Effect of storage on the quality of purified live Pacific and Sydney rock oysters

Effet du stockage sur la qualité des huîtres du Pacifique et de Sydney vivantes purifiées

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Abstract

In December, 1990, the NSW Minister for Agriculture and Rural Affairs removed the Pacific oyster (*Crassostrea gigas*) from the noxious fish list for the Port Stephens area, permitting it's cultivation. As Port Stephens Pacific oysters are grown intertidally, similar to Sydney rock oysters (*Saccostrea commercialis*), there was a belief that they may have different storage requirements to overseas Pacific oysters which are cultivated in deep water exclusively. Consequently, the keeping quality of this oyster and it's compliance with N.S.W. bacteriological regulations were examined. Port Stephens Pacific and Sydney rock oysters were purified, then stored at refrigeration and ambient temperatures. Levels of faecal coliforms, standard plate count and *Vibrio parahaemolyticus* were determined daily. Pacific oysters were found to require refrigeration to extend shelf life. At 5°C, Pacific oysters survived up to 14 days, whereas at ambient temperature (23°C) they started to gape and die after 4 to 8 days. The level of *V. parahaemolyticus* generally decreased with increasing storage time for Sydney rock oysters at 23°C and 5°C. No general decrease was observed with Pacific oysters, where 2.4×10^3 *V. parahaemolyticus*/g were detected after 14 days at 5°C.

Keywords: Oysters, *Crassostrea gigas, Saccostrea commercialis,* storage, *Vibrio parahaemolyticus, Escherichia coli,* faecal coliforms, purification.

Résumé

En décembre 1990, le ministère de l'Agriculture et des Affaires rurales de Nouvelle-Galles du Sud a rayé l'huître du Pacifique (*Crassostrea gigas*) de la liste des mollusques toxiques pour la zone de Port Stephens, autorisant ainsi son élevage. Les huîtres du Pacifique de Port Stephens étant élevées sur la zone intertidale, comme le sont les huîtres de Sydney (*Saccostrea commercialis*), on pensait que leurs conditions de conservation pouvaient différer des huîtres du Pacifique élevées à l'étranger exclusivement en eaux profondes.

Il a par conséquent été décidé d'étudier la qualité de conservation de cette huître et sa conformité à la réglementation bactériologique applicable en Nouvelle-Galles du Sud. Des huîtres du Pacifique de Port Stephens et des huîtres de Sydney ont été purifiées, puis stockées à température ambiante ainsi que sous réfrigération. Des analyses journalières furent effectuées pour déterminer les niveaux de coliformes fécaux, de *Vibrio parahaemo-*

lyticus, et un comptage sur plaque standard. Il a été constaté que les huîtres du Pacifique nécessitent une réfrigération pour prolonger leur durée de conservation. A 5 °C, les huîtres du Pacifique survivent jusqu'à 14 jours, alors qu'à température ambiante (23 °C), elles commencent à bailler et meurent au bout de 4 à 8 jours. Chez les huîtres de Sydney, le taux de *V. parahaemolyticus* diminue généralement à mesure que le temps de conservation augmente, à 23 °C et à 5 °C. Aucune diminution globale n'a été observée chez les huîtres du Pacifique dans lesquelles des grandeurs de 2.4×10^3 de *V. parahaemolyticus* ont été détectées après 14 jours à 5 °C.

Mots-clés : Huîtres, *Crassostrea gigas*, *Saccostrea commercialis*, conservation, *Vibrio parahaemolyticus*, *Escherichia coli*, coliformes fécaux, purification.

INTRODUCTION

Numerous species of oyster occur along the Australian coastline. The Sydney rock oyster (*Saccostrea commercialis*) accounts for the majority of oyster production, however farming of the Pacific or Japanese oyster (*Crassostrea gigas*) is gradually increasing.

Initially NSW opposed the introduction of Pacific oysters, as it was considered that if the species became well established it could seriously damage the local industry based on the Sydney rock oyster. In 1985, the Department of Agriculture declared the Pacific oyster a noxious fish, making the cultivation and presence of this oyster on leases an offence. However by 1990 the Pacific oyster had spread throughout Port Stephens, NSW (Holliday and Nell, 1990). Consequently in December 1990, the NSW Minister for Agriculture and Rural Affairs removed the Pacific oyster from the noxious fish list for Port Stephens thereby allowing oyster farmers to cultivate and sell Pacific oysters in this area.

