# Identification of Microbial Hazards in Oysters in Australia



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**Important Notice**

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# Summary

1      A Hazard Identification (HI) for microbial hazards associated with oysters has been undertaken to help inform a planned research program to validate various storage regimes for live oysters.

2      Enteric viruses, *Vibrio parahaemolyticus* and *Vibrio vulnificus* are identified as hazards reasonably likely to occur in oysters.

3      Enteric viruses have been associated with many large outbreaks of oyster-borne disease; their prevalence and concentration in oysters is not influenced by storage regime.

4      Vibrios have been associated with a small number of illnesses related to oysters.

5      Since temperature may influence the growth of vibrios in live oysters, two storage regimes are considered worthy of research to redress knowledge gaps:

- Growth and survival of *V. parahaemolyticus* in Sydney rock oysters should be monitored according to the existing dispensation (oysters stored no warmer than 25 °C for 72 hours, then colder than 15 °C thereafter) and for the ASQAP requirement (oysters must be stored colder than 10 °C after 24 hours).

- Growth and survival of *V. parahaemolyticus* in Pacific oysters should be monitored according to the ASQAP requirement (oysters must be stored colder than 10 °C after 24 hours).

6      Since *V. vulnificus* has a narrower temperature growth range than *V. parahaemolyticus* its behaviour will be captured by the above research.

7      The requirement for exported live oysters to be held at a temperature below 5 °C is considered untenable since it leads to mortality of the product.

8      It is recommended that an expert panel to regularise export of live oysters in the overall context of export of live seafood be convened by Seafood Services Australia (SSA).

9      The research work could be undertaken via the new Australian Seafood CRC Program 2 – Product Quality and Integrity or SSA/SIDF, depending on the scope of the work.

# 1   Background

The Australian oyster industry faces a range of regulatory requirements for storage and transport of live oysters:

- The Australian Shellfish Quality Assurance Program (ASQAP) stipulates that oysters must be stored below 10 °C, 24 hours after harvest.

- The current AQIS Export Control (Fish and Fish Products) Orders 2005 indicate that live oysters should be stored below 5 °C unless alternative storage arrangements can be validated and shown to not affect fitness for human consumption.

- New South Wales regulations currently have a dispensation for storage below 25 °C for 72 hours, then colder than 15 °C thereafter.

These apparent anomalies between ASQAP and the Export Control Orders stimulated the submission of FRDC Application TM003: 'Microbiological validation of current storage and transport temperatures for Pacific oyster industries in Australia'. The application was approved conditional on wider industry involvement. Subsequently, the New South Wales industry identified the above dispensation and asked that it be considered within the proposal. A teleconference on Friday 23rd Feb with New South Wales, Tasmanian and South Australian industry representatives, NSW Food Authority and Seafood Services Australia considered a background paper canvassing the above issues; no representative of AQIS was available. The meeting determined that, as a prelude to deciding the scope of work designed to close information gaps on storage temperatures and times, a Hazard Identification be undertaken for Pacific oysters (*Crassostrea gigas*) and Sydney rock oysters (*Saccostrea glomerata*).

The objectives of the study are to:

1. Conduct a Hazard Identification to clarify the food safety risks that the myriad of regulations and interpretations seek to mitigate.

2. Use the Hazard Identification to inform the technical interpretation of existing requirements with AQIS and New South Wales, Tasmanian and South Australian regulators and seek agreement on scientifically justifiable critical limits and define data gaps for validation of the equivalence of alternative temperature regimes.

3. Detail the scope of any research required.

The findings of the study are presented in this report.

# 2   Scope of the Present Study

Hazard Identification is defined as: *The identification of biological, chemical and physical agents capable of causing adverse health effects and that may be present in a particular food or group of foods.*

It is an important aspect of both HACCP and risk assessment. HACCP Principle 1 involves listing potential hazards while Hazard Identification is the first of four stages in risk assessment for which, in effect, it represents a Go/No Go stage.

The aims of this investigation were to:

- Identify those microbiological hazards reasonably likely to occur in oyster harvest, storage and processing of Pacific and Sydney rock oysters.

- Document their involvement in outbreaks of illness for each species.

- Identify knowledge gaps which can be closed by research.

- Inform regulator and industry consultations.

