

Rock Lobster Enhancement and Aquaculture Subprogram: Investigation of Tail Fan Necrosis in Live-Held Adult Southern Rock Lobsters

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**Final Report
Project No. 2000/211**

Rock Lobster Enhancement and Aquaculture Subprogram: Investigation of Tail Fan Necrosis in Live-Held Adult Southern Rock Lobsters

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Non-technical summary

20001/211	Rock Lobster Enhancement and Aquaculture Subprogram: Investigation of Tail Fan Damage in Live-Held Adult Rock Lobsters
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Objectives

- (1) To investigate potential causes of tail fan damage in live-held adult southern rock lobster.

Further objectives developed as a result of variation of the project

- (2) To characterize the microbial pathology of tail fan necrosis (TFN).
- (3) To evaluate the effectiveness of “bagging” lobsters post capture in minimizing the development of TFN.
- (4) To undertake a survey of the occurrence of TFN in major rock lobster fisheries in Australia.

Outcomes achieved to date

Several important conclusions can be made from this study.

- TFN develops at ambient summer temperatures under various holding conditions.
- TFN develops at 15°C although not as rapidly as at 23°C.
- Bagging at capture, limits physical damage and subsequent development of TFN in live-holding.
- Inflicting physical damage with sterile instruments does not promote TFN.
- Bacteria, especially several species of *Vibrio*, are associated with the TFN disease.
- Inflicting physical damage and inoculating *Vibrio* does induce TFN and the *Vibrio* inoculated can be recovered from the infection showing their causal role.
- The occurrence of TFN in fisheries in Australia has been recorded but not well documented.

Non-technical summary

An earlier project on liveholding of adult southern rock lobster, RLEAS 98/305, demonstrated that adult SRL could survive, feed, moult and grow when held in sea cages or raceways and fed an artificial diet developed in RLEAS 98/303. The major obstacle identified to such an industry was that lobsters live-held at ambient temperatures developed a tail fan disease involving melanization and loss of tissue. We have named this condition tail fan necrosis (TFN). Lobsters with advanced TFN are not acceptable for live export market and this limits any live-holding industry. There was a need to characterize the TFN condition, to see if it was correlated with environmental factors such as holding conditions (density and feeding patterns) and temperature. The microbial species involved in TFN needed to be identified and their role in TFN verified. Finally ways of limiting TFN needed to be proposed.

The present study comprised four experimental components including a survey of awareness and concern about TFN in industry.

- (1) Adult SRL were live-held in raceways for four months under various husbandry treatments. This trial investigated the effects of post-harvest handling, holding density and feeding pattern. The post harvest handling treatment involved a novel

regime where lobsters were placed in individual mesh bags at capture and kept in these bags through the post harvest period until released into the experimental system. This treatment was termed “bagging” and was expected to limit physical damage post harvest and the subsequent development of TFN.

This experiment showed that bagging lobsters did minimize physical damage post harvest and that this was reflected in lower levels of advanced TFN in the 4 month live-holding trial. Most of the lobsters in the other treatments developed TFN with no substantial differences at different holding densities (10 and 20 per cage) or different feeding regimes (fed daily, weekly or starved).

- (2) To investigate the effect of temperature on the development of TFN, lobsters were held individually in tanks at 15°C and 23°C for six weeks with samples taken each two weeks. At each temperature there were three groups of 20 lobsters. A group from the fishery with normal post harvest handling, a group that were bagged at capture and a third group that were bagged at capture and then inflicted with physical tail damage using sterile instruments. The bagged damaged treatment was included to see if physical damage would initiate TFN.

TFN developed in both the 15°C and 23°C treatments. A temperature effect was identified at the end of two weeks, however, by four and six weeks the temperature regimes had little apparent effect on TFN. There were significant bag effects at all sampling times with unbagged lobsters showing the highest level of advanced TFN. The results for the bagged and bagged-damaged treatments showed significantly lower occurrence of TFN and there were no significant differences between the bagged and bagged damaged treatments suggesting that physical damage with sterile instruments did not lead to development of TFN.

- (3) Further characterization of the bacteria responsible for TFN involved the isolation of bacteria from lobsters with TFN and the reinoculation into healthy tail fans to see if the disease could be induced. The bacterial flora of TFN tissue from field and laboratory experiments mostly comprised marine vibrios including *Vibrio vulnificus*, *Vibrio parahaemolyticus* and *Vibrio alginolyticus*. These bacteria were isolated from TFN tissue, grown in pure culture and inoculated into healthy tail fans. Inoculated tail

fans developed TFN demonstrating that the bacteria are responsible for the disease. Furthermore bacteria recovered from the diseased tail fans were generally the same species that was inoculated, although other common vibrios were also isolated from some of the cultures. These results show that the *Vibrio* bacteria are responsible for the TFN disease.

Overall, the experimental evidence from this project implicates physical damage and the contamination of damaged tissue with *Vibrio* bacteria as the principal cause of TFN. *Vibrio spp.* are commonly found in the marine environment and on lobster carapaces. Post-harvest is a key time when TFN can be initiated as during handling or while in close proximity lobsters can inflict wounds on themselves and others and these wounds involve inoculation of bacteria. Bagging immediately post capture can limit physical damage and minimize the development of TFN in live-holding.

- (4) The survey of the occurrence and awareness of TFN in various areas of the Australian rock lobster industry was undertaken by a questionnaire distributed to key industry members in Queensland, Victoria, Tasmania, South Australia, and Western Australia. The response was poor but indicated a general awareness of TFN throughout the industry. Most respondents indicated the occurrence of TFN was low (<5%), and they did not see TFN as a major wild fishery industry concern.

Acknowledgements

We wish to thank South Australian Seafoods (SAS), Port Lincoln, for the provision of raceway space and support in running the field experiment. We are very grateful to the staff at the SAS farm assisted with the construction of cages, the maintenance of lobsters and the two monthly assessments of the lobsters. Thanks especially to Mike Leech and Melissa Lorkin who coordinated the maintenance of the experiments and our visits.

Several rock lobster fishers assisted by capturing and bagging lobsters for use in the experiments. Lobster processors at Port Lincoln assisted by accumulating and holding appropriate lobsters for our experiments. In particular we thank Brenton Symons and Peter of Australian Southern Rock Lobster Exporters for assistance and space for handling and assessing lobsters throughout the experiment.

The lobster pellets used in these experiments were produced by Eyre Peninsula Aquafeeds at Cummins Mills, and we thank Tom Coote and Kym Heidenreich.

Much of the bacterial characterization was done by Damien May as part of his BSc (Hons) in Microbiology and Immunology at the University of Adelaide.

Finally thanks to the several volunteers at SARDI West Beach.

1. Background

The Fisheries Research & Development Corporation's Rock Lobster Enhancement and Aquaculture Subprogram (RLEAS) was formed in 1998. Project 5 (FRDC 98/305) of this subprogram was concerned with the determination of optimum environmental and system requirements for juvenile and adult rock lobster holding and grow-out. Part of this Project was focused on a small but expanding industry in South Australia based on the liveholding of wild-caught adult southern rock lobster, and on the development of an Australia-wide aquaculture industry based on the grow-out of juvenile rock lobster from pueruli. Project 5 has been completed and the Final Report- 1998/305 is available. The present research follows on from some of the findings of Project 1998/305.

Live-holding of wild-caught lobsters is seen as a means of value-adding to the existing commercial catch of southern rock lobsters in South Australia (worth approximately \$100 million export revenue). This value adding can occur in two ways: strategic marketing and product enhancement. By having access to holding facilities, fishers can strategically market their catch by holding lobsters at times of low prices and then selling them at times of higher prices. In addition, live-holding may also enable product enhancement (i.e. increases in weight through growth, and improvement in condition through feeding and growth of damaged, sick, and "white" lobsters). These white lobsters are captured in deep, offshore areas, and are paler in colouration than red inshore lobsters. The lack of pigmentation in white lobsters is believed to be due to a deficiency in their diet, and they are not well accepted in Asian markets. However, not only is their colour different, but their condition is poorer and they do not handle live export as well as inshore red lobsters. This then results in poorer prices and associated lower fishing effort for the white lobsters. Therefore the ability to change colour (for market acceptance) and to improve condition (for exportability) of white lobsters could lead to increased catches from this under-utilized resource in the Northern Zone Fishery.

In field trials during Project 98/305, high survival rates, good weight gains, and improved physiological and external condition (damage and colour) were observed in live-held lobsters. These results were promising for an industry based on the long-term live-holding of lobsters for strategic marketing alone. However, in order for

an industry to develop based on the live-holding of lobsters for product enhancement, improvements are required in the areas of weight gain, colour change, holding time, and feeding efficiency. In addition, tail fan damage and infections was found to be a major problem with long-term live-held southern rock lobster. In fact, the problem is seen to be a major obstacle to the future success of the live-holding industry and was subsequently identified by the RLEAS Steering Committee as a high priority for further investigation. The present proposal is therefore aimed at identifying the cause of tail fan damage and infection in live-held adult southern rock lobster. We have defined the condition as tail fan necrosis (TFN). TFN is characterized loss of tissue and progressive necrosis of the tail fan with associated melanisation of the area. By identifying the cause of tail fan necrosis, preventative strategies may then be employed during live-holding operations.

Despite the severity and importance of tail fan necrosis, little is known about its cause or progression. Observations from Project 98/305 suggest that the severity of tail fan necrosis may be related to seasonal water temperatures, with summer temperatures promoting the worst damage. This apparent relationship to temperature could in turn be related to bacterial numbers; bacterial infections (especially *Vibrio*) were identified from damaged tails in Project 98/305. However, it is not known if the bacteria are incidental or causative. Therefore, future research should focus on the effect of post-harvest handling, feeding and holding conditions and temperature on TFN. Bacteria and other agents involved in the infection should be identified to provide an understanding of the microbiology of TFN.

2. Need

FRDC Rock Lobster Enhancement and Aquaculture Subprogram Project 98/305 investigated the environmental and system requirements for juvenile and adult rock lobster holding and grow-out. Results from this project showed that adult southern rock lobster can be held long-term in industry facilities with high survival rates and that condition and weight can be improved. However, in order for a large-scale industry to develop in this area, there is a definite need for research aimed at reducing the incidence of tail fan necrosis in live-held adult southern rock lobster. Tail fan necrosis has also been identified by the Geraldton Fishermen's Co-operative as a problem with live-held western rock lobster prior to live export.

3. Objectives

- (1) To investigate potential causes of tail fan damage in live-held adult southern rock lobster.

Further objectives developed as a result of variation to the project

- (2) To characterize the microbial pathology of tail fan necrosis.
- (3) To evaluate the effectiveness of “bagging” lobsters post capture in minimising the development of TFN.
- (4) To undertake a survey of the occurrence of TFN in major rock lobster fisheries in Australia.

4. Technical Report

4.1 Methods

The factors investigated included the effects of post-harvest handling, feeding frequency, density of holding, and temperature on the development of TFN. In particular, a new post-harvest handling strategy of placing individual lobsters in mesh bags directly after capture and keeping them in those bags until they entered various long term holding experiments was trialed. This was termed “bagging”. All experiments with feeding used a feed formulation developed by Dr Kevin Williams for a previous Rock Lobster Enhancement and Aquaculture Subprogram (Program No. 2000/212), classified as RL35D (Table 1). Pellets were produced by Eyre Peninsula Aquafeeds at Cummins Mills, South Australia. In all feed treatments, lobsters were allocated 2% body weight per day of the diet.

Table 1. Rock lobster diet used in field and laboratory trials (RL35D)

Component	Percentage
Fish Meal	45.7
Wheat Gluten	6
Wheat Flour	22.9
Crustacean Meal	20
Aquabind	3
Banox E	0.01
Vitamin Mix	0.2
Vitamin C	0.1
Carophyll Pink	0.07
Cholesterol	0.2
Lecithin	1.2
Fish Oil	0.6
Total	100.00

Three groups of experiments were undertaken.

- (1) A field experiment where lobsters were held for four months in land-based raceways at different densities (10 or 20 lobsters per 1.6m² enclosure) and under different feeding regimes (fed daily, weekly, or starved) to follow the development of TFN. One group of lobsters were bagged at capture to test the effectiveness of this post-harvest strategy on the development of TFN.

- (2) A laboratory experiment over six weeks where individual lobsters were held at 15°C and 23°C to investigate the effect of holding temperature on the progression of TFN. In this experiment one group of lobsters were bagged at capture. Physical tail fan damage was inflicted on a subset of those lobsters at the start of the experiment. This allowed investigation of the effectiveness of bagging in restricting TFN and also tested whether physical damage of the tail fan led to TFN.

- (3) A study was undertaken to investigate the microbiology of TFN. This was done by isolating bacterial species from TFN infections, culturing the isolated strains and infecting the isolates into healthy lobster tails. This allowed evaluation of whether the isolated strains of bacteria were capable of inducing symptoms of TFN. Finally isolates were recovered from the induced infections to test whether the inoculated strains of bacteria were present in the induced TFN. If bacteria isolated from the diseased tail fan can be used to initiate TFN in healthy lobsters and can be recovered from the subsequent infection a causal role for the bacteria is demonstrated.

In addition, a survey was undertaken using a questionnaire to investigate the level of awareness of the TFN condition shown by the rock lobster industry and the perceived level of occurrence and importance of TFN to the industry.

4.1.1 The effects of holding density, feeding regime and bagging on the development of TFN in a raceway holding system

In November 2000, 450 lobsters were bought from local fishermen with 420 used for the experiment. The remainder were used to replace mortalities in the first two weeks of the experiment. The time from capture to release into onshore collection tanks varied between 7 and 14 days depending on the length of time a given fisher remained out fishing. This variation in time prior to entering the experiment applied equally to bagged (see below) and unbagged lobsters.

The experiment commenced on 27.11.00 in conjunction with FRDC 200/212 and was completed at the end of March 2001. It was set up in seven outside tanks at Southern Australian Seafoods at Port Lincoln. The tanks were rectangular, about four metres long and were continuously aerated and supplied with water (flow-through). They were situated within a shade cloth enclosure. Temperature was monitored using data loggers. Each tank contained 4 PVC/oyster mesh cages – 28 in total. There were three treatments of feeding frequency (daily feed, weekly fed and starved) and two of density (10 per cage (6.3/m²) and 20/cage (12.7/m²) (Fig. 1). Each treatment had four replicates, except for the starved treatment where there were two. In addition, a further treatment involved daily feeding of lobsters that had been bagged at capture; this was run at two holding densities and with four replicate cages. Lobsters were assigned randomly (within bagged and unbagged categories) to density and feeding treatments. Lobsters were placed in the cages and the cages secured in the raceways (Fig. 2).

