI. The increase in both the PI and LI appeared to reflect the increased bulk of the oyster as the gonads matured. The chemical composition of male and female scallop gonads is very different, since eggs are high in lipid-rich yolk (16% lipid and 34% protein; Gabbott, 1975), and testis is 67 - 80% protein (Ansell, 1974; Gabbott, 1975), and it is likely that oyster gonads follow the same pattern. In order to demostrate differences in chemical composition between whole meats from oysters of each sex, assuming that about 40% of the dry meat weight consists of gonad, The difference in percent protein between fully ripe male and female oysters will be about 13% to 18% of the dry weight (67 to 80% protein in males - 34% in females x 40% of the dry weight). The difference in percent lipid between ripe male and female oysters will be about 6% of the dry weight (16% lipid in female gonad x 40% body weight). The differences will be less for oysters with developing or partially spawned gonads, as may be seen from the smaller variance of the constituent indices immediately following a fall in condition index, which may be interpreted as evidence for spawning.

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Table 1. Composition (%) of meats of Sydney rock oysters (*Saccostrea commercialis*) and Pacific oysters (*Crassostrea gigas*) at four sites in Port Stephens. Mean, with lower and upper 95% confidence limits. The percentage of unidentified material was calculated for each oyster, and the sum of the means of protein, glycogen and lipid plus the mean unidentified material is not necesarily 100%.

Sydney rock oysters, raft (Tilligerry Creek, subtidal).

Date	Glycogen			Protein			Lipid			Unidentified material		
	mean	lower	upper	mean	lower	upper	mean	lower	upper	mean	lower	upper
28 Jul 88	16.7	14.1	19.5	47.9	40.5	55.3	8.6	7.2	10.2	25.8	19.8	32.2
9 Aug 88	18.0	14.3	22.1	39.8	30.5	49.8	7.6	5.7	9.7	34.1	25.3	43.4
25 Aug 88	16.1	12.5	20.1	58.1	48.1	67.8	10.9	8.7	13.4	20.7	12.9	29.8
20 Sep 88	20.2	16.2	24.4	70.6	61.1	79.3	9.3	7.2	11.6	6.1	1.8	13.0
11 Oct 88	19.0	15.2	23.2	69.4	59.9	78.2	6.5	4.8	8.4	7.2	2.7	13.5
21 Nov 88												
14 Dec 88	12.3	9.1	15.8	50.5	40.5	60.5	8.7	6.8	11.0	27.4	19.3	36.3
8 Feb 89	5.2	3.2	7.7	59.3	47.0	71.0	10.3	8.2	12.7	37.0	24.5	50.5
29 Mar 89	7.3	4.9	10.2	38.5	29.0	48.4	7.8	5.9	9.9	46.0	36.6	55.6
3 May 89	7.7	5.2	10.6	41.1	31.5	51.1	11.2	8.9	13.6	39.6	30.4	49.1
14 Jun 89	14.8	11.0	18.9	30.6	21.0	41.1	8.8	6.7	11.3	44.3	34.0	54.8

Pacific oysters, raft (Tilligerry Creek, subtidal).

28 Jul 88	16.4	13.1	20.0	62.8	50.3	74.5	9.4	7.8	11.1	32.0	20.3	45.0
9 Aug 88	16.2	11.6	21.3	50.0	33.2	66.8	9.3	7.1	11.8	25.2	13.7	38.8
25 Aug 88	15.6	11.1	20.6	82.2	60.7	96.3	11.4	9.0	14.0	27.3	4.8	59.2
20 Sep 88	14.9	10.6	19.9	50.4	32.0	68.7	10.6	8.2	13.1	15.8	5.9	29.4
11 Oct 88	6.3	3.5	9.8	67.8	49.5	83.7	11.3	8.9	14.0	15.1	5.4	28.5
21 Nov 88												
14 Dec 88	4.2	1.9	7.2	35.4	18.9	54.0	7.8	5.8	10.1	43.8	29.7	58.4
8 Feb 89	1.1	0.2	3.0	48.8	25.0	65.5	10.9	8.6	13.5			
29 Mar 89	8.7	5.3	12.7	34.3	19.3	51.1	6.8	4.9	8.9	55.1	41.7	68.0
3 May 89	14.6	10.2	19.5	48.4	30.2	66.8	8.0	5.8	10.6	38.2	23.1	54.4
14 Jun 89	19.2	13.2	25.9	44.3	21.8	68.1	7.4	5.0	10.1	36.4	19.6	55.2

(Table 1. cont.)
Sydney rock oysters, Tilligerry Creek (intertidal).

Date	Glycogen			Protein			Lipid			Unidentified material		
	mean	lower	upper	mean	lower	upper	mean	lower	upper	mean	lower	upper
28 Jul 88	16.0	13.5	18.6	40.5	35.1	45.9	8.4	6.6	10.4	34.1	27.5	41.0
9 Aug 88	21.9	18.0	26.2	36.2	29.0	43.9	9.5	.6.9	12.5	32.0	23.0	41.7
25 Aug 88	17.6	14.0	21.5	53.4	45.7	61.1	7.4	5.1	10.1	19.3	12.0	27.8
20 Sep 88	18.3	14.3	22.7	52.1	44.4	59.8	9.9	7.3	13.0	17.4	9.8	26.5
11 Oct 88	13.8	10.6	17.4	55.2	46.6	63.5	9.0	6.5	11.9	18.7	10.9	28.0
21 Nov 88												
14 Dec 88	9.2	6.6	12.3	50.8	43.0	58.5	7.1	4.9	9.8	30.8	21.9	40.4
8 Feb 89												
29 Mar 89	3.4	1.8	5.4	36.1	28.9	43.7	9.9	7.2	12.9	50.1	40.1	60.1
3 May 89	7.9	5.5	10.8	51.4	43.6	59.1	12.4	9.4	15.7	27.3	18.8	36.7
14 Jun 89	11.9	8.9	15.3	24.3	18.0	31.3	9.5	6.9	12.5	52.2	42.1	62.1
17 Jul 89	13.9	10.7	17.5	30.2	23.3	37.5	9.1	6.5	12.0	46.2	36.3	56.3
15 Aug 89	14.9	11.6	18.6	51.6	43.9	59.4	5.1	3.2	7.4	27.6	19.1	37.0
11 Sep 89	8.4	5.9	11.4	35.9	28.6	43.5	13.1	10.0	16.5	42.3	32.5	52.4

Pacific oysters, Tilligerry Creek (intertidal).

28 Jul 88	18.0	15.7	20.7	43.0	3.18	54.6	9.7	8.3	11.1	40.1	32.9	47.5
9 Aug 88	15.2	12.1	18.5	43.4	28.4	59.0	8.1	6.3	10.1	40.3	30.7	50.3
25 Aug 88	16.8	13.6	20.2	66.4	50.9	80.2	8.9	6.9	11.1	20.7	12.4	30.5
20 Sep 88	11.8	9.1	14.8	79.1	65.1	90.3	10.1	8.2	12.3	26.8	14.1	41.8
11 Oct 88	11.1	8.5	14.1	58.9	41.8	75.0	10.7	8.7	12.9	30.4	20.7	41.2
21 Nov 88												
14 Dec 88	1.9	0.9	3.4	37.9	23.5	53.5	9.4	7.5	11.5	49.9	4.07	59.0
8 Feb 89												
29 Mar 89	9.4	7.0	12.2	32.0	18.5	47.3	7.3	5.6	9.2	50.2	41.1	59.4
3 May 89	15.1	12.0	18.4	43.9	28.9	59.6	9.9	8.0	12.1	30.4	22.3	39.1
14 Jun 89	14.2	11.2	17.4	26.0	13.6	40.8	8.9	7.0	10.9	50.1	40.9	59.2
17 Jul 89	11.1	8.4	14.0	34.0	20.1	49.4	8.6	6.8	10.6	46.1	37.0	55.2
15 Aug 89	12.5	9.7	15.6	56.0	40.4	71.1	7.5	5.8	9.4	29.0	20.4	38.5
11 Sep 89	14.5	11.5	17.8	45.8	30.6	61.4	9.6	7.6	11.7	29.8	21.8	38.5

(Table 1. cont.)
Sydney rock oysters, Corrie Island.

Date	Glycogen			Protein			Lipid			Unidentified material		
	mean	lower	upper	mean	lower	upper	mean	lower	upper	mean	lower	upper
28 Jul 88	15.0	12.3	18.0	34.8	29.2	40.6	10.1	8.4	11.9	38.9	31.9	46.1
9 Aug 88	15.6	11.7	20.0	32.0	24.3	40.2	10.1	7.8	12.7	41.2	31.3	51.6
25 Aug 88	14.8	11.0	19.1	50.9	42.4	59.4	11.2	8.8	13.9	22.2	14.3	31.4
20 Sep 88	16.9	12.5	21.8	45.9	36.7	55.2	10.8	8.4	13.4	29.0	18.3	41.1
11 Oct 88	16.9	12.9	21.4	60.2	51.8	68.4	8.0	6.0	10.4	14.0	7.6	21.9
21 Nov 88	14.1	9.0	20.1	63.8	52.0	74.9	8.9	5.8	12.4	10.1	3.1	20.5
14 Dec 88	13.5	9.9	17.6	69.8	61.8	77.3	10.1	7.8	12.7	8.1	3.0	15.3
8 Feb 89	11.0	7.7	14.8	47.9	36.0	59.9	12.2	9.6	15.0	25.5	14.0	39.1
29 Mar 89	8.1	5.3	11.5	39.2	31.1	47.7	9.0	6.8	11.5	43.0	33.0	53.3
3 May 89	6.2	3.7	9.2	39.2	31.0	47.6	11.9	9.3	14.6	41.1	31.2	51.4
14 Jun 89	10.6	7.4	14.4	18.2	12.1	25.2	9.3	7.1z	11.8	59.3	49.0	69.2
17 Jul 89	4.2	2.2	6.7	22.1	15.4	29.5	9.5	7.3	12.1	63.2	53.0	72.9
15 Aug 89	5.7	3.4	8.7	56.8	48.3	65.1	7.8	5.7	10.1	28.9	20.0	38.6
11 Sep 89	9.8	6.7	13.4	44.1	35.8	52.6	10.3	8.0	12.9	35.3	25.8	45.4

Pacific oysters, Corrie Island.

28 Jul 88	15.3	12.1	18.8	45.4	37.5	53.5	9.4	7.9	10.9	31.6	23.7	40.0
9 Aug 88	13.6	9.4	18.4	33.0	22.8	44.1	8.9	6.9	11.0	43.8	32.3	55.7
25 Aug 88	13.0	8.9	17.7	48.2	35.9	60.6	10.5	8.4	12.7	26.8	16.2	39.0
20 Sep 88	10.4	6.7	14.7	60.1	46.1	73.2	9.0	7.1	11.2	17.7	8.1	30.0
11 Oct 88	5.7	3.0	9.2	55.5	44.2	66.7	8.9	6.8	10.9	28.5	18.4	39.7
21 Nov 88	2.5	0.9	5.0	39.6	28.8	51.0	10.4	8.3	12.6	46.9	35.2	58.8
14 Dec 88	2.4	0.8	4.9	41.2	30.3	52.5	10.3	8.2	12.6	45.0	33.4	56.9
8 Feb 89	1.6	0.4	3.7	53.2	39.3	66.8	9.7	7.7	11.9	34.1	21.1	48.4
29 Mar 89	13.7	9.5	18.6	39.3	28.5	50.6	10.0	7.9	12.2	35.6	24.7	47.3
3 May 89	12.4	8.4	17.1	33.0	22.8	44.0	8.4	6.5	10.5	46.1	34.5	58.0
14 Jun 89	11.5	7.6	16.0	34.8	24.4	46.0	9.7	7.7	11.9	49.9	37.0	62.8
17 Jul 89	12.3	8.3	17.0	31.6	21.6	42.6	8.4	6.5	10.5	46.9	35.2	58.7
15 Aug 89	11.2	7.4	15.7	72.3	61.6	81.9	6.9	5.2	8.8	12.6	5.3	22.4
11 Sep 89	10.9	7.2	15.4	42.6	31.6	54.0	7.9	6.1	9.9	38.3	27.1	50.0

(Table 1. cont.)
Sydney rock oysters, Karuah River.

Date	Glycogen			Protein			Lipid			Unidentified material		
	mean	lower	upper	mean	lower	upper	mean	lower	upper	mean	lower	upper
28 Jul 88	16.8	14.2	19.7	39.1	32.0	46.5	8.9	7.0	10.9	33.8	26.2	41.8
9 Aug 88	18.3	14.9	21.9	36.9	28.3	45.8	11.8	9.2	14.7	32.1	23.1	41.9
25 Aug 88	15.5	11.9	19.5	51.6	41.2	62.0	10.7	7.9	14.0	18.8	10.6	28.7
20 Sep 88	19.0	15.1	23.3	59.0	47.5	70.0	8.8	6.2	11.8	16.5	7.3	28.4
11 Oct 88	15.1	11.5	19.0	69.2	58.2	79.2	10.7	7.9	13.9	15.8	5.8	29.5
21 Nov 88												
14 Dec 88	7.6	5.0	10.6	56.1	45.6	66.3	7.9	5.4	10.7	27.6	17.8	38.6
8 Feb 89	6.1	3.8	8.8	75.0	61.3	86.6	13.7	10.5	17.2	46.4	19.9	74.1
29 Mar 89	6.7	4.3	9.5	28.3	19.4	38.2	11.3	8.4	14.6	51.9	40.2	63.5
3 May 89	7.6	5.1	10.6	28.9	19.9	38.9	13.2	10.0	16.7	49.0	37.4	60.6
14 Jun 89	9.8	6.9	13.2	20.5	12.7	29.6	9.7	7.0	12.7	58.8	47.1	70.0
17 Jul 89	4.7	2.6	7.4	23.3	14.3	33.6	8.8	6.0	12.1	62.2	49.5	74.2
15 Aug 89	8.3	5.6	11.4	64.0	53.6	73.7	9.9	7.1	13.0	15.0	7.7	24.3
11 Sep 89	6.1	3.8	8.9	29.9	20.8	39.9	12.2	9.2	15.6	51.5	39.8	63.1

Pacific oysters, Karuah River.

28 Jul 88	17.0	13.6	20.8	48.6	39.4	57.9	7.5	6.3	8.8	32.0	24.3	40.2
9 Aug 88	16.9	11.1	23.7	46.7	31.1	62.6	9.7	7.3	12.2	25.9	15.1	38.5
25 Aug 88	14.1	9.7	19.2	57.4	44.4	70.0	8.7	6.9	10.7	26.8	16.8	38.0
20 Sep 88	11.7	7.7	16.5	65.8	53.0	77.6	9.2	7.2	11.4	19.1	9.7	30.7
11 Oct 88	8.2	4.8	12.3	73.3	58.1	86.0	10.5	8.5	12.6	35.5	18.7	54.4
21 Nov 88												
14 Dec 88	1.2	0.2	3.2	34.1	22.4	46.9	10.3	8.3	12.4	54.3	43.2	65.1
8 Feb 89	2.7	0.9	5.4	54.5	38.5	70.0	9.5	7.6	11.6	31.8	20.0	44.9
29 Mar 89	10.7	6.8	15.3	32.9	21.3	45.7	8.6	6.8	10.6	47.6	36.7	58.6
3 May 89	7.7	4.4	11.7	36.0	24.1	49.0	10.2	8.2	12.3	44.3	33.6	55.3
14 Jun 89	7.6	4.4	11.6	26.2	15.6	38.4	8.7	6.9	10.7	56.1	45.1	66.8
17 Jul 89	5.9	3.1	9.5	29.3	18.2	41.8	6.0	4.5	7.7	57.7	46.7	68.3
15 Aug 89	8.4	5.0	12.6	67.8	55.0	79.3	8.5	6.7	10.5	21.7	12.7	32.4
11 Sep 89	5.9	3.1	9.5	52.5	39.5	65.4	10.3	8.3	12.4	30.9	21.1	41.5

Fig. 1. Location of experimental sites in Port Stephens, NSW. (1) Experimental raft moored in Tilligerry Creek. (2) Growing lease in Tilligerry Creek. (3) Catching lease at Corrie Island. (4) Depot lease in the Karuah River, about 3km upstream of the label in the figure.

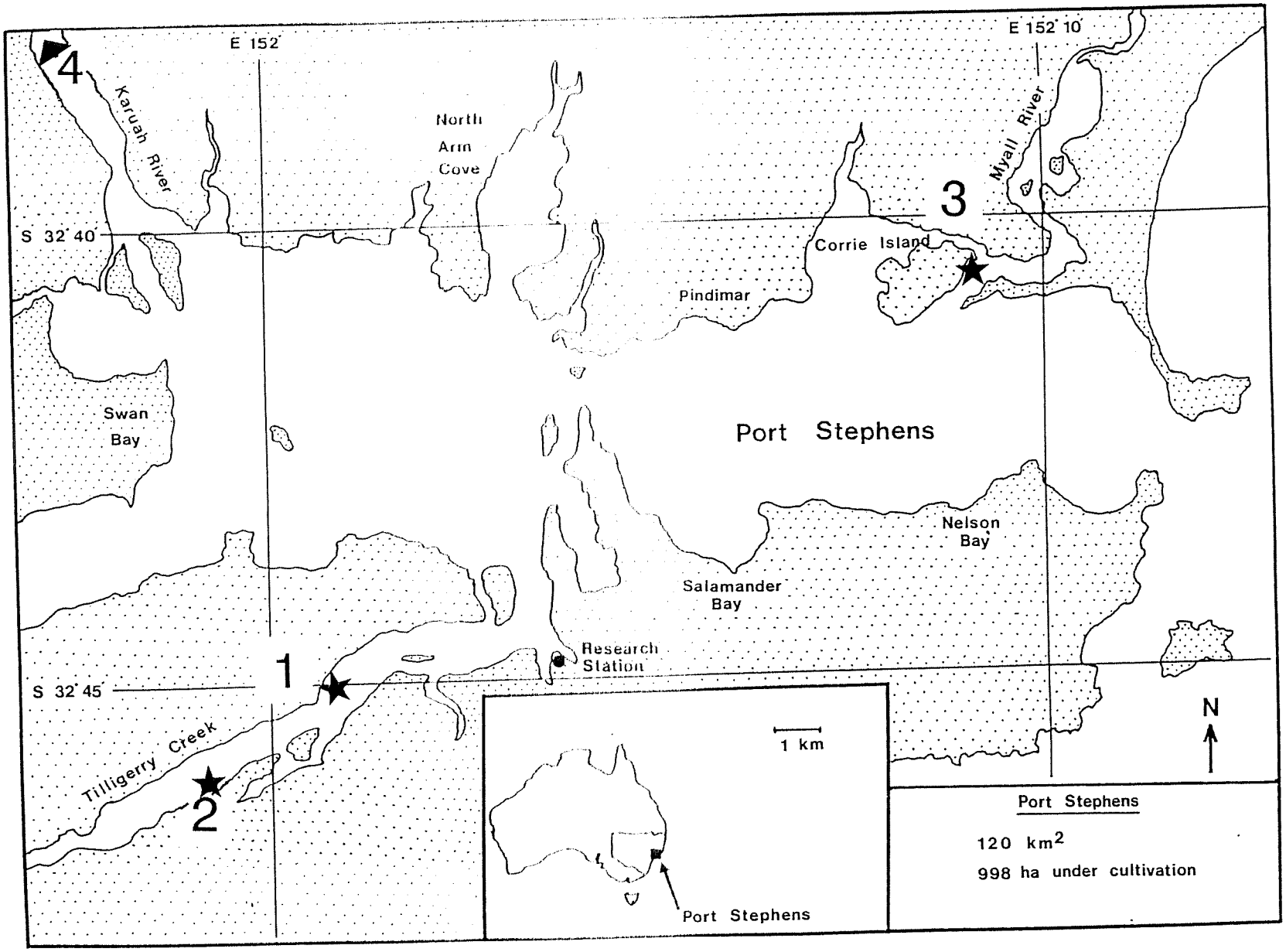
E 152
E 152 10
4
Karuah River
North
Arm
Cove
Myall River
3
S 32 40
Corrie Island
Pindimar
Swan
Bay
Port Stephens
Nelson
Bay
Salamander
Bay
Research
Station
1
S 32 45
Tilligerry Creek
2
1 km
N
Port Stephens
120 km2
998 ha under cultivation
Port Stephens

Fig. 2. Variation in water temperature (————) and salinity (— —) at the four experimental sites between July 1988 and October 1989. (A) Tilligerry Creek (includes the raft site). (B) Corrie Island. (C) Karuah River.

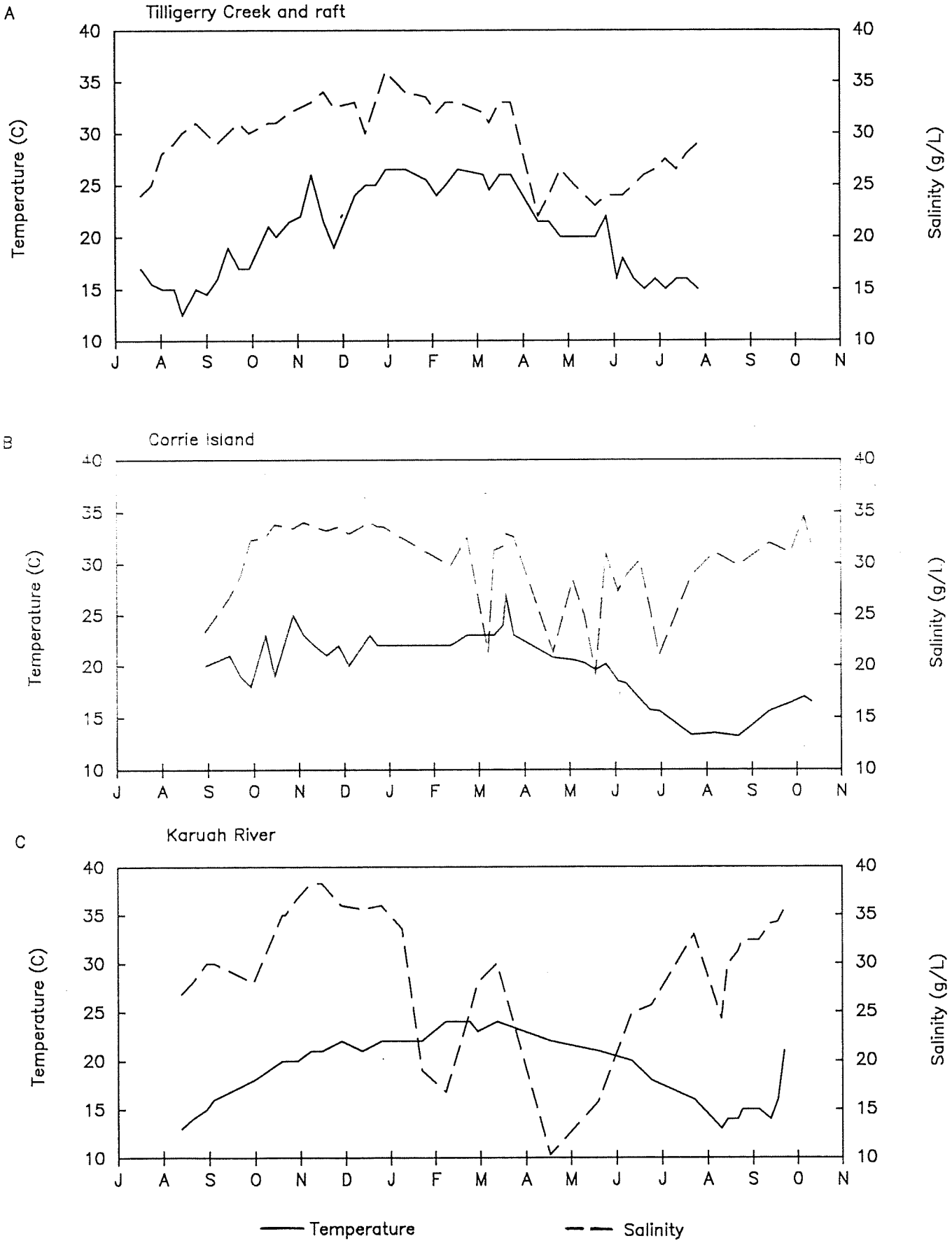
A
Tilligerry Creek and raft
Temperature (C)
Salinity (g/L)
40
35
30
25
20
15
10
J A S O N D J F M A M J J A S O N
B
Corrie Island
Temperature (C)
Salinity (g/L)
C
Karuah River
Temperature (C)
Salinity (g/L)
Temperature
Salinity

Fig. 3. Variation of constituent indices between July 1988 and June 1989 of Sydney rock oysters (*Saccostrea commercialis*) (○——○) and Pacific oysters (*Crassostrea gigas*) (●— —●) kept on a raft in Tilligerry Creek: (A) dry Condition Index (CI); (B) Glycogen Index (GI); (C) Protein Index (PI); (D) Lipid Index (LI); (E) Unidentified constituent Index (UI).

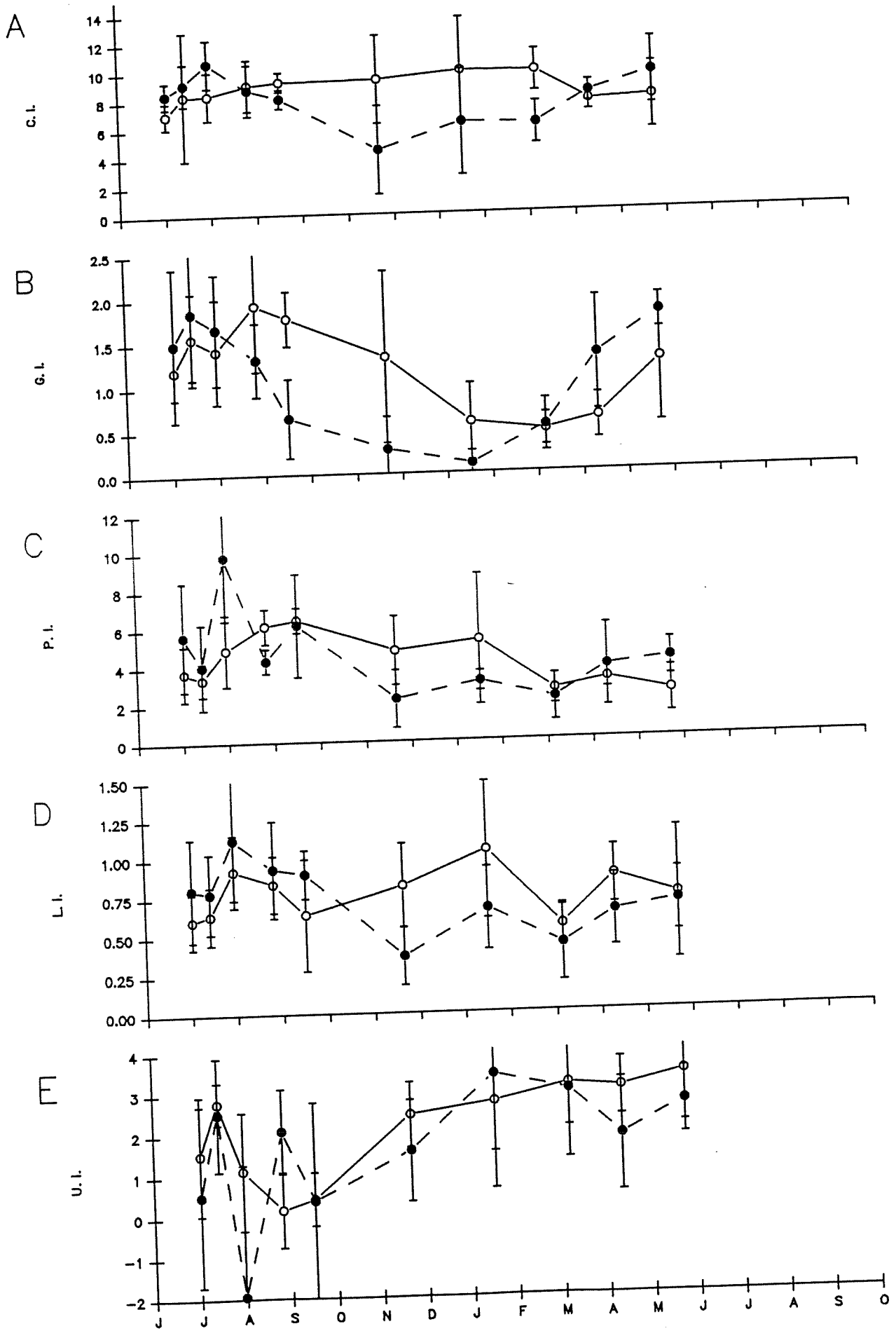
A
C. I.
14
12
10
8
6
4
2
0
B
G. I.
2.5
2.0
1.5
1.0
0.5
0.0
C
P. I.
12
10
8
6
4
2
0
D
L. I.
1.50
1.25
1.00
0.75
0.50
0.25
0.00
E
U. I.
4
3
2
1
0
−1
−2
J
J
A
S
O
N
D
J
F
M
A
M
J
J
A
S
O

Fig. 4. Variation of constituent indices between July 1988 and September 1989 of Sydney rock oysters (*Saccostrea commercialis*) (o——o) and Pacific oysters (*Crassostrea gigas*) (●— —●) kept on a growing lease (intertidal) in Tilligerry Creek: (A) dry Condition Index (CI); (B) Glycogen Index (GI); (C) Protein Index (PI); (D) Lipid Index (LI); (E) Unidentified constituent Index (UI).

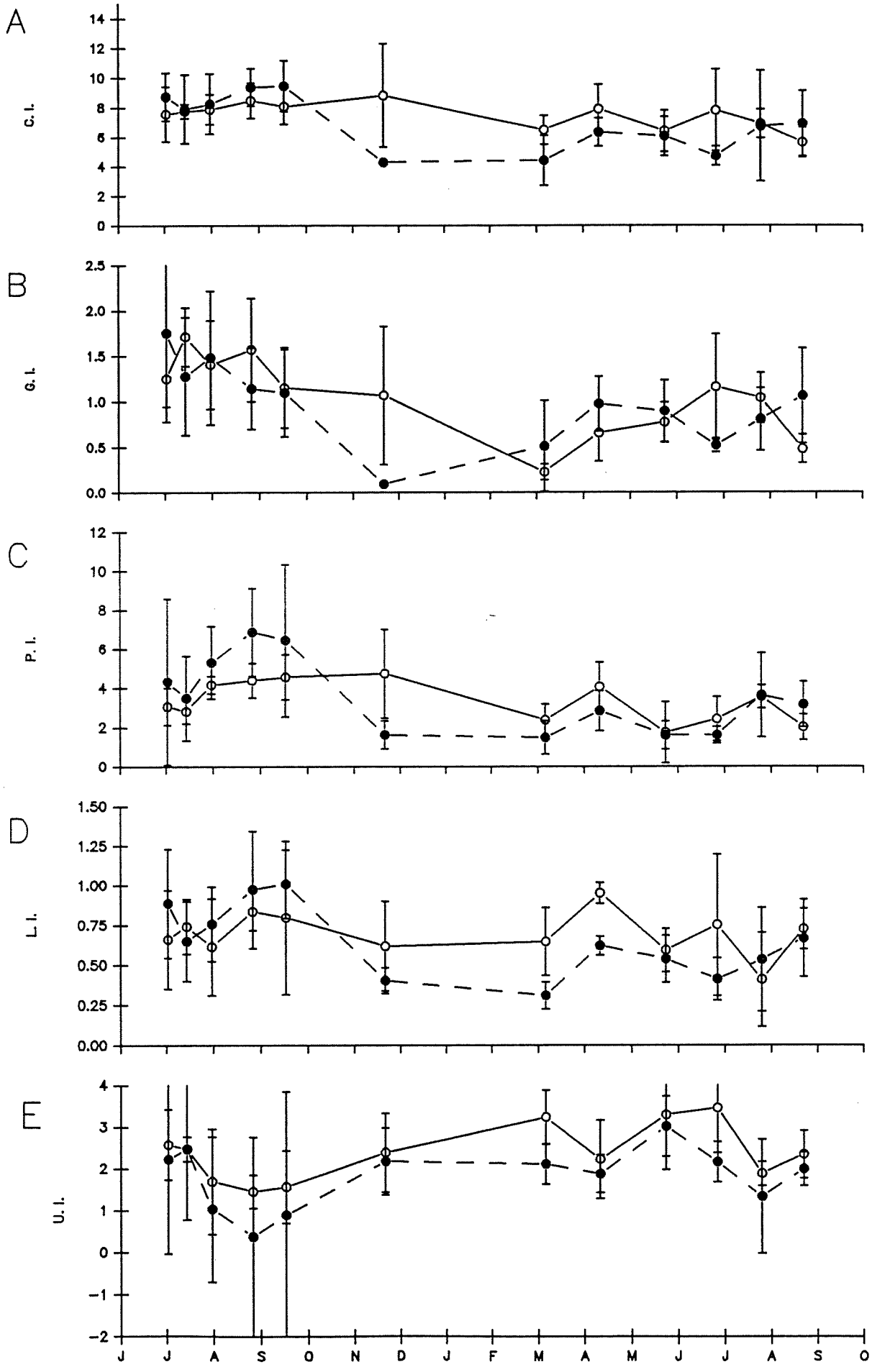
A
C.I.
14
12
10
8
6
4
2
0
B
G.I.
2.5
2.0
1.5
1.0
0.5
0.0
C
P.I.
12
10
8
6
4
2
0
D
L.I.
1.50
1.25
1.00
0.75
0.50
0.25
0.00
E
U.I.
4
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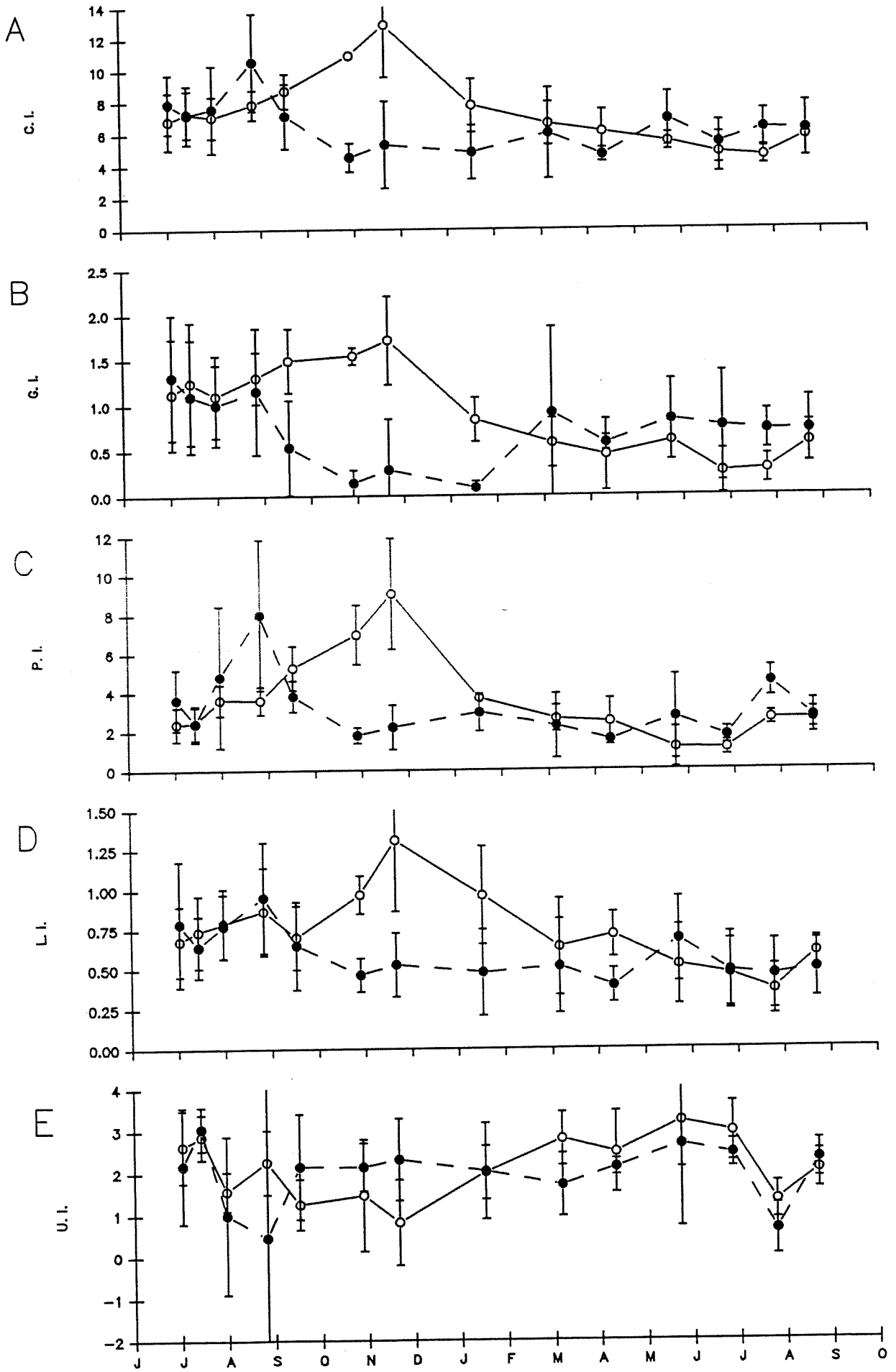
Fig. 5. Variation of constituent indices between July 1988 and September 1989 of Sydney rock oysters (*Saccostrea commercialis*) (○——○) and Pacific oysters (*Crassostrea gigas*) (●— —●) kept on a catching lease at Corrie Island: (A) dry Condition Index (CI); (B) Glycogen Index (GI); (C) Protein Index (PI); (D) Lipid Index (LI); (E) Unidentified constituent Index (UI).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P. I.
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L. I.
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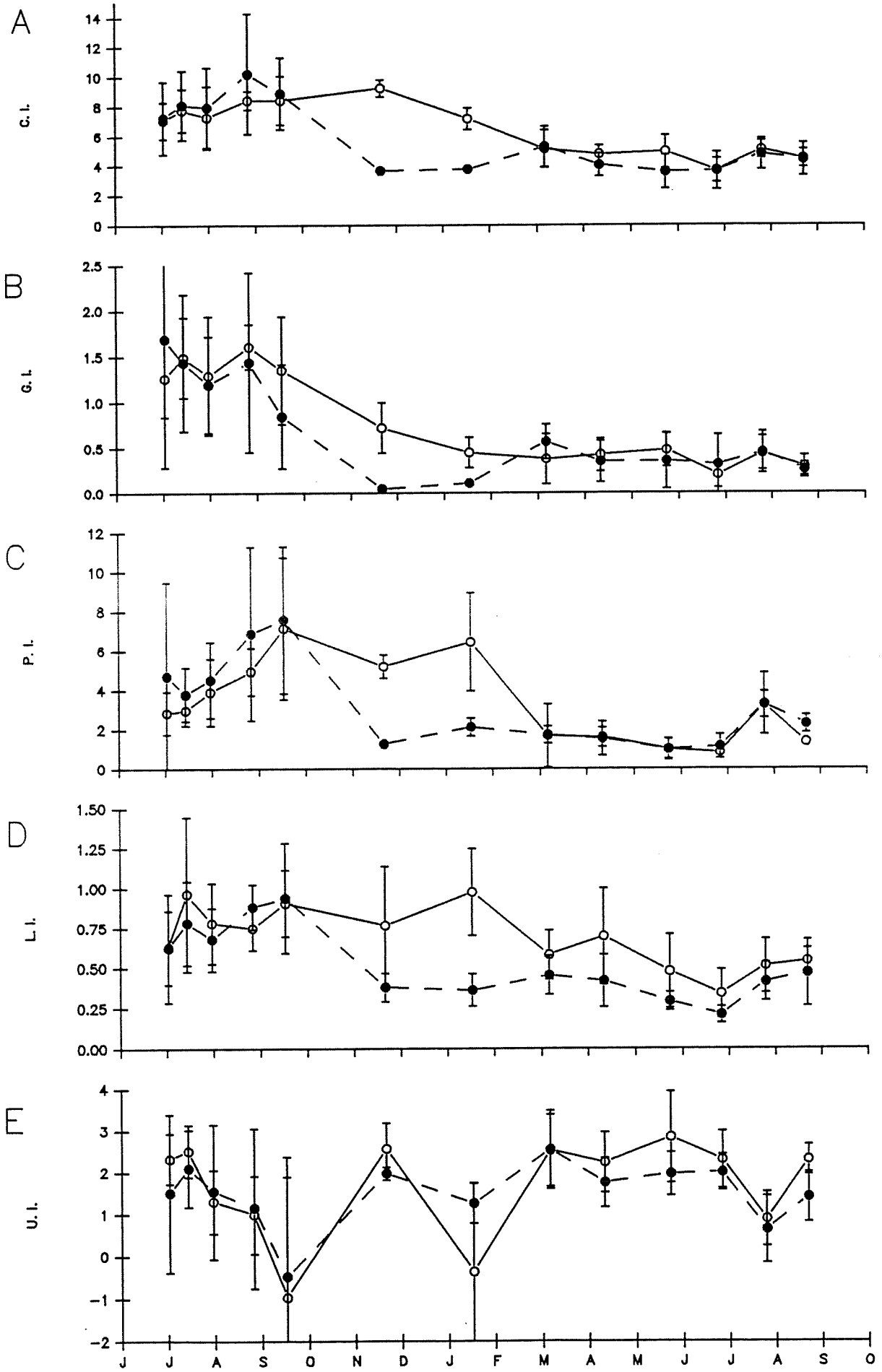
J J A S O N D J F M A M J J A S O

Fig. 6. Variation of constituent indices between July 1988 and September 1989 of Sydney rock oysters (*Saccostrea commercialis*) (○——○) and Pacific oysters (*Crassostrea gigas*) (●— —●) kept on a Depot lease in the Karuah River: (A) dry Condition Index (CI); (B) Glycogen Index (GI); (C) Protein Index (PI); (D) Lipid Index (LI); (E) Unidentified constituent Index (UI).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Acceptability of the Sydney rock and Pacific oyster

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Taste panels evaluated the sensory qualities of Sydney rock and Pacific oysters, both raw and cooked. Samples were assessed in two experimental designs, single and side-by-side presentation, using 9-point hedonic rating scales. Both species of oyster were found to be highly acceptable. In the single presentation condition, the raw Sydney rock oyster was liked slightly but significantly more than the raw Pacific oyster; however, this difference was not observed in the side-by-side condition, nor were there any detectable differences between species after cooking.

