

Development of a Method for Alleviating Leg Loss During Post-harvest Handling of Rock Lobsters.

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Australian Government
**Fisheries Research and
Development Corporation**

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Objectives:

- 1) 1a.) To identify a cold water immersion treatment that rapidly immobilises western rock lobsters, while allowing swift recovery from immobilisation upon return to ambient temperature seawater.
1b) To investigate the effect of season/acclimation temperature on effectiveness of cold-stunning in western rock lobsters.
1c) To investigate the use of seawater sprays versus immersion for inducing cold-water stunning in western rock lobsters.
- 2) To investigate, in captivity, the effectiveness of the preferred treatment (identified in Objective 1) for reducing leg loss in western rock lobsters during handling.
- 3) To test the accuracy of factory grading of cold-stunned western rock lobsters vs untreated controls.
- 4) To describe the occurrence of leg loss, morbidity and mortality of western rock lobsters subjected to cold-stunning prior to episodes of handling during the post-harvest process and to compare these to the performance of animals handled using current methods.

- 5) To investigate, in captivity, the effects of multiple simulated pot capture and release events, either with or without cold-stunning on growth, leg loss and survival of undersized western rock lobsters.
- 6) 6a) To compare, in captivity, the effects of handling,, with and without cold-stunning,, on the reproductive success of setose, tar spot and ovigerous female western rock lobsters.
6b) To investigate the effects of limb loss on the reproductive success of female western rock lobsters.
- 7) To conduct a survey to determine the extent and nature of leg loss in the southern rock lobster fisheries of Tasmania and South Australia.

Non-Technical Summary

Outcomes achieved to Date

A novel brief application of cold-stunning was identified as a practical method for preventing post-harvest leg loss at several points in the post-harvest handling chain. Using this method, there is potential for the western rock lobster industry to save in excess of \$2 million per season in lost catch weight attributable to leg loss. In addition, considerable value could be added to the catch through increases in the numbers of lobsters fit for exporting as premium product forms, such as live or whole frozen boiled.

Rates of leg loss occurring in different zones of the fishery have been quantified, providing an objective basis for conducting cost-benefit analysis of any preventative measures under consideration.

During the course of this study hypersaline-induced leg loss was identified as a significant cause of post-harvest leg autotomy. This finding led to a second study (FRDC Project 2001/255) which in turn identified potential six figure savings using simple, cost-effective methods.

Rates of post-harvest appendage loss in the southern rock lobster fisheries of South Australia and Tasmania have been documented as being very low. The small amount of antennal damage occurring on board commercial boats appeared to be largely preventable by making simple changes to handling practices. Further investigation of this issue does not appear warranted.

Leg loss is a major problem for the western rock lobster industry. At the start of this project it was conservatively estimated that up to \$3 million in weight of legs are lost each season. Additional losses are incurred through the downgrading and devaluing of damaged product.

This project set out to identify practical methods for preventing this post-harvest leg loss. Initially cold water stunning was tested as a potential preventative measure. It was shown the lobsters could be stunned in a matter of seconds by placing them in cold (0-10°C) seawater for very brief periods (5-10 sec.). After stunning, lobsters became very easy to handle and did not struggle when handled. When stunned lobsters were put back into normal temperature seawater, they recovered within 10 sec. From these observations, this treatment appeared to be very suitable for use at all points in the post-harvest handling chain.

It was found that western rock lobsters rapidly shed legs when they come into contact with concentrated seawater films. This response was studied more closely in the companion project, FRDC Project 2001/251. By using this concentrated seawater lobsters could be easily made to shed legs and this gave an opportunity to test methods for stopping the leg-shedding response. Placing lobsters in 5°C seawater for 5 sec. almost completely prevented this salt-induced leg shedding.

Cold water stunning was then tested as a method for preventing leg damage on board working commercial lobster boats. Cold-stunning was quick and easy for the crew to use and deckhands preferred to work with the stunned lobsters because they were so much easier to sort into size and undersize. After over 50 days of sea trials it was shown that, when applied correctly, cold-stunning could reduce on board leg loss by up to 80%. In addition, cold-stunning was shown to be effective over a wide area of the fishery and at all times of the year. When transported using best practice methods, cold-stunned lobsters were shown to be of higher quality when they were delivered to the processing factory than lobsters handled without stunning.

Cold-stunning was also applied to lobsters prior to factory grading in an attempt to reduce the damage that occurs during handling in the factory. It was shown that human graders could grade stunned and untreated lobsters equally well and were still able to identify those lobsters that were fit for live export. Cold-stunning before grading was also effective for reducing leg damage that occurs during freshwater drowning of reject lobsters after grading.

Stunning lobsters on board fishing boats raises concerns for the survival of undersized lobsters that are returned to the ocean in a stunned state and for the survival of eggs on berried females that are caught and returned. It was shown that removing berried lobsters from the water

and handling them in air would more than halve the numbers of larvae that a female would successfully produce. Berried females that were cold-stunned before handling in air produced just as many larvae as those handled without stunning, so there was no damage caused to the eggs by the stunning. Previous work has shown that leg loss results in breeding females producing fewer larvae. By preventing leg damage, cold-stunning may actually improve the numbers of larvae that commercially caught females can produce.

A survey of leg and feeler damage in the southern rock lobster fisheries of South Australia and Tasmania showed that only relatively minor post-harvest damage to legs occurs in these fisheries. Some damage occurs to antennae when lobsters are held by these appendages, but this can be easily prevented by handling lobsters with greater care.

In summary, cold-stunning is a practical method for preventing post-harvest leg damage in western rock lobsters. Further work is being undertaken to determine if it can be safely applied on board working commercial fishing boats without harming returned undersized lobsters.

Keywords:

Lobster, Autotomy, Cold-stunning, Post-harvest, *Panulirus cygnus*, *Jasus edwardsii*

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Background

In the late 1970s, Brown and Caputi (1983) investigated the effects of commercial handling practices on the growth and survival of captured and returned undersized western rock lobsters. At the time an estimated 18 million undersized animals were handled each season, suffering increased mortality and reduced growth after release.

Of the factors contributing to increased mortality and reduced growth of returned undersized animals, physical damage, predominantly in the form of leg loss, was identified as being significant. The walking legs are especially vulnerable due to the natural autotomy reflex that most decapods possess. Rock lobsters are handled numerous times during the post-harvest process: during the emptying of the pot and sorting onboard the fishing boat, at the point of landing of the catch, during grading upon arrival at the factory, at transfer into factory tanks, and during packing for live export. If the catch is landed at a remote site, ten additional handling, in the form of truck or carrier boat transport to the factory, is required. With each handling the probability of the animals being damaged increases.

Despite a significant reduction and improvement in handling of undersized animals resulting from the work of Brown and Caputi, leg loss remains a major concern to the western rock lobster industry (1998 FRDC Rock Lobster Post-Harvest Sub-Program Workshop, Hilary's Boat Harbour, Perth, WA). At the 1999 Annual FRDC Rock Lobster Post-Harvest Sub-Program Workshop, the same concern was voiced by representatives from the Southern Rock Lobster fisheries in Tasmania and South Australia.

The western rock lobster industry incurs costs from appendage loss in the following ways:

- **Loss of weight**

Mr Steven Hood of M.G. Kailis Ltd. Reported that during the 1998/1999 western rock lobster fishing season to June 15 1999, 0.3% of their total catch weight of size animals was lost as missing legs between sorting on board commercial boats and the completion of grading at the factory. This value was obtained by collecting and weighing detached legs at the grading table. Given the total catch in that season was 13 million kg and an average beach price of \$19.50/kg, the weight of product lost as legs would account for

\$760 500 in lost revenue. Note that this is an underestimate of the true cost as it does not include the weight of legs lost on board fishing boats prior to the animals being placed in the live holding tanks. The true cost could be up to 3 times this figure (Leo Zinetti, Geraldton Fishermen's Cooperative, pers. com.).

- **Loss of value/restricted marketing opportunities**

Leg loss renders lobsters unsuitable for certain product forms, such as live export or whole cooked, restricting processing options and limiting potential return. Mr Ross McGregor of Lobster Australia Ltd. Estimated that 5% of their 1.6 – 1.8 million kg annual catch of western rock lobsters is downgraded because of missing legs (> 3 missing legs) to lesser product forms, such as tails. At an estimated loss of \$2.00 – 3.00/kg, Mr McGregor estimates that Lobster Australia loses between %150 – 250 000/season.

- **Increased mortality of damaged returned lobsters (reproductive females and undersized)**

Tagged undersized western rock lobsters, damaged during capture and sorting and returned to the ocean, show reduced recapture rates, presumably due to increased mortality, compared to tagged, undamaged individuals, (Brown and Caputi, 1983). Leg loss presumably has similar consequences for returned reproductive (setose, tar spot or ovigerous) females.

- **Reduced growth of damaged returned lobsters (reproductive females and undersized)**

Damaged, undersized western rock lobsters also show reduced growth rates following return to the ocean (Brown and Caputi, 1986). This delays recruitment to the fishery and prolongs exposure to natural attrition (predation, etc.) before recruitment occurs. In *Panulirus argus*, Davis (1981) estimated that an extra 22 % of damaged 50 mm carapace length (CL) juveniles would die before being recruited to the fishery at 76 mm CL, this being additional to any mortality directly attributable to the physical damage.

- **Reduced reproductive success of damaged returned mature females**

Recent results suggest that female western rock lobsters with several missing legs fail to reproduce, presumably opting instead to direct energy to regenerating the missing limbs (Roy Melville-Smith, pers. com.). In addition, because sexual maturity is a function of

age rather than size (Chittleborough, 1974), slow-growing damage females will mature at a reduced size. A single 115 mm CL female produces as many eggs as three 77 mm CL females (Chubb et al., 1989), clearly demonstrating the impact of reduced female growth rate on fecundity. Given the specialisation of the last pair of walking legs in females (presumably for fertilising and tending eggs), leg loss may also impact on the reproductive capacity of females. There is no published information concerning this possibility.

The only formal documentation of the extent of leg loss occurring in Australian rock lobster fisheries is provided by a private consultancy conducted for the Geraldton Fishermen's Cooperative using western rock lobsters (Tod et al., 1990). The major findings of this work were:

1) Under the conditions of this study, at least 42% of the observed leg loss in western rock lobsters onboard coastal boats occurred after pot retrieval. The amounts to a significant cost to industry with the weight of legs lost in the cacka box alone accounting for approximately \$500 000 in lost revenue per annum. This cost is an underestimate as it does not include losses attributable to downgrading product and restricted marketing options for damaged lobsters. In addition, anecdotal evidence suggests that leg loss is positively correlated with wind speed and ambient temperature so that, under certain environmental conditions (eg. hot, dry easterly winds), western rock lobsters become especially sensitive to handling and many animals may shed several legs simultaneously. It is likely, therefore, that the 1990 survey underestimated the average rates of leg loss occurring onboard commercial vessels, due to mild environmental conditions prevailing during the survey period, which were as follows: wind speed 7-14 knots, temperature 18-24°C. During the fishing season in Geraldton, wind speed and temperature can exceed 40 knots and 45°C, respectively.

The percentage of pre-capture leg loss occurring on boats fishing at the Abrolhos Islands was reported to be greater than for coastal boats, accounting for an estimated 73% of leg loss attributable to fishing practices. However, given that only approximately 13% of the total annual catch of western rock lobsters comes from the Abrolhos Islands zone, the reported leg loss from this area was estimated to account for a small proportion (15-25%) of the total on board leg loss

occurring in any given season. It should be noted that meteorological conditions were not monitored during this part of the study.

2) Extrapolating from the data in the report, returned undersized animals lose approximately 1.8 million legs pre season (based on 18 million undersized animals handled each season (Brown and Caputi, 1983)) with resultant reductions in growth, survival and recruitment.

Using the above information in conjunction with the estimates provided by industry representatives, the direct cost to the western rock lobster industry of post-harvest leg loss is estimated to be \$2-3 million/season. This indicates there is scope for reducing post-harvest leg loss.

Leg loss and other damage usually occurs when lobsters show vigorous movement and tail-flipping when in air. This observation suggests that leg loss may be curtailed by reducing spontaneous movement during handling. In the development of this study, three potential methods for immobilizing lobsters during handling were considered: electro-shocking, anaesthesia and cold-stunning. All of these methods have been used previously on crustaceans (Gardner, 1997), but not necessarily in a commercial situation. Of these three methods, cold-stunning appears the simplest, safest and most benign, with a high likelihood of success.

A form of cold-stunning is presently widely used prior to packing of live western rock lobsters for export. Cold-stunning is used to reduce movement and leg loss and improve survival during transport. Cold-stunning is also used to immobilise blue manna crabs (*Portunus pelagicus*) during sorting on board commercial fishing boats and has been shown to reduce limb loss (Stevens, 1995).

A letter appearing in the April 1997 issue of *Prowest*, questioned the feasibility of using cold-stunning to reduce leg loss aboard commercial lobster boats. The main criticisms were that induction and recovery times were thought to be too long, resulting in increased mortality of returned animals. Initial experiments, conducted over a temperature range from 0 - 15°C, demonstrated the efficacy of cold water immersion for stunning lobsters and reducing spontaneous movement during handling (Davidson and Hosking, pers. obs.). Recovery from cold-stunning was very rapid upon return to ambient temperature seawater with no apparent detrimental effects. For example, at 10°C almost all movement ceased in 5-10 sec., and recovery (measured as righting time) in ambient temperature seawater was complete in 1 to 15 sec.

The initial proposal was to investigate the use of cold-stunning at several points in the western rock lobster post-harvest handling chain (for example, on board boats and prior to grading in the factory) to improve product quality and value, and to improve the growth, survival and reproductive success of undersize and breeding female lobsters that have been captured and released. A survey of the extent and nature of leg loss in the southern rock lobster fisheries of Tasmania and South Australia was also included at the request of the FRDC Rock Lobster Post-Harvest Subprogram Steering Committee. Once the usefulness of cold-stunning has been tested in captivity, and pending a successful outcome of this work, the intention was to submit a second proposal to FRDC to test the effects of cold-stunning on western rock lobsters in the field. This second proposal would include an extensive tag and recapture study to compare growth and survival of stunned and normally handled lobsters returned to the wild. This second study was approved as FRDC Project 2002/239 and the final report for this work is in preparation.

Need

At the 1998 FRDC Rock Lobster Post-harvest Sub-program annual workshop (Hilary's Boat Harbour, Perth, W.A.), fishers, processors and scientists identified post-harvest leg loss as a major problem. Industry incurs losses due to post-harvest leg loss in several ways:

- 1) Loss of catch weight.
- 2) Loss of value/restricted marketing opportunities for lobsters with too many missing legs.
- 3) Increased mortality of damaged returned undersized animals and reproductive females.
- 4) Reduced growth of damaged returned animals.
- 5) Reduced reproductive success of damaged returned breeding females.

Estimates of the costs to industry are provided in section Background (see above).

Objectives

- 1a.) To identify a cold water immersion treatment that rapidly immobilises western rock lobsters, while allowing swift recovery from immobilisation upon return to ambient temperature seawater.
- 1b) To investigate the effect of season/acclimation temperature on effectiveness of cold-stunning in western rock lobsters.
- 1c) To investigate the use of seawater sprays versus immersion for inducing cold-water stunning in western rock lobsters.
- 2) To investigate, in captivity, the effectiveness of the preferred treatment (identified in Objective 1) for reducing leg loss in western rock lobsters during handling.
- 3) To test the accuracy of factory grading of cold-stunned western rock lobsters vs untreated controls.
- 4) To describe the occurrence of leg loss, morbidity and mortality of western rock lobsters subjected to cold-stunning prior to episodes of handling during the post-harvest process and to compare these to the performance of animals handled using current methods.
- 5) To investigate, in captivity, the effects of multiple simulated pot capture and release events, either with or without cold-stunning on growth, leg loss and survival of undersized western rock lobsters.
- 6a) To compare, in captivity, the effects of handling,, with and without cold-stunning,, on the reproductive success of setose, tar spot and ovigerous female western rock lobsters.
- 6b) To investigate the effects of limb loss on the reproductive success of female western rock lobsters.
- 7) To conduct a survey to determine the extent and nature of leg loss in the southern rock lobster fisheries of Tasmania and South Australia.

Materials and Methods

Objective 1 1a.) To identify a cold water immersion treatment that rapidly immobilises western rock lobsters, while allowing swift recovery from immobilisation upon return to ambient temperature seawater. 1b) To investigate the effect of season/acclimation temperature on effectiveness of cold-stunning in western rock lobsters.

Lobsters that had been held in factory tanks for less than 15 days were used in the experiments. The night before being used in experiments, lobsters in a selected factory tank (140-180 kg) were fed at a rate of 3% of their total wet weight with frozen mussels (*Perna canaliculus*) on the half shell. This feeding was to simulate feeding of freshly caught lobsters on bait in pots. The mass of mussel used was calculated on the edible portion only (i.e. not including the shell).

The next morning, groups of 20-30 lobsters in the holding tank were gathered into submerged plastic baskets (Nally Lug Box; IH300, 66 L) prior to use in experiments. Individual lobsters were removed from the baskets by hand and were placed in another plastic basket (Nally Lug Box; IH300, 66 L) sitting in a 180 L insulated plastic bin (stun tank) filled with aerated Geraldton seawater (36 ‰) (Figure 1). The temperature of the seawater in the bin was either 0, 5, 10, 15°C, or ambient (control). Water temperature was regulated by adding chilled seawater or seawater ice. Up to 30 lobsters were individually exposed to each temperature for either 1, 5, 10, 20, 45 or 60 sec. Water in the stun tank was continuously aerated using a Sukuragawa 200GJ-H air pump. Dissolved oxygen (DO) was measured periodically using a WTW Oxi320 DO meter. DO never was maintained at >90% of saturation at all times. Water in the tank was renewed at regular intervals to prevent deterioration of water quality.



Figure 1) Lindsay McDonald (l) and Glen Davidson(r) conducting experiments addressing Objectives 1a and 1b. Note the insulated stun tank and aerator in the foreground. The basket used to measure recovery (righting) times can be seen in factory live holding tank in the background.

After stunning, lobsters were treated to one of two methods of handling. The first was intended to simulate stunning for use prior to on board sorting of the catch and the second was intended to simulate factory grading.

To simulate stunning prior to on board sorting of the catch, individual lobsters were transferred by hand from the factory tanks to the plastic stun tank. Following the stun treatment, the size (carapace length) of each lobster was checked using a standard aluminium lobster gauge. After checking the size, each lobster was inspected ventrally and the abdomen was extended in order to determine the gender and inspect the pleopods of females for the presence of setae. This standard task simulated the sorting of the catch onboard commercial lobster boats.

To simulate stunning prior to factory grading, lobsters in factory tanks were transferred to the stun tank and subjected to stun treatments as previously described. In this case the basket in the stun tank was lined with a plastic mesh basket. Following the stun treatment, the basket lining was used to lift the lobster out of the stun tank. The lobster was then shaken gently in the basket for 30 sec. to simulate the animal being transported down a conveyor belt during grading.

After the 30 sec. period the animals were removed from the basket by hand and inspected for vigour and for missing legs. This was intended to simulate factory grading procedures.

Control treatments consisted of placing lobsters in ambient temperature seawater for the prescribed period before subjecting them the prescribed handling treatment.

Recovery from stunning and boat sorting/factory grading was determined by placing lobsters upside-down in a plastic basket (Nally Lug Box; IH300, 66 L) submerged in aerated ambient temperature seawater to a depth of 150 mm. The time each lobster took to right itself was used as a measure of recovery time.

The experiments were repeated at two ambient seawater temperatures (18 (June 2000) and (November 2000) 22°C) spanning a large part of the range of temperature experienced by *P. cygnus* during the commercial fishing season (15 November - 30 June).

Objective 1c)

Objective 2 (see below) had to be completed before undertaking Objective 1c. The results and discussion of Objective 1c follow Objective 2.

Objective 2) To investigate, in captivity, the effectiveness of the preferred treatment (identified in Objective 1) for reducing leg loss in western rock lobsters during handling: Identifying “optimum” stun temperature.

Stun temperatures between 0°C and ambient (21°C) were tested in the sea trials. The results of these trials suggested that stun temperatures of between 0-10°C were most effective for preventing leg loss. However, large daily variation in leg loss makes it difficult to identify the optimal stun temperature. The logical solution was to identify the preferred stun temperature using a controlled trial in the factory.

In order to test the effectiveness of cold-stunning for reducing leg loss, we must first be able to induce it reliably. This provides some scope for testing improvements. In Objective 1 (the time/temperature matrix experiments) it was difficult to identify a preferred treatment for reducing leg loss because only very low rates of leg loss (less than 2 legs shed/100 lobsters handled) were observed.

Subsequently we began looking for an autotomy trigger that would allow us to compare in a controlled manner the effectiveness of a number of cold-stunning treatments for reducing leg loss. Previous in-house experimentation by the GFC research staff lead to the finding that hypersaline sea water solutions (i.e. SW + ≥ 30 g/L of added salt) were potent inducers of autotomy in western rock lobsters.

On the basis of induction and recovery times observed in experiments addressing Objective 1, a stun temperature of 5-10°C appeared suitable. The stun time was set at 5 sec., as this was found to be effective and workable during the sea trials (Objective 4). In the interim it was discovered that exposing lobsters to hypersaline water films induced leg loss (see FRDC 2001/255). Armed with method for reliably inducing leg loss the following experiment was conducted to further refine cold-stunning and try to identify the optimum stun temperature on the basis of leg loss prevention. Six hundred A-grade lobsters of either sex (mean weight = 455 g) with no more than one missing leg were removed from a factory live tank and were placed in baskets (Nally IH300 610 x 419 x 312 mm, 66 L) in groups of 6. These baskets were then submerged in aerated ambient seawater (22°C) in flow-through factory live tanks prior to use in experiments.

Each group of lobsters was subjected to one of 4 treatments: 1) 5 sec. immersion in 5°C seawater, 2) 5 sec. immersion in 10°C seawater, 3) 5 sec. immersion in 15°C seawater, and 4) 5 sec. exposure to 22.5°C air. After each treatment the basket of lobsters was left in air (22.5°C) to drain for 10 sec. before the lobsters were tipped from basket into tubs (Nally IH078 No 15 Crate; 68.2 L) containing 100 mL of a solution of Geraldton seawater + 55 g/L of butcher's salt. Concentrated salt solutions have previously been shown to induce autotomy in western rock lobsters. Lobsters were left in the tubs for 5 sec before each animal was removed individually and subjected to a simulated on board sorting which consisted of gauging each lobster for size, inspecting each ventrally for reproductive condition and counting the numbers of missing legs. The numbers of missing legs and the number of legs remaining in the tub after sorting the lobsters were recorded. The experiment was set up to complete one round of single replicates of each treatment before commencing the next round. This controlled for any systematic variation during the course of the experiment (Figure 2). After completing each replicate of a treatment, the solution in the tub was replaced and the investigator sorting the lobsters washed his gloves in seawater to prevent any interaction between treatments. For the purposes of statistical analysis,

each group of 6 lobsters was considered to be a single sample. Each treatment was repeated 20 times, giving a sample size of 20 for each treatment. In total, 480 lobsters were used in the experiment.



