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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 II

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EXECUTIVE SUMMARY

1. The objectives of Phase II of the Scottish *Nephrops* Survey have been achieved through a series of six work packages.

2. The processes that underlie quality loss in the whole animal compared to the tailed product have been identified. The following factors have been evaluated:

    o Evolution of the post-mortem biochemistry and bacterial growth

    o Physical damage impact due to trawl duration

    o The effect of capture on quality loss due to necrotic animals

    o Development of melanosis

    o Effective methodology for a rapid cooling rate in fish boxes containing whole langoustines

3. The effectiveness of gas flushing or MAP for prolonging shelf life has been determined. This has been achieved by:

    o Testing different gas mixes and testing their effect on reducing the rate of spoilage of whole langoustines under controlled conditions

    o Identifying the optimal gas mix (OGM) that maintains quality and freshness of whole langoustines for a longer period of time

    o Testing the OGM at different times to determine the maximum shelf life that can be obtained.

- o Monitoring the production of any un-desirable breakdown products including 7 different biogenic amines, hypoxanthine and trimethylamine.

- o Testing the efficiency of the OGM under several conditions:

  - - De-gutting or not de-gutting the animals

  - - Packing fresh or 24 h old animals stored under controlled conditions

  - - Testing the effectiveness of different chill storing temperatures in combination with MAP packaging on maintaining product quality

- o Identifying changes in the microflora due to the OGM

- o Determining and comparing laboratory results with a professional sensory panel in order to validate the results

- o Transferring the knowledge about MAP to the industrial partner and comparing results using two different and independent sensory evaluations.

4. The effects of melanosis dipping on product quality have been described using different commercially available additives, and these results have been compared with un-dipped animals. Furthermore, certain alternative treatments for inhibiting melanosis development in whole animals have been tested, namely:

  - o Icing alone

  - o MAP

  - o High hydrostatic pressure

5. A sensory scoring system to evaluate the general external appearance of un-cooked whole langoustines has been developed. This system has been adapted for whole langoustines that may have been treated with anti-melanotic dips.

6. Furthermore, a professional sensory panel has been established and trained for evaluating cooked langoustines. Results from laboratory tests have been cross-calibrated with sensory evaluations performed by the sensory panel.

7. A method has been developed for determining the sex and body size (carapace length) of animals from tailed product delivered to the industrial partner.

8. Immunological methods have been applied to determine the level of infection by the parasite *Hematodinium* sp. in langoustines, and its consequences for quality have been described.

On the basis of these findings, the following recommendations are made to Young's Seafoods Ltd.:

- When working with whole animals, it is important to reduce trawling time in order to increase the amount of un-damaged animals that can be sold as whole langoustines.

- Reducing stress during the handling of the catch is important in order to minimise the proportion of necrotic animals that can affect the quality of the catch.

- Melanosis develops in whole langoustines even if they are iced properly at all times. Therefore it is important to wash the animals

carefully and treat them with anti-melanotic formulations before entering the supply chain.

- During storage, it is important not only to leave the whole langoustines in a cold room but also to ice the box on top, which will considerably accelerate the cooling rate in the middle of the box.

- By MAP packing whole langoustines in the optimal gas mix identified by the University of Glasgow it is possible to reduce the spoilage rate and extend shelf life.

- Following MAP packing it is important to control the temperature throughout the whole storage process, since the modified atmosphere loses its effectiveness when packs are stored above 5 ºC.

- Determining levels of *Hematodinium* sp. infection throughout the year is important, since this parasite has been shown to affect the quality and the shelf life of the langoustines.

The final recommendation to Young's Seafoods Ltd. would be that by using the extensive amount of data obtained in the Phases I and II of of the Scottish *Nephrops* Survey it will be possible for them to exert effective control on the '**chain of custody**' of langoustines from each catch by incorporating key information into a monitoring system such as the "**Youngstrace**" traceability system. In this way the industrial partner will have all the information necessary both to determine the expected shelf life of the landed products and to incorporate measures to maximise it, thus obtaining significant commercial gains.

INTRODUCTION

Nephrops norvegicus or Norway lobster is commercially exploited throughout its geographical range, and is subject to two main fishing methods. The fine soft mud to muddy sand habitats for *N. norvegicus* make the species readily available to trawling and therefore this method of capture is the most common one. Trawling takes place using a specially designed light trawl which was derived from existing whitefish otter trawl gear. The second method is capture using baited traps, so called 'creels'.

Until 1950s *N. norvegicus* was considered a nuisance by-catch (O'Riordan, 1963). When unwanted, the lobsters were discarded at sea or occasionally landed without any commercial value. However, with the decline of the whitefish fishery in European waters the demand of *N. norvegicus* has increased gradually and a targeted trawling fishery has grown.

Nowadays, the *N. norvegicus* fishery is the most valuable fishery in the UK, having overtaken the main whitefish fisheries in recent years. The 50,000 tonne fishery is currently worth more than £89M p.a. at first sale, with the greatest proportion landed in Scottish waters (>29,000 t, worth £68M in 2004). Export value was estimated to be approximately £170M in 2006. The bulk of the catch is taken by trawlers. Creeling takes only approximately 7% of the tonnage, but contributes a higher proportion of the overall catch value (15%), because creel-caught animals are larger, on average, and therefore fetch a higher price. Fishing intensity on most grounds and by all methods is currently thought to be at sustainable levels.

The *N. norvegicus* trawl fishery is generally considered a high by-catch fishery where up to 86% of a total catch can be discarded as by-catch (Bergmann et al., 2002). The incidental capture of large numbers of juvenile fish species such as cod, hake, whiting, haddock or blue whiting is a feature common to all European *N. norvegicus* trawl fisheries. In Scottish waters, the by-catch varies seasonally and include poor cod *Trisopterus minutus*, lesser spotted dogfish *Scyliorhinus canicula*, cod *Gadus morhua*, haddock *Melanogrammus aeglefinus*, whiting *Merlangius merlangus*, conger eel *Conger conger*, edible crab *Cancer pagurus*, swimming crab *Liocarcinus depurator*, squat lobster *Munida rugosa*, hermit crab *Pagurus bernhardus*, other fish and crustaceans, various molluscs, echinoderms and gastropods (Bergmann et al., 2002). Invertebrates are considered to have a high survival rate when discarded, but teleost fish often suffer swim bladder damage and do not survive the ordeal of trawling and are discarded dead. Technical measures (e.g. square mesh panels) are used to reduce by-catch and this is an area of active research.

Norway lobsters are sold either as frozen tails, as whole animals (fresh or frozen) or as live animals. In Scotland creel-caught animals are traditionally exported live (in vivier transport or by air) to continental Europe, where they fetch the highest market prices. Creeling is thought to leave the animals in a better condition, thus enabling the lobsters to survive the transport more easily. Furthermore, creel-caught animals are considered to be of a better quality than trawl-caught animals. Trawling is known to cause stress and exhaustion in both fish and lobsters (Harris and Andrews, 2005, Ridgway et al., 2006). However, since the demand of fresh live lobsters is continually rising, there are an increasing number of vivier-transported trawl-caught lobsters. To ensure their sale the animals must be in good condition both during post-capture

treatment on arrival at the destined market, following transport. Biochemical changes during live storage, culture and vivier transport of many lobster species (including *N. norvegicus*) are well studied, and the effects of asphyxiation and other handling stresses on the state of the animals are known (Stentiford and Neil, 2000, Harris and Andrews, 2005). In contrast, when sold as tails or whole (but dead) animals, knowledge on post-mortem biochemical changes is sparse. Hence, effects of stressors on post-mortem changes and their effects on later quality are even less understood. There is a high demand for premium quality seafood in Europe and worldwide. However, fish and especially shellfish are highly prone to spoilage, with the main factors affecting the degree of spoilage in fish being the method of capture, on-board handling, processing and storage conditions (Huss, 1995; Dalgaard, 2000).

It is known that the post-mortem changes taking place during handling, processing and storage are important for the quality and shelf life of both meat and white fish. However, knowledge of the post-mortem changes in commercially important crustacean species is poor. More detailed knowledge of the post-mortem changes in crustaceans will lead to improvements in catching, handling, processing and storage, thus yielding longer lasting freshness and a longer shelf life. Knowledge of bacterial spoilage and shelf life of *N. norvegicus* is virtually non-existent and only few studies have ever been conducted, most of which are preliminary and are only published in leaflets and technical notes. As with other crustaceans, shelf life of *N. norvegicus* is relatively short, and spoilage is generally indicated after just 5 - 7 days. Moreover, when recommendations have been made for handling regimes and quality preservation, these have been based on data from finfish and not derived from experiments on the crustacean themselves.

In this Phase II of the Scottish *Nephrops* Survey, studies have concentrated in evaluating how improvements can be made in catching, handling, processing and storage of whole fresh langoustines, thus yielding longer-lasting freshness and a longer shelf life.

Having investigated in Phase I of the Scottish *Nephrops* Survey the quality deterioration in *N. norvegicus* tail meat during iced storage, and identified several effects on the quality, this Phase II will describe the development and evaluation of a modified atmosphere packing (MAP) procedure for whole *N. norvegicus*. Factors relevant to this are the process of MAP itself, the use of anti-melanotic agents to prevent shell blackening and the application of a suitable quality index to determine outcomes. These will be introduced first.

Modified atmosphere packing (MAP) of whole *N. norvegicus*

It is long known that chilling, along with other preservative measures such as smoking and drying, increases the shelf life of food. However, if the atmosphere surrounding the foodstuff is further modified an additional extension of the shelf life can be achieved in many cases. The main gases used in modified atmosphere packing (MAP) are oxygen (O_2), carbon dioxide (CO_2) and nitrogen (N_2). One of the main objectives of using a modified atmosphere is to reduce the amount of O_2 available both to the foodstuff itself and to the microorganisms present in and on the foodstuff. The lack of O_2 results, on the one hand, in a reduction of the rate of chemical oxidation - a main cause for spoilage in fatty foods - and, on the other hand, in the reduced growth of microorganisms. Furthermore, this reduction in microbial growth can be shown to be

even more pronounced if high levels of CO_2 are used. In fact, carbon dioxide is the major antimicrobial, bacteriostatic factor in MAP.

In 1877 Pasteur and Joubert showed that the agent of anthrax (*Bacillus anthracis*) was killed by high levels of CO_2 (Valley, 1928) and several years later a paper was published on the preserving effect of CO_2 on beef (Kolbe, 1882). The first 'real' MAP was recorded in 1927 where storing apples in a low O_2, high CO_2 atmosphere was found to greatly extend the shelf life (Phillips, 1996). Around the same time producers began to transport refrigerated beef carcasses over long distances in high CO_2 atmospheres, and bulk storage of produce in MA also became widely used. Later, from 1970 onwards, commercial retail MAP-packs were gradually introduced in Europe. The first MAP-packs in Britain were offered by Marks & Spencers (M&S) in 1979 and contained meat (Phillips, 1996) and today many fresh products, such as bacon, fish, poultry, sliced meats (e.g. ham), pasta, fruits and vegetables are MAP-packed.

As mentioned, it was found that CO_2 has a generally reducing effect on the growth of bacteria, and this reducing effect has been shown to be highly temperature dependent (e.g. Dhananjaya and Stroud, 1994). It was established that the antimicrobial effect of CO_2 was greater at lower temperature, since the solubility of the gas in the foodstuff is affected by the temperature. Water and fat content of the product, as well as partial pressure of the gas, are additional influencing factors (Ho et al., 1987). Devlieghere et al. (1998) could show that the anti-microbial effect of CO_2 is dependent on the amount of the gas dissolved: CO_2 is highly soluble in water and fat and its solubility

increases greatly with decreased temperatures amongst other factors. The main factors affecting solubility of CO_2 in water are:

1. The pressure of CO_2 in equilibrium with the solution: the solubility of CO_2 increases with increasing pressure

2. The temperature: the solubility of CO_2 decreases with increasing temperature.

3. The pH: the solubility of CO_2 increases with increasing pH.

4. The presence of other substances: the solubility of CO_2 tends to decrease with the increase of 'inert' ionic solutes such as sodium chloride, but may increase or decrease with increasing concentration of organic compounds, depending upon the compound.

The amounts of CO_2 that dissolve in 1 liter of water are 3.32 g at 1 ℃, 3.09 g at 2 ℃, 2.87 g at 4 ℃ and 2.68 g at 6 ℃. At 20 ℃ only 1.73 g is absorbed - a reduction of around 50 % compared to 1 ℃. Once a MAP pack containing CO_2 is opened or if the storage temperature is increased, the CO_2 is slowly released, but continues to express an antimicrobial effect for a period of time after the change. This effect is known as the CO_2 residual effect (Stammen et al., 1990). The amount of CO_2 that dissolves into the foodstuff is consequently removed from the surrounding atmosphere and eventually leads to package collapse. To prevent this collapse a high gas-to-product ratio (G/P ratio) is mandatory. Usually, if high CO_2 concentrations and low storage temperatures are used a G/P-ratio between 2:1 and 3:1 is recommended.

When CO_2 is absorbed into the water and fat content of the product, carbonic acid is formed and the ultimate pH is lowered. Since it was shown that the exclusion of O_2, by using N_2 as the sole filling gas, does not greatly affect the growth of spoilage-causing microorganisms, the pH decrease caused by the CO_2 was thought to be

responsible for the antimicrobial nature of the gas. However, Coyne (1933) had already stated that the acidification at the surface of products stored in a high CO_2 atmosphere couldn't entirely explain the bacteriostatic effects observed. Therefore, since the acidification caused by carbon dioxide and the exclusion of oxygen can be excluded as the prime reason, CO_2 must have a more complex impact on the microorganisms.

To date, at least 4 different effects have been identified to be caused by the presence of CO_2 and affect the growth of microorganisms (Sivertsvik et al., 2002):

1. The cell membrane is altered and nutrient uptake and absorption are inhibited

2. Enzymes are inhibited and rates of enzyme reactions are decreased

3. CO_2 penetrates the membranes of microorganisms and leads to intracellular pH changes

4. Properties of (cellular) proteins are altered

The efficiency of CO_2 not only depends on the previously mentioned factors, such as solubility and temperature, but also on the initial microflora present in the foodstuff (Phillips, 1996). Yeast growth, for examples is stimulated by the presence of CO_2.

In general, MAP (and therefore CO_2) is most effective in high fat, high water containing foods (e.g. meat) where the spoilage flora consists of aerobic, gram-negative, psychrotropic bacteria. Furthermore, it is important to point out that MAP-packing can only work if applied to fresh foods early post-harvest, and its use does not eliminate the need to control storage temperature and conditions, or processing hygiene.

MAP in fish and shellfish

Around 1930 it was found that fresh fish kept 2-3 times longer in 100 % CO_2, compared to an air control, when stored at low temperatures (Killeffer, 1930). Coyne (1932, 1933) found that haddock (*Melanogrammus aeglefinus*), cod (*Gadus morhua*), sole (*Solea solea*), whiting (*Merlangius merlangus*) and plaice (*Pleuronectes platessa*) stored under atmospheres containing 20 - 100 % of CO_2 at low temperatures were protected from early deterioration, and Stansby and Griffith (1935) described the effect of CO_2 on the quality of haddock (*Melanogrammus aeglefinus*) during handling and storage. Since then numerous papers have been published on MAP in fish and shellfish. In Table 1.1 the most important results are summarized and species, gas-mix, storage temperature, shelf life gain and references are given. The data are taken and modified from (Sivertsvik et al., 2002).

In general, a shelf life extension of 30-60 % can be observed in MAP packing of fresh fisheries products, when CO_2 levels are elevated (see Table 1.1). The differences in CO_2 concentrations reported are dependent on the species and the habitat of fish, the freshness grade of the fish and the hygiene during processing and handling, all of which affect the initial levels and composition of the microbial flora present in and on the seafood product. The different microflora are in turn affected in different ways by the distinct gas mixes used during MAP.

When a product is spoiled, the microflora will be composed of several different bacterial species (Huss, 1995) and off-odours and off-flavours will be released. It is known that not all of the bacteria present in a spoiled product are responsible for the

spoilage *per se*, and that only a specific group within the flora represents the causative agent for the spoilage. These are called specific spoilage organisms (SSOs). *Shewanella putrefaciens* was identified as the most important and abundant spoilage organism in chilled seafood from temperate waters (e.g. Gram et al., 1987). In order to produce energy during growth, the bacterium is capable of reducing TMAO to the fishy-smelling TMA, and produces H_2S from various sulphur sources within the product. When the storage temperature is changed other bacteria, eg. *Vibrio* sp., can become specific spoilers (Gram et al., 1987). *Pseudomonas* spp. are marine and terrestrial, gram-negative, psychrotropic, non-spore forming, aerobic bacteria also known to cause spoilage of various other foods (Gennar and Dragotto, 1992). Some species of the genus are also pathogenic in vertebrates (Lopez-Romalde et al., 2003). In high CO_2-MAP, however, it was shown that in many seafood products another bacterium, *Photobacterium phosphoreum*, becomes the most important SSO (Dalgaard et al., 1993, Dalgaard, 1995, 2000). This bacterium is highly resistant to the effects of CO_2 and dominates the microflora of chilled MAP packed seafoods. Furthermore, it was shown by Dalgaard (1995) that *P. phosphoreum* is $10 - 100$ times more effective at reducing TMAO to TMA, compared with *S. putrefaciens*, which makes this particular bacterium a potentially more hazardous spoilage organism. The anaerobic conditions in CO_2-MAP-packs are known to induce the production of TMA, since the conversion of TMAO to TMA is an essential part of anaerobic respiration of many bacteria. Therefore, the inclusion of a minimal proportion of O_2 within a MAP pack has been thought to be beneficial, on the basis that the production of TMA will be delayed or inhibited (Boskou and Bebevere, 1997).

Table 1.1 Gas mixes used for MAP-packing in fish and shellfish products

Table 10.1: Gas mixes used for MAP-packing of fish and shellfish

Species	Temperature [°C]	Gas mix [$CO_2 : N_2 : O_2$]	Shelf life gain [days]	References
	1	60 : 40 : 0	3	Woyevoda et al. (1984)
	2	40 : 60 : 0	2	Guldager et al. (1998)
	0	40 : 30 : 30	2.5	Cann et al. (1983)
	0	25 : 75 : 0	3.5	Villemure et al. (1986)
	2	100 : 0 : 0	1	Jensen et al. (1980)
cod (*Gadus morhua*)	2	2 : 98 : 0	5	
	2	3 : 97 : 0	4	
	2	29 : 71 : 0	7	Dalgaard et al. (1993)
	2	48 : 52 : 0	11	
	2	97 : 3 : 0	6.5	
crayfish (*Pacifastacus leniusculus*)	4	80 : 20 : 0	7	Wang and Brown (1983)
haddock (*Melanogrammus aeglefinus*)	0	40 : 30 : 30	2	Dhananjaya and Stroud (1994)
striped bass (*Morone saxatilis*)	0	60 : 34 : 6	6	Handumrongkul and Silva (1994)
spotted shrimp (*Pandalus platyceros*)	0	100 : 0 : 0	>7	Matches and Layrisse (1985)
Mullet snapper (*Lutjanus aratus*)	3	100 : 0 : 0	6.5	Scott et al. (1984)
tilapia (*Tilapia* spp.)	4	75 : 25 : 0	14	Reddy et al. (1995)
whiting (*Merlangius merlangus*)	4	100 : 0 : 0	11	Post et al. (1985)

In contrast to the beneficial effect of MAP, the change of the microflora induced by the gas mix can occasionally be a cause for concern. Fish and shellfish are reservoirs for foodborne diseases (Huss, 1995) and the growth of certain pathogens could be induced by MAP. Pathogens found in seafood include: *Clostridium botulinum*, *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Listeria monocytogenes*, *Bacillus* sp., *Salmonella* sp., *Escherichia* sp., etc. Most of these species are either intrinsic to fish or shellfish (e.g. *Listeria monocytogenes*) or are common in seawater (e.g. *Vibrio* sp.), but numbers are always low in the fresh product and pose no risk to the consumer. Others (like *Salmonella* sp. or *Escherichia* sp.) are introduced during processing by cross-contamination. Not all these pathogenic bacteria are eliminated or repressed by MAP. For example, *L. monocytogenes*, is known to be little affected by CO_2 atmospheres and can easily grow at low temperatures. However, an inhibition of *L. monocytogenes* was reported in 100 % CO_2 (Avery et al., 1994). The growth of *C. botulinum* was shown to be greatly reduced in MA containing high CO_2 levels (e.g. Reddy et al., 1999). However, there are two opinions on the effect of CO_2 on *Clostridium* sp. Various publications show a reducing effect on the growth, whereas numerous other publications have demonstrated the opposite. The bacteria of this genus, although many are not pathogenic, should therefore always be considered as a possible risk factor. Accordingly, food samples should always be monitored for the presence of these bacteria as demanded by the corresponding health and safety authorities. Hygiene and control during handling, transport and processing are essential to reduce the risk of MAP pack foodborne illnesses. Nevertheless, in the UK, it is recommended by the Advisory Committee on the Microbiological Safety of Food (ACMSF) that the shelf life of MAP packed products stored below 5 ºC be limited to 10 days, if chilled

storage is the only controlling factor, in order to eliminate the risk of evolving foodborne pathogens.

Melanosis and Anti-melanotic treatments

Melanosis (also known as enzymatic browning or blackspot syndrome) causes a blackening of food produce surfaces, shells and membranes and affects fruits, vegetables and seafood. In seafood, melanosis occurs primarily in crustaceans and is a major problem during post-harvest storage.

The blackening is caused by the action of the enzyme polyphenol oxidase (PPO) (1,2 benzenediol; oxygen oxidoreductase, EC 1.10.3.1), which to be functional needs two copper (Cu^{2+}) moieties as essential co-factors (Marshall et al., 2000). PPO catalyses two different reactions and is both a tyrosinase (monophenolase) and a catechol oxidase (diphenolase). In a first reaction monophenols such as tyrosine are transformed by a hydroxylation reaction to diphenols, and a second reaction catalyses the oxidation of diphenols to o-quinones. PPO is an inert enzyme and requires activation (Zotos and Taylor, 1997).

Generally, the enzyme is activated during the innate immune response of crustaceans (Terwilliger, 2007) by at least one or more factors (e.g. a protease) (Bartolo and Birk, 1998) and its products help to melanize introduced foreign matter (e.g. bacteria or parasite debris). Polyphenol oxidase is also activated during ecdysis and is involved in the sclerotization of the new exoskeleton (Montero et al., 2001, Bartolo and Birk, 1998). Cuticle hardening occurs when calcium (Ca^{2+}) is deposited in the new shell and when the quinones produced from diphenol oxidation form cross-links between

adjacent protein chains within the cuticula (Stevenson, 1985). The highly reactive o-quinones cross-link with histidyl residues of cuticular proteins and chitin, resulting in hardening of the exoskeleton (Xu et al., 1997).

The main monophenols in crustaceans are tyrosine and its derivates (e.g. thyramine), which are present in the greatest concentrations in the haemolymph during moult (Ferrer et al., 1989). Tyrosine is transformed to dihydroxyphenylalanine (DOPA) or dopamine (both diphenols), which are then oxidized to form dopaquinone. The dopaquinone is subsequently attacked by at least two other enzymes (carboxylase and transacetylase) and results in the formation of highly reactive molecules that polymerize, e.g. in the presence of light, to form a brownish, black coloured precipitate: melanin (Lerner, 1953).

It has also been shown that several additional, stress-related factors, other than an immune response or ecdysis, can activate the PPO enzyme. Capture, rough handling and in general 'traumatic' events (Bartolo and Birk, 1998), e.g. trawling, can activate the PPO - presumably by activating the proteases which in turn activate the enzyme – and post-harvest melanosis rates are increased. Once melanosis is triggered and becomes established, several factors affect the rate of blackening. These factors are:

- pH of the tissue

- temperature

- monophenol substrate levels

- enzyme levels

- oxygen

- copper levels

- exposure to light

Some authors (e.g. Chinivasagam et al., 1998) suggest, that certain spoilage bacteria can aggravate melanosis.

Since the process of melanization is complex and many steps, substrates and enzymes are involved, numerous points of attack are represented for intercepting or inhibiting the formation of melanin. Despite this fact, currently there are only two main methods in use within the crustacean processing industry. The most widely used method is based on the antioxidant nature of sulphites. The primary role of sulphites in the inhibition of enzymatic browning is the reduction of the melanin precursors (o-quinones) to colourless, less-reactive diphenols. To this end sodium metabisulphite (E-223) has been traditionally used to inhibit melanosis in Crustaceans.

Although sulphites are very effective in controlling browning, they are subject to regulatory restrictions owing to their potentially adverse effects on human health. Many reports have described allergic reactions in humans following the ingestion of sulphite-treated foods (Marshall et al., 2000). Allergic reactions also occur in workers who treat foods with sulphite-based anti-melanotics or handle large quantities of treated food (Mendes, 2002). However, for economic reasons the main anti-melanotic treatments used in *N. norvegicus* remain sulphite-based formulations, such as sodium metabisulphite, Freskor and Melacide-SC20.

The active ingredients in Melacide-SC20 are hydrogen sulphite (E-222) together with sodium ascorbate, potassium citrate and citric acid. Melacide-SC20 is subjected to

allergen labeling similarly to sodium metabisulphite. However, melacide is safer to handle due to its reduction of fumes, thus indicating a lower health risk for the fishermen and others involved in processing the product.

Another method commercialized under the name of Prawn-Fresh from Xyrex® uses 4-hexylresorcinol, a specific inhibitor of the PPO enzyme. The 4-hexylresorcinol binds specifically to the active site of the PPO, thus preventing it from being functional. It is also used in the cosmetic and pharmaceutical industries, and is the main active ingredient in many topical antiseptics (e.g. lozenges (Zhou et al., 2000) and ointments (Iga et al., 2006)).

In general, anti-melanotics are applied to the product by immersion into an aqueous solution for a certain period of time, depending on the size of the product.

Quality Index Method (QIM)

Freshness and changes in sensory attributes are critical parameters during food and seafood deterioration. In 1985 the Tasmanian Food Research Unit developed the first QIM as a scoring system for freshness and quality estimation in fisheries products (Bremner, 1985).

In general, QIM is based on objective evaluation of certain attributes (e.g. odour, skin or shell colour, appearance, etc.) of raw fish and crustaceans, depending on species. Usually, a 4-point scoring system is used, with points given for each attribute on a scale of 0 to 3, where 0 represents the quality of the attribute analyzed when fresh,

and the higher scores (1, 2 and 3) represent a deterioration of the quality of this certain attribute during storage. The scores are summed up to give an overall Quality Index score. Increase in the Quality Index score tends to be linear and to correlate with the time of iced storage. If the shelf life during iced storage of the monitored species is known, the score can also be used to calculate the remaining shelf life. For evaluation and to ensure reproducibility, guidelines for each species and parameter are documented, and since 1985 numerous QIM schemes for various types of fish have been introduced e.g. haddock, plaice, pollock, sole, turbot (Luten, 2000), cod (Bonilla et al., 2007), octopus (Barbosa and Vaz-Pires, 2004). QIM is in use for both farmed fish (e.g. Gonçalves et al., 2007) and wild caught fish (for more information, see: www.qimeurofish.com). In crustaceans, few articles have been published on QIM, e.g. in shrimp (Nielsen et al., 1997) and prawns (Chinivasagam et al., 1996). Crustaceans are highly valued seafood products, but their shelf life is limited because of melanosis. To avoid this, crustaceans are commonly treated with antimelanotics. However, the sensory assessment of crustaceans treated in this way is compromised due to the way the anti-melanotic compounds work, and QIM schemes have to be adapted.

A QIM scheme for *N. norvegicus* has been developed (Anonymous, 2002 and was validated validated for use by Phase I of the Scottish *Nephrops* Survey. The outer appearance of the claws, head, tail and the odour are used as attributes for the QIM assessment. However, the effects of anti-melanotics on the QIM have not been assessed so far and need to be clarified. Furthermore, it remains to be seen if the QIM can be correlated with a thorough sensory assessment of the cooked product. It could well be that the QIM is too conservative and that samples rejected this way could still

be perfectly edible once cooked. To clarify this, a more thorough study involving a professional sensory panel is needed.

