F.I.R.T.A, STUDY GRANT FINAL REPORT

"To Develop a Regime For The Long Distance Transport Of Live Abalone"

The aim of the study was to find the best possible regime for keeping abalone alive out of water, for a period of 48 hours. At the end of that time the abalone had to be able to be resuscitated to full strength and vigour by placing back in seawater environment.

It was felt that this would ensure the success of live abalone shipments to Japan.

The study faced the following problems:-

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- 1. What criterea should be used in selecting abalone for shipment?
- 2. What effect did temperature have on the animals and what was the optimum?
- 3. What effect did humidity have?
- 4. Was an anaerobic or aerobic environment better?
- 5. What effect did oxygen have on the animals?
- 6. Does the animal produce  $co^2$  and if so what effect does a build up of this gas have? (ie what are tolerance levels).
- 7. What are the effects of atmospheric pressure on the Abalone?
- What was the best packing material and what effect did sub-temperatures have on the insulation used in the packaging boxes.
- 1. WHAT CRITERIA SHOULD BE USED FOR SELECTION OF ABALONE FOR SHIPMENT.

The method used to answer this problem was as follows:-

- a) 100 Random samples were taken of animals from several divers catches.
- b) 100 Selected samples of the healthiest and most active were taken from the divers catch with the most apparently active animals.

These abalone were placed in boxes and held at a temperature of  $7^{\circ}$  C for 48 hours. At the end of that time they were examined for mortality and placed back in the sea in specially constructed baskets for a period of a further

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48 hours. The results after 48 hours were that abalance from category (a) had a mortality rate (not responding to touch or application of salt) of 30%. Abalance from category (b) had a mortality rate of 22%. After placing the survivors back in water for a further 48 hours, 9% of category (a) had died, and 11% of category (b).

It was concluded that this was too high a mortality rate to suffer if the venture was to be viable, so a further experiment was set up.

This involved issuing to selected divers, plastic fine mesh baskets into which the abalone were placed immediately on catching, and hung from the side of the boat in 3 metres of water.

The same selections were made as in (a) and (b) above, immediately on reaching port.

These samples (100 in each) were then placed in circulating seawater tanks. After 5 days 3 of sample (a) had died and one from sample (b). However this one had a small cut in the membrane of its gut that had escaped notice during selection.

The animals were kept in the tanks for a further 5 days during which no more deaths occurred.

At the completion of this period they were packed as before and held for 48 hours at  $7^{\circ}$ C. At the end of this time 5 from sample (a) had died and 3 from sample (b). They were then put back into water as before, and after 48 hrs a further 2 from sample (a) and one from sample (b) had died. Thus the mortality was 10% for (a) and 5% from sample (b).

This result we regarded as barely marginally acceptable, and decided on further experiments involving the other factors involved (2 to \* page 1)

2. WHAT EFFECT DOES TEMPERATURE HAVE? A number of tests were made using temperatures ranging from  $1^{\circ}C$  to  $12^{\circ}C$  in air, and in seawater ranging from  $10^{\circ}C$  to  $20^{\circ}C$  each experiment ran for 48 hours.

The most difficult task it proved was to ascertain the point of death, or the point at which the animal was judged to be past "the point of no return" which was when the animal did not respond immediately to poking. This was a subjective decision, but by attempting to resuscitate a sample of the latter category (unsuccessfully) it proved to be fairly accurate.

It appeared that in air, the tolerence of the animals to temperature change was fairly narrow. Ranging from  $5^{\circ}$ C on the low side to  $10^{\circ}$ C on the high side with the optimum being around 7.5°C. Above  $10^{\circ}$ C and below  $5^{\circ}$ C the animals

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reached mortality figures up to 50% in 48 hours. At the optimum 7.5  $^{\rm O}{\rm C}$  only 1% or 2% were recorded.

In water, the same mortality (1% - 2%) was recorded, but no apparent differences were recorded between  $10^{\circ}C$  and  $20^{\circ}C$ , is, there was a marked tolerance between these temperatures.

3. WHAT EFFECT DOES HUMIDITY HAVE? In this experiment we endeavoured to provide as much seawater in the boxes holding the aballone as possible, using various methods:-

These were -

- a) placing the animals in a plastic bag filled with seawater.
- b) placing them in a plastic bag with only a small amount of seawater.
- c) placing them in a plastic bag with plastic foam soaked in seawater.
- d) placing them in a plastic bag filled with wet seaweed.
- e) Placing them in a plastic bag filled with spagnum moss saturated with seawater.

The result of these experiments after 48 hrs at 7.5°C were -

a) 91% mortality

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- b) 83% "
- c) 35%
- d) 11%
- d) 5%

4. WAS AN ANAEROBIC OR AEROBIC ENVIRONMENT BETTER? As the results of tests (3) were so conclusively in favour of (e) ie spagnum moss saturated with seawater, we confined our attention to this method.

