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Neil, D.M., Coombs, G.M., Birkbeck, T.H., Atkinson, R.J.A., Albalat, A., McPherson, H., Westrop, G., Gornik, S., and Milligan, R.J. (2006) The Scottish Nephrops Survey Phase I: The Factors During Capture, Post-Capture Handling, Delivery and Factory Processing of Nephrops Tails that Affect Product Freshness and Quality. Project Report. University of Glasgow, Glasgow, UK.

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The Scottish *Nephrops* Survey

A joint venture to generate high quality *Nephrops* products from a sustainable fishery

Delivered through

A research partnership between Young's Seafood Ltd., the University of Glasgow and UMBS Millport*

Scientific Report on Phase I

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December 2006

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EXECUTIVE SUMMARY

1. The objectives of the Partnership Project have been achieved through a series of seven work packages.

2. The factors during capture, post-capture handling, delivery and factory processing of *Nephrops* tails that affect product freshness and quality have been identified. The following factors have been evaluated:
 - Mode of capture (trawl vs creel)
 - Trawl duration
 - Damage in trawl and during handling
 - Levels of stress after capture
 - Temperature abuse
 - Tailing procedures
 - Time to icing
 - Delivery time and transport conditions to factory
 - Comparison of catches from different ports and boats

3. The essential components that give the product its characteristic texture and flavour have been identified, and the ways in which these are altered as a result of both natural factors and production processes have been determined. This has been achieved by:
 - Analyses of tissue metabolites have been performed, including carbohydrates, fats, proteins, nucleotides, amino acids and amines
 - A description of the post-mortem processes in *Nephrops* tails in terms of:

- Changes in metabolites
 - pH changes in the muscle tissue,
 - The breakdown of nucleotides, yielding a “Freshness Index”
 - The formation of biogenic amines characteristic of flavour change
 - The production of ammonia and related nitrogenous end products (TMA), which contribute to off-flavours
 - The onset and release from rigor mortis
 - The breakdown of muscle tissue proteins measured by gel electrophoresis
- Identification of Specific Spoilage Organisms using microbiological and molecular techniques
 - Measurements of spoilage rates under different post-capture temperature/time combinations
 - Comparisons of spoilage in product from control trawls by RV *Aora* and from commercial trawls
 - Comparisons of spoilage in factory samples received from different ports/boats
 - Correlations between the microbiological measurement, flavour analysis and the sensory methods used at factory
4. The key features of the population biology of *Nephrops* that impact upon the maintenance of sustainable stocks in fisheries supplying Youngs Seafoods Ltd, and affect the body condition of the animals have been identified. These features, have been assessed through:

- A seasonal analysis of catch composition from the Clyde sea area in terms of size, sex, moult stage, body condition and the prevalence of infection by the *Hematodinium* parasite
 - Correlation of these factors with damage in catches of different durations
 - Co-correlation of these factors with rates of flavour change and spoilage in tailed product
5. On the basis of these findings, the following recommendations are made to Young's Seafoods Ltd.:
- When trawling, it is necessary take into account that greater trawling time is going to increase the number of damaged animals
 - However, quality parameters such as K-value are less dependent on the initial condition of the animal, and are little affected by trawling time, at least over the range of times tested in this part of the project (1-3h)
 - Handling procedures on board are crucial in terms of the quality that the product will have afterwards. In order to preserve quality, adequate icing must be performed within 4 hours of capture. This offers a handling window for the fishermen to work within without detriment
 - Icing the tails correctly is a key issue in quality control. If boxes are not iced correctly, the residual heat in the product will allow loss of freshness and spoilage to occur. Inadequate icing of a box also leads to there being tails of different quality within the same box
 - Washing the tails will decrease the bacterial load that develops in the tail meat, and so this procedure is beneficial to reducing spoilage

- It is important to work with hard animals, since damage is less and quality parameters are improved, compared with soft / 'jelly' animals. It would be particularly helpful to avoid periods during the year when high levels of 'jelly' animals are encountered
- It would be also convenient to avoid animals with heavy *Hematodinium* infection since the presence of the parasite produces alterations in several of the quality-related parameters

INTRODUCTION

The Nephrops fishery

European waters currently support a major fishery for the Norway lobster, *Nephrops norvegicus*. Until 1950 *Nephrops* were considered as a bycatch and were discarded, but then the demand increased continuously until in 1986 the first quotas and Total Allowance Catch (TAC) rates were introduced for the Irish Sea Fisheries Areas. In 1996 the North Sea Fisheries Areas followed. In 2006 the TAC is 54613 tonnes for European waters, and the UK *Nephrops* fisheries hold by far the largest share (48758 tonnes representing 89% of the European TAC).

With the decline in white fish catches all over Europe the *Nephrops* lobster has become the most valuable species within the UK area (Fish Industry Yearbook, 2005). UK export values exceeded £170M in 2004 (FAO, 2006), with the Scottish *Nephrops* fishery representing about 75% of this (£57.1M at first sale). There is, however, a strong static gear fishery on the west coast of Scotland with creelers taking up to twenty percent of the total catch in some years.

In Scotland trawling is the most common method of capture (Figure 1) accounting for 95% of the landings. The vast majority are taken by small inshore trawlers targeting the species, with some also taken by whitefish trawlers. The remaining 5% are traditional creel caught landings. The animals are either sold to processors as scampi tails or as whole animals (fresh or frozen) or live animals. Live animals are the most valuable and will generally achieve at least double the prize of frozen tail meat, depending on size.

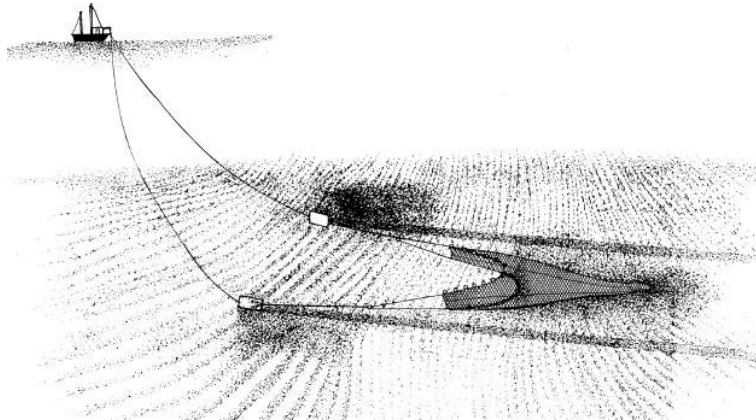


Figure 1. Bottom Trawl System for *Nephrops*: Towing vessel, warp, otter boards, ground gear, net headrope, sweep, and webbing.

In trawling the gear is towed along the bottom with the ground line in contact with the sea bed, forcing bottom dwelling organisms such as *Nephrops* into the net. The tow duration can be between one and six hours, dependent on several factors such as the size of the vessel and the weather conditions. As a general rule, larger vessels tow for longer as they take proportionally longer to shoot and haul their gear, and normally have larger crews which can cope with processing the greater bulk of catch from longer tows.

Threats to the body condition of the *Nephrops* begin at the point of capture, since the animals will be subjected to abnormal stress and also damage within the trawl net. When hauled onto the deck of the trawler, body condition will also be affected by such factors as the emersion into the air, causing asphyxiation, and the change of temperature.

After the net has been hauled, the catch is emptied into a boxed off area on the deck, where it is then shovelled into baskets and tipped onto a table for sorting. Modern

boats however are commonly equipped with a “hopper”, a structure designed to receive the catch and hold it off the deck while allowing the crew direct access for sorting. This effectively removes the need for shovelling and so reduces the potential for damage to the animals.

Sorting involves separating the usable part of the catch from the by-catch and grading the animals into baskets. The animals are graded onboard the boat into three sizes, small, medium and large, and those animals that are too small, damaged or soft are “tailed”. This involves removing the abdomen or “tail” of the animal from the carapace or “head”, with the tail being kept and the heads discarded over the side with the rest of the by-catch. In best practice, when the baskets of ‘whole prawns’ or tails are full they are washed by inserting the deck hose into the basket and shaking vigorously. When clean, the tails are tipped into fish boxes, covered in flake ice and transferred to the fish room (sometimes chilled, sometimes not) until the vessel is ready to land.

The final stages of delivery of the product in fish boxes to the processor involve transportation in one or more stages, using various types of vehicle (most often with refrigeration, but sometimes not), and taking different length of time, depending on location, distance and road conditions. Dwell times may be introduced if re-sorting and re-packing of the tails occurs at intermediate points along the supply chain.

Therefore, potential threats to quality exist from the point of capture and along the whole delivery chain, and any deviations from good practice can initiate or promote deterioration in the condition of the *Nephrops* tails. Identifying the most significant

dangers to product quality, and recommending ways in which to avoid them represent the main aims of the Partnership project.

Seafood deterioration

The deterioration of seafood occurs through a series of processes. The initial loss of freshness and quality is caused mainly by biochemical changes, due to autolysis within the tissues. These initial enzymatic processes lead to the onset of *rigor mortis*, which is then superseded by further enzymatic breakdown processes resulting in loss of freshness. The degradation products of these processes are then slowly utilized by growing populations of bacterial microflora. The bacteria inevitably cause spoilage by the accumulation of volatile and/or toxic products and by decomposition of tissue. If sensory quality analysis over time is plotted on a graph (Figure 2) and the biochemical and microbial changes are incorporated it can be seen that the initial biochemical changes cause a rapid loss in quality (phase 1 and 2). These first phases are followed by a slower but steady phase of degradation caused by both autolytic and microbial processes (phase 3). The final phase (phase 4) represents full bacterial spoilage, leaving the product unfit for consumption.

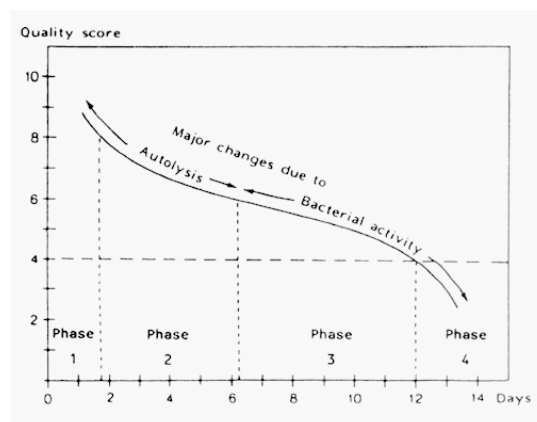


Figure 2. Correlation of *post mortem* and sensory changes

Loss of freshness

Although spoilage of seafood is invariably attributed to the activity of contaminating micro-organisms, the degradation begins post-harvest with enzymatic reactions. The loss of freshness which generally precedes microbial spoilage primarily involves these early enzymatic reactions (Ashie, 1996).

Adenine triphosphate (ATP) is main energy resource for metabolic activity and degrades rapidly *post mortem* (Figure 3) which leads in fish to *rigor mortis*. All biosynthetic activities come to a halt and the cells lose their ability to maintain integrity. The general route of ATP degradation in fish muscle involves a stepwise dephosphorylation (PI, step 1, step 2) to Adenine diphosphate (ADP) and Adenine monophosphate (AMP) and further deamination (NH₃, step 3) to inosine monophosphate (IMP) another dephosphorylation (PI, step 4) to inosine (Ino) and finally the formation of hypoxanthin (Hx).

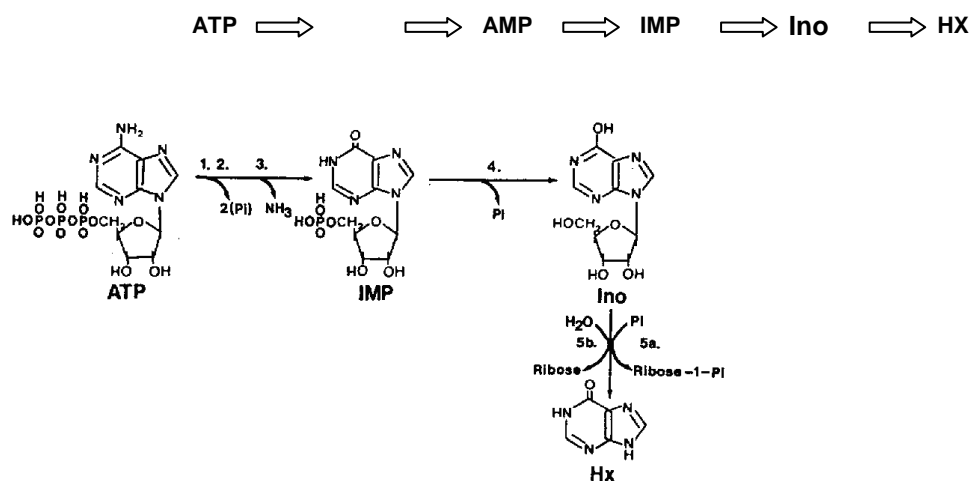


Figure 3. The sequence of ATP degradation

Some of these compounds are related to different tastes, and in this sense IMP has been described to give a nice, meaty taste to food and fish in particular while Hx has been attributed to give a bitter-off taste to fish products. In many fish species these changes correlate well with sensory assessment.

Taken together the concentrations of all these compounds make it possible to calculate the rate of ATP degradation. In many cases, the sensory loss of freshness correlates strongly with the rate of ATP degradation. Saito *et al.* (1959) were the first to observe this pattern and developed a formula for fish freshness based on the ATP breakdown process, now known as the K-index or K-value.

$$\text{K-index} = \frac{[\text{Ino}+\text{Hx}]}{[\text{ATP}+\text{ADP}+\text{AMP}+\text{IMP}+\text{Ino}+\text{Hx}]}$$

The higher the value of the K-index, the greater the loss of freshness. In several fish species a K-value of 20 % has been set as a freshness limit, although this index is species-dependent and therefore does not apply to all fish species. The K-index is widely used today; for example it is a standard freshness index in Japan.

Bacterial Spoilage of Seafood

All raw foods, including seafood, are initially contaminated with a wide variety of micro-organisms, and a fraction of these are able to contaminate and colonize the food product and grow to high numbers (Gram, 1996). The proliferation of these micro-organisms results in decomposition and the subsequent production of derivatives of

tissue metabolites. Such products are: fatty acids, aldehydes, ketones, trimethylamine (TMA) and ammonia.

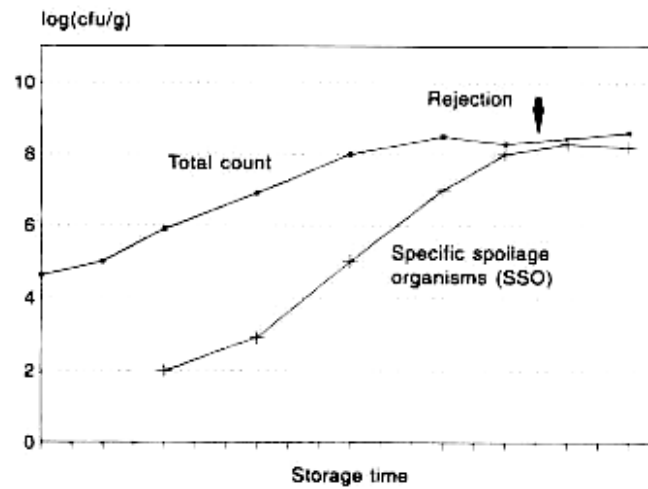


Figure 4. A comparison of total viable counts (TVCs) of bacteria and the growth rate of SSOs

The sensory quality deterioration of seafood is generally characterized by an initial loss of fresh fish flavour. After a period, where odour and flavour are described as neutral or non-specific, the first indications of off-flavours and odours become detectable. These will gradually become more pronounced and inevitable lead to the sensory rejection of the fish (Gram, 1996). Determination of bacterial numbers in seafood is widely used as indicator of spoilage, although it seldom correlates with the sensory quality or the expected shelf life of a product. Therefore, the bacterial numbers are taken as a general indicator of the hygiene status of a product instead. It is understood that usually only a part of the spoilage microflora participates in the actual spoilage process. The SSO (specific spoilage organism) concept (Dalgaard, 1995) explains the relationships between the overall microflora present and the microorganisms mainly responsible for the actual spoilage (Figure 4). SSOs are typically present in low numbers and constitute only a small fraction of the microflora found in

and on post harvest fresh fish. Only later during storage do they take over the microflora and become major spoilers.

Different SSOs can be found in different species of fish and seafood. Identification of SSOs is not easy, and uses the combined effort of many analytical and sensory methods. Seafood SSOs produce ammonia, biogenic amines, organic acids and sulphur compounds from amino acids, hypoxanthin from ATP breakdown products, acetate from lactate and TMA from intramuscular trimethylamine oxide (TMAO). TMA contributes mainly to the characteristic ammonia-like fishy off-flavour associated with spoiled fish. Important SSOs in seafood are:

- *Shewanella putrefaciens* (Jorgensen, 1989)
- *Photobacterium phosphoreum* (Daalgaard, 1997)
- *Vibrio spp.* (Gram, 1987)
- *Pseudoalteromonas sp.* (Gram, 1990)

Several factors influence spoilage of seafood, with initial handling techniques, temperature and pH being the most important (Ashie, 1996). Freshly caught fish have to be handled with great care and hygiene as soon as they are landed on the vessel. Poor post-harvest handling enhances the rate of spoilage. Immediate gutting also promotes the extension of shelf life in fish. Spoilage rates are highly influenced by storage temperature, and the time from catch to icing is most crucial. All biochemical and microbial processes are slowed down at low temperatures, and thus spoilage rates are lower. However, some psychrotrophic (cold tolerant) bacteria are able to continue growth under refrigerated condition at a much reduced rate. Spoilage rates are also influenced by post harvest pH changes (Ashie, 1996), and the low pH generated

during *rigor mortis* inhibits bacterial growth to some extent. The initial decline of the pH is reversed slowly during storage as a result of the decomposition of nitrogenous compound (e.g. AMP) first by intrinsic and later by bacterial enzymes.

Spoilage in Nephrops

In *Nephrops* not much is known about the deterioration of its muscle flesh. Thus, freshness loss, spoilage rates and SSOs are not established, and the ways in which post harvest changes in *Nephrops* are influenced by the method of catch, the handling procedures and storage temperature have received virtually no attention. Stroud *et al.* (1982) investigated TMA, TVBN, hypoxanthin and pH changes alongside sensory changes in *Nephrops* tails. The authors concluded that none of the biochemical methods gave a reliable prediction for product stored longer than 2 days and almost all their results were highly variable. Similar methods were used by Ruiz-Capillas and Moral (2004) to examine changes in modified atmosphere packed (MAP) *Nephrops* tails during frozen storage. Although significant changes could be seen between samples and the control group after 21 days of frozen storage, good chemical indicators could not be established for the early stages of freshness loss. No work on microbial spoilage of *Nephrops* exists.

There are two best practice notes released by Seafish (1994) and by the FRS (Torry Advisory note #29), but both lack scientific data since their purpose was to be helpful to fishermen. The need for icing and hygiene is mentioned but never fully explained.

The physical condition of *Nephrops norvegicus* and catch composition after trawl

As with many fisheries, the Scottish *Nephrops* fishery is subject to quota restrictions set by the EU. As such, it is important that landed catches should be of high quality in order to maximise the profit to the fishermen while keeping within the quota. One of the most obvious quality indicators is the presence of physical damage in the individuals. Trawling gear is known to cause significant damage to benthic organisms (Kaiser and Spencer, 1996, Tuck *et al.*, 1998, Collie *et al.*, 2000) but the effects can be highly variable between species and few studies have specifically examined the effects of trawling on the physical condition of *Nephrops*.

In a recent study by the Glasgow University group, a positive relationship was found between trawl duration and the extent of physical damage in *Nephrops norvegicus* from the Clyde Sea Area (Ridgway *et al.*, 2006a). Mortality was found to be higher in heavily damaged individuals compared to undamaged animals. Most other studies have focussed on the survivorship of discarded or escaped animals (Wileman *et al.*, 1999, Harris and Andrews, 2005a, b) rather than on the condition of the animals that are kept for sale, but a similar link between damage and mortality rates was found.

Physical damage and limb loss are known to affect survival in decapod crustaceans (Juanes and Smith, 1995) and may therefore affect the survival rates of animals that are being transported live, or of discarded individuals. The nature of the damage suffered is also believed to be important. For example, individuals have a higher mortality rate if limbs are forcibly removed, rather than being naturally autotomised (Bergmann and Moore 2001). In *Nephrops*, damage to the abdomen significantly reduces survival rates in discarded individuals and may also lead to necrosis of the tail

muscles (Wileman *et al.*, 1999, Stentiford and Neil, 2000, Harris and Andrews, 2005a). Such animals may be unsaleable and will reduce the overall value of the catch. Minimising the damage to individuals may therefore be important both in terms of increasing the value of a catch and minimising any effects to the wild population.

Another major aim for fisheries in recent years has been to reduce catches of non-target or 'bycatch' organisms. Capturing large quantities of bycatch can have significant direct economic implications for the fishermen, and can affect catches in other fisheries (Hall and Mainprize, 2005). Up to 90% of the total catch may be discarded from the Clyde *Nephrops* fishery, which often contains a high proportion of invertebrates and commercially important fish (Bergmann *et al.*, 2002).

Reducing the amount of discarded material is particularly important for stocks of roundfish such as cod, haddock and whiting that are already overexploited. The minimum mesh size restriction for these fish is currently set at 120mm, while it is 70mm for *Nephrops* (ICES, 2005b). The *Nephrops* fishery is therefore more likely to retain juvenile fish, or individuals below the minimum landing size (MLS) (Stratoudakis *et al.*, 2001). Roundfish rarely survive trawling due to distension or rupturing of the swim bladder and stomach, and estimated mortality rates for these species are typically around 100% (Catchpole *et al.*, 2005).

Several mitigation measures are already in place to reduce the amount of bycatch in the Scottish *Nephrops* fishery. These include the use of square-mesh panels, a minimum mesh size of 70mm in single-rig otter trawls (80mm in twin-rigs), a

minimum landing size of 20mm (carapace length) and the closure of the fishery at weekends (ICES 2005a). However, alterations in trawling practise may also serve to reduce the quantity or alter the composition of the bycatch, and thereby reduce the effects on these threatened stocks.

Overview of Hematodinium sp. Parasite infections

Of the 2000 recognised species of dinoflagellates, over 140 species are known to be parasites, and many have been reported to infect commercially important crustaceans from all over the world. *Hematodinium sp.* is a genus of parasitic dinoflagellates whose species predominantly infects the haemocoel (blood sinuses) and haemolymph (blood) of several of these crustaceans. The parasite inflicts substantial annual losses to crab and lobster fisheries. For example, the *Nephrops* industry around Scotland suffers estimated losses of £3 million annually due to mortality caused by *Hematodinium sp.*

Hematodinium sp. infection in *Nephrops* stocks occurs in Scotland, Norway and the Irish Sea (Briggs, 2002). The parasite is pathogenic and ultimately kills its host. In 1987, fishermen and lobster processing industries for the first time reported low quality catches as well as moribund and dead animals. A sampling programme was therefore instigated from 1990-1997 by Glasgow scientists to monitor the incidence and geographical extent of this condition on the West Coast of Scotland (Appleton, *et al.*, 1997).

Hematodinium infection is thought to be a progressive process, with early, moderate or suppressed infections being difficult to diagnose visually because blood-circulating

parasites resemble immature haemocytes (blood cells). In advanced infections the haemolymph (blood) is milky white in colour due to the vast numbers of parasites present. As the parasites proliferate, several types of host tissue degenerate and hence the animal's metabolism is affected. Gills and blood sinuses show congestion with parasites and a marked reduction of blood cells occurs, and the resulting lowered oxygen-carrying capacity makes the animals lethargic (Stentiford *et al.*, 2000). The infection also leads to a reduced swimming performance of the lobster. It has been shown that when the peak of infection is reached, when the number of parasites is highest within the animal, the host dies. However, prior to the death of the host, dinospores (see below) are released in large numbers from the gill chambers and ventral tail ridge into the surrounding seawater and afterwards the host seems to be completely empty of haemolymph and parasites (Appleton and Vickerman, 1996).

The results of several studies on the *Hematodinium* infection in *Nephrops* demonstrate that there is a seasonal pattern of parasite prevalence within the host between February and July, with peak numbers of infected animals in March and April (Stentiford *et al.*, 2001b). However, it is not known if the parasites are prevalent within the living lobster throughout the whole year and there is no knowledge of the fate of the parasites outside of the peak season of infection. Furthermore, there is no information about the possible effects of this parasite on the quality of the meat tail, in the case that infected animals go to sale.

OBJECTIVES

The lack of relevant scientific information in the different areas outlined above emphasises the importance and relevance of the Partnership Project between Young's Seafood Ltd, The University of Glasgow and the UMBS Marine Station Millport to the improvement and consistency in the quality of *Nephrops* tails delivered to Young's from its many supply vessels around Scotland. Accordingly the following objectives were identified for the Partnership programme.

1. To identify the factors during capture, post-capture handling, delivery and factory processing that affect product freshness and quality. These factors will include spoilage.
2. To determine the essential components that give the product its characteristic texture and flavour, and to identify how these are altered as a result of both natural factors and production processes.
3. To identify the key features of the population biology of *Nephrops* that impact upon the maintenance of sustainable stocks in fisheries supplying Youngs Bluecrest, and affect the body condition of the animals. These features will include moulting time and seasonal infections.

Scope of project

The project was achieved through a series of 7 workpackages (WP), which brought into use a suite of appropriate methodologies, and applied them to particular issues. The outcome of each these WPs is a set of identified deliverables.

WP1. Measures of stress during capture and handling

- a. Calibrate existing assays of key metabolites (eg. lactate) for assessing exhaustive stress
- b. Validate existing measures (total haemocyte counts – THCs) to assess the immunological status of the animal
- c. Develop a standard index for quantifying body damage
- d. Adapt existing methods for measuring the numbers of spoilage bacteria in the tail meat

Leading to a suite of methods for monitoring the effects of capture and handling stresses on product quality

WP2. Threats to quality during capture and handling

- a. Measure capture stresses in relation to trawling duration and other fishing practices. Identify patterns of seasonal variability.
- b. Assess handling damage on deck
- c. Relate post-capture stresses caused by exposure on deck to air and to high temperature to the initiation of spoilage. Identify periods of greatest vulnerability.
- d. Establish the extent to which spoilage bacteria are present in the tailed product
- e. Relate post-mortem changes in meat quality to handling, tailing, grading, packing and on-board storage procedures

Leading to the identification of the main factors during trawl capture, handling and storage that lead to degradation of product quality

WP3. Measures of freshness, flavour and texture

- a. Use methods for the proximate analysis of the composition of tail meat (protein, carbohydrate, fat, water content, minerals, ash)
- b. Develop an index of freshness, based on reliable alternatives to TVB_N (eg. Ornithine and FAAs determined using HPLC), and compare this with existing sensory measures
- c. Identify the factors that give scampi its characteristic flavour (sugars, amino acids)
- d. Identify post-mortem changes in the protein composition of the muscle using gel electrophoresis
- e. Identify post-mortem changes in the structure of the muscle using histopathological methods.
- f. Distinguish between post-mortem changes due to autolysis and those caused by spoilage bacteria

Leading to a suite of methods for monitoring product freshness, flavour and texture

WP4. Maintaining quality along the supply chain and during processing

- a. Determine the natural variation in the flavour and texture of tail meat with size, sex, moult condition, and season
- b. Monitor changes in the freshness, flavour and texture of tail meat along the supply and production chain, including the effects of frozen storage and the peeling & cooking processes

- c. Determine the extent to which spoilage bacteria and other infectious agents persist or multiply in the tail meat along the supply chain or during factory processing.
- d. Identify any changes in flavour and texture that are induced by spoilage bacteria and other infectious agents persisting in the tail meat along the supply chain or during factory processing.

Leading to recommendations for best practice along the supply chain and during factory processing to maintain freshness, flavour and texture in the final product.

WP5. Development of diagnostic test kits for infective agents and spoilage bacteria.

Based on the existing scientific knowledge and procedures that has been generated by researchers at the University of Glasgow and UMBS Millport concerning the infection of *Nephrops* by the dinoflagellate parasite *Hematodinium* and of the spoilage caused by *Vibrio* bacteria, this project will:

Develop, adapt, standardise and calibrate assays for detecting these pathogens within the tissues and derived products of *Nephrops*. For *Hematodinium*, the assays will be based on immunological and molecular techniques. For *Vibrios*, the assays will be based on bacterial cell counts and on proteolytic activity.

- a. Incorporate the assays into diagnostic test kits which can be used by various operators in both field and factory situations to determine whether infection levels exceed pre-determined standards.

WP6. Linking quality measures to a traceability scheme

- a. Identify best practices for landing and handling product that are necessary to maintain quality – eg. trawl time, deck temperature, humidity, time to tailing and icing

Leading to the incorporation of key proxy measures of product quality into an appropriate traceability scheme

WP7. Population biology of *Nephrops norvegicus*

- a. Determine recruitment, size at age and moulting times in the different sea areas that supply Young's Seafood Ltd.
- b. Determine the natural variation in the flavour and texture of tail meat with size, sex, moult condition, and season
- c. Determine the seasonality of the infection by *Hematodinium* in animals from different sea areas using existing immunological and molecular diagnostic methods.

Leading to recommendations for best fishing practices and effort to sustain stocks, to catch animals in good body condition and to avoid moulting periods when animals are most vulnerable to stress and infection.

Timetable for Years 1 & 2 of Partnership project (Months)

		0-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20	21-22	23-24
WP1 Measures of stress (effort =10%)	Metabolite assays												
	Immune measures												
	Body damage index												
	Bacterial counts & ID												

WP2 Threats to quality (effort =20%)	Seasonal variability												
	Necrosis & Spoilage												
	Trawl duration												
	Handling on deck												

WP3 Flavour & texture (effort =15%)	Flavour analysis												
	Texture analysis												
	Post-mortem changes												

WP4 Maintaining quality (effort =20%)	Texture changes												
	Flavour changes												
	Spoilage bacteria												
	Histopathology												

WP5 Diagnostic kits (effort =15%)	Necrosis												
	Proteolysis												
	Parasite infection												

WP6 Linking to Traceability (effort =10%)	Trawling												
	Deck conditions												
	Handling time												
	Transport												

WP7 Population Biology (effort =10%)	Infections												
	Age & moulting												
	Recruitment												
	Fisheries Modelling												

MATERIAL AND METHODS

I. EXPERIMENTAL DESIGNS

I. A) Post-mortem changes in *Nephrops*

Nephrops norvegicus lobsters were creel-caught around the island of Cumbrae in the Clyde Sea Area, Scotland, UK and transported back to University Glasgow in seawater tanks. The animals were then held solitary in temperature controlled aquarium tanks. The tanks are supported by a circulating bio filtered natural seawater system adjusted to a salinity of 35 ‰ and 8°C. The animals were fed at least twice a week with herring pieces. To acclimatize the animals to their new environment, no experiments were conducted within the first 2 weeks after catch.

To assess the post mortem changes in pH, metabolites and protein degradation, every animal was first sacrificed by detaching the head and carapace from the body by a quick twist and turn. Then a ~1g piece of tail flexor muscle was taken immediately to determine the starting values. The tail was then divided into five equal sagittal pieces with the shell still attached. The pieces were then placed in a humid chamber at RT, 8°C, 16°C or on ice. One piece was constantly monitored for pH changes by insertion of a pre-calibrated microelectrode. Following a timescale relative to the incubation temperature (3h intervals at 0°C (on ice), 1.5h intervals at 8°C, 1.0h intervals at 16°C for up to 5 times) a sample was taken for the determination of the metabolites (AEC system metabolites, glycogen, glucose, ammonia, arginine phosphate), pH values and

protein degradation. The intervals were defined in a preliminary trial run (data not shown).

For gel electrophoresis a high salt protein extraction of muscle samples was performed at different time points post-mortem (1,3,5 and 7 days), loading amounts were equalised (following Bradford assays) and applied to a 4-12% gradient SDS gel. Protein bands were visualised by Coomassie-staining followed by de-staining. Details of other methods are given elsewhere.

I. B) The effect of pre-slaughter stress

Nephrops escape swimming behaviour (exercise) was artificially induced and maintained until exhaustion (about 200 tail flips in ~5 minutes) and sampling of muscle tissue was performed immediately after sacrifice. For comparison a control group of rested animals was sampled in the same way.

I. C) Effect of capture method on the physiology of *Nephrops* and possible consequences for quality

In order to assess the effect of the capture method on the physiology of *Nephrops* and its possible consequences for quality animals were obtained from creels located in the Clyde area by the Research Vessel *Aplysia* (UMBSM Millport) and also from otter trawling. In the latter case, animals were trawled by the Research Vessel *Aora* (UMBSM Millport) for three different length of time: 15 min, 1 hour and 2 hours. In all cases, lobsters were rapidly tailed and washed after landing on board and tails were placed in plastic bags and stored on ice. On days 1, 3, 5 and 7 tails were taken, pH was analysed and muscle from all tails was frozen in liquid nitrogen and stored at -80°

until analysis. Furthermore, creoled animals were taken to the laboratory and were placed on tanks for 2 days to let the animals recover for any stress occurred during capture and transport and were killed by decapitation. Tails from these animals were also placed on ice and sampled on days 1, 3, 5 and 7.

In this experiment the parameters measured in order to assess the impact on capture method on the stress and quality were:

- Lactate levels in the muscle and in the haemolymph
- Phosphoarginine concentration in the muscle
- pH in the muscle
- Muscle ATP and its derivatives (ADP, AMP, IMP, INO and HX) which allowed us to calculate the Adenylate Energy Charge (AEC) and the K-values.

I. D) Effect of temperature abuse on *Nephrops* tails: bacterial spoilage

In order to characterize bacterial growth due to temperature abuse, fresh *Nephrops* tails were taken directly after landing on the RV *Aora* and held at different temperatures for various lengths of time in order to assess the effect on bacterial concentrations and quality. The temperatures chosen were on ice (control) and at 4°C for 6 days or at 8 and 16°C for up to 2 days.

The bacterial types present in tails from the temperature storage trials were identified to see if certain groups of bacteria were associated with the different storage temperatures, in particular the high temperatures, where high levels of bacteria were found. A selection of bacterial isolates was taken from each temperature storage group, (0, 4 and 16 °C) and identified using 16sRNA gene sequencing (see following section for methodology on 16sRNA gene sequencing).

I. E) Simulated Commercial Trial I – Different temperature abuses

Nephrops were obtained by otter trawling (70mm mesh size net) by the Research Vessel Aora (UMBSM Millport) from the Farlie channel, north of the island of Cumbrae in the Clyde Sea Area, Scotland, UK.

Lobsters were tailed immediately after landing on board by researchers and the crew. After a thorough wash tails were randomly sub sampled into sterile bags (20 tails each) and either stored on ice immediately or incubated at different temperatures using temperature controlled incubators for different time intervals (Figure 5).

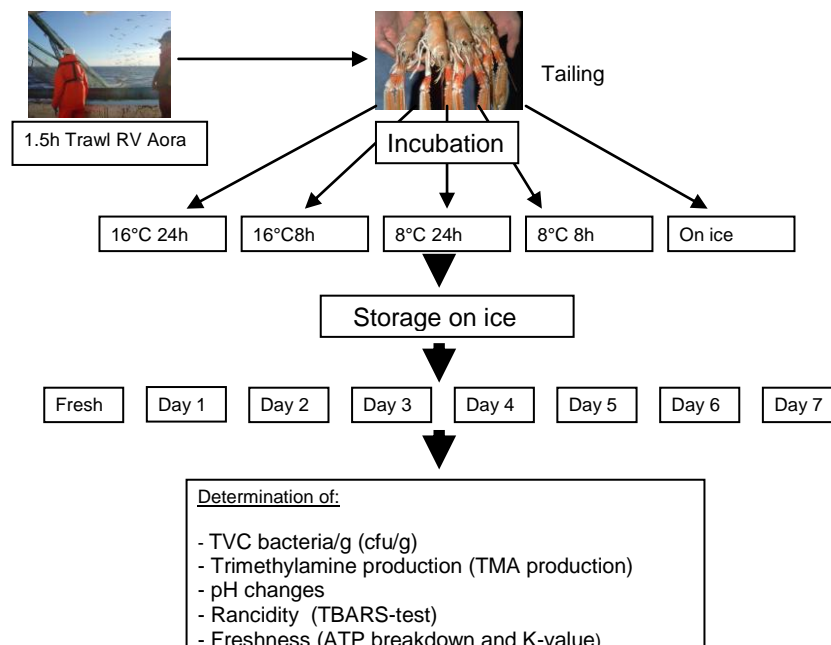


Figure 5. Experimental set-up in simulated commercial trial I.

The temperatures chosen correlate to the mean air temperature values in Scotland throughout the seasons (16°C in the summer and 8°C in spring and autumn) and therefore represent feasible threats for the quality of the tails during handling onboard a vessel. The 0°C value aims to represent the control for the best possible handling

practise from catching to icing (which would be achievable on a standard *Nephrops* Fishing Vessel). The chosen incubation times of 4 h, 8 h and 24 h are well within the timescale tailed *Nephrops* could be left untouched before being chilled or iced.

In this experiment the following parameters were analysed:

- pH in the muscle
- Total Viable Counts (TVC) or total number of aerobic bacteria
- Trimethylamine (TMA) and Ammonia levels in the muscle
- Thiobarbituric Reactive Substances (TBARs) in the muscle
- Muscle ATP and its derivatives (ADP, AMP, IMP, INO and HX) which permitted us to calculate the K-values

I. F) Simulated Commercial Trial II – Short term temperature abuse

In this experiment a similar approach compared to Simulated Commercial Trial I (I. E) was used. However, in this case the temperature abuse tested was 16°C for 4 h. Similarly, *Nephrops* were obtained by otter trawling (70mm mesh size net) by the Research Vessel Aora (UMBSM Millport) from the Farlie channel, north of the island of Cumbrae in the Clyde Sea Area, Scotland, UK.

Lobsters were tailed immediately after landing on board by researchers and the crew. After a thorough wash tails were randomly sub sampled into sterile bags (20 tails each) and either stored on ice immediately or incubated at 16°C for 4 h using temperature controlled incubators. After this time, all tails were placed on ice and stored on days 1, 3 and 5.

I. G) Simulated Commercial Trial III: The effect of washing

In order to study if washing tails on board promoted quality extension, an experiment was performed in which tails of *Nephrops* were washed or un-washed before placing them on ice. Samples on days 3 and 5 were taken and the bacteria load was quantified using the ‘Compact Dry’ system. As in previous experiments, *Nephrops* were obtained by otter trawling (70mm mesh size net) by the Research Vessel Aora (UMBSM Millport) from the Farlie channel, north of the island of Cumbrae in the Clyde Sea Area, Scotland, UK.

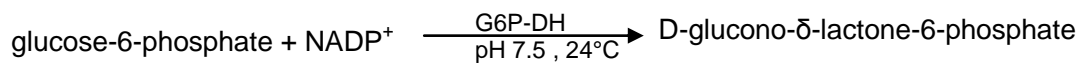
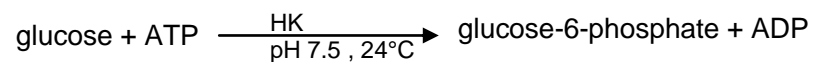
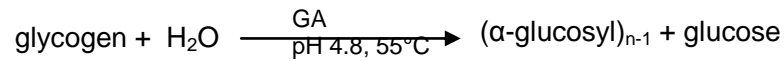
II. PARAMETERS AND METABOLITES MEASURED IN ORDER TO DETERMINE STRESS AND QUALITY IN NEPHROPS**II. A) Physiological measures for *Nephrops****Glycogen Assay*

An enzymatic method to determine the glycogen content in tissue is based on glycogen hydrolysis using the fungal enzyme glucoamylase, GA (EC 3.2.1.3) and subsequent analysis of the liberated glucose moieties. Glucoamylase hydrolyzes not only the α -1,4 linkages but also the α -1,6 glucosidic bonds of glycogen. The specificity of glucoamylase is such that glycogen can be determined in tissue homogenates without the need to extract the polysaccharides first.

For analysing the liberated D-glucose a UV-Method with hexokinase, HK (EC 2.7.1.1) and glucose-6-phosphate dehydrogenase, G6P-DH (EC 1.1.149) was used.

D-glucose is phosphorylated to glucose-6-phosphate by ATP in the presence of hexokinase. The glucose-6-phosphate produced is then reduced to D-glucono- δ -lactone-6-phosphate catalysed by glucose-6-phosphate dehydrogenase. NADP^+ acts as electron acceptor.

Principle:



The amount of glucose liberated by the hydrolysis of glycogen is proportional to the increase of NADPH measured by the change in absorbance at 340nm and standardized using samples of known D-glucose concentration. Muscle was thoroughly homogenized in 5x volume of 0.6M PCA using an Ultra Turrax Homogenizer. Immediately after homogenization, 0.2 ml of homogenate was centrifuged at 13.000 rpm for 15 min. The supernatant was used later for the determination of basal tissue glucose levels (tissue glucose background).

A fraction of the remaining homogenate (0.1 ml) was placed in a fresh Eppendorf tube and 0.05ml of 1M KHCO_3 was added. After this neutralization, 1 ml of glucoamylase solution was added and the mixture was incubated at 55°C for 2h with occasional shaking. After incubation, 0.5 ml of the solution was mixed with 0.5 ml of 0.6M PCA and centrifuged at 13.000 rpm for 15 minutes to precipitate any remaining proteins. The supernatant was then used to determine the tissue glycogen level by measuring glucose liberated during the hydrolysis.

