



Seafish Technology SR424

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## **Assessment of Stress and Mortality of the Prawn (*Nephrops norvegicus*) During Live Handling from Vessel to Market**

### **Summary:**

This report describes work undertaken as part of a series of studies assessing the factors that contribute towards the stress and mortality of crustacean shellfish during live transportation. The current research relates to the live trade sector of the creel fishing industry for *Nephrops* in North West Scotland.

Observational trips aboard fishing vessels and around live holding premises identified that the major sources of stress arise from aerial exposure, thermal and osmotic shock and that considerable physical damage results from abusive mishandling aboard the vessel and from aggressive behaviour between animals (claw damage).

Systems that successfully reduced stress and mortality aboard the vessel included vivier tanks whereby seawater is supplied by a deckhose and a racked spray cascade enclosure where seawater is supplied using a high pressure jet assembly. Both these designs maintain the animals in cool, wet and dark conditions similar to those experienced in their natural environment.

Mortality and stress at live holding premises was reduced by appropriate use of live holding tanks serviced with full salinity seawater which provided conditions allowing animals to regain condition and facilitated the selection of the strongest animals for airfreight to foreign markets.

The use of novel mist equipment to hold and transport animals significantly reduced stress and mortality compared to that experienced using the normal 'dry' method of

storage and transportation where animals are maintained in baskets and covered with sacking dampened with sea water. Use of this equipment would maintain intrinsic quality and provide for a higher quality product when transporting Nephrops between the vessel and storage facility.

Longer term storage experiments (72 hours duration) designed to assess the suitability of the equipment for a road transport system to the continent were not conducted; however, the mist equipment maintained animals in a satisfactory market condition for 36 hours. Longer term experiments are planned.

Of three packing mediums tested for air freight of live product to continental markets, sawdust dampened with seawater maintained animals in the best condition; far superior to the quality of animals maintained in either the ribbonwood packing medium or the newspaper and ice combination which is the current method employed.

This report provides a set of recommendations that, if implemented, should realise a marked reduction in stress and mortality compared to that resulting from current handling practices. This should increase financial returns from current operations and over the longer term would lead to a higher market profile and an improved reputation as a supplier of quality product.

## Table of Contents:

<b>1.</b>	<b>Introduction and Terms of Reference</b> .....	<b>1</b>
<b>2.</b>	<b>Aims</b> .....	<b>2</b>
<b>3.</b>	<b>Approach</b> .....	<b>2</b>
<b>4.</b>	<b>Current Practices</b> .....	<b>3</b>
<b>5.</b>	<b>Experiment Objectives</b> .....	<b>4</b>
	5.1 Introduction .....	4
	5.2 Objectives .....	4
<b>6.</b>	<b>Trials Summary</b> .....	<b>5</b>
	6.1 Experiment I .....	5
	6.2 Experiment II .....	5
	6.3 Experiment III .....	5
	6.4 Experiment IV .....	5
	6.5 Experiment V .....	6
<b>7.</b>	<b>Materials and Methods</b> .....	<b>7</b>
	7.1 Assessment of Stress and Condition .....	7
	7.2 Sample Size .....	7
<b>8.</b>	<b>Experiment Procedure</b> .....	<b>8</b>
	8.1 Experiment I .....	8
	8.1.1 Results .....	8
	8.2 Experiment II .....	10
	8.2.1 Results .....	10
	8.3 Experiment III .....	12
	8.3.1 Results .....	12
	8.4 Experiment IV .....	14
	8.4.1 Results .....	14
	8.5 Experiment V – Packing Regime.....	16
	8.5.1 Results .....	16
	8.5.2 Results .....	17
<b>9.</b>	<b>Discussion</b> .....	<b>19</b>
<b>10.</b>	<b>Conclusions</b> .....	<b>23</b>
<b>11.</b>	<b>Recommendations</b> .....	<b>24</b>
	11.1 On Vessel.....	24
	11.2 During Transport .....	24
	11.3 At Factory.....	24
<b>12.</b>	<b>Further Work</b> .....	<b>25</b>

### Appendices

Appendix I Methods

Appendix II Materials

### Figures

**Figure 1** Blood ammonia concentration and total percentage survival at different stages of Experiment I

**Figure 2** Blood ammonia concentration and total percentage survival at different stages of Experiment II

**Figure 3** Blood ammonia concentration and total percentage survival at different stages of Experiment III

**Figure 4** Blood ammonia concentration and total percentage survival at different stages of Experiment IV

<b>Figure 5</b>	Blood ammonia concentration and total percentage survival at different stages of Experiment V
<b>Figure 6</b>	Boat Vivier Tank
<b>Figure 7</b>	Boat 'Cascade' System
<b>Figure 8</b>	Mist Equipment (Static)
<b>Figure 9</b>	Side View of Misting Equipment and Operation in a Mobile Transport Unit
<b>Tables</b>	
<b>Table 1</b>	Describes the blood ammonia concentration, condition and percentage survival of animals after the recorded number of hours in each regime of Experiment I
<b>Table 2</b>	Describes the blood ammonia concentration, condition and percentage survival of animals after the recorded number of hours in each regime
<b>Table 3</b>	Describes the blood ammonia concentration, condition and percentage survival of animals after the recorded number of hours in each regime
<b>Table 4</b>	Describes the blood ammonia concentration, condition and percentage survival of animals after the recorded number of hours in each regime
<b>Table 5a</b>	Describes the blood ammonia concentration, condition and percentage survival of animals after the recorded number of hours in each regime
<b>Table 5b</b>	Describes the condition, temperatures and blood ammonia concentration before and after 24 hours duration in the various packing regimes in Experiment IV

## 1. Introduction and Terms of Reference

This report describes work done as part of a series of studies of the system of live transport for crustacean shellfish, particularly those sold on the continental market. To date those studies have investigated:

- Holding and transport conditions in land and lorry based tanks and ponds.
- Reception and conditions to the continent.
- The biological stresses induced on the shellfish by these conditions.
- The influence of bad handling and selection practices on mortality rates within the live system.

The information above is found in Seafish Report Nos. 259 (Reference 1) and 280 (Reference 2).

The background to the work conducted in this research relates to the live trade sector of the creel fishing industry for *Nephrops* in Northwest Scotland. Meetings with the industry identified considerable scope to expand the live export trade if:

- Excessive mortality could be reduced.
- Bad handling practices could be corrected.
- A cost effective method to transport *Nephrops* live to the continental markets could be designed.

Current handling practices compromise the condition and survival of animals destined for export and often a less than optimal product is transported to continental markets.

In physical terms this translates as mishandling of animals when caught, inappropriate storage onboard both vessel and truck and inadequate methods of packaging prior to air freight abroad. These practices result in high levels of stress and a loss of intrinsic quality such that the animals have to be airfreighted as a 'fresh' product with the minimum of delay so that they arrive at foreign markets as an acceptable product. All these factors serve to constrain the expansion of the market and represent a loss of profit and future opportunities.

A reduction in both stress and mortality would allow a better product to arrive at the market at more convenient timings. Using current methods of transport (airfreight) this would result in higher financial returns. It would also allow more cost effective methods of transportation to be considered.

## **2. Aims**

The two main aims of the research were:

- To investigate current handling conditions between the vessel and factory to identify bad handling practices and to recommend improvements which would reduce stress and mortality.
- To assess the viability of a mist system as a storage facility for live animals after capture and as a viable system to export live product by road to continental markets.

## **3. Approach**

The project was undertaken with the close cooperation of skippers, live holding and transport operators located on the West Coast of Scotland. The work was undertaken in two stages:

- Observation trips were made aboard fishing vessels and at live holding premises to assess current handling practices and to identify specific operations likely to contribute to stress and mortality. The findings are summarised in Section 4.
- Quantitative field experiments were conducted relevant to specific operations identified above. These are detailed in Section 6.