# 3   Hazard Identification - Potential Pathogens of Oysters

In considering micro-organisms associated with seafoods, Huss *et al.* (2000) cite three primary sources of pathogens:

- Indigenous in the marine environment, eg *Clostridium botulinum* Type E, *Vibrio* spp, *Aeromonas*

- Indigenous in the general environment, eg *Listeria monocytogenes*, *Clostridium botulinum* Types A and B

- From the animal/mammal reservoir, eg *Salmonella*, *Shigella*, *E. coli* O157, *Staphylococcus*, Norovirus (NOV), Hepatitis A virus (HAV)

The above organisms will be assigned to one of two categories: not reasonably likely to occur and reasonably like to occur.

## 3.1  Pathogens not reasonably likely to occur in Australian oysters

### 3.1.1  Aeromonas

This Gram-negative bacterium occurs in fresh and estuarine waters. It is a pathogen for some aquatic animals and has been implicated in a relatively small number of incidents of foodborne illness (see Kirov 2003 for summary). Kirov (2003) cites three incidents in the USA and the UK in which oysters were suspected, though since faecal samples from patients were not examined in 2/3 of the incidents the evidence is not overwhelming.

There is no epidemiological evidence linking Aeromonas with foodborne illness in Australia. In the context of oyster harvest the organism is inhibited by salt levels above 2% which, given that oyster management systems prohibit harvest following rain events, precludes Aeromonas as a hazard reasonably likely to occur.

### 3.1.2  Clostridium botulinum

Its strict anaerobic nature precludes this pathogen as a hazard for all but canned and smoked, vacuum-packed oysters.

### 3.1.3  Enteric pathogens

*Salmonella*, *Shigella* and pathogenic *E. coli* are possible contaminants of oyster leases in estuaries affected by human or animal pollution. They are precluded as hazards reasonably likely to occur in oysters because of site selection, Australian Shellfish Quality Assurance Programs (ASQAPs) and by depuration. Enteric bacteria have never been implicated in any incident of illness in Australia involving oysters.

### 3.1.4  Staphylococcus

This organism is a common commensal of humans, inhabiting the nose, ears and skin. Staphylococcal food poisoning almost always involves contact with hands and cooked product coupled with temperature abuse. In harvest, processing and retailing of raw oysters, staphylococci are not a hazard reasonably likely to occur.

### *3.1.5 Listeria monocytogenes*

The pathogen is ubiquitous and has been found in association with a wide variety of fish, birds and domestic and wild mammals (FDA, 1999). It is commonly carried in the human gut with Iida *et al.* (1998) reporting carriage rates of 1.3% in healthy adults and Notermans *et al.* (1998), 5-10% in humans and domestic animals. It is often present on ready-to-eat (RTE) fish products such as smoked fish and other vacuum packed seafoods. It has the potential to grow on seafoods at refrigeration temperatures and, given some RTE seafoods have relatively long refrigerated shelf-lives, the organism has a competitive advantage.

In Australasia there have been two shellfish-related outbreaks of listeriosis. In Tasmania three healthy people aged 83, 37 and 10 years became ill with symptoms limited to the gastrointestinal tract (Misrachi *et al.*, 1991; Mitchell, 1991; Eyles, 1994). The illnesses, in 1991, followed consumption of New Zealand smoked mussels which had been illegally repackaged with use-by dates over three months beyond their original and had *L. monocytogenes* >$10^6$/g.

A second listeriosis involving smoked mussels occurred in New Zealand in 1992, when newborn twin babies died as a result of *Listeria* infection (Eyles, 1994). The mother had received medical advice to consume smoked mussels to increase her iron count, as she was somewhat anaemic (Andrews and Young, 1993). It is therefore likely that her consumption rate was unusually high.

There is no epidemiology linking *L. monocytogenes* with raw shellfish, in general, or raw oysters in particular, making the hazard unlikely to occur.

### *3.1.6 Vibrio cholerae*

Outbreaks of cholera have been associated with consumption of seafood including oysters, crabs and shrimp (Oliver & Kaper, 1997). The largest outbreak was a pandemic in South America in the early 1990s when *V. cholerae* O1 caused more than 400,000 cases and 4,000 deaths, mainly in Peru (Wolfe, 1992). Contaminated water used to prepare food, including the popular, lightly fermented fish *ceviche*, was the cause of the outbreak.

The epidemiology of foodborne cholera in Australia has been reviewed by Desmarchelier (2003). Cholera has long been recognised as a waterborne disease but foodborne transmission is increasingly recognised as important. In Australia, cholera infections are acquired both locally and overseas. Foods including lettuce irrigated with contaminated well water and cooked prawns cooled in river water have been implicated. Only non-toxigenic *V. cholerae* 01 have been isolated from brackish and estuarine waters and oysters in Australia to date, and no cholera cases have been associated with local oysters.

