ISSN 0810-5804

STORAGE AND SHELFLIFE OF THE AUSTRALIAN NATIVE FLAT OYSTER (*OSTREA ANGASI*)

L. Mantzaris
N.J. Hickman
G.W. Grossel

Technical Report No. 79

September 1991

Marine Science Laboratories
Queenscliff, Victoria
Australia

CONTENTS

ABSTRACT

The shelf life of oysters (*Ostrea angasi*) depended on storage temperature, humidity and the extent to which the oysters gaped during storage. Oysters chilled to 1.5°C by means of ice meltwater and weighed down by a load of 1.5 kg had a shelf life of 23 days. By contrast, oysters stored at 20°C under dry conditions and without being weighed down had a shelf life of only 2 days. When oysters prechilled to 3°C were kept moist and tightly packed in polystyrene boxes stored at 4.5°C, they remained fresh for 14 days; their shelf life was not prolonged by the presence of 600 g of coolant (Thermosorb or Ice-bricks). Mortality and odour were the most reliable indicators of spoilage; standard plate counts, *E. coli* counts, and analysis of total reducing substances were no use as indicators of spoilage.

INTRODUCTION

Unlike most fresh chilled seafood, oysters are distributed live because of an assumption that if an oyster is alive it must be good enough to eat. This assumption is dangerously misleading because the palatability of shellfish (detected by sensory analysis) may deteriorate to below acceptable levels while the shellfish are still alive (Brooks and Harvey 1981; Warwick 1985).

Organoleptic scores (based on odour and flavour) for mussels have been found to correspond with total bacteriological counts (Brooks and Harvey 1981). This suggested that the sensory perception of spoilage is a response to the metabolic breakdown products formed during bacterial decomposition. Furthermore, results from total bacterial counts or Standard Plate Counts (SPCs) exhibited uniform, sigmoidal growth of bacteria with storage time. Such results have led to the use of SPCs as the standard test for quality control of shellfish marketed by Australian producers.

In contrast, most chemical methods of evaluating shellfish quality, such as pH and nucleotide analysis, have been reported to be unreliable indices of shellfish quality (Hoff *et al.* 1967; Boyd *et al.* 1980; Thrower 1983). More successful methods have included the Total Reducing Substances (TRS) test (Liuzzo *et al.* 1975) and the triphenyltetrazolium chloride (TTC) test (Monchinga 1971). The latter is a test of gill function and is used for quality assessment of oysters in the Tokyo fish market. A quick and reliable standard chemical test of the quality of oysters marketed in Victoria would be valuable in overcoming the delays associated with microbiological analysis.

Because the Australian flat oyster lives subtidally, its tolerance of storage and transportation stresses was not expected to be as high as that of intertidal species of oysters. Therefore the question of whether flat oysters could be transported long distances without spoilage needed to be answered. Overseas marketing, for instance, would require maintenance of a high quality product for at least one week after harvesting. Hence, in Victoria, there is a need to determine the best possible handling and storage procedures which would ensure that flat oysters remained alive and of high quality during distribution.

In this report, we describe a study in which we examined how the quality of live oysters changed in relation to the temperature and the methods used to store them. We also examined various methods of assessing spoilage in oysters.

We conducted three trials. The first was designed to monitor the effects of the oysters' orientation and storage temperature on the oysters' survival during dry storage. The second was designed to investigate the effectiveness of weighing down the oysters and the "curtain of ice" method of chilling oysters (Boyd *et al.*

1978) during moist storage. In the third trial, the effectiveness of two commercially available coolants in maintaining chilled temperatures within a typical shellfish packaging unit was compared. The effect of storage temperature on oyster quality was also determined and different methods of assessing oyster quality were compared.

DRY STORAGE

In this section we examined the effects of orientation and temperature on the shelf life of oysters exposed to dry air conditions.

MATERIALS AND METHODS

In May 1989, market size cultured oysters from a site near Port Albert were transported to Swan Bay for temporary holding before being taken to the Marine Science Laboratories (MSL), Queenscliff (Fig 1). At the laboratory, the oysters were thoroughly cleaned of fouling material, labelled and kept in unfiltered sea-water in 5000 L flow-through tanks until required.

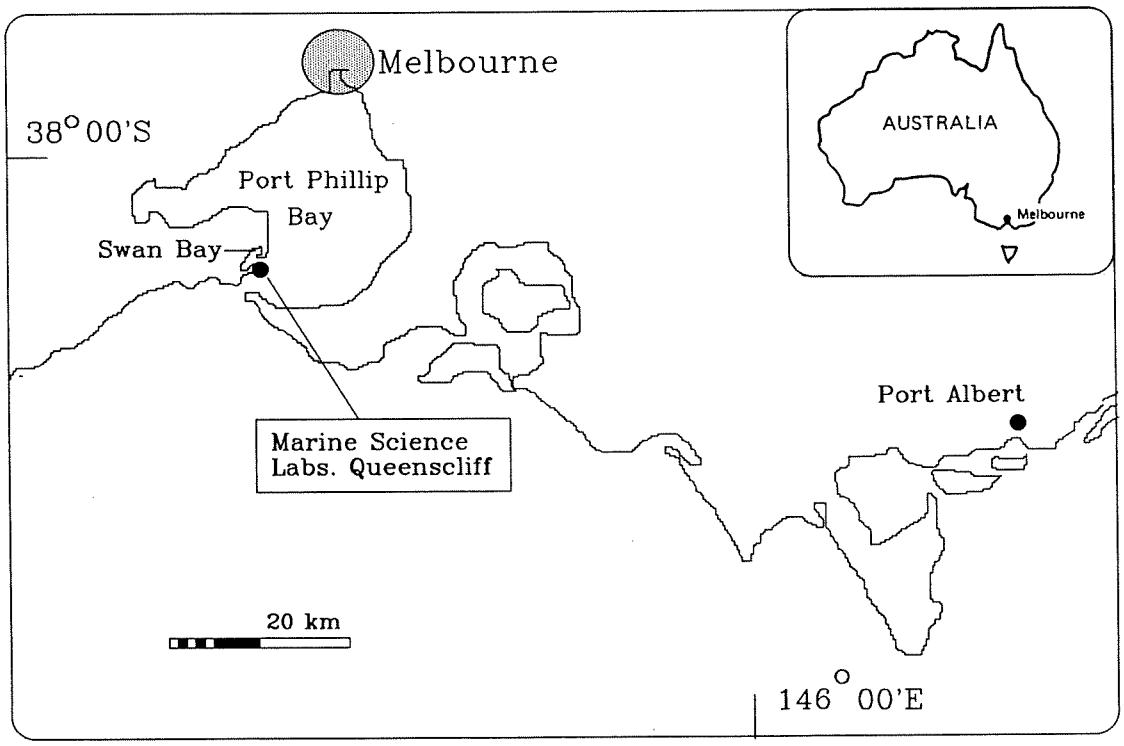
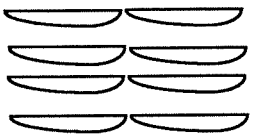
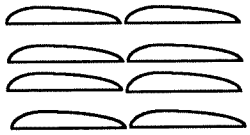
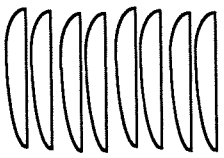
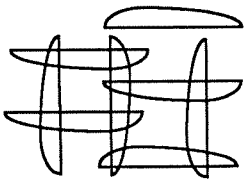


Figure 1. Location of laboratories and oyster holding sites.

Twelve groups of 20 oysters selected at random were exposed to air dry conditions at three different temperatures and four different orientations (Table 1). Storage facilities which provided the three temperatures included; a refrigerator (6 ± 1°C), a laboratory bench at ambient temperature (20 ± 2°C) and an oven (28 ± 1°C). Oysters were stored on metal racks so that any shell liquor would drain away. Temperatures around the oysters were recorded at 1 hour intervals throughout the experiment using probes linked to a data logger.

Table 1. Orientation of oysters.

Cup down		Resting on the cupped valve.
Cup up		Resting on the flat valve.
Vertical		Suspended from the hinge.
Random		Mixed in a plastic mesh bag in all possible positions.

Each oyster was measured (length) and weighed for whole live weight (before treatment) and dry meat weight (freeze dried after death). Twice daily, the number of dead oysters was assessed. A live oyster was one which was closed tightly or one which was gaping but showed an adductor muscle response when tapped. A dead oysters was one which gaped and showed no response to being tapped. The number of days each oyster survived was recorded. Oysters that had died but had not gaped were excluded from the data set because their time of death was not known.