The method of the experiment was simply to expell some air from the plastic bag using a vacuum pump to 5 inches hg and to inject oxygen in the case of the aerobic experiment. Again the experiment was repeated over a 48 hr period at  $7.5^{\circ}$ C.

The results were quite dramatic as the animals in the anaerobic environment showed an increase in mortality to 29% (the remainder were very sick) while those in the aerobic, oxygen enriched environment dropped to a mortality of 3%.

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5. WHAT EFFECT DID OXYGEN HAVE? In endeavouring to answer this question we found we were involved in question (6) also ie. does the animal produce  ${\rm CO}^2$ .

The experiment again involved the use of animals in plastic bags in which a partial vacuum had been created and others injected with oxygen A control sample using bags filled with air only was used. We used a Drager gas analyser to determine  $CO^2$  levels, and also a Ph meter to determine the Ph of the pedal sole, by placing the electrodes directing on the pedal sole. This method was used by James and Olley who demonstrated that the Ph of the animal is directly related to the physical condition and impending mortality.

The results of the experiment were to show that the animals in the anaerobic environment produced no discernable  $CO^2$  gas, those in the control (air) environment, a barely measurable discolouration of the Drager tube, while those in the Oxygen environment showed readings of up to 1.1% Vol % CO<sup>2</sup>.

Although it was mainly subjective judgement, the animals with the highest CO<sup>2</sup> readings appeared obviously the most active and vigorous.

Using the Ph meter we found by placing the electrodes across the pedal sole that the Ph varied as follows:

Dxygen environment Ph7.55 Air environment Ph7.25 Partial Vacuum Env. Ph6.95

These results coincided with subjective observations of healthiness of Abalone.

> The results of the experiment were:-Oxygen mortality Nil Air mortality 9% Partial vacuum Mort. 25%

Because of the obvious lessons to be drawn from the oxygen environment experiment we did not proceed with the tests of  ${\rm CO}^2$  tolerences in Abalone.

## CONCLUSION

Abalone should not be left out of the water for any appreciable length of time prior to shipping.

They should preferably be kept in a wet tank on the boat, failing this, hung in coffs over the side (with mesh small enough to exclude small fish) and left in the water until the last moment before leaving for port.

On landing they should be immediately selected and graded (rejecting obviously injured or weak ones) and returned to seawater either in tanks or natural sea conditions.

The water can be between  $10^{\circ}$ C and  $20^{\circ}$ C but obviously coffs in the sea itself from which the fish came are by far the better.

They should be left in water without food for 10 days. This allows them to recover from the shock of being taken from their home site. (We believe that shock plays a big part in mortality of abalone). This period also allows the gut to be cleaned out and prepares the animal for its journey without further selection.

They should then be packed into a poly-styrene foam box in a fully seawater saturated medium such as spagnum moss, using ice in plastic bags at the bottom (not touching the abalone). (We found about 2kg of ice to each 10kg of abalone sufficient.)

The box should then be sealed, and injected with oxygen to raise the partial pressure.

This should ensure the 100% safe arrival of abalone in live condition. However it did not, as trial shipments showed that abalone still had mortality of between 5% and 15%, which was unacceptable.

This set back directed us to a reappraisal of the effects of pressure on abalone, and the problems associated with gases, temperature and insulation materials, for which we set up further experiments.

Firstly enquiries were made of the airlines to find out the temperatures of their cargo holds, whether they were pressurised and to what pressures.

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We were informed that some holds were at cabin temperature and pressures, others were at ambient temperature. This means for an aircraft flying at 40,000 feet the ambient temperature can be  $-56^{\circ}$ C (using the International Civil Aviation Organisation's Standard Atmosphere) and that temperatures in the hold can be around  $-20^{\circ}$ C or lower pressurised to around 13,000 feet, or 10" hg vacuum.

The following experiments were set up.

1. What are the insulating properties of polystyrene boxes in an ambient temperature of  $-20^{\circ}$ C? We placed a box, packed in the usual way with abalone, ice etc in a  $-20^{\circ}$ C room, with a high-low thermometer. After 10 hrs, the temperature had fallen inside the box to  $-4^{\circ}$ C. As this had been demonstrated sufficient to kill the abalone, the obvious conclusion was to place the boxes in the cabin temperature section of the aircraft around  $\pm 20^{\circ}$ C. At these temperatures ambient the abalone box internal temperature remained between 7°C and  $10^{\circ}$ C.

2. What are the effects of atmospheric pressure on Abalone? An experiment was set up using a clear plastic dome with abalone weighing 250 grams placed inside. The animal could be observed at all times.