#### 4. Current Practices

The chain of handling from capture to export follows a general pattern. Animals are hauled aboard the vessel, emptied from the creel onto the sorting table and thrown distances of 2 to 3 metres into fish baskets where they remain on deck for up to 6 hours before being landed. Damp sacking is often placed over the animals to provide protection from wind, rain and sunshine. On landing, animals are transported in this 'dry' condition for periods of up to 12 hours before delivery at the factory where they can remain overnight under similar circumstances. At the factory the more robust animals are selected for low temperature treatment and airfreight to European markets.

Within these operations three main areas contributing to stress and mortality were identified:

- i. The extended periods of exposure to air and temperature shock at all stages along the handling chain.
- ii. The high incidence of shell and limb damage resulting from physical abuse aboard the vessel.
- iii. The evidence of puncture damage from claw activity that was increased by handling malpractices.

Of these three, aerial exposure and high temperatures, though less obvious, were thought to contribute significantly to stress and mortality.

Accordingly the major part of this report is concerned with the effects of aerial exposure on the levels of stress, condition and mortality of *Nephrops*. Although discussed within this report, detailed research findings and recommendations relating to Sections (ii) and (iii) are dealt with in greater detail in a report by R. F. Uglow (Reference 1, Appendix III).

## 5. Experimental Objectives

### 5.1 Introduction

In their natural environment crustaceans absorb oxygen and remove waste products from their blood across the gill membrane which operates most effectively when covered with seawater. When out of water the gills collapse and their transfer efficiency is greatly reduced. Under these conditions animals respire without oxygen and store the waste products in their blood. This condition causes extreme stress and if allowed to continue for extended periods will cause death by suffocation and poisoning of the blood.

Furthermore animals become stressed more rapidly at higher temperatures as their metabolic rate, which is determined by ambient temperature, is faster at elevated temperatures.

The objectives of the experiments within this report were to determine the extent to which aerial exposure contributed to stress and mortality. They are specified below.

### 5.2 Objectives

- To assess the background mortality of *Nephrops* held under 'idealised' conditions as controls.
- To determine levels of stress and mortality of *Nephrops* held in 'dry' conditions experienced aboard vessel and during transport.
- To determine levels of stress and mortality of *Nephrops* maintained in a boat vivier 'cascade' environment.
- To determine levels of stress and mortality of *Nephrops* maintained in a 'mist' environment.
- To determine the condition and mortality of *Nephrops* held in three different packing materials intended for use to airfreight animals to foreign markets.



## 6. Trials Summary

### 6.1 Experiment I

This trial was an attempt to assess background mortality from the boat to final destination. A sample of animals was subjected to the "best possible handling procedure" designed to minimise stress and mortality. The claws of animals were banded to prevent puncture damage and the animals placed into an onboard vivier tank (Fig. 6, Appendix 11) comprising a large tank with a deck hose. On despatch from the boat a mobile vivier was employed to transport animals to the live holding premises whereupon the animals were placed into a commercial live holding tank. After a period within the tank the animals were transported to Hull in the mobile vivier and installed within the aquarium for subsequent blood analysis and mortality assessment.

### 6.2 Experiment II

This trial was designed to identify stress and subsequent mortality of animals maintained in good condition aboard the vessel and subsequently subjected to 'dry' storage and 'dry' transport to the factory.

Accordingly, animals were maintained in a vivier tank aboard the vessel during the fishing trip but were exposed to 'dry' storage conditions at the quayside prior to collection and subsequent assessment in the static mist live holding unit.

### 6.3 Experiment III

This trial was designed to determine stress levels and mortality of animals maintained in a novel cascade regime aboard the vessel that was designed to maintain the intrinsic quality of the animals while at sea. The concept of the design was that the spray would provide a source of flowing water over the animals which would allow excretion of ammonia and uptake of oxygen. It would also provide evaporative cooling, prevent desiccation and maintain animals in a darkened environment.

Newly caught animals were placed into baskets that were located inside the cascade equipment (Fig. 7, Appendix II) for the duration of the fishing trip. On landing the animals were transported to the factory in a mobile mist regime and subsequently placed into the static mist live holding unit for stress and mortality assessment.

### 6.4 Experiment IV

This trial was designed to determine stress and mortality resulting from current handling practices and to assess the suitability of the static mist live holding unit (Fig. 8, Appendix II) for storage prior to export.

The design of the mist system contains two key elements - refrigeration and fine mist. Refrigeration reduces animal locomotion, body temperature and metabolic rate which as a consequence reduces oxygen demand and production of metabolic waste. The mist maintains a saturated humid environment which protects the animal from desiccation and maintains their membranes in a wetted functional condition.

Accordingly, animals were maintained in a 'dry' environment in baskets located on the deck but were covered with wetted sacks to provide a cool dark environment. On landing the animals were placed into a mobile mist regime and subsequently into the static mist live holding unit for stress and mortality assessment.

## **6.5 Experiment V**

This trial was designed to assess condition, mortality and blood ammonia of animals maintained in live holding tanks and subsequent packing regimes with the aim of increasing shelf life of animals packed 'dry' for air freight to Spain. Recently creel-caught prawns were placed into live holding tanks overnight to excrete waste products from the blood and to regain condition. The following day live animals were packed 'dry' using different materials to simulate different regimes for airfreight.

## **7. Materials and Methods**

The methods and materials used in the experiments are detailed in Appendices I and II respectively, but summaries are provided below:

### **7.1 Assessment of Stress and Condition**

Ammonia concentrations in the blood were analysed as they can be used as an index of stress levels that an animal is being subjected to.

Animal condition was assessed using a liveliness index which related to the position of the claws.

### **7.2 Sample Size**

The sample size for each assessment of blood ammonia was five animals. The value recorded in the results tables denotes the average value and its standard error.

## 8. Experimental Procedure

### 8.1 Experiment I

Fifty animals were selected during a fishing trip and their claws were restrained with rubber bands to prevent damage from pincer activity. A blood sample was taken when animals came aboard, after which they were placed into a vivier tank aboard the vessel. After 7 hours the prawns were landed, a blood sample taken and the animals placed into a mobile vivier. After 3 hours in the mobile vivier another blood sample was taken before the animals were placed into a commercial live holding tank. After 40 hours in the commercial tanks a blood sample was taken, after which the animals were placed into the mobile vivier for transport to Hull. At the end of this journey (9 hours) a blood sample was taken before the animals were placed into the University aquarium for long-term mortality assessment.

Results are shown in Table 1 and Fig. 1.

**Table I describes the blood ammonia concentration, condition and percentage survival of animals after the recorded number of hours in each regime of Experiment I.**

Stage	Time Cumulative (Hours)	Regime	Duration of Regime (Hours)	Blood Ammonia Concentration plus S. Error	Condition % Grade				Total % Survival A+B+C
					A	B	C	D	
1	0	Directly out of sea	0	576 ± 78	100	0	0	0	100
2	7	Vivier tank on vessel	7	618 ± 77	100	0	0	0	100
3	10	Mobile vivier tank	3	451 ± 82	100	0	0	0	100
4	50	Live holding tanks	40	577 ± 129	94	0	0	6	94
5	59	Mobile vivier tank	9	564 ± 164	91	0	0	9	91
6	83	University aquarium	24	601 ± 74	88	0	0	12	88

#### 8.1.1 Results

The results indicate that the blood ammonia concentration of animals at each stage of the experiment did not increase significantly over the blood ammonia concentration of animals directly out of the sea and that the concentrations are not significantly different from each other. The results indicate that if *Nephrops* are maintained in good quality seawater when undergoing different handling regimes the ammonia concentration in the blood does not differ significantly from that of animals sampled directly out of the sea.

The results also show that percentage total survival decreased from 100% to 88% and that all the surviving animals exhibited 'A' grade condition. Fig. 1 overleaf describes the data in graphical format.