The mortality data were subjected to univariate analysis which showed that for most treatments the data were not normally distributed. Because no assumptions could be made about the distribution of the data, nonparametric statistical methods were necessary. Therefore, subsequent analysis of variance involving a general linear models procedure was based on ranked versions of the mortality data and median values were used in plotting.

RESULTS AND DISCUSSION

Survival time can be regarded as an index of shelf life although useful shelf life ends before death. As expected, survival time increased significantly ($P < 0.001$) with decreasing temperature (Fig 2). Oysters survived for 11-15 days in a refrigerator at 6°C, for 6-7 days on the laboratory bench at an ambient

temperature of 20°C and for 2-4 days in an oven at 28°C. Therefore, at 6°C, shelf life was at least 4 days longer than that at 20°C and at least 7 days longer than that at 28°C. The extent to which shelf life is reduced by continuous exposure to high temperatures emphasizes the importance of keeping oysters cool at all times after harvesting. None of the size variables (length, whole live weight or dry meat weight) had any significant interactive effect on shelf life.

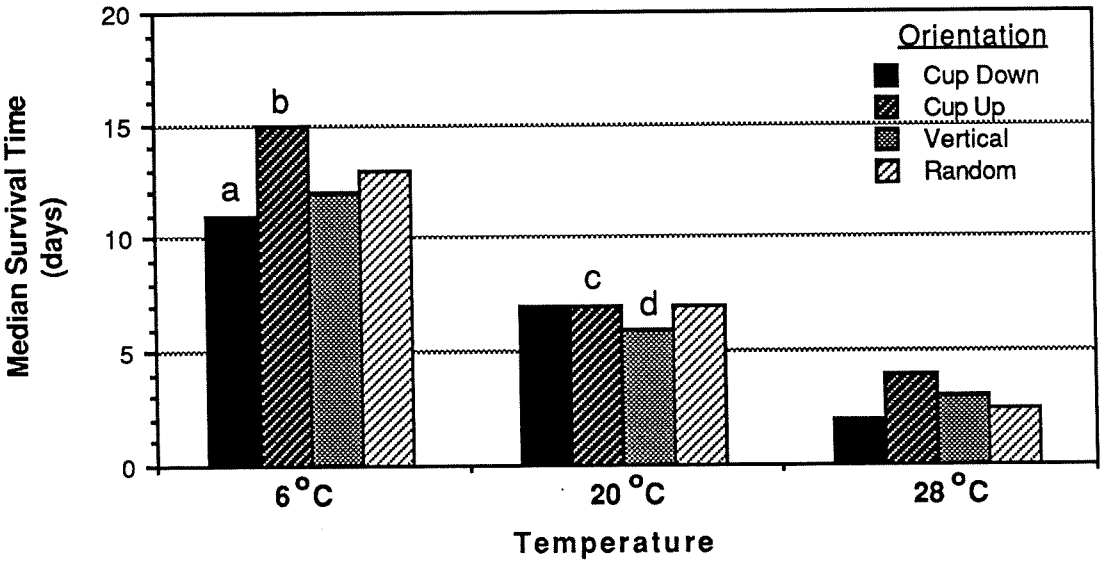


Figure 2. Duration of survival (median) during dry storage of live oysters at 6°, 20° and 28°C. Note: N=20 in all cases except for treatments a-d where N=16, 17, 14 and 18 respectively due to the omission of individuals which failed to gape.

The orientation of the oysters significantly ($P<0.05$) affected their survival in the refrigerator and the oven; median survival times were highest with the "cup up" orientation and lowest with the "cup down" orientation. Stroud (1981) has suggested that shellfish should be stored or packed "cup down" so that shell liquor is retained when gaping occurs and deterioration through dessication is minimised. Our findings provide evidence to the contrary.

An oyster closes when its adductor muscle contracts. At the same time, the hinge ligament is compressed. Gaping follows relaxation of the adductor muscle and the elasticity of the hinge ligament then allows the valves to separate. When gaping, the oyster loses moisture from its tissues and will die. When the oyster's heavier cupped valve is on top gaping may be more difficult than when the lighter flat valve is on top; hence, oysters may find it more difficult to gape when oriented "cup up".

Survival time probably depends on the time until gaping first occurs, the number of times gaping occurs, and the duration of gaping. To determine whether gaping does affect survival, we measured mortality in oysters physically prevented from gaping by heavy weights placed on top of the oysters (see next section).

MOIST STORAGE

In this section we examined the effect of cooling in conjunction with weighing down oysters with a weight of about 1.5 kg.

MATERIALS AND METHODS

Oysters harvested in late June were brought to the laboratory, cleaned thoroughly and kept in a holding tank for two days to recuperate from the stress associated with being cleaned. The oysters were divided into four groups of 50 and each group was kept under different conditions (Fig 3):

A. kept moist with tap water at ambient temperature (control);
B. kept moist and weighed down with 1.5 kg at ambient temperature (pressured);
C. kept under a "curtain of ice" in a cool room (chilled);
D. held under a "curtain of ice" and weighed down with 1.5 kg in a cool room (pressured and chilled).

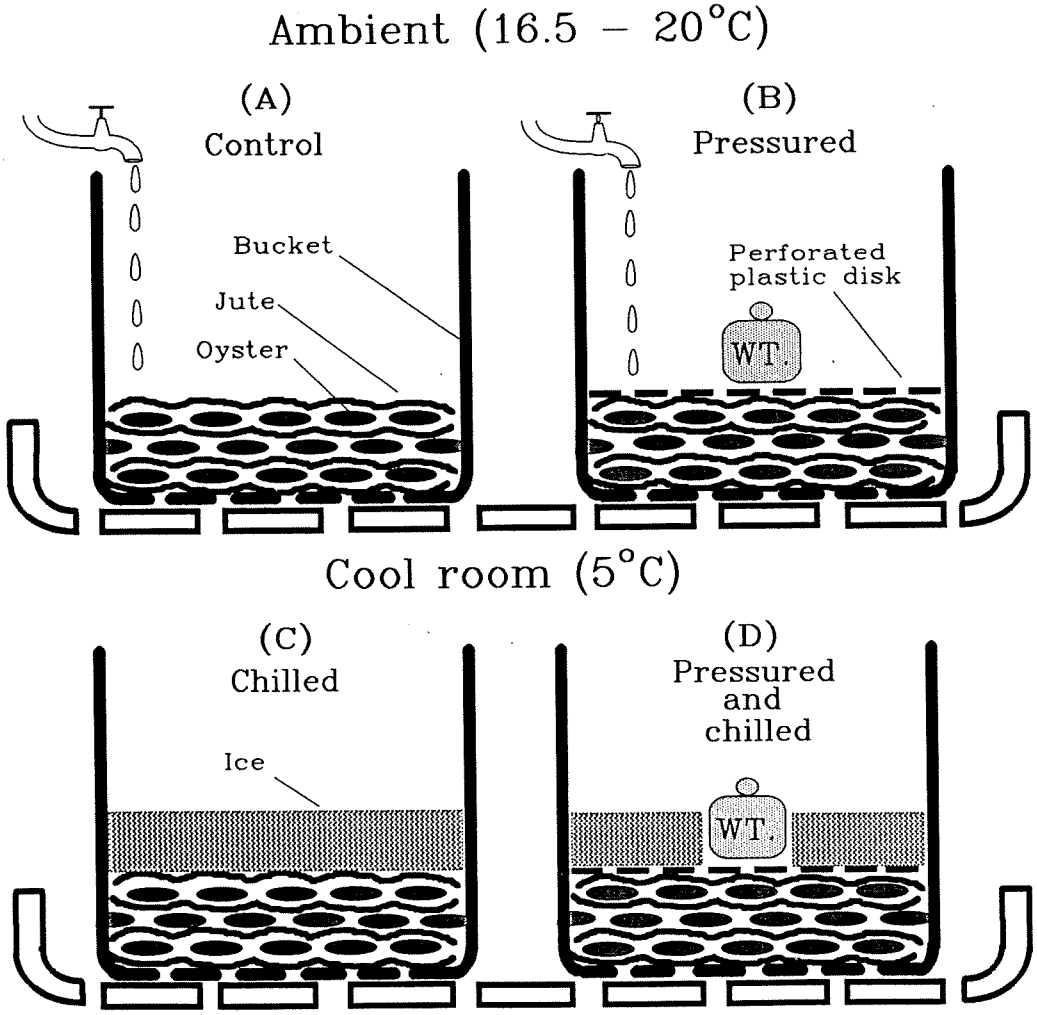


Figure 3. Experimental treatments for moist storage of live oysters.

The "curtain of ice" technique (Boyd and Wilson 1978) is a simple way of chilling oysters and keeping them damp at the same time. Ice is placed above the oysters so that the melt water can run down around them and drain away from the bottom of the storage container. Some type of porous material is needed to separate the ice from the top layer of oysters to prevent them from freezing.