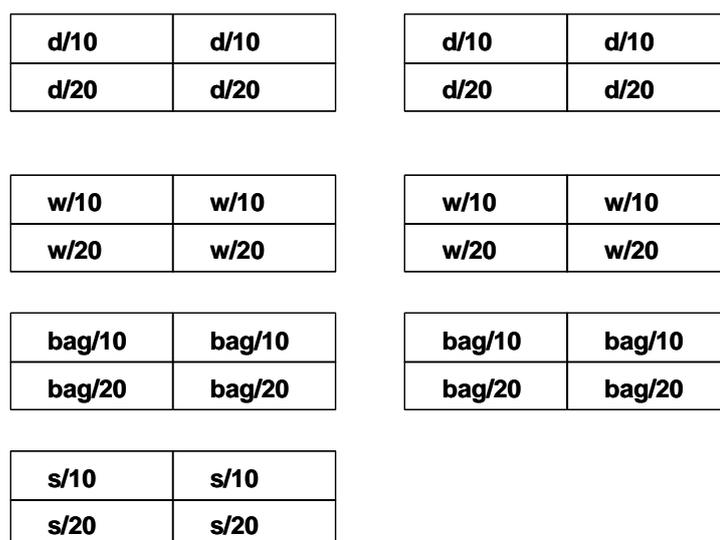


Fig. 1. Experimental design for feeding frequency density trial, d = daily feed, w = weekly feed, bag = bagged daily feed, and s = starved).

The bagged treatment contained lobsters that were put straight from the capture pot on the fishing vessel into individual fine mesh (0.5mm) nylon bags (Fig. 3). These bags were those used in the oyster industry as spat bags. The bags had a drawstring to allow closure, and retention of the lobster. The bags protected the animals until they could be released into the experimental cages at which point the bags were removed. This was intended to control for damage that might have occurred on the boat and during the transport and potentially lead to tail fan necrosis.

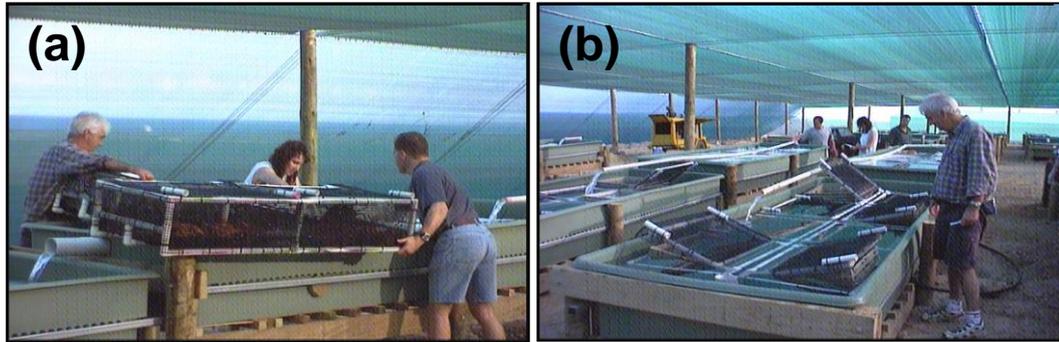


Fig 2. Cages and tanks used in the experiment, (a) cage being placed in a tank and (b) cages set up ready for the introduction of lobsters.



Fig 3. Fine mesh nylon bags used in the trial.

Tail fan state was assessed every two months as follows. Each of the five appendages of the tail were assessed individually for the presence of scratches, tears, blisters, holes and erosion (Fig. 4). Each damage category was classified as either large or small and assigned a letter accordingly: t or T = tears \leq or >7 mm; b or B = blisters \leq or >5 mm; s or S = scratches \leq or >7 mm; h or H = holes \leq or >2 mm; e = erosion of the limb margin not extending into the limb proper, E = erosion of the limb proper.

Percentage TFN was divided into seven categories as follows: no erosion; less than 25% of the margin of the limb; greater than 25% of the margin of the limb; affecting more than the entire margin but less than or equal to 25% of the whole limb; $>25\%$ to 50% ; $>50\%$ to 75% and >75 to 100% . All subdivisions were made based on previous observations of damage. Most analyses were undertaken on damage classified as TFN and for statistical analyses damage assessment was combined into one of three TFN erosion categories, nil, $<25\%$ and $>25\%$ erosion of the tail fan limb.

Erosion involved the loss of tissue, necrosis and melanisation and so is considered TFN in this study. Other damage, scratches, tears, blisters, and holes may be the

precursors of TFN. Photographs were taken of each tail fan at each assessment date, and the photos used to standardize assessment.

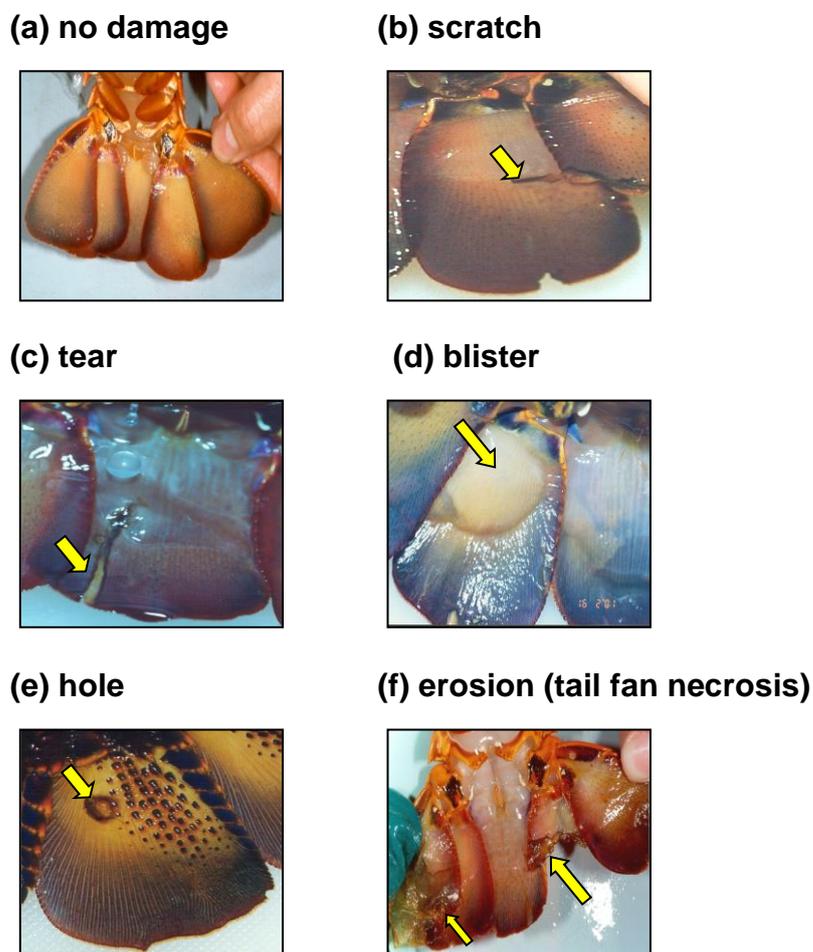


Fig. 4. Various degrees of damage to lobster tail fans.

4.1.1.1 Microbiology, SEM and TEM

Swabs of the surface of healthy tail fans and those showing TFN of randomly selected lobsters from each treatment were plated out onto TCBS (Oxoid) and Marine agar (1% w/v Oxoid Peptone, 1% Oxoid Lab Lemco powder, 3% w/v NaCl, 1.5% w/v Agar) for assessment of the microbial flora. Pure cultures of colonies of bacteria randomly selected from TCBS and Marine agar were prepared and identified where possible to species level by Dr Connor Thomas. The Gram reaction, motility status and oxidase reaction of pure cultures of individual isolates were determined. Isolates were then characterized using Microbact 24E Identification System (Medvet Diagnostics, Adelaide, SA). The results of these phenotypic tests were used to identify each isolate, where possible, to species level. Pure cultures of identified bacterial

isolates were stored at -70°C in 1ml volumes of a solution containing glycerol (32% v/v) and Difco Bacto Peptone (0.6%).

Plastic substrates were placed into each of the holding raceways and left for the four months of the experiment to allow the development of a bacterial flora. The substrates were swabbed and the bacteria plated and identified as above.

At harvest, three lobsters with evident TFN were selected and placed on ice and brought back to Adelaide. Discs (1cm diameter) of tail fan tissue were removed from unaffected (i.e. no TFN) and affected uropods from each individual, homogenised and plated on marine agar and TCBS agar to quantify the bacterial flora. The tail fan limbs were also examined by Scanning electron microscopy (SEM).

Fluid was also extracted from a number of intact tail fan blisters. This material was plated onto TCBS and Marine agar to look for the presence of a microbial flora

4.1.2 The effects of temperature, physical damage and bagging on the development of TFN in laboratory trials

In February 2001, 90 Lobsters were caught over a three-day period during normal fishing operations with the assistance of a commercial lobster fisherman. The pots were set in 30-50 feet of water off the south coast of Kangaroo Island. When pots were hauled up, males just over the legal limit (102mm Carapace Length) were separated from the catch. 65 of these lobsters were put into mesh bags and the remainder (25) left unbagged. The same technique was used in setting up the field experiment and had shown that bagged lobsters sustained much less tail fan damage, and damage in general, than those left without bags, the standard commercial practice.

Lobsters were then transferred to a processor's tanks on the mainland and, the following day, to the South Australian Aquatic Sciences Centre. During the journey they were treated in the same way as commercial lobsters, that is, housed in crates covered with damp sacking in a chiller truck ($10-12^{\circ}\text{C}$). Once at SAASC they were in then kept in communal tanks (15°C) for one week prior to the beginning of the experiment. At that point 60 lobsters were transferred to in individual 38L tanks. Lobsters were removed from bags just prior to tank assignment. The remaining

animals were used to replace any mortality during the first week of the experiment. During the acclimation period and the experiment the water supply to all tanks was flow-through and all lobsters were fed 3 times per week at 2% body weight/day. The experiment was run for six weeks.

Lobsters were kept in individual tanks for the experiment. The tanks were divided into two groups of thirty, kept at either 15°C or 23°C (Fig. 5). Within each group lobsters were randomly assigned to one of three bagging treatments: bagged, un-bagged, or bagged damaged, ten lobsters per treatment. Each tank had its own individual water and air supply.

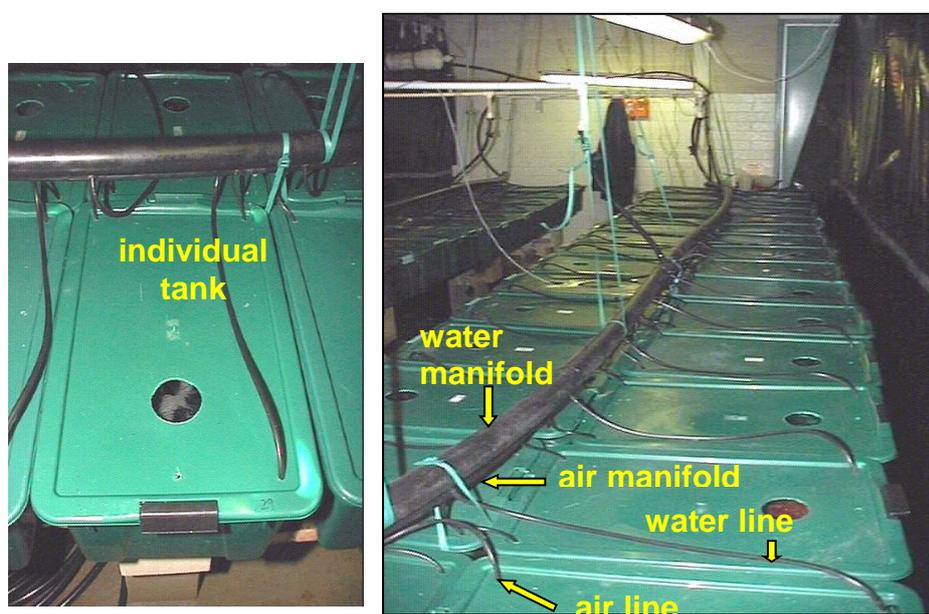


Fig. 5. Individual tanks used for laboratory experiment.

Bagged-damaged animals were purposely damaged before they were put into tanks. Four of the five tail fan appendages (the telson and three uropods (right to left)) of each animal had holes (2mm diameter) punched through them and cuts (7mm) made at opposite ends of the distal margins. All holes and cuts were made with sterile instruments.

The tail fan states of all lobsters were assessed each fortnight as described above and photographs taken of all fans. Swabs for microbial analysis were also taken from the same 18 lobsters each fortnight (three lobsters for each of the three bagging treatments from each temperature) and plated out onto TCBS and Nutrient agar for assessment of microbial flora.

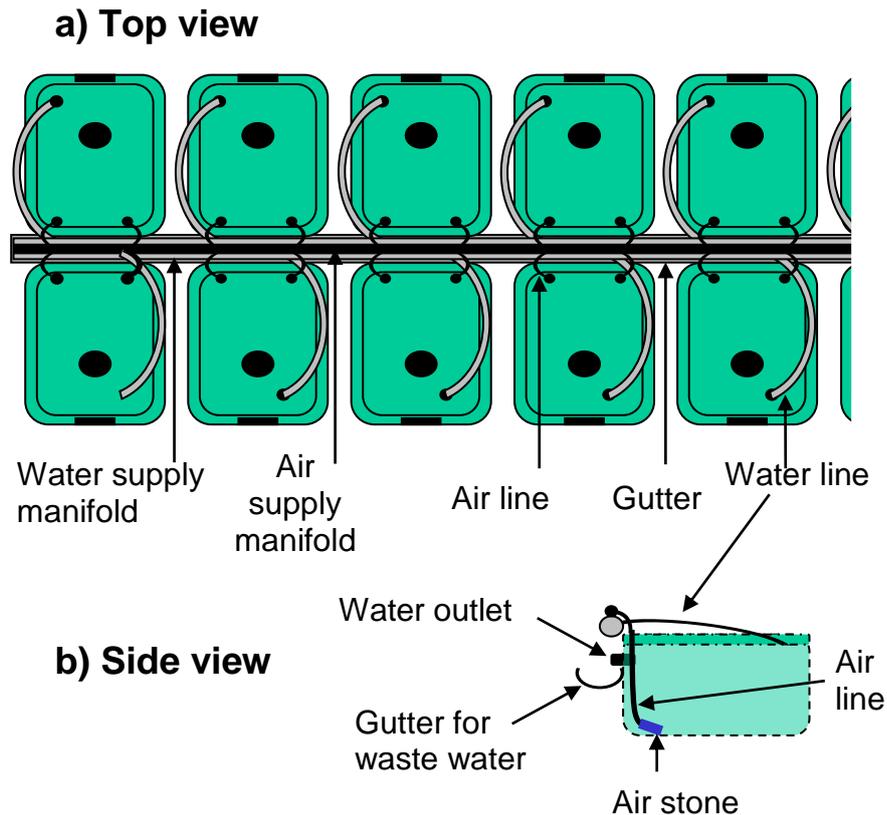


Fig. 6. Tank array used for laboratory experiment.

4.1.3 Data analysis

Initial damage of lobsters entering both the field and laboratory experiments was analysed using a One-tailed Mann-Whitney U Test (SPSS). Treatment effects on the development of tail fan damage, measured as erosion categories, were analysed with a Poisson Regression (Genstat) and significant differences tested using analysis of deviance (ANOD) Payne (2002).