Qadri *et al* (1976) found that Sydney rock oysters remain alive for longer than 3 weeks at 10°C, whereas trials performed on New Zealand Pacific Oysters (Boyd *et al*, 1980) found that their useful shelf-life was limited to 5.5 days at 11°C. There was a common belief held, however, that as Port Stephens Pacific oysters are grown intertidally similar to Sydney rock oysters they may have different storage requirements to overseas Pacific oysters which are cultivated in deep water only.

Hence this research project was commenced to address whether live Port Stephens Pacific oysters stored under either ambient conditions or under refrigeration, presented any likely problems such as generating bacterial levels in excess of regulatory standards. Both Port Stephens Pacific and Sydney rock oysters were therefore examined for the presence of faecal coliforms, *Escherichia coli*, standard plate count and *Vibrio parahaemolyticus*.

In NSW, microbiological standards for oysters are prescribed in the NSW Food Act 1989. It stipulates that the total content of the oyster's flesh and shell water shall not contain a faecal coliform density, determined by the most probable number (MPN) technique, of more than 230 MPN/100g or a plate count of more than 500,000 colony forming units (CFU)/g.

Although NSW regulations do not specify standards for *V. parahaemolyticus*, this organism has been shown to be responsible for incidents of food poisoning when present in large numbers (10^6 viable cells/g) in seafood. Also *V. parahaemolyticus* is a natural bacterial inhabitant of marine waters and

unlike bacteria introduced into estuaries as a result of pollution, is not completely eliminated during oyster purification. Hence its survival pattern in Pacific oysters during storage was also of interest.

Methods

Sampling and Storage

In contrast to studies in which high levels of bacteria were artificially inoculated into the shellfish, the shellfish examined in this study were collected from a range of environments, and the effects of storage were determined on the microorganisms present as a result of natural processes.

Undamaged oysters, both Sydney rock and Pacific, of marketable size were collected from commercial leases in Port Stephens in January, 1991. Oysters were purified for 36 hours in one of two commercial UV pool-type purification plants selected for use in this experiment. The plants were operated using a recirculation water system and had a parallel system or twin tanks where the different oyster species could be purified separately, under the same conditions.

Purified oyster samples for the storage trial were collected from each depuration tank at random. Samples of Pacific oysters were collected in lots of not less than 50 oysters and Sydney rock in lots of not less than 10 oysters. All samples were then transported in eskies to the NSW Health Department's Division of Analytical Laboratories for microbiological examination and subsequent storage trials. Oysters used in the storage trials were placed in covered plastic bins to prevent excessive drying, then stored under refrigeration (5°C) or at ambient temperature (23°C), until they were unfit for analysis. Microbiological testing of oyster samples was performed daily throughout storage.

Bacteriological Analyses

Pacific oysters were analysed in lots of five sample units comprising ten oysters each, whereas only one sample unit of ten Sydney rock oysters was analysed. Oysters were prepared for analysis according to AS1766.3.5 (SAA, 1983), and examined quantitatively for standard plate count, faecal coliforms, *E. coli* and *V. parahaemolyticus*. Standard plate count was performed according to AS1766.2.1.1 (SAA, 1976). Faecal coliforms and *E. coli* were enumerated using the MPN technique specified in AS1766.2.3 (SAA, 1987), where 3 tubes were inoculated. *V. parahaemolyticus* was enumerated using the MPN technique described by Eyles *et al* (1985).

Results

Storage life of oysters

Purified Pacific oysters stored at ambient temperature started to gape and die after 4 days storage, with well over 50% similarly affected after 8 days storage. Pacific oysters were not stored beyond 8 days at ambient temperature as they were deemed unfit for analysis. Sydney rock oysters remained sound throughout the 8 day storage period.

Storage of purified Pacific oysters at refrigeration temperatures allowed oysters to remain sound for 10 days. After 11 days storage 14% were found to be gaping or dead. The oysters were not stored beyond 15 days as more than 75% were gaping or dead by this time.