Figure 2) Investigators conducting experiments addressing Objective 2. The five parallel treatments can be seen extending into the background.

Objective 1c) To investigate the use of seawater sprays vs. immersion for inducing cold-water stunning in western rock lobsters.

Using an identical method to that described above, the relative efficacy of applying the cold water stun as a seawater spray or by immersion was tested.

A pump delivering water to at 11 L/min. was submerged in a 2000 L tank containing 5°C seawater (Figure 3). The water temperature was maintained using thermostatically controlled refrigerated plates in the tank. The temperature of the spray as it hit the lobsters was actually measured at 9°C. This may have been due to the mixing of the cold water with warm air, or heat from the pump delivering water to the sprays may also have contributed. Given this observation, exposure to seawater sprays (9°C) was compared with immersion in seawater at the same temperature. Apart from the different stun treatments, the experimental design was identical to that described above. Sample size was 20 groups of 6 lobsters for each treatment.



Figure 3) Cold-stunning being applied by spraying chilled seawater on lobsters.

Objective 3) To test the accuracy of factory grading of cold-stunned western rock lobsters vs. untreated controls.

Before commencing work on this objective we needed to identify a stun treatment that will reduce leg loss during grading but that will also allow accurate grading using the traditional indicators of vigour. As shown in Objective 1 experiments, increasing the duration and/or reducing the temperature of the stun treatment depresses these indicators of vigour (e.g. tail-flipping, struggling, etc). In the companion project (FRDC Project 2001/255, Davidson and Hosking, under review), it was found that appreciable leg loss occurs during “drowning” in ambient temperature fresh water. In addition, it was shown that prior cold-stunning in chilled seawater could reduce leg loss during ambient temperature FW drowning. A 15 sec. stun in 0°C seawater reduced leg loss during ambient temperature FW drowning by an average of 91.7 ± 5.4 % (n = 6). This treatment was nearly 100 % effective and was the most extreme, in terms of stun temperature and time. It was selected for testing in Objective 3, based on the assumption that if graders could still accurately grade these heavily stunned lobsters, then they would also be able to work with virtually any stun treatment.

Two hundred kilograms of fit for live export red A grade lobsters were taken as they arrived at the factory. Lobsters of both sexes with no more than 2 missing legs or 1 missing feeler were used.

Four hundred lobsters were tagged around base of one antenna with a small livestock tag and cable tie. The lobsters were placed in a tank overnight to recover from capture and tagging. The next day the lobsters were allocated to 8 prawn baskets sequentially, giving 50 lobsters in each basket. The tag number, gender and appendage loss of each lobster was recorded during transfer. Two baskets of lobsters were placed in each of following storage environments for 4 hours: Submerged in flow-through, aerated seawater, in humid air, under recirculating seawater sprays, and under flow-through seawater sprays (Figure 4). All treatments were run at ambient SW temperature (25.5°C). After four hours the baskets of lobsters were taken directly to the factory ramp to be graded (trial grading). One basket from each treatment was stunned at 0°C for 15 sec in aerated SW. Previous work that showed this treatment reduced leg loss during freshwater drowning by 90%. The remaining baskets (control) were dipped in ambient aerated SW for 15 sec. The order of the treatments was randomised they were applied out of sight of the graders.



Figure 4) The cubicles used to expose lobsters to different storage environments. For the spray environment (l), baskets of lobsters were placed under ambient seawater sprays. In the submerged treatment (r) baskets of lobsters were submerged in tight-fitting black tanks continuously supplied with seawater and constant aeration.

Each basket was then tipped onto the factory grading belt and sorted by two experienced factory graders as suitable/not suitable for live storage. All lobsters were then inspected for damage before being returned to a factory tank (Figure 5). During this time the lobsters were monitored to remove those that had died or were considered too weak to survive. Tag numbers of weak and dead animals were recorded and tags recovered.

After 9 days of storage, the lobsters were transferred from the tank into baskets. The baskets were transported by trolley to the load out area where they were placed in a 2000 L tank filled with 7°C seawater to stun the lobsters prior to grading. Each basket of lobsters was presented to a grader as if in preparation for live export (load out grading). The grader was instructed to grade on vigour only (i.e. not to reject lobsters due to appendage loss). Tag numbers of rejected and accepted lobsters were recorded. The numbers of missing appendages on each lobster was also recorded during grading.

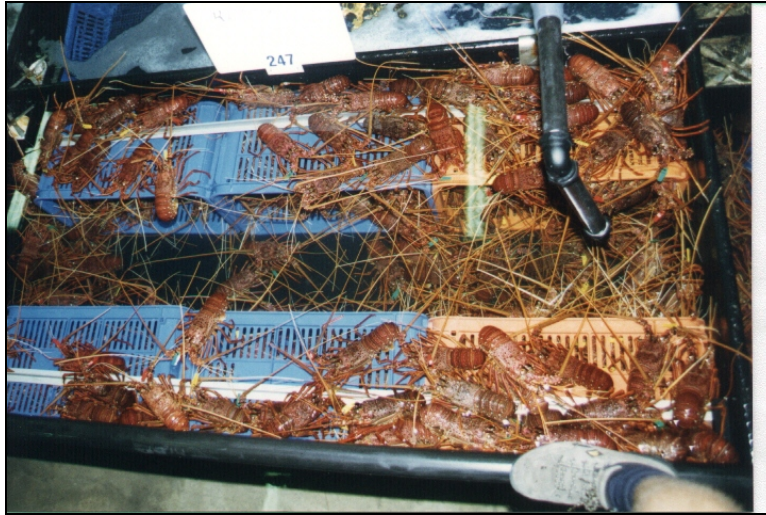


Figure 5) Typical density of lobsters held in factory live holding tanks prior to live export. Tagged lobsters used in experiments addressing Objective 3 were held in identical tanks after simulated transport and grading.

Objective 4) To describe the occurrence of leg loss, morbidity and mortality of western rock lobsters subjected to cold-stunning prior to episodes of handling during the post-harvest process and to compare these to the performance of animals handled using current methods.

Windjana Sea Trials (B Zone)

The commercial lobster boat, *Windjana*, which operated out of Port Denison, 60 km south of Geraldton was used in the trials. *Windjana* was fitted with a modified cacka box to allow controlled trials of cold water stunning and normal handling. The box consisted of two compartments; one being a refrigerated seawater bath (360 L, 750 x 600 x 800 mm). The other side was an aluminium pan (750 x 600 x 200 mm) of a similar design to that used widely throughout the industry (Figure 6). The seawater bath was chilled by a 1.5 hp chiller unit mounted below decks. Water in the bath was aerated constantly to prevent thermal stratification and to maintain high dissolved oxygen levels. A weather station (Davis WM918) was also installed on the boat to record atmospheric conditions during trials.



Figure 6) The modified cacka box installed aboard the commercial lobster boat, Windjana. The box consists of a refrigerated seawater-filled stun tank (aft) and a standard aluminium sorting tray (forward). Note the aeration in the stun tank and the 2 chutes (PVC pipes) on the inboard side of the sorting tray that lead down to below deck live tanks.

On days when trials were run, the cold bath was filled with fresh seawater immediately prior to departing from port and the chiller unit was turned on. This allowed the seawater to reach experimental temperature prior to pulling the first pot of the day. On days when the boat was working close to shore, there was less time for the water to reach temperature and the water was chilled more by rapidly adding seawater ice.

During trials, alternate pot catches were sorted using normal practice (control) or were subjected to cold-stunning prior to sorting (treatment). Normal practice (control) was to empty the contents of the pot into the dry aluminium box, where the lobsters waited to be sorted. After re-baiting and stacking the pot and pot rope, deckhands removed individual lobsters from the box and checked them for size using standard aluminium lobster gauges. Undersized lobsters were immediately returned to the ocean. Legal-sized lobsters were checked for reproductive condition before being slid down a PVC tube (150 mm dia.) and into below deck live tanks. The live tanks were supplied continuously with fresh seawater and the water in the tanks was aerated. Any size lobsters found to be in reproductive condition were returned to the ocean.

Lobsters from treatment pots were emptied directly into a plastic lug box (Nally IH300 610 x 419 x 312 mm, 66 L) in the sea water bath where they remained for a maximum of 5

seconds (Figure 7). Five seconds was the maximum exposure time permissible without causing substantial delays in retrieving pots. The exposure period was started from the time the first lobster entered the bath. After the 5 sec period, the basket containing the lobsters was removed from the bath and the lobsters were sorted as above. Legal-sized treatment lobsters were slid down a second PVC tube and into a separate basket (Viscount Produce Crate, 740 x 422 x 366 mm, 84 L) in the live tanks. In this way, the treatment and control lobsters were kept separate until they could be delivered to the factory.



Figure 7) Lobsters were tipped directly from the pot into the stun tank (top). Control and treatment lobsters were slid down PVC pipes into separate baskets in below deck live tanks (bottom).

On experimental days, an on board observer was present to ensure treatments were run appropriately. The observer timed the exposure to the cold water, recorded numbers of legal, breeding female and undersized lobsters in each pot, all legs lost during on board handling and tried to attribute a cause for each loss. The observer also took regular measurements of weather conditions (air temperature, relative humidity, barometric pressure, wind speed), sea depth and sea temperature and recorded the presence of predators, such as octopus, in pots.

Upon return to shore, all treatment and control lobsters were consigned to the factory in their respective baskets. For the lobsters to be delivered to the live holding factory in Geraldton, the lobsters were transported 1.5 km in air by utility vehicle to the Dongara depot where they were stored in an aerated recirculating seawater holding system (8000 L, 17°C) for between 1.5 and 24 hours. The lobsters were then transported to Geraldton by truck. During truck transport the lobsters were continually sprayed with 15°C recirculating seawater.

Upon arrival at the live factory, the catch was intercepted and the lobsters were placed in a refrigerated (10°C) seawater bath for 20-60 minutes. Chilling the lobsters made it possible to assess the catch without inducing additional leg loss in the process. All lobsters were then inspected for old (>24 h) and new (<24 h) limb autotomy wounds and missing antennae. The length of storage at the Dongara depot was taken into account when estimating the ages of autotomy wounds. The age of the autotomy wounds could be estimated from the degree of melanisation of the limb stump. The numbers of new wounds indicated the maximum possible amount of damage that could have been associated with the last pot-set and capture. The treatment effect was determined by comparing the numbers of new wounds in treatment and control lobsters. The error associated with this method is likely to be insignificant given that the daily rate of leg loss in the wild is extremely low.

The trials were run for 44 days at intervals throughout the season. Stun temperatures between 0-21°C (ambient) were tested. Standard lobster baits (a mixture of mackerel, Australian salmon and herrings) were used throughout the trial.

Best possible practice and treatment-induced mortality

To investigate potential mortality associated with the cold-stunning, on 4 days, treatment lobsters were repeatedly stunned during the post-harvest chain. This experiment was also an attempt to apply to the treatment lobsters what we presumed to be best possible conditions. On all other days, only the single on board stun was used and it may be possible to further reduce post-harvest leg loss by modifying the process further.

Using the on board stun bath, treatment lobsters were stunned (5°C for 5 sec) prior to on board sorting. Lobsters were again stunned (5°C for 120 sec) after removing them from the on board live tanks and prior to offloading the catch and transporting it to the Dongara depot.

During transport to the depot, baskets of treatment lobsters were covered with hessian covers wetted with seawater. Data from the sea trial suggested that lobsters transported in partially-filled baskets lose more legs than those in full baskets. This may be due to the increased freedom of movement of lobsters in the former. To overcome this, baskets of treatment lobsters were fitted with modified lids that could be tied down with plastic cable ties to restrict the movement of the lobsters within.

Lobsters were stored at the depot and transported to Geraldton by truck as above. Upon arrival at the live factory in Geraldton, all lobsters were placed in ambient temperature seawater in a factory tank for 90 min. to allow them to warm up and become responsive again. Treatment lobsters were then stunned (5°C for 120 sec) prior to grading. This treatment was sufficient to stop all movement during the 5 minutes it took to grade the lobsters. Control lobsters were graded without prior stunning. Grading involves tipping the lobsters onto a conveyor belt, which moves the lobsters past factory grading staff. In this case all lobsters were inspected for damage, and were then held in air in baskets (Nally IH300 610 x 419 x 312 mm, 66 L), prior to being placed in a factory tank. Treatment and control lobsters, were placed in separate live factory tanks (2 x 1 x 0.5 m) supplied continuously (2 L/sec) with fresh, aerated seawater. All lobsters were placed in tanks, regardless of condition or numbers of missing legs. The lobsters were left in the tanks for between 10 and 15 days and during this time factory tank monitors regularly checked the lobsters and removed any animals considered to be weak or dead. After the storage period all lobsters were packed as for live export.

The initial proposal was to trial cold-stunning under controlled conditions. These were the trials conducted aboard *Windjana*. After presenting the results of the initial trials, fishermen from other areas of the fishery commented that the conditions under which they worked were very different to those aboard *Windjana* and suggested that cold-stunning may not yield similar benefits under their unique operating conditions. In response additional sea trials were conducted in the southern C-zone aboard the commercial lobster boat Shark Raider II. A brief survey of the incidence of leg loss in the A zone (Abrolhos Islands) was also conducted.

Shark Raider II Sea Trials (C Zone)

In general the on board practices in the southern C Zone are quite different to those experienced in previous trials conducted in the more northern B Zone. For example, in B Zone most fishermen empty lobsters in the pot into a sorting box (cacka box) via a gate on the pot. In contrast many C Zone fishermen do not have gates on their pots and the lobsters are removed by hand from the pot through the pot neck. The various methods of fishing used in the different areas of the fishery seem to largely be hangovers from historical times, rather than have specific and unique benefits for a particular region. The Skipper of *Shark Raider II* and other C Zone fishermen suggested a couple of “reasons” for not having gates in the pots. The first suggestion was that seals are able to open pot gates to get to the lobsters inside, resulting in the loss of catch. So, by not having gates losses to seals are reduced. The second suggestion was that, if pots have gates, it was easier and faster for poachers to pull up someone else’s pot and empty without being caught in the act. The plausibility of these suggestions has yet to be determined, but given there is no perceived difficulty with using gates in other parts of the fishery, they don’t seem very convincing. In addition, after using pot gates during the cold-stunning sea trials, the skipper and crew of *Shark Raider II* were so impressed with their ease of use, compared to skinning through the neck, they continued to use the gates installed in the pots even after the trial was finished.

Given the above, the aim of these trials was to compare, in a controlled fashion, leg loss occurring during on board sorting after skinning through the pot neck with that during on board sorting after skinning the pot through a gate and cold-stunning. To achieve this, gates were fitted to all the batten pots used by the fisherman.

An existing day tank on board *Shark Raider II* was converted for use as the stun tank. As in the B Zone trials refrigeration equipment (1.5 hp chiller and heat exchanger) was installed below decks for the purpose of controlling the water temperature in the stun tank. However, the setup was quite different to that used previously in a couple of main ways. The boat used in the Zone B was equipped with a generator that supplied power for the refrigeration unit, whereas the C Zone boat did not have a generator and power for the refrigeration unit was generated by an alternator and delivered via an inverter. Secondly, in the Zone B trials the refrigeration coils were placed directly in the stun tank, whereas on the C zone boat, water from the stun tank was pumped through a heat exchanger unit below decks before being returned to the stun tank

(Figure 8). Because of the diverse range of boat designs within the industry it is unlikely that there will be a “one size fits all” stun tank design. The sea trials provided a unique opportunity to test different types of stun tank/refrigeration setups, to allow recommendations to be made for future applications.

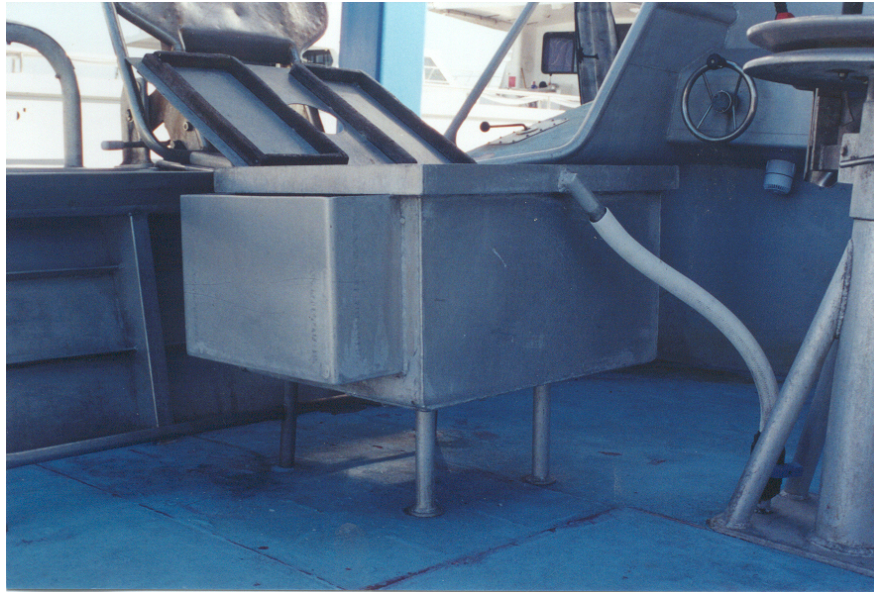


Figure 8) For the Zone C trials an existing day tank was converted to a stun tank. Chilled seawater was pumped between the tank and a refrigeration unit below decks.

On days when trials were being conducted an observer was on board the boat. The experimental method used was similar to that used in the B Zone trials. Every second pot was subjected to cold-stunning whilst control pots were skinned through the neck as per the standard practice aboard the boat.

The onboard observer ensured the treatment was run according to the experimental design, controlling stun time (5 sec.) and temperature. Based on the results of the Zone B trials (see below), stun temperature was set between 5 - 10°C. As with the earlier trials, the observer recorded numbers of legal, breeding female and undersized lobsters in each pot, numbers and causes of legs lost during on board handling. The observer also took regular measurements of weather conditions (air temperature, relative humidity, barometric pressure, wind speed) via an onboard weather station (Davis WM918), sea depth and sea temperature and recorded the presence of other species in pots.

After sorting, legal lobsters were placed in plastic produce baskets in below deck tanks via PVC pipe chutes similar to the set up on *Windjana*. Treatment and control lobsters were kept separate baskets in the live tanks. The live tanks were continuously supplied with fresh seawater.

The boat delivered its catch to Mindarie Keys in the northern suburbs of Perth. From there the catch was loaded onto a refrigerated truck for transport to the processing factory at Rous Head, Fremantle. This trip usually took 45 –60 minutes.

After transport to the processing factory, the baskets of lobsters were weighed and were placed under chilled (15 - 17°C) seawater sprays until they could be inspected. Before inspection, each basket was immersed in 5 - 10°C seawater for a minimum of 60 seconds. Stunned lobsters were inspected for old and new missing legs and antennae, the gender of each lobster and the numbers of detached legs in each basket were also recorded.

Abrolhos Leg Loss Survey (A Zone)

The aim of this work was to assess the commonly stated belief that most of the leg loss in Zone A occurs prior to capture and can therefore not be controlled by on board cold-stunning. Typically, densities of predators (eg. Bald-chin Groper (*Choerodon rubescens*), Dhufush (*Glaucosoma hebraicum*)) in the waters surrounding the islands are much higher than in inshore waters and predator damage on lobsters is likely to be significant in this area. In April 2003 a survey of leg loss aboard commercial boats working in Zone A (Abrolhos Islands) was undertaken. Over 7 days of fishing, an observer accompanied 6 commercial lobster boats operating in different areas of Zone A (2 boats from Southern Group and 3 boats from North Island). Two of the boats were fishing in relatively deep water (> 20 fms) and 4 in shallower waters (< 20 fms) closer to the islands.

Where possible the on board the observer attempted to record the following information for each lobster caught: size (legal-sized, undersized (< 77 mm CL) or oversized female (> 105 mm CL)) gender, no. of old (i.e. > 24 h) and new (< 24 h) limb stumps, no. of old and new missing antennae, reproductive condition of females (setose, tar spot, or ovigerous). On 2 boats the observer was unable to separate old and new wounds on size and undersize, and on one of these boats, he could only clearly count the missing legs on one side of each lobster. To account

for this, he estimated that the numbers of missing legs he observed should be multiplied by 1.3 to more accurately estimate the actual numbers of missing legs. Any other species caught in pots (fish, octopus, etc) were also recorded.

Objective 5) To investigate, in captivity, the effects of multiple simulated pot capture and release events, either with or without cold-stunning on growth, leg loss and survival of undersized western rock lobsters.

Experiments addressing Objective 5 were designed to investigate the effects of multiple simulated pot recaptures on the leg loss, growth and survival of undersized lobsters in captivity. For these experiments an experimental live holding system was built at a site in Jurien Bay in early 2002 (Figure 9)



Figure 9) The live holding system built at the Jurien Bay site. Each large blue tub housed 15 undersized lobsters for growth experiments (Objective 5).

With the assistance of a local commercial fisherman, undersized lobsters were collected in early February 2002 and taken to the site. This timing allowed the animals to be brought into captivity prior to the February moult before commencing the experiment. The first moult after capture is typically affected by capture stress. This is seen as a reduced moult increment. By

allowing the animals to moult before commencing the experiment, growth parameters are effectively “re-set” to a more “normal” pattern. Moulting of the undersized lobsters began in March and continued through to early May. This moulting activity coincided with a period of high mortality. Observations suggested that most lobsters were dying in the process of moulting – similar to the moult death syndrome reported in many culture situations. We have previously held western rock lobsters in captivity at high densities with no difficulties. The unexpected mortalities lead to the suspicion that some aspect of the holding conditions at the site was causing the mortalities. This information was presented to the RLPHS Steering Committee in Cairns in May 2002. In consultation with the Steering Committee, the experiment was abandoned, in favour of the tag and recapture work that was planned (FRDC 2002/239).

Objective 6a) To compare, in captivity, the effects of handling, with and without cold-stunning, on the reproductive success of setose, tar spot and ovigerous female western rock lobsters. 6b) To investigate the effects of limb loss on the reproductive success of female western rock lobsters.