Investigating a *Hematodinium* sp. parasite in Scottish stocks of *Nephrops norvegicus*

N. norvegicus is an important commercial species, as outlined above. This species is parasitized by a syndinid dinoflagellate organism of the parasitic genus *Hematodinium,* which are destructive parasites of decapod crustaceans (for review see Stentiford & Shields 2005). Parasite proliferation is progressive and debilitates the host lobster by competing for essential blood nutrients and by stimulating host mobilisation of energy reserves. As parasite load and therefore metabolic demand increase, visible patent disease (parasite aggregation in the pleopods; cuticle colour change) and a moribund state are preludes to host death as the endpoint of infection. All previous evidence (Field & Appleton, 1996) and also the results obtained in Phase I of the Scottish *Nephrops* Survey suggests that the duration of infection is one year. Patently-diseased animals which are visible by eye in late winter, spring and early summer show discolouration of the cuticle, and are unmarketable due to the degradation of meat quality. These animals are discarded at sea. In the Clyde Sea area in Scotland, incidence of patently-diseased animals varies from year to year (Field et al., 1992; Stentiford et al., 2001). Patent disease prevalence has been as high as 70% (in the late 1980's: Field et al., 1992), and Phase I of the Scottish *Nephrops* Survey revealed that patent disease prevalence in simulated commercial trawls in the Clyde Sea area peaked in March 2007 at 18 %) and in February 2008 at 10 %. Sub-patent

and lower severity patent *Hematodinium* infections are, however, detectable in the host lobster throughout the year.

Field and Appleton (1995) developed a more sensitive score of patent disease by scoring aggregation of parasites and haemocytes in the pleopods. Its application on a large scale by Stentiford et al., (2001) showed a seasonal peak similar to the peak of patent disease, but revealed a low prevalence in summer and winter that had previously eluded detection. This methodology was subsequently superseded by immunoassay and molecular diagnostic tools for the purpose of detecting infection in its early stages (Stentiford and Shields, 2005). The latest application of a molecular probe against the *Hematodinium* sp. found in *Nephrops* (a polymerase chain reaction (PCR) developed by Small et al., 2006) has been used in used in Phase I of the Scottish *Nephrops* Survey. It has been revealed that prevalence levels approaching the patent peak are in fact detectable in many months of the year, whereas the intensity of infection, as reflected by the ratio of haemocytes to parasite cells (Field and Appleton (1995), increases from extremely low levels for many months of the year to the patent peak in the spring as the infection progresses.

These immunoassay and molecular diagnostic tools therefore provide methods for providing an early warning of the disease prevalence that will exist at the peak in a given population or a particular year. In contrast, the only commercial screening tool currently used is the cuticle colour change mentioned above, which is only associated with the most advanced stage of the disease, and so does not give any early warning, For most of the time it also greatly underscores disease prevalence. It is used by fishermen to select out heavily diseased individuals, a practice also seen in the blue

crab (*Callinectes sapidus*), tanner crab (*Chionoecetes bairdi*) and opilio crab (*C. opiolo*) fisheries in N. America and the brown crab (*Cancer pagurus*) fishery in Ireland (Chualain et al., in press). It was also used by Briggs & McAlliskey (2004) to establish a spring seasonality of patent *Hematodinium* disease in *Nephrops* in the Irish Sea.

Many commercially-exploited populations of *Nephrops* in the North Atlantic and around Scotland are parasitised by *Hematodinium* parasites, but there exists little systematic sampling of these areas to monitor prevalence. Most data on *Hematodinium* in *Nephrops* are generated by research trawls in the Clyde Sea area and the Irish Sea, but research trawls cannot feasibly monitor all stocks. Considering the evidence that parasite prevalence can differ between and within the North Clyde populations (which differ in size and diet) (Parslow-Williams et al., 2001; Parslow-Williams et al., 2002; Parslow Williams, 1998), monitoring different populations could generate valuable datasets. Comparisons of parasite persistence in differing host populations, their population structures and associated fishing pressures are not available for multiple stocks throughout the year. The only vessels regularly fishing outside research in the Clyde Sea area for example are the commercial fleets.

Commercial landings of *Nephrops* in Scotland consist of two forms, whole animals and 'tailed' product. Eighty percent of Scottish landings are 'tailed', a process by which the cephalothorax is detached from the tail and discarded; only the tail meat is kept. The tails are usually from the smaller animals, whereas the larger animals are either sold alive or frozen whole, to more lucrative UK and continental European markets.

In order to relate infection levels in tailed product delivered to Young's Seafood Ltd from various supplying fleets operating on different fishing grounds, a number of procedures need to be established. Firstly, the traceability of the product in terms of the location and date/time of capture needs to be established. Secondly, the size and sex of the animals needs to be reconstructed from the anatomical features and the dimensions (biometrics) of the tails. Thirdly, a sensitive diagnostic method needs to be applied for testing the level of infection by the blood parasite *Hematodinium* sp. in *Nephrops.* Bringing into use the immunological methods developed in Phase I of the Scottish *Nephrops* Survey, and demonstrating that these methods can be applied successfully to tailed product arriving at Young's Seafood Ltd. premises will fulfil these requirements.

Using these procedures in conjunction with data from the 'Youngstrace' traceability systems on supplying vessels will also make it possible to determine the seasonal variations in catch composition, body condition and infection prevalence in the different sea areas that supply Young's Seafood Ltd. This will allow commercial samples to be used as a monitoring tool for *Hematodinium* in *Nephrops* stocks for which research cruises are prohibitively expensive, and the feasibility of this approach was assessed in this project.

OBJECTIVES

- To identify the main factors that affect the product quality of whole langoustines, in comparison to tailed product, and to determine the most effective handling and packing methods for prolonging the shelf life of this whole product.

- To establish objective benchmarks for the quality of both tailed and whole fresh products, by calibrating the developed biochemical and instrumental assays against sensory evaluations performed by an independent, professional sensory panel.

- To identify the key features of the population biology and ecology of *Nephrops* that impact upon the body condition of the animals and their post-capture quality, and to determine how these vary with location, fishing practices and transport.

Scope of project

The project was achieved through a series of 6 work packages (WP), that used the suite of methodologies developed in Phase I of the Scottish *Nephrops* Survey for assessing product quality, freshness and spoilage rates, and will apply these to different products and procedures.

WP1. Post-capture changes in the quality of whole langoustines

In order to exploit the expanding market opportunities for whole langoustines, supplied as a chilled fresh product, it is essential to understand the processes that underlie quality loss in the whole animal, compared to the tailed product. Using measures developed in phase 1 of the project for quality, freshness and spoilage, the

following factors will be assessed immediately on capture by research vessel, and for up to 7 days thereafter while the product held on ice or at different ambient temperatures.

- The extent to which degradation occurs due to autolysis by enzymes from the organs in the 'head'

- The production of any undesirable breakdown products (eg. Histamine)

- The effect of this autolysis on the texture and flavour of the tail meat

- The time course of post-mortem changes, including changes in tissue ph and the onset of rigor mortis

- The time course and extent of bacterial spoilage

WP2. Factors that affect the product quality of whole langoustines

- The effect of capture method (trawl vs. creel) on the quality of whole langoustines

- The effect of trawl duration on the quality of whole langoustines – is there any advantage in very short trawls for supplying this product in a better condition?

- The effect of capture damage on quality loss, including the occurrence of tail muscle necrosis.

- The effectiveness of icing procedures in maintaining product freshness

- Comparison of product quality from different supply ports and vessels in relation to post-capture handling procedures, icing methods and transportation conditions.

Leading to recommendations for the most appropriate and cost-effective methods for capturing langoustines for whole fresh product, and for best practice in handling and storing.

WP3. Establishing the effectiveness of gas flushing for prolonging shelf-life

- Determine the effectiveness of different $CO_2/O_2/N_2$ gas mixtures as inhibitors of the growth and activity of the known specific spoilage organisms for Nephrops when grown in culture

- Test the effect of different gas mixtures on reducing the rate of spoilage of Nephrops meat under controlled conditions

- Establish the optimal gas mixtures for maintaining quality and freshness of whole langoustines

- Using product obtained directly from the research vessel, prepare modified atmosphere packs (MAP) of whole langoustines using the optimal gas mixes

- Using the full suite of tests, measure the quality and freshness of the MAP product at different times, and determine the maximum shelf-life that can be obtained.

- Monitor MAP packed product for the production of any undesirable breakdown products (eg. Histamine).

- Determine whether a professional sensory panel can detect any differences in the sensory properties MAP packed product that represent loss of flavour

- Test the effectiveness of different chill temperatures in combination with MAP packaging on maintaining product quality.

- Compare the shelf-life of MAP packed product based on whole langoustines obtained from the normal supply chain with that of animals obtained directly from the research vessel

Leading to recommendations for the most effective gas mixture for MAP packs to give the longest shelf-life with acceptable quality.

WP4. The effect of melanosis dipping on product quality

- Do the various dip treatments for preventing melanosis of the shell of whole langoustines have and check any effect on the quality of flavour of the meat

- Analyse if melanosis dip treatments have an antibacterial action against spoilage bacteria

- Study if icing alone, if adequately applied and provide effective suppression of melanosis ever the required shelf-life of the product

- Compare if melanosis development is affected by MAP packing

Leading to conclusions about the influence of melanosis dips on product quality.

WP5. Calibration of assays for product quality against Sensory Evaluation

The biochemical assays and instrumental methods for quality and freshness become fully valid only when they are measured directly in relation to the sensory evaluation of flavour and texture of the meat. Without such cross-calibration it is not possible to properly interpret the values obtained form objective tests, or to stipulate appropriate thresholds for acceptability based on such measures alone.

- A professional sensory panel will be recruited by an appropriate food industry agency and will be trained in evaluating langoustine product

- A sensory scoring system, based on the existing QASAS scheme, will be established

- Whole langoustines and tailed product will be obtained under standard conditions using the research vessel, and will be subjected to controlled temperature abuse or held for defined periods of shelf-time.

- Duplicate samples will be prepared for simultaneous testing using the established laboratory procedures, and for evaluation by a trained professional sensory panel.

- The sensory evaluations will be repeated with a range of defined product types and qualities, until a consensus is obtained for the sensory evaluation

- The laboratory measures will then be correlated with the sensory measures, and appropriate thresholds will be established.

- Validation trials will be made both by laboratory measures and by sensory panel evaluations on product obtained form commercial supplies, to establish conformity

- Achieving conformity will validate the quality indices and thresholds based on the laboratory measures.

Leading to the establishment of benchmark values for the biochemical assays and instrumental measures that represent thresholds for acceptable product quality and freshness, or reflect good flavour and texture.

WP6. Variation in product quality with location and supplier

- Establish methods for determining the sex, body size and moult state of animals from either whole langoustines of from the tailed product delivered to Young's Seafood Ltd. premises.

- Bring into use the immunological methods developed in Phase I of the Scottish *Nephrops* Survey for testing the level of infection by the blood parasite *Hematodinium* sp. in *Nephrops*, and demonstrate that these methods can be applied successfully to either whole langoustines or to tailed product arriving at Young's Seafood Ltd. premises.

- Using these procedures in conjunction with data from the 'Youngstrace' traceability systems on supplying vessels, determine the seasonal variations in catch composition, body condition and infection prevalence in the different sea areas that supply Young's Seafood Ltd.

- Determine the natural variation in the flavour and texture of tail meat in relation to these seasonal factors

- By comparing product fished by different supply vessels from the same locations, identify any differences in product quality that may be attributable to differences in post-capture handling and transport procedures.

Leading to recommendations for best fishing practices and effort to catch animals in good body condition at given seasons and locations, and to avoid moulting periods when animals are most vulnerable to stress and infection.
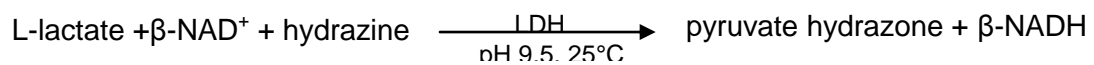
MATERIAL AND METHODS

I. Physiological measures in *N. norvegicus*

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:

$$\text{L-lactate} + \beta\text{-NAD}^+ + \text{hydrazine} \xrightarrow[\text{pH 9.5, 25°C}]{\text{LDH}} \text{pyruvate hydrazone} + \beta\text{-NADH}$$

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, 50μl 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. Quality related measures for *N. norvegicus*

pH in the muscle

In the quality experiments, in order to obtain the muscle pH, muscle 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 *N. 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). 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 N. norvegicus 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.
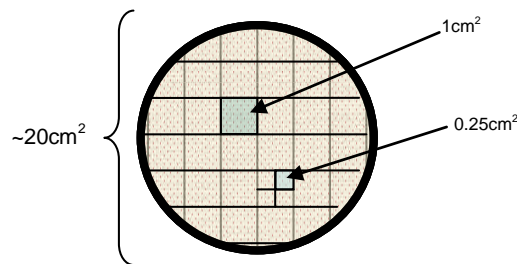
Colony Count: The bacteria form red colonies which can be counted by eye, if the

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 1cm^2 grid counted. By using a binocular numbers up to 50 colonies per 0.25cm^2 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 ºC 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 MgCl$_2$, 4 μl dNTPs, 0.4 μl *Taq* polymerase, 1 μl of each primer, 36.2 μl of dH$_2$O and 1 μl of template DNA. The amplification conditions were 94 ºC for 2 min, followed by 35 cycles of 94 ºC for 1 min, 55 ºC for 1 min and 72 ºC for 2 min with a subsequent extension cycle at 72 ºC 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.

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.

Determination of nucleotide breakdown products and K-values in N. norvegicus 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 C_{18} 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 KH_2PO_4 + 0.06 K_2HPO_4 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 1. 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

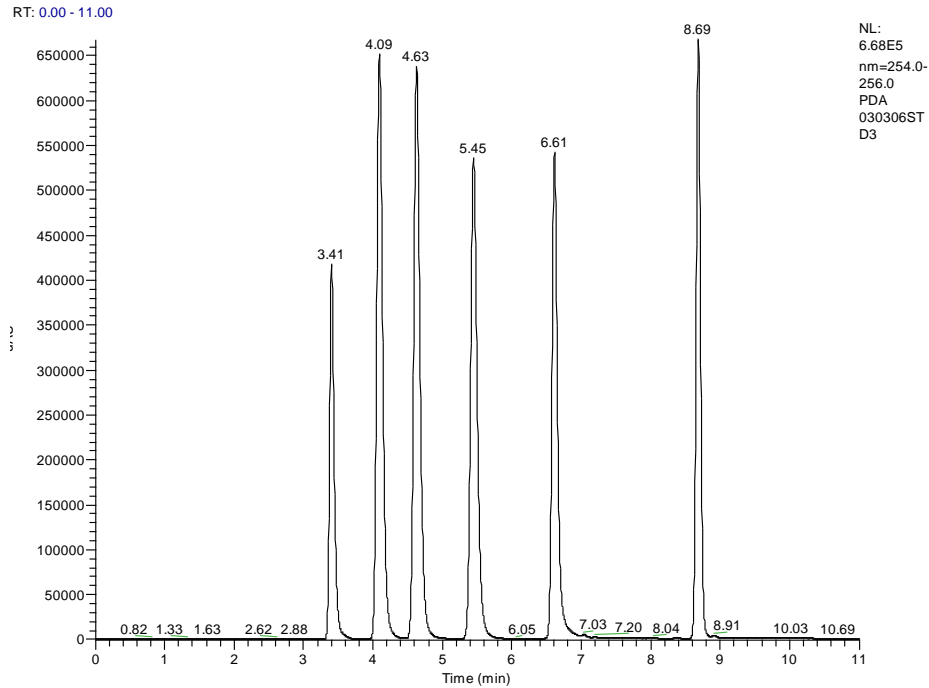process allows the contents to be expressed in µmol of nucleotide per g wet weight

tissue.

Figure 1. 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.

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

K-value (%) = [INO] + [Hx] / [ATP] + [ADP] + [AMP] + [IMP] + [INO] + [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

Determination of biogenic amines in Nephrops tail muscle

Biogenic amines (histamine, tyramine, cadaverine, agmatine, putrescine, spermidine and tryptamine) were determined according to Veciana-Nogues et al. (1995) with slight modifications. Samples of 1 g of *N. norvegicus* tail muscle were homogenized on ice in 5x volume of 0.6 M PCA using an Ultra Turrax Homogenizer. Immediately after homogenization, 5 ml the whole homogenate was centrifuged at 5.000 rpm for 10 min at 4 °C. Perchloric acid extracts were filtered through a 0,45 µm filter and stored at −80 ℃ until analysis.

Biogenic amines were analysed by high performance liquid chromatography (HPLC) with post-column derivatization. A SpectraSystem P2000 HPLC pump was used coupled to a fluorescence detector (FP-920) from Jasco. Separations were carried out using a Thermo Hypersil Gold column 250 × 4.60 mm, with an internal particle diameter of 5 μm. The mobile phase was composed of: solvent A (0.1 M sodium acetate and 10 mM sodium octanesulfonate, adjusted to pH 5.20 with acetic acid) and solvent B (a solution of 0.2 M sodium acetate and 10 mM sodium octane sulfonate adjusted to pH 4.50 with acetic acid mixed with acetonitrile in a proportion 6.6/3.4, v/v). Conditions used for the analysis are summarised in Table 2.

Table 2. Chromatographic conditions used for the analysis of biogenic amines in *Nephrops norvegicus* muscle extracts.

Time (min)	Solvent A (%)	Solvent B (%)	Flow rate (ml/min)
0.0	70	30	1.0
30.0	63	37	1.0
40.0	50	50	1.0
55.0	10	90	1.0
56.0	10	90	1.0
57.0	70	30	1.0
63.0	70	30	1.0

Post-column derivatization was performed by a precision mixer/splitter module form Reeve connected to zero dead volume mixing T installed between the column outlet and the detector. A coil of 200 cm long and 0.01 in. id stainless steel tubing was used to connect T with the detector. Post-column derivatizing reagent consisted in 15.5 g of boric acid and 13.1 potassium hydroxide in 500 mL of H_2O (if necessary pH was adjusted to 10.5-11 with 30 % of KOH). Then 1.5 ml of Brij-35 (30 %) and 1.5 ml of mercaptoethanol were added and finally 0.1 g of o-phthalaldehyde (OPT) dissolved in 2.5 mL of ethanol were all mixed. This reagent was prepared daily and degassed and protected from light. Flow rate of mobile phase was 1.0 mL/min as indicated in Table 2 and flow rate of derivatization reagent was 0.5 mL/min. Spectrofluorometric detector was set at excitation wavelength of 340 nm and emission wavelength of 445 nm. No changes in wavelengths were made to avoid troubles with baseline stability. Standard curves were prepared from histamine dihydrochloride, tyramine freebase, tryptamine hydrochloride, cadaverine hydrochloride, putrescine hydrochloride, agmatine sulphate and spermidine trihydrochloride all from Sigma Aldrich (Dorset, England, UK) in concentrations ranging from 0.25 mg/L to 8 mg/L. To obtain the concentrations of different products of biogenic amines, 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 2.
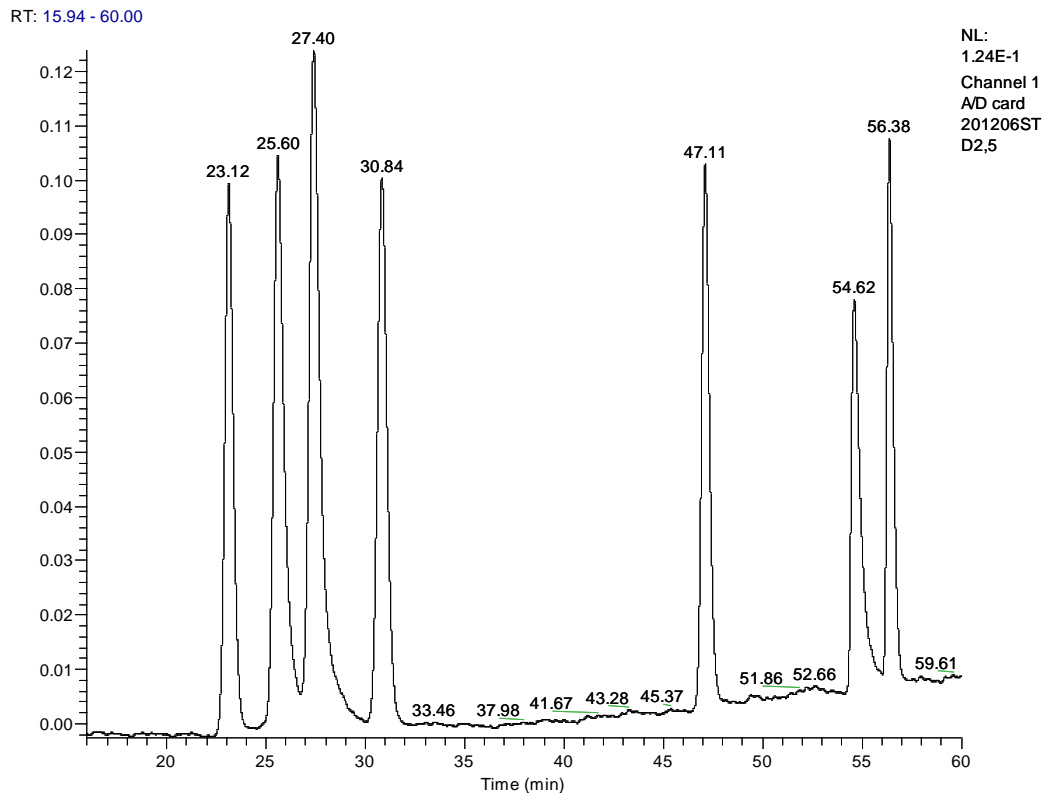
Figure 2. Chromatogram showing a typical standard of biogenic amines resolved by HPLC with post-column derivatization with OPT. Peaks were identified by their retention time and order of appearance was: tyramine, putrescine, cadaverine, histamine, agmatine, spermidine and tryptamine.

Determination of Melanosis Development in whole Norway lobster animals

The assessment of blackening or melanosis development was quantified using:

- Digital images
- The Melanosis Index Score
- Measuring the activity of the enzyme responsible for the blackening or polyphenol oxidase activity (PPO) in the cephalothorax of *N. norvegicus*.

Melanosis Index Score: This index has been already published for the quantification of blackening in *N. norvegicus* by Martinez-Alvarez et al. (2007) (Table 3). This index was applied separately to the different parts of the Norway lobsters (Figure 3):

- Cephalothorax dorsal
- First clawed legs

- Cephalothorax ventral and pereiopods

- Pleopods

- Abdomen or tail

- Tail fan

Table 3. Melanosis Index Score used to evaluate blackening or melanosis development in whole Norway lobsters

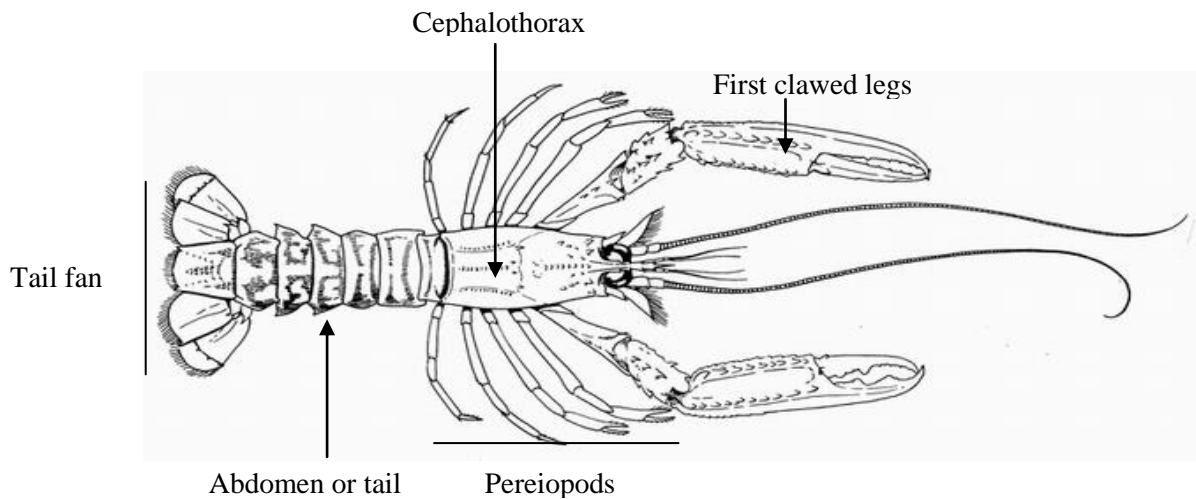| Scoring	Attributes
1.0	Total absence of black spots or blackening
2.0	Few black spots or blackening (less than 30 %)
3.0	Considerable blackening (between 30-70 %)
4.0	Substantial blackening (more than 70 %)

Figure 3. Diagram taken from showing the different parts of the body of a Norway lobster (*Nephrops norvegicus*) or langoustine.

PPO Activity: PPO activity was measured in the cephalothorax of whole *N. norvegicus* as this is the body part were melanosis is more pronounced once the animals are removed from the water. Crude enzyme extracts from the cephalothorax (always stored at −80 ℃) were prepared according to Wang et al. (1992). Between 30 and 40 g of cephalothorax was added to 2 x of 0.1 M sodium phosphate buffer pH 6.4 and homogenized in an Ultra Turrax-homogenizer for 2 min on ice. Homogenates were then centrifuged at 5 0000 x g for 30 min at 4 ℃. Supernatants were used as the

crude poplyphenoloxidase preparations and were immediately frozen at −80 ℃ to minimise alterations before determination.

The enzyme activity was measured using the proline-catechol spectrophotometric assay at saturating conditions as in (Martinez-Alvarez et al., 2005). The reaction mixture contained 480 µl of 30 mm catechol, 480 µl of 30 mm L-proline and 40 µl of crude enzyme preparation or buffer in blank condition. Catechol and L-proline were prepared in 0.1 M sodium phosphate buffer pH 7. Changes in absorbance at 530 nm were monitored at 25 ℃ for 8 min in a spectrophotometer with a thermostat controller. Enzyme activity was expressed as the increase in optical density (OD)/min/mL as in Yan et al. (1990). At least triplicates per condition were always performed.

Damage index

In order to assess the physical damage during trawling in WP2, a random sample of approximately 250-300 individuals of *N. norvegicus* was taken from the catch and the sex, carapace hardness and level of damage were recorded. Carapace hardness, which corresponds to moulting stage (Ridgway *et al.* 2006) was estimated by squeezing the sides of the carapace just behind the head. The carapace was considered to be 'hard' if there was no noticeable compliance when squeezed, and 'soft' if squeezing caused a clear distortion. Finally, animals were recorded as 'jelly' if the entire exoskeleton gave no resistance to pressure. The level of damage was scored against a three-level index, previously introduced by Ridgway et al. (2006) and presented in Table 4.

Table 4. Damage index used to classify the extent of damage of *N. norvegicus* after being captured by trawl.

Damage category	Criteria
Undamaged	No visible damage to external structure and no loss of limbs
Lightly damaged	*Exhibit no more than two of:*
	Loss of two or fewer walking legs (pereiopods 2-5)
	Loss of not more than one claw
	Soft tissue punctures or small puncture to the shell
	Loss of the tip of the rostrum
Highly damaged	*Exhibit at least one of:*
	Loss of more than two walking legs (pereiopods 2-5)
	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

III. Prevalence of the parasite *Hematodinium* sp. in *N.norvegicus*

Haemolymph enzyme linked Immunosorbent assay (ELISA)

Prevalence of *Hematodinium* sp. infection in the simulated commercial trawls was investigated using an ELISA. All 100 animals from the December to July research trawls were tested. The procedure was similar to that described by Small *et al*. (2002), with some minor modifications. The haemolymph and anticoagulant mixtures were diluted 1:500 and used to coat duplicate wells of a high-binding ELISA plate (COSTAR) at 50 µl per well for 0.5 h. After washing 5 times with PBS with 0.05 % Tween-20 (PBST) the plates were blocked with 10 % low-fat milk powder in PBST (Marvel) for 45 min and washed a further 5 times with PBST. Anti-*Hematodinium* sp. antibody (Field and Appleton, 1996) was diluted 1:5000 with the blocking solution and coated at 50 µl per well for 0.5 h. The plate was subsequently washed 5 times

with PBST and goat antibody against rabbit IgG conjugated with horseradish peroxidase (Sigma-Aldrich) was diluted in blocking solution at 1:10000 and coated at 50 µl for 0.5 h. After 5 washes with PBST, 100 µl of Tetramethlybenzidine ultra slow ELISA substrate for horseradish peroxidase (Sigma-Aldrich) was added at 100 µl per well and the colour was allowed to develop for 5 min. Colour intensity was read at a wavelength of 560 nm using Biolinx software (version 2.1) a spectrophotometer. Positive and negative controls were run in duplicate on each plate. Positive control was the haemolymph from patently diseased *N. norvegicus* and negatives were haemolymph from animals in captivity for a year without showing any sign of disease. Blank wells that received no homogenate were run in parallel with test samples and on every plate.