For each treatment, the oysters were oriented "cup up" and layered between pieces of jute in a bucket with a perforated base. The "cup up" orientation was used in an attempt to minimise the oysters' tendency to gape. The buckets were placed on gratings above drip trays that collected excess water. Treatments C and D were carried out in a cool room at a mean temperature of 5°C to conserve ice and to prevent it from melting completely overnight. The unchilled treatments (A and B) were carried out at ambient temperature and the oysters were kept moist by potable water dripping from a tap. Pressure applied with weights (treatments B and D) was a method of minimising the oysters' gaping. Temperature probes linked to a data logger were used to record the temperatures in each bucket and in the ambient air every 2 hours.

Mortality

After various time intervals, the number of dead oysters in each bucket was assessed and recorded. The criterion of death was that described in the preceding section. During the 10-15 minutes required to count the dead oysters in each bucket, continuity of pressurization and chilling was briefly compromised. This discontinuity would also be necessary in a commercial situation because dead oysters have to be removed to prevent them from contaminating live oysters.

From the total number of deaths per treatment the percentage mortality was calculated. Data from the previous experiment were included so that results from dry and moist storage could be compared.

Bacterial Counts

At various time intervals three oysters from each bucket were taken for individual bacteriological analysis by means of the SPC technique required by Commonwealth export regulations (Australian Standard AS 1766: Methods for the Microbiological Examination of Food). Only live oysters were analysed because bacterial numbers rise very rapidly after death (Boyd *et al.* 1980).

The mean number of total bacteria per gram of oyster tissue was calculated for oysters from each bucket. The means from each treatment were plotted against storage time. Bacterial counts could not be made after 13 and 15 days of storage for treatments A and B, respectively, because no oysters remained alive after this

time.

RESULTS AND DISCUSSION

For the duration of the experiment, the ambient temperature in the laboratory varied from 16.5 to 20°C with an average of 18°C. Within buckets A and B, the dampened layers of jute brought the temperature down to 15.5°C (range 14.5 - 16.5°C). Under a "curtain of ice" (treatments C and D), the temperature averaged 1.5°C (range 1.0 - 2.5°C).

Mortality

As expected, the mortality rate of chilled oysters was lower than that of oysters at ambient temperature (compare A with C and B with D in Fig 4). Figure 4 also shows a decrease in mortality rate associated with the application of pressure (compare A with B and C with D). This result supports our suggestion that gaping affects survival; preventing the oysters from gaping increases survival and could be used to advantage when oysters are being stored.

Preliminary trials carried out alongside this experiment showed that, out of a sample of five oysters kept moist at 0.4°C for 2 days, all survived and were still alive after a further week in seawater. Green mussels cannot tolerate being chilled below 2°C (Warwick 1985). The tolerance of the flat oyster to very low temperatures is useful to handlers who know that they can safely chill oysters to near freezing temperatures to maximise shelf life.

The time taken for mortality to reach 10% was taken as a measure of useful shelf life and is referred to hereon as T_s. Ten percent mortality could conceivably occur as a result of weaker oysters dying from physiological stress. At mortalities higher than 10%, bacterial decomposition of oyster tissue becomes a more likely cause of death.

For treatments A, B, C and D, the T_s values were 6, 7, 11 and 23 days respectively (estimated from Fig 4). Therefore, T_s was extended 1 day by application of pressure, 5 days by chilling and 17 days by combining pressure and chilling. The effect of the chilling and pressure combined was therefore greater than the sum of the two effects. Weighing down the oysters is a simple and inexpensive method for longer term storage of oysters and is applicable to commercial operations.

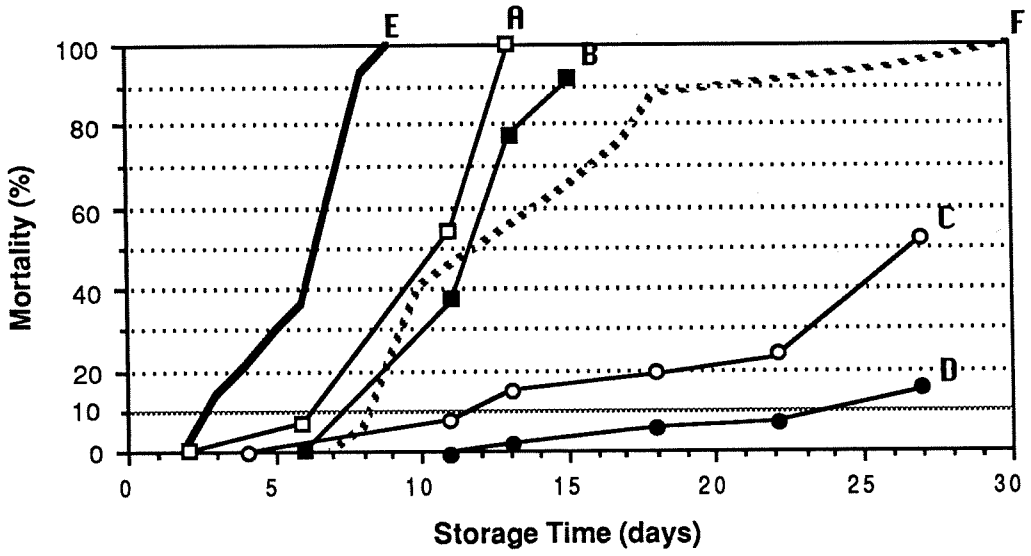


Figure 4. Changes in mortality during moist storage of live oysters at (A) 15.5°C, (B) 15.5°C and under pressure, (C) 1.5°C, and (D) 1.5°C under pressure. E and F are derived from data in the first section and represent dry storage of "cup up"-oriented oysters at 20°C and at 6°C respectively.

Results from the dry storage treatments (Fig 4, E and F) showed that Ts was 2 days at ambient temperature and 8 days in the refrigerator. Under moist conditions Ts was 6 days at ambient temperature and 11 days when oysters were chilled. Therefore, moisture is another factor which contributes to increased shelf life.

Results from similar studies on the Sydney rock oyster, *Crassostrea commercialis* are conflicting. Quadri *et al.* (1976) found that under dry conditions mortality in the Sydney rock oysters was not significant after 3 weeks at 10°C, but Boyd *et al.* (1980) found that Sydney rock oysters survive for no longer than 1 week at 10°C in dry conditions. Pacific oysters (*Crassostrea gigas*) in dry storage survived for only 8 days at 11°C but for 13 days under a "curtain of ice". These results suggest that Sydney rock oysters and Pacific oysters, although they are intertidal species, have a shelf life similar to that of the subtidal flat oyster.

Bacterial Counts

Trends in the results from bacterial counts (Fig 5) differed from those of the mortality data. There was no distinct difference between bacterial counts in oysters exposed to the various storage conditions and the SPC limit (1 x 10^5 microorganisms per gram according to state public health legislation) was reached within about 10 days for each treatment. After 10 days, bacterial growth rate decreased and then rose again in a bimodal fashion. This pattern could be attributed to a succession of bacterial populations within the oysters (B. P. Keogh, formerly of CSIRO, division of food research, *pers. comm.*). Oysters from treatments A and B did not live long enough to reveal this pattern.

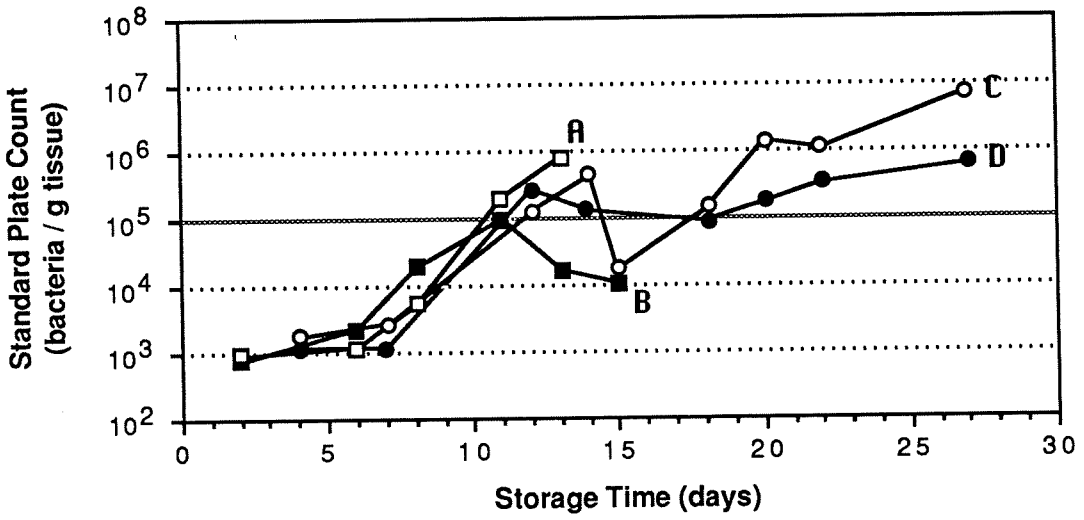


Figure 5. Changes in standard plate count during moist storage of live oysters at (A) 15.5°C, (B) 15.5°C and under pressure, (C) 1.5°C , and (D) 1.5°C under pressure.

