Methods of Transportation for Scallops (*Pecten maximus*)

Seafish Report SR 481

October 1996

Seafish Aquaculture

Marine Farming Unit, Ardtoe

Methods of Transportation for Scallops (*Pecten maximus* (L.))

A report prepared for Highlands and Islands Enterprise

Seafish Report No. 481

October 1996

Authors: D. Cashmore, J.T. MacMillan and M. Learmouth

Seafish Aquaculture

Marine Farming Unit, Ardtoe

Methods of Transportation for Scallops (*Pecten maximus* (L.))

A report for Highlands and Islands Enterprise

Summary

The relationship between various forms of transportation and physiological stress and subsequent mortality in scallop spat, overwintered and two year old scallops was investigated. Laboratory experiments assessed survival after immersed and emersed storage and data were related to the stresses of pH decline, depleted oxygen level and increased ammonia level during holding. The use of buffers and 'lowered' temperatures significantly enhanced survival, without any discernible detrimental side-effect, by maintaining pH levels and reducing the rate of oxygen depletion and ammonia accumulation in holding media. Field work, using commercial numbers of scallops, monitored the survival of scallops on the seabed and in suspended cultivation following different types of transportation. Future project objectives and recommendations are discussed.

List of Tables

List of Figures

Table of Contents

2.1 The Stress Responses of Scallops During Transportation ....

5. Discussion, recommendations and further work

Seafish Aquaculture

Marine Farming Unit, Ardtoe

Methods of Transportation for Scallops (*Pecten maximus* (L.))

A report prepared for Highlands and Islands Enterprise

Seafish Report No. SR 481

October 1996

Authors:
D. Cashmore
J.T. MacMillan
M. Learmouth

1. Introduction

Historically Great Scallop (*Pecten maximus*) cultivation has been exclusively conducted in locations along the West coast of Scotland. Although scallop populations are located and exploited throughout UK waters, to date only the west coast of Scotland provides the topography and hydrographic conditions necessary for the collection and initial cultivation stages of the juvenile scallops. Similarly, only specific areas along the coastline experience settlement in quantities that are commercially viable. As there are presently no hatcheries operating on a commercial scale in the UK, the scallop farming industry to date has been developed entirely from naturally produced scallop seed (spat). The growth cycle which allows sufficient time for the scallop to obtain optimum market size is presently 5 years. This now means that to economically produce scallops, seabed ranching is the only option left open to the industry, as a full growth cycle in suspended cultivation is prohibitive in terms of maintenance and equipment costs.

The concept and practice of seabed cultivation has extended the feasibility of potential site development to areas that were previously considered unworkable for suspended cultivation techniques. Conventional seabed cultivation usually involves the transfer of scallops from suspended cultivation once they have attained a size at which they are considered to be robust enough to endure the rigours associated with life on the seabed. Transfer of stock is carried out from subsurface ongrowing units which are located reasonably close to the residing site. The close proximity of these ongrowing units to the reseeding area means that aerial exposure of the scallops, with the associated trauma it causes, is kept to a minimum. Latterly, the development of sites remote from areas traditionally recognised as having an abundant and reliable supply of scallop spat has resulted in the transfer of scallop stock over substantial distances. This transportation of scallop spat/juveniles, has enjoyed only limited success, the overall condition of transported stock being totally dependent upon the vagaries of distance, temperature, method of transport, quantity and any number of factors that may or may not be within the control of the supplier or recipient of stock. The transportation of spat from collection to ongrowing areas has been almost entirely abandoned by the industry due to unacceptably high losses experienced during early transfer attempts. The failure of suppliers to guarantee a reliable provision of spat, has meant that stock transfer is at present restricted to older, more robust scallops, which while they offer growers better survival levels than spat, may still suffer higher mortalities than is commercially acceptable. Over-wintered scallops are presently more attractive to growers than spat since the mortalities suffered by the spat during the initial period of over-wintering (approximately 10-15%) are encountered before the stock are purchased. However there is still a lack of tangible data on which to base an effective transportation technology. The successful transportation of juvenile scallops is not strictly limited to the commercial demands of the wild collector sector; it would be an essential element in the supply of hatchery produced stock, should industry expansion justify it.

J.T. MacMillan

2. The Stresses of Transportation: a mini review

General Introduction

With the expansion of the *Pecten maximus* cultivation industry in the UK and increasing demand from the continental market for juvenile scallops, there is urgent need to develop methods for the reliable long haul transport of large numbers of scallops. Some understanding of the nature of the stresses from handling and transportation is required in order to define appropriate methods for scallop transportation. While the stresses upon scallops from handling and transportation are applicable to all age classes, the tolerance and susceptibility of different age groups to these stresses will vary, giving different recommendations for handling and transportation for each age class. The duration of transportation and scale of the operation is also an important consideration in the choice of transportation method. For relatively short periods, the addition of costly precautionary measures may not be justifiable. However, for longer periods of transportation and/or larger scale operations, expensive technology may be cost effective in terms of greater post-transport quality of stock.