4.1.4 Identification and characterisation of bacteria associated with TFN

Isolates of bacteria of lobsters with TFN were routinely cultured on Marine agar and Marine broth (1% w/v Oxoid Peptone, 1% w/v Lab Lemco powder and 3% w/v NaCl) and grown overnight at 30°C. Pure cultures of bacterial isolates were stored at -70°C in 1ml volumes of a solution containing glycerol (32% v/v) and Difco Bacto Peptone (0.6%). Four of these pure cultures were used in the inoculation experiments (below) to investigate causal agents in TFN. The species/strains *Vibrio* sp, *V. vulnificus* and *V. parahaemolyticus* were thought to be pathogens involved in TFN while the *V.*

alginolyticus treatment was included to investigate whether this bacteria may act as a probiotic against other bacterial infection.

Twenty lobsters were used in the study; four lobsters in each treatment. Puncturing with a sterile nail infected tail fans, or cutting with fine scissors both of which had been inoculated with bacteria previously isolated from TFN lesions. On each lobster, four of the tail fan limbs were damaged in this way and the left-most uropod fan remained undamaged. The nail punch produced a hole in the middle of the tail fan limb of approximately 2mm in diameter; the box-cutter blade made a 7mm long incision in the outer perimeter of the tail fan limb at opposite ends of the distal margin. The four bacterial isolates chosen for the study were tentatively identified as *Vibrio sp.*, *Vibrio vulnificus*, *Vibrio parahaemolyticus*, and *Vibrio alginolyticus*, all common bacteria associated with tissue from TFN lesions. The fifth group of lobsters were damaged with sterile implements as a control. The lobsters were held in individual tanks at ambient temperature for six weeks and fed once a week. At the end of the experiment, the damaged tail fan lobes were examined to evaluate the extent of TFN by estimating the area of TFN damage around the holes.

At the end of the six weeks a sterile hole-punch was used to remove samples of tissue from the damaged tail fans. The tissue was homogenised in PBS, the homogenate serially diluted and each dilution spread onto Marine agar and TCBS agar. The plates were incubated overnight at 30°C and four colonies were chosen for further analysis. The Gram reaction, motility status, oxidase and catalase reaction and chitinase activity of pure cultures of individual isolates were determined. Isolates were then characterized using Microbact 24E Identification System (Medvet Diagnostics, Adelaide, SA). The results of these phenotypic tests were used to identify each isolate, where possible, to species level. Confirmation of the phenotypic analysis was performed by comparison of DNA sequence data derived from PCR amplified DNA encoding 16s rRNA, with sequences stored in the Ribosomal Database project website (<http://rdp.cme.msu.edu>). The presence of restriction length fragment polymorphisms within the amplified DNA encoding 16s rRNA was used to differentiate clonal populations of the same species. A more complete description of these microbiological and molecular methods is described in May (2002).

4.1.5 Lobster tail fan necrosis survey

A survey was undertaken to investigate the extent of knowledge and the occurrence of what we have termed tail fan necrosis in the Australian rock lobster industry. The survey was in the form of an information sheet and a questionnaire. The questionnaire asked for information about the respondent's role in the industry, asked whether they had seen tail rot (TFN) and asked when and to what extent tail rot was observed. For respondents in the processing sector, it asked whether tail rot developed/progressed in the holding tanks.

Hard copies and electronic copies were sent, with a request that the survey be distributed to appropriate people in each state. The following survey was sent to at least two key contacts in Queensland, Victoria, Tasmania, and South Australia:

Lobster Tail Fan Necrosis Survey; Queensland, Victoria, Tasmania, and South Australia

Tail fan necrosis or tail rot is probably caused by bacterial infection of the tail fan. It appears likely that bacteria, which occur naturally in seawater, get into damage (small tears and holes) that happens when lobsters flap about on deck or are placed in high-density storage wells or crates on board the boat. When these lobsters are kept on tanks for a period (weeks to months) the infected area may blacken (called melanisation) as the lobster's immune system responds and there is usually some degree of erosions or rotting away (necrosis) of the fan. This necrosis can extend to the point where individuals limbs of the fan are eroded away completely.

This questionnaire is intended to determine if the condition is found in all the major lobster fisheries in Australia. Your help would be appreciated.

First of all, what does it look like? The following series of photos of the Southern Rock Lobster, *Jasus edwardsii*, taken after the lobsters had been held for anywhere between 4 weeks and 5 months in various live-holding trials in South Australia.

(a) Normal tail fan



(b) Tail fans with various degrees of tail fan necrosis (tail rot)



Secondly, there are a few questions we would like to ask regarding your experience with the condition. All answers will be treated in confidence.

1. What is your primary involvement with the lobster industry? Processor / Scientist?

2. If you are a lobster processor, what is the average turnover of your operation in tonnes of lobsters per year?

<100 101-500 500-1,000 1,001-5,000 5,000-10,000

3a. Do you see lobsters coming from the fishery with tail rot? Yes / No / Don't know

3b. If 'yes', how much of a problem do you consider tail rot to be overall? (1 = not a problem, 5 = serious problem, circle one) 1 2 3 4 5

3c. Does tail rot get bad enough that lobsters get rejected from shipments? Yes / No

3d. If so, how bad does the tail rot have to be, in terms of percentage of tail missing, before a lobster will be rejected from a shipment? 10 20 30 40 50 60 70 80
90 100

3e. What percentage of the lobsters would be rejected during sorting prior to shipping because of tail rot?

<5 5-10 11-20 21-30 31-40 41-50 51-60 61-70 71-80 81-90 91-100

4a. If you do get lobsters coming into your facility with tail rot, do you get them

All season / Sporadically

4b. If sporadically, any particular month (circle one or more)

Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

4c. Are there any peaks in the appearance of lobsters with tail rot in the catch? (circle one or more).

Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

4d. Are some size grades of lobster more likely to develop tail rot than others? Yes / No

4e. If so – which grade(s)

4f. Of the lobsters that are brought to your facility in a season, what percentage would have tail rot?

0 1-5 6-10 11-20 21-30 41-50 51-60 61-70 71-80 81-90

4g. What has been the trend over the last 10 years Remained constant / Increased / Decreased

4h. If there have been peaks when were they (circle one or more)

1990/91 1991/2 1993/4 1994/5 1995/6 1996/7 1997/8 1998/9 1999/00 2000/01

5a. Do lobsters develop tail rot in the holding tanks in your facility? Yes / No / Don't know

5b. How long do you typically hold lobsters in your tanks? (a range will do)

5c. If you answered 'yes' for Q5a, how long does it take to develop tail rot in the holding tanks?

5d. What range of percentage of lobsters would develop tail rot within the tank system in any given year?

0 1-5 6-10 11-20 21-30 41-50 51-60 61-70 71-80 81-90

5e. What has been the trend over the last 10 years Remained constant / Increased / Decreased

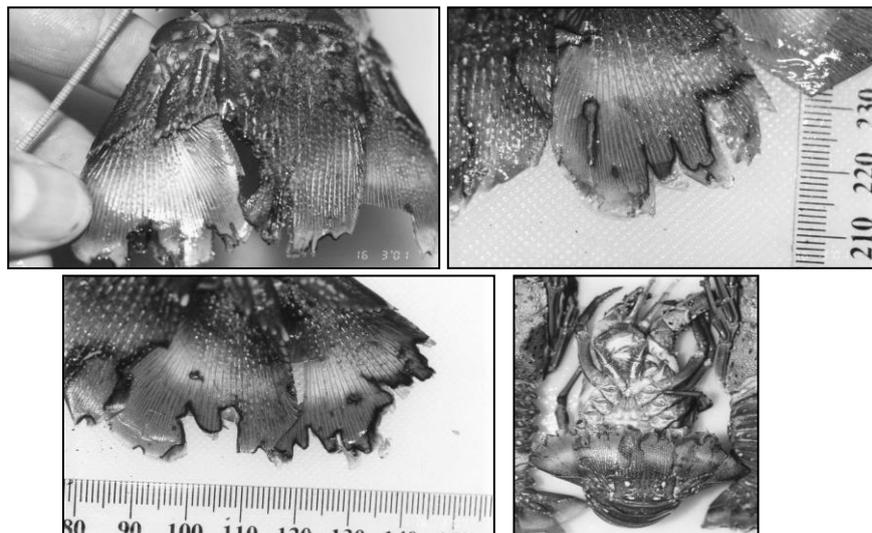
5f. If there have been peaks when were they? (circle one or more)

1990/91 1991/2 1993/4 1994/5 1995/6 1996/7 1997/8 1998/9 1999/00 2000/01

Any other comments?

Lobster Tail Fan Necrosis Survey, Western Australia

A slightly different survey form, but with the same questionnaire, was prepared for Western Australia. We had received specimens of *Panuluris cygnans* showing tail fan necrosis presented a little differently from in *Jasus edwardsii*. We were also aware that in 2001/2002 there had been some concern about tail rot in lobsters caught at the changeover of legal length in the season. The Western Australian survey was improved by input from key industry members in Western Australia during 2001. The survey sent to Western Australia included photos of TFN on *P. Cygnus*. The photos were taken from lobsters caught during normal fishing operation in WA in 2001.



4.2 Results

4.2.1 The effects of holding density, feeding regime and bagging on the development of TFN in a raceway holding system

Water temperature in the raceways were unusually high in the summer of 2001-2002 during the when the experiment was undertaken (mean 21.5 and max 23.3°C), averaging 2.5°C warmer than the following summer (18.99°C) (Fig. 7).

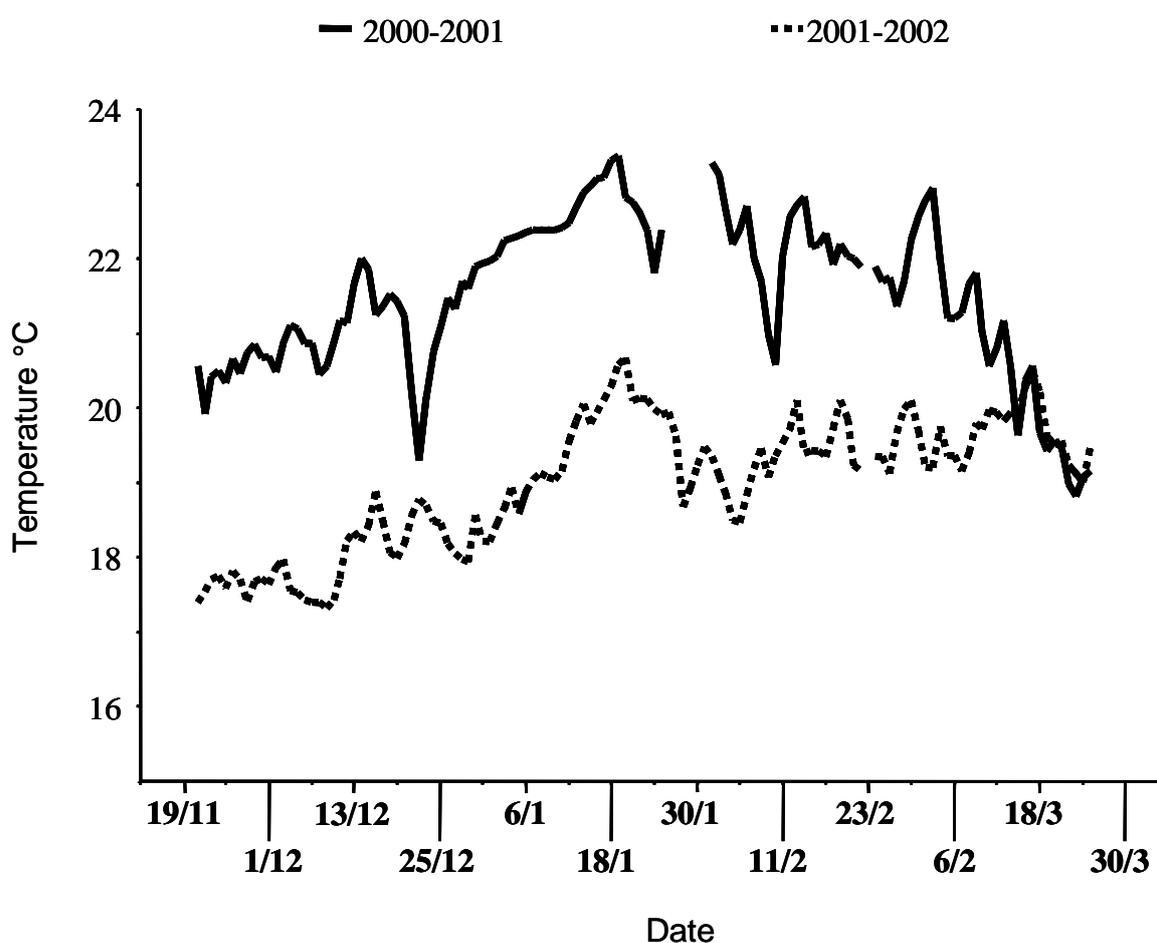


Fig. 7. Water temperatures (°C) in raceways at the Southern Australian Seafoods, Port Lincoln, over the summers of 2000-2001 and 2001-2002.

The survival of lobsters throughout the four month experiment was highly variable with high water temperatures and associated poor water quality probably contributing to much of the mortality (Table 2). A failure of the flow-through system early in the experiment also contributed to the high mortality.

The extent of initial damage at the commencement of the experiment, after lobsters had been held for varying periods from 14 to 21 days from capture, is shown in Fig. 8. Minor damage was recorded on lobsters from unbagged and bagged post-harvest handling groups, but advanced erosion (e.g. Fig. 4) which represents TFN was only observed on unbagged lobsters with 10.8% of unbagged lobsters showing TFN compared with none for bagged lobsters (Fig. 8). This suggests that post-harvest bagging minimizes physical damage that may lead to TFN.

The progress of TFN has been analysed by looking at the occurrence of TFN on lobsters at the start of the experiment (Fig. 9a), after 2 months (Fig. 9b), and at the completion of the trial after 4 months (Fig. 9c). Holding density of lobsters (10 or 20 per cage) had no effect on TFN ($P=0.832$) so the data were pooled within feeding and bagged treatments. Data from replicates within treatments were also pooled, as there were no significant differences between replicates in any treatment ($P>0.05$). The level of TFN (defined by erosion categories of nil, <25% and >25% loss of tail fan tissue) increased significantly during the trial ($P<0.001$) and showed significant two-way interactions with time and with treatment ($P\leq 0.001$) (ANOD). Bagged treatments showed significantly less advanced (i.e. >25%) erosion than weekly or daily fed treatments throughout the trial (Fig. 9) with 51% of bagged lobsters showing no TFN at the four-month termination of the experiment ($P<0.001$). In the unbagged treatments, 46-50% of lobsters showed erosion in the <25% category and 25-33% showed erosion of more than 25% of the tail fan, the latter being two to three times that recorded for bagged lobsters (11%). There were only minor differences between the groups fed daily, weekly and starved with the starved lobsters showing slightly lower rates of advanced TFN (>25%) than the fed treatments.

