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# FINAL REPORT

## Development and validation of reduced thermal processing requirements for canned abalone

FRDC Fisheries Research and Development Corporation

Abalone Council Australia Ltd

Abalone Association of Australasia Inc.

Government of South Australia, Department of Primary Industries and Regions

SARDI South Australian Research and Development Institute

ENDFOODWASTE COOPERATIVE RESEARCH CENTRE

Australian Government, Department of Industry, Science and Resources

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End Food Waste Cooperative Research Centre is funded through the Australian Government's CRC Program administered by the Department of Science, Industry and Resources.

## Panel Review Statement

End Food Waste CRC recognises the value of knowledge exchange and the importance of objective peer review. It is committed to encouraging and supporting its research teams in this regard. The author(s) confirm(s) that this document has been reviewed and approved by the Project Leader and Industry Partner.

This project has also been evaluated by the End Food Waste CRC publication review panel. These reviewers evaluated its:

- Methodology articulated clearly
- Positioning of findings within the current literature
- Acknowledged compliance with food safety standards
- Conclusions against results
- Relevant human and/or animal ethic approvals obtained



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## Industry Partner Foreword

Australian commercial abalone production is comprised of a combination of wild-caught and farmed products and sold in various formats including live, frozen (whole or meat only), canned (in metal, glass or plastic containers and pouches), in brine or sauce, and dried. Some markets prefer canned abalone due to convenience of storage because many small restaurants don't have refrigerated storage for frozen, affordability and extended shelf-life. During canning, abalone lose weight through a partial loss of the haemolymph (blood). The extent of loss is impacted by product handling and the thermal process used for commercial sterilising of the canned product. The current reduction in product yield (meat weight) during canning is typically between 12% and 30%. This project aimed to provide robust scientific evidence to allow the abalone industry to reduce the thermal process requirement of applying a 12D thermal process to the centre of piece of abalone meat, (the foot), for Australian/New Zealand wild-caught and farmed abalone whilst enabling the industry to continue to meet the regulatory requirements of providing a commercially sterile, safe and shelf-stable product. The project aimed to challenge the principle that abalone can be contaminated with pathogenic heat-resistant bacterial spores throughout the meat (or abalone foot).

A literature review confirmed that the haemolymph/blood of many invertebrates (including abalone) can contain a range of bacteria, however there was no evidence to either confirm or refute that abalone can be internally contaminated with *Clostridium botulinum,* (spores of which are considered the target or reference point for establishing thermal processing of all canned products). An abalone husbandry study exposed live abalone to high concentrations of *Clostridium sporogenes*. *Clostridium sporogenes* is a causative agent of food spoilage and is often used as the nontoxigenic surrogate for *Clostridium botulinum*. The study did not recover any anaerobic vegetative cells or anaerobic spores from the haemolymph, which is a promising finding. However, the abalone only consumed a low amount of feed during the trial which hampered the robustness of the findings. The potential for uptake of heat-resistant spores into the abalone foot was not determined and further research would be required.

The project experienced a series of delays, and the project team and industry stakeholders agreed to cease the project prior to completing all objectives; as such, there is no direct reduction in food waste from the outcomes of this project.

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Alex Ziolkowski, Mark Webster and Kane Williams

Abalone Association of Australasia

22 June 2026





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## Executive Summary

Abalone (*Haliotis* spp.) are a high-value seafood product harvested from wild and aquaculture fisheries. Australia is a global supplier of wild-caught and farmed abalone with current combined production levels of about 2,500 tonnes (live weight) per annum, worth AU$108M farmgate. Whilst the production of Australian canned abalone has decreased in volume over recent years, canning remains important for industry viability and market access. Canned abalone are sold on a drained-weight basis, and during the canning process, abalone lose weight through partial loss of the haemolymph (or blood). Yield losses of 12-30% are commonly reported and are influenced by handling practices and thermal processing severity. Thermal processes are designed to achieve commercial sterility whilst maintaining acceptable sensory qualities over long-term storage at ambient temperatures.

This project aimed to generate scientific evidence to determine whether the thermal processing requirements for Australian and New Zealand wild-caught and farmed abalone could be safely reduced whilst maintaining regulatory and market access compliance. The project aimed to challenge the assumption that pathogenic heat-resistant bacterial spores could be present throughout the meat (or abalone foot) and that the point of microbiological concern may not be located at the slowest heating point (geometric centre of the abalone).

A literature review was undertaken which confirmed that haemolymph/blood of many invertebrates (including abalone) can contain bacteria; however, there was no evidence in the literature that demonstrated internal contamination of abalone foot muscle with *Clostridium botulinum* spores. A dye penetration study on thawed Blacklip and Greenlip abalone showed slow migration of dye towards the centre of the foot, with faster penetration through the sinus structures of abalone.

An experimental approach was also developed and independently reviewed to investigate uptake of heat-resistant bacterial spores by live abalone. In a subsequent husbandry study, Blacklip abalone were exposed to *Clostridium sporogenes* spores and vegetative cells through a feed and emersion challenge study. *Clostridium sporogenes* is a causative agent of food spoilage and is often used in thermal process validation studies as the nontoxigenic surrogate for *Clostridium botulinum*. The abalone consumed a low amount of feed during the trial. No anaerobic vegetative cells or anaerobic spores were recovered from the abalone haemolymph post exposure. Variable levels of anaerobic vegetative cells and anaerobic spores were recovered from the visceral mass. Additional microbiological profiling was not undertaken to confirm if there were any other types of bacteria in the haemolymph or abalone foot. The potential for uptake and distribution of heat-resistant bacterial spores into the abalone foot was not determined and remains a key knowledge gap. Further research would be required, however, the lack of detectable heat-resistant spores in the haemolymph is not an evidence of absence.

Given the inconclusive findings from the feed challenge study, coupled with the existing safe-harbour approach, and that any absence of evidence is not evidence of absence, the project team and industry stakeholders decided to cease the project before completion of all stated objectives. Therefore, reductions in thermal processing for canned abalone were not achieved. Nevertheless, the project has established national capability in anaerobic *C. sporogenes* spore production and analysis, strengthened thermal processing expertise, and developed collaborative partnerships between researchers and the abalone industry, providing a foundation for future evidence-based optimisation of canned abalone processing.



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## Glossary

| | |
|---|---|
| Canned food | A food that has been packed in a container which has been hermetically sealed and heated to achieve commercial sterility. |
| *Clostridium botulinum* | A group of gram-positive, motile, obligate anaerobic, rod-shaped bacteria that produces heat-resistant endospores. It can produce a highly potent neurotoxin that causes botulism and is the primary target with respect to food safety when producing shelf-stable low-acid canned foods. |
| Commercially sterile | A condition achieved by application of heat to render the product free of viable microorganisms including spores of public health significance and microorganisms capable of growing in the product under normal non-refrigerated conditions of distribution and storage. |
| Haemolymph | The circulatory fluid in molluscs, arthropods and other organisms where the fluid is not confined to blood vessels. |
| Low-acid canned foods | Any food product in which any one or more components had a pH value greater than 4.6 and a water activity greater that 0.85 at the end of the thermal process. |
| Pathogenic microorganism | A microorganism capable of causing disease in its host. |
| Retort | A pressure vessel used in the food manufacturing industry to commercially sterilise food after it has been placed in a container and the container has been hermetically sealed. |
| Scheduled thermal process | The heat process chosen by the processor, validated for a given product and container size, to achieve commercial sterility. |
| Slowest heating point | A specific location within a container that receives the lowest amount of heat energy during a heat treatment process. |
| Spoilage microorganism | A microorganism that can produce changes in food products that impact on the composition and sensory characteristics. |
| Endospore (spore) | Resistant state of bacteria to which the vitality of the vegetative cell is transferred to the spore. During endospore formation, the spore forms within the mother cell prior to release. The resultant spore increases the organism's capacity to survive in unfavourable environments. *Clostridium botulinum* spores are the most heat resistant from all pathogenic spore formers. |
| Vegetative cell | A food-consuming, waste-eliminating and reproducing form of bacteria. |



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# 1. Introduction

Abalone are large marine gastropods in the family *Haliotidae* and are considered a delicacy, especially in Asian countries. Australian wild-caught and farmed abalone production is currently about 2,500 tonnes (live weight) per annum, worth AU $108M (ABARES 2025). Most of Australia's abalone is exported to Asian countries in live, frozen or canned/pouch formats (ABARES 2020). Canning is a process whereby food is packed into a hermetically sealed container and subjected to a thermal process for the purpose of extending the useful shelf-life of the food. In 2019, approximately 40% of Australia's abalone production volume was retorted/canned yielding 370 tonnes per annum of drained meat weight, worth AU $71.9M (M. Webster and A. Ziolkowski, personal communication, 15 March 2019). However, this volume is reducing, principally due to abalone fishing quota restrictions, and is placing greater financial pressures on the industry. Whilst there are thirteen species of abalone found in Australian waters, only four are commercially harvested. These are the Blacklip abalone (*Haliotis rubra rubra*), Greenlip abalone (*Haliotis laevigata*), Brownlip abalone (*Haliotis rubra conicopora*) and Roe's abalone (*Haliotis roei*). In addition, *H. laevigata* and the Tiger abalone (a hybrid of *H. laevigata* and *H. rubra rubra*) are farmed in Australia. New Zealand's abalone species, Paua (*Haliotis iris*), is also imported into Australia to a limited extent for canning. Blacklip abalone is the species that is most frequently canned in Australia.

Canned abalone are sold on a drained weight basis. During thermal processing operations abalone meat lose some of their haemolymph which reduces product yields (Young and Olley 1974, Warne and Brown 1982). The extent of haemolymph loss is chiefly governed by the severity of the scheduled thermal process (Young and Olley 1974, Warne and Brown 1982). Product losses through the reduction in yield (meat weight) during canning operations are between 12-30% (Warne and Brown 1982). Some canned abalone are also marketed as 'fully-bled'. It was anticipated that reducing the thermal processing requirements may reduce abalone processing/haemolymph losses during the canning operating by at least 1-2% (by weight). Less abalone would be required per retorted can/pouch for the same drained weight. On current canning production levels this would equate to an increased annual export tonnage of at least 3.4-6.8 tonnes, worth approximately AUD $0.7-$1.4M per annum.

There is a general regulatory requirement that food businesses must take all practicable measures to supply safe and suitable foods. Management of microbiological food safety should be based on wholesome raw materials and sound design, and implementation of appropriate processes and procedures. For canning foods, the thermal process must be sufficient to achieve commercial sterility. The generalised minimum thermal lethality requirement is 121°C for 3 minutes (or equivalent). Whilst final product testing can be considered as a control measure, it generally provides limited information on the safety of the food (Zwietering *et al.* 2016). For instance, if a pathogenic organism is detected in a product, it can be meaningful; whereas a lack of detection, does not mean the pathogen is absent. Without a clear and well-established hurdle, proving that *Clostridium* spp. cannot transfer into the meat/tissue of abalone under all situations, will be challenging.

## 1.1 Objectives

The aim of this research was to provide robust scientific evidence to allow the abalone industry to reduce the thermal processing requirements for Australian and New Zealand wild-caught and farmed abalone whilst enabling the industry to continue to meet the regulatory requirements. In order to achieve this objective, the project aimed to challenge the principle that abalone can be contaminated with pathogenic heat-resistant bacterial spores throughout the meat (or abalone foot) and that the point of microbiological concern may be closer to the edge of the abalone foot and not at the slowest heating point (geometric centre of the abalone).





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# 2. Methodology

## 2.1 Literature reviews

The published scientific and grey literature[1] was reviewed to provide background information on thermal processing of foods (including how it has evolved and manufacturer's requirements), the importance of inactivating *C. botulinum*, potential for microbiological contamination in abalone through understanding abalone physiology, post-harvest handling processes and documented bacterial internalisation in abalone and extent of bacterial migration. Further details on the methodology of these reviews are included in Appendix B, Appendix C, Appendix D and Appendix E, respectively.

## 2.2 Dye penetration in abalone meat

### *2.2.1 Preparation of abalone meat*

Commercially farmed Greenlip abalone (and wild-caught Blacklip abalone meat was sourced from Western Abalone (Port Lincoln, SA, Australia) and had previously been shucked and individually quick frozen. The meat was partially thawed overnight in a commercial coolroom before being manually scrubbed with a Nylon brush to remove any external mucus, pigmentation, debris left after shucking and evisceration. Some of the whole cleaned abalone meat was cut in half to expose the internal structure. The cleaned whole and half abalone meats were dipped in a 1% solution of Red Pillar Box Powder 30% dye (Australian Food Ingredient Suppliers) for 1 min before being allowed to drain. The dyed abalone meat (Blacklip abalone: 1x whole and 2x half pieces; Greenlip abalone: 3x whole and 6x half pieces) were placed into tinplate cans, filled with a commercial brine solution and sealed. Cans were retorted under a commercial process and stored under ambient conditions or chilled (non-retorted) and kept refrigerated (4°C). The lids of the chilled cans were pierced in order to prevent the formation of an anaerobic environment.

### *2.2.2 Assessment of dye penetration in processed abalone meat*

Retorted samples were assessed on weeks 1, 4, 18 and 26 (post-processing), whilst chilled samples were assessed on days 1, 3, 5 and 7. After the required storage interval the cans were opened, liquor drained, and meat sliced transversely at 2-3 mm intervals along the length of each animal. The cut surfaces were physically examined for the depth of dye penetration measured in approximately 1 mm unit intervals and photographed.

## 2.3 *Clostridium* internalisation in abalone – A feed and emersion challenge study

Abalone can become infected with a range of bacteria, fungi, viruses and protozoans that can cause clinical disease (Handlinger *et al.* 2006, Gavine *et al.* 2009, Day *et al.* 2010, Georgiades *et al.* 2016). However, the results from the literature search (see Appendix D) did not reveal any previous study that investigated or recovered *C. botulinum* from abalone (of any species). Only one article, Dixon *et al.* (1991), reported association of *Clostridium* with abalone. This was an isolation of *C. lituseburense* from tissue samples of diseased abalone which might have been a result of environmental contamination and proliferation of the bacteria under anaerobic conditions within necrotic tissue.