In February 2002, sixty tar spot females were collected for use in experiments by a Jurien Bay commercial fisherman. During transport to shore, the lobsters were held in on board live holding tubs continuously supplied with fresh seawater. Upon reaching shore, the lobsters were transported in air briefly to the Jurien Bay holding facility described above. Once the lobsters had been collected they were temporarily held *en masse* in three 1000 L tanks that were continuously supplied with aerated seawater. Individual lobsters were removed from these tanks and the following data recorded; weight, carapace length and numbers of missing legs and antennae. After weighing lobsters were transferred to individual clear plastic 40 L tanks (Figure 10). Each tank was continuously supplied with seawater (2 L/min) and water in each tank was continuously aerated. After all lobsters had been placed in tanks, each tank was randomly allocated to one of 4 treatment groups:

Treatment 1: No treatment - transfer with minimal handling (negative controls).

Treatment 2: Subjected to cold-stunning (5°C for 5 seconds) before handling in air to simulate sorting aboard a commercial boat (i.e. gauged and inspected ventrally).

Treatment 3: Subjected to handling in air to simulate sorting aboard a commercial boat (dropped from a small height into a dry tub (cacka box), then gauged and inspected ventrally) (positive control).

Treatment 4: One of the fifth sub-chelate legs was removed by inducing autotomy before transfer to the holding tanks. No further disturbance.

A small number of these animals proceeded to go to the “berry” stage. All but one of these lobsters proceeded to shed the eggs. On February 22, the lobsters began to moult. The one remaining berried female moulted, discarding the egg mass with the exuvium. By early May almost all the remaining lobsters had moulted, shedding the tar spot with the exuvium. The researchers consulted widely with a number of other scientists who had worked with breeding female western rock lobsters to determine the cause of the shedding of eggs and tar spots. The widely held belief at that time was that our observations were atypical and probably a result of sub-optimal holding conditions.



Figure 10) The Jurien Bay holding facility. The small clear tubs used to individually house the 60 females can clearly be seen on the racks above the large blue tubs..

Because of the poor results described above, the Jurien site was abandoned. The WA Department of Fisheries WA Marine Research Laboratory at Waterman was used instead.

For the second attempt of this experiment, sixty tar spot female lobsters with developed ovaries were to be collected by WA Department of Fisheries staff during the Annual Broodstock Survey, conducted in October-November each year. In November 2002, 31 females bearing tar spots were collected during the broodstock survey. The lobsters were held in on board live tanks supplied continuously with flow through seawater until they could be returned to shore. Lobsters were transported in air by vehicle from the jetty at Fremantle to the Waterman Research Laboratory. Of the 31 lobsters collected, 27 had fewer than 3 missing legs (not including the 5th legs) and four were received with one of the 5th legs already missing. These latter lobsters were automatically allocated to the treatment investigating the effect of losing one of the specialised 5th walking legs on hatched brood size. Two groups of 2 lobsters with missing 5th legs were placed in 2 of 11 tanks (1500 x 600 x 300 mm; Figure 11) set aside for the experiment. Individuals from the remaining 27 lobsters were randomly selected, before being tagged, weighed and inspected for damage before being subjected to one of the allocated to one of the remaining 9 tanks in rotating fashion to give 3 lobsters per tank. The 11 tanks were all continuously supplied with ambient temperature (19.5 - 23.0°C) seawater. Water in each tank was also aerated. After allocating lobsters there were nine tanks each holding 3 lobsters and 2 tanks each holding 2 lobsters. During the experiment lobsters were fed live mussels (*Mytilus edulis*) daily *ad libitum*.



Figure 11) The tanks used to hold breeding female lobsters during experiments addressing Objective 6.

The day after allocating lobsters to the tanks the experimental treatments 1-3 described above for the Jurien Bay experiments were each assigned randomly to the 9 tanks containing 3 lobsters, giving 9 lobsters per treatment. Obviously treatment 1 required no further action until the lobsters extruded their eggs. For each tank of lobsters receiving treatment 2 (stunning and handling), the animals were caught from the tank using a mesh hand net and were tipped into a tub of seawater at the required temperature (5°C). After 5 sec. the lobster was lifted out by hand and gauged using a standard lobster gauge and inspected ventrally. Lobsters receiving treatment 3 were tipped into a dry tub before being gauged and inspected ventrally.

After transfer to the holding tanks, the lobsters were inspected daily for deposition of the eggs under the tail. These inspections were carried out with minimal handling and no exposure to air. Usually the first sign that one of the females had deposited eggs was the appearance of eggs on the floor of the tank. It was usually apparent which female had deposited her eggs from her posture and the way the abdomen was being held. Treatments 2 and 3 were repeated 1 and 3 weeks after extrusion of the eggs was first noted. After the third treatment, ovigerous lobsters

were transferred with minimal disturbance to 40 L buckets fitted with "windows" of 200 μm filter mesh (Figure 12). The buckets sat in the tanks and were continuously supplied with aerated seawater the water in the buckets was also aerated continuously. Lobsters subjected to treatments 1 and 4 were transferred to buckets after a similar time following egg deposition.

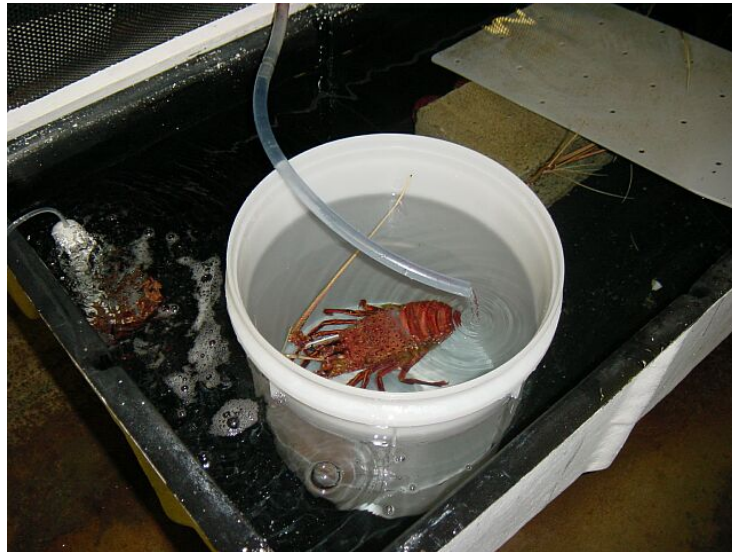


Figure 12) Egg-bearing female housed in bucket fitted with filter mesh "windows" for collecting larvae.

Upon hatching, the larvae from each female were retained within the buckets for estimation of total number of larvae hatched. This was determined by taking a 10 mL subsample of water containing larvae from within the bucket. A sample of 20 larvae was also taken for measurements of larval size. An attempt was also made to assess larval viability using a challenge test based on the work of Smith *et al.* (2003). Samples of 20 larvae were subjected to a challenge of lowered salinity (10 ppt) and increased temperature (31°C) in a series of wells sitting in a water bath (Figure 13). The number of actively swimming larvae was counted at 3 minute intervals for 1 hour. The test score was obtained by summing the number of non-motile larvae at each of the 20 determinations. A high score (up to a maximum of 400) indicates poorer larvae. This test was performed in triplicate for each hatch.



Figure 13) Apparatus for applying combined osmotic and temperature challenge test to larvae. Each cell housed 20 larvae during the test and the cells sat in a controlled temperature water bath.

Objective 7) To conduct a survey to determine the extent and nature of leg loss in the southern rock lobster fisheries of Tasmania and South Australia.

South Australia

The survey was conducted over two 10 day periods; one in January 2000, the other in May 2000. These 2 periods were nominally designated as “summer” and “winter”, respectively.

Summer

Four commercial lobster boats were surveyed over a ten day period. These boats were *Jo Be II*, *Fine Time*, *Ocean Image* and *Sara Fay*. All the boats were fishing out of Avoid bay, which is situated between Coffins Bay and Port Lincoln. The boats used were recommended as ‘research friendly’ and likely to do their best to co-operate, the boats were not chosen at random. Skippers that are willing to be involved in research of this nature tend to be more aware of quality and handling issues. Therefore the results obtained from these boats may underestimate the average levels of appendage loss occurring more widely in the fishery.

The general fishing practice of these boats involved setting the pots early in the afternoon using depth sounders and GPS to locate appropriate spots. The depths pots were set at varied between 10-40 fms. The cray pot design was similar for all boats. All pots had an extra rail around the bottom of the pot to lift the floor of the pot about 15mm off the tipper. This prevents legs protruding from the pot from being crushed on the tipper. The walls of the pot were either stainless steel mesh wire or rope mesh. Australian Salmon (*Arripis trutta*) was the most commonly used bait, although pilchards were used in the berley pot of one boat and carp were used as secondary bait in another. Bycatch was also extensively used as bait. The following morning the pots were individually pulled between 06:00 and 12:00, depending on steaming time required to reach the pots. The pots were then set again that afternoon. The fishermen are not allowed to “double pull” their pots and only pull once a day, leaving the pots in water when they take their shore breaks to check the following morning.

The on board observer observed the pot as it left the water and came across the tipper. Once pulled all lobsters were removed from the pots by hand via the neck of the pots. After removal and sorting, legal-sized lobsters were placed in below deck live tanks. Any protected lobsters (undersized and breeding females) were immediately returned to the sea. As each lobster was removed from the pot the crew held for the on board observer to count the numbers of old, recent and new missing legs (old = black or very thick hard amber crust on stump; recent = glazed thin amber layer over stump; new = moist flesh or congealed blood on stump.) and antennae and to record the gender and whether lobsters were legal or protected. Legs were recorded as missing only if they had been shed at the plane of autotomy. There may have been some legs broken at a more distal position that are generally not considered to be a problem in terms of lobster being in a fit for live export condition.

After removal from the pot, legal lobsters were packed in small square bins. These bins have long slits in the sides and bottom. The boats observed in this study placed lobsters of similar size in the same bin avoiding placing large lobsters with small ones. Some of the boats even used PVC dividers to split the bin horizontally so lobsters would not be stacked one on another. These dividers were thought to prevent fighting amongst the lobsters which is believed to result in cut tails. Once packed, a canvas tarpaulin cap was placed on top of each bin. These bins sat on deck until reaching the Coffins Bay or Port Lincoln jetty. During steaming the skippers made an effort to minimize the amount of wind reaching the bins. Bins were unloaded

at jetties and into refrigerated lorries for transport to processing factories. The bins unloaded at Avoid bay were stacked in rubber inflatable boats and run into the beach. The bins were then loaded onto four wheel drive utility vehicles with tarps and ice or an insulated box with ice to hold bins for the road trip back to the processing factories.

Data was collected from four factories. These factories were: The Fish Factory, Australian Bight Seafood Ltd, Tapley's and Southern Ocean Rock Lobster.

To examine the impact of holding in boat tanks and transportation, lobsters were again inspected upon arrival at the factory before being placed in holding tanks. The observer worked in with the factory graders who inspect every lobster upon arrival at the factory. Graders inspected each lobster for missing legs and determined if they were new, recent and old for the observer to record.

The observer also recorded the numbers of legs missing from each lobster as they were removed from tanks for packing for live export ('pack out'). This gave an indication of the amount of legs lost during factory holding and handling.

Winter

The winter sampling was carried out in similar fashion to the summer sampling. However, on board observations were made on only 2 boats, because boats were out at sea for considerable longer than during the summer survey.

Tasmania

See Appendix 3 for an details of the leg loss survey conducted in Tasmania.

Statistical Analysis

Statistical analysis of all results was performed using the computer packages MSEXcel ver. 8.0, SigmaStat ver. 1.01 or JMP ver.3.2.2 for Macintosh.

Results and Discussion

Objective 1 1a.) To identify a cold water immersion treatment that rapidly immobilises western rock lobsters, while allowing swift recovery from immobilisation upon return to ambient temperature seawater. 1b) To investigate the effect of season/acclimation temperature on effectiveness of cold-stunning in western rock lobsters.

As is typical of data from behavioural studies, the tail flip data were very variable between lobsters. In an attempt to standardise the data for analysis, non-reactive lobsters (ie. those that did not tail flip at anytime before or after the stun treatment) were not included in the analysis. By doing this, lobsters that may have been unable to react (for whatever reason, such as lack of vigour, etc) were excluded. To further reduce variation between reactive lobsters, the remaining data were expressed as:

$$\frac{\text{no. of tail flips prior to stunning} - \text{no. of tail flips after stunning}}{\text{total number of tail flips.}}$$

This transformation yielded values ranging from +1 to -1. If the stun treatment had no effect on tail flip activity, the resulting value was close to 0, if stunning suppressed tail-flipping, the result was a positive value and if stunning actually increased tail flip activity, a negative value resulted.

The data from the experiment are presented in Figures 14 and 15. Control values from lobsters not subjected to a stun treatment were typically close to 0, suggesting that lobsters were able to react with approximately equal vigour to the first and second handling episodes. This is important to note and suggests that any suppressive effect on tail flip activity seen after cold stunning is due to the treatment *per se* and not due to some other unintended effect, such as exhaustion after the first handling when lobsters were captured from the holding tank and transferred to the stun treatment tank.

For both the simulated onboard sorting and grading handling, the values of the standardised tail flip activity measure tended to increase as stun time increased and temperature decreased (Figure 14). In plain terms, the colder and longer the stun, the less active the lobsters were during handling. Due to the similarity of the responses with different handling treatments at different times of the year, representative examples of responses are shown in Figure 14. Lobsters that were subjected to the simulated factory grading showed similar suppression of tail-flipping after cold-stunning, despite being held in ambient air with disturbance for an additional 30 sec. before handling. This indicates that the lobsters had not begun to recover from the stunning despite being held in warm air for that short time. Subsequent experiments conducted as part of FRDC Project 2001/251 (Davidson and Hosking, under review) showed that lobsters could be immersed in 5°C seawater for as little as 10 sec. and still appeared stunned when handled after being held in ambient air for 3 min.

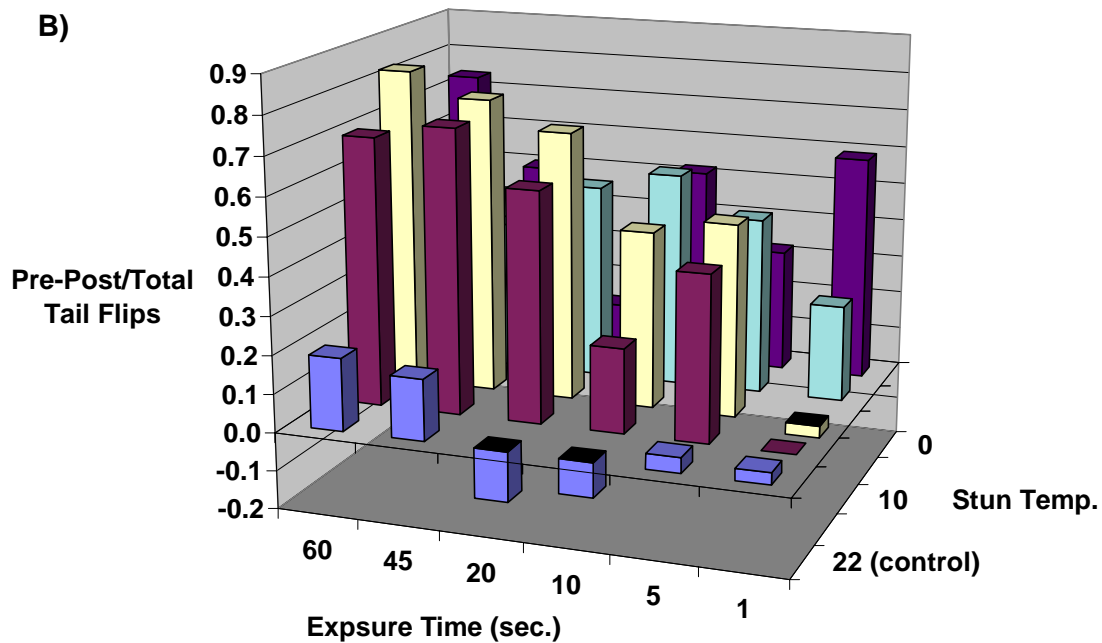
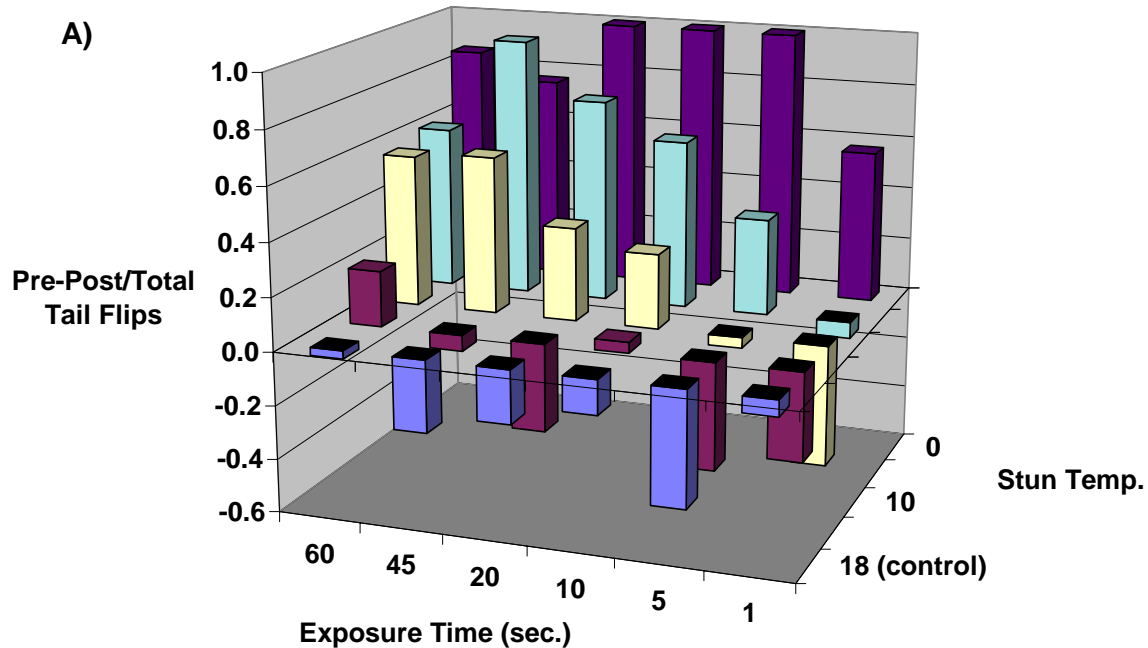


Figure 14) Examples of standardised tail flip activity from lobsters treated with a range of cold-stunning treatments. A) Results from lobsters treated to simulated on board sorting in June 2000, when the ambient water temperature was 22°C (control). B) Results from lobsters treated to simulated factory grading in November 2000. See text for details.

Figure 15 shows examples of righting times of A grade lobsters upon return to ambient temperature seawater after stunning and simulated sorting or grading. Lobsters from all control treatments recovered equally quickly. This recovery was extremely rapid with lobsters righting themselves almost instantly once they were placed back in ambient seawater. On average, as stun temperature decreased and time increased, lobsters took progressively longer to recover from stunning. However, in general recovery from stunning was still very rapid. For example at 0-10°C for 5-10 seconds, lobsters righted themselves in less than 10 seconds. It should be noted that using these brief stun times, the lobsters are not chilled significantly by immersion in cold water, rather they appear to be momentarily shocked by the cold.

Interestingly these lobsters still showed very rapid righting times when returned to ambient seawater, suggesting that they required a shock to rouse them from the stunned state.

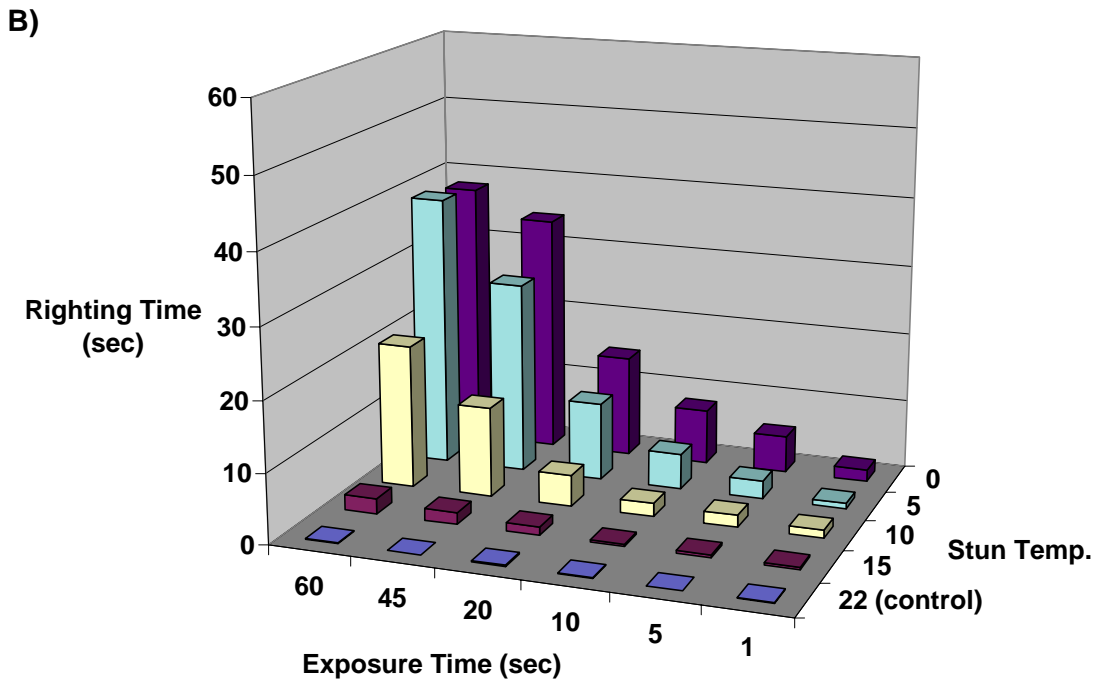
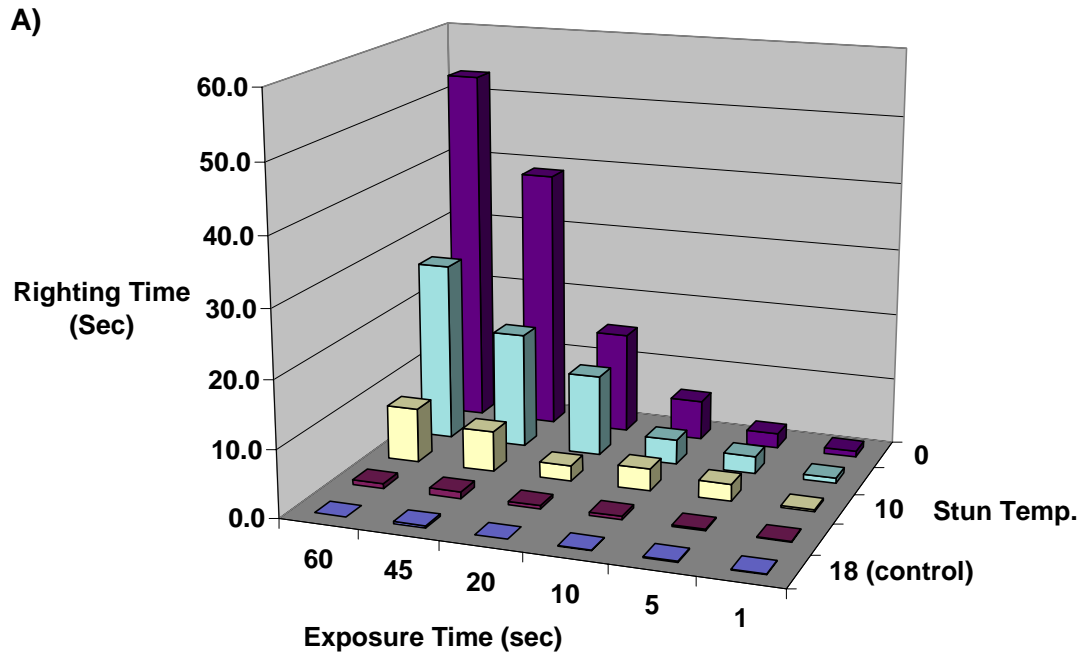


Figure 15) Examples of righting times from lobsters treated with a range of cold-stunning treatments. A) Results from lobsters treated to simulated factory grading in June 2000, when the ambient water temperature was 22°C (control). B) Results from lobsters treated to simulated factory grading in November 2000. See text for details.