Glucose Determination

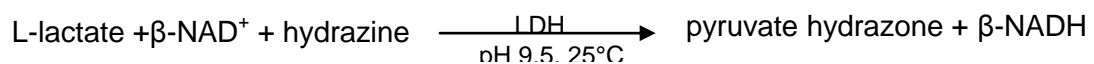
A volume of 0.05 ml of sample or standard was mixed with 0.95 ml of ATP/NADP⁺/G6P-DH solution. The increase in absorbance at 340nm was recorded (A₁) and 5µl hexokinase suspension was added and the change in absorbance was followed and read A₂ when constant. To obtain the D-glucose content of a sample the absorbencies before and after hexokinase addition have to be subtracted first (A₂ - A₁ = ΔA). D-glucose levels are then calculated by extrapolation of ΔA using the standard curve.

The D-glucose content measured in the homogenate after subtraction of the free glucose present (tissue glucose background) before hydrolysis corresponds to the glycogen content of the tissue sample. Multiplication by the dilution factors of the tissue in the homogenate and in the later process allows the glycogen content to be expressed in mmol glycosyl units per g wet weight. If results are to be expressed in mg glycogen per g of tissue the molecular weight of the glycosyl moiety in glycogen (M_r 162) must be included in the calculation.

L-lactate Assay

An enzymatic method to determine the L-Lactate content in tissue is based on direct analysis of L-lactate moieties. For analysing the L-lactate content a UV-Method with lactate dehydrogenase, LDH (EC 1.1.1.27) and hydrazine was used.

Principle:



In general, the reduction of pyruvate by NADH to form L-lactate is catalysed by lactate dehydrogenase. A disadvantage of this reaction is the unfavourable equilibrium of reaction (a). It is far in favour of L-lactate. However, lactate is dehydrogenated completely by LDH if pyruvate is withdrawn from the equilibrium by conversion to pyruvate hydrazone by means of the reaction of hydrazine and pyruvate to pyruvate hydrazone. This reaction functions only as a trapping reaction for pyruvate. The amount of L-lactate is proportional to the increase of NADH concentration measured by the change in absorbance at 340 nm and standardised using samples of known L-lactate concentration.

Tail muscle was homogenized at high speed in 5x volume of 0.6M PCA using an Ultra Turrax Homogenizer. Immediately after homogenization, 0.2 ml of homogenate was delivered into a fresh Eppendorf tube and centrifuged at 13.000 rpm for 15 min. The supernatant was then used for the determination of tissue L-lactate levels. A volume of 0.05 ml of supernatant or standard was mixed with 0.95 ml of Hydrazine Buffer and the increase in absorbance was followed at 340nm until constant (A_1). Then, 50 μ l of diluted lactic dehydrogenase suspension was added and the change in absorbance was followed until constant (A_2).

To obtain the L-lactate content of a sample the absorbencies before and after lactic dehydrogenase addition has to be subtracted first ($A_2 - A_1 = \Delta A$). L-lactate levels are then calculated by extrapolation of ΔA using the standard curve.

II. B) Quality related measures for *Nephrops*

pH in the muscle

In the post-mortem experiments in order to measure pH changes in the muscle tails continuously pH was measured by placing directly a pre-calibrated microelectrode into the *Nephrops* tails (ISFET, Sentron Corp.). In the quality experiments, in order to obtain the muscle pH, samples were homogenised in distilled water in a ratio 1:10 (w/v) and measurements were carried out using a pH meter (Jenway model 3510).

*Bacteriology of the *Nephrops* tails*

The Total Viable Count (TVC) is an estimate of the total number of aerobic bacteria present in a sample that are capable of growth on a Standard medium plate when incubated at a fixed temperature for a fixed amount of time. The theory behind the Total Viable Count is that individual bacteria (or tight groups or “clumps” of bacteria) will multiply and grow on the plate to form a visible, countable colony (i.e. a colony forming unit or CFU). Colonies counted are e.g. expressed as the number of CFU per gram of sample (CFU/g) at the time of sampling. In order to achieve the TVC or CFU/g in *Nephrops norvegicus* tails two different methods were used:

- Traditional method with marine agar plates
- ‘Compact Dry System’

*Determination of TVC in *Nephrops* tail muscle using the traditional method with marine agar plates*

Isolated tails had pleopods removed and were surface sterilised by immersion for two minutes in 0.1% Benzalkonium chloride made up in seawater. Meat was dissected

from the underside of the tail using sterile instruments. A small piece (~0.4-1.0 g) of meat was placed aseptically into sterile Stomacher bags and weight recorded. A 10 ml volume of sterile seawater (SSW) was added to the Stomacher bags containing the meat and sets of 5 bags were homogenised in the Stomacher machine for 2 x 120 sec on high-speed setting. The homogenised material was transferred into sterile plastic universals and an appropriate dilution series was set up using SSW as diluent. A 100 µl volume of these dilutions were spread inoculated in duplicate onto Marine agar plates. Plates were incubated for 48 h at 20°C and bacterial numbers recorded as colony forming units per gram of meat (cfu g⁻¹). Using rankit plots results were found to follow a non-normal distribution therefore, data was normalised by converting it into LOG values.

Determination of the total viable count of bacteria in Nephrops tail muscle using the 'Compact Dry System'

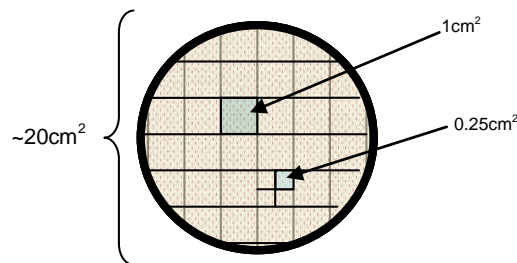
A commercial available ready-to-use 'Compact Dry' system was used (see picture). 'Compact Dry' plates are coated with a dehydrated film of growth media which contains nutrient standard agar and the redox indicator tetrazolium salt. The plates are rehydrated by direct addition of 1ml of the solution or homogenate which is to be checked for its bacterial load. After incubation colonies grown are easily identified by colour due to the redox indicator tetrazolium salt.



If working with bacteria from the marine environment the growth medium must represent the mineral and salt composition of the sea. Therefore samples were prepared in sterile seawater instead of sterile water.

The tail of a *Nephrops* lobster was dissected and a meat sample was aseptically taken and transferred to a sterile “stomacher” bag. The weight of the sample was measured and 10x volume of sterile seawater (SSW) was added to the sample (w/v). The bag was then put into the “stomacher” and mechanically homogenized for 2x240s at highest speed.

Bacteria plates were taken out of their sterile container. The plate cap was carefully removed and 1ml of the homogenate was pipetted in the middle of the dry media sheet. The sample solution diffuses automatically and thereby transforms the dry sheet into a gel. Every sample was at least done in duplicate. The plates were incubated at 20°C for 96h.



Colony Count: The bacteria form red colonies which can be counted by eye, if the numbers are few. The growth area is 20 cm². The back of the plate has a grid carved of 1 cm x 1 cm. In case of a higher density due to large number of colonies grown, total viable count can be obtained by multiplying 20 by an average number of colonies per 1cm² grid counted. By using a binocular numbers up to 50 colonies per 0.25cm² can be counted.

To obtain the CFU/g content of the sample all dilution factors and the sample weight have to be taken into account. Usually the CFU/g represents a large number so the LOG of the value is calculated and plotted to clarify the result.

Identification of bacteria using 16S rRNA sequencing

Template DNA was prepared by suspending a bacterial colony harvested from a fresh Marine agar plate in 100 µl of sterile distilled water in a microcentrifuge tube and heating at 100 °C for 10 min. In all cases, 1 µl of the resultant template preparation was used in subsequent 50 µl PCR reactions. The respective DNA template preparations were subjected to PCR amplification using the two universal eubacterial 16S rRNA primers (27F, 5'-AGA GTT TGATCM TGG CTC AG-3'; 685R, 5'-TCT ACG CAT TTC ACY GCT AC-3'). The PCR mixture was set up in 50 µl aliquots with 5 µl of 10X buffer, 3 µl MgCl₂, 4 µl dNTPs, 0.4 µl *Taq* polymerase, 1 µl of each primer, 36.2 µl of dH₂O and 1 µl of template DNA. The amplification conditions were 94 °C for 2 min, followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min with a subsequent extension cycle at 72 °C for 10 min. The PCR products were visualised on a 0.7 % agarose gel stained with ethidium bromide. The 50 µl volume of the PCR reactions were purified using MicroSpin columns (Amersham Pharmacia) following the supplied protocol. Purified PCR products were sent for sequencing to MWG Biotech in 20 µl volumes containing ~30 ng DNA µl⁻¹, along with the universal eubacterial 16S rRNA primer, 685R at 10 pmol µl⁻¹ in 10 µl. Sequences were analysed using the software programme BioEdit and entered into the BLAST database on the EBI website to identify sequence matches. ClustalW was used to align the sequences and phylograms were produced using Clustal Graphics 7 software.

TMAO and H₂S assay (taken from Gram et al., 1987 and personal communication with Prof. L. Gram)

TMAO-medium was used to screen bacterial strains for their ability to reduce TMAO and produce H₂S: 2 % peptone, 0.3 % Lab Lemco powder, 0.3 % yeast extract, 0.03 % ferric citrate, 0.03 % sodium thiosulphate, 0.4 % NaCl, 0.4 % KH₂PO₄, 0.575 % K₂HPO₄, 0.05 % MgSO₄, 2 resazurin tablets and 0.4 % technical agar. The pH was adjusted to 6.8. The medium was sterilised at 121 °C for 15 minutes. Before use, sterile 0.04 % cysteine was added and medium boiled to expel oxygen. After cooling to 45 °C, sterile 0.5 % TMAO, 2H₂O was added. The medium was dispensed aseptically in 10 ml volumes into sterile 15 mm test tubes and allowed to solidify. Tubes were stab inoculated with bacterial growth and overlaid with a thin layer of sterile paraffin oil. The tubes were incubated at the appropriate temperature for 48 h. TMAO reduction was indicated by the redoxindicator in the medium changing from red to yellow, and H₂S production was indicated by the formation of a black precipitate of FeS from the cysteine.

Determination of trimethylamine (TMA) concentration in Nephrops tail muscle

TMA was determined by the method of Dyer (1945) with some minor modifications introduced by Stroud *et al.* (1982) in order to suppress interferences with dimehylamine. TMA from muscle portions (15 g) was extracted with 50 ml of trichloroacetic acid, TCA (7.5%). The extract was centrifuged and 1 ml of the supernatant was taken and pH was adjusted to 7.6 with NaOH and then made up to 5 ml using distilled water. This diluted mixture was then mixed with 1 ml of formaldehyde (4%), 10 ml of toluene and 3 ml of KOH (40%), vortexed for 2 min and

left to stand for another 2 min. A portion of the toluene layer was then reacted with picric acid, which reacts with primary and secondary amines to produce yellow picrates with a maximum absorbance at 410 nm.

Thiobarbituric acid reactive substances (TBARS) determination

TBARS determination was based on the spectrophotometric quantification of the pink complexes formed after the reaction of secondary compounds of lipid oxidation with thiobarbituric acid. Basically, 1 g of muscle was homogenised in 3 ml of TCA (7.5%) and was centrifuged at 3000 g for 10 min at 4°C. After centrifugation, 500 µl of the supernatant were mixed with 500 µl of thioarbituric acid (0.02 M) and the mixture was boiled for 30 min. Absorbance was recorded spectrophotometrically at 530 nm. Malonaldehyde (MDA) was used as standard in order to quantify lipid oxidation in the samples. Results are expressed as mg of MDA equivalents per 100 g of tissue.

Determination of nucleotide breakdown products, adenylate energy charge (AEC) and K-values in Nephrops tail muscle

Nucleotide extracts were prepared as described in Ryder (1985) and kept at -80°C until HPLC analysis was performed. Basically, tail muscle is weighted and thoroughly homogenized at high speed on ice in 5x volume of 0.6M PCA using an Ultra Turrax Homogenizer. Immediately after homogenization, 5 ml of homogenate were pipetted into a fresh centrifuge tube and centrifuged at 5.000 rpm for 10 min at 4°C. The supernatant was transferred to a glass beaker on ice. The pH was adjusted to pH 6.5 – 6.8 with 1M KOH. The sample was left on ice for 30 min and potassium perchlorate was removed by filtration through sintered glass and volume was added with

phosphate buffer up to a final volume of 10 ml. The diluted filtrate was then used for the determination of ATP and its breakdown products.

ATP and breakdown products were analysed by high performance liquid chromatography (HPLC). A SP8800 ternary HPLC pump was used coupled to a PDA detector from ThermoFinnigan set to monitor at 254 nm. Separations were carried out using a reverse-phase C₁₈ SYNERGY MAX-RP 80 A column 250 × 4.60 mm, with an internal particle diameter of 4 µm. The mobile phase was composed of: solvent A (0.04 M KH₂PO₄ + 0.06 K₂HPO₄ pH 7) and solvent B (methanol). Conditions used for the analysis are summarised in Table 1. Standard curves were prepared from adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), inosine 5'-monophosphate (IMP), inosine (INO) and hypoxanthine (Hx) all from Sigma Aldrich (Dorset, England, UK) in concentrations ranging from 0 to 1 mM. To obtain the concentrations of different products of ATP-breakdown, levels are calculated by extrapolation of ΔA using the standard curve. An example of a typical standard curve using this method is shown in Figure 6. Linearity of each compound was verified by analysis of variance of regression and r values ranged from 0.9921 to 0.9995.

Multiplication by the dilution factors of the tissue in the homogenate and in the later process allows the contents to be expressed in μmol of nucleotide per g wet weight tissue.

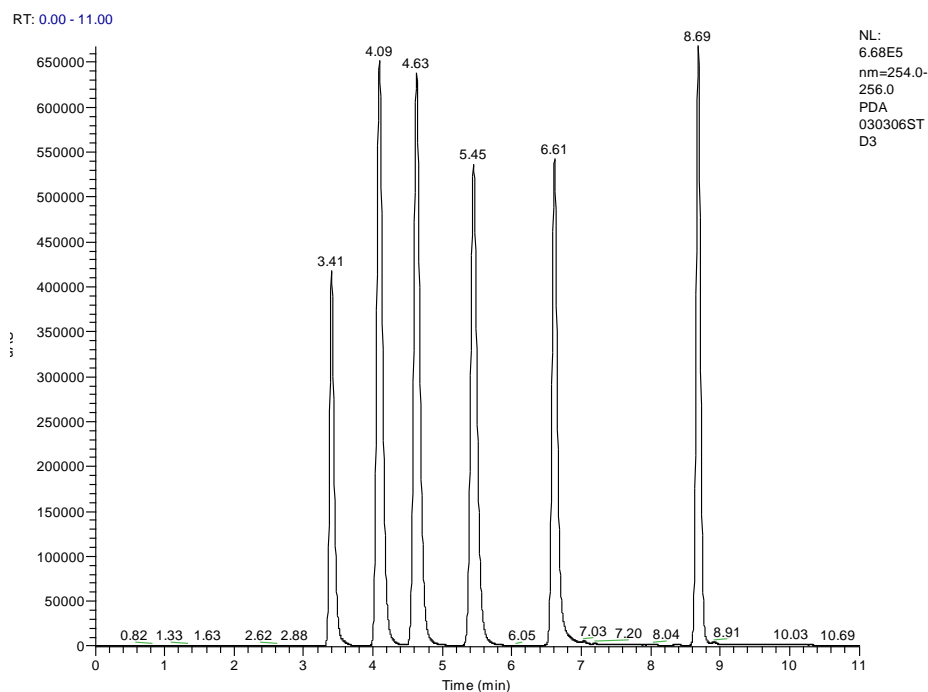


Figure 6. Chromatogram showing a typical standard of ATP and its breakdown products resolved by HPLC. Peaks were identified by their retention time and order of appearance was: IMP, ATP, ADP, AMP, HX and INO.

The AEC was calculated by using the molar concentrations of ATP, ADP and AMP per weight of sample with the formula introduced by Atkinson and Walton (1967) as an index for the amount of metabolically available energy in an organism.

$$\text{AEC} = [\text{ATP}] + [1/2\text{ADP}] / [\text{ATP}] + [\text{ADP}] + [\text{AMP}]$$

K-values were calculated following the formula proposed by Saito *et al.* (1959) where

$$\text{K-value (\%)} = [\text{INO}] + [\text{Hx}] / [\text{ATP}] + [\text{ADP}] + [\text{AMP}] + [\text{IMP}] + [\text{INO}] + [\text{HX}].$$

Table 1. Chromatographic conditions used for the analysis of nucleotide breakdown products in *Nephrops norvegicus* muscle extracts.

Time (min)	Solvent A (%)	Solvent B (%)	Flow rate (ml/min)
0	100	0	1.1
8	84	16	1.1
8.5	40	60	1.1
13.5	40	60	1.1
14	100	0	1.1
24	100	0	1.1

III. DETERMINATION OF CATCH COMPOSITION AND DAMAGE INDEX IN NEPHROPS TAILS AFTER TRAWLING

Following the recovery of each trawl onto the deck, the entire catch was shovelled into containers and the total wet weight was recorded. The catch was washed in seawater to remove excess mud from the organisms, and any weed or debris (e.g. plastics and rocks) were also removed before weighing. Care was taken to limit the amount of damage caused at this stage to avoid biasing the results. It was occasionally only possible to analyse a sub-sample of the catch due to time constraints and the large number of organisms that were often caught. In such cases, approximately half the containers were selected for complete analysis. It was assumed that these were representative of the entire catch, and were selected as randomly as possible.

Nephrops lobsters were then separated from the rest of the catch into baskets and the wet weight was recorded. The remaining organisms were then sorted to the most appropriate taxonomic level, and the wet weights recorded for each group. All

organisms were identified to species to produce a qualitative list. The invertebrate components were not weighed or identified from trawls Aoral-S and Aoral-L as a different sorting method was used. This did not affect the analyses of *N. norvegicus* or fish species.

A sub-sample of approximately 5 kg (300 individuals) of *Nephrops* was taken from a single basket from each trawl for further analysis. This sample was taken by lifting several organisms at a time by hand, which would have avoided selecting for a particular size class. Additionally, the baskets contained individuals from the entire catch, and should not have been biased towards a particular section of the trawl. On return to the laboratory the carapace length, sex, carapace hardness and level of damage were recorded for the sample.

Carapace length was measured from the base of the orbit to the mid dorsal posterior edge using callipers, as indicated in Figure 7. Carapace hardness was estimated by squeezing the sides of the carapace just behind the head (figure 7). The carapace was considered to be 'hard' if there was no noticeable give when squeezed, and 'soft' if squeezing caused a clear distortion. The entire exoskeleton of 'jelly' animals was 'papery' and gave no resistance to pressure. This method is somewhat objective, but was suitable for this study as only one person was involved in the analysis.

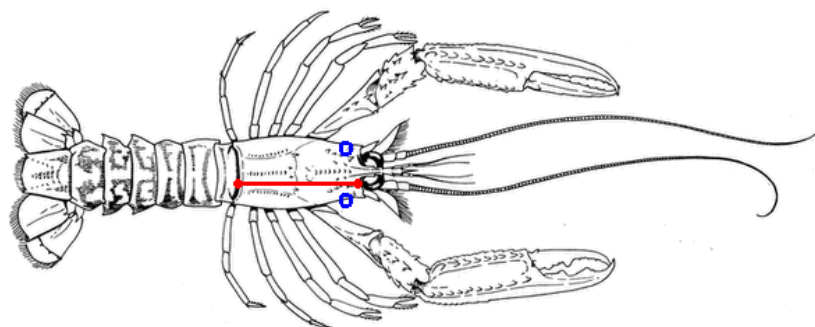


Figure 7. Diagram of *Nephrops norvegicus* (modified from FIGIS website). The red line shows the carapace length. The blue circles show the points that were tested to assess carapace hardness.

Damage was scored against a three-level index, which is presented in Table 2. A score of 0 indicated no damage, 1 indicated minor damage, and 2 indicated severe damage. Examples of each damage category are shown in figures 8 and 9.

Table 2. Damage criteria used for the determination of Damage Index in *Nephrops* after trawling

Damage Category	Criteria
0 (No damage)	No visible damage to external structure and no loss of limbs
1 (Minor damage)	Exhibit no more than two of: <ul style="list-style-type: none"> • Loss of two or fewer walking legs • Loss of not more than one claw • Soft tissue punctures or small puncture to the shell • Loss of the tip of the rostrum
2 (Major damage)	Exhibit at least one of: <ul style="list-style-type: none"> • Loss of more than two walking legs • Loss of both claws • Loss of an eye • Compressed or cracked body parts\segments • Major soft tissue punctures • Exhibit three or more criteria of category one animals



Figure 8. Dorsal view of *Nephrops* showing an example of each damage category. From left to right: Undamaged (0), slightly damaged (1) and severely damaged individuals (2).
The scale is indicated by a 10p piece.



Figure 9. Ventral view of *Nephrops* showing an example of each damage category. From left to right: undamaged (0), slightly damaged (1) and severely damaged individuals (2).
The scale is indicated by a 10p piece.

Possible effects of the carapace hardness on the quality of *Nephrops* tails

In June 2006 *Nephrops* were obtained by otter trawling (70mm mesh size net) by the Research Vessel Aora (UMBSM Millport) from the Farlie channel, north of the island of Cumbrae in the Clyde Sea Area, Scotland, UK.

Trained personnel classified lobsters after landing on board as hard or jelly. Immediately, animals were tailed and after a thorough wash tails were sampled (Fresh) or were placed into sterile bags (20 tails each) and stored on ice until further analysis. Tails were sampled on days 1, 3, 5 and 7 and several quality measures were analysed (bacterial load and nucleotide breakdown products, K-values).

IV. PREVALENCE OF THE PARASITE HEMATODINIUM SP. IN NEPHROPS AND EFFECTS ON QUALITY**Prevalence data**

During the first year of this project prevalence data were collected during monthly boat trips to add to the 10 years of infection prevalence knowledge within the work group. The method of choice was a recently developed ELISA method targeting parasite proteins present in blood samples of *Nephrops* hosts (Small, 2002).

Nephrops were obtained by otter trawling (70mm mesh size net) by the Research Vessel Aora (UMBSM Millport) from the Farlie channel, north of the island of Cumbrae in the Clyde Sea Area, Scotland, UK on a monthly basis.

Then a sub-sample of ~300 animals was taken randomly and put into tanks with circulating seawater. Then haemolymph was taken from 100 animals. On return to the laboratory the haemolymph was assessed for parasite infection by ELISA.

***Hematodinium* Enzyme-Linked Immunosorbent Assay (*Hematodinium* ELISA)**

Haemolymph was withdrawn from the base of the 5th pleopod using a 26³/₈ G needle and a 1ml syringe. 250µl of fresh haemolymph are transferred to an Eppendorf tube containing 250µl of marine anticoagulant (0.4 M NaCl, 0.1 M glucose, 30 mM tri-Na-citrate, 25 mM citric acid, 9 mM EDTA, pH xx), mixed and stored at -20°C until analysis. To increase the difference between *Hematodinium* -positive and -negative haemolymph samples 2µl of haemolymph sample (in anticoagulant) were diluted in 1ml H₂O dd. prior to the actual assay procedure.

Immediately after dilution, 0.1ml of each sample, a positive control and a blank were pipetted onto a “high protein binding” 96-well plate (Imm lux HB “high protein binding” 96-well Microplates (Dynex Cat. # 1010) in duplicate. The 96-well plate was then incubated for at least 20 minutes at RT. After incubation the wells were emptied and washed 4 x 5 minutes with PBS-T solution (200 ml of PBS containing 100µl Tween-20). Meanwhile the primary antibody solution (1µl rabbit polyclonal α-*Hematodinium* AB (Appleton *et al.*, 1997) in 5ml PBS-T) was prepared. 100µl of the AB solution was added onto each well and incubated for 20 min at RT or over night at 4°C. Again after incubation the wells were washed 4 x 5 minutes with PBS-T solution. After washing, the secondary antibody solution (2µl goat-α-rabbit-IgG-horseradish-conjugated AB. (Sigma # A 9169) in 10ml PBS-T) was applied and

following a third 20 minute incubation the wells were finally washed 4 x 5 minutes in PBS-T and were ready for the final chromatic development.

A volume of 100µl of TMB *Sure Blue*TM-substrate (KPL SureBlueTM (KPL 52-00-01) was added per well and the plate was incubated 5-10' at RT until a blue colour precipitate appeared. The plates were read at 630 nm with a micro plate-reader. Positive control: A mix of ten previously tested infection (ELISA) positive haemolymph sample was used as a positive control in the process.

Possible effects of the parasite on the quality of *Nephrops* tails

On March and April of 2006 *Nephrops* were obtained by otter trawling (70mm mesh size net) by the Research Vessel Aora (UMBSM Millport) from the Farlie channel, north of the island of Cumbrae in the Clyde Sea Area, Scotland, UK.

Lobsters were classified after landing on board as visually infected or visually non-infected by trained personnel. Immediately, animals were tailed and after a thorough wash tails were sampled (Fresh) or were placed into sterile bags (20 tails each) and stored on ice until further analysis. Tails were sampled on days 1, 3, 5 and 7 and several quality measures were analysed (muscle pH, bacterial load, TMA concentrations and nucleotide breakdown products, which allowed us to calculate the K-values).

RESULTS

I. POST-MORTEM CHANGES IN *NEPHROPS* AND THE EFFECT OF PRE-SLAUGHTER STRESS

I. A) *Post mortem changes in muscle metabolites*

Typical values for metabolites in the muscle of resting live *Nephrops* are shown in Table 1.1.

Table 1.1. Metabolite concentrations in muscle of resting live *Nephrops*

Metabolite	Average	S.E.
ATP [$\mu\text{mol/g}$]	6 - 9	
AEC	0.96	0.01
Glycogen [$\mu\text{mol/g}$]	19.7	2.24
L-lactate [$\mu\text{mol/g}$]	1.73	0.72
Phosphoarginine [$\mu\text{mol/g}$]	25.6	2.2
Ammonia [$\mu\text{mol/g}$]	1.3	0.2
Other measures	Average	S.E.
Muscle pH	7.6	0.1
Bacteria load LOG_{10} [cfu/g]	2.4	0.3

Following separation of the tail from the head, the initial glycogen concentrations, L-lactate production, pH changes and ATP breakdown processes were closely linked. The glycogen stores in the muscle were consumed and lactic acid was produced (Figure 1.1), and these glycolytic processes continued until the muscle stores of glycogen were totally depleted, which occurred after ~12h at 10°C. Measurements of tail muscle pH showed that values fell by around 1 pH unit over the same time period (Figure 1.2), and stores of phosphoarginine were also depleted (Figure 1.3).

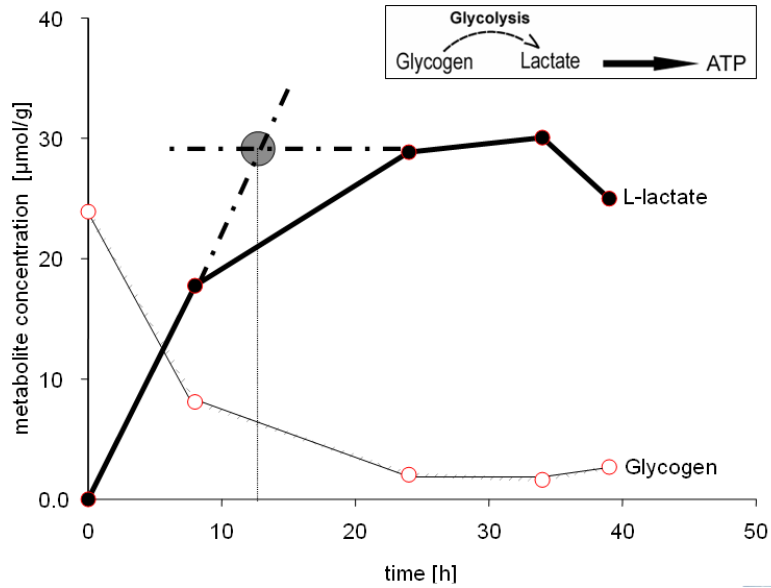


Figure 1.1. Typical result showing the loss of glycogen and the accumulation of L-lactate due to post mortem glycolysis in *Nephrops* tail muscle when held at 10°C. The tail was separated from the head at time=0. The dashed lines indicate that glycolysis is complete after ~ 12h.

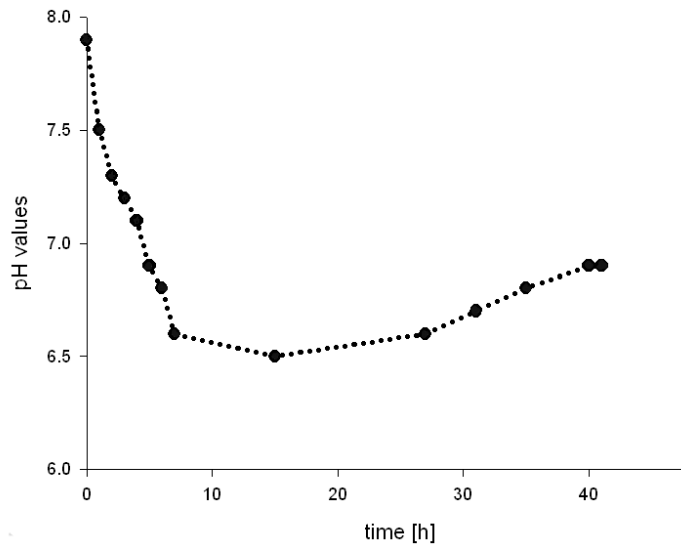


Figure 1.2. Typical result showing changes in pH of the *Nephrops* tail muscle when held at 10°C. The tail was separated from the head at time=0.

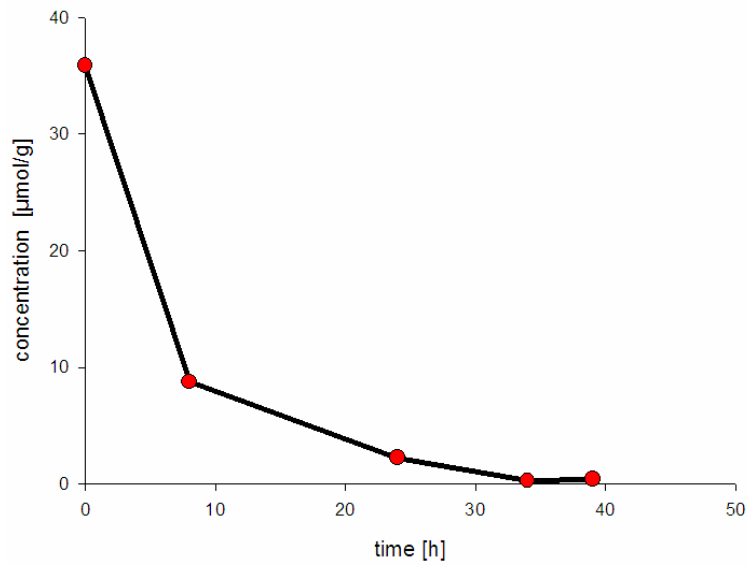


Figure 1.3. Typical result showing post mortem reduction of phosphoarginine in *Nephrops* tail muscle when held at 10°C. The tail was separated from the head at time=0.

Storage temperature had a significant effect on these post-mortem changes in energy metabolites, as reflected by the pH profiles of the tail meat (Figure 1.4). The rate of this acidification was temperature-dependent, with the peak being reached within 2h at 20°C, ~12h at 10°C and >36h at 0°C.

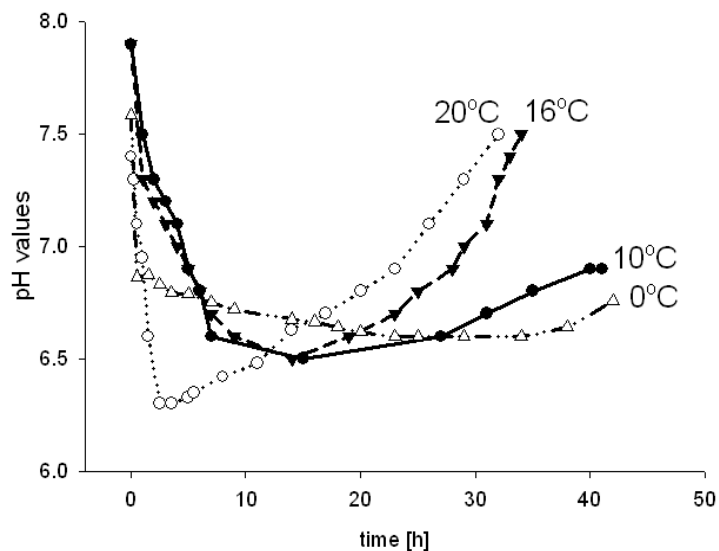


Figure 1.4. Typical results showing changes in pH of the *Nephrops* tail muscle when held at different temperatures. The tail was separated from the head at time=0.

The reversal of the pH shift also showed temperature dependence (Figure 1.4), and since lactic acid concentrations remained high over this period (Figure 1.1) this must reflect the simultaneous accumulation of end products of muscle breakdown and spoilage that promote alkalinity.

I. B) Factors determining rigor mortis

Analysis of the nucleotide breakdown products provides a direct measure of the post mortem changes in the concentration of ATP in the tail muscle (Figure 1.5). From an high initial value (9-10 $\mu\text{mol.g}$) the ATP concentration declined rapidly, due to conversion to ADP. In *Nephrops* tail muscle held at 10°C it was still measurable at 8 hours post mortem (arrow in Figure 1.5), but was totally absent thereafter.

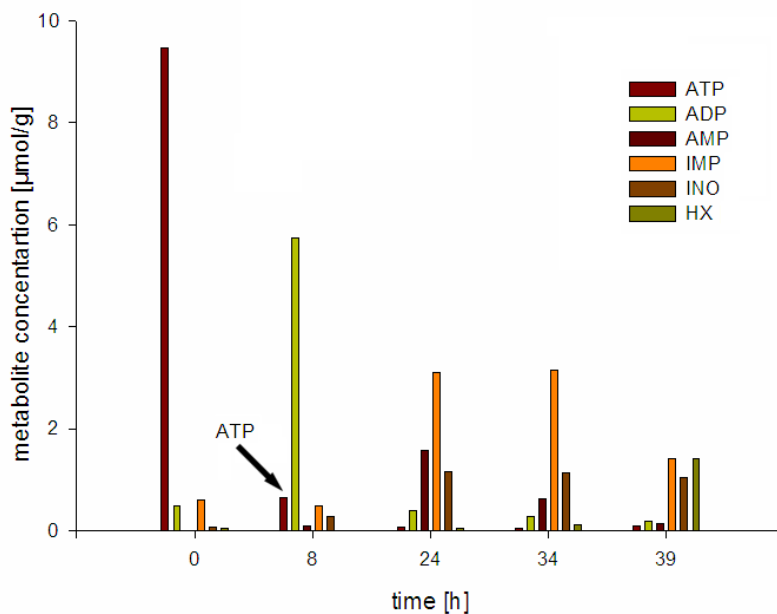


Figure 1.5. Nucleotide breakdown products in *Nephrops* tail muscle when held at 10°C measured at different intervals of time post mortem. The tail was separated from the head at time=0. Data from 4 samples at each time point.

SDS PAGE gel electrophoresis of muscle samples taken immediately post mortem (Fresh in Figure 1.6A) allowed the main contractile and regulatory proteins to be identified. In comparison, samples taken from tail meat held at 10°C for 20h (Figure

1.6B) show the appearance of numerous additional bands of lower MW than the myosin heavy chain (MHC) (arrow Figure 1.6). The identity of these bands was investigated by Western blotting, using antibodies against MHC (Figure 1.6B). These react against both MHC and also paramyosin (which forms the core of myosin tails in crustacean muscle) (Fresh in Figure 1.6B). After 20h at 10°C the MHC and paramyosin signals were much reduced, while new signals appeared in the range of the additional bands found with SDS Page (arrows in Figures 1.6A and 1.6B). These can therefore be identified as breakdown products of MHC and paramyosin, leading to the conclusion that myosin is the main target of protein degradation in *Nephrops* tail muscle.

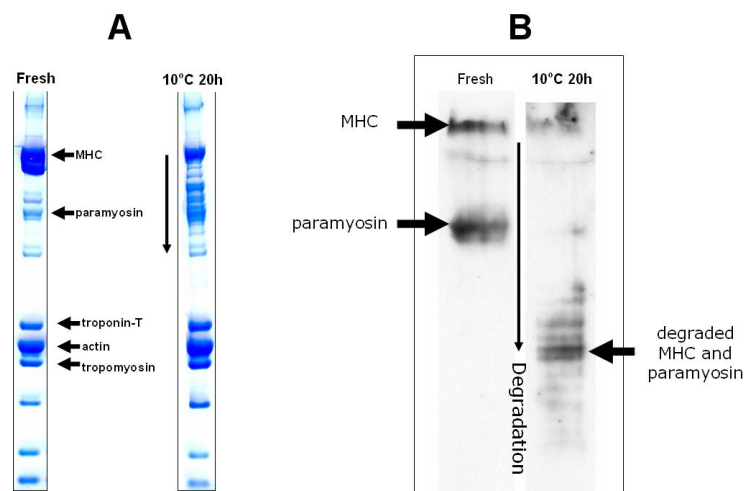


Figure 1.6. A. SDS PAGE gel electrophoresis (4%-12% gradient gel) of muscle proteins in the main flexor muscle of the *Nephrops* following high salt extraction. Samples taken immediately after separation of the tail from the head (Fresh) and after holding the tail at 10°C for 20h. B. Western blot of same samples using Rabbit- α -Myosin-AB. Arrows in A and B indicate the range of additional breakdown bands

In order to investigate the apparent absence of muscle stiffness (rigor) in *Nephrops* tail muscle post mortem, simultaneous measurements of the rate of depletion of ATP and the rate of muscle protein degradation were performed at shorter time intervals (Figure 1.7).

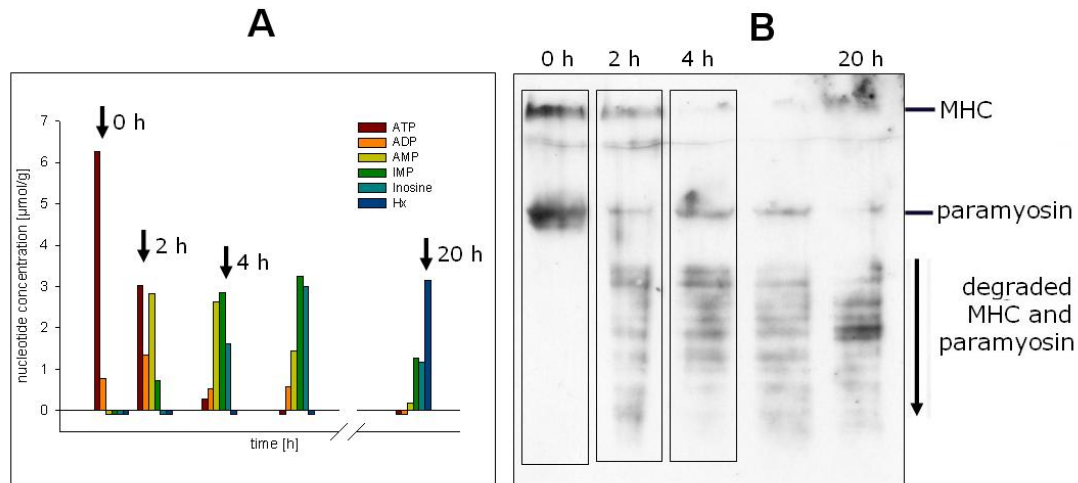


Figure 1.7. A Nucleotide breakdown products in *Nephrops* tail muscle when held at 10°C measured at different intervals of time post mortem. The tail was separated from the head at time=0. Data from 4 samples at each time point. B Western blot of same samples using Rabbit- α -Myosin-AB. Arrow indicates the range of additional breakdown bands

Extensive breakdown of myosin occurred within 2 hours, judged both by the loss signal in MHC and the development of signals in lower molecular weight breakdown bands (Figure 1.7B). At the same time point, however, the muscle ATP concentration remained at around 50% or its initial value (Figure 1.7A). This rapid breakdown of a key myofibrillar protein before ATP is significantly depleted may explain why the characteristic stiffness of muscle in the rigor mortis state is not apparent in this species.

I. C) The effect of pre-slaughter stressors

The above results refer to changes occurring in animals sacrificed when in a fully rested state, but post mortem changes in commercially trawled *Nephrops* follow the stressful process of trawl capture, which involves induced swimming (possibly to exhaustion) crowding in the net (which will restrict ventilation) and possible damage. Several of these factors have been investigated in newly landed and in discarded

animals (Harris and Andrews, 2005a,b; Ridgway *et al.*, 2006a,b) but their effects on post mortem events have not been studied.

Two approaches have been taken to address this deficiency: a study of the metabolite changes associated with exhaustive swimming (to simulate this aspect of trawl capture), and a study of the subsequent changes in post mortem processes in animals sacrificed after simulated trawling. *Nephrops* escape swimming behaviour (exercise) was artificially induced and maintained until exhaustion (about 200 tail flips in ~5 minutes) and sampling of muscle tissue was performed immediately after sacrifice. For comparison a control group of rested animals was sampled in the same way. Figure 1.8 shows that capture stress caused significant changes in the concentrations of all major metabolites in the tail muscle. This is consistent with the increased energy demand of exercise, with the phosphoarginine store being depleted, and glycolytic activities taking place to consume glycogen and produce lactic acid.