RESULTS

WP1. Post-capture changes in the quality of whole langoustines

One of the first objectives in this Phase II of the Scottsih *Nephrops* Survey was to focus on issues relating to whole animals rather than tails. In recent years, there have been increased market opportunities for whole fresh *N. norvegicus*, sold under the agreed name of 'langoustines'. This move from tails, to be used for scampi production (breaded tails of *N. norvegicus*), to langoustines implicates a move from low to high value products.

Implications of this new marketing strategy for the fishing industry in the UK and especially in Scotland could be very significant since it would mean adding value to an existing product. However, seafood and in particular shellfish are very prone to spoilage. The main factors affecting the rate of spoilage in fish include the method of capture, on-board handling and processing and storage conditions (Huss 1995; Dalgaard, 2000). All this processes are potential bottleneck or restricting factors since fishing ports are often far from very populated areas where fish is consumed and therefore extension of shelf life becomes essential.

During Phase I of the Scottsih *Nephrops* Survey the main factors affecting the rate of spoilage in *N. norvegicus* tails were identified. The main factors studied were the effect of capture method, on-board handling and storage conditions. Results of these trials were explained in detail in the Report from Phase I of the Survey.

The prime objective of this WP1 was to determine the key differences in these measures between whole fresh product and isolated tails. To this end, the first comparison made was between tails stored on ice and whole animals stored on ice.

The methodology of this trial was very similar to the one used during the Phase I of the Scottsih *Nephrops* Survey. To obtain tails or whole animals, *N. norvegicus* were caught by otter trawl using the Research vessel 'Aplysia' from the University Marine Biological Station at Millport (UMBSM). Once on board, animals were carefully washed with running seawater and left as whole animals or tailed. In both cases, specimens were transported to the University of Glasgow on ice and stored at 0-1 ºC for up to 7 days. In order to assess if degradation takes places at a different rate depending on the product type bacterial load, pH in the muscle and K-values used as freshness indicator (results on the usefulness of this parameter in *N. norvegicus* was explored in Phase I of the Scottsih Nephrops Survey) were obtained and compared. Also any other observations were recorded in order to identify the main differences when working with whole animals compared to working with tails.

The two variables measured in order to evaluate if spoilage rate is more rapid in whole animals compared with tails were bacterial load and the pH in the muscle. Total bacterial load in the muscle was very similar in both groups, indicating a similar bacterial growth in the tail muscle independently of having the cephalothorax attached to the tail or not (Figure 1.1).
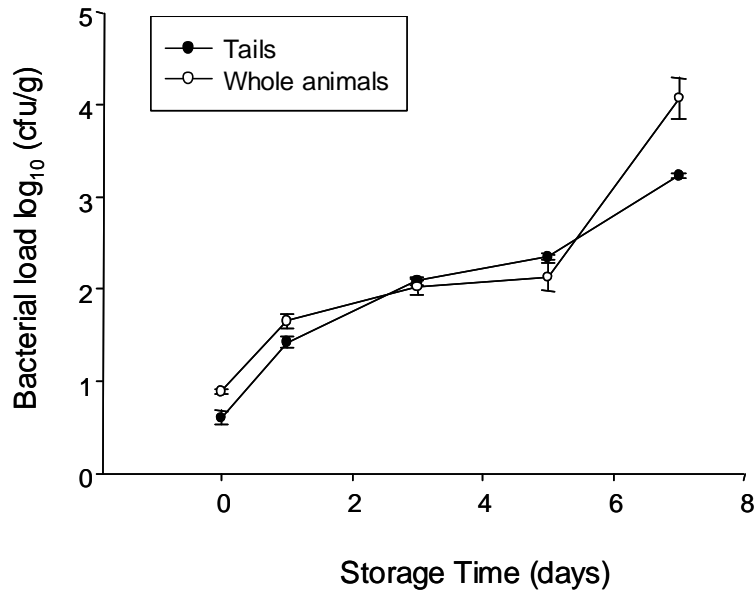
Figure 1.1 Total bacterial load in the muscle from tails or whole animals stored at 0-1 ºC for up to 7 days. Values are the mean ± S.E.M of 3 different samples each one containing 3 muscles from 3 different individuals.

Similarly, pH in the muscle extracted from stored tails or stored whole animals was not different (Figure 1.2). In both cases muscle pH increased with storage time and on day 7 similar values were obtained for both groups (pH in the tails was 7.85 ± 0.07 and in whole animals was 7.79 ± 0.10).
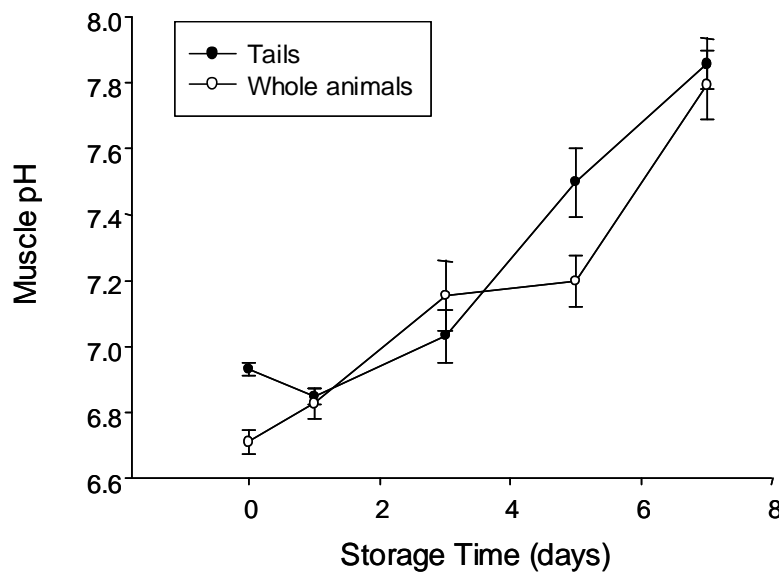
Figure 1.2 Muscle pH in the muscle from tails or whole animals stored at 0-1 ºC for up to 7 days. Values are the mean ± S.E.M of 10 independent samples for each group.

K-values were also determined as a freshness indicator. This value is based on a ratio

of the ATP and its breakdown products, as explained in detail in the report of Phase I

of the Scottsih *Nephrops* Survey. This breakdown of ATP is a chain reaction that

takes place during early post-mortem phases due to autolytic enzymes present in the

muscle. K-values in the muscle of stored whole animals were very similar to the

values obtained in the muscle of stored tails (Figure 1.3).
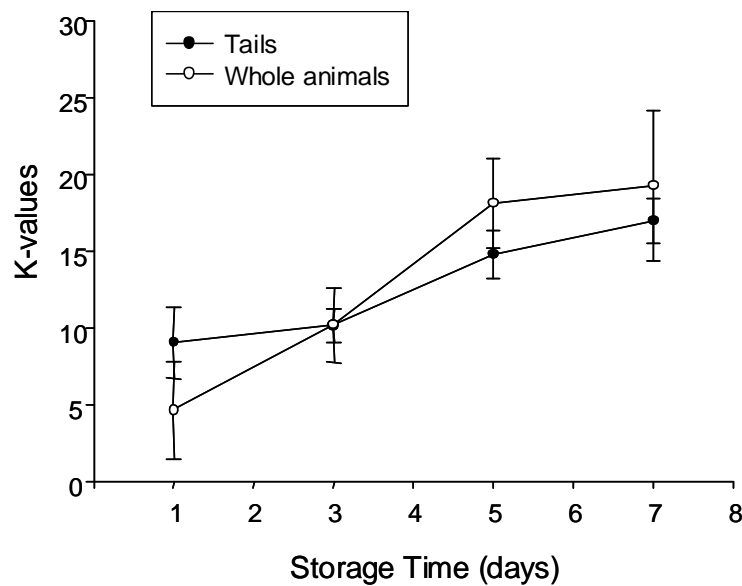
Figure 1.3 K-values in the muscle from tails or whole animals stored at 0-1 ºC for up to 7 days. Values are the mean ± S.E.M of 5 independent samples for each group.

However, when looking at the individual nucleotides over storage time a slight

difference was obtained in the first 24-48 hours of storage. To clarify this point ATP

and its breakdown products are represented individually in Figure 1.4. In both groups

AMP was the main nucleotide immediately after the animals were killed, which is

consistent with results previously obtained from animals captured by trawling (Report

I of the Scottsih Nephrops Survey). On Day 1, IMP was the main nucleotide in tails,

while AMP was still high in whole animals. These results indicate that on Day 1 the

processes of autolysis were more advanced in tails compared to whole animals. These differences between the nucleotide profiles of the two groups were only observed in the early storage stages, but from Day 3 onwards there was no difference between them. Therefore, it would appear that the autolytic phase is more protracted in whole animals compared to tails, while the bacterial phase is very similar between both types of product.
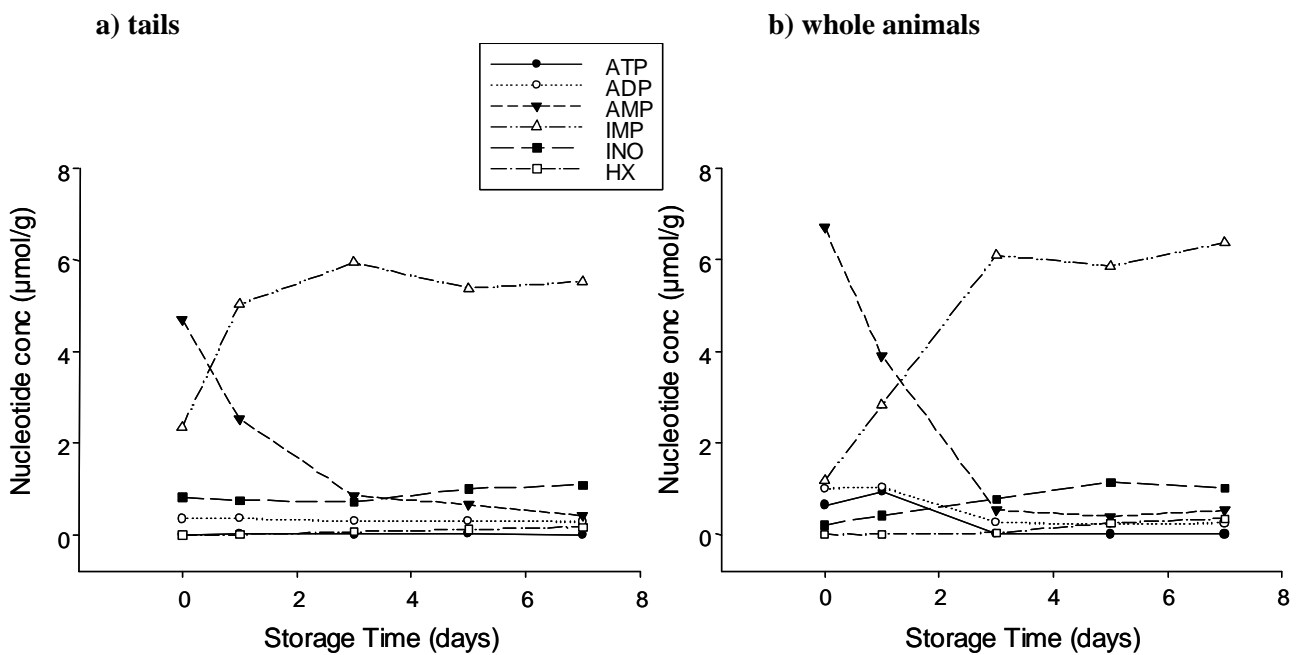

Figure 1.4 ATP and its breakdown products in the muscle from tails or whole animals stored at 0-1 ℃ for up to 7 days. Values are the mean ± S.E.M of 5 independent samples for each group.

The results from this trial suggest that having the cephalothorax attached to the tail does not have any negative impact on the biochemistry or on bacterial growth in the meat of the tail. On the contrary, the autolytic phase is prolonged since under these circumstances the animals die very slowly on the ice and metabolism is slowed down producing retardation on the post-mortem phase of the animal.

However, it was consistently observed that melanosis development was more apparent in whole animals, suggesting that this could be a limiting factor when commercialising whole animals compared to tails. Tails are in fact one of the last parts of the body to be affected by melanosis development (Figure 1.5). Therefore, when working with tails alone it could be unecessary to treat this product with anti-melanotics, if temperature is low and strictly controlled. However, in whole animals melanosis developed extensively and a high rate in the dorsal and ventral parts of the cephalothorax and in the appendages, and so anti-melanotic treatments should be considered.
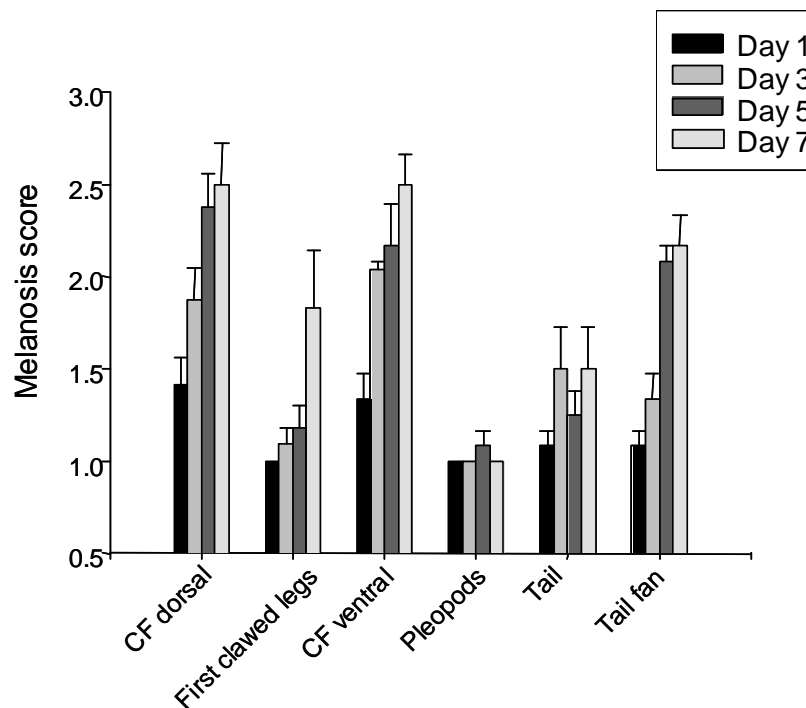
Figure 1.5 Melanosis development (expressed as Melanosis score) of whole animals stored at 0-1 ºC for up to 7 days. Values are the mean ± S.E.M of 10 independent samples per sampling time.

The results obtained in this trial indicate that when moving from tails to whole animals:

- Melanosis should be studied in depth and anti-melanotic protocols should be put onto place

- A methodology to assess the external appearance of the whole animals should be added to the trials since laboratory results do not reflect the external appearance of the animals.

These two observations have been pursued in this Phase of the Scottsih *Nephrops* Survey in the following work packages:

- Melanosis studies are covered in WP4

- The development of a method to assess the external appearance of the animals or a sensory scoring system (QIM) is described in WP5

WP2. Factors that affect the product quality of whole langoustines

The results from the previous WP1 indicated that the limiting and more important factor to be considered when changing to work from tails to whole animals is the development of melanosis. However, other factors to be considered when working with whole animals compared to tails would be:

1. Damage due to trawl duration and handling on the fishing vessel

2. The effectiveness of icing procedures

3. The occurrence of tail muscle necrosis

1. Damage due to trawl duration and handling on the vessel

In this section we will present the data concerning the damage found in whole animals caught by otter trawl on the same day using different trawl durations. The scientific results from this trial have been reported in an International Journal (Albalat et al., 2009).

Norway lobsters (*N. norvegicus*) were caught by otter trawl in the Largs-Fairlie Channel of the Clyde Sea area, Scotland, UK (55.41˚ N, 04.56˚ W). Trawls of 15, 60 and 150 min were performed using RV 'Aora' from the University Marine Biological Station Millport (UMBSM) during the month of June 2006.

The characteristics of the animals recorded for the three trawl durations were similar (Table 2.1). Females were more abundant than males, and a high proportion of animals had a 'hard' carapace (between 62.83 and 72.95 %). Both these features are characteristic for a study conducted in early summer at this location (Ridgway et al.

2006). Information on catch composition monthly over a year can be found in Report

I of this Scottsih *Nephrops* Survey.

Table 2.1. Total number of animals, sex (%) and carapace hardness (%) of *N. norvegicus* from the three different trawls recorded using the Damage Index (Table 4).

Trawl duration (min)	15	60	180
Total Animals	281.00	266.00	339.00
Sex (%)			
Male	44.84	41.73	39.82
Female	55.16	58.27	60.18
Carapace hardness (%)			
Hard	72.95	66.17	62.83
Soft	20.28	25.56	31.27
Jelly	7.12	8.27	5.90

When looking at the total damage data (Table 2.2, 'Total'), a decrease in undamaged

animals was observed in the longest trawl (Table 2.1). However, since 'soft' and

'jelly' animals are usually discarded, from a commercial point of view and especially

when targeting the market of whole fresh/frozen langoustines it was necessary to

separately assess the damage to 'hard' animals (Table 2.2, 'Hard'). For this category

the longest trawl produced greater damage, as indicated by the reduced percentage of

undamaged animals and the increased percentage of lightly and heavily damaged

animals, compared with the shorter trawls.

Table 2.2 Extent of damage categorised using the Damage Index (Table 4) and expressed as percentage of Norway lobsters according to trawl duration and carapace hardness**.**

	Hard	Soft	Jelly	Total
15 min trawl				
Undamaged (%)	64.88	24.56	0.00	52.31
Lightly damaged (%)	32.68	29.82	0.00	29.89
Highly damaged (%)	2.44	45.61	100.00	18.15
60 min trawl				
Undamaged (%)	72.16	38.24	0.00	57.52
Lightly damaged (%)	24.43	39.71	27.27	28.57
Highly damaged (%)	3.41	22.06	72.73	13.91
180 min trawl				
Undamaged (%)	52.58	32.08	15.00	43.95
Lightly damaged (%)	37.56	39.62	10.00	36.58
Highly damaged (%)	9.86	28.30	75.00	19.47

During trawling, physical damage can take place due to abrasion and compression within the cod-end (Lancaster and Fridd 2002) and during hauling, due to differences in pressure (ICES 1994). Previous studies have observed that up to 50 % of the catch can be affected physically by the trawl (Harris et al. 1997; Ridgway et al. 2006). Similar proportions were found in the present study. Furthermore, increased trawling time did have an effect on the percentage of damaged animals. The consequences of this physical damage during long tows were accentuated by the fact that 'hard' animals, important from a commercial perspective, were the most affected. Increasing damage occurs with trawl duration, which could be detrimental from a commercial point of view if long tows are performed. Furthermore, physical damage could affect mortality, which could be an important point to consider if animals trawled for a long time are to be kept alive for sale. In this sense, Ridgway et al. (2006) found higher mortalities associated with long tows when assessed after 24 hours but not if recorded after 7 days. Therefore, if trawl-caught animals are aimed at the live market, further studies should be carried out to elucidate the impact of longer tows on mortality rates.

Therefore, the conclusion from this experiment was that short duration trawls (60 min) are recommended over longer duration trawls (150 min) in order to achieve a higher proportion of 'hard' animals undamaged that will be commercially very valuable to be sold as whole langoustines.

2. The effectiveness of icing procedures in maintaining product freshness

Once the impact of trawl duration was assessed in whole animals another critical point examine, following results from Phase I of Scottsih *Nephrops* Survey, was to assess how the cooling mechanisms differed between having tails in a box or having whole animals.

In Phase I of the Scottsih *Nephrops* Survey one of the trials performed highlighted the importance of cooling the product as quickly as possible ('Thermal properties of boxes of *Nephrops* tails'). In this sense, it was demonstrated that leaving a box full of tails in a cold room (5-6 ºC) without ice on top of it was not sufficient to cool the whole box effectively, and therefore the quality of the tails situated in the middle of the box was seriously compromised.

Given these premises, one of the points to study was if the cooling pattern in a box of whole animals would be similar to a box of tails, when left in a cold room. To this end, a comparison was done where both, tails or whole animals were stored in containers of different sizes (Table 2.3) and left in a cold room at 5-6 ºC.

Table 2.3 Extent of damage categorised using the Damage Index (Table 4) and expressed as percentage of *N. norvegicus* according to trawl duration and carapace hardness**.**

Box Type	Length (mm)	Width (mm)	Height (mm)	Volume of container (l)
Small	70	70	70	0.32
Medium	120	120	125	2.01
Large	295	190	150	9.46

For this trial, animals were collected by otter trawl in the Clyde sea area by the Research Vessel 'Aora' from UMBSM. After the catch was on deck, animals were carefully washed with running seawater. Afterwards, some animals were tailed and some were left as whole animals. *N. norvegicus* were packed into different sized plastic boxes which were brought aboard the vessel. Containers were then taken back 3-4 h after collection to the facilities at the University of Glasgow. Temperature loggers (Stowaway Tidbit Temperature Loggers) were placed in the middle of each container to monitor changes in temperature. Finally, boxes were placed in a cold room set at 5 ℃ and left for 48 h.

The pattern of cooling in a box of whole animals was exactly the same as for a box of tails (Figure 2.1). Therefore, the results of studies on the cooling rate in tails could be applied equally to whole animals.
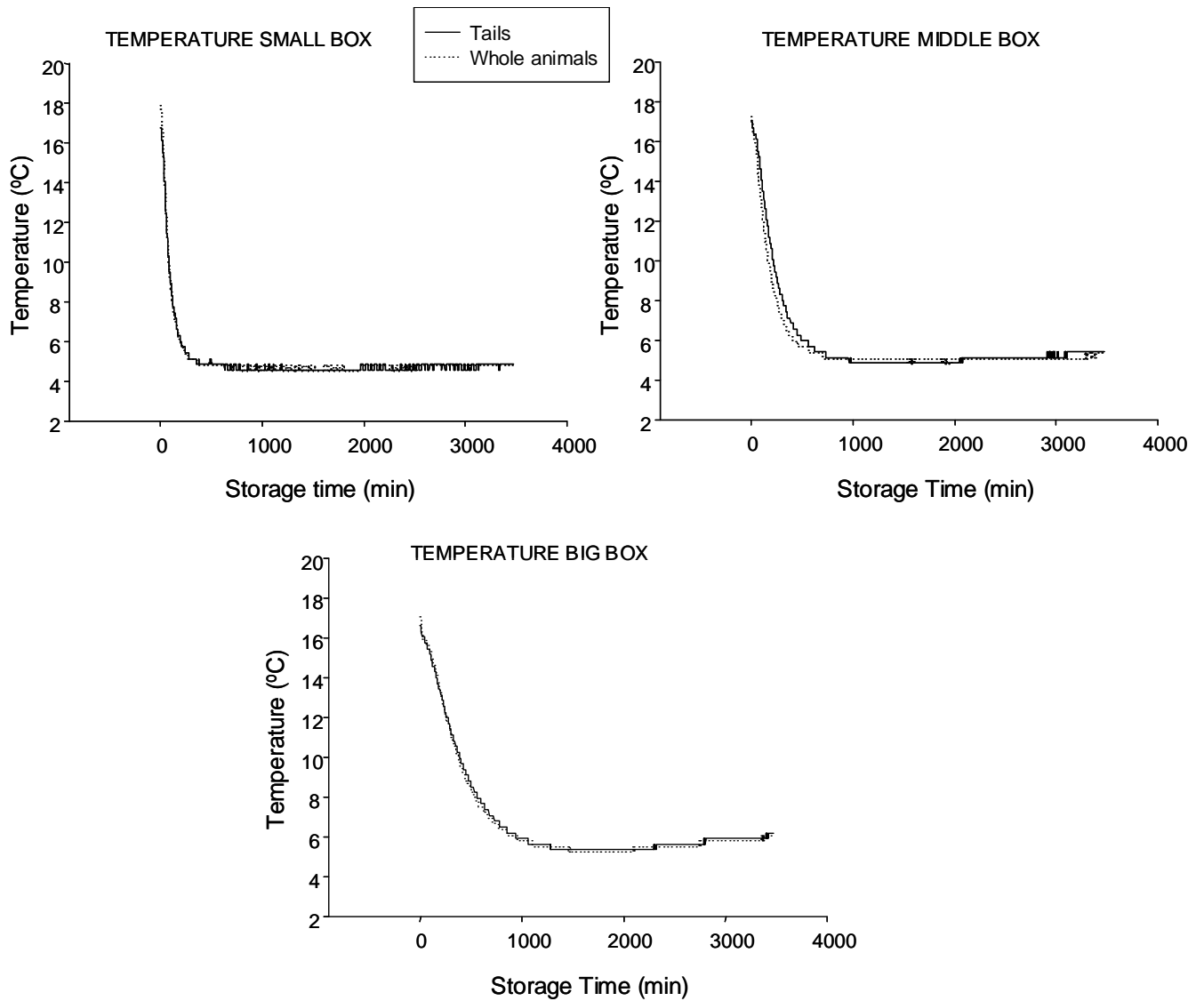
Figure 2.1 Temperature profiles measured in the middle of containers full of tails or whole animals stored in 3 containers of different size in a cold room set at 5-6 ℃.

Another interesting point was to compare the cooling effect due to the container size. As shown in Figure 2.2 it took a longer time to cool down the animals in the middle as the container size increased. It would appear that the tissues of the lobsters act as an insulator to slow down the cooling of the lobsters in the centre. Therefore, larger boxes that can hold more animals will increase the insulation of the animals in the middle.
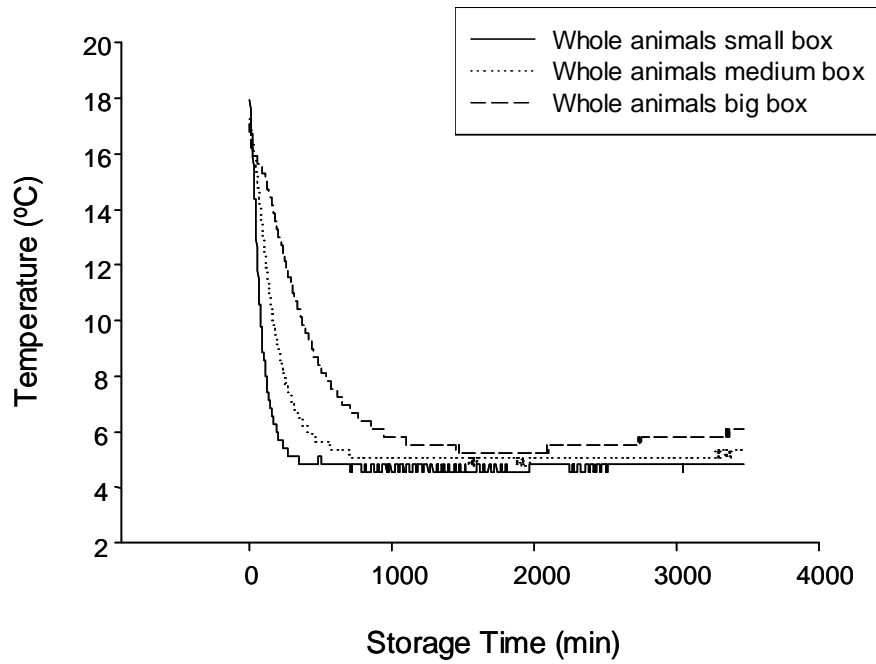
Figure 2.2 Temperature profiles measured in the middle of containers of different size full of whole animals stored in a cold room set at 5-6 ℃.