Because no differences in bacterial counts could be detected in oysters exposed to different conditions and because bacterial growth was irregular, the SPC could not be relied on as an indicator of quality. For instance, after 15 days of storage, mortality in oysters exposed to treatment B was 90% but bacterial counts in the live oysters were within statutory requirements. Hence, the suitability of the SPC as a test for quality control of oysters is suspect.

PACKAGED OYSTERS

In this section we examined the effectiveness of two commercially available coolants, Thermosorb and Ice Brick, in maintaining chilled temperatures within packages. The effect of storage temperature on packaged oysters was also assessed and different methods of analysing oyster quality were compared.

MATERIALS AND METHODS

Experiment 1 - Preliminary Study

Temperature changes of water in plastic bags were used to assess the refrigerant capacity of the coolants. Three different weights of each coolant were tested to determine how cooling efficiency varied with weight.

A 15 L polythene bag full of water chilled to 3-5°C was placed in each of twenty-one 20 L polystyrene boxes. A temperature probe was inserted into every bag and frozen coolant was placed on top of 18 of the bags; three of the water bags were used as a control group. There were three replicates for each coolant type and weight (745 g, 1460 g, and 2240 g). The boxes were sealed and kept at ambient temperature on a laboratory bench for 72 hours. Temperature was recorded at 2 hourly intervals. For each set of replicates, the mean temperatures were plotted to illustrate the effects of coolant type and weight.

Experiment 2 - Live Oyster Study

This experiment was carried out during August and September when local oysters are in best condition (Hickman and O'Meley 1988). Clean live oysters (30 per box) prechilled to 3°C were layered between dampened jute in a polystyrene box lined with a thick plastic bag at the bottom of which was placed a pad of dry Thermosorb to absorb excess liquid. A bag of water (approximately 10 L) which had also been prechilled to 3°C was placed on top of the oysters. Frozen coolant (600 g) was placed on top of the bag. Each of the boxes was sealed and the lid taped down firmly. Each box was labelled with the treatment, storage time and date to be sampled. Temperatures within the boxes were recorded by a probe inside the bag of water. The packaging technique is shown diagramatically in Figure 6.

Four boxes were opened each sampling day (Table 2) and the oysters were assessed using the following forms of analysis:
-Percentage mortality;
-Bacterial counts (SPC and *Escherichia coli* count) and presence of *Salmonella*

spp (5 oysters per box);
-Chemical analysis (Total Reducing Substances [TRS] concentration) (5 oysters per box);
-Sensory analysis (appearance, odour, texture and flavour scores) (14 oysters per box).

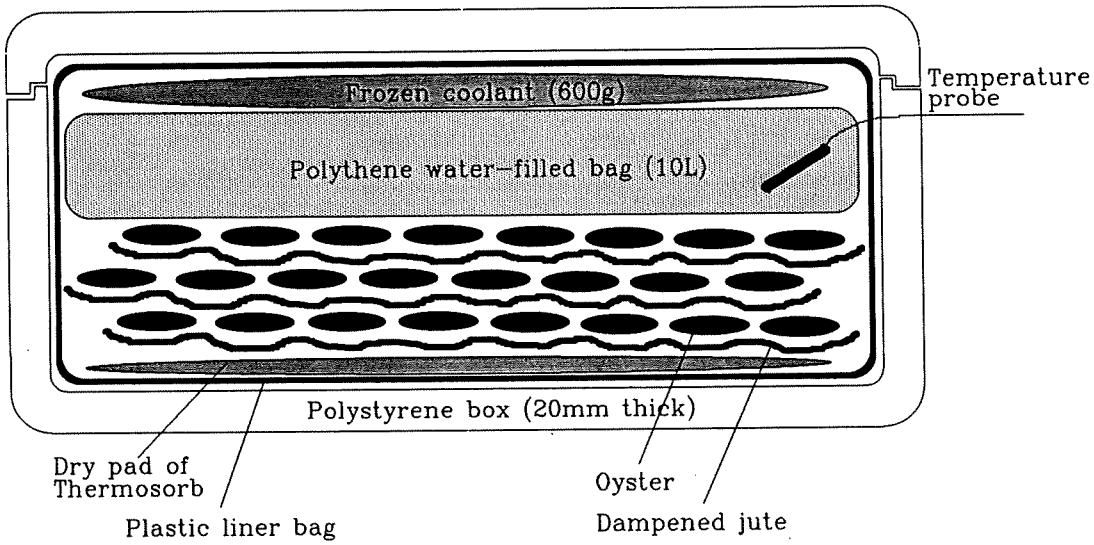


Figure 6. Diagram of experimental packaging unit.

Table 2. Schedule for sampling of packaged oysters stored under various conditions.

Storage conditions		Sampling day								
Temperature	Coolant									
Cool Room	Thermosorb	1	4	7	10	15	18	20	24	
(4.5°C)	Ice Brick	1	4	7	10	14	18	21	24	
	No Coolant	1	4	7	10	13	15	17	21	24
Ambient	Thermosorb	1	3	5	8	9	12	15	17	
(15.5°C)	Ice Brick	1	3	5	8	10	12	14	16	
	No Coolant	1	3	5	7	9	11			
Hot Room	Thermosorb	1	3	5	7	9				
(24.5°C)	Ice Brick	1	3	5	7	8	10			
	No Coolant	1	3	4	5	6	8			

Mean results from the assessments were plotted and, where possible, a limit was assigned which defined the point of rejection or spoilage. The time taken for each treatment to reach this limit was used as a measure of useful shelf life and was refered to as T_S. A two-way analysis of variance was applied to the T_S figures to determine whether the treatments had any significant effect.

Mortality

Dead oysters in each box were counted and removed so that only live oysters were used for quality assessment. The time taken for mortality to reach 10% was taken as a measure of useful shelf life (Ts).

Bacterial Counts

A random sample of five live oysters from each box was scrubbed and aseptically shucked. Meat from the five oysters was macerated into one sample which was analysed in accordance with the current Australian standard method described in AS1766: Methods for the Microbiological Examination of Food.

Chemical Analysis

Five live oysters selected at random were asceptically shucked. The meats were analysed for TRS concentration using the method described by Luizzo *et Al.* (1975). By this method TRS capable of reducing a solution of alkaline potassium permanganate are measured. TRS are metabolic by-products which accumulate during spoilage.

Sensory Analysis

Seven people (volunteers from staff at MSL) assessed the appearance, odour, texture and flavour of the test oysters using the seven-point Smiley scale (Street and Carroll 1972). At each sampling, each volunteer was given eight oysters (two from each of four boxes) placed at random in eight cells labelled 1-8, on a tray.

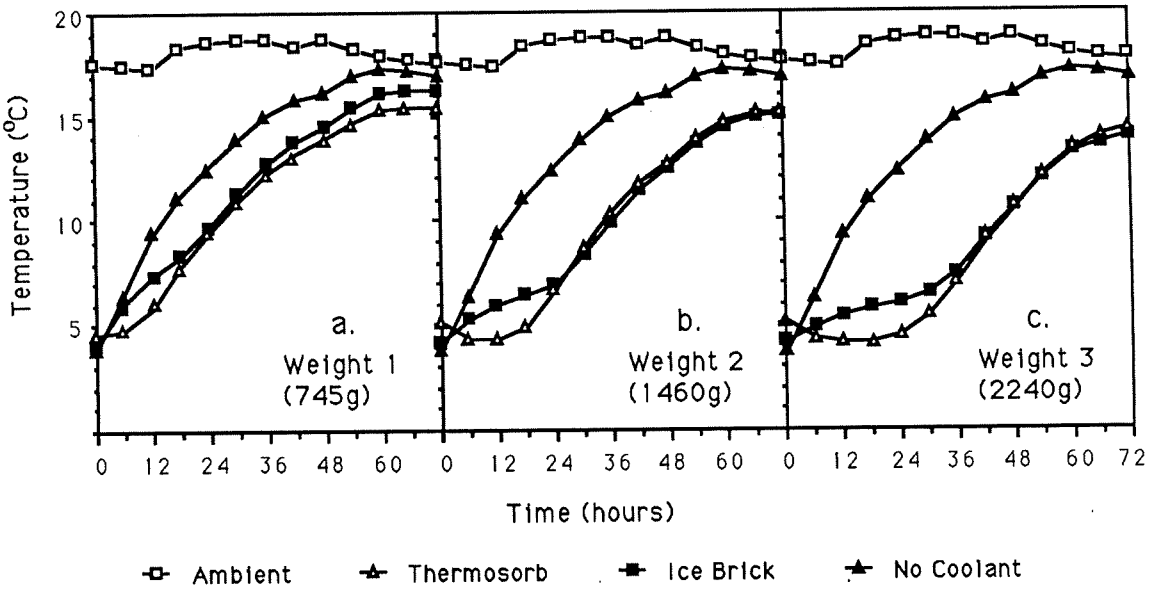
A score of seven indicated that the characteristic being assessed was excellent. A score of four was an indication that the characteristic being assessed was beginning to go "off". A score of one meant that the oyster was totally unacceptable. A mean from fourteen scores per treatment was calculated for each characteristic. Ts was taken as the last recording before the score fell below four.