After being used in these experiments, the vigour of the lobsters appeared similar to pre-experimental levels. In addition, in the ensuing 48h after the experiment removal of dead and weak lobsters from the holding tanks by monitoring staff, did not appear different from pre-experimental baseline rejection rates.

At the two ambient water temperatures used (18°C and 22°C), the responses of lobsters to any given treatment did not appear substantially different. It is possible that at lower ambient seawater temperatures, the reduced difference between ambient and stun temperatures may reduce the effectiveness of stunning. During the commercial lobster season, in most areas the ambient seawater temperature does not fall below about 16°C and it is therefore likely that cold-stunning would be effective over the entire geographic area of the fishery and throughout the season. This was confirmed during sea trials using cold-stunning aboard commercial lobster boats fishing in different areas of the fishery and at different times of the year (see below).

The responses of the larger C and G grade lobsters, in terms of tail flip activity and righting times, to the limited range of cold-water stunning treatments were similar to those exhibited by smaller A grade lobsters (data not shown). Approximately 20% of the catch is comprised of these larger lobsters, but their somewhat smaller contribution to the catch is offset by their relatively greater contribution to the breeding stock.

Rates of appendage loss were very low during the experiments. Out of approximately 3000 lobsters handled, only 30 legs were shed. This is probably unremarkable given that each lobster was handled individually and in the humid environment of the live holding factory where the development of hypersaline films, which contribute to leg loss, would be minimal.

Based on the results of this experiment, a stun treatment of 0-5°C for 5-10 sec appears suitable for use on board fishing boats prior to sorting of the catch. At these temperatures, induction of a stunned state is very rapid (5-10 sec.) and tail flip activity during handling is reduced dramatically when compared to unstunned controls. This treatment could also be used prior to handling in a factory. In the companion project (FRDC 2001/255), it was shown that hypersaline-induced leg loss could be virtually eliminated by immersing lobsters in 5°C seawater for 5 sec. prior to hypersaline exposure. Leg loss during standard freshwater “drowning” can also be significantly reduced by prior immersion in 0°C seawater for 5 sec. However, a 15 sec. stun time resulted in even greater suppression of leg loss during “drowning”. Extended stun

times can be applied in a factory situation because many handling procedures in the factory are not subject to the same strict time constraints as sorting of the catch on board fishing boats. As shown by the experiments, longer stun times result in longer recovery times (10 - 15 sec.), but this is also acceptable in the factory environment and may even prove beneficial by allowing lobsters to be graded or transferred to tanks whilst still in a stunned and immobile state.

In summary, these experiments have identified stun treatments that appear potentially suitable for applying to lobsters at all points in the post-harvest chain in an attempt to reduce appendage loss.

Objective 2) To investigate in captivity the effectiveness of the preferred treatment (identified in Objective 1) for reducing leg loss in western rock lobsters during handling.

In this experiment lobsters were treated in groups of 6, rather than individually. This was done for two reasons. Firstly, there are usually several lobsters caught in each pot and they are tipped into the cacka box as a group where they freely interact. This interaction may be an important contributor to leg loss because the activity of one lobster will often cause the others in the group to become active. This activity usually takes the form of tail-flipping and general defensive behaviour. Active lobsters are much more difficult to handle and are more prone to being dropped or mishandled, both of which can damage legs. By using groups of lobsters it was possible to mimic these effects in the experiment.

By inducing autotomy using hypersaline solutions, controlled trials were conducted in the factory to rank the stun temperatures in order of effectiveness for reducing leg loss, and also to test the effectiveness of applying the cold water as a spray, rather than by immersion (Objective 1c). Figure 16 shows the results of this experiment. Leg loss in the control group was 2.36 ± 0.35 legs/lobster. Immersing lobsters in 15°C seawater for 5 sec. did not significantly reduce leg loss when compared to controls (One way ANOVA; $p > 0.05$). Immersion of lobsters in 10°C seawater or colder did significantly reduce leg loss compared with controls (One way ANOVA; $P < 0.05$). In addition, immersion in 5°C seawater was significantly more effective for preventing leg loss than 10°C.

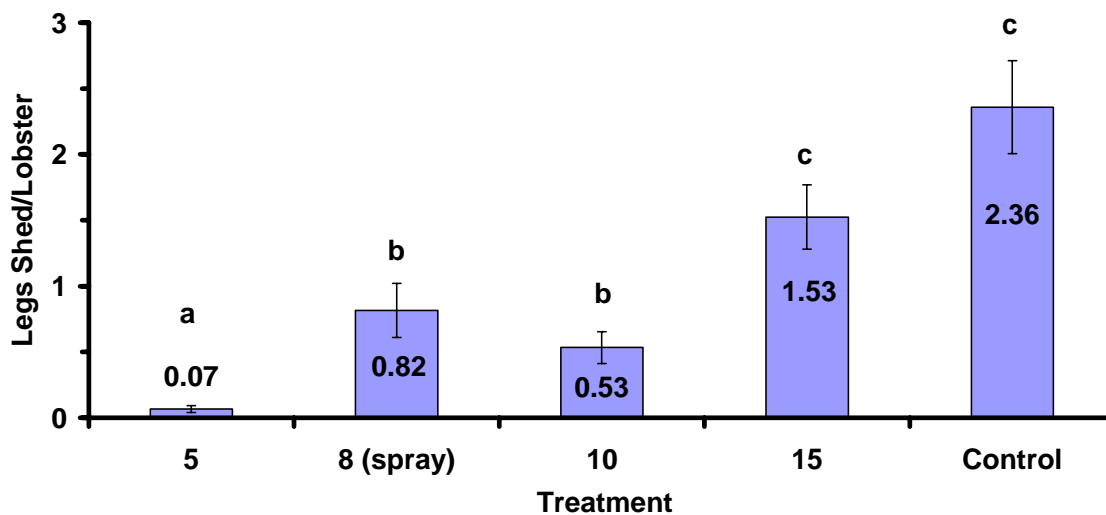


Figure 16) The effectiveness of different cold-stunning treatments was tested in captivity. All treatments were applied for 5 seconds. Leg loss was induced by exposing groups of 6 lobsters to hypersaline seawater (SW + 55 g/L of salt) following cold-stunning treatments. For the purpose of analysis, each group of 6 lobsters represents one experimental unit. Treatments with the same letters are similar (ANOVA; $P > 0.05$). Sample size = 20 for all treatments.

Objective 1c was to investigate the usefulness of applying the cold water as either a spray or by immersion. In the initial experiment an attempt was made to test 5°C sprays vs. 5°C immersion, but it was found that seawater at 5°C delivered to sprays via a pump was actually 8°C by the time it reached the lobsters. This was probably due to mixing of the cold water with warm air and heating of the water by the pump itself. The 8°C spray did significantly ($p < 0.05$) reduce leg loss when compared to the controls, but without an 8°C immersion treatment it was impossible to determine the effect of sprays vs. immersion alone. For this reason a second small trial was run with 9°C sprays and 9°C immersion (Figure 17). At this temperature sprays were found to be significantly less effective than immersion for preventing leg loss (unpaired t-test, $t = 3.136$, $P < 0.05$, $df = 22$).

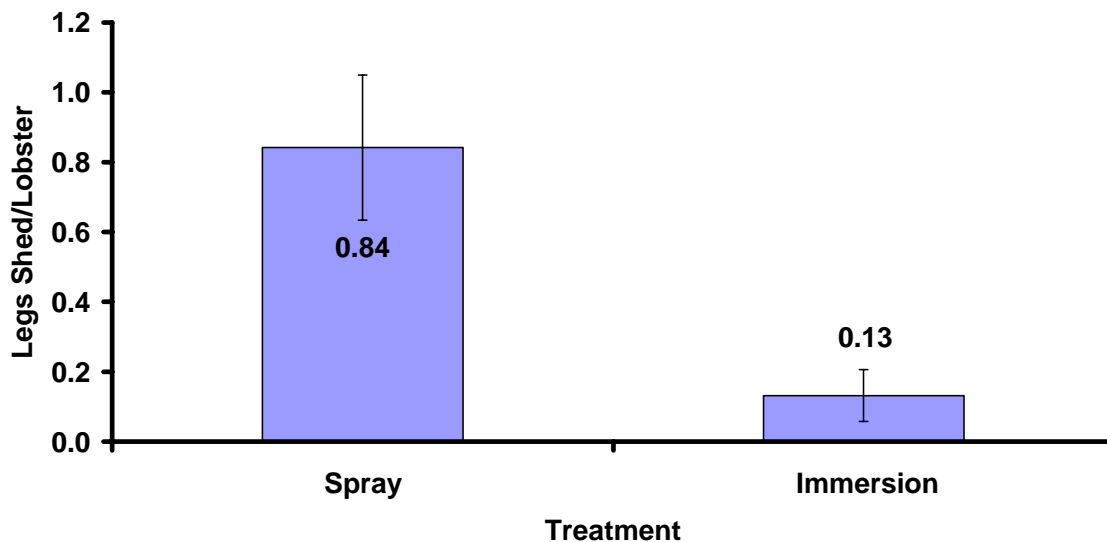


Figure 17) Comparison of cold water (9°C) stunning by immersion, or by chilled seawater spray. Sprays are clearly less effective than immersion. The treatments are significantly different ($P < 0.01$). The sample size = 20 for both groups.

In addition to being superior for preventing leg loss, stunning by immersion may have other advantages over cold water sprays. Firstly, sprays rely on seawater being circulated by a pump of some kind and during C Zone sea trials aboard *Shark Raider II* it was shown that this is not practical on a commercial boat where pump impellers and intakes are readily blocked by material suspended in the water, such as seaweed and scales from bait. Secondly, with a spray system the lobsters would be tipped out of the pot and onto a hard surface, such as the floor of the cacka box, rather than into water in an immersion stun tank. Whether this would have a major impact on leg loss is unknown, but intuitively one might expect the latter to be less damaging to the lobsters.

In summary, the preferred stun time and temperature identified in Objective 1 significantly reduced hypersaline-induced leg autotomy in western rock lobsters. Furthermore it was shown that immersing lobsters in cold water was significantly more effective for reducing

leg loss than spraying cold water over lobsters. The identified treatment could then be tested in the field aboard commercial lobster fishing boats (Objective 4).

Objective 3) To test the accuracy of factory grading of cold-stunned western rock lobsters versus untreated controls.

The results of grading stunned and control lobsters were very similar (Table 1). The proportions of correctly and incorrectly classified animals in the two groups were not significantly different ($\chi^2 = 0.0525$, $df = 1$, $P > 0.05$). Indeed, there were no differences between the two groups in any of the criteria used to assess the performance of the graders. This shows that cold-stunning as applied under the conditions of this trial did not affect the ability of graders to grade lobsters. During the initial grading, almost identical numbers of lobsters were graded as accepted, rejected and “dead” in the two treatment groups. Lobsters considered to be “dead” were generally still moving slightly. Out of interest one of these animals was returned to the live holding tanks and went on to pass the final load out grading as acceptable for live export. This shows that even the weakest of lobsters may recover from exposure to severe stressors.

In a group of cold-stunned lobsters, graders may be able to differentiate between weak and strong animals based on muscle tone. Weak lobsters tend to exhibit poor muscle tone, and when picked up, the legs and tail hanging down limply. Cold-stunning does not modify this appearance. After cold-stunning, strong lobsters continue to exhibit firm legs and tail sections and it may be these cues that the graders are picking up on. In many cases, stunned strong lobsters exhibit a characteristic “clawed” posture, with the legs tightly flexed under the body and this requires considerable muscle contraction that weak lobsters are unable to maintain.

Table 1) Summary of results from experiments investigating the ability of graders to accurately grade cold-stunned lobsters. “Accepted” and “rejected” refer to lobsters acceptable or not considered acceptable for future live export. “LO grading” refers to the load out grading. (** = $P < 0.05$, n.s. = not significantly different).

	Control	Stun
# Accepted at first grading	124	127
# Rejected at first grading	56	58
# “Dead” at first grading	20	14
Total	200	199
Overall Accuracy (%)	79.5	80.9 n.s.
% Falsely Accepted	13.7	11.0
% Falsely Rejected	42.9	39.7
Legs shed during treatment and first grading (legs/100 lobsters)	7 ± 2	48 ± 8**
Legs shed during live storage and LO grading (legs/100 lobsters)	18 ± 4	15 ± 3

Based on the numbers of lobsters rejected as “dead” at first grading, removed from live tanks as weak during live storage or LO grading, the overall accuracy of the initial grading was about 80% for both stunned and control lobsters (Table 1). This figure agrees well with those found by Paterson et al. (2001) in FRDC Project 96/345. In that study the accuracy of grading was determined on 3 occasions and varied between 67-82%. The percentages of lobsters that were accepted falsely at the initial grading were between 11-13%, whilst the percentages of lobsters falsely rejected were about 40% in both cases. This suggests that it is easier for graders to accurately identify vigorous lobsters that will go on to survive to export, whilst it is more difficult to determine if a weaker lobster is irreversibly exhausted or will recover if placed in live holding tanks. This result may also suggest that the graders are fairly conservative in their estimation. If a lobster may have even a slight chance of being rejected further down the post-harvest chain, the grader is likely to reject it. This is consistent with the aim of grading upon

receipt of freshly caught product, which is to store only lobsters fit for live export in the holding tanks.

Surprisingly, the number of legs shed during treatment and the trial grading (determined by recording the numbers of missing legs on lobsters as they were placed in the storage tank after the trial grading) was significantly higher in the stunned animals than in the controls (one way ANOVA, $F = 25.029$, $df = 1$, $P < 0.05$). This was unexpected because in every previous experiment cold-stun treatments applied to individuals or groups of lobsters significantly reduced leg loss resulting from a wide range of handling practices and experimental manipulations, including exposure to hypersaline films, freshwater drowning and on board sorting. As a precaution, before implementing cold-stunning prior to factory grading trials should be conducted to determine the net benefits, in terms of reduction in leg loss and the payback time on the installation, and to establish an efficient flow of product.

At the time the lobsters were inspected and placed into the live tanks (after 4 h of storage and trial grading), 108 additional legs were missing when compared with the number observed when lobsters were first inspected upon receipt at the factory. Thirty five detached legs were left in the baskets used to carry the lobsters from the grading area to the live tanks. This leaves 73 legs unaccounted for. This damage probably occurred during transfer into and out of the storage environments, during tipping onto the grading belt and grading itself.

Amongst the lobsters surviving to reach the load out (LO) grading, the numbers of legs lost since the time the lobsters were placed in live storage was similar in controls and stunned lobsters (Table 1; one way ANOVA, $F = 0.2672$, $df = 1$, $P > 0.05$). In total these losses amounted to 48 legs. Thirteen of these were found detached in the baskets used to transfer the lobsters to the load out area for grading. The remaining 35 legs unaccounted for, were probably shed during transfer to the live holding tank and during live storage.

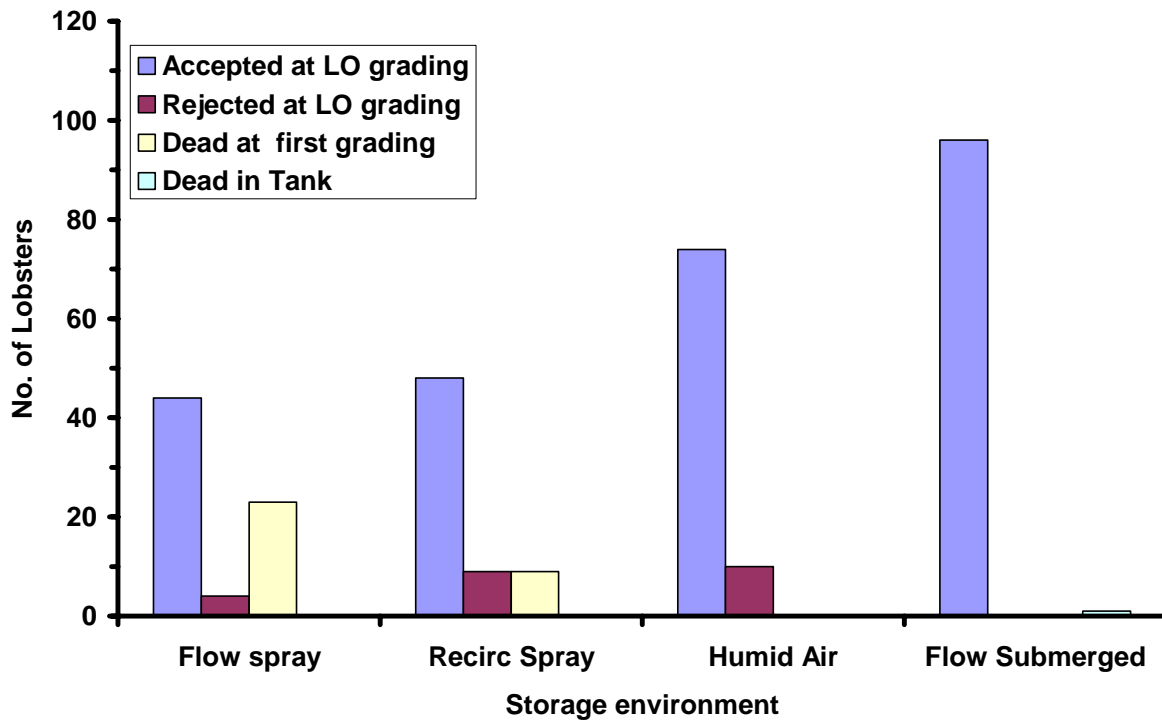


Figure 18) Performance of lobsters subjected to different storage treatments. “Accepted” and “rejected” refer to lobsters acceptable or not considered acceptable for live export. “LO grading” = load out grading

This experiment also provided an opportunity to compare the four storage environments used. The proportions of lobsters considered acceptable for live export at the load out grading varied significantly between treatments (Figure 18). The flow through submerged treatment was significantly better than the next best treatment (humid air), with 98% of lobsters being considered suitable for live export at the end of the experiment ($\chi^2 = 0.19.6$, $df = 1$, $P < 0.05$). Humid air was the next best with 75% of lobsters making it to export. This was significantly better than for the next best treatment (recirculating spray; $\chi^2 = 14.1$, $df = 1$, $P < 0.05$). The proportions of animals fit for live export in the two spray treatments were the poorest and were similar ($\chi^2 = 0.131$, $df = 1$, $P > 0.05$).

While it is interesting to compare the percentages of lobsters from each storage treatment that were considered fit for live export on the basis of vigour, it is important to note that none of the animals were graded for leg loss at any stage. If the storage treatments affected leg loss in different ways then this would alter the outcome of grading in a real factory situation.

To summarise, upon receipt at a live holding facility, cold-stunning can be applied to lobsters prior to grading without affecting the graders' abilities to correctly identify lobsters that are suitable for live storage in preparation for live export.

Objective 4) To describe the occurrence of leg loss, morbidity and mortality of western rock lobsters subjected to cold-stunning prior to episodes of handling during the post-harvest process and to compare these to the performance of animals handled using current methods.

Windjana Sea Trials (Zone B)

The B Zone cold-stunning sea trials were run over 44 days during the 2000/2001 season. During this time, data were collected from over 5200 pot lifts and 24444 lobsters were handled.

Stun temperatures from 0 – 10°C appeared to be approximately equally effective for reducing on board leg loss. In this stun temperature range, the daily reduction in leg loss was 70-80% (Figure 19). At 15°C, the reduction in leg loss was less ($49.1 \pm 11.2\%$, $n = 4$). After 5 sec immersion in ambient temperature seawater in the stun tank, leg loss in treatment lobsters was actually greater than in control animals ($183.4 \pm 9.0\%$, $n = 2$). Given the small sample size, this result should be interpreted with some caution.