Table 2. Percent survival of lobsters in raceway system over four months for lobsters fed daily, weekly, bagged, and starved. Bagged lobsters were fed daily. Pooled over replicates within treatments. Four replicate cages of 10 or 20 for each daily, weekly, and bagged treatments, two replicate cages for each starved treatment

	Number of lobsters			Percentage survival	
	Initial	2 months	4 months	2 months	4 months
daily 10	40	35	21	88	53
daily 20	80	66	48	83	60
Total	120	101	69	84	58
weekly 10	40	34	22	85	55
weekly 20	80	69	37	86	46
Total	120	103	59	86	49
bagged 10	40	25	20	63	50
bagged 20	80	43	35	54	44
Total	120	68	55	57	46
starved 10	20	16	14	80	70
starved 20	40	38	35	95	63
Total	60	54	39	90	65

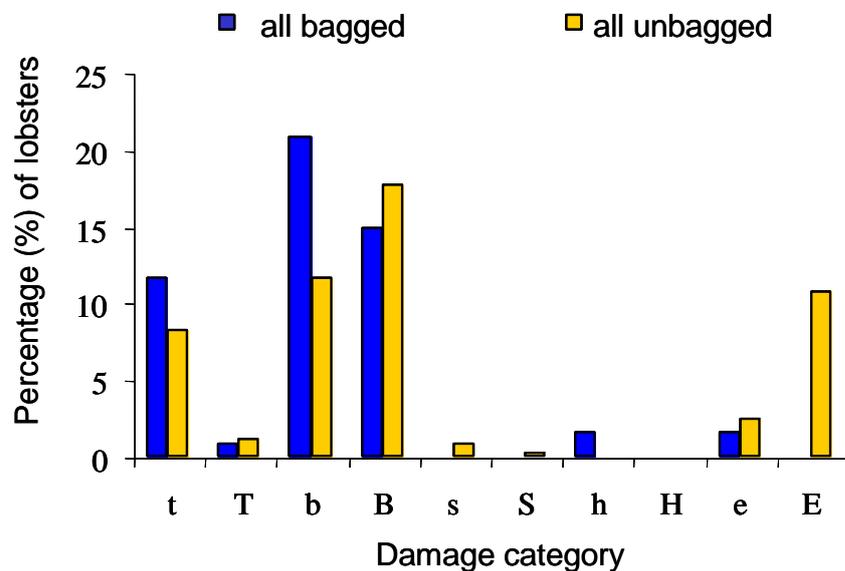


Fig. 8. Field percentage of lobsters with pre-existing tail fan damage/necrosis. t or T = tears \leq or $>$ 7 mm; b or B = blisters \leq or $>$ 5 mm; s or S = scratches \leq or $>$ 7 mm; h or H = holes \leq or $>$ 2 mm; e = erosion of the limb margin but not extending into the limb proper, E = erosions of the limb proper. N = 120 bagged, 300 unbagged.

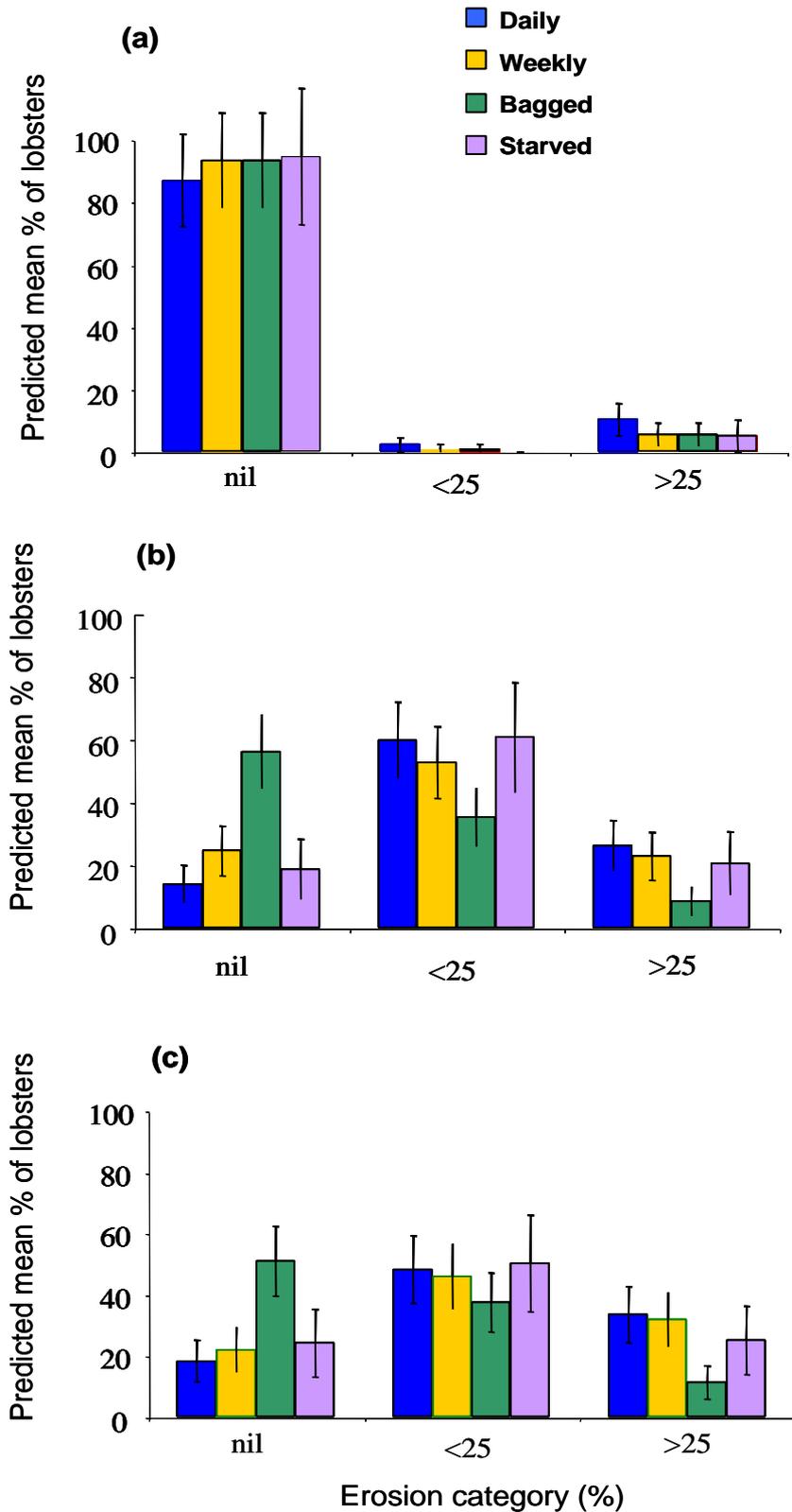


Fig. 9. Mean percentage of lobsters (\pm SE) with each erosion category within each treatment from the field experiment. Data pooled across densities within feeding and bagged treatments. TFN at (a) commencement (n daily=120, n weekly=120, n bagged=120, n starved=60); (b) at 2 months (n daily=101, n weekly=103, n bagged=68, n starved=54); (c) at 4 months (n daily=69, n weekly=59, n bagged=55, n starved=39).

4. 2. 1. 1 Microbiology, SEM and TEM

Samples of tissue were taken for microbiology, SEM and TEM from TFN lesions, tears and normal intact tail fans. Swab samples were taken from the surfaces of tail fans in areas with and without TFN.

Bacterial cells were only occasionally located on the surface of normal tail fan tissue. Bacteria from these swabs included vibrios and showed similar growth patterns on TCBS agar as bacteria from lesions. They were not further identified.

Significant concentrations of rod shaped cells of bacterial size (ca. $1\mu\text{m} \times 2\mu\text{m}$) were visible on the surface of tissue taken from lesions and tears (Fig. 10). Larger spherical structures, cocci in Fig. 10, were often found associated with these cells.

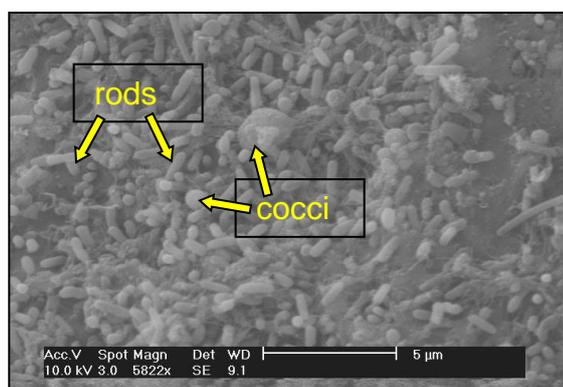


Fig. 10. Bacterial cells on the surface of TFN lesions observed under SEM. Scale bar is $5\mu\text{m}$.

Swab samples from TFN lesions were cultured and the isolates investigated. Most isolates from tail fan lesions were catalase and oxidase positive, gram-negative rods, motile by polar flagellum, able to grow in the presence of 3% NaCl, and ferment glucose, suggesting they were *Vibrio* spp. Further testing confirmed that most isolates were marine species of *Vibrio* (*V. vulnificus*, *V. parahaemolyticus*, and *V. alginolyticus* and other *Vibrio* spp.). All but *V. alginolyticus* can be considered potentially pathogenic. The incidence of each type of bacteria isolated from TFN lesions are shown in Table 3.

Table 3. Incidence of types of bacteria isolated from TFN lesions

Genus /species	Percent of all isolates (%)
<i>V. alginolyticus</i>	30%
<i>V. vulnificus</i>	11%
<i>V. parahaemolyticus</i>	11%
<i>Vibrio spp</i>	37%
Other	11%

Transmission electron microscopy (TEM) of samples of TFN lesion tissue clearly showed concentrations of two types of granulocytes in tissue surrounding lesions (Fig. 11). Bacterial cells were not found in any of the limited numbers of samples examined using TEM.

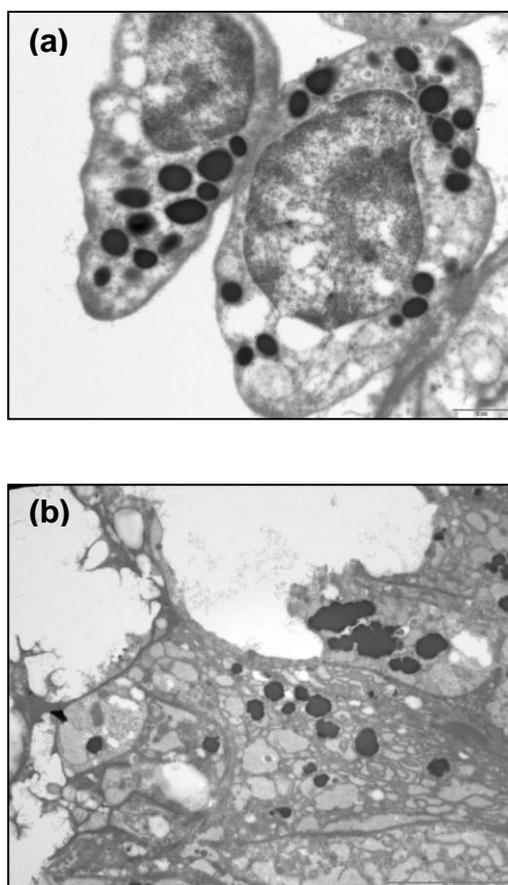


Fig. 11. Transmission electron microscopy of sections through TFN lesions, (a) TEM of large granulocytes found within the diseased tissue, scale bar 2 microns and (b) TEM cross-section of semi granulocytes. Note the dense granules, which degranulate to induce encapsulation and cellular defence reactions, scale bar 10 microns.

Total viable counts of bacteria colonising plastic surfaces immersed in seawater in holding tanks were similar irrespective of the feeding regime used for lobster

experimental groups (Fig. 12). However, the numbers of sucrose fermenters capable of growing on TCBS agar (an indication of *Vibrio* bacteria) were significantly greater for the daily and weekly fed experimental groups of animals. This result indicated that feeding increased the potential for growth of marine vibrios on surfaces in the holding tanks and that the plastic surfaces of the tanks may act as a reservoir of these organisms.

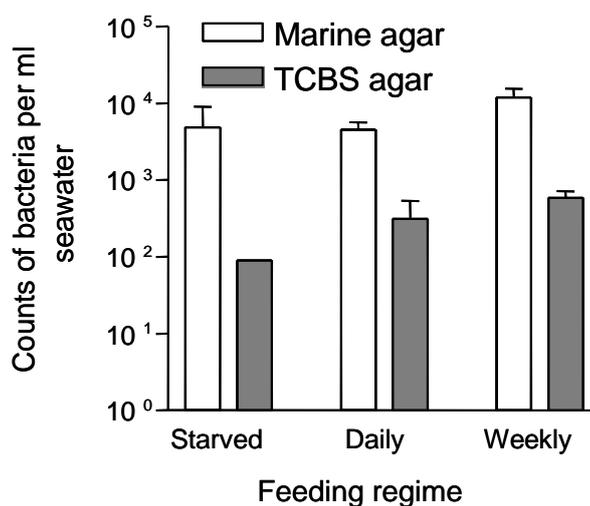


Fig. 12. Numbers of bacteria colonising plastic surfaces immersed in seawater in lobster holding tanks for 18 weeks.

Tail fans from three lobsters were studied quantitatively by plating of homogenised tail fan tissue from lobes of the tail fan with and without TFN lesions.

Microbiological analysis showed that greater numbers of bacteria were recovered from tail fan lobes with TFN compared to those without the condition (the controls) (Table 4). Moreover, the number of isolates capable of producing acid from sucrose (sucrose fermenter) was undetectable on healthy tissue samples, whereas significant numbers of these bacteria were obtained from tissue samples from tail fan lobes displaying TFN. Sucrose fermentation to acid is characteristic of vibrio bacteria on TCBS culture medium. This observation suggested that formation of TFN lesions was associated with colonisation of tissue by *Vibrio sp.*

Contents of tail fan blisters were cultured on a variety of media. Tail fan blister contents proved to be sterile, suggesting the fan infection is externally derived, not originating from a more general systemic infection.

Table 4. Counts of bacteria on Marine agar and TCBS agar from control (or normal) tail fans and tail fans displaying TFN. Counts on TCBS agar are shown counts of bacteria capable of producing acid from sucrose and counts of bacteria unable to produce acid from sucrose. nd = none detected.

Animal ID	Sample	Marine Agar	Counts per tissue disc	
			TCBS agar	
			Acid from Sucrose	No acid from Sucrose
D10-4/8	Control fan	5.10E+03	nd	nd
	Lesion	3.60E+05	4.00E+03	1.10E+04
D10-3/1	Control fan	2.80E+03	nd	nd
	Lesion	1.33E+06	1.80E+05	5.40E+05
B10-1/10	Control fan	1.30E+03	nd	nd
	Lesion	1.62E+05	2.30E+04	8.50E+04

4.2.2 The effects of temperature and physical damage on the development of TFN in laboratory trials

The extent of initial damage at the commencement of the experiment, after lobsters had been held for 9 to 11 days, is shown in Fig. 8. Unbagged lobsters showed significantly more initial damage ($P < 0.01$, one-tailed Mann-Whitney U test – Zarr, 1984, pg 141) in most categories compared to those that had been transported to the facility in mesh bags (Fig. 13). A single lobster in the bagged treatment showed erosion of the margin of a tail fan limb and one lobster showed erosion of less than 25% of a limb (EL25). Data were pooled over all categories for the analysis.