This factor should be taking into account when whole *N. norvegicus* are placed in a fish box and stored in a cold room. One of the possibilities studied in order to address this problem was to put ice on top of the box and then stored it in a cold room. When comparing the temperature profile of a fish box left in a cold room with ice on top or not the cooling rates were quite different (Figures 2.3 and 2.4).
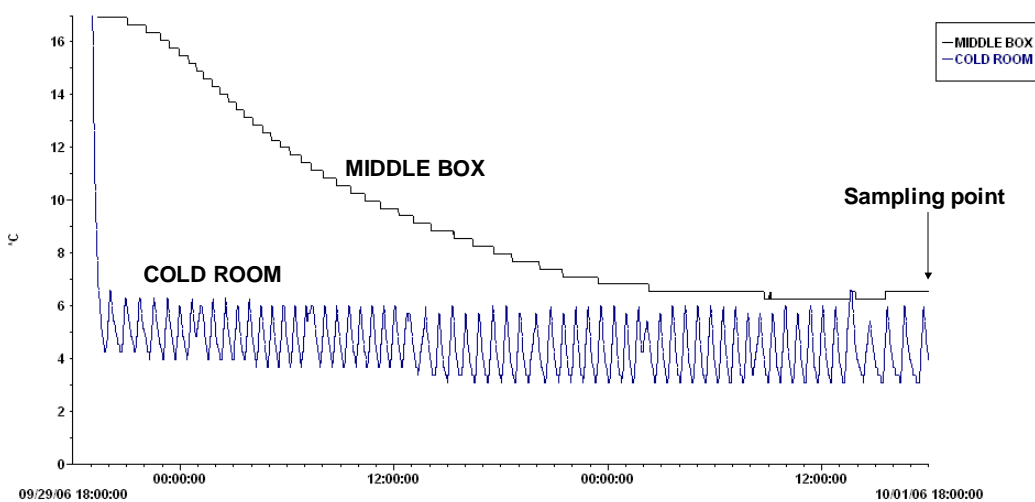
Figure 2.3 Temperature profiles measured in the middle of box of *N. norvegicus* tails stored in a cold room set at 5-6 ℃ without ice on top.

When the box is not iced on top, from an initial temperature of 18 ºC the material situated in the middle of the box reach the temperature of the cold room approximately 42 h later. On the other hand, if the fish box is iced on top the material in the middle reaches the temperature of the cold room within 3 h, due to the action of the ice and its percolation through the box.
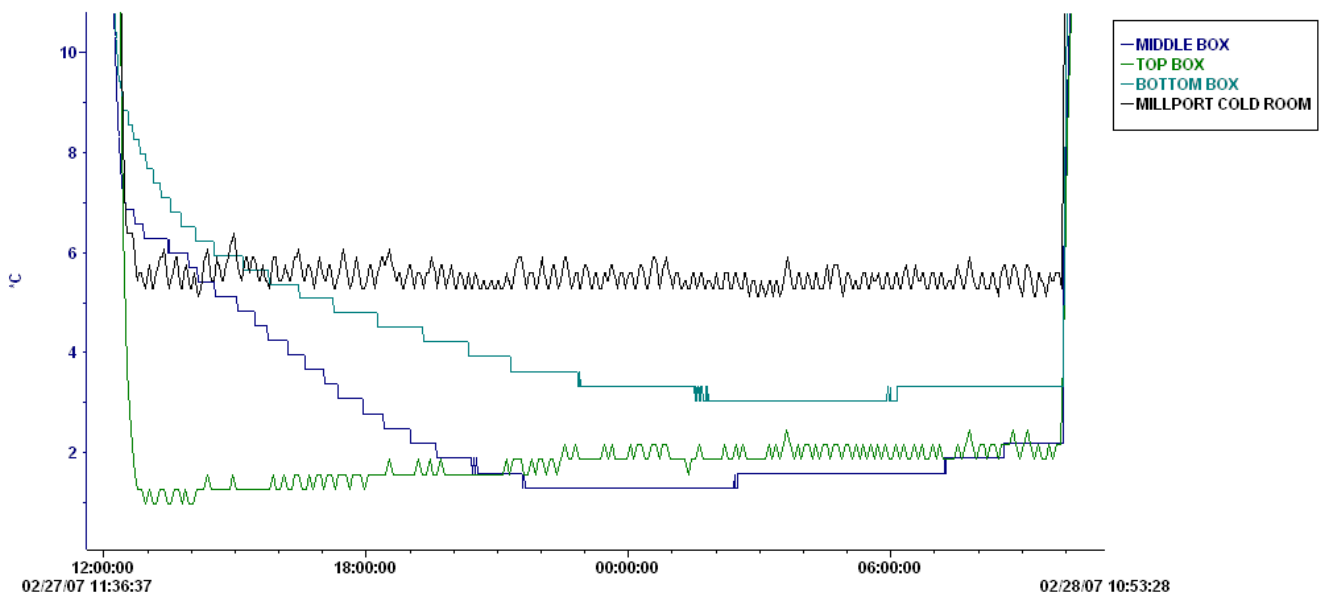
Figure 2.4 Temperature profiles measured in the middle of box of *N. norvegicus* tails stored in a cold room set at 5-6 ºC with ice on top.

Therefore, conclusions from this section would be:

- The cooling pattern of a box full of whole animals works a very similar manner to a box full of tails, and therefore same principles may apply.

- In order to increase cooling effect it is sufficient to apply ice on top of a fish box, since the melt water from the ice percolates through the animals, thus accelerating the cooling process throughout the box.

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3. The occurrence of tail muscle necrosis

Idiopathic muscle necrosis is characterised by focal to gross opaque lesions in the muscle of the tail. Affected animals show a characteristic whitening of individual muscle fibres and fibre bundles of the abdomen within hours of capture (Stentiford and Neil, 2000). As mentioned in the Report on Phase I of the Scottsih *Nephrops* Survey, in *N. norvegicus*, damage to the abdomen significantly reduces survival rates in discarded individuals and may also lead to necrosis of the tail muscles (Stentiford and Neil, 2000, Harris and Andrews, 2005).

Such animals may be unsaleable and the overall value of the catch will be reduced. Minimising the damage to individuals may therefore be important both in terms of increasing the value of a catch and minimising any effects on the wild population. So far, research into this condition has failed to identify its aetiology, although factors that can initiate necrosis are the exposure of animals to stress conditions such as trawl capture, temperature and salinity changes, handling and air exposure (Venkataramaiah, 1971; Lakshmi et al., 1978; Stentiford and Neil, 200).

In this sense it was interesting to study in further detail the differences in the tail meat quality of necrotic animals, compared to animals that do not present necrosis. To this end, animals were caught by otter trawl in the Clyde sea area and rapidly placed in vertically set tubes in running seawater to recover from the stress of the trawl. Animals were transported to the University of Glasgow and left overnight in a tank containing running seawater. The following morning necrotic (judged by them

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presenting with tail meat of white appearance) and non-necrotic animals were sampled and the following parameters measured: L-lactate in the muscle (as an indicator of stress) and nucleotide concentrations that allowed us to calculate the K-values (used as indicator of recovery and freshness respectively).

Muscle L-lactate was higher in necrotic animals when compared to non-necrotic animals (Figure 2.5). This value is generally used as an indication of stress and therefore the results indicate that necrotic animals are more stressed than non-necrotic animals.
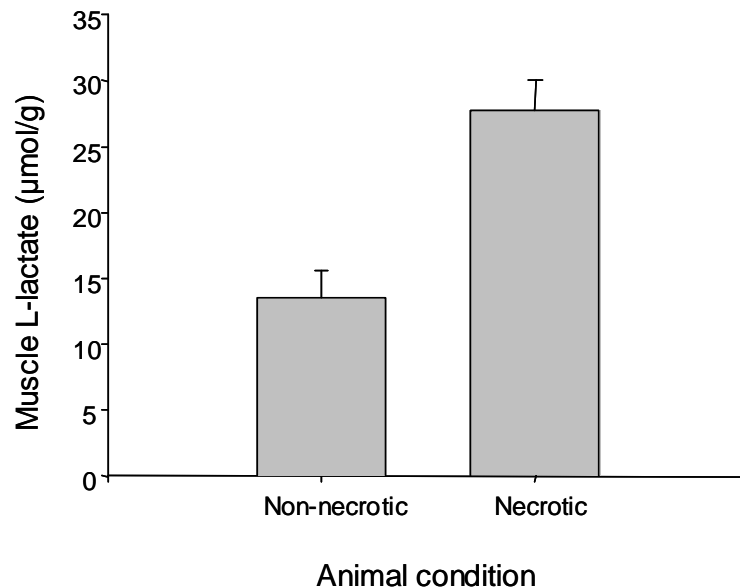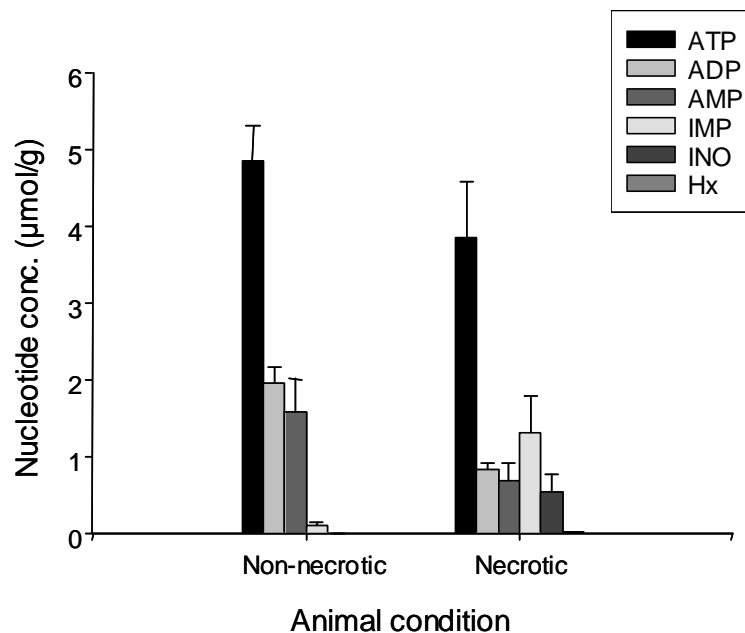

Figure 2.5 Muscle L-lactate measured in *N. norvegicus* trawled and stored in running seawater for 24 h. Values are the mean ± S.E.M. of 10 different animals for each group.

Nucleotides were also measured in the tail muscle. As demonstrated in Phase I of the Scottsih *Nephrops* Survey, the main nucleotide of a rested animal is ATP. However, after the trawl capture the animals are exhausted and the main nucleotide becomes AMP. The animals of the present trial were caught by trawl but they were sampled 24 h following holding in running seawater. Non-necrotic animals had recovered by this point, and the main nucleotide was ATP followed by lower concentrations of ADP

and AMP (Figure 2.6). In necrotic animals the main nucleotide was also ATP. However, in these animals IMP and INO were also present in the muscle. IMP has been found to accumulate in the tissues of dying and dead crustaceans (Chen et al., 1990) and thus it appears that deamination of AMP to IMP only occurs after inordinate levels of stress (Paterson, 1993) while INO is normally found once the animal is dead (at the post-mortem stages). These different nucleotide profiles suggest that in necrotic animals part of the muscle must be in a post-mortem stage, even though the animal is still alive.

Figure 2.6 Muscle nucleotide concentrations measured in *N. norvegicus* trawled and stored in running seawater for 24 h. Values are the mean ± S.E.M. of 10 different animals for each group.

According to these values it was possible to calculate the K-values as a freshness indicator (more information in Material and Methods section). This value should be zero if animals are still alive. However in necrotic animals K-values were around 6 % indicating that the muscle has lost part of its freshness, although surprisingly the animals were still alive (Figure 2.7).
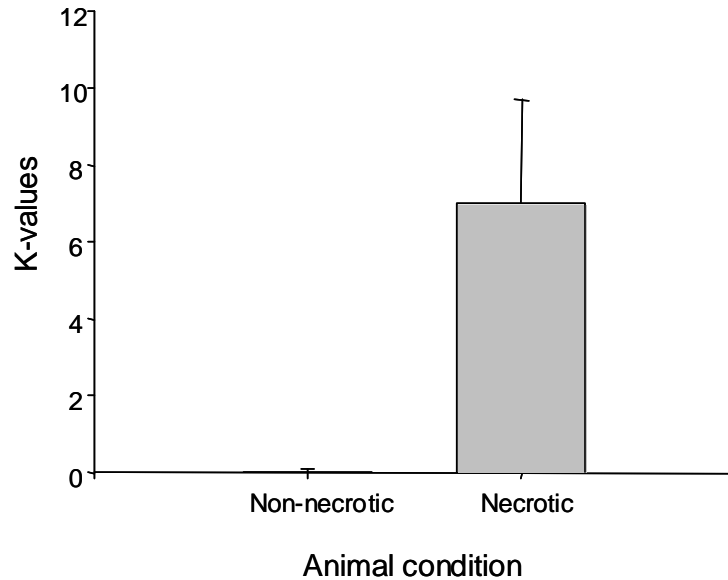
Figure 2.7 K-values measured in *N. norvegicus* trawled and stored in running seawater for 24 h. Values are the mean ± S.E.M. of 10 different animals for each group.

These results indicate that necrotic animals are seriously compromised, and therefore further procedures should be conducted to evaluate the fitness of these animals if they are to be directed for live transport. On the other hand, if these necrotic animals are sold as whole fresh langoustines the fact that K-values and the nucleotide inter-conversions are much more advanced compared to non-necrotic animals could have an impact on the shelf life of the product.

The list of recommendations presented in Reports I and II of this Scottsih *Nephrops* Survey should be followed in detail in order to minimise the percentage of necrotic animals, since biochemical parameters indicate that these animals are much more stressed and potentially therefore will spoil at a more rapid rate.

WP3 Establishing the effectiveness of gas flushing for prolonging shelf-life

<u>Initial selection process to find the most suitable MAP combination –</u>

<u>PHASE I</u>

It has been known for a long time that modified atmosphere packing (MAP), together with chilling, can greatly influence and reduce the growth of microbial organisms in all foods, including seafood. In general the following rule applies: "increased carbon dioxide levels enhance the shelf life and quality of food by retarding microbial growth" (Stiles, 1991). However, exceptions to this general rule are known (Villemure et al., 1986). No information about acceptable MAP packing conditions for *N. norvegicus* has previously been generated, and no quality assessment methods of the sophistication and sensitivity of those developed in Phase I of the Scottish *Nephrops* Survey have previously been available. Therefore, in this phase of the project these methodologies have been brought together to determine the optimal gas mix for *N. norvegicus* for extending shelf life while retaining high quality.

In order to find the best suitable gas mix of CO_2, O_2 and N_2 for the MAP-packing of whole *N. norvegicus* a triangular approach was chosen using the gas mixes shown in Table 3.1. The mixes were chosen to encompass the whole triangular range (Figure 3.1).
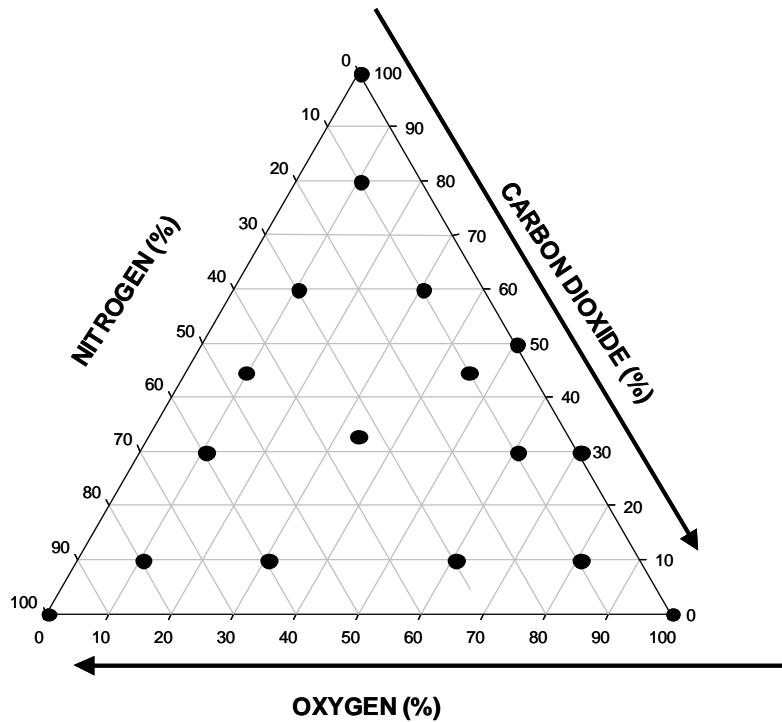
Table 3.1: Gas mixes used to identify a suitable MAP for the shelf life extension of whole Norway lobsters treated with Melacide® SC20.

Number	Gas Mix (O_2:CO_2:N_2)	Number	Gas Mix (O_2:CO_2:N_2)
1	100 : 0 : 0	9	10 : 10 : 80
2	0 : 100 : 0	10	10 : 80 : 10
3	33.5 : 33.5 : 33.5	11	60 : 30 : 10
4	30 : 10 : 60	12	60 : 10 : 30
5	30 : 60 : 10	13	80 : 10 : 10
6	10 : 30 : 60	14	70 : 30 : 0
7	10 : 60 : 30	15	50 : 50 : 0
8	45 : 45 : 10	16	10 : 45 : 45

Figure 3.1. Ternary plot of gas mixes used to identify a suitable MAP mix for shelf life extension on whole Norway lobsters dipped with Melacide® SC-20.

Methodology in Phase I

The method used in this phase to determine the effect of the gas mix on the shelf life of the fresh whole *N. norvegicus* utilised the following quality related parameters:

- Sensory assessment (QIM) (detailed information in WP5)

- Bacterial load using the rapid Compact Dry® TC plates

- Melanosis development using 'Melanosis Index Score' and in some cases analysing the PPO activity in the cephalothorax.

N. norvegicus were caught by otter trawl in the Clyde sea area with the research vessel 'Aplysia' from UMBSM. Once on board, animals were carefully washed and once in the Marine Biological Station at Millport animals were stored in a fish box with ice on top that was placed in a cold room set at 5-6 ºC. Temperature loggers (StowAway® Tidbit® from Onset) were placed every time an experiment was performed at the top, middle and bottom of the box and on a table near the fish box in the cold room. Temperature profiles obtained in a typical experiment are shown in Figure 3.2. Since it is common that lobsters do not arrive at a processor earlier than 24 h after landing, animals were stored in this condition until the following morning.
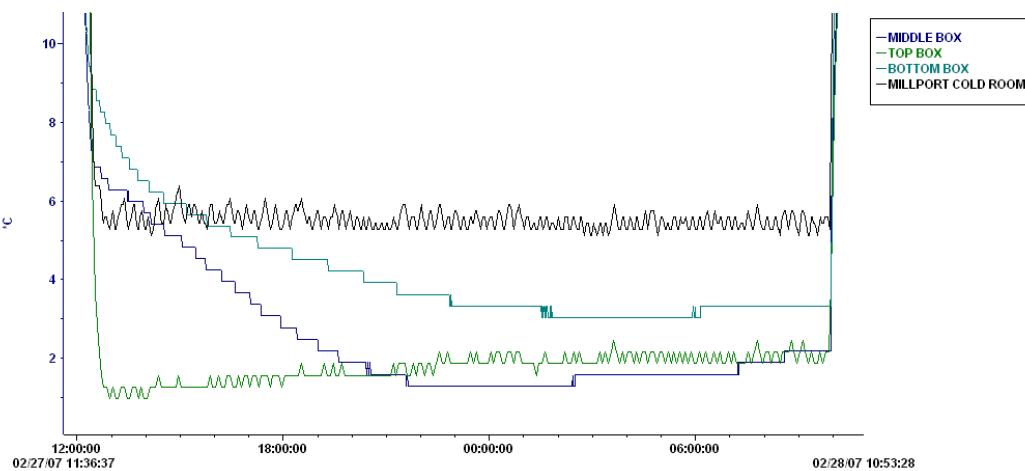

Figure 3.2. Temperature profiles in a fish box of Norway lobsters iced on top and stored in a cold room at Millport overnight for MAP experiments.

Next day, all animals were washed again with running seawater and treated with the commercially available anti-melanotic additive Melacide-SC20 at the concentration of 2 % (w/v) for 15 min. After dipping, animals were ready to pack and after being packed they were stored in the cold room at Millport (5-6 ºC).

To pack the animals, around 200 ± 20 g of whole *N. norvegicus* (usually 5-6 animals) were placed into Clearfresh® MAP packs (R15-45, 260 x 177 x 45 mm, from Linpak, UK) and packed using a PA1200 MAP vacuum packing machine (Packaging Automation Ltd.) (Figure 3.3). Gas mixes were delivered to the packing machine through a WITT 3800 gas mixer from three isolated gas cylinders containing food-grade N_2, O_2 and CO_2 (BOC). The CO_2 was pre-heated using a heated valve unit (BOC) to prevent icing up of the valve. The sealing film was Esteerpel PS2+ AF that had a transmission rate of 8 cm^3 O_2 per m^2 day $^{-1}$ and an anti-fog property. In all the experiments, 3 packs full of air were also prepared as controls.

Figure 3.3. Image showing the MAP machine with the gas cylinders used in the MAP trials.

The gas composition in the headspaces was analysed every 10 packs with a hand-held analyser fitted with a metal needle inlet (Oxybaby, WITT) in order to verify that the delivery of the gases was correct. The analyser can detect both O_2 and CO_2. A fine needle was punctured through the seal-film of a pack and a gas sub-sample was automatically drawn form the headspace of the pack. The machine measured the gas and the proportion was given as a percentage of the total volume analysed (Figure 3.4).
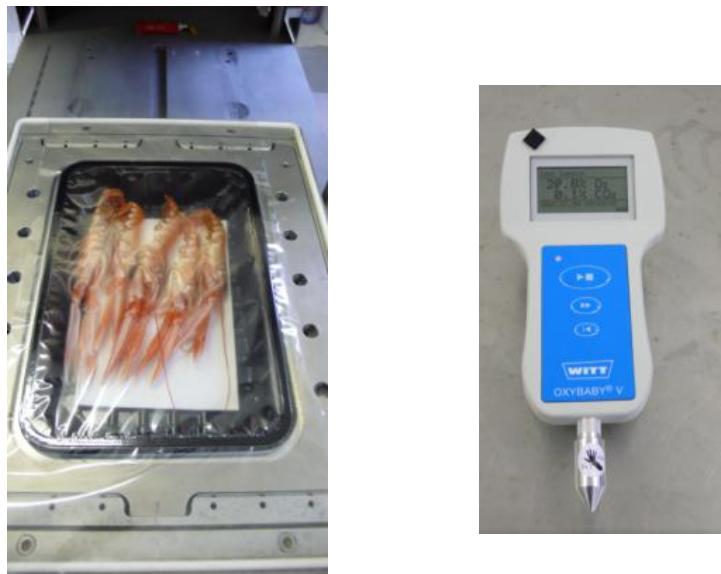

Figure 3.4. Images showing a MAP pack containing whole fresh *N. norvegicus* and the Oxybaby portable gas analyser used to measure gas composition within the MAP packs.

Once all packs were prepared, they were transported to the facilities at the University of Glasgow and stored at 3 ± 1 ºC for 5 days. On days 3 and 5 after packing, 3 packs per selected MAP and storage time were opened and a sensory assessment and a melanosis index score were conducted. At the same time, the remaining packs were transferred to 5-6 ºC and were sampled again on day 8 after packing. The gas content of each pack was measured prior to opening, using the Oxybaby (from WITT) to ensure that packs had not leaked during storage. Packs with leaks (composition similar to air) were discarded immediately.

QIM scores obtained using the different gas compositions in this first phase are shown in Figure 3.5. From these results mixes: 0:100; 30:60; 10:60; 10:80; 50:50 and 10:45 (O_2:CO_2) were identified as potential beneficial gas mixes as QIM scores were lower than those obtained in packs containing air.
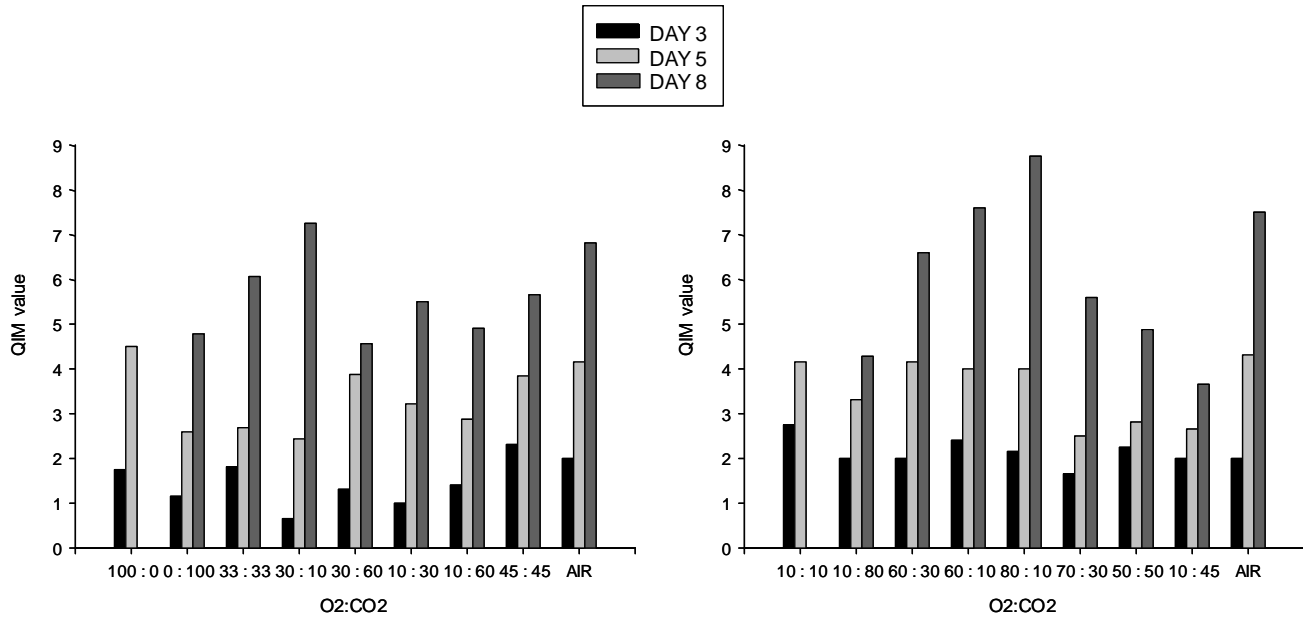

Figure 3.5. QIM scores obtained in whole *N. norvegicus* packed with different MAP gas compositions on days 3, 5 and 8 of storage. Values are the mean of 3 different packs for each condition and storage time; each one was evaluated by 3 different persons.

Furthermore, the melanosis index score was used to determine blackening development in the *N. norvegicus* using the different gas mixes, and results are shown in Table 2.2. According to these results it would appear that gas mixes with high CO_2 (above 45 %) are beneficial (Table 3.2).

Table 3.2: Melanosis development scored in whole *N. norvegicus* packed with different MAP gas compositions on day 8 of storage. (+++) melanosis very apparent, (++) melanosis started, (+) slight melanosis and (–) no melanosis. Signs represent the mean of 3 different packs for each condition.