The objective of the current feed and emersion challenge study was to expose live Blacklip abalone to *C. sporogenes* (a surrogate to *C. botulinum* for thermal processing studies) for two weeks before sampling the haemolymph and viscera for the presence of anaerobic vegetative cells and anaerobic spores. The media used for the detection or enumeration of anaerobic bacteria was non-selective and it was assumed that any anaerobic bacteria in the haemolymph or visceral mass would be culturable. The findings intended to provide new data on the potential for *Clostridium* to transfer into and circulate within the haemolymph of abalone and may lead to the identification of a site of microbiological concern for thermal processing, which is not located at the slowest heating point.

[1] Grey literature: is information produced by businesses, organisations, governments and academics outside of traditional commercial publishing and usually has not been peer-reviewed.





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### 2.3.1 Vegetative cell and spore preparation

*Clostridium sporogenes* PA 3679 (ATCC 7955) was purchased from the American Type Culture Collection (ATCC) as a freeze-dried pellet and stored between 2-8°C. *Clostridium sporogenes* cells were revived in 5 mL of tryptone peptone glucose yeast (TPGY) growth media and incubated at the optimal growth temperature of 35±2°C for 2 days based on the method of Yang *et al.* (2009). Culture incubation occurred under strict anaerobic conditions in anaerobic jars using anaerobic gas generating pouches (AnaeroGen sachets, Oxoid) and grown up to a suitable volume and density. The anaerobic status was monitored using anaerobic Resazurin indicator strips (Oxoid). Glycerol stocks of the culture were prepared in nutrient broth with 15% glycerol for long term storage in -80°C.

For vegetative cell preparation for subsequent spore crop production, *C. sporogenes* glycerol stocks were reactivated in 10 mL TPGY broth under anaerobic condition for 2 days in 35±2°C. After which 1 mL of this culture was transferred to 100 mL fresh TPGY broth and incubated under the same conditions. Then the 100 mL culture was equally dispensed in two sets of 500 mL TPGY broths and incubated under the same conditions for 3 days. The culture was then centrifuged (15,000 *g* for 10 min at 4°C) and the pellet washed three times with 0.1% peptone water and resuspended in an M/15 Sörensen's phosphate buffer (pH 7.0). This culture harvest was used for subsequent spore crop preparation based on the method of Mah *et al.* (2009).

For sporulation, the harvested bacterial culture was transferred to a sporulation medium which consisted of 6% tryptone, 0.1% dextrose, 0,1% sodium thioglycolate, and 0.5% calcium carbonate pH adjusted to 5.0 with 1M HCl (Mah *et al.* 2009). The culture was then incubated at the sub-optimal temperature of 30°C for 10 days. The resultant bulk of endospores were harvested by centrifugation (15,000 *g* for 30 min at 4°C) and washed once with 0.1% peptone water and resuspended in M/15 Sörensen's phosphate buffer (pH 7.0). The concentration of spores in the spore crop was determined by heat shocking an aliquot of spore crop for 10 min at 90°C, serial dilution, spread plating on Sheep Blood Agar plate for colony-forming unit (CFU) count after 48 to 72 hrs incubation at 35±2°C. Vegetative cell concentration was determined by the same method skipping the heat shock step.

### 2.3.2 Basal and experimental feed preparation

The basal and experimental diets were formulated to contain approximately 35% crude protein, 6% crude fat and 15.5 MJ $kg^{-1}$ gross energy (Bansemer *et al.* 2016). The diets were prepared by milling and blending the dry mash ingredients together with the fish oil (see Table 1) before sieving to 1 mm. Pellets were formed using a twin-screw extruder (Thermo Scientific™ TSE 24 MC). For the basal diet potable water was added to the extruder, whilst for the experimental feed the potable water was spiked with the prepared *C. sporogenes* spore concentrate to deliver a final spore concentration in the dried pellet of approximately $1x10^5$ spores $g^{-1}$. The wet pellets from the extruder were dried at 45°C until the average moisture content was less than 10% and a flat, sinking pellet (14.6 x 13.6 x 3.2 mm) produced. Feed pellets were stored at -18°C for approximately 2 months until required. The proximate composition of the feed was measured and reported in Table 2.

**Table 1 Ingredient composition of the abalone feed. Based on Bansemer *et al.* (2016)**

| Ingredients | Composition (%) |
|---|---|
| Tuna meal | 6.00 |
| Soy protein concentrate | 8.00 |
| Soybean meal | 28.44 |
| Wheat flour | 27.53 |
| Lupins | 22.40 |
| Ulva meal | 5.00 |
| Fish oil | 1.00 |
| Vitamin/mineral premix | 0.50 |





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| | |
|---|---|
| Monosodium phosphate | 0.61 |
| Sodium alginate | 0.30 |
| Calcium sulphate | 0.22 |

**Table 2 Nutrient composition of feed**

| | Proximate composition (g 100 $g^{-1}$ diet as fed) |
|---|---|
| Moisture | 8.4 |
| Crude protein | 34.4 |
| Fat | 5.4 |
| Ash | 6.9 |
| Carbohydrate [a] | 45.0 |
| Gross energy (MJ $kg^{-1}$) | 15.5 |

[a] Calculated by difference

The concentration of anaerobic vegetative cells and anaerobic spores in the basal and experimental feed were analysed and reported in Table 3. Pellets (25 g) were homogenised in 0.1% peptone water (to give a 1 in 10 suspension) using a stomacher (BagMixer 400P, Interscience) for 3 min. The initial suspension was serially diluted in sterile 0.1% peptone water and plated on Sheep Blood Columbia Agar (in duplicate). The plates were incubated anaerobically at 35±2°C for 24-48 h. For spore enumeration, samples were heat-shocked for 10 min at 90°C in a water bath prior to plating on Sheep Blood Columbia Agar plates.

**Table 3 Anaerobic vegetative cell and spore load in the basal and experimental feeds**

| Feed | Anaerobic vegetative cells | Anaerobic spores |
|---|---|---|
| Basal diet | $2.8x10^4$ CFU $g^{-1}$ | <10 spore $g^{-1}$ |
| Experimental diet | $3.0x10^6$ CFU $g^{-1}$ | $4.3x10^4$ spores $g^{-1}$ |

### *2.3.3 Experimental animals and initial animal husbandry*

Wild Blacklip abalone (average weight 526±133 g; average shell length 144±20 mm) were collected from the Western Zone Abalone Fishery, near Port Lincoln, South Australia on 31 July 2025 under ministerial exemption ME9903364. Animals were held overnight in Port Lincoln, before being airfreighted to Adelaide and then transported to the South Australia Aquatic Biosecurity Centre (SAABC) (Roseworthy, SA, Australia).

Upon arrival at the SAABC the abalone were transferred to two 450 L holding tanks (100 animals/tank) containing seawater (collected from SARDI Aquatic Sciences at West Beach) at 15.2°C and 36 psu. Seawater in the holding tanks was constantly filtered and recirculated (~2,000 L $h^{-1}$) through canister filters (Aqua One Ocellaris 3000UV). Air was supplied to each tank through two 30 cm airstones. To buffer any potential pH fluctuations, the seawater was supplemented with sodium bicarbonate to obtain an alkalinity of 150 ppm. All routine husbandry operations were conducted under red light. Whilst in the holding tanks (5 days) the abalone were maintained without feed. Water temperature, pH, salinity, dissolved oxygen (DO) and ammonia concentrations were





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measured daily, whilst nitrite concentrations were measured every second day. Solid waste (faeces from natural feed consumed by the abalone prior to wild-harvest collection) was removed daily via siphon. Daily water exchanges (15-60% by volume) were also conducted in response to the recorded water quality parameters in an attempt to maintain the following conditions: ammonia <1 ppm, nitrite <0.4 ppm, salinity 35 psu, temperature 15.1-18.3°C and pH 8.0-8.2 (as recommended in Seger *et al.* (2020)).

### 2.3.4 Experimental design and experimental system

Internalisation potential of heat-resistant spore-forming microorganisms was assessed through feed and emersion exposure of the abalone described above to *C. sporogenes* spores and vegetative cells over a two-week duration in static tanks (i.e. no seawater flow, 100% water exchange every 24 h). The vegetative *C. sporogenes* cells and spores were incorporated within the experimental pellets and the exposed abalone fed in excess daily. Unexposed (control) abalone were fed the basal diet in excess on a daily basis. The internalisation experiment was conducted at a nominal temperature of 16°C in 52 L treatment tanks (485 x 320 x 270 mm) containing 35 L of seawater. Tanks were covered with perforated opaque lids (~40% perforation) and light supplied at an average intensity of 13 Lux by LED lights (5000 K, 20 W) on a 12:12 h light/dark cycle. These static tanks were arranged in two blocks to provide a separation between the exposed and unexposed (control) abalone. Each block consisted of 12 tanks (four rows of three tanks). There were ten replicate tanks per treatment, leaving two tanks in each block to monitor nutrient leaching loss from the feed into the water. Tanks within each block were randomly allocated one of the treatment or nutrient leaching replicates.

### 2.3.5 Abalone stocking and acclimation

After five days in the holding tanks an abalone was chosen at random, weighed and measured (shell length) and placed into one of the 20 treatment tanks. This random allocated was repeated until each treatment tank contained two abalone, apart from the four tanks to monitor nutrient leaching that did not contain any abalone. Air was supplied to each treatment tank through a 5 cm airstone (Aqua One). The abalone were provided either a 12 or 14 day acclimation period after introduction to the treatment tanks, during which the abalone were fed the basal diet at 0.2% body weight (bw) daily. Routine husbandry operations were undertaken daily as reported below.

### 2.3.6 Feeding, bacterial application and animal husbandry

During the internalisation trial, abalone were fed their allocated basal or experimental diets to excess (~1.0 bw $day^{-1}$) at 4:00pm daily. The concentration of *C. sporogenes* spores and vegetative cells in the experimental feed was approximately $4.3x10^4$ spores $g^{-1}$ and $3.0x10^6$ cells $g^{-1}$, respectively. This is well in excess of reported environmental levels for *C. botulinum*, where the highest spore load in soils and sediments is 25 spores $g^{-1}$ and 1.2 spores $g^{-1}$, respectively (Austin and Dodds 2001).

Throughout the study, water quality was maintained at levels appropriate for the abalone. Husbandry operations commenced at 8:30am by measuring the water temperature, pH, salinity, DO, and ammonia concentrations daily whilst nitrite concentration was measured every second day. Any moribund or dead abalone were recorded and removed each morning, and feed rates were adjusted to compensate for biomass changes to individual tanks arising from mortalities.

When routine environmental measurements were completed, any uneaten feed was removed from the tanks and transferred to containers and stored at -20°C. Immediately after the collection of the uneaten feed, all seawater and faeces were removed by inversion of the tanks, and the tanks briefly rinsed with and refilled with fresh seawater. To calculate nutrient leaching loss from the feed, the same method was used with the four nutrient leaching tanks (containing no abalone).

### 2.3.7 Sample collection and analysis

Following the two-week exposure, the abalone from each replicate were removed and haemolymph sampled for the presence of anaerobic vegetative cells and spores. Approximately 2 mL of haemolymph was collected using a 5 mL sterile syringe with a 23-gauge needle attached from the pedal sinus of each animal after disinfection of the epithelium over lying the needle entry point. The disinfection protocol involved wiping the foot of the abalone with a single-use disposable paper towel, bathing the surface of the foot with 12.5% sodium hypochlorite for 5 mins, followed by rinsing with autoclaved MilliQ water. The disinfected surface of each abalone was then swabbed with a sterile moist cotton swab and the swab immersed into tubes containing 10 mL TPGY broth. The collected haemolymph (100 µL) was also inoculated into 10 mL of enrichment broth media (TPGY). The inoculated broths were





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incubated anaerobically at 35°C for 24-48 h. Any turbid cultures were deemed presumptive positive for vegetative *C. sporogenes* cells. Non-turbid tubes were heat-shocked (90°C for 10 min) and re-incubated under anaerobic conditions to detect spore germination.

Following haemolymph collection and sample processing for microbiology, the abalone were shucked, and the visceral mass (including gills, gonads, stomach and digestive tract) from the paired abalone homogenised. A 25 g sample of the homogenised visceral mass was blended in 0.9% saline (to give a 1 in 10 suspension). The initial suspension was serially diluted in sterile 0.1% peptone water and/or plated on Sheep Blood Columbia Agar (Thermo Fisher Scientific Australia Pty Ltd) in duplicate and incubated anaerobically for 24-48 h at 35±2°C for presumptive vegetative *C. sporogenes* cells. Additionally, the total number of presumptive *C. sporogenes* spores were determined by the same procedure after being heat-shocked (90°C for 10 min).

At the end of the experiment, all uneaten feed samples were oven-dried (16 h at 105°C) to obtain the dry weights. Apparent feed intake was calculated by subtracting the uneaten feed (dry weight) and the average amount lost due to leaching (dry weight), from the total amount of feed delivered to each tank and then converted to an “as fed” basis using the original moisture content of the diets.





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# 3. Results and Discussion

## 3.1 Literature review on thermal processing of foods

Literature reviews were completed in 2020, that included summarising the history of thermal processing of foods and the underlying science and technology, including the instigation for heat penetration and requirements for thermal inactivation of pathogenic and non-pathogenic microorganisms, background on *C. botulinum*, and use of *C. sporogenes* as a surrogate for *C. botulinum*. Copies of the reviews are contained in Appendix B and Appendix C. The reviews identified that the first successful experiments for the preservation of food in sealed containers by means of heat were achieved in 1795 by Nicolas Appert. The findings from the reviews also underline that as the science underpinning thermal processing of foods has evolved, the assumption that heat-resistant microorganisms could be located at the centre or the slowest heating point has become deeply ingrained. Furthermore, the food industry has observed that a 12-logarithmic reduction in *C. botulinum* spores to be an acceptable and minimum food safety target for thermal process requirements (Anderson *et al.* 2011). However, some scheduled thermal process may target a process lethality that is greater than the minimum required for food safety, often for textural considerations or to reduce the likelihood of spoilage from non-pathogenic bacteria. The 12-logarithmic reduction of *C. botulinum* is mathematically calculated based on the time-temperature profile achieved during the thermal process, coupled with thermal death kinetics of *C. botulinum*. The thermal death kinetics of bacteria are dependent on the intrinsic properties of the food being processed. Abalone have also exhibited an inhibitive reaction towards *C. botulinum* spores (Lang and Dean 1934) but it is unclear if this has been exploited.