The marked differences in TFN between treatments, especially between bagged and unbagged lobsters, are shown in Fig. 14. There were no mortalities and so analysis was for $n=20$ for each treatment. Temporal and temperature effects were analysed using Poisson Regression on Genstat. TFN data were re-categorized as either “nil”, “ $\leq 25\%$ ” or “ $> 25\%$ ”. Analysis was carried out using these categories for three bagged treatments (bagged, bagged-damaged and unbagged) and two temperature treatments (15 and 23°C at 4 time intervals (T_0 , $T_{2\text{weeks}}$, $T_{4\text{weeks}}$ and $T_{6\text{weeks}}$)). Analysis of Deviance showed an overall significant treatment effect ($P < 0.001$), with unbagged lobsters showing highest levels of advanced TFN, $> 25\%$ tail erosion (Fig. 15). There were significant interactions between bag treatment and TFN level ($P < 0.001$) and time and TFN level ($P < 0.001$). Temperature had little effect on TFN, significant ($P = 0.05$) only at the end of Week 2 ($P < 0.001$) via an interaction with treatment (Fig. 15). At four

and six weeks there were no significant temperature effects between 15 and 23°C. There were significant bag effects at each, individually analysed, sampling date ($P \leq 0.001$); in each case the unbagged treatment showed significantly higher levels of advanced TFN.

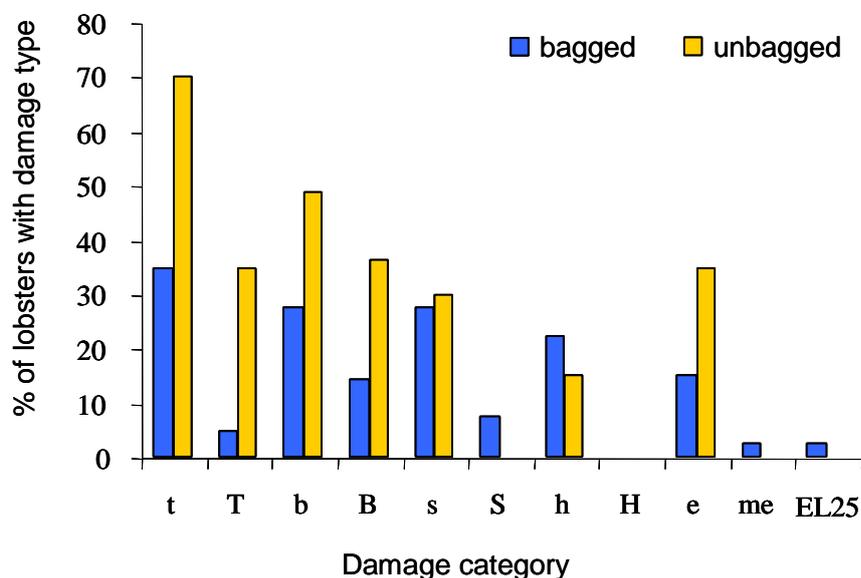


Fig. 13. Initial damage found in bagged and unbagged lobsters brought into laboratory. t or T = tears \leq or >7 mm; b or B = blisters \leq or >5 mm; s or S = scratches \leq or >7 mm; h or H = holes \leq or >2 mm; e = erosion $\leq 50\%$ of the limb margin but not extending into the limb proper; me = erosion $>50\%$ of the limb margin but not extending into the limb proper; EL25 = erosion of $<25\%$ of the limb proper. Bagged n = 40, unbagged n=20.

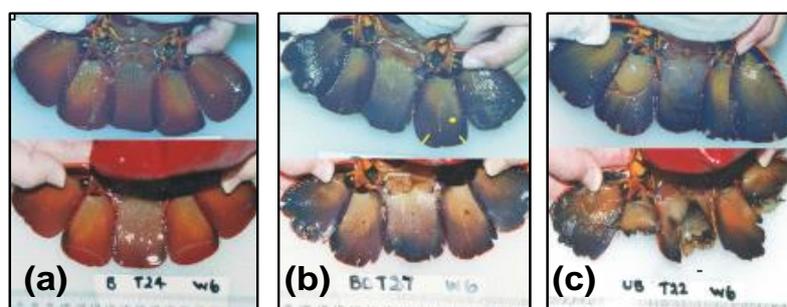


Fig. 14. Tail fan necrosis by treatment. Each pair of photographs shows the tail fan of a typical lobster (a) bagged, (b) bagged (damaged), where the position of the damage inflicted all but one limb of each tail fan, indicated by yellow lines and dot and (c) unbagged. Beginning (T0) and end (T6 weeks) of experiment.

The highest incidence of TFN was in the lobsters given the normal post harvest handling of communal holding in boat holds and processor trays and tanks. These conditions are associated with physical damage inflicted on the lobsters by each other. Such damage will involve both physical damage and presumably the infection of wounds by the bacterial flora of the crayfish exoskeleton. The bagged and bagged-damaged treatments had no advanced TFN after the six week period. This suggests

that post-harvest bagging minimizes TFN and it also shows that inflicting physical damage to lobster tail fan with aseptic instruments does not lead to the development of TFN.

The lack of influence of temperature on the progression of TFN in the present experiment was surprising. Over the extended period of the experiment any differences in the rate of progress of TFN may have been masked as sufficient time was available for TFN to develop at both temperatures.

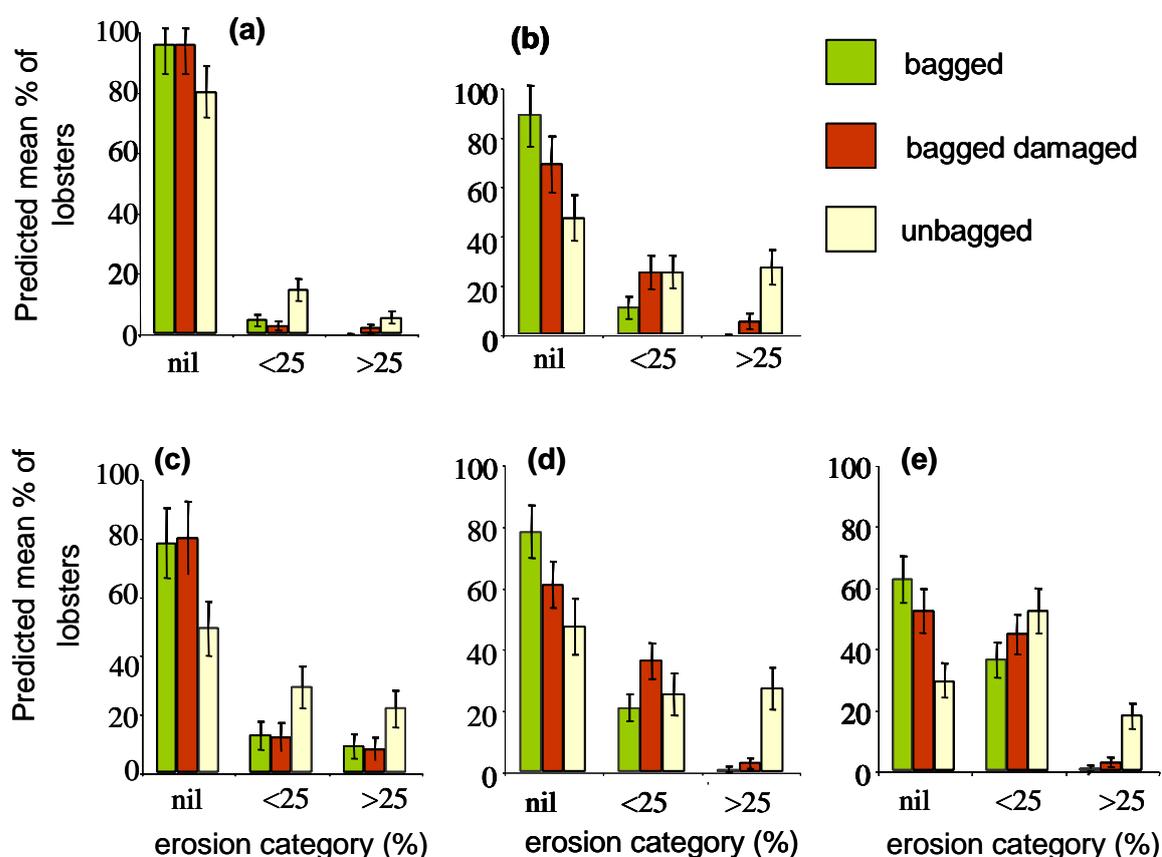


Fig. 15. Laboratory experiment, predicted mean percentage of lobsters (\pm SE) expected to have each erosion category within each treatment. Tp = temperature pooled. N = 20 per treatment for each sample. There were no mortalities.

4.2.3 Identification and characterisation of bacteria associated with TFN

To determine the role of physical damage and the role of bacteria in development of TFN, we used laboratory experiments in which groups of animals were either damaged with sterile instruments or damaged with instruments intentionally contaminated with pure cultures of *Vibrio* spp. isolated from TFN lesions. Lobsters

damaged with sterile instruments did not develop the disease. However, we did observe a greater than one order of magnitude increase in numbers of bacteria on tail fans during the six week period of the damage experiment (Fig. 16).

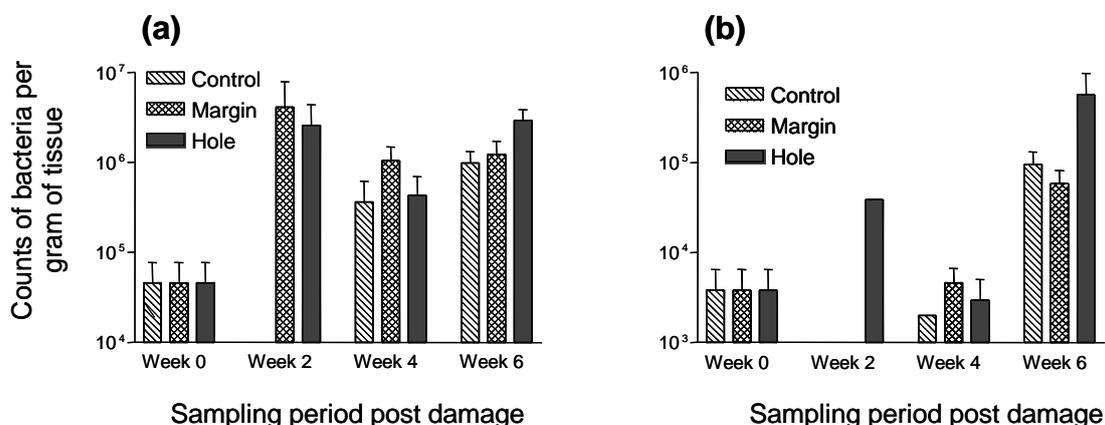


Fig. 16. Changes over a six week period in numbers of bacteria on the surface of normal control tail fans and tail fans damaged by sterile cuts or holes in tail fan limbs. Data as counts of bacteria on Marine agar and sucrose fermenting bacteria on TCBS agar.

By contrast, all of the damaged tail fans that were infected with pure cultures of bacteria isolated from lesions, showed some degree of melanization and necrosis (Fig. 17). This is especially seen around cuts made in the tail fans with the perimeter of the tail fans suffering erosion that made them quite ragged (Fig. 17b, c, d, e). The holes also showed erosion surrounded by melanization (Fig. 17b). This offered the opportunity to quantify the extent of erosion. The area of the lesion around the puncture wound was measured as in index of the extent of TFN.

The index of the extent of TFN as measured by the size of the hole after the six week incubation period, is shown in Fig. 18. Each point represents the mean area of the holes made in each of the four tail fan lobes damaged. The numbers were small with only four lobsters per treatment. There is considerable individual variability but almost all lobsters infected with bacteria developed larger lesions than the control sterile damaged lobsters. The area of the hole for control lobsters was not substantially larger than that initially made by the 2 mm hole punch. These infection experiments implicate bacteria in the development of TFN. However, the extent of TFN (size of hole, swelling of the tail fan) was considerably less than that observed for lobsters held for long periods in holding tanks at Port Lincoln exhibiting TFN. This suggests that while a range of types of bacteria are capable of inducing TFN-like

symptoms in rock lobsters, other types of bacteria may be required to induce the chronic disease characteristic of TFN.

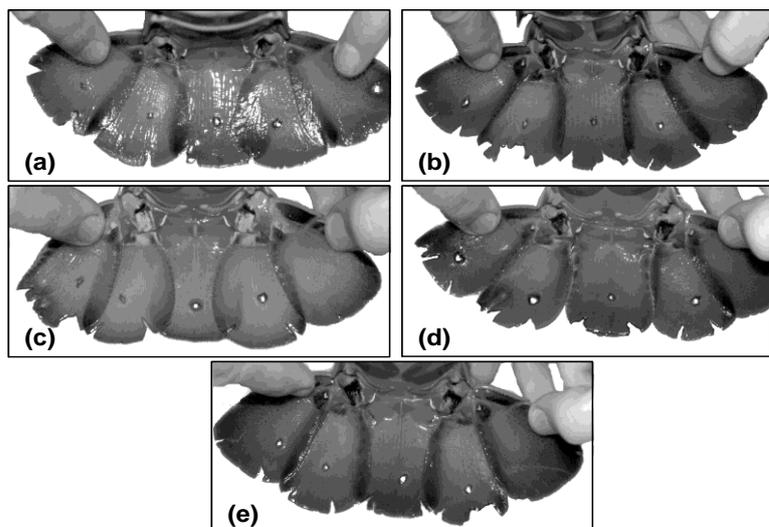


Fig. 17. Typical damage from (a) the control group that were cut and punctured with sterile instruments. Typically, only mild erosion and necrosis is seen. Note that in this example, the control fan (far right as seen below) has also suffered erosion. This is a very distinct square hole, (b) the *A. caviae* inoculated group. Moderate to significant damage was seen for all cut sites as well as the puncture sites, (c) the *V. vulnificus* infected group. Damage seen was typically mild to moderate erosion around the cut and puncture sites, (d) the group inoculated with *V. parahaemolyticus*. Resultant necrosis was typically moderate to significant in nature especially for the cut sites, and (e) the *V. alginolyticus* group. Damage was typically mild to moderate although one trial lobster displayed particularly high levels of erosion and necrosis observed at the end of the inoculation experiment.