If mortality was more than 10% on any sampling day the panelists would score the oysters for appearance and odour only. As a further precaution against food poisoning, if any of the oysters being assessed were suspected by either the shucker or the panelist of being spoiled, the oysters would again be scored for appearance and odour only. These safeguards were strictly enforced because one oyster can cause serious illness.

RESULTS AND DISCUSSION

Experiment 1 - Preliminary Study

The time-temperature curves (Figs 7a-c) reveal that Thermosorb kept down the temperature in an insulated box for longer than the same weight of Ice Brick but only for a length of time which depended on the weight of coolant used. Thermosorb cooled more effectively than the Ice Bricks for 18 hours with 745 g, 24 hours with 1460 g and 42 hours with 2240 g. Beyond these times both coolants behaved similarly and allowed the temperature within the boxes to reach 10°C after 27, 35 and 46 hours for the three respective weights. The temperature within the control boxes took only 13 hours to reach 10°C. Ambient temperature during the experiment was 17-19°C.



Figures 7a-c. Temperature changes within packages containing (a) 745 g, (b)1460 g, and (c) 2240 g of coolant.

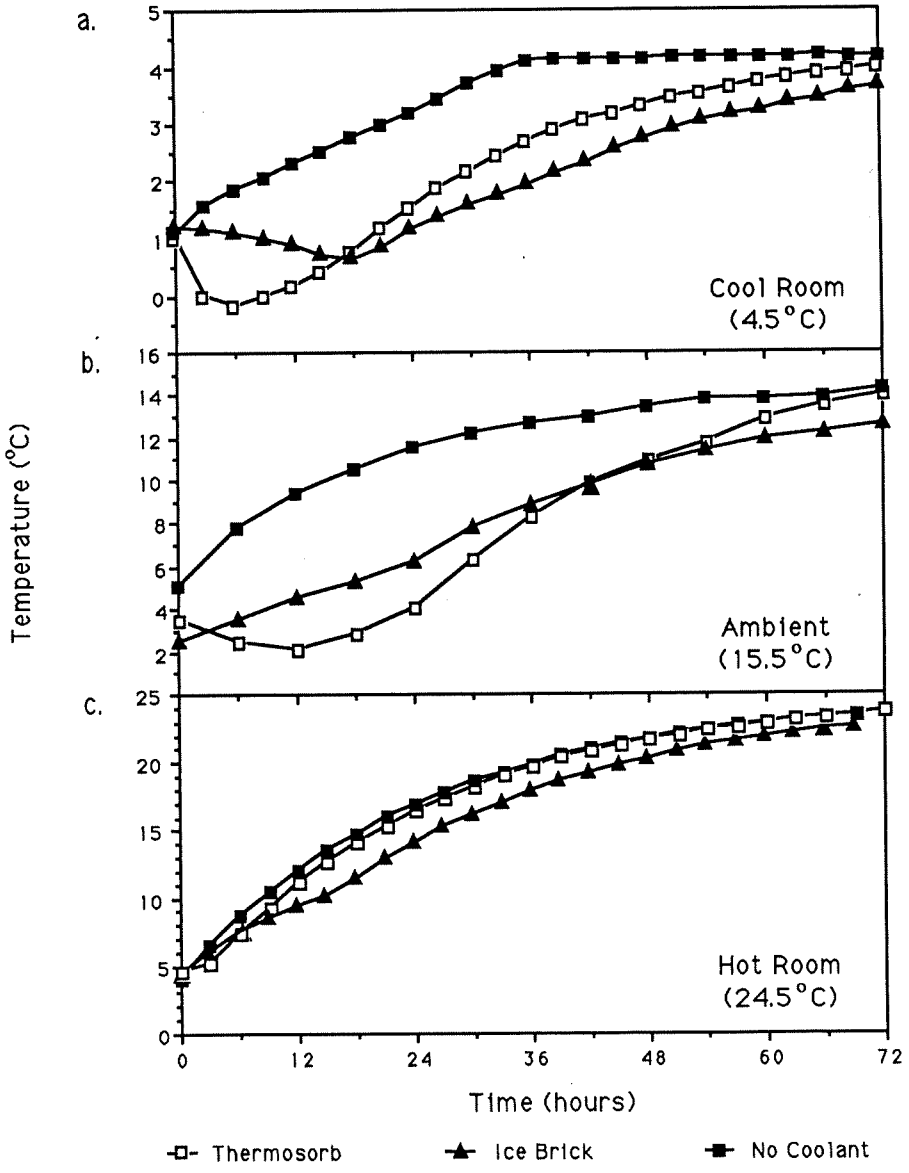
Therefore Thermosorb initially did provide more effective chilling than did the Ice Bricks but both kept the temperature below 10°C for the same length of time. However, Thermosorb was only 25% of the cost of the Ice Bricks and was therefore more cost effective.

The amount of coolant required to maintain chilled temperatures within a package would depend on the duration of unrefrigerated storage or transport, the ambient temperature at that time and the cost of displacing shellfish weight with coolant weight. For instance, at an ambient temperature of 18°C, at least 1.5 kg of coolant would be required to keep the temperature of a package below 10°C for 36 hours. In this experiment, the coolants contributed about 4, 7 and 11% of the package's total weight.

Experiment 2 - Live Oyster Study

Temperatures

The effectiveness of 600 g of coolant varied with temperature. At the highest of the three storage temperatures, the coolant was ineffective; each package warmed up at about the same rate (Fig 8c). At the lowest storage temperature both coolants were effective (Fig 8a) but little would be gained from using a coolant at such a low temperature. However, at an ambient temperature of 15.5°C, the effectiveness of the coolants was more pronounced (Fig 8b). After 18 hours at 15.5°C the temperature in the Thermosorb boxes was only 3°C while temperature in the boxes without coolant had risen to 10.5°C.



Figures 8a-c. Temperature changes within packages containing live oysters and stored at (a) 4.5°C, (b) 15.5°C, and (c) 24.5°C.
Note: Coolant weight = 600 g in each treatment.

At 15.5°C, the cooling capacity of Thermosorb surpassed that of the Ice Brick for the first 42 hours but when the ambient temperature was 18°C with 145 g more coolant (Fig 7a), this period was only 18 hours. Moreover, at 15.5°C, the boxes with coolants took 15 hours longer to reach 10°C than did boxes at 18°C ambient. Thus, at higher ambient temperatures, even slight increases in temperature can severely reduce the effectiveness of the coolants and the amount of coolant required to keep boxes chilled could become too expensive.

Results from the various analyses showed that the presence of coolant did not increase shelf life. Only the mortality rate data showed a slight distinction between coolants at 15.5°C (Fig 9) although Ts for each treatment was similar (between 5-6 days). Therefore, the capacity for 600 g of coolant to temporarily maintain chilled temperatures was of no clear advantage to oyster shelf life. However, the use of more coolant might have produced detectable differences between coolant treatments.