Based on the foregoing, *V. cholerae* is not considered a hazard likely to occur in oysters in Australia.

## 3.2 Pathogens reasonably likely to occur in oysters

### *3.2.1 Enteric viruses*

Two groups of viruses have caused numerous problems in oysters in Australia. One group are Noroviruses, formerly known as Norwalk or Norwalk-like viruses (NLV) and as Small, Round, Structured Viruses (SRSV); the other group is Hepatitis A virus (HAV). All outbreaks of viral illness associated with oysters have been associated with oysters harvested from NSW leases (Table 1); note estimates of those affected vary according to Kraa (1990a,b; 1995).

**Table 1: Selected outbreaks of viral illness linked with oyster consumption**

| Year | Origin of oysters | Agent | Cases | Reference |
|------|------------------|-------|-------|-----------|
| 1978 | Georges River | Norovirus | >2000 | Kraa 1990a,b; Kraa 1995 |
| 1989 | Georges River | Norovirus | 370-412 | Kraa 1990a,b; Kraa 1995 |
| 1990 | Georges River | Norovirus | 461-752[a] | Kraa 1990a,b; Kraa 1995 |
| 1996 | Terranora | Norovirus | 97 | Stafford *et al.* 1997; Dalton 1997 |
| 1997 | Wallis Lake | Hepatitis A virus | 467 | Conaty *et al.* 2000 |

[a] Includes 18 cases from Darwin (Ruben *et al.,* 1992)

Several norovirus outbreaks have been reported (Cross *et al.*, 1979; Kraa, 1990a,b; Bird and Kraa, 1995; Dalton, 1997; Grohmann, 1997; Linco and Grohmann, 1980: Murphy, *et al.*, 1979; Stafford *et al.*, 1997). The first problem occurred in 1977 when oysters harvested from Georges River in Sydney and frozen on the half-shell caused problems in the UK. Product conformed to the microbiological standard of <2.3 *E. coli*/g and no bacterial pathogens were isolated at levels which would cause gastroenteritis. The oysters had been harvested during rainfall events when the *E. coli* count had exceeded the standard, though freezing may have killed *E. coli* but not viruses. Oysters from Georges River were also implicated in an outbreak of gastroenteritis involving more than 2,000 consumers in 1978 and, though Norwalk-like viruses were isolated from patients, they were not isolated from oysters.

These incidents resulted in changes to oyster regulation in NSW with the mandating of depuration for all oysters before sale (Ayres, 1991). However, in 1984, norovirus was implicated in an outbreak of gastroenteritis in Tamworth, NSW (Kraa, 1990a) and also in 1996, when oysters harvested from estuarine waters in northern NSW and depurated were implicated in an outbreak of gastroenteritis involving 96 people. The virus was isolated from one sample of oysters but not from faecal samples.

The first case of HAV from shellfish in Australia was attributed to incompletely cooked mussels from contaminated waters in Victoria with 7 out of the 10 consumers who ate the mussels developing symptoms of Hepatitis A (Locarnini & Gust, 1978).

The largest outbreak of Hepatitis A in Australia occurred during 1996-97 following consumption of oysters from the Wallis Lake region of Australia (CDI, 1997) when almost 500 people were affected and one died. Oysters from the area tested positive for HAV (by PCR) and also for enterovirus and adenovirus, but not for norovirus. The *E. coli* level was <2.3/g and the oysters had passed through a depuration process. However, a coronial enquiry established several point sources of faecal contamination into growing areas and the question of bacteriological criteria for oysters was again questioned (Wilcox, 1999).

Viral hazards associated with consumption of seafood were the topic of a number of reviews; Fleet *et al.* (2000) and Lees (2000) presenting up-to-date reviews of viral contamination of Australian oysters.

In summary, the main conclusions of these reviews were that:

- Virus particles can remain detectable for several months under certain conditions in seawater and in food.

- Shellfish depuration techniques do not totally eliminate viral particles.

- Infectious doses are presumed to be low, ie 10-100 virus particles.

- Human enteric viruses do not replicate in seafood products so that time and temperature of storage/handling are not risk factors.

- Viruses are resistant to moderate heat and pH conditions.

### 3.2.2  Vibrios

Vibrios are Gram-negative, facultatively anaerobic rod-shaped bacteria. The genus contains twelve species that can cause foodborne illness (Table 2), most caused by *V. cholerae*, *V. parahaemolyticus* or *V. vulnificus* (Oliver & Kaper, 1997, Dalsgaard, 1998). Some species are associated with gastrointestinal illness (*V. parahaemolyticus*) while others cause non-intestinal illness, such as septicaemia (*V. vulnificus*).