A good example of this approach can be found in the established transportation technology for commercial species of crustacean (Whitely and Taylor, 1989). While green crab (*Carcinus maenas*), brown crab (*Cancer pagurus*) and lobster (*Homarus gammarus*) have all been shown to have some tolerance to the stresses of emersion during transportation (24 hours), the velvet swimming crab (*Necora puber*), spider crab (*Maja squinado*) and Dublin Bay prawn (*Nephrops norvegicus*) have reduced tolerance to emersion (4 hours). Consequently, immersed transportation is only recommended for the former group for lengthy periods of transportation. However, the latter group of species must always be transported in an immersed state.

2.1 The Stresses and Responses of Scallops During Transportation

The responses of *Pecten maximus* to handling and transportation stresses are complex. Reduced fitness and death is ultimately the result of respiratory dysfunction, brought about by reduced oxygen (O_2) availability. Concomitant stresses, caused by an accumulation of excretory waste products and bacteria, fluctuating temperatures, desiccation and handling, act to further reduce respiration efficiency. *Pecten maximus* can be greatly stressed during loading and transportation as a result of reduced oxygen availability.

2.1.1 Respiratory stress

The aerobic respiratory systems of scallops are poorly adapted to function during emersion. Loss of hydrostatic support results in the clumping of gill filaments, reducing the surface area for oxygen uptake and the impairment of respiratory gas exchange, and cardiac arrest.

Scallops can also experience stress from lack of oxygen during immersed transportation as a result of depleted oxygen levels in the water. Extensive work has been done on the responses of scallops to depleting oxygen levels in seawater. Aquatic animals may be categorised as either oxyregulators or oxyconformers (Bricelj and Shumway, 1991). An oxyregulator is able to efficiently maintain levels of oxygen uptake independently of declining oxygen saturation levels. An oxyconformer is unable to regulate its rate of oxygen uptake which consequently declines with declining levels of oxygen saturation. *Pecten maximus* is both an oxyregulator and an oxyconformer. Brand and Roberts (1973) reported that juvenile *P. maximus* maintained at 10°C could regulate their rate of oxygen uptake down to an oxygen saturation level of 48-56%. At levels below this critical point the rate of oxygen uptake declined with decreasing oxygen saturation. Seawater usually contains 80% saturated oxygen, which is well within the capabilities of *P. maximus*. However, as oxygen is depleted from a finite supply *P. maximus* loses control and becomes stressed.

The rate of oxygen uptake (VO_2) of scallops in water is affected by temperature, body size, oxygen saturation (PO_2), food concentration, reproductive state, activity level and physiological condition (See Bricelj and Shumway, 1991 for review). Relationships between body size and rate of oxygen uptake have been determined for several pectinid species, including *P. maximus*. The important implication forthcoming from these experiments is that while the rate of oxygen uptake increases with increasing scallop body size, the rate of oxygen uptake per unit scallop weight decreases (by a rate of approximately 0.7) with increasing body size. Consequently the amount of oxygen consumed for a defined wet weight of scallop spat is likely to be greater than that of an equivalent weight of adult scallop.

Pecten maximus can temporarily switch to anaerobic respiration during low oxygen availability. However, this method of respiration imposes a physiological stress and results in a massive degradation of carbohydrate (glycogen) stored in the adductor muscle (Gabbot, 1983; Hochachka and Somero, 1984; Duncan, 1993). There is evidence to suggest that *P. maximus* is not so well adapted to respire anaerobically when aerially exposed as when exposed to anoxic seawater. The normal responses of some bivalve species, such as *Mytilus edulis* to respiratory stress caused by sudden aerial exposure are rapid brachycardia (a reduction in heart rate), valve closure and a switch to anaerobic respiration. This response incurs an oxygen debt which manifests itself in a temporary elevated rate of respiration once conditions for aerobic respiration are resumed. Brand and Roberts (1973) reported that this was the typical response of juvenile *P. maximus* when exposed to anoxic seawater, resulting in a 33% reduction in heart beat rate after 150 minutes of exposure. However, aerially exposed scallops were observed to respond by violent adductions of the shell and tachycardia (an increase in heart rate), followed by valve gaping and a reduced rate of oxygen uptake below normal levels upon immersion. It has been suggested that the poor respiratory response of scallops during aerial exposure is caused by an uncontrolled elevated metabolic rate in response to the stresses associated with being out of water. Inability to close the valves when out of water further makes *P. maximus* more vulnerable to desiccation and low salinity stress, especially at high temperatures (Brand and Roberts, 1973; Duncan, 1993).

2.1.2. The accumulation of waste products

Another cause of stress for aquatic animals during transportation is the accumulation of respiratory waste products. Normally these are diluted in the seawater upon release and are rendered harmless to the scallop. However, during transport, CO_2 from aerobic respiration and organic acids from anaerobic respiration can accumulate in the mantle cavity, tissues and hemolymph (hypercapnia) of scallops (De Zwann, 1976). This produces a condition called acidosis, which is the alteration of pH below normal values. A drop in pH can affect enzymatic activity, permanently damage the gills and reduce the efficiency of oxygen uptake (Whitely and Taylor, 1992). Some bivalve species can internally buffer a potential acidosis by mobilising shell carbonates (Byrne *et al.*, 1991) which may also increase the affinity of respiratory pigments to O_2 (Taylor and Whitely, 1989; Duncan 1993). However, *P. maximus* is poorly adapted to effectively buffering a potential acidosis during transportation (Duncan 1993).