The Sydney rock oyster *(Saccostrea commercialis)* is cultivated in estuaries on the eastern seaboard of Australia and is prized for its excellent flavour. Over recent years, some leases of Sydney rock oysters have been encroached by the more vigorous Pacific oyster *(Crassostrea gigas)* (Holliday & Nell 1985). The two species are slightly different in appearance: the Pacific oyster has a darker mantle and whiter meat than the creamy-coloured Sydney rock oyster.

Is there a difference in eating quality between the two species? In a triangle test on raw (natural) oysters, conducted under subdued lighting to mask the difference in appearance, panelists detected a difference between species but no significant preference was noted (Australian Consumers Association 1981). In contrast, Longley (1982) in a more comprehensive series of acceptability tests found the Sydney rock oyster markedly preferred over its Pacific counterpart.

Some of the oysters in those studies were frozen, not fresh, and there was little control over the source of the samples. This communication reports a sensory comparison of Sydney rock and Pacific oysters grown in the same estuary under the same conditions.

Method

Raw oysters and cooked oysters (oysters Kilpatrick) were evaluated in two separate experiments, six months apart.

Raw oysters

Oysters of both species were grown on intertidal sticks in the Port Stephens estuary, NSW. Only plate oysters in good condition (weight range 40–60 g) were used for the sensory evaluation. After harvest (November 1986) oysters were depurated for 36 h in water sterilised by flaring ultra-violet light. They were held in tanks at the Brackish Water Fish Culture Research Station, Port Stephens, NSW, before being transported 200 km at ambient temperature to the CSIRO Food Research Laboratory, North Ryde, a suburb of Sydney.

The taste panel comprised 32 men and women employees of the CSIRO Food Research Laboratory, all of whom professed to like raw oysters. Most had had some previous experience in the sensory evaluation of food.

Oysters were shucked just before the first tasting session; those not required for immediate used were stored in the half-shell at 4°C. There were three tasting sessions, one session a day for three consecutive days. Panelists were given no information about the samples to be presented. At the first session, 16 panelists each received a set of Sydney rock oysters in the half-shell; the others received sets of 4 Pacific oysters. This order was reversed at the second session. At the third session, sets of each species (two oysters per set) were presented simultaneously for evaluation; the two sets were coded for identification and the order of tasting of the sets was balanced. Thus, the first two sessions correspond to *single presentation*, the third to *side-by-side presentation* (McBride 1986).

The oysters were served at approximately 10°C. Panelists were required to assess appearance, flavour, texture and general acceptability of each set, using standard 9-point hedonic scales (9 = like extremely, 5 = neither like nor dislike, 1 = dislike extremely; Peryam & Girardot 1952).

Cooked oysters

Oysters were harvested in May 1987. Subsequent methodology was as for the raw oysters, except for sample preparation. Immediately before the tasting session, oysters in the half-shell were garnished with finely-chopped, cooked bacon, and a mixture of melted butter in Worcestershire sauce (450 g butter/kg of food preparation). They were then cooked in a convection oven for 10 min at 180°C, and served hot to 30 assessors, 20 of whom had taken part in the previous evaluation of the raw oysters.

Results

Mean hedonic scores for the raw oysters are given in Table 1. For the single presentation condition, analysis of variance revealed significantly higher scores for the Sydney rock oyster in flavour ($p < 0.01$), texture ($p < 0.05$) and general acceptability ($p < 0.05$), but not in appearance ($p < 0.15$). In the side-by-side condition, none of the differences between scores was statistically significant, although the scores for the Sydney rock oyster are higher on all four sensory attributes.

Table 2 contains the mean hedonic scores for the cooked oysters. There were no significant differences between oyster species on any sensory attribute, either in the single or side-by-side presentation conditions.

Table 1. Mean hedonic scores for raw Sydney rock and Pacific oysters in the single and side-by-side presentation conditions

	Single		Side-by-side	
	Sydney rock	Pacific	Sydney rock	Pacific
Appearance	7.5	7.1	7.2	6.8
Flavour	7.5**	6.8	7.1	7.0
Texture/mouthfeel	7.6*	7.0	7.2	6.8
General acceptability	7.4*	6.8	6.9	6.8

Scores within pairs are significantly different ($p < 0.01$; * $p < 0.05$)*

Table 2. Mean hedonic scores for cooked Sydney rock and Pacific oysters in the single and side-by-side presentation conditions

	Single		Side-by-side	
	Sydney rock	Pacific	Sydney rock	Pacific
Appearance	7.0	7.0	7.0	7.4
Flavour	7.3	7.5	7.6	7.7
Texture/mouthfeel	7.3	7.1	7.5	7.5
General acceptability	7.4	7.4	7.5	7.7

Discussion

Overall, the scores in Tables 1 and 2 are high, indicating that oysters of both species were of good quality; indeed, ratings of 7 or more on a 9-point hedonic scale are uncommon from laboratory panels. The differences between varieties are much smaller than those reported by Longley (1982).

When served raw in the single presentation condition, the Sydney rock oysters were perceived to have slightly, but significantly, better flavour and texture than the Pacific oysters; this was reflected in the ratings for general acceptability. But after garnishing and cooking the sensory difference disappeared: most likely the garnish flavour tended to mask the oyster flavour, and the cooking process itself would have rendered the species less distinguishable.

The sensory differences between the raw oysters were significant in the single presentation condition but not in the side-by-side condition. This is somewhat counter-intuitive: single presentation allows only a notional comparison, i.e. comparison against a remembered level of oyster quality, whereas the side-by-side condition permits direct discrimination and might be predicted to be the more sensitive mode. However, further analysis revealed an order bias in the side-by-side presentation. For the attributes of appearance, flavour, and general acceptability, there was a significant ($p < 0.05$) preference for the set evaluated *first*. For example, in the evaluation of flavour, the first set was rated higher in 18 of the 24 pairs of scores that were untied. There was neither order bias in the single presentation of raw oysters, nor in any evaluation of the cooked oysters.

Order bias is an unpredictable, idiosyncratic feature of side-by-side presentation (McBride 1986) and might occur for a number of reasons. Notwithstanding its cause, the net effect of order bias is to obscure any genuine difference and thereby desensitise the comparison. Had only the side-by-side condition been employed in this investigation, no significant differences would have been obtained.

Single presentation is not as susceptible to order bias and has the additional advantage of more closely simulating normal eating — people do not routinely taste two species of oyster, side-by-side. Consequently, a difference obtained in the single presentation is more likely to be of practical as well as statistical significance.

One proviso should be noted before generalising from the present results. There is now evidence to suggest that mere exposure to a particular product can induce preference for that product (Zajonc 1968). So, given that panelists in this study — and in the studies cited in the introduction — were Sydney residents, accustomed to the Sydney rock oyster, their predilection is perhaps not surprising. To confirm superiority of the Sydney rock oyster in any 'absolute' sense would require that it also be preferred by consumers accustomed to the Pacific oyster.

Acknowledgement

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The effect of bis-tributyltin oxide (TBTO) and copper on the growth of juvenile Sydney rock oysters *Saccostrea commercialis* (Iredale & Roughley) and Pacific oysters *Crassostrea gigas* Thunberg

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ABSTRACT

The growth of juvenile (spat)(1.25-2.62 mg dry weight) Pacific oysters *C. gigas* and Sydney rock oysters *S. commercialis* was reduced by only 5 ng bis-tributyltin oxide (TBTO) L^{-1} seawater. The reduction in growth of the Pacific oysters was more severe than that of the Sydney rock oysters. When Sydney rock oyster spat were exposed to both copper and TBTO they suffered a greater reduction in growth than those exposed to only one toxicant. There was no significant interaction ($P>0.05$) between the two toxicants, but an additive effect instead. Copper accumulation in Sydney rock oyster spat increased significantly ($P<0.05$) 1.2 times in the presence of 20 ng TBTO L^{-1}.

INTRODUCTION

Annual production of Sydney rock oysters *Saccostrea commercialis* (Iredale & Roughley) in New South Wales (NSW), by far the largest oyster industry in Australia (1), has declined from a peak of 14 million dozen in 1979/81 (2) to 11 million dozen in 1988/1989 (1). This industry is beset by a wide range of problems affecting production (1) and oyster lease experiments demonstrated that tributyltin (TBT) in water was a contributing factor in some estuaries (3). High concentrations of bis-tributyltin oxide (17.5-170.0 TBTO ng L^{-1}) had been reported for the Hawkesbury and Georges Rivers in NSW, two of the main oyster growing estuaries in NSW, which are characterised by high boat densities (4). Shell deformities and reduced tissue weights have been associated with Sydney rock oysters exposed to elevated TBT concentrations (4), although no studies have been conducted to determine the minimum concentration which reduced growth of this species.

The use of antifouling paints containing TBT had a severe impact on the culture of Pacific oysters *Crassostrea gigas* Thunberg in France and the subsequent ban on such paints in 1982 allowed this industry to recover (5, 6). Reduced growth and shell thickening in Pacific oysters, in the presence of elevated concentrations of TBTO has also been reported for many farming areas in the United States (7, 8), England (9) and in Scotland (10). Weight gain of Pacific oyster spat (2.5 g) was reduced and pronounced thickening of the upper shell occurred, at 240 ng TBT L^{-1} (11). Length increases of much smaller (0.02 mm) Pacific oyster spat were

reduced by as little as 20 ng TBT L^{-1} (12), indicating that smaller or younger oysters are more sensitive to TBT in the environment.

Copper based antifouling paints are a commonly used alternative to TBTO based paints. Although copper based paints are less toxic to adult Pacific oysters than TBTO based paints (13), both Sydney rock (14) and Pacific oysters (15) accumulate copper from their environment. In many estuaries both types of paints may have been used on boats and consequently any interaction effects of the two toxic compounds are of interest to oyster farmers.

The objectives of this study were to determine the minimum concentration of TBTO that would significantly reduce the growth of juvenile Sydney rock and Pacific oysters (spat) and to determine the combined effects of TBTO and copper on the growth of Sydney rock oyster spat.

MATERIALS AND METHODS

Three experiments were conducted to examine the effects of TBTO on the growth of oyster spat. All experiments were conducted with spat held in nylon mesh bags in 5 L borosilicate glass beakers filled with 4 L of lightly aerated seawater with a salinity of 35‰ and at a temperature of 24±2°C for a period of four weeks. All beakers were kept in the dark to avoid photolysis of TBTO by ultraviolet light (16). There were four replicate beakers with 40 spat in each for Experiments 1 and 3, and 30 spat in each for Experiment 2. At the start of each experiment oyster spat were graded and four samples of spat were taken to determine initial dry weights. At the end of each experiment, all live spat were counted, dried in a forced draught oven at 110°C for 24h and weighed. Average individual weight gain was calculated on a whole (meat and shell) dry spat basis.

Water in the beakers was changed daily and excess food in the form of the algae Tahitian *Isochrysis* aff. *galbana* Green (clone T. iso; termed Tahitian *Isochrysis*) and *Palova lutheri* Droop (Green) were added to the aquaria to give a final concentration of 150,000 cells mL^{-1} for each species. These species are an excellent food source for Sydney rock oyster larvae (J. A. Nell, unpublished data, 1990). To each beaker, a volume (0 - 40 μL depending on the treatment) of stock solution (10 mg TBTO L^{-1} in ultra pure glacial acetic acid) was added. The volume of added acetic acid was then made up to 40 μL per beaker by adding blank acetic acid. A copper stock solution of 1 g L^{-1} was used for the copper additions in Experiment 3.

The background concentration of TBTO in the seawater and the algal cultures was <1.25 ng L^{-1} (n=4). The background concentration of Cu in the seawater and algal cultures was 17±0.5 and 5.2±1.9 μg L^{-1} (n=4) respectively. The fate of TBTO in 4 beakers containing 20 ng TBTO L^{-1}, treated as other experimental beakers but without spat, over 24 h was determined and a 52% reduction was observed. This test was carried out to determine if the spat were actually exposed to the TBTO concentration that was added to the beakers. Because of their small weight, no TBTO analysis of spat was possible. TBTO in water was determined on samples extracted with tropolone in hexane by atomic absorption spectrophotometry after

hydrization, trapping and thermal desorption (4). Copper analysis of seawater, algal cultures and spat was carried out by atomic absorption spectrophotometry. Because of the small sample weight, the four replicate spat samples were pooled for each treatment in Experiment 3, prior to analysis for copper concentration.

Experiment 1

The effect of TBTO on the growth of Sydney rock oyster spat was determined using concentrations of 0, 5, 10, 15, 20, 25, 50, 75 and 100 ng TBTO L^{-1}.

Experiment 2

A comparison of sensitivity to TBTO between Sydney rock and Pacific oyster spat was carried out using concentrations of 0, 5, 10, 15 and 20 ng TBTO L^{-1}. Spat of the two species were held in different beakers.

Experiment 3

The combined effects of TBTO and copper on the growth of Sydney rock oyster spat were determined using copper concentrations of 0, 8, 16, 32, and 64 μg L^{-1} both with and without an addition of 20 ng TBTO L^{-1}.

STATISTICAL ANALYSIS

Homogeneity of variance was confirmed using the Cochran test (17). Percentage mortalities were transformed using arcsin $x^{0.5}$ and dry weight gain data for Experiments 2 and 3 were transformed using $\log_{10}$ before the data were subjected to analysis of variance (18). A two factor analysis of variance was used in Experiment 3 to determine if there was a significant interaction between TBTO and copper on the growth or mortality of spat. In all experiments mean values were compared using Tukey's *w* or the T-method (18). No statistical comparison was made between the weight gain data for the Sydney rock and Pacific oyster spat in Experiment 2, because of the difference in average initial spat weight for the species. The effect of TBTO on copper concentration in spat (Experiment 3), was assessed with the non-parametric Wilcoxon's signed ranks (two-tailed) test (18). All data are expressed as means ± SD.

RESULTS

Experiment 1

Weight gains of Sydney rock oyster spat decreased with increasing TBTO concentrations (Table 1). There was a large and significant decrease ($P<0.05$) in growth (39%) with the addition of only 5 ng TBTO L^{-1}. Growth reductions at higher TBTO concentrations were more gradual. There was no significant treatment effect on mortality ($P>0.05$) and the average mortality was 7±3%.

Experiment 2

There was a large and significant decrease ($P<0.05$) in growth of spat of both species with the addition of only 5 ng TBTO L^{-1} (Table 2). The decrease was 58% for Sydney rock and 79% for Pacific oyster spat. Weight gains of both Sydney rock and Pacific oyster spat decreased with increasing TBTO concentrations, although the differences from 5 to 20ng TBTO L^{-1} were not significant ($P>0.05$). There was no significant effect ($P>0.05$) of TBTO concentration on the mortality of the spat of either species. The mortality of Pacific oyster spat ($16\pm14\%$) was, however, significantly higher ($P<0.05$) than that of the Sydney rock oyster spat ($1\pm3\%$).

Experiment 3

For all copper concentrations tested, growth of Sydney rock oyster spat was reduced with the addition of 20 ng TBTO L^{-1} (Table 3). Weight gains decreased with increasing copper concentrations. Weight gains of spat exposed to the two toxicants were reduced more than those exposed to only one. The addition of 20 ng TBTO L^{-1} decreased growth by 66%, but when 8 μg Cu L^{-1} was added they were reduced by 74%. Copper accumulation in spat increased significantly ($P<0.05$) 1.2 times in the presence of 20 ng TBTO L^{-1} (Table 4). In the absence of TBTO, copper concentrations in spat increased from 29 to 307 mg kg^{-1} with increasing copper concentrations in seawater from 0 to 64 μg L^{-1} (Table 4). In the presence of 20ng TBTO L^{-1} copper concentrations in spat increased from 41 to 370 mg kg^{-1}. There was no significant interaction ($P>0.05$) between copper and TBTO on weight gain, but there was an additive effect. Both copper and TBTO reduced weight gains of oyster spat, but if the two toxicants were added together, weight gains in oyster spat were reduced more than those exposed to only one toxicant. The effects of a low concentration of TBTO (5 ng L^{-1}) caused a much greater reduction in weight gains of oyster spat than a much higher concentration of copper (8 μg L^{-1}; Table 3). There was no significant effect on mortality ($P>0.10$) and the average mortality rate was $5\pm4\%$.

DISCUSSION

The concentration of TBTO (5 ng L^{-1}) required to produce a significant reduction of weight gain in small Sydney rock and Pacific oyster spat was much lower than the smallest concentration previously reported to reduce growth of Pacific oyster spat (11, 12). The smaller Sydney rock and Pacific oyster spat used here (1.25-2.62mg) compared with those used by others (11, 12) (0.15-2.5g) are likely to have been more sensitive. Although it should be noted that during this study oysters were cultured for maximum growth at a relatively high temperature ($24\pm2°C$) and fed to excess.

There were relatively large differences in the weight gains of spat amongst the three experiments. Weight gains of Sydney rock oyster spat on the control treatment (no added TBTO or copper) were 5.38, 30.4 and 5.42mg for experiments 1, 2 and 3 respectively. These differences could only be partly explained by differences in initial weights indicating that more research about the factors

controlling the growth of oyster spat is required.

The concentration of TBTO (5 ng L^{-1}) which produced a significant reduction in growth of both Sydney rock and Pacific oyster spat was much lower than the high TBTO concentrations (17.5-170 ng L^{-1}) reported for the Hawkesbury and Georges Rivers in NSW (4). This may help to explain, the reduction in production of Sydney rock oysters in NSW from a peak of 14 million dozen in 1979/81 (2) to 11 million dozen in 1988/1989 (1).

Although growth of Sydney rock and Pacific oyster spat were shown to be reduced by as little as 5 ng TBTO L^{-1}, in fact the spat would have been exposed to much lower concentrations than this as it was demonstrated that there was a 52% reduction in the TBTO concentration in experimental beakers without spat within a 24 h period. This reduction in TBTO concentration in the beakers was presumably due to adsorption (16) and is often overlooked.

Pacific oyster spat appeared to be more sensitive to TBTO as they suffered a much larger reduction (79%) in weight gain than Sydney rock oysters (58%) when exposed to 5ng TBTO L^{-1} (Table 2). Pacific oysters, exposed to the same TBTO concentrations on a commercial oyster lease, displayed 2-3 times the TBTO concentrations in their meat than Sydney rock oysters (4). The greater reduction in weight gain in Pacific oysters may be related to the higher accumulation of TBTO in their meats.

The additive effect of copper and TBTO in reducing the growth of Sydney rock oysters could be an important factor in oyster growing areas with high boat densities. Although copper based antifouling paints are less toxic to adult Pacific oysters than TBTO based paints (13), low copper concentrations (LC_{50} 33 μg L^{-1}) are still toxic to American oyster *C. virginica* (Gemlin) larvae. As copper accumulates in the soft tissue rather than in the shell of oysters (19), high copper concentrations in the water of oyster growing areas is undesirable.

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TABLE 1

Effects of bis-tributyltin oxide (TBTO) concentration in seawater on the weight gain of Sydney rock oyster (*Saccostrea commercialis*) spat over 4 weeks. Experiment 1

TBTO concentration (ng L^{-1})	Whole spat dry weight gain[1] (mg)
0	5.38 ± 1.04^{a}
5	3.27 ± 1.04^{b}
10	2.94 ± 0.32^{bc}
15	2.53 ± 0.55^{bc}
20	2.87 ± 0.49^{bc}
25	2.65 ± 0.26^{bc}
50	2.29 ± 0.92^{bc}
75	1.85 ± 0.30^{bc}
100	1.54 ± 0.22^{c}

ANOVA

Source	SS	df	MS	F
Between groups	38.9653	8	4.8707	11.37
Within groups	11.5620	27	0.4282	
Total	**50.5273**	**35**		

[1] Means ± SD. Means with a common superscript do not differ significantly ($P>0.05$). Initial average whole dry weight of the spat was 1.25 ± 0.11 mg.

TABLE 2

Comparison of sensitivity to bis-tributyltin oxide (TBTO) between Sydney rock (*Saccostrea commercialis*) and Pacific oyster (*Crassostrea gigas*) spat over 4 weeks. Experiment 2

TBTO concentration (ng L^{-1})	Whole spat dry weight gain[1] (mg)	
	Sydney rock oyster	Pacific oyster
0	30.4±3.5[a]	27.1±11.7[a]
5	12.9±3.7[b]	5.7± 1.6[b]
10	11.7±2.3[b]	6.0± 1.3[b]
15	9.7±2.4[b]	5.9± 1.0[b]
20	9.6±1.9[b]	4.2± 1.3[b]

ANOVA Sydney rock oysters

Source	SS	df	MS	F
Between groups	118.9450	4	29.7363	3267.71
Within groups	0.1360	15	0.0091	
Total	**119.0810**	**19**		

ANOVA Pacific oysters

Source	SS	df	MS	F
Between groups	1.5823	4	0.3956	26.03
Within groups	0.2276	15	0.0152	
Total	**1.8099**	**19**		

[1] Means ± SD. Within each column, means with a common superscript do not differ significantly ($P>0.05$). Initial average whole dry weights of Sydney rock and Pacific oyster spat were 2.62±0.14 and 1.48±0.15 mg respectively. For statistical analyses a $\log_{10}$ transformation of the dry weight gain data was used.

TABLE 3

Effects of copper and bis-tributyltin oxide (TBTO) on weight gain in Sydney rock oyster (*Saccostrea commercialis*) spat over 4 weeks. Experiment 3

Copper concentration ($\mu g\ L^{-1}$)	Whole spat dry weight gain[1] (mg)	
	0 ng TBTO L^{-1}	20 ng TBTO L^{-1}
0	5.42 ± 1.96^{a}	1.85 ± 0.37^{c}
8	5.03 ± 3.09^{b}	1.41 ± 0.21^{c}
16	3.94 ± 0.85^{ab}	1.56 ± 0.14^{c}
32	2.34 ± 0.87^{bc}	1.22 ± 0.28^{c}
64	0.36 ± 0.09^{d}	0.26 ± 0.08^{d}

ANOVA

Source	SS	df	MS	F
TBTO	1.2603	1	1.2603	74.14
Copper	5.5340	4	1.3835	81.38
TBTO x Copper	0.1632	4	0.0400	2.40
Pesidual	0.5101	30	0.0170	
Total	**7.4676**	**39**		

[1] Means ± SD. Means with a common superscript do not differ significantly ($P>0.05$). Initial average whole dry weight of spat was 1.33 ± 0.04 mg. For statistical analyses a $\log_{10}$ transformation of the dry weight gain data was used.

TABLE 4

Effects of bis-tributyltin oxide (TBTO) concentration in seawater on copper accumulation by Sydney rock oyster (*Saccostrea commercialis*) spat over 4 weeks. Experiment 3

Copper concentration in seawater (μg L^{-1})	Copper concentration in whole dry spat[1] (mg kg^{-1})	
	0 ng TBTO L^{-1}	20 ng TBTO L^{-1}
0	29	41
8	95	111
16	149	174
32	246	263
64	307	370

HYBRIDISATION OF SYDNEY ROCK OYSTERS (*Saccostrea commercialis*) WITH PACIFIC OYSTERS (*Crassostrea gigas*)

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ABSTRACT

Crosses were attempted between Sydney rock oyster (*Saccostrea commercialis*) males x Sydney rock oyster females, Sydney rock oyster males x Pacific oyster (*Crassostrea gigas*) females, and Pacific oyster males x Pacific oyster females and Pacific oyster males x Sydney rock oyster females. Fertilisation and development to "D" stage occurred in the pure species controls. No viable larvae were observed in the cross using Pacific oyster sperm and Sydney rock oyster eggs. D-stage larvae were produced by the cross using Sydney rock oyster sperm and Pacific oyster eggs, however electrophoretic analysis of the larvae showed only loci characteristic of Pacific oyster larvae. It is concluded that it is unlikely that a true cross occurred, and that the larvae may have been the result of gynogenesis induced in the Pacific oyster eggs by the Sydney rock oyster sperm.

INTRODUCTION

Fertilisation between oysters of different species has been reported for six attempted crosses (Table 1), but the development of metamorphosed oysters or settlement of spat was reported for only three crosses. Successful hybridisation was also reported for a cross between *Crassostrea gigas* and *C. angulata* (Imai and Sakai, 1961), but it was subsequently demonstrated that they are the same species (Menzel, 1974).

Fertilization without metamorphosis for a cross between Pacific oysters *C. gigas* and New Zealand rock oysters *Saccostrea glomerata* (*S. glomerata* is considered to be a subspecies of *S. commercialis*; Buroker *et al*, 1979) was reported by Menzel (1986). A successful cross between *S. glomerata* and *C. gigas* was reported by Dinamani and Swindlehurst (1982). This was later refuted when electrophoretic examination of the alleged hybrids demonstrated that some were "pure" New Zealand rock oysters (*S. glomerata*) while others were "pure" Pacific oysters (Smith, 1985). An experiment was carried out to determine whether or not Sydney rock and Pacific oysters could be successfully hybridised.

MATERIALS AND METHODS

Two each of male and female Sydney rock and Pacific oysters were selected and stripped of their gametes. Gametes were obtained by stripping, because eggs obtained from natural

spawnings are invariably contaminated with sperm (Mason, 1986). Stripping of ripe oysters does not affect the viability of the eggs. Larvae produced from eggs obtained by stripping have been reared successfully to metamorphosis (J. A. Nell, personal observation, 1989). Eggs and sperm were kept separate to prevent premature or accidental fertilisation. The mixing of eggs and sperm for fertilisation was arranged according to a Latin sqaure design (Sokal and Rohlf, 1981) and took place within 2h after stripping. The mixtures of eggs and sperm were stocked in 40l aerated tanks filled with seawater with a salinity of 30‰ maintained at 26°C. Larvae were examined microscopically 24h after mixing of eggs and sperm. A sample of eggs from each female oyster was kept aside for 24h to check for self-fertilisation or contamination with sperm.

Viable D-stage larvae (Table 1) were transferred to a 1000l tanks 24h after fertilisation and maintained as above. They were fed on equal mix of the algae Pavlova lutheri and Isochrysis galbana at 10,000 cells/ml per day. Viable D-stage larvae were collected on nylon mesh, washed with distilled water and frozen in liquid nitrogen, 3 days after fertilisation.

RESULTS

No self-fertilisation nor contamination with sperm was observed in the eggs after 24h. Viable D-stage larvae were obtained from all within species fertilisation attempts and also from those between the Pacific oyster females and the Sydney rock oyster males. All of these treatments developed normally when fed for 2 days. In two out of four attempts some fertilisation and larval development was observed as a result (Table 2) of the mixing of Sydney rock oyster eggs with the sperm of Pacific oysters. The few larvae had all died after 24h.

Electrophoretic examination of larvae resulting from the mixing of Pacific oyster eggs with Sydney rock oyster sperm showed only Pacific oyster genotypes (appendix 1).

DISCUSSION

The sperm of the Pacific oysters may have fertilised some of the Sydney rock oyster eggs (Table 2), but no viable larvae developed. Larvae of this cross were produced by Menzel (1986) who also failed to rear them to metamorphosis.

The larvae produced from the fertilisation of Pacific oyster eggs by Sydney rock oyster sperm were shown to be of Pacific oyster genotype. These larvae may have been the result of parthenogenesis, as accidental fertilisation by sperm of Sydney rock oysters was unlikely because a cross check of the controls showed no fertilisation. Gynogenesis of C. virginica eggs with X-irradiated sperm was observed by Stiles (1978).

TABLE 1

Hybridisation of oysters

	Fertilisation and metamorphosis	
C. gigas x C. virginica		(Menzel, 1986)
C. angulata x C. rizophorae		(Menzel, 1986)
C. angulata x C. virginica		(Menzel, 1986)
	Fertilisation without metamorphosis	
C. gigas x C. rivularis		(Imai and Sakai, 1961)
C. gigas x C. echinata		(Imai and Sakai, 1961)
C. gigas x S. glomerata		(Menzel, 1986)

TABLE 2

Two day old larvae produced[1] from crossing attempt of Sydney rock oysters[2] (Saccostrea commercialis) with Pacific oysters[3] (Crassostrea gigas)

Attempted fertilisation Male	Female	Larvae development
PO(1)	x PO	Viable D stage larvae
"	x PO	" " " "
"	x SRO	" " " "
"	x SRO	" " " "
PO(2)	x PO	" " " "
"	x PO	" " " "
"	x SRO	" " " "
"	x SRO	" " " "
SRO(1)	x PO	some larvae but not viable
"	x PO	no larvae
"	x SRO	viable D stage larvae
"	x SRO	" " " "
SRO(2)	x PO	no larvae
"	x PO	some larvae but not viable
"	x SRO	viable D stage larvae
"	x SRO	" " " "

1 Larvae were examined 24h after mixing sperm and eggs
2 SRO is Sydney rock oyster
3 PO is Pacific oyster

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SECTION 3

PACIFIC OYSTER ABUNDANCE AND SPATFALL STUDIES

PACIFIC OYSTER (Crassostrea gigas) ABUNDANCE SURVEY OF PORT STEPHENS, NSW

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ABSTRACT

The number of Pacific oyster (Crassostrea gigas) spat caught on sticks in the inner harbour of Port Stephens increased from 0.6/10 sticks in 1986 to 112.8/10 sticks in 1988. There were estimated to be 25 million Pacific oysters in Port Stephens in July 1988. The number of Pacific oysters found on the caught sticks in the outer harbour of Port Stephens increased from 0.07/10 sticks in 1986 to 6.8/10 sticks in 1989.

INTRODUCTION

Pacific oysters (Crassostrea gigas) were first found in Port Stephens NSW in 1973 (Wolf and Medcof, 1974). Although the numbers found in 1973 were very small, Medcof and Wolf (1975) said that when a breeding colony of Pacific oysters has established itself in an estuary it is simply impossible to eradicate. From 1974-1984 only a few Pacific oysters were seen in Port Stephens from time to time (J A Nell, personal communication with local oyster farmers, 1989). Large numbers of newly caught Pacific oyster spat suddenly appeared in Big Swan Bay in the inner harbour of Port Stephens in November 1984 (Holliday and Nell, 1985). Spat were found to settle from November to May (Holliday and Nell, 1985) and there was a larger spatfall observed in the inner harbour of Port Stephens in the summer of 1985/86.

The appearance of large numbers of Pacific oysters in Port Stephens was of great concern to the NSW oyster industry as Port Stephens is a very important supplier of spat and other oysters for on-growing in other estuaries (Espinas et al., 1988). Spat caught in Port Stephens account for approximately 85% of the oyster production in New South Wales (Anonymous, 1986).

The concern about Pacific oysters is best illustrated by the rapid explosion of the oyster in the North Island of New Zealand, which has a similar latitude to New South Wales. The Pacific oyster was accidentally introduced into New Zealand around the late 1960's (Dinamani, 1971). By the latter half of the 1970's, the oyster had spread rapidly to all oyster growing estuaries, leading to the almost total replacement of the native rock oyster by the newly arrived Pacific oyster on farms by 1978 (Dinamani, 1987).

Because of the concern about the spread of Pacific oysters, comprehensive surveys in Port Stephens, NSW were carried out in 1986, 1987 and 1988. A survey restricted to the catching leases in the outer harbour of Port Stephens was carried out in 1989.

MATERIALS AND METHODS

The surveys were carried out during the months of July and August each year. This was considered to be the most suitable time, when the catch of spat of Pacific oyster (J A Nell, personal observations from 1986-1989) and Sydney rock oysters (Holliday and Goard, 1988) was finished and before caught and depot sticks (Malcolm, 1987) were moved by farmers.

The number of Pacific oysters on sticks and trays were counted and recorded on data sheets (Appendix 1). If in doubt oysters were opened and the shells examined for the presence or absence of hinge teeth. Sydney rock oysters (Saccostrea commercialis) have hinge teeth on both shells, whereas Pacific oysters do not have any (Holliday and Nell, 1985).

The 1986 survey was an exercise in identifying the heavily infested areas of the inner harbour of Port Stephens. More attention was given to the heavily infested areas than to the less infested areas. This survey was therefore not a representative survey for the whole of the inner harbour and the average number of Pacific oysters for the depot and nailed out sticks of the inner harbour were inflated. A total of 24,745 sticks were examined in 1986. Blocks of sticks were not always opened for examination.

Great care was taken with both the 1987 and the 1988 survey to ensure that it was representative for the whole of Port Stephens. All areas of the port were examined with equal effort allocated to both the heavily infested and the less infested areas. The number of sticks examined in 1987 and 1988 were 9,828 and 7,558 respectively. Blocks of sticks were opened and sticks examined individually.

The 1989 survey was restricted to the caught sticks in the outer harbour of Port Stephens. Blocks of sticks were opened and sticks (2,688) examined individually.

RESULTS

The 1986 survey showed a very heavy catch of Pacific oysters on the depot sticks in the inner harbour of Port Stephens (Table 1 and 5), where there was an average of 99.3 Pacific oysters per 10 depot sticks (Table 1 and 5). In 1986 the numbers on the caught sticks were 0.07 (Table 4) and 0.6 (Table 5) for the outer and inner harbours respectively. The average number of Pacific oysters on the depot and nailed out sticks in 1986 were inflated (Table 1 and 5) as too much emphasis was placed on the heavily infested areas.

The 1987 survey (Table 2) showed a large increase in the number of Pacific oysters on caught sticks (Table 4 and 5), compared to 1986. The number of Pacific oysters on the caught sticks in the inner harbour had increased from 0.6/10 sticks in 1986 to 6.3/10 sticks in 1987 (Table 5). A large increase was also observed on the caught sticks in the outer harbour where the number of Pacific oyster increased from 0.07/10 sticks in 1986 to 4.8/10 sticks in 1987 (Table 4). The average number of Pacific oysters on the depot sticks in 1987 (Table 2; 8.9/10 sticks) was much lower than that in 1986 (Table 1; 99.3/10 sticks). The average number of Pacific oysters on the nailed out sticks in 1987 (Table 2; 6.9/10 sticks) was much less than that in 1986 (Table 1; 30.5/10 sticks).

The 1988 survey (Table 3) showed a dramatic increase in the number of Pacific oysters on the caught sticks of the inner harbour (Table 5) as compared to those in 1987 and 1986. The number of Pacific oysters on the caught sticks in the inner harbour had increased from 6.3/10 sticks in 1987 to 112.8/10 sticks in 1988 (Table 5). The number of Pacific oysters on the caught sticks in the outer harbour however, had decreased from 4.8/10 sticks in 1987 to 0.9/10 sticks in 1988 (Table 4). The number of Pacific oysters in the inner harbour (Table 5) on the depot sticks (56.1/10 sticks) and nailed-out sticks (48.6/10 sticks) in 1988 were much higher than those on the depot (8.9/10 sticks) and nailed-out sticks (7.2/10 sticks) in 1987. There were estimated to be 25 million Pacific oysters in Port Stephens in July 1988, based on an average of 5.0 Pacific oyster per depot and nailed-out stick and there being 5 million of sticks in Port Stephens.

The 1989 survey (Table 6) showed a substantial increase in the number of Pacific oysters on the northern shore of Port Stephens compared to the period from 1986-1988. The numbers of Pacific oysters on the southern shore of Port Stephens were much greater than those in 1988, but not as high as those in 1987.

DISCUSSION

The Pacific oyster in Port Stephens, NSW has become well established since it was first found in large numbers in November, 1984 (Holliday and Nell, 1985). Pacific oysters have also established themselves on the rocks and the mangroves on the foreshores of the inner harbour of Port Stephens (J A Nell, personal observations, 1989). The number of Pacific oysters on caught sticks in the inner harbour has increased dramatically from 0.6/10 sticks in 1986 to 112.8/10 sticks in 1989 (Table 5). Medcof and Wolf (1975) believed that once a breeding colony of Pacific oysters has established itself in an estuary it is humanly impossible to eradicate it.

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TABLE 1

Port Stephens Pacific oyster (Crassostrea gigas) abundance survey (July - August, 1986)

	Oysters per stick					
	Caught		Depot		Nail-out	
Site	Pacific Oysters	Sticks	Pacific Oysters	Sticks	Pacific Oysters	Sticks
Inner harbour						
Soldiers Point					53	20
" "					8	20
Tilligery Creek					47	10
Little Swan Bay			27	1.5		
" "			62	11		
Big Swan Bay					23	7.5
" "					259	15
" "			296	8		
Wirrung Island	170	2880	176	36		
Outer harbour						
Corlette	0	1170				
Wanda Point	25	1050				
Salamander Bay	15	4960				
" "	24	4158				
" "	29	1452				
" "	0	1750				
Kore Kore Creek					0	20
Corrie Island	26	2200				
Duckhole Creek	1	2290				
Pig Station Creek	28	2166				
Hawkes Nest Bridge					14	40
" "	2	480				
Total	320	24556	561	56.5	404	132.5
Average	0.1/10 sticks		99.3/10 sticks		30.5/10 sticks	

Adapted from D Reid, 1986.