### *Vibrio parahaemolyticus*

*Vibrio parahaemolyticus* is a marine micro-organism occurring in estuarine waters throughout the world. The organism was first identified as a foodborne pathogen in Japan in the 1950s (Fujino *et al.*, 1953). By the late 1960s and early 1970s, *V. parahaemolyticus* was recognized as a cause of diarrhoeal disease worldwide, although most common in Asia and the United States. A recent history of seafood consumption is quite a consistent aspect of *Vibrio* infection. Vibrios concentrate in the gut of filter-feeding molluscan shellfish such as oysters, clams and mussels where they multiply and cohere. Although thorough cooking destroys these organisms, oysters are often eaten raw and, at least in the United States, are the most common food associated with *Vibrio* infection (Hlady, 1997).

**Table 2: Vibrios which cause, or are associated with, human infections (after Dalsgaard, 1998)**

| | Occurrence in human clinical specimens[a] | |
| --- | --- | --- |
| | **Intestinal** | **Non-intestinal** |
| *V. cholerae* O1 | ++++ | + |
| *V. cholerae* non-O1 | ++ | ++ |
| *V. parahaemolyticus* | ++++ | + |
| *V. fluvialis* | ++ | - |
| *V. furnissii* | ++ | - |
| *V. hollisae* | ++ | - |
| *V. mimicus* | ++ | + |
| *V. metschnikovii* | + | + |
| *V. vulnificus* | + | +++ |
| *V. alginolyticus* | - | ++ |
| *V. carchariae* | - | + |
| *V. cincinnatiensis* | - | + |
| *V. damsela* | - | + |

[a] The symbols (+) refer to the relative frequency of each organism in clinical specimens and (-) refers to not found

During 1997 and 1998 there were more than 700 cases of illness due to *V. parahaemolyticus* in the United States, the majority of which were associated with the consumption of raw oysters. In two of the 1998 outbreaks a serotype of *V. parahaemolyticus,* O3:K6, previously reported only in Asia, emerged as a principal cause of illness for the first time. Subsequent studies on these strains have revealed their pandemic spread. It was suggested that warmer than usual water temperatures were responsible for the outbreaks.

Four cases involving *V. parahaemolyticus* and oyster consumption are recorded in Australia. The first two cases (one of whom died) occurred in 1992, with Sydney rock oysters the suspected food vehicle

(Kraa, 1995). A further two cases of gastrointestinal illness occurred in 2005 where temperature abuse at the retail level was implicated (Anon, 2005). Kraa (1995) notes that in the same period there were large outbreaks of *V. parahaemolyticus* in NSW, involving chilled, cooked prawns imported from Indonesia. In 1990 more than 100 people were affected and one died and, in 1992, two separate outbreaks involving more than 50 people occurred following consumption of cooked prawns received from the same wholesaler on the same day.

In summary, illness due to *V. parahaemolyticus* in seafood has occurred on several occasions in Australia but only four cases have involved oysters.

### *Vibrio vulnificus*

*Vibrio vulnificus* is a naturally occurring marine bacterium. While no major outbreaks of illness have been attributed to *V. vulnificus*, the bacterium is responsible for 95% of all seafood-related deaths in the United States (FDA, 1989) because of sporadic cases which also occur in many parts of the world. Illness is most often associated with the consumption of raw oysters by susceptible members of the population.

*V. vulnificus* is present in Australian waters (Myatt & Davis, 1989) and cases of wound sepsis have been reported (Maxwell *et al.*, 1991). A 1990 survey in NSW found 40% of oysters were contaminated with *V. vulnificus* (McAnulty, 1990). However, there is little published data on the levels of *V. vulnificus* in Australian seafoods or seawater. In Australia, four cases of *V. vulnificus* foodborne disease were reported in the period 1988-1992, all in NSW. All cases consumed oysters, were aged 50-74, suffered from chronic liver disease and presented with primary septicaemia; there were two deaths (McAnulty, 1990; Kraa, 1995). Brady and Concannon (1984) also reported an Australian case believed to be linked to consumption of raw oysters.

In summary, illness from *V. vulnificus* in Australia is often associated with people with impaired immune function who enter estuarine waters.