It has been postulated that traditional methods of determining safe or commercially sterile processing schedules may provide more than adequate lethality for non-comminuted meats[2] (Schack *et al.* 1959). Pflug (1987)[3] considered existing research and regulatory practices regarding thermal processing and concluded that the term “commercial sterility” is an empirical term, not numerically defined in regulations, and suggested that the endpoint of heat preservation processes should be expressed as a specification, such as the probability of a nonsterile unit. Later, a US Committee on the *Review of the Use of Scientific Criteria and Performance Standards for Safe Foods* (Institute of Medicine and National Research Council 2003) also concluded that although the use of the *12D* thermal process has a long history of safe use, its appropriateness should be scientifically re-evaluated. From our awareness and understanding the present safe-harbour approaches for canned foods have not been rigorously challenged, with heat penetration data currently being determined under the most adverse conditions for all product types and the assumption that microorganisms targeted in heat penetration studies are located at the slowest heating point.

## 3.2 Post-harvest handling, physiology and microbiological contamination in abalone

Abalone live in a range of marine environments and can be exposed to various microorganisms and stress factors during the primary production process and from the point of capture to and during food processing procedures. Physical damage to the abalone can also cause a potential point of entry for microbes. As abalone lack an acutely effective clotting mechanism, any lacerations of external and/or internal tissues during production, harvesting or processing can result in the injured abalone losing enough haemolymph to cause death by hypovolaemia (loss of blood or bodily fluids) (Loeher and Moore 2020). Abalone Council Australia has developed *A Quality Assurance Code of Practice for the Australian Wild Abalone Fishery* which includes the need to handle abalone gently to avoid scratching or puncturing the abalone foot (Abalone Council Australia Ltd 2013). During harvesting a speciality designed tool (referred to as an “abalone iron” or “ab iron”) is used to gently remove the abalone from its substrate. These abalone irons typically have rounded edges to help protect the abalone from cuts. Despite recommendations and the use of appropriate tools surface abrasions/grazes, shallow slices/nicks or ripped frills may inevitably occur during harvest, transport, shucking or preparation of tissue for canning. Additionally, the core temperature of abalone may be measured during processing which results in deep puncture wounds.

[2] Comminuted products are products which are chopped, diced, minced, ground, or otherwise reduced to small pieces.

[3] Irving Pflug is an Emeritus Professor of Food Science and Nutrition and has been a researcher, teacher and consultant in sterilization, microbiology and Food Sciences





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Abalone material destined for canning may be supplied to canneries live, chilled or frozen (with or without viscera and shell) formats. After shucking (removal of the abalone from its shell), viscera are removed and the foot muscle is generally rumbled/abraded in a process designed to remove any external mucus, pigmentation, debris left after shucking and evisceration, and any other foreign material. Following the rumbling stage, trained factory staff manually remove the abalone's mouth, grade product based on appearance and inspect product for signs of damage. Any identified loose flaps of tissue from slices/nicks are physically removed by manual trimming, whereas any product that is identified with larger cuts/stabs is further cut and used as "pieces". These surface cuts effectively become an "external surface" and tissue within the vicinity would consequently receive a more severe thermal process; however, it is unclear if the cuts would provide a portal or gateway for bacterial migration deeper into the tissue, or what would happen if animals with un-identified cuts/nicks are processed.

The initial descriptions of the cardiovascular anatomy of abalone were described by Crofts (1929), and have been further developed or informed by Russell and Evans (1989) and Bourne *et al.* (1990). The cardiovascular anatomy contains characteristics of both semi-open and closed systems. These studies have shown that the lacunar tissue spaces appear to lack an endothelial lining which enables the haemolymph to freely move through the foot muscle; the sinuses within the abalone are simply larger channels where the fluid can move more rapidly (Russell and Evans 1989, Day *et al.* 2010).

The gut microbiota of marine organisms is shaped by ontogeny, diet, and bacteria in the surrounding aquatic environment (Huang *et al.* 2020). Whilst being shaped, the microbial composition of abalone intestines can also differ significantly from the surrounding seawater, and is altered when abalone become ill or suffers abnormal mortality (Zhang *et al.* 2022). The intestinal/gut microbiota of abalone can also impact their immune system function, production/growth characteristics and animal health (Gobet *et al.* 2018, Danckert 2019). However, the muscle tissues and internal organs of freshly caught, healthy finfish and molluscan shellfish have historically been considered to be normally sterile (ICMSF 2005). Although, the internal microbiota, or microbial communities, of marine animals, including abalone, are generally poorly understood and have only recently begun to be characterised (Gobet *et al.* 2018, Nel *et al.* 2018, Danckert 2019, Offret *et al.* 2019a, Wang *et al.* 2021).

Abalone and other invertebrates, have innate immunity as a principal line of defence against a broad spectrum of microbiological pathogens. This innate immunity consists of physical barriers, (e.g. shell, skin, epithelium) and cellular and humoral (cell-free) responses (Dang *et al.* 2013, Pratiwi *et al.* 2025). Cellular immunity is centred on the activity of haemocytes (or circulating immune cells) within the haemolymph. These haemocytes act through phagocytosis, followed by the generation of reactive oxygen species including superoxide anion, hydrogen peroxide, and hydroxyl radicals to eliminate pathogens. Humoral responses involve the production of defensive enzymes and other compounds with antimicrobial properties (Hooper *et al.* 2007a).

Stressors, whilst inhibiting growth performance, can also lead to immunosuppression that increases the susceptibility to opportunistic infections and mortality (Hooper *et al.* 2007a, Hooper *et al.* 2011, Cardinaud *et al.* 2014). These stressors can be induced by numerous factors including sub-optimal water temperature, reduced concentrations of DO (hypoxia), increased concentrations of metabolic wastes (especially ammonia and nitrite), elevated stocking densities, exposure to pathogenic microorganisms and physical handling (Hooper *et al.* 2007a, Cardinaud *et al.* 2014). Various studies including Dang *et al.* (2012), Stone *et al.* (2014) and Lange *et al.* (2014) have shown that elevated water temperatures can reduce the immune system and make abalone more vulnerable to bacterial infections. Elevated water temperatures can also damage the gill epithelium providing a portal of entry for some bacteria (Hooper *et al.* 2014).

### 3.3 Literature review on bacterial internalisation in abalone

The concentration and taxa of bacterial and other microorganisms on/in marine organisms can fluctuate according to a variety of factors, such as the microenvironments provided by skin/shell surfaces, gill areas, digestive tract, and habitats. Bacteria are present in the digestive gland and intestines of abalone and are essential in the digestion of feed (Yu *et al.* 2022). *C. botulinum* (the target pathogen for thermal processing of low-acid foods) are also known to inhabit aquatic environments, including sediments, and is considered to be ubiquitous in nature. A review of the published literature was initially undertaken to identify if *Clostridium* or botulism had been associated with abalone. A copy of this review is contained in Appendix D. There is growing evidence indicated by the literature that abalone may have an endemic microbiota within the haemolymph. Isolation of bacteria from the haemolymph





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in diseased abalone is commonly reported and can be of a primary or secondary nature. However, this defined literature search did not reveal any work undertaken that found an association of *C. botulinum* with abalone (of any species). Only one article reported any association of *Clostridium* with abalone. This was an isolation of *C. lituseburense* from tissue samples of diseased abalone which may have been a result of environmental contamination and proliferation of the bacteria under anaerobic conditions within necrotic tissue.

Most studies investigating abalone haemolymph and/or bacterial infections have focused on understanding and trying to mitigate mass-mortality events and have used microbiological techniques specific for these purposes. The normal approach being: sample collection – processing for aerobic bacterial isolation – identification of cultured organisms. Techniques for isolating anaerobic bacteria are not routinely used in these circumstances and are not indicated in the literature. There is a particular paucity of publications undertaking the isolation of *Clostridium* from normal or diseased abalone.

### 3.4 Literature review on bacterial migration studies in animal tissues

The findings from the literature review on bacterial migration studies in animal tissues is reported in Appendix E. The review determined that bacterial migration studies in food products are primarily conducted to help to ensure food safety, extending shelf-life and preventing spoilage. A range of quantitative and qualitative methods have been used depending on the nature of the study. The application of dye-based particles as a visual tool to determine the potential impact processing conditions may have on bacterial migration have been reported in a range of terrestrial animal muscle tissues including those from beef and pork. These studies reported that extent of bacterial migration into muscle tissue after slaughter and during processing and/or storage is dependent on a number of intrinsic and extrinsic factors including the structural integrity and fibre orientation of the muscle(s). No publications were identified that contained information on the potential for, or the extent of, bacterial migration in abalone tissue during any post-harvest processing. However, the tissue structure of abalone differs from that of terrestrial animal protein sources, and this characteristic may limit the extent of bacterial migration.

### 3.5 Dye penetration in abalone meat (experimental findings)

Red Pillar Box Powder was selected for the trial because it is a water-soluble dye and exhibited satisfactory heat-stability and provided good colour contrast against the abalone tissue. In contrast, a preliminary trial using the hydrophobic Blue Lake dye produced poor colour contrast and visual attributes (data not reported). This may be because the Blue Lake dye is less compatible against lean, high moisture seafood matrixes, such as abalone, providing limited opportunity for the dye to associate with the sample surface.

The combination of the freeze-thaw cycle and manual scrubbing to remove surface pigmentation was intended to provide a worst-case scenario for tissue damage, through potential ice-crystal related disruptions and surface scratches or abrasions. Figure 1 shows examples of the dye penetration in chilled and retorted Blacklip abalone pieces at various time points.

In chilled (non-retorted) Greenlip and Blacklip abalone samples, the dye penetrated to a depth of 1-2 mm across all surfaces after 1 day and gradually increased to 3-4 mm after 7 days. There was no apparent difference in penetration depth between the farmed and wild-caught abalone samples. Dye was also observed in some of the pedal sinuses and occurred more frequently in the abalone pieces, rather than whole abalone. It was likely due to cutting the abalone in half which increased exposure of the internal sinus structure, thereby allowing the dye to move more readily into the tissue.

In retorted samples, the dye penetration was more extensive, reaching approximately 5 mm after 1 week, 8-10 mm after 4 weeks, and full penetration by 18 weeks. Similar to the chilled samples, the dye also penetrated some of the pedal sinuses. The retorting process may have increased tissue permeability, disrupted muscle structure and opened internal spaces, allowing the dye to migrate further during storage.





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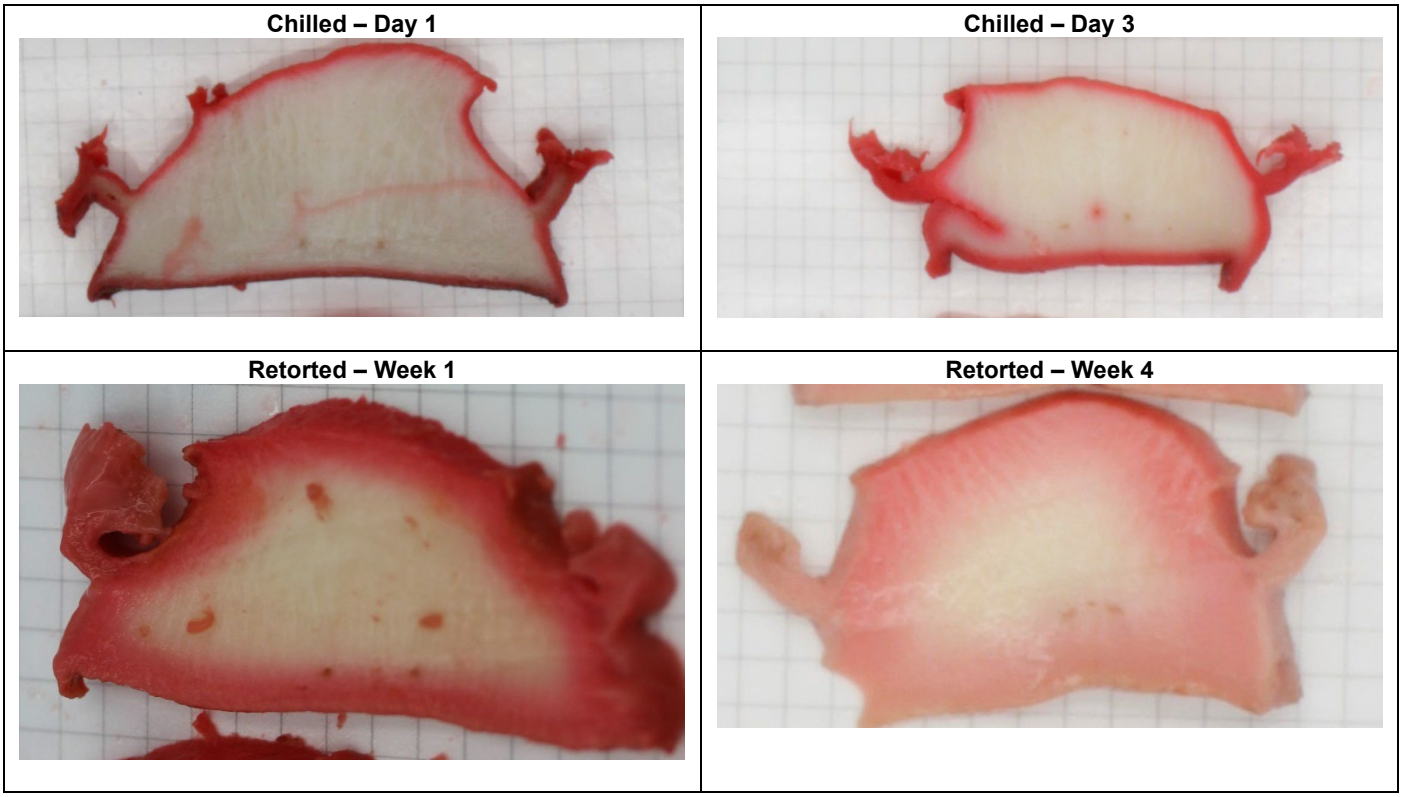


**Figure 1 Penetration of Red Pillar Box Powder dye in chilled and retorted Blacklip abalone**

### 3.6 *Clostridium* internalisation in abalone (experimental findings)

At the commencement of the trial, abalone exhibited normal behaviour, remaining submerged with the muscular foot firmly attached to tank surfaces. Similar behavioural observations under static tank conditions have been reported previously by Seger *et al.* (2020). However, as the experiment progressed, a small number of abalone displayed reduced attachment strength. Three mortalities occurred during the internalisation trial; one abalone fed the basal diet died on day one, while two abalone fed the experimental diet died on day 13 in separate tanks. The cause(s) of the mortalities were not investigated further.