Gas Mix (O_2:CO_2:N_2)	Melanosis	Gas Mix (O_2:CO_2:N_2)	Melanosis
100 : 0 : 0	+++	10 : 10 : 80	
0 : 100 : 0	-	10 : 80 : 10	-
33.5 : 33.5 : 33.5	++	60 : 30 : 10	+++
30 : 10 : 60	+++	60 : 10 : 30	+++
30 : 60 : 10	-	80 : 10 : 10	+++
10 : 30 : 60	++	70 : 30 : 0	++
10 : 60 : 30	-	50 : 50 : 0	+
45 : 45 : 10	+	10 : 45 : 45	+

Furthermore, in packs that had good sensory assessment scores and no melanosis total, the bacterial load was measured as total viable counts (TVC). As shown in Figure 3.6 bacterial load is significantly reduced in all the mixes that scored positively on sensory and melanosis score and in some cases a more than 2-fold log reduction was obtained on day 8 of storage compared to animals packed on air.
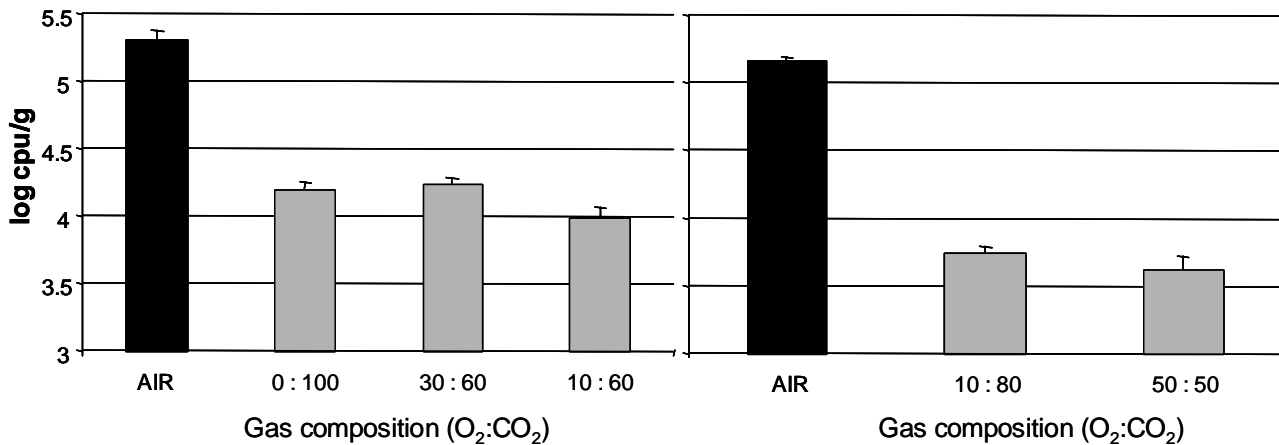

Figure 3.6 Total Viable Counts obtained in whole *N. norvegicus* packed with different MAP gas compositions on day 8 of storage. Values are the mean of 3 different packs for each condition and storage time.

By taking all these results together it would appear that the most effective gas mixes for shelf life extension in whole fresh *N. norvegicus* are composed by high CO_2 levels (at least 50 %) and low O_2 levels (less than 50 %) (Figure 3.7).
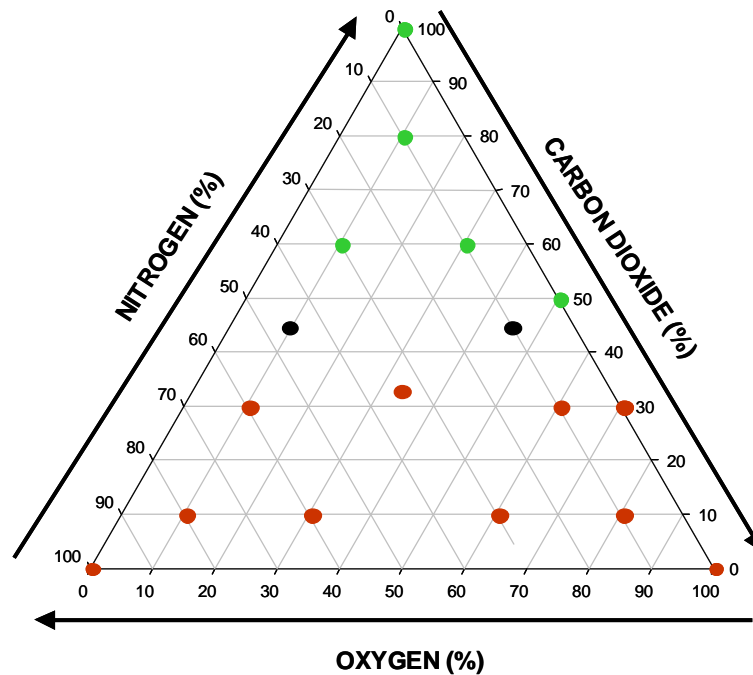
Figure 3.7 Ternary plot of gas mixes used to identify a suitable MAP mix for shelf life extension on whole Norway lobsters dipped with Melacide® SC-20. In red mixes that did not score well in at least one of the parameters measured, in green mixes that are promising and in black MAP mixes with mixed results.

Pack collapse

Another observation taken from this phase of MAP experiments was 'pack collapse'. This is an effect already described in the bibliography and accounts for the negative pressure that appears when MAP packs contain high concentrations of CO_2. This gas is absorbed into the tissues, and therefore, CO_2 concentration in the packs decreases as time of storage increases (Figure 3.9). This loss of gas from the pack leads to a lower than atmospheric (i.e negative) pressure inside the pack, and produces a visual appearance of the sealing film being stretched inwards (Figure 3.8).. This stretching can cause pack collapse that gives distortions even in the tray itself. From our observations pack collapse was clearly observed in the packs that contained between 80-100 % of CO_2 while negative pressure in the packs was visually clear in packs that

contained between 60-80 % of CO_2. However, at this point of the study negative

pressure or pack collapse was not taken into consideration when evaluating a suitable

gas mix since this effect can be mitigated by various measures such as using stronger

tray packs, increasing the ratio of gas/weight of product or by re-packing the product

(these possibilities were checked at a later stage in the project).

Figure 3.8. Pack of 200 g of whole *N. norvegicus* packed on a gas mix of 10:80 ($CO_2:O_2$) and stored at 2-3 ºC for 5 days. Note the negative pressure in the pack.
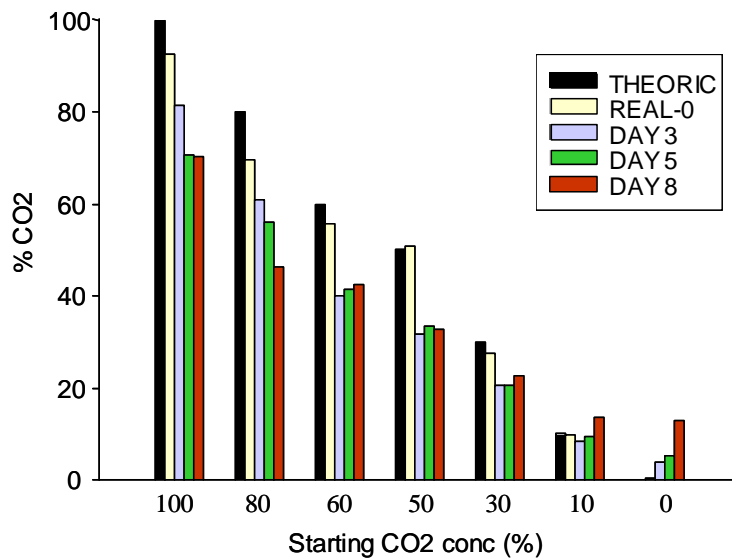
Figure 3.9. CO_2 concentration in the MAP packs containing 200 ± 20 g of whole *N. norvegicus* packed on different concentrations of CO_2 and stored for up to 8 days. Values are the mean of the CO_2 concentration in 3 different packs for each condition and time of storage.

Optimal time for dipping whole animals before being packed in MAP

At this point and before going further it was also important to determine the best time
for dipping the animals. Young's Seafood Ltd. had already stated their intention to use
Melacide-SC20 as the anti-melanotic product for MAP fresh whole langoustines, and
therefore experiments were carried out using this compound. In the following
experiment the most effective time for dipping with Melacide-SC20 was tested. The
protocols were:

A) Dipped after catch + packed 24 h later

B) Dipped after catch + washed 24 h later + packed

C) Dipped after catch + washed 24 h later + dipped again + packed

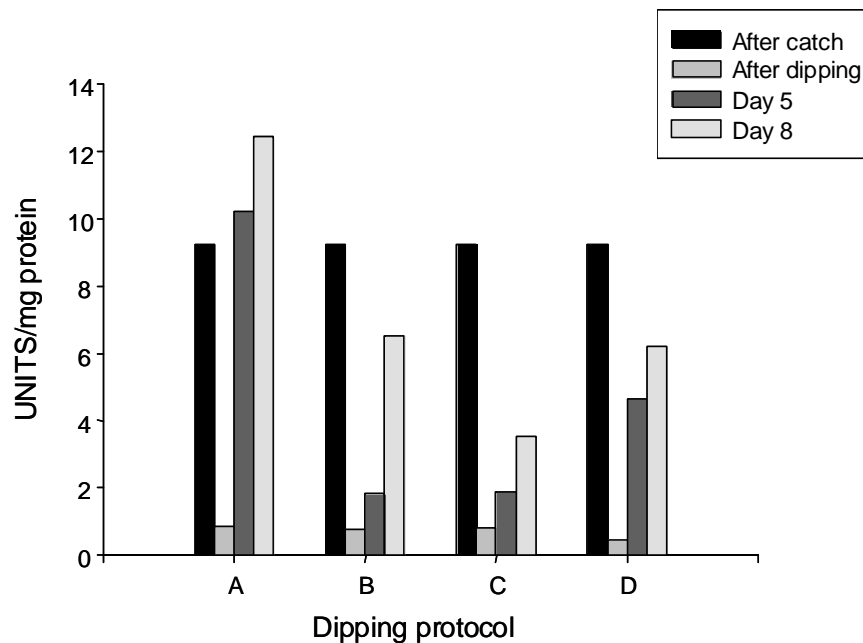D) Only washed after catch + washed 24 h later + dipped + packed

Figure 3.10. PPO activity in cephalothorax of whole *N. norvegicus* dipped with Melacide-
SC20 using different time protocols. (A) Dipped after catch + packed 24 h later (B) Dipped
after catch + washed 24 h later + Packed (C) Dipped after catch + washed 24 h later + dipped
again + packed (D) Only washed after catch + washed 24 h later + dipped + packed In red
mixes. Values represent the mean of 3 independent measures.

It would appear that the least effective combination would be to dip the animals after catch and pack them the next day without being extra-washed, while the best combination would be to dip the animals twice, once after catch and another time 24 h later just before packing (Figure 3.10). The problem with this last combination would be that it would require the use of double the Melacide-SC20 product and would also mean that the operation time required would increase. Similar results were obtained with dipping after catch + washing the animals at 24 h, just before packing. This option had already been used in previous MAP experiments where animals were kept on ice for 24 h + washed + dipped before being packed. Therefore, the last approach was used in the remainder of the MAP experiments, although it is necessary to emphasize that other timing protocols for dipping could be possible and would give similar results.

Further selective process to find the most suitable MAP combination–

PHASE II

According to the results from the first phase, the most suitable MAP gas combination would contain: more than 40-50 % of CO_2, less than 50 % of O_2 and the rest would be N_2 acting as a filling gas to make it up to 100 % (Figure 3.7). Therefore, another ternary plot was designed to cover this area of possible gas combinations (Figure 3.11).
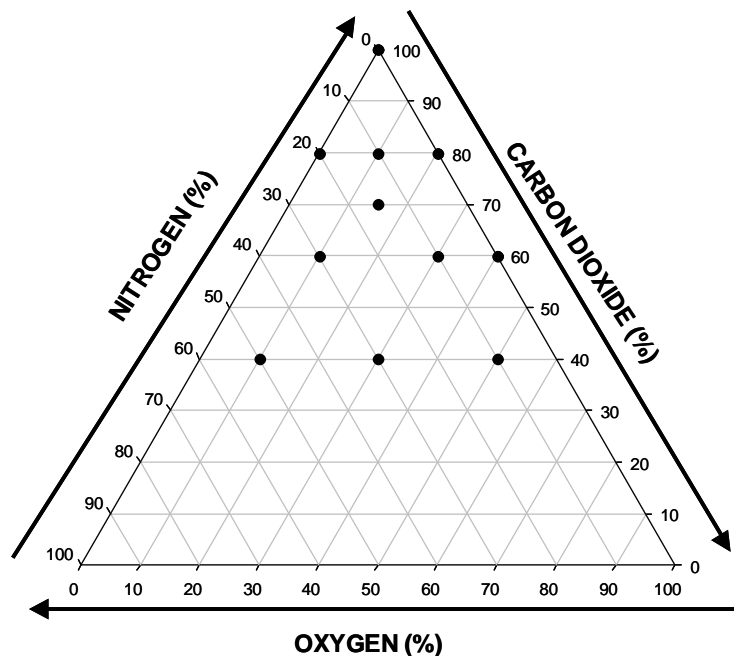

Figure 3.11 Ternary plot of gas mixes used in Phase II to identify a suitable MAP mix for shelf life extension on whole Norway lobsters dipped with Melacide® SC-20. A more comprehensive analysis was performed in order to gain more information on the effect of these mixes to the quality of the Norway lobsters.

Methodology

In order to gain a further insight into the action of these possible gas combinations the following quality related parameters were measured:

-      Sensory assessment (QIM)

- Bacterial load more into detail using the traditional method for TVC determination

- K-value used as freshness indicator based on the ATP and its breakdown products

- Biogenic amines: Tyramine, putrescine, cadaverine, histamine, agmatine, spermidine and tryptamine.

The methodology used to catch, store and dip the animals was exactly the same used in the first phase. In summary, *N. norvegicus* were caught by otter trawl in the Clyde sea area with the research vessel 'Aplysia' from UMBSM. Once on board, animals were carefully washed and once in the Marine Biological Station at Millport animals were stored on ice in a fish box that was placed in a cold room set at 5-6 ℃. Next day, all animals were washed again with running seawater and treated with Melacide-SC20 at the concentration of 2 % for 15 min. After dipping, animals were ready to pack and they were stored afterwards in the cold room at Millport (5-6 ℃).

Once all packs were prepared, they were transported to the facilities at the University of Glasgow and stored at 3 ± 1 ℃ for 5 days. On days 3 and 5 after packing, 3 packs per selected MAP and storage time were opened. At the same time, the remaining packs were transferred to 5-6 ℃ and were sampled again on day 8 after packing Temperature profiles obtained with temperature loggers during storage time were recorded in each experiment (Figure 3.12). The gas content of the packs was measured prior to opening using the Oxybaby gas analyser , to ensure that packs had not leaked during storage.
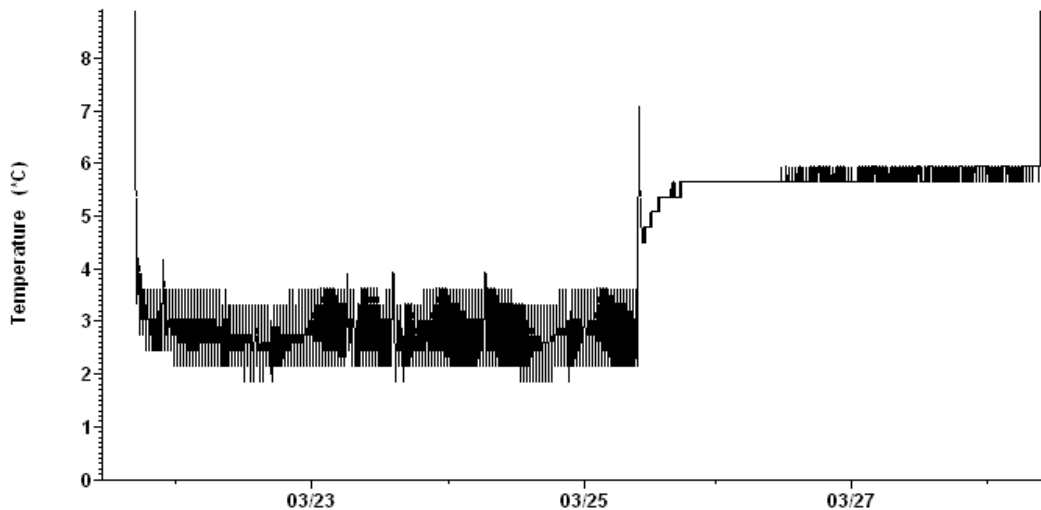
Figure 3.12. Temperature profiles in the packs stored for up to 8 days in the university of Glasgow for MAP experiments (phases I and II).

Sensory assessment results expressed as QIM values indicated that most of the gas mixes tested had a positive effect on the product, except the mix 50:40 (O_2:CO_2) which had a very similar QIM score than the packs stored on air (Figure 3.13).
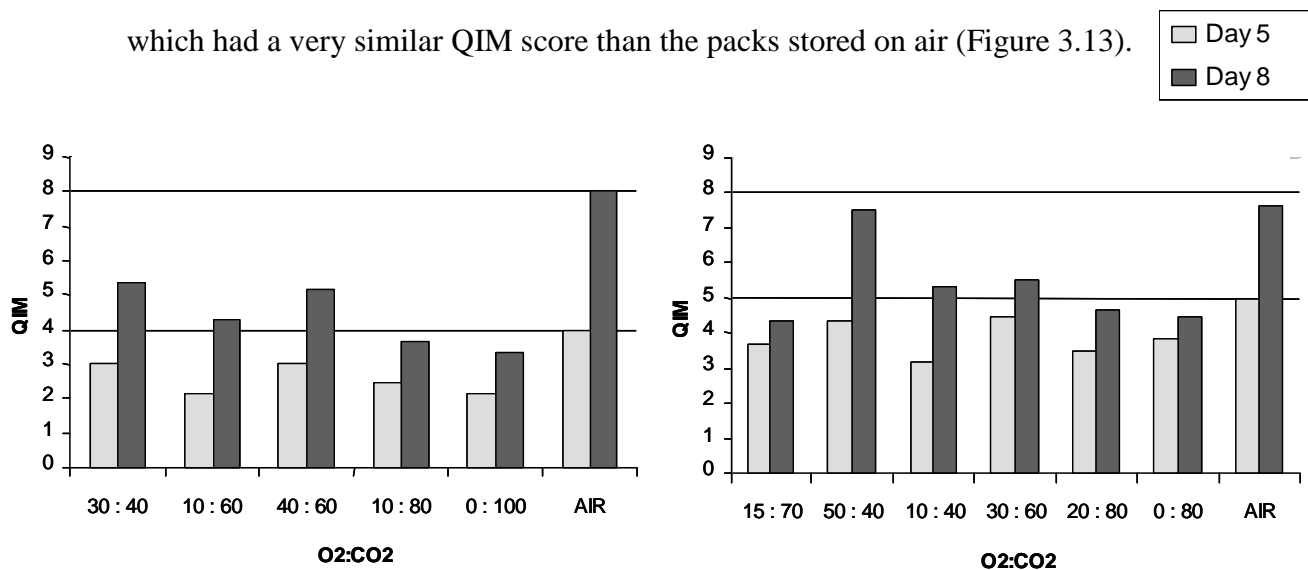

Figure 3.13 QIM scores obtained in whole Norway lobsters packed with different MAP gas compositions on days 5 and 8 of storage. Values are the mean of 3 different packs for each condition and storage time each one evaluated for 3 different persons.

However, when melanosis development was examined closely it was observed that some gas mixes had a negative impact on the appearance of the animals (higher melanosis, i.e. the mixes 30:40 and 50:40 (O_2:CO_2) (Table 3.3). In contrast mixes with low O_2 levels (between $0 - 15$ %) in conjunction with high concentrations of

CO_2 (between 60-100 %) had an inhibitory effect on melanosis development when compared to packs containing air. This effect would be very positive, as consumers perceive melanosis very negatively even if the product is still safe for consumption.

Table 3.3 Melanosis development scored in whole Norway lobsters packed with different MAP gas compositions on day 8 of storage. (+++) melanosis very apparent, (++) melanosis started, (+) slight melanosis and (–) no melanosis. Signs represent the mean of 3 different packs for each condition.

Gas Mix (O_2:CO_2:N_2)	Melanosis	Gas Mix (O_2:CO_2:N_2)	Melanosis
30 : 40 : 30	+++	15 : 70 : 15	-
10 : 60 : 30	-	50 : 40 : 10	+++
40 : 60 : 0	++	10 : 40 : 50	+
10 : 80 : 10	-	30 : 60 : 10	++
0 : 100 : 0	-	20 : 80 : 0	++
		0 : 80 : 20	-

In this series of experiments the total bacterial load was also analysed using not only the rapid Compact Dry® TC counting method but also the traditional marine agar plate method. Moreover, parameters of autolysis such as the K-value and the products mainly produced by spoilage bacteria (hypoxanthine and biogenic amines) were also measured.

Using marine iron agar plates (MIA plates) it was possible to quantify:

- Total bacterial load or TVC

- H_2S producing bacteria

- Luminous bacteria

Furthermore, as detailed in the Material and Methods section *Pseudomonas* sp. were also determined by CFC plates.

By taking in consideration bacteria numbers obtained using both methods it appeared that some gas mixes were more beneficial than others (Figures 3.14 and 3.15). On day 5, the most effective gas mixes in reducing TVC were 10:80, 0:100 and 15:70.

However, when looking at TVC on day 8 of storage after the storage temperature had increased from 3-4 ℃ to 5-6 ℃ the best gas mix was 10:80. A reduction of almost 2 $\log_{10}$ units was obtained using this gas mix compared to air. This 2 x $\log_{10}$ decrease on day 8 would mean than if in packs on air the bacterial load is 10 million bacteria/g then the in 10:80 (O_2:CO_2) mix the bacterial load would be 100 thousand bacteria/g. This reduction is quite significant and if spoilage bacteria are affected then this reduction could mediate a significant shelf life extension.
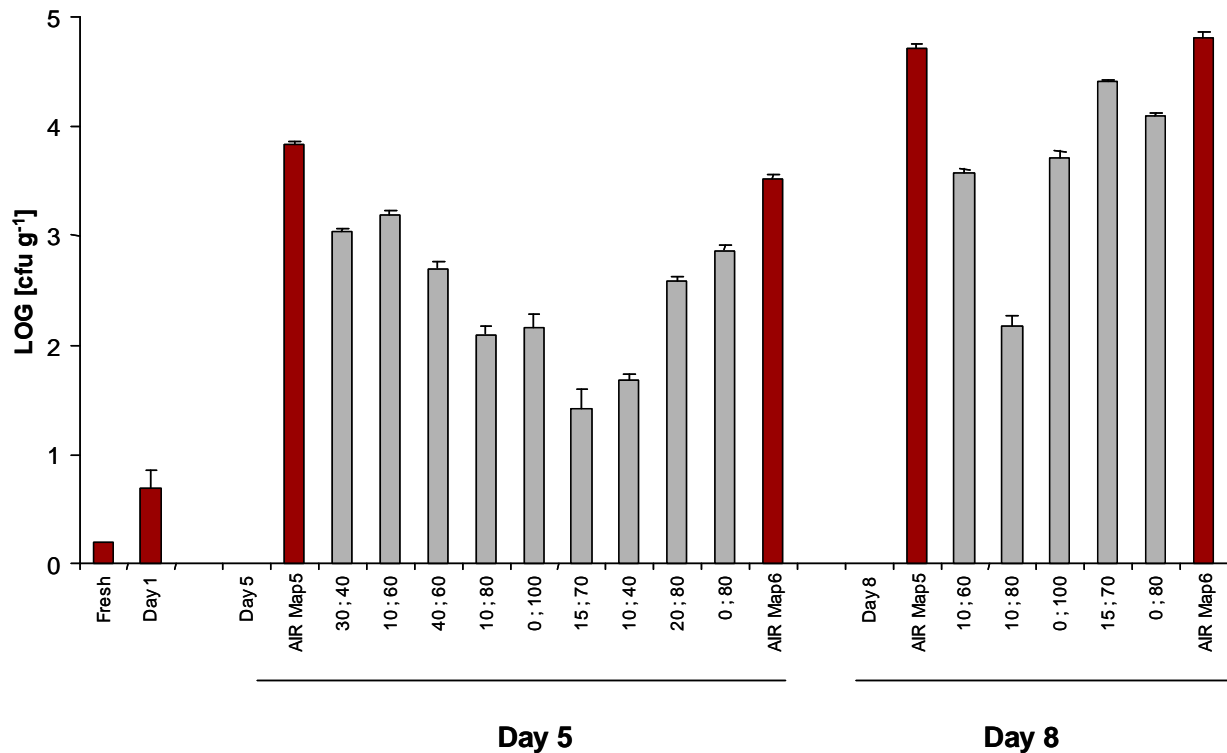

Figure 3.14. Total Viable Counts using the rapid rapid Compact Dry® TC plates obtained in whole Norway lobsters packed with different MAP gas compositions on day 5 and 8 of storage. Values are the mean of 3 different packs for each condition and storage time.

From the type analysis of bacteria present in the different packs some observations can be drawn (Figure 3.15). Firstly, MAP gas combinations had a very negative impact in the growth of *Pseudomonas* sp. This seemed to be the case for all the gas combinations tested in this phase, except for those combinations in which no O_2 was added. So it would appear that this MAP gas combination has an inhibitory effect on

Pseudomonas by either directly affecting the growth of this genera of bacteria or by promoting the growth of other bacteria types that prevent *Pseudomonas* sp. from growing. However, in totally anoxic conditions *Pseudomonas* sp. overcomes this inhibitory effect. Therefore, even if it were at very low levels, it would seem advantageous to have some O_2 in the mix to avoid the growth of *Pseudomonas* sp. and other anaerobic growing bacteria.

The other groups of bacteria studied (luminous and H_2S producing bacteria) seemed to follow the trends observed in TVC according to the different gas mixes. Effective mixes showed a reduction in these two types of bacteria. However, since this is a general approach to determining bacteria composition, a more exhaustive study regarding the possible changes in bacteria types due to changes in gas composition was carried out using molecular techniques and results will be shown later in this report.
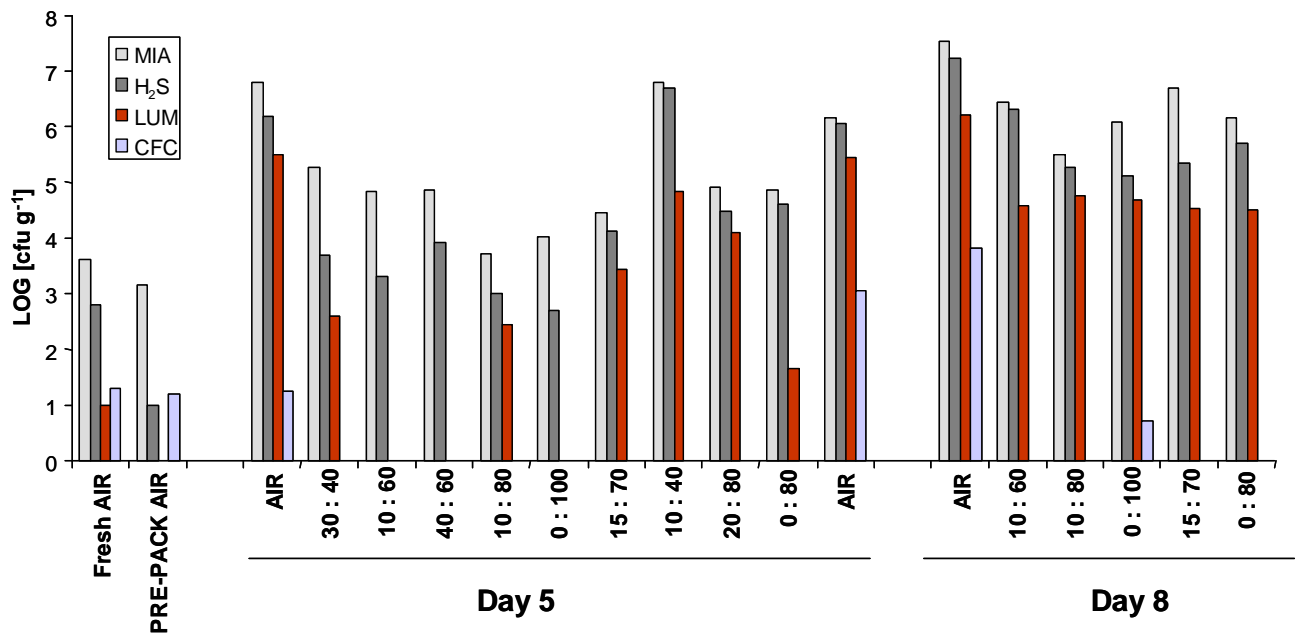

Figure 3.15. Total Viable Counts, H_2S producing and luminous bacteria using the traditional MIA plates and *Pseudomonas* sp. using CFC plates obtained in whole Norway lobsters packed with different MAP gas compositions on day 5 and 8 of storage. Values are the mean of 3 different packs for each condition and storage time.