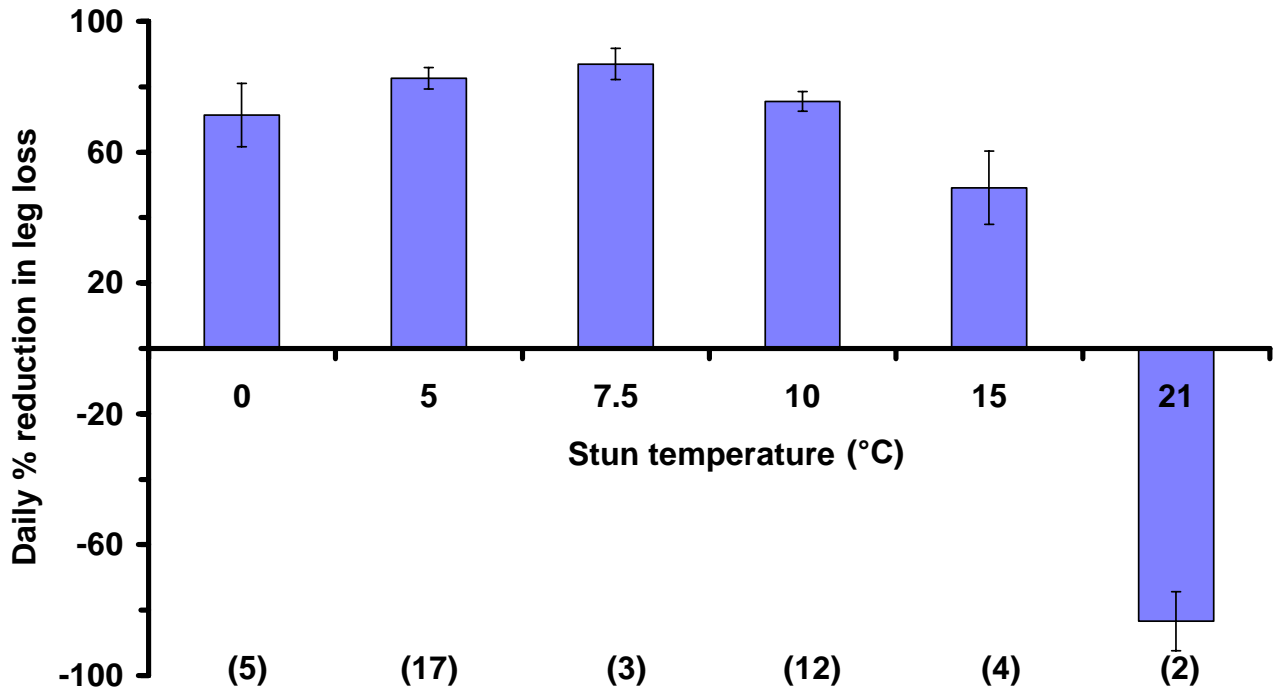


Figure 19) Percentage reductions in on board leg loss with different stun temperatures used during cold-stunning sea trials in Zone B. All treatments were applied for 5 sec. only.

Given that it was shown in Objective 1 that recovery times from stunning were longer after exposure to colder temperatures, it was assumed the “best” temperature for use in the field is the highest effective temperature for preventing leg loss. The highest temperature will presumably allow the most rapid recovery amongst protected lobsters that have been caught, stunned and returned to the ocean. This would be important for returned lobsters faced with evading predators and seeking shelter. For this reason, and because refrigeration systems regulate temperature to within a couple of degrees either side of a set point, subsequent experiments focused on stun temperatures between 5 - 10°C. Stun temperatures in this range were also used in FRDC Project 2002/239 to test in the wild the effect of on board cold-stunning on recapture rates of caught and released lobsters (Davidson, in preparation). The results presented below are for the days where stun temperatures between 5-10°C were used.

The daily average on board leg loss in control lobsters was 0.145 ± 0.014 legs/lobster ($n = 32$). This was significantly reduced to 0.023 ± 0.002 legs/lobster (paired t -test, $t = -9.045$, $P < 0.01$; $n = 32$) by immersing animals in 5 - 10°C seawater for 5 sec. prior to on board sorting. The average daily reduction in leg loss for these lobsters was $80.3 \pm 2.2\%$.

The rate of leg loss in protected (i.e. primarily undersized) control lobsters (0.155 ± 0.016 legs/lobster) was significantly greater (paired t -test, $t = 1.926$, $P < 0.10$; $n = 32$) than in legal-sized control animals (0.135 ± 0.014 legs/lobster). This suggests smaller lobsters are more likely to shed legs. This is consistent with anecdotal reports from fishermen. Controlled experiments conducted as part of the companion project (FRDC Project 2001/255; Davidson and Hosking, under review) were unable to demonstrate a significant relationship between hypersaline-induced autotomy and size. The average daily percentage reductions in leg loss resulting from stunning legal and protected control lobsters ($81.4 \pm 2.2\%$ and $77.3 \pm 4.0\%$, respectively) were similar (paired t -test, $t = 1.414$, $P > 0.05$, $n = 32$). This suggests lobsters in the two size ranges respond equally to cold-stunning.

From the fishermen's and processor's point of view, the commercial measure of success of on board cold-stunning is the reduction in leg loss evident at the point of change of ownership of the catch (i.e. where the processor purchases the catch from the fisher). In the B Zone trials, the lobsters were subjected to several additional handling steps (and opportunity for further leg loss), before the catch was received by the processor at Geraldton.

The results of the catch assessments at the Geraldton factory are presented in Table 2. The numbers of new (< 24 h old) leg stump wounds in control lobsters were significantly greater than in stunned lobsters (paired t -test, $t = 5.398$, $P > 0.01$, $df = 22$). This is to be expected given the reduced on board leg loss observed in the stunned lobsters. Likewise, the number of newly broken antennae in control lobsters was also significantly greater than in stunned animals (paired t -test, $t = -2.218$, $P > 0.05$, $df = 22$). This was somewhat surprising, but given the large sample sizes, it would appear to be a real beneficial effect of the stunning. The loss of antennae is also a big problem. Not only do these appendages contribute to catch weight but, by law, processors are unable to export in a whole form (i.e. live, or frozen boiled or raw) any lobster with more than one missing antenna. Therefore any treatment that prevents their loss has great benefit to both the catching and processing sectors.

Upon delivery to the factory, the catch was assessed in terms of the proportions of lobsters suitable for different product forms. Lobsters with fewer than 1 missing leg + 1 missing antenna were considered fit for live export (FFL). Lobsters with fewer than 3 missing legs + missing antenna were considered fit for whole cooked/raw (FFW) and lobsters with greater than 3 missing legs + 1 missing antenna were considered fit for tailing (FFT). These assessments were made on the basis of damage alone, and no consideration was given to the vigour of the lobsters arriving at the factory. A subsequent experiment looking at the mortality effects of the treatment showed that cold-stunning had no detrimental impact on the subsequent survival of lobsters in factory holding tanks (see below).

The average daily proportion of stunned lobsters that were intact (i.e. no missing appendages) was significantly greater than in control lobsters (paired *t*-test, $t = -4.919$, $P < 0.01$, $df = 22$). Likewise, the average daily proportion of stunned lobsters that were delivered in FFL condition was also significantly greater than in control lobsters (paired *t*-test, $t = -3.053$, $P < 0.01$, $df = 22$). The average daily proportion of stunned lobsters that were FFW was significantly greater than in control lobsters (paired *t*-test, $t = 2.063$, $P < 0.10$, $df = 22$). The average daily proportion of stunned lobsters that were FFT was significantly less than in control lobsters (paired *t*-test, $t = 1.856$, $P < 0.10$, $df = 22$).

Table 2) Summary of factory assessment data for treatment (on board cold-stun 5-10°C) and control lobsters consigned by the Windjana. n = 23 for all samples. The asterisks indicate significant differences

between the 2 groups (** = $P < 0.05$, * = $P < 0.10$). %FFL = percentage fit for live export, %FFW = percentage fit for processing as a whole product (frozen whole raw or boiled), %FFT = percentage fit for tailing only. See text for details.

	Control	Treatment
New leg wounds (wounds/lobster)	0.547 ± 0.064***	0.311 ± 0.043
New Antenna wounds (wounds/lobster)	0.106 ± 0.012**	0.085 ± 0.010
% Intact	61.3 ± 2.8***	72.3 ± 2.6
% FFL	84.0 ± 1.9***	88.7 ± 1.7
% FFW	10.8 ± 1.4*	7.8 ± 1.2
% FFT	5.1 ± 0.9*	3.5 ± 0.7

Old damage

Old damage (> 24 hours old) was recorded for each lobster received at the factory. This damage is not associated with the final capture, but rather reflects events the lobsters were exposed to previously. Because damaged legs are often regenerated at the next moult, the old damage presumably largely reflects damage inflicted since the last moult.

The numbers of old leg wounds/lobster in the treatment group were not significantly different from those in the control group (paired *t*-test, $t = -1.345$, $P > 0.10$, $df = 28$). Overall (combining treatment and control lobsters), the average number of old leg wounds was 37.0 ± 7.9 wounds/100 lobsters. The numbers of old antennal wounds was also similar in the 2 groups (paired *t*-test, $t = 0.812$, $P > 0.10$, $df = 28$). Overall (combining treatment and control lobsters), the average number of old missing antennae was 5.8 ± 1.3 /100 lobsters. These findings are to be expected and simply shows that all lobsters caught during the trials were drawn from a single population and that treatments were applied to randomly selected individuals from within this population.

Figure 20 shows the numbers of old limb wounds/lobster for all lobsters consigned to the factory during the trials plotted against time. Although the distribution of points is patchy, the numbers of old wounds/lobster increases between the earliest factory assessment (December 15

2000) and February 1 2001. On February 1, old damage was greatest at 13.8 old leg wounds/100 lobsters. After Feb 1, there was a rapid decrease in the numbers of old wounds/lobster. By February 27, the numbers of old wounds were similar to values recorded at other times of the year. The increase in the numbers of old leg wounds seen on February 1 results from sub 77 mm lobsters appearing in the catch consigned to the factory. During the early part of the season (November 15 – January 31) these lobsters are protected and must be returned to the ocean if caught. The net effect of multiple capture and release events on this group of animals is evident as accumulated damage to appendages and erosion of the tail fans (see below). The numbers of old leg wounds declines rapidly following February 1, suggesting these lobsters are rapidly exploited. Animals in this size range also show widespread moulting activity in February and March during which time missing legs are regenerated. This would also contribute to the observed decline in old leg wounds.

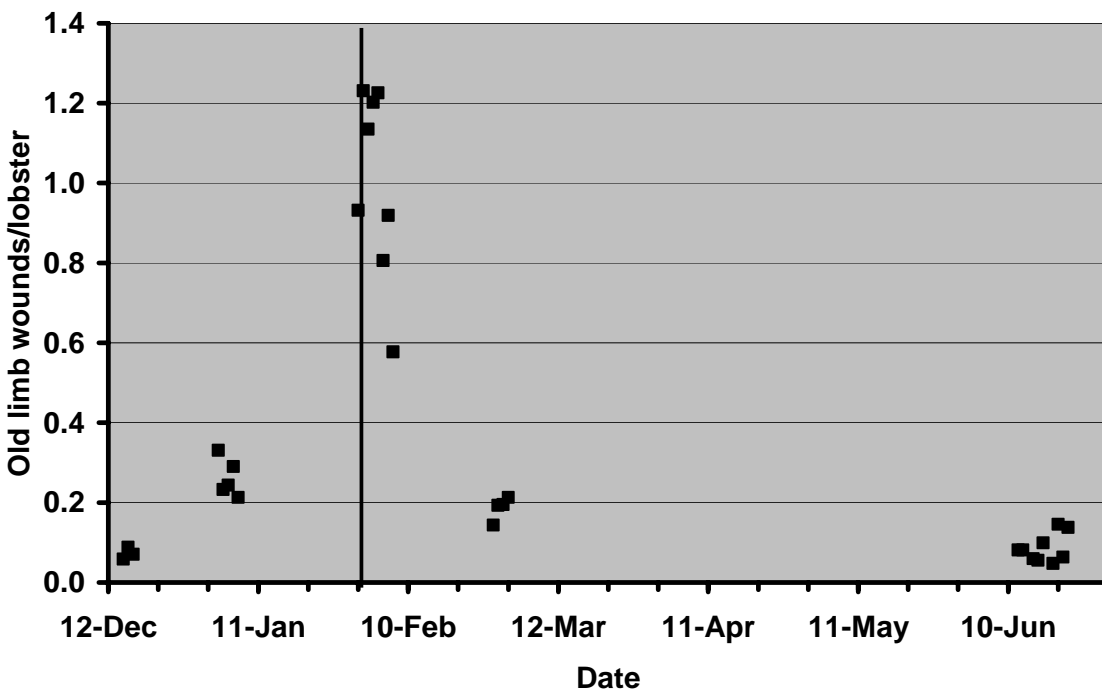


Figure 20) The numbers of old limb stumps/lobster plotted over time during cold-stunning sea trials in the B Zone. The vertical line indicates February 1 when the gauge size changes each year from 77 mm to 76 mm.

Causes of leg loss

In addition to the parameters already discussed, the on board observer also attempted to determine the cause of loss of each leg. The types of damage were categorised as follows:

- **Over board.** Legs lost from returned undersized and breeding females after they had been cast over board. Legs were observed to become detached as the animals travelled through the air before landing in the water.
- **Glove.** Legs lost during handling by deckhands.
- **Chute.** Legs lost from retained lobsters as they were placed in the chutes leading to the on board live tanks.
- **Tipper.** Legs lost when returned lobsters struck the tipper as they were thrown over board.
- **On board.** Legs found in the cacka box or on the deck after sorting a pot-load of lobsters. A specific cause of loss could not be determined for these legs.

Figure 21 shows the breakdown by cause of leg loss in control lobsters. When comparing the percentage amounts of leg loss in each category, one must always be mindful of the vast difference in the absolute numbers of legs lost in control (1346 legs) and treatment lobsters (209 legs). Interestingly, in control lobsters, the single greatest amount of leg loss (35%) was that which occurred over board after the animals had been released. The non-specific ‘On board’ leg loss was the next largest amount, accounting for 28% of observed leg loss. ‘Glove’ and ‘Chute’ accounted for approximately equal proportions of observed leg loss (19% and 17%, respectively). A minor proportion of leg loss (1%) was attributable to returned protected lobsters striking the tipper as they were thrown over board.

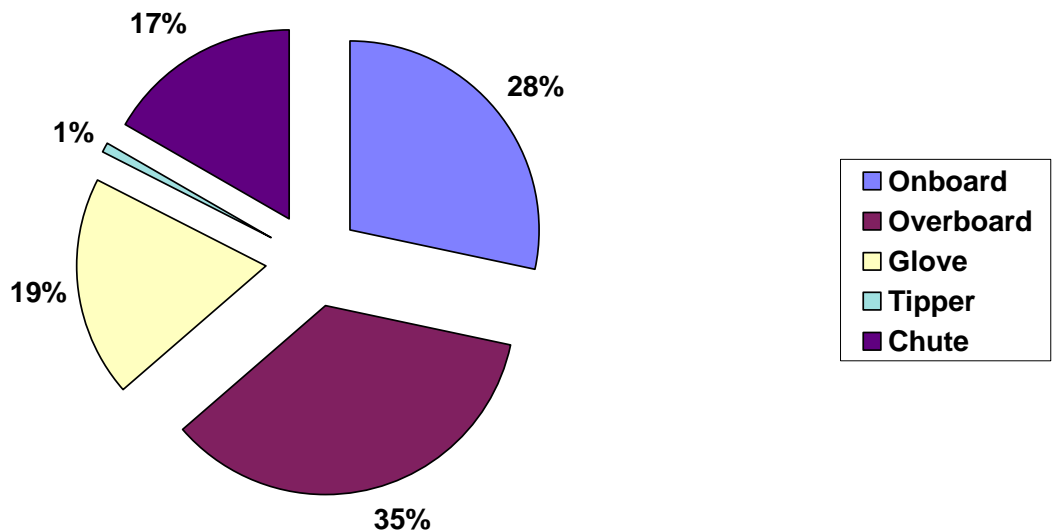


Figure 21) Causes of on board leg loss in control lobsters caught during B Zone cold-stunning sea trials. These data represent the percentages of a total of 1346 legs observed to be shed on board.

In contrast to control lobsters, the bulk of the leg loss (57 %) in treatment lobsters (stunned for 5 sec. at 5-10°C) was categorised as non-specific “On board” damage. This was accompanied by relative reductions in damage classed as “Over board”, “Glove” and “Chute”. As with control lobsters, a very minor fraction of damage (1 %) occurred when returned protected lobsters struck the tipper (Figure 22).

Part way through the day on March 3, the on board observer became aware that some legs were being broken off when the gate on the pot was removed. In the action of pushing the pot gate down to free it, legs projecting between the lower edge of the gate and the adjacent pot batten were sometimes broken off. This type of damage was recorded on 2 of the 11 days of trials after March 3. On these 2 days, a total of 8 legs were lost and all the damage occurred in the treatment lobsters. This last observation raises the question of whether or not this cause of damage occurred equally in both groups, or whether it was over represented in the treatment group. Each treatment pot had to be slid along the pot rail on the leading edge of the cacka box

before the pot was emptied. This gave additional time for lobsters to fall to the front of the pot and allow their legs to project through the battens around the gate. Therefore it might not be unreasonable to expect greater damage in the treatment group. Prior to March 3 any damage attributable to this cause would have been included in the non-specific “On board” category.

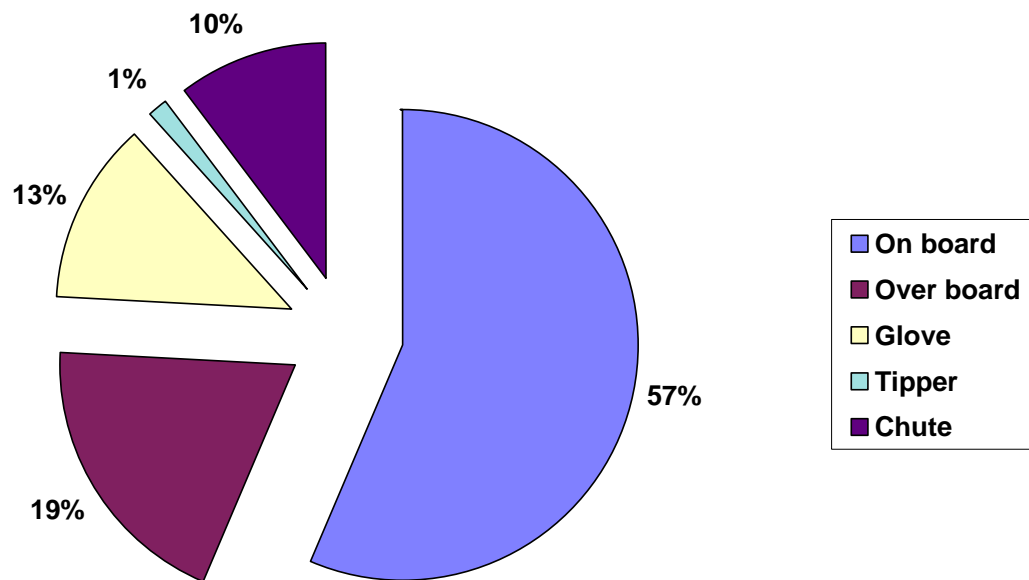


Figure 22) Causes of on board leg loss in cold-stunned (5-10°C for 5 sec.) lobsters caught during B Zone cold-stunning sea trials. These data represent the percentages of a total of 209 legs observed to be shed on board.

Post-consignment leg loss

It was found during factory assessments, that lobsters stored and transported in partially filled baskets showed significantly greater numbers of new leg wounds/lobster than those transported in “full” baskets (Figure 23; unpaired *t*-test, $t = 2.161$, $P < 0.05$, $df = 14$). Generally the maximum numbers of lobsters in a basket was about sixty and, for the purpose of this analysis, baskets containing more than 40 lobsters were considered to be full, whereas baskets containing less than this number were considered to be partially filled. Caution is urged when considering these data as they were not collected under controlled conditions.

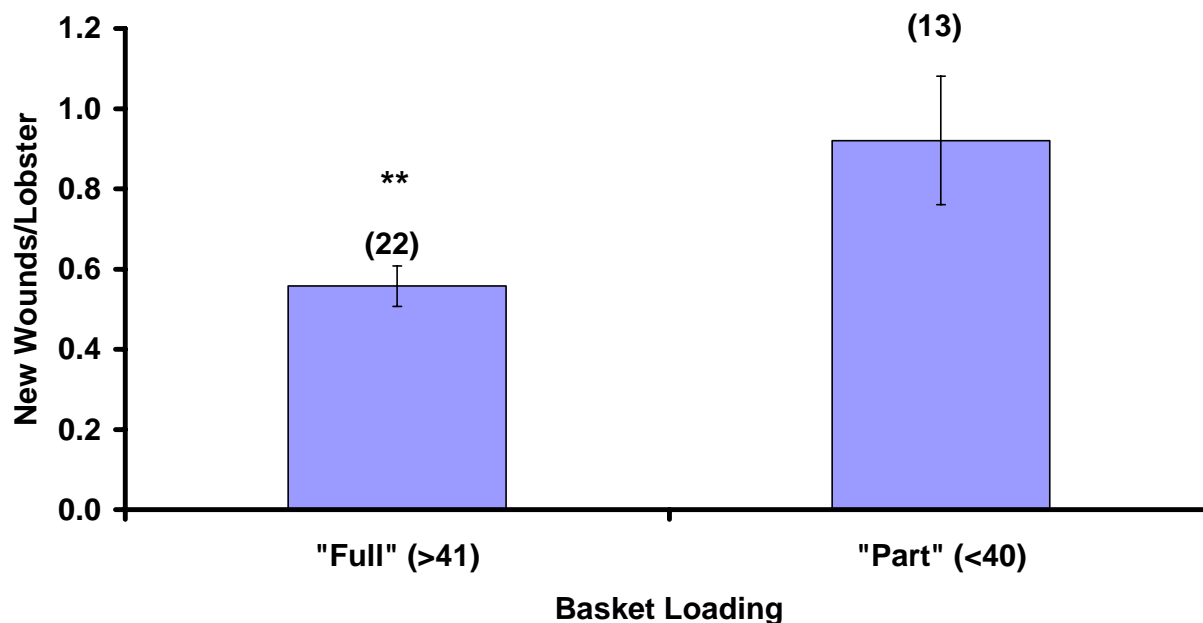


Figure 23 The apparent effect of basket loading on leg loss during transport and handling of lobsters. "Full" baskets contained ≥ 40 lobsters and "part" baskets contained ≤ 40 lobsters. The numbers in brackets indicate sample sizes. The treatments are significantly different ($P < 0.05$).

Additional: Evidence of Cumulative Handling Damage in Undersized Lobsters

On February 1 each year the gauge size changes from 77 mm to 76 mm. Randomly selected lobsters arriving at the GFC lives factory on February 1 and 2 were measured (carapace length) and the numbers of missing legs were counted. This data enabled investigation of the effects of the repeated capture, handling and release experienced by sub-77 mm lobsters during the period between the start of the season (November 15) and the gauge change on February 1. From these figures, the **average** number of previous capture events for the sub-77 mm lobsters was estimated to be approximately 3. Individual damage was very variable, indicating that many lobsters may have escaped previous capture altogether, while others may have been caught many more times this average.