Ammonia, which is the excretory waste of many aquatic organisms, can also accumulate in the mantle water and tissues of *P. maximus* during transportation. Ammonia in solution exists as two compounds in equilibrium; ionized ammonium (NH_4^+) and unionised ammonia (NH_3). While the ionized form is relatively harmless to animals, the unionised form is extremely harmful. Unionized ammonia can irreversibly damage gills and reduce the efficiency of gaseous exchange (Duncan, 1993). The relative proportion of these two forms is determined by pH and temperature; the higher the pH or temperature the higher the concentration of unionized ammonia. The capacity of scallops to buffer ammonia concentrations to acceptable levels will be greatly affected by the stresses of transportation. Further to this, there is some evidence that the rate of ammonia excretion increases with aerial exposure and that this in turn can increase the rate of oxygen consumption above normal levels (De Vooys and De Zwann, 1978; Chen and Lin, 1995).

2.2. Measures for reducing transportation stresses

The types and effects of transportation stress upon scallops described above are common for many other commercial marine species. Hence much work has already been undertaken to establish measures which will reduce these transportation stresses.

2.2.1. Cooling

Cooling reduces the rate of metabolism, the rate at which energy is utilised, and hence the rate of oxygen utilisation and excretory waste production. In addition, chilling reduces the rate of bacterial growth and increases the oxygen saturation level in seawater. Duncan (1993) reported that a reduction in storage temperature from 20°C to 5°C caused a 62% increase in maximum survival time in market sized *P. maximus.*

2.2.2. Humidity

Maintaining high levels of moisture during transportation delays the onset of desiccation, and helps to keep aerobic respiratory systems functional by maintaining moisture on the gills. Duncan (1993) reported that a reduction in storage humidity from 95% to 75% resulted in a 27% reduction in the maximum survival time of market sized *P. maximus.*

2.2.3 Respiration relief

Tolerance to anoxia is thought to be related to the quantity of glycogen stored in the adductor muscle, glycogen being the main respiratory substrate during anaerobic respiration (Hochachka and Somero, 1984). The glycogen content of scallops varies throughout the year, reaching maximum levels in early winter. Manipulation of this seasonal variation in glycogen level has been suggested (Duncan, 1993). There is also some evidence suggesting that the pre-treatment of scallop spat in a glucose solution can boost energy stores and enhance chances of survival during transit (Basurco, 1988).

2.2.4 The control of bacteria

The use of antibiotics such as Streptomyocin Sulphate and Penicillin G has been shown to reduce the rate of bacterial accumulation and gill deterioration in scallops during transportation (Duncan, 1993).

2.2.5 Controlling pH

Buffers can offset fluctuations in pH. Although routinely implemented in the transportation systems of flat-fish fry, the use of buffers in transportation systems for scallops has not been investigated.

2.2.6 Preparation and handling

It is important that scallops are selected for quality before transport. Weak, damaged or diseased scallops are likely to die during transit and reduce the survival of others in the system. Loading and handling should be kept to the minimum and disconnections between different forms of transport should be avoided. The pooling of scallops for some time before transport will enable selection of healthy individuals, allow the gut and stomach contents to partially empty and allow the scallops to recover from the initial stress of being taken out of the water.

3. Methods and Materials

3.1. Measurement of pH, oxygen and ammonia

pH, oxygen concentration (mg. l^{-1}), and ammonia concentration (mg.$^{-1}$ l) were monitored *in situ* in holding media. pH was monitored using either a WTW pH probe (Burmarc UK) or a narrow range pH Test Kit (HH/01470-06 pH range 5.5-8.5, Camlab). Oxygen concentration (mg. l^{-1}) was measured by an 'Oxyguard' handy meter (Dryden Aquaculture Ltd.). Ammonia concentration (mg. l^{-1}) was measured by the ammonia-nitrogen (NH_3-N), salicylate method using either a HACH DR-2000 spectrophotometer (Camlab) or an 'Easy Test' ammonia test kit (Interpet). Measurement of pH, oxygen and ammonia in the mantle cavity fluid of scallops, using the equipment detailed above was found to be unreliable because of insufficient quantities available to obtain replicate samples and cloudiness of the mantle fluid caused by the deterioration of gills. Preliminary investigations had shown that ammonia concentration could not be measured in treatments which included buffer because the buffer interfered with the chemical reactions of the test.

3.2. Laboratory based storage experiments

Three preliminary storage experiments were conducted at the MFU using two-year-old scallops to develop protocols for future transportation work and to initially compare the merits of emersed and immersed transportation for juvenile stock. Methods of storage included emersed in polystyrene boxes and immersed in seawater filled containers. The potential use of buffers to maintain appropriate pH levels during transportation and thereby enhance the survival of scallops was also examined both in the immersed storage of scallops and as a pre-treatment for scallops stored in an emersed state. A fourth storage experiment was conducted using hatchery produced scallop spat. Animals were stored as for the experiments described above although the effects of storage temperature and scallop spat densities were also investigated.

3.2.1. Experiment One: Methods of storage for two year old scallops

80 two year old scallops, with a mean (n=20) shell height of 63 mm and a mean wet weight of 49g, were collected from the seabed in Ceann Traigh Bay (Ardtoe, Argyll) on the 26 April 1996. They were healthy and relatively free of fouling organisms (less than 30% cover). The stock were kept in a flow-through seawater tank for three days prior to the experiment (seawater temperature - 8 °C - 9° C; pH - 8.1; O_2 concentration - 9.1 mg. l^{-1}).