A very successful methodology used in Phase I of the Scottsih *Nephrops* Survey was the determination of the K-value as a freshness indicator. As shown there, the K-value increases with storage time and reflects any temperature abuses to the product. However, many authors have found the K-value not to be a suitable measure to assess the freshness of a product under MAP conditions. This fact is because MAP does not necessarily affect the activity of autolytic enzymes, in contrast to a change in temperature that will definitively affect the activity of such enzymes. However, as well as K-values the hypoxanthine concentrations were also separately determined. This compound (the last product in the inter-conversion of ATP) is produced mainly by spoiling bacteria and therefore reflects changes in bacterial load. As shown in Figure 3.16 K-values were similar in air and MAP packs, although a reduction was obtained in the gas mix 10:80 (O_2:CO_2) on day 8 of storage.
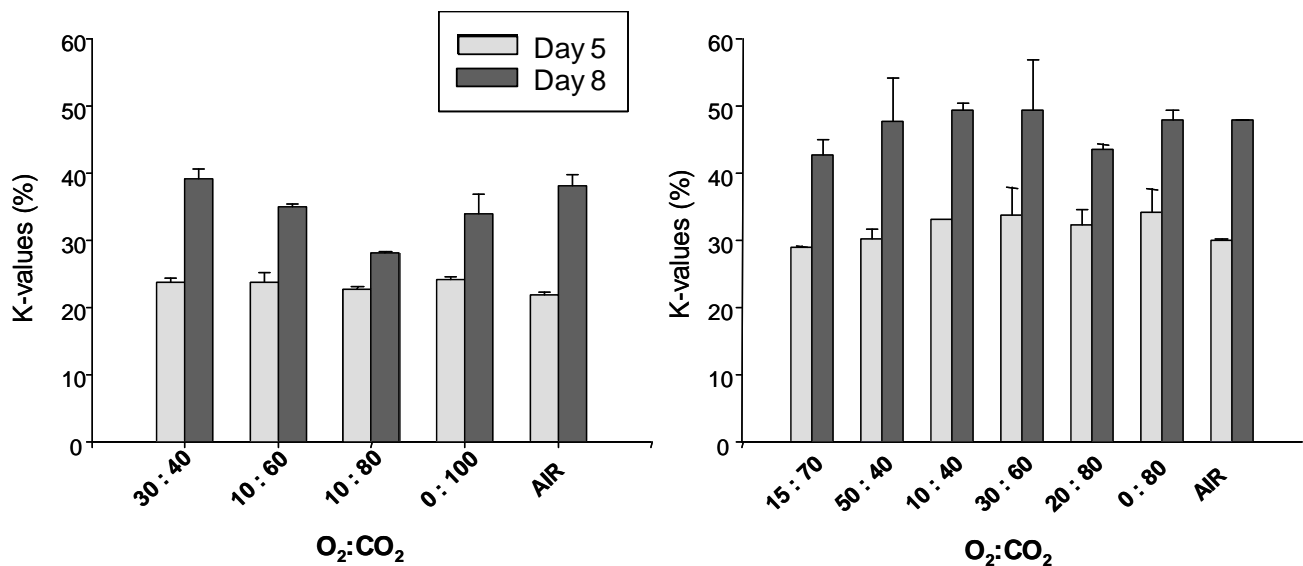

Figure 3.16 K-values (%) in whole *N.norvegicus* packed with different MAP gas compositions on day 5 and 8 of storage. Values are the mean ± S.E.M of 3 different packs for each condition and storage time.

Correspondingly, the hypoxanthine concentration in tail meat in this 10:80 (O_2:CO_2) gas mix was significantly lower than in the air packs (Figure 3.17). Lower

concentrations of hypoxanthine were also observed in the gas mix 0:100 (O_2:CO_2) although more variability was detected.
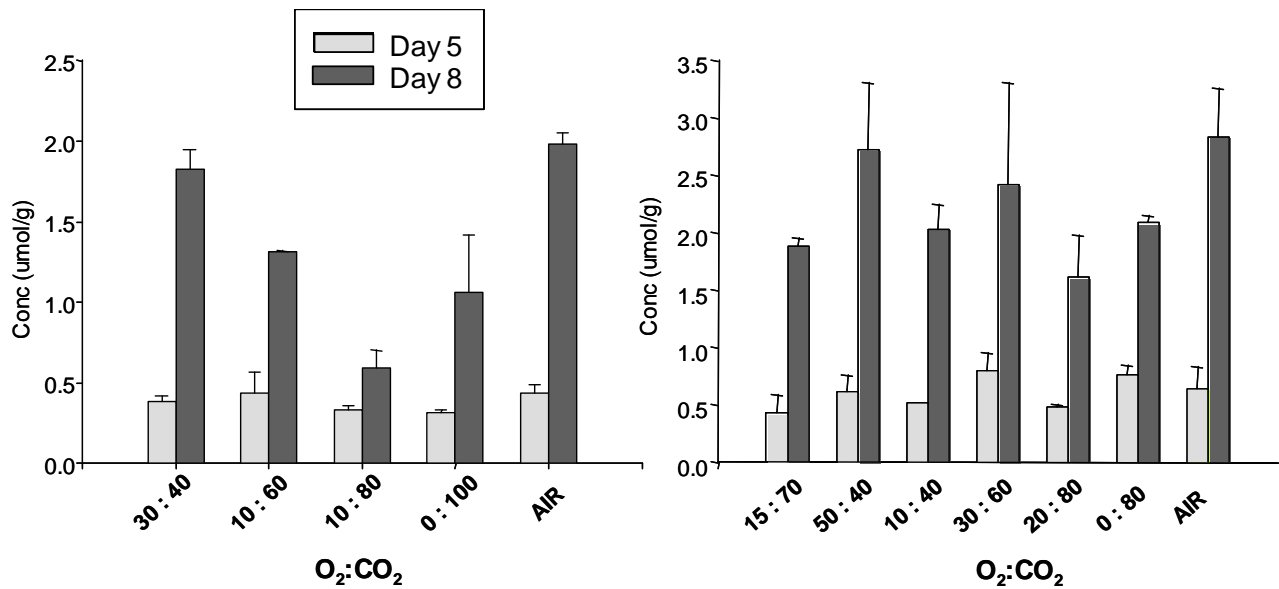

Figure 3.17 Hypoxanthine concentrations (µmol/g) in whole Norway lobsters packed with different MAP gas compositions on day 5 and 8 of storage. Values are the mean ± S.E.M of 3 different packs for each condition and storage time.

Finally, results on biogenic amines pointed in the same direction. These compounds are produced by bacteria and have various toxicological implications. Biogenic amines are found in fresh seafood products at very low levels and therefore their presence is directly associated with spoilage. Biogenic amines were measured on days 5 and 8. However, only on day 8 were significant amounts of certain biogenic amines found (Table 3.4). The main biogenic amines present in *N. norvegicus* meat were agmatine followed by putrescine. Other important biogenic amines such as histamine were not detected, or detected at very low levels (maximum contents for histamine in fish and fish products by the European Community have been fixed at 100 mg histamine/Kg of fish and fish products). The most effective gas mix in terms of biogenic amines inhibition was again 10:80 (O_2:CO_2).

Table 3.4: Biogenic amines concentrations (µg/mL) in whole Norway lobsters packed with different MAP gas compositions. Values are the mean ± S.E.M on three independent measures per condition

	30 : 40	10 : 60	10 : 80	0 : 100	Air
Tyramine	1.73 ± 1.73	2.05 ± 2.05	0.04 ± 0.04	1.23 ± 1.23	0.93 ± 0.17
Purescine	21.81 ± 10.04	15.07 ± 12.30	n.d	n.d	22.74 ± 10.20
Cadaverine	1.59 ± 0.74	0.40 ± 0.40	1.17 ± 0.36	2.58 ± 1.55	1.30 ± 0.88
Histamine	n.d	n.d	0.19 0.19	0.63 0.4	n.d
Agmatine	96.25 ± 1.55	66.15 ± 21.40	18.28 ± 9.00	66.43 ± 46.71	63.09 ± 2.27
Spermidine	2.31 ± 0.01	1.23 ± 0.76	0.65 ± 0.02	0.32 ± 0.13	1.63 ± 0.88
Tryptamine	n.d	n.d	0.23 ± 0.13	0.33 ± 0.08	1.24 ± 0.89

The conclusions from these first two phases to find the most suitable MAP gas combination can be summarised as follows:

- MAP is capable of increasing shelf-life in fresh whole langoustines

- High O_2 levels facilitate melanosis on-set

- Gas mixes including high CO_2 levels together with low O_2 levels were the most effective gas mixes

- The use of QIM determination and other simple measures can help to define the most suitable conditions, but they have to be supplemented with more detailed analysis (bacterial load, K-value, hypoxanthine and biogenic amine concentrations)

- From all the tests used in the present work it would appear that the best and most effective gas mix to provide shelf-life extension in whole fresh langoustines would be 10:80:10 (O_2:CO_2:N_2).

Confirmation on the precise composition of the optimal MAP mix around 10:80 (O_2:CO_2)

A further experiment was conducted in order to elucidate how precisely the optimal gas mix of 10:80 (O_2:CO_2) needed to be. To this end, the following MAP gas mixes (O_2:CO_2) were tested: 10:80; 5:80 and 15:80. As in the other experiments, sensory assessment (QIM) and bacterial load using the traditional marine agar method were used to evaluate the effect of the different gas mixes.

As shown in Figure 3.18 the sensory properties were very similar between the different MAP gas mixes. However, on day 5 a slightly lower QIM score was obtained in packs on 15 : 80 suggesting that the lower the O_2 the better for the visual appearance of the animals. However, this difference disappeared by day 8, when all the packs scores were very similar.
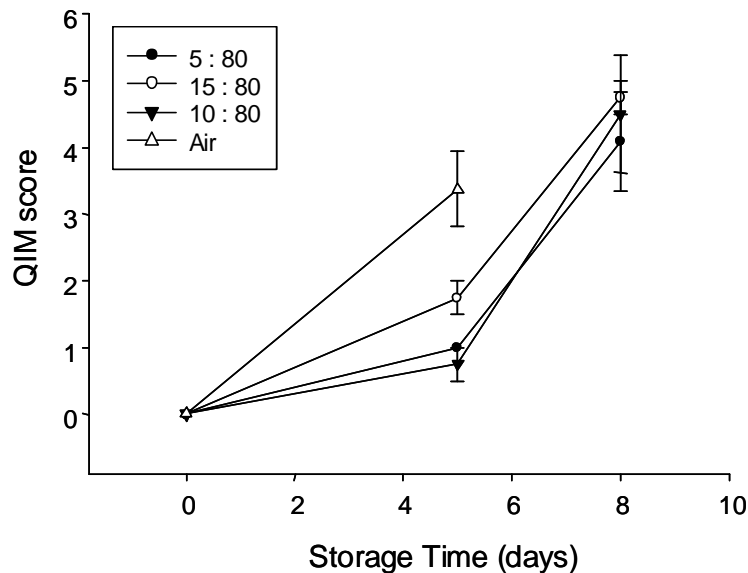

Figure 3.18 QIM values in whole *N. norvegicus* packed with different MAP gas compositions around the 10:80 (O_2:CO_2) mix on day 0, 5 and 8 of storage. Values are the mean ± S.E.M of 4 different packs for each condition and storage time each one evaluated for 3 different persons.

Total bacterial load was measured on day 5 of storage (Figure 3.19). All MAP combinations gave a reduction of total bacteria counts of approximately 2 log units. No differences were found by changing the concentration of O_2 between 5% and 15 % if the CO_2 was kept at 80 %. As in previous experiments, *Pseudomonas* sp. was totally inhibited at all the different MAP combinations used, while more than 10,000 cfu/g of tissue of *Pseudomonas* sp. were found in air packs stored for 5 days. The H_2S producers and luminous bacteria were lower in all MAP packs compared to air packs. However, small decreases in these types of bacteria was found in the mix 10:80 compared to the other MAP combinations, indicating the suitability of this gas mix for the shelf life extension of fresh whole Norway lobsters.
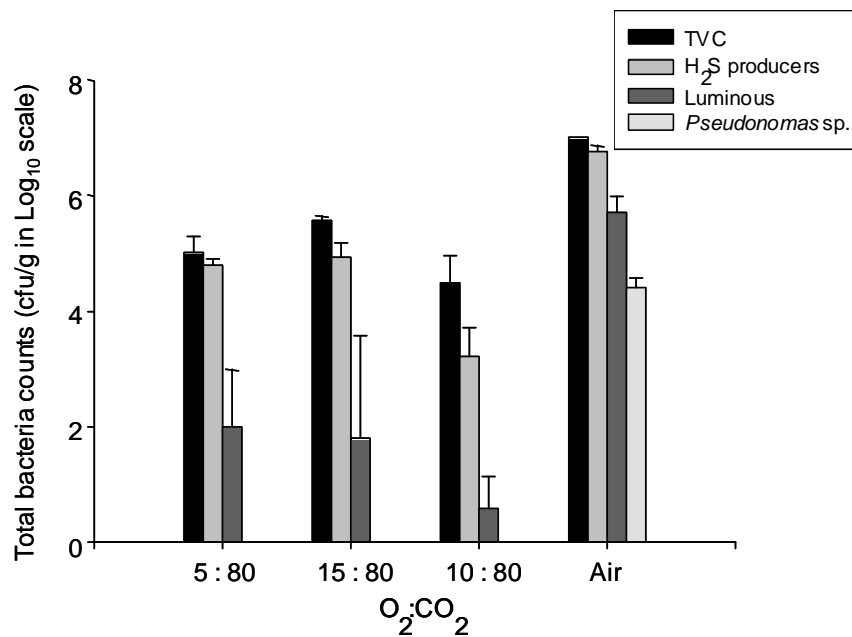

Figure 3.19 Total Viable Counts, H_2S producing and luminous bacteria using the traditional MIA plates and *Pseudomonas* sp. using CFC plates in whole *N. norvegicus* packed with different MAP gas compositions around the 10:80 (O_2:CO_2) mix on day 5 of storage. Values are the mean ± S.E.M of 4 different packs for each condition and.

Therefore, it was concluded that the MAP gas combination of 10:80:10 (O_2:CO_2:N_2) was the most suitable to obtain shelf-life extension in fresh whole *N. norvegicus,* and in the next phase of this WP the potential of this MAP gas mix in different conditions

was evaluated in order to gain a deeper understanding of its full potential. This MAP

mix will hereafter be referred to as the OGM (Optimal Gas Mix).

Evaluations on the potential of the OGM 10:80 (O_2:CO_2) for shelf-extension of whole *N. norvegicus* – PHASE III

Whereas in the experiments leading towards the identification of the OGM a 2-step temperature regime was used, in the following experiments more complex temperature regimes were followed. According to the trial involved, either a 1-step and a 3-step regime was used. The 1-step regime was used as a positive control, where packs were held at low temperatures (around 1 ℃) for the whole period of storage. The 3-step regime was suggested by Young's Seafood Ltd. in order to closely match the temperatures in a commercial retail chain from processor to market (supermarket or fishmonger) and then to the costumer. After an initial storage at 1-2 ℃ for 3 days (simulating condition in the chill store of the processor) the temperature was stepped up to 3-4 ℃ for an additional 2 days (simulating the temperature in the chilled display cabinet of a retailer) and then up to 5-6 ℃ for the remaining time (simulating the temperature in a domestic refrigerator). During every trial of either regime, temperature profiles were recorded with temperature loggers that accompanied the packs.

The effects of the two different temperatures regimes on the overall performance of the optimal MAP mix were tested. Prior to further analysis, a sensory analysis (based on the new QIM developed and modified as explained in WP5 of this report) was carried out for each pack. Additionally, microbiological analysis was performed, and samples were also kept for the determination of the K-value, the TMA concentrations and the biogenic amine levels. Samples were also stored at different time points and an organoleptic evaluation was performed by a professional sensory panel trained for

evaluating *N. norvegicus* (at the Food Innovation Institute, Edinburgh). Only the key results for each experiment will be given here, but the full reports of all the sensory evaluations are provided in the Annexe section to this report

Furthermore, for the packs that were held at a constant low temperature, the changes in microflora types were analysed using molecular techniques. For comparison, the microflora in the air packs used as controls were analysed likewise. Since temperature has a large effect on the performance of the MAP (Phillips, 1996), the microflora composition was analysed only in packs from the 1-step temperature regime. Generally, a lower storage temperature increases the effectiveness of MAP on the product and, if a change in the microflora has occurred, the changes would be expected to be more pronounced at lower temperatures.

Other conditions tested in this phase of this WP were:

- The effect of de-gutting the animals in conjunction with MAP, to further extend shelf-life,

- The difference between packing fresh and 1 day old product

- Changes in the step temperatures imposed, again in comparison to maintaining the product at low temperature for all the storage time.

Bacterial flora and bacteria dispersion in *N. norvegicus* tail meat: The effect of de-gutting on shelf-life extension

The bacteria flora in temperate water fish is dominated by psychotrophic Gram-positive, rod-shaped bacteria belonging to genera *Pseudomonas*, *Moraxella*,

Acinetobacter, *Shewanella*, *Flavobacterium*, *Vibrionacea*e (*Vibrio* and *Photobacterium*) and *Aeromonaceae* (Gram *et al*, 1996).

The Norway lobster (*Nephrops norvegicus*) is a non-migratory bottom-dwelling burrowing decapod crustacean. Being a temperate seawater animal, the bacteria flora might be expected to be similar to that of temperate fish, but their specific identity was not known until the work performed in Phase I of the Scottish *Nephrops* Survey . In this study the Specific Spoilage Organisms (SSOs) in *N. norvegicus* were isolated from the spoilt meat, and were identified as belonging to three main groups: *Vibro* species, *Photobacterium phosphorium*, and *Pseudoalterodomonas haloplanktonis*.

Like other seafood products, the microbiological complexity in *N. norvegicus* depends on various factors such as environmental habitat, growth condition, handling and processing practice etc. The initial bacteria contamination in the animal is, however, one of the potential factors that can greatly affect such contamination in the product. Understanding the dispersion of localized bacteria either in muscle or organs particularly, in the gut, can provide the basic knowledge for the further development of bacterial reduction methods in Norway lobster products.

Gram stain and colony morphology of bacteria flora in N. norvegicus

Seven different colonies from a MIA plate showed different morphologies under the light microscope (Fig 3.20). Six of them showed the gram-negative characteristic (red staining), whereas the cocci shaped bacteria of the remaining colony appeared slightly

orange (Figure 3.20 a-f). On the other hand, gram-positive bacteria showed the characteristic purple staining (Figure 3.20 g).
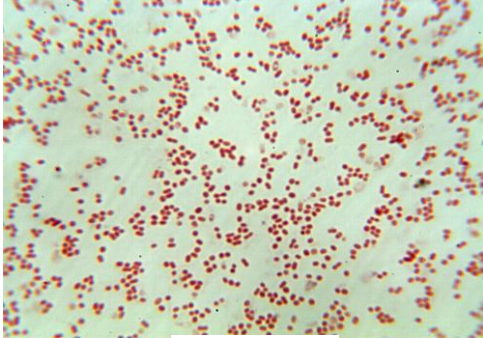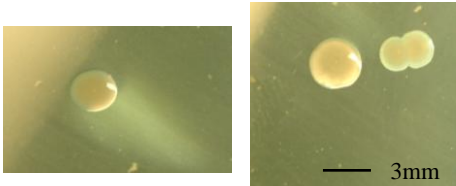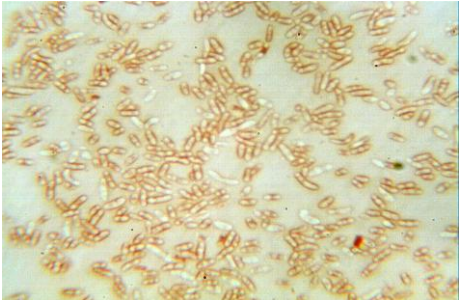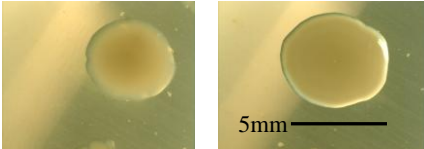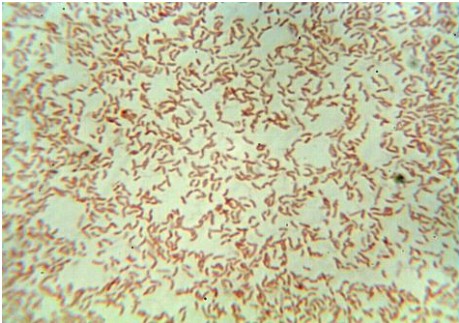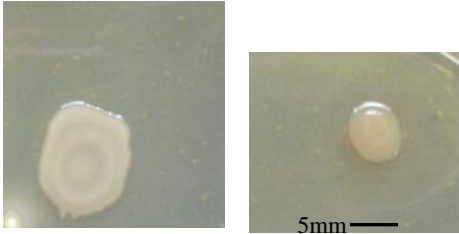
Gram stain morphology (100X)	Colony morphology
Fig 3.20.1a	3mm
Fig 3.20.1b	5mm
Fig 3.20.1c	5mm

Figure 3.20 Gram stain and colony morphology of *N. norvegicus* bacteria flora
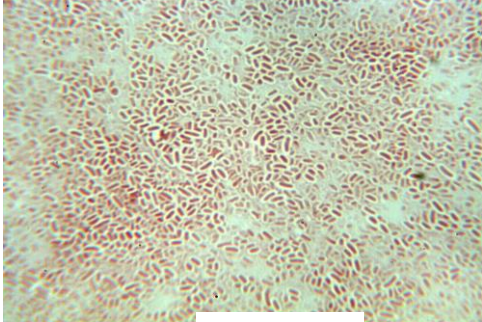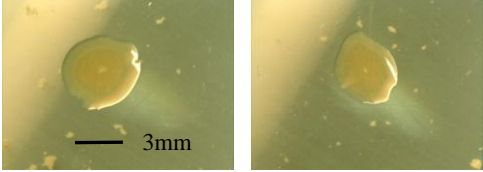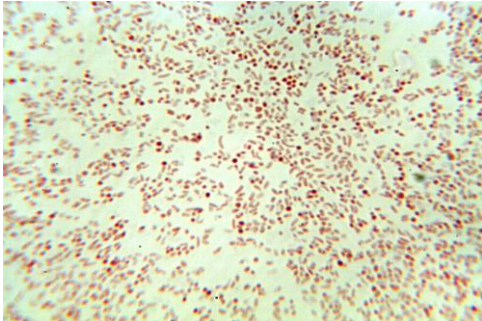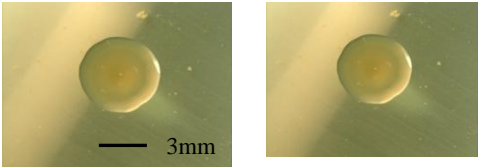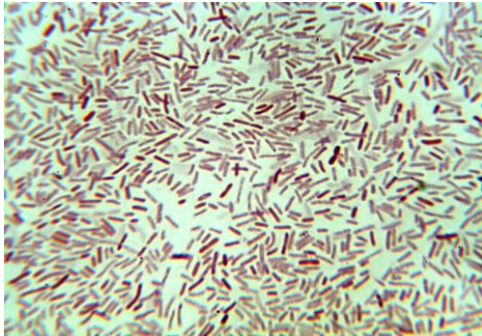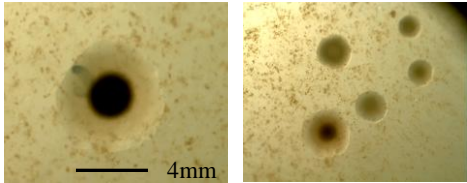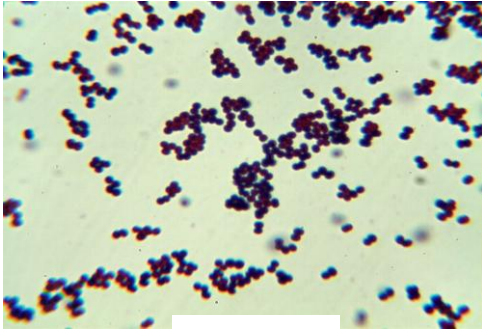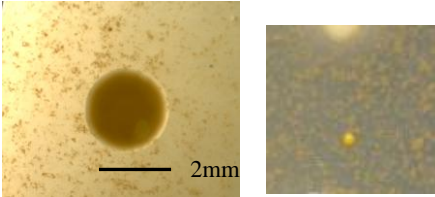
Gram stain morphology (100X)	Colony morphology
 Fig 3.20.1d	 3mm
 Fig 3.20.1e	 3mm
 Fig 3.20.1f	 4mm
 Fig 3.20.1g	 2mm

Figure 3.20 Gram stain and colony morphology of *Nephrops* bacteria floras

Cross-sections of the *N. norvegicus* tail (Fig. 3.21 and 3.22) show the tissue structure within the meat. The muscle appears as a red shade by Eosin staining, and the midgut (Yonge, 1924) lies in the midline relatively close to the dorsal part. Its brown shade probably derives from the unstained food remains. It is assumed that this undigested food in the midgut contain a high load of the normal microbial flora, and it is separated from the surrounding muscle by only a thin membrane.
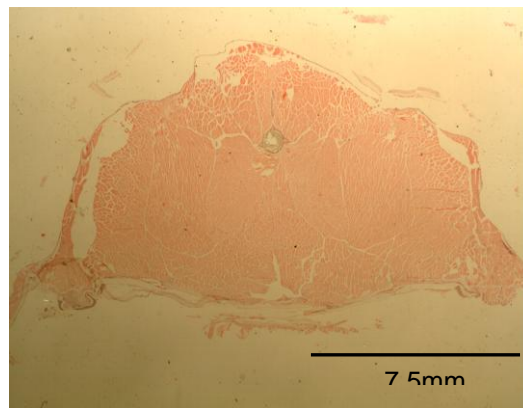
Figure 3.21 Whole cross section of *Nephrops* tail, Eosin stain
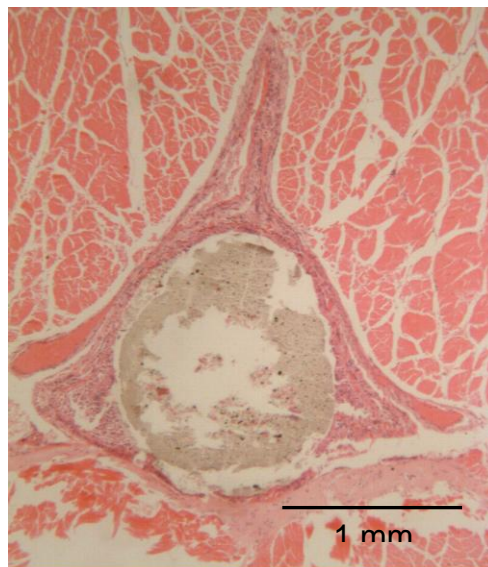
Figure 3.22 Gut section of *Nephrops* tail, Eosin stain

The determination of bacteria dispersion in the tail meat was conducted using a tissue gram stain, with aurantia as a counterstain. Bacteria will take up the stain and show a different colour according to the physiology of their cell wall. The positive result of tissue gram stain will be seen by the clump of bacteria, which are a purple shade as Gram-positive bacteria and a red shade as Gram-negative bacteria. Both colours are distinguished from the tissue by the yellowish shade shown by the background stain, aurantia.

In order to determine whether the bacteria load on later days of storage, or in spoiled samples, originally come mainly from the gut, animals that had been stored at 2-3 ℃ in air 8 day were examined (Figure 3.23). The gut area was stained strongly red for Gram-negative bacteria (the normal flora of fish and shellfish, Gram and Huss, 1996), whereas the muscle sector appeared not to show any signs of bacteria clumps.

At higher magnification of the gut sections (Figure 3.24) there are red strips caused by the presence of Gram-negative bacteria within the gut (arrows), but there in the muscle there is only pale yellow from the counterstain, with no evidence of bacteria. Although some bacteria tend to be rod-shape like, the shape of bacteria cannot be identified precisely in this section due to over-staining.