The following cases currently under investigation are typical: *Three people have died after contracting a rare flesh-eating disease in the Northern Territory: Two of them were tourists who became ill after fishing near Borroloola in the Gulf of Carpentaria and the third was a 19 year old local girl who had been swimming in a coastal tidal creek. All three had other health problems.*

*The disease, necrotizing fasciitis, can be caused by marine bacteria, which get in through cuts in the skin and into the deep tissues and blood. Survivors often have to have limbs amputated. Menzies School of Health Research professor Bart Currie announced an investigation into what caused the Vibrio bacteria to proliferate near Borroloola. The world's biggest lead and zinc mine is close to the township. Professor Currie said the mine and the infections were not linked.*

*The deaths occurred between July 2000 and October 2005. The NT Government's chief health officer Tarun Weeramanthri said flyers had been put up in Borroloola. 'We have to tell people what the risk is and what precautions they should take without giving a message that no one should go fishing or launch their boats and get their feet wet', he said. An article published in a medical journal linked high levels of zinc in the McArthur River and an increase in the flesh-eating bacteria.*

*The first case to be recorded in the Territory was in 1988, in Darwin.*

(downloaded at http://www.theaustralian.news.com.au/story/0,20867,21311721-5006790,00.html> (Ralph and Currie, 2007).

# 4    Occurrence of Pathogenic Vibrios in Australian Oyster-Growing Waters

In two surveys in Australia, Desmarchelier (1978) surveyed *V. parahaemolyticus* in oysters from eight sites. In the first survey, 41/60 (68%) samples were positive and in the second, 128/633 (20%) samples were positive for *V. parahaemolyticus*. The author noted a direct relationship between *V. parahaemolyticus* population and temperature (Table 3).

**Table 3: Numbers of *V. parahaemolyticus* in Sydney rock oysters**

| Temp (°C) | Log Vp/100g |
|-----------|-------------|
| <16       | Not detected |
| 16-20     | < 1         |
| 21-24     | 1-2         |
| 25        | 3           |

Davey *et al.* (1982) detected *V. parahaemolyticus* in three subsamples of undepurated oysters at a level of 4-6/g and in one subsample of depurated oysters at 0.8/g while Eyles *et al.* (1985) found 19/21 oyster meat samples were positive with a geometric mean MPN 7.3/g and range 0.3-50/g. The New South Wales Department of Health examined samples of Sydney rock oysters between April 1989 and April 1990 for *V. parahaemolyticus* and *V. vulnificus* (Table 4).

**Table 4: Prevalence of vibrios in Sydney rock oysters**

|       | *V. parahaemolyticus* | *V. vulnificus* |
|-------|-----------------------|-----------------|
| **Date** | **Number Positive/total** | **Number Positive/total** |
| **1989** | | |
| April | 8/13  | 0/11  |
| May   | 1/2   | 0/2   |
| June  | 3/4   | 2/3   |
| July  | 0/1   | 0/1   |
| Aug   | 4/5   | 1/5   |
| Sept  | -     | -     |
| Oct   | 3/3   | 0/3   |
| Nov   | 5/9   | 1/7   |
| Dec   | 11/12 | 0/11  |
| **1990** | | |
| Jan   | 10/11 | 0/11  |
| Feb   | 42/62 | 35/61 |
| Mar   | 12/19 | 1/19  |
| April | 31/44 | 35/44 |
| May   | 12/19 | 16/16 |

In the summer of 2001-02, Lewis *et al.* (2002) undertook a pilot study of prevalence of total and pathogenic *V. parahaemolyticus* from leases in NSW, SA and Tasmania (Table 5). The organism was isolated from 16/20 (80%) of oysters from NSW, 6/10 (60%) from Tasmania and 2/10 (20%) from SA.

**Table 5: Prevalence of total and pathogenic *V. parahaemolyticus* in Australian oysters**

|  | Total *V. parahaemolyticus* | | | Pathogenic *V. parahaemolyticus* | | |
|---|---|---|---|---|---|---|
|  | Positive /total | Mean log/g (SD) | Max count/g | Positive /total | Mean log/g (SD) | Max count/g |
| **NSW** | 16/20 | 2.4 (0.5) | 900 | 4/20 | 2.0 (0.4) | 350 |
| **Tasmania** | 6/10 | 2.5 (0.5) | 2000 | 2/10 | 2.4 (0.1) | 300 |
| **SA** | 2/10 | 3.0 (0.4) | 2000 | 1/10 | 2.3 | 200 |

Previous reports had not mentioned the presence of pathogenic strains of *V. parahaemolyticus* in Australian waters or in marine products (see review by Desmarchelier, 2003). In the pilot study of Lewis *et al.* (2002) pathogenic *V. parahaemolyticus* were isolated from oysters from all three states, with between 10% and 20% of samples having pathogenic strains (TDH[+]). In NSW, 4/20 samples from the Wallis Lake growing area were positive; in SA one sample from Denial Bay was positive and in Tasmania, samples from Dunalley and Little Norfolk Bay contained pathogenic strains. Pathogenic *V. parahaemolyticus* ranged between 50/g (the limit of detection) and 350/g.