Microbiological quality

The standard plate counts obtained for both Pacific and Sydney rock oysters stored under refrigeration (5°C) and ambient (23°C) conditions are shown in figures 1 and 2. The faecal coliform density for Pacific oysters decreased from 0.9MPN/g before purification to non detectable (less than 0.3MPN/g) after purification. On storage of Pacific oysters, apart from two samples (levels: 0.9 and 0.4MPN/g), no faecal coliforms could be detected throughout the trial. Sydney rock oysters yielded similar results throughout storage. *E. coli* counts in both Pacific and Sydney rock oysters throughout storage under both temperature conditions were less than 0.3MPN/g in all samples except one (0.9 MPN *E. coli*/g).

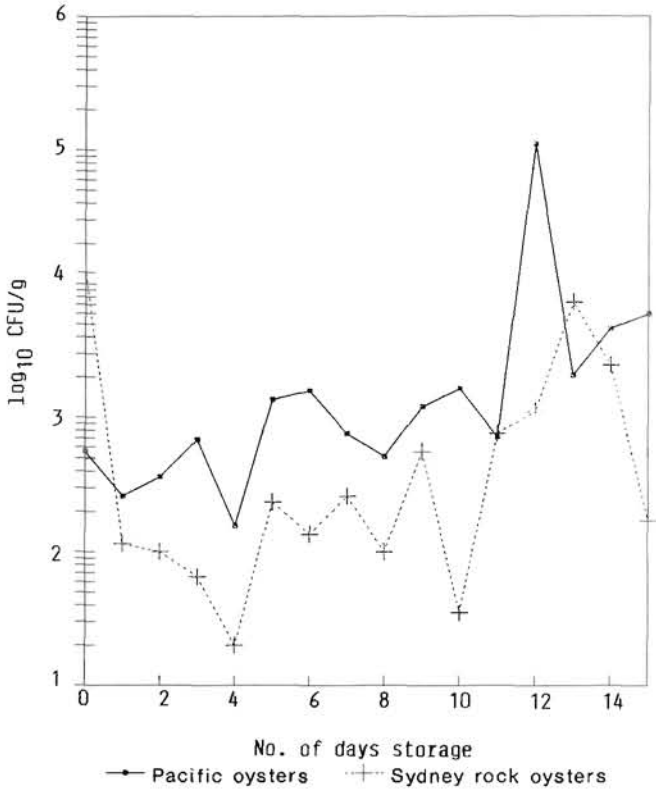

Figure 1: Standard plate count for oysters stored under refrigeration

The levels of *V. parahaemolyticus* detected in Pacific and Sydney rock oysters stored under both refrigeration and ambient conditions are shown in figures 3 and 4.

DISCUSSION

Both Pacific and Sydney rock oysters grown in Port Stephens were successfully purified using normal commercial purification procedures. Microbiological examination of the oysters after purification yielded results which complied with regulations contained in the NSW Food Act.

Storage trials using both species of oyster revealed marked differences in their shelflife requirements. Sydney rock oysters remained sound for a much longer period at ambient temperature than Pacific oysters. Refrigeration (5°C) extended the shelflife of Pacific oysters, however it reduced the storage life of Sydney rock oysters. Hence, different storage conditions are required for each species of oyster so they should not be mixed together during harvest. Pacific oysters should be transported and stored under refrigeration for maximum shelf life, while Sydney rock oysters should not be stored at temperatures below about 8°C.