TABLE 2 Port Stephens - Pacific oyster (*Crassostrea gigas*) abundance survey (July 6-9, 1987)

Site	Caught sticks		Depot sticks		Nailed-out sticks		Trays	
	Pacific Oysters	Sticks	Pacific Oysters	Sticks	Pacific Oysters	Sticks	Pacific Oysters	Trays
Outer harbour								
Myall River	101	554	-	-	60	78	52	30
Corrie Island	79	844	-	-	-	-	-	-
Pindimar	307	1688	-	-	25	210	-	-
Salamander Bay, Wanda Head								
Kangaroo Point	1596	1254	-	-	-	-	-	-
Inner harbour								
Dowardee Is. (Soldiers Point)	34	100	6	48	1	72	6	6
Shag Is. One Tree Is.								
(Rocks Awash)	-	-	-	-	60	228	-	-
Cromarty Bay	-	-	18	51	24	44	-	-
Tilligery Creek	-	-	122	278	339	143	0	4
Snapper Island	-	-	-	-	534	370	-	-
Big Swan Bay	-	-	-	-	401	186	0	2.5
Cockle Shell Pt to Carcair Pt	-	-	60	102	56	34	0	3
Little Swan Bay & Reedy Creek	-	-	344	467	163	151	-	-
North Arm Cove	-	-	114	150	750	1349	29	1
North Arm Cove to Karuah River	-	-	91	150	53	522	3	1
Karuah River	-	-	524	185	144	374	302	10
Wirrrung Island	107	114	39	50	5	32	-	-
Total	2224	4554	1318	1481	2615	3793	392	57.5
Average	4.9/10 sticks		8.9/10 sticks		6.9/10 sticks		6.8/tray	

TABLE 3 Port Stephens - Pacific oyster (Crassostrea gigas) abundance survey (27-6-88 to 13-7-88)

Site	Caught sticks		Depot sticks		Nailed-out sticks		Trays	
	Pacific Oysters	Sticks	Pacific Oysters	Sticks	Pacific Oysters	Sticks	Pacific Oysters	Trays
Outer harbour								
Myall River	6	330	-	-	2	85	-	1
Corrie Island	4	578	-	-	-	-	-	-
Pindimar	41	1536	7	40	51	53	-	-
Salamander Bay, Wanda Head & Kangaroo Point	368	2170	-	-	-	-	-	-
Inner harbour								
Dowardee Is. (Soldiers Point)	-	-	45	72	22	12	-	-
Shag Is. One Tree Is. (Rocks Awash)	-	-	-	-	32	15	-	-
Cromarty Bay	-	-	996	80	72	39	-	-
Tilligery Creek	920	120	2453	102	167	56	-	-
Snapper Island	-	-	0	95	1159	111	-	-
Big Swan Bay	-	-	3630	373	460	30	-	-
Cockle Shell Pt to Carcair Pt	-	-	-	-	717	164	11	12
Little Swan Bay & Reedy Creek	-	-	1036	453	457	128	-	-
North Arm Cove	-	-	140	121	408	135	58	2
North Arm Cove to Karuah River	-	-	50	60	324	137	35	1
Karuah River	-	-	150	117	115	79	0	10
Wirrung Island	546	10	985	218	710	49	4	2
Total	1885	4744	9492	1731	4696	1083	108	28
Average	4.0/10 sticks		54.8/10 sticks		43.4/10 sticks		3.9/tray	

TABLE 4

Pacific oysters (Crassostrea gigas) in the outer harbour of Port Stephens

	oysters/10 sticks		
Year	Caught	Depot	Nailed-out
1986	0.07	No data	2.3
1987	4.8	No data	3.0
1988	0.9	1.8	3.8
1989	6.8	No data	No data

TABLE 5

Pacific oysters (Crassostrea gigas) in the inner harbour of Port Stephens

	oysters/10 sticks		
Year	Caught	Depot	Nailed-out
1986	0.6	99.3*	53.8*
1987	6.3	8.9	7.2
1988	112.8	56.1	48.6

* Overestimated

TABLE 6

Pacific oysters (Crassostrea gigas) in the outer harbour of Port Stephens

	spat/10 caught sticks	
Year	Northern shore	Southern shore
1986	0.08	0.06
1987	1.6	12.7
1988	0.2	1.7
1989	4.5	11.8

Appendix 1

DATE: _____ NAME OF SURVEYORS: _____ ESTUARY: _____

Location	Lease No.	Position on Lease	Category	No./Unit	Size of P.O.	Catch of S.R.O*	Comments

Position on Lease

Lease front

Mid Lease

Rear lease (closest to shore)

Category

1. Caught
2. Depots
3. Nailed-outs
4. Trays
5. Tumblers

Catch of S.R.O.

None
Light
Medium
Heavy

* Catching leases only
- Count all P.O. on all sides of sticks.
- Give No. of P.O. per full length stick or whole tray or tumbler.

PACIFIC OYSTER (CRASSOSTREA GIGAS) SPATFALL SURVEY OF PORT STEPHENS, NSW.

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ABSTRACT

The survey clearly showed that the vast majority of Pacific oyster (Crassostrea gigas) spat are caught in the more estuarine inner harbour and that most Sydney rock oyster (Saccostrea commercialis) spat are caught in the more oceanic outer harbour of Port Stephens. The main spatfall season of the Pacific oysters in Port Stephens occurred from November to May, but some light settlements were recorded throughout the year.

INTRODUCTION

The first sighting of the Pacific oyster (Crassostrea gigas) in New South Wales was reported from the Pambula River in 1967 (Wolf and Medcof, 1974; Medcof and Wolf, 1975). The first report of sightings in Port Stephens was made in 1973 (Wolf and Medcof, 1974; Medcof and Wolf, 1975). Farmers were advised by Medcof and Wolf (1975), that once Pacific oysters are established as a natural population in an area, they would be impossible to eradicate. The first sighting of large numbers of Pacific oyster spat was made in Big Swan Bay in the inner harbour of Port Stephens, NSW in the spring of 1984 (Holliday and Nell, 1985). Serious concern about the spread of Pacific oysters in Port Stephens was raised in the spring of 1985 when sightings of Pacific oyster spatfall became more numerous. The Pacific oyster spatfall survey of Port Stephens using stacks of slurry coated (Table 1) conical discs was established in the same year with the cooperation of oyster farmers.

MATERIALS AND METHODS

Stacks of slurry (Table 1) coated conical PVC discs of 350mm diameter (ARMEP, Lane Industrielle, 54603 Auzay Cedex, France) were used to monitor the spatfall of Pacific oysters in Port Stephens, NSW. The initial survey from December, 1985 to January 1986 was restricted to one stack of 7-12 discs at 13 sites in the inner harbour of Port Stephens (Fig 1). This survey was subsequently extended with an additional 6 sites in the outer harbour of Port Stephens from January, 1986 to May, 1986 (Fig 1). No work was carried out on the survey from May, 1986 to October, 1986 when it was recommenced with 3 stacks of 8 discs placed in the water at least one month apart at 20 sites in Port Stephens (Fig 2).

The total number of Pacific oyster and Sydney rock oyster (Saccostrea commercialis) spat (>5mm) were counted. Only those spat positively identified as Pacific oysters were included in the data. This often involved opening some

oysters for correct identification ie. hinge teeth on shells of Sydney rock oysters and no hinge teeth on shell of Pacific oysters (Holliday and Nell, 1985).

RESULTS

Spatfall of Pacific oyster spat in the inner harbour was much greater than that of the outer harbour (Tables 2 and 3), whereas Sydney rock oyster spat settled more heavily in the outer harbour (Tables 4 and 5). During the survey, the main spatfall period for Pacific oysters was November - May, but light catches were also recorded outside this period (Tables 2 and 3).

DISCUSSION

The Pacific oyster has become well established in Port Stephens since its first reported sighting in 1973 (Wolf and Medcof, 1974). However, industry concern was raised only after large numbers were reported in Port Stephens on the Sydney rock oyster crop (Holliday and Nell, 1985). A survey of all the bays in Port Stephens (J A Nell, personal observation, 1988) showed that they were spread throughout the Port and that they were also found on the mangroves and the rocks of the foreshores of the inner harbour. In 1988, the number of Pacific oysters in Port Stephens was estimated to have reached 26 million (Nell, 1989). It is clear that a large breeding population of Pacific oysters exists in Port Stephens. Medcof and Wolf (1975) considered that once such a breeding population was established, it could not be possible to eradicate the species.

A spatfall survey of the other major oyster growing estuaries in NSW (Wallis Lake, Brisbane Water, Georges and Hawkesbury Rivers) found only a few locally caught Pacific oyster spat over the survey period 1986-1988 (J. A. Nell, unpublished data, 1989). A similar result was obtained from a survey of the minor oyster growing estuaries (Lake Merimbula, Wagonga Inlet, Nambucca, Macleay, Hastings, Manning, Crookhaven and Clyde Rivers) over the survey period 1986 -1987 (J. A. Nell, unpublished data, 1989). It would appear that during the above spatfall periods, no major breeding population of Pacific oysters had become established in any of the above estuaries. Although Pacific oysters were first found in the southern growing estuaries of NSW (Wolf and Medcof, 1974), no explanation can be given as to why they have established large numbers in Port Stephens and not elsewhere in NSW.

The large number of Pacific oyster spat caught in Port Stephens (Tables 2 and 3), are of great concern. A similar situation existed in the North Island of New Zealand in the late 1960's (Dinamani, 1971), but by 1978, the newly arrived Pacific oyster had replaced the rock oyster (S. glomerata) on the farms (Dinamani, 1987). The rock oyster still exists in the oyster growing areas in New Zealand, with the Pacific oyster occupying the lower catching zone (Curtin, 1986). The North Island of New Zealand has a similar latitude to New South Wales. However, there is a difference between the New

Zealand approach to the Pacific oyster problem. In New Zealand, no attempt was made to control Pacific oysters (P. Dinamani, personal communication, 1987), but in New South Wales, strict control measures are in place (Anonymous, 1989)

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TABLE 1

Slurry mixture composition

Ingredients	Parts
Hydrated lime	5.0
Cement	1.5
Fireclay	1.0
Sand	1.5

TABLE 2 Pacific oyster (Crassostrea gigas) spatfall survey of Port Stephens (inner harbour)

Period	Number (mean ± S.D.) of spat per stack of slurried discs (350mm diam)				
	1985	1986	1987	1988	1989
Jan - Mar		370 ± 428(13)[2]			
Jan - Apr			108 ± 263(12)		181 ± 236(12)
Feb - May			12 ± 18(11)	531 ± 614(11)	59 ± 114(12)
Mar - May		0 (13)[2]			
Mar - Jun			<1 (11)		
Apr - Aug			<1 (11)		
Jun - Sep				39 ± 58(11)	
Jul - Oct				3 ± 3(12)	
Jul - Nov			7 ± 10(11)		
Oct - Dec		1 ± 4(12)		13 ± 17(12)	
Oct - Jan			57 ± 73(11)		
Nov - Jan				171 ± 429(12)	
Nov - Mar		101 ± 262(12)			
Dec - Jan	171 ± 174(12)[2]				
Dec - Mar			488 ± 497(11)	284 ± 1(12)	

1 The number of sites per period is shown in brackets.
2 These counts were based on 7 - 12 discs per stack, and others were based on 8 discs per stack.

TABLE 3 Pacific oyster (Crassostrea gigas) spatfall survey of Port Stephens (outer harbour)

Period	Number (mean ± S.D.) of spat per stack of slurried discs (350mm diam)			
	1986	1987	1988	1989
Jan - Mar	2 ± 2(6)[2]			
Jan - Apr		12 ± 19(8)		16 ± 17(7)
Feb - May		4 ± 4(8)	2 ± 2(7)	5 ± 5(7)
Mar - May	<1 (5)[2]			
Mar - Jun		1 ± 1(8)		
Apr - Aug		< ± (8)		
Jun - Sep			5 ± 4(7)	
Jul - Oct			0 (7)	
Jul - Nov		0 (8)		
Oct - Dec	70 (8)		<1(7)	
Oct - Jan		1 ± 2(6)		
Nov - Jan			2 ± 1(7)	
Nov - Mar	1 ± 1(8)			
Dec - Jan				
Dec - Mar		11 ± 16(7)	11 ± 11(7)	

1 The number of sites per period is shown in brackets.
2 These counts were based on 7 - 12 discs per stack, and others were based on 8 discs per stack.

TABLE 4 Sydney rock (Saccostrea commercialis) oyster spatfall survey of Port Stephens (inner harbour)

Period	Number (mean ± S.D.) of spat per stack of slurried discs (350mm diam)				
	1985	1986	1987	1988	1989
Jan - Mar		1016 ± 1213(13)[2]			
Jan - Apr			62 ± 56(12)		212 ± 63(7)
Feb - May			1 ± 1(11)	53 ± 60(11)	489 ± 421(12)
Mar - May		28 ± 54(13)[2]			
Mar - Jun			36 ± 37(11)		
Apr - Aug			23 ± 68(11)		
Jun - Sep				270 ± 371(11)	
Jul - Oct				172 ± 180(11)	
Jul - Nov			5 ± 7(11)		
Oct - Dec		<1 (12)		11 ± 18(12)	
Oct - Jan			14 ± 10(11)		
Nov - Jan				33 ± 38(12)	
Nov - Mar		16 ± 19(12)			
Dec - Jan	128 ± 298(12)[2]				
Dec - Mar			141 ± 272(11)	2 ± 1(12)	

1 The number of sites per period is shown in brackets.
2 These counts were based on 7 - 12 discs per stack, and others were based on 8 discs per stack.

TABLE 5 Sydney rock oyster (Saccostrea commercialis) spatfall survey of Port Stephens (outer harbour)

Period	Number (mean ± S.D.) of spat per stack of slurried discs (350mm diam)			
	1986	1987	1988	1989
Jan - Mar	9437 ± 10648(6)[2]			
Jan - Apr		442 ± 33(8)		1131 ± 1140(7)
Feb - May		472 ± 274(8)	313 ± 243(7)	1930 ± 1805(7)
Mar - May	23 ± 52(5)[2]			
Mar - Jun		399 ± 209(8)		
Apr - Aug		1182 ± 1911(8)		
Jun - Sep			887 ± 458(7)	
Jul - Oct			500 ± 210(7)	
Jul - Nov		27 ± 54(8)		
Oct - Dec	70 ± 13(8)		1 ± 2(7)	
Oct - Jan		49 ± 54(6)		
Nov - Jan			342 ± 297(7)	
Nov - Mar	377 ± 255(8)			
Dec - Jan				
Dec - Mar		287 ± 197(7)	723 ± 528(7)	

1 The number of sites per period is shown in brackets.
2 These counts were based on 7 - 12 discs per stack, and others were based on 8 discs per stack.

FIG 1. Pacific oyster (Crassostrea gigas) Spatfall monitoring sites in Port Stephens from 1985 - 1986

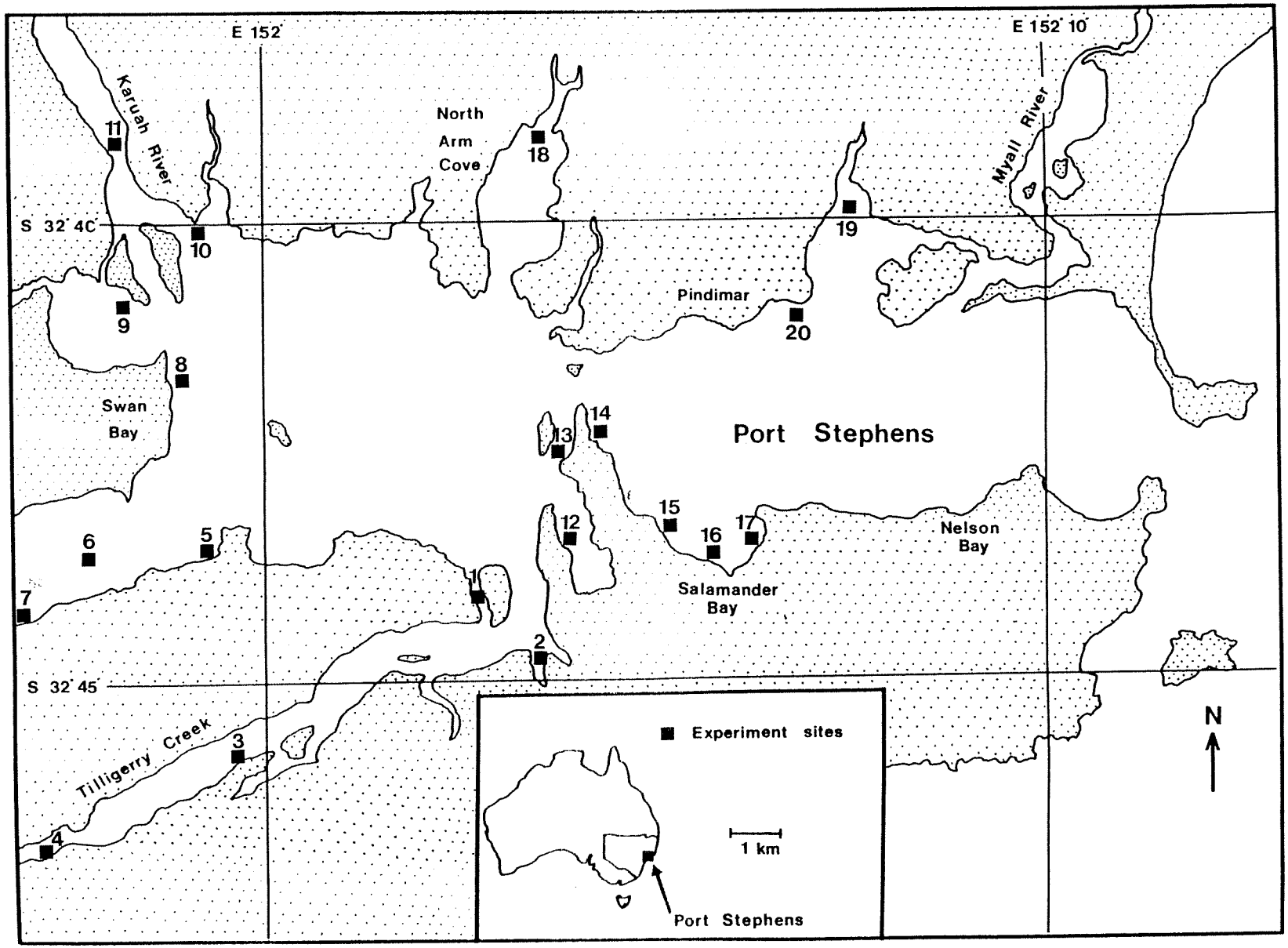
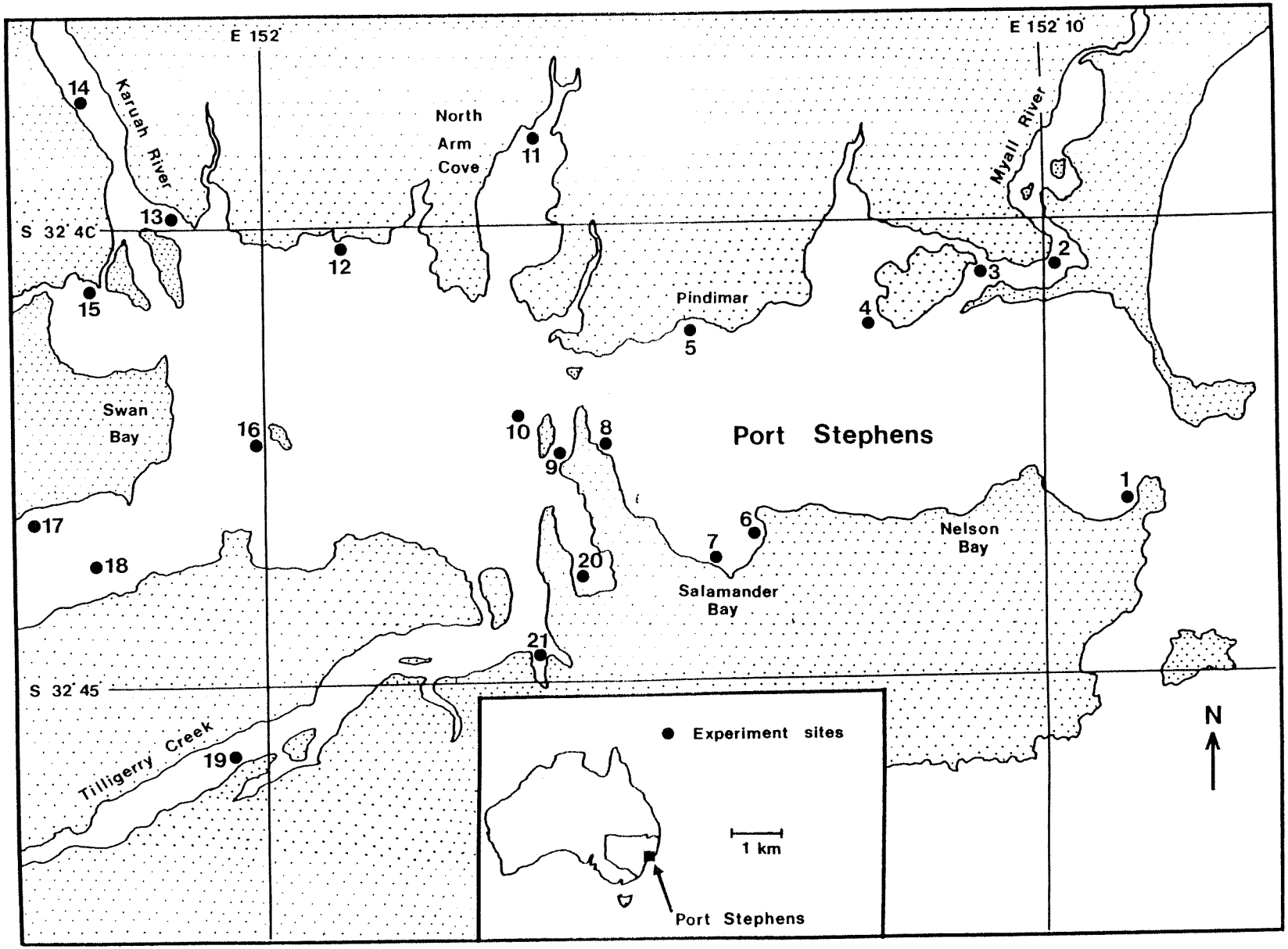

E 152
E 152 10
Karuah River
North
Arm
Cove
Myall River
S 32 4C
Pindimar
Swan
Bay
Port Stephens
Nelson
Bay
Salamander
Bay
S 32 45
Tilligerry Creek
Experiment sites
1 km
Port Stephens
N

FIG 2. Pacific oyster (Crassostrea gigas) Spatfall monitoring sites in Port Stephens from 1986 - 1989


E 152
E 152 10
S 32 4C
S 32 45
Karuah River
Myall River
North
Arm
Cove
Pindimar
Swan
Bay
Port Stephens
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Bay
Salamander
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17
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19
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21
N
Experiment sites
1 km
Port Stephens

THE EFFECT OF HEIGHT ON THE RECRUITMENT OF PACIFIC OYSTER (Crassostrea gigas) SPAT IN PORT STEPHENS, NSW

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ABSTRACT

Recruitment of Pacific oyster (Crassostrea gigas) spat in Port Stephens, NSW (1988) was heaviest on blocks of tarred hardwood sticks at approximately 150mm below the conventional intertidal growing height for Sydney rock oysters.

INTRODUCTION

Sydney rock oysters (Saccostrea commercialis) in Port Stephens are traditionally caught on blocks of tarred sticks placed at intertidal rack height, (Malcolm, 1987). This height is approximately the same as the mid range of the height at which Sydney rock oysters are found on the rock and mangroves of the foreshores of Port Stephens (J. A. Nell, personal observation, 1989). The appearance of large numbers of Pacific oysters (Crassostrea gigas) in Port Stephens in November, 1984 (Holliday and Nell, 1985), prompted studies into the biology of the Pacific oyster.

MATERIALS AND METHODS

Tarred hardwood (turpentine) sticks were put out at the conventional intertidal growing height (Malcolm, 1987) and at heights of 150mm and 300mm above and below the conventional growing height (rack height) at Tanilba Point, in the inner harbour of Port Stephens, NSW. There were two blocks of sticks at each of the five heights. The blocks consisted of 5 frames of 12 sticks each. The sticks were 1.8m long by 22mm x 22mm and were nailed 36mm apart. The blocks were 885mm wide and 210mm high. The blocks were left in place from November, 1987 to July, 1988. Recruitment was defined as the total number of live spat on the sticks after 8 months. Pacific oyster spat on 4 randomly selected sticks per frame were counted ie. 20 sticks per block. A logarithmic transformation of the number of spat per stick [$\log_{10}$ (no. of spat + 1)/stick] was carried out.

Homogeneity of variance was confirmed using the Cochran test (Winer, 1971). The data was then submitted to a nested analysis of variance and mean values were compared by least significant difference (Sokal and Rohlf, 1981).

RESULTS

A level of 150mm below rack height gave the highest spat recruitment number per stick (Table 1). The variation in recruitment between sticks from the same height was very large.

DISCUSSION

Recruitment of Pacific oyster spat was the heaviest at approximately 150mm below the conventional growing or rack height for Sydney rock oysters. This finding was confirmed by the observation of some settlement of Pacific oyster spat on the plastic mesh of trays placed 150mm below rack height compared to the settlement of Pacific oyster spat on trays placed at rack height (J. A. Nell, personal observation, 1989). The heavier recruitment at 150mm below rack height is likely to have been the result of heavier spatfall at this height.

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TABLE 1

Effect of height on the recruitment of Pacific oyster (*Crassostrea gigas*) spat at Tanilba Point, Port Stephens, NSW, from November 1987 to June 1988.

Catching height	Number of spat per stick[1]	$\log_{10}$ (No. of spat + 1)/stick[1]
+300mm	4± 5	1.27±0.91[a]
+150mm	14±14	2.39±0.72[bc]
rack height	10± 6	2.28±0.50[b]
-150mm	38±30	3.29±0.92[d]
-300mm	15± 8	2.60±0.74[c]

[1] Values are mean ± S.D. Within columns, means with a common superscript do not differ significantly (P>0.05).

SECTION 4

SALINITY STUDIES

SALINITY TOLERANCE OF PACIFIC OYSTERS (Crassostrea gigas) FROM PORT STEPHENS, NSW

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ABSTRACT

Adult (55g) Pacific oysters (Crassostrea gigas) have a wide salinity tolerance range of 5-55‰.

INTRODUCTION

Adult Sydney rock oysters (Saccostrea commercialis) have a salinity tolerance range of 15-50‰ (Nell and Gibbs, 1986). Sydney rock oysters are osmoconformers over their salinity tolerance range and the uptake of L-methionine over this range was not affected by salinity (Nell and Dunkley, 1984). The salinity tolerance range of the indigenous flat oyster (Ostrea angasi), a more oceanic species, was 20-45‰ (Nell and Gibbs, 1986). Good growth rates of Sydney rock and Pacific oyster (Crassostrea gigas) spat (average whole dry weight greater than 0.6g) were obtained at salinities from 20-40‰ and 15-45‰ respectively (salinities outside this range were not tested) (Nell and Holliday, 1988). The objective of the study was to determine at what salinities adult Pacific oysters may survive on oyster leases.

MATERIALS AND METHODS

Adult Pacific oysters were collected in Port Stephens, NSW. Oysters were transferred from a salinity of 35‰ to the experimental salinities 5-55‰ with 5‰ intervals. Oysters were held in 40L aquaria for 14 days. There were four aquaria (each holding 4 oysters) for each salinity. Salinities were measured with a temperature-compensated refractometer. Precise salinities were obtained by adding an artificial sea salt mixture (Wood and Ayres, 1977) or rainwater as required to sand-filtered seawater with a salinity of 35‰. Temperatures were maintained at 22°C and the water was changed each day. Pooled mantle fluid samples of all oysters from individual aquaria were used to measure osmolarities on a vapour pressure osmometer. Mantle fluid was used to measure tissue fluid osmolarities rather than pericardial fluid because mantle fluid is easier to collect and no difference was reported between mantle and pericardial fluid osmolarities in Sydney rock oysters over a wide range of salinities (Nell and Gibbs, 1986).

RESULTS

Very few oysters died within the range of salinities tested (5-55‰) over the 14 day experimental period (Table 1). The oysters were not effective osmorequlators, although they remained slightly hyperosmotic to the external medium (Fig 1). The mantle fluid showed osmoconformity over the whole range of salinities tested (5-55‰).

DISCUSSION

Adult Pacific oysters have a very wide salinity tolerance range (5-55‰), which is wider than the Sydney rock oyster (15-50‰) and the flat oyster (20-45‰; Nell and Gibbs, 1986). The wide salinity tolerance range of Pacific oysters was confirmed by Nell and Holliday (1988) who demonstrated good growth rates with Pacific oysters at salinities from 15-45‰. In the case of the Sydney rock oyster the salinity range (20-40‰) for good growth rates (Nell and Holliday, 1988) was substantially narrower than the salinity tolerance range.

TABLE 1

Pacific oysters (Crassostrea gigas) surviving within the salinity tolerance range after 14 days (n=16).

Salinity (‰)	Number of oysters surviving
5	13
10	15
15	16
20	15
25	16
30	15
35	15
40	13
45	15
50	14
55	13

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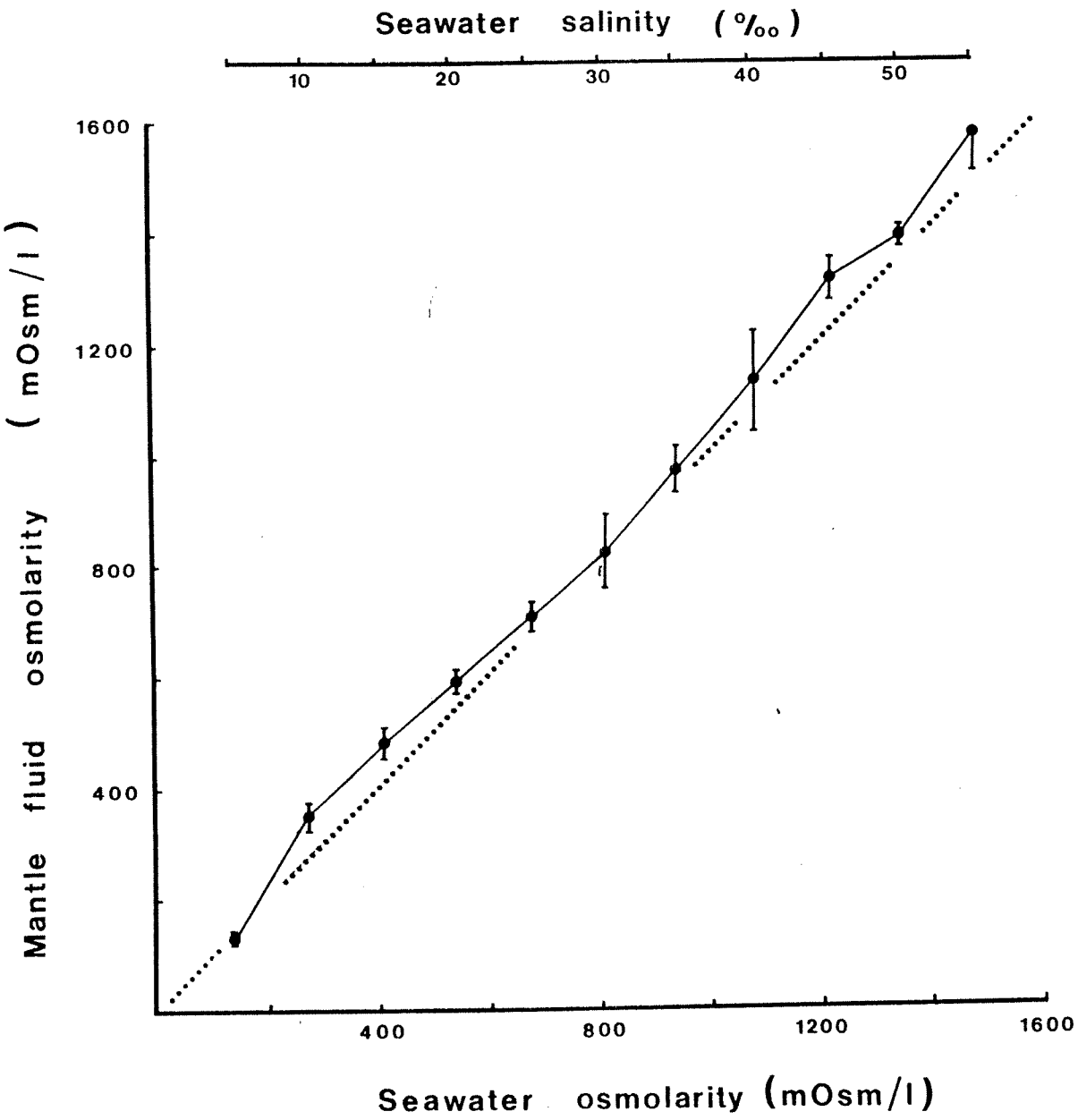
Wood, P. C. and Ayres, P. A., 1977. Artificial seawater for shellfish tanks. Great Britain Ministry of Agriculture, Fisheries and Food, Division of Fisheries Research, leaflet no. 39, 11pp.

FIG 1. Effects of seawater salinity on the mantle fluid osmolarity of Pacific oysters (Crassostrea gigas). Mean ± S.D. Broken line indicates isosmolarity.


Seawater salinity (‰)
10
20
30
40
50
1600
1200
800
400
Mantle fluid osmolarity (mOsm/l)
400
800
1200
1600
Seawater osmolarity (mOsm/l)

Aquaculture, 68 (1988) 39–44
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Effects of Salinity on the Growth and Survival of Sydney Rock Oyster (*Saccostrea commercialis*) and Pacific Oyster (*Crassostrea gigas*) Larvae and Spat

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(Accepted 27 July 1987)

ABSTRACT

Nell, J.A. and Holliday, J.E., 1988. Effects of salinity on the growth and survival of Sydney rock oyster (*Saccostrea commercialis*) and Pacific oyster (*Crassostrea gigas*) larvae and spat. *Aquaculture*, 68: 39–44.

Sydney rock oyster larvae had the highest growth rates at salinities of 23–39‰, and survival rates at 27–39‰. Two groups of Sydney rock oyster spat with average initial weights of 1.3 mg and 0.61 g grew best at salinities of 25–35‰ and 20–40‰, respectively. The equivalent optimum salinity ranges for the growth of Pacific oyster larvae, 1.1 mg spat and 0.68 g spat were 19–27, 15–30 and 15–45‰, respectively. Salinity had no significant effect ($P > 0.10$) on survival of spat of either species.

INTRODUCTION

Both the Sydney rock oyster *Saccostrea commercialis* (Korringa, 1976) and the Pacific oyster *Crassostrea gigas* (Korringa, 1976; Bourne, 1979; Ventilla, 1984) are cultivated over a wide range of conditions. The optimum salinity for development of Pacific oyster larvae was suggested to be from 23 to 28‰ (Korringa, 1976) and experimentally shown to be approximately 25‰ (Helm and Millican, 1977). However, little was known about the optimum salinity for growth of spat and adult oysters of either species. Pacific oysters were successfully cultivated in hypersaline ponds of 41‰ in Israel (Hughes-Games, 1977) and in southern Australia (King, 1977). The salinity tolerance or survival ranges of adult Sydney rock oysters and Pacific oysters are 15–50‰ and 5–55‰, respectively (Nell and Gibbs, 1986). However, the salinity tolerance range provides no indication as to the optimum salinity for growth. Therefore, a series of experiments was conducted to determine the optimum salinity range

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for growth and survival of the Sydney rock oyster and Pacific oyster. The former is native to Port Stephens, N.S.W., Australia, and the latter is an exotic species which was used to establish new oyster industries in southern Australia in the nineteen-fifties and found its way accidentally to Port Stephens (Holliday and Nell, 1985). The salinity range for good growth and survival of oysters is important in the selection of hatchery and field culture sites.

MATERIALS AND METHODS

All experiments were randomised with four replicates per treatment. Salinities were measured with a temperature-compensated refractometer. Precise salinities were obtained by adding an artificial sea salt mixture (Wood and Ayres, 1977) or rainwater as required to sand-filtered seawater with a salinity of 35‰. A salinity range of 15–45‰ was chosen for the spat experiments. Lower salinities were not used, because of the high salinity level (30–35‰) of the algal cultures used in the experiments; it would have been difficult to reduce salinities much below 15‰, whilst still providing enough algae to feed the oysters. A narrower salinity range of 15–39‰ was chosen for the larval experiments as the larvae were not expected to tolerate very high salinities.

Homogeneity of variance was confirmed using the Cochran test. The data were then submitted to analyses of variance and mean values were compared by least significant differences (Sokal and Rohlf, 1981). The larval survival data were transformed by arcsin $\sqrt{x}$ before analysis of variance, because the percentages covered a wide range of values.

Oyster larvae

One-day-old D-stage oyster larvae were stocked in 8-l nonaerated aquaria at a density of 5 larvae/ml. Water in the aquaria was maintained at 26°C and changed every 48 h with the larvae being retained on a 45-μm (diagonal) screen. The algae, *Pavlova lutheri* (Droope) and *Isochrysis galbana* (Parke), were added to the aquaria at the rate of 25 000 cells per ml per species at the start of the experiment and after every water change. The experiments were terminated after 6 days and the larvae were preserved in a solution containing 10% formalin in seawater. The percentage of live and dead larvae at the time of preservation was assessed by microscopic examination. The term survival in the text describes the percentage of larvae alive at the end of the experiments as not all larvae stocked could be accounted for. The length (greatest distance parallel to the hinge) of ≥ 50 preserved larvae per aquarium was measured using a microscope and a micrometer slide. The initial average lengths of four samples of 50 preserved Sydney rock oyster and Pacific oyster larvae were 72 ± 0.8 and 73 ± 1.4 μm, respectively.

Oyster spat

Small hatchery-reared spat were held on nylon mesh trays in 8-l aerated aquaria with one tray of 50 spat per aquarium. Water in the aquaria was maintained at 24°C and changed daily; the algae, *Pavlova lutheri* and *Isochrysis galbana*, were added daily at the rate of 150 000 cells/ml of each species. This high algal concentration was used to ensure that the quality of food would remain unlimiting during the experiment. The experiments were terminated after 3 weeks. The initial average whole dry weights of four samples of 50 small Sydney rock oyster and Pacific oyster spat at the start of the experiments were 1.3 ± 0.1 and 1.1 ± 0.2 mg, respectively, with an initial weight range of 0.5–1.5 mg.

Larger hatchery-reared spat were held on plastic mesh trays in 40-l aerated aquaria (one tray of 10 spat per aquarium). Water in the aquaria was maintained at 24°C and changed daily and the algae, *Pavlova lutheri* and *Isochrysis galbana*, were added daily at the rate of 150 000 cells/ml of each species. The experiments were terminated after 3 weeks. The initial average whole spat weights of four samples of 50 larger Sydney rock oyster and Pacific oyster spat at the start of the experiments were 0.61 ± 0.04 and 0.68 ± 0.02 g, respectively, with an initial weight range of 0.5 ± 1.0 g.

RESULTS

The growth increments for Sydney rock oyster larvae increased with salinity from 15 to 27‰, but further increases in salinity (31–39‰) did not significantly improve the growth rates (Table 1). Larval survival was highest at salinities from 27 to 39‰ and was reduced at the lower salinities of 15–23‰.

TABLE 1

Effect of salinity on the growth and survival of Sydney rock oyster larvae over 6 days[1]

Salinity (‰)	Length increase[2] (μm)	Survival[3] (%)
15	7 ± 6^{a}	9.7 ± 4.4^{a}
19	19 ± 2^{b}	20.1 ± 13.3^{ab}
23	34 ± 2^{c}	22.5 ± 13.6^{b}
27	37 ± 1^{c}	30.6 ± 7.9^{bc}
31	36 ± 5^{c}	40.0 ± 5.6^{c}
35	34 ± 3^{c}	28.8 ± 4.7^{bc}
39	30 ± 10^{c}	28.2 ± 2.8^{bc}

[1]Values are means ± SD. Within each column, means with a common superscript do not differ significantly ($P > 0.05$).
[2]Initial average length of the 1-day-old D-stage larvae was 72 ± 0.8 μm.
[3]For statistical analyses an arcsin $\sqrt{x}$ transformation was used.

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TABLE 2

Effects of salinity on the growth in length of Pacific oyster larvae over 6 days[1]

Salinity (‰)	Length increase[2] (μm)
15	30 ± 2^{ab}
19	53 ± 4^{c}
23	56 ± 7^{c}
27	55 ± 2^{c}
31	41 ± 5^{bc}
35	36 ± 11^{b}
39	16 ± 3^{a}

[1]Values are means ± SD. Means with a common superscript do not differ significantly ($P > 0.05$).
[2]Initial average length of the 1-day-old D-stage larvae was 73 ± 1.4 μm. There were no significant salinity effects ($P > 0.10$) on survival and the average survival rate was 96 ± 2.8%.

The optimum salinity range for growth of Pacific oyster larvae was 19–27‰, with much lower increments being recorded at the lowest salinity (15‰), and a very marked reduction in growth being obtained as salinity increased in the range 31–39‰ (Table 2). Pacific oyster larvae survived very well at all salinities tested. There were no significant salinity effects ($P > 0.10$) on survival and the average survival rate was 96 ± 2.8%.

The optimum salinity for growth of small hatchery-reared Sydney rock oyster spat was 25–35‰ with growth steadily increasing with salinity in the range 15–25‰ and decreasing in the range 35–45‰ (Table 3). These results differ

TABLE 3

Effect of salinity on the growth of small hatchery-reared Sydney rock and Pacific oyster spat over 3 weeks, measured as weight gain of whole dry spat[1]

Salinity (%)	Weight gain (mg)[2]	
	Sydney rock oyster	Pacific oyster
15	6.8 ± 2.7^{ab}	24.4 ± 7.0^{c}
20	8.7 ± 1.9^{bc}	26.8 ± 2.5^{c}
25	11.7 ± 2.4^{cd}	22.1 ± 6.3^{c}
30	12.5 ± 3.7^{d}	21.1 ± 3.0^{c}
35	9.6 ± 3.5^{cd}	11.2 ± 3.3^{b}
40	5.5 ± 0.9^{ab}	4.2 ± 1.9^{a}
45	3.5 ± 0.1^{a}	0.4 ± 0.3^{a}

[1]Values are means ± SD. Within columns, means with a common superscript do not differ significantly ($P > 0.05$).
[2]Initial average whole dry weights of the Sydney rock oyster and Pacific oyster spat were 1.3 ± 0.1 and 1.1 ± 0.2 mg, respectively.