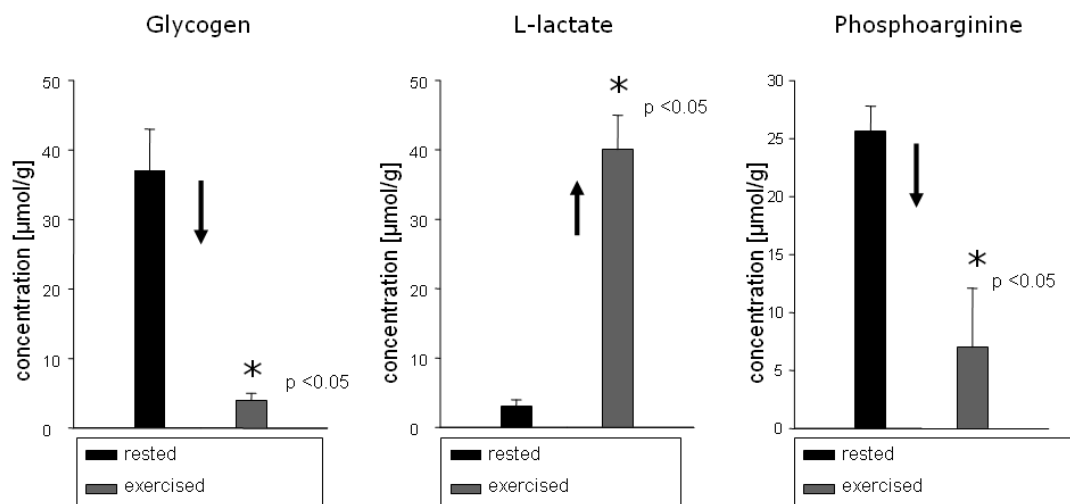


Figure 1.8. The concentrations of glycogen, L-lactate and phosphoarginine in the tail muscles of *Nephrops* at rest and immediately following a period of exhaustive exercise (~200 tail flips in 5 minutes).

This altered metabolic status of trawled *Nephrops* would be expected to have an influence on the timing and rate of post mortem events, and this has been tested by monitoring the pH in the tail muscle of animals that have performed exhaustive exercise immediately before being sacrificed (Figure 1.9).

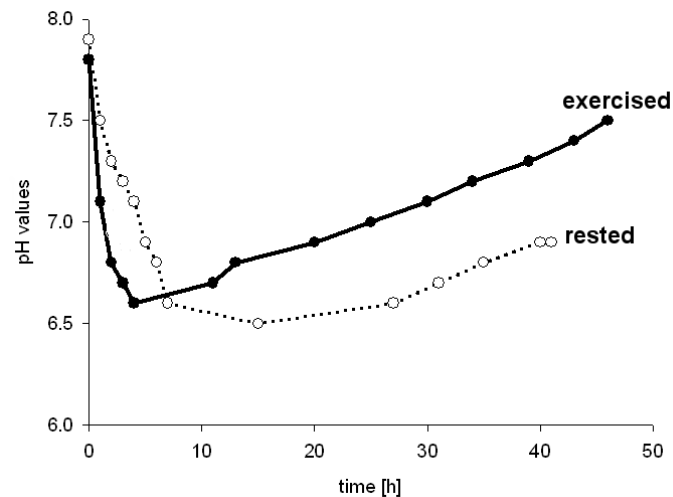


Figure 1.9. Comparison of the pH of the *Nephrops* tail muscle of a rested animal and an animal exercised to exhaustion (~200 tail flips in 5 minutes). The tail was separated from the head at time=0, and was then held at 10°C.

These results indicate that muscle pH falls more rapidly post mortem in exercised *Nephrops*, compared to rested controls, reaching a peak in the former group around 2h rather than at 12h for the latter group at the test temperature of 10°C. This is consistent with there being a greater cumulative production of lactic acid in the exercised group. A feature of this result that still requires explanation, however, is the more rapid increase in pH displayed by the exercised group following the acidic peak.

An independent measure of the effect of exercise on the energy status of *Nephrops* was provided by the analysis of nucleotide breakdown products. This is conveniently expressed in the adenylate energy charge (AEC) value (Figure 1.10). A significant

reduction in AEC occurred following exhaustive exercise, which will predispose the tail muscle to more rapid further breakdown of nucleotide end products post mortem.

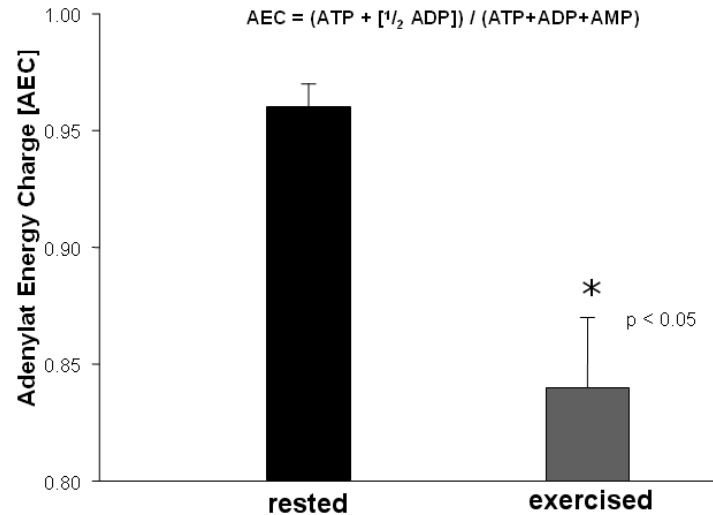


Figure 1.10. The AEC for the tail muscles of *Nephrops* at rest and immediately following a period of exhaustive exercise (~200 tail flips in 5 minutes).

I. D) The link between post mortem events and spoilage

The initial events occurring in post mortem *Nephrops* tail meat are described here for the first time. These results provide new insights into the condition of the *Nephrops* tail meat over a critical period, corresponding to the time from when they are caught in the trawl net to being landed on the fishing vessel, and for the few hours thereafter.

Knowledge of the processes occurring over this period has informed the studies of the later events in the post-harvest after capture that lead to loss of freshness and spoilage. Thus the post mortem events lead on to further biochemical breakdown processes (autolysis) within the muscle, and subsequently to a phase of bacterial spoilage. These various processes produce numerous end products (eg. ammonia, TMA) that contribute to the muscle alkalinity detected by the pH measurements seen in Figures

1.2, 1.4 & 1.9. Further inter-conversions of nucleotide breakdown products also occur, as shown in a preliminary way in Figures 1.5 & 1.7.

These later processes have been the main focus of other work packages in this Partnership project, which have involved systematic trials on *Nephrops* trawled under controlled conditions by the research vessel RV Aora (see Section II). Taken together, these various approaches have provided a relatively complete picture of muscle degradation and spoilage and their relationship to freshness and shelf life.

II. THREATS TO QUALITY DURING CAPTURE AND HANDLING

II. A) Effect of capture method on the physiology of *Nephrops* and possible consequences for quality

Effect of capture method on haemolymph and muscle lactate

As shown in Figure 2.1A lactate levels in the muscle were lowest in rested animals and increased significantly in creeled and especially in trawled animals, which presented the highest levels of muscle lactate. For trawled animals there was no difference between the different trawling times, indicating that even if animals are trawled for as short as 15 min, lactate is already significantly elevated in the muscle. This indicated a mobilization of muscle glycogen reserves. On the other hand, lactate concentrations in the haemolymph (Figure 2.1B) were proportionate to trawl duration, suggesting that there is a considerable lag between lactate production in the muscle and its appearance in the haemolymph. Creeled animals showed low lactate levels in the haemolymph, similar to those found in the muscle.

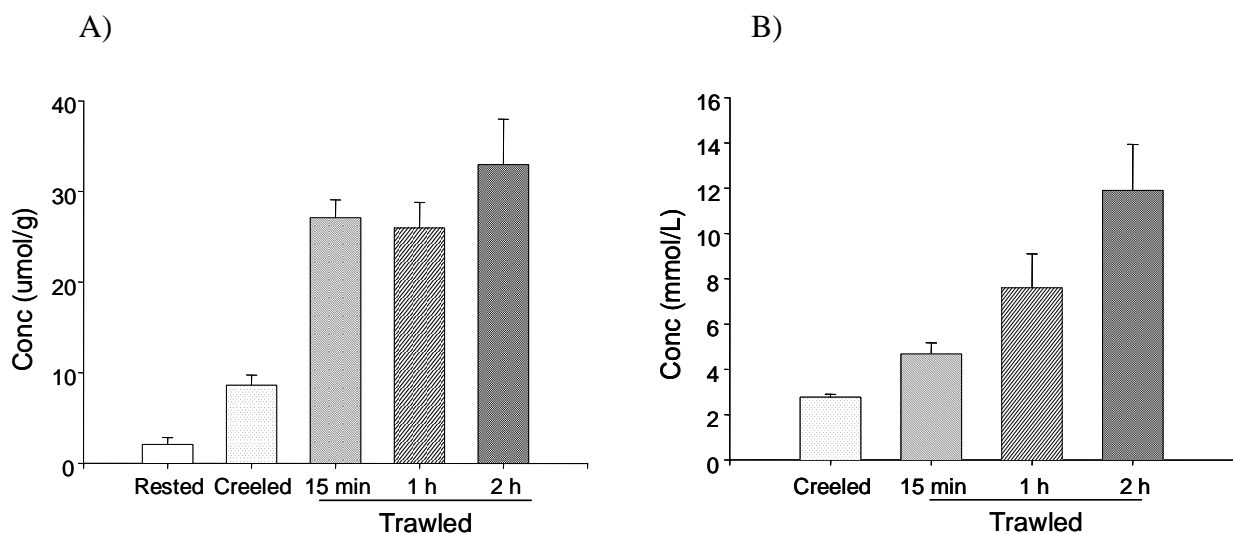


Figure 2.1. Changes in muscle lactate (A) and haemolymph lactate (B) in *Nephrops* after landing the animals with different capture methods. Values are the mean \pm S.E.M. of five determinations.

Phosphoarginine, nucleotide profiles and AEC values changes due to different capture methods

Phosphoarginine was highest in rested and unstressed animals, but was significantly lower in creeled animals (Figure 2.2). In trawled animals, muscle phosphoarginine was completely depleted, even if animals were trawled for as short as 15 min. This indicates that phosphoarginine was totally consumed in order to sustain the concentration of ATP.

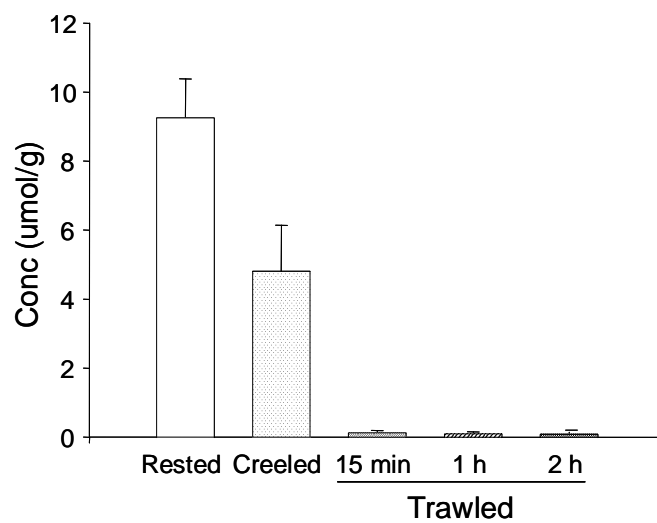


Figure 2.2. Changes in muscle phosphoarginine in *Nephrops* muscle after landing the animals with different capture methods. Values are the mean \pm S.E.M. of five determinations.

The nucleotide profiles are shown on Figure 2.3. Immediately after removal from an aquarium tank and sacrifice, the main nucleotide in the tail muscle of unstressed animals was ATP, with only very low levels of ADP and AMP being recorded. In creeled animals, similar profiles were obtained, with ATP being the main muscle nucleotide. However, in this case higher concentrations of ADP and AMP were recorded. Finally, in trawled animals, even if animals were trawled for as little as 15 min, the main nucleotide in muscle samples taken just after catch was AMP rather than ATP, suggesting a rapid ATP breakdown in this species. Taken together, the concentrations of ATP, ADP and AMP allow the AEC to be calculated (Figure 2.4).

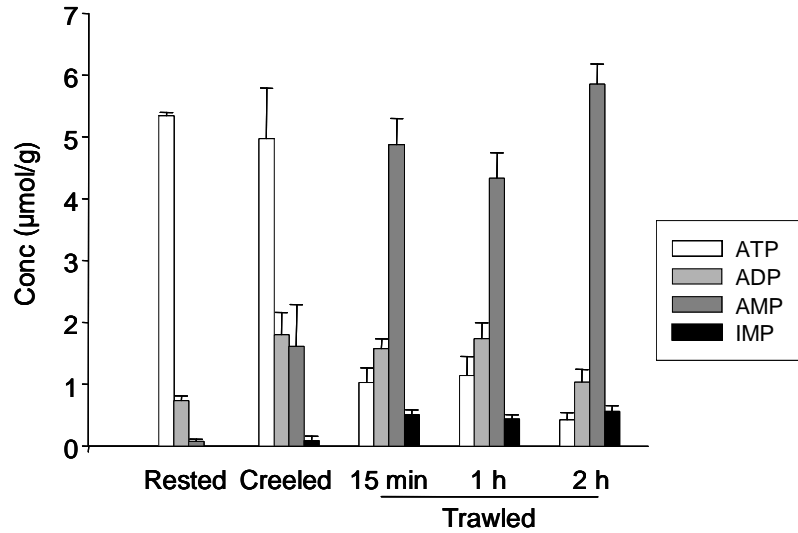


Figure 2.3. Nucleotide profiles in *Nephrops* muscle after landing the animals with different capture methods. Values are the mean ± S.E.M. of ten different determinations.

The AEC ratio was highest in rested animals taken from a tank (0.92 ± 0.01) and decreased to 0.77 ± 0.07 in creeled animals. Animals caught by trawl had the lowest AEC ratios, ranging from 0.24 ± 0.04 in animals trawled for 15 min, to 0.13 ± 0.03 in animals trawled for 2 hours.

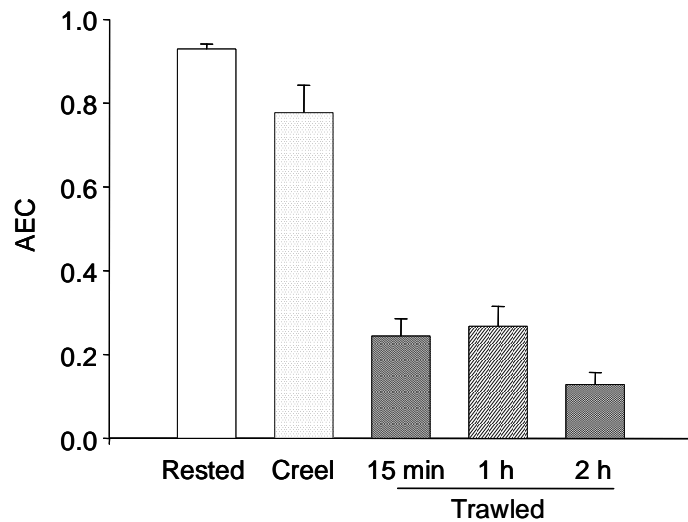
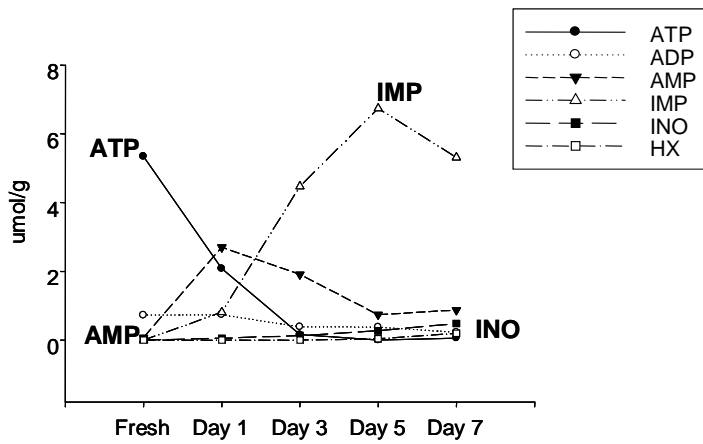


Figure 2.4. AEC ratios in *Nephrops* muscle after landing the animals with different capture methods. Values are the mean ± S.E.M. of ten different determinations.

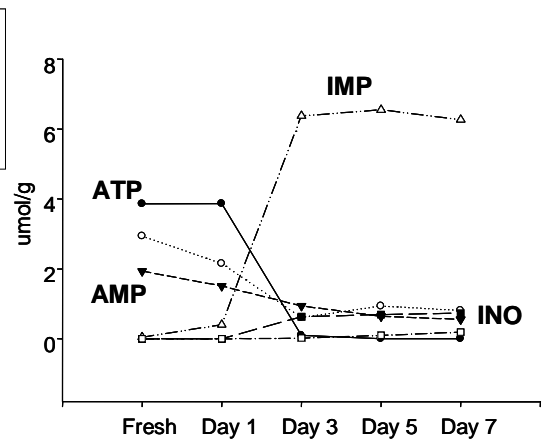
Effect of capture method on the nucleotide profiles and K-values when tails are stored on ice

Nucleotide profiles when tails were stored on ice following different capture methods are shown in Figure 2.5. In rested and creeled animals ATP levels dropped within 2 days and were at undetectable levels on day 3 in both cases. For trawled animals the main nucleotide after catching was AMP (Figure 3.3), and when tails were stored on ice this AMP decreased considerably in the first 2 days. In all cases, IMP increased with chilling storage time and maximum levels occurred between days 3-5. In fact, apart from their different nucleotide profiles in fresh samples, the curves for the different capture methods were very similar when tails were left on ice, and by day 7 similar concentrations were obtained for the main nucleotides detected in all cases.

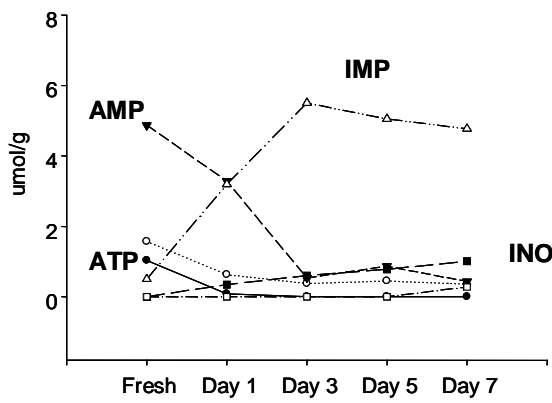
A) Rested



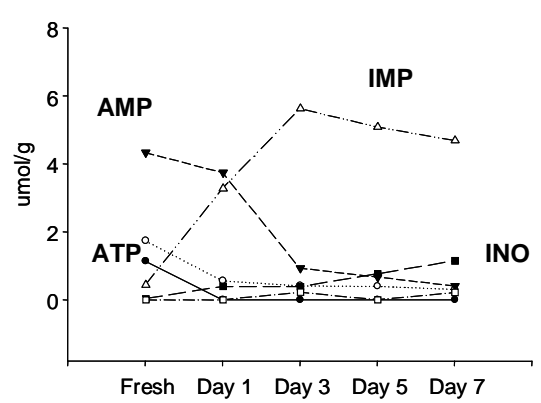
B) Creeled



C) Trawled 15 min



D) Trawled 1 h



E) Trawled 2 h

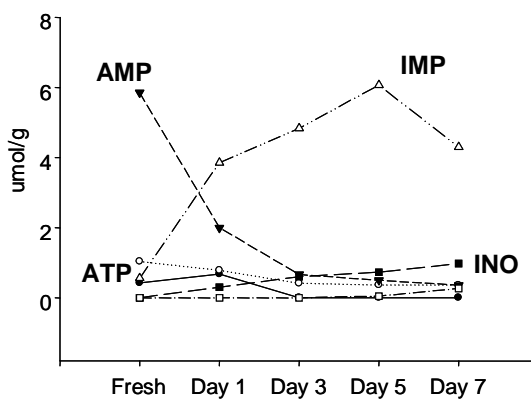


Figure 2.5. Nucleotide profiles in *Nephrops* tails when stored on ice up to 7 days following different capture methods: A) Rested B) Creeled C) Trawled for 15 min D) Trawled for 1 h and E) Trawled for 2 h. Values are the mean \pm S.E.M. of three different determinations.

Taken together, the concentrations of the different nucleotides allow the K-value to be calculated. The K-value was significantly higher in trawled animals on day 1, compared to rested and creeled animals (Figure 2.6), but after this time no significant differences were found between trawled and creeled animals. Furthermore, after day 3 no significant differences were found in K-values between trawled, creeled and rested animals. These results suggest that K-values are not strongly dependent on the initial state of the animal.

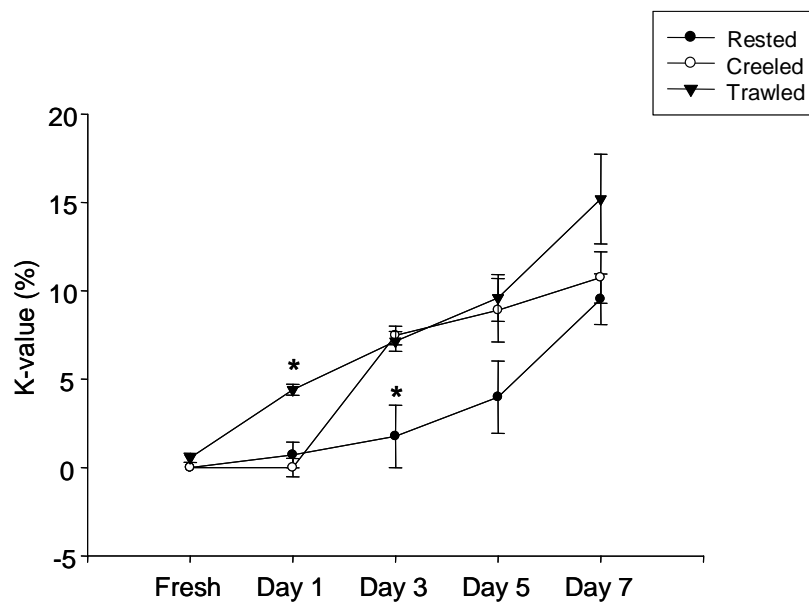


Figure 2.6. K-values in *Nephrops* tails when stored on ice up to 7 days following different capture methods. Values are the mean \pm S.E.M. of three different determinations.

K-values were also calculated for tails from animals trawled for different periods of time (Figure 2.7). No differences were found between the 3 groups, suggesting that trawling time does not have an effect on the K-values if the tails are subsequently stored on ice.

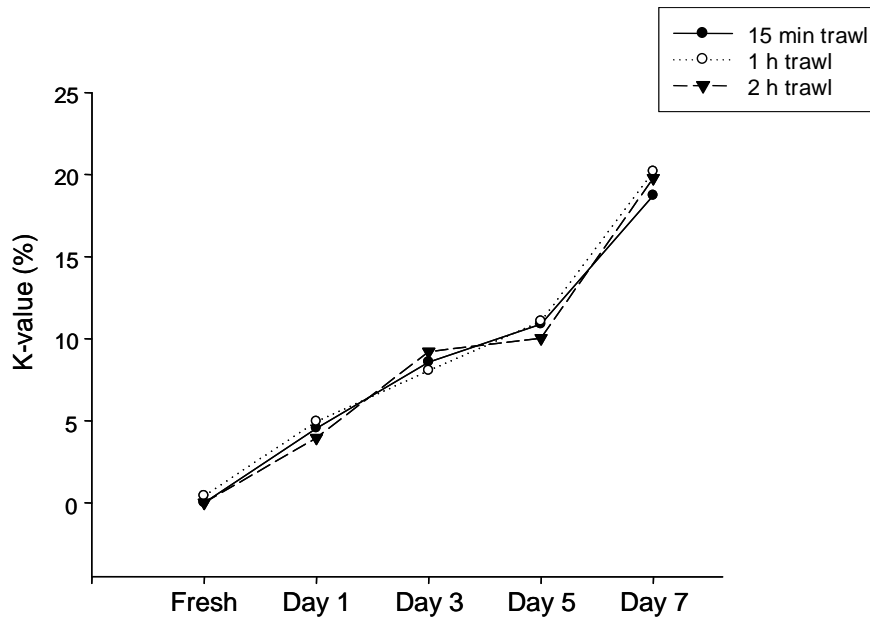


Figure 2.7. K-values in *Nephrops* tails when stored on ice up to 7 days after being trawled for different periods of time. Values are the mean \pm S.E.M. of three different determinations.

II B). Effect of temperature abuse on *Nephrops* tails: bacterial spoilage

Characterization of bacterial growth in tails held at different temperatures

The basal level of bacteria present in fresh tails was approximately 10^3 cfu g⁻¹ of meat. This basal level of bacteria was maintained for up to 6 days at 0°C, for up to 24h at 4°C and 8°C, but for only up to 8 h at 16°C. As shown in Figure 2.8, storage on ice arrested microbial proliferation, but *Nephrops* tails still supported a population of psychrotrophic bacteria. Furthermore, keeping *Nephrops* tails at temperatures ranging between 8-16°C for 2 days produced a high growth of bacteria, and concentrations increased up to approximately 10^7 cfu g⁻¹ in both cases.

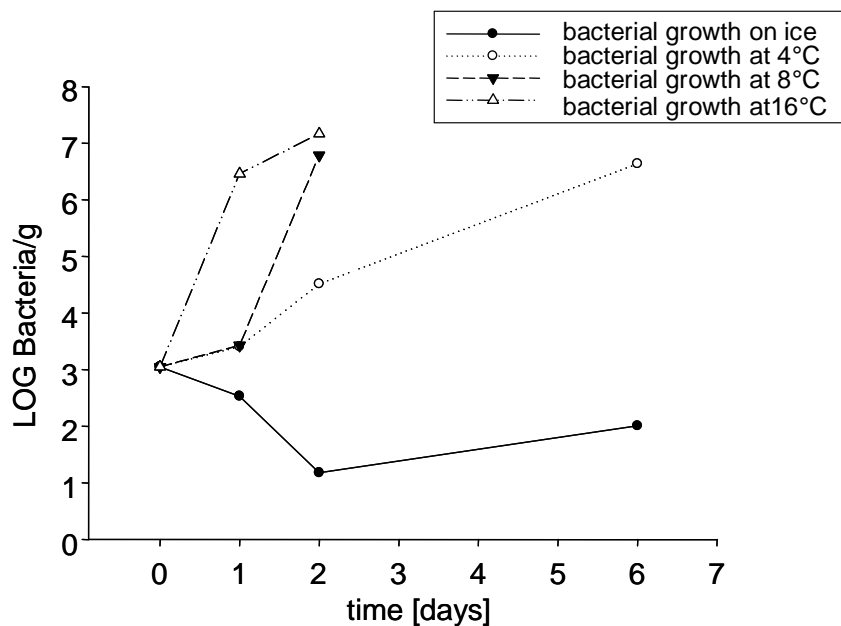


Figure 2.8. Changes in TVC for *Nephrops* tails kept at different temperatures for up to 6 days (tails on ice or at 4°C) or up to 2 days if temperatures were higher (8 and 16°C). Values are the mean \pm S.E.M. of five determinations.

Identification of the bacterial species implicated in the spoilage

To provide a better understanding of the spoilage mechanism in the tails, the possibility that specific bacteria were responsible for spoilage was investigated. The bacterial types present in tails in the temperature storage trials were identified to determine whether certain groups of bacteria were associated with the different storage temperatures, in particular the high temperatures, where high levels of bacteria were found.

A selection of bacterial isolates was taken from each temperature storage group, (0, 4 and 16°C) and identified using 16sRNA gene sequencing. Figure 2.9 shows a phylogram of the bacterial isolates identified. The phylogram shows selected bacterial isolates from each of the temperature groups alongside the type strains. It is clear that the bacterial isolates are separated into four main groups of bacteria - *Photobacterium* spp., *Vibrio splendidus*, *V. logei*, and *Pseudoalteromonas* spp. These are all Gram-negative bacteria, which predominate in marine environments. No single group was identified as being solely responsible for spoilage, as all of these bacterial types were identified throughout the three temperature groups.



Figure 2.9. Phylogram of bacterial isolates from the temperature storage trial compared with type strains. Four main groups are identified as indicated. (Bacterial isolates highlighted in colour are from the following isolation temperature groups: red=A, 0 °C; blue= B, 4 °C; green= F, 16 °C, Black =Type strains)

Capacity for TMAO reduction and H₂S production in bacterial isolates

Spoilage bacteria can use TMAO in anaerobic respiration to produce trimethylamine (TMA) using the enzyme trimethylamine N-oxide reductase. Additionally, *Nephrops* meat contains a rich source of free amino acids and other soluble nitrogenous compounds (Ruiz-Capillas and Moral, 2004), and potential spoilage bacteria may be able to utilise these amino acids as a source of nutrients for growth and multiplication. The amino acids (nitrogen source) are converted through oxidative deamination to ammonia (NH₃), which can be incorporated into the bacteria or can accumulate as volatile amines and free fatty acids in the tail meat of the *Nephrops*. Both TMA (trimethylamine) and TVB (total volatile bases) can be used as indicators of bacterial spoilage. In fact, pH values would provide an index of bacterial spoilage, as the basic compounds produced during bacterial spoilage would cause the pH in tail meat to increase.

The capacity for TMAO reduction and H₂S production was determined in the bacterial isolates from temperature storage trials using simple laboratory tests. Table 2.1 shows the results for TMAO reduction and H₂S production at 20°C by a selection of bacterial isolates identified from the temperature storage trial. Both activities occurred in a selection of isolates of all the types of bacteria identified and was not confined to any one of the four groups identified in Figure 2.9. However, overall, only 6 out of 21 in group A (0 °C) were positive for TMAO reduction and 4 out of 21 for H₂S production, whereas, in group F (16 °C), 23 out of 30 were positive for TMAO reduction and 18 out of 30 for H₂S production, which are significantly higher with p-values of <0.005. Even isolates of group B (4 °C) had higher activity than the

A (0 °C) group, with 11 out of 26 positive for TMAO reduction and 8 out of 26 for H₂S production. Therefore, it does suggest that these activities are involved in spoilage and can be prevented by keeping the storage temperature at 0 °C. Low temperatures are known to reduce or stop TMA production, with spoilage being prevented if fish are stored below 2 °C (Valle *et al.*, 1999).

To further this work, each of the bacterial isolates identified as being positive for TMAO reduction and H₂S production were incubated at their respective isolation temperatures, to determine if this altered their activities (Table 2.2). For TMAO reduction, the bacterial isolates from the lower temperatures of 0 °C and 4 °C either did not produce the activity or it was significantly reduced compared to the activity at 20 °C. When the isolates were exposed to room temperature (20°C) after incubation at isolation temperature the TMAO activities recorded as weak (for isolates B24-4 and B44-9) became more pronounced, while the others remained negative. The only difference in TMAO reduction with bacterial isolates from the higher temperature group of 16 °C was that one isolate (F29-13M) was negative at this temperature while all others remained positive.

For H₂S production, those isolates from Group A (isolated at 0 °C) and Group B (isolated at 4 °C) that were positive at 20 °C, subsequently lost their ability when held at their isolation temperatures, with only one isolate (B24-4) from group B producing activity at R.T. This is probably due to the fact they were held at R.T. for only 24 h compared to 48 h (Table 2.2). The exception in this low temperature group was isolate B44-9, identified as *Vibrio logei*, which was negative for H₂S at 20 °C, but

produced weak activity at 4 °C, which subsequently increased further when returned to R.T.

For group F (16 °C) four isolates (F12-6, F31-7, F47-10 and F8-10M) that were positive for H₂S at 20 °C remained so at their isolation temperature, whereas six other isolates positive for H₂S at 20 °C were negative at their isolation temperature, with the activity returning when the isolates were subsequently exposed to R.T. All other isolates that were positive at 20 °C remained negative at their isolation temperature and subsequently at R.T. Again, as with isolate B44-9, the isolates F10-5, F23-6, F40-9 and F17-4M, all *V. splendidus*-like, were exceptions to these trends, being negative for H₂S at 20 °C but producing it at their isolation temperature.

Therefore, from these results it can be concluded that the isolates from 0 °C (Group A) did not reduce TMAO or produce H₂S when held at 0 °C, but they were capable of these activities at 20 °C. The same was true for the isolates from 4 °C (Group B), apart from a few exceptions. This would be of particular concern if this temperature (4°C) was used for storage, as the potential for spoilage to occur exists even under these conditions. With group F (16 °C), the TMAO activity was unaffected and remained throughout the isolation temperature of 16 °C. Therefore, it does suggest that these activities are important in spoilage with these types of bacteria, but it is preventable by storing the *Nephrops* on ice.

Table 2.1. TMAO reduction and H₂S production of selected bacterial isolates from the temperature storage trial identified by 16sRNA sequencing. 0 = negative, 1 = positive. Isolates are highlighted according to their isolation temperatures: A, 0 °C; B, 4 °C; F, 16 °C.

Bacterial isolate	Storage time	TMAO reduction	H ₂ S production	16S rRNA match
A 13-7	0 h	0	0	<i>Photobacterium phosphoreum</i>
A 9-5	0 h	0	0	<i>Photobacterium phosphoreum</i>
A 4-2M	0 h	0	0	<i>Photobacterium phosphoreum</i>
A 25-6M	24 h	1	0	<i>Photobacterium phosphoreum</i>
A 24-15M	72 h	0	0	<i>Photobacterium phosphoreum</i>
A 19-6	72 h	1	0	<i>Photobacterium phosphoreum</i>
B 14-4	16 h	0	0	<i>Photobacterium phosphoreum</i>
B 8-3	16 h	0	0	<i>Photobacterium phosphoreum</i>
B 1-11	48 h	0	0	<i>Photobacterium phosphoreum</i>
B 14-2	48 h	0	0	<i>Photobacterium phosphoreum</i>
B 25-16	6 d	0	0	<i>Photobacterium phosphoreum</i>
B 26-17	6 d	0	0	<i>Photobacterium phosphoreum</i>
F 9-1M	16 h	1	1	<i>Photobacterium phosphoreum</i>
F 14-2M	16 h	0	0	<i>Photobacterium phosphoreum</i>
F 19-7	16 h	0	0	<i>Photobacterium phosphoreum</i>
F 34-9	16 h	1	1	<i>Photobacterium phosphoreum</i>
F 3-2	16 h	1	1	<i>Photobacterium phosphoreum</i>
F 5-3	16 h	1	1	<i>Photobacterium phosphoreum</i>
F 39-12	16 h	1	1	<i>Photobacterium phosphoreum</i>
F 8-4	16 h	1	1	<i>Photobacterium phosphoreum</i>
F 12-2	24 h	0	0	<i>Photobacterium phosphoreum</i>
F 16-3	24 h	0	0	<i>Photobacterium phosphoreum</i>
F 11-1	24 h	0	0	<i>Photobacterium phosphoreum</i>
F 17-4	24 h	1	1	<i>Photobacterium phosphoreum</i>
F 20-5	24 h	1	0	<i>Photobacterium phosphoreum</i>

Table 2.1. Continued

Bacterial isolate	Storage time	TMAO reduction	H ₂ S production	16S rRNA match
A 17-9	0 h	0	0	<i>Vibrio logei</i>
A 5-3	0 h	0	0	<i>Vibrio logei</i>
B 6-1M	16 h	1	0	<i>Vibrio logei</i>
B 9-2M	16 h	1	0	<i>Vibrio logei</i>
B 12-3M	16 h	0	0	<i>Vibrio logei</i>
B 4-1	16 h	1	0	<i>Vibrio logei</i>
B 48-10	24 h	0	0	<i>Vibrio logei</i>
B 28-1	24 h	0	0	<i>Vibrio logei</i>
B 30-7M	24 h	0	0	<i>Vibrio logei</i>
B 24-4	48 h	1	0	<i>Vibrio logei</i>
B 44-9	6 d	1	0	<i>Vibrio logei</i>
B 42-8	6 d	0	0	<i>Vibrio logei</i>
B 40-7	6 d	0	1	<i>Vibrio logei</i>
F 27-6M	16 h	1	0	<i>Vibrio logei</i>
F 35-14M	24 h	1	0	<i>Vibrio logei</i>

Table 2.1. Continued

Bacterial isolate	Storage time	TMAO reduction	H ₂ S production	16S rRNA match
A 18-5	72 h	1		1 <i>Vibrio splendidus</i> -like
A 22-22	72 h	0		0 <i>Vibrio splendidus</i> -like
A 1-1	72 h	1		1 <i>Vibrio splendidus</i> -like
A 45-10	6 d	0		0 <i>Vibrio splendidus</i> -like
A 14-9M	72 h	1		1 <i>Vibrio splendidus</i> -like
B 38-9M	24 h	0		0 <i>Vibrio splendidus</i> -like
B37-15M	6 d	1		0 <i>Vibrio splendidus</i> -like
F 17-4M	16 h	1		1 <i>Vibrio splendidus</i> -like
F 45-8M	16 h	1		1 <i>Vibrio splendidus</i> -like
F 10-5	16 h	1		0 <i>Vibrio splendidus</i> -like
F 12-6	16 h	1		1 <i>Vibrio splendidus</i> -like
F 23-6	24 h	1		0 <i>Vibrio splendidus</i> -like
F 40-9	24 h	1		0 <i>Vibrio splendidus</i> -like
F 8-10M	24 h	1		1 <i>Vibrio splendidus</i> -like

Table 2.1. Continued

Bacterial isolate	Storage time	TMAO reduction	H ₂ S production	16S rRNA match
A 3-2	72 h	1		1 <i>Pseudoalteromonas</i> sp.
A 10-19	72 h	0		0 <i>Pseudoalteromonas</i> sp.
A 38-7M	24 h	0		0 <i>Pseudoalteromonas</i> sp.
B 16-4M	16 h	1		1 <i>Pseudoalteromonas</i> sp.
B 43-10M	24 h	0		0 <i>Pseudoalteromonas</i> sp.
B 5-12	48 h	0		0 <i>Pseudoalteromonas</i> sp.
B 22-14	48 h	0		0 <i>Pseudoalteromonas</i> sp.
B 35-6	6 d	0		0 <i>Pseudoalteromonas</i> sp.
F 16-3M	16 h	0		0 <i>Pseudoalteromonas</i> sp.
F 25-11M	24 h	0		0 North Sea bacterium (<i>Pseudoalteromonas</i>)
F 31-7	24 h	1		1 <i>Pseudoalteromonas</i> sp.
F 47-10	24 h	1		1 <i>Pseudoalteromonas</i> sp.
F 29-13M	24 h	1		0 <i>Pseudoalteromonas</i> sp.
F 8-10M	24 h	1		1 <i>Vibrio splendidus</i> -like
B 19-5M	16 h	1		1 <i>Vibrio</i> sp.
F 24-5M	16 h	1		1 <i>Vibrio</i> sp.
F 29-7M	16 h	1		1 <i>Vibrio</i> sp.
F 46-9M	16 h	1		1 <i>Vibrio</i> sp.
F 26-12M	24 h	1		1 <i>Vibrio</i> sp.
F 43-15M	24 h	1		1 <i>Vibrio</i> sp.
A 1-1M	0 h	0		0 <i>Shewanella woodyi</i>
A 14-5M	0 h	0		0 <i>Shewanella woodyi</i>
B 23-6M	16 h	1		1 <i>Shewanella woodyi</i>
A 6-3M	0 h	0		0 <i>Psychrobacter</i> sp.
A 11-4M	0 h	0		0 <i>Psychrobacter</i> sp.
A 32-10M	6 d	0		0 <i>Aeromonas schuberti</i>

Table 2.2. Bacterial isolates that are positive for TMAO reduction and H₂S production at 20 °C were tested for the same activity at their isolation temperature. Isolates are highlighted according to their isolation temperatures: A, 0 °C; B, 4 °C; F, 16 °C.

Bacterial isolate	Storage time	TMAO reduction at 20°C after 48 h	TMAO reduction at isolation temp after 48 h	TMAO reduction at room temp 24h after being held at isolation temp	H ₂ S production at 20°C after 48 h	H ₂ S production at isolation temp after 48 h	H ₂ S production at room temp 24h after being held at isolation temp.	16S rRNA match
A25-6M	24h	1	0		0	0		<i>Photobacterium phosphoreum</i>
A19-6	72h	1	0	0	0	0	0	<i>Photobacterium phosphoreum</i>
A18-5	72h	1	0	0	1	0	0	<i>Vibrio splendidus</i> -like
A1-1	72h	1	0	0	1	0	0	<i>Vibrio splendidus</i> -like
A14-9M	72h	1	0	0	1	0	0	<i>Vibrio splendidus</i> -like
A3-2	72h	1	0	0	1	0	0	<i>Pseudoalteromonas</i> sp.
B16-4M	16h	1	0	0	1	0	0	<i>Pseudoalteromonas</i> sp.
B23-6M	16h	1	0	0	1	0	0	<i>Shewanella woodyi</i>
B19-5M	16h	1	0	0	1	0	0	<i>Vibrio</i> sp.
B4-1	16h	1	0	0	0	0	0	<i>Vibrio logei</i>
B6-1M	16h	1	0	0	1	0	0	<i>Vibrio logei</i>
B9-2M	16h	1	0	0	1	0	0	<i>Vibrio logei</i>
B24-4	48h	1	1 (weak)	1	1	0	1	<i>Vibrio logei</i>
B44-9	6d	1	1 (weak)	1	0	1 (weak)	1	<i>Vibrio logei</i>
B37-15M	6d	1	0	0	0	0	0	<i>Vibrio splendidus</i> -like
F34-9	16h	1	1	1	1	0	1	<i>Photobacterium phosphoreum</i>
F3-2	16h	1	1	1	1	0	1	<i>Photobacterium phosphoreum</i>
F5-3	16h	1	1	1	1	0	1	<i>Photobacterium phosphoreum</i>
F39-12	16h	1	1	1	1	0	1	<i>Photobacterium phosphoreum</i>
F8-4	16h	1	1	1	1	0	1	<i>Photobacterium phosphoreum</i>
F9-1M	16h	1	1	1	1	0	0	<i>Photobacterium phosphoreum</i>
F17-4M	16h	1	1	1	0	1	1	<i>Vibrio splendidus</i> -like
F45-8M	16h	1	1	1	1	0	0	<i>Vibrio splendidus</i> -like
F10-5	16h	1	1	1	0	1	1	<i>Vibrio splendidus</i> -like
F12-6	16h	1	1	1	1	1	1	<i>Vibrio splendidus</i> -like
F24-5M	16h	1	1	1	1	0	0	<i>Vibrio</i> sp.
F29-7M	16h	1	1	1	1	0	0	<i>Vibrio</i> sp.
F46-9M	16h	1	1	1	1	0	0	<i>Vibrio</i> sp.
F27-6M	16h	1	1	1	1	0	0	<i>V. logei</i>
F17-4	24h	1	1	1	1	0	1	<i>Photobacterium phosphoreum</i>
F20-5	24h	1	1	1	0	0	0	<i>Photobacterium phosphoreum</i>
F23-6	24h	1	1	1	0	1	1	<i>Vibrio splendidus</i> -like
F40-9	24h	1	1	1	0	1	1	<i>Vibrio splendidus</i> -like
F8-10M	24h	1	1	1	1	1	1	<i>Vibrio splendidus</i> -like
F35-14M	24h	1	1	1	1	0	0	<i>V. logei</i>
F43-15M	24h	1	1	1	1	0	0	<i>Vibrio</i> sp.
F26-12M	24h	1	1	1	1	0	0	<i>Vibrio</i> sp.
F31-7	24h	1	1	1	1	1	1	<i>Pseudoalteromonas</i> sp.
F47-10	24h	1	1	1	1	1	1	<i>Pseudoalteromonas</i> sp.
F29-13M	24h	1	0	0	0	0	0	<i>Pseudoalteromonas</i> sp.