Emersed storage - The effect of pre-soaking in a buffer solution upon scallop survival

Four 10 l. plastic containers were filled with 5 litres of seawater (9 ^{0}C, 33 ppt.). The seawater in each of two containers was then either (1) treated by the addition of 10g of a commercially available biological buffer, 'Antitox' (Tris hydoxymethyl aminoethane hydrochloride and Tris hydroxymethyl aminoethane) (2g. l^{-1}), which was obtained from Dryden Aquaculture Ltd, or (2) left untreated (non-buffered seawater treatment, NBS).

Ten scallops were placed in each container and left for 30 min, which was assumed to be sufficient time for some exchange between the treated seawater and the mantle cavity fluid of the scallops. The containers were continuously aerated and there was no discernible change in either the pH (8.1 - 8.2) or temperature (9 ^{0}C) during this period.

Scallops from each container were then transferred to a lidded polystyrene box (internal capacity - 15 l). Each box had a false bottom for drainage and a hole in the lid, large enough for insertion of a thermometer (glass/mercury type, Commercial Grade, B.D.H.). The scallops were covered with a heavy cotton cloth, which had been previously soaked in the appropriate treatment, and left for 24 h. Air temperature was 10.5 to 12.6 °C during the experiment.

Immersed storage - The effect of storing in buffered solution upon scallop survival

Ten scallops were placed in each of four containers containing 5 li. of seawater (0.1 kg (wet weight). l^{-1}) which had been treated as described. Two additional containers of seawater, one for each treatment, were set up as controls. Seawater temperature, pH, oxygen concentration and ammonia concentration were monitored in each immersed treatment throughout the experiment (24 h) and were compared to data obtained in the controls. After 24 h experimental scallops were placed into mesh bags and transferred to the seawater flow-through tank. Survival of scallops was observed for a period of two weeks after the trial.

3.2.2. Experiment Two: Methods of storage for two year old scallops

Based on the results obtained from Experiment One, the experiment was repeated on 6 May 1996 following the same protocol and under the same conditions but with several amendments. Only one animal died in

Experiment One and no difference in survival was discernible. The duration of the experiment was therefore extended to 48 h to subject the scallops to stress closer to the extremes of their tolerance than those in Experiment One. Further, a Tris/HCl based buffer which has been used in the transportation of halibut fry (Duncan, 1993) was included as both an immersed and emersed treatment (4g of Trishydroxymethlyamine. l^{-1}, adjusted to a pH of 8.1 by addition of hydrochloric acid, both obtained from Sigma Chemicals). An additional amendment to the previous protocol was that scallops in emersed treatments were pre-soaked in the various treatments for 1 hr rather than 30 min. to ensure greater exchange of treatment water with the mantle fluid of the scallops.

3.2.3. Experiment Three: Further investigations into the effects of using buffers in the emersed storage of two year old scallops

Data obtained from the previous two experiments suggested that buffers used as a pre-soak treatment before emersed transportation could significantly enhance the survival of scallops. An experiment was therefore conducted on 3 June 1996 which specifically investigated the potential of buffers as a pre-treatment for the emersed transportation of scallops. The number of scallops used in this experiment was increased to twenty animals in per replicate. Only Antitox was used as a buffer treatment since this was the most economical.

The pre-treatments were (1) soaked in Antitox (2g. l^{-1}) for 1 h (2) soaked in seawater for 1 h (3) soaked in Antitox (2g. l^{-1}) for 20 min (4) soaked in seawater for 20 min (5) soaked in non-buffered seawater. There were two replicates for each treatment. Ten animals were removed from each box after 24 hours and the remaining ten were removed after 48 hours. Survival was observed over a two week period. The air temperature during the experiment is shown in Table 1.

Table 1. Temperature (°C) during Experiment three.

Hours	Air	Seawater
0	14.5	10
12	16	10
24	13	10
36	15	10
48	13.5	10.5

3.2.4. Experiment Four - Transportation methods for scallop spat

A preliminary investigation into the transportation methods for scallop spat was conducted, including the effects of buffers, air storage temperature and spat densities.

Five thousand scallop spat (with a mean shell height of 12 mm and a mean wet weight of 14 mg) were transported at a density of 60 spat l^{-1} from CEFAS (Conwy) to the MFU Ardtoe (9 h) in four seawater filled polyethylene bags (0.5 m x 0.8 m) on the 25th of July, 1996. Ice packs wrapped in rags were placed in between the bags to cool the seawater which was initially at 19 °C. Upon arrival the spat were transferred to a flow-through seawater tank (15 °C). The temperature drop experienced by the spat during their transferral from the bags to the tank had no discernible detrimental effect upon the spat. The spat were put into pearl nets (50. level.l^{-1}) the following day and suspended on a longline in Loch Ceann Traigh.

Spat were retrieved on the 13 August 1996 and kept overnight in a flow-through seawater tank (15 °C). The spat had grown by 2mm in shell length and mortality upon retrieval was less than 1%. The experiment was conducted the following day.