Water quality parameters (temperature, DO, pH, salinity, ammonia, and nitrite) monitored during the internalisation trial are presented in Figure 2. Aside from the water temperature during the first week of the trial and pH generally throughout, the water quality parameters were within the desired ranges. The increase in water temperature between days 1 and 6 resulted from an adjustment to the setpoint of the SAABC air conditioning system. Despite increasing the alkalinity of the incoming seawater from approximately 135 ppm to 150 ppm, pH generally remained below the target value of 8.0. Seawater pH is known to influence feed consumption, growth and mortality in juvenile Blacklip abalone. Harris *et al.* (1999) reported that growth was greatest between pH 7.76 and 8.27, whereas mortality rates increased significantly when pH decreased below 7.16.





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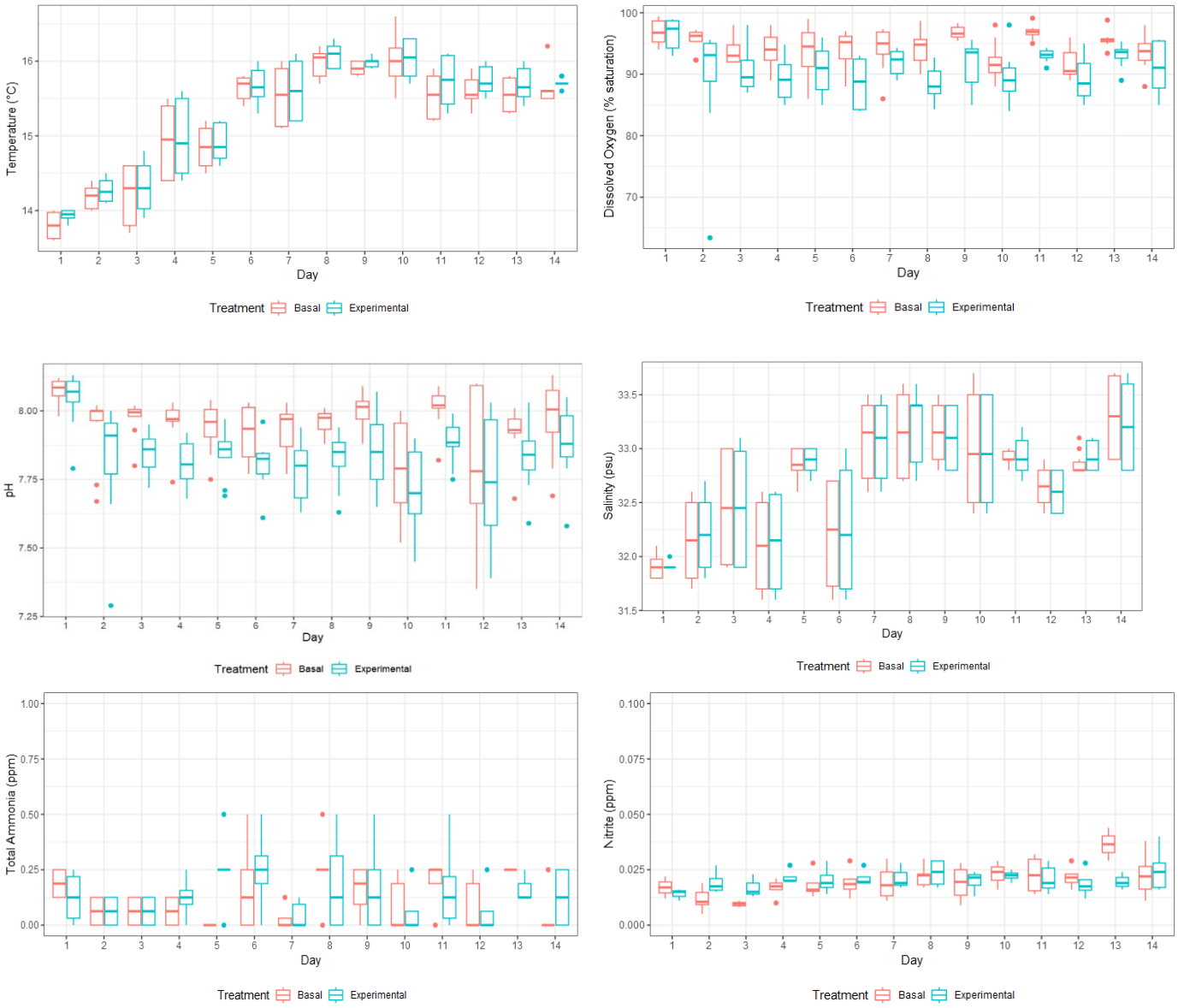


**Figure 2 Water quality parameters throughout the *Clostridium* internalisation trial**

The amount of feed consumed by the abalone is shown in Figure 3. The abalone consumed more feed in the first week than the second week; however, for both the basal and experimental diets, the amount of feed consumed was lower than anticipated. The reduced feed consumption of the experimental diet likely had a direct impact on the amount of vegetative *C. sporogenes* cells and spores available for incorporation into the viscera. Approximately 30% of the feed weight added was not recovered from the control tanks and was determined to be due to losses from nutrient leaching. Nutrient leaching would likely have resulted in an increased microbiological load within the seawater, and therefore additional exposure of the abalone to the *C. sporogenes* cells and spores, through their gills.





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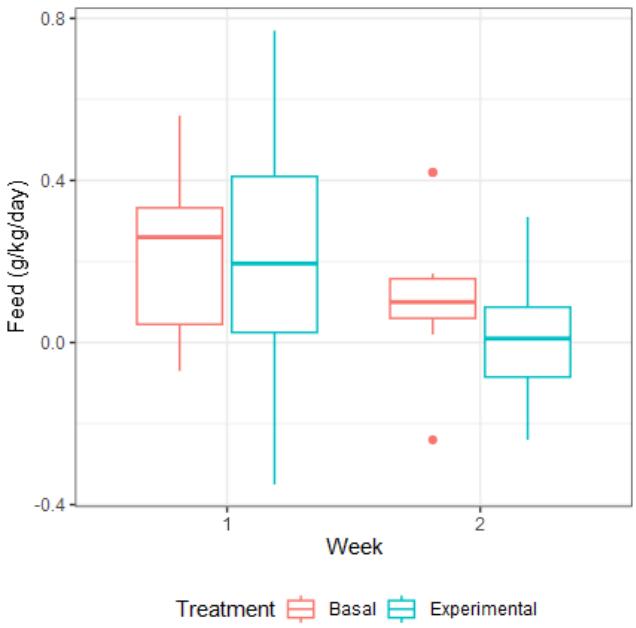


**Figure 3 Average feed consumption (grams feed consumed per kilogram body weight per day) in week one and week two of the internalisation trial**

The concentrated solution of sodium hypochlorite was used to disinfect the surface of the abalone foot prior to haemolymph collection, owing to its well-established sporicidal activity. No microbial growth was detected from any swabs collected following disinfection (data not shown), indicating effective surface decontamination. After hypochlorite treatment, the abalone foot was rinsed with autoclaved MilliQ water; however, no assessment was undertaken to confirm the absence of residual hypochlorite. It is acknowledged that the efficacy of hypochlorite solutions can be reduced in the presence of organic material (Omidbkhsh 2010). Nevertheless, the use of concentrated sodium hypochlorite for surface disinfection prior to microbiological analysis in a *Clostridium* challenge study has also been reported by Newell *et al.* (2015), supporting the appropriateness of this approach.

Sufficient haemolymph was collected from each of the live abalone. The results from the microbiological analysis are reported in Table 4. No growth of anaerobic vegetative cells or spores of *C. sporogenes* was observed from the sampled haemolymph. Anaerobic vegetative cells or spores were not detected from the visceral mass of the abalone fed the basal diet, whilst seven visceral mass samples from the experimental diet reported quantifiable anaerobic vegetative cells and one sample was confirmed to contain anaerobic spores (400 CFU $g^{-1}$). The lack of consistent detection of anaerobic vegetative cells and spores in the visceral mass recovered from the abalone fed the experimental diet could be due to a combination of factors including low feed consumption, a potential inhibitory effect associated with the abalone, and contamination of the visceral mass with hypochlorite. No attempt was made to neutralise any residual hypochlorite prior to collecting the visceral mass.

Previous method development and testing (data not shown) from *in-vitro* studies demonstrated satisfactory recovery and growth of *C. sporogenes* cells and spores from samples of artificially spiked abalone haemolymph and spiked visceral mass. *In-vivo* exposure of live abalone to *C. sporogenes* through direct injection into the animals was not trialled, but in future could help elucidate whether abalone possess a natural mechanism to inactivate or prevent the growth of these vegetative cells or spores. Furthermore, no assessment of the abalone's immune response (i.e. haemocyte concentration or phagocytic activity) was performed, due to limitations in the number of abalone able to be accommodated into the tanks, which may have assisted in determining whether *C. sporogenes* from the experimental feed translocated into the haemolymph.





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**Table 4 Microbiological analysis of any anaerobic vegetative cells and anaerobic spores from the abalone haemolymph and visceral mass**

| Treatment | Tank | Abalone | Haemolymph | | Visceral mass | |
|---|---|---|---|---|---|---|
| | | | Anaerobic vegetative cells (growth/no growth) | Anaerobic spores (growth/no growth) | Anaerobic vegetative cells (CFU $g^{-1}$) | Anaerobic spores (CFU $g^{-1}$) |
| Basal | 1 | A<br>B | No growth<br>No growth | No growth<br>No growth | Not detected | Not detected |
| | 2 | A<br>B | No growth<br>No growth | No growth<br>No growth | Not detected | Not detected |
| | 3 | A<br>B | No growth<br>No growth | No growth<br>No growth | Not detected | Not detected |
| | 4 | A<br>B | No growth<br>No growth | No growth<br>No growth | Not detected | Not detected |
| | 5 | A<br>B | No growth<br>No growth | No growth<br>No growth | Not detected | Not detected |
| | 6 | A<br>B [A] | No growth<br>Not sampled | No growth<br>Not sampled | Not detected | Not detected |
| | 7 | A<br>B | No growth<br>No growth | No growth<br>No growth | Not detected | Not detected |
| | 8 | A<br>B | No growth<br>No growth | No growth<br>No growth | Not detected | Not detected |
| | 9 | A<br>B | No growth<br>No growth | No growth<br>No growth | Not detected | Not detected |
| | 10 | A<br>B | No growth<br>No growth | No growth<br>No growth | Not detected | Not detected |
| Experimental | 1 | A<br>B [A] | No growth<br>Not sampled | No growth<br>Not sampled | Not detected | Not detected |
| | 2 | A<br>B | No growth<br>No growth | No growth<br>No growth | Not detected | Not detected |
| | 3 | A<br>B | No growth<br>No growth | No growth<br>No growth | 200 | Not detected |
| | 4 | A<br>B | No growth<br>No growth | No growth<br>No growth | 300 | 400 |
| | 5 | A<br>B | No growth<br>No growth | No growth<br>No growth | 300 | Not detected |
| | 6 | A<br>B | No growth<br>No growth | No growth<br>No growth | 500 | Not detected |



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| | | | | | | |
|---|---|---|---|---|---|---|
| | 7 | A<br>B | No growth<br>No growth | No growth<br>No growth | 400 | Not detected |
| | 8 | A<br>B | No growth<br>No growth | No growth<br>No growth | 200 | Not detected |
| | 9 | A<br>B [A] | No growth<br>Not sampled | No growth<br>Not sampled | Not detected | Not detected |
| | 10 | A<br>B | No growth<br>No growth | No growth<br>No growth | 2,500 | Not detected |

[A] Tissues from this abalone was not sampled as the abalone had died during the trial





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## 4. Conclusions

Canned abalone remains an important value-added product for the Australian abalone industry, supporting market access and industry viability despite recent declines in production volumes. Canned abalone is sold on a drained-weight basis and the product's commercial value is strongly influenced by processing yield. Typical processing losses during canning are reported to be between 12-30% and are associated with a partial loss of haemolymph.

The project attempted to address whether thermal process requirements for Australian and New Zealand wild-caught and farmed abalone could be safety reduced while maintaining commercial sterility, regulatory compliance and market access. The literature review and experimental work did not identify evidence that *C. botulinum* spores internally contaminated abalone. However, the project also did not conclusively determine whether heat-resistant spores can be taken up and distributed into the abalone foot, or if there would be regulatory and market access support to base the thermal process lethality on a site of microbiological concern that is not the slowest heating point for the product. The absence of recovered *C. sporogenes* anaerobic vegetative cells or spores from haemolymph in the husbandry study is encouraging, but it cannot be interpreted as an evidence of absence, particularly given low feed intake during the trial, variable recovery of anaerobic cells and spores from the visceral mass, and the absence of further microbiological profiling of haemolymph or foot tissue, or any immune responses.

As the project was terminated before completion, it did not provide sufficient scientific evidence to support immediate reductions in thermal processing severity or associated reductions in yield loss and food waste, or if the existing safe-harbour approach can be challenged. Nevertheless, the work delivered important enabling outcomes, including development of national capability in anaerobic *C. sporogenes* spore production and analysis, strengthened thermal processing expertise, and improved collaboration between researchers and the abalone industry. These outcomes provide a foundation for any future research to reduce processing losses associated with canned abalone.



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## 5. Recommendations

The following recommendations have been made.

**1) Maintain current validated thermal processes until further evidence is available**

Current scheduled thermal processes for canned abalone should remain in place until robust scientific evidence demonstrates that any reduction in processing severity can achieve commercial sterility under worst-case product and process conditions. Any proposed process change should be validated against the relevant target hazards, critical factors, regulatory and market access requirements.