Therefore in this histological study there was no evidence that bacteria had migrated from the gut to the muscle during the storage period.
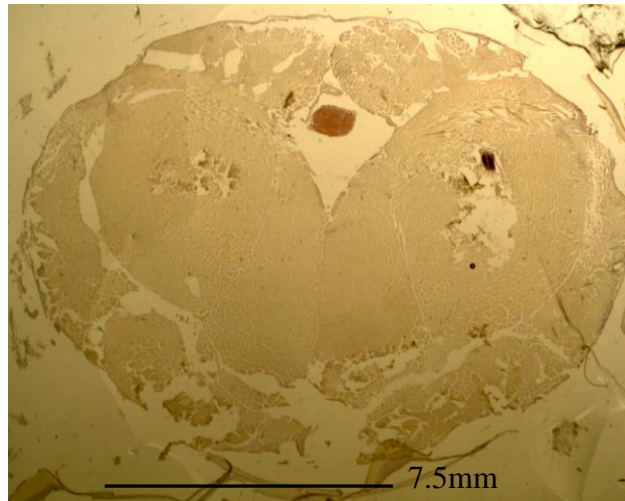
Figure 3.23 Whole section tissue Gram stain of *Nephrops* tail on Day 8
after storage at 2-3° C
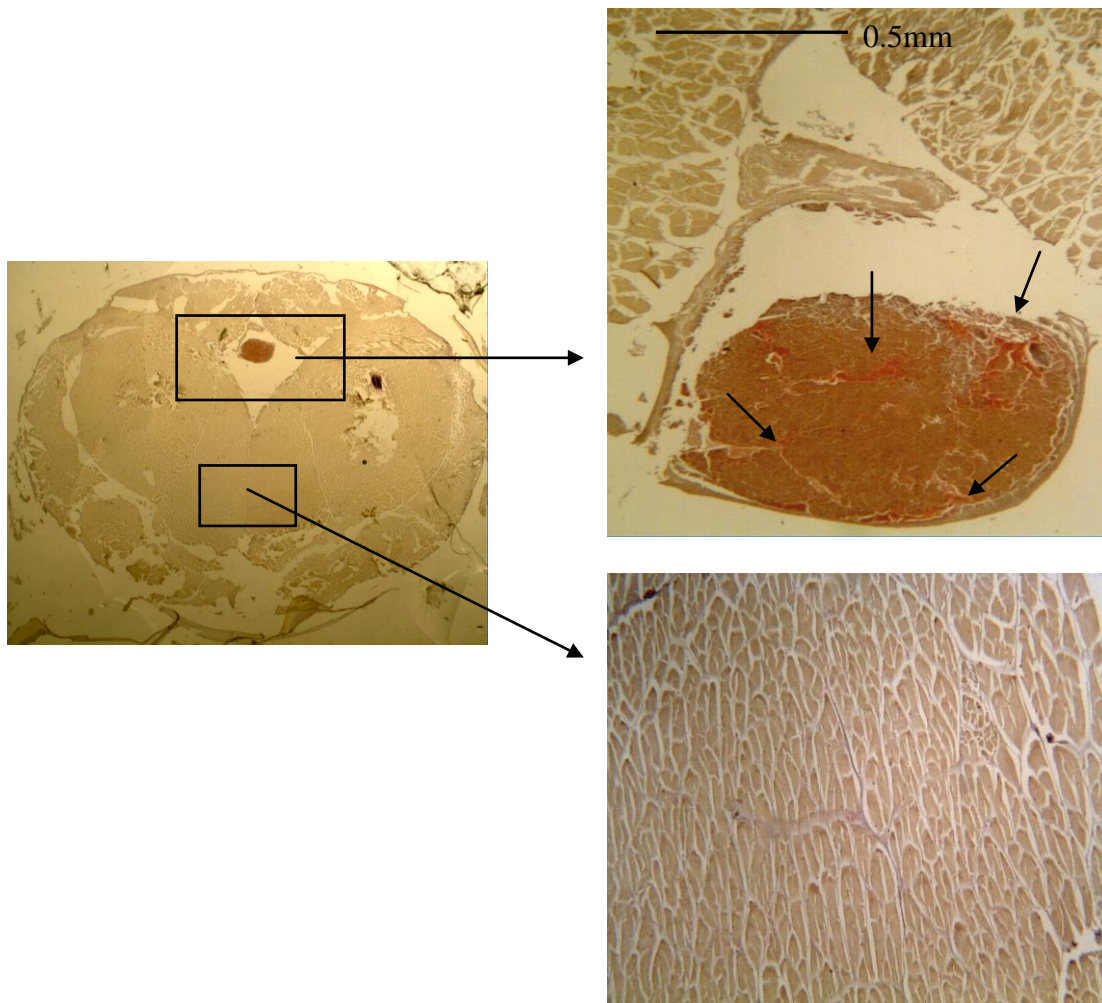
Figure 3.24 Gut tissue Gram stain of *Nephrops* tail on Day 8 after storage at 2-3° C
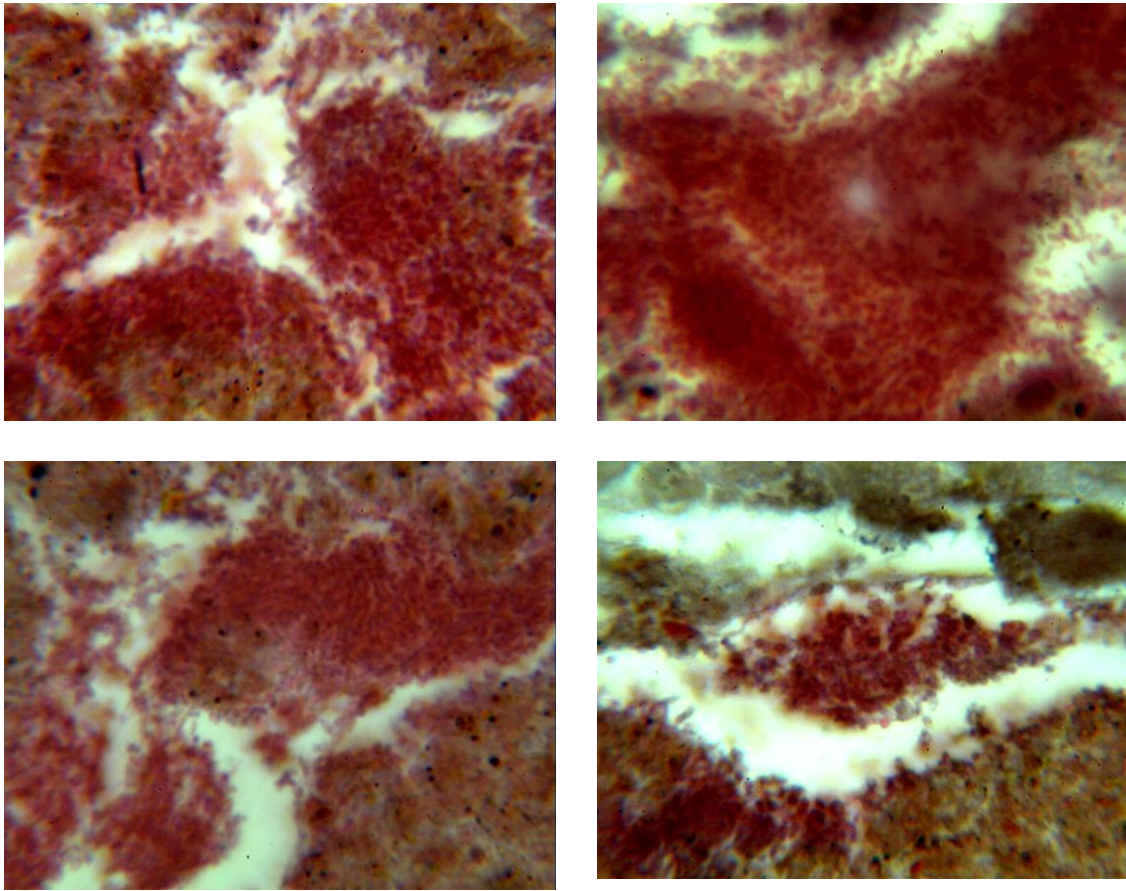
Figure 3.25 Bacteria clump in gut section under x100 magnification

The enumeration of bacteria in the tail meat during the storage period focused on three main SSO; *Vibro* species, *Photobacterium phosphorium*, and *Pseudoalterodomonas haloplanktonis*, Figure 3.26 shows that the total number of bacteria in the tail meat, including H_2S producers, luminescence bacteria and *Pseudomonas* sp., obviously increase after storage at 2-3 ^{o}C for 8 day. The increases in bacterial numbers differ for each type of bacteria. Thus for total bacteria there is a 10,000 fold increase (4 Log units), for H_2S producers there is a 100,000 fold increase (5 Log units), for luminescence bacteria it is 1,000,000 (6 Log units), and *Pseudomonas* spp. it is 1,000 (3 Log units).
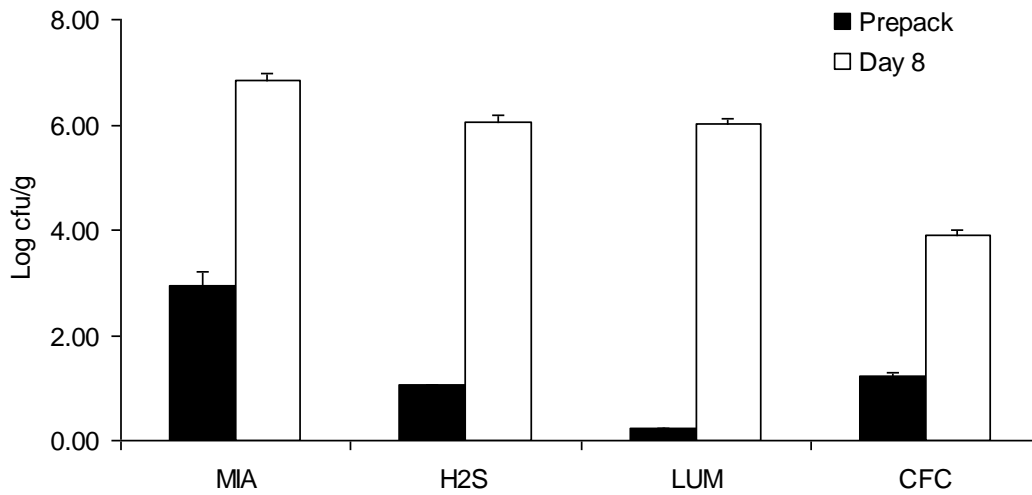
Figure 3.26 Bacteria count in *Nephrops* tail meat before packing and the 8th day after storage

The growth of each bacterial species during the storage period follows a common pattern (Fig. 3.27). The crucial period of bacteria growth occurs between days 1 to 5 of storage. A large number of total bacteria (10^7-10^8 cfu/g) was found on day 5 and was slightly higher on the day 8. The growth of H_2S producer and *Pseudomonas* spp. slightly decreased from the day 5 to the day 8.
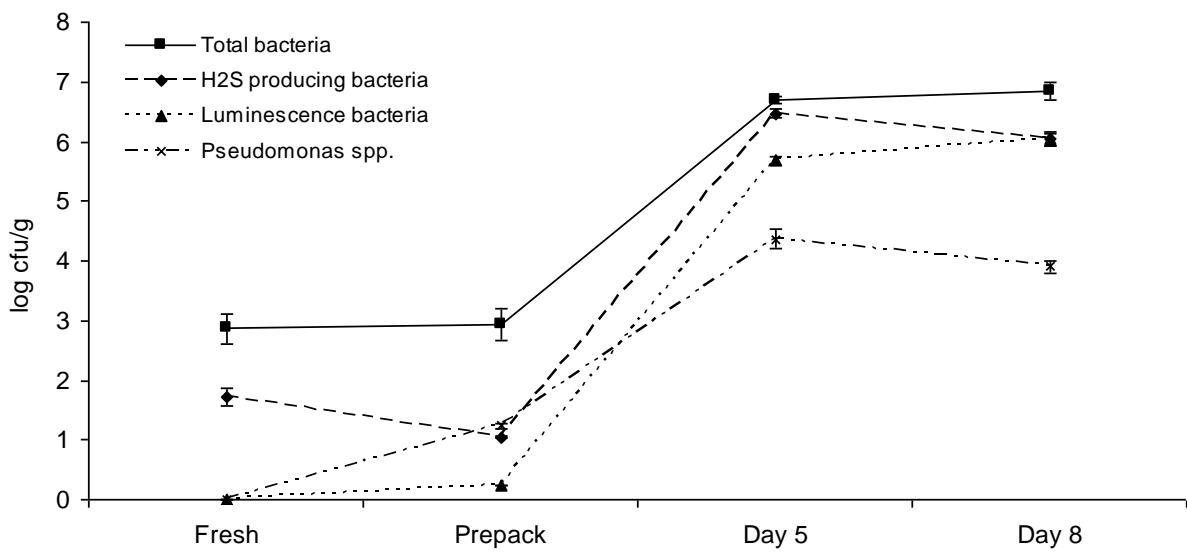
Figure 3.27 Bacterial growth during storage period under air at 2-3°C

Figures 3.28 and 3.29 show the difference between bacteria number in the gut and the tail muscle separately at different time intervals, immediately after catching and after storage for 8 day. The bacteria numbers in the muscle of fresh animal are much lower than those in the gut. The luminescence bacteria and *Pseudomonas* sp. are not detectable in fresh meat, but they both tend to increase in numbers in the meat during the storage period and reach 10^4-10^5 cfu/g at day 8. However, after storage to day 8, the bacteria numbers in the gut remain higher than those in muscle, except for *Pseudomonas* sp.
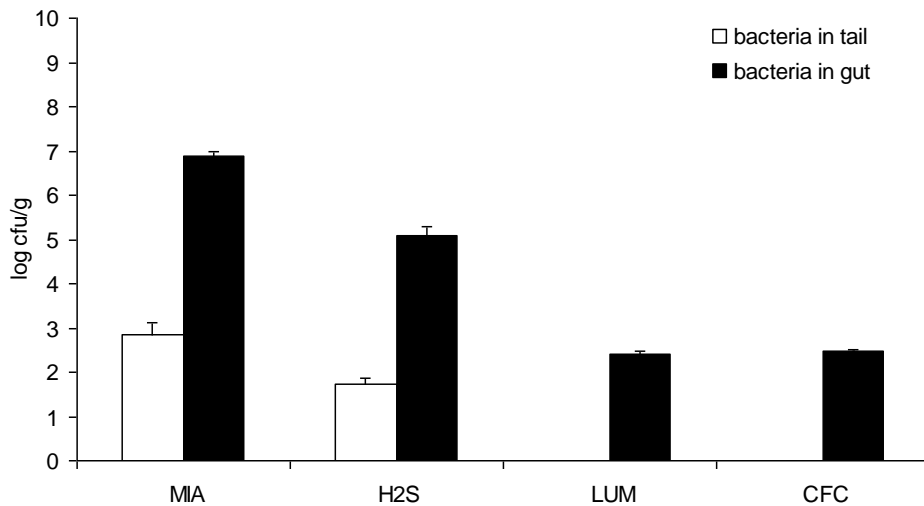
Figure 3.28 Bacteria count of fresh *Nephrops* in the gut and tail meat after catching
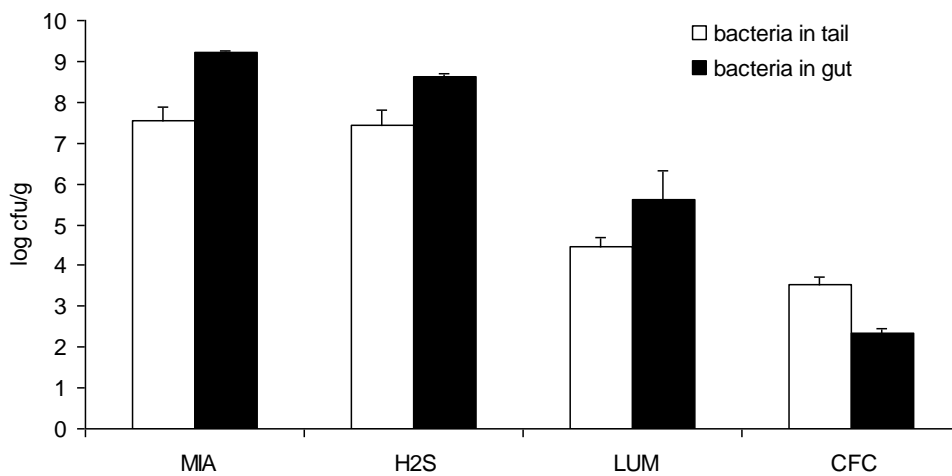
Figure 3.29 Bacteria count of *Nephrops* in gut and tail meat on the day 8 of storage at 2-3°C

Due to the absence of any increased density of bacteria in the tail meat around the gut, it must be assumed that the bacteria that grow during storage do not come mainly from the gut.

Luminescence bacteria grow at a dramatically high rate, and it can therefore be assumed that they represent the largest proportion of micro flora which grows during storage time in *N. norvegicus* tail meat stored under air. This rate of growth is followed by H_2S producer and *Pseudomonas* sp. respectively. Thus the group of *Nephrops* SSOs can be narrowed down to *Photobacterium phosphorium* and *Vibrio* species, because the *Photobacterium phosphorium* and some *Vibrio* species eg. *Vibrio logei* act as luminescence bacteria. *Vibrio* species produce the highest amount of H_2S compare with *Photobacterium* sp. and *Pseudoalteromona*s sp. during storage. Therefore, in the present study, *Vibrio* sp. which display luminescence can be assumed to be the majority of *Nephrops* SSO.

Bacterial examination from degutted sample under OGM MAP condition

Bacteria numbers in the tail meat of *N. norvegicus* that were degutted before packing under OGM MAP conditions have been compared with the bacteria number in the tail meat from non-degutted animal.

Certain trends apparently occur, as follows. On day 3 after packing, the total number of bacteria from the degutted animals is slightly higher than those in the intact animal. These numbers then remain until day 8, when they are approximately 10^5-10^6 cfu/g

(Fig 3.30). This trend also occurs in H$_2$S producing bacteria (Fig 3.31). The growth of luminescence bacteria and *Pseudomonas* sp. from degutted *N. norvegicus* appeared to be lower than the number from animals with intact gut on day 5 (Fig 3.32, Fig 3.33)., although the day 8 this difference is reversed.

However, statistical analysis, one-way ANOVA, shows that, in fact, there is no significant different between the bacterial number in degutted and non-degutted *N. norvegicus* tail meat (P=0.487).
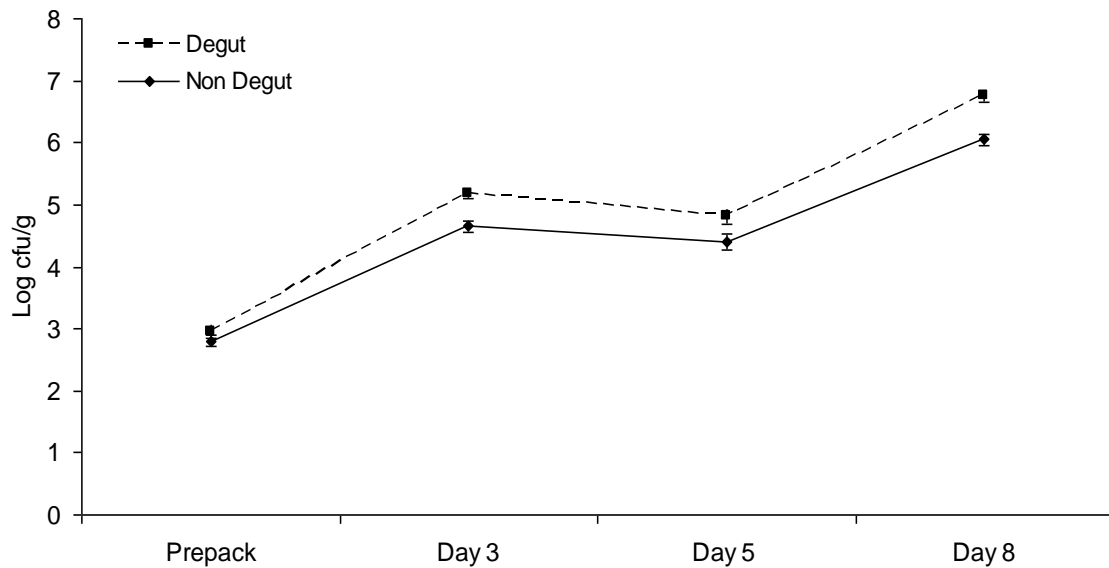
Figure 3.30 Total bacteria growth in degutted and non-degutted *Nephrops* tail meat at 2-3°C after packing up to 5th day and then change to 5-6°C up to the 8th day
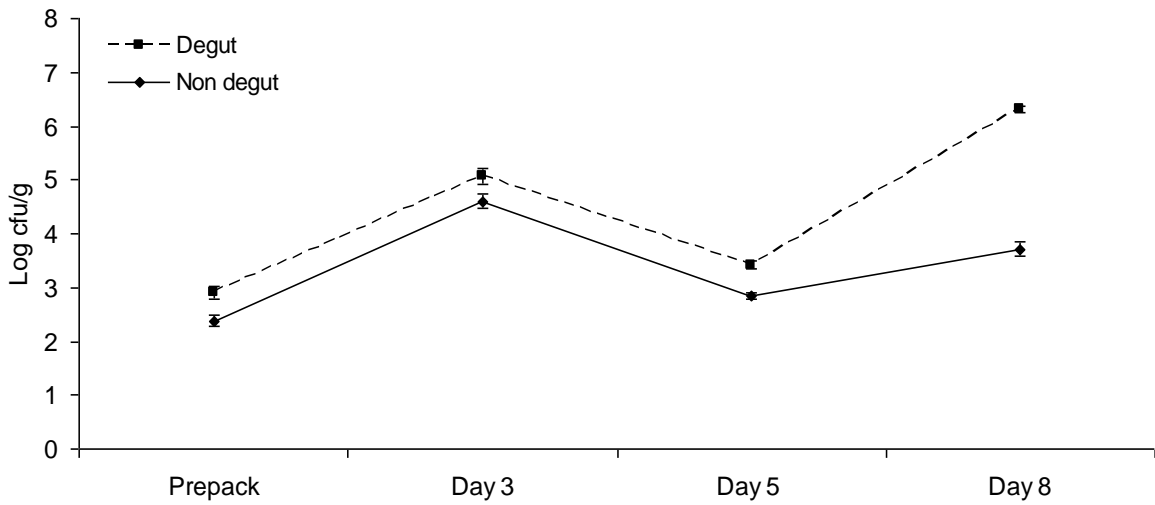
Figure 3.31 H_2S producing bacteria growth in degutted and non-degutted *Nephrops* tail meat at 2-3°C after packing up to 5th day and then change to 5-6°C up to the 8th dav
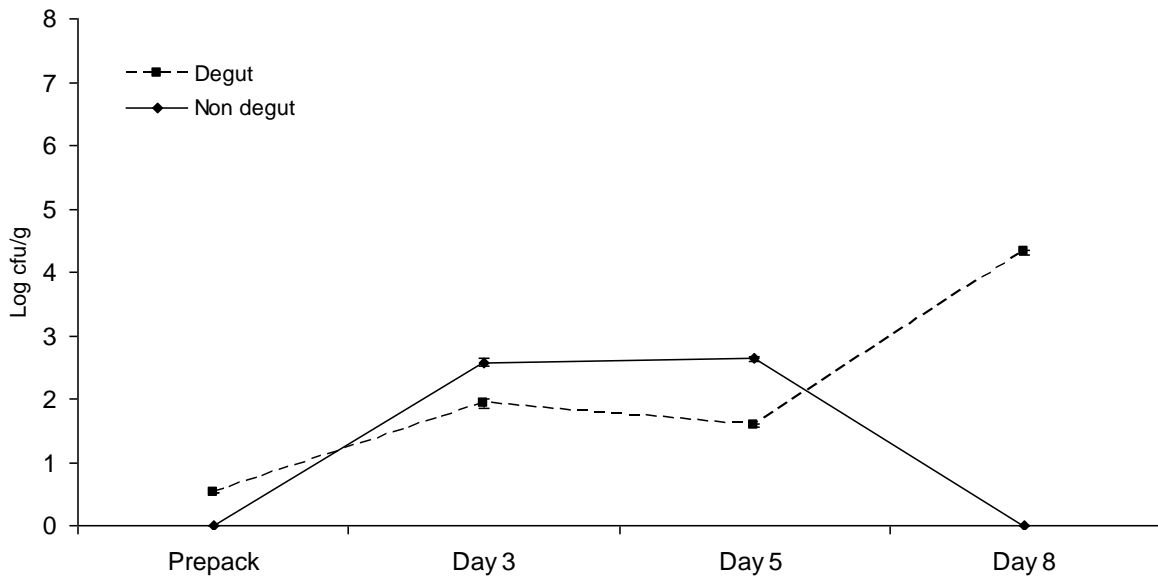
Figure 3.32 *Pseudomonas* sp. bacteria growth in degutted and non-degutted *Nephrops* tail meat at 2-3°C after packing up to 5th day and then change to 5-6°C up to the 8th day

In conclusion, we have demonstrated that bacterial growth in *N. norvegicus* tails meat is inhibited by OGM MAP packing. However, it appears that removing the gut from *N. norvegicus* before packing does not reduce the bacterial load in the tail meat following storage. In contrast, the bacterial numbers from the degutted animals actually appeared to be slightly higher than those from animals containing an intact gut.

A possible explanation for this apparently paradoxical result is that the gutting procedure may cause damage to the gut membrane, thus allowing some gut fluid containing bacteria to be spread over the surface of the adjacent muscle tissues. As a result, following the gutting procedure the initial bacteria in the tail meat will actually be increased, rather than reduced, as might have been assumed.
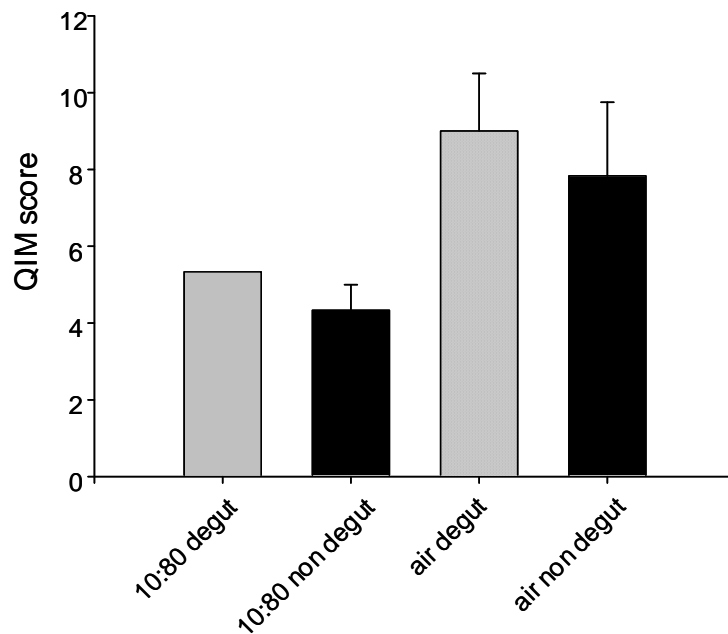
Figure 3.34 QIM values in whole Norway lobsters degutted or non degutted and packed with the OGM or with air on day 8 of storage. Values are the mean ± S.E.M of 3 different packs for each condition and storage time each one evaluated for 3 different persons.

These results were further confirmed by the fact that sensory assessment (QIM score) of the animals degutted or with intact gut showed very similar scores on day 8 of storage, indicating that removing the gut does not have any positive impact on the shelf extension of whole *N. norvegicus*. As shown in Figure 3.34 this seemed to be the case both for packs containing the OGM and for packs containing air.

Differences between packing fresh and 1 day old product

So far one of the premises in this WP was that Young's Seafood Ltd. would pack whole *N. norvegicus* in MAP at approximately 24 h after landing. This assumption was taken as it was considered to be the most common situation in their commercial operations. However, one of the factories from Young's Seafood Ltd. is situated in Stornoway (Isle Lewis, North West coast of Scotland). In this factory, due to its proximity to the landing boats (the main port is just 5 min drive from the factory) it could be possible to pack the animals very soon after landing them, which would be on the same day as they are caught. This possibility was assessed at this point of the project.

The question was: *Would it be better to pack animals just after catch rather than the next day?*

To this end, the following experiment was performed where fresh animals and 24-h old caught animals were packed in the OGM and in air and their quality was assessed

after different storage times. Furthermore, this experiment was carried out with two different temperature regimes:

- Packs always stored at temperatures between 2-3 ℃

- Packs stored using the 2-step temperature regime used in earlier phases of this WP3: at 2-3 ℃ for 5 days and then to 5-6 ℃ from day 5 to day 8.