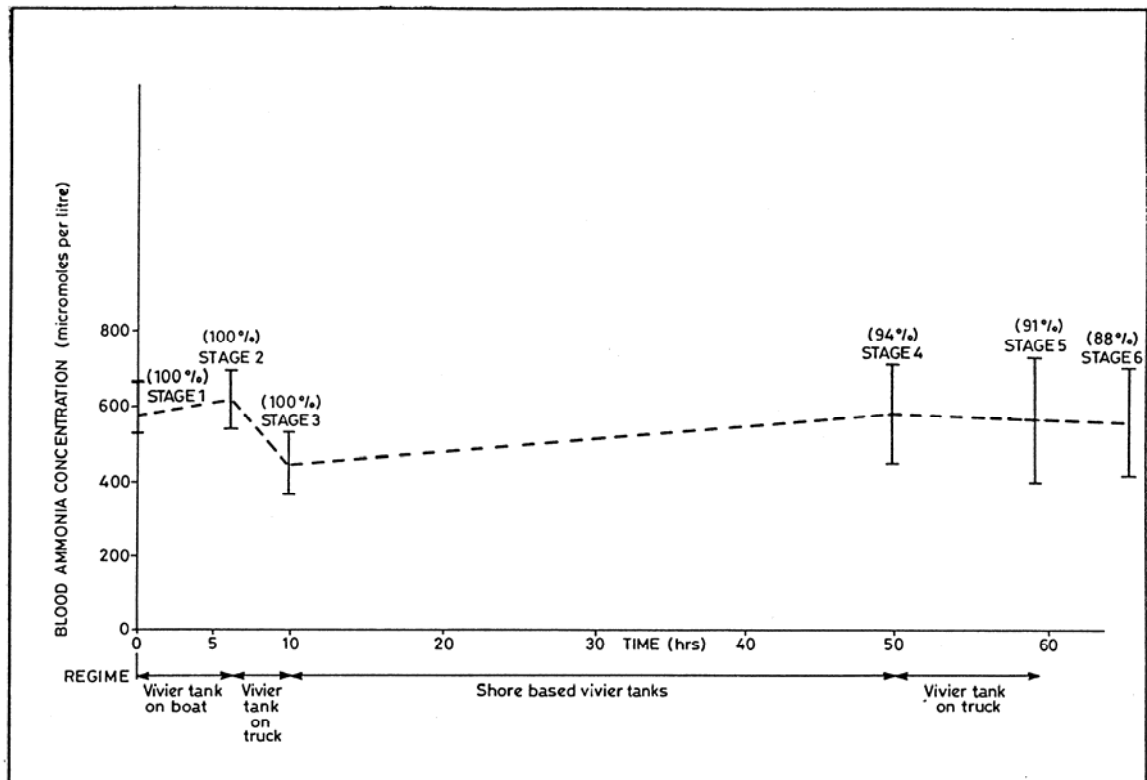


Fig. 1 - Blood ammonia concentration and total percentage survival at different stages of Experiment I

The graph illustrates that low blood ammonia concentrations were exhibited by animals at all stages of the experiment and that the total percentage survival decreased from 100% to 91 % after 60 hours immersion and subsequently to 88% after 83 hours immersion in different regimes.

## 8.2 Experiment II

Forty-three creel caught animals were stored (unbanded) in a vivier tank aboard the vessel for 2 hours. After landing they were subjected to 2 hours 'dry' storage conditions where they were exposed to wind and sun. They were then transported for 3 hours in baskets covered in damp sacking. The animals were then placed into the static live holding mist regime for 15 hours (5°C). Blood samples, condition and survival were assessed after each stage of the experiment.

**Table 2 describes the blood ammonia concentration condition and percentage survival of animals after the recorded number of hours in each regime.**

Stage	Time Cumulative (Hours)	Regime	Temp °C	Duration or Regime (Hours)	Blood Ammonia Concentration plus S. Error	Condition % Grade				Total % Survival A+B+C
						A	B	C	D	
1	0	Vivier tank	-	2	973 ± 62	100	0	0	0	100
2	2	'Dry' storage	18	2	6805± 892	99	0	0	1	99
3	5	'Dry' transport	12	3	5639 ± 817	87	12	0	1	99
4	22	Static mist	5	15	3862± 376	123	27	28	121	179

### 8.2.1 Results

The results indicate high levels of blood ammonia in animals in Stages 2, 3 and 4 and that these levels are not significantly different from each other. However, they are all significantly different from the blood ammonia concentration of animals in Stage 1 where animals are immersed in good quality seawater. The most dramatic increase is after 2 hours 'dry' storage which gave a reading of 6805 micromoles ammonia. It is interesting to note that this high ammonia concentration did not increase significantly over a further 3 hours exposure to 'dry' conditions during transport indicating that some equilibrium or upper limit had been reached. The blood ammonia concentration is significantly lower after 15 hours in the mist regime indicating that *Nephrops* exhibit some ability to remove ammonia from their blood when placed in this environment.

The results also indicate that the total percentage survival decreased from 100% to 79% and that the survivors were divided into 23% grade A, 27% grade B and 28% grade C.

The results have been presented in graphical format in Fig. 2 overleaf.

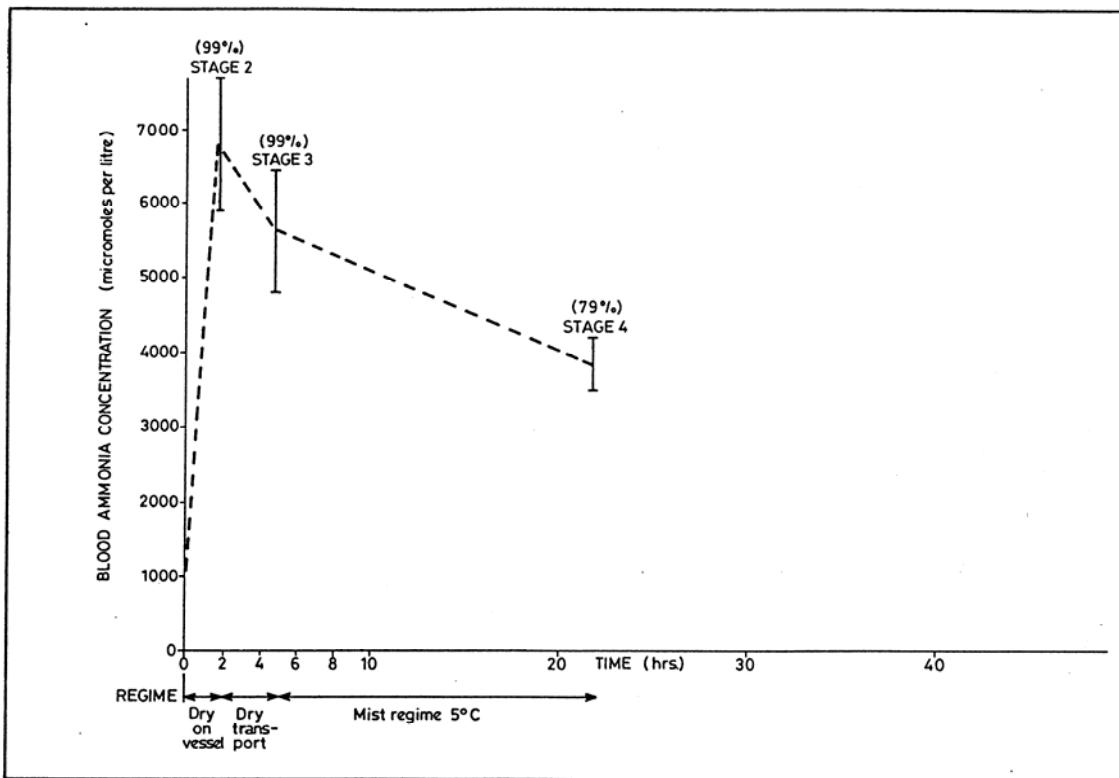


Fig. 2 - Blood ammonia concentration and total percentage survival at different stages of Experiment II

The graph illustrates the rapid rise in blood ammonia concentration after 2 hours 'dry' storage (Stage 2) and a similar high concentration after a further 3 hours 'dry' transport (Stage 3). The blood ammonia concentration exhibits a gradual and significant decline after 15 hours storage in the static mist regime. The figures in brackets denote the total percentage survival of animals. This was 99% after 5 hours in 'dry' storage conditions and 79% after a further 15 hours in the static mist regime.