Emersed storage of scallop spat - Fifty spat were placed in each of two polystyrene boxes and covered with tissue paper wetted with seawater. One box was placed in a controlled temperature room (10 °C) and the other placed in an area where the temperature fluctuated with ambient air temperature (15.1 - 17.5 °C). Twenty five spat were removed from each box after 24 h and the remainder were removed after 48 h. The spat were placed in pearl nets and suspended in a flow-through seawater tank (15.0 °C) for two weeks to observe survival.

Immersed storage of scallops - Scallop spat were packaged at either 50. l^{-1} or 100. l^{-1} in 3 l of seawater (15° C) in 390 x 270 mm polyethylene bags. Bags were then placed in polystyrene boxes (internal capacity - 15 litres). The treatments were Antitox (2g. l^{-1}) and non-buffered seawater which were maintained in either a controlled temperature room (10 °C) or at ambient temperature (15.0 - 17.5 °C), with two replicates of each density per treatment. Spat at 50. l^{-1} were stored for either 24 or 48 h but spat at 100. l^{-1} were stored for only 24 h.

Table 2. Temperature (°C) of seawater in immersed treatments during experiment four.

Time of Experiment (hrs)	0	2	6	24	48
Immersed treatments in the controlled temperature room (10 °C)	14.7	13.5	12.1	9.8	9.1
Immersed treatments maintained at ambient temperature	14.7	15.1	15.3	15.8	16.1

3.3. Field studies

Data from the storage experiments described above suggested that the effects of different types of transportation varied for different age classes of scallops. However, data for maximal duration times and densities were not considered to be applicable to commercial practice since the transportation of large numbers of scallops would invoke greater stress reflecting greater disturbance in handling, sorting, packing and unloading, together with vehicle noise and vibration during transport. The next section of the project therefore involved several case studies which focused upon the monitoring of small scale commercial transportation runs of one and two-year old scallops between various sites in the UK. A comparison between the transportation of two year old scallops by vivier lorry and in an emersed state was conducted. Post-transportation survival was assessed in both lantern nets and on the seabed, where scallops were also vulnerable to predation. Because of uncertainty regarding legislation covering the use of buffers in the transportation of animals destined for human consumption, buffers were not used in this case.

Transportation of one and two year old stock in polystyrene boxes for durations of less than 6 h.

Five consignments of 5000 two year old scallops were transported from Skye to Ardtoe on the 9, 11, 18, 26 July and 7 August, 1996. Scallops were packed directly into lidded polystyrene boxes of 15 li. internal capacity at an approximate density of 600 per box. The boxes were taped and transported on an open truck to Ardtoe (4 h). Upon arrival, scallops were hosed down with ambient seawater to remove accumulated waste products before being placed in a flow-through seawater tank for one day. The scallops were than placed into lantern nets at 30 per level and transferred to a longline, either in the North Channel or Ceann Traigh Bay (Ardtoe). Survival was monitored for 2 months.

A second series of trials involved the transportation of 5000 two year old and 30,000 one-year-old scallops in polystyrene boxes from Skye to Loch Ewe (6 h). The two-year-old scallops were transported in 8 boxes (600 per box), one year old stock were transported in 12 boxes (2,500 per box).

Transportation of one and two year old stock for durations of greater than 6h.

13,000 one-year-old and 10,000 two year old scallops were transported from Raasay Sound to Hugh Wiltshire of Quest Holdings in Portland (16 h. journey time) on the 4 September 1996. The mean shell heights of the two- and one-year-old stock were 51 mm and 33 mm respectively and the mean wet weights were 18g and 4 g. Scallops were loaded on to a 32 tonne vivier lorry (Tarbert Shellfish) at Broadford Pier, Skye, between 11.00 am and 3.30 pm.

All of the one-year-old stock and approximately 9,000 of the two year old stock were packed in Northwest Plastic Trays (650. tray^{-1} and 250. tray^{1} respectively), which were roped in stacks of ten and immersed in the tanks (1.3 m^3) of the vivier lorry. The stacks took up less than a third of the vivier lorry, the unused tanks being used as controls. The air temperature in the vivier lorry was maintained at 10 °C and the seawater in the tanks was continuously aerated. The temperature of the seawater in Skye and in Portland and in the tanks are shown in Table 3. The pH, ammonia concentration and temperature in the tanks were monitored by the vivier driver during loading, transportation and unloading of scallops.

For comparison, six hundred two year old scallops were transported in three half-sized polystyrene boxes (200/box) on the floor of the vivier lorry. The vivier set off at 3.30pm, arriving in Portland at 4.00 am the following morning. The scallops were unloaded at 11.00 am and placed in lantern nets, which were suspended from the pier for 6 h. All one-year-old stock remained in these nets. Two year old stock were transferred to the seabed the following day except for some which were used to assess mortality in lanterns.

Table 3. Air and seawater temperature (°C) recorded during vivier transportation of scallops

Location	Time (hrs)	Air	Seawater
Skye	0	20	12.2
Vivier lorry	0 4 8 16	15.6 12 9.5 9.8	13.1 13.6 12.1 11.2
Portland	20	23	17.5

Long-line suspended cultivation

Two labelled lantern nets (14 levels) were used to hold scallops of both age classes for convenient assessment of mortality due to transport stress. There were three replicates (50. level^{-1}) for each treatment, namely one-year-old and two year old scallops transported by the vivier and two year old scallops transported in polystyrene boxes. Survival was assessed after one day and twenty days.