II. C) Simulated Commercial Trial I – Different temperature abuses

Changes in muscle pH

Muscle pH in a catch of *Nephrops* trawled for 1.5 h was 6.91 ± 0.03 and increased to 8.08 ± 0.07 on day 7 when tails were stored on ice. Tails stored at 16°C for 24 h showed a more rapid increase in muscle pH compared to tails kept on ice all the time. By day 2, pH values in tails kept on ice were 6.91 ± 0.04 while the pH of tails kept at 16°C for 24 h and then on ice was 7.52 ± 0.14 . This pattern was similar to the one obtained in tails incubated at 8°C for 24 h and then stored on ice. However, after 2 days values between the different groups were more similar, and finally on day 7 there was no significant difference in muscle pH between the different groups (Table 2.3).

Table 2.3. Changes in muscle pH in *Nephrops* tails during ice storage after different temperature/time abuses. Values are the mean \pm S.E.M. of ten different tails.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
On ice	7.05 ± 0.03	6.91 ± 0.04	7.70 ± 0.11	7.54 ± 0.12	7.91 ± 0.07	8.14 ± 0.07	8.08 ± 0.07
8°C 24 h	6.75 ± 0.06	7.48 ± 0.10	7.52 ± 0.06	7.97 ± 0.06	8.00 ± 0.07	8.11 ± 0.06	8.16 ± 0.06
16°C 8 h	7.15 ± 0.02	7.01 ± 0.04	7.53 ± 0.06	7.78 ± 0.08	7.89 ± 0.09	7.98 ± 0.09	8.17 ± 0.06
16°C 24 h	7.12 ± 0.05	7.52 ± 0.14	8.02 ± 0.09	7.82 ± 0.11	8.09 ± 0.08	8.08 ± 0.05	8.34 ± 0.04

Total Viable Counts (TVC)

The results for TVCs in tails which have been stored on ice continuously did not show any increase in bacterial numbers during the time course of the experiment (Figure 2.10). The bacteria numbers stayed around a value of $10^2 - 10^3$ bacteria per gram wet weight. No apparent spoilage due to bacterial growth is therefore to be expected. Tails which were incubated at 8°C for 24 h before being stored on ice were similar to the tails which were incubated at 8°C for 24 h. Although no apparent effect could be

detected over the first 2 days after the incubation, between days 2-3 the effects of the temperature abuse became apparent and the numbers rose from 10^3 to 10^5 within 2 days. After 5 – 6 days of incubation the numbers reached the same levels as in the heavily spoiled 16°C 24 h group. On the other hand, tails which had been incubated at 16°C for 8 h before being stored on ice were somewhat different to the 24 h group. Here, the temperature abuse caused no rapid rise in bacteria per gram of wet weight within the tail muscle sample, and the bacterial numbers decreased somewhat during the first 4 – 5 days. After 5 – 6 days of incubation the numbers finally rose, but never reached the numbers seen in the 16°C 24 h group. Finally the results for TVCs in tails which had been incubated at 16°C for 24 h before being stored on ice show that this degree of temperature abuse caused a rapid rise in bacteria per gram of wet weight within the tail muscle sample.

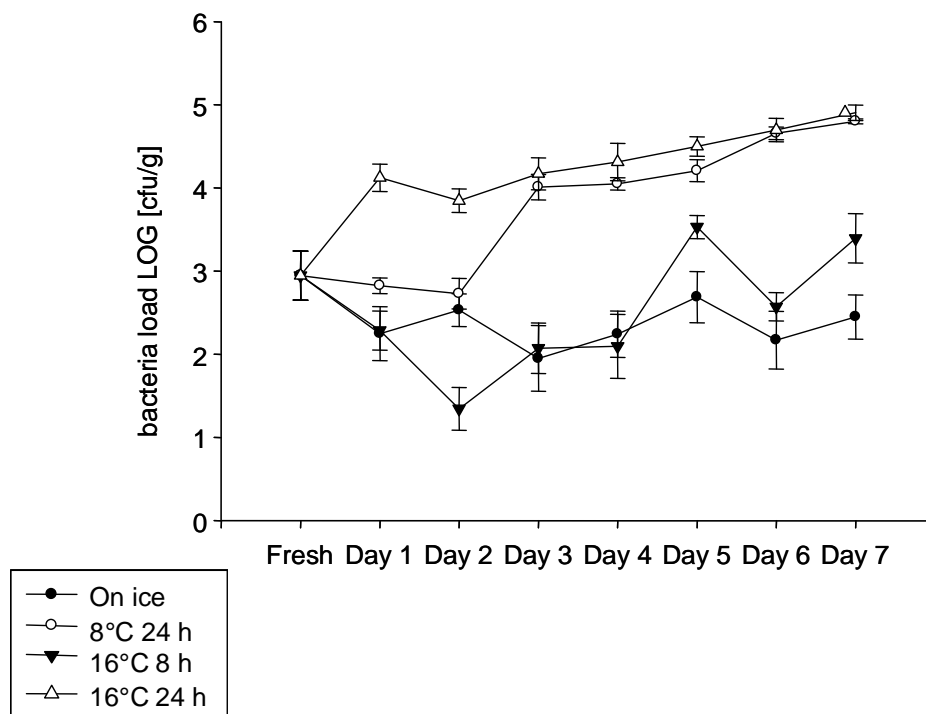


Figure 2.10. Changes in TVC for *Nephrops* tails kept on ice up to 7 days after different temperature/time abuses. Values are the mean \pm S.E.M. of five determinations.

The bacterial load before the incubation (day 0) was within the standard range of 10^3 bacteria per gram, but 24 h after the incubation (day 1) the numbers were already as high as 10^5 bacteria per gram, and continued to rise slowly over the subsequent days.

Trimethylamine (TMA), Ammonia and Thiobarbituric Reactive Substances (TBARs) concentrations in the muscle

TMA values for tails kept on ice after different temperature abuses are shown in Figure 2.11. TMA did not increase significantly after 7 days if tails were kept on ice all the time, or had suffered temperatures abuse of 16°C for no more than 8 hours. However, if tails were left at 16°C for 24 hours TMA values were significantly higher from day 1 and increased over time to reach values of 7.76 ± 1.04 mg TMA/100 g muscle on day 7. Interestingly, TMA in tails kept at 8°C for 24 h did not increase up to 5 days but by 7 days had increased to similar values (7.90 ± 1.41 mg TMA/100 g muscle) of those tails kept at 16°C for 24 h.

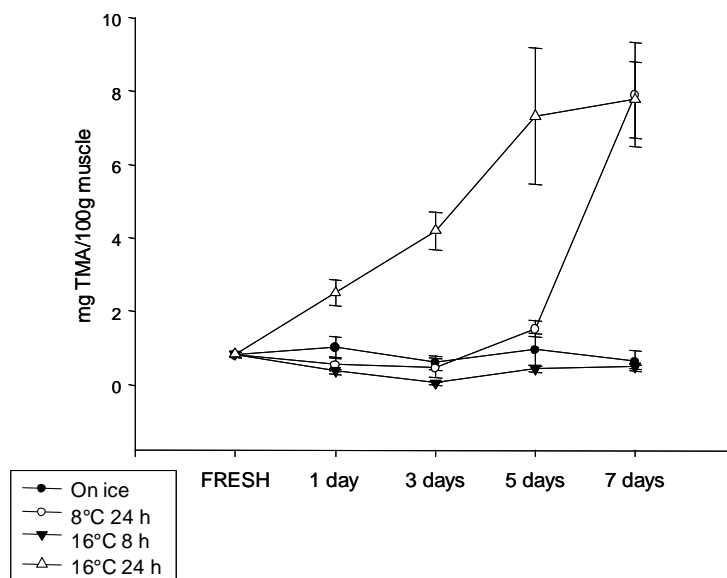


Figure 2.11. Changes in TMA concentration for *Nephrops* tails kept on ice up to 7 days after different temperature/time abuses. Values are the mean \pm S.E.M. of five determinations.

Ammonia changes in the muscle were also analysed and results are presented in Figure 2.12. In this case, a slight increase in ammonia levels was observed even if tails were kept on ice all the time. Values after capture were 4.53 ± 0.64 mg NH₃/100 g muscle, while after 7 days on ice ammonia concentration was 13.37 ± 1.85 mg NH₃/100 g muscle. However, higher values were obtained when tails were held at 16°C for 24 h before being placed on ice, and in tails held at 8°C for 24 h (but in this case only on day 7).

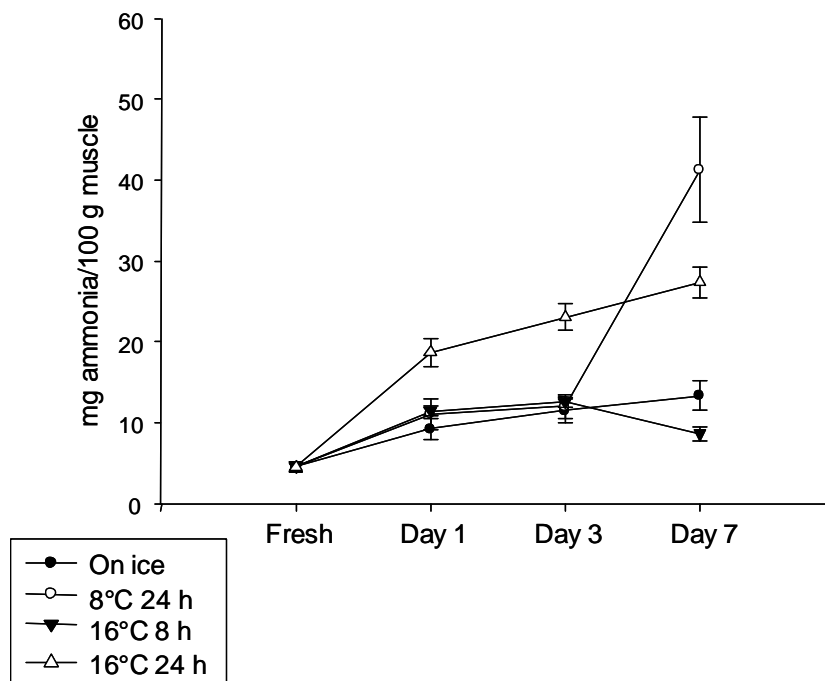


Figure 2.12. Changes in ammonia concentration for *Nephrops* tails kept on ice up to 7 days after different temperature/time abuses. Values are the mean \pm S.E.M. of five determinations.

TBARs were also analysed, and no difference was found between tails held on ice all the time compared to tails that suffered different temperature/time abuses (Figure 2.13). This indicates that lipid oxidation would be not a key issue in tails of *Nephrops* during ice storage.

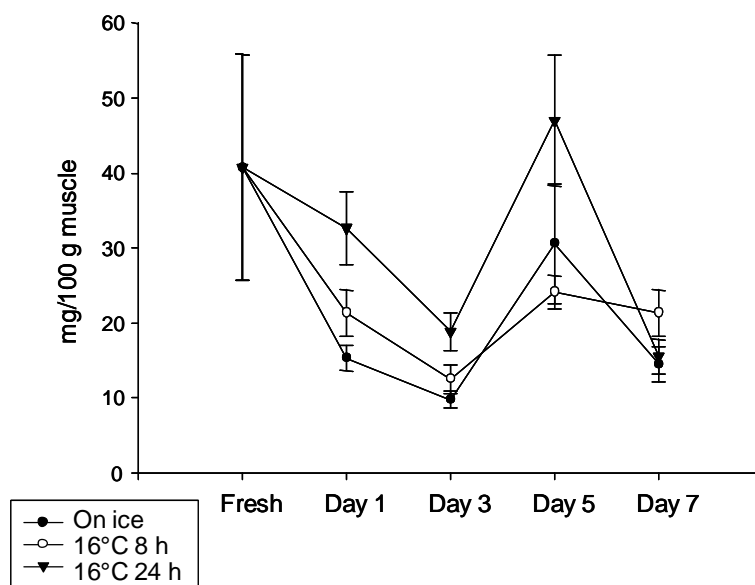


Figure 2.13. Changes in TBARS, expressed as mg MDA per 100 g of muscle in *Nephrops* tails kept on ice up to 7 days after different temperature/time abuses. Values are the mean \pm S.E.M. of five determinations.

ATP breakdown products and K-values

ATP breakdown products were analysed by HPLC and the results are shown in Figure 2.14. Immediately after trawling the main nucleotide in *Nephrops* tails was AMP rather than ATP, indicating a rapid breakdown of ATP in this species. In the tails held on ice all the time, IMP concentrations increased rapidly over the first 3 days of ice storage and maximum values were reached on day 5 ($5.87 \pm 0.82 \mu\text{mol/g}$ muscle). After this time IMP values started to decrease at a slow rate. INO was formed from day 1 of ice storage and increased steadily in concentration to $0.69 \pm 0.10 \mu\text{mol/g}$ muscle by day 7. *Nephrops* tails stored on ice formed Hx (related to a bitter-off taste in fish products) at a relatively slow rate, reaching a concentration of $0.08 \pm 0.03 \mu\text{mol/g}$ muscle on day 7. Several differences were found between tails kept on ice all the time and tails exposed to different temperature/time abuses before placing them on ice, especially those kept at 16°C for 24 h (Figure 2.14D).

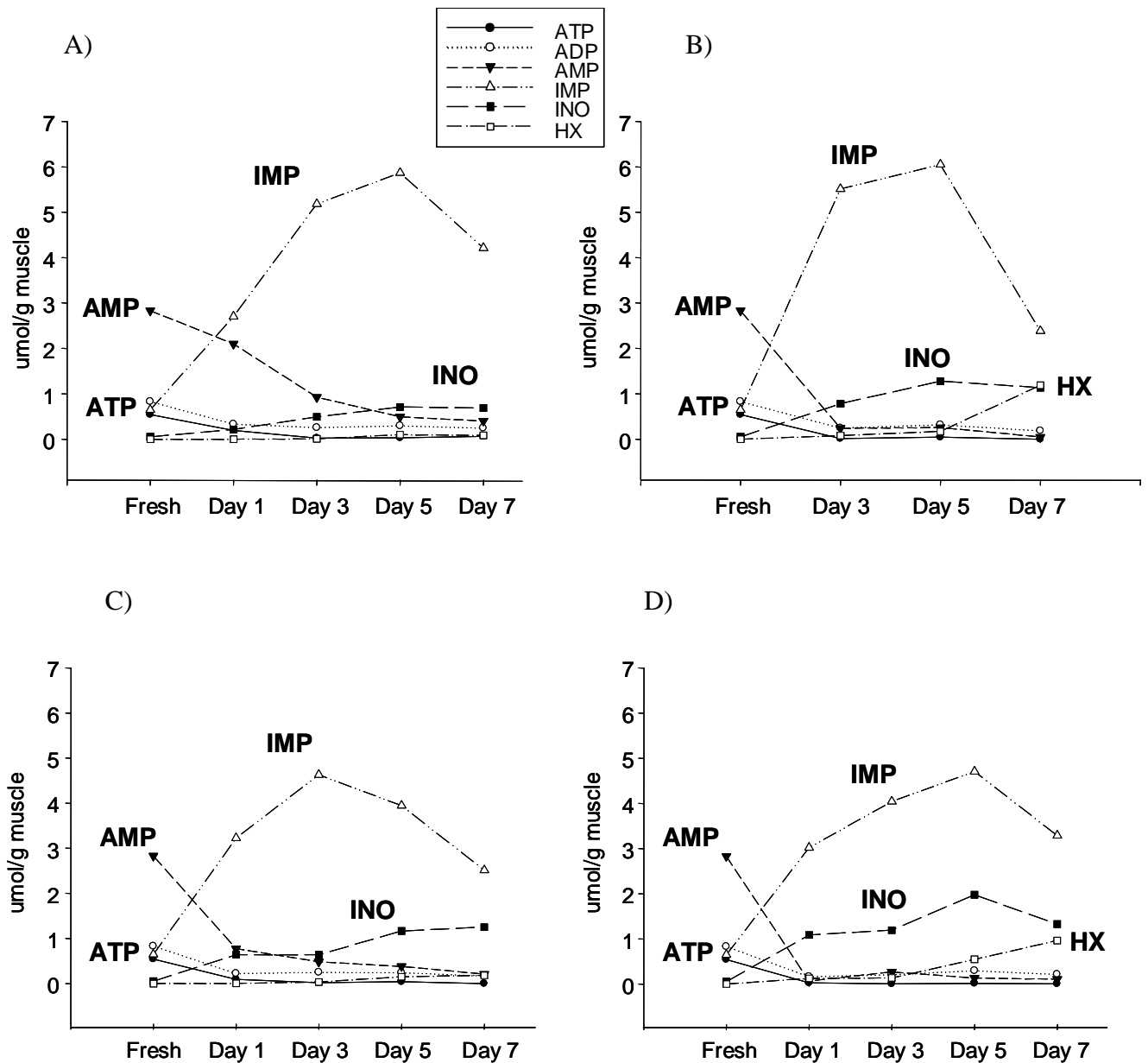


Figure 2.14. Changes in ATP breakdown products in *Nephrops* tails kept stored on ice up for 7 days on ice after different temperature/time abuses. A) tails kept on ice from start; B) tails exposed to 8°C for 24 h; C) tails exposed to 16°C for 8 h and D) tails exposed to 16°C for 24 hours. Values are the mean \pm S.E.M. of three determinations.

In this case AMP disappeared more rapidly and although IMP increased up to day 5, maximum values at this time were lower ($4.71 \pm 0.01 \mu\text{mol/g}$ muscle) than those obtained with tails kept on ice all the time. INO levels also increased more over storage time (INO levels on day 7 were $1.33 \pm 0.18 \mu\text{mol/g}$ muscle) and finally Hx

reached values around 10 times ($0.96 \pm 0.40 \mu\text{mol/g}$ muscle) higher on day 7 compared to tails held on ice all the time.

K-values were also calculated (Figure 2.15) and results indicate an increase over storage time, even if tails were kept on ice throughout. However, the K-values on ice were much lower than those obtained when tails suffered different temperature/time abuses. These differences were apparent from day 1, when tails kept continuously on ice showed a K-value of $4.23 \pm 1.08 \%$ while tails exposed initially to a temperature of 16°C for 24 h had a K-value of $27.01 \pm 0.27 \%$ (above 20%). In many fish species, K-values of around 20% are related as a freshness limit. However, this limit is species dependent, and the freshness limit for *Nephrops* remains to be determined.

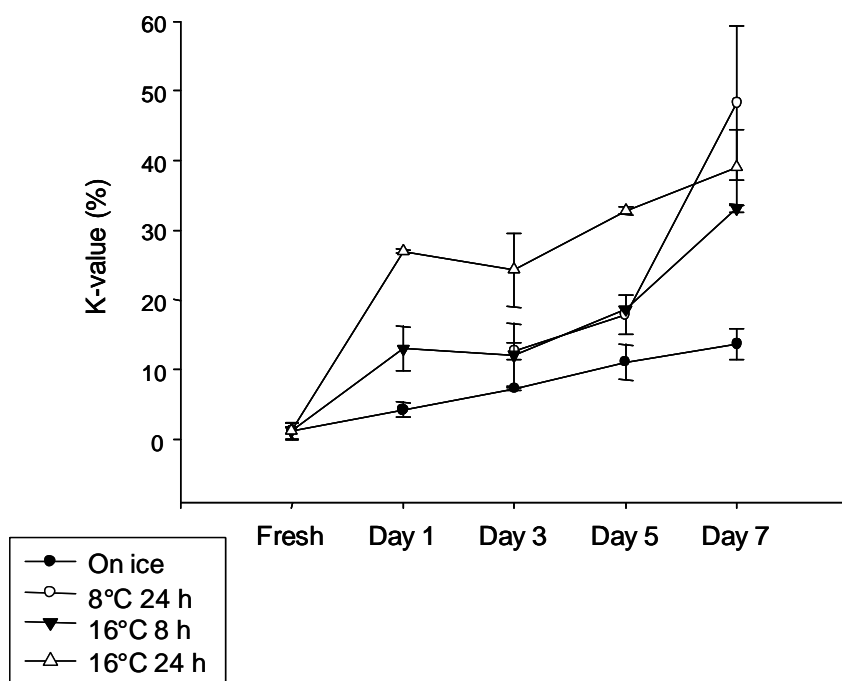


Figure 2.15. Changes in K-value percentages for *Nephrops* tails kept on ice up to 7 days after different temperature/time abuses. Values are the mean \pm S.E.M. of three determinations.

In order to visualise all the results, a summary table has been constructed (Table 2.4).

The only parameter that changed significantly in tails stored continuously on ice was

the pH. In contrast, all parameters measured in tails held at 16°C for 24 h prior to placing them on ice were affected from day 1. For the other two temperature/time abuses applied these parameters were affected after 3 days on ice, which represents a delay, compared to tails held initially at 16°C for 24 h.

Table 2.4. Summary table including all the parameters measured in *Nephrops* tails held on ice all the time or after suffering different temperature/time abuses. ■ Indicates not significantly different from fresh samples and ✗ indicates that the parameter is different from fresh samples.

	On ice				8°C 24 h				16 °C 8 h				16 °C 24 h			
DAY 1	■	■	■	■	■	■	■	■	■	■	■	■	✗	✗	✗	✗
DAY 3	■	✗	■	■	✗	✗	■	■	■	✗	■	■	✗	✗	✗	✗
DAY 5	■	✗	■	■	✗	✗	■	(✗)	✗	✗	■	(✗)	✗	✗	✗	✗
DAY 7	■	✗	■	■	✗	✗	✗	✗	✗	✗	■	✗	✗	✗	✗	✗
	Bacteria load	pH	TMA	K-value	Bacteria load	pH	TMA	K-value	Bacteria load	pH	TMA	K-value	Bacteria load	pH	TMA	K-value

II. D) Simulated Commercial Trial II – Short term temperature abuse

Changes in muscle pH

As shown in Figure 2.16, no differences were found in muscle pH between tails kept on ice all the time and tails incubated at 16°C for 4 h and then placed on ice. Starting pH was 6.76 ± 0.02 and reached values around 7.35 and 7.53 after 5 days, the differences being not significantly different.

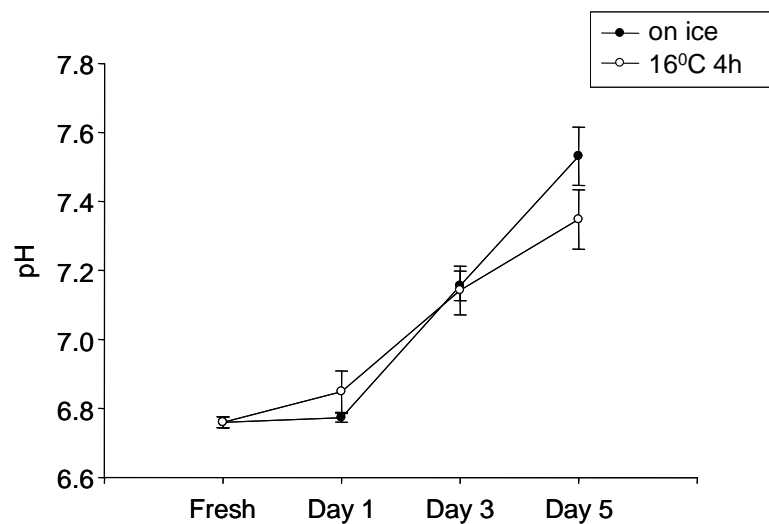


Figure 2.16. Changes in muscle pH in *Nephrops* tails kept on ice up to 5 days after being incubated for 4 h on ice or at 16°C for 4 h. Values are the mean \pm S.E.M. of ten different determinations.

Total Viable Counts (TVC)

The results for TVCs in tails which were incubated at 16°C for a period of 4 h were not significantly different from a control which was iced continuously (Figure 2.17). Therefore, no difference in spoilage would be expected between these two groups due to bacterial growth.

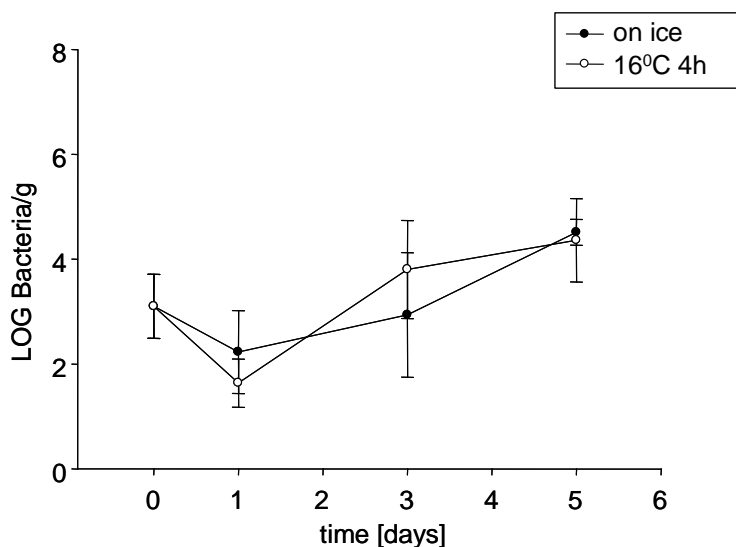


Figure 2.17. Changes in TVC for *Nephrops* tails kept on ice up to 7 days after being kept on ice all the time or incubated at 16°C for 4 h and then stored on ice. Values are the mean \pm S.E.M. of five determinations.

ATP breakdown products and K-values

In order to determine if a temperature abuse of 16°C for 4 h would have an effect on the freshness of tails kept on ice, nucleotide concentrations were determined and K-values were calculated. Nucleotide concentrations are shown in Figure 2.18. Profiles between both groups were similar, although ATP and AMP appear to drop more rapidly in tails that were incubated at 16°C for 4 h before being placed on ice. In both cases, IMP increased significantly from day 1 and maximum levels were reached on day 5. INO concentrations were detectable from day 1 and increased similarly with storage time in both treatments while very low levels of HX were recorded (values ranged from 0 to 0.02 $\mu\text{mol/g}$ on day 5).

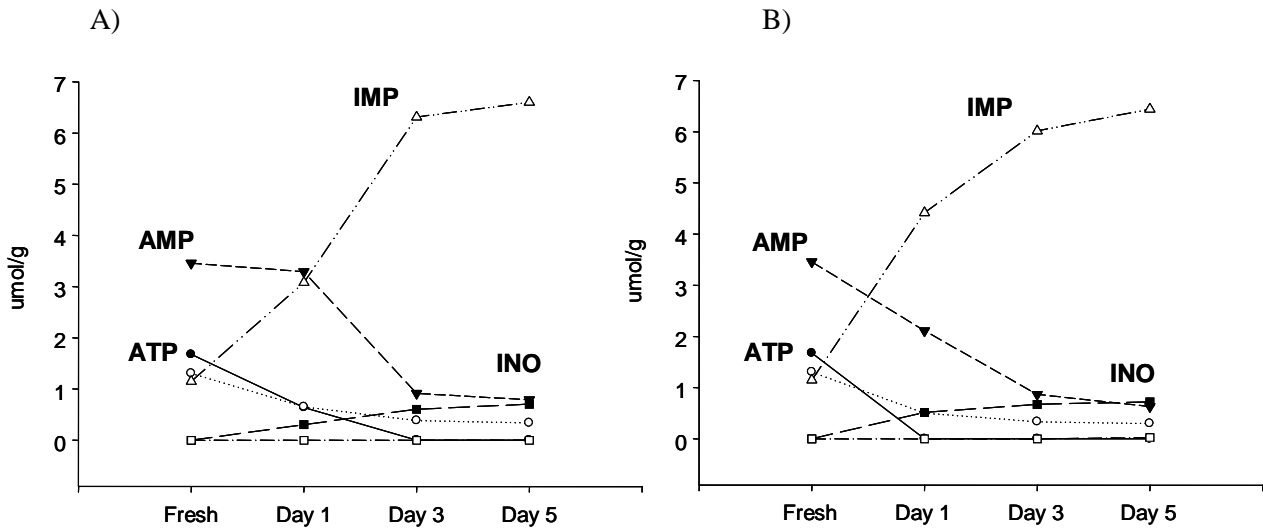


Figure 2.18. Changes in ATP breakdown products in *Nephrops* tails A) kept on ice all the time or B) incubated at 16°C for 4 h before being placed on ice. Values are the mean \pm S.E.M. of three determinations.

Based on the concentrations of these nucleotides, K-values were obtained for both treatments (Figure 2.19). The K-values for the two treatments were different on day 1, but after this time no significant differences were found, indicating that an incubation of 16°C for 4 h would not have a detrimental effect on the K-values if tails were kept on ice after day 1.

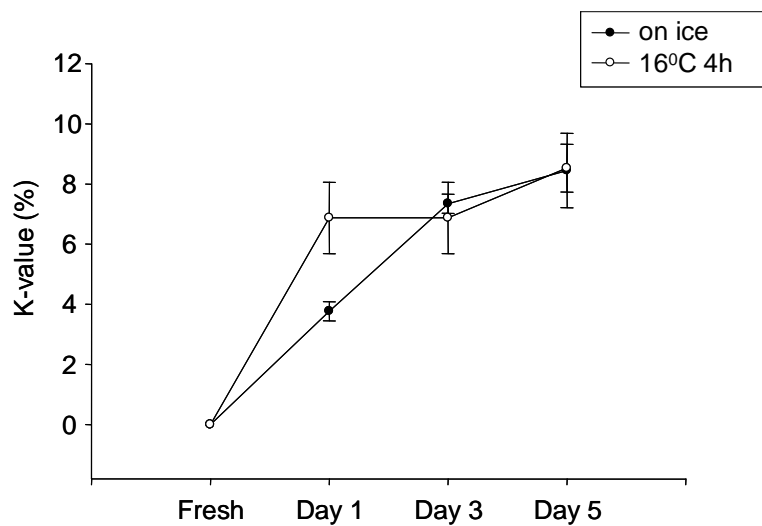


Figure 2.19. Changes in K-value percentages for *Nephrops* tails kept on ice all the time or B) incubated at 16°C for 4 h before being placed on ice. Values are the mean \pm S.E.M. of three determinations.

II. E) Simulated Commercial Trial III: The effect of washing

In order to determine whether the washing the tails on landing has any effect on quality or on spoilage rate, the effect of this procedure on bacteria load was measured. Unwashed tails showed significantly higher levels of bacteria on day 3 and especially on day 5 of ice storage, compared to washed individuals (Figure 2.20). This highlights the value of washing as a measure to prevent early spoilage, and suggests that it should be recommended as a routine post-capture treatment.

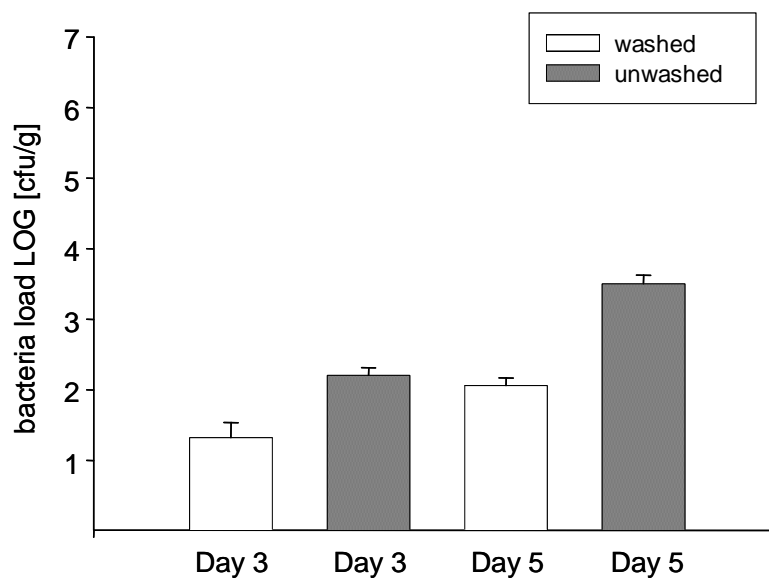


Figure 2.20. Bacteria load measured as TVC in *Nephrops* tails which were washed or unwashed on board and stored on ice for up to 5 days. Values are the mean \pm S.E.M. of five different tails.

III. DETERMINATION OF CATCH COMPOSITION AND DAMAGE INDEX IN *NEPHROPS* TAILS AFTER TRAWLING

Carapace Length

Overall, in trawls made from October 2005 to October 2006, male *Nephrops norvegicus* were significantly larger (mean carapace length (MCL) = 29.0mm) than the females (MCL = 27.1mm), as shown in figure 3.1.

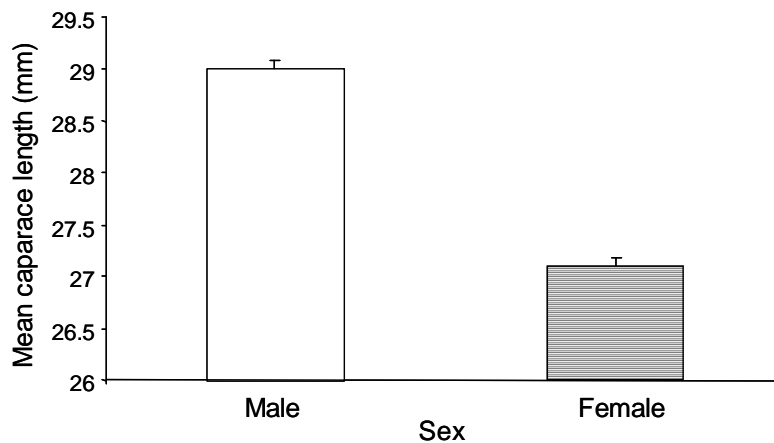


Figure 3.1. Mean carapace length of male and female *Nephrops norvegicus* from all trawls. Error bars = one standard error. Mann-Whitney test: $W = 9847385.0$, $p = 0.0000$

Trawl duration appeared to affect significantly the MCL of *N. norvegicus* since individuals from long trawls were slightly smaller (MCL = 27.7mm) than those from short trawls (MCL = 28.5mm). There was also a significant difference between the carapace lengths over time. The Kruskal-Wallis multiple comparisons test showed significant differences between several months. Generally, the median carapace length appeared to be greatest in December 2005, May 2006, June 2006 and September 2006, and least in October 2005, November 2005 and October 2006. The median carapace length was greatest in May 2006 (30mm), and was significantly different from every other month except September 2006 (Figure 3.2).

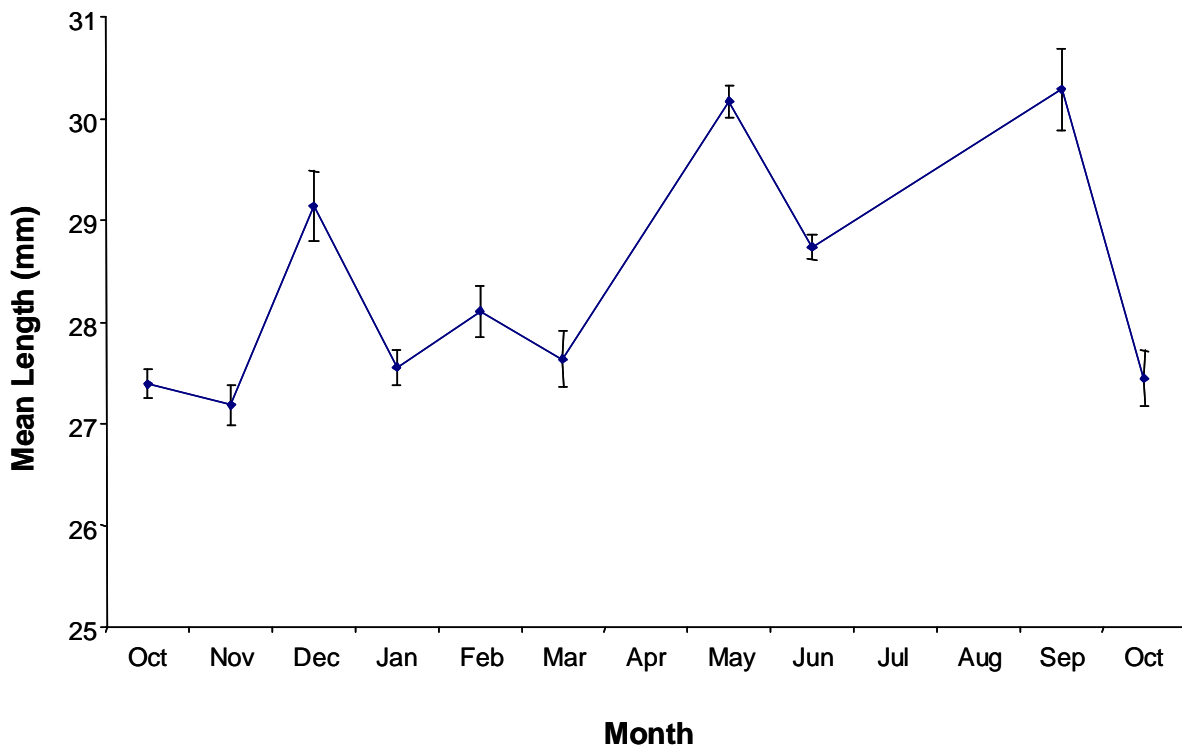


Figure 3.2. Mean caparace length of trawled *Nephrops norvegicus* between October 2005 and October 2006.

Sex

Overall, male *Nephrops norvegicus* were more abundant than females ($W = 467$, $p = 0.0068$). Males comprised between 57.5 - 80.8% of the catches until May 2006 (Aora9-S), when females became more abundant (72.4%). The ratio of males to females then remained at approximately 1:1 for the remainder of the samples (Figure 3.3). The proportion of males and females did not vary significantly with trawl duration.

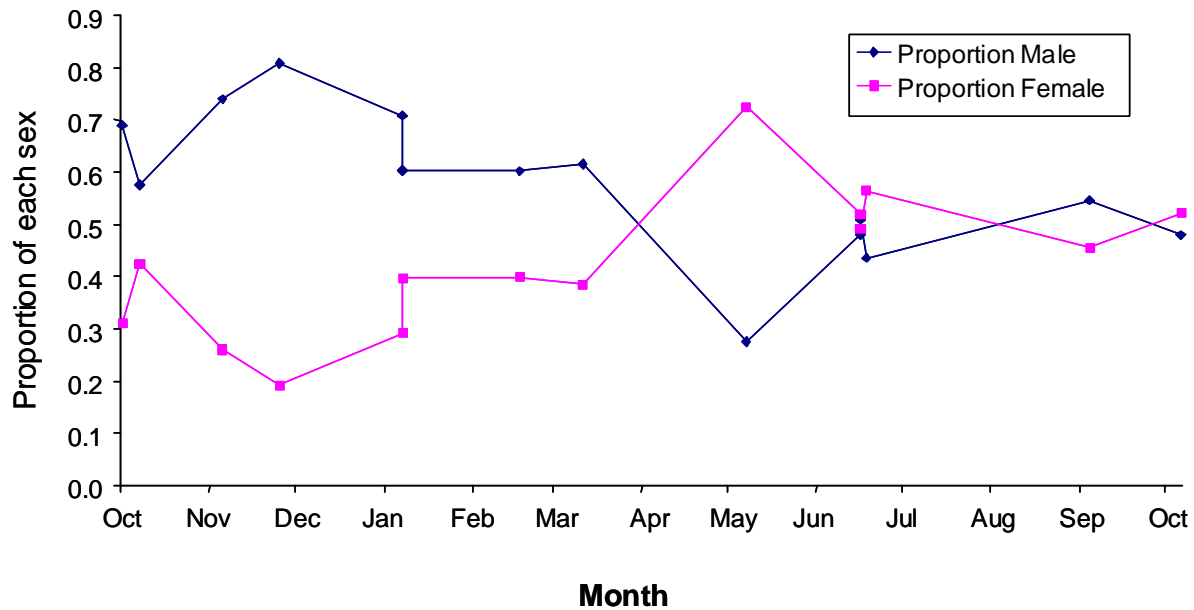


Figure 3.3. Mean proportion of males and females from all trawls

Carapace Hardness

Carapace hardness appeared to vary over time, as shown in figure 3.4. A significantly higher proportion of ‘jelly’ animals were caught in May than any other month

($\chi^2 = 543.108$, $df = 18$, $p = 0.000$).