**2) Complete targeted research on spore uptake and distribution in abalone tissues**

Further research should be prioritised to determine whether heat-resistant spores can enter, survive or distribute within the abalone foot muscle and haemolymph. This work should include improved exposure protocols that ensure active feeding. However, whilst building evidence, it is acknowledged that the outcomes may continue to stumble against the absence of evidence is not evidence of absence.

**3) Undertake microbiological profiling of abalone tissues**

Future studies should include broader microbiological profiling to characterise the natural bacterial communities of abalone haemolymph, foot muscle and visceral mass. This would help determine whether internal tissues are typically sterile, intermittently contaminated, or associated with particular bacterial groups of relevance to spoilage or food safety.

**4) Refine dye penetration and heat-transfer studies**

The dye penetration work suggests that the abalone foot structure, particularly the sinuses, may influence the extent or depth of bacterial migration. Further studies could link dye penetration, tissue anatomy and heat-transfer data to determine whether the point of microbiological concern aligns with the slowest heating point or at some other specific anatomical position.

**5) Quantify the relationship between processing severity, yield and product quality**

The project had assumed that changes to the minimum thermal processing requirements may result in product drained weight increases of between 0.5-2.0%. Further work should quantify how different thermal process conditions affect not only drained weight, but also the textural and sensory quality of the final product. This would help allow industry to assess the economic and food-waste benefits of any future process optimisation against food safety and market access requirements.

**6) Review the pathway for acceptance**

Final or intermediate product testing, whilst it can be considered as a control measure, provides limited information on the safety of the food and the absence of evidence is not evidence of absence. The pathway to challenge the current safe harbour process that has developed as canned food products have evolved needs to be reconsidered. An alternative pathway would be to consider remapping the thermal death kinetics of *C. botulinum* in abalone-specific matrices. This would determine whether the current minimum thermal processes ($F_0$=2.4) are more severe than required for abalone.



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## 6. Impact and Ongoing Monitoring

The project was terminated before completion and has not provided sufficient scientific evidence to support immediate reductions in thermal processing severity or associated reductions in yield loss and food waste. Nevertheless, the project has developed national capability in anaerobic *C. sporogenes* spore production and analysis, strengthened thermal processing expertise, and improved collaboration between researchers and the abalone industry. There are no ongoing monitoring measures that will be used to determine if there is any impact from this project.



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## Appendix A – Project Summary



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# Development and validation of reduced thermal processing requirements for canned abalone

## KEY POINTS

- Abalone (*Haliotis* spp.) are a high-valued seafood product harvested from wild and aquaculture systems, and are sold in various formats including live, fresh, frozen, canned and value-added.
- Abalone processors typically lose between 12-30% product yield (meat weight) during thermal processing within cannery operations. This weight loss is associated with the partial loss of haemolymph and is equivalent to 40-102 tonnes per annum.
- Thermal processing of low acid canned foods (such as abalone) remains a very critical and highly regulated operation. Scheduled processes must be established by competent persons having expert knowledge of thermal processing and having adequate facilities for making such determinations.

Figure 1. Canned abalone.

## THE CHALLENGE

Canned abalone (like all canned products) are heat-treated to eliminate the potential presence of pathogens and reduce the incidence of spoilage organisms. As the science underpinning thermal processing of foods has evolved, the assumption that heat-resistant microorganisms could be located at the center or the slowest heating point has become deeply ingrained.

## THE OPPORTUNITY

The aim of this research was to provide robust scientific evidence to allow the abalone industry to reduce the thermal process requirements for Australian and New Zealand wild-caught and farmed abalone whilst enabling the industry to continue to meet the regulatory requirements.

The project aimed to challenge the principle that abalone can be contaminated with pathogenic heat-resistant bacterial spores throughout the meat (or abalone foot) and that the point of microbiological concern may be closer to the edge of the abalone foot and not at the slowest heating point which is generally considered to be the geometric centre of the abalone.

The underlying requirement is to continue to produce commercially sterile foods – a condition achieved by application of heat to render the product free of viable microorganisms including spores of public health significance and microorganisms capable of growing in the product under normal non-refrigerated conditions of distribution and storage.

## OUR RESEARCH

The project reviewed published data to provide background information on the evolution and science behind thermal processing of foods and the potential for microbiological contamination in abalone through understanding abalone physiology, post-harvest handling processes and bacterial studies.

Two laboratory investigations were completed that assessed the potential for bacterial spore uptake in live abalone and dye migration or penetration in prepared abalone meat.



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Figure 2. Photograph taken drawing the abalone blood (haemolymph) for microbiological assessment.

## OUTCOMES

The literature review concluded that the haemolymph (blood) of many invertebrates (including abalone) can contain a range of bacteria, however there was no evidence to confirm or refute that abalone can be internally contaminated with *Clostridium botulinum*, the causative agent of botulism.

The dye penetration study demonstrated that the dye particles slowly migrated towards the centre of the abalone foot and could penetrate faster through the sinuses.

The live abalone husbandry trial did not recover any anaerobic vegetative cells or anaerobic spores of *Clostridium sporogenes* (a surrogate for *C. botulinum*) from the haemolymph. However, the abalone only consumed a low amount of feed during the trial and variable levels of anaerobic vegetative cells and anaerobic spores were recovered from the visceral mass.

## IMPACT

The project ceased prior to confirming that abalone cannot be contaminated with heat-resistant bacterial *C. sporogenes* spores throughout the meat (or abalone foot) or if the existing safe-harbour approach can be challenged and that it would be acceptable to reduce the thermal processing requirements for canned abalone.

This project has established additional national capability in the production and analysis of anaerobic *C. sporogenes* spores, thermal processing expertise and built collaborative partnerships between the abalone industry and researchers.

## NEXT STEPS

Food safety has a significant influence on food markets, consumer trust and is fundamental in helping to protect human health. However, the current absence of evidence is not evidence that heat-resistant bacterial spores could be absence from the centre of the abalone foot.

Further *in-vitro* and *in-vivo* trials could be taken to help determine if there is any potential for, or mechanism to prevent, heat-resistance bacteria being present at the slowest heating point; and to complete a microbiological risk assessment.

Alternative approaches to modify the minimum thermal processing requirements could be to review and redraw the thermal death kinetics for *C. botulinum* in abalone tissue.

## PROJECT TEAM

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## PROJECT REPORTS/PUBLICATIONS

Nil

## PROJECT WEBPAGE

https://endfoodwaste.com.au/reducing-canning-losses-in-the-abalone-industry/



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# Appendix B – Literature Review on Thermal Processing of Foods

## A Historical Perspective of Thermal Processing Requirements

As early as 1745 there was recognition that food could be preserved through a combination of heating and exclusion of air (Pearson 2016). However, it wasn't until 1795 that the first successful experiments for the preservation of food in sealed containers by means of heat were achieved by Nicolas Appert. Appert believed that exposure to air was the main cause of spoiled food. May (1937) reported that Appert's preservation method was simple, in that he sealed fresh or cooked meat, fish, fruits, eggs and vegetables in air-tight bottles, immersed the bottles in boiling water for varying periods of time and was able to keep his "processed" foods in edible conditions. In 1809 Appert earnt a reward of 12,000 francs from the French Government, and in 1810 as a condition to the award published the book "*The Book for all Households; or, The Art of Preserving Animal or Vegetable Substances for Many Years*", which subsequently became a foundational text of modern food preservation.

From the time of Appert's discovery, the basis of the canning industry has been trial and error (Adam 1960) and technologies have developed in both the application of heat and in the packaging of the food products in hermetically sealed containers. Ball (1938) published a comprehensive summary of the evolution of thermal processing. Canning of food products was first developed on an extensive commercial scale in the United States of America in 1819 (Busch 1981). Before the manufacture of canned food became feasible, food preservation relied on natural mechanisms (such as freezing, dehydration, and storage at low temperatures) or artificial methods that introduced a physiochemical process (such as salting, sugaring, spicing or pickling) or treatment (i.e. smoking) designed to resist fermentation or putrefaction. Prior to the development of tin or metal containers, heavy glass jars were used. The earliest types of metal containers were made exclusively by hand with side seams and can-ends sealed with solder. Food was often filled into the can through a circular hole or opening (around one inch in diameter) in one end of the can which was subsequently covered by a disc or cap and soldered in place. The disc or cap often had a small vent hole which was later closed with more solder after sufficient amount of steam had escaped during processing. These 'hole-and-cap' containers came with several innate disadvantages; they were time consuming to make and fill, inherently difficult to pack large pieces of food through the relatively small hole and lacked efficient cleansing of the can prior to filling (Baumgartner 1949). Hand-soldered cans were gradually replaced by machine-soldered cans during the 1880's. Modern "open-top" or sanitary containers were developed and commercially implemented around the early 1900's (May 1937). These new style of cans were considered more sanitary because they were only soldered on the outside. They also paved the way for technological developments that led to high-speed economically can production and enabled the end cap being crimped onto the can after filling. This enabled the container to hold larger pieces of food than 'hole and cap' cans (Busch 1981).

In the 1860's some canneries increased the processing temperature from 100°C to 116°C by adding calcium chloride to the cooking water which resulted in reduced processing times (Tucker and Featherstone 2021). It wasn't until the 1860's and 1870's that Louis Pasteur with his pioneering bacteriology studies proved that some microbes were responsible for deadly diseases. Pasteur's findings were subsequently applied to canning in the 1890's (Pearson 2016). Pasteur concluded that spoilage and putrefaction in canned and bottles products was not exposure to air, but the bacteria present in the environment. It was also understood that different foods required different amounts of heat due to the properties of the food and presence of different bacteria. The early canning industry relied on "rule of thumb" procedures and canneries were secretive about the best heat and exposure time to preserve various foods (National Canners Association 1937).

The earliest known scientific reference to heat-penetration studies occurred in the late 1890s. In 1896 W. Lyman Underwood (a third generation canner) began to study bacteriology under the instruction of Samuel C Prescott from Massachusetts Institute of Technology (May 1937). Prescott and Underwood (1898) whilst investigating spoilt canned sweetcorn kernels reported that sufficient energy must be applied to heat and maintain an adequate temperature throughout the contents of the can. It is likely that following the findings from Prescott and Underwood (1897) and Prescott and Underwood (1898) it has been assumed that the heat-resistant microorganisms may be located at the centre, or the slowest heating point of the can. Prescott and Underwood's research on microbiology of canned foods and the subsequent application of principles that were established, marked the introduction of science into the preservation of foods by thermal processing (Stumbo 1973). Heat penetration tests of various food products have become more common and various methods have been used to describe the lethal effects of time-temperature relationships. In 1920 the book "*Heat Penetration in Processing Canned Foods*" was published by Bigelow *et al.* (1920) and established the





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scientific foundation for modern thermal processing. During process validation thermocouples or other devices can be used to measure the temperatures during heating and cooling of foods. Bigelow *et al.* (1920) established the General (or graphical) Method for thermal process calculations, whereby the lethal effects of various time-temperature relationships in a container of food are integrated during processing. Bigelow *et al.* (1920) also used the point of greatest temperature lag during heating and cooling of the product for these calculations. Over time, these calculations were refined and improved (Stumbo 1949). Around the same time Baumgartner (1949) reported that it is always assumed that the heat-resistant microorganisms will be located at the centre or the slowest heating point of the can contents.

Bacteria, such as *Bacillus* and *Clostridium* species, can develop a resistant stage known as an endospore or spore. These spores are a dormant form of the vegetative bacterial cell and retain the vitality of the vegetative cell under adverse conditions. Spore formation (sporulation) is influenced by many factors, but often in response to stress, such as nutrient deprivation and other unfavourable conditions (Wohlgemuth and Kämpfer 2014, Gauvry *et al.* 2017). Spores are much more resistant to heat, pressure, low pH, germicides, irradiation and desiccation than their vegetative counterparts. The pH and temperature ranges within which sporulation occurs are usually narrower than that which permits growth (Kaplan and Williams 1941). Heat resistance of spores within processed foods varies with microorganism species and strain, and the intrinsic factors of food. These intrinsic factors include parameters such as pH, water activity ($a_w$), protein type, fat content and consistency of the canned product (ICMSF 1996). Spores can remain dormant for very long periods of time. Spore germination is triggered in response to a number of nutrient, non-nutrient, enzymatic and physical stimuli that can provide favourable growth conditions (Paredes-Sabja *et al.* 2008).

The thermal inactivation of various pathogenic and non-pathogenic microorganisms has been extensively studied in a range of food products (van Asselt and Zwietering 2006). The D-value is a measure of the heat-resistance of a microorganism and is the time required to bring about a one logarithmic reduction in the concentration of the target microorganism (as illustrated in Figure 4a). D-values are affected by a number of factors including the food matrix, age of the spores/cells, environment in which the spores/cells were produced, incubation temperature and the recovery system. The z-value (as illustrated in Figure 4b) is the change in temperature required to bring about a one logarithmic change in the D-value and provides a mechanism to convert time-temperature heat penetration data into a lethality value. The D-values of bacterial spores important in the manufacture of low-acid canned foods are frequently specified at a reference temperature of 121.1°C (250°F). The range in D-values of several of these bacterial spores are reported in Table 5.