### 8.3 Experiment III

Eighty-seven animals were placed into a novel spray cascade aboard the vessel (Fig. 7, Appendix 11) for 6 hours. Upon landing the animals were transferred to a mobile mist system at 5°C for 2 hours and then into the static mist regime at 5°C for 21 hours.

Blood samples were taken as prawns were removed from the sea, after 6 hours in the spray cascade, after 2 hours transportation in the mobile mist regime and after 21 hours in the static mist regime. A condition and survival assessment was conducted at each stage of the experiment.

The results are shown in Table 3 and Fig. 3.

**Table 3 describes the blood ammonia concentration, condition and mortality of animals after the recorded number of hours in each regime.**

Stage	Time Cumulative (Hours)	Regime	Temp °C	Duration of Regime (Hours)	Blood Ammonia Concentration plus S. Error	Condition % Grade				Total % Survival A+B+C
						A	B	C	D	
1	0	Out of sea	-	0	541 ± 60	100	0	0	0	100
2	6	Spray on boat	13	6	407 ± 51	100	0	0	0	100
3	8	Mobile mist	5	2	440 ± 79	98	0	0	2	98
4	29	Static mist	5	21	1583 ± 67	52	17	20	12	88

#### 8.3.1 Results

The results indicate that the low blood ammonia concentrations exhibited at Stages 2, 3 and 4, when animals are not immersed in water, are not significantly different from each other and are all similar to that of animals directly out of the sea (Stage 1). These results suggest that under certain conditions *Nephrops* are able to maintain low blood ammonia concentrations when out of water.

The results also show a 98% total survival after 8 hours exposure to the boat spray cascade and mobile mist regime and an 88% total survival after a further 21 hours in the mist regime. The results show that the surviving animals were 52% grade A, 17% grade B and 20% grade C.

The results are presented in graph format in Fig. 12 overleaf.



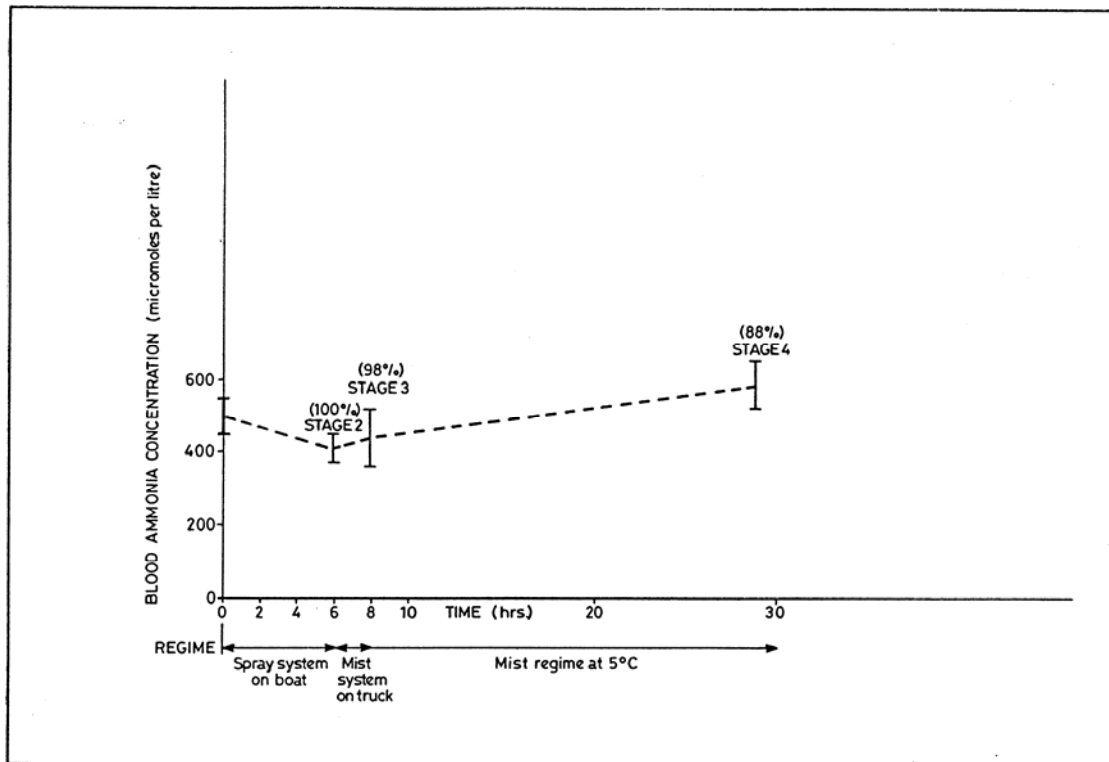


Fig. 3 - Blood ammonia concentration and total percentage survival at different stages of Experiment III

The graph illustrates that the blood ammonia concentration did not increase significantly over the duration of the experiment after 29 hours throughout the three different immersed handling regimes.

### 8.4 Experiment IV

Creel caught prawns were held 'dry' onboard the boat for 8 hours. The animals were covered with sacks wetted with seawater to provide a cool dark environment. At reception at the factory a blood sample and mortality count were taken. Eighty animals were then placed into the static mist regime at 5°C for a total duration of 41 hours. Blood samples and survival assessments were taken after 20 and 41 hours. Results are shown in Table 4 and Fig. 4.

**Table 4 describes the time, blood ammonia concentration and condition of animals after the recorded number of hours in each regime.**

Stage	Time Cumulative (Hours)	Regime	Temp °C	Duration of Regime (Hours)	Blood Ammonia Concentration plus S. Error	Condition % Grade				Total % Survival A+B+C
						A	B	C	D	
1	0	Out of sea	10	0	625 ± 52	100	0	0	0	100
2	8	Dry onboard	12	8	6206 ± 383	100	0	0	0	100
3	28	Static mist	5	20	7117 ± 1462	68	9	0	23	77
4	69	Static mist	5	41	6174 ± 190	3	2	4	91	9

#### 8.4.1 Results

The results indicate that the high levels of blood ammonia in animals in Stages 2, 3 and 4 are not significantly different from each other but are significantly different from the blood ammonia concentration of animals in Stage 1.

The results illustrate that high concentrations of ammonia were detected in the blood after 8 hours 'dry' storage aboard the vessel and that this high concentration did not reduce significantly after a subsequent 61 hours storage within the static mist regime.

The results also indicate the percentage survival was 68% grade A and 9% grade B after 20 hours in the mist regime and 3% grade A, 2% grade B and 4% grade C after 21 hours in the mist regime.

The results have been presented in graphical format overleaf in Fig. 14.