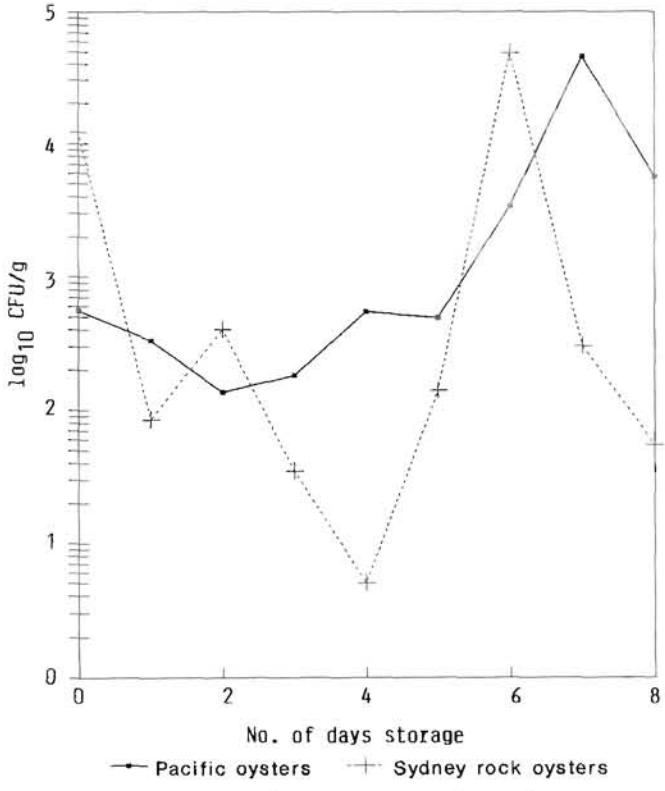

Figure 2: Standard plate count for oysters stored at ambient temperatures

Microbiological examination of Pacific and Sydney rock oysters throughout storage under both refrigeration and ambient temperatures yielded bacterial levels which complied with NSW Food Act regulations, for both the standard plate count and faecal coliform density. Furthermore standard plate count, faecal coliform and *E. coli* counts performed on the oysters throughout their shelflife remained fairly stable.

The survival pattern of *V. parahaemolyticus* differed between Pacific and Sydney rock oysters during storage (figures 3 and 4). Prior to storage the level of *V. parahaemolyticus* detected in the Pacific and Sydney rock oysters were similar, 1300 MPN/g and 1500 MPN/g respectively. The level of *V. parahaemolyticus* in Sydney rock oysters stored under both ambient and refrigeration conditions generally decreased with increasing storage time. In contrast the *V. parahaemolyticus* counts in Pacific oysters stored under ambient conditions ranged from 120 to 4,300 MPN/g during the first four days of storage and then levels greater than 11,000 MPN/g were detected after 5 to 7 days storage. Sydney rock oysters stored under refrigeration had levels of *V. parahaemolyticus* under 100 MPN/g after 2 days and remained at that level for the 15 day storage period. The level of *V. parahaemolyticus* in Pacific oysters stored under

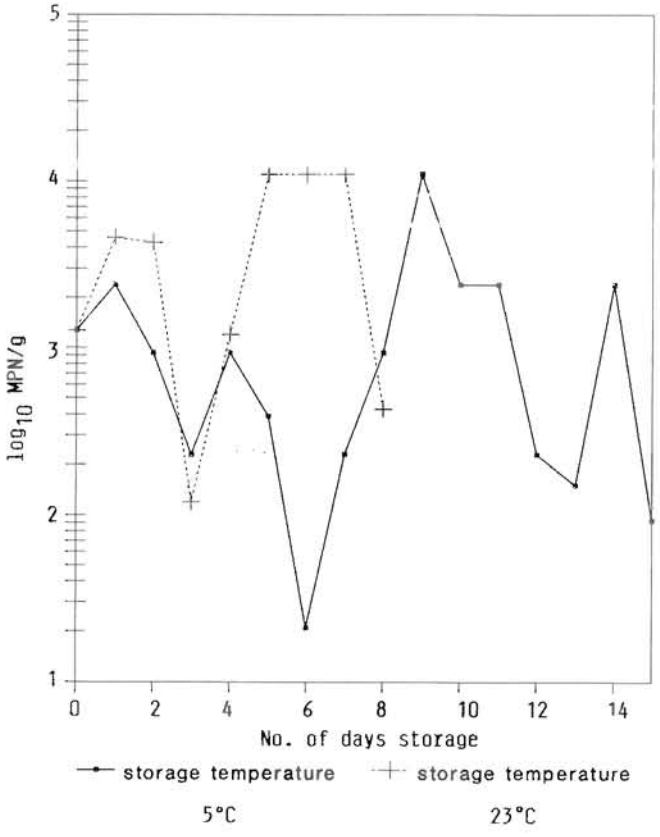

Figure 3: *Vibrio parahaemolyticus* counts in stored Pacific oysters

these conditions ranged from 21 to greater than 11,000 MPN/g, with levels under 100 MPN/g occurring only 15% of the time. *V. parahaemolyticus* was detected at a level of 2.4 x 10^3 MPN/g even after 14 days storage at refrigeration temperatures.