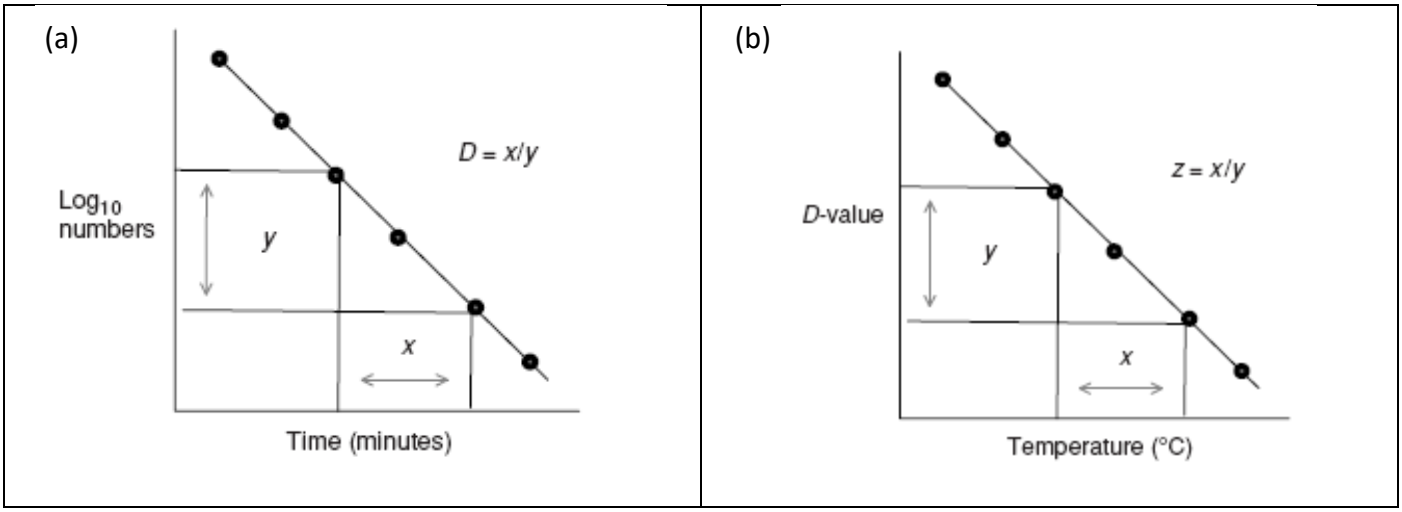


**Figure 4 a) Typical plot used to calculate the D-value of a microorganism; the D-value is the time required in minutes to bring about a one logarithmic (90%) reduction in the concentration of the target microorganism. b) Typical plot used to calculate the z-value of a microorganism; the z-value is the temperature change required to bring about a one logarithmic change in the D-value.**





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**Table 5 Typical range in D-values of pathogenic and non-pathogenic bacteria important in low-acid canned foods. Adapted from FAO (1985) and CSIRO (2019).**

| Organism | D-value (minutes at 121.1°C) |
|---|---|
| *Geobacillus stearothermophilus* (formally *Bacillus stearothermophilus*) | 4.0 - 5.0 |
| *Clostridium thermosaccharlyticum* | 3.0 - 4.0 |
| *Desulfotomaculum nigrificans* (formally *Clostridium nigrificans*) | 2.0 - 3.0 |
| *Clostridium botulinum* (botulinum toxin types A & B) | 0.1 - 0.23* |
| *Clostridium sporogenes* (Putrefactive Anaerobe 3679; ATCC 7955) | 0.1 - 1.5 |

* Several authors including Anderson *et al.* (2011), EFSA (2005) and Huss *et al.* (2004) have reported that the highest known D-value for proteolytic *C. botulinum* spores is 0.25 minutes at 121°C

It remains uncertain when abalone was first commercially canned or retorted. However, in April 1902, a canning company in California (USA) was reported to be handling between two and ten tons of abalone per week (Santa Cruz Island Foundation 2019) and by 1917 four or five plants in the United States of America were producing canned abalone (Cobb 1919). At the time, the abalone meat was sliced and minced, or cut into cubes, prior to retorting (Cobb 1919). Lang and Dean (1934) studied the heat-resistance of *C. botulinum* spores in a range of seafood, including abalone, and concluded that suspension of abalone exhibited a marked inhibitive reaction towards these spores. Lang and Dean (1934) reported that it took 1.57 minutes at 250°F (121.1°C) to destroy *C. botulinum* spores. Since the 1920s significant changes have occurred in the commercial manufacturing of canned foods and relatively few botulism cases have been attributed to commercially-produced low-acid canned foods (Anderson *et al.* 2011).

### Manufacturer's Requirements for Canned Foods

Microbial spoilage in canned foods can be caused by pre-process spoilage, under-processing or post-process leaker contamination. For shelf-stable low-acid canned foods (e.g. canned abalone) *Clostridium botulinum* spores are the primary target microorganism with respect to food safety during the thermal processing operation as they are the most heat-resistant pathogenic bacteria known. *Clostridium* species are commonly found in soil, the aquatic environment, and the intestines of fish and animals (Austin and Dodds 2001, EFSA 2005). The minimum thermal process required to make shelf stable low-acid canned foods (i.e. food with an equilibrated pH greater than 4.6; such as abalone) commercially sterile[4], depends on several factors including the initial microbial load, storage temperature, the presence of various preservatives, products composition, water activity, and both size and type of container.

[4] Commercially sterile is a condition achieved by the application of sufficient heat (alone or in combination with other appropriate treatments) to render the food free from microorganisms capable of growing in the food at normal non-refrigerated conditions at which the food is likely to be held during distribution and storage.





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Food processors, when producing low-acid canned foods focus on achieving 'commercial sterility', rather than 'absolute sterility'[5]. However, the presence and survival of any non-pathogenic mesophilic and thermophilic spores, if not reduced to acceptable levels, can result in food spoilage and substantial commercial losses. Good Hygienic Practices and Good Manufacturing Practices also provide fundamental proactive measures to reduce the initial load of *Clostridium* species by preventing or minimising contamination with soil, dust and faecal matter into the food chain.

The thermal processing of low-acid foods in hermetically sealed containers remains a very critical operation. Scheduled processes for low-acid canned foods must be established by competent persons having expert knowledge of thermal processing and having adequate facilities for making such determinations (Codex Alimentarius Commission 1979). A scheduled thermal process if properly executed is sufficient to eliminate, or to reduce to satisfactory remote levels, all pathogenic microorganism and other spoilage microorganism (Codex Alimentarius Commission 1979). The food industry as a whole has found a 12 logarithmic reduction of *C. botulinum* spores to be an acceptable target level of public health risk for low acid canned foods (Anderson *et al.* 2011). This process is often referred to as a 'botulinum cook', or the *12D* process and is considered the minimum thermal process requirement for food safety. Heat penetration must be determined under the most adverse conditions that are likely to be met in production (Codex Alimentarius Commission 1979). Good manufacturing practices (GMP) currently assumes that *C. botulinum* spores will be present in foods to be retorted at a concentration of one spore per gram, and which requires at least a 12 log reduction in order to bring the probability of their survival to below acceptable levels (i.e. no more than one in a trillion, or ≤1 in $10^{12}$). In practice, the probability of *C. botulinum* spores surviving in the container at points other than the slowest heating point will be less than 1 in $10^{12}$. An alternative food safety assurance level could potentially be based on a food safety objective or the maximum frequency and/or concentration of a microbiological hazard in a food at the time of consumption, or the probability of a nonsterile unit. Pflug (1987) adapted the probability of a nonsterile unit concept and recommended an endpoint probability of a viable *C. botulinum* spore surviving in a container of food should be of the order $10^{-9}$. Currently accepted safe harbours in low-acid canned foods are 12-log reduction in *C. botulinum* or probability of a viable spore being present $<10^{-9}$ per can (Bean *et al.* 2012). However, the food industry has not adopted this approach for establishing sterilisation processes (Anderson *et al.* 2011). Alternatives to safe harbours can only be considered when it is demonstrated that less severe processes provide an acceptable level of public health (Bean *et al.* 2012).

For abalone, the currently adopted minimum required $F_0$ value is typically 2.4 minutes (Warne 2005)[6]. Various microbiological and food safety reference books including Austin and Dodds (2001), Huss *et al.* (2004) and Ryder *et al.* (2014) have reported that for thermal inactivation of foods the canning industry generally uses a D-value of 0.2 minutes at 121°C. However, D-values for *C. botulinum* greater than 0.2 minutes have also been reported by others. Hazzard and Murrell (1989) quoted a $D_{121.1}$ value of 0.23 minutes, and more recently Huss *et al.* (2004), EFSA (2005) and Anderson *et al.* (2011) have reported that the highest known D-value for proteolytic *C. botulinum* spores is 0.25 minutes at 121°C. To achieve process safety for low-acid foods Campden & Chorleywood Food Research Association (CCFRA) (now known as Campden BRI) has recommended that the minimum severity of a thermal process should be equivalent to 3.0 minutes at 121.1°C (i.e. $F_0$ = 3.0 min) when no other mechanism contributes to preservation (May and Archer 1998). Food Standards Australia New Zealand also recommends that for canning low-acid foods (pH>4.6) then the minimum thermal process should also be 121°C for 3 minutes (or equivalent) (Food Standards Australia New Zealand 2024).

[5] Absolute sterility is defined as the complete absence of all viable microorganisms. It requires a more severe thermal process than that to achieve commercial sterility and is unrealistic for canned foods.

[6] An $F_0$ value of 2.4 minutes can be calculated from a $D_{121.1}$ value of 0.2 minutes. This minimum requirement is believed to be based on the review by Stumbo (1973) where it was quoted $D_{121.1}$ values for spores of proteolytic *C. botulinum* are in the order of 0.10 to 0.2 minutes. At the same time Stumbo (1973) also reported that the most heat-resistant *C. botulinum* spores so far studied are characterised by a $D_{121.1}$ value of about 0.21 minutes.





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The *12D* process whilst considered acceptable for the destruction of *C. botulinum* spores, may not ensure the destruction of all spoilage organisms and $F_0$ values greater than 3.0 minutes are commonly used in commercial practices for a range of products. Higher $F_0$ values not only provide a greater margin for safety with respect to *C. botulinum* spores survival, but also reduce the survival levels of other bacterial spores with greater heat resistance (CSIRO 2019). For mesophilic spores other than those of *C. botulinum* a *5D* process is generally regarded as adequate (CSIRO 2019). For thermophilic spores, the scheduled process is generally assessed in terms of the probability of spore survival that is commercially acceptable (CSIRO 2019). A balance that must be considered, when developing a scheduled process, is that lower $F_0$ values (but still greater than the minimum for food safety) can increase the risk of product spoilage, whilst higher $F_0$ values can be damaging to product quality, increase energy requirements and reduce throughput within a retort. For many shelf-stable low-acid canned products, $F_0$ values are likely to be three to four time the allowable minimum (Warne 2005). Canneries must ensure that their thermally processed products are commercially sterile. Levels of non-pathogenic spoilage below 1 in 50,000 containers are often considered to be a minimum goal (May and Archer 1998) whilst spoilage rates of less than one in every million units are frequently achieved (May 2008). Whilst the use of the *12D* thermal process has a long history of safe use, its appropriateness should be scientifically reevaluated (Institute of Medicine and National Research Council 2003). Any under processing of low-acid canned foods can have significant implication for public health and irreversible damage the reputation and financial viability of the food manufacturer.

There are various Codes of Practice and regulatory requirements for thermal processing of low-acid foods. Codex Alimentarius Commission's Code of Hygienic Practice for Low and Acidified Low Acid Canned Foods (CAC/CRP 23-1979) states that the "*heat penetration into the product must be determined under the most adverse conditions that are likely to be met in production*" and that when establishing scheduled processes the *"temperature in the slowest heating point in the container contents should be monitored"*, and *"if accurate heat penetration data cannot be obtained, alternative methods acceptable to the agency having jurisdiction should be used*" (Codex Alimentarius Commission 1979). The Australian Government's Export Control (Fish and Fish Products) Rules 2021[7] stipulates that unless the approved arrangement specifies otherwise, the canning of fish and fish products must result in fish and fish products that are commercially sterile.

The Australian Government's Export Control (Fish and Fish Products) Rules 2021 also includes the following interpretations:

- ***canned*** means thermally processed and enclosed in a hermetically sealed can.
- ***commercially sterile*** in relation to fish and fish products, means free of pathogens that are capable of growing under the conditions the fish or fish products are likely to encounter during storage and distribution at ambient temperature.
- ***site of microbiological concern****,* in relation to fish or fish products, means:
    a) if the site for fish or fish products where pathogens are likely to be located is known—that site; or
    b) in any other case—the thermal centre of fish or fish products.
- ***thermal centre,*** of fish or fish products or an immediate container of fish or fish products, means the last point in the fish or fish products, or container, at which a change in temperature occurs.
- ***thermal process***, in relation to fish or fish products, means the heat sterilisation process in which the fish or fish products, or immediate containers of fish and fish products, are exposed to a defined heating medium at a specified temperature for a specified time for the purpose of making the fish or fish products commercially sterile.

[7] Based on content from the Federal Register of Legislation at 27 February 2026. For the latest information on Australian Government legislation please go to https://www.legislation.gov.au.





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# Appendix C – Literature Review on *Clostridium botulinum* and testing considerations

## *Clostridium botulinum*

*Clostridium botulinum* is a Gram-positive, rod-shaped, obligate anaerobic, spore-forming bacteria. Breed *et al.* (1957) reported that the morphology of vegetative cells of *C. botulinum* type B strains are rods 0.5-0.8 µm in width by 3.0-8.0 µm in length, whereas Smith and Sugiyama (1988) reported straight to slightly curved rods (0.5-2.0 µm in width by 2-10 µm in length). Curved rods (20-45 µm in length) can occur in some cultures when the media is barely adequate (Smith and Sugiyama 1988). The vegetative cells are motile and usually occur singly, less often in pairs or short chains.

*Clostridium botulinum* is considered to be ubiquitous in nature, and its spores are naturally present in soil and sediments of lakes and oceans. This bacteria is a public health risk as it can produce highly potent neurotoxins under low-oxygen conditions leading to botulism. Botulism resulting from exposure to the *C botulinum* toxins, is a rare but potentially fatal illness. The botulinum toxin is one of the most powerful toxins known. The toxins block nerve function and can lead to respiratory and muscular paralysis. Botulism is classified according to exposure and transmission route and can be caused by one of five scenarios, namely:

- foodborne botulism via intoxication from the ingestion of pre-formed neurotoxin in foods;
- wound botulism in which toxin is produced and absorbed from a wound infected with *C. botulinum;*
- infant botulism in which spores are ingested, germinate, and lead to neurotoxin formation in the intestines;
- adult intestinal toxemia botulism caused by intestinal germination, leading to growth and toxin formation; and
- iatrogenic botulism caused from accidental overdose of the botulism toxin.

Foodborne botulism is a significant international hazard especially in reduced oxygen packaged products and needs to be managed accordingly. It is due to this hazard that canned and retorted products are subjected to stringent processing requirements. Neurotoxin levels as little as 30-100 ng is sufficient to cause illness and even death, unless treated appropriately (Peck 2006, Peck 2009). *Clostridium botulinum* strains are classified into one of four physiological groups (I-IV) based on the serological properties of the neurotoxins produced. Most human botulism cases are caused by Group I or Group II strains, and for the canning industry the thermal inactivation of the proteolytic Group I are targeted due to their higher heat resistance.