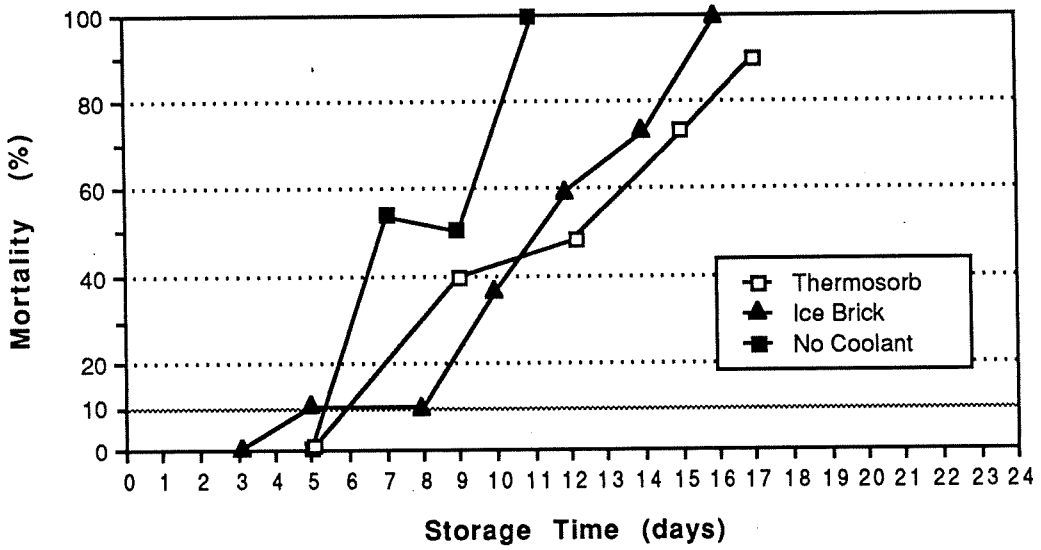


Figure 9. Changes in mortality of packaged oysters stored at 15.5°C with different coolants.

Mortality and Sensory Analyses

Because there was no effect of coolant, the results from one of the coolant treatments (Thermosorb) were used to compare the effects of storage temperature on quality. The mortality and sensory analysis data clearly showed the effect of storage temperature on quality. Mortality rate was much lower (Fig 10) and organoleptic quality was maintained for a much longer time (Figs 11a-d) at lower storage temperatures. Because of the precautions taken to avoid food poisoning amongst the taste panelists, the texture and flavour data (Figs 11c and d) are not as complete as the appearance and odour data (Figs 11a and b) but presumably the scores for texture and flavour were also unacceptable (ie, < 4) beyond the last recorded points.

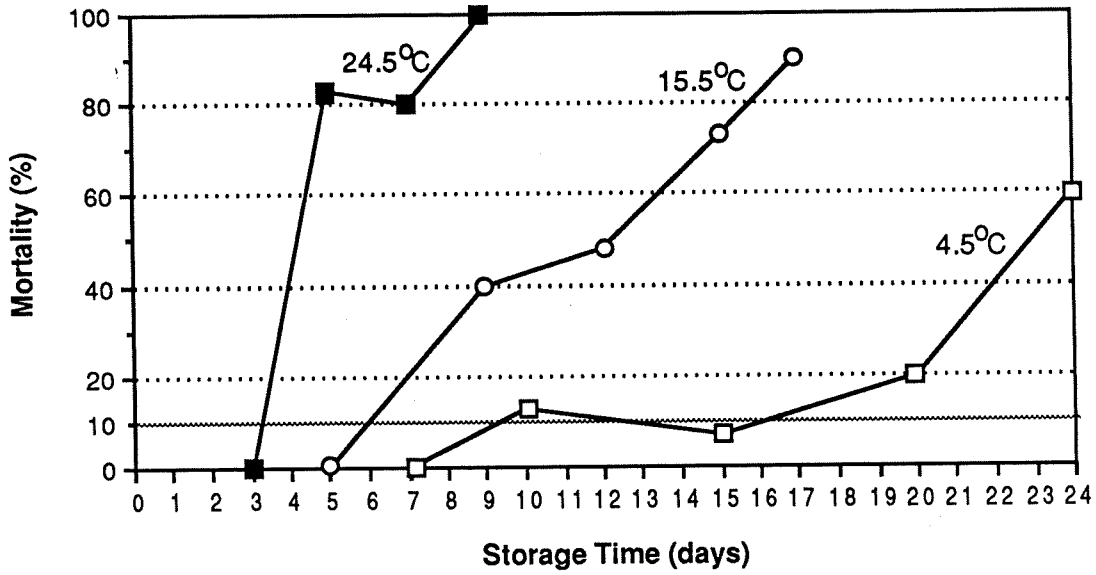
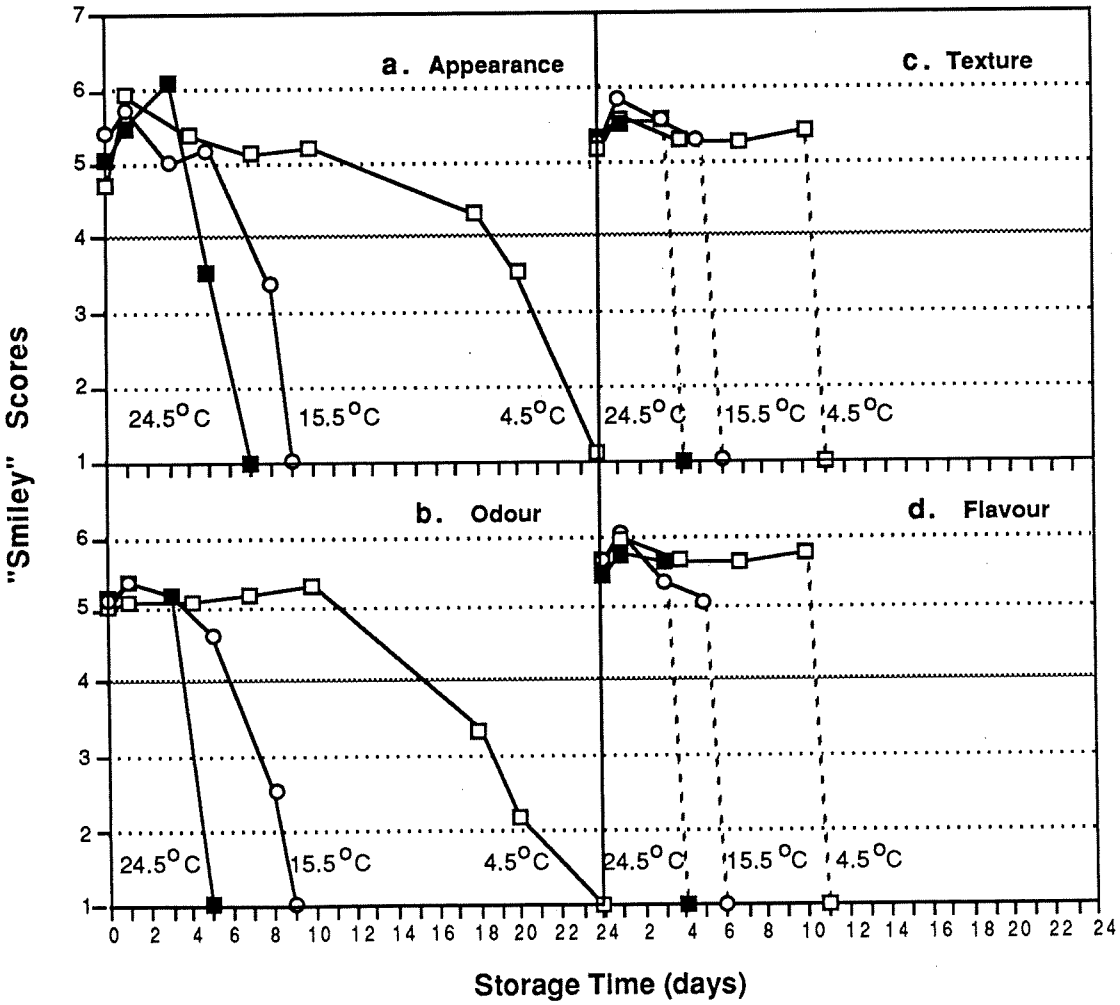


Figure 10. Changes in mortality of packaged oysters at different storage temperatures.



Figures 11a-d. Changes in (a) appearance, (b) odour, (c) texture, and (d) flavour of packaged live oysters at different storage temperatures.

Ts was calculated from the mortality and sensory data only (Table 3) because no other data showed any spoilage pattern. An analysis of variance (Table 3) revealed a significant difference in Ts at different temperatures. The level of significance was greater for odour ($P<0.001$) than for appearance or mortality ($P<0.01$), consequently odour was the most sensitive indicator of oyster quality. Results from assessments of texture and flavour, although being the same as for odour, are less reliable because spoilage was assumed and not recorded.

Table 3. Useful shelf life (Ts) of packaged live oysters stored under various conditions. Ts was determined by mortality and sensory analysis.