The prevalence and levels of pathogenic *V. parahaemolyticus* established in the Lewis study were lower than those recorded in the USA where pathogenic strains were in the range 10,000-100,000/g compared with 100-1,000/g in Australian oysters. The study by Lewis *et al.* (2002), based on only 40 samples of oysters from three states, was not regarded as definitive in the quantitative sense and a longitudinal study over an annual cycle was recommended. However, the study did isolate pathogenic strains, a finding of qualitative importance, especially for those areas where water temperatures are high for several consecutive months.

A study was undertaken in the summer of 2006-7 when Madigan *et al.* (2007) investigated South Australian oysters for presence of pathogenic vibrios. In 25 samples, each of twelve oysters, *V. parahaemolyticus* was isolated from four while *V. vulnificus* was not detected in any sample. Of the four isolates of *V. parahaemolyticus* three were trh+ and none was tdh+. Interestingly, while sucrose-negative vibrios (a category which contains pathogenic strains) were relatively high ($10^3$-$10^4$/g) during warmer months, *V. parahaemolyticus* was isolated only after oyster samples were pre-enriched and molecular techniques were employed; when samples were enumerated the researchers considered pathogenic *V. parahaemolyticus* was present at below the limit of detection (<10/g) in oyster meat.

# 5 Effect of Temperature on Growth and Death of Vibrios

The growth ranges of *V. parahaemolyticus* and *V. vulnificus* are presented in Table 6.

**Table 6: Growth ranges of pathogenic vibrios (ICMSF, 1996)**

| Species | Temperature (°C) | Aw | NaCl (% w/w) |
|---|---|---|---|
| *V. parahaemolyticus* | 5 – 43 | 0.940 – 0.996 | 0.5 – 10 |
| *V. vulnificus* | 8 – 43 | 0.960 – 0.997 | 0.5 – 5.0 |

With respect to temperature, vibrios are typical mesophiles, have optima around 35 °C and lack the ability to grow at refrigeration temperatures. It should be emphasised that temperature minima are usually determined in favourable culture media and have long lag phases and generation times. Thus the temperature minimum of 5 °C quoted for *V. parahaemolyticus* should be balanced by the quotation that the organisms *dies when exposed to temperatures <5-7 °C; the rate of mortality is highest between 0 and 5 °C* (ICMSF 1996). As seen from Table 7, although *V. parahaemolyticus* is theoretically capable of growth at 5 and 8 °C, the length of lag phase and of generation time on oyster substrates are probably so long that death occurs.

**Table 7: Death of *V. parahaemolyticus* at 5 ° and 8 °C in oysters**

| Substrate | Temperature (°C) | Initial log count/g | Log kill/days | Reference |
|---|---|---|---|---|
| Live | 5 | 5 | 1.5/14 | Johnson & Liston (1973) |
| Shucked | 5 | 6 | 6/10 | Goatcher *et al.* (1974) |
| Live | 8 | 1.9 | 1.9/21 | Hood *et al.* (1983) |
| Shucked | 8 | 1 | 1/14 | Hood *et al.* (1983) |

Growth of *V. parahaemolyticus* at temperatures commonly used in harvest and storage of oysters in Australia are presented in Table 8, based on growth in Tryptic Soy Broth (TSB) with 2.5% sodium chloride (Jackson, 1974).

Son and Fleet (1980) reported that *V. parahaemolyticus* increased slightly after 4 days of storage and Eyles *et al.* (1985) reported that *V. parahaemolyticus* grew in unopened Sydney Rock oysters at 30 °C, but not to levels higher than $10^4$ cfu/g. Bird *et al.* (1995) observed that 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 $2.4 \times 10^3$/g were detected after 14 days at 5 °C. However, depurated Pacific oysters could not be stored under the conditions used for Sydney rock oysters, as many gaped after only 4 days when stored at 23 °C.

By contrast, significant increases in *V. parahaemolyticus* have been shown when the American oyster (*Crassostrea virginica*) is held at ambient (Gooch *et al.* 2002). After harvest *V. parahaemolyticus* multiplied rapidly in live oysters held at 26 °C, increasing by 1.7 log cfu/g after 10 hours and 2.9 log cfu/g after 24 hours.