Table 3 shows the numbers of old leg stump wounds for the two size classes. Old wounds are defined as those greater than 24 hours old, and this can be estimated visually with a high degree of accuracy. The mean number of old leg wounds/lobster in 76 mm animals was

significantly greater than in 77 + mm lobsters (unpaired *t*-test, $t = 11.053$, $P < 0.01$, $df = 1043$). On the basis of old appendage loss alone, 53% of sub-77 mm lobsters were fit for live export, compared to 81% of 77+ mm lobsters. Presumably the sub-77 mm lobsters would be more similar to the 77+ group if they had not been subjected to this repeated capture and release.

Likewise the number of old antennal wounds/lobster was also significantly greater in 76 mm lobsters (unpaired *t*-test, $t = 10.556$, $P < 0.01$, $df = 1059$).

Many more of the sub-77 mm lobsters were considered unfit for live export due to poor appearance, mainly resulting from tail fan necrosis and the incidence of this type of damage was again greater in this size class. These lobsters would have limited value even as tails, because of the unsightly damage to the tail fan. This supports the theory that tail rot may largely result from lobsters tail-flipping in air without the cushioning effect of water. Contact with the rough underside of the lobsters would lead to physical damage of the tail fan, allowing entry of ubiquitous pathogens. A reduction in tail-flipping in air, resulting from cold-stunning could conceivably reduce this damage. If cold water stunning were to be widely adopted, a significant portion of this cumulative damage might be alleviated, yielding further benefits to the industry.

Table 3) The occurrence of old (>24 h) damage to appendages in 76 mm and 77 + mm lobsters delivered to the factory on February 1, 2001.

	76 mm	77+ mm
Sample size	699	365
Old leg wounds/lobster	1.17 ± 0.06	$0.29 \pm 0.05^{***}$
Old antennae wounds/lobster	0.30 ± 0.02	$0.07 \pm 0.01^{***}$
Incidence of tail fan necrosis (%)	33	4

Shark Raider II Sea Trials (Zone C)

In general the on board practices in the southern C Zone are quite different to those experienced in previous trials conducted in the more northern B Zone. For example, most B Zone fishermen empty lobsters from the pot into a sorting box (cacka box) via a gate on the pot. In contrast, many C Zone fishermen do not have gates on their pots and the lobsters are removed by hand from the pot through the pot neck. The various methods of fishing used in the different areas of the fishery seem largely to be carried over from historical times, rather than have specific and unique benefits for a particular region. Through informal discussions with C Zone fishermen the following “reasons” for not having gates in the pots were put forward. The first suggestion was that seals are able to open pot gates to get to the lobsters inside, resulting in the loss of catch. By not having gates, losses to seals are reduced. The second suggestion was that, if pots have gates, it was easier and faster for poachers to pull up a pot and empty and this somehow made it easier for the offenders to evade detection. The plausibility of these suggestions has yet to be determined, but given there is no perceived difficulty with using gates in other parts of the fishery they seem unconvincing. In addition, after using pot with gates during the cold-stunning sea trials, the skipper and crew of *Shark Raider II* were so impressed with their ease of use that they continued to use the gates even after the trial had finished.

A series of technical difficulties with the refrigeration unit and associated hardware were experienced during the trials and as a result, only 7.5 of the planned 20 days of trials were completed. The problems encountered were related to the set up of the refrigeration, which differed from that used during the Zone B trials in two main ways. Firstly, the boat used in the Zone B trials was equipped with an auxillary generator that supplied power for the refrigeration unit. The C Zone boat did not have a generator and power for the refrigeration unit was generated by an alternator on the engine and delivered via an inverter. Despite professional advice and installation, the alternator did not generate sufficient power for the refrigeration unit when the main engine was at idle. As a result, the skipper had to run the engine at slightly higher revs when pulling the gear. This affected the speed of the pot rope winch, which in turn caused problems when pulling gear and disrupted the normal routine of the crew. Secondly, in the Zone B trials the refrigeration coils were placed directly in the stun tank and refrigerant was circulated through them, whereas on the C zone boat, water from the stun tank was pumped through a refrigeration unit below decks before being returned to the stun tank. The circulation

of water through the system was impaired frequently due to build up of material in the lines and pump. This affected the cooling of the stun tank water and resulted in variable stun temperatures. It is not known if the variable stun tank temperatures produced an inferior result compared with constant regulated temperatures.

Although frustrating for all concerned, this experience proved very useful for making recommendations regarding the preferred installation of refrigeration equipment aboard commercial vessels. Clearly the setup installed aboard the boat in Zone B is superior. Aside from the reliability issues, this design is more efficient for cooling the water in the stun tank (i.e. shorter pull down times) due to the direct contact of seawater with the refrigeration coils.

In total nearly 5000 lobsters were handled during the Zone C trials. The data collected showed that rates of on board leg loss in control lobsters were similar to those recorded during the Zone B trials at similar times of the year (Figure 24). The overall average daily leg loss in control lobsters was 10.7 ± 2.9 legs/100 lobsters handled. This is interesting because the two trials operated under very different conditions. Compared to the B Zone boat, the C zone boat was, on average, catching larger lobsters (the average size of legal lobsters was ~610 g), was skinning control pots through the neck as rather than through gates, and was operating further offshore (~ 30 km) and in deeper water (> 40 fms compared with mostly < 20 fms in Zone B). Anecdotally larger lobsters are thought to be less likely to shed legs during handling (Davidson and Hosking, under review). In addition, boats working further offshore are less likely to experience the hot dry winds coming off the mainland, which are related to high rates of on board leg loss in western rock lobsters (Davidson and Hosking, under review).

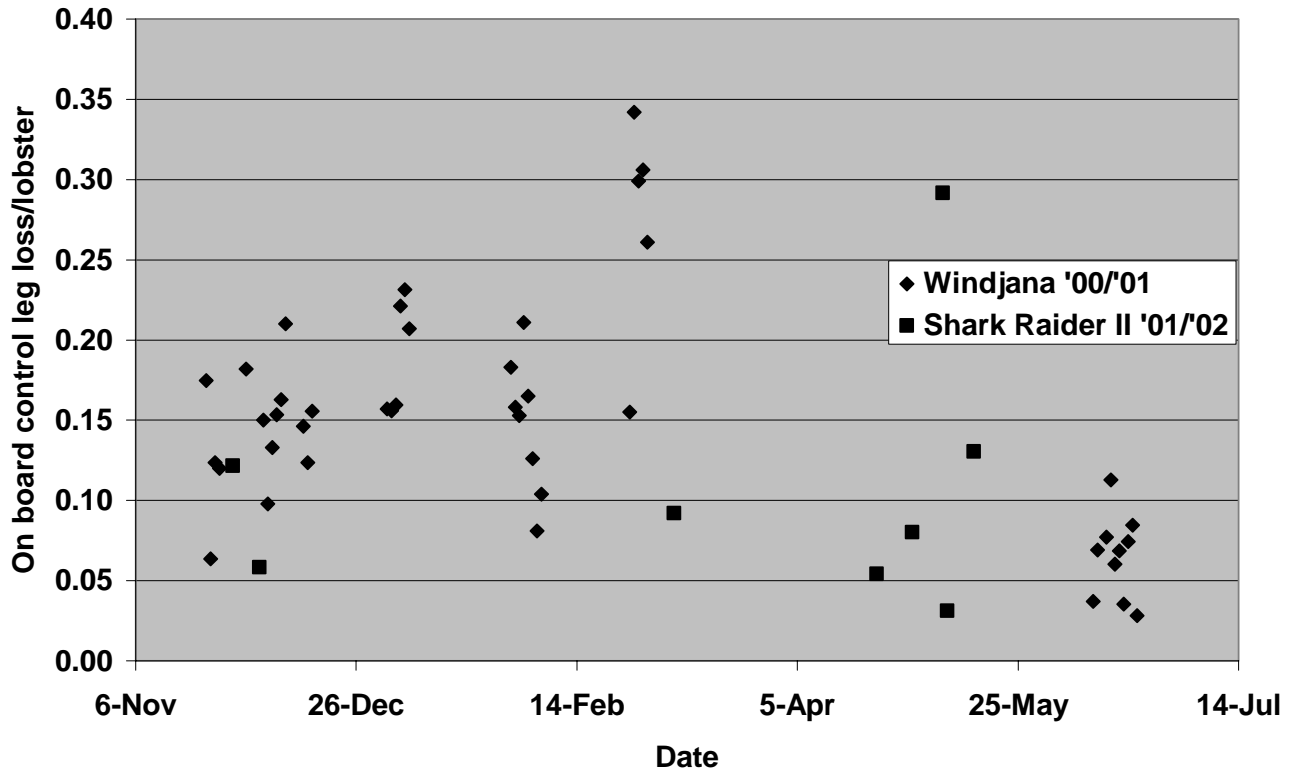


Figure 24) Leg loss in control lobsters aboard two commercial lobster boats during sea trials of cold-stunning.

More importantly, onboard leg loss during the C Zone trials was reduced by a similar amount to that seen during B Zone trials (Figure 25). Stunning lobsters for 5 sec at 5-10°C reduced overall on board leg loss by 66 %. This demonstrates the very wide applicability of cold-stunning. This figure would be expected to increase if data from the whites run of the season were collected. Anecdotally, the post-moult white lobsters are very susceptible to shedding legs during handling, and based on the results of the Zone B trials (see above), this increases the scope for reducing leg loss through the use of cold-stunning. The average onboard leg loss in the treatment group was 3.7 ± 0.5 legs/100 lobsters.

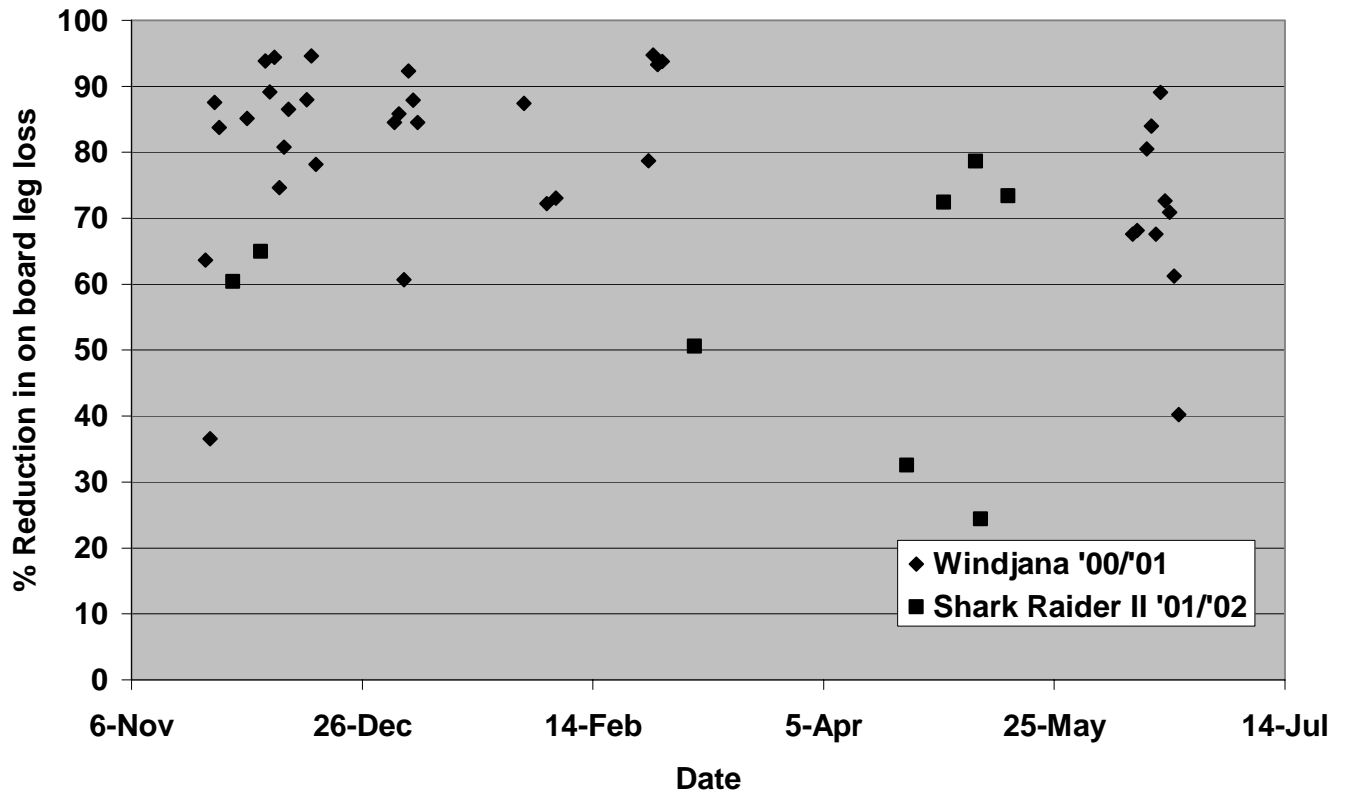


Figure 25) Percentage reductions in daily leg loss occurring during commercial lobster fishing due to the use of cold-stunning (5-10°C for 5 sec.) prior to on board sorting of the catch on two commercial lobster boats.

Causes of Onboard Leg Loss

Leg loss observed onboard *Shark Raider II* was classified into the following categories:

- **Basket.** These legs were found in the basket in the stun tank and were either shed in the stun tank or were shed prior to the lobsters entering the tank and fell into basket without being noticed by the observer
- **Glove.** Came off in the deckie's glove during sorting.
- **Pot.** Found in the pot. May have been caught between the tipper and the pot or shed when control lobsters were removed from the pot via the pot neck
- **Air.** Legs shed by returns in midair as they are tossed overboard.

- **Chute.** Legs broken off when the lobsters are placed at the entrance to the chutes to the below deck live tanks.
- **Deck.** Detached legs found on the deck. These are legs that were not noticed by the observer at the time the pot was skinned. Most are likely to have been shed in the pot and dropped through the base of the pot.

Note that these categories vary slightly from those in the Zone B trials. This is due to differences in the operating conditions aboard each vessel restricting the observer's ability to see what was happening.

Figure 26 shows the breakdown by cause of observed leg loss in control and treatment lobsters onboard *Shark Raider II*. Because the two vessels used in sea trials were operating under very different conditions, the aim of this breakdown is not to draw comparisons between them, but simply to describe the types of damage observed in each trial. As for the *Windjana* data, one must always be mindful that the absolute numbers of legs shed are very different in the stunned and control groups.

In the control group the greatest numbers of shed legs were found on deck (Figure 26). It is likely that most of these had fallen through the bases of the pots as they rested on the slide rail while the lobsters were removed via the pot neck. This is supported by the observation that the next greatest number of detached legs was found in the pot itself. Together, legs found on the deck or in the pot accounted for nearly 80% of the observed damage. If one includes damage occurring whilst the lobsters are held in the gloved hand, 90 % of all damage is caused during skinning of the pot through the neck. Without conducting a specific controlled trial to investigate this further, it is not possible to say if skinning pots via the neck causes more or less damage than skinning via a gate and into a cacka box.

When one considers the damage in the stunned lobsters, the first thing to note is the greatly reduced overall numbers of detached legs (Figure 26). It is interesting to note the damage attributed to “pot” and “deck” in the treatment lobsters. These categories account for 50% of all damage in this group. It occurs prior to the lobsters being emptied into the stun tank and therefore cannot be controlled using cold-stunning as it is applied here. A further 44% of detached legs were found in the basket in the stun tank. Given the relatively high amount of damage occurring in the pot prior to lobsters being tipped into the stun tank, together with our

observations of the typically insignificant loss of limbs during cold-stunning in factory-based experiments, it is likely that a substantial portion of the legs found the stun tank basket may have been shed in the pot and tipped into the cacka box with the lobsters. This is supported by the observation that some legs were found in the stun tank basket after sorting of control animals where the lobsters were not even tipped into the stun tank.

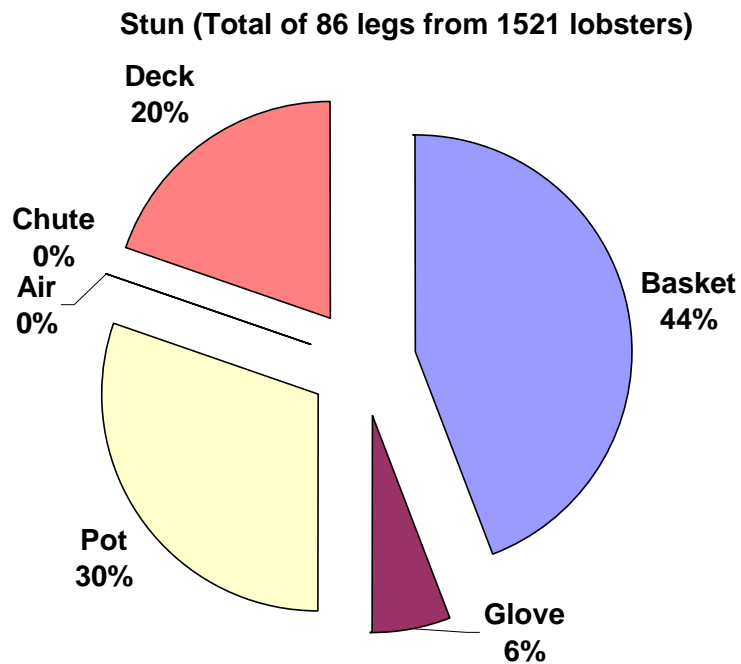
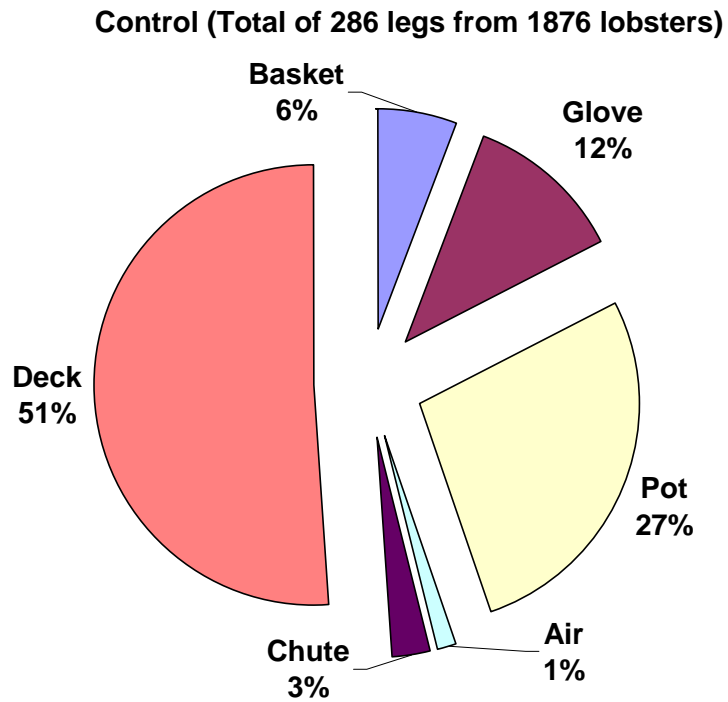


Figure 26) Analysis of the causes of on board leg loss in control and cold-stunned (“stun”; 5-10°C for 5 sec.) lobsters caught during C Zone cold-stunning sea trials aboard the commercial lobster boat Shark Raider II.

After landing the catch, lobsters were transported by truck to the processing factory in Fremantle. Upon arrival at the factory, the lobsters were chilled before being assessed for new and old appendage damage. Table 4 shows the results of the factory inspections. There were no significant differences ($P > 0.05$) between the control and treatment groups in any of the measures taken. The number of new wounds (less than 24 h old) gives an estimate the damage occurring since the pots were set the day before and is the measure of interest when comparing the treatment and control groups. Control lobsters had 37.2 ± 7.9 new limb wounds/ 100 lobsters (Table 4). The numbers of new wounds on treatment lobsters were not significantly different from this (31.1 ± 7.2 new wounds/100 lobsters paired t-test, $t = 0.5774$, $df = 6$, $P > 0.05$). Given that the rate of onboard leg loss in the control group was only 11.8 legs/ 100 lobsters, these figures suggest that there was a major amount of leg loss occurring other than during the onboard sorting of the catch. At the present time the causes of this additional damage are unknown, but it would have been a combination of pre-capture damage (caused by predators etc.) and damage occurring during transfer to and from the truck. It is worthwhile noting that the baskets used to transport the lobsters were supplied under a basket exchange program by the processor. These baskets were not lined with plastic “oyster” mesh, as is common practice in the northern part of the fishery. From personal observation, significant appendage damage occurs when lobsters are transported in unlined baskets. The baskets used in the Zone B trials were all completely lined with mesh. It is also interesting to note that the lobsters from *Shark Raider II* were transported to the factory in a “dry” air refrigerated truck. The refrigeration unit will lower the relative humidity in the truck and will dry the lobsters. It is possible that such a drying atmosphere will cause hypersaline films to develop in the truck, on baskets and the lobsters themselves, thereby inducing leg autotomy (Davidson and Hosking, under review). Salinity surveys conducted as part of FRDC Project 2001/255 showed that water on the floor of trucks delivering to this particular factory could indeed be hypersaline, with salinities up to 85 ppk (Davidson and Hosking, under review).

Table 4) Summary of factory assessment data for treatment (on board cold-stun 5-10°C for 5 sec.) and control lobsters (untreated) consigned to the factory by Shark Raider II. n = 7 for all samples. The

asterisks indicate significant differences between the two groups (paired t-test; *** = $P < 0.01$, ** = $P < 0.05$, * = $P < 0.10$, n.s. = not significant). %FFL = percentage fit for live export, %FFW = percentage fit for processing as a whole product (frozen whole raw or boiled), %FFT = percentage fit for tailing only. See text for details.

	Control	Treatment
New leg wounds (wounds/100 lobsters)	37.2 ± 7.9	31.1 ± 7.2 n.s.
New Antenna wounds (wounds/100 lobsters)	7.7 ± 2.1	8.9 ± 2.6 n.s.
% Intact	55.5 ± 3.6	55.9 ± 5.2 n.s.
% FFL	83.9 ± 1.6	83.9 ± 3.5 n.s.
% FFW	12.1 ± 1.4	12.9 ± 2.4 n.s.
% FFT	4.0 ± 0.8	3.1 ± 1.3 n.s.

Old damage

Since old appendage damage occurred prior to capture, the level of damage could not have been affected by onboard cold-stunning under the conditions of the trial. For this reason, the old damage data were not separated into treatment and control groups for analysis. The daily average number of old wounds recorded on all lobsters received at the factory from *Shark Raider II* was 20.7 ± 4.0 /100 lobsters. This figure is somewhat lower than that recorded during the Zone B trials. This is, perhaps, not surprising because the average size of lobsters was much bigger in the Zone C trials, therefore it is unlikely that they would have been caught and released (and suffered cumulative appendage loss) in the months leading up to their capture by *Shark Raider II*. This is in contrast to the situation in the Zone B trials where the great majority of the lobsters were very close to minimum size and were likely to have some old appendage damage from previous capture and release events prior to the gauge change on February 1.