Carapace hardness varied with sex ($\chi^2 = 122.229$, $df = 2$, $p = 0.000$), and there were more ‘jelly’ females than expected overall. Carapace hardness also varied with length ($H = 33.34$, $df = 2$, $p = 0.000$). A *post hoc* analysis showed that ‘hard’ animals were significantly smaller (median = 27.7mm) than both ‘soft’ and ‘jelly’ animals, while ‘jelly’ animals were the largest (median = 29.4mm). No significant difference was

found in carapace hardness between the long and short trawls ($\chi^2 = 5.436$, $df = 2$, $p = 0.066$).

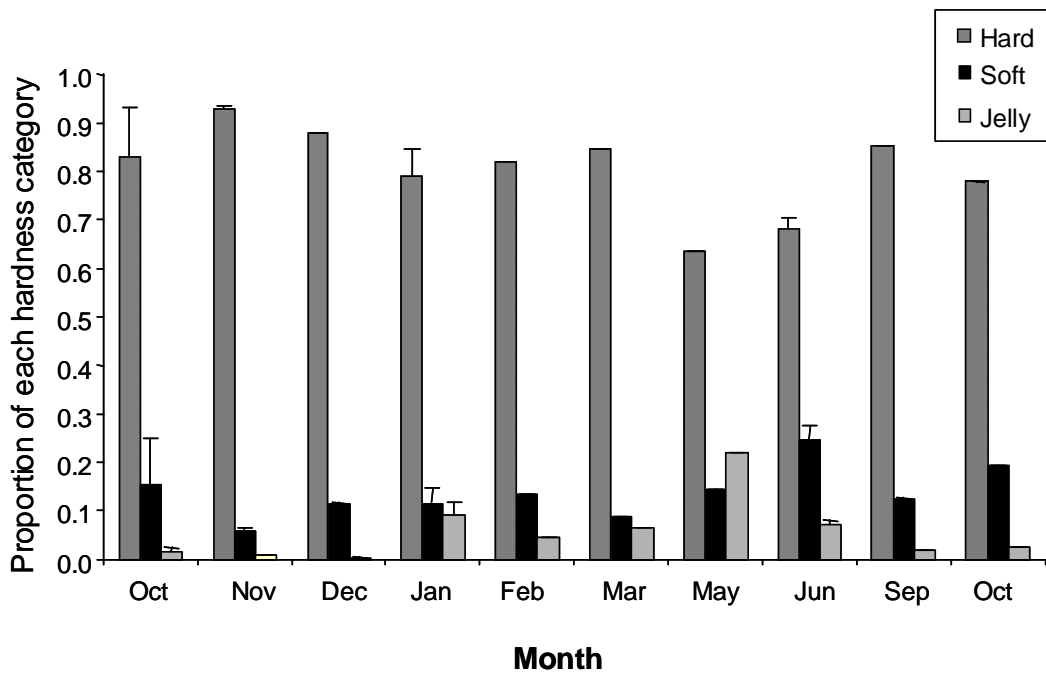


Figure 3.4. Mean proportion of each category of carapace hardness per month. Error bars = one standard error. $\chi^2=543.108$, $df = 18$, $p = 0.000$.

Damage

The mean damage scores (MDS) for short and long trawls are shown in figure 3.5. There was a significant difference between them ($t = -2.39$, $p = 0.033$), with a higher mean damage score occurring in long trawls.

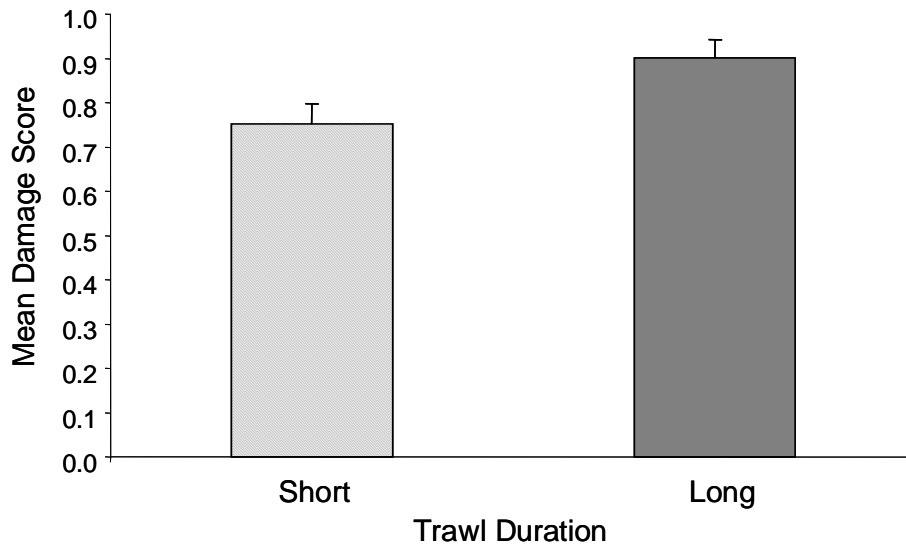


Figure 3.5. Comparison of mean damage score of *Nephrops norvegicus* from long and short trawls. Error bars = one standard error.

This was also seen in the individual damage categories. More undamaged individuals were present in the short trawls than expected, while the converse was true for long trawls ($\chi^2 = 38.699$, $p = 0.000$). The MDS of short trawls were more variable than long trawls, ranging from 0.514 (Aora1-S) to 1.138 (Aora9-S) which are both the lowest and highest values recorded.

A strong relationship was apparent between carapace hardness and the extent of damage to *Nephrops norvegicus* ($\chi^2 = 1123.954$, $p = 0.000$). ‘Hard’ *N. norvegicus* generally appeared less damaged than ‘soft’ or ‘jelly’ animals. In the case of ‘jelly’ animals, only 3 undamaged individuals were found.

Since carapace hardness appeared to influence the amount of damage sustained by an individual and since the proportion of ‘jelly’ individuals varied significantly over the study period, it was necessary to analyse ‘hard’, ‘soft’ and ‘jelly’ animals separately against damage. Figure 3.6 shows the proportion of *Nephrops norvegicus* in each damage category according to carapace hardness. Chi-squared analysis suggested that

longer trawl durations resulted in greater damage to ‘hard’ *N. norvegicus* ($\chi^2 = 70.079$, $df = 2$, $p = 0.000$). For long trawls, more animals than expected were found to have slight or severe damage, while in short trawls there were more undamaged animals. This trend was not seen in the ‘soft’ and ‘jelly’ *Nephrops norvegicus* however, and there did not appear to be a relationship between trawl duration and damage in these categories ($\chi^2 = 3.933$, $df = 2$, $p = 0.140$ for ‘soft’ *N. norvegicus* and $\chi^2 = 0.165$, $df = 1$, $p = 0.685$ for ‘jelly’ *N. norvegicus*).

There was a difference in the extent of damage between sexes ($\chi^2 = 48.302$, $df = 2$, $p = 0.000$). More males occurred in the undamaged category than would be expected, while more females were severely damaged.

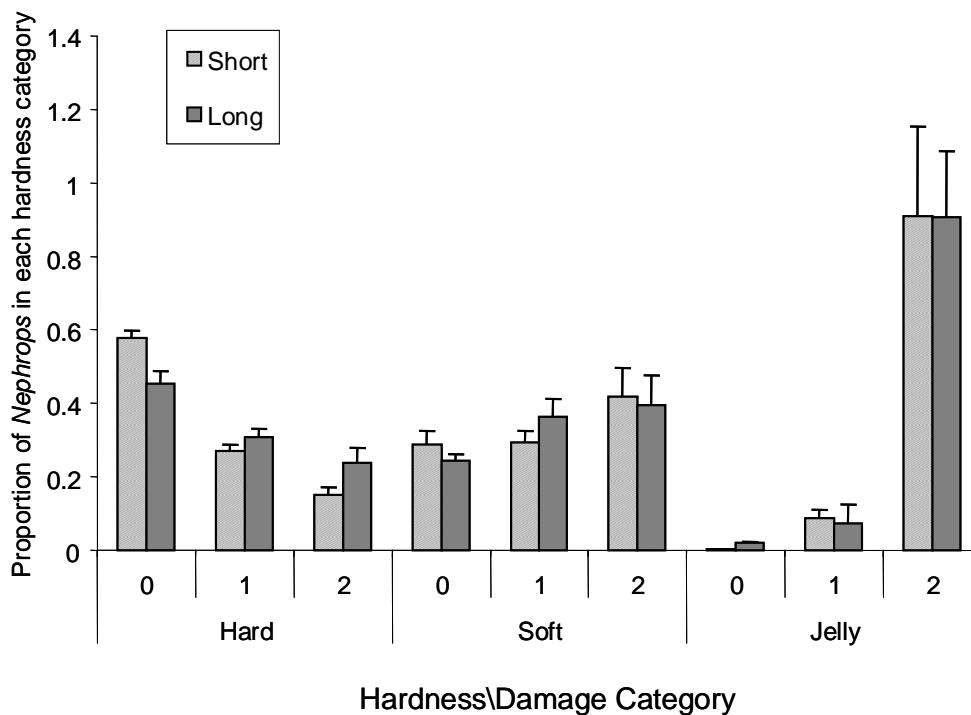


Figure 3.6. Mean proportion of *Nephrops norvegicus* in each damage category (0-2) according to carapace hardness in short and long trawls. Error bars show one standard error.

Possible effects of carapace hardness on the quality of *Nephrops norvegicus* meat

Apart from this effect of hardness on the damage of the animals (Figure 3.6) at this point it was important to see if this hardness would also affect the quality of the muscle of the tails if jelly animals were sold as tails at this time of the year. Jelly animals had higher levels of bacterial on days 1 and 3 but eventually bacteria from hard animals reached similar values on day 5 (Figure 3.7).

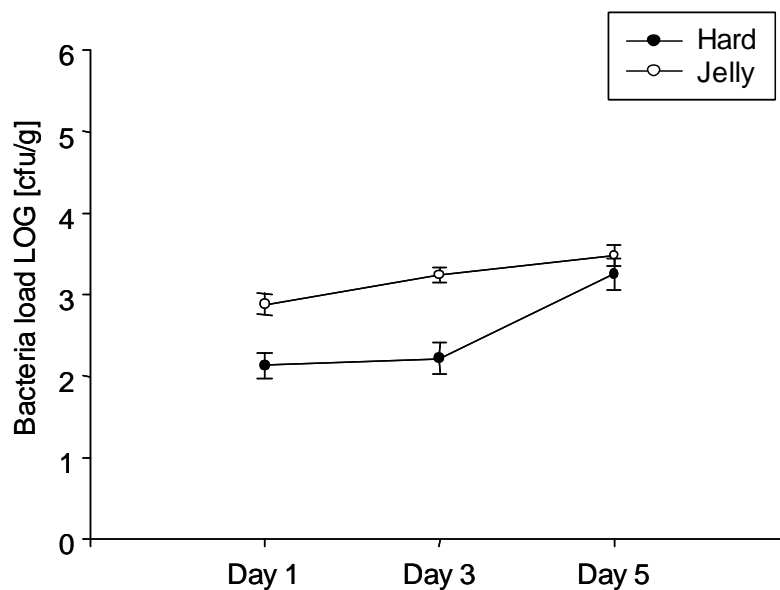


Figure 3.7. Bacterial load on tails from hard and jelly animals when stored on ice up to 5 days. Values are the mean \pm S.E. of five different determinations.

Nucleotide breakdown products were analysed in fresh samples and AEC values were accordingly calculated (Figure 3.8). Interestingly, jelly animals had higher levels of ATP than hard animals after trawl, and therefore AEC values were higher in jelly animals. A possible explanation for this effect is that jelly animals have not re-established their insertions onto the exoskeleton of the newly moulted animals and therefore the muscle is not fully functional and do not react as expected when the animal is in the trawl.

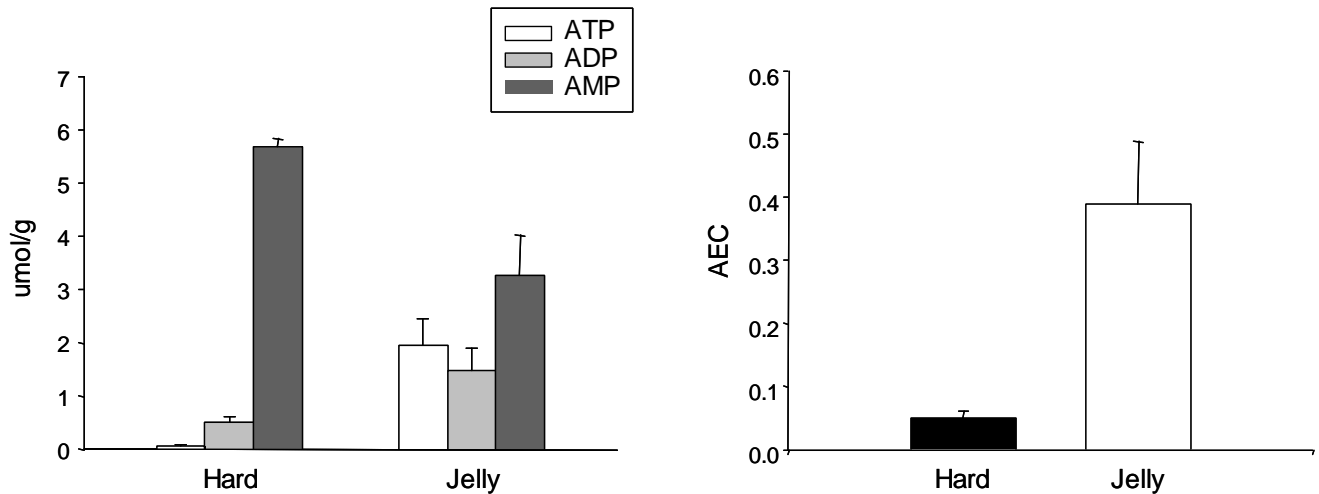


Figure 3.8. Nucleotide breakdown products on fresh samples taken from hard and jelly animals after landing on board. Results are the mean \pm S.E. of four different determinations.

Finally, K-values were obtained from tails kept on ice for 7 days (Figure 3.9). Jelly animals showed similar K-values compared to hard animals up to day 5, although on day 7 K-values of jelly animals were significantly higher (K-values were 46.6 ± 6.82 on jelly animals and 20.55 ± 1.69).

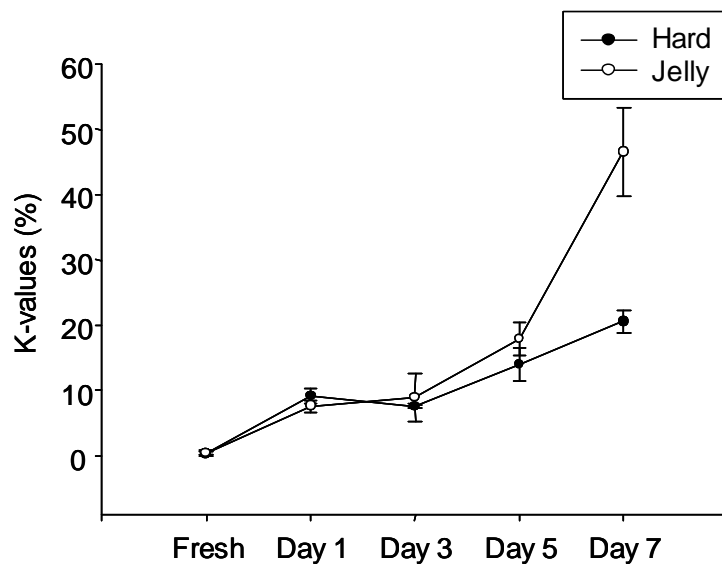


Figure 3.9. K-values on tails held on ice taken from hard and jelly animals. Results are the mean \pm S.E. of four different determinations.

Catch Composition

A total of 52 species were identified from seven trawls (45 species in five short trawls; 40 species in two long trawls). The vertebrate and invertebrate species lists are presented in Tables 3.1 and 3.2 respectively, along with the wet weights for each major taxon and for species of ‘commercial fish’. Figure 3.10 shows the total mass of each trawl, and the mass of each major group within them. Long trawls did not recover a significantly higher mass of organisms than the short trawls ($T = 2.31, p = 0.147$), but this appears to be due to the low mass recovered in Aora1-L. *Nephrops norvegicus* was recovered in all trawls, comprising 18-56% (mean = 37%) of the total catch from short trawls, and 34-55% (mean = 42%) of the catch from long trawls by wet weight. There was no significant difference in the proportion of *N. norvegicus* caught during different trawl durations ($W = 16.5, p = 0.796$).

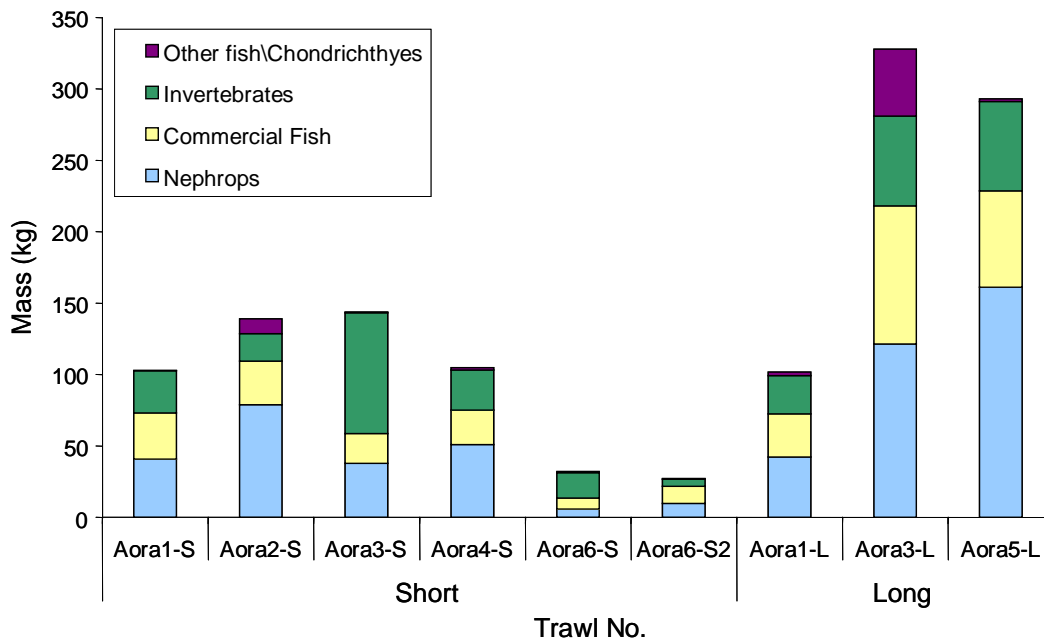


Figure 3.10. Total mass (wet weight) of each trawl, showing the major groups of organisms. $T = 2.31, p = 0.147$.

The commercial fish species were generally the next most common group, and comprised 14-44% (mean = 22.5%) of the total catch from short trawls, and 23-31% (mean = 28.0%) of the total catch from long trawls. *Merlangius merlangus* (whiting), *Merluccius merluccius* (hake), *Melanogrammus aeglefinus* (haddock) and *Pleuronectes platessa* (plaice) were caught in every trawl. *Solea solea* (sole) was present in six of the nine trawls, and cod was present in five. *Glyptocephalus cynoglossus* (witch flounder) was also caught, but only in two trawls. Of the non-commercial fish species, *Hippoglossoides platessoides* (long rough dab) was caught in all nine trawls, *Scyliorhynchus canicula* (dogfish) in seven, and *Clupea harengus* (herring) in five trawls. No other species were collected in more than three trawls.

The invertebrates (excluding *Nephrops norvegicus*) comprised 12-59% (mean = 33.4%) of the total catch from short trawls and 19-22% (mean = 21%) of the long trawls by wet weight. The proportions of each major taxon varied considerably between trawls, although bivalves, crabs, urchins and starfish often made up a relatively large proportion of the total catch.

Table 3.1. Species composition of vertebrates from short and long trawls. Wet weights for each major taxon and species of 'commercial fish' are shown in kg. √ = species present.

Major Taxon	Short Trawls					Long Trawls	
	Aora2-S	Aora3-S	Aora4-S	Aora6-S	Aora6-S2	Aora3-L	Aora5-L
Osteichthyes							
'Commercial Fish' (kg)	30.32	20.54	24.38	7.64	12.00	96.75	67.54
<i>Gadus morhua</i> Linnaeus 1758	0	0	2.46	0.18	0.62	5.72	5.81
<i>Melanogrammus aeglefinus</i> (Linnaeus 1758)	11.4	7.18	6.46	0.96	8.26	31.27	28.44
<i>Merluccius merluccius</i> (Linnaeus 1758)	2.8	0.42	0.82	3.06	1.04	5.5	4.69
<i>Pleuronectes platessa</i> Linnaeus 1758	3.4	1.22	2.58	1.12	0.66	4.32	5.25
<i>Solea solea</i> (Linnaeus 1758)	0	2.03	1.4	1.16	0	3.84	2.12
<i>Merlangius merlangus</i> (Linnaeus 1758)	10.4	9.69	10.66	1.02	8.26	46.9	21.23
<i>Glyptocephalus cynoglossus</i> (Linnaeus 1758)	2.2	0	0	0	0	0	0
'Non-Commercial Fish' (kg)	1.20	0.38	1.36	0.76	0.22	9.61	1.79
<i>Agonus cataphractus</i> (Linnaeus 1758)				√			
<i>Aspitrigla cuculus</i> (Linnaeus 1758)							√
<i>Callionymus lyra</i> Linnaeus 1758			√	√			√
<i>Clupea harengus</i> Linnaeus 1758		√		√	√	√	
<i>Enchelyopus cimbrius</i> (Linnaeus, 1766)	√		√			√	
<i>Hippoglossoides platessoides</i> (Fabricius 1790)	√	√	√	√	√	√	√
<i>Lumpenus lampretaeformis</i> (Walbaum, 1792)	√						
<i>Pollachius pollachius</i> (Linnaeus 1758)						√	
<i>Trisopterus esmarkii</i> (Nilson, 1855)			√				√
<i>Trisopterus minutus</i> (Linnaeus 1758)	√						
Chondrichthyes (kg)	9.00	0	0.36	0	0.18	37.12	Not recorded
<i>Leucoraja garmani</i> (Whitley, 1939)						√	
<i>Scyliorhinus canicula</i> (Linnaeus, 1758)	√		√		√	√	√

Table 3.2. Species composition of invertebrates from short and long trawls. Wet weights for each major taxon are shown in kg. √ = species present.

Major Taxon	Short Trawls					Long Trawls	
	Aora2-S	Aora3-S	Aora4-S	Aora6-S	Aora6-S2	Aora3-L	Aora5-L
Cnidaria (kg)	0.1	0	0	0	0	0	0.5
<i>Rhizostoma octopus</i> (Linnaeus 1758)			√				
<i>Urticina eques</i> (Gosse 1859)	√						√
Mollusca							
Bivalvia (kg)	0.02	20.39	0.56	1.06	0.12	14.32	3.02
<i>Acanthocardia echinata</i> (Linnaeus 1758)	√	√		√			
<i>Aequipecten opercularis</i> (Linnaeus 1758)	√	√	√		√	√	√
<i>Arctica</i> sp. Schumacher 1817			√	√		√	
Gastropoda (kg)	2.57	5.19	3.12	0.56	1.16	7.82	27.88
<i>Aporrhais pespelecani</i> (Linnaeus 1758)		√					
<i>Buccinum undatum</i> Linnaeus 1758	√	√	√	√	√	√	√
<i>Neptunea antiqua</i> (Linnaeus 1758)	√	√	√	√	√	√	√
Cephalopoda (kg)	1.30	0	2	0	0	1.75	0
<i>Eledone cirrhosa</i> (Lamarck 1798)	√						
<i>Loligo forbesii</i> Steenstrup 1856			√				
<i>Sepietta oweniana</i> (D'Orbigny 1839-1841)	√					√	
Annelida (kg)	0	1.18	0.26	0	0.16	3.93	0
<i>Aphrodita aculeata</i> Linnaeus 1761		√	√		√	√	
Crustacea							
Anomura: Nephropidae (kg)	79.00	38	50.9	5.72	9.72	121.40	161.17
<i>Nephrops norvegicus</i> (Linnaeus 1758)	√	√	√	√	√	√	√
Anomura: Paguridae (kg)	2.66	2.03	2.32	0.2	0.42	6.03	4.47
<i>Pagurus bernhardus</i> (Linnaeus)	√	√	√	√	√	√	√
<i>Pagurus prideauxi</i> Leach	√	√					
Anomura: Galatheididae (kg)	0.62	0	0.42	0.2	0	4.41	2.74
<i>Munida rugosa</i> (Fabricius 1775)	√		√	√		√	√
Caridea (kg)	0.30	0	0.44	0	0	1.00	1.06
<i>Crangon</i> sp. Fabricius 1798	√		√				
<i>Dichelopandalus</i> sp. Caullery 1896	√		√			√	
<i>Pandalus</i> sp. Leach 1814							√
Brachyura (kg)	7.64	10.05	9.38	2.76	0.64	12.14	5.25
<i>Cancer pagurus</i>		√	√	√	√	√	√
<i>Carcinus maenas</i> (Linnaeus 1758)							√
<i>Liocarcinus depurator</i> (Linnaeus 1758)	√	√	√	√	√	√	√
Majidae sp. 1 Samouelle 1819							√
<i>Necora puber</i> (Linnaeus 1767)	√					√	√
Echinodermata							
Asteroidea (kg)	2.10	4.43	2.72	3.46	2.06	6.38	14.47
<i>Asterias rubens</i> Linnaeus 1758	√	√	√	√	√	√	√
<i>Astropecten irregularis</i> (Pennant)	√	√	√	√	√	√	√
<i>Luidia ciliaris</i> (Philippi)		√					
<i>Marthasterias glacialis</i> (Linnaeus)	√	√	√	√	√	√	√
<i>Porania</i> sp (Muller)	√			√			
Ophiuroidea (kg)	2.05	10.47	1.78	0	0.38	5.15	1.45
<i>Ophiura ophiura</i> (Linnaeus 1758)	√	√	√		√	√	√
Echinoidea (kg)	0	30.99	4.86	9.76	0	0	1.62
<i>Brissopsis lyrifera</i> (Forbes)		√	√	√			√
<i>Echinus esculentus</i> Linnaeus 1758							√

Bycatch

The mass of bycatch organisms was generally high, comprising 44-82% (mean = 61%) of the total catch by wet weight. Long trawls returned a greater mass of bycatch than short trawls ($W = 15$, $p = 0.037$), but there was no difference between the proportions of bycatch ($W = 31.5$, $p = 0.796$).

Undersize *Nephrops norvegicus* and ‘Heads’

The minimum landing size for *Nephrops norvegicus* is 20 mm, and undersize individuals were present in all trawl samples. These animals were not included as part of the bycatch, although they would have been discarded from the haul. However, the proportion of undersize individuals did not vary between short and long trawls ($W = 128.5$, $p = 0.301$), and they were unlikely to influence the trends seen in the levels of bycatch.

Commercial Fish

The mass of commercial fish species in each trawl is shown in Figure 3.11. Whiting and haddock typically made up the highest proportion of the commercial fish in each trawl (mean = 39% and 32% respectively). The total mass of commercial fish varied between long and short trawls ($W = 21$, $p = 0.028$). However, this was not necessarily true for individual species. The wet weights of whiting were significantly greater in long trawls than short trawls ($W = 21$, $p = 0.028$), but the wet weights of other species were not linked with trawl duration ($p > 0.05$).

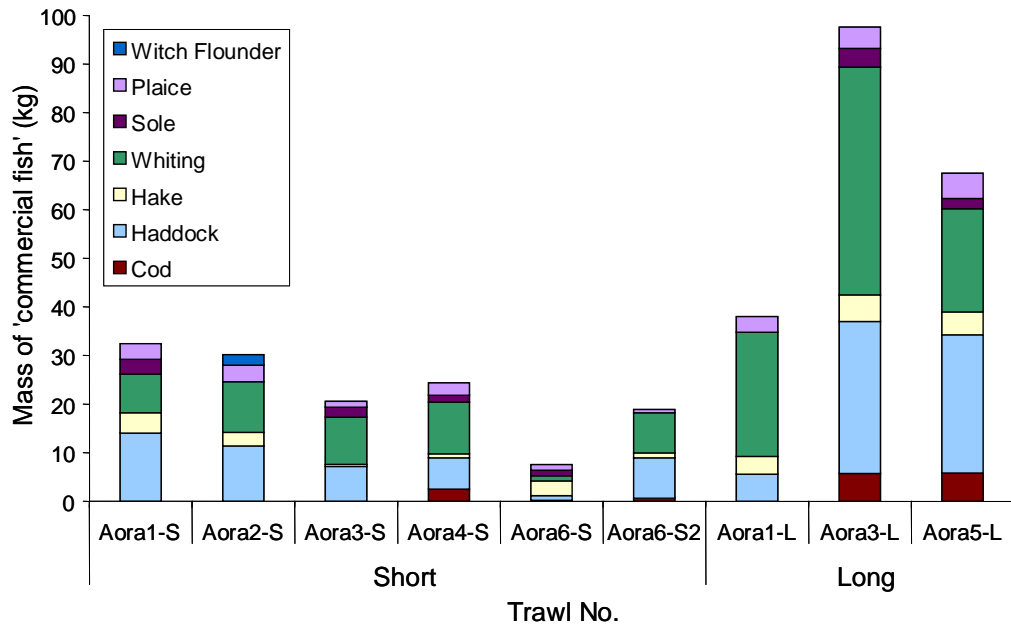


Figure 3.11: The wet weight of 'commercial fish' in each trawl, showing individual species.
 $W = 21, p = 0.028.$

IV. PREVALENCE OF THE *NEPHROPS* PARASITE *HEMATODINIUM* SP. AND EFFECTS ON QUALITY

IV. A) Prevalence data

The prevalence data for *Hematodinium* sp. in *Nephrops* from January 2005 to January 2006 was determined using the ELISA method (Figure 4.1A). Values of ~15% were recorded from January to May. June and July showed few or no infected animals (0 to 3%), but during the late summer and autumn the numbers increased again, reaching 9%-11% in October-December. These results correspond to those obtained previously using Western blotting with the same polyclonal antibodies (Stentiford *et al.*, 2001a), which detect not only the full expression of the parasite (patent infection), but also the low levels of infection (sub-patent) which precede the overt seasonal peaks in March-April (Figure 4.1B).

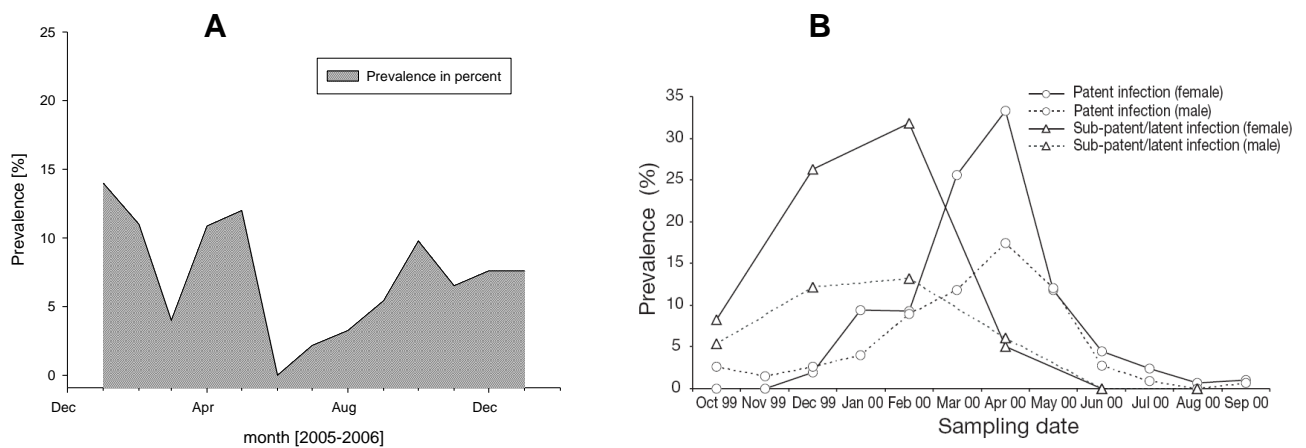


Figure 4.1. A. *Hematodinium* sp. prevalence in *Nephrops* in 2005 from representative monthly samples taken in the Clyde area. Data based on ELISA. B. Immunoassay-derived (sub-patent) infections and pleopod-derived (patent) *Hematodinium* infection prevalence for female and male *Nephrops* in 2000 (from Stentiford *et al.*, 2001a).

IV. B) The effect of *Hematodinium* sp. on the quality of *Nephrops* tails

Changes in muscle pH

Muscle pH in the tail meat of parasitized animals just after catch was higher (7.10 ± 0.04) compared to non-parasitized animals (6.93 ± 0.02) (Figure 4.2). These differences were maintained in day 1 when tails were stored on ice. However, the pH between both groups then converged progressively, and from day 3 onwards there was no difference in muscle pH between infected and non-infected animals.

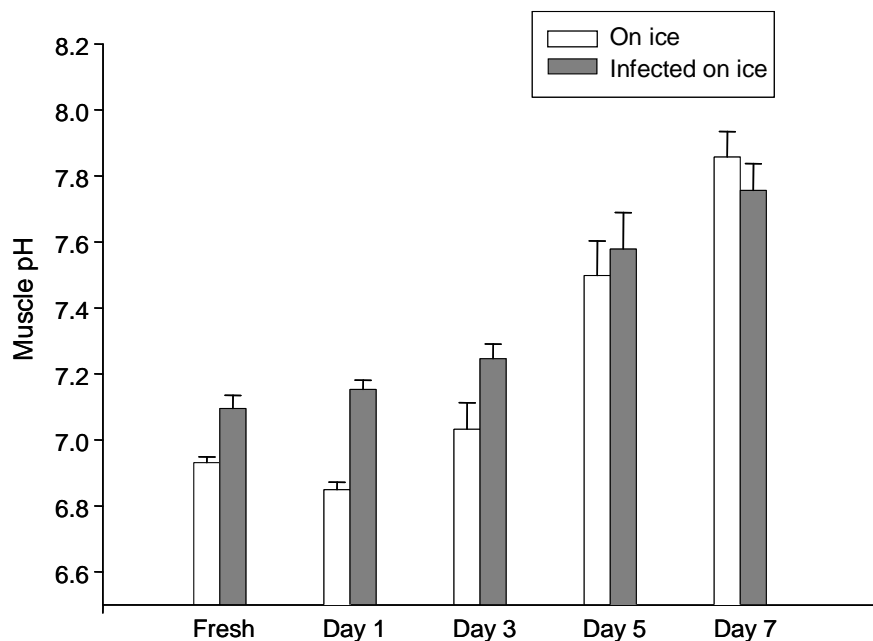


Figure 4.2. pH variation in *Nephrops* tails between non-infected and infected animals during ice storage. Values are the mean \pm S.E.M. of ten different tails.

Total Viable Counts (TVC)

The bacterial numbers (TVCs) in both parasitized and non-parasitized (standard) tails stored on ice for up to 5 days increased slowly and did not differ significantly from each other during the storage period (Figure 4.3).

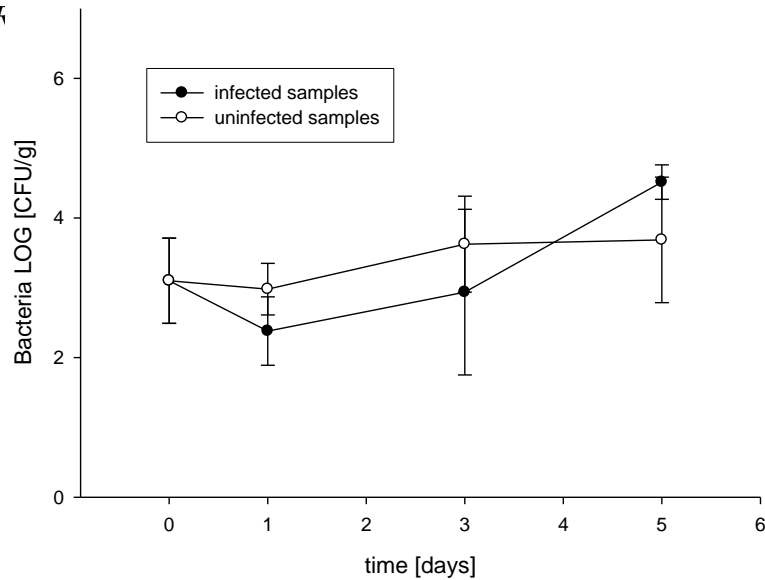


Figure 4.3. Bacteria load in *Nephrops* tails between uninfected and infected animals during ice storage. Values are the mean \pm S.E.M. of five different tails.

Trimethylamine (TMA) concentrations in the muscle

There was no significant difference in TMA concentrations between infected and non-infected animals during ice storage up to day 7 (Figure 4.4). These results are consistent with the similarity in their bacteria loads (Figure 4.3).

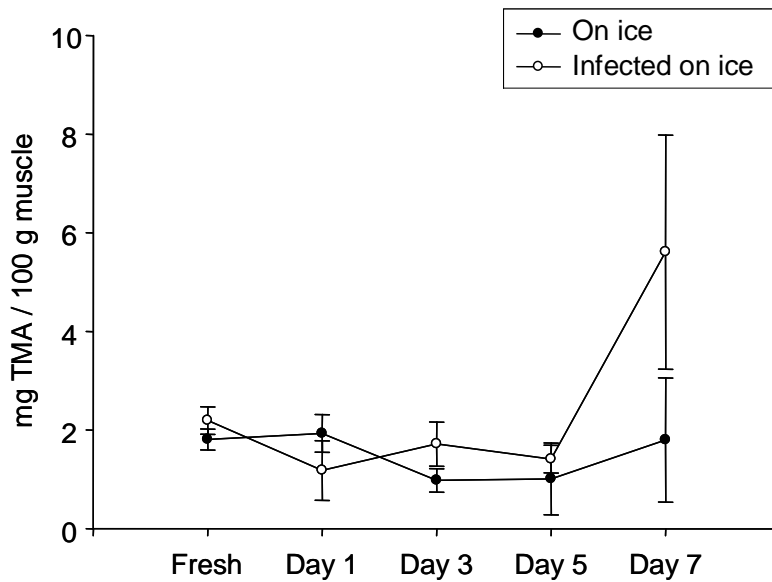


Figure 4.4. TMA concentration in *Nephrops* tails in non-infected and infected animals during ice storage. Values are the mean \pm S.E.M. of five different tails.

Effect of the parasite on AEC ratio, ATP breakdown products and K-values in Nephrops tails

The AEC ratio, which is an indicative index for the amount of metabolically available energy in an organism, was lower in infected animals compared to non-infected animals just after trawl capture (Figure 4.5). This suggests that the infected animals have less metabolic energy available than non-infected animals after the trawl process, due to the presence of the parasite in the animal.

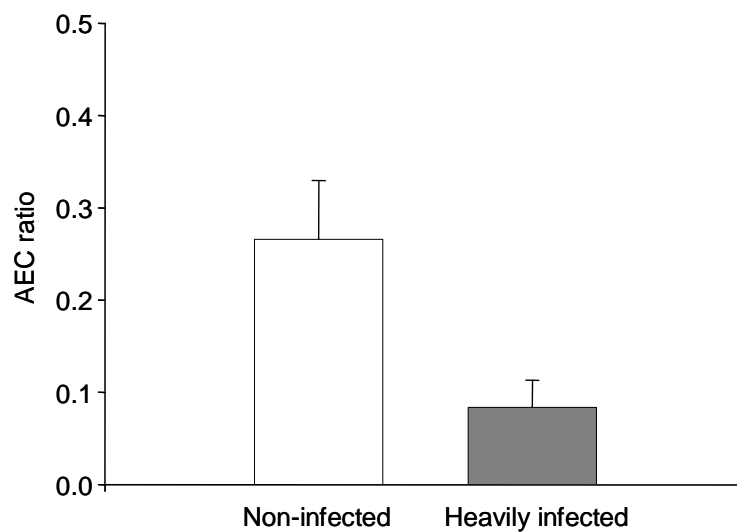


Figure 4.5. AEC ratio in *Nephrops* tails just after landing on boat in non-infected and infected animals. Values are the mean \pm S.E.M. of four different tails.

When tails from both lots of animals were then left on ice the nucleotide breakdown processes occurred (Figure 4.6). The inter-conversion of AMP to IMP took place earlier and more rapidly in infected animals. INO appeared in the muscle of infected animals from day 1, and reached final concentrations of $2.1 \pm 0.1 \mu\text{mol/g}$ on day 7 compared to $0.96 \pm 0.01 \mu\text{mol/g}$ in non-infected animals. This shift in the breakdown processes in infected animals resulted in changes in the K-values (Figure 4.7).