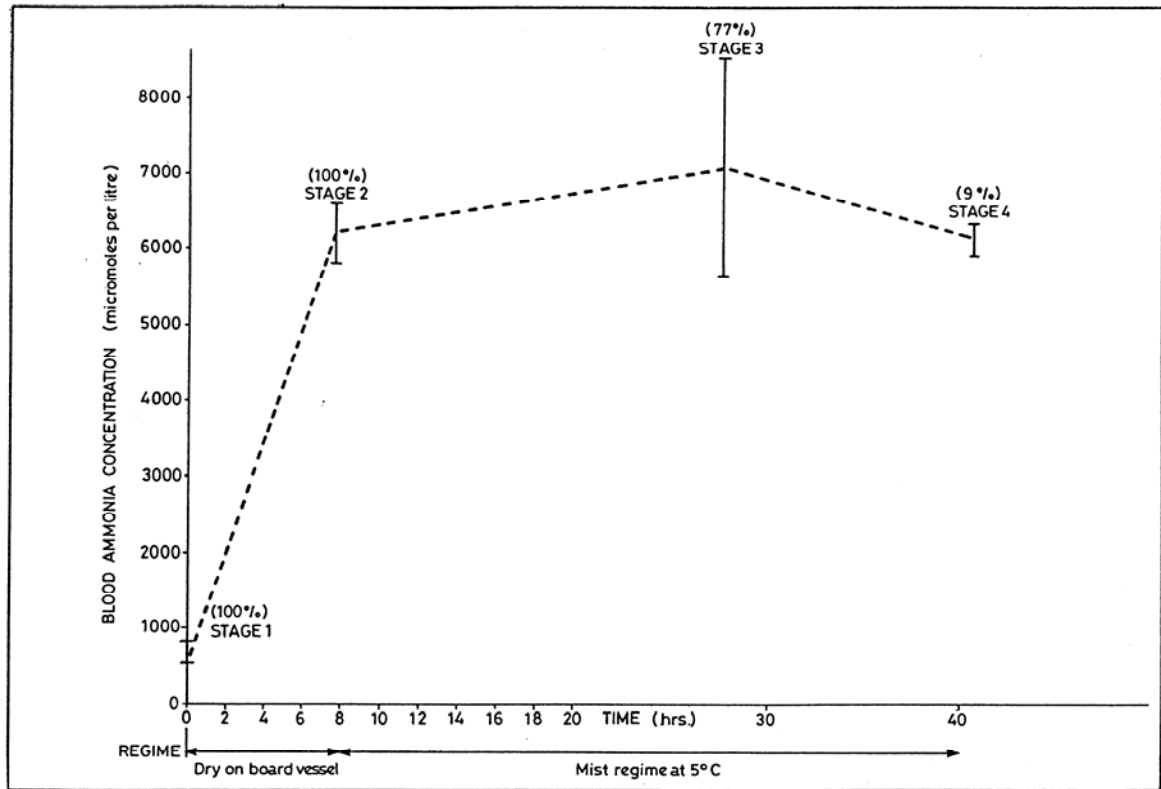


Fig. 4 - Blood ammonia concentration and total percentage survival at different stages of Experiment IV

The figure illustrates the rapid rise in blood ammonia for animals held dry aboard the vessel (Stage 2) and that this level did not reduce significantly while animals were maintained in the mist regime (Stages 3 and 4). The number in brackets indicates the total percentage survival at each stage of the experiment.

### 8.5 Experiment V - Packing Regime

Creel caught prawns which had been stored 'dry' onboard under wet sacking for 8 hours were placed into live holding tanks overnight to regain condition and remove waste products from the blood. After 20 hours in the live holding tanks at 3°C, 32 small and medium animals were packed into each of three different 'dry' transport regimes in polystyrene boxes. The experiment was conducted for 24 hours to simulate air freight times to the continent. Blood samples were analysed before and after the packing operation to assess stress levels. Condition and survival was assessed at the end of each experiment.

The experimental packing regimes were:

- Sawdust wetted with seawater at 10°C.
- Ribbon wood fibre wetted with seawater at 10°C.
- Newspaper wetted with seawater with freshwater ice on top at 2°C.

Results are described in Tables 5a, 5b and Fig. 5.

**Table 5a describes the blood ammonia concentration of animals after the recorded number of hours in each regime.**

Stage	Time Cumulative (Hours)	Regime	Duration of Regime (Hours)	Blood Ammonia Concentration plus S. Error
1	0	Directly out of water	0	523 ± 80
2	8	Dry on boat	8	1518 ± 558
3	9.5	Live holding tank	1.5	468 ± 59
4	29.5	Live holding tank	20	673 ± 66

#### 8.5.1 Results

The results illustrate high blood ammonia concentration after 8 hours 'dry' storage and a significantly reduced concentration after 1.5 hours immersion in the live holding tank containing good quality seawater.

The results also show that the increase in ammonia concentration from this low level was slightly significant after a further 20 hours storage in the live holding tanks.

**Table 5b describes the condition, temperatures and blood ammonia concentration before and after 24 hours duration in the various packing regimes in Experiment IV.**

	Packng Regime	Initial Blood Ammonia Concentrate plus S Error	Initial Sample Temp °C	Final Blood Ammonia Concentration plus S Error	Final Sample Temp °C	Condition % Grade				Total % Survival A+B+C
						A	B	C	D	
S T A G E 5	Sawdust	673 ± 66	3	2756± 313	9.5	13	44	31	12	88
	Ribbonwood	673 ± 66	3	*8709±495	10.2	0	0	0	100	0
	Newspaper	673 ± 66	3	2996± 915	1.9	0	6	13	81	19

\* Sampled from dead animals

### 8.5.2 Results

The results indicate that the final blood ammonia concentration of animals packed in sawdust and newspaper are not significantly different from each other but that they are both significantly different from the blood ammonia concentration of animals packed in ribbonwood.

The final condition assessment indicates that animals packed in sawdust exhibited significantly better condition and survival then animals packed in either of the other two materials, giving an 88% total percentage survival compared to a 19% total percentage survival for the newspaper and ice regime and a 0% survival for the ribbonwood packing regime.

The results from Tables 5a and 5b are represented in graphical format in Fig. 5 overleaf.

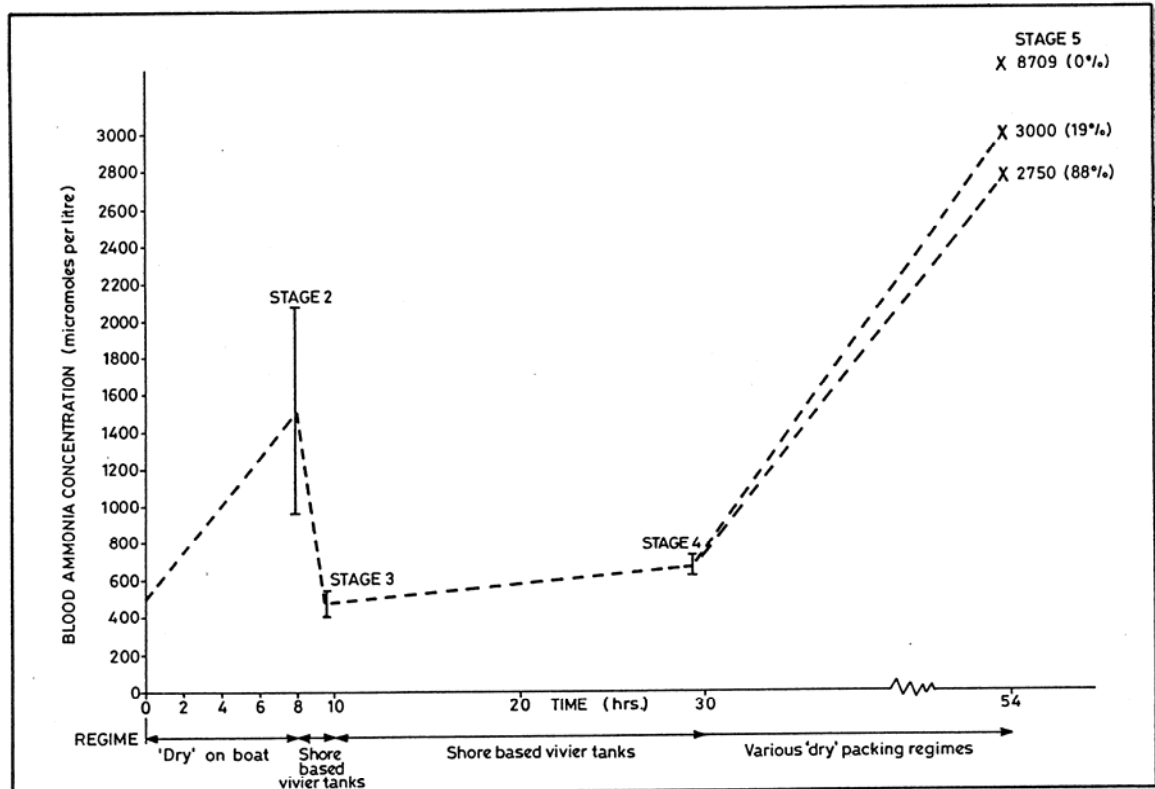


Fig. 5 - Blood ammonia concentration and total percentage survival at different stages of Experiment V

The graph shows the initial increase in blood ammonia concentration after dry storage aboard the vessel (Stage 2), the rapid reduction on immersion and storage in live holding tanks (Stages 3-4) and the subsequent increase under the different packing regimes (Stage 5). The final blood ammonia concentration of the ribbonwood regime (8709) should not be regarded as significant as the blood samples were taken from dead animals.