**Table 8: Effect of temperature on generation time of *V. parahaemolyticus***

| Temperature (°C) | Generation time (min) |
|:---:|:---:|
| 18 | 60 |
| 20 | 50 |
| 22 | 42 |
| 24 | 39 |
| 26 | 32 |
| 28 | 29 |
| 30 | 25 |
| 32 | 22 |
| 34 | 20 |
| 36 | 19 |
| 38 | 19 |
| 40 | 22 |
| 42 | 29 |

There is considerable anecdotal evidence that Sydney rock oysters are extremely hardy and are often held out of water at ambient temperature for some days before release to the market. It is often stated that the Sydney rock oyster will remain alive for weeks at ambient without gaping or loss of sensory quality. While it is good handling practice to store this species at less than 20 °C as soon as possible in the warmer months (November-April), the Sydney rock oyster may (by regulation) be held up to 72 hours at 25 °C.

# 6 *V. parahaemolyticus* in Australian Oysters - Summary

Two recent studies by Lewis *et al.* (2002) and Madigan *et al.* (2007) have demonstrated the presence of pathogenic strains of *V. parahaemolyticus* on both Pacific and Sydney rock oysters, though at prevalence and concentration well below those found in USA oysters.

It may be that levels of the organism are influenced by growing methods. In the USA, oysters are harvested directly from the floor, whereas in Australia they are taken from racks well off the bottom. Since *V. parahaemolyticus* is associated with substrates this may account for the lower levels in Australia.

The practice of managing harvesting according to rainfall events, although intended for control of faecal pathogens, also has importance for controlling *V. parahaemolyticus* by not harvesting at salinities <23 ppt.

Finally, the Sydney rock oyster is a particularly hardy species which can remain alive out of water for at least two weeks at ambient temperature. It may be that this species, because it is alive, also can eliminate *V. parahaemolyticus* from its tissues and shell liquor.

Taken together, the foregoing account for the extremely low incidence of illness caused by ingesting *V. parahaemolyticus* or *V. vulnificus* in oysters.

# 7 Effect of Regulatory Temperature:Time Regimes on Hazard Control in Australian Oysters

The present hazard analysis has established that the Australian oyster industry has two microbiological hazard categories which are reasonably likely to occur:

- Enteric viruses of mammalian origin

- Vibrios indigenous to the aquatic habitat, in particular *V. parahaemolyticus* and, to a lesser extent, *V. vulnificus*.

## 7.1 Likely effect of existing regulatory regimes on microbial hazards in live oysters

Regulatory regimes and their likely effect on microbial hazards identified for each hazard are summarised in Table 9.

**Table 9: Regulatory regimes (current and proposed) and their likely impact on microbial hazards**

| Regulatory regime | Temperature /time | Hazard | | |
|---|---|---|---|---|
| | | **Enteric viruses** | *V. parahaemolyticus* | *V. vulnificus* |
| **Pacific oyster** | | | | |
| ASQAP | <10°C/24h | No effect | Growth unlikely | No growth |
| AQIS | <5°C | No effect | No growth | No growth |
| **Sydney rock oyster** | | | | |
| Current dispensation | 25°C/72h then <15°C | No effect | Growth possible | Growth possible |
| ASQAP | <10°C/24h | No effect | Growth unlikely | No growth |
| AQIS | <5°C | No effect | No growth | No growth |

### 7.1.1 Enteric viruses

In the case of enteric viruses, current regulations surrounding holding temperatures and times have no effect. Virus particles are incapable of growth outside host cells and their numbers will remain static in oysters, post-harvest.

### 7.1.2 Vibrio vulnificus

**Storage at 5 °C and 10 °C**

The organism has a temperature minimum in culture media at 8 °C and is unlikely to be capable of multiplication in live oysters at 10 °C because:

- oysters present a less favourable growth medium than selected culture broths

- long generation time at 10 °C

- long lag time (rule-of-thumb: lag is 5-times the generation time at the specified temperature).

Kaspar and Tamplin (1993) found that *V. vulnificus* died at 12 °C in sterile seawater adjusted to 10 ppt to mimic low salinity.

Storage at 5 °C, while it will have no effect on growth of *V. vulnificus*, will have a significant deleterious effect on sensory quality of both Pacific and Sydney rock oysters.

### Storage at 15 °C

It is likely the pathogen can grow slowly at this temperature.

### Storage at 25 °C

The pathogen will grow at this temperature.