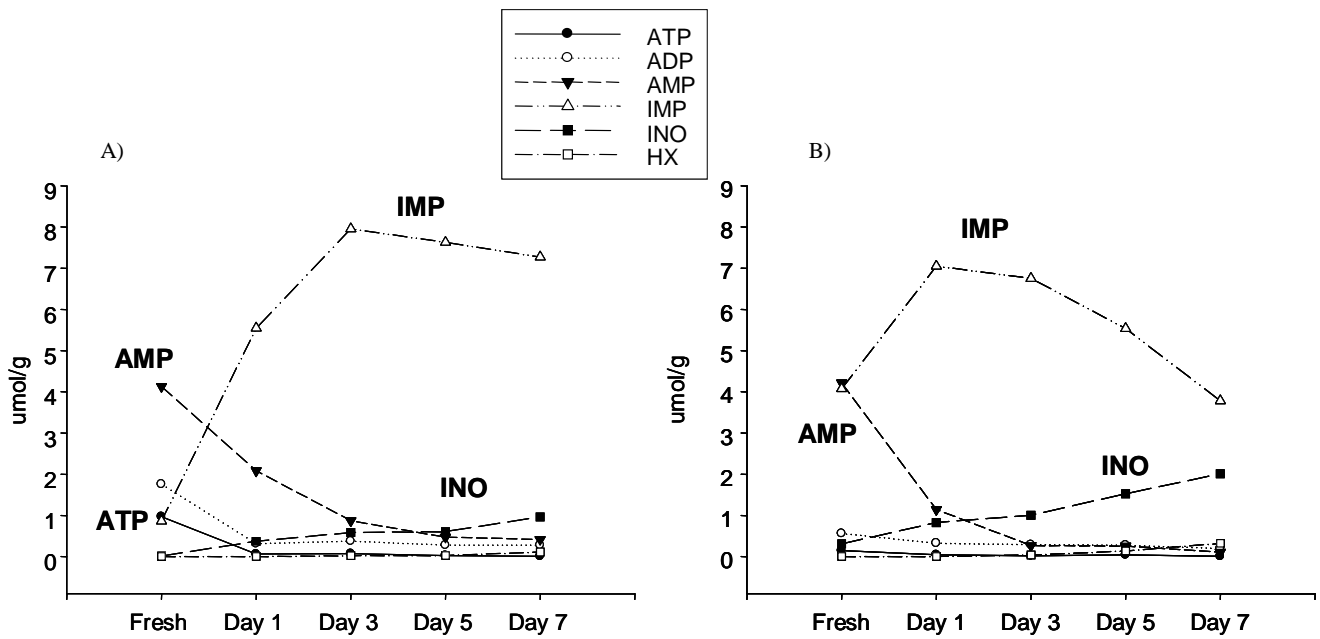


Figure 4.6. Changes in ATP breakdown products in *Nephrops* tails A) tails from non-infected animals kept on ice and B) tails from infected animals kept on ice. Values are the mean \pm S.E.M. of three determinations.

K-values were higher in infected animals from the outset (just after catch) and the difference increased as storage on ice progressed. Thus on day 7 the K-values for tails from non-infected animals were around 13 (i.e. within the freshness limit of 20 proposed for many fish species) while the K-values for tails from infected animals were around 40 (well above the proposed freshness limit).

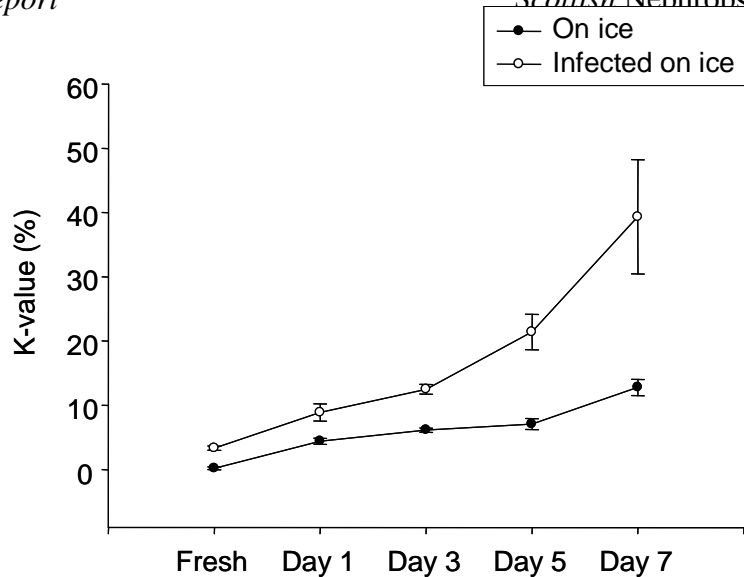


Figure 4.7. Comparison of K-values in *Nephrops* tails kept on ice from non-infected and infected animals. Values are the mean \pm S.E.M. of three determinations.

Table 4.1 summarises all the results. Tails from infected animal showed higher muscle pH values from just after catch, and K-values were higher than 20 from day 5. The latter measure indicates that the freshness limit in these tails is reached more rapidly than in the tails of non-infected animals. In contrast, the bacteria loads and TMA concentrations were similar between both lots.

Table 4.1. Summary table including all the parameters measured in *Nephrops* tails held on ice from non-infected and infected animals. ■ Indicates not significantly different from fresh samples and ✗ indicates that the parameter is different from fresh samples

	On ice				Infected On ice			
FRESH	■	■	■	■	■	✗	■	■
DAY 1	■	■	■	■	■	✗	■	■
DAY 3	■	✗	■	■	■	✗	■	■
DAY 5	■	✗	■	■	■	✗	■	✗
DAY 7	■	✗	■	■	■	✗	■	✗
	Bacteria load	pH	TMA	K-value	Bacteria load	pH	TMA	K-value

These findings demonstrate that the seasonal infection of *Nephrops* by *Hematodinium* has a direct effect on product quality, that this can be detected by appropriate measures of freshness (the K value), but that it does not necessarily involve the induction of spoilage bacteria. This combination of results highlights the value of the multifactorial quality assay approach, since the different measures do not always change in corresponding ways.

V. IMPLEMENTING QUALITY CONTROL

A major objective of the Research Partnership between Young's Seafood Ltd. the University of Glasgow and UMBS Millport was for the scientists to work closely with the company to implement changes in practice in order to guarantee quality and to improve profitability within a fishery that promotes sustainability. This has been achieved by performing the following:

1. Surveying samples that had been delivered to Young's Annan factory from various ports and then forwarded to the testing laboratory at GU. Threats to quality were assessed according to bacterial load (TVC).
2. Combining the logging of product temperature along the capture and supply chain with traceability data derived from the Youngstrace system. This allowed us to determine the effects of various time-temperature combinations on the quality of product delivered to the Annan or Stornoway factories of Young's. In some cases quality assessment was also performed using the suite of assays developed by the partnership.

I. Screening of bacterial concentrations in factory tails landed at different ports

Initially, samples were received on ice from Young’s Seafood factory at Annan and these had been separated into two groups of “healthy” and “necrotic” animals based on a visual assessment of tail colour devised by the company. On visual assessment in Glasgow, however, no obvious difference was seen in the colour between the two groups (Figure 4.1). Quantification of bacterial levels in these two groups of tails indicated no significant difference between them (Figure 4.2). Therefore, visual assessment of the tails was not considered a useful method to identify poor quality product and it was concluded that a thorough quality assessment scheme was required for this purpose.



Figure 4.1. Visual assessment of factory tails in the laboratory at Glasgow

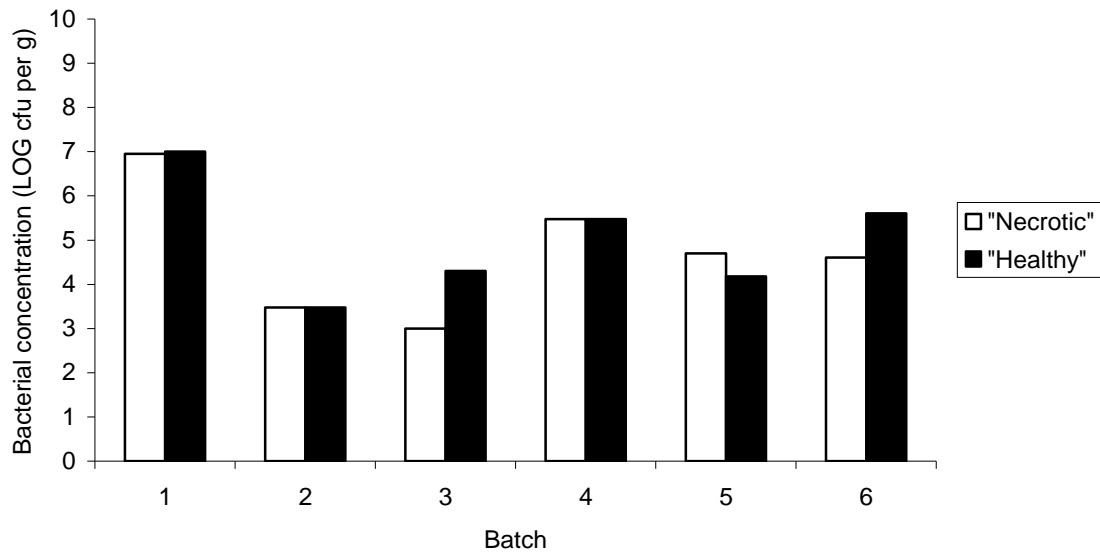


Figure 4.2. Bacterial concentrations in 6 batches of factory tails separated into two groups based on visual assessment of the tail colour

Such a quality assessment scheme has been developed by Youngs Seafood Annan factory using freshly caught samples from the RV *Aora* as a high quality standard, rather than on tail samples from the factory, due to their variable quality.

Screening of the factory samples was carried out on a monthly basis in order to investigate seasonal and geographical variation of bacterial levels within the tail meat. In 2004, tails were screened only during July to September, with only two ports of origin, Campbeltown and Fraserburgh (Figure 4.3). A more comprehensive monthly screening regime began in March 2005 and included a greater number of ports (Figures 4.4 - 4.6).

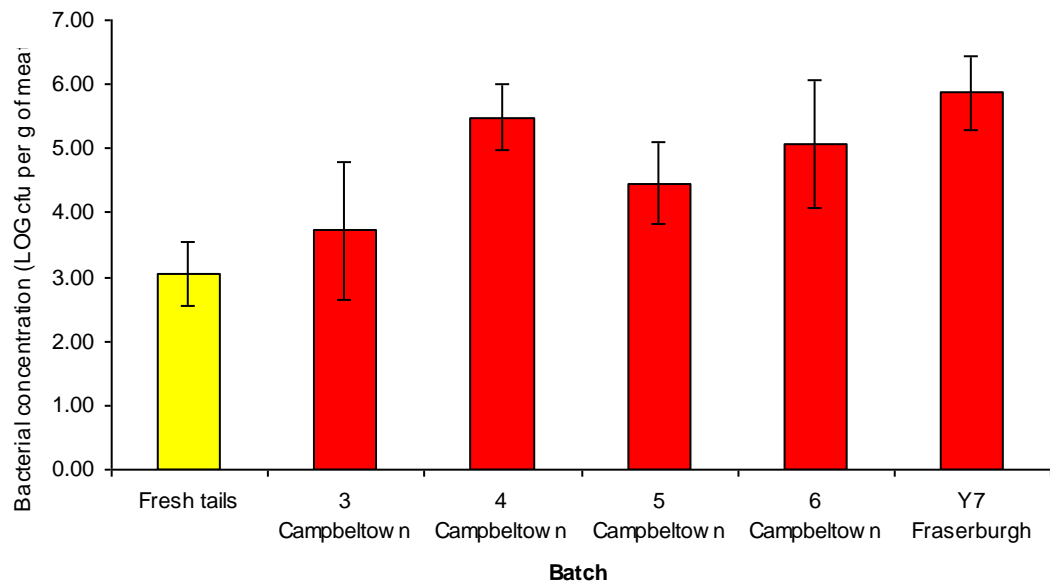


Figure 4.3. Comparison of bacterial concentration in fresh tails taken on the RV Aora and from factory tails landed at Campbeltown and Fraserburgh.

The bacterial concentrations in the factory tails from each of the ports in March 2005 were close to the basal level of 10^3 cfu g^{-1} obtained from fresh tails (Figure 4.4). Although there was an apparent trend for batches from later months to have higher bacterial levels, there were several exceptions to this. Thus in April samples from Oban on 02/04/05 had much higher loads (Figure 4.4). This high level of bacteria may have resulted from the tails being stored at 20°C for 2 h in the factory prior to onward dispatch to Glasgow on ice. Also, two batches with low bacteria occurred in May-July 2005 reflecting that a high variation occurs between the batches (Figure 4.4).

Overall, we conclude that there do not seem to be consistent differences in the bacterial loads in tails from the different ports (Figure 4.5). However, a re-analysis of these data according to transportation time from the port and via the factory to the sampling laboratory indicates that transit and storage time is a very influential factor in determining the measured bacterial load (Figure 4.5). Bacterial concentrations

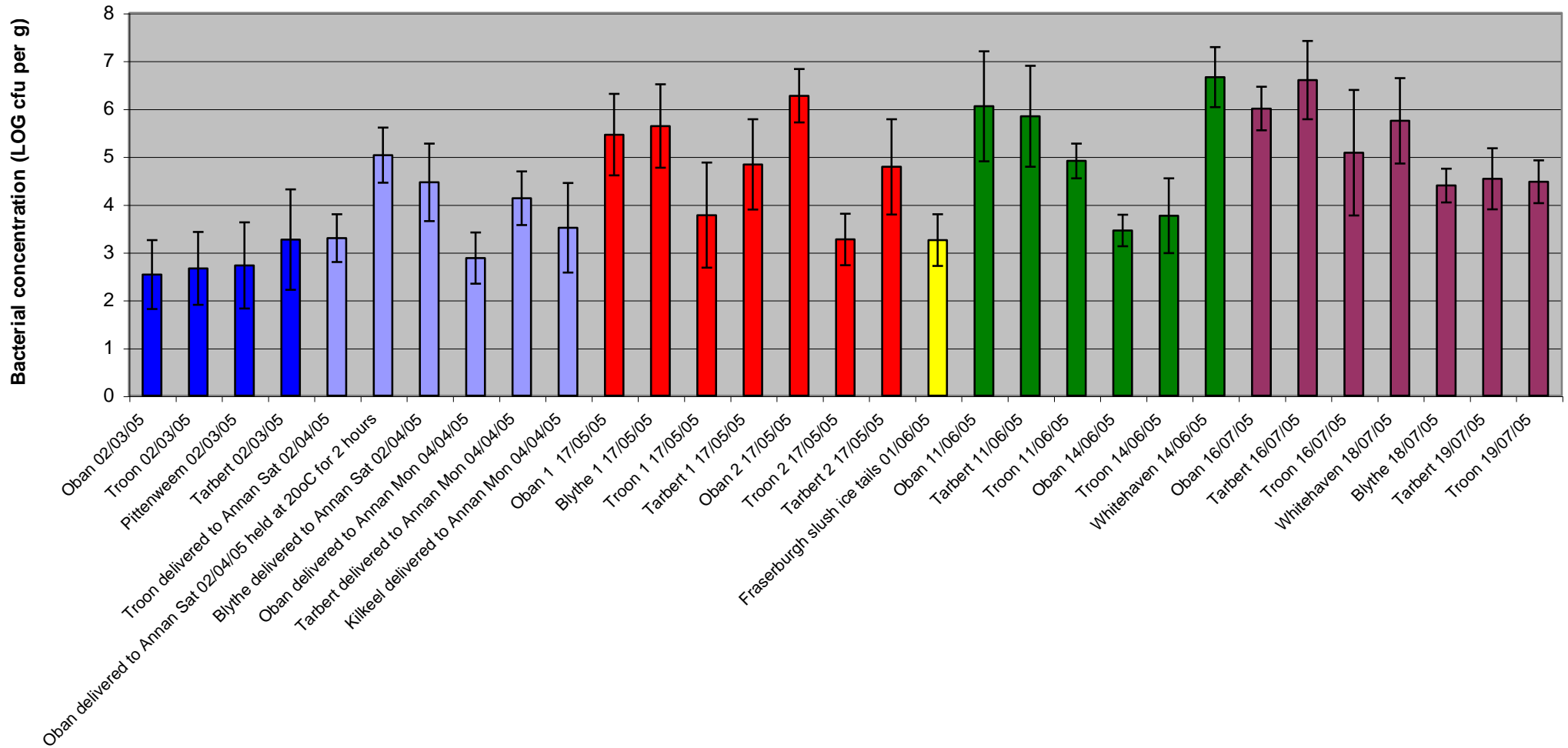


Figure 4.4. Bacterial concentration in factory tail meat from different ports between March to July 2005

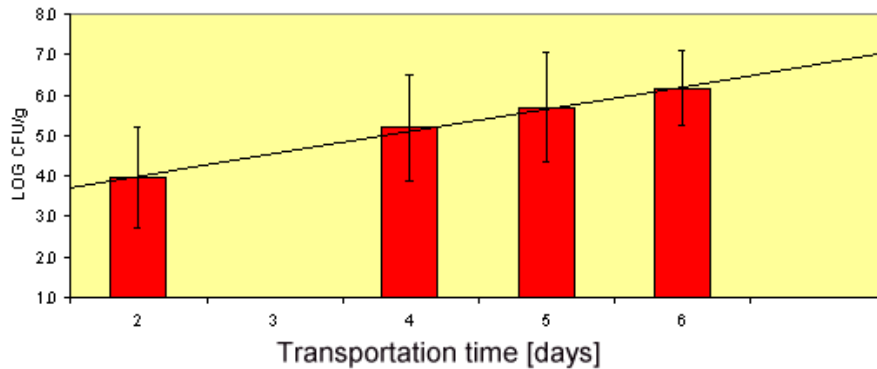


Figure 4.7. Amalgamated data from samples received from all ports in 2005 showing the correlation between bacterial load in *Nephrops* tails and the transportation time to the testing laboratory. Bacterial load expressed as colony forming units

A batch of tails was received from Fraserburgh in June 2005 from a fishing boat that had slush ice storage facilities. The tails were placed into the slush ice after trawling until landing on-shore and were transported directly to the testing laboratory on ice, thereby maintaining a low storage temperature. As shown in Figure 4.8, the level of bacteria in these tails was close to basal level, unlike the high level found in the previous batches from Fraserburgh.

Therefore, these results provide evidence that in commercial fishing practices the bacterial levels are kept low when tails are chilled,. This supports the findings of the temperature storage trials carried out with the fresh tails from the RV *Aora*, thus emphasising the need for strict control of the temperature during storage and handling on the boat and throughout the delivery chain.

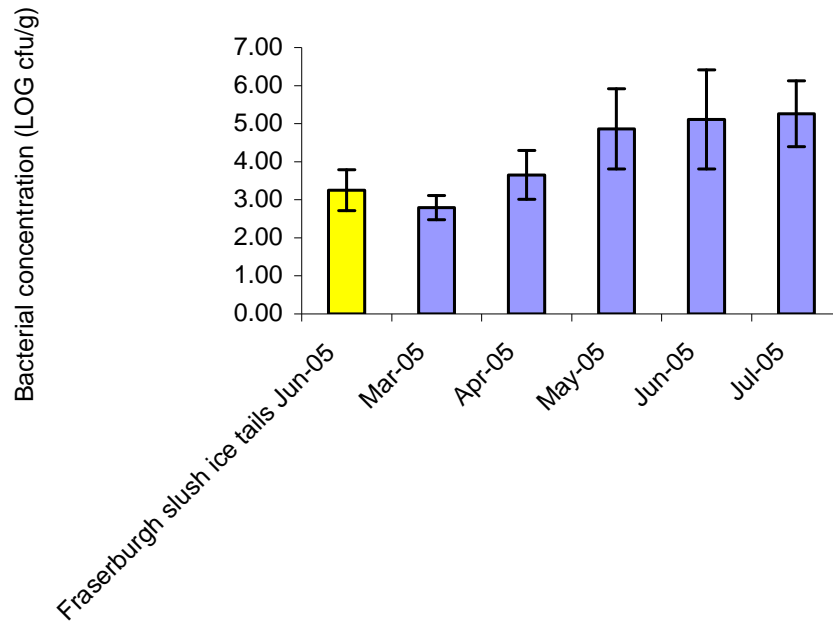


Figure 4.8. The bacterial load in *Nephrops* tails received from Fraserburgh in March-July 2005, including a batch that had been held on slush ice from the time of trawling, and on ice during transportation (left bar). Bacterial load expressed as colony forming units

II. Integrating traceability and temperature logging

A number of trials were conducted in which the temperature of the *Nephrops* was monitored from source (fishing boat) to destination (processing factory) and the quality of the product was then determined by appropriate assays.

Preliminary trial: Troon July 2006

In a preliminary trial, temperature loggers were placed in boxes of *Nephrops* from two vessels based at Troon harbour, the *Trust* and *Our Venture*, which trawl for *Nephrops* in the Clyde Sea Area. The YoungTrace record for the fishing activity of the *Trust* is shown in Figure 4.9.

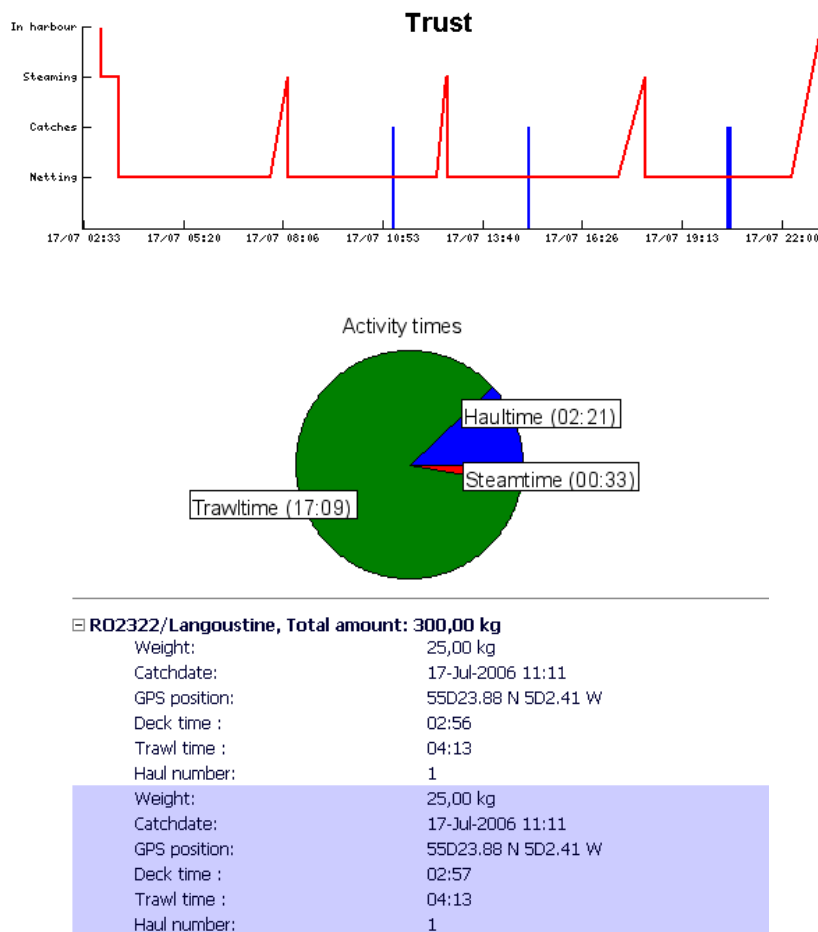


Figure 4.9. Traceability record of the fishing activity of the vessel *Trust* on 17/07/06.

Air temperatures on the day of the trial were around 21°C. Temperature loggers were to be placed in two boxes from each vessel while at sea, but in fact they were inserted only on landing at port. The recordings therefore represent only the land-based stage of post-capture handling and transport (Figure 4.10). Temperatures recorded within the product in the chilled transporter were around 4°C, and in the factory chill room were in the range 0 - 2°C (depending on the position of the boxes in the room).

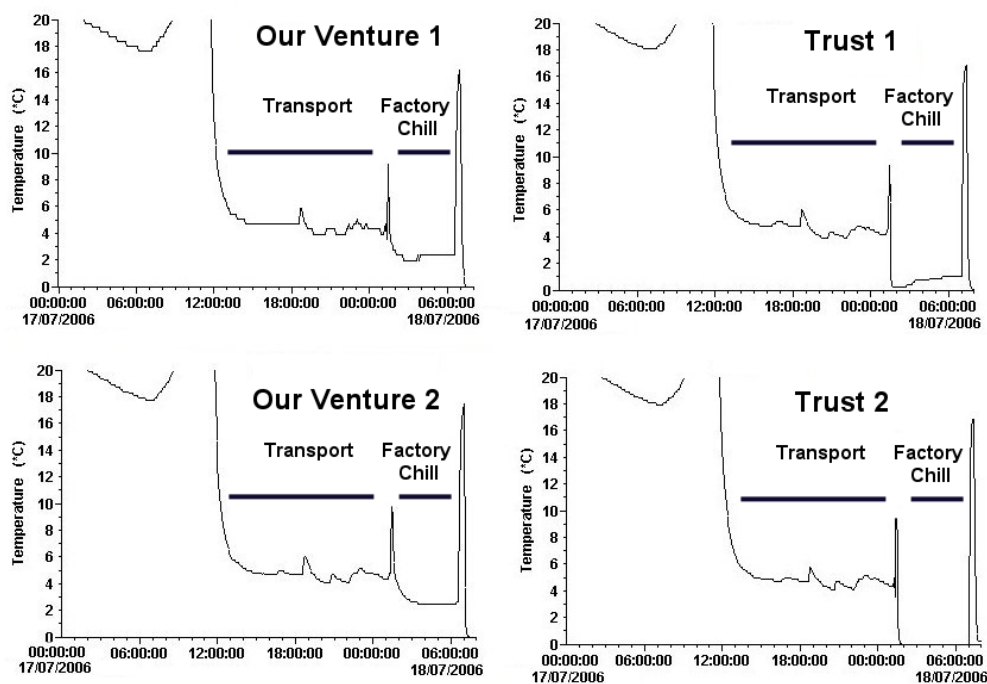


Figure 4.10. Temperature logs from loggers placed in two boxes of *Nephrops* tails from each fishing vessel on 17/07/06. The loggers were retrieved from the boxes at 7.00h on 18/07/06 at the Annan factory.

Samples of 50 tails were taken from the boxes at the factory, were held on ice for up to 7 days, and groups of 10 were assayed at intervals for pH, TMA and nucleotide breakdown products. The pH of the tail meat was initially around 7.0 for product from both vessels (Fresh in Figure 4.11), but became significantly greater for the *Trust* samples after 7 days.

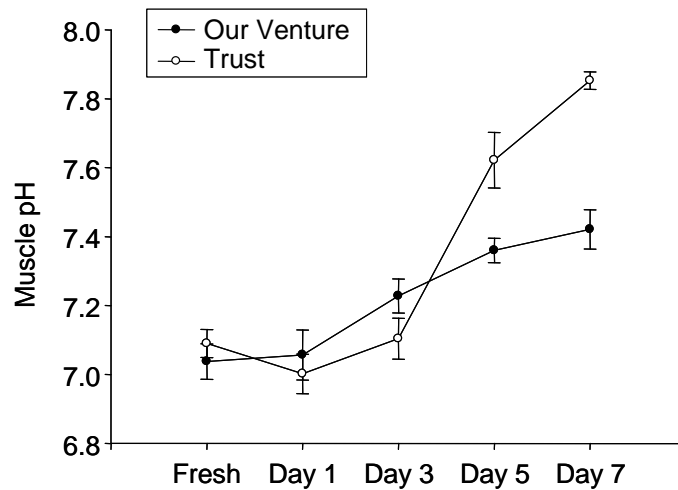


Figure 4.11. Changes in muscle pH in *Nephrops* tails kept on ice up to 7 days after being sampled from boxes at Young’s Annan factory on 18/07/06. Values are the mean ± S.E.M. of ten determinations.

The bacterial load of the samples was first measured on day 2, when the values for the *Venture* were within normal range (10^3 bacteria per gram), whereas those for the *Trust* were significantly higher (Figure 4.12). Over the following days, TVCs increased in both groups, and reached values greater than 10^5 bacteria per gram by day 7.

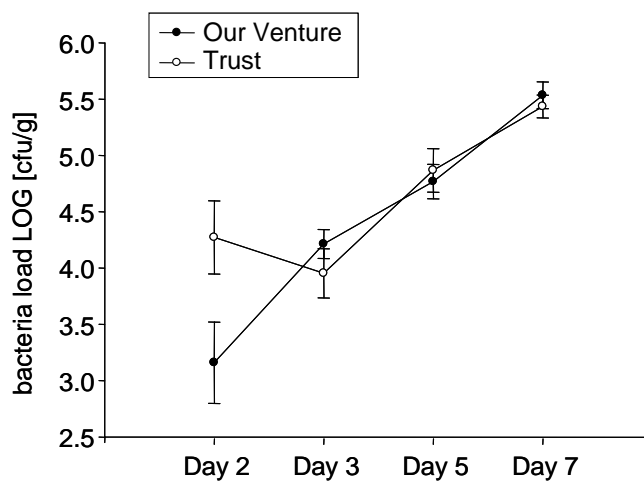


Figure 4.12. Changes in TVC for *Nephrops* tails kept on ice up to 7 days after being sampled from boxes at Young’s Annan factory on 18/07/06. Values are the mean ± S.E.M. of ten determinations.

Nucleotide breakdown products of tails from both boats held on ice for 7 days are presented in Figure 4.13. 'Fresh' samples correspond to the tails taken at 6am, soon after they arrived in the factory, and in these IMP and not AMP was the main nucleotide. This compares with typical fresh samples taken immediately after capture by the Aora research vessel, in which the main nucleotide is AMP (Figure 2.14). In tails from Trust the second highest nucleotide was AMP, whereas in tails from Our Venture the second highest nucleotide was INO. This indicates a clear difference in freshness.

When held on ice, IMP dropped very quickly in tails from Our Venture and highest concentrations were observed in fresh samples, compared with days 3-5 for tails from Aora (Figure 2.14) or for the Stornoway commercial trial (see later). In tails obtained from the Trust, IMP levels were higher than in tails from Our Venture and peaked between days 1 and 3. Interestingly, on tails from Our Venture the main nucleotide from day 5 onwards was HX (related with a bitter-off taste in fish products) and the same was true on tails from Trust from day 7. These profiles indicate a greater loss of freshness than occurs in the controlled trials on the research vessel Aora., even for tails incubated at 16°C for 24 h and then stored on ice (Figure 2.14 D).

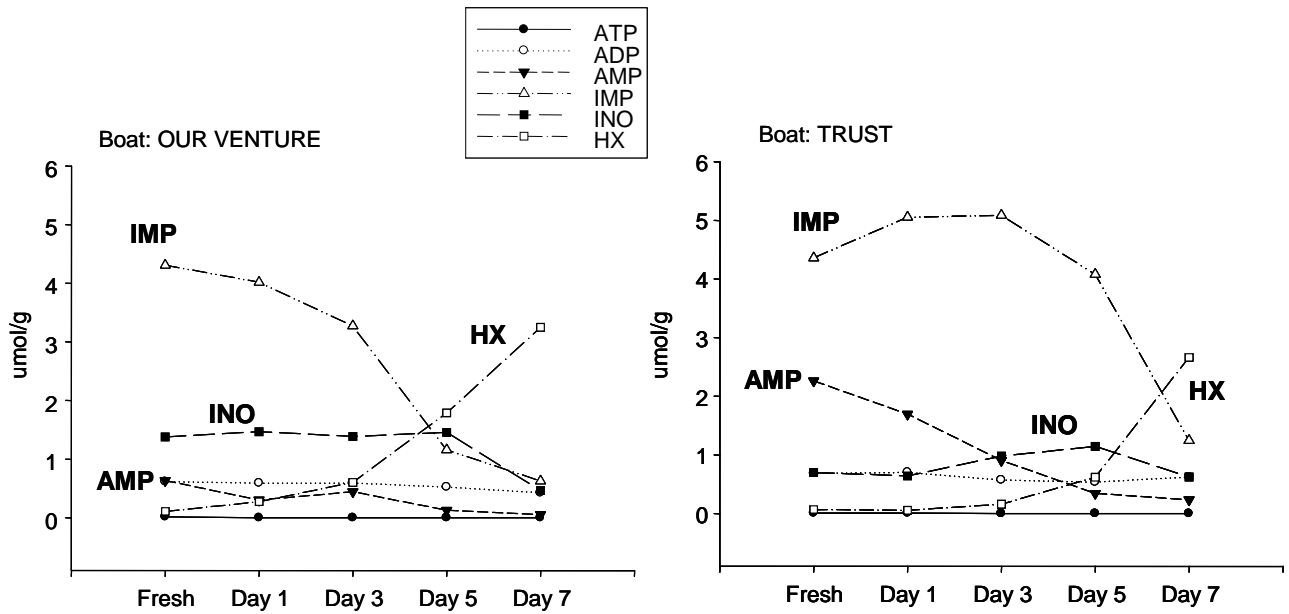


Figure 4.13. Changes in ATP breakdown products in *Nephrops* tails from Our Venture (left) and Trust (right) kept on ice up for 7 days after being sampled from boxes at Young’s Annan factory on 18/07/06. Values are the mean \pm S.E.M. of ten determinations.

The K values derived from these data were within the acceptable range (~ 20) for the *Trust* samples for up to 5 days of holding on ice, but were greater for the Our Venture samples, with values of >20 over the whole period (Figure 4.14).

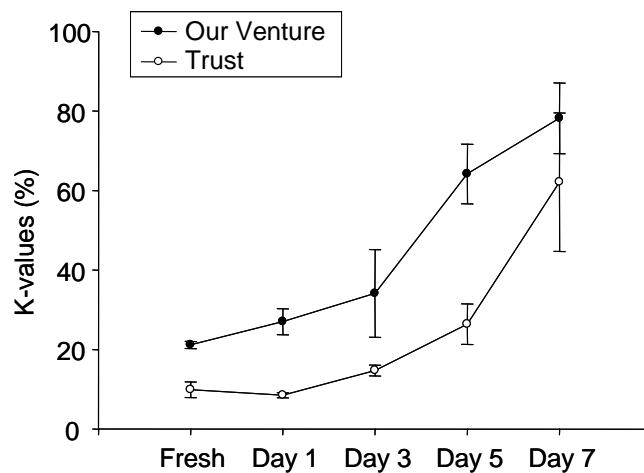


Figure 4.14. Changes in K-value percentages for *Nephrops* tails from Our Venture and Trust kept on ice up for 7 days after being sampled from boxes at Young’s Annan factory on 18/07/06. Values are the mean \pm S.E.M. of four determinations.

Taken together, the results from nucleotide breakdown products indicate that the *Nephrops* tails from the Trust were in good condition on arrival at the Annan factory on the morning following catch and landing, although higher levels of bacteria were already present. On the other hand, the product from Our Venture had a K-value already at the limit of the acceptable range, and although bacteria load was better than in tails from Trust on day 2, they reached similar levels of those recorded in tails from Our Venture within 24 hours. Differences in the freshness index (K-value) persisted over the following holding period on ice and bacterial spoilage became established in both groups, with consequent effects on muscle pH.

Trial at Stornoway, August 2006

Objectives

- Using temperature loggers, to measure the temperature changes in boxes of tailed *Nephrops* following catch by a commercial vessel and standard icing procedures
- To integrate this temperature information with the output of the Youngstrace traceability system for the fishing trip by the commercial vessel
- To measure the rates of temperature change in boxes of *Nephrops* tails during storage at ambient temperature and following transfer from the vessel to an on-shore cold store
- To evaluate the use of an IR thermographic camera for measuring the temperature of product in fish boxes at different times after landing
- To apply a range of quality measures to product sampled at different times following the catch

Introduction

Within the Partnership project between Young's Seafood Ltd. and GU, an extensive series of trials has been conducted on the RV Aora fishing in the Clyde Sea Area, for which traceability data for each fishing trip were fully available and the location and duration of the catch were replicated on each trip. Following each catch the product was handled in a precisely controlled manner with regard to the time of tailing and the holding temperature. Sampling of the tailed product was carried out using a series of

assays developed at GU to determine a range of measures relating to the flavour, freshness, shelf-life and spoilage of the tailed product.

In order to relate the results obtained under the strictly controlled conditions on the RV Aora to the commercial situation, a series of trials was arranged using vessels that regularly supply product to Young's Seafood Ltd.. The purpose of these trials was to establish procedures for monitoring the temperature of the tailed product following the normal fishing, post-catch handling, grading and tailing practices operated by these boats. These trials also provided an opportunity to obtain product from the commercial supply chain and to compare its quality with the 'benchmark' values established from the Aora catches.

The commercial vessel chosen was a day boat, the *Wavecrest*, equipped with standard prawn trawl gear and fitted with the Youngstrace traceability system. It had a chilled fish storage room, but this was inoperative on the day of the trial. The trial was carried out on August 10-12th 2006.

Methods

Traceability

Traceability records for each fishing trip were generated by the Youngsdtrace traceability system. The data included the time, duration and location (GPS position) of each trawl, the time between landing the catch and icing the tails, and the catch

weight. These data were made available by arrangement with the YBC management (Mr Malcolm Blanthorn at Annan and Mr John Nicolson at Stornoway).

Temperature logging

Ambient air temperatures and the temperature at various points within the boxes of prawn tails were measured using ‘Stowaway’ and ‘Tidbit’ continuous temperature logging devices (Onset Computer Corp.). These were programmed to take measurements at intervals of 1 minute for the period of the trial.

For on-board measurements, by arrangement with the YBC management (Mr Malcolm Blanthorn at Annan and Mr John Nicolson at Stornoway) the skippers of the fishing vessels were provided with logging devices for insertion into the boxes of tailed product after catching and tailing (2 devices per box). These recorded the temperatures within the box during on-board storage, and continued to record temperatures during the land-based transportation to the factory. The loggers were retrieved from the boxes on arrival of the product at the factory, and their temperature data were downloaded and analysed using the ‘Boxcar Pro 4.3’ software package (Onset Computer Corp.).

For on-shore measurements, boxes of tailed product obtained from the commercial supply were equipped with up to 4 loggers positioned at different points through the product, and a further logger was attached externally to monitor the ambient

temperature. These boxes were stored at different temperatures within the factory premises, either in the reception bay (10°C - 15°C) or in the chill store (0°C - 3°C).

Thermography

The use of an infra red (IR) thermographic camera (Fluke 1200) was evaluated in the Stornoway trial. This device produces a medium-high resolution image of a surface, which can be represented as a colour-coded image or as a temperature profile.

Images were taken of the external surfaces of the fish box and of the top surface of the product. To allow measurements to be taken within the product, half of the tails were re-packed into a wire basket, which occupied the upper half of the box and allowed the upper and lower layers of product to remain in close contact. Removal of the basket for a short period (30s) allowed thermographic images to be obtained across the central plane of the product.

Quality measures

Samples of tails were obtained at various times after catch and storage, and were subjected to a series of assays for pH, bacterial load, spoilage indices (TMA), nucleotide breakdown products and the Freshness indicator (K index). The methods for these assays are given in Section I.

Results

The results were obtained for a fishing trip by the Stornoway-based vessel Wavecrest (Figure 4.15) on Thursday 10th August 2006. As recorded by the Youngstrace traceability system (Figure 4.16), the vessel fished in the Inner Minch and conducted two trawls. The first was from 04.30h – 09.35h (trawl time 5:05h) at GPS position 58D5.25N 6D19.46W, with the tails boxed and iced at 13.00h, and the second was from 11.00h – 16.30h (trawl time 5:30h), with the tails boxed and iced at 18.00h. The vessel landed at port at 23.30h.



Figure 4.15. The Wavecrest (black) and Sharon Rose (green) in Stornoway harbour. Both vessels supply *Nephrops* to Young's Seafood Ltd.

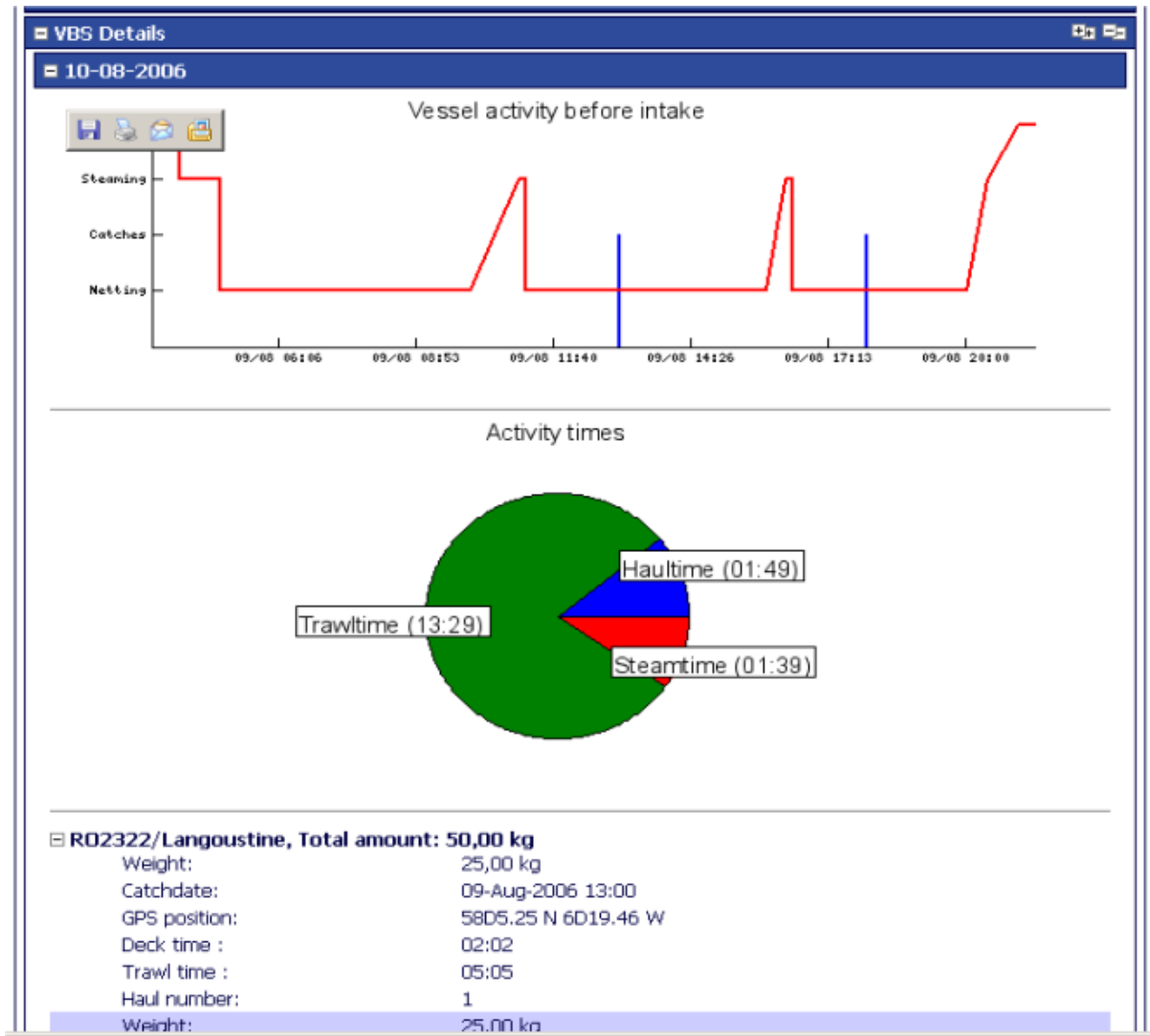


Figure 4.16. Youngstrace traceability record for Wavecrest on August 9th 2006. Vessel activity trace shows trawl and haul times (red) and times when tailed product was weighed (blue)



Figure 4.17. Boxes of prawn tails from Haul 1 (left) and Haul 2 (right) on landing at Stornoway quay at 23.30h. Note the loss of ice at the edges of the box from Haul 1.