Storage conditions		Useful shelf life (days) assesed as				
Temperature	Coolant	% Mortality	Appearance	Odour	Texture	Flavour
Cool room (4.5°C)	Thermosorb	12.5	18.0	15.0	15.0	15.0
	Ice brick	15.0	14.0	14.0	14.0	14.0
	No coolant	18.5	17.0	17.0	17.0	17.0
Ambient (15.5°C)	Thermosorb	6.0	5.0	5.0	5.0	5.0
	Ice brick	6.5	8.0	5.0	5.0	5.0
	No coolant	5.0	7.0	5.0	5.0	5.0
Hot room (24.5°C)	Thermosorb	3.0	3.0	3.0	3.0	3.0
	Ice brick	3.0	5.0	3.0	3.0	3.0
	No coolant	3.0	6.0	4.0	4.0	4.0
P for Temperature		**	**	***	***	***
P for Coolant		NS	NS	NS	NS	NS

Key: ** = $P<0.01$ *** = $P<0.001$ NS = Not significant (ie, $P>0.5$)

According to the odour index, oysters can be safely kept in the described package for 2 weeks at 4.5°C before spoiling. Ts was reduced to 5 days at 15.5°C and 3 days at 24.5°C which highlights the importance of maintaining a cold chain from the time oysters are harvested until they reach the consumer.

Bacterial Counts

SPC (Fig 12) of bacteria in live oysters did not respond uniformly to storage temperature or time. There was no indication of significant spoilage except at the very end of the treatments when the counts were often very high. By this time only a few oysters had survived and they would have been moribund. There was also a possibility that survivors had been contaminated by the dead oysters

decomposing around them. The SPC data tended to follow a bimodal trend which may have been due to population succession within the bacterial flora of the oysters. Because of this irregular growth Ts figures could not be determined.

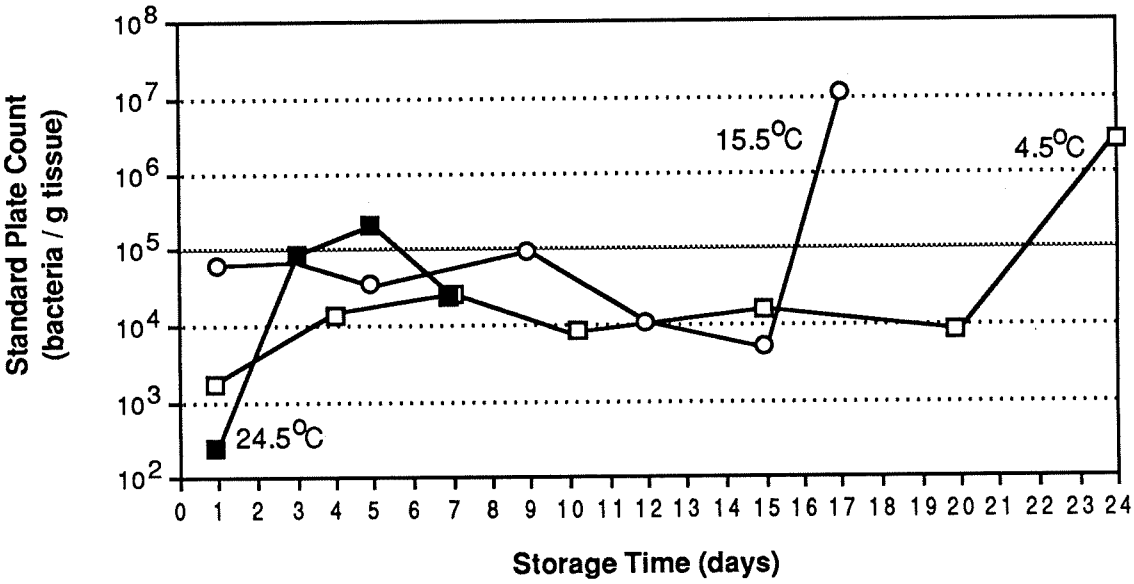


Figure 12. Changes in standard plate count of packaged live oysters at different storage temperatures.

Long after the taste panel had begun to detect "off" odours (Fig 11b), the SPC still indicated the oysters to be fit for consumption. Our results contradict those of Hoff *et Al.* (1967) and Boyd *et Al.* (1980), who reported that the SPC of harvested live shellfish changed in response to storage conditions and provided a good indication of freshness. For these reasons the SPC is currently used to determine whether or not shellfish are fit for export. Our study casts doubt on the reliability of the SPC as an index of quality. As Ayres (1975) suggested, the type of bacteria causing decomposition is probably more important than the number of bacteria.

Export regulations also require the number of *Escherichia coli* to be determined in oysters destined for overseas markets; the health limit for *E.coli* is 2.3 microorganisms per gram of tissue. In our study, the *E. coli* counts never exceeded 0.2 per gram and there was no indication that the number of *E. coli* varied in response to storage conditions. These results are consistent with the observations of Brooks and Harvey (1981) who showed that *E. coli* numbers in green mussels are not affected by post-harvest conditions. Our results therefore support the view that *E. coli* determinations are useful only as indicators of oyster quality at the time of harvest and imply that the oysters we examined were free from contamination when harvested.

None of the samples tested positive for *Salmonella* spp.

Chemical Analysis

No distinct trends were evident in the TRS data (Fig 12). TRS concentration did not seem to be an indicator of oyster quality despite a claim (Liuzzo *et Al.* 1975) that TRS concentration increased uniformly during the storage of live oysters and provided a reliable index of oyster quality. Our results may differ from those of Liuzzo *et. al.* (1975) because of methodology or the different species.

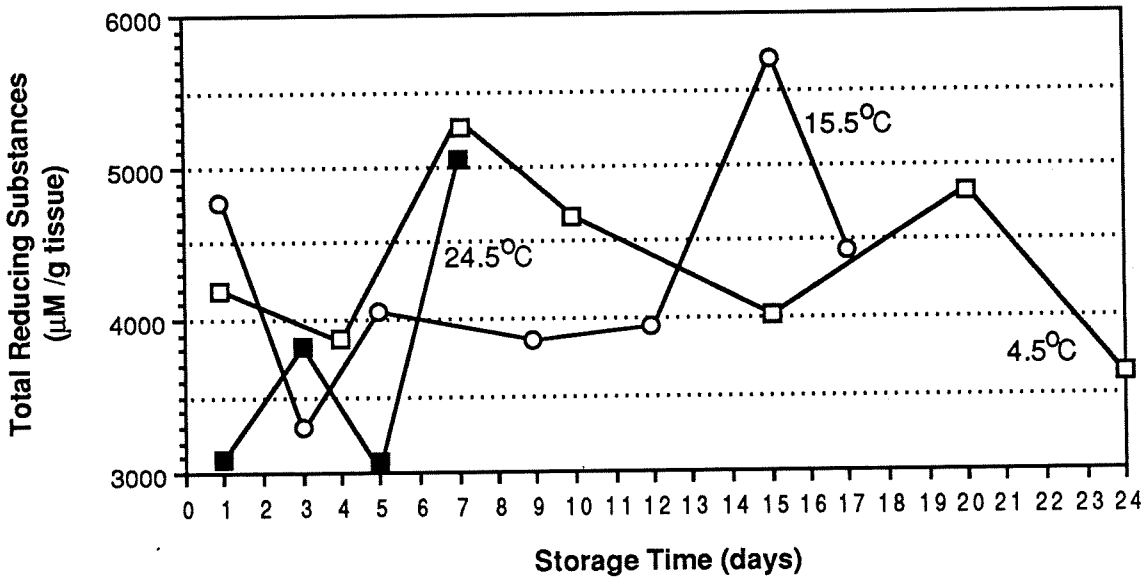


Figure 13. Changes in total reducing substances concentration of packaged live oysters at different storage temperatures.

CONCLUSIONS

These storage trials have revealed that flat oysters can be kept for three weeks under pre-packaged conditions and for two weeks in a package without spoiling. To achieve this degree of shelf life three key factors have been identified as being essential: continuous chilling; a moist storage environment; applying pressure to prevent oysters from gaping. All three factors influence the oysters' useful shelf life but the main emphasis should be on continuous chilling. A period of unchilled storage or transport will hasten spoilage and therefore reduce the amount of time available for the planning of product dispatch by farmers, processors and distributors.

Our results suggest that sensory analysis (especially odour) can be used as an index of the quality of flat oysters. The main disadvantage of sensory evaluation is its subjectivity; therefore, it should be used in conjunction with an objective index. Mortality is the most suitable objective index; neither the microbiological nor the chemical analyses had merit as indicators of quality. Justification for the use of mortality data in conjunction with sensory analysis is that Ts figures for both analyses were closely correlated.

The current acceptance of bacterial counts as the standard index of oyster quality needs to be questioned. Our results showed that bacterial counts did not increase uniformly with storage time or respond to temperature. This inconsistent growth, along with the inconvenience of waiting for results (approximately 3 days), detracts from the importance of describing spoilage of live oysters in terms of bacterial numbers. Although the TRS method was not a suitable indicator of quality, a quick and accurate chemical method for evaluating quality would be valuable and warrants further investigation.