Seabed cultivation

The survival of two year old scallops on the seabed was also monitored. Six alkathene hoops (0.75m in diameter) were anchored in a line on the seabed at Portland. Fifty scallops were seeded into each plot with three plots being seeded with scallops that had been transported by vivier and the other three being seeded with scallops that had been transported in polystyrene boxes. Survival was assessed the following day.

4. Results

4.1 Laboratory based storage experiments

4.1.1. Experiment One: immersed and emersed storage of two year old scallops

Figure 1a shows *in situ* pH recorded in non-buffered seawater (NBS), Antitox treatments and in the controls during the 24 h of the experiment. The initial pH in both the NBS treatment and the seawater control was 8.1. Addition of buffer increased the pH of the seawater to 8.2. While the pH in both the control and Antitox treatment remained close to intial values during the experiment, the pH in the NBS treatment declined from 8.1 to 7.35 over the first eight hours, remaining relatively constant thereafter for the remainder of the experiment.

Data for oxygen concentration (mg. l^{-1}) in immersed treatments and controls are presented in Figure 1b. While the oxygen concentration in both controls remained constant throughout the experiment, scallops (0.1 kg. l^{-1}) in both NBS and Antitox treatments depleted the oxygen from a concentration of 8 mg. l^{-1} down to less than 2 mg. l^{-1} within the first eight hours. Likewise, the ammonia concentration in the NBS treatment increased from 0.01 to over 3.0 mg l^{-1} during the first 12 h of the experiment (see Figure 1c). Thereafter, the rate of ammonia accumulation was less rapid, increasing from 3.0 mg l^{-1} to 4.0 mg. l^{-1} during the remaining 12 h of the experiment.

Mortality of scallops in emersed and immersed treatments

At the end of the experiment (24 h), the scallops in the emersed treatments were gaping and their mantle margins were fully retracted. Scallops were observed for a further two weeks to determine whether they would recover from the stress of the experiment. For the first three days the scallops were still gaping but the mantle edges were starting to extend to the edges of the shell. All the scallops survived the experiment except for one scallop from the NBS treatment which died after one day. Scallops in the immersed treatments showed no signs of stress and all survived.

4.1.2. Experiment Two: immersed and emersed storage of two year old scallops.

Measurement of *in situ* pH, oxygen and ammonia

Data for pH in the NBS treatment in Experiment One suggested that the rate of change in pH occurred rapidly within the first 8-12 h but remained relatively constant thereafter. To determine whether further declines in pH would occur the immersed experiment was repeated again, scallops being left in immersed treatments for 120 h instead of 24 h. Again, the results were similar to those obtained in Experiment One. The pH in the NBS treatment declined rapidly down to 7.4, remaining at this value for the remainder of the experiment (see Figure 2a). The pH in both the immersed buffer treatments and in the controls remained constant throughout.

Oxygen in immersed treatments also showed a similar pattern of decline to those observed in Experiment One; declining rapidly at first but then at a decreasing rate (see Figure 2b). As before, ammonia initially rapidly accumulated in the NBS treatment, increasing from negligable concentrations to 4 mg. l^{-1} during the first 24 h (see Figure 2c).

Mortality of scallops in emersed and immersed treatments

All scallops in immersed treatments (120 h) survived the experiment. However, high mortalities of scallops in the emersed treatments (48 h) were observed in the two weeks following the experiment. Figure 2d presents the mean percentage mortality of scallops in the various emersed treatments. One-way analysis of variance and Tukey's test on arc-sine transformed percentage data showed that the mean percentage mortality of scallops in the emersed seawater treatment (100 %) was significantly greater than those for the emersed buffer treatments (< 30 %) (see Figure 2d legend).

4.1.3. Experiment Three: emersed storage of two year old scallops.

Mortality of scallops in emersed treatments

The mean percentage mortality of scallops in the various emersed treatments (24 or 48 h exposure) during the 2 weeks after the experiment are shown in Figures 3a and 3b. In contrast to results obtained in Experiment Two, the soaking of scallops in buffer before emersion did not significantly increase the survival of the scallops.

4.1.4. Experiment Four: immersed and emersed storage of scallop spat

Figure 4a. and 4b. show data for pH in the various immersed treatments during the experiment (48 h). As before, the pH in the control and buffered treatment remained close to initial values, although there was a slight decline in pH during the latter part of the experiment, especially in those which were maintained at the higher temperature. The pH in the NBS treatment rapidly declined to 7.5 during the first 24 h but remained constant thereafter. The rate of decline in pH was especially marked in those treatments which contained higher densities of spat and which were maintained at the higher temperature.

Dissolved oxygen rapidly declined in all immersed treatments, although differences in the rate of decline were observed with respect to the density of spat in the treatments and to storage temperature (see Figures 4c. and 4d.). The oxygen concentration in treatments containing 100 spat. l^{-1} declined more rapidly than those containing 50 spat. l^{-1}. However, while the oxygen concentration declined continually in treatments maintained at the higher temperature, the rate of decline of oxygen in treatments maintained at the lower temperature decreased during the latter half of the experiment. Similarly, ammonia accumulated more rapidly in treatments maintained at the higher temperature than in those maintained at the lower temperature (see Figures 4e. and 4f.).