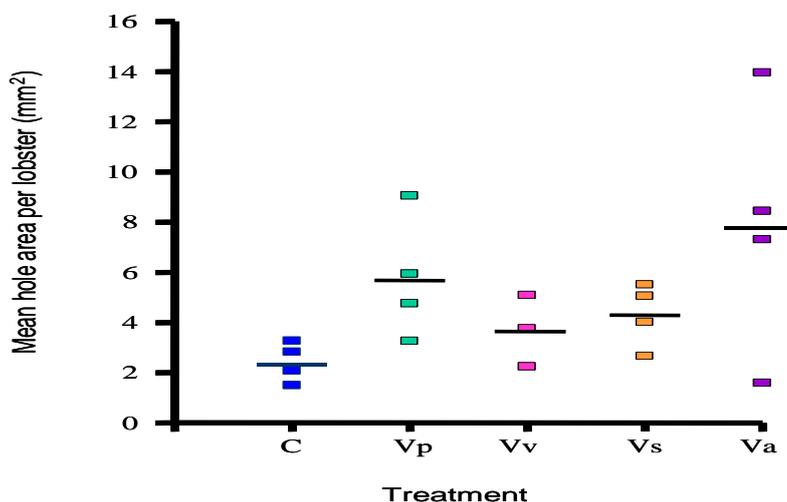


Fig. 18. TFN lesion area as an indicator of disease. Treatment: C = control, Vp = *V. parahaemolyticus*, Vv = *V. vulnificus*, Vs = *Vibrio sp.*, Va = *V. alginolyticus*. Horizontal bars within each treatment represent means

Counts of bacteria associated with tissue surrounding the site of damage were generally at least one order of magnitude greater than counts for uninfected control animals (Table 5). This was true for the counts of bacteria on TCBS agar, which is a culture medium specific for vibrios including all of the test species in this study.

Table 5. Mean counts of bacteria per gram of tail fan tissue from experimental animals used in infection experiments

Treatment group	Mean Counts of bacteria per gram of tail fan tissue	
	Marine Agar	TCBS agar
<i>V. alginolyticus</i> strain <i>P12</i>	2.5x10 ⁴	1.7x10 ⁴
<i>A. caviae</i> strain <i>L5</i>	1.2x10 ⁶	9.4x10 ⁵
<i>V. vulnificus</i> strain <i>L2</i>	2.6x10 ⁵	1.7x10 ⁵
<i>V. parahaemolyticus</i> strain <i>L21</i>	3.2x10 ⁵	2x10 ⁵
Uninfected, damaged control	3.3x10 ⁴	6.7x10 ³

Scanning electron microscopy of sections of damaged tail fans with TFN shows that bacteria are present in colonies within the damaged area where *Vibrio* were inoculated (Fig. 19a). Some of these bacteria appear to be boring into the tissue (Fig. 19b). In addition to the rod-shaped bacteria, probably vibrios, other cocci and spiral shaped unidentified bacteria were also present (Fig. 19c). Other areas of the tail fan surface were free of colonies (Fig. 19d).

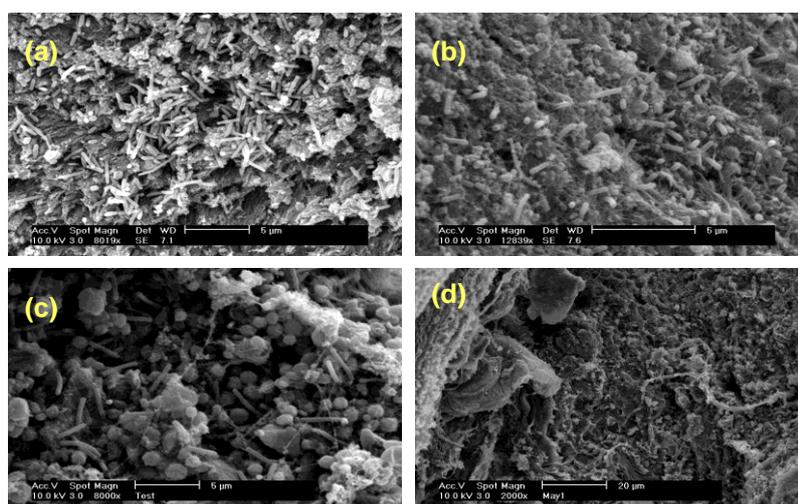


Fig. 19. Scanning electron micrographs showing bacteria present in damaged areas of tail fans. (a) micro-colonies such as this were common amongst the samples taken from lobsters inoculated with the test isolates, (b) area of moderate density bacteria again taken from the above-mentioned sample. In this instance, all of the bacteria are rod shaped and are likely to be of the same genus as the test isolates. Note that some bacteria appear to be ‘boring’ into the tissue (white arrows), (c) small cocci such that these were often present amongst the rod shaped bacteria, and (d) areas such as this with no obvious signs of bacterial colonisation were seen in all of the samples tested by SEM. Some samples were void of any surface bacterial colonisation.

We investigated whether bacteria isolated from infected tissue of experimentally infected animals were the same species/strains as those used to infect damaged tissue. Randomly selected colonies of bacteria that had been taken from the homogenate of the tissue sample from the wounded and inoculated area were grown on TCBS and Marine Agar. They were then isolated and identified using the Microbact 24E identification system to investigate whether the inoculated species/strain of bacteria was present and thus associated with the TFN around the wound. From each tissue sample two to eight individual colonies were chosen for further analysis. In general isolates of bacteria from each lobster treatment group were of the same type as those used to infect the damaged tissue (Table 6). These species of bacteria were also the most common recovered from the uninfected control group showing that they are widely present. A wide variety of different types of bacteria were isolated from the lobsters infected with *V. alginolyticus*, with only 2 of 16 isolates identified as *V. alginolyticus*.

Table 6. Identification of bacteria isolated for further microbiological analysis from each treatment group

Treatment Group	Isolates identified from lesions on experimental animals				
	<i>V. parahaemolyticus</i>	<i>V. vulnificus</i>	<i>V. alginolyticus</i>	<i>Vibrio</i> spp.	Other
<i>V. alginolyticus</i> strain P12	5	0	2	5	4
<i>Vibrio</i> sp strain L5	2	2	2	8	0
<i>V. vulnificus</i> strain L2	0	6	0	4	0
<i>V. parahaemolyticus</i> strain L21	13	0	0	0	1
Uninfected, damaged control	3	1	1	3	0
Total	23	9	5	20	5

We confirmed the identity of these isolates by DNA sequence analysis of PCR amplified DNA (ca 1600 bases) encoding 16s rRNA. This was achieved by comparison of the sequence data with known sequences in the Ribosomal RNA Project database (<http://rdp.cme.msu.edu/html/>). All sequences have been lodged with the Genbank DNA database. Genbank Accession numbers for 19 of these sequences are as follows: AY245178, AY245179, AY245180, AY245181, AY245182, AY245183, AY245184, AY245185, AY245186, AY245187, AY245188,

AY245189, AY245190, AY245191, AY245192, AY245193, AY245194, AY245195, AY245196.

To determine whether the bacteria isolated from lesions of experimentally infected animals were genotypically identical to the infecting strains used, we employed Pulsed Field Gel Electrophoresis (PFGE) of bacterial genomic DNA and restriction fragment length polymorphism analysis (RFLP analysis) of PCR amplified DNA encoding 16s rRNA as a means of identifying clonal groups of isolates. Amplified DNA was digested with restriction enzymes that cut frequently within DNA (ie. restriction enzymes which recognize 4 bp sequences). Enzymes which produced at least four DNA fragments were used for RFLP analysis.

Production of extracellular DNases by most isolates of bacteria tested limited the utility of PFGE. In view of this result we relied on RFLP analysis to identify clonal groups of isolates. At least two distinct RFLP groups for each species were identified depending on the restriction enzyme used. In particular, this analysis showed that bacteria capable of colonising lobster shell and tissue are likely to be clonal and that bacteria of the same clonal group as the infecting strain could be identified from developing lesions of experimentally infected lobsters. Thus the bacteria used to inoculate wounds in the tail fans were capable of colonising this tissue for long periods of time and were associated with the TFN that developed.

4.2.4 Survey on occurrence of tail fan necrosis in the Australian rock lobster industry

The response to the survey questionnaire was patchy and poor overall. In all states there was an awareness of the condition tail rot (TFN) in the industry. Responses and comments were received from Victoria, South Australia, Queensland, and Western Australia. Some of the respondents from Victoria also had fished around Tasmania. An outline of the state responses is given below.

Victoria and Tasmania. In all, five responses from commercial fishers were compiled and returned. They indicated that TFN was seldom seen and then only of minor severity. Most respondents had held lobsters in coffs (sea cages) for short periods and did not see TFN develop in these lobsters. It was further noted that

fishers regularly caught lobsters that had been previously released as undersize and so had been in pots and on decks, yet they had not gone on to develop tail rot.

South Australia. There were three responses from processors in South Australia. All were aware of tail rot, two said that it was rare, with less than 1% occurrence. Perhaps the occurrence was higher in larger lobsters. They considered tail rot only a minor problem. One respondent felt that tail rot occurred in up to 10% of lobsters in some years.

Queensland. There was one response from Queensland. This processor had seen tail rot in the industry but felt that the occurrence was low, under 1%. The awareness of the condition by Queensland processors shows that some form of TFN can occur in the tropical ornate rock lobster, *Panulirus ornatus*.

Western Australia. No formal responses were received from Western Australia. However discussions with scientists and industry showed that there was an awareness of tail rot. There seemed to be differences as to the occurrence of TFN in the industry, with it being encountered in some areas of the wild- fish industry. There was no response from the processing industry.

Overall these responses suggest that there is an Australia-wide awareness of tail rot, the condition we have called TFN. In most areas the occurrence was seen as very low and the issue not a major one for the industry. Each state did note that tail rot caused some rejection of lobsters for the export market. There seem to be a few reports of situations where the occurrence may be greater and the condition more advanced and of significant concern to the industry.

4.3 Discussion

There is little record in the literature on lobsters of what we have termed TFN. Recent studies on southern rock lobsters, *Jasus edwardsii*, in live-holding systems have identified TFN as a major problem in live-holding (Lorkin et al 1999; Geddes et al 2001) and have identified *Vibrio* and *Aeromonas* species associated with the disease. Other reviews (Evans and Bock 1994; Evans 1997) have reported that the majority of diseases observed in live-held and cultured lobsters are opportunistic infections caused by microscopic organisms, especially *Vibrio* spp, that are widely distributed in

the marine environment. Overall Australian rock lobsters have been seen to be free of serious disease threats (Evans 1997).

In the North American clawed lobster, *Homarus americanus*, three main types of disease have been identified in lobsters that are live held (Bayer et al 1999; Cawthorn 1999). The major problem is gaffkemia, an infection of the tail muscle caused by the bacteria *Aerococcus viridans* breaching the integument through wounds. Heavy infection leads to the disease gaffkemia or “red tail”. This systemic infection can be treated with antibiotics. The second disease involves the ciliate protist *Anophryoides haemophila*, which invades lobster tissue and haemal spaces through perforations in the integument and can lead to “box car” disease. A third group of diseases are termed shell diseases, a catch-all term for lesions associated with bacteria of the genera *Vibrio*, *Aeromonas* and *Pseudomonas*. Shell disease can occur over widespread areas of the carapace, the tail, and the claws (Bayer et al 1999). Interestingly it is not noted as prevalent on the tail fan of *Homarus*.

A recent study on the Caribbean spiny lobster *Panulirus argus* identified necrotic lesions on the uropods and telson (tail fan) of captive lobsters (Porter et al 2001). They studied the bacterial flora associated with the exoskeleton of the lobsters and the necrotic lesions. Microbial investigation showed that the genera *Vibrio* and *Pseudoalteromonas* were common both on the exoskeleton of healthy lobsters and in the tissue in the area of the shell disease. A tail fan disease very similar to that described here for *J. edwardsii* has been identified in the western rock lobster *Panulirus cygnans* (unpublished data but see Western Australia survey form). The microbiology has not been characterized. It seems that among the spiny lobsters, at least those studies to date *P. argus*, *P. cygnans* and *J. edwardsii*, tail fan infections and TFN is the most common form of “shell disease”. There does not seem to be widespread occurrence of shell disease on general areas of the carapace as seen in *Homarus*

The reason for the particular susceptibility of the tail fan of spiny lobsters to shell disease and necrosis, TFN, may relate to the structure of the uropods and telson. They have a thin carapace and so physical damage can be easily inflicted. Furthermore it is likely that the uropods and telson are not well perfused with hemolymph limiting any immunological response.

Our limited microbiological studies indicated that live-holding results in gradual increases in numbers of bacteria present on external surface of lobsters. On tail fans in particular, we observed significant increases in numbers of bacteria capable of growth on TCBS agar, a medium used for selective isolation of *Vibrio* spp. Moreover, the number of these bacteria present on surfaces of live-holding tanks was higher for groups of animals fed on a regular basis. We therefore conclude that even with adequate flow of fresh seawater, feeding increases the nutritional status of the water to an extent that supports larger populations of bacteria associated with holding tank surfaces. This was reflected in the slightly lower rates of advanced TFN in lobsters in the starved treatment. This observation may have an important impact on development and incidence of TFN in live-holding lobster operations.

We can propose mechanisms by which TFN may be initiated and develop. One possibility is that it may be initiated via a systemic infection and a compromised immune response perhaps associated with stress of live-holding, leading to the blisters often observed in the early stage of TFN. However not all TFN starts with blisters and more importantly samples of fluid from the blisters showed they were sterile with no bacteria cultured from them. We believe that TFN is initiated externally by damage to the integument, which allows the entry of common marine bacteria, especially vibrios. These bacteria are present on the carapace of lobsters and it is likely that in most cases TFN is initiated by physical damage to the tail fan by self-damage or by another lobster. This would have the effect of breaching the integument and inoculating/injecting a mix of bacteria from the lobster carapace into the wound. Such damage is highly likely to occur in the post-harvest handling of southern rock lobsters. During transfer from the pot to the holding well lobsters flap in air and can easily inflict wounds. They are stored at high density in the well in close proximity to other lobsters that can cause them damage. During processing they are transported in crates in air where lobsters can easily rub and scratch against each other. If lobsters are subsequently live held at ambient temperatures there is a high probability that TFN will develop.

The bacterial infection generally elicits a melanization response from the immune system of the lobster which results in blackening of lesion associated tissue. However the bacteria continue to multiply in the tissue. As most vibrios possess chitinase

activity (Murray et al 1984 in May 2002), it is possible that they can continue to break down the integument and spread across the tail fan. From an initial wound more and more of the tail fan is infected and the tissue lost. As the bacteria continue to multiply the tissue melanizes and becomes necrotic. Migration of granulocytes to the site of infection probably leads to the typical swelling associated with chronic TFN. These granulocytes may act to restrict the growth of lesion-associated bacteria and limit spread of bacteria via the hemolymph to other parts of the lobster.

Potentially, the progress of TFN might be related to temperature and the associated activity of the bacteria. The field experiment where temperatures were 17 to 24°C showed rapid advancement of TFN. In the laboratory the initial progression of TFN was faster at 23°C than 15°C, but after four weeks TFN was well progressed at 15°C. It is likely that lower temperatures, such as those generally maintained in SRL processor holding tanks, would greatly limit the progress of TFN.