- Group I: proteolytic and mesophilic; produces toxin types A, B and F;
- Group II: non-proteolytic and psychrotrophic; produces toxin types B, E and F;
- Group III: produces toxin types C and D (generally effects animals; however there have been several documented human cases from type C); and
- Group IV: produces toxin type G (no cases of foodborne botulism reported, however the organism and toxin have been detected in autopsy material).

The distribution and initial concentrations of *C. botulinum* in aquatic and general environments are reported in Table 6. In Australia a small number of surveys have been undertaken to isolate *C. botulinum* from the local environment:

- 183 soil samples from Victoria, four contained *C. botulinum* type A (Eales and Gillespie 1947);
- 22 marine mud samples from Tasmania, two samples were positive for *C. botulinum* type B (Ohye and Scott 1957);
- 528 samples of mud, cultivated soil, fish intestines and potato washings collected between 1965-1970 from New South Wales, Tasmania and Queensland. No *C. botulinum* was detected (Christian 1971);
- C. *botulinum* type A was detected in a Sydney suburb and 16-20% of rural areas surveyed in New South Wales were contaminated with either *C. botulinum* type A or B spores (Murrell and Stewart 1983); and
- 368 samples from ship ballast waters, harbour sediments, port sediments and estuarine sediments. One ballast sample (out of 281 ballast samples) contained *C. botulinum* type C, all other samples were negative (Gibson *et al.* 1994).





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Whilst *C. botulinum* is present in the Australian environment, botulism is a very rare disease (McCallum *et al.* 2015). Targeted sampling has also reported a very low prevalence in New Zealand marine sediments (Fletcher *et al.* 2008).

**Table 6 Natural habitat of *C. botulinum*. Adapted from Ryder *et al.* (2014).**

| *C. botulinum* type | Distribution | Levels in fish at primary production stage |
|---|---|---|
| Group I (proteolytic toxin types A and B) | Worldwide, soil | Generally low |
| Group II (non-proteolytic toxin types B, E, and F) | Worldwide, higher incidences in temperate waters | <0.1 – 5.3 spores/g fish |

Control of *C. botulinum* can be achieved by inactivation of vegetative cells and spores or by the inhibition of growth. The main factors that control the growth of *C. botulinum* in foods are temperature, pH, salt, $a_w$, redox potential and the presence of any preservatives. The limiting growth factors for Group I-IV strains are reported in Table 7, however, these factors are seldom independent.

**Table 7 Widely accepted limiting growth factors of *C. botulinum*. Adapted from McLauchlin and Grant (2007), Peck (2009) and Ryder *et al.* (2014).**

| Parameters | Group I | Group II | Group III | Group IV |
|---|---|---|---|---|
| Neurotoxin types | A, B, F | B, E, F | C, D | G |
| Human disease | Yes | Yes | No[a] | No |
| Growth temperatures<br>Minimum<br>Optimal<br>Maximum | <br>10 °C<br>35-40 °C<br>48 °C | <br>3 °C<br>18-25 °C<br>45 °C | <br>15 °C<br>40 °C<br>ND | <br>ND[b]<br>37 °C<br>ND |
| Minimum pH for growth | 4.6 | 5.0 | 5.0 | ND |
| Inhibitory $a_w$ for growth | 0.9353 | 0.97 | ND | ND |
| Inhibitory by NaCl | 10% | 5% | 2% | ND |
| $D_{100\ °C}$ of spores | ~25 min | <0.1 min | 0.1-0.9 | 0.8-1.12 |
| $D_{121\ °C}$ of spores | 0.21 min | <0.005 min | ND | ND |





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| | | | | |
|---|---|---|---|---|
| Related non-neurotoxigenic *Clostridium* species | *C. sporogenes*, *C. putrificum* | *C. beijeriinkii* | *C. novyi*, *C. haemolyticum* | *C. subterminale*, *C. histolyicum*, *C. linosium* |

[a] *C. botulinum* Group III generally effects animals, however, there have been several documented human botulism cases from type C

[b] ND, not determined

### Testing considerations for *C. botulinum*

In Australia *Clostridium botulinum* is categorised as a Tier 2 Security Sensitive Biological Agent (SSBA) (those of a high security concern) and is managed through the Security Sensitive Biological Agents Regulatory Scheme (Australian Centre for Disease Control 2026). Soloman and Lilly (2001) and Lindström and Korkeala (2006) review the complexed laboratory diagnostic methods for isolation and characterisation of *C. botulinum* and for the diagnosis of botulism, respectively indicating that laboratory detection methods for *C. botulinum* and botulism are not suited to routine food microbiological laboratories. The same would apply to research institutions. This is because of the complexity of the anaerobic methods for bacterial isolation and the need to test for the neurotoxin for the confirmation of botulism by mouse bioassay. Added to this is the need for enhanced regulated safety precautions for staff within the laboratory (EFSA 2005). The use of *C. botulinum* in experimental studies adds a number of complexities (particularly legal and occupational health and safety) that makes its use expensive and prohibitive in many situations. To help overcome many of the occupational, food safety and logistical challenges and constraints when handling bacterial pathogens, non-toxigenic organisms can be and are frequently used as surrogates. Suitable surrogates must be carefully selected and justified (Hu and Gurtler 2017). An organism that is closely related, and with similar microbial features, to *C. botulinum* is *C. sporogenes* and its use is substantially less complexed.

*C. sporogenes* and *C. botulinum* are both Gram-positive, rod-shaped bacteria that exhibits spore production and flagellar motility. Morphological comparisons between vegetative cells and spores of *C. botulinum* and *C. sporogenes* are reported in Table 8.

**Table 8 Comparisons in the morphology of *C. botulinum* and *C. sporogenes* cells and spores**

| State | *C. botulinum* (type B) | *C. sporogenes* |
|---|---|---|
| Vegetative cell | Gram positive<br><br>Rods with rounded ends, occurring singly, in pairs and in short to occasionally long chains<br><br>- 0.5-0.8 µm in width by 3.0-8.0 µm in length (Breed *et al.* 1957)<br><br>Straight or slightly curved rods<br><br>- 0.5-2.0 µm in width by 2-10 µm in length (Smith and Sugiyama 1988)<br><br>Motility by peritrichous flagella | Gram positive<br><br>Rods with rounded ends occurring singly, in pairs or less frequently, in short to long chains and filaments<br><br>- 0.6-0.8 µm in width by 3.0-7.0 µm in length (Breed *et al.* 1957)<br><br>Motility by peritrichous flagella |





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| | | |
|---|---|---|
| Spore | Ovoid, central, subterminal, terminal at maturation, swelling the cells<br><br>- ~1.8°µm in diameter (Hodgkiss *et al.* 1967) | Ovoid, eccentric to subterminal, swelling the cell<br><br>- 2.4±0.3 µm in diameter (Yang *et al.* 2009) |

PA 3679 is a nontoxigenic putrefactive anaerobe, whose heat resistance is greater than the maximum recorded for spores of *C. botulinum*. It was first isolated in 1927 from spoiled canned corn in the research laboratories of the National Canners Association and was considered to be *C. sporogenes*. Various other putrefactive anaerobes culturally similar to PA 3679, have subsequently been isolated from various foods referred to as PA 3679 or as strains of PA 3679 (Brown *et al.* 2012). Brown *et al.* (2012) confirmed by 16S rRNA sequencing that PA 3679 from the American Type Culture Collection (ATCC) (listed as ATCC 7955) is a strain of *C. sporogenes*. Despite the development of a nontoxigenic mutant, *C. botulinum* 62A, *C. sporogenes* PA 3679 is considered to remain the more suitable thermal processing surrogate for inoculated pack studies (Brown *et al.* 2012). The degree of relatedness of other *C. sporogenes* isolates to *C. botulinum* Group I are reported to vary (Brown *et al.* 2012). Various strains of *C. sporogenes* have also been used as a surrogate for *C. botulinum* in growth models (Koukou *et al.* 2022).





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## Appendix D – Literature Review on Bacterial Internalisation in Abalone

In order to study or understand the mechanisms and likelihood of internalisation of microbes of concern in prepared/canned abalone products it is best to understand the relationship of abalone and their microbes under natural circumstances and commercial farming and holding circumstances. A search of the published reviewed literature using the following search streams: "(haliot* OR abalone OR paua OR "bao yu" OR ormer) AND (clostri* OR botul*)" in the title, abstract, keywords and keywords plus was undertaken on 7 November 2019 through Web of Science (all databases). The search yielded ten publications since 1864 to present, however, only four of these papers directly involved studies on abalone, and a summary from these papers are below.

- Nel *et al.* (2018) investigated the gut-bacterial communities in juvenile South African abalone *Haliotis midae* after they were fed different feeds during weaning. Whilst the authors did not directly assess any haemolymph samples, bacterial DNA was isolated from pooled samples that consisted of all soft tissues including the foot muscle and viscera. Consequently, it is unknown if any of the bacterial DNA would have originated from the foot or haemolymph, or was solely based on the viscera. The authors reported that anaerobic *Clostridium* species dominated the gut microbiota of abalone fed kelp, whereas a variety of bacterial genera were prevalent in the gut of abalone fed a commercial formulated fed.

- Dixon *et al.* (1991) recovered *Clostridium lituseberense*[8] and *Vibrio alginolyticus* from 'tissue samples' of moribund juvenile *H. midae* during an experimental feeding trial. The feeding trial was to establish the apparent digestibility of several macroalgae, however the juvenile animals started dying of an unknown cause. The 'tissue samples' were reportedly collected following the procedure described by Elston and Lockwood (1983), although on review Elston and Lockwood (1983) described that they collected microbiological samples using a bacteriological loop from a shallow aseptic incision into the pedal muscle which had been swabbed with 70% ethanol. Dixon *et al.* (1991) plated the 'tissue samples' onto blood agar (non-selective; one aerobic and one anaerobic plate), nutrient agar (non-selective; aerobic), MacConkey agar (differential agar for Gram-negative and enteric bacteria; aerobic) and deoxycholate citrate agar (selective for Gram-negative bacteria; aerobic) plates. Plates were incubated at 37°C for 48 h and in all cases microbial growth was not observed. A tissue sample was then inoculated into tryptone soya broth (an enrichment media), incubated overnight and re-plated. Microbial growth was subsequently observed on some of the plates, including the blood agar plate incubated under anaerobic conditions. Cultures were purified and later identified as *C. lituseberense* (identification method not reported) and *V. alginolyticus* (identified using API 20E strip system for *Enterobacteriacaea*). Dixon *et al.* (1991) postulated that the abalone probably harboured *C. lituseberense* in their digestive tract which produced toxins when the abalone were subjected to stressful conditions during capture and transport.

- Warne (1982) assessed abalone canning-cooking processes in Australia and highlighted the thermal lethality requirement of $F_0$ values of 2.8 min to be achieved in all parts of the product in order to be safe from *C. botulinum*.

- Lang and Dean (1934) reported that abalone exhibit a marked inhibitive reaction towards the spores of *C. botulinum*. This study aimed to determine the thermal resistance of *C. botulinum* spores in various canned seafood products. The authors also stated that abalone from California's commercial fisheries is canned either minced or in chunks immersed in its own broth.

A broader search of "(bacteria OR bacteremia OR bacteraemia OR sepsis OR septic*) AND (hemolymph OR haemolymph)" in the title, abstract, keywords and keywords plus was undertaken on 10 January 2020 through Web of Science (all databases) to identify relationships or interactions with bacteria in the haemolymph of all invertebrates. The search yielded 5,421 results, which when refined to also include "(haliot* OR abalone OR paua OR "bao yu" OR ormer)" in the title, abstract, keywords and keywords plus yielded 64 results. Restricting the initial search to only the Web of Science Core Collection yielded 1,523 results. A scan of the shortlisted publications identified that abalone were linked to several of these studies for a variety of reasons including abalone

[8] Older literature often used the spelling *Clostridium lituseberense*, whilst some more modern literature spelt this species as *Clostridium lituseburense*. However, the current nomenclature is *Romboutsia lituseburensis.*





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being a carrier of some of the bacterial pathogens, animal-health related diseases or infections, or abalone haemolymph or haemolymph components potentially being a source of antimicrobial compounds. Several *in-vivo* studies also demonstrated that crustaceans and bivalves can clear bacteria from the haemolymph (Hartland and Timoney 1979, Martin *et al.* 1993, Martin *et al.* 1996, Van de Braak *et al.* 2002, Macey *et al.* 2008, Montalvo *et al.* 2016). However, several other investigations, including those summarised below, have recovered microorganisms or bacterial DNA from haemolymph samples abalone.

- Offret *et al.* (2019a) investigated the diversity of bacteria and other microorganisms with antimicrobial activity present in the coelomic fluid and haemolymph of wild and healthy echinodermata and mollusca. They recovered cultivable *Pseudoalteromonas* sp., *Cellulophaga* sp. and *Psychrobacter* sp. (all Gram-negative bacteria) from the haemolymph of healthy European abalone *Haliotis tuberculata*. No yeasts or moulds were detected. The authors used Marine Agar (for heterotrophic marine bacteria) and Potato Dextrose Agar (for yeasts and moulds). The Marine Agar and Potato Dextrose Agar plates were incubated at 18°C for 5 day and 25°C for 3 weeks, respectively. The DNA from purified colonies was isolated and the recovered cultivable microorganisms were identified using partial 16S rRNA gene sequencing. The concentration of bacteria in the haemolymph of *H. tuberculata* was 2.37 ±1.04 $\log_{10}$ CFU $mL^{-1}$ (n=15) and were more abundant than the seawater that was collected at the same time. Offret *et al.* (2019a) reported a larger variability in the observed bacterial concentration in the haemolymph of *H. tuberculata* when compared to the other invertebrates in the study. Bacterial concentrations in *H tuberculata* ranged from 0 to 3.79 $\log_{10}$ CFU $mL^{-1}$. Comparatively, the concentration of bacteria in the seawater that was collected at the same time was 1.57 ± 0.13 $\log_{10}$ CFU $mL^{-1}$.