The drop in pH and oxygen and the accumulation of ammonia recorded in the immersed treatments did not appreciably stress the spat at the densities they were packaged because the mortality of scallops after two weeks did not exceed 4% (see Figures 4g. and 4h.). Nevertheless, observed patterns of mortality could be related to experimental treatment. As expected, greater mortalities were observed at higher spat densities and longer duration's of storage. Perhaps the most important differences were those between NBS and buffer treatments, especially at the higher temperature. Table 4 shows the results of three-way analysis of variance on arc-sine transformed percentage mortality data (factors were spat density, the inclusion of a buffer and storage room temperature). As observed from Figures 4g. and 4h., significant differences were shown among spat density treatments and among buffered and unbuffered treatments. Temperature was shown to significantly interact with spat density and the use of a buffer in affecting scallop spat mortality.

Figure 4i. presents the mean percentage mortality of scallops in emersed treatments following 24 and 48 h exposure at the two experimental temperatures. The mortality in emersed treatments greatly exceeded those for immersed treatments. Patterns were also observed with respect to temperature and storage duration. For example, while spat stored for 48 h at the higher temperature all died, only 65 % mortality was recored for spat stored at the lower temperature.

4.2. Field Studies

Experiment Five: Vivier transportation of one and two year old scallops

The mean pH and ammonia level in the tanks of the vivier lorry during the trip from Skye to Portland are presented in Figures 5a. and 5b.. Unlike results from laboratory experiments the pH only began to decline after the first four hours had elaped, decreasing to a pH of 7.5 over the following 8 hours. Ammonia accumulated almost linearly over time, increasing from negligible quantities to 2 mg. l^{-1} over 16 h.

Figure 5c. shows the mean percentage mortality of two year old scallops in lantern nets after one and twenty days following transportation for 16 h in either an immersed state in the vivier tanks or in an emersed state in polystyrene boxes. One-way analysis of variance on data collected after twenty days showed that the mean percentage mortality of scallops transported in an emersed state was significantly greater than that for scallops transported in an immersed state (25 % and < 5 % respectively). Comparisons of mortality between one-year-old stock and two year old stock following transportation in the vivier are shown in Figure 5d. One-way analysis of variance did not show any significant differences between age classes. Data for mortality of two year old stock on the seabed following both immersed and emersed transportation are presented in Figure 5e.. Again, significantly greater numbers died in emersed treatments.

5. Discussion, recommendations and further work

5.1. Spat transportation

The results from Experiment Four clearly show the advantages of transporting spat in seawater filled polyethylene bags. Notwithstanding the low percentage mortality of spat, preliminary results suggested that the inclusion of a buffer could significantly enhance survival and potentially be used to increase viable transport densities. Additional improvements may include the use of filtered seawater, so as to reduce the rate of bacterial accumulation, superoxygenated water, refrigeration and flat-bottomed polyethylene bags for ease of packing.

5.2. Over-wintered and two year old scallops

The success of transportation of overwintered and two year old scallops clearly depends upon the duration and scale of the operation. Transportation by vivier is at present the only practical option for transporting commercial quantities of scallops (>10,000) for durations longer than 12-14 h. The advantage of vivier transportation is that aerial exposure during the loading and unloading of scallops can be minimised, allowing scallops to be seeded on to the seabed after a shorter recovery period. Large numbers could be transported, allowing for loading from several areas in one shipment. The vivier trial to Portland was successful although it was apparent that modifications to the vivier could make it better suited for transporting scallops. Further work would have to include trials from Skye to locations on the continent. It is recommended that stock should be stacked in NWP trays several days in advance of transport.

5.3. Reducing the stresses caused by waste product accumulation

One of the main problems to arise during transportation was the accumulation of potentially lethal levels of ammonia. Although ammonia levels were only measured *in situ* in holdingt media, it is assumed that there were greater levels in the mantle fluids of scallops in emersed treatments (see Duncan, 1993). Likewise, the drop in pH was likely to be more extreme for scallops transported in an emersed state. There are a number of ways in which ammonia and acidic conditions can be reduced, including, violent aeration, minimising the stress of scallops during loading (eg. pooling), buffers and reducing temperature. It is essential that levels of ammonia and pH are monitored continuously so that problems can be quickly resolved.

The most obvious use for a buffer is in the transportation of scallop spat in water filled polyethylene bags and for juveniles transported in a vivier, especially for long haul operations. Results for using buffers as a pre-treatment to emersed transportation are too tenuous to make any recommendations at present. The legislation for using buffers is not particularly restrictive (see schedule 5 of the Bivalve Molluscs regulations 1992 (SI 3164) and the Bivalve Molluscs (Import Controls and Miscellaneous Provisions) Regulations 1994 (SI 2782)), although some stipulations under the Shellfish Hygiene Directive regarding the tainting of shellfish may apply. If the buffer is readily depurated after transportation the proposed process would be acceptable (I Davies, SOAFED, pers. comm.). In view of the short duration for which scallops would be exposed to the buffer, and the fact that they would not be destined for human consumption for at least three years after use, it is unlikely that there would be any risk.