TABLE 4

Effect of salinity on the growth of larger hatchery-reared Sydney rock and Pacific oyster spat over 3 weeks, measured as weight gain of whole dry spat[1]

Salinity (‰)	Weight gain (g)[2]	
	Sydney rock oyster (g)	Pacific oyster (g)
15	0.26 ± 0.12^{a}	0.67 ± 0.13^{a}
20	0.30 ± 0.10^{ab}	0.66 ± 0.10^{a}
25	0.38 ± 0.10^{ab}	0.69 ± 0.12^{a}
30	0.40 ± 0.12^{ab}	0.69 ± 0.12^{a}
35	0.46 ± 0.05^{b}	0.79 ± 0.12^{a}
40	0.31 ± 0.14^{ab}	0.70 ± 0.21^{a}
45	0.25 ± 0.10^{a}	0.71 ± 0.12^{a}

[1]Within each column, means with a common superscript do not differ significantly ($P > 0.05$).
[2]Initial average whole dry weights of the Sydney rock and Pacific oyster spat were 0.61 ± 0.04 and 0.68 ± 0.02 g, respectively.

somewhat from those obtained for small hatchery-reared Pacific oyster spat. The optimum salinity for growth of Pacific oyster spat was 15–30‰; however, growth declined rapidly as salinity increased from 30–45‰. Survival rates for Sydney rock oyster and Pacific oyster spat were very high ($98.9 \pm 1.8\%$ and $98.1 \pm 2.4\%$, respectively) and not significantly affected ($P > 0.10$) by salinity.

Salinity levels had much less effect on growth rates of larger hatchery-reared spat for both species (Table 4). The optimum range for Sydney rock oysters was 20–40‰ with some reduction in growth at 15 and 45‰. Growth rates of Pacific oysters were relatively uniform with no significant differences ($P > 0.10$) being obtained over the whole range tested (15–45‰). The only mortality recorded among these larger spat was one Sydney rock oyster.

DISCUSSION

Sydney rock oyster larvae appear to grow most rapidly at salinities of 23–39‰ and had the highest survival rate at 27–39‰, whereas Pacific oyster larvae grow best at lower salinities of 19–27‰. This concurs with field observations by Holliday and Nell (1985) who reported that the settlement of Pacific oyster larvae predominantly occurred in the estuarine environment of the inner harbour, whereas Sydney rock oyster larvae settle in the more oceanic environment of the outer harbour of Port Stephens, N.S.W., Australia. The optimum salinity range (19–27‰) determined for Pacific oyster larvae from Port Stephens, Australia, is very similar to the 25‰, which was found to be best for maximum growth of Pacific oyster larvae from England (Helm and Millican, 1977).

The salinity range for good growth and survival of 0.6-g spat of both species was wider than that for the 1-mg juvenile spat, which in turn was wider than that for the larvae of both species investigated. For all age groups studied, growth of Pacific oysters at optimum salinities was considerably faster than that for Sydney rock oysters.

This study confirms the reports by Hughes-Games (1977) and King (1977) that adult Pacific oysters may be cultivated in hypersaline ponds of 40‰, whereas adult Sydney rock oysters appear to be less suited to hypersaline conditions. However, oyster hatcheries that are producing Pacific oyster spat should operate with dilute seawater (Holliday, 1985) of 25‰, whereas Sydney rock oyster hatcheries should preferably operate at full oceanic salinities (35‰).

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SECTION 5

SELECTIVE KILLING AND CULTIVATION OF OYSTERS

SELECTIVE KILLING OF PACIFIC OYSTERS (Crassostrea gigas)

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ABSTRACT

Pacific oysters (Crassostrea gigas) may be killed selectively (range 17-99%) by continuous exposure to air for up to 17 days. Substantial losses of Sydney rock oysters (Saccostrea commercialis) were recorded (range 4-42%) after the same period of exposure. Further losses of Sydney rock oysters must be expected from oysters dropping off, if sticks are nailed out again on racks after drying. It is better to knock oysters off sticks after drying and then place them on trays to avoid losses caused by oysters dropping off.

INTRODUCTION

A heavy spatfall of Pacific oysters (Crassostrea gigas) in the inner harbour of Port Stephens occurred during the summer of 1984/85 (Holliday and Nell, 1985). This raised great concern amongst oyster farmers, because of the catch of small Pacific oysters (second catch) on the trays and sticks of Sydney rock oysters (Saccostrea commercialis). Subsequently the NSW Department of Agriculture placed restrictions on the movement of Sydney rock oysters from Port Stephens to other estuaries (Anonymous, 1985). This made the removal or killing of Pacific oysters a necessity if farmers were to continue the transfer of oysters from Port Stephens to other estuaries.

The transfer of oysters between estuaries in New South Wales is an important feature of this industry. Port Stephens is an important source of spat for other estuaries (Marshall and Espinas, 1987); several large producers transfer oysters to other estuaries for growing or fattening purposes. The selective killing/drying of Pacific oysters by leaving oysters out of water was investigated to assist farmers with the transfer of Sydney rock oysters from Port Stephens to other estuaries and for the continued survival of the world renowned Sydney rock oyster industry in Port Stephens.

MATERIALS AND METHODS

The terminology used in oyster farming in New South Wales is described by Korringa (1976) and Malcolm (1987). Spat are caught on tarred hardwood sticks (1.8m long and about 25mm^2) nailed up 10cm apart and five or six layers (frames) deep (Malcolm, 1987), to make a block. After settlement, which usually takes place between January and May, blocks of sticks are transferred to other racks further upstream and retained there until the following winter, a process known as depoting (Malcolm, 1987). These sticks are known as depot sticks. All-

ins are three year old oysters that have been knocked-off sticks and not been sorted into different size grades.

For the purpose of this study, dead oysters were oyster shells with no meat, or with dried or decomposed meats in them. Gaping oysters with apparently good meats were considered as live oysters, because some will recover when placed back in water (J. A. Nell, personal observation, 1985).

Experiment 1

Depot sticks (in total 55 frames of 15 sticks) with 1 year old oysters were taken by oyster farmers in Port Stephens and stood upright in a shore depot, exposed to prevailing weather conditions for 6-17 days during May and July, 1985. Five oyster farmers placed depot sticks on their shore depots. Mortality of Pacific oysters was assessed, before sticks were returned to the lease.

Experiment 2

Hatchery spat (1000 Sydney rock and 100 Pacific oysters) ranging from 5-10mm were exposed in shaded conditions in March 1986 for 7 days.

Experiment 3

Two year old oysters were taken from three different leases in Port Stephens in April, 1986. Sticks were stood upright under a tarpaulin that covered them from rain, but still exposed them to some direct sunlight early in the morning and late in the afternoon. Oysters were left out of the water for 4, 8, 12 or 16 days. At the end of the drying period, they were knocked off the sticks and placed on trays in Port Stephens for at least 14 days. There were 5 half frames of 6 or 7 sticks from each of three leases in Port Stephens. After all the oysters had been back in the water for at least 14 days, they were sorted into live and dead Sydney rock and Pacific oysters.

Experiment 4

Two year old oysters on sticks were taken from a lease in Port Stephens and stood in an upright position in an open (northern side) farmer's shed for 10 or 12 days in April, 1986. There were 7 frames of sticks for each drying period. Mortality of oysters was assessed before they were returned to the lease.

Experiment 5

Two year old oysters on sticks were taken from a lease in Port Stephens, stood in an upright position in a open (northern side) farmer's shed for 10 or 12 days in June, 1986. There were 7 frames of 20 sticks each per drying period. Oysters were knocked off the sticks and placed on trays in water for 10 days. One third of the oysters were deployed to each of the following estuaries: the Georges River, Port Stephens and the Tweed River.

Experiment 6

All-ins from a lease in Port Stephens were placed on trays in a well ventilated shed to dry for 11 or 13 days in June, 1986. Sticks were placed between the trays for ventilation. There were 1.5 bags of oysters per drying period.

Two year old oysters freshly knocked off sticks (all ins) from a lease in Port Stephens were placed on trays and the trays stacked on top of one another under shadecloth and left to dry for 11 or 13 days in June, 1986. The trays were staggered for ventilation. There were 15 bags of oyster per drying period.

RESULTS

Experiment 1

The length of time required to kill Pacific oysters was highly variable (Table 1). Drying depot sticks out in the open for 6-11 days in winter time killed only up to 45% of the Pacific oysters, whereas drying for 12-15 days in winter time killed up to 79% of the Pacific oysters, and a 100% kill of Pacific oysters was obtained with drying oysters for 17 days (Table 1).

Experiment 2

Drying of small (5-10mm) hatchery spat for 7 days in autumn killed all Pacific oysters and no Sydney rock oysters (Table 1).

Experiment 3

Drying of 2 year old sticks for 4-7 days out in the open in autumn killed up to 17% of the Pacific oysters and only 4% of the Sydney rock oysters (Table 1). Drying for 8-12 days in autumn killed 76-99% of the Pacific oysters and 22-26% of the Sydney rock oysters. Drying the same sticks for 16 days in autumn killed 100% of the Pacific oyster and 42% of the Sydney rock oysters.

Experiment 4

Drying 2 year old sticks in an open shed for 10-12 days in autumn killed most Pacific oysters and only a few Sydney rock oysters (Table 1).

Experiment 5

Drying 2 year old sticks in an open shed for 12-14 days in winter time killed most Pacific oysters and only a few Sydney rock oysters (Table 1).

Experiment 6

Drying all-ins in a closed, well ventilated shed or under shade cloth for 11-13 days killed more than 80% of the Pacific oysters and only a few Sydney rock oysters (Table 1).

DISCUSSION

The selective killing/drying of Pacific oysters by leaving oysters out of water for up to 17 days may kill a large percentage of the Pacific oysters, but losses of Sydney rock oysters may also be very substantial. Farmers should carefully watch their oysters when drying and terminate drying when 75% of the Pacific oysters have died or 25% of the Sydney rock oysters, or whichever comes first.

Depot sticks should be handled very gently after drying to avoid excessive losses from oysters dropping off sticks. After drying when the sticks have shrunk, oysters drop off very readily. Oysters on 2 year old sticks should be knocked off and placed on trays after drying.

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TABLE 1 Pacific oyster (*Crassostrea gigas*) drying/killing experiments

Experiment	Type of oysters	Time of year	Conditions	Number of days to kill Pacific oysters	% kill	
					Pacific oysters	Sydney rock oysters
1	Depot sticks	May 1985	Outside open to sun	17	100	7
"	" "	" "	" "	5	11	?
"	" "	July 1985	" "	6	0	?
"	" "	" "	" "	7	0	?
"	" "	" "	" "	9	44	?
"	" "	" "	" "	10	45	?
"	" "	" "	" "	11	19	?
"	" "	" "	" "	12	79	?
"	" "	" "	" "	13	26	?
"	" "	" "	" "	15	64	?
2	Hatchery spat	March 1986	" "	7	100	0
3	2 year old sticks	April 1986	" "	7	0	?
"	" "	" "	" "	4	17	4
"	" "	" "	" "	8	76	26
"	" "	" "	" "	12	99	22
"	" "	" "	" "	16	100	42
4	" "	" "	Open shed	10	most	few
"	" "	" "	" "	12	100	few
5	" "	June 1986	" "	12	>90	few
"	" "	" "	" "	14	100	few
6	All ins on trays*	" "	Shed or shade cloth	11	>80	few
"	" "	" "	" "	13	>90	few

* Large Pacific oysters were difficult to kill.

MORTALITY OF SYDNEY ROCK OYSTERS (*Saccostrea commercialis*) AND PACIFIC OYSTERS (*Crassostrea gigas*) KEPT OUT OF WATER AT DIFFERENT TEMPERATURES AND RELATIVE HUMIDITIES

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ABSTRACT

Sydney rock oysters and Pacific oysters were exposed to air temperatures from 3°C to 40°C for varying lengths of time. For temperatures above 8°C, survival time for both species was inversely related to air temperature. Pacific oysters died faster than Sydney rock oysters at all temperatures above 8°C, but Sydney rock oysters died faster than Pacific oysters when kept at 3°C. The relative humidity had no significant effect on the rate at which oysters of either species died. The recent growing history of the oysters affected the rate at which the oysters died, as Sydney rock oysters from environments with restricted food availability usually survived for longer than oysters from good enviroments with high availability of food. 35°C initially appeared to be a useful temperature for the selective killing of Pacific oysters amongst Sydney rock oysters, however subsequent experiments showed that its commercial implimentation would be impractical. It is suggested that 20°C would be a more appropriate temperature for the selective killing of Pacific oysters, however, there would be a cost in terms of the increased time required to kill the Pacific oysters.

INTRODUCTION

Pacific oysters, *Crassostrea gigas*, are a fast growing species that has been introduced to many parts of the world (see Mann, 1978). The species now accounts for 80% of the world oyster production. Pacific oysters were introduced to Australia between 1947 and 1970 by the CSIRO (Thomson, 1959; Medcof and Wolf, 1975). Pacific oysters introduced to Western Australia did not survive, but a breeding population eventually established itself in Tasmania, whence further introductions were made to South Australia and Victoria. The culture of Pacific oysters is now a viable industry in Tasmania, which had no native oyster suitable for cultivation. Pacific oysters were first identified in New South Wales in 1967 (Wolf and Medcof, 1974). By 1973, they

had been found in Port Stephens, NSW (Wolf and Medcof, 1974), and had spread as far north as Moreton Bay, Qld, by the early 1980's (C. Lucas, pers. comm., 1988).

In New Zealand, the first observations of live Pacific oysters were reported in 1971 (although shells later identified as belonging to Pacific oysters had been collected in 1958) (Curtin, 1973). By 1978, the cultivation of Pacific oysters had virtually supplanted that of the slow-growing native rock oyster, *Saccostrea glomerata* (Dinamani, 1981). The New Zealand rock oyster is thought to be a sibling species of the Sydney rock oyster *Saccostrea commercialis* (Buroker *et al*, 1979).

Pacific oysters were discovered in large numbers in Port Stephens (NSW) in 1984, and it is believed that they became established as a viable breeding population in the summers of 1984-85 and 1985-86 (Holliday and Nell, 1986).

In 1985, in an attempt to prevent the spread of the Pacific oyster throughout the rivers of New South Wales and to reduce its abundance in Port Stephens, the then Department of Agriculture declared the species a Noxious Fish. The presence of Pacific oysters on oyster leases became an offence (although the sale of Pacific oysters imported from Tasmania or New Zealand was not prohibited). Pacific oysters do not survive for as long out of water as Sydney rock oysters. This is the basis of attempts by Port Stephens oyster farmers to kill Pacific oysters overcaught on a crop of Sydney rock oysters, by leaving sticks or trays out of the water for 10 to 14 days. Although this treatment was successful in killing many of the Pacific oysters, the number of each species killed by the treatment varied with temperature, and windy weather caused heavy losses of Sydney rock oysters from the sticks when the sticks dried out.

The following series of experiments were designed to find

(i) how variations in temperature and humidity might affect the selective killing of Pacific oysters disributed among Sydney rock oysters,

(ii) how size and condition affects the susceptiblity of each species to drying, and

(iii) the optimum conditions to achieve maximum kill of Pacific oysters, with minimum loss of Sydney rock oysters.

MATERIALS AND METHODS

Seventeen experiments were carried out, under a range of experimental conditions. Sydney rock oysters and Pacific oysters were obtained from local oyster farmers, and kept on an intertidal lease until used in the experiments (unless specified otherwise).

In tables and figures, SRO refers to Sydney rock oysters, and PO to Pacific oysters.

Relative Humidity (RH) for any temperature was determined with a wet-and-dry bulb hygrometer.

The precision of the temperature that the oysters were held at varied with the equipment used. In particular, although the thermostats used in the hot-room were capable of reacting to temperature changes of less than 1°C, the heaters were not powerful enough to maintain the higher temperatures during the winter nights.

The oysters used in the experiments varied widely in size (whole weight). In experiments where the weight of the oysters is not explicitly given, "spat" refers to oysters between <1 - 10g; "small oysters" to oysters in the range 10 - 20g; "medium oysters" to oysters in the range 20 - 30g; and "large oysters" to oysters larger than 30g.

Experiment 1 (Refrigeration)

Forty spat of each species were marked so they could be identified individually, and weighed. Twenty of each species were placed in a refrigerator at 3°C, and twenty at 8°C. The spat were weighed individually every 2 - 3 days. The temperature in the refrigerators was monitored with maximum-minimum thermometers. Twenty days after the oysters were put in the refrigerators, ten of each species from each temperature were removed and placed in running sea-water. The remaining spat were taken from the refrigerators 8 days later and placed in running sea-water.

Experiments 2, 3 and 17 (Temperature)

Oysters of both species were placed in six temperature controlled cabinets at the Gosford Post-harvest Research Station, Gosford, NSW. The cabinets were kept in an insulated room maintained at 10°C. The humidity within the cabinets was not controlled, and equilibrated to the absolute humidity of the insulated room. The temperature and relative humidity within the cabinets was measured at two-day intervals when the oysters were inspected and collected.

Experiment 2

Five cabinets were used. Cabinet temperatures were set to 15, 25, 30, 35, and 40°C. Ten large Pacific oysters, 15 small Pacific oysters and 15 small Sydney rock oysters were placed into each cabinet. The oysters were inspected 2 days and 4 days after being put in the cabinets, and gaping oysters removed, counted and weighed. At the 4 day inspection it was discovered that the oysters kept at the higher temperatures (30, 35 and 40°C) did not necessarily gape when they died. It is thought that this is because the hinge ligament loses its elasticity when it dries out, thus preventing the valves from gaping. The remaining oysters in the 30°C cabinet were brought back to the research station and put into running sea-water to allow the ligament to re-hydrate, so that the dead oysters could be recognised. Oysters in the 15 and 25°C cabinets were left in the cabinets and carried over into Experiment 3, which was started 3 days later.

Experiment 3

Thirty each of large Pacific oysters, small Pacific oysters and small Sydney rock oysters were placed in each of the 30, 35 and 40°C cabinets. The oysters remaining in the 15 and 25°C cabinets from Experiment 2 were included in the results. Oysters that were obviously dead were removed when the oysters were inspected at 2 day intervals. A number of oysters of each species was removed from the cabinets when the oysters were inspected, brought back to the research station and put into running sea-water to assess mortality.

Experiment 17

Three cabinets were used, set at 15, 20 and 25°C. The actual temperatures measured in the cabinets remained at 18, 20 and 24°C. Thirty-six each of large Pacific oysters and large Sydney rock oysters were placed in each cabinet. The oysters were divided into 6 groups of 6. One group of each species was collected every few days and placed in running sea-water until no groups remained in the cabinets.

Experiment 4 (Humidity)

Oysters of each species were placed in sealed 20L polyethylene drums, with air of predetermined humidity flowing through at a rate of 4 to 5L per minute. The humidity of the air flowing through the drums was adjusted by mixing dry air and moist air in the appropriate ratio. The drums were kept in an insulated room maintained at 25°C. At this temperature and air flow rate, the lowest humidity that could be achieved (by passing air over anhydrous $CaCl_2$) was 35% RH, and the highest (by bubbling air though water), 90% RH Six drums were used for the experiment, two at 35% RH,

two at 70% RH, and two at 90% RH For ease of handling, the oysters in each drum were divided amongst 5 lightweight polyethylene mesh spat bags, and suspended in the drum. Each bag contained 8 small Pacific oysters (2 - 5g), 4 medium Pacific oysters (8 - 15g) and 8 small Sydney rock oysters (2 - 7g). One bag was collected from each drum 2, 5 and 7 days after the start of the experiment, and the remaining two bags were collected on day 9 after the start of the experiment. The bags were put into running sea-water so that mortality could be assessed. Oviously dead oysters were removed from bags remaining in the drums during the course of the experiment to reduce the smell of putrefaction. The actual RH in each drum was checked during the course of the experiment with a wet-and-dry bulb hygrometer, and was found to be within 5% of the nominal RH in each drum.

Experiments 5 and 6 (Incubator experiments)

In these experiments, oysters of each species were kept in a water-jacketed incubator at the research station. A number of the oysters was removed at intervals and put immediately into running sea-water so that mortality could be assessed. The number of dead oysters was recorded. It was assumed that all oysters mortally damaged by the heating would be dead after 7 days in running sea-water.

Experiment 5

The incubator was set to 35°C, and the RH was found to be approximately 40% at the begining of the experiment. Pacific and Sydney rock oysters in three size ranges each were divided into four groups. One group was removed at 12h, 24h, 36h and 48h.

Experiment 6

The incubator was set to 35°C, and the RH was found to be approximately 40% at the begining of the experiment. Pacific and Sydney rock oysters in two size ranges each were divided into four groups. Two groups each were removed at 36h and at 48h.

Experiments 7, 9, 10 and 12 (Hot room)

In these experiments, oysters of each species were kept on trays supported by trestles in a room 3m X 3m with a 2.4m ceiling. The room was heated by two 1kW fan heaters controlled by seperate thermostats. Groups of oysters were taken from the room at intervals, and (except for experiment 10) put immediately into running sea-water.

Experiment 7

The thermostats were set to 35°C, and the RH (50% at the start of the experiment) stayed at 44% for the rest of the experiment. 200 Spat of each species in this experiment had been kept sub-tidally or at commercial inter-tidal growing height for five weeks before the start of the experiment. 25 spat of each species from each environment were taken at 24h, 42h, 45h, and 54h after the start of the experiment, and 50 spat of each species from each environment were taken at 48h and 51h after the start of the experiment.

Experiment 9

The thermostats were set to 37°C. The RH remained between 40 and 44% for the duration of the experiment. 200 small Pacific oysters (7.4g $\pm$ 2.2g), 70 large Pacific oysters (more than one year old, with heavy overspatting of Pacific and Sydney rock oysters), and 200 small Sydney rock oysters (3.0g $\pm$ 0.7g) were divided into 7 groups. One group was removed at 36h, 38h, 40h, 42h, 44h, 46h and 48h after the start of the experiment.

Experiment 10

The oysters used in this experiment had been kept for 18 weeks in trays suspended at 3 heights under the wharf at the research station. One tray (subtidal) was suspended at a depth that was only exposed at extreme low spring tides. The second was suspended at rack height (Malcolm, 1987), and the third was suspended 450mm above the second (rack height +450). The conventional rack height used in Port Stephens has been found to leave the oysters exposed for about 30% of the tidal cycle (I. R. Smith, pers. comm. 1989). The trays were originally stocked with large Pacific oysters (>40g), small Pacific oysters (<20g), large Sydney rock oysters (>30g) and small Sydney rock oysters (<15g). The trays were removed from the wharf and hosed to remove mud. Oysters that had died during the 18 weeks were counted and removed. Three hours after the trays had been taken from the water, they were put into the hot room, with the thermostats set to 35°C. They were kept in the room for 47h, and then removed and left in cool air outside for a further 17h before being put into running sea-water.

Experiment 12

Plate grade (>35g) Sydney rock oysters from four different environments were kept at 35°C for 45h. Group A consisted of 37 fat oysters from an inter-tidal lease in Tilligerry Creek, Port Stephens. Group B was 74 oysters, originally from the same lease in Tilligerry Creek as group A, which had been kept in filtered sea-water at 24°C for a month without food. Group C was 48 fat oysters from a raft in the Clarence River on the North coast of NSW. Group D was 22 oysters

originally from the same lease in Tilligerry Creek as groups A and B, which had been kept in a tank supplied with water from a 0.1ha pond for 4 hours per day, and remained drained for the remaining 20 hours.

Experiment 8 (Growth rate after heating)

Surviving Sydney rock oysters from Experiment 7 and control oysters were put into a tank supplied with running sea-water pumped from a fertilised, 0.1ha pond at the Research Station. Control spat were from the same population as the survivors, but had not been subjected to heating at 35°C. 160 survivors (initial weight 3.2 ± 0.9g) and 164 controls (initial weight 2.9 ± 1.0g) were weighed at weekly intervals for a month.

Experiments 11, 13, 14, 15 and 16 (Sticks)

These experiments were carried out in the same hot room as experiments 7, 9, 10 and 12, but two 2kW radiant heaters combined with two domestic fans were substituted for the two 1kW fan heaters. The oysters in these experiments were Sydney rock oysters of various ages on blocks of sticks, with a heavy (younger) Pacific oyster overcatch. The RH in the room was measured with a wet-and-dry bulb hygrometer, and 8 maximum-minimum thermometers were placed between sticks in the blocks. After heating for a pre-determined time, individual frames of sticks were taken from the blocks. The frames were then put on a section of rack at mide-tide level in the tidal canal supplying the Reseach Stations ponds, or into a pond, immersed in water. After a week on the rack or in the pond, the frames were brought up onto the bank and the number of live and dead oysters of each species remaining on the sticks recorded.

Experiment 11

Heavily over-caught depot sticks (Sydney rock oysters about 27 months old) were taken from a lease and placed horizontally on trestles in the hot room 4-5 hours after being exposed by the dropping tide. The thermostats were set to 35°C and the probes suspended between sticks at each end of the block. The the maximum-minimum thermometers placed between the sticks indicated that the actual temperature rose to 37-38°C by 9 hours after the sticks were put in the hot room. The temperature had fallen to 34-35°C by 24h, and fallen still further to 30-32°C by 42 hours after the sticks were put in the hot room. Four frames of 4 sticks each were removed at 43h, 48h and 50h; two frames were put on the canal rack, and the other two into the pond.

Experiment 13

This experiment was similar to experiment 11, except that two frames of 4 sticks each were removed at 30h, 36h, 42h and 48h; one frame was put on the canal rack, and the other into the pond. The 30h frames were put out immediately, but the 36h and 42h frames were put out at the same time as the 48h frames, and so spent the intervening time out of water in cool air.

Experiment 14

This experiment was almost identical to experiment 13, except that the thermostat probes were suspended on the ends of the block of sticks for the first 15h after the sticks were put in the hot room, and then between the sticks for the rest of the experiment. The temperature remained at about 34°C for most of the experiment. One frame was removed at 42h, 45h and 48h and put into the pond.

Experiment 15

Caught sticks about 8-10 months old with a heavy overcatch of Pacific oyster spat (probably 4-8 months old) were put in the hot room. The thermostats were set up as for experiment 14, and set to 35°C. The actual temperature remained at 33-34°C for most of the experiment. One frame of 4 sticks was removed at 36h, 42h, 45h and 48h after being put in the hot room, and put into the pond.

Experiment 16

This was identical to experiment 15, except that the thermostats were set to 33°C. One frame was removed at 24h, 36h, 42h, 45h and 48h, and put into the pond.

RESULTS

Experiment 1

Most of the oyster spat of each species at both 3°C and 8°C were dead after 28 days out of water (Table 1). The effect of storage of spat out of water at low temperatures on survival was more apparent when spat were replaced in running sea-water 20 days after the start of the experiment (Table 1). Sydney rock spat survived better at 8°C (1 of 9 dead) than at 3°C (8 of 10 dead). For the Pacific oyster spat, the situation was reversed, with better survival at 3°C (1 of 9 dead) than 8°C (7 of 11 dead). After an initial weight loss of about 2.5% caused by drying of moisture on the shell in the first two days, the spat continued to lose weight at a steady rate. The rate of water loss depended both on the

species and temperature. Sydney rock spat at either temperature lost the least weight, and Pacific oyster spat at 3°C lost the most weight (Table 2). There was a good correlation between weight loss and mortality for Pacific oyster spat kept at 8°C ($0.05 > p > 0.02$) and at 3°C ($0.02 > p > 0.01$). The single dead Sydney rock spat that was dead after 20 days of storage at 8°C lost more weight (11.6%) than the survivors ($8.4 \pm 1.9\%$), however the difference was not significant ($p > 0.1$). The two surviving Sydney rock spat kept at 3°C lost more weight than the dead spat, but the difference was not significant ($0.1 > p > 0.05$). The two survivors were also the two smallest spat of the 10 spat in the group. Weight loss in individual spat of each species was steady during the experiment, and the gradient of the weight loss could be used as predictor of mortality at 20 days in the Pacific oyster spat.

Experiments 2, 3, and 17

Because the oysters did not gape immediately when they died in air at high temperatures, it was not possible to determine whether the oysters in Experiment 2 had died. Oysters kept at 30, 35 and 40°C were discarded, and those at 25 and 15°C were combined with Experiment 3 (Table 3). The length of time the oysters could spend out of water was inversely related to the temperature. Sydney rock oysters survived for longer than Pacific oysters at all temperatures tested in these experiments. Larger Pacific oysters tended to last for longer than small Pacific oysters (Table 3).

Experiment 4

Spat were kept at three different humidities at 25°C for up to 9 days. Sydney rock oyster spat survived for longer than Pacific oyster spat at all humidities tested (Table 4). There were no significant differences in mortality between spat of the same species at different humididdities, nor in large Pacific oyster spat compared to small Pacific oyster spat (Table 4).

Experiments 5 and 6

Pacific oysters died faster at 35°C than Sydney rock oysters. The relative size of the oysters did not affect the rate at which the oysters died (Table 5).

Experiment 7

Sydney rock oysters from a sub-tide environment appeared to die faster than spat from a rack height environment when kept

in air at 35°C. There was no difference in the mortality of Pacific oysters from either environment (Table 6).

Experiment 8

There was no difference in growth rates over two weeks of Sydney rock oyster spat that had survived being heated to 35°C for between 42 and 51h, and control spat which had not been heated (Table 7).

Experiment 9

Heating at 37°C caused heavy mortality amongst the Sydney rock oyster spat, and was fatal to all Pacific oyster spat tested. A few large Pacific oysters survived for up to 40h (Table 8). Sydney rock "jockeys" overcaught on the large Pacific oysters survived relatively well, with only 15% dying until 42h at 37°C (Table 8).

Experiment 10

There was some mortality of oysters on the trays during the three months the oysters were kept under the wharf (Table 9). The post-heating mortality of the Sydney rock oysters was relatively high compared to previous experiments at 35°C, but this was probably caused by the 17h delay before the oysters were put into running sea-water. The general trend was for the mortality of each species to be highest on the middle (rack height) tray, followed by the top tray (rack height + 450mm), and the lowest mortality in the bottom tray (sub-tidal) (Table 9). Many of the small Pacific oysters and some of the large Pacific oysters had chambered shell valves. Sydney rock oyster spat had tubular "frills" on the upper valve. These abnormal shell morphologies are characteristic of stressed oysters for the respective species.

Experiment 11

Depot sticks that had been heated at 35°C for 43h, 48h and 50h were put out on an intertidal rack or in a pond. One week later, the number of live and dead oysters of each species on the sticks were counted. It was observed that the percent mortality of the Sydney rock oyster spat differed at each end off the stick for all frames; the number of dead spat was greater on the left-hand ends of the sticks than on the right-hand end, and was found to be significantly different ($p =< 0.02$ for each frame, Table 10). The orientation of the frame of sticks in the hot-room could be deduced from the position of the numbered tag identifying the frame. It was concluded that although the heating seemed to be even, the right-hand side of the block of sticks, which was nearer the heater, had experienced higher temperatures

during the heating period than the left-hand half of the block. The Pacific oyster spat suffered 100% mortality on all sticks except for the left-hand end of the frame removed after 50h. These Pacific oyster spat may have been protected by a very heavy density of Sydney rock oyster spat caught on the sticks at this position.

Experiment 12

The Sydney rock oysters from four different environments were heated at 35°C for 46.5h on a tray in the hot room. The temperature was monitored, and remained between 33 and 36°C for the duration of the experiment. The relative positions of each group of oysters were changed at 19.5h and 43h. The number of dead and live oysters in each of the three sub-groups of each group was counted. The mortality of oysters from the two environments with low food availability (groups B and D) was significantly lower ($p < 0.02$) than that of oysters from the two environments with high food availability (groups A and C) (Table 11).

Experiment 13

Mortality of the Sydney rock oysters was low. The number of dead spat per stick ranged from 0 to 5, and there was no pattern to the mortality, which was considered to have been randomly distributed (Table 12). Mortality of the Pacific oysters was also low and highly variable, with between 0 to 15 dead per stick, and 0 to 100% dead per stick (Table 13). The percent mortality of Pacific oysters increased with the length of time spent at 33°C.

Experiment 14

As with Experiment 13, the mortality of the Sydney rock oysters kept at 34°C was low and there was no particular pattern to the mortality (Table 14). The mortality of the Pacific oysters was also low, ranging from a minimum of 38% to a maximum of 84% (Table 14).

Experiment 15

Mortality of spat of both species on caught sticks was high. No Pacific oyster spat survived more than 42h of heating at 34°C, and the percent mortality of Sydney rock oyster spat went from 34% after 36h at 34°C, to 69-85% after 42-48h at 34°C (Table 15).

Experiment 16

Mortality of spat of both species on caught sticks was high. The mortality of Pacific oyster spat was variable; 14% of the Pacific oysters survived 42h at 35°C, but no Pacific oysters survived on the crates of sticks removed at 24, 36, 45 or 48h after the start of the experiment. Mortality of the Sydney rock oyster spat ranged from 24 to 56% (Table P). The pattern of the mortality of spat of both species suggests that spat on the sticks on the outside of the block (that is, the top and bottom crates) were more affected by the heating process than the spat on the middle crates of the block.

DISCUSSION

This series of experiments confirmed the observations of oyster farmers that Sydney rock oysters last for a considerably longer time out of water than Pacific oysters. The temperature at which the oysters are held influences the survial time. Sydney rock oysters of all sizes survived for longer out of water than Pacific oysters for all temperatures tested except 3°C. The mortality after 19 days for Sydney rock oysters was higher at 3°C than at all temperatures below 35°C (Tables 1 and 3). Roughley (1926) found that Sydney rock oysters kept on ice for between 24 and 72h suffered about 20% mortality. It appears that prolonged exposure to the low temperature killed the Sydney rock oysters, so chilling to below 8°C is not a suitable method of storing Sydney rock oysters, although it is entirely suitable for the short-term storage of Pacific oysters (Seaman, 1991).

Pacific oysters behave differently to Sydney rock oysters when exposed to air; Pacific oysters gape within a few hours of being removed from the water (C. Mason, personal observation 1988), and may lose a substantial amount of the mantle fluid, apparently without any detrimental effects if they are replaced in the water promptly. In contrast, Sydney rock oysters do not relax the adductor muscle significantly until they are *in extremis*, and rarely recover once they have gaped (C. Mason, personal observation 1988). The loss of weight of Sydney rock oysters when kept at low temperatures is evidence that they do open the shell slightly when kept out of water, as reported by Medcof (1959), but to a lesser extent than the Pacific oysters, which lost weight faster. Bivalve molluscs survive for long periods out of water by switching from aerobic metabolism (in which the end products are H_2O and CO_2), to anaerobic metabolism in which the end product is mostly succinic acid, buffered by calcium ions disolved from the shell. Deschger (1990) found that glycogen was the most important metabolic substrate during anaerobiosis in the bivalves *Astarte borealis* and *Artica islandica*. Unlike the anaeobic metabolism of vertebrate white muscle, in which the lactic acid produced as an end product may be recovered and converted back to glucose, the succinate and calcium is flushed out when the animal returns

to the water (for a comprehensive review of anaerobiosis in bivalve mollusks, see De Zwaan, 1977). Since the relative humidity had no significant effect on the rate at which either species died when kept out of water, it seems likely that death is caused by a build-up of toxic metabolites during anaerobiosis, rather than dehydration. Seaman (1991) however, found that Pacific oysters stored at 7°C survived for longer when irrigated with seawater than when they were kept dry.

The length of time that Sydney rock oysters survive being kept out of water at high temperatures depends on the environment in which they were kept before the experiment. Three possible explainations for the longer survival of oysters from environments with low availability of food are as follows:

(a) Enzymes used in anaerobiosis are induced by anaerobic conditions, thus enabling oysters that have been exposed to long periods out of water to cope with anaerobic conditions better than inexperienced oysters. However this does not explain why the oysters kept continuously submerged in filtered sea-water survived better than oysters from a mid-tide lease in Experiment 12, nor does it account for the high survival of the continuously submerged oysters in Experiment 10.

(b) "Thin" oysters have a smaller metabolic load to bear during anaerobiosis than the fat oysters from more favourable environments. The apparently paradoxical high survival of the oysters from the continuously submerged tray in Experiment 10 could be explained by assuming that these oysters had spawned, and were in poorer condition than oysters from the mid-tide tray which had not yet spawned.

(c) Fat (sexually mature) oysters have a smaller reserve of glycogen, which is the substrate for anaerobic respiration, than oysters with developing gonads, or even winter condition oysters (Gabbott, 1975;Littlewood and Gordon, 1988; Mason and Nell, 1991; Perdue and Erickson, 1984; Whyte and Englar, 1982).

The ability of Sydney rock oysters to survive for long periods in the absence of oxygenated seawater may be a response to the periodic fresh-water flooding of NSW rivers; Ivanovici et al (1982) found evidence of a shift to anaerobic respiration in Sydney rock oysters kept in water at 10 ppt. salinity. Ahmad (1986) and Descher (1990) found a good correlation between the anaerobic facility of bivalve molluscs and the likelyhood of their experiencing anaerobic conditions. Medcof (1959) reported that American oysters *Crassostrea virginica* were routinely overwintered in cold air, and Hidu et al (1988) kept American oyster spat for 6 months in humid air at 0 - 6°C with 80% survival; the native environment of the American oyster is also subject to periodic flooding, although unusually prolonged immersion in nearly freah water causes heavy mortalities (Andrews, 1982). The relatively poor survival of Pacific oysters kept in air

(see also Seaman, 1991) may be a result of the evolution of Pacific oysters in a less uncertain environment than Sydney rock oysters or American oysters.

During preliminary experiments, 48h at 35°C seemed the most promising temperature for a potential commercial method for killing Pacific oysters amongst a crop of Sydney rock oysters, because of the good survival of the Sydney rock oysters, and because of the short time it took to kill the Pacific oysters, thus allowing a quick turn-around of the oysters being treated. Further work confirmed the useful difference in the time at 35°C required to kill each species, but also showed that the time taken to kill the oysters was related to the meat condition of the oysters. The major problem with the method however was the extreme sensitivity of the oysters to slight variations in temperature from 35°C; 48h at 33°C killed only 80% of the Pacific oysters, and 48h at 37°C caused up 91% mortality of Sydney rock oysters. This seriously limits the practical use of the method, since the capital cost of equipment required to heat large quantities of oysters to 35°C within 1°C would be too great for oyster farmers to bear.

Given that the degree of control required to impliment drying at 35°C effectively is out of the reach of oyster farmers, the preferable method would be to leave the oysters at 20°C for two weeks. This has the advantage of being less sensitive to small variations in temperature and time than drying at 35°C. No information is available for the effect of oyster condition on mortality at this temperature, although it would be wise to err on the conservative side if the Sydney rock oysters were particularly fat. The major disadvantages of drying at 20°C would be related to the economics of the greater length of time required to kill the Pacific oysters (as growing time forgone, and the smaller number of oysters that could be processed per unit time).

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Table 1. Experiment 1. Percent mortality of Pacific oyster (*Crassostrea gigas*) spat and Sydney rock oyster (*Saccostrea commercialis*) spat kept at 3°C and 8°C.

Species	Temperature	Mortality (%) 20 days	28 days
Pacific	3°C	10%	90%
Pacific	8°C	64%	90%
Sydney rock	3°C	80%	100%
Sydney rock	8°C	11%	70%

Table 2. Experiment 1. Mean percent weight loss (± S.D.) from day 2 to day 20 for Pacific oyster (*Crassostrea gigas*) spat and Sydney rock oyster (*Saccostrea commercialis*) spat kept at 3°C and 8°C.

Species	Temperature	Mean weight loss (%) Live spat (n)	Dead spat (n)
Pacific	3°C	21.5 ± 6.2 (9)	40.5 (1)
Pacific	8°C	13.2 ± 3.4 (4)	33.6 ± 7.8 (7)
Sydney rock	3°C	17.2 ± 3.9 (2)	8.4 ± 3.1 (8)
Sydney rock	8°C	8.4 ± 1.9 (8)	11.6 (1)

Table 3. Experiments 2, 3 and 17. Percent mortality of Pacific oysters (*Crassostrea gigas*) and Sydney rock oysters (*Saccostrea commercialis*) kept in air at different temperatures with time.