### 7.1.3 Vibrio parahaemolyticus

Gooch *et al.* (2002) found that large increases in *V. parahaemolyticus* occurred when *C. virginica* was stored at 26 °C; a 790-fold increase after 24 hours and 630,000-fold increase after 48 hours

**Table 10: Count/g of *V. parahaemolyticus* in *C. virginica* at 26 °C**

| Time at 26°C (hours) | Starting level 1/g |
|---|---|
| 5 | 6 |
| 10 | 50 |
| 24 | 790 |
| 48 | 630,000 |

This storage temperature almost exactly matches that allowed under the current dispensation for Sydney rock oysters and would be expected to lead to large contamination levels of *V. parahaemolyticus*. Clearly, from the epidemiology in NSW, this does not happen since almost no cases linked with live oysters over the last two decades have been notified to authorities. It must be concluded, then, that the Sydney rock oyster, while it remains alive, prevents growth of *V. parahaemolyticus*.

### Storage at 5 °C

The organism has a temperature minimum in culture media at 5 °C, at which no growth will occur. Storage at 5 °C is likely to have a great commercial impact on sensory quality.

### Storage at 10 °C

The organism is likely to multiply very slowly in live oysters at 10 °C because:

- oysters present a less favourable growth medium than selected culture broths. Gooch *et al.* (2002) found that observed growth of *V. parahaemolyticus* in oysters was 4-times slower than that predicted by the model (Miles *et al*., 1997).

- generation time at 10 °C is probably around 7 h

- lag time is probably of the order of 30 h.

Given the foregoing it is theoretically possible that *V. parahaemolyticus* would grow at 10 °C over normal marketing and retailing times and this storage regime should be followed for both Sydney rock and Pacific oysters.

**Growth at 15 °C**

In oysters:

- generation time at 10 °C is probably around 4 h
- lag time is probably of the order of 20 h.

Thus growth is theoretically possible and this storage regime should be followed for Sydney rock oysters.

**Growth at 25°C**

In oysters:

- generation time at 10 °C is probably around 2 h
- lag time is probably of the order of 10 h.

Thus growth is theoretically possible and this storage regime should be followed for Sydney rock oysters.

# 8   Storage of Oysters at <5 ℃

The Export Control (Fish and Fish Products) Orders 2005 indicate that live oysters being exported should be stored at less than 5 °C unless alternative arrangements can be validated and shown to not affect fitness for human consumption. This position appears anomalous for several reasons:

- Storing oysters <5 °C will kill them, making them commercially unacceptable

- Other seafood products, eg prawns, are exported live without regulated temperature regimes

- AQIS is a signatory to ASQAP, which stipulates that live oysters be held at <10 °C after no more than 24 hours

- The AQIS document 'Validation and verification: a guideline to compliance with the Export Control (Fish and Fish Products) Orders 2005' contains the statement that live fish do not have a preservation step and therefore there is nothing to validate (Section 2.6).

It is recommended that Seafood Services Australia convene an expert panel to regularise the position of export live oysters within the overall context of live seafood export.

# 9    Elements of a Research and Development Program

The proposed R&D program should follow a number of storage regimes for both Pacific and Sydney rock oysters, as summarised in Table 11.

**Table 11: Storage regimes to be followed in the R&D project**

|  | <5 °C | <10 °C/24 h | 25 °C/72 h then <15 °C |
|---|---|---|---|
| Pacific | No | Yes | No |
| Sydney rock | No | Yes | Yes |

Storage at 5 °C has been excluded from the R&D project because it has no food safety impact compared with storage at <10 °C, but will have deleterious effects on sensory quality.

Storage regimes should be studied for *V. parahaemolyticus* only (since *V. vulnificus* has a narrower growth range).

Consideration should be given to a joint program involving the South Australian Research and Development Institute (SARDI) and the Australian Food Safety Centre of Excellence (AFSCoE). Each group has strengths which overlap to some extent with the other.

SARDI has:

- expertise in isolating and characterising vibrios from Pacific oysters
- close working ties with the SA oyster industry.

AFSCoE has:

- expertise in predictive microbiology, in general, and in predictive microbiology of *V. parahaemolyticus* in particular
- close working ties with the Tasmanian oyster industry.

**Funding**

Depending on the final scope and budget for this work, the CRC model may be a more appropriate funding mechanism than the existing 'capped' SSA/SIDF process.

The new Australian Seafood CRC Program 2 – Product Quality and Integrity provides an option for co-investment by sectors on 'common' issues.

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