5.4. Temperature shock

While it is recommended that vivier transportation is the most appropriate method, the high cost of vivier transportation will still encourage improvements to emersed transportation. We therefore suggest further investigation into the use of refrigerated lorries for transport at low temperatures. This would greatly reduce the rate at which waste products accumulate during transportation but further work on the effects of transport shock upon scallops would also be required. Data from the present work indicated that scallops are tolerant to rapid changes in temperature but the initial condition of the scallops may be an important factor.

6. Acknowledgements

We would like to thank Peter Millican (CEFAS), David Oakes, Bob Parry, Sandra Parry (Raasay Sound Growers Association), Hugh Wiltshire (Quest Holdings, Portland) and the Tarbert Shellfish Company for their co-operation in the field trials and for supplying the scallops. We also gratefully acknowledge Dr Ian Davies (Scottish Office) for supplying information regarding the legislation for using buffers.

7. References

Basurco, F. (1988), Effects of glucose treatment on glycogen accumulation and resistance to air exposure during transport in scallop spat (*Pecten maximus* L.). M.Sc. Dissertation (unpublished). Institute of Aquaculture, University of Stirling.

Brand, A., R. and Roberts, D. (1973), The cardiac responses of the scallop *Pecten maximus* (L.) to respiratory stress. J. Exp. Mar. Biol. Ecol., 13: 29-43.

Bricelj, V., M and Shumway, S., (1991) Physiology: Energy Acquisition and utilisation. In: Scallops: Biology, Ecology and Aquaculture, ed. S. E. Shumway, Elsevier publ., pp 305-347.

Byrne, R., A., Shipman, B., N., Smatresk, N., J., Dietz, T. H. and McMahon, R., F., (1991). Acid base balance during emergence in the freshwater bivalve *Corbicula fluminea*. Physiol. Zool., 64 (3): 748-766.

Chen, J-C. and Lin, C-Y., (1995) Responses of oxygen consumption, Ammonia-N excretion and Urea-N excretion of *Penaeus chinensis* exposed to ambient ammonia at different salinity and pH levels. Aquaculture, 136: 243-255.

De Vooys, C., G., N. and De Zwaan, A. (1978), The rate of oxygen consumption and ammonia excretion by *Mytilus edulis* after periods of exposure to air. Biochem. Physiol., 60: 343-347.

De Zwann, A. and Wijsman, T,. C., M. (1976), Anaerobic metabolism in bivalvia (mollusca). Characteristics of anaerobic metabolism. Comp. Biochem. Phys., 54B: 313-324.

Duncan, P., F. (1993), Post-harvest physiology of the scallop *Pecten maximus* (L.). Ph. D. Dissertation (unpublished) University of Glasgow.

Gabbott, P. A., (1983), Developmental and seasonal activities in marine molluscs. In: The Mollusca, 2, Environmental Biochemistry and Physiology, P. W. Hochachka (ed.), Academic Press, New York, pp. 165-219.

Hochachka, P., W. and Somero, G., N. (1984), Limiting oxygen availability. In: Biochemical Adaptation. Princeton University Press, New Jersey, pp-145-181.

Scott, W., L., G. (1986), Physiological measurements on the effects of simulated transportation on scallop spat (*Pecten maximus*). B. Sc. Hons. Project (unpublished). Heriot-

Watt University, Edinburgh.

Taylor, E., W., (1989) Oxygen transport and acid base balance in the haemolymph of the lobster, *Homarus gammarus*, during aerial exposure and resubmersion. J. Exp. Mar. Biol. Ecol., 144: 417-436.

Whitely, N., M and Taylor, E., W., (1989) Handling, transport and storage of live crabs and lobsters. An open learning module for the Sea Fish Industry Authority. Her Majesty's Stationary Office, London England, pp 1-104.

Whitely, N., M and Taylor, E., W., (1992) Oxygen and acid base disturbances in the haemolymph of the lobster *Homarus gammarus*. J. Crust. Biol., 12: 19-30.

Appendix

Definitions

acid-base balance - the correct ratio of acids to bases in the haemolymph which maintains a suitable pH.

acidosis - condition in which the pH of the haemolymph becomes abnormally acid eg. pH 7 rather than the normal pH 8.1.

aerobic respiration - respiration occurring in the presence of oxygen with the release of carbon dioxide.

anaerobic respiration - respiration occurring in the absence of oxygen with the release of partially oxidised waste products.

ATP - adenosine triphospate - an energy rich molecule which is an important source of energy for metabolic reactions in all living cells.

buffer - a salt solution which minimises changes in pH when an acid or alkali is added thereby reducing the impact of changes in the system.

glycogen - branched chain polysaccharide made up of glucose units which have an energy storage function in animals.

glycolysis - anaerobic breakdown of glucose to pyruvate in living cells, with the production of ATP.

oxidative phosphorylation - process in aerobic organisms in which ATP is formed from ADP and orthophosphate. This process is driven by the energy released from the oxidation of food stores.

oxygen debt - a deficit in stored chemical energy which builds up during periods of low oxygen availability. It then consumes oxygen above the normal rate for some time until energy supplies are restored by aerobic respiration.

pH - The measure of the acidity of a solution, which is the negative $\log^{10}$ of the hydrogen ion concentration. The pH of a neutral solution is 7, that of acid solutions less than 7 and of alkaline solutions greater than 7.