Species and mean weight	Temp.	Mortality % (n)							
		Day 2	Day 5	Day 7	Day 8	Day 13	Day 14	Day 15	Day 19
SRO	15°C								
8.4g		-	-	-	-	47% (15)	-	-	
22g		0% (6)	0% (6)	-	0% (6)	0% (6)	-	0% (6)	17% (6)
PO	15°C								
8.3g		-	-	-	-	-	100% (15)	-	-
28g		0% (6)	33% (6)	-	67% (6)	100% (6)	-	100% (7)	100% (6)
77g		-	-	-	-	-	88% (8)	-	-
SRO	20°C								
22g		0% (6)	0% (6)	-	17% (6)	0% (6)	-	0% (6)	17% (6)
PO	20°C								
28g		0% (6)	100% (6)	-	83% (6)	100% (6)	-	100% (6)	100% (6)
SRO	25°C								
8.0g		-	-	0% (15)	-	-	-	-	-
22g		0% (6)	0% (6)	-	0% (6)	33% (6)	-	50% (6)	67% (6)
PO	25°C								
7.8g		-	-	73% (19)	-	-	-	-	-
28g		0% (6)	100% (7)	-	100% (6)	100% (6)	-	100% (6)	100% (6)
57g		-	-	20% (10)	-	-	-	-	-

Table 3. (cont).

Species and mean weight	Temp.	Mortality % (n) Day 2	Day 4	Day 7
SRO 8.0g	30°C	0% (14)	43% (14)	94% (16)
PO 8.1 64g	30°C	11% (9) 13% (8)	100% (9) 75% (8)	100% (10) 88% (8)
SRO 7.8g	35°C	0% (14)	8% (14)	-
PO 7.4g 63g	35°C	100% (10) 78% (8)	100% (10) 100% (8)	- -
SRO 7.5g	40°C	100% (18)	100% (15)	-
PO 7.1g 66g	40°C	100% (11) 100% (12)	100% (15) 100% (15)	- -

Table 4. Experiment 4. Percent mortality of Pacific oysters (*Crassostrea gigas*) and Sydney rock oysters (*Saccostrea commercialis*) kept at 25°C at three different levels of relative humidity (RH).

			Mortality % (n)			
Time at 35°:			Day 2	Day 5	Day 7	Day 9
RH	Species	Size				
90%	P.O.	Med.	0% (4)	75% (4)	100% (4)	100% (8)
	P.O.	Sml.	0% (8)	88% (8)	100% (8)	92% (16)
90%	P.O.	Med.	0% (4)	75% (4)	100% (4)	100% (8)
	P.O.	Sml.	25% (8)	50% (8)	100% (8)	92% (16)
90%	S.R.O.	Sml.	0% (8)	13% (8)	13% (8)	25% (16)
	S.R.O.	Sml.	0% (8)	0% (8)	0% (8)	13% (16)
70%	P.O.	Med.	13% (4)	50% (4)	75% (4)	100% (8)
	P.O.	Sml.	0% (8)	75% (8)	100% (8)	100% (16)
70%	P.O.	Med.	0% (4)	75% (4)	100% (4)	100% (8)
	P.O.	Sml.	25% (8)	38% (8)	100% (8)	92% (16)
70%	S.R.O.	Sml.	13% (8)	0% (8)	0% (8)	44% (16)
	S.R.O.	Sml.	0% (8)	13% (8)	0% (8)	35% (16)
32%	P.O.	Med.	0% (4)	100% (4)	75% (4)	100% (8)
	P.O.	Sml.	0% (8)	63% (8)	88% (8)	100% (16)
32%	P.O.	Med.	0% (4)	25% (4)	100% (4)	100% (8)
	P.O.	Sml.	13% (8)	38% (8)	100% (8)	100% (16)
32%	S.R.O.	Sml.	0% (8)	0% (8)	13% (8)	25% (16)
	S.R.O.	Sml.	13% (8)	0% (8)	13% (8)	13% (16)

Table 5. Experiments 5 and 6. Percent Mortality of Pacific oysters (*Crassostrea gigas*) and Sydney rock oysters (*Saccostrea commercialis*) kept in air at 35°C.

		Mortality % (n)			
Time at 35°C:		12 h	24 h	36 h	48 h
Species	Mean Wt.				
P.O.	22g	0% (6)	33% (6)	67% (6)	100% (6)
	7.5g	13% (8)	75% (8)	63% (8)	100% (8)
	3.5g	13% (8)	63% (8)	75% (8)	100% (8)
P.O.	>35g	-	-	95% (20)	80% (20)
	8.5g	-	-	80% (10)	80% (10)
S.R.O.	13g	0% (6)	0% (6)	0% (6)	0% (6)
	8.0g	25% (8)	0% (8)	13% (8)	25% (8)
	3.5g	13% (8)	0% (8)	13% (8)	13% (8)
S.R.O.	>35g	-	-	5% (20)	5% (20)

Table 6. Experiment 7. Percent mortality of Pacific oysters (*Crassostrea gigas*) and Sydney rock oysters (*Saccostrea commercialis*) from two different growing environments after heating in air at 35°C.

	Mortality % (n)					
Time at 35°C:	24h	42h	45h	48h	51h	54h
Subtidal						
P.O.	0% (25)	64% (25)	95% (25)	98% (50)	100% (25)	100% (25)
S.R.O.	0% (25)	24% (25)	48% (25)	76% (50)	80% (25)	100% (25)
Mid-tide						
P.O.	6% (25)	89% (25)	100% (25)	100% (50)	100% (25)	100% (25)
S.R.O.	0% (25)	0% (25)	3% (25)	5% (50)	14% (25)	4% (25)

Table 7. Experiment 8. Weekly increases in weight (Mean ± S.D.) of Sydney rock oyster (*Saccostrea commercialis*) spat after being heated to 35°C for 42-51h.

	Weight (g)		
	Initial	One week	Two weeks
Heated spat	3.16 ± 0.96	3.38 ± 0.96	3.55 ± 0.96
Control spat	2.86 ± 0.95	3.01 ± 0.91	3.09 ± 0.92

Table 8. Experiment 9. Percent mortality of Pacific oysters (*Crassostrea gigas*) and Sydney rock oysters (*Saccostrea commercialis*) kept in air at 37°C with time.

	Mortality % (n)						
Time spent at 37°C:	36h	38h	40h	42h	44h	46h	48h
SRO spat 3.0g	46% (26)	42% (26)	62% (26)	81% (26)	93% (30)	80% (30)	86% (51)
PO spat 7.4g	100% (26)	100% (27)	100% (27)	100% (26)	100% (24)	100% (26)	100% (72)
SRO jockeys	0% (30)	11% (19)	15% (26)	15% (26)	64% (36)	75% (17)	91% (46)
large PO	67% (9)	80% (10)	90% (9)	100% (10)	100% (9)	100% (10)	100% (10)

Table 9. Experiment 10. Percent mortality of Pacific oysters (*Crassostrea gigas*) and Sydney rock oysters (*Saccostrea commercialis*) from three different growing heights. The oysters were kept on trays under a wharf for 3 months. They were heated at 35°C for 48h, and then kept at ambient temperatures (15 - 20°C) for 17h before being replaced in running sea-water.

		Mortality (%)		
Species and size	Original number	Before heating	After heating	Total
Top Tray				
large SRO	98	21%	61%	69%
small SRO	347	1.4%	39%	40%
large PO	97	7.2%	99%	99%
small PO	227	16%	100%	100%
Middle Tray				
large SRO	100	16%	76%	80%
small SRO	532	1.7%	64%	65%
large PO	90	2.2%	99%	99%
small PO	205	5.9%	94%	95%
Bottom tray				
large SRO	115	16%	13%	30%
small SRO	420	5%	5%	6%
large PO	105	17%	83%	75%
small PO	187	20%	94%	95%

Table 10. Experiment 11. Percent mortality of Pacific oyster (*Crassostrea gigas*) spat and Sydney rock oyster (*Saccostrea commercialis*) spat on Depot sticks heated at 35°C. Uneven heating caused heavier mortality on the right hand side (RHS) of the crates of sticks than the left hand side (LHS). The sticks were placed on an intertidal rack or in a pond after heating.

	% Mortality ± S.D. (number of oysters, number of sticks)		
Time at 35°C:	43h	48h	50h
P.O. (RHS) Rack	-	100% (61, 12)	-
	-		-
P.O. (RHS) Pond	100% (22, 12)	100% (28, 12)	100% (103, 20)
P.O. (LHS) Rack	-	100% (17, 12)	-
	-		-
P.O. (LHS) Pond	100% (42, 12)	100% (50, 12)	87% ± 26% (115, 20)
P.O. (whole stick) Rack	100% (71, 8)	100% (78, 12)	-
			-
P.O. (whole stick) Pond	100% (64, 12)	100% (106, 12)	93% ± 20% (218, 20)

Table 10. (cont.)

	% Mortality ± S.D. (number of oysters, number of sticks)		
Time at 35°C:	43h	48h	50h
S.R.O. (RHS)	-	75% ± 22%	-
Rack	-	(356, 12)	-
S.R.O. (RHS)	34% ± 20%	57% ± 19%	89% ± 10%
Pond	(300, 12)	(310, 12)	(687, 20)
S.R.O. (LHS)	-	5.0% ± 4.4%	-
Rack	-	(282, 12)	-
S.R.O. (LHS)	5.4% ± 3.8%	5.0% ± 5.2%	6.6% ± 4.8%
Pond	(287, 12)	(315, 12)	(449, 20)
S.R.O. (whole stick)	12% ± 7%	40% ± 38%	-
Rack	(404, 8)	(638, 12)	-
S.R.O. (whole stick)	16% ± 18%	30% ± 29%	48% ± 42%
Pond	(539, 12)	(625, 12)	(1136, 20)

Table 11. Experiment 12. Percent mortality of Sydney rock oysters (*Saccostrea commercialis*) from four different environments after heating at 35°C for 46.5h.

	% Mortality ± S.D. (n)			
Environment:	Tilligerry Ck. (fat)	24°C, (poor)	Sub-tidal (fat)	4h water (poor)
	28% ± 8% (37)	3.9% ± 3.3% (74)	25% ± 5% (48)	0% (22)

Table 12. Experiment 12. Analysis of Variance of percent mortality of Sydney rock oysters (*Saccostrea commercialis*) from four different growing environments, after heating at 35°C for 46.5h.

Source of Variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. level
Between groups	1825.4	3	608.5	16.425	0.0009
Within groups	296.4	8	37.0		
Total	2121.7	11			

Table 13. Experiment 13. Percent mortality of Pacific oyster (*Crassostrea gigas*) spat and Sydney rock oyster (*Saccostrea commercialis*) spat on Caught sticks heated at 33°C. The sticks were placed on an intertidal rack or in a pond after heating.

	% Mortality ± S.D. (number of oysters, number of sticks)			
Time at 35°C:	30h	36h	42h	48h
P.O.				
Rack	67% ± 41% (10, 4)	68% ± 23% (42, 4)	41% ± 26% (78, 4)	74% ± 18% (19, 4)
Pond	40% ± 11% (11, 4)	41% ± 31% (38, 4)	65% ± 23% (44, 4)	100% (7, 4)
Rack + Pond	53% ± 33% (22, 8)	54% ± 31% (80, 8)	53% ± 28% (122, 8)	87% ± 18% (26, 8)
S.R.O.				
Rack	0% (218,4)	0.5% ± 0.9% 207, 4)	0.5% ± 0.9% (199, 4)	0% (239, 4)
Pond	0.7% ± 1.0% (232, 4)	0.5% ± 0.8% (249, 4)	1.9% ± 2.0% (332, 4)	2.5% ± 2.7% (166, 4)
Rack + Pond	0.5% ± 0.8% (450, 8)	0.5% ± 0.8% (456, 8)	1.2% ± 1.7% (531, 8)	1.7% ± 2.5% (405, 8)

Table 14. Experiment 14. Mortality of Sydney rock oysters (*Saccostrea commercialis*) and Pacific oysters (*Crassostrea gigas*) on depot sticks kept at 34°C. Replicates are individual frames of sticks.

	Mortality % (n)			
Time at 34°C	SRO		PO	
42 h	1%	(205)	71%	(24)
	1%	(245)	68%	(22)
45 h	0%	(201)	67%	(24)
	1%	(205)	77%	(70)
	1%	(171)	38%	(42)
48 h	2%	(200)	84%	(51)
	4%	(156)	70%	(24)
	2%	(168)	83%	(6)

Table 15. Experiment 15. Mortality of Sydney rock oyster spat (*Saccostrea commercialis*) and Pacific oyster spat (*Crassostrea gigas*) on 8-10 month old caught sticks kept at 33-34°C. Replicates are sticks within frames.

	Mortality % (n)			
Time at 33-34°C	SRO		PO	
36 h	57%	(95)	100%	(3)
	39%	(112)	100%	(6)
	28%	(128)	100%	(5)
	30%	(112)	100%	(5)
	15%	(85)	87%	(7)
42 h	62%	(115)	100%	(3)
	66%	(186)	100%	(1)
	67%	(141)	100%	(6)
	57%	(190)	100%	(7)
	62%	(155)	100%	(5)
45 h	66%	(209)	100%	(4)
	68%	(219)	100%	(3)
	72%	(246)	100%	(1)
	66%	(240)	100%	(2)
	71%	(215)	100%	(3)
48 h	77%	(146)	100%	(5)
	86%	(187)	100%	(6)
	88%	(177)	100%	(3)
	87%	(193)	100%	(5)
	89%	(150)	100%	(6)

Table 16. Experiment 16. Mortality of Sydney rock oyster spat (*Saccostrea commercialis*) and Pacific oyster spat (*Crassostrea gigas*) on 8-10 month old caught sticks kept at 33°C. Replicates are sticks within frames.

	Mortality % (n)			
Time at 33°C	SRO		PO	
24 h	74%	(87)	50%	(2)
	50%	(110)	-	(0)
	59%	(97)	-	(0)
	54%	(116)	-	(0)
	47%	(115)	-	(0)
36 h	27%	(143)	100%	(3)
	42%	(147)	100%	(3)
	34%	(151)	100%	(3)
	34%	(138)	100%	(2)
	35%	(96)	100%	(1)
42 h	16%	(58)	86%	(7)
	12%	(112)	80%	(5)
	34%	(86)	83%	(6)
	26%	(98)	100%	(5)
	42%	(69)	80%	(5)
45 h	13%	(92)	100%	(4)
	16%	(125)	100%	(1)
	37%	(143)	-	(0)
	23%	(142)	100%	(2)
	27%	(117)	100%	(3)
48 h	47%	(86)	100%	(3)
	39%	(108)	100%	(3)
	54%	(138)	100%	(1)
	43%	(105)	100%	(5)
	40%	(138)	100%	(3)

THE EFFECT OF FREEZING ON THE SUBSEQUENT SURVIVAL OF PACIFIC OYSTERS (*Crassostrea gigas*) AND SYDNEY ROCK OYSTERS (*Saccostrea commercialis*).

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ABSTRACT

Live Pacific oysters (*Crassostrea gigas*) and Sydney rock oysters (*Saccostrea commercialis*) were frozen rapidly in a freezer, and kept at -20°C for 22 hours. The oysters were thawed in sea-water at 17°C. Ten hours after thawing commenced, all of the Pacific oysters were gaping. The adductor muscle was easily shredded, and the gills took on a greyish appearance, however, the taste of the meat was not impaired. Many of the Sydney rock oysters (61%) gaped 10 hours after thawing commenced, but 5 oysters (6%) did not gape until 5 days after thawing, and 3 oysters (4%) did not gape until 8 days after thawing. The flesh of these oysters did not smell of putrefaction, and it is suggested that the oysters survived the freezing but gaped because of damage caused to the adductor muscle by the freezing process.

INTRODUCTION

Australian quarantine regulations require that shellfish (mussels and oysters) imported into Australia from New Zealand should be dead (Anon, 1984). This is acheived by exporting the shellfish "chilled killed", that is, frozen. Some doubt was raised as to whether the frozen shellfish could survive the freezing (J. Humphrey, above); Roughley (1926) found that a number of Sydney rock oysters (*Saccostrea commercialis*) that had been frozen hard enough to prevent a thermometer being pushed into their flesh survived for several months after being replaced in the Georges River. There is a risk that people might be tempted to replace imported shellfish in Australian waters if it was found that significant numbers of shellfish do survive freezing, with attendant problems of introduction of exotic diseases and organisms. This experiment was designed to discover whether Pacific oysters (*Crassostrea gigas*) and Sydney rock oysters would survive being frozen under commercial conditions.

MATERIALS AND METHODS

Pacific oysters (*Crassostrea gigas*) and Sydney rock oysters kept in a pond at the research station were scrubbed and weighed. Approximately 40 each of large and small Pacific oysters and Sydney rock oysters (Table 1) were put into an empty commercial prawn freezer set at -20°C. The temperature of the oysters was not measured, but it is likely that they reached -20°C less than an hour after being placed in the freezer. The oysters were left in the freezer for 22 hours, then brought back to the research station in an insulated container. Less than 90 minutes after being removed from the freezer, the oysters were put into running sea-water at 17°C to thaw. The oysters were examined one hour, and nine hours after thawing commenced, and thereafter daily until eight days after thawing. Oysters that were gaping and unable to close their shell were considered to be dead or moribund.

RESULTS

Pacific oysters

Almost all of the small, and 75% of the large Pacific oysters were gaping an hour after thawing commenced. The remainder were gaping nine hours later (Table 2). On gross examination, the shells of the Pacific oysters (which are thinner and more fragile than Sydney rock oyster shells) had become crumbly in texture. The meat condition of the oysters seemed to be reasonably good. The shell liquor was opaque, with fragments of tissue floating in it. The adductor muscle was easily torn apart, and the gills, (which are more highly pigmented that the gills of Sydney rock oysters) took on a greyish appearance. The taste of the meat did not seem to be different to that of fresh Pacific oysters from the pond.

Sydney rock oysters

Only two of the large and three small Sydney rock oysters were gaping one hour after thawing commenced. Most of the small oysters and 50% of the remaining large oysters were gaping nine hours after thawing (Table 2). Most of the remaining Sydney rock oysters gaped over the next two days, except for three large and two small oysters that were able to close their shells until five days after thawing, and three large oysters which lasted for eight days after thawing (Table 2). The shells of the Sydney rock oysters were weakened by the freezing and thawing, but not to the same extent as those of the Pacific oysters. The meats were in good condition. There were some fragments of tissue in the shell liquor (but not as much as the Pacific oysters). The adductor muscle was easily torn, like that of the Pacific oysters. The gills were not discoloured. The taste of the

frozen meats was not affected. There was no observable smell of putrefaction in any of the Sydney rock oysters that did not start to gape until several days after thawing.

DISCUSSION

It seems probable that the risk that frozen Pacific oyster would be replaced in the water by people wishing to keep them for a future market is low, since the oysters were apparently killed (or at least damaged) by the freezing and thawing. What is perhaps more interesting is the prolonged survival of a percentage of the Sydney rock oysters after freezing and thawing. Roughley (1926) froze a number of Sydney rock oysters in an ice and salt bath, and found that the flesh of one of the oysters he opened had frozen too hard to insert a thermometer. He reported that one or two of the oysters survived in the Georges River for several months after thawing. Since oysters do not have a centralised nervous system, it is rather difficult to define when an oyster is dead; as part of a study of the processes of decay in oysters, it was discovered that the gut and digestive diverticulae (which started to decay very rapidly) might be completely necrotic, while the tissue of the mantle and gills remained relatively unnaffected and still pumped water. During the experiment described above, it was observed that the adductor muscles of the oysters of both species that were frozen became very soft and tore easily; little pressure was required to force open the valves as the muscle fibres separated from each other and the shell. There was no smell of putrefaction in the Sydney rock oysters that did not gape for several days after thawing, and the meat of the oyster did not appear to be damaged upon gross examination. It is therefore possible that the process of freezing and thawing did not kill the oysters, but the oysters gaped because the adductor muscle was damaged and became unable to hold the oyster closed against the pressure of the shell ligament.

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Table 1. Number and weight (mean ± S.D.) of Pacific oysters (*Crassostrea gigas*) and Sydney rock oysters (*Saccostrea commercialis*) frozen during the experiment.

Size and Species	Weight of oysters (g)	Number
Large Pacific oysters	54.7 ± 13.0	38
Small Pacific oysters	7.2 ± 1.8	41
Large Sydney rock oysters	45.0 ± 7.9	40
Small Sydney rock oysters	8.1 ± 1.6	40

Table 2. Number of Pacific oysters and Sydney rock oysters gaping after thawing.

	Number of oysters gaping			
Time after thawing	Large Pacific oyster	Small Pacific oyster	Large Sydney rock oyster	Small Sydney rock oyster
1 hour	28	39	2	3
10 hours	10	2	19	30
1 day	-	-	8	4
2 days	-	-	5	1
5 days	-	-	3	2
8 days	-	-	3	-
Total number of oysters	38	41	40	40

GROWTH AND SURVIVAL OF PACIFIC OYSTERS (*Crassostrea gigas*) AND SYDNEY ROCK OYSTERS (*Saccostrea commercialis*) ON DEPOT STICKS AT DIFFERENT GROWING HEIGHTS

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ABSTRACT

Blocks of Sydney rock depot sticks were placed on racks at the conventional growing height (nominally mid-tide), and 150mm and 300mm above mid-tide. The number of Pacific oyster (*Crassostrea gigas*) spat recruited on the sticks decreased with growing height, with the greatest number of Pacific oysters per stick occurring in the middle layers of the mid-tide block. The mean shell length of the Pacific oyster spat decreased from 38mm in the mid-tide block to 21mm in the block 300mm above mid-tide. There appeared to be two age classes of Sydney rock oyster (*Saccostrea commercialis*) spat on the sticks, the 1985 catch and a second (1986) catch. The total number of spat (1985 + 1986) per stick did not vary with growing height. The number of 1985 spat per stick decreased with growing height. There was no significant difference between the mean lengths of 1985 spat in the mid-tide block and 1985 spat in the +150mm and +300mm blocks.

INTRODUCTION

An overcatch of Pacific Oyster (*Crassostrea gigas*) spat on Sydney rock oyster sticks (*Saccostrea commercialis*) depot sticks is undesirable. It is illegal to grow Pacific oysters in New South Wales (Anonymous, 1989), so farmers incur the costs of removing the Pacific oysters from the sticks, and risk losing a proportion of the Sydney rock oysters because of the extra handling of the sticks. Pacific oysters grow much faster than Sydney rock oysters (Nell, 1989a), and a heavy over-catch easily out-competes the Sydney rock spat, overgrowing, and often smothering the slower growing spat.

The slower growth rate of Sydney rock oysters is offset by their greater hardiness out of water (Mason and Nell, 1989), and it has been observed that settlement and survival of Pacific oyster spat is poor compared to Sydney rock spat at growing heights above the mid-tide level (Nell, 1989b). This cultivation experiment was conducted to determine whether there is an optimum growing height at which settlement and growth of Pacific oyster spat is severely limited, while still allowing reasonable growth of Sydney rock oysters.

MATERIALS AND METHODS

A section of depot (Malcolm, 1987) rack in Tilligerry Creek, in the inner harbour of Port Stephens, was chosen as a growing site. Two sections of the rack were modified by being elevated 150mm and 300mm respectively above the normal depot rails, nominally at mid-tide (Fig. 1). Two depot blocks of sticks were placed on each of the modified sections of rack, and two blocks at the normal growing height were set aside for the experiment. The depot blocks consisted of double frames of 24 sticks (20 sticks across, Fig. 2) wired together in a block of four (Fig. 3). The sticks were 1.8m long by 25mm x 25mm so the underside sufaces of the sticks were separated by 50mm, and the underside surface of the top frame was 150mm above the underside surface of the bottom frame (Fig. 3). The spat were found mostly on the underside surfaces of the sticks of each frame except the bottom frame, which was turned upside down with respect to the other frames to prevent fish predation of the exposed spat.

The Sydney rock oyster spat were originally caught early in 1985 in Salamander Bay, and transferred to depot in Tilligerry Creek at the end of 1985. The six depot blocks were brought ashore from Tilligerry Creek in July 1986, after 8 months in depot. Each block was taken apart, and the double frames laid out with the underside of the sticks uppermost for ease of counting. The number and species of spat on each stick was recorded.

The spat were measured with calipers. The length of the shell is the distance between the "beak" of the shell at the hinge end, and the the furthest edge of the shell margin. Measurements of Sydney rock spat were made on one stick chosen at random from each frame at each growing height, and every spat on the stick was measured. Because there were relatively few Pacific oyster spat, the length of every Pacific spat in the six blocks was measured.

The effect of frame height and the position of the stick within the frame (1 to 20) on the numbers of spat caught per stick was examined using a two-way anova. Ranked normal deviates (Sokal and Rohlf, 1969) were calculated for the lengths of Sydney rock oyster spat on each frame of each block.

RESULTS

Pacific oysters

The number of Pacific oyster spat recruited per stick was affected by the growing height. The number of spat per stick ranged from 6.15 ± 3.16 ($x \pm$ S.D.) on the third frame of the mid-tide blocks to 0.25 ± 0.63 on the top frame of the +300mm blocks (Table 1). There was a slight but significant

($p = 0.0104$) effect of stick position within the frame on numbers of Pacific spat per stick, but this disappeared if the outermost sticks (numbers 1 - 3 and 18 - 20) were excluded from the anova.

The mean length of the Pacific oyster spat decreased with increase in growing height from 39.0 $\pm$ 8.0mm on the bottom frame of the mid-tide blocks to 18.3 $\pm$ 6.7mm on the top frame of the +300mm block (Table 2).

Sydney rock oysters

While counting and measuring the Sydney rock oyster spat on the sticks, it became apparent that there were two age classes of spat present, corresponding to the original 1985 Salamander Bay catch and an overcatch from Tilligerry Creek in 1986. The overall number of Sydney rock spat per stick decreased slightly with increasing growing height. The two age classes of spat present on sticks in the +150mm and +300mm blocks could be distinguished by plotting the ranked normal deviates (rankits) against length (Fig. 4). The discontinuity in the plot was used to estimate a length that could be used to separate the two age classes for each growing height. When the two age classes were separated on the basis of size, it became apparent that the number of the original 1985 set of spat per stick decreased from 93 per stick on the second frame of the mid tide blocks, to 33 per stick on the third frame of the $\pm$300mm blocks (Table 3). The 1986 overcatch was distinguishable in all frames above the bottom frame of the +150mm blocks (Table 4).

The overall mean length of the Sydney rock oyster spat decreased with growing height from 39.1 $\pm$ 10.2mm on the bottom frame of the mid-tide blocks, to 32.9 $\pm$ 8.7mm on the top frame of the +300mm blocks. This reduction in mean size was apparently caused by the increasing proportion of the smaller 1986 age class spat, as the mean size of the 1985 spat on the top frame of the +300mm blocks was 38.6mm once the 1986 spat had been subtracted (Table 5).

DISCUSSION

As expected, the number and size of Pacific oyster spat decreased with increasing growing height. The number decreased from around 100 per frame in the mid-tide block to fewer than 20 per frame in the +300mm block. The mean length was reduced by two thirds to a half between the lowest and highest growing heights, which corresponds to a three- to eight-fold reduction in spat weight (C. Mason, unpublished data, 1988). The reduction in size was not caused by different age classes of Pacific oyster spat, as the spat length distribution at each height was normal and not bimodal, as was the case for the Sydney rock spat.

The interpretation of the number and length of the Sydney rock oyster spat at the different growing heights was complicated by the presence of a second catch of spat. These spat were obviously different to the 1985 catch, as the length of the shells was smaller, and the spat were still growing flat over the substrate. The 1985 spat were starting to build up depth, and the growing edges had grown up from the substrate (Fig. 5). Depot sites are chosen to avoid overcatch as much as possible, but the summer and autumn of 1986 were very dry, so the salinity in Tilligerry Creek was much higher than usual. It is believed that this allowed the Sydney rock oyster larvae to penetrate and settle in the inner port areas normally outside their salinity preferences of 27 - 39 ‰(Nell and Holliday, 1988).

The number of Sydney rock oyster spat identified as 1985 catch per stick decreased markedly with growing height. Since the blocks of spat would have originally been caught at the same height, this was presumably caused by subsequent mortality of spat in depot at the higher growing heights. The length of these spat did not vary much with growing height, however. The 1986 overcatch apparently settled in the spaces left on the upper sticks by loss of the original spat, thus bringing the total number of Sydney rock spat on the +150mm and +300mm blocks to nearer the number on the mid-tide blocks.

It seems likely that although significant reductions in the number and size of overcaught Pacific oysters could be acheived by raising the growing height of the sticks in depot, the advantage would be lost in the higher mortality experienced by the Sydney rock oysters.

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Table 1. Number (mean ± S.D.) of Pacific oyster spat per stick at different growing heights.

Block	Frame	Height above mid-tide (mm)	Nmber of spat per stick
Mid-tide	bottom	0	3.20 ± 1.73
	third	50	4.88 ± 1.86
	second	100	6.15 ± 3.17
	top	150	4.05 ± 1.99
+150mm	bottom	150	2.85 ± 1.79
	third	200	3.53 ± 2.10
	second	250	3.43 ± 2.24
	top	300	1.75 ± 1.88
+300mm	bottom	300	0.83 ± 1.13
	third	350	0.95 ± 0.88
	second	400	1.05 ± 1.45
	top	450	0.25 ± 0.63

Table 2. Length (mean ± S.D.) of Pacific oyster spat at different growing heights.

Height above mid-tide (mm)	Spat length (mm)	Number of spat measured
0	40.0 ± 8.0	41
100	39.4 ± 9.2	105
200	35.5 ± 7.8	57
300	28.4 ± 6.5	47
350	25.3 ± 5.8	20
400	26.2 ± 4.7	15
450	18.3 ± 6.7	6

Table 3. Number (mean ± S.D.) of Sydney rock oyster (*Saccostrea commercialis*) spat per stick at different growing heights.

Block	Frame	Height above mid-tide (mm)	Number of spat per stick (Total)	Number of spat per stick (1985 catch only)
Mid-tide	bottom	0	70.3 ± 22.6	*
	third	50	87.4 ± 27.4	*
	second	100	93.3 ± 19.7	*
	top	150	79.7 ± 13.8	*
+150mm	bottom	150	87.2 ± 21.1	*
	third	200	97.0 ± 35.1	63%, 61/stick
	second	250	86.6 ± 23.0	no data
	top	300	79.4 ± 18.1	39%, 31/stick
+300mm	bottom	300	67.4 ± 16.9	no data
	third	350	56.1 ± 18.1	67%, 37/stick
	second	400	79.1 ± 19.7	53%, 40/stick
	top	450	62.6 ± 19.4	66%, 41/stick

* size classes not separable.

Figure 5. Cross section from hinge to lip of 1985-caught Sydney rock oyster (*Saccostrea commercialis*) spat and 1986-caught spat.

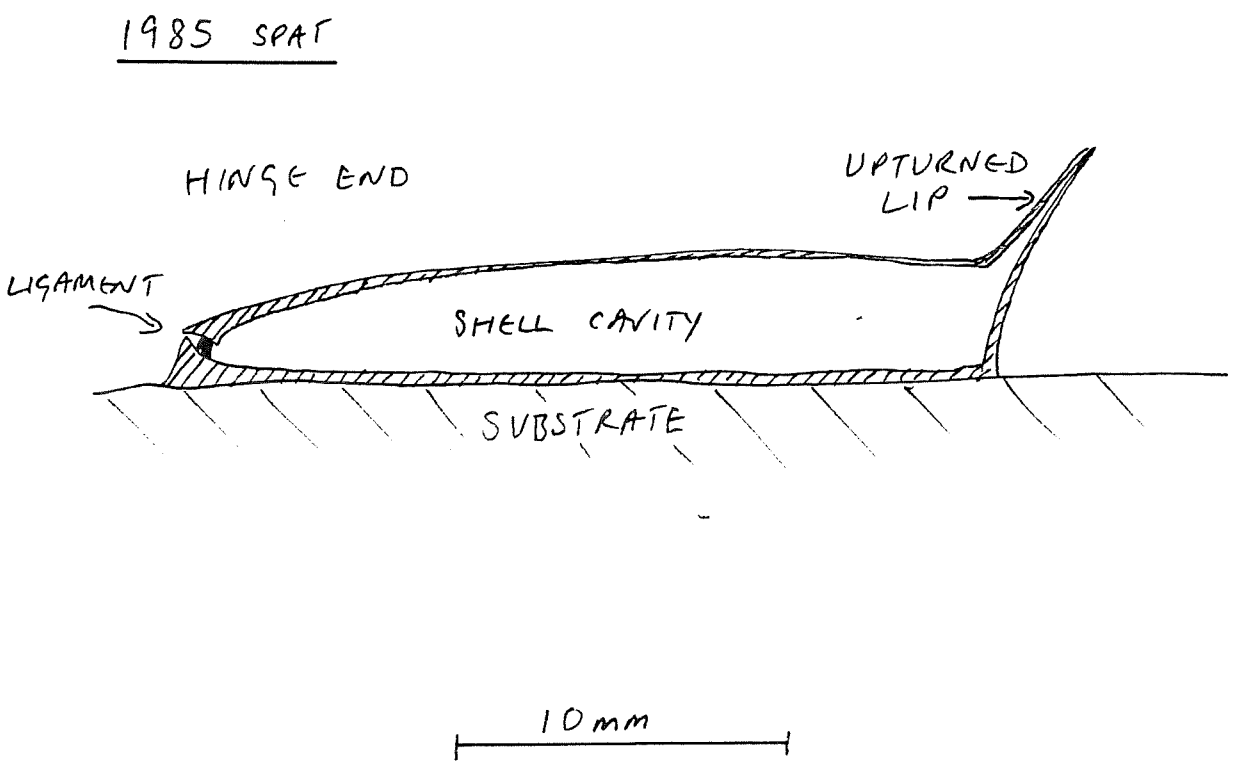


Table 4. Frequency distribution of Sydney rock oyster spat length with growing height.

Class limits length (mm)	Growing Height (mm above mid-tide)							
	0	100	150	200	300	350	400	450
5 < 10	0	0	0	**1**	**1**	**1**	**0**	**0**
10 < 15	0	1	2	**3**	**13**	**8**	**2**	**1**
15 < 20	0	4	2	**5**	**16**	**7**	**6**	**3**
20 < 25	1	5	4	**12**	**16**	**6**	**7**	**3**
25 < 30	4	7	5	**8**	**4**	10	**4**	**5**
30 < 35	7	12	10	12	16	7	9	**2**
35 < 40	19	17	14	6	13	9	11	14
40 < 45	9	22	8	8	14	5	5	6
45 < 50	5	19	9	6	7	0	2	1
50 < 55	2	10	3	4	3	1	0	0
55 < 60	0	4	1	0	1	0	0	0
60 < 65	0	1	0	0	0	0	0	0
Total:	47	102	58	65	104	54	46	35

Frequencies printed in bold type have been classified as 1986 second catch.

Table 5. Length (mean ± S.D.) of Sydney rock oyster (*Saccostrea commercialis*) spat at different growing heights.

Height above mid-tide (mm)	Spat length (all spat)	Number of spat measured	Separation length (mm)	Spat length (1985 only) (mm)	Number of spat measured
0	38.0 ± 6.7	47	*	-	-
100	39.1 ± 10.2	102	*	-	-
150	35.8 ± 9.9	58	*	-	-
200	31.5 ± 11.1	65	26	38.5 ± 7.4	40
300	29.0 ± 12.2	104	23	38.0 ± 7.5	60
350	26.8 ± 10.4	20	27	35.4 ± 5.5	27
400	29.9 ± 9.6	46	27	36.0 ± 5.1	30
450	32.9 ± 8.7	35	29	38.6 ± 2.9	23

* Size classes not separable.

Figure 1. Modified sections of depot racks.

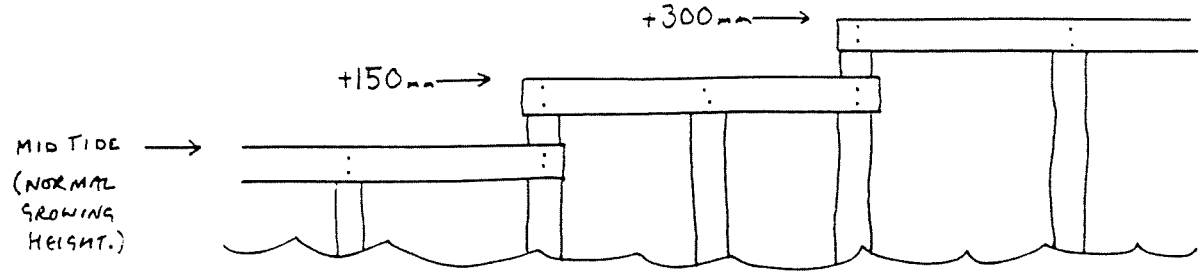


Figure 2. Double frame of 20 + 4 sticks nailed together.

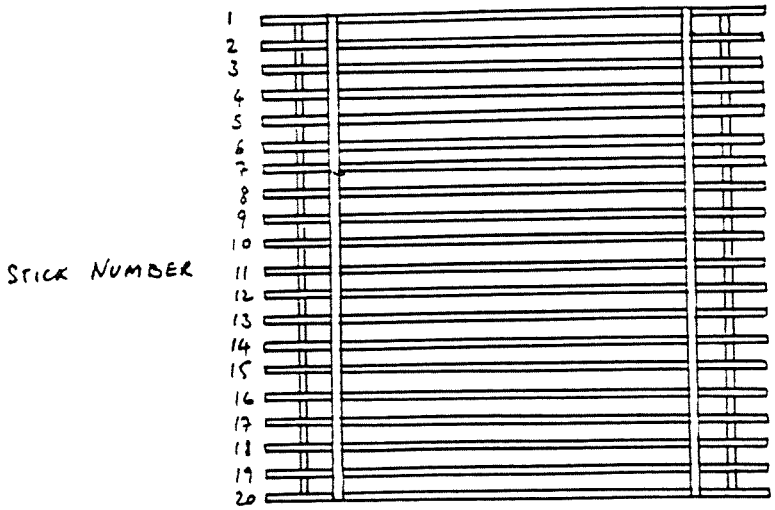


Figure 3. Depot block consisting of four frames wired together.

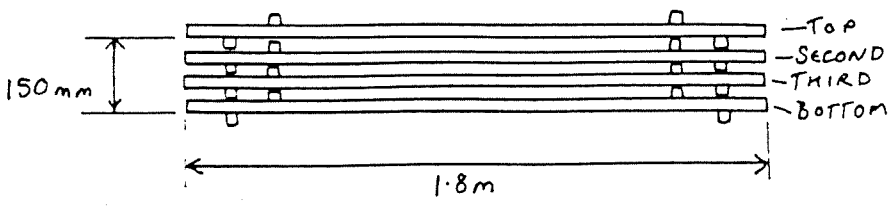
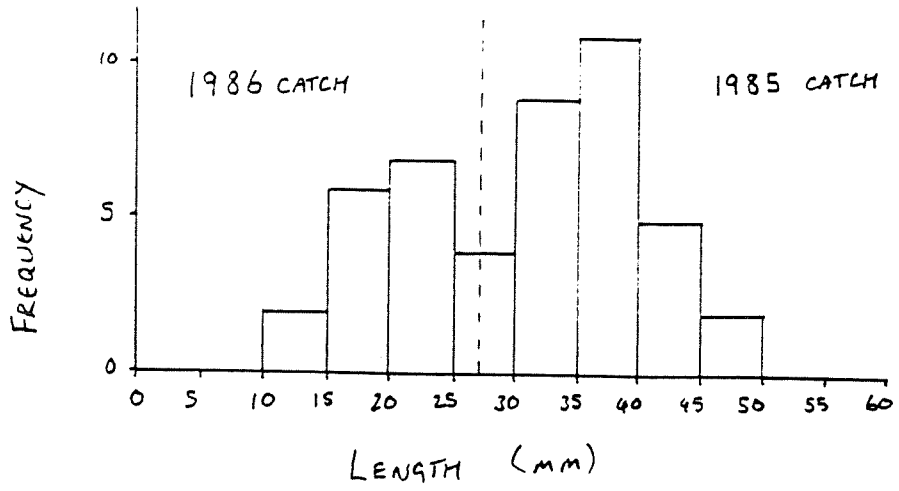
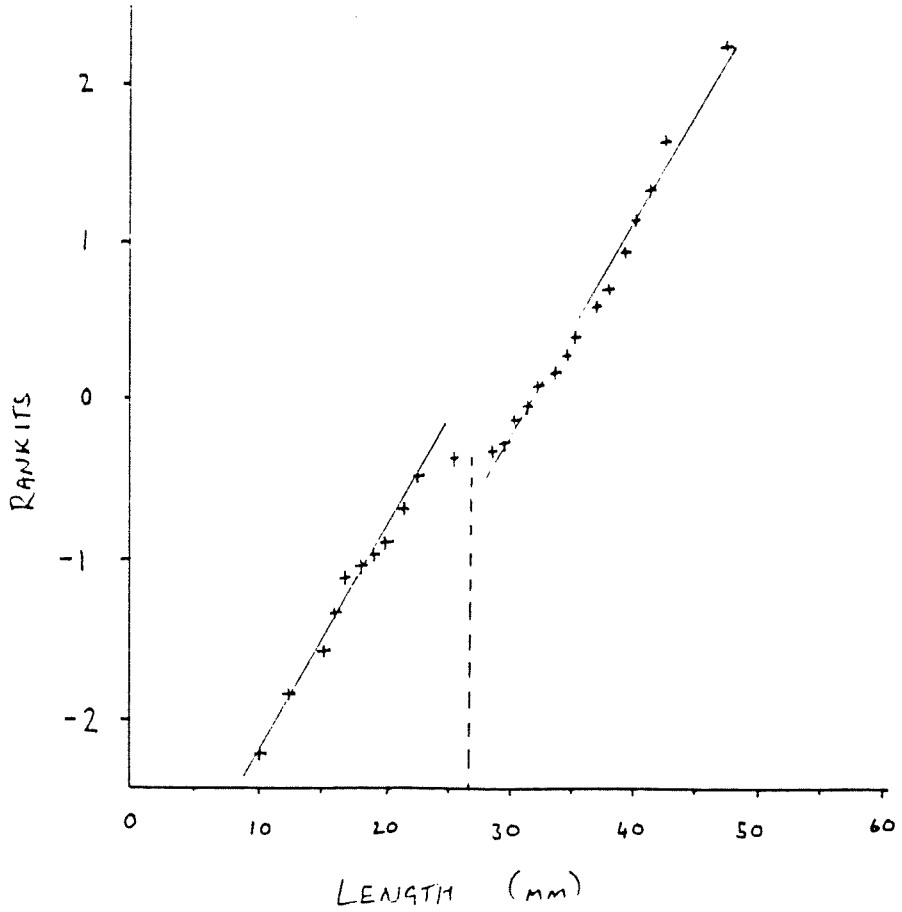


Figure 4. Size distribution of Sydney rock oyster (*Saccostrea commercialis*) spat on the second frame of the +300mm blocks (400mm above mid-tide).