A variety of bacteria have been identified in recent studies on TFN. In studying the bacterial flora of *P. argus*, Porter et al (2001) used partial DNA sequencing of the 16s rRNA gene and identified four subgroups of *Vibrio* and *Pseudoalteromonas*. The further identity of the vibrios could not be determined but it was suggested that they may be a flora specific to *P. argus*. The studies on *Jasus edwardsii* indicate the difficulty in confidently identifying species and strains. Reuter et al (1999) have identified *Vibrio alginolyticus*, *Aeromonas hydrophila* and *Plesiomonas shigelloides* from TFN lesions. Lorkin et al (1999) reported *Vibrio alginolyticus*, *Aeromonas caviae* and *A. hydrophila*. In the present study, *V. vulnificus*, *V. parahaemolyticus* and *V. alginolyticus*, and other *Vibrio spp.* were commonly associated with TFN lesions. Further characterization of selected strains by genomic analysis of DNA encoding the 16s rRNA gene(s) confirmed isolates as *Vibrios* and further confirmed the identification of *V. vulnificus* (strain L2) and *V. parahaemolyticus* (strain L21). In laboratory experiments we used RFLP analysis to confirm that bacteria, which were used to artificially infect, damaged tail fan tissue, could be recovered from developing TFN lesions. These observations showed that marine vibrios are capable of colonising lobster tissues and shell surfaces for long periods of time. Consequently, it is possible that probiotic strains of bacteria might be used to treat animals prior to holding as a means of limiting TFN.

The present study investigated physical methods of limiting or controlling the development of TFN in live held rock lobsters. The mesh bags developed for the “bagging” treatments, where lobsters were placed in bags at capture, limited physical damage and resulted in lower levels of TFN in live-holding. Lobsters spent up to 20 days in bags from when they were placed in them from the pots, held in wells of fishing vessels and then in processors tanks before assessment at the start of experiments. Lobsters immediately became inactive in the bags, did not interact with other lobsters and were easy to handle during processing. This post harvest technology will increase the feasibility of live-holding by minimizing TFN and is worthy of further investigation at a commercial scale.

Another approach to controlling TFN might be by use of probiotic bacteria. There may be non-pathogenic bacteria that might colonize damaged tissue and preclude the establishment of strains capable of initiating TFN. In the present study an isolate of *V. alginolyticus* was tested for ability to initiate TFN in intentionally damaged lobsters. This organism is not regarded as pathogenic and we were interested to determine whether this organism would elicit TFN to the same extent as other *Vibrio* spp. Interestingly, lobsters inoculated with *V. alginolyticus* developed TFN, which involved a mixture of *Vibrio* spp. We therefore conclude that this strain of *V. alginolyticus* cannot be used as an effective probiotic treatment.

5. Benefits

This research will be of immediate benefit to rock lobster processing and especially to any section of the rock lobster industry interested in long term holding of lobsters. The bagging developed here minimizes physical damage post-harvest and is of benefit to fishers and processors. For long term holding the minimization of post harvest damage leads to lower rates of development of TFN. When feed is provided in holding systems there is an increase in the abundance of *Vibrio* bacteria, which will probably lead to higher rates of occurrence of TFN.

In the longer term, there is significant value in developing an understanding of the progress of TFN and a basic description of the microbiology of the TFN condition. We now have an understanding of the relationship of TFN to the general condition of “shell disease” that has been described in rock lobsters. This is the first step in moving towards control of the TFN disease.

An understanding of the microbiology of TFN has led to identification of potential public health risks associated with animals in long-term live-holding that are affected by TFN, as TFN involves potentially pathogenic bacteria. Developments in the global trade of food have exposed primary producers to a new set of opportunities and risks. Estimating ‘equivalence’ is now the process used to determine whether or not Australian products can penetrate foreign markets, and whether or not products produced abroad can penetrate Australian markets. This involves an appraisal of whether the imported product presents the same or lesser magnitude of human-health risk as posed by the domestic product. Under the guidelines produced by the World Trade Organisation (WTO), the assessment of equivalence demands the conduct of a food safety risk assessment by the importing country. A country can deny the entry of a product if it fails to meet the equivalence standard. It needs to be emphasized that advanced TFN in *Jasus edwardsii* has only been identified in experimental long-term live-holding systems and is not prevalent in the wild fishery or the normal processing and export path. However, vigilance needs to be maintained.

As lobster aquaculture becomes established in future years it is likely that juvenile lobsters, especially larger advanced juveniles, will show some form of TFN. Thus this study is relevant to the proposed new rock lobster aquaculture industry.

The benefits and beneficiaries of the project are much as proposed in the original application. They include the South Australian wild lobster fishery sector via their interest in live-holding, rock lobster processors and all of the sectors of the proposed aquaculture industry for rock lobsters.

6. Further developments

This study has established an understanding of post harvest damage and TFN and proposed technologies that can be taken up immediately. In particular the bagging developed in this project could go to a commercial scale as a means of protecting against physical damage in the normal handling, processing and marketing chain. Interest has been shown in such commercial development and it should be pursued. Bagging should be a key component of future initiatives in live-holding. A proposal for further work on bagging was prepared but did not gain funding (see Appendix 3).

The survey of the occurrence of TFN in wild rock lobster fisheries and in processing systems throughout Australia undertaken in this project has not provided a good understanding of the situation. A future survey would need to be better resourced and include personal interviews.

The study has opened many lines of further inquiry into the microbiology of surfaces of lobsters and the microbiology of TFN. A full understanding of the microbiology of lobster surfaces and TFN will require molecular analysis of the bacterial flora of healthy and damaged lobster tissue of wild lobster populations. In particular, the development and succession of bacterial floras needs to be identified in the progression of the TFN disease. Future studies should also look at ways of controlling the disease, including use of probiotics.

Finally, because TFN involves potentially pathogenic bacteria the public health aspects of bacterial flora of healthy and diseased rock lobsters need to be considered. There is potential for transfer of pathogens from live lobsters to other foods during food handling and preparation. Subsequent growth of these pathogens in foods would pose a public health risk to consumers and a risk to export of live lobsters.

7. Planned Outcomes

- (1) Enhanced knowledge of the symptoms/presentation of tail fan damage that we have defined as tail fan necrosis, TFN.
- (2) An evaluation of the relationship between certain environmental factors and the occurrence of TFN. Higher water temperatures promote TFN, but occurrence was noted at 15 and 23°C; bacteria on surfaces in the holding environment are related to extent of TFN; and especially, TFN is promoted by physical damage to the tail fan.
- (3) Demonstration of the effectiveness of bagging lobsters at capture to reduce the extent of physical damage to the tail fan and subsequent development of TFN.
- (4) Initial identification of the causal agents of TFN, especially strains of several species of *Vibrio*, including *V. vulnificus*, *V. parahaemolyticus*, *V. alginolyticus* and other *Vibrio* spp.
- (5) Drawn attention to the need for vigilance in assessing and understanding the microbiology of live lobster body surfaces as a potential public health risk.
- (6) Provided an awareness of the limited, but occasional, occurrence of TFN in the rock lobster industry.

8. Conclusions

The Objectives of Project 2000/211 were:

- (1) To investigate potential causes of tail fan damage in live-held adult southern rock lobster.

Further Objectives

- (2) To characterize the microbial pathology of tail fan necrosis
- (3) To evaluate the effectiveness of “bagging” lobsters post capture in minimising the development of TFN
- (4) To undertake a survey of the occurrence of TFN in major rock lobster fisheries in Australia.

These have generally been met and the following conclusions have been reached.

- (1) The cause of TFN appears to be bacterial infection that is initiated by bacteria, especially species of *Vibrio*, given access to tissue via physical damage or a wound. This is most likely to happen post-capture when lobsters are handled and kept at high density. TFN progresses faster at 23°C than at 15°C, but still develops to an advanced stage at 15°C. Lower temperatures, such as those in most lobster processing plants, would be expected to inhibit development of the disease. Different holding densities and feeding regimes had little effect on the occurrence and progression of TFN. The lobsters in the starved holding treatment showed slightly lower levels of advanced TFN which is consistent with the observation that numbers of *Vibrio* on surfaces in the holding system were higher in the more heavily fed treatments.
- (2) The microbial pathology of TFN has been characterized to involve several species of *Vibrio* including *V. vulnificus*, *V. parahaemolyticus*, *V. alginolyticus* and other *Vibrio* spp. Culture of pure strains, re-infection to healthy lobster tail fans and re-isolation of bacteria from diseased tissue implicate *Vibrio*

species in the development of TFN. However, this infection experiment did not produce the large lesions and advanced TFN seen in the field trials. Other types of bacteria may be required to induce the chronic disease characteristics of TFN.

- (3) The novel practice of placing lobsters in mesh bags at capture and keeping them in the bags through transport and processing was shown to minimize physical damage. This may be useful in the normal processing path to export of rock lobsters. The bagging minimized tail fan damage such as tears, scratches and holes in the tail fan, and this resulted in lower rates of TFN in the extended live-holding of bagged lobsters.
- (4) We developed a survey that can be used to make lobster fishers and processors aware of TFN and to investigate the occurrence of TFN from their responses. The survey was distributed to fishers and processors in South Australia, Western Australia, Victoria, Tasmania and Queensland. There were very few returns. From the limited information we have it seems there is an awareness of TFN in the lobster industry in South Australia, Western Australia and Victoria but the occurrence of TFN in wild populations or in lobster processors cannot be quantified.

9. References

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APPENDIX 1 - INTELLECTUAL PROPERTY

The purpose of the work was to conduct public domain research so that all stakeholders can benefit from the findings.

Research results have been/are being published and have/are being presented at national and international forums. It is not anticipated that patents or commercial intellectual property will arise from the project.

APPENDIX 2 - PROJECT STAFF

Name and affiliation	Position	FTE on Project
<i>University of Adelaide</i>		
MC Geddes	Asoc Prof	5%
C. Thomas	Assoc Prof	3%
D. May	Hons student	40% for 8 months
<i>SARDI Aquatic Sciences</i>		
RJ Musgrove	Senior Research Scientist	30%
<i>Casual Staff</i>		
M Lorkin	Field support, Port Lincoln	5%
Various	Technical assistance SARDI Aquatic Sciences, West Beach	20%

APPENDIX 3 - FRDC R&D FUNDING APPLICATION, FEBRUARY 2002

PART A ADMINISTRATIVE SUMMARY

A1 Value-adding rock lobsters: A pilot study of a new packaging method

A2 APPLICANT

Organisation	--South Australian Research and Development Institute	
Unit	-Aquatic Sciences	
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-	-	
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Legal Status: Registered Research Agency		

A4 PRINCIPAL INVESTIGATORS

Name	Mr Greg Ward-
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A5 CO-INVESTIGATOR

A6 PLANNED START AND END DATE

Start Date	March 2002-
End Date	June 2002

A3 ADMINISTRATIVE CONTACT

Name	-Mr Dennis O'Malley	
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	AUSTRALIA -	AUSTRALIA
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A7 PROJECT BUDGET SUMMARY

Contribution by the FRDC (C1 - C4)

Year	Salaries	Travel	Operating	Capital Total
01/02	12,059	4,300	21,162	1,20038,721
02/03	-	-	-	--
03/04	-	-	-	--
Total	12,059	4,300	21,162	1,20038,721

Contribution by the Applicant (C5)

Year	Salaries	Travel	Operating	CapitalTotal
01/02	12,059	-	-	--
02/03	-	-	-	--
03/04	-	-	-	--
Total	12,059	-	-	--

Contribution by Other Sources (C6)

Year	Salaries	Travel	Operating	CapitalTotal
01/02	7,000	-	2,000	5,00014,000
02/03	-	-	-	--

03/04	-	-	-	--
Total	7,000	-	2,000	5,00014,000

Budget Totals	31,119	4,300	23,162	6,20064,781
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A8 SPECIAL PROJECT BUDGET CONSIDERATIONS

A9 EXTERNAL REVIEW

A10 CERTIFICATION

The Applicant and the Principal Investigator warrant that all information contained in and forming part of this R&D Application to the FRDC is complete, accurate and provided in good faith at the date submitted to the FRDC and that any changes to circumstances will be notified to the FRDC as soon as possible. They also warrant that the Principal Investigator, key research staff and research agency funding inputs will be available for the duration of the project.

Signed for and on behalf of the Applicant

(Print Name and Position) (Signature and Date)

Signed by the Principal Investigator

(Print Name and Position) (Signature and Date)

A11 TIME BOX (Applicable to applicant organisations with less than 20 employees)

PART B PROJECT DESCRIPTION

The Project Description should provide all the information necessary to enable the R&D Application to be fully evaluated

B1 PROJECT IDENTIFICATION

FRDC Programs: Industry Development
 Strategies -Fishing Technology
 -Value adding
 -

[Species] Southern Rock Lobster (*Jasus edwardsii*)

B2 BACKGROUND

The South Australian Southern Rock Lobster industry is worth \$75 million to \$80 million annually. The annual harvest is about 2600 tonnes and the average beach price around \$30/kg, ranging from \$23 to \$50 depending on area and month of the season. In recent years the quota in the fishery's Southern Zone has remained unchanged and the Northern Zone harvest has declined steadily from 1016 tonnes in the 1997/98 season to 847 tonnes in the 2000/2001 season. In the coming season the NZ harvest is expected to be between 700 and 800 tonnes (J. Prescott, pers. comm.).

In the face of this, and similar concerns in the other states, there has been increasing national interest in aquaculture and post-harvest handling/value-adding in Australian lobster fisheries, with most of the research done under the Rock Lobster Enhancement and Aquaculture Subprogram (RLEAS). In South Australia such work had an initial focus in determination of the optimum environmental and system requirements for long-term holding of adult rock lobsters (Geddes *et al*, 2001, FRDC 98/305).

In the context of the present proposal, Geddes *et al* identified tail-fan damage as the single greatest impediment to the development of the rock lobster live-holding industry in South Australia. During field trials up to 82% of the lobsters in some treatments were recorded as having some form of tail fan damage with up to 45% developing erosion, now called Tail Fan Necrosis (TFN, Musgrove 2001).

This led to the initiation of FRDC project 2000/211 (Investigation of tail fan necrosis in live-held adult rock lobsters). During 2000/211 it was found that damage to lobsters in transit from the pot to the factory was virtually eliminated by putting them in individual fine mesh bags. Once in the bags, the lobsters did not move and could be handled easily. Once released from the bags they showed significantly less damage (both tail fan and leg loss) and were extremely lively compared to their un-bagged con-specifics which had been in the same boat wells, crates and tanks for the same period – up to two weeks in some cases. In subsequent field and laboratory trials the lobsters that had been bagged developed significantly less TFN than those in un-bagged treatments.

At present there is a project (FRDC 2000/251) in Western Australia looking to minimize damage through leg loss, in particular that caused by contact with hypersaline water on boat decks. This spontaneous shedding of legs (hypersaline-induced autotomy) is a significant problem for the western rock lobster (*Palinurus cygnus*) fishery but has not been recorded from the South Australian fishery where leg loss appears to be caused more by multiple handling and crowding. While the cold-stunning approach under development in WA to reduce leg loss may be of use here, the differences in marketing strategy between the two fisheries suggests that a different approach may be more beneficial. About 95% of the SA product is exported live compared with only 35% of the WA product (pers. comm. R. Stevens).

During the journey from pot to market, whether it be direct or via a live-holding facility, each live lobster is handled multiple times, each occasion an opportunity for leg loss. They are also kept in crowded boat wells, crates and tanks during the process. If live-handling can be reduced by appropriate packaging then leg loss will be reduced and the value of the product increased, clearly an advantage in an increasingly competitive market. This project proposes the development of an apparatus for bagging lobsters at the pot to be taken through to market with minimal handling.