Abrolhos Island Leg Loss Survey

The aim of this work was to assess the commonly stated belief that most of the leg loss in Zone A occurs prior to capture and therefore cannot be controlled by on board cold-stunning. This statement is a bit misleading, because if there is a great deal of pre-capture leg loss, there may still be a significant amount of preventable on board damage. The data from the cold-stunning sea trials carried out in Zones B and C, showed that in both regions most of the observed damage occurred pre-capture, but there was still a significant benefit to be had by preventing the substantial on board leg loss that also occurs. The situation is confused even more because at certain times of the year, such as prior to the February 1 gauge change, a large portion of “pre-capture” damage is actually the result of leg loss occurring during previous capture and release events, and so is also preventable using cold-stunning.

Overall, onboard leg loss on commercial lobster boats operating in Zone A was 2.2 ± 0.7 legs/100 lobsters handled ($n = 6$). This is very low compared with the rates of leg loss recorded previously on coastal boats (5-35 legs/100 lobsters). Based on this rate of leg loss and assuming an annual catch of 1500 t from the Islands, the total weight of legs lost during handling during the island season would only be approximately 350 kg.

Table 5 shows that observed on board leg loss accounted for between 1.9 and 31.9% of all recorded damage. On average, pre-capture damage accounted for $89.7 \pm 5.2\%$ of all recorded damage. This agrees with the findings of Tod, et al. (1990) who showed that on average 73% of damage observed up to the time of transportation to the mainland was accounted for by pre-capture damage. It is likely that most, if not all, pre-capture damage was caused by predators. Densities of large predatory fish species, such as Baldchin Groper (*Choerodon rufescens*) are much higher in the waters surrounding the Islands than in coastal waters. Lobsters trapped in pots overnight are susceptible to attack by such fish species. Perhaps not surprisingly, the total numbers of leg wounds were lowest in deeper waters (>20 fms) where densities of these reef-dwelling predatory species are likely to be lower.

Table 5) Summary of leg damage recorded aboard commercial lobster boats fishing in the A (Abrolhos Islands) Zone.

Boat	1	2	3	4	5	6
Fishing Depth	> 20 fms	> 20 fms	10-20 fms	10-20 fms	<10 fms	<10 fms
% of catch as legal	66.9	66.5	39.8	47.8	48.2	46.7
Total # wounds/100 lobsters (A)	15.1	14.4	54.7	30.0	54.6	120.0
Observed onboard leg loss/100 lobsters (B)	4.8	3.1	1.2	0.6	1.7	2.8
% A explained by B	31.9	20.3	2.2	1.9	3.1	2.3

As expected, the two boats fishing in deeper water (> 20 fms) caught only small numbers of undersized lobsters, catching between 5-10 legal-sized lobsters for every undersized returned to the water. These are areas where greater numbers of large lobsters (>600 g) are caught. These boats however, caught fairly large numbers of breeding females (1 breeding female for every 2-3 legal-sized animals). In contrast, boats fishing in shallower water (10-20 fms) caught size and undersized lobster in approximately equal numbers, with fewer breeding females appearing in the catch. In shallow waters (< 10 fms) greater numbers of undersized lobsters will be caught and returned to the ocean, accumulating damage with each capture. This contributes to the higher levels of damage observed on boats fishing in shallow to mid depths.

As reported in the companion project (FRDC 2001/255), on board leg loss was correlated with environmental factors, such as sea surface temperature (SST), relative humidity and air temperature. In that project, SST gave the best correlation with on board leg loss. If SST actually does influence on board leg loss, one might expect levels of leg loss at the Abrolhos Islands to be higher than on the coast due to the influence of the warm southward flowing Leeuwin Current. Clearly this was not found to be the case, suggesting the relationship between on board leg loss and SST is not causal. FRDC Project 2001/255 also showed that on board leg loss was correlated with a number of other factors, including air temperature and relative humidity (Davidson and Hosking, under review). Values of both of these factors tend to be more moderate at islands without the local influence of the mainland. Moderate temperatures and high relative humidity do not favour the development of hypersaline films, and this may help to explain the low observed rates of on board leg loss.

The low rates of on board leg loss recorded in the present study indicate that cold-stunning would have only a small benefit, in terms of preventing leg loss, and on this basis, its use at the Islands is probably not warranted. This recommendation is reinforced by the finding that, at the Southern Group of the Abrolhos Islands, lobsters returned to the ocean after on board cold-stunning were recaptured at a significantly lower rate than those returned to the water without stunning (Davidson, in preparation). There is however, adequate justification to recommend installing cold-stun cacka boxes on “island’ boats to cover the first four months of the season when all boats (including “island” boats) intensively work inshore waters and where catches of size and undersized are high and leg loss significant.

The significant pre-capture damage observed at the Abrolhos Islands may be at least partially preventable through other strategies, such as increasing the size of the escape gaps from 54 to 55 mm. Work done by WA Fisheries has shown that this significantly reduces the catch of undersized lobsters (Brown and Caputi, 1986), thereby reducing their exposure to predators whilst trapped in pots. Despite the large catch of undersized lobsters in coastal waters, the larger escape gaps were not implemented in these zones due to an apparent reduction in the catch of size lobster during seasons of low abundance. In seasons of high abundance, the larger escape gaps appeared to enhance the catch of legal-sized lobsters, resulting in increased inter-annual variation in catch. This would not be such a concern in the A zone, because the catch (and presumably the abundance of lobsters) varies little from year to year. However, as each islands season progresses, the abundance of lobsters will be reduced through exploitation and the catch of legal sized lobsters may fall as this occurs. However, this is likely to be partially offset by a reduction in incidental mortality resulting from predator attacks on lobsters trapped in pots and increased catch weights in due to reduced leg loss. Further research is required to determine if increasing the escape gap size used at the Islands would have the desired effect of reducing the capture of and damage to undersized lobsters without affecting the catch of legal-sized lobsters.

Objective 6a) To compare, in captivity, the effects of handling, with and without cold-stunning, on the reproductive success of setose, tar spot and ovigerous female western rock lobsters. 6b) To investigate the effects of limb loss on the reproductive success of female western rock lobsters.

Of the 31 tarspot females obtained from the wild, 19 (61%) successfully produced broods. Figure 27 shows the results for the 19 lobsters from which broods were collected. Larval production of the groups was compared by ANCOVA after log-log transformation of the data. The ANCOVA showed that the numbers of larvae successfully hatched by lobsters handled without stunning were significantly lower than those from undisturbed lobsters (ANCOVA; $F = 9.8986$; $P > 0.05$; $df = 1$). Lobsters that were stunned before handling showed similar numbers of larvae to those from the animals that were handled without stunning (ANCOVA; $F = 1.4905$; $P > 0.05$; $df = 1$). If one considers the predicted hatch sizes for an 84 mm CL female in each of the groups, one can see that an unhandled lobster would produce 297 000 larvae. This figure agrees very well with egg counts from wild caught ovigerous females (Chubb et al., 1989). The predicted number of larvae produced by an 84 mm lobster that was handled without stunning was reduced by 64 % to 108 000. This seems a very drastic reduction in egg production and suggests that the impact of capture and release of breeding females may have a much greater impact than previously thought. The animals in this experiment were handled twice when they were in berry. This was selected as a reasonably severe treatment. However, discussions with commercial fishermen who have conducted their own crude mark and recapture experiments suggest that in the wild, ovigerous females may be handled many more times than this (P. Auguston, pers comm; H. White, pers. comm.). The predicted number of larvae produced by an 84 mm female that was stunned before handling (146 000 larvae) was similar to that produced by a female handled without prior stunning. This suggests that cold-stunning did not adversely affect the breeding success of female western rock lobsters. Finally, the number of larvae hatched from the single female with one of the 5th legs missing was much lower than would be predicted for any of the other treatment groups. If the loss of one of the 5th legs was indeed responsible for the poor performance of this female, then by preventing leg loss, cold-stunning would presumably improve the reproductive success of such individuals. Further data is required to draw firm conclusions, but it is sufficient to say that, at the very least, cold-

stunning did not adversely affect egg production in breeding females and may actually have a beneficial effect in this regard.

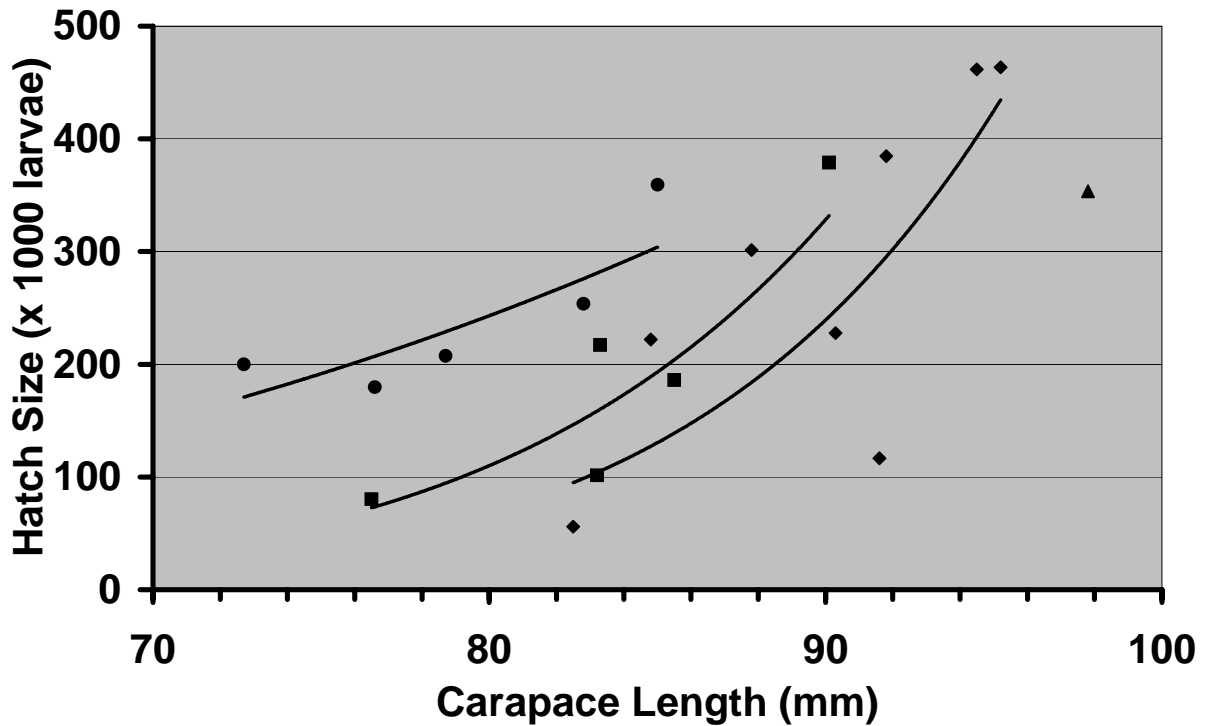


Figure 27) A graph of hatch size (10^3 larvae) against size (CL, mm) of breeding female western rock lobsters. Four treatments were applied to the lobsters: handled (diamonds); stunned and handled (squares); undisturbed (circles); and one 5th leg removed (triangle). See text for details.

Having determined the numbers of larvae produced in each of the treatment groups, it was of interest to then attempt to assess the quality of those larvae. This was done by challenging the larvae with a combined high temperature/low salinity stressor. Analysis of the groups by one-way ANOVA showed the groups were significantly different ($F = 3.7282$; $P < 0.05$; $df = 2$). Larvae from the group of unhandled lobsters and the cold-stunned lobsters scored similarly (331.9 ± 6.9 and 328.5 ± 7.9 sec., respectively). Despite producing significantly fewer larvae, the larvae from lobsters handled without cold-stunning performed better under the conditions of the challenge test (294.0 ± 13.1). One possible explanation for this is that the

stress of handling the breeding females in air had already weeded out the weaker eggs/larvae, so that only the strongest larvae made to hatch and the challenge test. However, if this were the case, one might expect the larvae from cold-stunned lobsters, which also showed reduced brood sizes, to score similarly and this was not apparent.

Caution must be exercised when drawing inferences about the performances of larvae in the field, based on the results of this challenge test. No work has been done to correlate survival during the challenge test with future performance of the larvae. Despite other studies having shown good correlation between a challenge test and future performance of *Jasus edwardsii* larvae under culture conditions (Smith *et al.*, 2003), it remains speculative to argue a similar case for larvae growing in the wild.

The size of the larvae produced by the unhandled lobsters was 1.71 ± 0.01 mm. The mean size of larvae from the other two groups were not significantly different from this (one-way ANOVA; $F = 0.7251$; $P > 0.05$; $df = 2$). Larval size at hatch has important implications for subsequent growth in other palinurid species, such as *Jasus edwardsii*, with smaller larvae growing more slowly (Smith, 2003).

Objective 7) To conduct a survey to determine the extent and nature of leg loss in the southern rock lobster fisheries of Tasmania and South Australia.

The first stage of this work involved sending a questionnaire to members of the southern rock lobster industries of Tasmania and South Australia (SA) seeking information on the extent and possible causes of appendage loss.

South Australia

In SA the survey was sent to a number of key participants in the fishery by Mr Roger Edwards, the Executive Officer of the South Australian Rock Lobster Advisory Council (SARLAC). Of the 14 completed surveys returned, 9 were from processors, 1 was from a fisher, and 4 were from fisher/processors. According to Mr Edwards, the combined production from these individuals and companies would cover more than 70% of the catch in that state.

In response to the question “Is post-harvest appendage loss in southern rock lobsters a problem?”, 13 respondents (93%) replied yes and 1 (a processor) replied no. All respondents who considered appendage loss a problem were then asked to rate the how severe the problem was considered to be on a scale from 1 (a minor problem with no further work required) to 5 (a major problem requiring specific research). The average value of these answers was 3.5 ± 0.3 , indicating appendage loss was considered a moderate problem.

The major suggested causes of appendage loss are presented in Table 6. Poor handling of lobsters by crew was suggested to be the primary cause of appendage loss. Much of this presumably occurs when lobsters are removed from pots by hand via the pot neck. Comments regarding gear design mainly related to pot and tipper designs, but there were also some comments regarding designs of holding baskets in live tanks. Basket design is also an area of interest in the western rock lobster fishery. Many fishermen in this fishery line their baskets with plastic mesh to prevent legs and antennae from protruding through the ventilated walls of the baskets and being damaged when baskets are moved.

Table 6) Summary of responses to a questionnaire on the nature and prevalence of appendage loss from members of the South Australian rock lobster industry.

Suggested Factors	No. of respondents
Handling/Poor handling	13
Octopus/Predation	5
Gear design	5
Hot/adverse weather conditions	3

When asked which appendages were shed more often (i.e. legs or antennae), five respondents stated antennae, and eight stated legs were lost more often (one respondent did not answer this question).

Six respondents thought that appendage loss did not vary seasonally, six thought that it did, one did not answer the question and one did not know. All respondents who thought appendage loss varied seasonally identified the hottest months of the year (October-March) as the times when appendage loss was worst. This is similar to anecdotal suggestions regarding the prevalence of leg loss in the western rock lobster fishery.

Tasmania

With the assistance of Mr Rodney Tregloggen, who is the Executive Officer of the Tasmanian Rock Lobster Fishermen's Association (TRLFA), 330 questionnaires were sent out to members of the industry. Fifty five responses were received.

In response to the question "Is post-harvest appendage loss a problem?", the respondents were fairly equally divided. Thirty-one respondents (56%) replied "no" and twenty-four replied "yes". This is a much lower percentage than was noted in SA. It is noteworthy that, as in SA, Tasmanian respondents noted warm weather conditions as a suggested contributing factor (see below) and it is tempting to suggest that the cooler climate in Tasmania may result in less appendage loss. This is however, highly speculative.

Experiments conducted as part of the companion project FRDC 2001/255 showed that *J. edwardsii* is not susceptible to hypersaline-induced leg autotomy as occurs in the western rock lobster. Therefore it would appear that if there is indeed increased appendage loss in southern rock lobsters during periods of warm weather, evaporative concentration of seawater is not mechanism by which this occurs.

The average score when rating the problem of appendage loss on a scale of 1 to 5 was 3.0 \pm 0.3, indicating it is perceived as a moderate problem.

Interestingly the major supposed causes of appendage loss identified by Tasmanian respondents (and their order of importance) were almost identical to those identified by SA respondents (Table 7).

Table 7) Summary of responses to a questionnaire on the nature and prevalence of appendage loss sent to members of the Tasmanian rock lobster industry.

Suggested causes	No. of respondents
Handling/ Poor handling	17
Octopus predation	6
Gear design	6
Recently moulted	5
Hot, windy weather	3

Octopus predation is well documented as a significant cause of mortality of pot-caught lobsters in both the southern rock lobster fisheries and the western rock lobster fishery. Often lobsters remaining in pots containing octopus show considerable leg loss, presumably from being harassed by the octopus. Some work has been done by Brock et al. (2003) to develop an octopus-proof pot for the SA rock lobster fisheries. The final design successfully reduced octopus predation by 45-48%, but also reduced the catch of lobster by 28%. The inclusion of escape gaps in the pot was also found to be effective for reducing octopus predation by allowing undersized lobsters to escape.

The number of respondents who thought that legs were the appendages shed most frequently (13 respondents, 54%) was similar to those who thought antennae were shed most frequently (11 respondents, 46%).

Fifteen of the Tasmanian respondents thought that appendage loss varied seasonally, eight did not and one respondent did not answer this question. Unlike the SA respondents, however, there was no clear pattern of seasonality identified by the respondents.

Appendage Loss Field Monitoring, South Australia

During the “summer” monitoring, observations were made on four commercial boats over 10 days of fishing between January 24 and February 5, 2001. A total of 715 lobsters were caught from 463 pot pulls during this time. Fifty one of the lobsters caught (7.1%) were undersized.

During the “winter” monitoring observations were made aboard only two boats over 12 days of fishing between May 3 to 22. Fewer boats used because during May the catches were lower and boats stayed at sea for longer periods. In the cases of the two boats observed, both stayed at sea for six days during the monitoring period. During the “summer” monitoring, boats were at sea for 1-2 days only. A total of 411 lobsters were caught from 391 pot pulls. Forty eight (11.7%) of the lobsters were undersized.

When considering the nature and occurrence of appendage loss in southern rock lobsters, there are 2 key considerations. Firstly, it is of interest to know the total amount of appendage loss that is observed on lobsters, and secondly it is important to identify how much of this damage is preventable.

Figure 28 shows the leg loss recorded at various points in the post-harvest chain during the two field trips. When considering these data it is important to bear in mind that the same groups of lobsters were not followed from point of capture to pack out. In addition, the data collected on board include both legal-sized and undersized lobsters, whereas data collected in the factory were taken from legal-sized lobsters only. As a result, the total amount of leg loss appears to vary, but does not indicate actual cumulative damage during the post-harvest chain.

Pre-existing leg loss (i.e. pre-capture leg loss) observed on board commercial lobster boats was very similar during the two survey periods, with values of approximately 16 legs/100 lobsters handled. This compares with an average pre-capture leg loss observed in western rock lobsters caught in the Abrolhos Islands (A) Zone of 45.8 legs/100 lobsters caught. So it would appear that pre-capture leg loss in southern rock lobsters is relatively modest when compared to that in western rock lobsters. Undoubtedly some of this damage occurs when lobsters trapped in pots are harassed by predators, such as octopus (*Octopus maorum*). The use of escape gaps in

pots would be largely ineffective for reducing this damage, because out of the 1178 lobsters caught in total only 99 (8.4%) were undersized that could potentially escape from pots via escape gaps. Recent work by Brock *et al.* (2003) on the development of a 2-chambered “octopus-proof” pot may go some way to reducing pre-capture damage, but this pot design significantly reduced the catch of size lobsters, and requires further refinement to achieve catches on a par with current pot designs.

Leg loss observed during sorting of southern rock lobsters on board commercial boats was also low. During the “summer” monitoring, on board leg loss occurred at a rate of 1.4 ± 0.5 legs/100 lobsters handled ($n = 4$). During the “winter” monitoring, only 0.7 ± 0.0 legs were shed for every 100 lobsters handled. These values are very low compared to those recorded on inshore boats in the western rock lobster fishery during sea cold-stunning sea trials (see above), but are similar to leg loss rates recorded on boats working at the Abrolhos Islands, where on board leg loss was also found to be very low. Based on a total annual catch of 2525 t and assuming each leg accounts for 1% of total body weight, average beach price of \$28/kg the damage occurring on board would cost the industry between \$5 000 and \$10 000 per annum in lost catch weight.