Plot of Ranked Normal Deviates (Rankits) of Sydney rock oyster spat length on the second frame of the +300mm blocks (400mm above mid-tide).



SECTION 6

GROWTH STUDIES OF SYDNEY ROCK OYSTERS

THE EFFECT OF STUNTING, TEMPERATURE AND SIZE ON THE GROWTH OF SYDNEY ROCK OYSTER (*Saccostrea commercialis*) SPAT.

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ABSTRACT: (*Saccostrea commercialis* Iredale & Roughley, 1933) spat were obtained from a single spawning of four female and two male oysters. The spat were divided into six size classes by weight. Spat of each size class were distributed among twelve treatments. The difference between treatments consisted of the length of time the spat spent in poor growing coonditions, followed by good conditions, or in good growing conditions followed by poor conditions. The growth rate of spat of any size was very low when they were kept in poor growing conditions. The growth rate of spat kept in good conditions depended on the water temperature and on the size of the spat, but not on the original size grading of the spat nor on the length of time the spat had originally spent in poor growing conditions. A temperature-corrected von Bertalanffy model was applied to the growth of the spat. The difference in relative growth rates seen between spat with the largest and smallest initial sizes was thought to be an artefact caused by the temperature correction of the growth model, and the slowing of growth as the larger spat divert energy into gonad production. Differences in size between spat were caused by random environmental effects causing the temporary stunting of a proportion of the spat, not by genetic differences in relative growth rate.

INTRODUCTION

Growth of bivalves is affected by environmental variables such as temperature and particulate matter (Malouf and Breese, 1977; Incze et al, 1980; MacDonald and Thompson, 1985; Brown, 1988; Spencer, 1988), water flow rate (Rodhouse and O'Kelly, 1981; Wildish and Kristmanson, 1985) and the amount of time the animals spend exposed to air (Spencer et al, 1978; Gillmor, 1982). The effect of salinity appears to negligible (Brown and Hartwick, 1988; Nell and Holliday, 1988) (for reviews of bivalve growth studies, see Winter, 1978 and Bayne and Newell, 1983). If a true assessment of the effect of genotype on the growth rate of oysters is to be obtained, the effect of the environment on the phenotype should be measured and accounted for.

A number of field studies have demonstrated the effect of environmental variables on bivalve growth: Instantaneous growth rates (dW/dt)/W of American oysters *Crassostrea virginica* taken from an intertidal oyster bar decreased with increasing size and lower temperatures (Dame 1972). Brown (1988) attributed the differences between medium and good growing sites for Pacific oysters *Crassostrea gigas* in British Columbia (Canada) to differences between the amount of food present in the water at the sites. In this case temperature differences between sites were less important than food supply. Spencer *et al* (1978) reported that growth of Pacific oysters held on intertidal trays was markedly reduced at exposures to air between 10-30% of the tidal cycle, and the degree of exposure at zero growth was found to vary substantially between two sites because of differences in wave action.

Another approach is to conduct controlled studies, in which only one variable is changed at a time. In American oyster spat fed with different rations of two algal species over a three week period, maximum growth occurred at the highest algal densities tested (Urban *et al* 1983). Maximum assimilation efficiency however, occurred at a much lower algal density. In a similar study (Laing *et al* 1987), three species of juvenile clams were fed diets of microalgae at four different temperatures. The clams grew fastest at higher temperatures, but growth efficiency was greatest near the middle of the temperature range tested. The authors observed an increased deposition-of shell relative to organic growth at higher temperatures.

Theoretical growth models may be used to predict bivalve growth in situations for which no data is available. Menesguen *et al* (1984) used a von Bertalanffy growth curve modified by a trigonometric funtion to model the seasonal effect of temperature. This model accounted for the winter decrease in growth in Manilla clams *Tapes phillipinarum*, and enabled the authors to compare the growth of the clams under different rearing conditions. By calculating the von Bertalanffy coeffient K, they were also able to compare growth rates of clams of different sizes. The growth of flat oysters *Ostrea edulis* in the Beaulieu River (England) measured from year to year fitted a von Bertalanffy growth curve (Rodhouse 1978). The author used an empirical estimate of the proportion of the year's growth achieved in each season to predict seasonal growth rate during the year. Another type of modification to the von Bertalanffy growth curve, involving the subtitution of a day x temperature term for the time term, closely approximated data on American oyster growth affected by seasonal changes in temperature (Dame, 1975).

Bivalves appear to have some peculiarities of growth that will affect the interpretation according to which variable is being measured. For instance, the growth of soft tissues and

of shell in mussels *Mytilus edulis* is uncoupled, as the mussels put on more shell growth compared to meat in winter than in summer (Hilbish 1986). The irregular shell growth habit of oysters is well known, the relative shell dimensions depending on the environment in which the oyster has been grown. Hatchery produced, cultchless Sydney rock oyster spat (*Saccostrea commercialis*) have a much more regular shell form than wild-caught spat (Nell and Mason, 1991). The growth of Sydney rock oyster spat also appears to be uncoupled to some extent, as they can rapidly extend the shell margins to make a deeply cupped, thin shell under some circumstances, and then thicken the shell and increase the volume of the soft tissues later (C. J. Mason, unpublished data, 1990). Thus, the weight of spat of the same length and area may differ greatly, depending on the volume enclosed by the upper and lower valves of the oyster.

In contrast to the the substantial literature on the effects of environmental variables on bivalve growth, there has been little work reported on the effects of genotype on growth of bivalves. Newkirk and Haley (1983) reported that first generation flat oysters *O. edulis* selected for fast growth had a weight gain averaging 23% greater than control lines at approximately two years of age. The advantage was lost in the second generation as the selected offspring were not significantly heavier than the controls. The poor performance of the second generation of selected spat was attributed to inbreeding depression. In two separate studies, American oyster *C. virginica* larvae settling on different days were caught on collectors provided on a daily basis. The spat were measured a few months later, and "early setters" were found to be significantly larger than "late setters" (Newkirk *et al* 1977, and Losee 1978). In each case the authors concluded that larval growth rates and spat growth rates were highly correlated, and that it should be possible to select for fast spat growth rate by culling slower growing larvae.

Our experiment was designed to determine whether the difference in size between the largest and smallest spat in a batch is genetic or caused by local environmental factors, and to see if spat kept under poor growing conditions would remain stunted when given good growing conditions. Thus it answered the question "Are the largest spat in a cohort large because they have genetically superior growth rates, or because they have had more favourable growing conditions?"

MATERIALS AND METHODS.

Oyster spat were obtained from a spawning of four female and two male Sydney rock oysters *Saccostrea commercialis* in July 1987. After two months in the nursery as cultchless spat, the 20,000 surviving spat were transferred to a single

outside upweller unit in September 1987. Size variability between the spat was deliberately increased by keeping them at a high density in the upweller for three months. Under these conditions the larger, heavier spat settled to the bottom of the upweller where they were the first to feed in the incoming water, while the lighter spat at the top received a mixture of well-filtered water and faeces. In January 1988 the spat were graded by weight into six size classes and tranferred to baskets with nylon fly-screen bottoms (Table 1). Six baskets (one of each size class) were allotted to each of twelve treatments, totalling seventy-two baskets. Spare graded spat were held in additional baskets and used as replacements for dead spat during the course of the experiment.

To examine the effect of initial size and growing conditions on the growth rate of the spat, two different growing environments were set up. Each consisted of a short concrete raceway (2.3m x 0.95m x 0.80m deep, volume = 1750L), fitted with plywood lids to prevent growth of filamentous algae. The raceways were supplied with water from a 0.1 ha pond pumped at 1.5 to 2.5Ls^{-1}. The pond was fertilised regularly to maintain a phytoplankton bloom. One raceway received water for 24 h per day (the "fully-fed" raceway), while the other received water for only 4 h per day, and remained drained for the rest of the time (the "starved" raceway). Initially, treatments 1 to 6 were stocked in the fully-fed raceway (a total of thirty-six baskets), and treatments 7 to 12 were stocked in the starved raceway. One basket of spare spat of each size class was also stocked in each raceway; they were referred to as the fully-fed spares and starved spares.

The various treatments differed as to the length of time the spat spent in each raceway: Two months after the start of the experiment, the six baskets of treatments 1 and 7 were exchanged; thus by the end of the experiment, the history of treatment 1 consisted of 2 months in good conditions, followed by 10 months of poor conditions, while treatment 7 had the converse history, 2 months poor conditions followed by 10 months of good conditions. This process was repeated every two months (Table 2), and by the end of the year, all treatments except 6 and 12 had been removed from the raceway in which they had originally been placed. As treatments 6 and 12 spent the year in their original raceways, they represented the control treatments for the fully-fed and the starved raceways, respectively.

The raceways were drained and the baskets removed and hosed as often as was necesary to prevent a build-up of mud and faeces; during the winter this was every two to three days, but the spat had to be washed daily during the summer. The positions of the baskets in the raceways were changed on a rotational basis so that any differences in availability of food in different parts of the raceways would be minimised.

By April 1988 it was apparent that the spat in the fully-fed raceway were growing much faster than expected. In order to prevent overcrowding affecting the growth rate of the spat, a second fully-fed raceway was set up with each treatment being equally represented in each fully-fed raceway. When spat in a standard basket reached or exceeded a total weight of 200g at a monthly weighing, they were tranferred into a larger basket with a larger mesh size. There was a small set of barnacles and wild oyster spat on the walls of the raceway and on some of the experimental spat during the summer, however these were easily identified and scraped off before the spat was weighed.

Water temperature was monitored daily with a maximum-minimum thermometer located in the fully-fed raceway. At monthly intervals the spat were dried and weighed individually. Dead spat were recorded. Dead spat from baskets still in their original raceway were replaced with spat of the same size class from the spares baskets. Monthly mortality normally ranged from 0 to 4.5%. Class 2 of treatment 3 suffered 19% mortality at the 6 month weighing, possibly because of exessive exposure to the sun while drying.

Statistical methods

A mean monthly water temperature was obtained for each weighing by summing the mean daiły temperature ((minimum + maximum)/2) over the period between monthly weighings, and dividing by the number of days between weighings. To assess the effect of water temperature on growth rate, the following calculation was carried out: Mean weights for spat of all classes and treatments in the fully-fed raceway were assigned to three ranges; 1 - 2g, 6 - 8g and 10 - 13g. The relative spat growth rate (weight at month(n) divided by weight at month(n-1)) for each range was then plotted against water temperature. The relative growth rate was extrapolated to zero for each size range at between 9.5 and 11°C, mean 10°C (Table 3). Because fitted growth rate is zero at about 10°C, the water temperature minus 10 was used in calculations for temperature corrections of growth rates.

Average relative growth (ARG) is defined by:

$$ARG = (\ln(W_2)-\ln(W_1))/time$$

where W_1 is the weight (g) measured at the start of the month, W_2 is the weight measured at the end of the month, and time is the time between measurements in days.

A day x degree temperature adjustment was employed by substituting (days x temperature-10) for days in the formula for ARG to obtain temperature-corrected average relative growth (CARG):

$$CARG = (\ln(W_2)-\ln(W_1))/days \times (temp-10)$$

RESULTS.

Mean monthly temperature ranged from 13.90°C to 24.26°C (Table 4). The mean weights of spat from treatment 6 and treatment 12 are shown in Table 5. Plots of water temperature and log weight for treatments 6 and 12 against time (Figs 1, 2 and 3) indicate that the growth rate of the spat was strongly influenced by water temperature.

Growth rate of the spat is dependant on temperature, with the regression of ARG on the mean log weights of all classes of treatment 6 spat over the year showing a reasonably good fit ($r^2 = 0.81$). The fit for the regression of CARG against the mean log weight for all classes was slightly improved ($r^2 = 0.91$), Table 6. Analysis of covariance showed that the slopes and intercepts of the regressions of CARG on mean log weight for each size class of treatment 6 were not significantly different, ie the temperature corrected average relative growth is not significantly different between classes (Table 6). The residuals of the regression indicate a trend towards reduced relative growth rate with increasing initial size. Thus, the day x degree temperature correction was not perfect, as it caused the CARG of the smaller spat to be underestimated, particularly at low temperatures.

An improved fit was obtained by estimating the multiple regression of ARG against log weight and a squared temperature term (Table 7). These regressions are not as useful as the CARG versus log weight, as both the coefficients for the log weight and the squared temperature terms differ between classes, with the classes are being directly comparable. There was a trend for the log weight coefficient to decrease with increasing initial size, as for the CARG-log weight regression. There was also a trend for the squared temperature coefficient to decrease with increasing initial weight, which implied that changes in temperature have a greater effect on the growth rate of the smaller spat than the larger spat.

Since growth rates of spat in the starved raceway were substantially less than for spat in the fully-fed raceway, the explanatory power of the regressions was less.

That the growth rate of spat initially graded into different size classes was not significantly different betweeen classes, is confirmed by comparing the mean log weights of [treatment 7/class 1], [treatment 9/class 5] and [treatment 10/class 6] from when they were exchanged to the end of the experiment (Table 8): The weights of these size classes were very similar when the spat were exchanged, and despite different histories of grading and previous growing conditions between the three sets of spat, the growth curves are closely matched.

Figures 4 and 5 illustrate the effect on spat growth of the

exchange between the fully-fed and the starved raceway, and vice versa. For clarity, only class 3 from each treatment is shown: Spat exchanged from the starved raceway to the fed raceway at warm temperatures appeared to grow faster than expected, but if the exchange took place during the cold months, they appeared to lag in growth rate for a couple of months. The most recently exchanged spat were the smallest in the raceway, and thus were the most affected by temperature.

A day x degree temperature adjustment was employed by substituting (days x temperature-10) for days in the formula for ARG to obtain temperature-corrected average relative growth (CARG):

$$CARG = (\ln(W_2)-\ln(W_1))/\text{days} \times (\text{temp}-10)$$

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DISCUSSION

The genetic composition of the spat used in the experiment was a mixture of full-sibs, half-sibs, and unrelated spat. Had the differences in spat size at the initial grading been caused by differences in growth rate, the monthly average relative growth rates of the large size classes would have been greater than those of the small classes. It was not possible to distinguish between the effects of genetically inferior growth rates and differences in growth rate caused by permanent environmentally induced stunting. Some of the spat were kept for up to 10 months under very poor growing conditions. Reduced relative growth rates in these spat once transferred to good conditions would very likely have been caused by some degree of permanent stunting.

Since the temperature varied seasonally throughout the duration of this experiment, a temperature corretion was applied in order to compare the growth rate of spat of a given size measured at different temperatures. When the temperature correction was used, the smaller size classes appeared to have a relative growth rate [(dWt/dt)/Wt] greater than the larger size classes. A possible explanation is that the smaller spat, having been stunted, are now compensating for the period of stunting by "catching up" with a faster

relative growth rate. However, it is difficult to postulate a mechanism for such compensation. Another explanation is that the larger spat become sexually mature during the warm months and divert energy that in immature spat would have been used in somatic growth, into developing gonads; the reduction in relative growth rate of the larger spat in the summer months is over-compensated by the temperature correction, so that their relative growth rate over the year appears to be less than the smaller spat.

The second hypothesis is supported both by direct observation and by the literature. Sydney rock oyster spat as small as 15 mm (which corresponds to a whole weight of 2 - 3g) often have mature sperm or ova in their gonads in the summer months (C. J. Mason, personal observation 1988). Rodhouse (1979) carried out a detailed breakdown of the energy budget of flat oysters *O. edulis* in the estuary of the Beaulieu River (England) using a von Bertalanffy growth model. He did not apply the model to recently settled spat, as he believed that it did not give a good fit in early parts of the growth curve. He found that in the flat oyster, gonad output increased with increasing size, and eventually accounted for almost all soft tissue production. Given the assumptions of the von Bertalanffy growth equation (ie that the growth rate of an organism depends on the balance between the rate of anabolism and the rate of catabolism) the von Bertalanffy coefficient K of an organism will depend on whether or not it is diverting energy into gonad production. This implies that immature oyster spat would have a faster growth rate than spat with developing gonads, and provides a possible explanation for the decrease in relative growth rates in the larger size classes of spat. The transition between the immature and the mature growth curves is either gradual, or masked by the temperature correction factor. It is possible that development of gonads is stimulated by temperature above some threshold level.

The results of this experiment confirm the findings of other authors, firstly, that a simple temperature correction can largely explain seasonal changes in growth rate (Ursin, 1962; Dame, 1975; Menesguen *et al*, 1984), and secondly that weight increases under a given set of conditions are exponential (Urban *et al*, 1983; Laing *et al*, 1987) (thus relative growth rates must be used to compare the growth of spat of different weights). The important finding is that the relative growth rate of spat originally graded into different (weight) size classes can be fitted to the same growth curve. Thus, the growth rates of the largest and smallest spat were not significantly different. This suggests that the original size differences were caused entirely by temporary environmental stunting, and that once growing conditions were equalised, the stunted spat took up growing at the same relative rate as their larger peers. A field experiment carried out during 1990 (C. J. Mason and J. A. Nell, unpublished data) confirmed this; four groups of spat from

three different year-classes with an initial weight between 8 and 10g (4 to 6g for the youngest year-class) were grown on trays on an oyster lease for a year. The two groups of spat from the oldest year-class had previously been kept under conditions that had stunted then considerably. The middle year-class were normal stick oysters, and the youngest year-class were the largest spat from the 1989 set. Despite initial differences in shell morphology between the groups, after one year of growth the difference in growth rate between the oldest and middle year-classes was not significant ($P > 0.5$). The youngest year-class grew significantly faster than the older year-classes, however the faster growth rate relative to the larger spat fitted the same pattern of growth as the spat in the raceways.

It is possible that despite an apparently low degree of variation in growth rates of oyster spat, selective breeding for fast growth rate may be a feasible method of making the aquaculture of oysters more profitable. The remarks of Wilkins (1981) on the relevance of genetics in aquaculture are still entirely relevant. However, given the pitfalls associated with the large effect of environmental variables on growth and the non-linear growth habit of oysters, great care must be taken in the interpretation of results of selective breeding trials.

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Table 1. Initial weights and numbers of size classes of spat.

Class	Initial weight (g) Min.	Max.	Mean	Number stocked per basket
1	0.03	0.06	0.05	100
2	0.07	0.20	0.13	100
3	0.21	0.40	0.30	100
4	0.41	0.60	0.50	100
5	0.61	0.80	0.69	50
6	0.81	1.11	1.03	25

Table 2. Exchange history of treatments.

Groups	Exchanged at:	History of growing conditions
1	2 months	2 months fed then 10 months starved
& 7	.....	2 months starved then 10 months fed
2	4 months	4 months fed then 8 months starved
& 8	.....	4 months starved then 8 months fed
3	6 months	6 months fed then 6 months starved
& 9	.....	6 months starved then 6 months fed
4	8 months	8 months fed then 4 months starved
& 10	.....	8 months starved then 4 months fed
5	10 months	10 months fed then 2 months starved
& 11	.....	10 months starved then 2 months fed
6	no exchange	12 months fed
& 12	.....	12 months starved

Table 3. Regressions of relative growth ($W_t - W_0/t$) against temperature °C.

Spat Size (g)	Intercept	Gradient	Relative Growth = 0	r^2
1 - 2	-0.48	0.046	10.5°C	84%
5 - 7	-0.25	0.027	9.1°C	95%
10 - 13	-0.16	0.019	8.4°C	60%

Table 4. Mean monthly temperature, °C. *Temperature readings in February 1986 consisted of a single measurement taken at midday.

Month	Days	Temperature (x ± S.D.)		Range
February*	1 - 24	24.26	± 0.76	24 - 26
March	25 - 52	22.36	± 2.34	20 - 29
April	53 - 95	18.41	± 3.34	14 - 29
May	96 - 149	15.84	± 3.26	10 - 25
June	150 - 179	14.40	± 3.00	8 - 17
July	180 - 212	13.90	± 3.29	10 - 24
August	213 - 239	15.39	± 4.19	12 - 25
September	240 - 274	15.48	± 3.44	12 - 27
November	275 - 304	19.22	± 4.80	13 - 30
December	305 - 333	22.76	± 3.24	21 - 31
January	334 - 364	23.40	± 3.58	21 - 31
February	364 - 375	24.34	± 4.25	15 - 30

Table 5. Mean weights (g) ± standard deviation of Treatment 6 spat (fed) and Treatment 12 (starved) over the year.

Treatment 6.

Day	class 1	class 2	class 3	class 4	class 5	class 6
1	0.05 ± 0.02	0.14 ± 0.04	0.31 ± 0.05	0.50 ± 0.06	0.68 ± 0.05	1.01 ± 0.31
25	0.32 ± 0.10	0.67 ± 0.18	0.94 ± 0.17	1.41 ± 0.20	1.69 ± 0.18	2.06 ± 0.47
53	0.97 ± 0.33	1.94 ± 0.50	2.64 ± 0.53	3.35 ± 0.55	3.82 ± 0.53	4.39 ± 0.86
96	2.38 ± 0.79	3.45 ± 0.91	4.46 ± 0.87	5.16 ± 0.92	6.22 ± 0.91	7.35 ± 1.48
116	2.90 ± 0.93	4.06 ± 1.06	5.06 ± 1.02	5.91 ± 1.05	7.08 ± 1.07	8.23 ± 1.64
150	4.01 ± 1.29	5.39 ± 1.32	6.52 ± 1.24	7.45 ± 1.30	8.56 ± 1.24	9.99 ± 1.66
180	4.65 ± 1.35	6.05 ± 1.37	7.30 ± 1.37	8.26 ± 1.41	9.49 ± 1.37	11.16 ± 1.70
213	5.19 ± 1.39	6.79 ± 1.46	7.90 ± 1.42	8.88 ± 1.53	10.22 ± 1.59	12.15 ± 1.67
240	5.66 ± 1.48	7.40 ± 1.57	8.56 ± 1.57	9.38 ± 1.64	11.06 ± 1.69	13.13 ± 1.58
275	7.50 ± 1.81	8.97 ± 1.86	10.25 ± 1.81	11.38 ± 1.97	12.85 ± 1.91	14.83 ± 1.84
305	10.13 ± 2.53	11.57 ± 2.47	13.17 ± 2.27	14.39 ± 2.50	16.17 ± 2.67	18.04 ± 2.06
334	13.24 ± 2.77	14.78 ± 2.88	16.68 ± 2.85	18.01 ± 3.18	19.33 ± 3.55	21.19 ± 2.01
364	14.36 ± 2.90	15.99 ± 2.99	17.82 ± 2.33	19.16 ± 3.39	20.78 ± 3.71	22.32 ± 2.25

Treatment 12.

Day	class 1	class 2	class 3	class 4	class 5	class 6
1	0.05 ± 0.02	0.14 ± 0.04	0.30 ± 0.05	0.49 ± 0.06	0.70 ± 0.06	1.06 ± 0.39
25	0.07 ± 0.03	0.17 ± 0.04	0.25 ± 0.07	0.61 ± 0.08	0.86 ± 0.07	1.25 ± 0.43
53	0.09 ± 0.03	0.22 ± 0.05	0.40 ± 0.07	0.67 ± 0.07	0.95 ± 0.08	1.25 ± 0.46
96	0.14 ± 0.05	0.30 ± 0.06	0.57 ± 0.10	0.76 ± 0.08	1.10 ± 0.17	1.54 ± 0.52
116	0.14 ± 0.05	0.32 ± 0.07	0.60 ± 0.10	0.81 ± 0.08	1.14 ± 0.10	1.59 ± 0.52
150	0.17 ± 0.07	0.42 ± 0.09	0.70 ± 0.11	0.98 ± 0.10	1.36 ± 0.13	1.84 ± 0.59
180	0.20 ± 0.07	0.45 ± 0.09	0.73 ± 0.12	1.01 ± 0.10	1.38 ± 0.17	1.93 ± 0.60
213	0.21 ± 0.08	0.47 ± 0.10	0.74 ± 0.12	1.04 ± 0.15	1.41 ± 0.18	1.91 ± 0.47
240	0.24 ± 0.09	0.49 ± 0.10	0.74 ± 0.13	1.06 ± 0.12	1.42 ± 0.19	1.91 ± 0.48
275	0.25 ± 0.10	0.52 ± 0.11	0.75 ± 0.14	1.09 ± 0.14	1.51 ± 0.21	2.07 ± 0.54
305	0.32 ± 0.13	0.65 ± 0.15	0.88 ± 0.14	1.17 ± 0.15	1.60 ± 0.32	2.22 ± 0.62
334	0.40 ± 0.15	0.74 ± 0.15	1.00 ± 0.16	1.24 ± 0.17	1.80 ± 0.23	2.37 ± 0.63
364	0.46 ± 0.16	0.79 ± 0.15	1.08 ± 0.17	1.31 ± 0.18	1.84 ± 0.24	2.51 ± 0.66

Table 6. Regressions of corrected average growth rate (CARG) against log weight for spat of treatment 6.

Class	Intercept x 10^{-3}	Slope x 10^{-3}	r^2
1	2.90	-1.08	0.94
2	3.09	-1.12	0.94
3	2.81	-0.96	0.89
4	2.88	-0.96	0.89
5	2.86	-0.91	0.93
6	2.71	-0.81	0.89
All	2.91	-0.97	0.91

Table 7. Coeficients of the regression of log weight and a temperature correction factor on the average growth rate (ARG) of spat of treatment 6.

Class	k1 x 10^{-3}	k2 x 10^{-3}	k3	r^2
1	17.6	-11.1	94.4	0.97
2	21.0	-11.5	84.8	0.98
3	20.2	-9.85	72.4	0.97
4	21.0	-9.74	69.2	0.98
5	22.2	-9.42	58.0	0.98
6	22.3	-8.52	44.0	0.97

where ARG = k1 + (k2 x log weight) + (k3 x $(temp-10)^2$)

Table 8. Increase in mean log weight of three treatment/classes with time. " * " denotes measurements taken before exchange into the fed raceway.

	ln(mean weight)		
Day	Tr 7/cl 1	Tr 9/cl 5	Tr 10/cl 6
1	*	*	*
25	*	*	*
53	-2.55	*	*
96	-0.74	*	*
116	-0.39	*	*
150	0.12	*	*
180	0.29	0.31	*
213	0.58	0.47	*
240	0.78	0.52	0.68
275	1.14	0.86	1.12
305	1.73	1.55	1.70
334	2.01	1.90	1.95
364	2.11	2.02	2.06

Fig. 1. Variation in water temperature over the period of the experiment. (— — —) mean daily temperature, (● —— ●) monthly mean temperature.

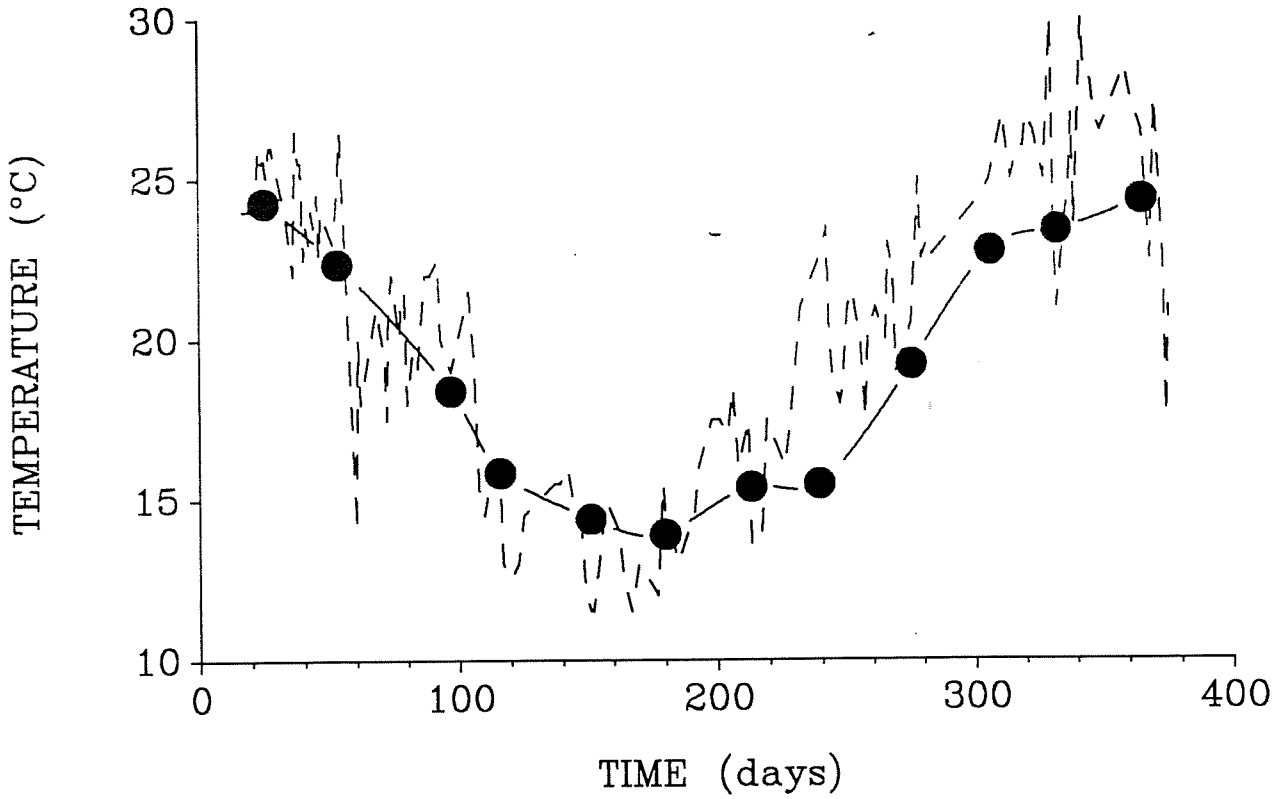
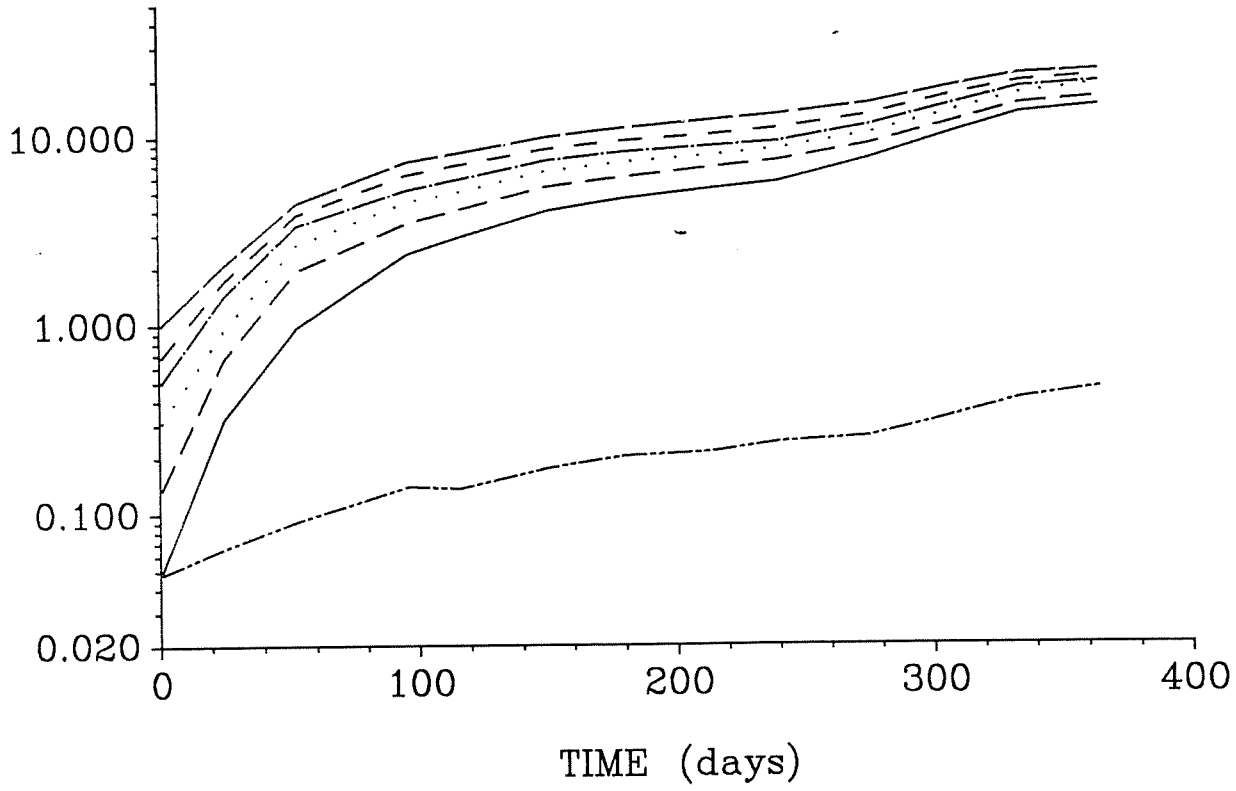

30
25
20
15
10
TEMPERATURE (°C)
0
100
200
300
400
TIME (days)

Fig. 2. Weight increases of spat of the six size classes in treatment 6, with class 1 of treatment 12 for comparison.
(————) Tr. 6, class 1; (— — —) Tr. 6, class 2;
(·····) Tr. 6, class 3; (—·—·—) Tr. 6, class 4;
(— — — —) Tr. 6, class 5; (——— -) Tr. 6, class 6;
(—--—--) Tr. 12, class 1.


10.000
1.000
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100
200
300
400
TIME (days)

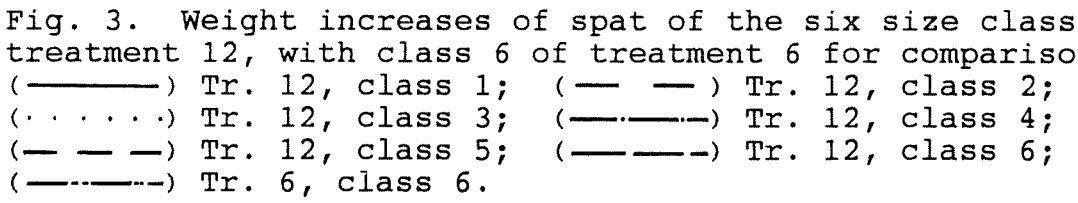


Fig. 3. Weight increases of spat of the six size classes in treatment 12, with class 6 of treatment 6 for comparison. (————) Tr. 12, class 1; (— —) Tr. 12, class 2; (· · · · · ·) Tr. 12, class 3; (—·——·—) Tr. 12, class 4; (— — —) Tr. 12, class 5; (——·——·–) Tr. 12, class 6; (——··——··–) Tr. 6, class 6.

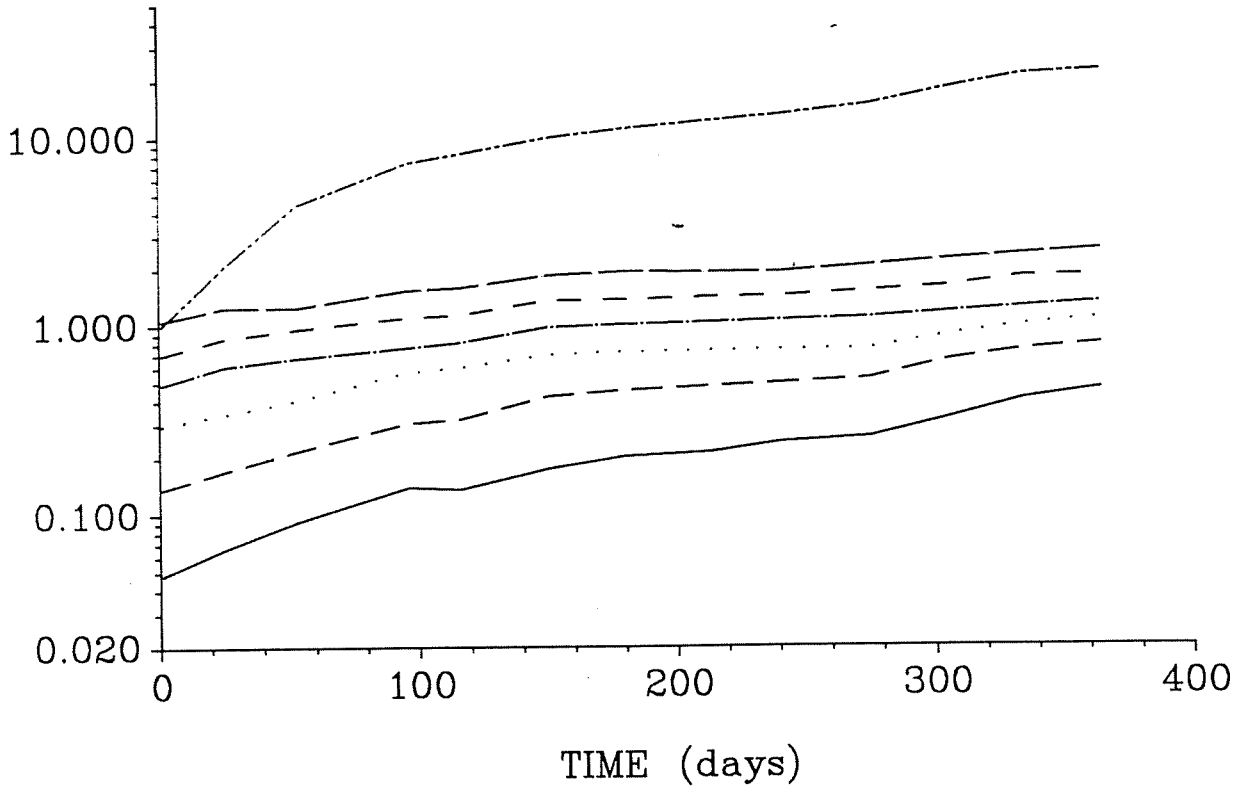

10.000
1.000
0.100
0.020
0
100
200
300
400
TIME (days)

Fig. 4. Weight increases in spat moved from the fully-fed raceway to the starved raceway. (— — -) Tr. 1, class 3; (————) Tr. 2, class 3; (——·——·) Tr. 3, class 3; (— — — -) Tr. 4, class 3; (——··——) Tr. 5, class 3; (· · · · ·) Tr. 6, class 3 (fully-fed control); (— — —) Tr. 12, class 3 (starved control).