The percentage number in brackets (%) after the figures for blood ammonia concentrations indicate the total percentage survival (grades A+B+C) of animals in each packing regime at the end of the experiment.

## 9. Discussion

Table 1 describes the blood ammonia concentrations of prawns maintained under 'ideal' handling conditions. The objective of this experiment was to determine the stress and mortality of animals held in conditions most similar to those experienced in their natural environment. Thus for all the handling procedures, banded animals were maintained immersed in a regime of fully oxygenated seawater and darkness for the majority of the time. Under these conditions animals could respire effectively, absorbing oxygen and removing waste products across the gill surfaces.

The results indicate that despite undergoing five different handling regimes for a total period of 83 hours, the concentration of ammonia in the blood of sample animals did not differ significantly from that recorded in the blood of animals immediately out of the sea.

Survival was high (94%) after 40 hours in the live holding tank and although some mortality was experienced during transport to Hull the overall survival was 91 % for the 60 hour trip. A further 3% mortality was experienced over the subsequent 24 hours when animals were maintained in the University aquarium. Presumably this was the result of stress incurred during the trip which manifested itself as mortality later on. Thus, at the end of this experiment 88% of the animals were in a condition suitable for export to the European markets.

Although it must be assumed that the process of multi-handling and exposure to different environmental conditions (temperature and pressure change, exposure to daylight, desiccation etc.) would impart some stress upon the animals, the results suggest that if such animals are provided with oxygenated seawater and a darkened environment, subsequent stress and mortality levels are low. This suggests that *Nephrops* are physiologically robust and will survive if provided with conditions that allow normal physiological functions to occur.

It is possible to conclude that undamaged animals can withstand the stress associated with general boat and truck handling operations if damage from claws is prevented and the animals are maintained in suitable environmental conditions. It would have been useful to repeat the experiment with unbanded animals to determine the mortality element from 'claw damage'. Unfortunately this was not possible due to commercial and weather constraints.

The results from Experiment 11 and V provide insight into the high stress levels created by the current handling practice of 'dry' storage and transport. Table 2 illustrates that the ammonia concentration in the blood increases rapidly after two hours unprotected 'dry' storage in warm and windy conditions; whereas similar concentrations of blood ammonia were found in animals that had been stored for eight hours (Experiment IV, Table 4) under wet sacking which had provided some protection against drying out, precipitation and rain, and temperature shock. It is possible that the ammonia reached this concentration after only two hours and then did not increase further until the blood was sampled after 8 hours. However, other studies (Reference 4) indicate that under similar environmental conditions the concentration of ammonia in the blood increase with time.

The high ammonia levels measured in both these conditions demonstrate the extreme rapidity with which immersed animals can become stressed, especially when several stress factors act simultaneously. The inference from this is that aerial exposure should be kept to a minimum in all situations.

It is also evident from the results in Experiments II and N that high concentrations of ammonia in the blood reduce slowly with time for animals maintained in the mist regime compared to those held in a full immersion regime (Experiment I). This suggests that the animals are unable to remove ammonia from their blood at a rapid rate presumably due to the unsupported condition of the gills. In Experiment III, animals with a low initial blood ammonia were held in the mist regime for a period of 21 hours. The results show that the ammonia levels did not change significantly from the original value, indicating a low rate of ammonia production presumably due to the low holding temperature. Together these results indicate that animals maintained in the mist regime at low temperatures do not produce large amounts of ammonia but neither are they able to remove it from their blood rapidly.

It will also be seen from these experiments that when maintained under similar conditions in the mist regime animals with a low initial blood ammonia concentration exhibit better condition and survival compared to those with high initial levels. A logical assumption would be that significant mortality resulted from the high stress levels caused by the high levels of ammonia in the blood and the animals' inability to remove it.

Although similar levels of blood ammonia were recorded for both experimental regimes, survival was not as good in the mist regime compared to animals immersed in seawater. This suggests that although able to maintain survival, the mist regime is not as successful as full immersion - where animals can excrete ammonia and take in oxygen. However, since similar blood ammonia concentrations were recorded at the conclusion of both experiments there must be other causes of mortality not identifiable by analysis of blood ammonia. It would have been useful to conduct this experiment for a longer duration to determine mortality over 72 hours, but this was not possible due to commercial constraint. It is intended to send a consignment to Spain to assess mortality over a commercial trip of two to three days.

The results from Experiment III indicate that the blood ammonia concentration after 6 hours storage in the boat cascade was not significantly different from that of animals directly out of the sea and was similar to that of animals maintained in the boat vivier tank. The inference from this is that either the cascade regime imposes little stress on the animals during storage and that the animals do not produce high levels of ammonia, or that the high flow rate of water supports the gills adequately allowing animals to remove ammonia. It can also be seen that the survival of animals maintained in the boat cascade (and subsequent mist regime) was much higher than animals maintained in any other 'dry' regime. A further benefit of the spray cascade compared to the vivier tank was that without the support of water around the limbs the animals were less able to cause damage with their claws. It was noticed that animals in the vivier tank would have been able to cause extensive damage if their claws had been unbanded.



The aim of the final experiment was to determine the most suitable packing material to airfreight live animals to European markets. To achieve this it was necessary to use unstressed animals in prime condition. Accordingly, only animals which had been rested overnight in live holding tanks were used for experimentation.

The results from Table Sa illustrate that the high blood ammonia concentration of animals which had been stored in 'dry' conditions onboard the vessel for 8 hours, was reduced to that of animals directly out of the sea in only 11h hours when the animals were placed into good quality seawater.

This rapid reduction suggests the potential use of live holding tanks after 'dry' boat handling and prior to export. Immersion for short periods of time would allow animals to remove ammonia and regain condition whereas longer periods of storage would allow selection of the strongest animals for export and provide a buffer against sporadic supplies of stock.

The results of blood ammonia concentrations and mortality for different packing regimes are shown in Table Sb. There was considerable difference in both the blood ammonia concentrations and condition of animals maintained in the three regimes. Wet sawdust appeared to effect least stress and least mortality compared to the ribbonwood or newspaper/ice combination. When examined at the end of the experiment 13% of animals in the sawdust regime flicked their tails (escape reaction) indicating excellent condition. This material is probably the most suitable because it was able to pack intimately around each animal conferring cushioning, insulation and moisture to all exposed membranes and delicate organs. This would prevent desiccation of membranes and the cushioning would reduce physical damage. The main problem for a commercial operation would be the tendency for this material to adhere to the animals requiring considerable time and effort for its successful removal. Other materials, which provide similar conditions but are easier to remove, should be considered as viable alternatives.