While *V. parahaemolyticus* counts in Pacific oysters were not excessive, Pacific oysters could pose a risk of *V. parahaemolyticus* food poisoning if harvested and sold in the summer period, when levels of *V. parahaemolyticus* are highest in marine waters. This risk would be considerably reduced however, if Pacific oysters are harvested during winter, the off-peak season for Sydney rock oysters.

Acknowledgements

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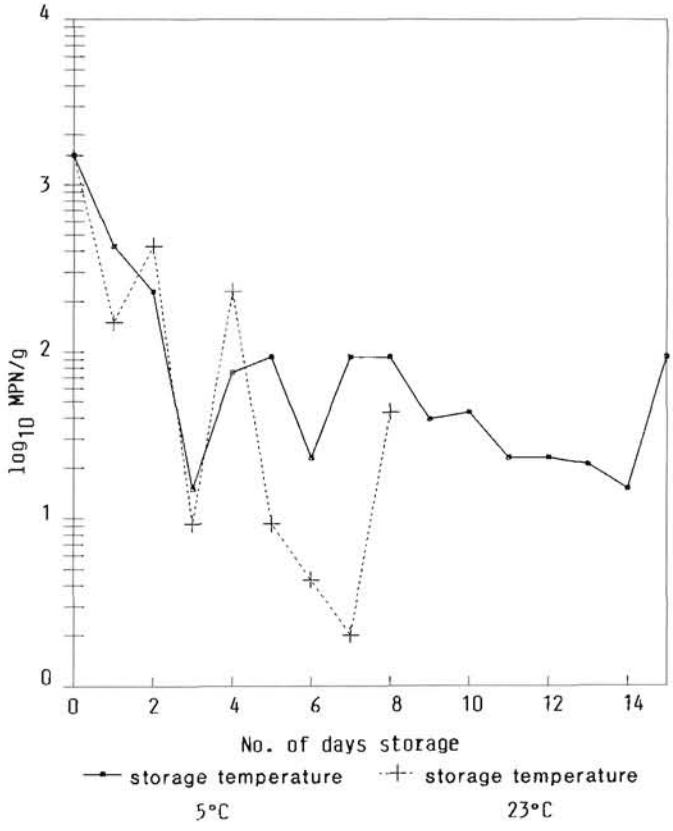

Figure 4: *Vibrio parahaemolyticus* counts in stored Sydney rock oysters

REFERENCES

Boyd N.S., N.D.C. Wilson, B.I. Hall, 1980. Storage of live Pacific oysters out of water. *N.Z.J. of Science*, **23**, 171-176.

Eyles M.J., G.R. Davey, G. Arnold, 1985. Behaviour and incidence of *Vibrio parahaemolyticus* in Sydney rock oysters (*Crassostrea commercialis*). *Int. J. Food Microbiol.*, **1**, 327-339.

Holliday J.E., J.A. Nell, 1990. Pacific oysters in New South Wales. Agfact, F2.1.3, 2nd ed, *NSW Agriculture and Fisheries, Sydney, NSW Aust.*, 4p.

N.S.W. Food Act, 1989. Food Standards Code D1(7).

Qadri R.B., K.A. Buckle, R.A. Edwards, 1976. Bacteriological changes during storage of live and shucked oysters. *Food Technol. Aust.*, **28**(8), 283-287.

Standards Association of Australia, 1976. Australian Standard 1766.2.1.1. Methods for the microbiological examination of food - Standard plate count.

Standards Association of Australia, 1983. Australian Standard 1766.3.5. Methods for the microbiological examination of food - Molluscs, crustaceans and fish, and products thereof.

Standards Association of Australia, 1987. Australian Standard 1766.2.3. Methods for the microbiological examination of food - coliforms and *Escherichia coli*.