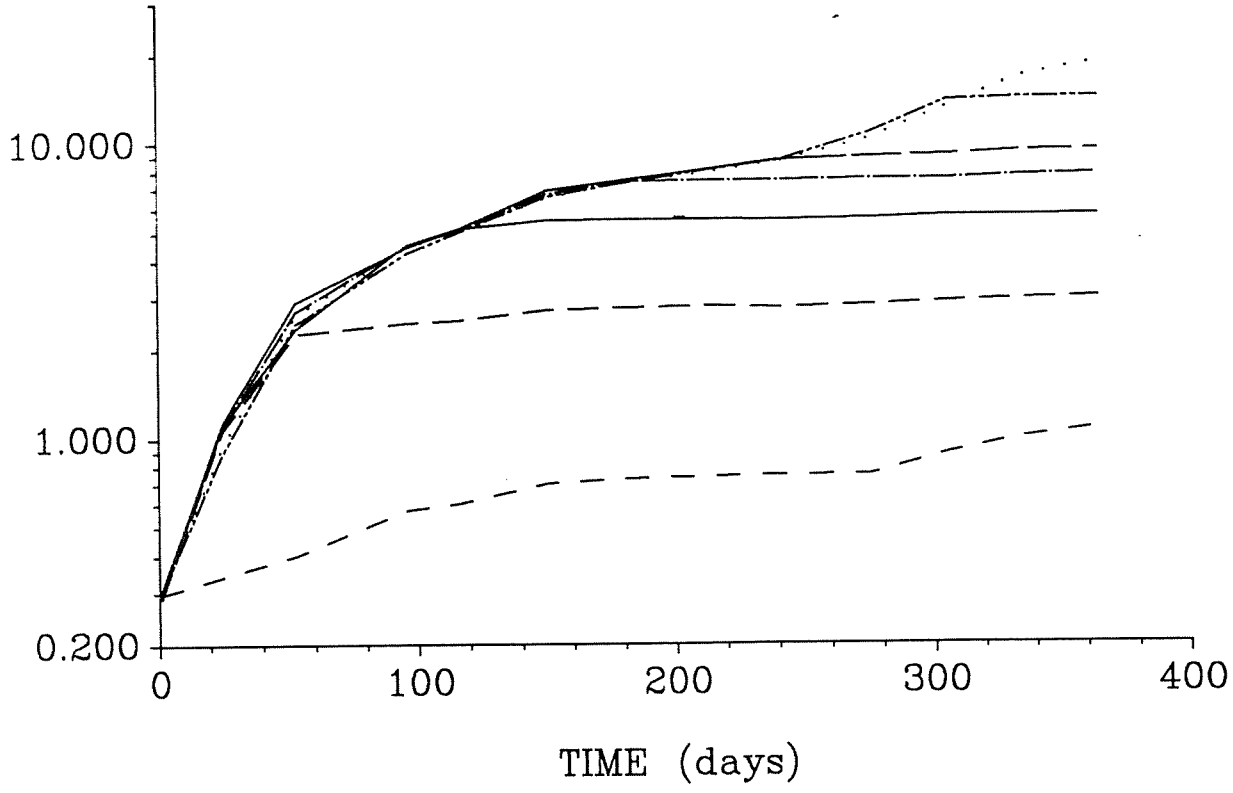
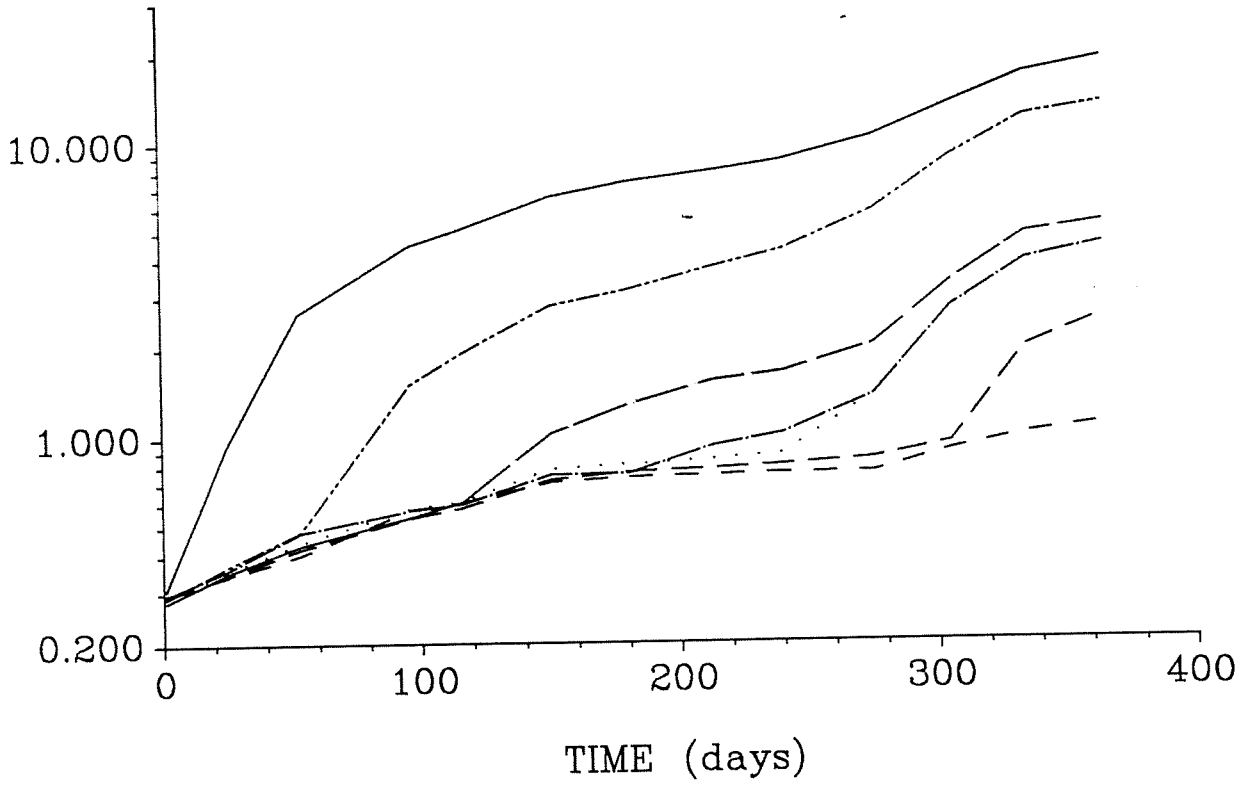

10.000
1.000
0.200
0
100
200
300
400
TIME (days)

Fig. 5. Weight increases in spat moved from the starved raceway to the fully-fed raceway. (——··——··) Tr. 7, class 3; (—— ——) Tr. 8, class 3; (——·——·—) Tr. 9, class 3; (· · · · · · ·) Tr. 10, class 3; (—— ——) Tr. 11, class 3; (— — —) Tr. 12, class 3 (starved control); (————) Tr. 6, class 3 (fully-fed control).


10.000
1.000
0.200
0
100
200
300
400
TIME (days)

COMPARISON OF GROWTH AND MORTALITY OF SYDNEY ROCK OYSTERS (SACCOSTREA COMMERCIALIS) GROWN ON TRAYS, CYLINDERS AND STICKS IN PORT STEPHENS, NSW.

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ABSTRACT

In Port Stephens, oysters on sticks grew faster than those on trays, which in turn grew faster than those in cylinders. Good growth rates of oysters on trays in Port Stephens can be achieved, provided that suitable protection from wave action is provided. For this estuary, cylinders appeared to be suitable only for small oyster spat (<1.0g). Oyster losses can be minimised by scraping oysters off sticks at approximately 5mm and growing them on trays or in cylinders.

INTRODUCTION

Sydney rock oysters (Saccostrea commercialis) have traditionally been grown on sticks until they are between three and four years old (Malcolm, 1987). When they are knocked off the sticks, the plate grade (>40g) oysters are sold and the smaller grades put on trays for on-growing (Malcolm, 1987). Since 1985 oyster farmers have been stocking smaller oysters (4-10mm) on sectionalised trays (Holliday, 1985). These trays are subdivided into 6 rather than 2 or 3 sections to reduce oyster movement. The optimum stocking densities for sectionalised trays to achieve maximum biomass gain for oysters of 1.2 - 1.6g was found to be at a tray coverage of approximately 70% (Holliday et al., 1990). Rotating mesh cylinders were developed during the past decade for culturing oysters (Anonymous, 1985). The optimum stocking density for maximum biomass gain was found to be 2.5L of oysters per cylinder (Holliday, 1987). Although the optimum stocking density for maximum biomass gain in sectionalised trays (Holliday et al., 1990) and in cylinders (Holliday, 1989) was determined for specific grades, there had been no comparison of oyster growth and mortality rates using the varying methods.

MATERIALS AND METHODS

Four blocks of tarred hardwood sticks with Sydney rock oyster spat caught between February and May 1986 (Holliday and Goard, 1986) were taken from a catching lease in Salamander Bay, Port Stephens, NSW in December, 1986. The blocks, which had been placed at intertidal rack height (Malcolm, 1987), were made of six frames. Each frame consisted of 13 sticks separated by 4 lateral sticks nailed 120mm apart. The sticks were 1.8m long by about 25mm x 25mm.

Two blocks of sticks were place in depot (Malcolm, 1987) in North Arm Cove, Port Stephens, from December 1986 - August, 1987. The spat on the other two blocks of sticks were scraped

off. In total 18L of spat was collected. Four sectionalised trays (3mm mesh) were stocked with 3L of spat each and 6 cylinders (3mm mesh) with 1 litre each. The trays and cylinders were placed on an intertidal rack at North Arm Cove until April, 1987, when spat were graded, culled into single oysters if necessary and counted (Table 1). An average of 399 live oysters were harvested per stick (Table 1).

Spat for restocking in trays and cylinders (1500 per cultivation technique per site) were chosen from the two middle size grades (9-12mm and 12-20mm; Table 1). The proportion of spat selected from each of the two grades was the same as their proportions after grading (Table 1). Spat were restocked at the same proportion and in the same growing system after grading (Table 1). Trays were restocked with oysters at the recommended densities for maximum biomass gain (70% tray coverage; Holliday et al., 1990) and cylinders at 2.5L (Holliday, 1987). Additional trays and cylinders were used to stock the excess oysters.

It was not considered to be necessary to change the mesh size (3mm) of the cylinders during the course of the experiment as the mesh did not foul up with algae and silt. Oysters grown on trays (3mm) were restocked on 9mm mesh as the smaller mesh had a tendency to block with algae and silt.

The intertidal growing areas chosen in Port Stephens were Karuah River, North Arm Cove, Big Swan Bay and Tilligerry Creek (Fig 1). The two blocks of sticks were taken out of depot in North Arm Cove and four-frames of sticks nailed out at each of the four growing sites in July, 1987. The sticks, trays and cylinders were all placed at the conventional intertidal rack height, which meant that the cylinders were immersed for a longer period of time than the trays or sticks.

All oysters in the trays and cylinders were counted and the total weight of the oysters taken at every weighing time (Table 2). Oysters on the sticks were not touched until the final weighing in June, 1989, when they were separated into single oysters before weighing. Average oyster weights and mortalities at each weighing time were compared with a t-test (Sokal and Rohlf, 1981). Oysters were not grown to plate size (40g) and treatments were terminated at different times, because of time constraints.

Salinity measurements were taken weekly with a salinity dip meter (inductive coupled cell sensor, model 605; Yeo-kal Electronics Pty Ltd, Brookvale, NSW, 2100) at 0.3m below the surface. Water temperatures were taken at the same time.

RESULTS

Over the 27 months experiment, oysters in trays grew much faster (16.6±1.1g) than those in cylinders (9.1±0.5g; Table 2; Fig 2). The growth rates of the oysters in the cylinders did not vary much with growing site as indicated by the small variation in oyster weights (9.1±0.5g) in March, 1989 (Table 2). However, the growth rates of the oysters in the trays

varied greatly with growing site (Table 3).

Oysters on sticks grew faster than those on trays (Table 2). In June, 1989, oysters on sticks had an average weight of 27.4±3.3g, whereas those on trays weighed 19.4±0.4g (Table 2). The Karuah River site produced the highest average oyster weight (32.3g) for oyster grown on sticks in June, 1989 (Table 4), whereas for oysters grown on trays the highest oyster weights were found at Tilligerry Creek (23.7g) and North Arm Cove (21.4g) in March, 1989 (Table 3). At all sites tested in Port Stephens, oysters on sticks (Table 4) grew faster than oysters on trays (Table 3). The oysters from the sticks in the Karuah River were heavily overcaught with spat, which had to be removed before weighing.

There were no significant differences ($P>0.05$) in the percentage mortalities between oysters held on trays and those held in cylinders (Table 2). On average there were 47 live oysters surviving per stick at harvest time (Table 4) from the 399 per stick present at scrape-off time (Table 1), ie. a loss of 88.2%. On the trays there were 288 live oysters left from the 399 on each stick at the start of the experiment, a loss of 27.8%.

There was a little variation in water temperature (n=116) and salinity (n=116) between sites which were 21±4°C, 27±8‰, ranges 13-28°C, 0-36‰ at Karuah River; 21±4°C, 30±6‰, ranges 12-28°C, 0-37‰ at North Arm Cove; 21±4°C, 30±5‰, ranges 12-28°C, 7-39‰ at Big Swan Bay and 21±5°C, 28±5‰, ranges 11-30°C, 8-37‰ at Tilligerry Creek. At any site salinity levels <15‰ did not last longer than 2 weeks.

DISCUSSION

It is likely that the reduced growth rates experienced by the oysters in trays and cylinders compared to those grown on sticks was caused by the regular rumbling of the oysters in the trays (Holliday et al., 1990) and cylinders, which causes new shell growth to break off. In the case of the trays, oyster growth rates appeared to be reduced the most where wave action was the most severe as was indicated by the wide range of average oyster weights on trays from 14.7 - 23.7g at harvest time (Table 3). Site selection is very important for the growing of oysters in trays and leases in "choppy" water should be avoided or protection should be provided for oysters on trays. Wave action can be reduced by constructing fences around oyster leases or by using floating pipes, tubes or tyres to break up wave action. Alternatively a rack of sticks may be left in place for protection.

Oysters in the cylinders had the slowest growth rates, despite their longer immersion time. Growth rates of oysters in the cylinders were reduced because of the rather exposed nature of the oyster leases in Port Stephens, which caused excessive rumbling. In river systems where there is less wave action and where fine mesh (3mm) trays often block up with silt, cylinders are the preferred growing technique for small

up with silt (J. E. Holliday, personal communication, 1989). In Port Stephens, cylinders appeared to be only suitable for (1.0g) oyster spat.

The high loss of stick oysters (88.2%) from the experiment was probably largely caused by oysters dropping off sticks. High losses of stick oysters (90%) were also reported by Holliday and Goard (1986).

The scrape-off technique, in which small oysters are scraped off sticks and placed in trays or cylinders (Holliday and Goard, 1986), greatly increases the number of oysters that survive per stick at harvest time. This offers farmers the opportunity to put out fewer sticks to catch, if they want to maintain the same level of production, or to fill all their racks with oysters on trays to increase production. Unfortunately, current oyster farming techniques are not likely to permit the successful conversion of all stick leases to tray leases on Port Stephens, since it will not be practical and economically feasible to provide protection against wave action at the more exposed leases in Port Stephens.

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TABLE 1

Grades of Sydney rock oyster (Saccostrea commercialis) scrape-off spat after 4 months growth in trays or cylinders at North Arm Cove, Port Stephens, NSW from December 1986 - April 1987.

Size ranges	Trays			Cylinders		
	Total spat weight (g)	number of spat	average weight of spat (g)	Total spat weight (g)	number of spat	average weight of spat (g)
> 20mm	11,945	7,143	1.67	4,733	3,062	1.55
12 - 20mm	18,421	22,934	0.80	9,827	11,389	0.86
9 - 12mm	3,805	8,790	0.43	1,999	5,277	0.38
< 9mm[1]	3,131	2,974	-	971	621	
Total	37,302	41,841		17,530	20,349	

1 This grade contained a lot of shell grit.
There were 62,190 live oysters harvested from two blocks of sticks, ie. 399 oysters/stick.

TABLE 2

Comparison of growth[1] and mortality[1] of Sydney rock oysters (Saccostrea commercialis) grown on trays, cylinders and sticks in Port Stephens, NSW from December 1986 - June 1989.

Month and Year	Cultivation	Oyster weight (g)	Cumulative mortality (%)
April 1987	Cylinders	0.91	no data
	Trays	1.09	no data
August 1987	Cylinders	1.7±0.2*	4.9±0.2 NS
	Trays	2.2±0.2	9.6±3.5
November 1987	Cylinders	2.5±0.3*	7.4±2.8 NS
	Trays	3.6±0.8	10.3±4.9
February 1988	Cylinders	4.1±0.5*	11.2±7.8 NS
	Trays	6.5±1.0	14.4±7.1
June 1988	Cylinders	5.4±0.8*	12.8±7.9 NS
	Trays	10.6±0.6	18.2±3.3
November 1988	Cylinders	7.4±1.1*	13.8±8.6 NS
	Trays	14.0±1.3	20.1±4.2
March 1989	Cylinders	9.1±0.5*	16.9±9.6 NS
	Trays	16.6±1.1	20.8±1.4
June 1989	Cylinders	terminated	terminated
	Trays	19.4±4.0*	27.8±7.3
	Sticks	27.4±3.3	no data

1 Data presented as mean ± S.D. The statistical comparison of oyster weights and cumulative mortalities for each weighing time are indicated [NS = no significant difference ($P>0.05$); * = significantly different ($P<0.05$)]. An arcsin $x^{0.5}$ transformation was applied to the mortality data prior to statistical analyses. Spat were caught in Salamander Bay, Port Stephens from February - May 1986. Spat were scraped off the tarred hardwood sticks and placed in trays and cylinders in December, 1986. There were also 4 frames of 13 sticks each per site. Trays were stocked at 70% tray coverage and cylinders at 2.5L each. Initial stocking density was 1500 oysters/tray and cylinder at each site (n=4)

TABLE 3

Weights and mortalities of Sydney rock oysters (Saccostrea commercialis) grown on trays at various sites on Port Stephens, NSW from April 1987 - March 1989.

Site	Average oyster weight (g)	Mortality (%)
Karuah River	17.7	33.6
North Arm Cove	21.4	34.5
Big Swan Bay	14.7	20.3
Tilligerry Creek	23.7	22.6

Spat were caught on sticks in Salamander Bay, Port Stephens from February - May 1986. They were scraped off in December 1986 and placed on trays in North Arm Cove until April 1987. Then they were graded and distributed over the four growing sites. Oysters were grown at those sited from April 1987 - March 1989.

TABLE 4

Numbers and weights of Sydney rock oysters (Saccostrea commercialis) grown on sticks at different sites in Port Stephens, NSW from August 1987 - June 1989.

Site	Number of live oysters per 52 sticks	Average oyster weight (g)
Karuah River	1113	32.3
North Arm Cove	2964	26.0
Big Swan Bay	3214	25.0
Tilligerry Creek	2408	26.2

Spat on the sticks were caught in Salamander Bay, Port Stephens from February - May 1986. They were placed in depot in North Arm Cove from December 1986 - August 1987. Sticks were nailed out at the four growing sites from August 1987 - June 1989. Four frames of 13 sticks were nailed out at each site.

FIG 1. Location of experimental oyster cultivation sites (Karuah River, North Arm Cove, Big Swan Bay and Tilligerry Creek) in Port Stephens, NSW, from April 1987 - June 1989.

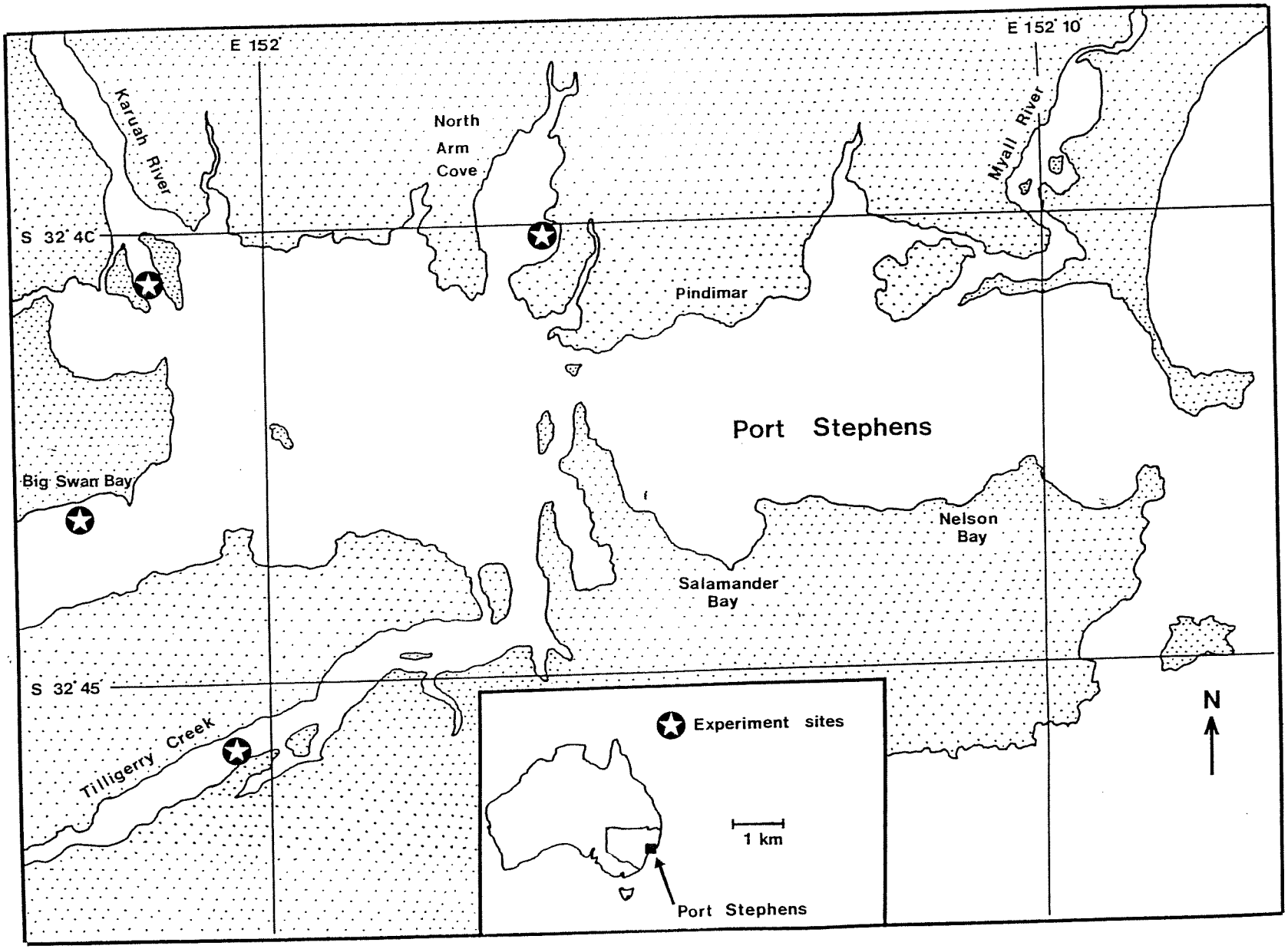
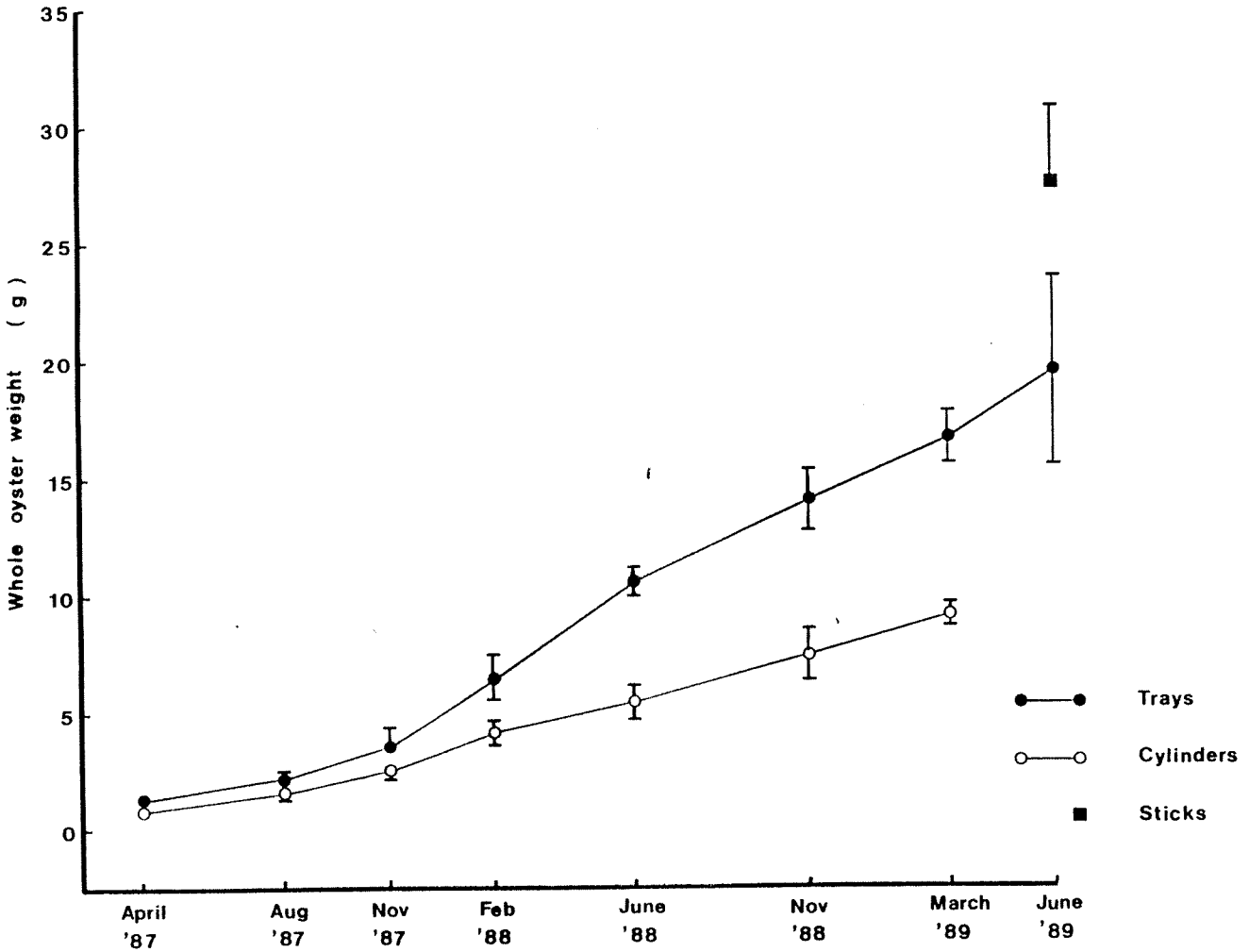

E 152
E 152 10
Karuah River
North
Arm
Cove
Myall River
S 32 4C
Pindimar
Port Stephens
Big Swan Bay
Nelson
Bay
Salamander
Bay
S 32 45
Tilligerry Creek
N
Experiment sites
1 km
Port Stephens

FIG 2. Comparison of growth rates of Sydney rock oysters (Saccostrea commercialis) grown on trays, cylinders and sticks in Port Stephens, NSW, from December, 1986 - June, 1989. Mean ± S.D.


Whole oyster weight (g)
35
30
25
20
15
10
5
0
April '87
Aug '87
Nov '87
Feb '88
June '88
Nov '88
March '89
June '89
Trays
Cylinders
Sticks

SECTION 7

QX DISEASE

MORTALITY OF THE PROGENY OF SYDNEY ROCK OYSTERS (*Saccostrea commercialis*) THAT SURVIVED AN OUTBREAK OF QX DISEASE

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ABSTRACT

Sydney rock oysters (*Saccostrea commercialis*) that had survived an outbreak of QX disease (60 to 80% mortality) were spawned, and the larvae reared in 7 family groups. Five surviving families of spat were ongrown in the Richmond River. Spat caught in the Brunswick River were obtained as control spat. Two of the 5 selected families were lost. The remaining 3 families and the control spat were divided between 4 trays and put out on rafts in the Clarence River, to be exposed to QX. Family A had significantly ($p < 0.05$) lower mortality than the large control group. Histological examination of surviving spat failed to show any signs of QX. No QX was reported for the Clarence River during the period the spat were exposed. It is probable that the mortality was not caused by QX, and no conclusion can be drawn about any improvement in resistance to QX in the selected spat.

INTRODUCTION

QX is a disease of Sydney rock oysters (*Saccostrea commercialis*) caused by a protistan parasite, *Marteilia sydneyi*. QX affects oysters growing in the rivers of Queensland and New South Wales north of the Macleay River. The disease usually occurs after periods of heavy rain, between February and May, and may cause up to 100% mortality of affected oysters, although less severe mortality is normal in New South Wales. The risk of losing a large proportion of their crop has forced oyster farmers in affected areas to change their traditional cultivation practices; rather than growing the oysters through from spat to market size over three years, they buy mixed grades of oyster ("all-ins") from the southern rivers in the winter for fattening and ongrowing. All oysters must be removed from the river by February. This is less profitable for the northern farmers, and leaves them at the mercy of fluctuations in supply of all-ins.

It has been noted that American oyster (*Crassostrea virginica*) stocks grown in areas affected by MSX, a disease caused by a parasite (*Haplosporidium nelsoni*) related to M. sydneyi, suffer less mortality than stocks imported from MSX-free areas (Haskin and Canzonier, 1969). A selective

breeding programme to produce MSX-resistant oysters was established, and acheived significantly improved MSX resistance in four generations (Haskin and Ford, 1978).

This experiment was set up to determine whether QX resistance in Sydney rock oysters is likely to be genetic in origin.

MATERIALS AND METHODS

Eight dozen oysters that had survived QX (60 to 80% mortality) in the Clarence River (T. Phillips, personal communication, 1987) were induced to spawn at the Research Station in November 1987. A total of 33 females and 9 males spawned. The sperm suspension from the 9 males was combined, and the eggs were divided into seven groups; five being the combined eggs of 5 females each (families A to E), and the remaining two being the combined eggs of 4 females each (families F and G). The combined sperm suspension was used to fertilise the seven maternal families. Families F and G were discarded at day 10 of the larval rearing, because of heavy dinoflagellate blooms in the rearing tanks, but the remaining five families A to E survived to settlement.

The spat were settled on disk collectors, flat, conical PVC spat collectors 140mm wide, with a lightly sculpted surface. The disks were placed in the tanks when pediveligers appeared, and replaced when a suitable density of spat had caught. The disks were kept in the nursery at the Research Station for a short time, and then suspended lengthwise inside spat cylinders and taken up to the Richmond River in December 1987. The cylinders containing families B and E were lost from the lease during floods in April 1988.

As the spat grew on the disks, they tended to become dislodged, and drop into the spat cylinders. Spat remaining on the disks were flexed off in August 1988 and transferred to sectionalised trays in individually marked compartments.

Spat caught on PVC slat collectors in the Brunswick River in January to February 1988 were obtained as controls in August 1988. The control spat were flexed off the slats and placed in compartments of the sectionalised trays. In March 1989, the selected and the control spat were sorted, counted, and the control spat divided into large (> 20mm length) and small (< 20mm length) groups. The four trays were sealed, and put onto rafts at two sites in the Clarence River, to await the expected QX outbreak (Table 1).

In August 1989 the trays were collected from the Clarence River, and brought back to the Research Station. The number of live and dead spat in each compartment were counted, and twenty live specimens from each compartment were fixed, sectioned and stained with Haematoxylin & Eosin.

The percent mortality for each compartment was arcsin (x^{-1}) transformed (Sokal and Rohlf, 1969). Homogeneity of variance was confirmed using Cochran's C-test (Winer, 1971), and differences between the mean mortalities of the selected families and control groups detected by one way analysis of variance (Sokal and Rohlf, 1969). Differences between means were identified using Tukey's Honestly Significant Diferences technique (Sokal and Rohlf, 1969).

RESULTS

Mortality in the compartments ranged from 2.8% to 28.2% over the period the spat were kept on the Clarence River rafts (Table 2). The percent mortality varied widely within the families and control groups, but could not be shown to be inhomogenous ($p = 0.15$). Only family A and the large control oysters had significantly different mean percent mortality ($p < 0.05$) (Table 3).

No signs of current nor old QX infection were found in any of the stained sections.

Round holes were observed in the lower valve in the majority of the dead shells from all tray compartments (Fig. 1).

DISCUSSION

The two control groups (large and small) had higher mean mortality than the spat bred from QX survivors, and the mean mortality of family A was significantly less than that of the large control group. However, histological examination of the spat from each compartment failed to show any evidence of QX infection in the selected spat or the controls. Nor was there any evidence of QX in the Clarence River during the autumn of 1989 despite extensive and prolonged flooding (T. Phillips, personal communication, 1989).

Upon examination, the majority of the dead shells of the spat were found to have a hole in the lower valve near the hinge (Fig. 1). Spat growing onto a smooth plastic catching substrate appear to deposit a very thin layer of shell on the upper valve compared to lower valve (S. McOrrie, personal communication, 1989). When spat are flexed off the substrate, the lower valve is considerably thinner than the upper valve (C. Mason, personal observation, 1989). During periods of anoxia, such as during low tide or when oysters are covered by fresh water, calcium is disolved from the inner surface of the shell to buffer organic acids generated during anaerobic glycolysis (Dugal, 1939). Mortality caused by holes in the lower valve has been observed in spat kept on trays in the Wallis Lake during extended periods of

flooding (I. Smith, personal communication, 1989), and it seems likely that the shell has simply been disolved away from the inside.

In the absence of any evidence of exposure of the spat to QX, it is probable that the mortality of spat on the trays was caused indirectly by flooding. Differences in mean mortality between the control groups and selected families probably reflect differences in cultivation history and shell growth rather than physiological differences between the spat.

No conclusion can be drawn about QX resistance of the selected families of spat compared to the control groups.

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Table 1. Spat were divided between sections of the trays and put out at two sites in the Clarence River, NSW.

		Number of spat				
		Family			Controls	
Site	Tray	A	C	D	small	large
1	1	200	-	200	500	250
1	2	-	260	200	500	250
2	3	200	-	200	500	250
2	4	-	260	200	500	250

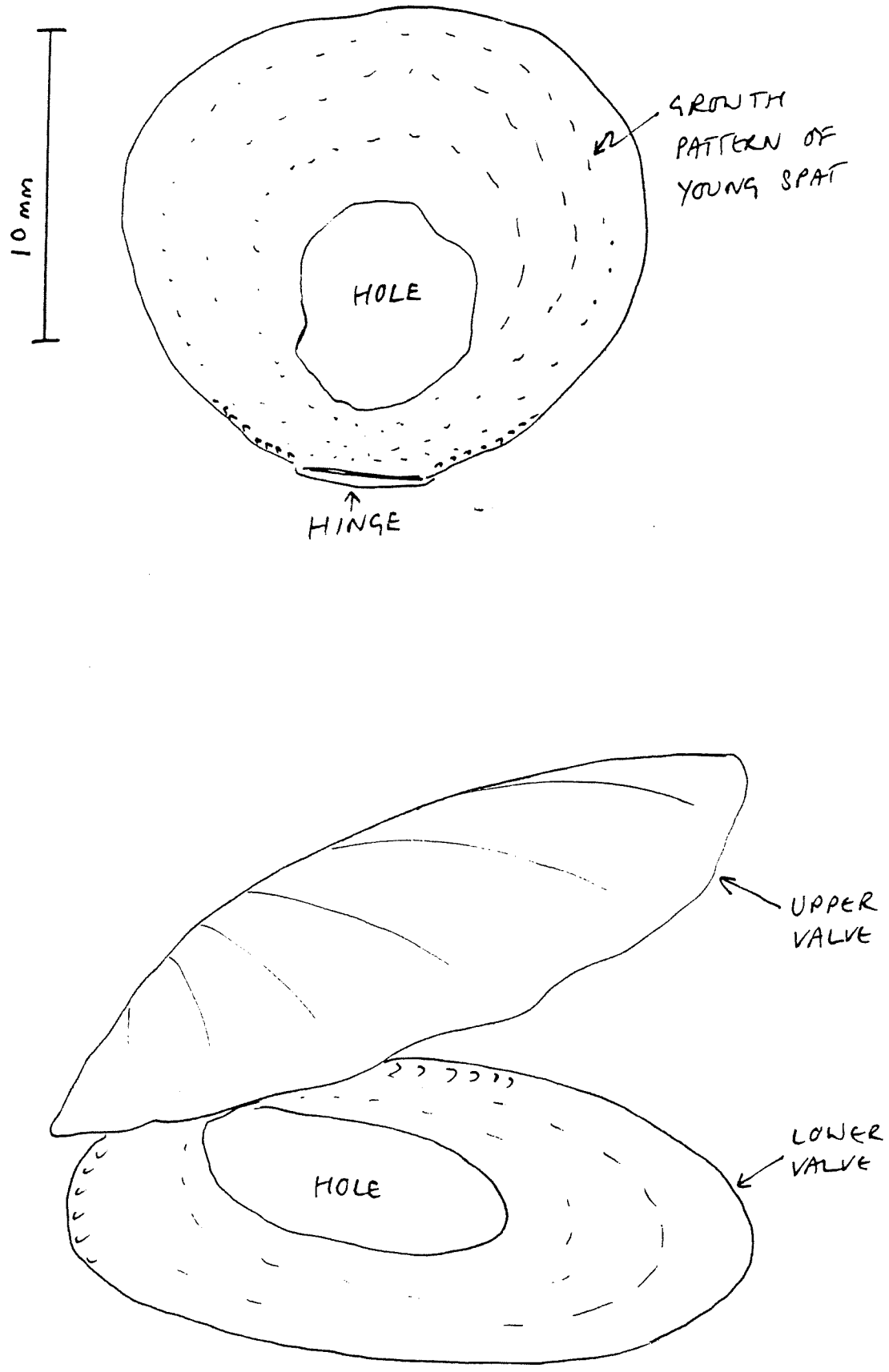
Table 2. Mortality of spat after 6 months on rafts in the Clarence River, NSW

		Mortality (%)				
		Family			Controls	
Site	Tray	A	C	D	small	large
1	1	4.6	-	4.3	16.7	25.5
1	2	-	2.8	10.9	22.1	18.9
2	3	4.9	-	11.1	20.5	25.9
2	4	-	17.5	28.2	12.7	27.9

Table 3. Mean Mortality of selected families and control groups. Means that do not share a superscript differ significantly ($p < 0.05$).

Spat	% mortality
Family A	4.8[a]
Family C	10.2[ab]
Family D	13.6[ab]
Small controls	18.0[ab]
Large controls	24.6[b]

Figure 1. Holes typically found in the lower valve of the shell of dead spat.



SECTION 8

CONCLUSION

CONCLUSION

It is most unfortunate that Pacific oysters were introduced into Port Stephens, NSW and that their eradication and control proved impossible. This study has shown that it is difficult to selectively kill Pacific oysters. In Port Stephens they suffer more from heat kill than Sydney rock oysters, but both species suffer from mudworm (*Polydora websteri*) infestation. Pacific oysters in Port Stephens grew at approximately twice the rate of Sydney rock oysters and Pacific oysters are readily accepted by the consumer.

The Pacific oyster however, has added to the existing overcatch (young oyster spat settling on larger oysters) problem in Port Stephens. Previously this problem was largely confined to the catch of Sydney rock oysters in the outer harbour of Port Stephens, but in addition to the farmers now also have to cope with a Pacific oyster catch in the inner harbour of Port Stephens.

Pacific oysters can be cultivated in Port Stephens, but the use of triploid Pacific oysters should be investigated both to overcome problems with overcatch of Pacific oyster spat and to lengthen the marketing season of Pacific oysters.

Oyster farmers are a very innovative group of people, who have adapted their growing techniques to a wide range of conditions and there is no doubt that they will apply the same ingenuity for the development of suitable farming techniques for Pacific oysters.

SECTION 9

APPENDICES

OYSTER HYBRIDISATION EXPERIMENT 20/1/'88

Pacific Oyster * Sydney Rock Oyster
(*C. gigas*) (*S. commercialis*)

- Electrophoretic Identification.

REPORT NUMBER 2

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February 1988

ELECTROPHORETIC DETECTION OF HYBRIDS

We were provided with digestive gland samples of 8 parental oysters, 2 males and 2 females each of the Pacific oyster (*Crassostrea gigas*), and the Sydney Rock oyster (*Saccostrea commercialis*). Gametes were stripped from the gonads, and we were provided with 12 larval samples (at D-stage in development, 20h old) resulting from the interspecific and intraspecific crosses...(see Table 1).

TABLE 1: Larvae from interspecific and intraspecific crosses. (Tube numbers indicated here).

		FEMALES			
		SRO1	SRO2	PO1	PO2
MALES	SRO1	23	17	24	12
	SRO2	31	25	26	36
	PO1	-	-	33	32*
	PO2	-	-	29*	28

KEY:

SRO = Sydney Rock oyster (*S. commercialis*)
PO = Pacific oyster (*C. gigas*)
\- = no fertilization

All samples were stored cryogenically until electrophoresed.