The least successful packing medium was the ribbonwood material as demonstrated by very high concentrations of ammonia in the blood and complete mortality. Although these animals experienced a similar temperature profile to those maintained in the moist sawdust, it is possible that the ribbonwood material did not afford a similar degree of moisture retention or cushioning. Another possibility is that toxic substances used during the industrial preparation of the fibre leached out and caused mortality. Excessive importance should not be attached to the high blood reading as it relates to blood sampled from dead animals that could be the result of cell damage or decomposition.

The newspaper and ice combination, which is the current method of packing, resulted in blood quality and mortality levels midway between those found for the other two methods. Although the inclusion of ice maintained the animals at 2°C considerable mortality resulted. This could have been caused by desiccation, chemical leachate from the newspaper, blood loss or osmotic cell damage. Either hypothesis is feasible as a volume of malodorous grey brown liquor was present in the base of the polystyrene box when it was opened at the end of the experiment.

Thus the main points to be drawn from these experiments are, in order of priority:

- Keep aerial exposure to minimum. Maintain animals in a cool, dark and humid environment, immersed in good quality seawater if possible.
- Prevent physical damage.

From the work carried out there are several methods to minimise aerial exposure, stress, loss of condition and subsequent mortality.

The best option onboard the fishing boat is to use a vivier tank or the spray cascade arrangement. Both of these methods maintain low blood ammonia concentrations and afford protection from the main stress factors - rain, wind, sunshine and temperature shock. Greater survival can be expected if claws are restrained at time of capture. However banding all animals is impracticable in a commercial operation due to the labour element and the low value of the medium and small sized animals. As such, consideration should be given to banding the large high value specimens to ensure their best chance of survival and maximum price. Another option would be to devise a method that isolates whole animal from each other.

For transport between the boat and holding facility the most suitable option is a standard refrigerated truck customised to produce a mist regime at 5°C, which would maintain the low blood ammonia concentrations achieved on the vessel. If the cost implication of this is too high the minimum requirement is to cover animals with an absorbent layer dampened with seawater to retain moisture and transport them in containers that provide protection from the wind, rain and sun.

To improve animal condition and minimise mortality at the factory, the most suitable option is to use live holding tanks. This would allow storage of animals enabling them to excrete ammonia and to regain condition before export. However, successful storage within the tank would require the prevention of claw damage.

To ensure maximum survival of animals airfreighted to European markets the best option is to pack animals in wet sawdust or in a more suitable material that confers the same survival characteristics.

## 10. Conclusions

- Nephrops can be kept alive for 73 hours in good quality seawater if maintained in a suitable condition (uncrowded and banded).
- Nephrops can remove blood ammonia rapidly when placed into good quality seawater.
- Levels of ammonia accumulate quickly in the blood (2 hours) when animals are exposed to aerial conditions (wind and sun).
- The concentration of ammonia in the blood of Nephrops held in the mist regime does not change significantly with time.
- Animals with low levels of ammonia in the blood exhibited less mortality and loss of condition than animals with high levels when held under the same conditions in the mist regime.
- For animals exhibiting similar concentrations of blood ammonia, those maintained in fully aerated seawater exhibited less mortality than those maintained within the mist regime.
- Wet sawdust was the most suitable packing medium tested for 'dry' air shipment.

## **11. Recommendations**

Keep aerial exposure to a minimum. Reduce physical damage to a minimum.

### **11.1 On Vessel**

Store animals in baskets within a vivier tank or in a spray cascade system. Ensure water is of full salinity by taking water well below the surface especially in times of heavy rainfall.

Do not mishandle animals - place them into baskets or other receptacles.

Restrain the claws of large valuable animals.

Consider other methods to isolate animals from each other to prevent claw damage.

### **11.2 During Transport**

Transport animals using a mobile mist unit. If this is not possible, maintain baskets of animals within a solid walled container and cover animals with an absorbent layer of material saturated with seawater.

### **11.3 At Factory**

Store animals in tanks of good quality seawater.

Pack animals in sawdust saturated with seawater (or other material that confers similar survival characteristics) for airfreight of animals to Europe.

## 12. Further Work

- Conduct banded and unbanded trials to assess mortality from claw damage.
- Assess mortality of animals maintained in the mist regime for 48 hours and 72 hours.
- Identify survival and condition profiles for other species maintained within the mist regime.
- Produce simple guidance notes for good storage and handling practices for live Nephrops.
- Consider other methods of disarming claws or preventing animals from damaging each other.

## **Appendices**

# **APPENDIX I**

## **Methods**

### **Condition Assessment**

At each stage of an experiment animal condition was assessed into one of four categories:

- A - Claws raised high
- B - Claws raised level
- C - Claws drooped
- D - Dead

This was designed to give an index of the range of animal condition that would be exhibited over the various trials. It allowed the overall suitability of each handling regime to be assessed. The numbers in each grade are recorded as a percentage of the total number of animals employed in the experiments.

The summation of categories of live animals (A, B and C) allowed quantification of the numbers of animals that would survive each experiment in a condition that would be at or better than the purchase specification of the European markets.

### **Blood Analysis**

Ammonia is a constituent of the blood that increases in concentration with increasing levels of stress. As such the ammonia concentration in the blood can be used as an index of the level of stress that an animal is being subjected to.

Blood was removed from either the first or second pair of walking legs via the thin membrane where the leg is joined to the thorax. The point of a glass tube was inserted into the joint and a small aliquot (5ml) of blood was removed.

The blood sample was then frozen to prevent deterioration before analysis. Ammonia concentrations were determined in terms of micromoles of ammonia per millimetre using laboratory equipment.

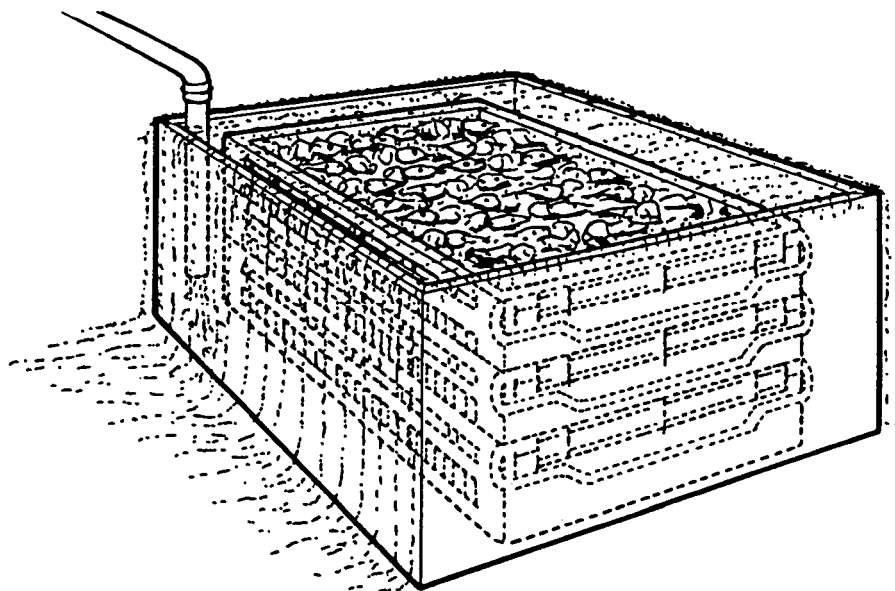


## **APPENDIX II**

### **Materials**

### **Mobile Vivier Tank**

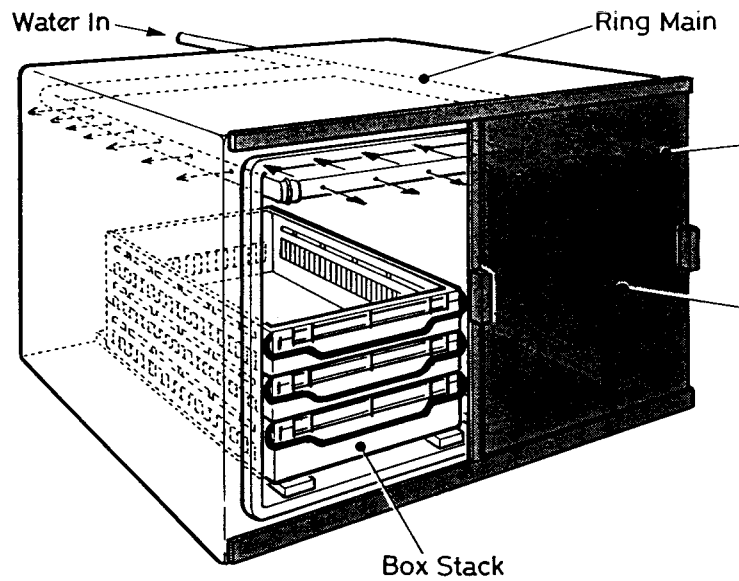
This comprised a 115 litre tank of approximate size 1.0m x 0.5m x 0.5m. Water was maintained using a 12v DC bilge pump of capacity 25 litres per minute. A small volume of biofilter material was incorporated into the design to process waste products from the animals. Temperature was maintained at approximately 10°C by use of picnic freezer packs. Adequate aeration was provided by the action of the pumped water cascading into the holding tank.