Temperature changes in boxes of tailed *Nephrops* following catch

Temperature loggers were placed in one box of *Nephrops* tails from each of the two trawls performed by the Wavecrest on August 10th. One logger was placed at the bottom of the box, and the other was positioned in the middle of the product. The boxes were iced on top according to normal commercial practice, and the boxes were stored in the fish hold until landing (Figure 4.17). No chill facility was operational in the fish hold on that day.

The temperature records obtained (Figs. 4.18 and 4.19) indicate that the initial temperature of the tails was around 13.5°C, reflecting the body temperature of the animals in the sea.

For haul 1 the temperature in the middle of the product fell from 13.5°C to around 7.0°C over the 9.5h before landing at port (a rate of fall of 0.70 °C . h⁻¹). The temperature at the bottom of the box fell much less rapidly, from 13.5°C to 11.0 °C, over the same period (a rate of fall of 0.26°C . h⁻¹). This indicates that the ice layer on top of the product has some cooling effect on product down to the middle, but that the tails at the bottom of the box were subjected to very little cooling.

For haul 2 the temperature in the middle of the product fell from 13.5°C to around 8.5°C over the 5.0h before landing at port (a rate of fall of 1.0 °C . h⁻¹). This rate is somewhat higher than that from haul 1. In greater contrast, however, the temperature at the bottom of the box from haul 2 fell much more rapidly (by a factor of 7) than that at the bottom of the box from haul 1, from 13.5°C to 4.5°C (a rate of fall of 1.8 °C

. h⁻¹). Moreover, this temperature drop was greater at the bottom of the box than in the middle of the box, which is opposite to the case from haul 1.

The difference between the rates of cooling of the product in the middle of the boxes from haul 1 and haul 2 may reflect either the positioning of the loggers or the amount of ice used. The greater difference between the measures at the bottom of the boxes from haul 1 and haul 2 may be explained if the second box was stacked on top of the first (or another iced box), so that it received a cooling effect from the ice below, through the floor of the box. It is not known if this was the case, but it is the most plausible explanation.

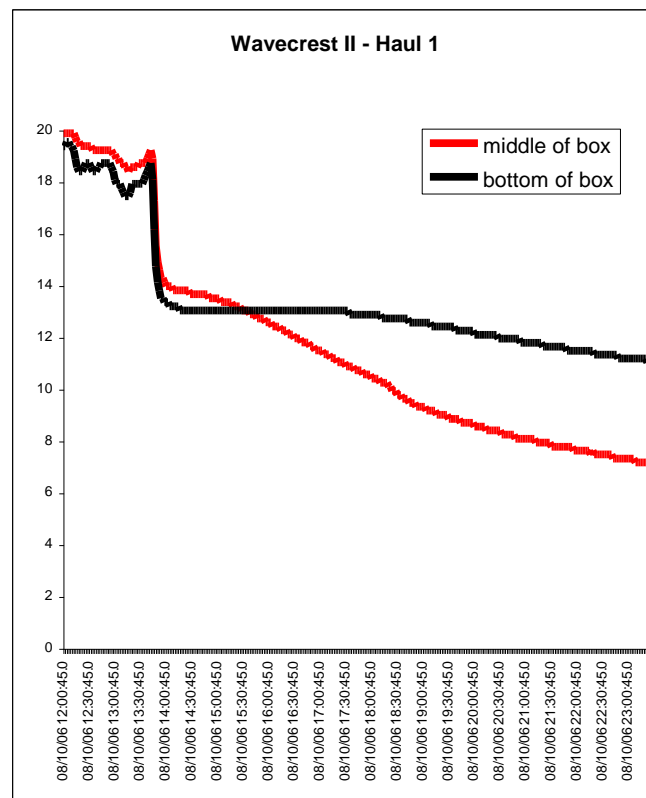


Figure 4.18. Temperature logs for Wavecrest haul 1. Before 1.30h the loggers reflect ambient temperature on the bridge. After 1.30h they report the temperature in the box of iced tails.

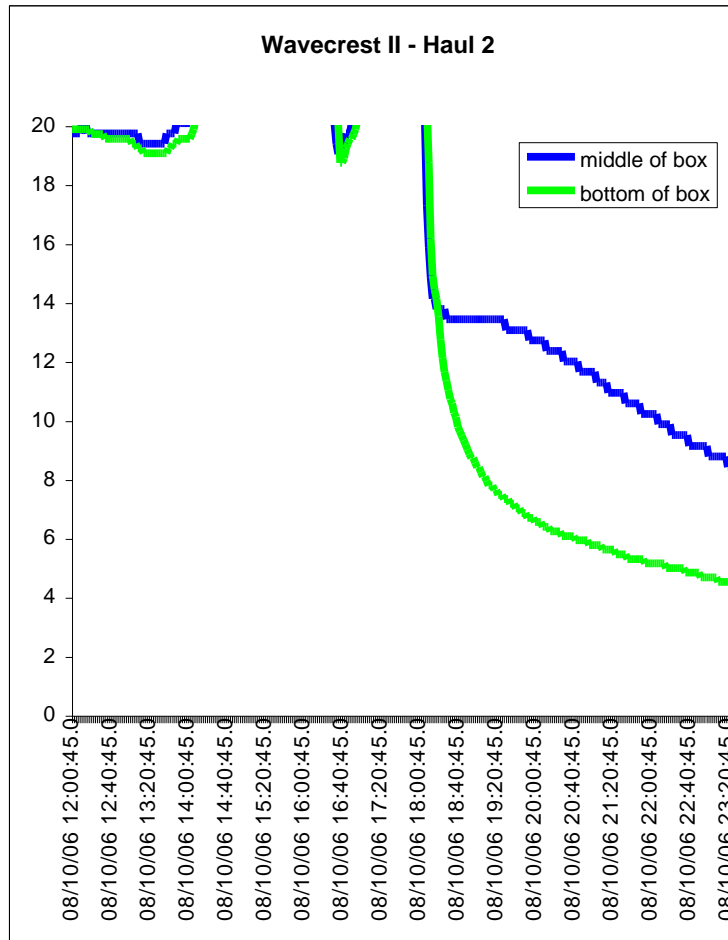


Figure 4.19. Temperature logs for Wavecrest haul 2. Before 18.15h the loggers reflect ambient temperature on the bridge. After 18.15h they report the temperature in the box of iced tails.

Temperature changes in boxes of tailed *Nephrops* following storage at different temperatures

On arrival at the factory at 23.45h on the day of catch, the boxes containing the temperature loggers were each treated in the following way:

the ice remaining above the product was removed;

- the positions of the two temperature loggers were confirmed and the loggers were removed for downloading the data;
- samples of tails totalling around 50 were removed and preserved for the quality assays (see below)

Product in the upper half of the box was then removed into a wire mesh basket, designed to fit tightly into the dimensions of the box. Two temperature loggers were inserted at the centre and at the edge of the basket, at mid-depth. Two other temperature loggers were inserted into the lower layer of the product at equivalent positions.

At 02.00h the box from haul 1 was placed in the factory reception bay (varying between 6-12°C), and the box from haul 2 was placed in the cold store room (controlled to 1-3°C). The boxes remained undisturbed in these places until 14.00h on the following day (total of 12 h). After this time further tail samples (totalling around 50 from each box) were removed and preserved for the quality assays (see below).

The temperature records obtained (Figures 4.20 and 4.21) indicate that the contents of the boxes became equilibrated with the surrounding air temperatures over these periods of storage. For the box from haul 1 the range of temperatures through the product measured on landing at port (7°C at the top to 11°C at the bottom – Fig. 14) became smaller, but remained around a value of 7°C reflecting the ambient temperature in the reception bay overnight. For the box from haul 2, the product in the upper layer started at ~8°C (Fig. 4.21) and took up to 6 hours to equilibrate with the chill room temperature (1-3°C), while product in the lower layer equilibrated in less time, since it started from a lower temperature (~5°C).

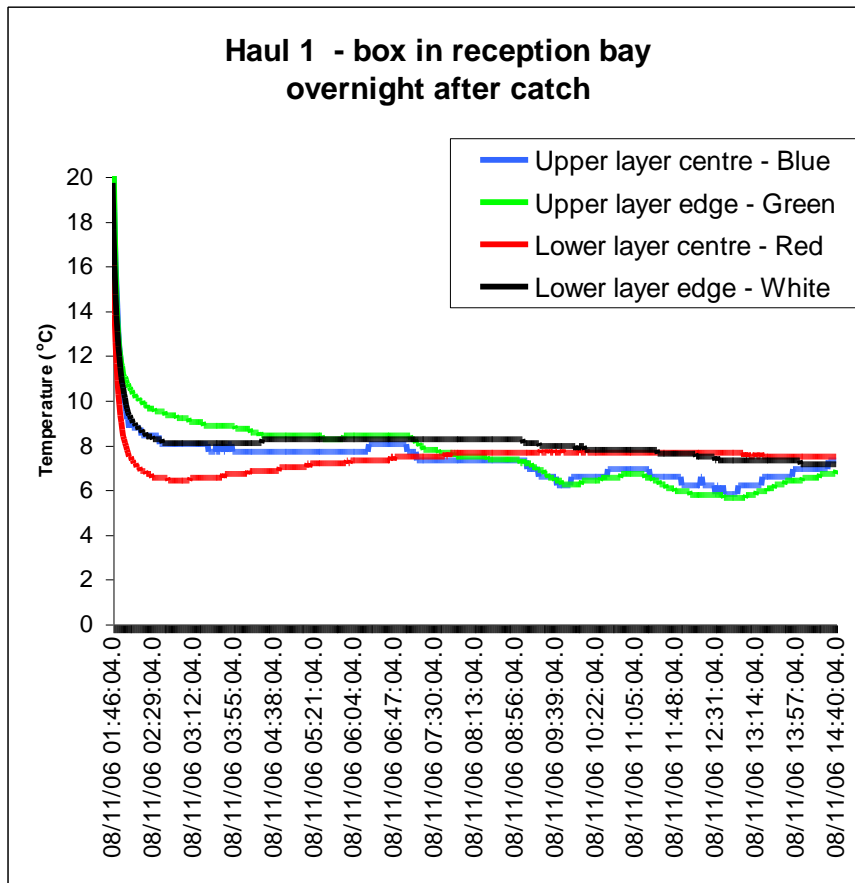


Figure 4.20. Temperature logs for overnight storage of box from haul 1 in reception bay. The temperature in the bay varied between 6-12°C.

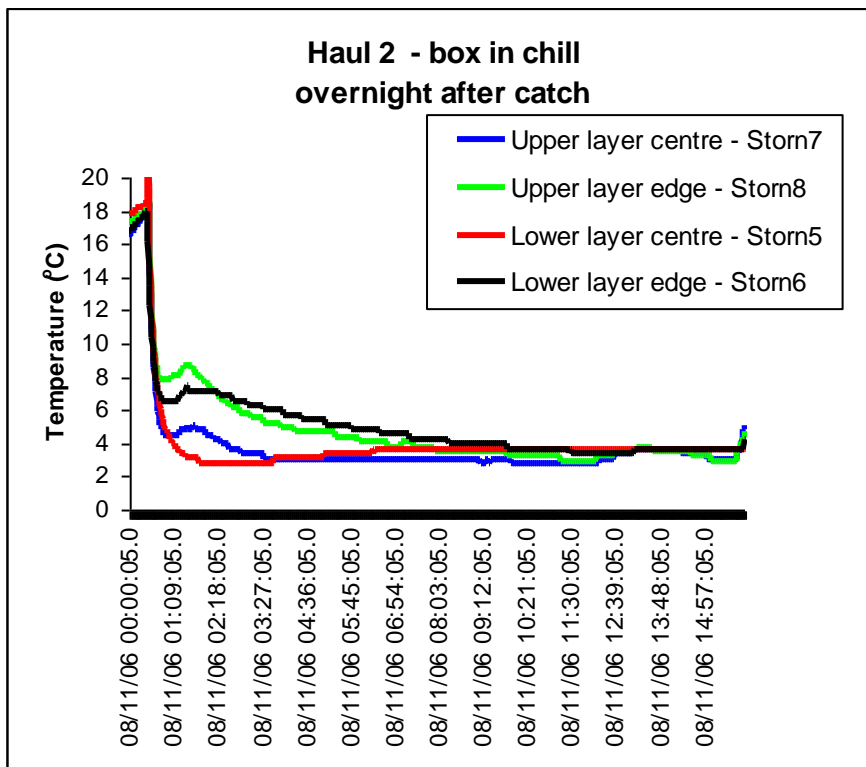


Figure 4.21. Temperature logs for overnight storage of box from haul 2 in chill store room. The temperature in the chill was controlled at 1-3°C.

The effect of further imposed temperature shifts on product temperature throughout the box

A number of further short-term measures were performed on the boxes of *Nephrops* tails in order to study the thermal properties of the product. These involved moving the boxes between the chill store room (where they were exposed to a narrow range of 1-3°C, and an outside location where they experienced ambient air temperatures (14-17°C). For these trials, in addition to two temperature loggers per layer in each box, another logger was attached to the outside of each box, so that it gave a continuous record of ambient temperature.

In order to facilitate the collection of thermographic images from the centre of the product, removal and replacement of the wire basket containing the upper layer of product was necessary. This caused some direct exposure of the product in the centre of the box to ambient temperatures, but no significant effect of this was registered by the *in situ* loggers.

Figure 4.22 shows the changes occurring during the first hour after transferring the box from haul 1 from ambient (~16°C) to the chill store room, a temperature drop of around 15°C. The largest response was by the loggers in the upper layer, which were close to the top surface. Even these however registered only about half of the imposed temperature shift. The loggers in the lower layer, furthest from the top surface, reported virtually no change in temperature during this period.

Figure 4.23 shows the opposite imposed temperature shift, when the box from haul 2 was transferred from the chill store room to an outside location, representing a temperature increase of around 15°C. The equivalent pattern of temperature response occurred, with the loggers near the surface of the product showing the most rapid response, although again only by about half of the imposed shift, while those in the lower layer responded less rapidly and completely. Indeed over the 2h of this trial the centre of the product changed by no more than 2°C, despite the ambient temperature increasing by 15°C.

These and other results demonstrate that a box of *Nephrops* tails has a high level of thermal inertia, and responds only slowly and partially to imposed changes in the surrounding air temperature. This indicates that such product has a high level of heat insulation, and consequently a low rate of heat conduction.

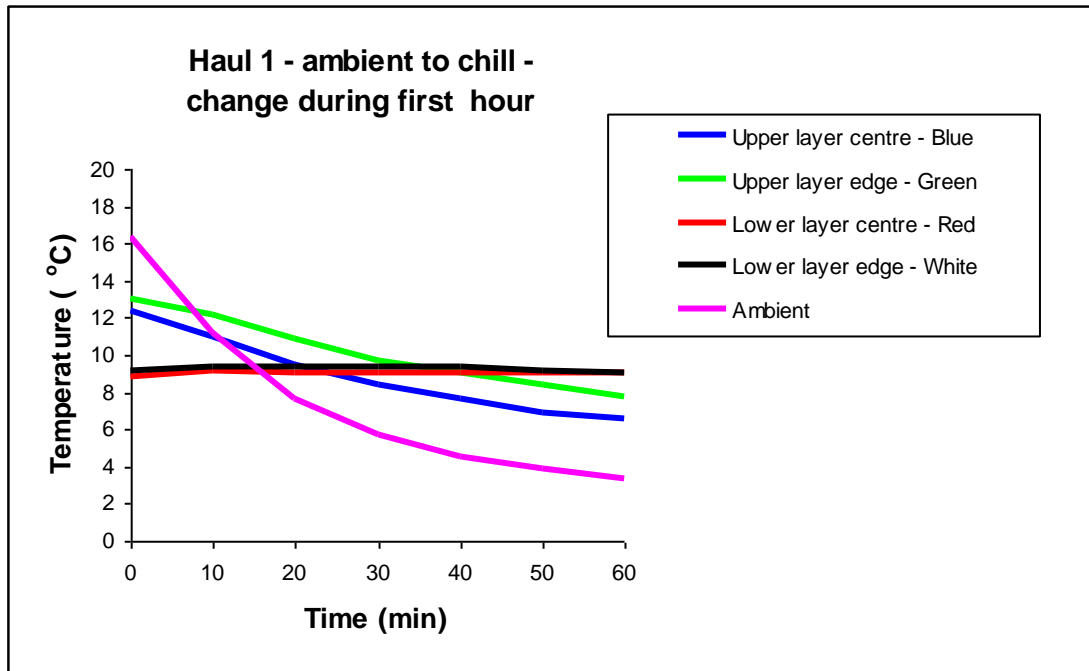


Figure 4.22. Temperature logs for transfer of box from haul 1 from ambient to chill store room. The temperature in the chill was controlled at 1-3°C.

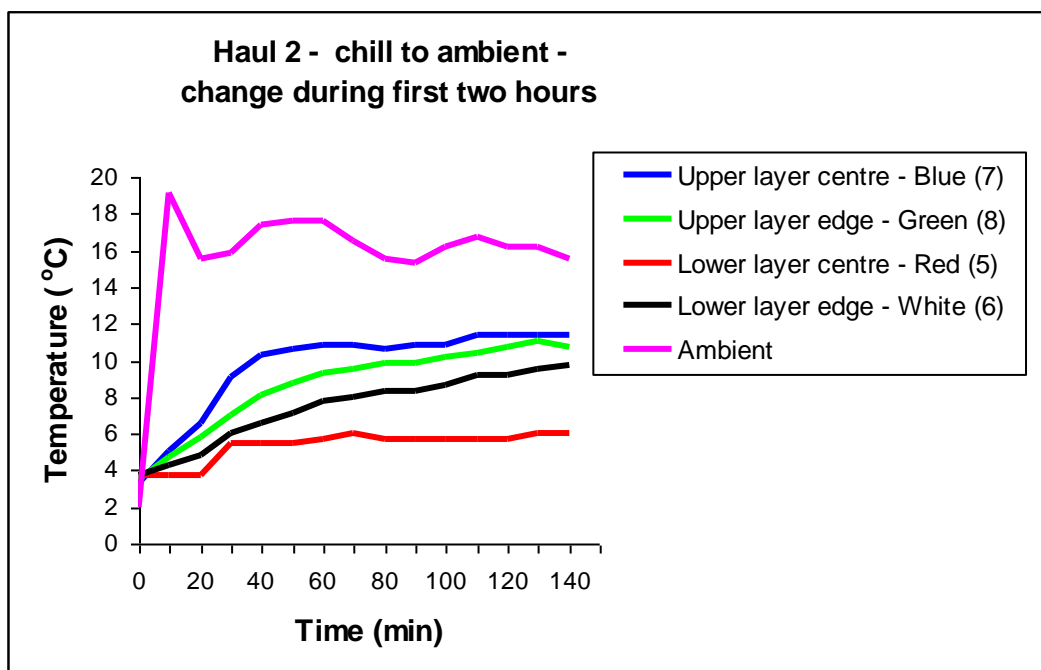


Figure 4.23. Temperature logs for transfer of box from haul 2 from chill store room to ambient.

Temperature changes associated with spoilage

The temperature in the box of *Nephrops* tails from haul 1 was monitored for up to 72 hours after catch, in order to determine how spoilage affects product temperature. The box was stored over the second night after capture in the chill room, and then at ambient temperatures varying between 15°C and 20°C during subsequent transport and storage (Fig 4.24).

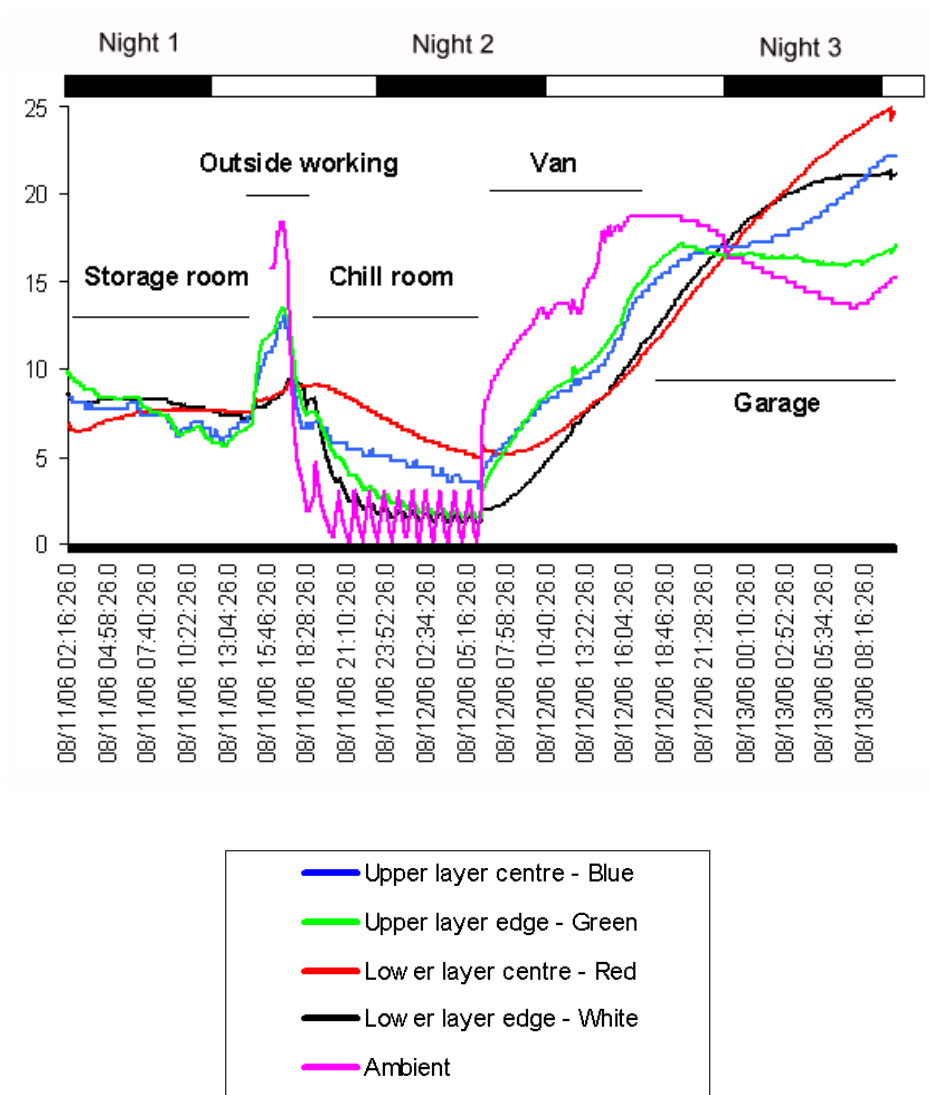


Figure 4.24. Temperature logs for the box from haul 1 from the night of landing for the next 2.5 days.

During night 2 the temperatures recorded at the edges of the box dropped into the range held in the room (1-3°C) within ~4h, whereas in the centre of the box the temperatures remained between 4-6°C even after 12 h. This may reflect the fact that the product at the centre of the box had reached the initial stages of spoilage, with some associated heat production.

During the following 12h period the box was transferred to a vehicle and experienced ambient temperatures up to 20°C. The product at the edges of the box responded more rapidly to this temperature shift than did the product in the centre, but the rate of increase in the centre was itself more rapid than might have been expected from the earlier shift (Fig 4.21). This again suggests that internal heat production was involved. Off-odours produced by the material at this time were consistent with the occurrence of spoilage.

At the beginning of the third night, a nodal point was reached where all loggers recorded the same temperature, and after that time there was significant increase in the temperature of the product in the box, particularly at the centre, taking it up to 10°C above ambient. This reflects a large amount of heat production associated with the advanced stages of spoilage.

IR Thermography

Thermographic images were taken of the surface of the upper layer, the surface of the lower layer and/or the sidewall of the boxes of *Nephrops* tails at different times after catch and landing.

Immediately after landing on the quay, the image of the side walls of the boxes gives an indication of the extent of the icing, and of the temperature of the product beneath (Figure 4.25).

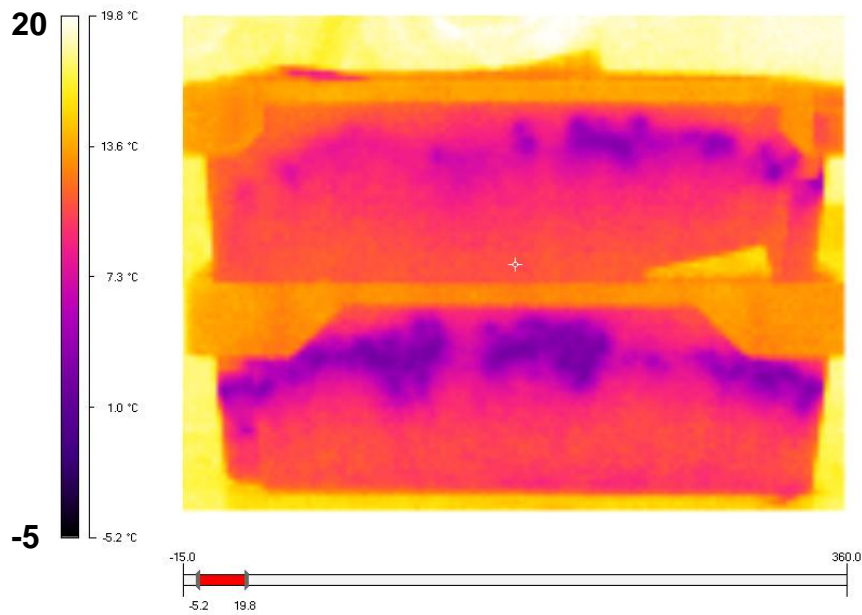


Figure 4.25. Thermographic image of Box from haul 2 (above) and box from haul 1 (below) stacked (23.00h on day of catch)

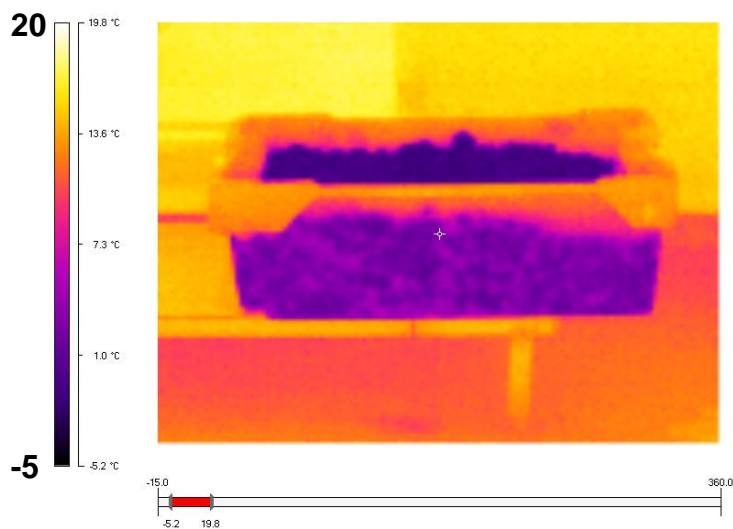
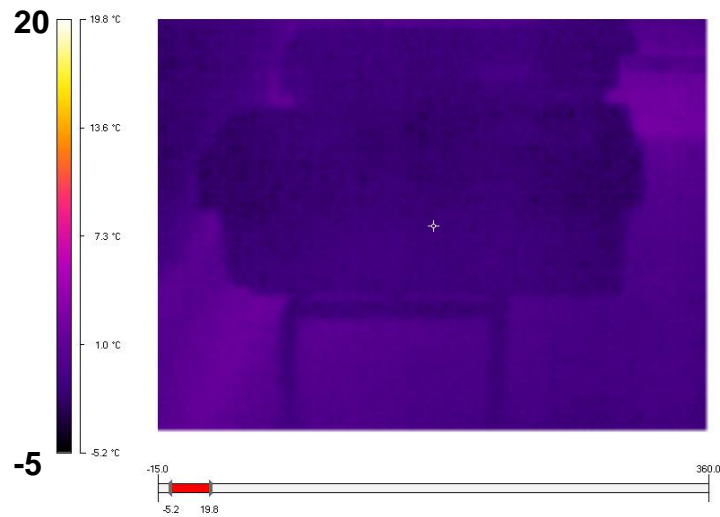


Figure 4.26. Thermographic image of a box of ice at ambient temperature

For comparison, Figure 4.26 shows the image of a box containing only ice, standing at ambient temperature ($\sim 12^{\circ}\text{C}$). This shows the difference between the directly measured temperature of the surface of the ice, and the temperature of the side wall.

No temperature differences are apparent through the side wall of the box of *Nephrops* tails after it has been stored in the chill room for 12h. (Figure 4.27).



Quality measures

Muscle pH

Muscle pH was recorded in tails from hauls 1 and 2 when samples were stored on ice for up to 7 days. Muscle pH was similar between animals from hauls 1 and 2 (Figure 4.28). In this experiment, pH was measured directly with a pre-calibrated ISFET microelectrode, with inoperative temperature compensation, which may explain the differences in absolute values obtained, compared with previous trials.

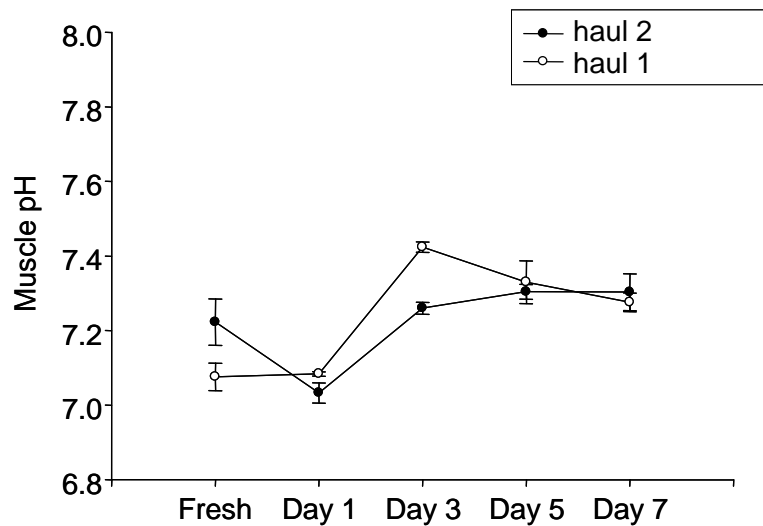


Figure 4.28. Changes in muscle pH in *Nephrops* tails kept on ice up to 7 days after being sampled from boxes at Young's Stornoway factory on 10/08/06.

Bacterial load

Samples from both hauls held on ice were also tested for bacterial load. No differences in total bacteria counts were found between tails from hauls 1 and 2 (Figure 4.29). Interestingly, initial values and values obtained after storing tails on ice for up to 5 days were similar to values obtained with the RV Aora.

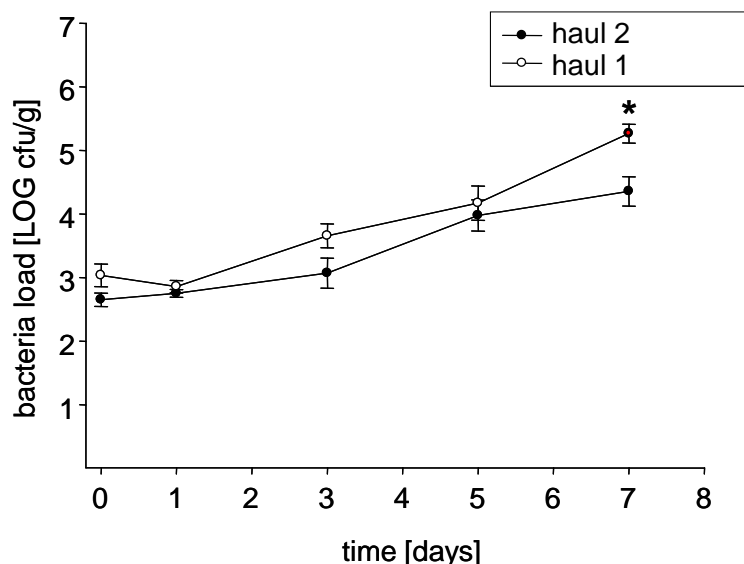


Figure 4.29. Changes in TVC for *Nephrops* tails kept on ice up to 7 days after being sampled from boxes at Young's Stornoway on 10/08/06. Values are the mean \pm S.E.M. of five determinations.

Nucleotide breakdown products and K-values

Changes in ATP breakdown products when tails from the 2 boxes were stored on ice are shown in Figure 4.30. Fresh samples corresponded to the condition of tails routinely taken into the Stornoway factory, which is adjacent to the landing port. At this sample time, the main nucleotide in tail muscle from both hauls was IMP instead of AMP (the highest nucleotide scored on fresh samples from Aora vessel). This might be due to the fact that fresh samples in Aora are taken when animals are landed on board, whereas the samples taken from Stornoway were at times of 9h (haul 1) and 5h (haul 2) post capture (arrival time at the factory). Higher levels of IMP were found in haul 1 compared to haul 2, corresponding to the different times of these catches. In both cases, AMP dropped with storage time, although it seemed to fall more rapidly in tails from haul 1. Finally, IMP rose from day 1 and high concentrations were maintained throughout the experiment. On day 7, tails from haul 2 showed a slightly better nucleotide profile, with higher levels of IMP ($5.2 \pm 1.1 \mu\text{mol/g}$ in haul 1 compared to $4.4 \pm 0.4 \mu\text{mol/g}$ in haul 2) and lower levels of HX ($0.3 \pm 0.08 \mu\text{mol/g}$

and $1.1 \pm 0.4 \mu\text{mol/g}$ in haul 1 and 2 respectively) although these differences were not significant.

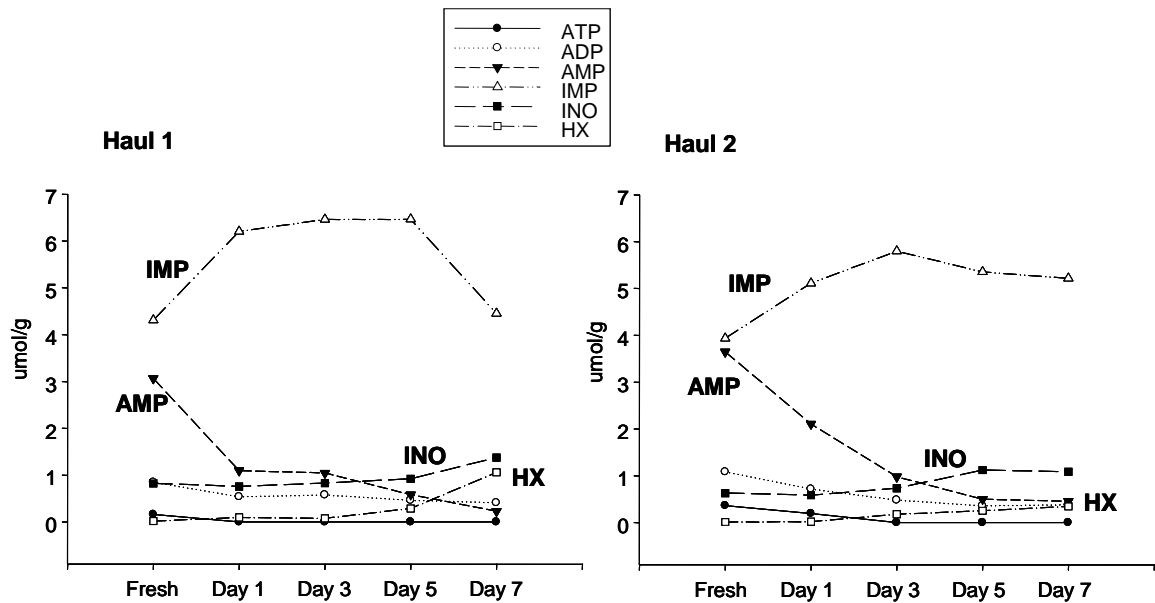


Figure 4.30. Changes in ATP breakdown products in *Nephrops* tails kept on ice up for 7 days after being sampled from boxes at Young’s Stornoway factory on 10/08/06. Values are the mean \pm S.E.M. of four determinations.

According with these results, K values between tails from the two hauls were similar and although higher levels were observed in tails from haul 1 on day 7, the differences were not statistically significant. Nevertheless, tails from both hauls had K-values lower than 20 (freshness limit proposed for several fish species) for up to day 5 stored on ice.

For comparison, a sample of product that had been frozen, thawed and de-shelled using standard factory procedures at Stornoway was taken from the conveyor belt and processed in the same way in order to obtain the K-values (isolated symbol in Figure 4.31). In this sample, which was analysed in triplicate, the K-value was 22.60 ± 2.62 . This value is around the freshness limit of 20, if this is considered applicable in

Nephrops. Moreover, TMA values were also measured in this sample, and values (2.95 ± 0.45 mg TMA/100 of muscle) were similar to those obtained from fresh tails (hauls 1 and 2 showed TMA ranging from 2.24-2.78 mg TMA/100 g muscle).

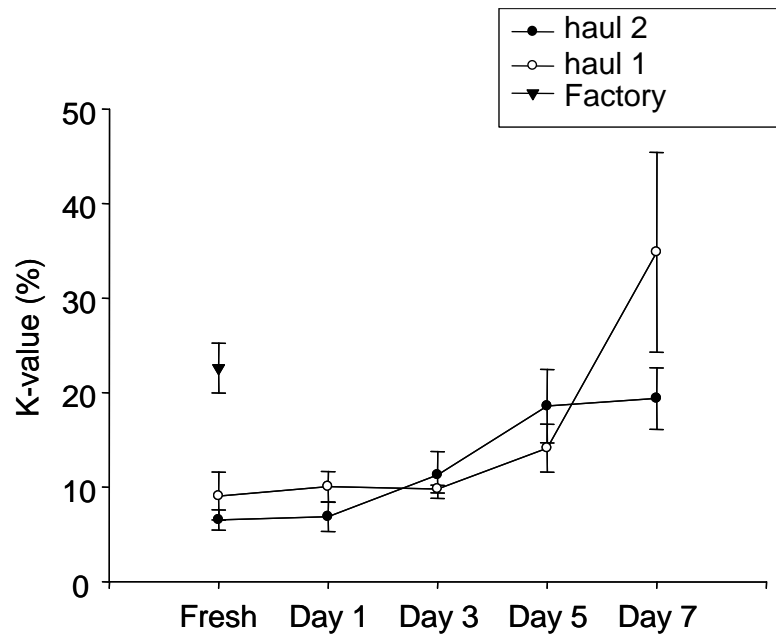


Figure 4.31. Changes in K-value percentages for *Nephrops* tails kept on ice up for 7 days after being sampled from boxes at Young's Annan Stornoway on 10/08/06. Values are the mean \pm S.E.M. of ten determinations.

Taken together these results suggest that samples from Stornoway, if stored on ice adequately, would have a good quality for at least 5 days. In fact, the bacteria load up to day 5 was similar to that obtained in reference samples taken from Aora, and the K-values were lower than 20 (although it needs to be established by sensory trials that this value is applicable to *Nephrops*).

The thermal properties of boxes of *Nephrops* tails

The results obtained in the Stornoway trials highlighted the importance of the thermal properties of *Nephrops* tails when packed in boxes in determining the effects of imposed icing or chilling on the stored product. A series of controlled trials was therefore undertaken to define these thermal properties in more detail, by measuring the rate of chilling of product when boxes of different sizes were held in a chill facility.

For each trial, tails obtained from standard trawls by the RV *Aora*, were transported to the University of Glasgow and were laid out in a single layer for 1h to allow them to equilibrate to the ambient temperature (typically ~16°C). They were then packed into plastic containers of different sizes (Table 4.1), with a temperature logger embedded centrally within the product (and in some cases also at other positions). The containers, which were completely filled with tails, were then placed in a controlled temperature room, the ambient temperature of which was monitored by another temperature logger. The trials were started within 6 h of the catch being landed on the vessel, and it ran for at least 48 h, and in some cases for 6 days.

Table 4.1. The dimensions of the containers, and the weight and number of tails.