Rapid instrumental methods of assessing shellfish quality and residual shelf life have been and are still being developed. These instruments include those used to measure impedance (Firstengerg-Eden and Eden 1985) and temperature function integration (McMeekin and Olley 1990). Impedance is the resistance to the flow of an alternating electrical current. As microorganisms multiply they change the chemical composition of the growth medium (eg., oyster tissue) which, in turn, alters the impedance of the medium and so provides an indirect measure of microbial growth. Impedance analyzers can be constructed in the laboratory or obtained commercially.

A temperature function integrator is a device for predicting shelf life on the basis of the temperature history of the product. A known relationship between spoilage rate and temperature is required and this is provided by a mathematical model which is programmed into the circuitry of the device. An added advantage with this technique is that the time of temperature abuse can be recorded to

implicate a particular stage during handling.

Either of these techniques could be applied to flat oysters and would be useful as a means of obtaining immediate information on the shelf life of a batch of live oysters.

RECOMMENDATIONS

Our recommendations are based on results and observations from this study and are designed to promote the highest possible standards to extend the shelf life of live flat oysters. If these standards are compromised, so is the time available for storage and transport.

General Storage Conditions

The most important factor to consider when storing flat oysters is the use of effective chilling to minimize the growth of decomposing and food poisoning bacteria.

Oysters should be chilled to just above 0°C (0.5-2.0°C) immediately after harvest by means of either a high humidity chiller or a "curtain of ice".

During storage or transportation chilling should be continued until the product reaches the consumer.

Oysters should also be kept moist and weighed down to prevent gaping. This practice extends the shelf life of the oysters to at least 2 weeks.

Potable water should be used in all cleaning, dampening or ice making.

During handling, mechanical shock should be kept to a minimum.

Pre-packaged Storage

Deterioration of oyster quality begins at the time of harvest. Therefore it is important to provide chilling facilities on board the harvest vessel if shelf life is to be maximised.

The "curtain of ice" should be used for chilling on board as it is the most cost-effective method. Crushed ice should be held on board the harvest vessel for this purpose.

Harvested oysters should be placed into drainable containers and covered with a porous material which prevents the oysters from coming into direct contact with

the ice but allows sufficient meltwater to run over them.

A thick layer of ice should then be placed on top of the covering and topped up when necessary. The ice layer should never be allowed to melt completely.

The oysters should be weighed down in some way to minimize gaping.

Meltwater should be allowed to drain through and away from the base of the container.

Oysters should be cleaned thoroughly and any fouling organisms removed.

Onshore, oysters should be stored in a high-humidity chiller or under a "curtain of ice".

Packaging

Oysters should be packaged and dispatched as soon as possible after harvesting because although chilling slows down spoilage it does not stop it altogether.

Oysters should be packed at a temperature not exceeding 3°C and so may require prechilling by dipping the oysters in an ice slurry for 2-3 seconds.

Oysters should be packed quickly so that they remain chilled.

Refrigeration should not be relied on to bring the temperature of packaged oysters down to chilled temperatures. The insulating effects of the packaging will retard cooling.

Polystyrene boxes are extemely effective containers for maintaining chilled conditions. Alternative containers which could be used include lined and wax-impregnated cardboard boxes or stackable double-walled plastic boxes which could be returned relatively cheaply to the processors.

More "high tech" packaging systems specifically developed for heat-sensitive seafood products are also available (Anon. 1989, 1990). Whatever the choice, the container must be sturdy, leakproof and thermally insulated.

An absorbant pad (Thermosorb is recommended) should be placed beneath the oysters to absorb excess liquid.

Oysters should be layered into the container. The material used to separate layers should be dampened to maintain high humidity within the package and should also provide cushioning to prevent shell damage.

The container should be filled to a point where the lid has to be forced down to shut the container. This will ensure that the oysters are packed under pressure. The lid should be lined with some type of compressible material (e.g. "bubble wrap") to stop the contents from moving.

The package should be thoroughly sealed and bound to prevent leakage and to maintain pressure.

Packaged containers awaiting despatch should be kept in cool storage.

Transport

Temperature histories during the distribution of live oysters will vary depending on the season and destination of the consignment.

The handling of containers should be scheduled so that the packages are not exposed to ambient temperatures for longer than is absolutely necessary.

For road transport, a refrigerated vehicle is necessary to maintain chilled temperatures within the packages.

During air freighting, temperatures down to 8°C can be maintained in the cargo hold while the plane is in flight but during loading, unloading and stop-overs, packages may be left unrefrigerated for long periods of time. Maintaining a cold chain is most difficult during air-freighting.

The addition of a coolant is recommended for packages that are to be air freighted. A coolant will slow down the rate at which the oysters warm up but the duration of the coolant's effectiveness will depend on the amount of coolant used and the external temperature.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the advice and assistance given by: R. Cowdell, S. Frlan, M. Mickelson, N. Dow, T. Walker, G. Arnott, D. Palmer, N. Coleman and D. Evans of the Marine Science Laboratories; the taste panelists, S. Palmer, D. Palmer, R. Barrow, K. Smith, J. Nicholson, B. Ponterfract and C. Grundy; A. Tan of the Melbourne University Microbiological Diagnostic Unit; T. Scheickowsky of the Royal Melbourne Institute of Technology and B. Keogh for microbiological advice. Thanks also go to the Fishing Industry Research and Development Committee for their financial support.

REFERENCES

Anon. (1989). Air packs can be reused. *Fish Farming Int.* 28 (7) : 45.

Anon. (1990). New radiant barrier packaging system. *Austasia Aquacult.* 4 (7) : 23.

Ayres, P.A. (1975). The quantitative bacteriology of some commercial bivalve shellfish entering British markets. *J. Hygiene.* 74 : 431-40.

Boyd, N.S. and Wilson, N.D.C. (1978). Handling and processing of raft-farmed mussels. Symposium on Fish Utilization Technology and Marketing in the Indo-Pacific Fishery Commission Region, Rome: Food and Agriculture Organisation of the United Nations. IPFC/78/SYMP/52.

Boyd, N. S., Wilson, N.D.C., and Hall, B.I. (1980). Storage of live pacific oysters out of water. *N. Z. J. Sci.* 23 : 171-76.

Brooks, J.D. and Harvie, R.E. (1981). Quality changes during storage of the green-lipped mussel, *Perna canaliculus*. *Food Technol. Aust.* 33 (10) : 490-95.

Firstenberg-Eden, R. and Eden, G. (1985). Impedence Microbiology. p.123. Research Studies Press Ltd., Letchworth, England.

Hickman, N.J. and O'Meley, C.M. (1988). Culture of Australian flat oysters (*Ostrea angasi*) in Victoria: final report. Department of Conservation, Forests and Lands; Mar. Sci. Lab. Internal Report No. 172.

Hoff, J.C., Beck, W.J., Erickson, T.H., Vasconcelos, G.J., and Presnell, M.W. (1967). Time-temperature effects on the bacteriological quality of stored shellfish. *J. Food Sci.* 32 : 121-24.

Liuzzo, J.A., Lagarde, S.C., Grodner, R.M., and Novak, A.F. (1975). A total reducing substances test for ascertaining oyster quality. *J. Food Sci.* 40 : 125-28.

McMeekin, T.A. and Olley, J. (1986). Predictive microbiology. *Food Technol. Aust.* 38 (8) : 331-34.

McMeekin, T.A. and Ross, M. (1990). Temperatures can spoil the fresh seafood market. *Aust. Fish.* 49 (2) : 34-5.

Monchinga, T. (1971). The TTC test for evaluating the freshness of shucked oysters. In; "Fish Inspection and Quality Control". (Ed.) R. Krenzer. 206-207. FAO.

Quadri, R.B. (1976). How to keep the "oomph" in stored oysters. *Aust. Fish.* 35 (12) : 19.

Quadri, R.B., Buckle, K.A., and Edwards, R.A. (1976). *Food Technol. Australia.* 28 (8) :283-87.

Stroud, G. D. (1981). Handling and processing oysters. *Torry Advisory Note.* No. 84.

Street, E. and Carroll, M.B. (1972). Preliminary evaluation of a new food product. In; "Statistics: A Guide to the Unknown". (Ed.) J.M. Tamur. p.220. Holden-Day Inc., San Fransisco.

Thrower, S. (1983). Curtain of ice extends mussels' life. *Fintas.* 6 (1) : 12-15.

Warwick, J.C. (1985). A Code of Practice for Mussel Processing. New Zealand Fishing Industry Board, Wellington.

Warwick, J.C. (1985). Handling and transport of New Zealand green mussels. *Infofish Marketing Digest.* No. 3 : 33-6.