**Fig. 6 - Boat Vivier Tank**

### **Boat Vivier Tank**

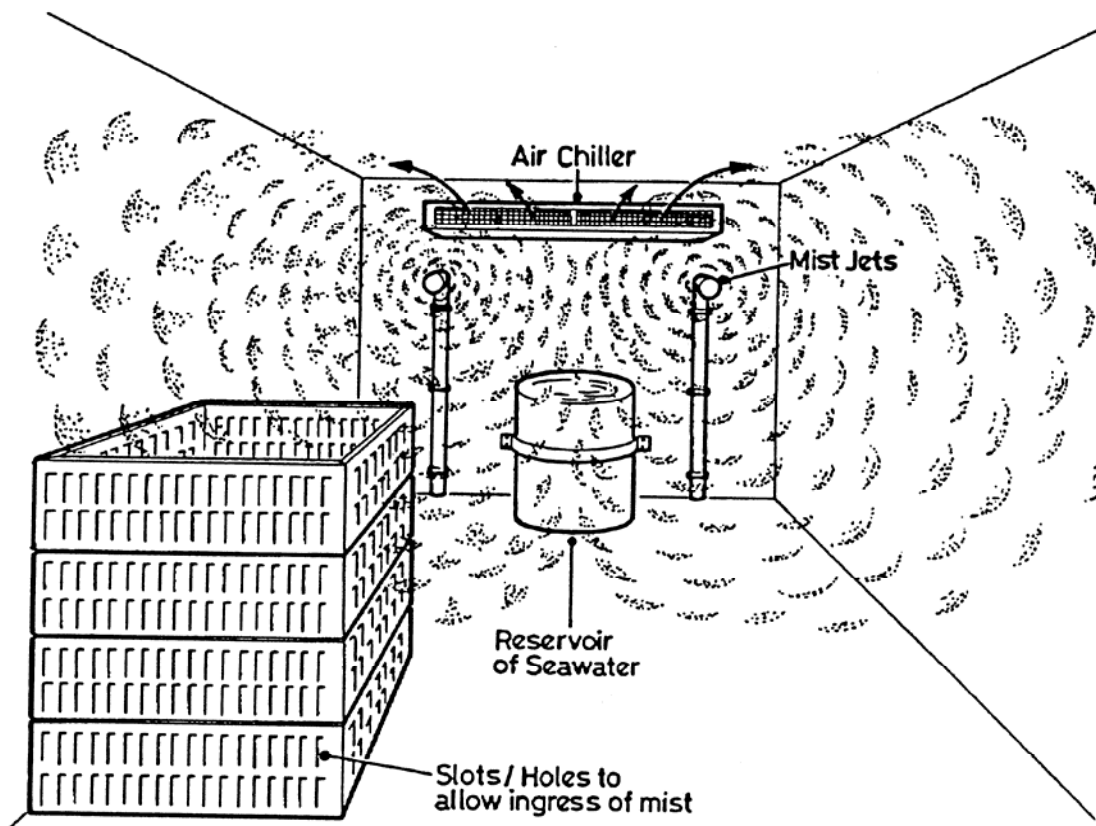
Fig. 6 describes the tank used for the onboard trials. It comprised a fibreglass tank and lid, approximately 1.3m x 1m x 1m and 1300 litres capacity. The trays used to contain the animals were the stack/nest variety with perforated sides and bases. Seawater was supplied at a rate of approximately 120 litres a minute from the boat's deckhose. Animals are placed within baskets which are then stacked on top of each other.



**Fig. 7 - Boat 'Cascade' System**

### **Boat 'Cascade' System**

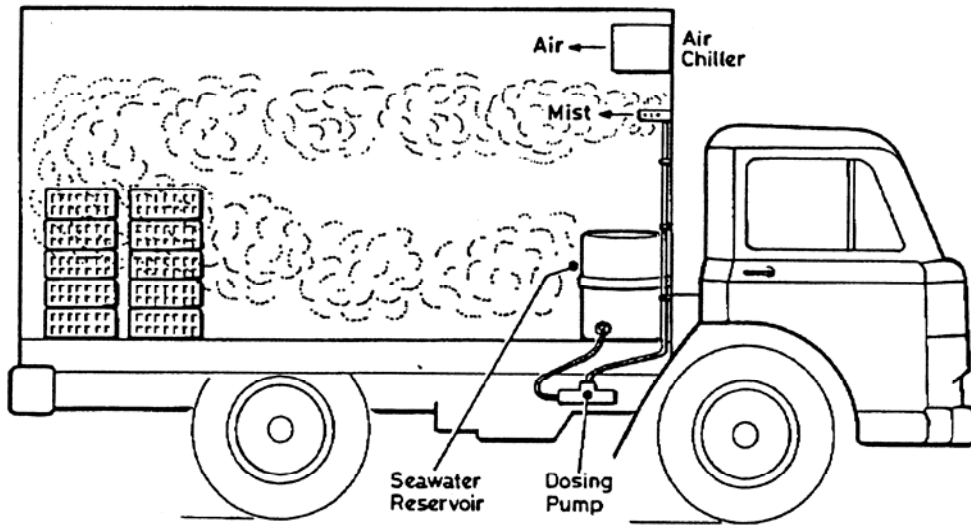
Fig. 7 describes the spray system used for holding animals onboard the vessel. The plywood container measured approximately 1.3m x 1m x 1m within which the trays and animals were held. Sliding doors allowed easy access to the trays and afforded protection from wind, rain and insolation. Mounted at the top of the container was an ABS plastic ring main the sides of which were perforated with small holes (2mm). These provided horizontal jets of water which, when they contacted the sides of the container broke up into a spray/mist combination which maintained the animals in a wet cool condition. The water was supplied by a 12v DC centrifugal pump with a flow rate of approximately 40 litres per minute.



**Fig. 8 - Mist Equipment (Static)**

**Mist Equipment (Static)**

Mist for these experiments was produced from stainless steel nozzles working at 150 psi producing droplets of 160 microns, each requiring a flow rate of approximately 0.16 litres per minute. Artificial seawater was used and the water was supplied to the nozzles by a 150 psi precision dosing pump which was able to supply low volumes of water at high pressure. The nozzles were located inside a refrigerated container box specified to maintain the temperature to plus or minus 1°C (Fig. 8).



**Fig. 9 - Side View of Misting Equipment and Operation in a Mobile Transport Unit**

## **Appendix III**

### **References**

1. Seafish Report No. 259. A Study of Mortality Rates of the Velvet Crab During Holding and Transport. (MacMullen, Uglow, Wyman).
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3. The Prawn (*Nephrops norvegicus*) as a Candidate for Vivier Transport - A Feasibility Study. (R. F. Uglow, 1990).