* Container 1 was tubular

	Length (mm)	Width (mm)	Height (mm)	Volume of container (l)	Average distance from edge to centre (mm)	Weight of product (kg)	Number of tails
Container 1*	70		70	0.32	35	0.21	25
Container 2	120	120	125	2.01	60	1.18	147
Container 3	295	190	150	9.46	106	4.65	559
Container 4	410	300	240	30.00	160	11.30	1750

The temperature profiles for the containers over the first 48 h of a typical trial are shown in Figure 4.32. It can be seen that the temperature in the chill room remained at 3-5°C throughout (Ambient in Figure 4.32). The rate of temperature drop in the middle of the containers differed according to their size, with the logger in container 1 reaching ambient temperature within 6 h, while ambient temperature was not achieved until 18 h in container 2, and 30 h in container 3.

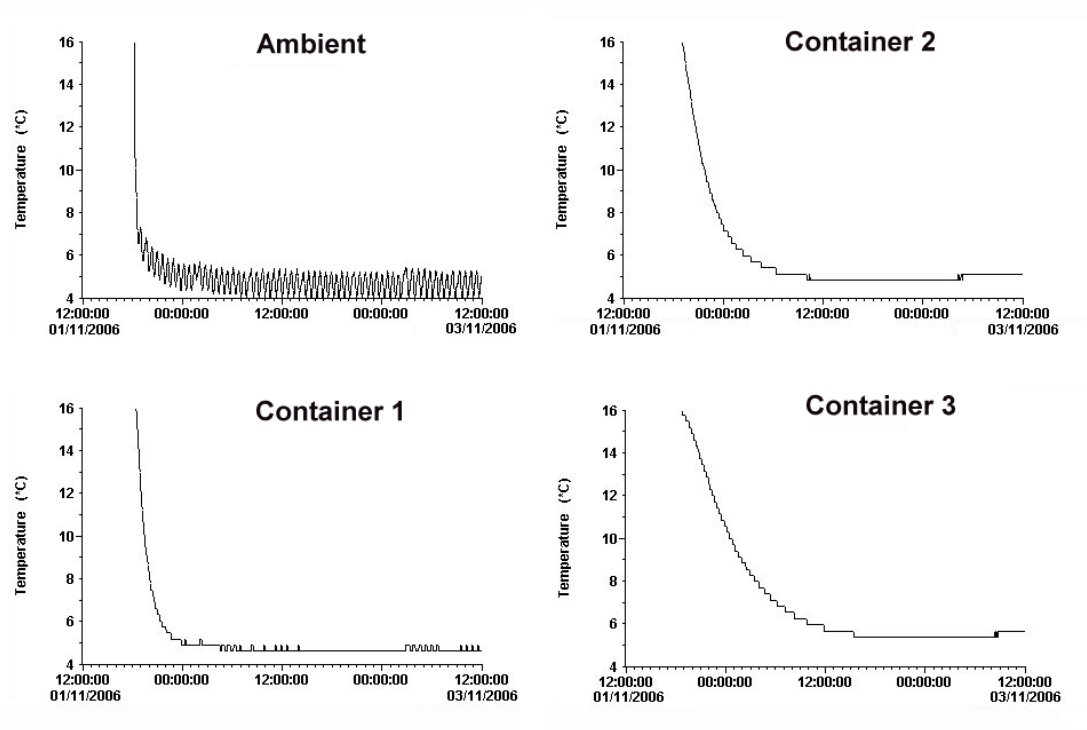


Figure 4.32. Recordings from temperature loggers embedded in the centre of *Nephrops* tails which completely filled different sized containers. These were held in a chill room (Ambient). The product had an initial temperature of 16°C.

A separate trial was conducted with a larger container, container 4, which had a capacity for ~11 kg of product (Table 4.1). This was separated into upper and lower layers by a wire mesh. Starting from the same initial temperature (~16°C) the equilibration time of tails in the centre of the box to the ambient temperature in the chill room was ~42 h, while near the edge of the box it was ~26 h (Figure 4.33). In

this case, after 48 h of storage samples of tails were taken from the surface of the product, and also from the middle and were subjected to a series of quality assessment measures (pH in the muscle, bacteria load, TMA values, K-values).

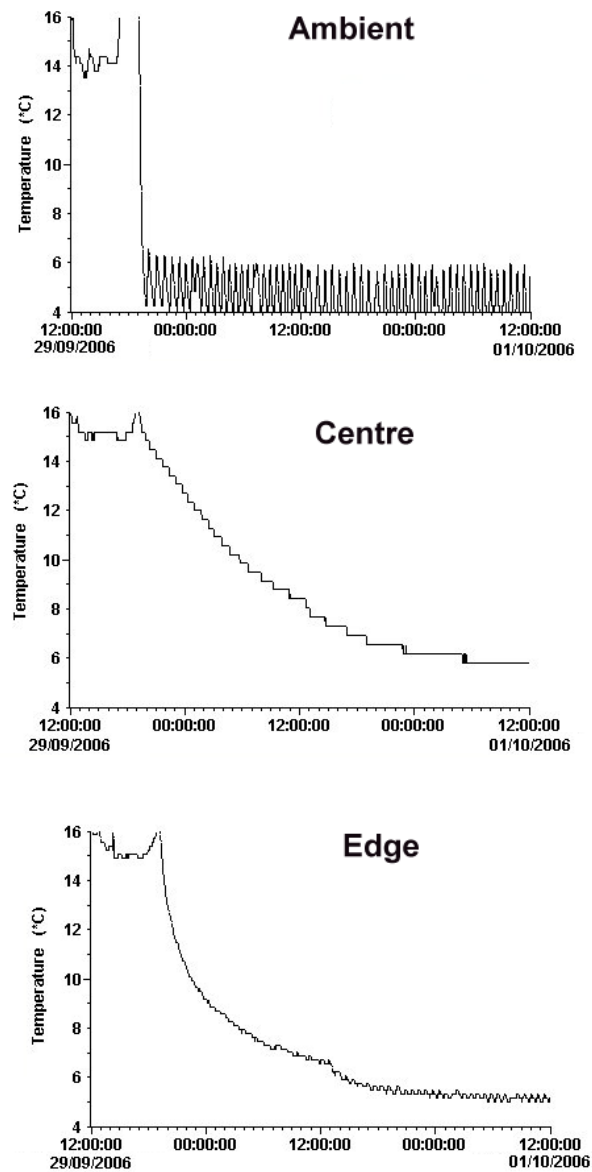


Figure 4.33. Recordings from temperature loggers embedded in the centre and near the edge of *Nephrops* tails which completely filled container 4. This was held in a chill room (Ambient). The product had an initial temperature of 16°C.

Thermographic images from the surface of the upper layer and from the surface of the lower layer (i.e. the middle of the container) were taken at the start of the trial, and after 16h and 30h (Figure 4.34). They confirm that the rate of cooling of the product in the middle is lower than that at the surface.

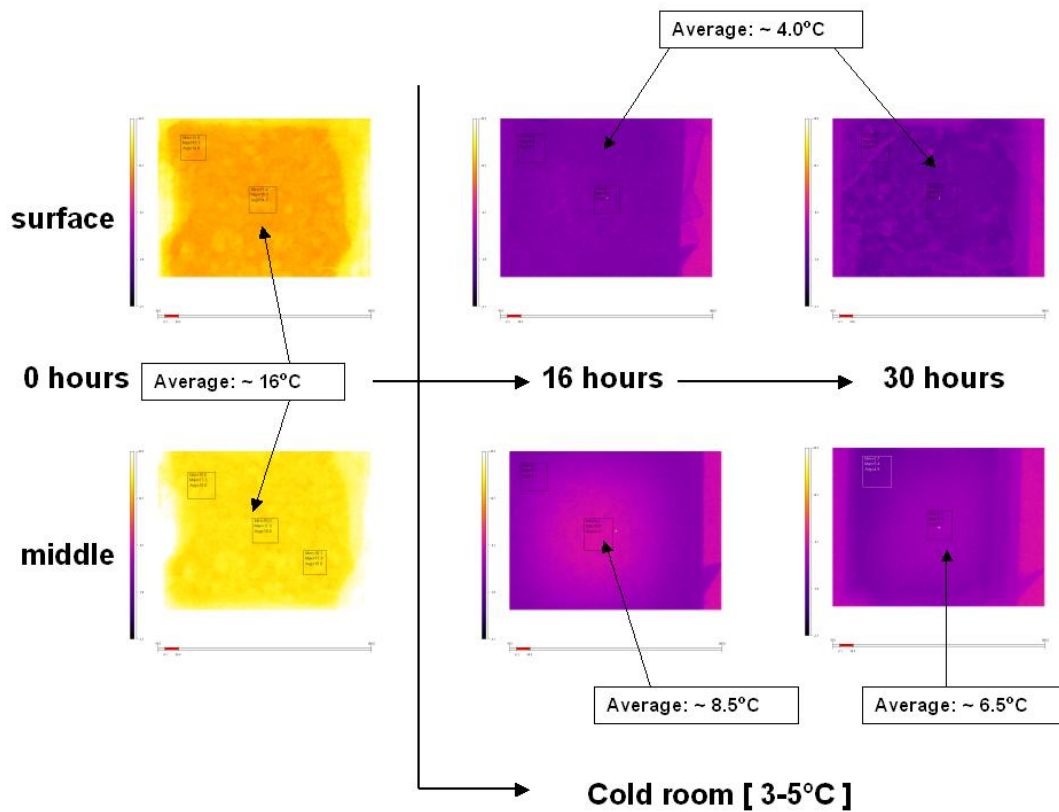


Figure 4.34. Thermographic images from the surface and the middle of container number 4 taken at different times. The product had an initial temperature of 16°C and was stored at 3-5°C.

Quality Assessment Measures

In this trial, after 48 h of storage samples of tails were taken from the surface of the product, and also from the middle and were subjected to a series of quality assessment measures (pH in the muscle, bacteria load, TMA values, K-values).

Muscle pH was higher in tails from the middle compared to tails from the top of the box (Figure 4.35).

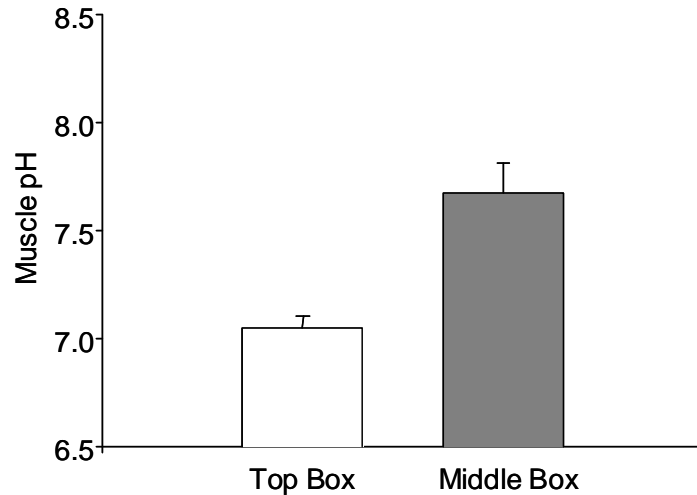


Figure 4.35. Values of muscle pH in tails sampled from the top and the middle of the box after 48 hours storage. Values are the mean \pm S.E. from ten different samples.

Bacterial load was also measured (Figure 4.36). Total bacteria counts were significantly higher in tails from the middle of the box compared to tails sampled from the top of the box. Furthermore, when TMA was measured corresponding results were obtained and higher levels of TMA were found in tails from the middle of the box (Figure 4.37).

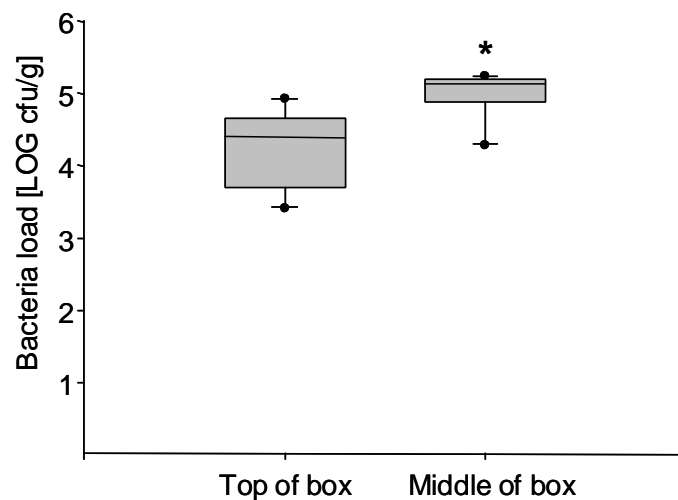


Figure 4.36. Bacterial load measured in tails sampled from the top and the middle of the box after 48 hours storage. Values are the mean \pm S.E. from five different samples.

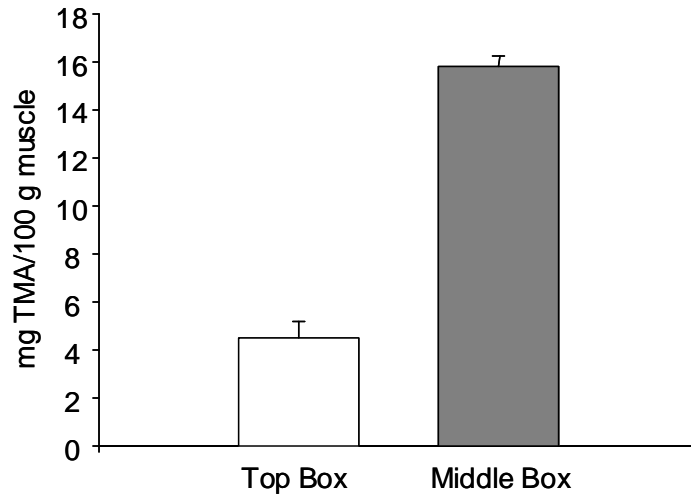


Figure 4.37. TMA concentrations in tails sampled from the top and the middle of the box after 48 hours storage. Values are the mean \pm S.E. from five different samples.

Finally, ATP breakdown products were measured and K-values were calculated accordingly (Figure 4.38). K-values were around 40 in tails from the middle of the box while tails from the top of the box had K-values around 20.

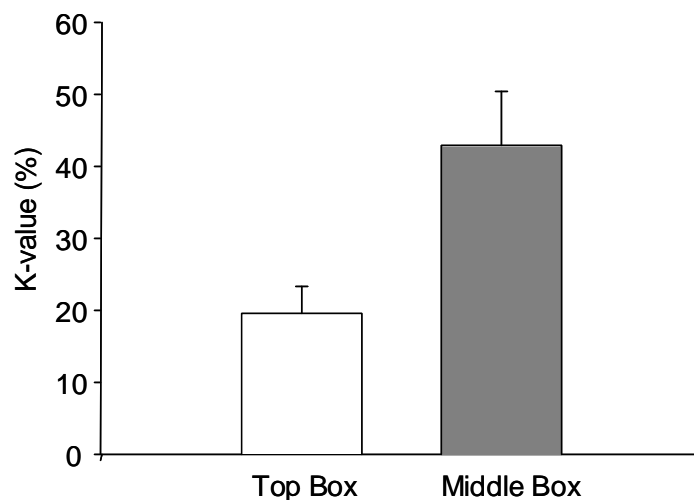


Figure 4.38. K-values obtained in tails sampled from the top and the middle of the box after 48 hours storage. Values are the mean \pm S.E. from four different samples.

These results indicate not only the importance of maintaining a correct temperature throughout the whole box, but also that it is necessary to take into account that tails from different parts of the box may differ in their condition. For this reason it is important that quality assessment procedures performed in the factory (either sensory

or instrumental) are performed on a selection of tails from different parts of the box. If routine sampling is restricted to tails taken only from the top of each box, this could give misleading results, especially if the boxes have been iced sparingly on their top surfaces.

Long term monitoring of temperature in a chill room

In some trials, the temperatures in the centres of containers 1-2 were monitored for up to 6 days, and following the initial equilibration to ambient, it was found that the temperatures increased again, most noticeably in containers 2 & 3 (Figure 39) and container 4 (data not shown). Thus in container 3, a temperature 2°C above ambient was reached after 6 days.

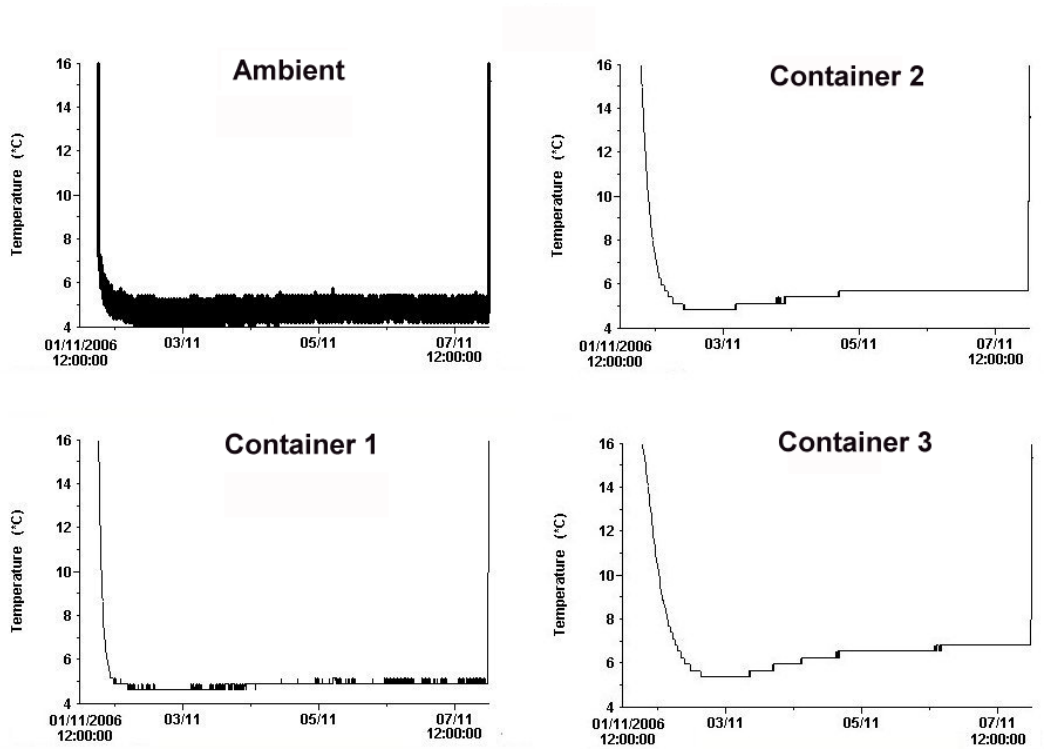


Figure 4.39. Extended recordings from temperature loggers embedded in the centre of *Nephrops* tails for the 6 days of the complete trial in a chill room (Ambient). The product had an initial temperature of 16°C.

Bacterial fermentation is the most plausible explanation for these temperature increases, especially since it was found that even by day 2 the bacterial load had increased within the tails at the centre of a similar box (Figure 4.36).

Taken together, the results obtained by monitoring temperatures within the boxes of *Nephrops* tails identify a chain of events that leads to an acceleration in the spoilage of the product. Thus the heat of tails, which derives initially from the environment, may be only slowly and incompletely removed by the combined effects of icing and chilling. This is due to the low thermal conductivity of this biological material. Such incomplete chilling, which will affect the tails in the middle of the box most extensively, allows more rapid proliferation of spoilage bacteria, which in turn produce heat of fermentation. This positive feedback effect then spreads throughout the box, increasing the temperature of adjacent tails and bringing them more rapidly into the bacterial phase of spoilage.

DELIVERABLES FROM THE RESEARCH PARTNERSHIP BETWEEN YOUNG'S SEAFOOD LTD., THE UNIVERSITY OF GLASGOW AND UMBS MILLPORT.

The deliverables from the project are listed below (*in italics*) against the original objectives and tasks for the individual workpackages. Summaries of the outcomes of each workpackage are also given (*in underlined text*), and have also been brought together in a further section (p. 169) as a LIST OF RECOMMENDATIONS BASED ON SCIENTIFIC DATA to the industrial partner.

Objectives

1. To identify the factors during capture, post-capture handling, delivery and factory processing that affect product freshness and quality. These factors will include spoilage.

- *The following factors have been evaluated:*
 - *Mode of capture (trawl vs creel)*
 - *Trawl duration*
 - *Damage in trawl and during handling*
 - *Levels of stress after capture*
 - *Temperature abuse*
 - *Tailing procedures*
 - *Time to icing (data not shown)*
 - *Delivery time and transport conditions to factory*
 - *Comparison of catches from different ports and boats*
 - *Effect of freeze/thawing (data not shown)*

2. To determine the essential components that give the product its characteristic texture and flavour, and to identify how these are altered as a result of both natural factors and production processes.

- *Analyses of tissue metabolites have been performed, including carbohydrates, fats, proteins, nucleotides, amino acids and amines.*
- *A description of the post-mortem processes is provided in terms of:*
 - *changes in metabolites*
 - *pH changes in the muscle tissue,*
 - *the breakdown of nucleotides, yielding a “Freshness Index”*
 - *the formation of biogenic amines characteristic of flavour change (in progress)*
 - *the production of ammonia and related nitrogenous end products (TMA), which contribute to off-flavours*
 - *the onset and release from rigor mortis*
 - *the breakdown of muscle tissue proteins measured by gel electrophoresis*
 - *A pilot study of instrumental texture measurement (data not shown)*
 - *A pilot study employing an electronic nose instrument to detect off-odours(data not shown)*
- *Specific Spoilage Organisms have been identified using microbiological and molecular techniques.*
- *Spoilage rates have been measured under different post-capture temperature/time combinations.*
- *Comparisons have been made of spoilage in product from control trawls (RV Aora) and from commercial trawls.*
- *Comparisons have been made of spoilage in Factory samples received from different ports/boats.*
- *Correlations have been made between the microbiological measurement/flavour analysis and the sensory methods used at factory.*

3. To identify the key features of the population biology of *Nephrops* that impact upon the maintenance of sustainable stocks in fisheries supplying Youngs Seafoods Ltd., and affect the body condition of the animals. These features will include moulting time and seasonal infections.

- *A seasonal analysis has been performed of catch composition from the Clyde sea area in terms of size, sex, moult stage, body condition and the prevalence of infection by the Hematodinium parasite.*
- *These factors have been correlated with damage in catches of different durations.*
- *These factors have also been co-correlated with rates of flavour change and spoilage in tailed product.*

Scope of project

The project will be achieved through a series of 7 workpackages (WP) which will bring into use a suite of appropriate methodologies, and apply them to particular issues. The outcome of each these WPs will be a set of identified deliverables.

WP1. Measures of stress during capture and handling

Calibrate existing assays of key metabolites (eg. lactate) for assessing exhaustive stress

- *An assay for L-lactate has been adapted and calibrated for use with Nephrops blood*
- *A measure of total carbohydrate based on existing protocols has been used, and specific assays for tissue glycogen and for blood glucose have also been developed*
- *A measure of the Adenylate Energy Charge (AEC) based on HPLC separation of various adenylate compounds has been adopted as a further estimate of exhaustive stress*

Validate existing measures (total haemocyte counts – THCs) to assess the immunological status of the animal

- *THC counts have been performed on blood samples taken over the year (data not shown)*

- *Blood protein levels have been measured using an established method (The Bradford Assay) and this has been cross-calibrated with a field method based on measurement with a refractometer. This has provided a measure of body condition over the year*

Develop a standard index for quantifying body damage

- *An index for quantifying body damage has been established, which scores on a 3-point scale the extent of claw loss, limb damage and puncture wounds.*
- *This has been used to compare the effect of different trawl durations, and also to assess the relative susceptibility to damage of animals at different stages in their moult cycle*

Adapt existing methods for measuring the numbers of spoilage bacteria in the tail meat

- *A standardised method for measuring the numbers of bacteria (total viable count – TVC) using homogenates in the tail muscle has been developed using standard media plates.*
- *An alternative method for measuring bacteria numbers has been developed using “Compact Dry” plates coated with a dehydrated growth medium containing the redox indicator tetrazolium salt. These pre-prepared plates can be used both in the laboratory and at factory or field locations.*
- *It has been found that the processes of bacterial spoilage include the production of trimethylamine (TMA) and hydrogen sulphide (H₂S).*
- *The percentage of bacteria in spoiled tail meat producing these compounds has been measured by techniques which visualise them on the growth plates (iron agar media; luminescence of colonies).*
- *Identification of selected isolates of the TMA- and H₂S-producing bacteria has been performed using molecular methods, yielding an estimate of the most significant Specific Spoilage Organisms (SSOs).*
- *A separate assay for the TMA produced by the SSOs in tailed product has been developed.*

Leading to a suite of methods for monitoring the effects of capture and handling stresses on product quality

This suite of measures is now being deployed to survey changes in product quality along the supply chain from capture to the processor.

WP2. Threats to quality during capture and handling

Measure capture stresses in relation to trawling duration and other fishing practices. Identify patterns of seasonal variability.

- *Trawling induces both exhaustion and stress, as measured by L-Lactate, the metabolic intermediate of anaerobic respiration, and by the levels of the stress hormone CHH in the blood. These measures are highly elevated in trawled animals compared with their natural values, but differences between these measures for short (<1.5 hour) and long (>3.0 hour) trawls are not significant. We interpret these data in terms of the exhaustion from capture being partly reversed by subsequent aerobic respiration while the animals are held for long periods in the net.*
- *An independent assessment of capture stress has been performed, based on the adenylate energy charge (AEC), which reflects the extent of intense activity performed. This measure is significantly reduced in trawled animals, compared to its value in unstressed animals.*
- *Seasonal variation has been established in catch composition. Over the period June-April the catch is dominated by males (~80%), which are mostly at the intermoult stage (“hard”). However, in the spring months April-June the catch comprises a larger percentage of females (~50%), which have emerged to feed and moult and are “soft” or “jelly”.*
- *Measures of body condition based on blood protein and on tissue carbohydrate reserves show small seasonal fluctuations, but with a very high inter-individual variability (data not shown).*

Assess handling damage on deck

- *The assessment of damage (using the developed index) has been performed in relation to season, to the state of shell hardness and to the duration of trawling. Significant differences have been identified between all these categories, with damage being greater in soft-shell animals and in catches from long (>3 h) trawls.*
- *The relationship between the extent of damage to the catch due to the trawling procedure and the subsequent rate of quality loss to the tail meat under standard storage conditions has been further investigated by measuring quality loss in product from separate groups of undamaged and highly damaged animals from given trawls.*

Relate post-capture stresses caused by exposure on deck to air and to high temperature to the initiation of spoilage. Identify periods of greatest vulnerability.

- *An extensive survey has been performed of the relationship between the exposure conditions on deck, the subsequent multiplication of bacteria (particularly the SSOs) within the tail meat, and the consequent spoilage produced, as indicated by the TMA assay.*
- *A range of temperature/time combinations has been investigated, from 4°C-16°C with 0°C (on ice) as a control, over periods from 1-7 days (“Temperature Abuse Trials”)*
- *A further series of trials has been performed which simulates commercial practices more closely. These involved aerial exposure of tails for periods of 4-24 hours to temperatures of between 4°C-16°C, with subsequent periods of storage on ice for up to 14 days (“Simulated Commercial Trials”).*

Establish the extent to which spoilage bacteria are present in the tailed product

- *Baseline values of 10^2 - 10^3 per gram for bacterial numbers have been established in the tail meat of animals in a natural state, i.e. those which experienced minimal stress during capture (by creeling), and were subsequently held in recovery tanks for at least two weeks.*
- *Temperature Abuse Trials at 0°C-16°C have demonstrated that at 0°C bacterial numbers in the tailed product remain at or below baseline values, but at all higher temperatures they rise over a period of 7 days in a*

temperature-dependent manner. Below 10°C they remain close to baseline values for at least 24 hours, but at 16°C they remain at these values for only 8 hours, and reach values of 10⁶–10⁷ per gram after 24 hours.

- *In the Simulated Commercial Trials, after 7 days on ice following an initial temperature abuse of >8h, the bacterial numbers in tailed product were proportional to the initial temperature of exposure, but never reached the numbers seen in the Temperature Abuse trials. However, if the prior temperature abuse was limited to 8 hours (even at 16°C), after a subsequent 7 days on ice bacterial numbers remained close to baseline values*

Relate post-mortem changes in meat quality to handling, tailing, grading, packing and on-board storage procedures

- *The first full description of post-mortem changes in Nephrops tailed product is provided.*
- *After tailing, anaerobic respiration continues in the muscle tissues, with the following consequences:*
 - *stored glycogen is metabolised to glucose*
 - *L-Lactate is produced*
 - *tissue pH is reduced*
- *The starting point for these processes is dependent both on the pre-capture body condition of the animal (including its starvation level) and on the stresses imposed during the capture and handling processes before tailing is performed. These processes then continue for many hours at a rate that is dependent on storage temperature.*
- *At a later stage in these processes the intermediate energy compound arginine phosphate is also consumed. Only after this time is the state of rigor-mortis entered, corresponding to the time when the ATP is totally consumed.*
- *Continuous measurement of tissue pH provides an effective way to monitor the progression of these post-mortem changes. They are completed within 8 hours at 16°C but continue for around 48 hours at 0°C.*
- *Production of both TMA and ammonia begins at some point during these processes, and they both contribute to an increase in tissue pH.*

- *Measures made using HPLC of the inter-conversion of nucleotide energy compounds (ATP>ADP>AMP>IMP>Inosine>Hypoxanthine) provide an independent time scale for the progression of post-mortem changes, and for the production of a flavour-enhancing product (IMP) and subsequently a bitter-off flavour component (Hypoxanthine).*
- *The initial steps in these inter-conversions are highly dependent on the pre-capture state of the animal and on the stresses imposed by the capture process.*
- *The relative amounts of these energy compounds also yield a “Freshness Index” (K value), which can be used to compare the state of tailed product after different post-capture procedures.*
- *Using a threshold for the K-value derived from fish, Nephrops tailed product held on ice from the point of capture remains within acceptable levels for at least 7 days. Temperature abuse causes this period to be reduced in a time-dependent manner.*
- *Trials have been performed with trawls of different durations (~0.3h, ~1.5 h, ~3.0h) on the same day and over the same ground. Tailed product from these trawls has been handled and stored under a common range of conditions, and analysed using the suite of developed methods for measuring stress, quality and spoilage.*
- *Immediately after capture, tails from animals trawled for all durations have reduced values for the adenylate energy charge (AEC), compared to unstressed animals. The fact that this occurs after only 20 minutes trawling suggests that the chasing phase of trawl capture is very significant in inducing the stressed condition.*
- *The AEC value is significantly reduced in animals from the ~3h trawl, compared with those from the ~1h trawl, indicating that long trawls cause a greater physiological exhaustion.*
- *However tailed product from trawls of different durations held on ice for up to 7 days have similar freshness/shelf-life (i.e similar K values).*

Leading to the identification of the main factors during trawl capture, handling and storage that lead to degradation of product quality

The trawl capture process is stressful and results in:

body damage

changes in blood metabolites

alterations in the tissue energy charge

Increased trawl duration causes:

increased body damage

greater physiological exhaustion

BUT

no significant increase in tissue L-Lactate

no measurable effect on the rate of tail meat degradation, when product is held on ice

Post-capture handling. If animals are tailed immediately on landing:

for tails iced immediately, product has acceptable freshness and low spoilage for up to 7days

At air temperatures below 16°C, icing of tails within 4h of capture also yields product with acceptable freshness and low spoilage for up to 7 days

At air temperatures above 8°C, aerial exposure of tailed product for >8h before icing induces a loss of freshness and an earlier onset of spoilage

WP3. Measures of freshness, flavour and texture

Use methods for the proximate analysis of the composition of tail meat (protein, carbohydrate, fat, water content, minerals, ash)

- *Although a complete proximate analysis has not been performed, values have been obtained for tissue carbohydrate, for total tissue lipids and for lipid oxidation using a TBARS assay.*

Develop an index of freshness, based on reliable alternatives to TVB_N (eg. Ornithine and FAAs determined using HPLC), and compare this with existing sensory measures

- *A Freshness Index (K-Value) has been developed which is based on measures made using HPLC of the nucleotide energy compounds (ATP>ADP>AMP>IMP>Inosine>Hypoxanthine).*
- *It has been demonstrated that monitoring tissue pH provides a simple method for establishing the post-mortem state of the meat.*
- *Measurement of the certain amines (cadaverine and putrescine) using HPLC is being evaluated as a further indicator of spoilage*
- *The assay developed for TMA production provides a convenient index of the degree of spoilage in the meat. It has been calibrated against the current practice for the sensory evaluation of an “ammonia” odour in product arriving at the processing factory*

Identify the factors that give scampi its characteristic flavour (sugars, amino acids)

- *A flavour-enhancing product (IMP) and subsequently a bitter-off flavour component (Hypoxanthine) are produced during the breakdown of nucleotide energy compounds.*
- *Tissue glycogen contributes sweetness to the flavour of scampi, and its loss from the tissues due to post-mortem processes may lead to blandness in the flavour.*

Identify post-mortem changes in the protein composition of the muscle using gel electrophoresis

- *Post-mortem changes in muscle protein composition have been quantified using SDS PAGE gel electrophoresis.*
- *The appearance of additional low molecular weight bands, indicative of breakdown, occurs after a post-capture period of 24 h on ice, but within 8 h at 16°C.*

Identify post-mortem changes in the structure of the muscle using histopathological methods

- *Post-mortem changes in muscle structure, including the demonstration of infiltration by bacteria, have been identified by standard light microscopy and also by electron microscopy.*

Distinguish between post-mortem changes due to autolysis and those caused by spoilage bacteria

- *Monitoring of both the bacterial numbers and the Spoilage Potential in the muscle tissues provides an indication of the timing of the onset of the bacterial phase of spoilage. Before that time, post-mortem changes in the freshness index and in muscle protein composition are indicative of autolysis.*

Leading to a suite of methods for monitoring product freshness, flavour and texture

This suite of measures is now being deployed to survey changes in product quality along the supply chain from capture to the processor.

WP4. Maintaining quality along the supply chain and during processing

Determine the natural variation in the flavour and texture of tail meat with size, sex, moult condition, and season

- *From monthly samples collected by RV Aora, variations in tissue glycogen and the flavour-enhancing nucleotide breakdown product IMP with season and sex have been measured. Comparisons have also been made between hard and soft animals to account for moult condition*
- *Tailed product from “jelly” animals spoils more rapidly than the tails of hard animals*

Monitor changes in the freshness, flavour and texture of tail meat along the supply and production chain, including the effects of frozen storage and the peeling & cooking processes

- *Changes in freshness along the supply chain have been measured using the Freshness Index based upon nucleotide breakdown products*

- *A preliminary evaluation of an instrumental method to determine changes in texture of the meat has been conducted. Significant differences can be detected between control (on ice) and temperature-abused product in terms of the compressibility and cohesion of the meat.*
- *A comparison has been made between the bacterial numbers in the fresh and in the frozen/thawed tailed products. After 7 days of freezing at -20°C, bacterial numbers decrease by 90%.*

Determine the extent to which spoilage bacteria and other infectious agents persist or multiply in the tail meat along the supply chain or during factory processing.

- *Bacterial numbers and Spoilage Potential have been monitored in tailed product held in iced storage over 1-14 days in the lab, simulating commercial storage conditions*
- *Bacterial numbers have been measured in commercial samples from 6 different ports with different lengths of supply chain*
- *The ability to detect infection by the Hematodinium parasite in tail meat along the supply chain using an immunological technique (ELISA) has been confirmed.*

Identify any changes in flavour and texture that are induced by spoilage bacteria and other infectious agents persisting in the tail meat along the supply chain or during factory processing.

- *Changes in flavour induced by spoilage bacteria have been estimated using the TMA assay on commercial samples with different lengths of supply chain*
- *The rate of spoilage in Hematodinium-infected tailed product, compared with uninfected meat, has been measured using the TMA assay and also the production of nucleotide breakdown products*

Leading to recommendations for best practice along the supply chain and during factory processing to maintain freshness, flavour and texture in the final product.

WP5. Development of diagnostic test kits for infective agents and spoilage bacteria.

Based on the existing scientific knowledge and procedures that has been generated by researchers at the University of Glasgow and UMBS Millport concerning the infection of *Nephrops* by the dinoflagellate parasite *Hematodinium* and of the spoilage caused by *Vibrio* bacteria, this project will:

Develop, adapt, standardise and calibrate assays for detecting these pathogens within the tissues and derived products of *Nephrops*. For *Hematodinium*, the assays will be based on immunological and molecular techniques. For *Vibrios*, the assays will be based on bacterial cell counts and on proteolytic activity.

Project 2.

For *Hematodinium*, the transfer of the ELISA principle for detecting the parasite (which is based on an antibody reaction to a cell coat antigen) into a lateral flow test, is being pursued.

- For the spoilage bacteria (SSOs) a Spoilage Potential Assay is being developed in which the rate of conversion of TMAO to TMA is indicative of the number and activity of SSOs present in the tail meat.
- A novel quick test for the rate of loss of freshness and the progress of spoilage, based on a new-generation ISFET pH electrode, has been piloted and is being validated.
- The ability of a commercial Electronic Nose instrument to be trained to detect off-odours indicative of spoilage is being evaluated in small-scale trials with the manufacturer.

Incorporate the assays into diagnostic test kits which can be used by various operators in both field and factory situations to determine whether infection levels exceed pre-determined standards.

Project 2. The principles for these diagnostic and rapid detection test kits will be delivered by July 2007

WP6. Linking quality measures to a traceability scheme

Identify best practices for landing and handling product that are necessary to maintain quality – eg. trawl time, deck temperature, humidity, time to tailing and icing.

Running tests with Aora traceability system.

- *Acceptable trawl times for avoiding excessive damage and for retaining acceptable body condition have been defined*
- *Acceptable ambient temperature / time combinations during handling and sorting of tailed product for retaining freshness and preventing the initiation of spoilage have been defined*
- *Maximum time to icing has been defined*
- *The importance of complete icing for the retention of freshness and the prevention of spoilage has been identified*
- *The potential for incorporating these defined values into the Youngstrace traceability system has been demonstrated*

Leading to the incorporation of key proxy measures of product quality into an appropriate traceability scheme

WP7. Population biology of *Nephrops norvegicus*

Determine recruitment, size at age and moulting times in the different sea areas that supply Young's Seafood Ltd.

- *The data obtained from the Clyde Sea Area for 2004-2006 add to the historical data for different Scottish fishing zones made available to the project by FRS.*

Determine the natural variation in the flavour and texture of tail meat with size, sex, moult condition, and season

- *See deliverables for WP4. These measures require to be extended by more survey work on hard/soft, male/female & seasonal samples*

Determine the seasonality of the infection by *Hematodinium* in animals from different sea areas using existing immunological and molecular diagnostic methods.

- *Results obtained for Clyde Sea Area for the season 2005-06 confirm the historical data obtained by GU over 15 previous years.*

Leading to recommendations for best fishing practices and effort to sustain stocks, to catch animals in good body condition and to avoid moulting periods when animals are most vulnerable to stress and infection.

Recommendations can be made for the Clyde Sea Area. Comparison of population biology in other exploited stocks awaits further surveys, but methods are in place to perform this work by factory-gate sampling of the delivered product.

LIST OF RECOMMENDATIONS BASED ON SCIENTIFIC DATA

From the experimental work performed by the University of Glasgow and UMBS at Millport it is possible to draw some specific recommendations to the industrial partner, Young's Seafood Ltd.:

- When trawling, it is necessary take into account that greater trawling time is going to increase the number of damaged animals.
- However, quality parameters such as K-value are less dependent on the initial condition of the animal, and are little affected by trawling time, at least over the range of times tested in this part of the project (1-3h).
- Handling procedures on board are crucial in terms of the quality that the product will have afterwards. In order to preserve quality, adequate icing must be performed within 4 hours of capture. This offers a handling window for the fishermen to work within without detriment.
- Icing the tails correctly is a key issue in quality control. If boxes are not iced correctly, the residual heat in the product will allow loss of freshness and spoilage to occur. Inadequate icing of a box also leads to there being tails of different quality within the same box.
- Washing the tails will decrease the bacterial load that develops in the tail meat, and so this procedure is beneficial to reducing spoilage.
- It is important to work with hard animals, since damage is less and quality parameters are improved, compared with soft/jelly animals. It would be particularly helpful to avoid periods during the year when high levels of jelly animals are encountered.
- It would be also convenient to avoid animals with heavy *Hematodinium* infection since the presence of the parasite produces alterations in several of the quality-related parameters measured in the present work.

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ACKNOWLEDGEMENTS

This Research Partnership was conducted with a contract from Young's Seafoods Ltd., and was funded in part by a grant from the EU Financial Instrument for Fisheries Guidance (FIFG), administered through The Scottish Executive.

The technical assistance of staff at Young's Seafoods Ltd. was essential in performing this study. Particular thanks to Michael Mitchell, Malcolm Blanthorn, Elizabeth Reynolds-Lewis and Paul Hart at Annan, and John Nicolson at Stornoway.

The skill and practical assistance of the skipper, Mick Parker, and his crew of the RV *Aora* and of Howard McKindle at UMBS Millport were crucial to the execution of this project, and are gratefully acknowledged.

The efforts of the crews of fishing vessels involved in the commercial trials, particularly those of the *Trust*, *Our Venture* and *Wavecrest* contributed significantly to project, and they are thanked.

Technical assistance was provided at the University of Glasgow by June Freel, Pat McLaughlin, and Graham Adam, and at UMBS Millport by Steve Parker and Tom Stevenson.

A number of undergraduate and postgraduate students contributed scientific data to the project: William Butler, Kevin Donnelly, Xu Feng, Deborah Laughland, Avril McRobb, Paul Ross and Joseph Ruarty.