B3 NEED

Given that mesh bags have been shown to reduce both TFN and leg loss, their usefulness to the lobster-live-holding industry can not be overstated. The development of a practical bagging device to transport lobsters to the live-holding facility will significantly reduce leg-loss and TFN and maximize the return from each lobster held. The reduction in TFN is critical as the condition is rare in the wild and live-held product is competing with wild-caught lobsters on the open market. TFN can render a lobster unsaleable.

Competition and reduced margins are forcing many processors to be more selective of the lobsters they buy from fishermen. This is largely due to losses that processors face as a result of the post-harvest treatment of the catch. The following is a typical example. Lobsters are brought in to the processors in plastic crates. The scales are tared using an empty crate which is then replaced with a full one and the weight of the lobsters recorded. The fisher is paid on that weight. However, when the lobsters are removed from the crate there are always legs left in the bottom, unattached. Recently one processor monitored this throughout the season and, at the end, found that he had paid \$20,000 for unattached legs which he then couldn't sell. He sees this as an on-going cost to his operation. Given that he gets about 20% of the SA Northern Zone catch, and that all processors in the zone use this system for weighing lobsters, that equates to about \$100,000 per year for the SA Northern Zone and about \$400,000 per year for the whole South Australian rock lobster industry.

In addition to this, most SA lobsters go to the Chinese market. If lobsters are missing three legs or more they are tailed, and the frozen tails are sold for a reduced price on the US market. Up until recently processors have been paying fishers the live price for lobsters which were only fit for the tail market. With the current exchange rate, a mid season live price is about \$30/kg compared to a tail price of \$25/kg. At the end of the season the live price can be \$46/kg, the tail price \$25/kg.

Because of the above losses and financial pressures, it is becoming more common for processors to pay tail price for damaged lobsters, reducing the money that fishers make from their catch. Clearly there is an increasing incentive to take care of lobsters. In monetary terms, if 5% of the annual SA catch was bagged and the premium for such lobsters was \$5/kg, assuming an average price of \$30/kg, the fishery would increase in value by \$650,000/year.

In summary, there is an increasing demand from processors and markets for a better-presented product with all legs and feelers intact. A "pot to market" packaging system would reduce such losses significantly and, if combined with a labelling system, would allow those processors/fishers, who take the initiative, to increase earnings by becoming identified with top quality product. Our intention is to

develop a system that achieves benefits in a scalable fashion – here we are anticipating future commercialisation activity within national and international (i.e. New Zealand) markets. Out of necessity a quality assurance scheme would accompany the commercialisation activity with the product quality certification taking the form of FRDC and SARDI logos added in some form to the fisher’s label. It is intended that there be a levy on some part of the technology (e.g. the bag) which would be collected by the manufacturer and funnelled back to SARDI to fund further research aimed at achieving a quality assurance (QA) standard for the industry.

B4 OBJECTIVES

1. Develop and build a prototype packaging system that can be used easily on board lobster vessels.
2. Test the effectiveness of the apparatus by quantifying the reduction in leg loss and tail fan necrosis achievable through improved product quality.
3. Determine the optimum commercialisation strategies for the bagging system, primarily focusing on a system of market delivery for effective distribution and uptake of the technology.

B5 OUTPUTS & EXTENSION

A prototype bagging table and manual on its use will be produced. The final report will include optimum commercialisation strategies developed in consultation with the Technology Commercialisation Group, a company retained by SARDI to advise on commercialisation of research outputs.

B6 PLANNED OUTCOMES

Reduction in leg loss and improvements in condition of lobsters leading to an improved average price paid for product in the marketplace.

B7 FLOW OF BENEFITS

Fishery (including aquaculture) Commercial Sector	Recreational Sector	Traditional Fishing (by Strait Islander people)
Managed by: Aboriginal & Torres Strait Islander people		
Sector		
New South Wales	--	-
Northern Territory	--	-
Queensland	--	-
South Australia	80--	-
Tasmania	-10-	-
Victoria	-10-	-
Western Australia	--	-
Australian Fisheries Management Authority		
AFMA - Bass Strait Scallop	--	-
AFMA - Southern & Western Tuna and Billfish	--	-
AFMA - Great Australian Bight Trawl	--	-
AFMA - South Tasmanian Rise (outside of AFZ)	--	-
AFMA - Torres Strait	--	-
AFMA - Heard and McDonald Islands	--	-
AFMA - Southern Bluefin Tuna	--	-
AFMA - Eastern Tuna and Billfish	-	-
AFMA - Northern Prawn	-	-
AFMA - Southern Shark	-	-
AFMA - South East (trawl and non-trawl)	-	-
AFMA - Southern Squid	-	-
AFMA - Other	-	-
Total	-	-

Summary Flow of Benefits	
Sub Total Commercial Sector	%
Sub Total Recreational Sector	%
Sub Total Traditional Fishing Sector	%
Summary Flow of Benefits	100%

B8 INDUSTRY AND MANAGEMENT CONSULTATION

Letters of support are attached from the Fish Factory Pty Ltd Famazos, Australian Southern Rock Lobster Exporters Pty Ltd and the Rock Lobster Enhancement and Aquaculture Subprogram. Greg Ward, a PI on the project, has had 32 years experience as a rock lobster fisher and has been involved in collaborated in research for many years, most recently in the Rock Lobster Enhancement and Aquaculture Subprogram (RLEAS) on determination of the optimum environmental and system requirements for long-term holding of adult rock lobsters (FRDC 98/305). He is also on the RLEAS steering committee.

B9 METHODS

1. Construction of prototype
 - i. Related objective: Objective 1
 - ii. Personnel: G. Ward, R Musgrove
 - iii. Schedule: March 2002 – April, 2002
 - iv. Budget: R. Musgrove (0.1 FTE) \$2312; Institutional levy \$462; Travel and accommodation \$550; Materials and manufacture \$5000; tags, label development and printing costs \$1750
 - v. Location: SAASC and G Ward’s vessel, Jewel Seas, based at Vivonne Bay, Kangaroo Is.
 - vi. Protocol: The “prototype” refers to the bagging system which includes bagging frame and purpose-modified plastic crates. The prototype will be designed to fit on the deck of a lobster boat and fold away when no longer needed. The frame will be constructed from plastic and high quality stainless steel.
 - vii. Output: Bagging system and initial report on performance.
2. Field prototype test and pack-out trial
 - i. Related objective: Objective 2
 - ii. Personnel: G. Ward, R Musgrove
 - iii. Schedule: April 2002 – June, 2002
 - iv. Budget: R. Musgrove (0.1 FTE) \$4,625; TBA \$5122; Institutional levy \$1949; Travel and accommodation \$3750; Statistical analysis \$1000
 - viii. Location: G Ward’s vessel, Jewel Seas, and his storage tank facility based at Vivonne Bay and Kingscote, respectively, on Kangaroo Is.
 - v. Output: Report on prototype performance and pack-out-trial.
 - vi. Protocol:

Experiment 1: Field Prototype Test

Lobsters will be bagged using the prototype on 2 occasions during April and May 2002. During each trip, on the lobster fishing vessel “Jewel Seas”, 20-30 lobsters (depending on the day’s catch rate) will be bagged and placed in the modified crates which will be stored with the rest of the catch in the boat’s well. 20-30 un-bagged lobsters will be placed in separate modified crates for comparison. A third un-bagged group will be placed in regular crates as a control. Crates will be triplicated within treatments. All treatment and control lobsters will be assessed for damage (antennal/leg loss, tail fan damage) immediately upon removal from the pot and tagged (pleopod clip). Well-water temperature will be monitored during each trip.

Experiment 2: Pack-out Trial

Lobsters from Experiment 1 will be bought back to the factory using standard industry practices, graded and packed out in treatment-specific eskies, i.e. bagged, unbagged/modified crate, unbagged/regular crate. Water temperature will again be monitored with data loggers during transport. After a time corresponding to transit time to the market lobsters will be removed from eskies and bags and graded with respect to mortality, leg loss/other damage and liveliness (sliding scale 1-5) as assessed by staff of the Fish Factory. The liveliness assessment will be blind, that is, the latter will not be told from which treatment a given lobster originated.

Experiment 3:

Lobsters from Experiment 1 will be bought back to the factory using standard industry practices. From there 10-15 bagged and the same number of un-bagged lobsters will be transferred to SAASC where bags will be removed and all lobsters assessed for damage and tail fans photographed. Groups of 5 to 7 lobsters from each of the bagged and un-bagged treatments will be placed in separate 500l tanks (i.e. duplicate bagged and unbagged tanks) with aeration and flow-through seawater supply. Lobsters will be fed cockles at 2% body weight/day, 3 times a week for four weeks then reassessed for damage and TFN. The four week period was selected as it is the length of time TFN generally takes to develop.

Data Analysis

Treatment effects will be analysed using Poisson regression on Genstat™ and liveliness and causative effects on injury frequencies within treatments (ie. the effect of existing or new leg loss on subsequent loss) will be analysed using Chi Square.

3. Commercialisation Strategy.

- vii. Related objective: Objective 3
- viii. Personnel: G. Ward, R Musgrove, TCG Staff
- ix. Schedule: December 2002 – May, 2002
- x. Budget: TCG consultancy fee \$5000
- xi. Location: SAASC, Adelaide
- xii. Output: Report on optimum commercialisation strategy.
- xiii. Protocol:

TCG will provide advice on the optimum commercialisation strategy, including the likelihood of a commercial application, the possible path to market and method of capturing a fund stream. They will also assist in identifying future development requirements. Early indications from fishers and processors suggest that there is commercial merit in the idea of a bagging system and that a premium would be paid for lobsters in good condition with all limbs intact. This work will proceed as follows but it is important to note that these activities do not necessarily occur in a linear fashion as set out.

1. Determine the degree of support for the above observations for the industry in general (including processors). Determine the value that processors and buyers would attach to a bagging system that provided consistent, quality lobsters in a way that was readily identified within the market (e.g. a red bag).
2. Develop an understanding of what the system needs to conform to current processes for each member of the industry to benefit. This will occur by consultation with industry and by working their needs into the prototype that is developed.
3. Identify future development requirements with regard to the overall objective, a QA standard for the industry.

B10 RISK ANALYSIS

1. Threat: The prototype will not function well
Contingency: The design process will be collaborative and care will be taken to ensure the most practical design is achieved.
2. Threat: Lobsters will be difficult to grade while in the bags. Lobster movement will be restricted within bags so the usual liveliness indicators (holding up or flipping of the tail and leg movement) will be harder to use.
Contingency: If necessary, the grading procedure will be modified in collaboration with the processor to whom the fish will be taken. Gill bailer movement may prove useful in this situation.
3. Threat: Lobsters will lose legs while in bags. Leg will then rot in the bag, compromising the product.
4. Contingency: Lobsters lose legs because they are handled roughly. Care will be taken during capture and transport to minimize this effect. Also, bags with loose legs will be found during the grading process.

B11 PERFORMANCE INDICATORS

- Production of the prototype

- Fitting prototype to the vessel
- Completion of the Experiment 1
- Completion of the Experiment 2
- Completion of the Experiment 3
- Uptake by at least one industry member

B12 MILESTONES

Production of the prototype	30 April, 2002
Completion of the trials	30 June, 2002
Completion of final report, including assessment of commercial potential	30 July 2002

B13 OTHER RELATED PROJECTS

FRDC 2000/251: Rock Lobster Post Harvest Subprogram: G. Davidson and W. Hosking (in progress) Development of a method for alleviating leg loss during post-harvest handling of rock lobsters.

Dr Musgrove has discussed the proposal with Dr. Glen Davidson and it has been agreed that the projects are complimentary and non-overlapping. Reviews of relevant databases have not shown any other projects in the available literature which address the leg loss issue.

Other projects from which the Dr Musgrove has gained experience in working with southern rock lobsters and/or post-harvest handling issues include:

FRDC 96/160: R Musgrove (submitted 2000) Condition and its assessment in the southern rock lobster *Jasus edwardsii*. ii. Field application of techniques for condition assessment and moult staging in the laboratory..

FRDC 99/422: R. Musgrove, S. Grauf, H. Williams and B. Goodrick (2000). Demonstrating the capacity of western king prawns for live export.

FRDC 2000/211: M. Geddes, R. Musgrove and C. Thomas :(Final report in preparation) Investigation of tail fan necrosis in live-held adult rock lobsters.

B14 FACILITIES

This project will take place in the facilities and vessels of Jewel Fisheries Pty. Ltd. and the South Australian Aquatic Science Centre.

B15 STAFF

Name	Position	Qualifications	Time
Greg Ward	Lobster Fisherman		20-
Richard Musgrove	Senior Scientist	PhD-	20-
TBA	OPS3		30

APPENDIX 4 - PUBLICATIONS AND PRESENTATIONS

Publications

- Geddes MC, Musgrove RJ, and Thomas C (2001). Investigations of tail fan necrosis in live -held adult rock lobsters. In RLEAS Publication No 6. RLEAS Workshop 2001. III ed. R van Barneveld Developments in Rock Lobster Enhancements and Aquaculture III
- Geddes MC, Musgrove RJ, and Thomas C (2002). Causes of tail fan necrosis in southern rock lobster (*Jasus edwardsii*). In RLEAS Publication No 7 .RLEAS/RLPHS Joint Workshop 2002, eds.R van Barneveld and B Phillips Developments in rock lobster enhancement, aquaculture and post harvest practices.
- May DG (2002). Identification and characterization of bacteria associated with Tail Fan Necrosis of Southern Rock Lobsters (*Jasus edwardsii*). BSc(Hons) Thesis, Department of Microbiology and Immunology, University of Adelaide.
- Musgrove RJ, MC Geddes, and C Thomas (in press). Causes of tail fan necrosis in southern rock lobster, *Jasus edwardsii*. New Zealand Journal of Marine and Freshwater Research; Lobster Biology and Management Special Issue.

Presentations

- 2001 Wellington NZ RLEAS Workshop. Causal factors of tail fan necrosis in live-held rock lobsters. MC Geddes, RJ Musgrove, and C Thomas.
- 2001 Industry presentation, Lincoln Marine Science Centre, Port Lincoln. MC Geddes and RJ Musgrove.
- 2001 Cairns RLEAS Workshop. Tail fan necrosis in rock lobsters: pathology, causes and minimization strategies. MC Geddes, RJ Musgrove, and C Thomas.
- 2004 7th International Conference and Workshop on Lobster biology and Management, Hobart Tas. TFN title. RJ Musgrove, MC Geddes and C Thomas.
- 2004 7th International Conference and Workshop on Lobster biology and Management, Hobart Tas. The microbiology of tail fan necrosis in southern rock lobsters subjected to live holding at ambient temperatures. D May, RJ Musgrove, MC Geddes, and C Thomas.
- 2004 37th Australian Institute of Food Science and Technology Annual Convention and Exhibition, Brisbane QLD. The role of marine vibrios in establishment of Tail Fan Necrosis in southern rock lobsters and implications for Public Health. D.May, RJ Musgrove, MC Geddes, and C Thomas.