- Offret *et al.* (2019b) demonstrated that a *Pseudoalteromonas* species (designated hC*g*-6), originally isolated from the haemolymph of oysters and mussels, could be rapidly detected in the haemolymph of the European abalone *H. tuberculata* following exposure by short-term immersion in seawater spiked with the bacterial culture. The animals (3-4 years old) were exposed to the *Pseudoalteromonas* sp. by immersion for 4 h at 19°C. The *Pseudoalteromonas* was added to the seawater such that the final concentration in the tank was $1x10^6$ CFU $mL^{-1}$. The concentration of *Pseudoalteromonas* in the haemolymph was quantified by qPCR; baseline concentration was approximately 63 CFU $mL^{-1}$ and then increased to a mean of approximately $2.5x10^3$ CFU $mL^{-1}$ after the 4 h exposure. Whilst the mechanism of bacterial internalisation was not investigated in the study, it is acknowledged that it could have been through the gills (C. Offret, personal communication, 27 April 2020). The health status of the abalone was checked but not reported. The *Pseudoalteromonas* provided the animals with a protective effect against *Vibrio harveyi*. The authors used 70% ethanol to disinfect the sampling zone, and the haemolymph was collected from the pedal sinus. Offret *et al.* (2019b) also exposed *H. tuberculata* to *Pseudoalteromonas* sp. by immersion at $1x10^8$ CFU $mL^{-1}$, without any animal mortality or abnormal behaviour.

- Dubief *et al.* (2017) recovered *Vibrio harveyi* DNA from the haemolymph of the European abalone *H. tuberculata* (shell length approximately 80 mm) following an immersion challenge study with a known virulent strain that had previously been recovered from diseased abalone. The study was designed to identify potential mechanisms of disease resistance between two geographically distinct populations (namely Saint-Malo and Molène, France). For each population, the animals (22 per tank) were exposed to no bacteria (control treatment) or $10^4$ CFU $mL^{-1}$ of *V. harveyi* for 24 h followed by a repeat exposure 28 days later. Water temperature was 18°C and the total haemocyte count, haemocyte viability and phagocytosis index was also determined. The experiment was undertaken in triplicate, and any dead animals were removed from the system. Haemolymph was collected from non-moribund abalone using a sterile syringe and the animals dissected to recover the gills. No disinfection method was reported prior to sampling. The sensitivity threshold of qPCR was estimated to be $7.5x10^2$ CFU $mL^{-1}$ and $2.5x10^2$ CFU $mL^{-1}$ for the haemolymph and gills, respectively. At the end of the challenge study there was nearly a 50% mortality rate of the Molène population when exposed to *V. harveyi*. *V. harveyi* was detected in the gills of 21 individuals (15 individuals exposed to *V. harveyi* from Molène, 5 individuals exposed to *V. harveyi* from Saint-Malo and 1 individual unexposed control from the Molène population) and in the haemolymph of 7 individuals (4 individuals exposed to *V. harveyi* from Molène, 1 individual exposed to *V. harveyi* from Saint-Malo, and 1 unexposed control from Molène and Saint-Malo populations). Those that were positive had a *V. harveyi* concentration in the haemolymph near the detection threshold of the qPCR method. Dubief *et al.* (2017) also demonstrated that two *V.*





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*harveyi* strains (one pathogenic and the other non-pathogenic) were able to grow in an acellular fraction of the abalone haemolymph.

- Cardinaud *et al.* (2015) analysed the response of European abalone *H. tuberculata* haemocytes, at the cellular and molecular levels during the first 24 h of *V. harveyi* infection. The exposure method was reported to follow that of Travers *et al.* (2009) and was based on an 24 h immersion process with an inoculum of approximately $10^5$ CFU $mL^{-1}$. The authors detected and quantified *V. harveyi* in the haemolymph by qPCR 9 h after exposure, whilst the abalone exhibited an overexpression of the Rel/NF-kB gene after 3 h of exposure. The detection threshold of the qPCR method was 10 CFU $mL^{-1}$.

- Cardinaud *et al.* (2014) reported that *V. harveyi* was detected in the haemolymph of the European abalone *H. tuberculata* after 3 h exposure; although 1 h of exposure was sufficient to induce mortalities. In this investigation sexually mature *H. tuberculata* (weight/shell length not reported) were preconditioned to 19°C and exposed to *V. harveyi* at either $1x10^5$ CFU $mL^{-1}$ or $1x10^6$ CFU $mL^{-1}$ by immersion. No details were provided on the method to prevent sample contamination during haemolymph collection, whilst the central portion of the pedal muscle and other tissues were dissected under sterile conditions and rinsed in 70% ethanol followed by sterile seawater to quantify the concentration of penetrated bacteria. The authors reported that *V. harveyi* first colonizes the mucosal area between the gills and the hypobranchial gland, then penetrates the whole gill-hypobranchial gland tissue, before invading other tissues. The authors also stated that *V. harveyi* is a frequently reported pathogen of marine vertebrates and invertebrate species and is believed to be responsible for a number of mass mortalities during summer spawning in both natural and farmed stocks of *H. tuberculata*.

- Givens *et al.* (2013) stated that it has been assumed that, based on studies of vertebrates, the haemolymphs of health invertebrates are sterile. The authors cited two studies (Tanaka *et al.* 2004, Huang *et al.* 2010) where it was reported that *Mycoplasma* sp. have been associated with the gut microbiota of abalone. No mention was made on any microbiological contamination of the haemolymph in abalone.

- Kamaishi *et al.* (2010) recovered *Francisella* sp. from the haemolymph of moribund Giant abalone *Haliotis gigantean* following a mass mortality event. No details were provided on the method of haemolymph collection. The DNA was amplified using universal 16S rRNA bacterial primers (20F and 1500R).

- Hooper *et al.* (2007b) whilst investigating the cause of an abalone mass mortality event, which was subsequently attributed to AVG, found no culturable bacteria in the haemolymph of three morbid or dead animals when plated onto Horse Blood Agar, TCBS (for oxidase positive organisms such as *Vibrio* spp.), and McConkey's Agar (for enteric pathogens such as *Salmonella* spp.), but a light growth of mixed bacteria was isolated from one of the animals (study did not report which agar exhibited the mixed bacteria). No further investigations were reportedly undertaken on the bacteria recovered from this animal.

- Lee *et al.* (2001) assessed the virulence of *Vibrio alginoltyicus* and *Vibrio parahaemolyticus* on the small abalone *Haliotis diversicolor supertexta* at different temperatures following intra-mantle injections with the pathogens. The vibrio strains had originally been isolated from the haemolymph of moribund *H. diversicolor supertexta*. The authors re-isolated the corresponding *Vibrio* species from the haemolymph of the moribund animals and demonstrated that elevated temperatures play a critical role in the development of vibriosis.

- Liu *et al.* (2000) reported that two *V. parahaemolyticus* strains were isolated from the haemolymph of moribund *H. diversicolor supertexta* during vibriosis outbreaks. No details were provided on the method of haemolymph collection. The *V. parahaemolyticus* strains were recovered using tryptic soy agar supplemented with 3% NaCl and/or thiosulphate citrate bile salt sucrose agar and partially characterised through biochemical tests.





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- Dixon *et al*. (1991) reported that *V. alginolyticus* can readily colonise the substratum of abalone which enables intimate contact with the foot. The authors also state that *V. alginolyticus* can rapidly colonise the musculature of the foot if there is any open wound, and once the foot is invaded the bacteria colonises the vascular system and nerve fibre sheaths.

- Elston and Lockwood (1983) reported isolating *V. alginolyticus* from moribund juvenile Red abalone *Haliotis rufescens* (shell length 0.2 mm to 1.5 cm). Microbiological samples were collected after swabbing the ventral foot surface with 70% ethanol. A shallow aseptic incision was made into the pedal muscle and a bacteriological loop inserted into this incision. Samples were plated on both Marine agar and TCBS agar. Physically moribund animals showed a loss of yellow cephalic tentacle pigmentation, red oral pigmentation and black pigmentation of the epipodium and foot. The cephalic and epipodial tentacles were short, flaccid and bulbous rather than long, slender and actively groping as in the normal animal. Some animals exhibited localised swellings of the foot or epipodium. The mouth was frequently flared-out and flaccid. Where the disease was apparently further advanced, the animals showed a general visceral shrinkage and retraction of the foot muscle further into the shell than normal.

A subsequent search of "(bacteria OR bacteremia OR bacteraemia) AND (hemolymph OR haemolymph) AND (*Clostridium* OR *clostridia*)" in the title, abstract, keywords and keywords plus on 10 January 2020 through Web of Science (all databases) yielded 19 results. These papers were reviewed but were not considered to be relevant as they did not report any studies on abalone.

A subsequent search of "(bacteria OR bacteremia OR bacteraemia OR sepsis OR septic*) AND (hemolymph OR haemolymph) AND (spore)" in the title, abstract, keywords and keywords plus on 10 January 2020 through Web of Science (all databases) yielded 118 results. These papers were reviewed but were not considered to be relevant as they did not report any studies on abalone.



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# Appendix E – Literature Review on Bacterial Migration Studies and Abalone Muscle Structure

Bacterial migration studies can be used to help understand how a pathogen or other microorganism moves, invades tissues, spreads through the environment or travel between hosts. Bacterial migration studies can be completed through either bacterial enumeration of various tissue samples, utilisation of histological techniques or visual analysis. Bacterial migration studies in foods are primarily conducted to help ensure food safety and to understand and prevent microbial spoilage and cross-contamination. Blue Lake is a water insoluble food-grade dye and has been used to model the potential for bacterial migration and/or penetration in various foods including meat and eggs (de Zuniga *et al.* 1991, Anderson *et al.* 1992, Kim and Slavik 1996, Bosse *et al.* 2017). One of the key advantages of utilising this technique is that the insoluble dye is easy to visualise, and that the particle size is similar to most bacteria. The particle size of Blue Lake dye is quoted variably in the literature. de Zuniga *et al.* (1991) and Anderson *et al.* (1992) both stated that the mean particle size was 0.6 µm in diameter, whereas Bosse *et al.* (2017) reported a median diameter of 3.18 µm and a mean diameter of 6.18 ± 0.32 µm.

Blue Lake dye can generally penetrate intact bovine tissues to a depth of 1 to 2 mm during pressurised carcass washing (de Zuniga *et al.* 1991), whilst penetration depths up to 5-6 mm were possible on cut surfaces (Anderson *et al.* 1992). Similarly, Bosse *et al.* (2017) reported that once pork muscle has been “injured” (e.g. through an injection), its structural integrity is lost, and it becomes possible for particles and thus likely also for bacteria to spread into the “uninjured” parts of the muscle utilising the intermediate spaces with sarcoplasm, between the muscle fibres. Mechanical treatments including tumbling, or vacuum can lead to additional tissue damage and enhanced particle migration (Warsow *et al.* 2008, Bosse *et al.* 2017).

There were no identified publications containing information on the potential for, or the extent of, bacterial migration in abalone tissue during any post-harvest processing. The migration and distribution of bacteria has been studied in a range of terrestrial animal muscle tissues (as reported above). These studies have highlighted that bacterial migration is dependent on a number of intrinsic and extrinsic muscle factors, such as fibre orientation, hydration of meat proteins and the availability of pores and canals in the muscle tissue (Bosse *et al.* 2015). The tissue structure of abalone is different to other land-based animal protein sources as the muscle fibres in abalone are arranged in a isotropic 3-dimensional weave, with collagen surrounding both the fibres and fibre bundles (Øiseth *et al.* 2013). For land-based animals the muscle tissue are generally assembled in parallel fibrous bundles. An example of scanning electron microscopy images of the muscle structure from the foot and adductor tissues from farmed Red abalone (*H. rufescens*) are shown in Figure 5.

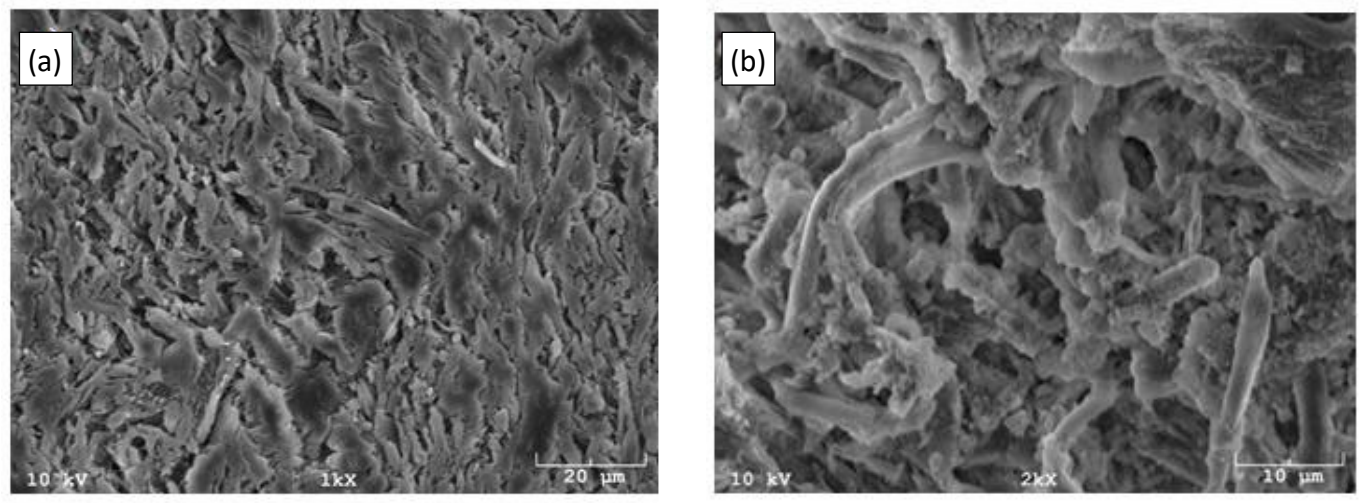


**Figure 5 Scanning electron microscopy images of uncooked farmed Red abalone (*Haliotis rufescens*) (a) muscle tissue and (b) adductor tissue. Reproduced from Hughes (2014).**





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Further information is required in order to help understand the impact of post-harvest processing on abalone tissue and the extent of bacterial migration (specifically spore forming bacteria) under normal circumstances, as well as under these enhanced situations during the canning process.



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