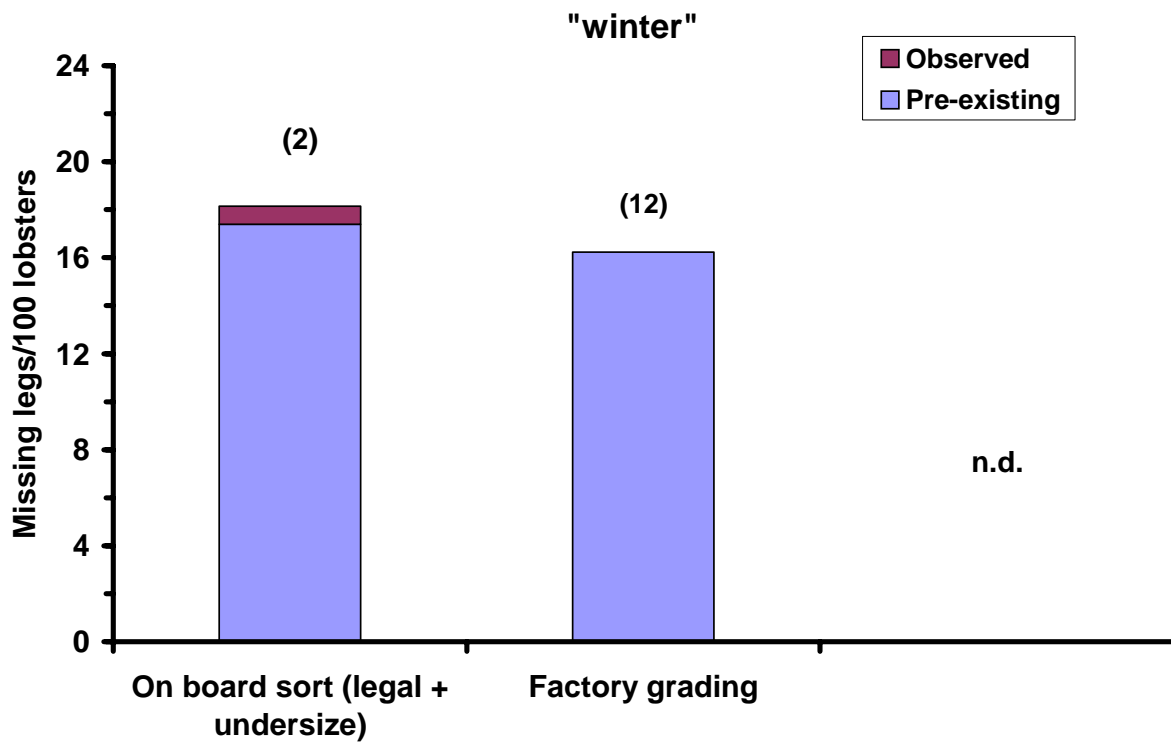
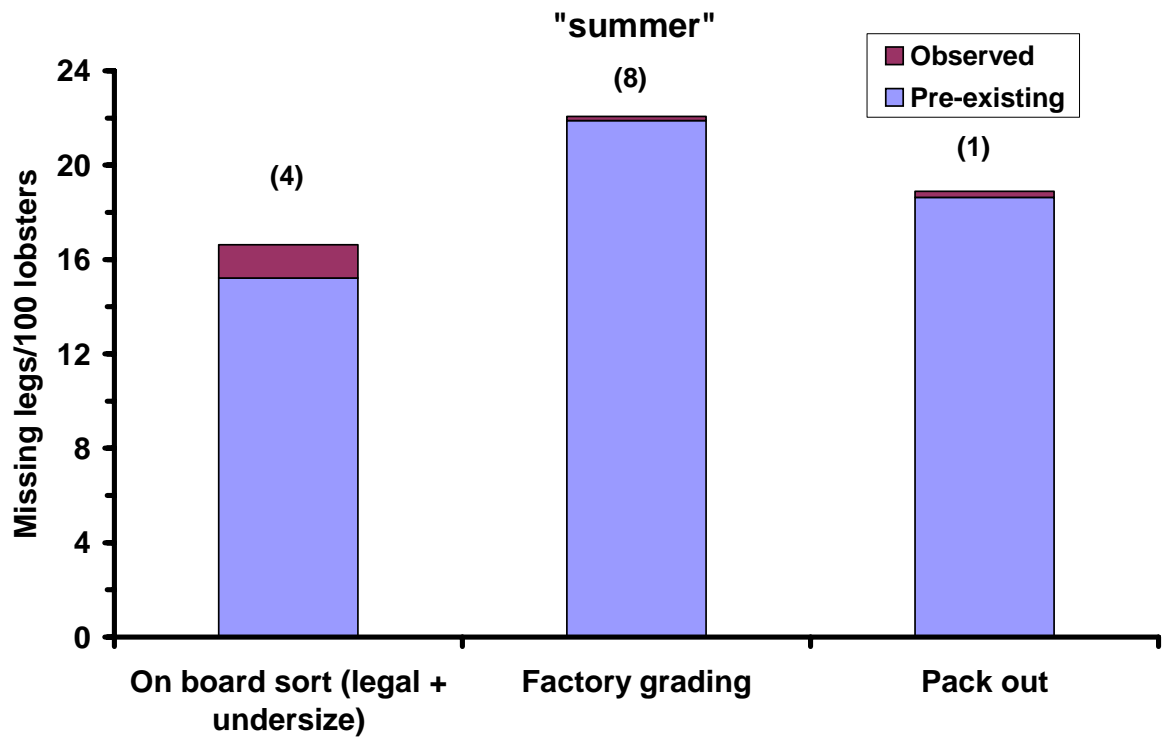


Figure 28) Seasonal variation in leg damage recorded during post-harvest handling of southern rock lobsters aboard commercial lobster boats and in processing factories in South Australia. n.d. = no data.

After delivery to the processing factories, any leg loss occurring during grading and pack out was negligible. This is interesting given that appreciable leg loss occurs in western rock lobster processing factories. A substantial proportion of the damage occurring in factories can be attributed to hypersaline-induced autotomy (Davidson and Hosking, under review) however, the same study showed that southern rock lobsters are not susceptible to hypersaline-induced leg loss.

Unlike the situation with damage to legs, the amount of damage to antennae occurring during on board handling was appreciable relative to the amount of pre-capture damage (Figure 29). Whilst, it is important to bear in mind that the absolute amounts of damage observed were still comparatively minor, this finding is worthy of further investigation because much of this damage may be preventable. Since these appendages do not possess an autotomy reflex, there must be a physical force applied to the antennae to break them off. Analysis to determine the main causes of damage to antennae showed that during both field trips, the bulk of this damage occurred whilst the lobsters were being held (Figure 30). The common practice amongst southern rock lobster fishermen is to hold the lobsters by the antennae whilst they are being removed from the pot, measured and inspected and this was the primary cause of the observed damage. It is convenient for the purpose of measuring the carapace length of lobsters to hold them by the antennae, but to avoid breaking these appendages the lobsters should be held around the basal segments and not by flagella. In the western rock lobster industry it is common practice to hold lobsters by the bases of the antennae and this can be done easily and quickly with minimal damage to the animals.

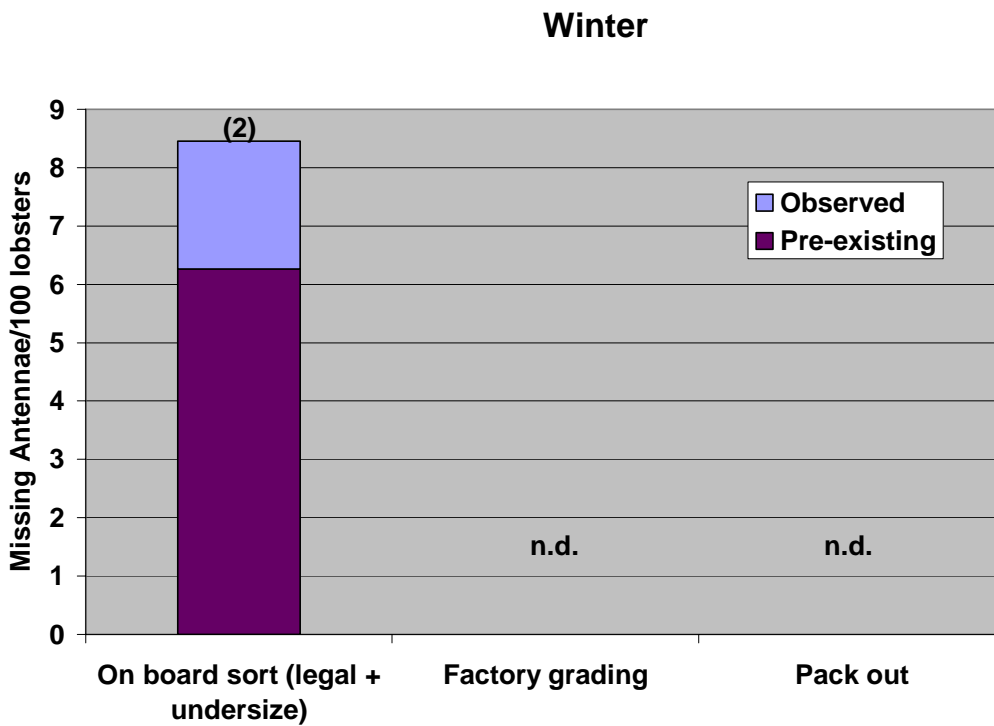
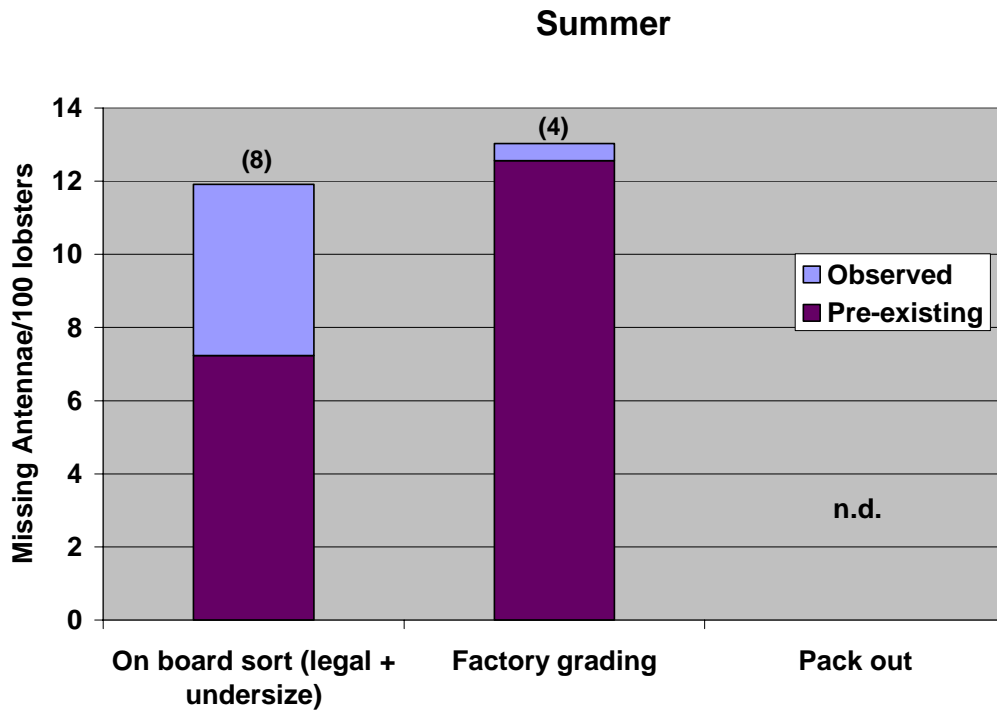


Figure 29) Seasonal variation in antennal damage recorded during post-harvest handling of southern rock lobsters aboard commercial lobster boats and in processing factories in South Australia. n.d. = no data.

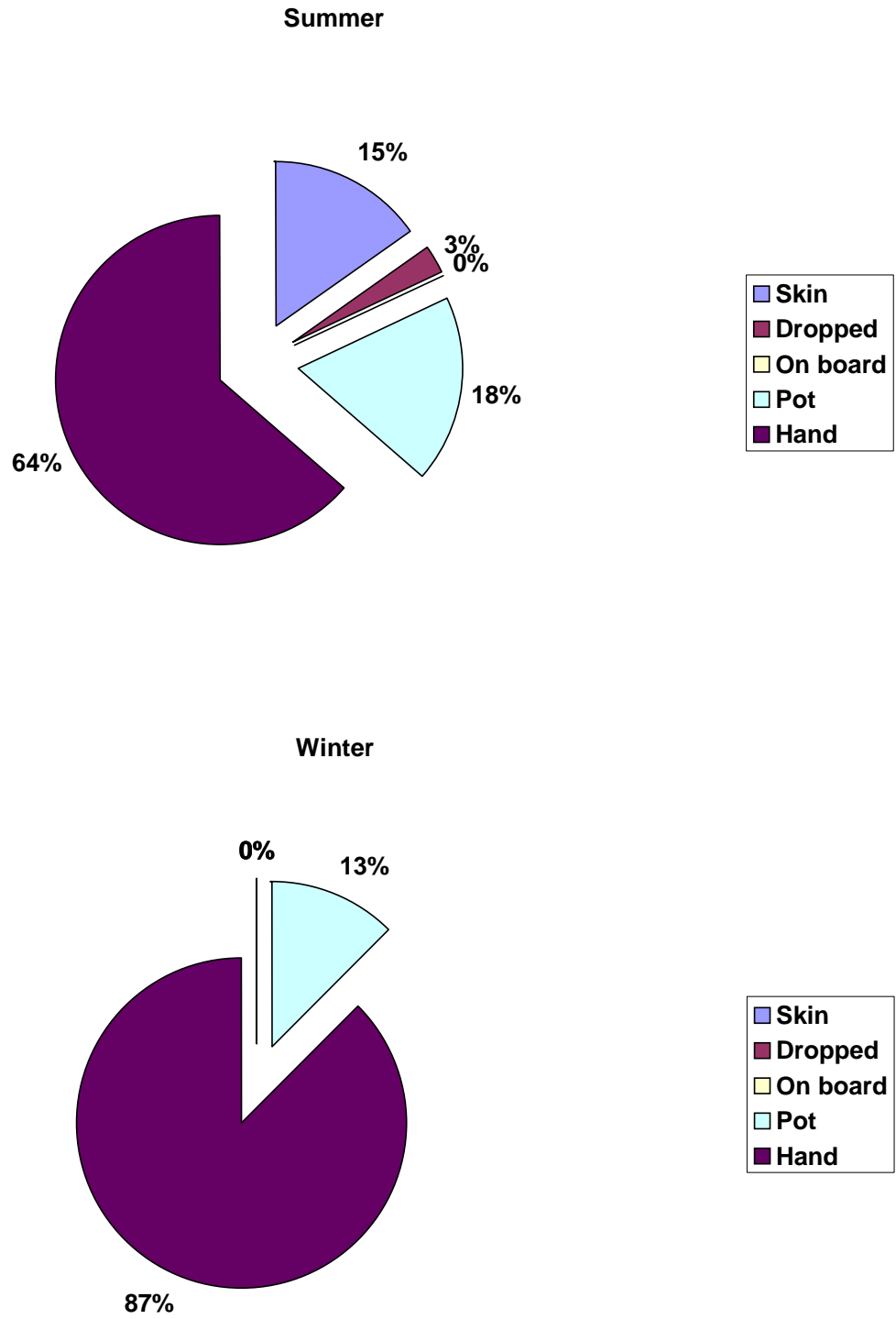


Figure 30) Seasonal variation in the causes of on board antennal damage in southern rock lobsters.

Appendage Loss Field Monitoring, Tasmania

For an analysis of the appendage damage in the southern rock lobster fishery in Tasmania, please see Appendix 3.

Benefits and Adoption

As envisaged in the original proposal, the catching and processing sectors of the western rock lobster industry are the primary beneficiaries of this research. It is clear that leg loss is a major problem for this industry and, as stated above, costs the industry up to \$3 million per annum in lost catch weight alone. Using cold-stunning up to 80% of this damage might be prevented, saving the industry more than \$2 million per annum. This is a very conservative figure because it does not take into account the improved market value of lobsters that would otherwise be downgraded due to the severity of the appendage loss. Neither does it consider benefits to be gained by applying cold water stunning at other points in the handling chain, such as prior to grading and drowning upon receipt of lobsters at processing factories. In the companion project FRDC 2001/251, it was shown that up to \$200 000 worth of legs could be saved by cold-stunning lobsters prior to drowning for processing.

Despite widespread dissemination of the results of this research, adoption of on board cold-stunning has been very limited. To the best of our knowledge only four fishermen have attempted to install cold water stunning systems on board their fishing boats. However, this may have been fortuitous, because there are still doubts about the effects of the stunning treatment on the survival of caught and returned undersized lobsters. Tag and recapture studies addressing this question have returned mixed results (Davidson, in preparation) and further work to clarify the situation is planned for the 2005/2006 season. If nothing else, this project will clarify the usefulness of on board cold water stunning, which under the terms of the current legislation can be applied freely. It may come to pass that cold-stunning is legislated against as a result of this research. Such an outcome should still be viewed as a positive finding, and would further our understanding and refinement of commercial fishing practices.

There has also been limited adoption of cold-stunning in processing factories. However, the installation of refrigeration equipment and tanks required to effect cold-stunning is no minor undertaking and requires significant capital expenditure. Using cold-stunning will also alter the flow of product through the factory and requires careful thought before making permanent changes to factory practices. Given these considerations, it is likely that cold-stunning will be

increasingly used as new processing factories are built around known best practice for handling western rock lobsters.

The appendage loss monitoring work undertaken in South Australia and Tasmania has shown that post-harvest appendage loss is a relatively minor issue for these fisheries and does not warrant further research. Again, this is a positive outcome advancing our knowledge of these fisheries. Having said this, some minor alterations to handling practices could reduce antennal damage and product quality with no additional effort or expenditure.

Further Development

Further development and refinement of cold-stunning as a method for preventing post-harvest hypersaline-induced leg autotomy has been carried out in the companion project FRDC 2001/255 (Davidson and Hosking, under review).

Investigation of the effects of on board cold-stunning on the recapture rate of captured and released undersized lobsters has been carried out in FRDC Project 2002/239 (Davidson, in preparation).

In order to disseminate the results of this research further, the information is to be included in the Rock Lobster Code of Practice Manual developed in FRDC Project 2002/237.

It is clear from the sea trials conducted in this study that more needs to be done to reduce the handling of undersized lobsters. A significant amount of leg loss occurs amongst caught and returned undersized. Given the clearly demonstrated negative effects of leg loss on the survival and growth of these animals (Brown and Caputi, 1983), any measures that can reduce leg loss in this group should be investigated. The most obvious way to achieve this is to increase the size (and number) of escape gaps fitted to pots. The Western Australia Department of Fisheries has done significant amount of work on this issue (Bowen, 1963; Brown and Caputi, 1986) and it is likely those studies are adequate to allow a decision to be made without the need for further research. However, if required, further work should be done to examine the effect of increasing the escape gap size to 55 mm and the minimum number of escape gaps used on pots. This would eliminate the handling of large numbers of undersized and associated leg loss. There is also the option of making every gap between slats on a pot the escape gap width. This would make escape of undersized lobsters even more probable.

It also appears that the 77 mm gauge size is not well matched to the pot escape gap size. The pots are designed to retain 76 mm lobsters and larger. As a result, large numbers of 76 mm lobsters are caught and released multiple times during the early part of the season, incurring significant cumulative damage and probably suffering increased mortality in the process. Two things could be done to avoid this cumulative damage. Firstly, the gauge size could be set at 76 mm for the entire season, but this would increase fishing pressure during the whites migration.

The effect on the stock of taking 76 mm lobsters all season would need to be assessed. Taking 76 mm lobsters all season would also increase the variation in the catch during the season, increasing the peak catches of the whites and reducing the catch during the quieter months of January and February. Secondly, if the current two size gauge is deemed necessary, two different sized escape gaps size could be used in pots in conjunction with the two gauge sizes. During the early part of the season the escape gap size could be set to allow sub-77 mm lobsters to escape from pots. As mentioned above, the information required to determine the size selectivity of different sized escape gaps may already be. Clearly altering/changing escape gaps would involve some effort on the part of the fishermen. Changing escape gap size could be achieved by one of several methods. Firstly fishermen could have duplicate gear; one set with large escape gaps and a second set with smaller escape gaps. Many fishermen already have duplicate sets of gear for use under different fishing circumstances (i.e. inshore and at the Abrolhos Islands). Alternatively, modular escape gap inserts could be used. These inserts could either be changed on board during fishing leading up to the date of the gauge change or the gear could be brought to shore to effect the change. Many fishermen pull their gear from the water during January when the catch drops to very low levels, then recommence fishing prior to the gauge change on February 1. This period of inactivity would allow time for fishermen to change their escape gaps.

Planned Outcomes

All but one of the planned outcomes identified in the initial proposal have been achieved. The one outcome that was not achieved was the investigation in captivity of the effect of repeated simulated capture and cold-stunning on the growth and survival of undersize lobsters (Objective 5). This outcome was not achieved due to technical difficulties with the holding system in which experiments were conducted.

The development and refinement of the novel cold-stunning method provides a practical and cost-effective solution for preventing leg loss during post-harvest handling of western rock lobsters. This was the primary planned outcome of the initial proposal (Objectives 1 and 2).

It has been shown that the performance of factory graders, in terms of their ability to correctly identify lobsters fit for live export, is not altered when lobsters are first stunned by the recommended method (Objective 3).

Cold-stunning was shown to be effective for preventing leg loss during handling of lobsters on board commercial fishing boats (Objective 4).

Handling ovigerous females in air was shown to reduce the number of larvae produced per brood by a great amount. Cold-stunning was shown to have no adverse impact on eggs attached to breeding females over and above the negative effect of handling alone (Objective 6).

Representatives from southern rock lobster fisheries of South Australia and Tasmania requested that a survey of appendage loss in these fisheries be conducted as part of the study. This has been completed and, as planned, the causes and extent of appendage loss has been documented (Objective 7).

Conclusion

This project has successfully developed a practical method for alleviating leg loss during post-harvest handling of western rock lobsters. Cold water stunning can be successfully applied at all points in the post-harvest chain to reduce both handling-related and hypersaline-induced leg loss. Induction of a stunned state was extremely rapid (5-10 sec.). Similarly rapid recovery times were observed upon return to ambient seawater. The rapidity of the response makes the technique readily applicable aboard commercial fishing boats, where speed of operation is key to the success of any method.

It was demonstrated that cold-stunning could be applied prior to factory grading to reduce the movement of lobsters during the grading process, presumably minimising any physical damage in the process. Reducing the outward appearance of vigour during grading did not limit the human graders' ability to successfully differentiate between lobsters that were fit for live storage and subsequent live export and those that were too weak to survive the live export process.

Cumulative damage to caught and released undersized lobsters during the first months of the fishing season (Nov 15 – Jan 31) was shown to be significant. This damage largely occurs due to the fact that the size selectivity of pot escape gaps is not matched to gauge size. Strategies are discussed for alleviating much of this damage. Assessment of the impact of some of these strategies may be required to allow evaluation of the strategies presented.

In captive experiments, cold-water stunning *per se* did not negatively impact upon the breeding success of reproductive females. In addition, the limited data collected suggest that cold-stunning may indirectly improve the breeding success of these animals by preventing the loss of legs from breeding females and removing the energy impost associated with re-growing the lost limbs at the expense of egg production.

Appendage loss in the southern rock lobster fisheries of Tasmania and South Australia occurred at much lower rates than found in the western rock lobster fishery. A survey of industry participants, including fishermen and processors, showed that most considered post-harvest appendage loss to be an insignificant problem. On board monitoring of damage occurring post-harvest largely confirmed the results of the survey. Nonetheless some potential

for improvement, particularly with regard to damage to antennae was identified by the on board monitoring. This damage was mainly caused by fishermen pulling lobsters from pots by holding onto the antennae, a practice that can be easily avoided.

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Appendix 1: Intellectual Property

None. All information generated by this project is in the public domain and has been made freely available to industry.

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Appendix 3: Appendage Damage in Harvested Tasmanian Southern Rock Lobster (*Jasus edwardsii*).