A vacuum pump was used to create pressure of 2/3 atm or 10" hg vacuum (ie. approx 13,000 ft in an aircraft).

The abalone was noticed to at once lift its shell up on its pedal sole about 1 cm. Liquid was exuded rapidly, which amounted to 15 mls in 10 minutes.

This equalled the bleeding rate one would expect had the animal been shucked.

In 5 hrs it was judged to be dead.

This experiment was repeated several times with similar results  ${\rm CO}^2$ , Ph of liquid and pedal sole were measured, and the average result from 10 Abalone was as follows.

Condition	CO <sup>2</sup> of air	Ph Liquid	Ph Foot
Dead	0.02% vol	б.2	6.4

## CONCLUSION

Animals are adversely affected by negative pressure, and should be carried in holds which are temperature controlled and pressurised. Furthermore the boxes should be well sealed to maintain positive pressure, and the abalone placed inside a sealed poly bag for the same purpose.

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Concerning the effect of CO<sup>2</sup> and its build up on abalone, we did not continue our experiments as we achieved our aim of 100% of abalone arriving alive. However, some questions remain in this area.

For example - In man at 2% volume concentrations, CO<sup>2</sup> toxicity will be noticeable 5% will bring noticeable discomfort, while at 10% concentrations and above unconsciousness and death will result. CO<sup>2</sup> has the effect in man of dilating blood vessels, increasing blood pressure and heart rate.

All these clinical problems involving  ${\rm CO}^2$  could be very important to the health of abalone during transportation remembering that concentrations of 1.7% vol were recorded in experiments.

However this remains outside the scope of our experiments.

We conducted numerous experiments of simulated flights and many actual flights, returning the abalone to the water in our holding coffs in the sea at Mallacoota. We succeeded in establishing as a matter of course 100% survival rates after placing back in water for 7 days.

Nothing then remained but the marketing of the animals in Japan.

This proved more difficult that the air transport problem.

## ACCESS TO JAPANESE MARKET

To be able to properly penetrate the Japanese market, the supply must be regular, constant and assured.

In practice this means that the Australian supplier must have a system of holding and preparing live abalone for shipment capable of supplying (say) 3 tonne of abalone per week regularly despite weather or other conditions.

In our experiments, on two occasions storms completely carried way our buoy lines from which abalone was suspended and on another occasion a raft was lost.

It means also, that Japanese company must establish holding facilities to rescusitate and feed the abalone, restoring them to full health before placing on the market, as and in the quantities required by market forces. Uh)

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These holding facilities must take account of Australian conditions and the water must be temperature controlled, Ph controlled, salinity controlled, oxygen controlled. As Japanese seawater temperature varies from around  $1^{\circ}$  to  $2^{\circ}$ C in winter to  $29^{\circ}$ C in summer, this is no mean problem.

It means also that if Australian live abalone is to be marketed in the proper manner, it will come into very real competition with Japanese abalone, most of which is marketed alive. This could probably have considerable repercussions for their local industry.

Whatever the reasons, we have found it impossible to this date to find a Japanese Company prepared to invest in plant and equipment for holding abalone to our requirements, and market it in thebest possible way to obtain the maximum prices.

There are plenty of course, who will take our live abalone, but only to place it on the market next day, with no resuscitation and build up period, simply for "a quick yen".

Abalone so presented is not in good condition and consequently does not realise a good return. Some poor Australian abalone reaching Japan in indifferent condition, in fact depressed the market to levels returning no more than if the abalone had been processed either frozen or canned.

Our conclusion therefore is that until the marketing problem (which includes resuscitation etc.) is solved, it is pointless proceeding any further. It may well be that the Australian Fishing Industry should set up a company in Japan with the aim of importing, marketing and distributing Australian seafood products, as this is an area where most difficulties appear to occur with many of our seafoods.

## SUMMARY

Abalone can be successfully transported by air. They can be held out of water in a humid aerobic oxygen enriched environment for a period of 48 hrs at temperatures around 7.5<sup>0</sup>C and successfully resuscitated when placed back in a oroper seawater environment.

Before transporting, the abalone require 10 days packed in suitable containers in seawater without food to allow for shock to recede and to condition them for close packing encountered during transport.

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If the abalone are then returned to normal seawater conditions the success rate can be as high as 100% survival.

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The main problem to be overcome in penetrating the Japanese market, is to establish a proper regime in Japan for resuscitating and storing the abalone prior to marketing in an orderly manner. If this is not done the price differential between Live abalone and frozen and canned abalone is not likely to be sufficient to warrant the very considerable extra expense of preparing, packing and transporting the abalone, and to accept the undoubted risks of mortality due to accident, strikes and other shipping mishaps.