Crude protein extracts were obtained from digestive gland and larval samples by homogenization with distilled water followed by centrifugation at 1000 x g for 45 minutes. These extracts were separated on 12% Sigma electrostarch gels in 3 buffer systems: POULIK, TM pH 7.8 and CAM pH 6.1. A total of 12 enzymes, representing 19 presumptive loci, were screened for their usefulness in the detection of hybrid patterns in the larvae ...(see Table 2).

Aat, Est-1 and Me (Poulik) showed no activity in the larvae, and Acph showed very poor resolution and warping. Idh, Mdh-1,2 (Cam pH 6.1), Me (Cam pH 6.1) and Pgm (Cam pH 6.1) showed larval loci that were not present in the adult tissue, with Pgm (Cam pH 6.1) showing a number of loci with a pattern too complex for useful hybrid detection. Two polymorphic loci, Lap and Gpi, showed some mismatch between observed and expected genotypes...(see Tables 3 and 4). This may, again, be an indicatation of larval loci.

The remaining 5 diagnostic loci Mdh-1,2 (Poulik), Mpi, Pep-GL and Sod clearly showed fixed differences between the two species, however no hybrid patterns were detected...(see Table 5). The larvae resulting from the interspecific cross between S. commercialis males and C. gigas females consistently showed across all loci screened only C. gigas genotypes...(see Figure 1).

Since only the female (C. gigas) genotype was carried through to the larvae resulting from the interspecific crosses it may be considered that parthenogenesis may have been induced. Possibly due to the incompatability of the gametes of the two species, a haploid intercross may have been produced. If larvae survived longer than D-stage, it may be possible that diploidization followed.

We suggest,

1/ Experimental design be checked for the possibility of contamination of material.

2/ The larvae,
(a) should be checked for chromosome number,
and (b) you may consider chromosome banding to try to identify each species.

3/ For electrophoresis,
(a) both the digestive gland and adductor muscle should be sampled from the adults to check for larval or tissue specific (muscle) loci,
and (b) larval samples should be washed free of food prior to collection so that the possibility of extraneous enzyme activity (although not observed) may be eliminated.

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TABLE 2: Enzyme loci screened.

Enzyme	E.C.	Subunit Structure	Buffer	No. of Loci		Comments
Acph	3.1.3.2	M	3	2	P	fixed difference, larval locus.
Aat	2.6.1.1	D	2	1	P	no larval activity.
Est	3.1.1.1	M	2,3*	2	P	no larval activity.
Gpi	5.3.1.9	D	2	1	P	genotype mismatch.(larval locus?)
Idh	1.1.1.42	D	2	2	P	larval locus.
Lap	3.4.11.1	M	2	1	P	genotype mismatch.(larval locus?)
Mdh	1.1.1.37	D	1,3	3	M	fixed differences, larval loci.
Me	1.1.1.40	T	1,3	1	P	no larval activity at high pH.
Mpi	5.3.1.8	M	1	1	M	fixed difference.
Pgm	5.4.2.2	M	1,3	3	P	multiple loci.
Pep(GL)	3.4.11	D	1	1	M	fixed difference.
Sod	1.15.1.1	D	1,2	1	M	fixed difference.

KEY:

M = monomer, D = dimer, T = tetramer.
1 = POULIK, 2 = TM pH 7.8, 3 = CAM pH 6.1.
* indicates best buffer system.
P = polymorphic, M = monomorphic.

TABLE 3: Observed and expected genotypes for Gpi. (Mismatches underlined).

		FEMALES (bc) SRO1		(aa) SRO2		(bb) PO1		(bb) PO2	
		E	O	E	O	E	O	E	O
	(bb) SRO1	bb		ab	aa	bb	bb	bb	bb
		bc	bc						
MALES	(bb) SRO2	bb		ab	aa	bb	bb	bb	bb
		bc	bc						
	(bb) PO1	bb		ab	-	bb	bb	bb	bb
		bc	-						
	(bb) PO2	bb		ab	-	bb	bb	bb	bb
		bc	-						

TABLE 4: Observed and expected genotypes for Lap.

		FEMALES (cc) SRO1		(bc) SRO2		(ab) PO1		(ab) PO2	
		E	O	E	O	E	O	E	O
	(bc) SRO1	bc		bb		ab	aa	ab	aa
		cc	cc	bc		ac		ac	
				cc	cc	bb		bb	
						bc		bc	
MALES	(cc) SRO2	cc	cc	bb		ac	aa	ac	ac
				bc		bc		bc	
				cc	cc				
	(ab) PO1	ac		ab	-	aa		aa	aa
		bc	-	ac		ab	ac	ab	
				bb		bb		bb	
				bc					
	(ab) PO2	ac		ab	-	aa	aa	aa	aa
		bc	-	ac		ab		ab	
				bb		bb		bb	
				bc					

FIGURE 1: Zymograms for: (a) Mdh-1,2 (Poulik), (b) Mpi, (c) Pep-GL and (d) Sod.

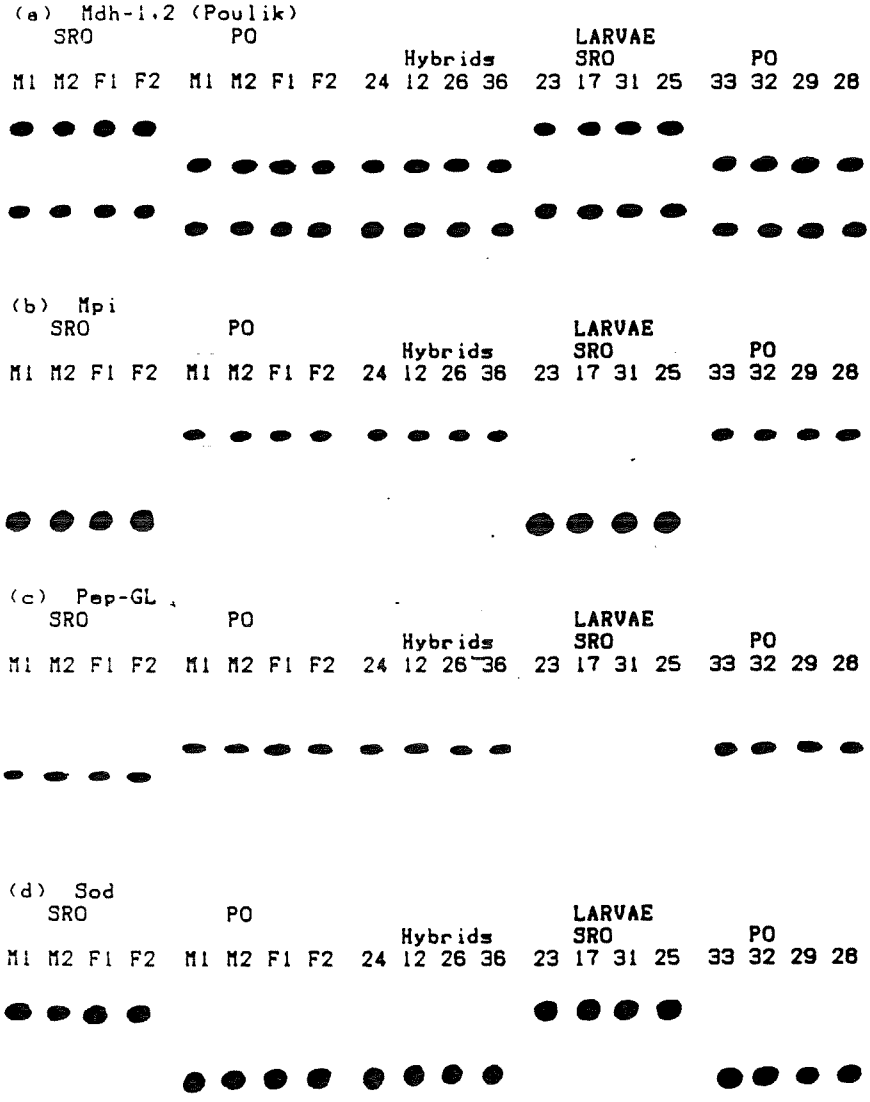

TABLE 5: Enzyme loci selected for the detection of hybrid larvae. Parental and offspring genotypes, resulting from inter- and intra- specific crosses, are given.

	ENZYME LOCI				
	Mdh-1	Mdh-2	Mpi	Pep-GL	Sod
PARENTS					
SROM1	aa	aa	bb	bb	aa
SROM2	aa	aa	bb	bb	aa
SROF1	aa	aa	bb	bb	aa
SROF2	aa	aa	bb	bb	aa
POM1	bb	bb	aa	aa	bb
POM2	bb	bb	aa	aa	bb
POF1	bb	bb	aa	aa	bb
POF2	bb	bb	aa	aa	bb
OFFSPRING					
SROM1.POF1	bb	bb	aa	aa	bb
SROM1.POF2	bb	bb	aa	aa	bb
SROM2.POF1	bb	bb	aa	aa	bb
SROM2.POF2	bb	bb	aa	aa	bb
SROM1.SROF1	aa	aa	bb	-	aa
SROM1.SROF2	aa	aa	bb	-	aa
SROM2.SROF1	aa	aa	bb	-	aa
SROM2.SROF2	aa	aa	bb	-	aa
POM1.POF1	bb	bb	aa	aa	bb
POM1.POF2	bb	bb	aa	aa	bb
POM2.POF1	bb	bb	aa	aa	bb
POM2.POF2	bb	bb	aa	aa	bb

KEY:

Genotypes designated alphabetically.
- = no staining activity.
F = female, M = male.
PO = Pacific Oyster (*C. gigas*).
SRO = Sydney Rock Oyster (*S. commercialis*).

A METHOD FOR ASSAYING CONDITION IN CULTURED PACIFIC OYSTER LARVAE (*Crassostrea gigas) USING LIPID SPECIFIC STAINS.*

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N.S.W.

February, 1988.

ABSTRACT

This study evaluates the use of lipid specific stains for assaying condition in cultured Pacific oyster larvae.A new method is presented for the paralysis of the larvae using $MgCl_2$which differs from those of similar studies.Lipid content of the digestive gland of larvae was found to be related to nutritional status.The study concluded that the lipid staining technique may be a useful way of monitoring larval development in cultured conditions.

INTRODUCTION.

Lipid plays an essential energetic role in the normal pattern of growth and metamorphosis in bivalve larvae (Holland 1978). Helm,Holland,and Stephenson (1973) found a direct relationship between total lipid content of newly released bivalve larvae and subsequent viability and larval growth rate.They also found that healthy adults gave rise to larvae with a high endogenous lipid content. Holland (1978) concluded that neutral lipid,especially triacylglycerol was most important as a storage medium for use during nutritional or environmental stress.

Measuring changes in lipid content and distribution has been found to be a cheap and effective means of monitoring larval condition in several bivalve species including *Crassostrea viginica, Ostrea edulus,Mercenaria mercenaria* (Gallager,Mann &Sasaki 1986) and *Teredo navalis* (Gallager &Mann 1981).The technique involves staining subsamples of culture populations with a lipid specific stain and microscopic examination of whole larvae.Larvae must also be anaesthetised and fixed in a special way so that the soft body parts are accessible to the stain.

In this study the technique is used to detect lipid in cultured larvae of the pacific oyster *Crassostrea gigas.*

MATERIALS AND METHODS.

ANIMALS.

Pacific oyster larvae were taken from large tanks of sand filtered seawater maintained in an enclosed hatchery building at the Brackish Water Fish Culture Research Station,Salamander Bay, NSW. Larvae were fed twice daily on a diet of three species of algae-*Tahitian isochrysis, Pavlova lutheri* and *Navochlaris atomus.* All lavae used came from the same brood stock.Some larvae were taken from a small population kept

in a second tank of filtered seawater.These larvae had been starved for two days.

ANAESTHETISING THE LARVAE.

Two drops of a 3.75M (saturated) solution of magnesium chloride was added under a dissecting microscope to a petri dish containing 50-100 larvae.This is a modification of the method of Gallager & Mann (1981). Anaesthetised larvae with velums extended were then fixed in a 10% solution of formalin in filtered sea water.

STAINING FOR LIPID

Two different stains were used- Oil Red O and Sudan Black B.The latter was prepared by dissolving 0.75g of Sudan Black in 100ml ethylene glycol, heating to about 60 degrees C and then hot filtering through Whatman no.2 paper. Oil Red O stain was prepared using the modified method of Gallager et al (1986) and involved substituting dimethyl-sulfoxide (DMSO) for ethylene glycol as the carrier solvent.

About ten fixed larvae were stained at a time.Staining was done in shallow petri dishes by adding 1ml Sudan Black or Oil Red O stain to the larvae and then leaving to stand for a minimum of 1 hour. After this time a clearing solvent was added to clear excess stain (ethylene glycol for Sudan Black and DMSO for Oil Red O).Clearing took about 3-5 mins for Oil Red O and up to 4 hours for Sudan Black. Stained larvae were mounted on slides using an aqueous mounting media consisting ofgelatin(70g),distilled water(60ml) glycerin(70ml) and phenol(0.25g) and then photographed under a light microscope. The difference in lipid content between fed and starved larvae were then compared.

RESULTS.

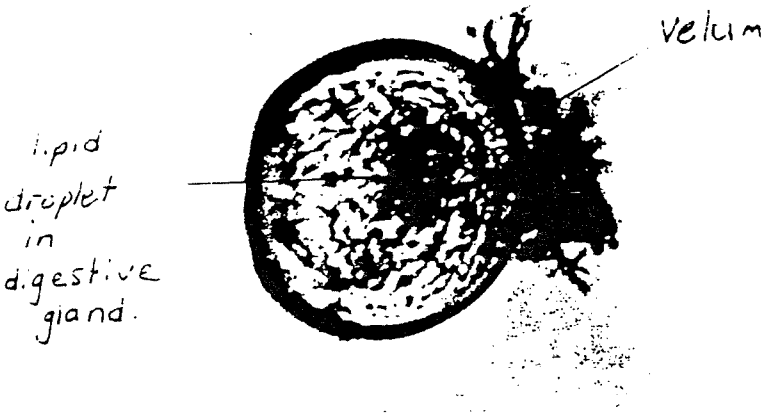
EFFECTIVENESS OF ANAESTHETIC.

One or two drops of a 3.75M solution of $MgCl_2$ caused immediate paralysis of larvae and prevented the withdrawal of the velum into the shell of most larvae (plate 4&5). Some larvae withdrew their velums upon contact with $MgCl_2$.These larvae were allowed to recover by placing them in plain sea water.They could then be reanaesthetised with $MgCl_2$.This process could be continued until larvae were anaesthetised with velums extended. Excessive $MgCl_2$ was toxic and caused larvae to secrete a mucous -like substance which accompanied the death of the organism.

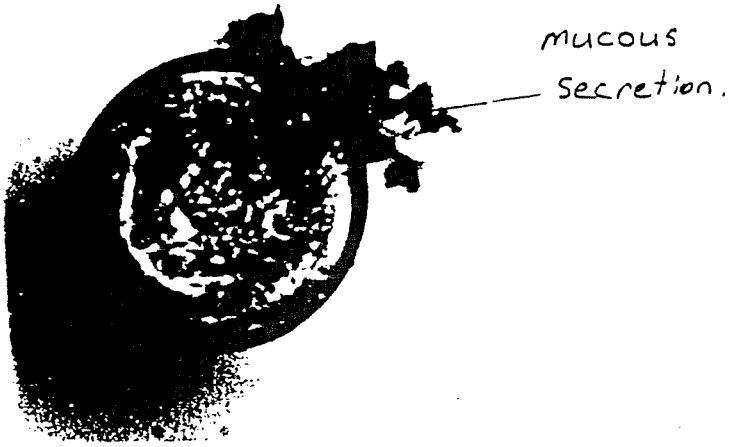
EFFECTIVENESS 0F LIPID STAINS.

Larvae that were anaesthetised with velums extended were more effectively stained than larvae with retracted velums(plates 1&2). Both stains showed the presence of lipid droplets in the larvae. In the six day larvae control, lipid was stained in the digestive gland and the velum (plate 3). There was less lipid present in the digestive gland of the six day larvae that had been starved for two days (plate 3). In the 18 day larvae control stained with Sudan Black,lipid was heavily stained in the digestive gland and velum (plate 4). There was less lipid in the digestive gland and none in the velum of the 18 day larvae that had been starved for two days(plate 5).

(Plate 1) Six day fed larva stained with Oil Red O - Velum retracted.

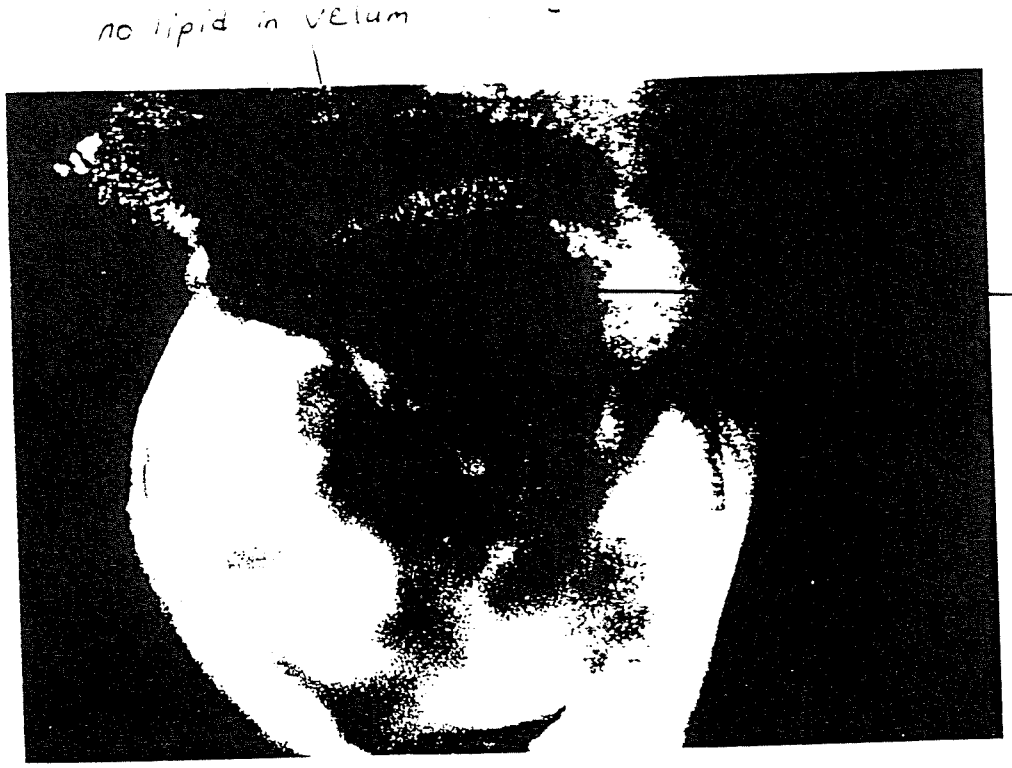


(Plate 2) Six day fed larva stained with oil red O - Velum extended.



(Plate 3) Six day starved larva Stained with Oil Red O - Velum extended.

(Plate 4) Eighteen day, fed larva stained with Sudan Black B - Velum extended. Photographed under phase contrast microscopy.



(Plate 5) Eighteen day, starved larva stained with Sudan Black B - Velum extended (Phase Contrast microscopy)

DISCUSSION.

The modified method developed in this study was a more effective method for anaesthetising Pacific oyster larvae than that of Gallager et al (1981).The anaesthetising method of Gallager et al (1981) involved the use of excessive amounts of $MgCl_2$.This caused a mucous secretion from the Pacific oyster larvae in this study and death.Larvae died with their velums extended,but the mucous secretion interfered with the later microscopic inspection of the lipid stained larvae.The modified method minimises the chance of the $MgCl_2$ building up to toxic levels and so anaesthetises the larvae without killing them.The only disadvantage of this method is that it should be carried out under the microscope so that anaesthetised larvae can be placed in formalin before they begin to recover from the effects of the $MgCl_2$.

The Oil Red O stain using the modified method of Gallager et al(1986) was superior to the Sudan Black stain because of it's intense red colour which showed the presence of lipid droplets very well under the microscope. The Oil Red O stain was also quicker to use because of its shorter clearing time.The only disadvantage with the Oil Red O stain was that it required constant filtering to remove particles of stain as these particles made locating larvae in the stain difficult.

In the fed control larvae there is an accumulation of lipid droplets in the velum and digestive gland and this lipid store is found to increase with the development of the larvae.Waldock and Nascimento (1979) showed that accumulation of lipid in the digestive gland of pacific oyster larvae occurred after the development of a functional mechanism for particulate feeding.Prior to this the larvae utilize an endogenous lipid supply provided to it at the egg stage by the parent.Thus monitoring the lipid content in

the digestive gland of the Pacific oyster larvae may be an effective means of determining whether the larvae is feeding normally.

In the six and 18 day larvae that had been starved for two days there was less lipid present in the digestive gland compared to that of the control.Thus the larvae may have responded to the lack of food by utilizing the lipid reseves it had built up.Hence a small lipid content in the digestive gland may be an indication of nutritional or environmental stress.A depleted lipid store may affect the ability of the larvae to metamorphose into a benthic adult because it is thought that the energy requirements during this transition are met by lipid reserves stored prior to metamorphosis (Holland 1978).Thus a knowledge of the amount of lipid in the digestive gland of Pacific oyster larvae may be used to predict the likelihood of survival of larvae through their development. Waldock et al (1979) also showed that the algal species that promoted the fastest growth of *C.gigas* larvae also promoted accumulation of the greatest triacylglycerol reserve.This finding suggests a relationship between good growth and accumulation of lipid.This relationship makes the lipid specific staining technique a quick and accurate means for assaying condition in cultured Pacific oyster larvae.

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SEROTONIN- INDUCED SPAWNING IN PACIFIC OYSTERS (*Crassostrea gigas*) *NARCOTIZED WITH MAGNESIUM SALT.*

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ABSTRACT

A method is described for the artificial induction of spawning in Pacific oysters (*Crassostrea gigas)* without killing the organism.The gonad is made accessible to injection with serotonin by firstly paralysing the oyster in magnesium salt.The paralysed oyster was found unable to spawn until it was removed from the magnesium salt solution,suggesting some muscular involvement with the act of spawning.

It was concluded that this technique may have potential as a non destructive method for the routine sampling of spawn from oysters.

INTRODUCTION.

It is important to be able to assess the reproductive condition of an oyster in a particular breeding program. A common technique called stripping,kills the oyster and this is undesirable if the oyster is needed for breeding purposes.Injection of serotonin(5-hydroxytryptamine,creatine sulphate complex) has been found to artificially induce spawning in several species of bivalve molluscs including the bay scallop *Argopecten irradians*, the American oyster *Crassostrea virginica,* the ribbed muscle *Geukensia demissa*, and the hard clam *Mercenaria mercenaria* (Gibbons and Castagna 1984).The mechanism of action of serotonin on spawning is unclear but serotonin has been identified as a neurotransmitter in the nervous system of molluscs, stimulating heart beating and ciliary movements (Leake and Walker 1980).

Gibbons et al (1984) injected serotonin through notches filed between the shell margin of bivalves.The damage to the mantle probably killed the organisms and so this method is not applicable to oysters required for subsequent breeding programs.

Magnesium salt has been found to paralyse muscular activity in oyster larvae (Gallager and Mann 1981) and adult oysters (Galtsoff 1964) so that the oyster is left in a state where the body organs are accessible to injection with serotonin.In this study adult Pacific oysters (*Crassostrea gigas)* treated with a magnesium salt are injected with serotonin with the aim to induce spawning.

MATERIALS AND METHODS.

ANIMALS.

Adult Pacific oysters were taken from large outdoor tanks located at the Brackish Water Fish Culture Research Station, Salamander Bay , N.S.W.The tanks were constantly flushed with unfiltered sea water the temperature of which was not regulated.

NARCOTIZING THE OYSTERS.

Two different magnesium salts were tested-magnesium chloride and magnesium sulphate. The method of Galtsoff (1964) was used to narcotize the oysters.Oysters were washed and scrubbed to remove fouling organisms and then each was placed in a small plastic container half filled with filtered sea water. Crystals of either magnesium salt were added to each container gradually over a 24 hr period until a concentration of 5-10% was reached.No salt was added to controls. The oysters were then left for a further 24 hours to allow the salt to take effect.Completely paralysed oysters did not close their shells upon touching them.

INJECTION OF SEROTONIN.

Anaesthetised oysters were injected with 0.4 ml of 2mM serotonin solution in filtered (1μm) seawater into the gonad mass (Gibbons et al 1984).A new needle was used for each oyster to prevent transference of gonadal products.

The seawater in which the oysters were immersed was maintained at the same temperature throughout the experiment to eliminate the possibility of spawning due to temperature change. Controls were injected with 0.4 ml of 1μm filtered seawater.

Notches were filed into the sides of oysters between the shell margin

of those that could not be successfully paralysed,and serotonin was injected via this route. The oysters were watched for signs of spawning and any spawn obtained was sexed and assessed for ripeness.

RESULTS.

EFFECTIVENESS OF MAGNESIUM SALTS.

One out of the two oysters was narcotized using magnesium chloride while no oysters could be narcotized with shells open using magnesium sulphate (table 1). All controls remained unanaesthetised(table 1).

Table 1. Numbers of Pacific oysters fully narcotized by salt treatment.

Treatment	Number tested	Number fully narcotized
Mg sulphate	2	0
Control	2	0
Mg chloride	2	1
Control	2	0

EFFECT OF SEROTONIN ON SPAWNING.

The oyster that was fully narcotized in magnesium chloride did not respond to an injection of 2 mM serotonin.After an hour it was placed in a second container with fresh filtered seawater and allowed to recover some muscle function (indicated by movement of mantle feelers).It was then reinjected with 2 mM serotonin and this time spawning occured within 30 seconds of injection.Spawning behaviour was characterised by gaping of the shells followed by a jet of spawn released from the site of injection.The spawn was found to consist of ripe female eggs(table 2).

Serotonin induced spawning in six out of eight oysters where the serotonin was injected into the gonad through a notch filed into the side of the oyster at the cell margin (table 2).Spawning generally occured within five to thity seconds of injection.

Table 2. Numbers of Pacific oysters induced to spawn by serotonin injection.

Treatment	No tested	No spawned	No males	No females
Serotonin	8	6	2	4
Controls	3	0	0	0

The seawater injected controls did not gape or release spawn (table 2). The gonads of the two oysters that did not spawn were removed and the sex cells were found to be in an unripe condition.The spawn of the six oysters that were induced to spawn were all found to be in a ripe condition. Inspection of the gonad and spawn of the controls showed them to be in a ripe condition as well.

DISCUSSION.

Too few oysters were tested to conclude which salt is the more effective narcotizing agent.It is probably the magnesium ion which interferes with muscle contraction in the oyster and so both salts would be expected to be equally as effective.But oysters were more prone to close their shells with the introduction of a crystal of magnesium sulphate than magnesium chloride which may suggest that the chloride ion is less irritating or toxic to the oyster than the sulphate ion. Further experiments are required to determine this conclusively.The method of Galtsoff(1964) was found to be time consuming and tedious and so may require some modification if it is to be used for routine laboratory work.

Since injection of serotonin into the gonad of the completely paralysed oyster failed to induce spawning while injection after it had regained some muscular activity led to spawning, then spawning may depend on the action of particular muscles.These muscles may be specifically stimulated by serotonin. When the muscles are paralysed by magnesium salt, serotonin may not be able to exert its effect.This assumes that serotonin after injection into the gonad is transferred to other tissues of the oyster via its vascular system.

Serotonin caused gaping and the production of water jets and the release of spawn in ripe oysters while the saltwater injected controls did not gape or produce water jets.Similar findings were found in the American oyster *Crassostrea virginica* (Gibbons et al 1984). This also suggests that serotonin may act by stimulating certain muscle groups

invoved with the release of spawn.Since gaping and water jets also occured in unripe oysters injected with serotonin, then the action of serotonin on muscles may occur independently of the condition of the gonad.

Since spawn was always released from the site of injection of serotonin, then the rupturing of the membrane surrounding the gonad may be a normal event during spawning,to provide an exit point for the spawn. Thus injection of serotonin into the gonad mass may mimick two naturally occuring processes-the rupturing of the gonad mass and the stimulation of muscles involved with the release of spawn from the gonad.

In conclusion it appears that serotonin may only induce spawning in nonparalysed ripe oysters.Hence oysters may need to be removed to fresh seawater after narcotizing in magnesium salt to allow them to recover slightly before injection of serotonin. This would need to be carefully timed as too greater period of recovery may enable the oyster to close its shell and prevent injection of serotonin.Otherwise this technique has good potential as a method for the artificial induction of spawning for breeding programs and for the routine testing of condition and sex of spawn without the need to kill the oyster.

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A Comparison of the biology of the Sydney rock oyster (*Saccostrea commercialis*) and the Pacific oyster (*Crassostrea gigas*) in Port Stephens, NSW.

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ABSTRACT

A study of the comparative biology of Sydney rock oysters (*Saccostrea commercialis*) and Pacific oysters was carried out by request of the NSW oyster industry, from July 1986 to June 1989. Pacific oysters (*Crassostrea gigas*) were cultivated in Port Stephens, where they grew at approximately twice the rate of Sydney rock oysters (*Saccostrea commercialis*), although Pacific oysters were more susceptible to heat kill than Sydney rock oysters. Both species of oysters suffered from mudworm (*Polydora websteri*) infestation, which caused heavy mortality. Adult Pacific oysters grew well at salinities from 15 to 45%, whereas the salinity range for maximum growth of Sydney rock oyster ranged from 20-40%

Pacific oysters intertidally cultured in Port Stephens had a peak meat condition from August 1988 to October 1988 whereas intertidally cultured Sydney rock oyster were in peak condition from December 1988 to February 1988, although there were large site to site differences in meat condition of oysters of both species in Port Stephens.

Both species of oyster were readily accepted for consumption by a CSIRO taste testing panel. Single seed adult Pacific oysters had a shell cavity volume of 56% (expressed as a percentage of whole oyster volume) compared to Sydney rock oysters which had a cavity volume of 40%.

INTRODUCTION

The first reports of Pacific oysters (*Crassostrea gigas*) in New South Wales came from the Pambula River in 1967 (Wolf and Medcof, 1974; Medcof and Wolf, 1975). In 1973, Pacific oysters were recorded in Port Stephens (Wolf and Medcof, 1974; Medcof and Wolf, 1975). Farmers were advised by Medcof and Wolf (1975) that once Pacific oysters are established as a natural population in an area, they would be impossible to eradicate. The first sighting of large numbers of Pacific oyster spat was made in Big Swan Bay in the inner harbour of Port Stephens, NSW, in the spring of 1984 (Holliday and Nell, 1985). Serious concerns about the spread of Pacific oysters in Port Stephens were raised in the spring of 1985 when sightings of Pacific oyster spatfall became more numerous.

The study of the comparative biology of the Sydney rock oyster and Pacific oyster has found differences in their biology which might be useful in the control and management of the Pacific oyster in Port Stephens.

Comparison of growth and mortality of oysters

In Port Stephens, NSW, Pacific oysters grew at approximately twice the rate of Sydney rock oysters in both intertidal and deepwater (raft) culture (Table 1; Nell, 1991a). If not protected with a plastic shade mesh, small intertidally grown Pacific oyster spat suffered a higher mortality rate from heat kill than Sydney rock oyster spat of the same size. Heat kill of Pacific oysters was particularly severe at growing heights greater than the conventional rack height. Subtidally cultured oysters of both species suffered a high incidence of mudworm (*Polydora websteri*) infestation.

Effects of tributyl tin oxide on growth of spat

TBTO (tributyl tin oxide) was found to affect both species of oysters. Weight gains of Pacific oyster spat were reduced from an average of 27 to 6 mg dry weight and those of Sydney rock oyster spat from an average of 30 to 13 mg dry weight, by the addition of 5 ng TBTO (bis-tributyl tin oxide) per litre over a 4 week period (Nell and Chvojka, 1991). These were reductions in dry weight gain of 78 and 57% for Pacific and Sydney rock oysters respectively. This suggests that Pacific oysters are more sensitive to TBTO than Sydney rock oysters. Pacific oysters exposed to the same TBTO concentrations on a commercial oyster lease, displayed 2 to 3 times the TBTO concentration in their meat than Sydney rock oysters (Batley, et al., 1989). The greater reduction in weight gain in Pacific oysters may be related to the higher accumulation of TBTO in their meats.

Mortality of oysters out of water

Sydney rock and Pacific oysters were exposed to air temperatures from 3°C to 40°C for varying lengths of time (Mason and Nell, 1991a). For temperatures above 8°C, oyster mortality increased with increasing air temperature. Pacific oysters died faster than Sydney rock oysters at all temperatures above 8°C, but Sydney rock oysters died faster than Pacific oysters when kept at 3°C. The relative humidity had no significant effect on the rate at which oysters of either species died.

The safety margin for selective killing of Pacific oysters at high temperatures is too narrow to be a practical proposition for oyster farmers. If however farmers wish to selectively kill Pacific oysters at ambient temperatures in a shed or out in the open they have to risk a substantial loss of Sydney rock oysters (Nell, 1991b).

The spat catch of Pacific oysters

The catch of Pacific oyster was heavier in the inner harbour than in the outer harbour of Port Stephens (Nell and Gwynne, 1991). The catch of Pacific oyster spat was substantially higher at 150 mm below the conventional rack height (Nell, 1991c) and decreased with increasing height (Mason and Nell, 1991b).

Attempts to cross oysters

To determine the likelihood of wild crosses occurring between the two species, crosses were attempted between Sydney rock oyster males x Sydney rock oyster females, Sydney rock oyster males x Pacific oyster females, Pacific oyster males x Pacific oyster females and Pacific oyster males x Sydney rock oyster females. Fertilisation and development to "D" stage occurred only in the Sydney rock oyster males x Sydney rock oyster females and Pacific oyster males x Pacific oyster females crosses. No viable hybrid larvae were produced (Nell and Mason, 1991a).

The optimum salinity for oysters

Sydney rock oyster larvae had the best growth and survival at salinities of 27-39‰, whereas Pacific oyster larvae had the best growth and survival at the lower salinities of 19-27‰ (Table 2; Nell and Holliday, 1988). The optimum salinity range (19-27‰) determined for Pacific oyster larvae from Port Stephens was very similar to the 25‰ which was found to be best for maximum growth of Pacific oyster larvae in England (Helm and Millican, 1977). Adult Pacific oysters can survive in water with salinities ranging from 5 to 55‰, whereas adult Sydney rock oysters can survive in salinities from 15 to 50‰ (Table 2; Nell and Gibbs, 1986).

The range of salinities at which oysters survived and grew well increased with the size of the oysters in both species from larvae to spat with increasing size up to 600 mg (Nell and Holliday, 1988). For all age groups studied, growth of Pacific oysters at optimum salinities was considerably faster than that for Sydney rock oysters.

This study confirms the reports by Hughes-Games (1977) and King (1977) that adult Pacific oysters may be cultivated in hypersaline ponds of 40‰. Adult Sydney rock oysters appear to be less suited to hypersaline conditions. Oyster hatcheries that are producing Pacific oyster spat should operate with dilute seawater of about 25‰, whereas Sydney rock oyster hatcheries should preferably operate at full oceanic salinities (35‰).

Comparison of meat condition of oysters

Intertidally cultured Pacific oysters in Port Stephens had a peak meat condition from August 1988 to October 1988 whereas intertidally cultured Sydney rock oysters were in peak condition from December 1988 to February 1989 although there are large site to site differences in meat condition of oysters of both species (Mason and Nell, 1991c).

There was a trend for the meat condition of Sydney rock oysters at each site to be greater than that of the Pacific oysters, except just before the period in which Pacific oysters spawned. The post-spawning drop in meat condition was less abrupt for Sydney rock oysters than Pacific oysters.

Comparison of whole oyster and shell measurements

Adult Pacific oysters had a larger shell cavity volume (Table 3; Nell and Mason, 1991b) expressed as a percentage of whole oyster volume (56 and 49% for single seed and stick oysters respectively) than adult Sydney rock oysters (40 and 36%). Pacific oysters contained less shell expressed as a percentage of whole oyster weight (63 and 65% for single seed and stick oysters respectively) than Sydney rock oysters (73 and 77%).

Comparative taste tests of oysters

A series of comparative taste tests on fresh and cooked Sydney rock oysters and Pacific oysters grown in Port Stephens, were carried out by Dr R L McBride, CSIRO, Division of Food Research. Oysters of both species used for the fresh oyster taste test (November, 1986) were all in peak condition, whereas oysters of both species used for the cooked oyster taste test (May, 1987), were in a good but not peak condition. All oysters were depurated before taste testing. The results indicated that fresh oysters of both species are very acceptable to the public, although there was a slight taste preference for Sydney rock oysters (McBride et al., 1988). There was no preference however, when the oysters were cooked (oysters Kilpatrick). These results showed that the promotion of the Sydney rock oyster as a gourmet product for the fresh oyster market has potential for improving sales.

CONCLUSION

The original purpose of the studies summarised in this review was to identify differences in biology which might lead to control, a "know your enemy" philosophy. It is most unfortunate that Pacific oyster control in Port Stephens has now been shown to be uneconomical, but what we have learned can assist the industry to plan its future. It is of great importance for the NSW oyster industry to control the Pacific oysters in estuaries other than Port Stephens, as the problems of Pacific oyster overcatch will make the farming of oysters in NSW very difficult and there may be many problems yet to come with the cultivation of Pacific oysters in Port Stephens. These problems may not become apparent until large scale farming of this oyster has become established.

The studies reviewed have shown that Pacific oysters can be cultivated in Port Stephens, but the use of triploid Pacific oysters, which maintain condition (Allen and Downing, 1986) and do not produce viable spawn (Allen and Downing, 1990), should be investigated both to limit future increases in overcatch of Pacific oyster spat and to lengthen the marketing season of Pacific oysters.

In the past NSW oyster farmers have adapted their growing techniques to a wide range of conditions. There is no doubt that they will apply the same ingenuity for the development of suitable farming techniques for Pacific oysters.

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TABLE 1

Growth rates[1] of Sydney rock oysters (*Saccostrea commercialis*) and Pacific oysters (*Crassostrea gigas*) in Port Stephens NSW, from August 1986 - May 1988

	Oyster weights (g)			
Cultivation[2]	13 months	24 months	28 months	33 months
Sydney rock oysters				
Rack height (4)	7±1 (13±5)	18±5 (16±6)	22±3 (18±7)	25±5 (20±5)
Deepwater (1)	14 (3)	28 (24)	terminated[3]	
Pacific oysters				
Rack height (4)	16±2 (27±26)	38±6 (29±25)	harvested	
Deepwater (1)	43 (9)	harvested		

1 Data are expressed as mean±SD. The percentage cumulative mortality is shown in brackets. The experiment was commenced with 1000 Sydney rock and 1500 Pacific oyster spat per site. The initial average weight of spat of both species was 0.2 g. The number of months indicate the length of the experimental period, not the age of the oysters

2 Number of sites under cultivation is shown in brackets

3 Treatment terminated because of mudworm infestation

TABLE 2

Salinity preferences and tolerances of oysters. Salinity levels are expressed as parts per thousand by weight (‰). Full sea water has a salinity of 35‰.

Species	Salinity ranges for maximum growth and survival[1]			Salinity ranges for survival of adult oyster[2]
	Larvae	Juvenile spat (1 mg)	Oysters (>600 mg)	
Sydney rock oyster *Saccostrea commercialis*	27-39	25-35	20-40	15-50
Pacific oyster *Crassostrea gigas*	19-27	15-30	15-45	5-55

1 Nell and Holliday, 1988

2 Nell and Gibbs, 1986

TABLE 3

Comparison[1] of shell density, percentage cavity volume and shell weight of Sydney rock oysters[2] (*Saccostrea commercialis*) and Pacific oysters[2] (*Crassostrea gigas*)

Species	Cultivation	Number of oysters measured	Cavity volume[3] (%)	Shell weight[4] (%)	Shell density (g/ml)
Sydney rock oyster	single seed	185	40 ± 5^{b}	73 ± 4^{c}	1.7 ± 0.1^{a}
Sydney rock oyster	stick	179	36 ± 4^{a}	77 ± 3^{d}	1.8 ± 0.2^{b}
Pacific oyster	single seed	220	56 ± 6^{d}	63 ± 6^{a}	2.2 ± 0.2^{d}
Pacific oyster	stick	201	49 ± 9^{c}	65 ± 5^{b}	2.1 ± 0.4^{c}

1 Mean±SD within columns, mean with a common superscript do not differ significantly ($P>0.01$)

2 All oysters (weight range 25-76 g) were grown intertidally in Port Stephens, NSW from August 1986 - May 1989

3 Expressed as a percentage of whole oyster volume

4 Expressed as a percentage of whole oyster weight