However, the first step was to check if there were any differences in the bacterial load in the tail meat at these different times. Therefore, the total bacteria from *N. norvegicus* tail meat was examined immediately after landing on the quay (within 2 h of capture), defined as fresh, and 24 hour later, defined as pre-pack. The data from two trawls (Figure 3.35) show that there is no difference in the bacteria counts in the fresh and pre-pack samples (STATS, P=0.979).
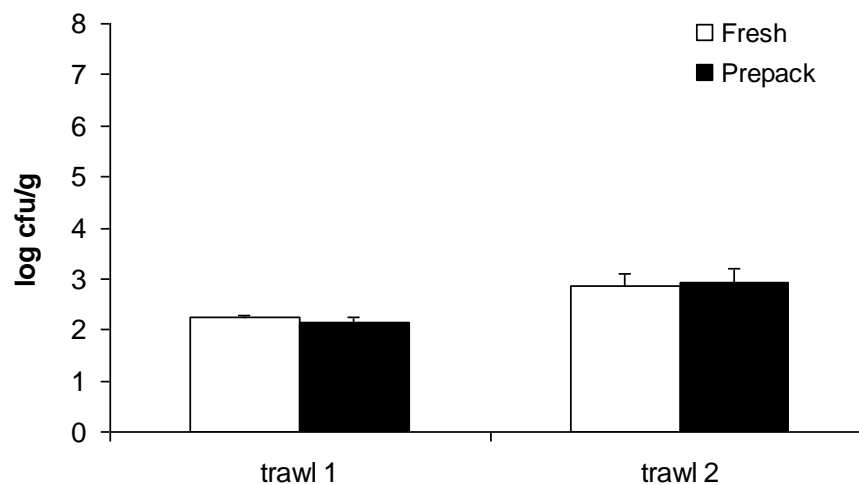
Figure 3.35 Bacterial load in *Nephrops* tail meat after landing on the quay (2h after capture) and before packing (24 h stored in ice after landing on the quay)

When air- and OGM-packs were stored at a constant temperature of 2-3 ℃ a clear difference was obtained on day 8 of storage between them. However, no differences between packing fresh animals or 24 h old animals were observed (Figure 3.36).

When stepping the temperature to 5-6 ℃ after day 5, QIM scores were generally higher and therefore the differences between packs containing the OGM and air were shorten.
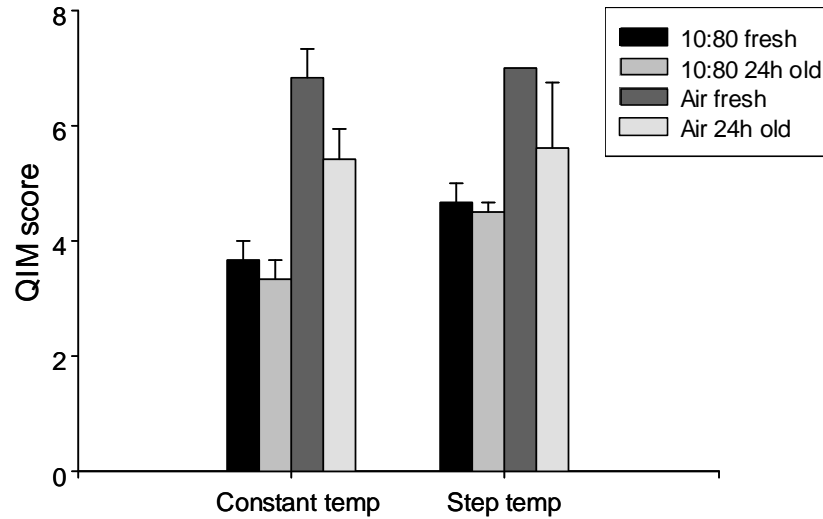
Figure 3.36 QIM scores in fresh and 24-h old *Nephrops* on the day 8 of storage after being kept at 2-3℃ all the time or after being stored at 2-3℃ for up to day 5 and then transferred to 5-6 ℃.

When packs were stored at a constant temperature of 2-3 ℃ bacterial loads on day 8 of storage were greatly affected by the pack gas composition: OGM packs had lower TVC than packs on air (Figure 3.37). However, packing fresh animals or packing 24-h old animals had only a minor impact on total bacteria load. When looking at the types of bacteria present, again no large differences were obtained. Slightly higher numbers in luminous bacteria were obtained in 24-h old animals packed in MAP compared to fresh animals packed in MAP. However, especially in the case of packs containing the OGM, the counts were all low in absolute terms, and therefore the significance of this result is questionable.
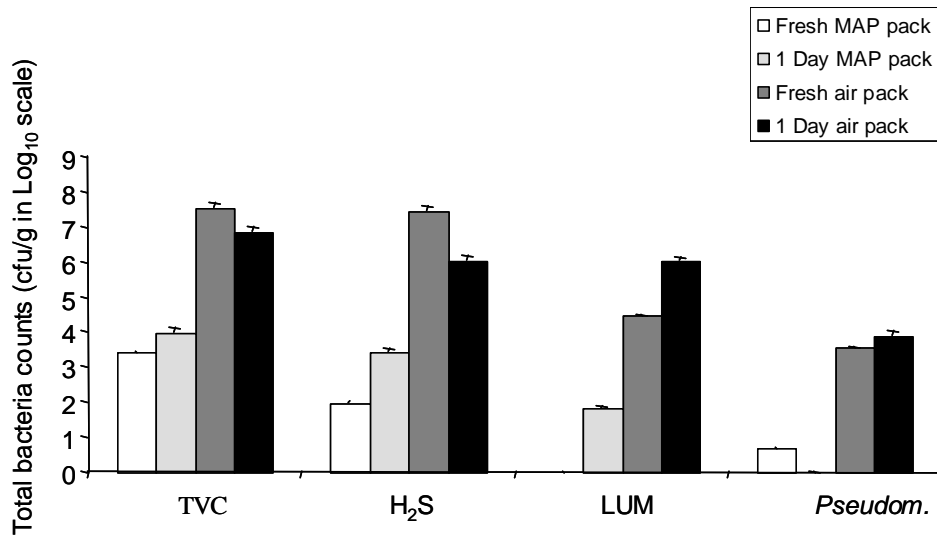
Figure 3.37 Bacteria count in fresh and 24-h old *Nephrops* on the day 8 of storage at 2-3°C.

When a different temperature regime was used, with the temperature being stepped up to 5-6 °C on day 5 onwards, a similar pattern in bacterial load was observed (Figure 3.38). Total bacteria numbers were higher in air packs than in pack containing the OGM. However, again, no differences were obtained if packs contained fresh animals or 24-h old animals.
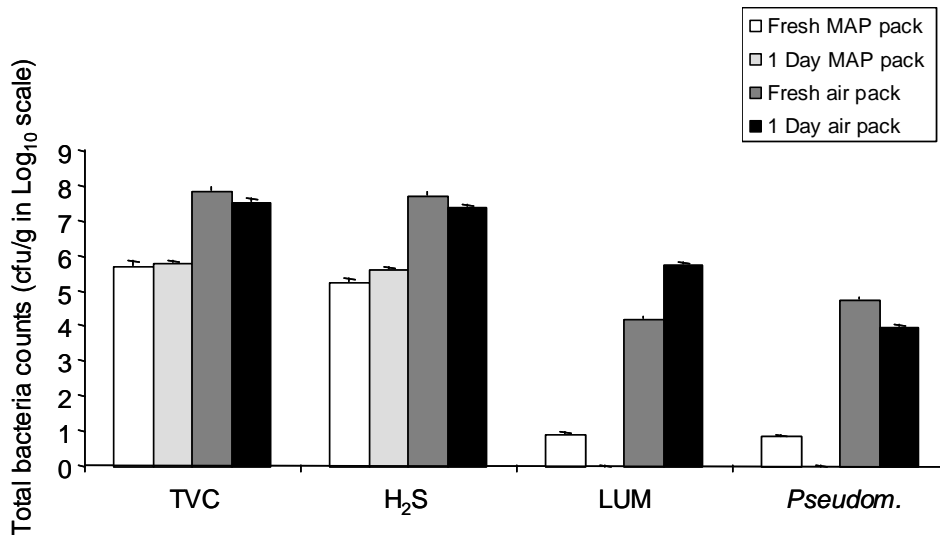
Figure 3.38 Bacteria count in fresh and 24-h old *Nephrops* on the day 8 of storage after being kept at 2-3°C for up to day 5 and then transferred to 5-6°C.

Another important observation made was the difference in the bacteria numbers resulting from using the two different temperature regimes. The differences in the TVC between MAP packs and air packs was reduced when these packs were stored at 5-6 ℃ after day 5 (Figure 3.39 and 3.40 respectively). This seemed to be the case for fresh packed animals and also for 24-h old animals. This change in the effectiveness of MAP is a very relevant result. Fish spoils twice as fast at 5 ℃ compared with the rate at 0 ℃. Therefore, very small changes in absolute temperature can have a great impact on the shelf-life of any seafood product. Some authors have shown that any benefit from a modified atmosphere will be much reduced when storage temperatures are above 5 ℃ (Cann, 2001).
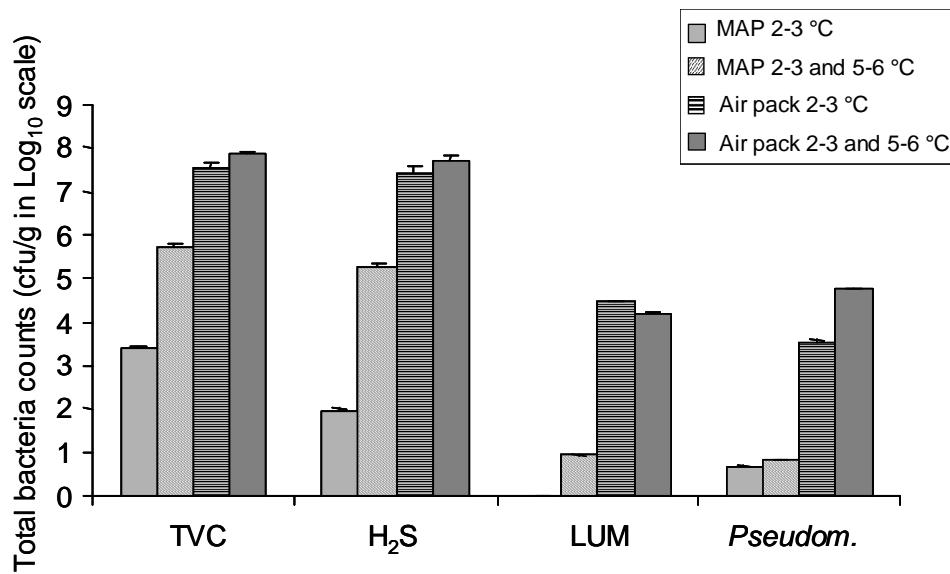
Figure 3.39 Bacteria count in fresh packed *Nephrops* on the day 8 of storage after being kept all the time at 2-3 ℃ or being stored at 2-3 ℃ for up to day 5 and then transferred to 5-6 ℃.
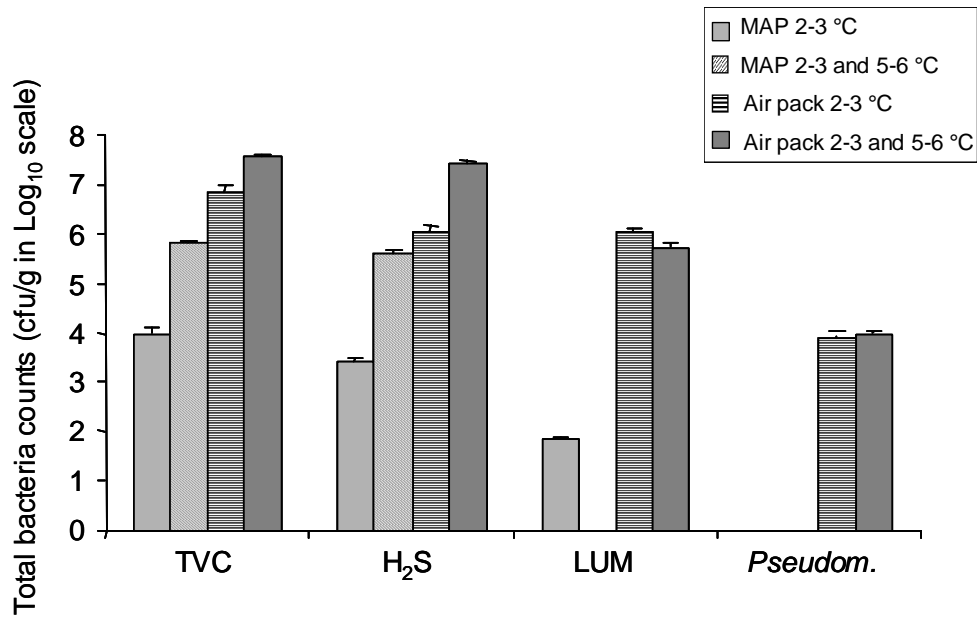
Figure 3.40 Bacteria count in 24-h old packed *Nephrops* on the day 8 of storage after being kept all the time at 2-3 °C or being stored at 2-3 °C for up to day 5 and then transferred to 5-6 °C.

In order to further confirm these results, hypoxanthine concentrations were measured in samples from all the different packs (Figure 3.41). No differences were found between packing fresh or 24-h old animals. In this case, stepping the temperature after day 5 of storage to 5-6 °C produced an increase in hypoxanthine concentration on air packs. In packs containing the OGM no changes were detected. Since this compound is produced by bacteria a delay between the increase in bacterial numbers and the production of hypoxanthine could probably explain why although higher bacteria were obtained in MAP packs with OGM stored in stepping temperature conditions no differences are observed in hypoxanthine concentrations.
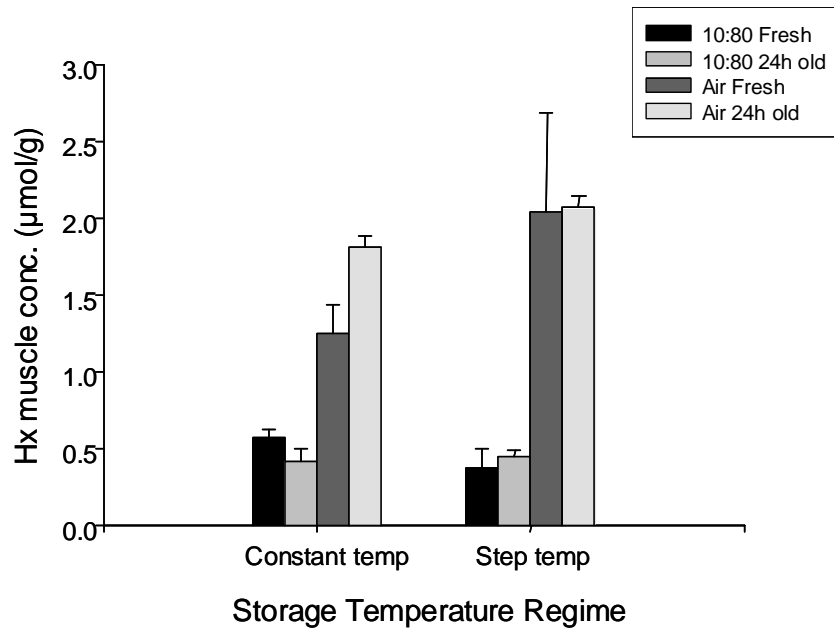
Figure 3.41 Hypoxanthine concentrations in the muscle of fresh and 24-h old packed *Nephrops* on the day 8 of storage after being kept all the time at 2-3 °C or being stored at 2-3 °C for up to day 5 and then transferred to 5-6 °C.

From these results it would appear that under the packing conditions employed a similar shelf extension would be obtained when packing fresh or 24 h old animals. On the contrary, storage temperature has a clear impact on the effectiveness of the OGM and therefore very small changes in temperature can lead to dramatic changes in quality. For this reason it was decided to explore more into detail the effect of different temperatures on the shelf life extension of MAP packed product.

The effect of different temperature regimes using MAP

Storage at a constant temperature of 1-2 ºC

In the same way as in previous experiments, in this series of experiments animals were caught by otter trawl by the Research vessel 'Aplysia' from UMBSM, washed with running seawater and stored overnight in a cold room 5-6 ºC in Millport. The next day, the remaining ice was removed; animals were washed with seawater and dipped in Melacide-SC20 dilution 1/1000 for 15 min. Animals were then packed in the OGM or in air and stored at 5-6 ºC until packing was finished. Once at the facilities in the University of Glasgow, animals were stored at a constant temperature 1-2 ºC for up to 13 days (Figure 3.42).
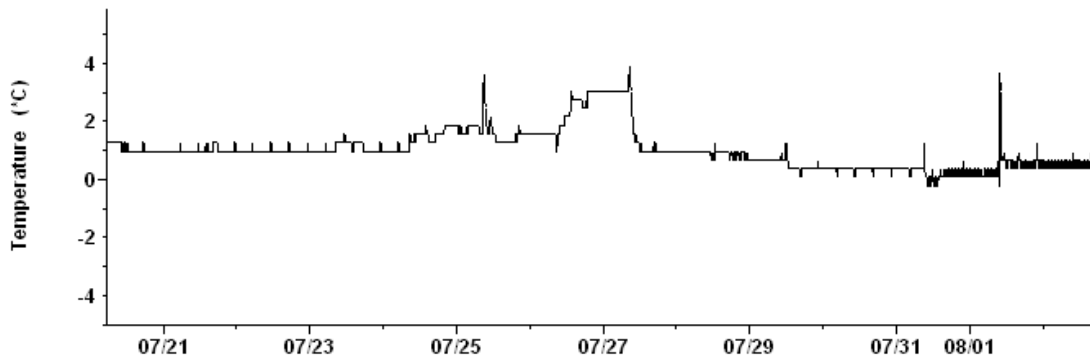
Figure 3.42 Temperature profiles in the packs stored for up to 13 days in the University of Glasgow at a constant temperature of 1 ± 1 ºC.

During storage at 1 ± 1 ºC the total bacterial count in whole *N. norvegicus* was strongly reduced compared with the air control (Figure 3.43 (a)). An initial decrease from 4.02 LOG_{10} cfu/g to 2.60 LOG_{10} cfu/g was observed in the first 3 days in the

MAP-packs. From day 3 onwards the total bacterial count increased to 4.24 LOG_{10} cfu/g on day 7 and later to 5.09 LOG_{10} cfu/g on day 13 in the MAP-packs, but never reached the values found in the air control. In the air control, on the other hand, the total bacterial count rose continually from day 1 onwards with a degree of variability and reached values of 7.84 LOG_{10} cfu/g on day 13.

Similar trends were found in the counts of H_2S-producing bacteria and the luminous bacteria. However, these counts were lower in absolute terms as these types of bacteria represent only a fraction of the total count, being on average an order of magnitude lower than the total count (Figure 3.43 (b) and (c)).

The most pronounced effect of the OGM was found on the *Pseudomonas* sp. counts. In the MAP-packs no *Pseudomonas* sp. were present above the detection limit of 2.0 LOG_{10} cfu/g. On the other hand, in the air control from day 3 onwards, and more especially from day 5 onwards, the *Pseudomonas* sp. numbers increased gradually from 2.0 LOG_{10} cfu/g to approximately 4.0 LOG_{10} cfu/g and became a considerable proportion of the total count (Figure 3.43 (d)).

(a) Total bacteria count

(b) H₂S-producing bacteria count

(c) Luminous bacteria count

(d) *Pseudomonas* sp. bacteria count
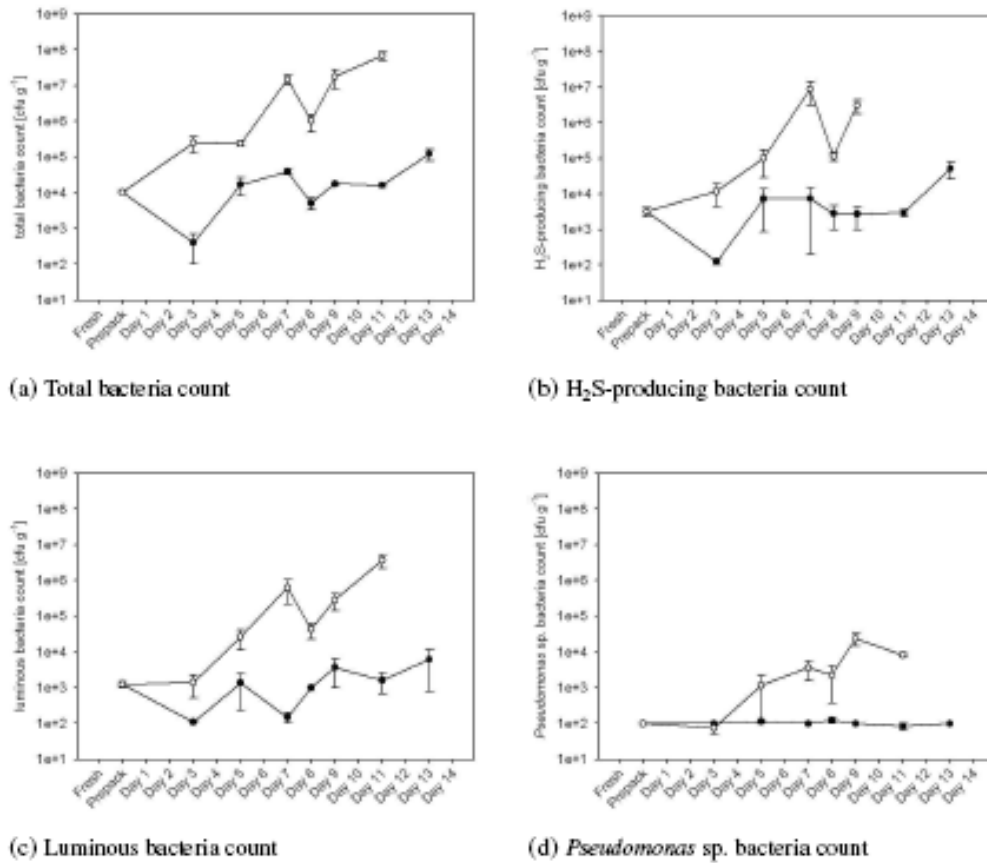
Figure 3.43: Bacteria counts in whole *N. norvegicus* stored at
1 ± 1ºC (●) bacterial numbers in the OGM-MAP-packed whole *N. norvegicus*; (○) bacterial
numbers in whole *N. norvegicus* stored in air. Bacteria numbers are plotted on a logarithmic
scale and each point is the mean ± SEM of 3 x 5 samples.

Lobsters that were packed in the OGM-MA and stored at 1 ± 1 ºC gave prime scores
(0) in the QIM [odour] until day 3 (Figure 3.44). A score of zero indicates a marine,
fresh, hay-like odour (for more information on the QIM [odour] please consult WP5).
Between day 3 and day 5 these odour attributes were lost and on day 5 the packs were
given a score of 1, having a less fresh or neutral odour. During the next 8 days the
MAP-pack gradually changed odour and by day 13 they were scored somewhere
between less fresh or neutral (a score of 1) and old seaweed-like, musty and slightly
ammoniacal, but never reached a score of 2 in the QIM, and were never rejected nor
described as releasing explicit off-odours (sour, musty, ammonia-like). The air
control, on the other hand by day 5 (and day 7) had reached its acceptable limit and it

was described as old seaweed-like, musty and ammoniacal (score 2). By day 9 all air control packs released strong, sour, ammonia-like, musty off-odors (the maximum score of 3).
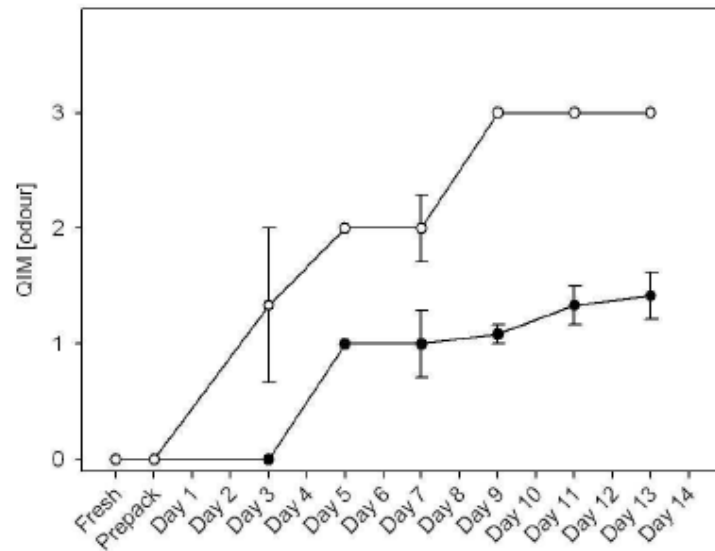
Figure 3.44 QIM results of whole *N. norvegicus* during storage at 1 ± 1 ℃ (●) QIM results of whole OGM-MAP-packed *N. norvegicus*; (○) QIM results of whole air-packed *N. norvegicus*. Each point is the mean ± SEM of 3 x 3 packs.

The bacterial microflora in the muscle of whole, Melacide SC20-treated *N. norvegicus* previously stored in the OGM-MA at 1 ± 1 ℃ for 8 days was analyzed and compared with air control packs. The samples were isolated, analyzed and identified by molecular tools. In the OGM-MAP-packs (after 8 days of storage) two main groups of bacteria were found: *Vibrio* sp. (22 %) and *Photobacterium* sp. (78 %). Within the *Vibrio* sp. no *V. logei* were found and all *Vibrio* spp. belonged to other groups such as for example *V. wodanis*. Within the *Photobacterium* sp. clade virtually all isolates found belonged to the *P. phosphoreum* species (94 %) and only 6 % being unidentified species of the *Photopbacterium* sp. clade (Table 3.5). An approximate number of bacteria was calculated from the total bacterial count of approximately 1.8 x 10^4 cfu/g established previously, and the proportion within the molecular analysis

(Table 3.6). The phylogenetic relationships between the isolates in comparison to various type strains based on partial 16S rDNA genes sequences is shown in a phylogram in Figure 3.45. Isolates cluster with their type strains into distinct groups, reflecting their common root and thus revealing their identity. The majority of isolates from 8 day old OGM-MAP-packed *N. norvegicus* held at 1 ± 1 ℃ were identified as *P. phosphoreum* (Table 3.6).

Table 3.5 Identity, abundance and proportion of bacteria isolated from the muscle of whole, Melacide-SC20 treated, OGM-MAP-packed *N. norvegicus* held at 1 ± 1 ℃ for 8 days

Clade	Proportion [%]	Species	Proportion [%]
Shewanella sp.	-		-
Vibrio sp.	22	*V. logei*	-
		others	100
Photobacterium sp.	78	*P. phosphoreum*	94
		others	6
Pseudomonas sp.	-		-

Table 3.6 Identity, abundance and approximate numbers of bacteria isolated from the muscle of whole OGM-MAP-packed *N. norvegicus* held at 1 ± 1 ℃ for 8 days

Clade	cfu g^{-1}	Species	cfu g^{-1}
Shewanella sp.	-		-
Vibrio sp.	4×10^3	*V. logei*	-
		others	4×10^3
Photobacterium sp.	1.4×10^4	*P. phosphoreum*	1.3×10^4
		others	1×10^3
Pseudomonas sp.	-		-

In the air control packs the bacterial microflora was composed differently. Although again only two different bacteria clades were found to dominate the microflora: *Vibrio* sp. And *Photobacterium* sp., here they were in different proportions. No clade dominated, with *Vibrio* sp. representing 45 % and *Photobacterium* sp. 55 % of the total. Clade composition was also different for the two species. Within the *Vibrio* clade *V. logei* represented 90 % and others (like *V. splendidus*) were only present with

10 %. In the *Photobacterium* sp. clade two main species were found. *P phosphoreum* represented a slight majority (55 %) and another *Photobacterium* sp., *P. profundum*, was present at 45 % (Table 3.7). An approximate number of bacteria calculated from a total bacterial count of 1.8×10^7 cfu/g established previously and the proportion within the molecular analysis is given in Table 3.8. The phylogenetic relationships between the isolates in comparison to various type strains based on partial 16S rDNA genes sequences is shown in a phylogram in Figure 3.45. Isolates cluster with their type strains into distinct groups reflecting their common root and thus revealing their identity. Congruent to the *LE*BIBIBLAST results (Table 3.8), two different bacteria clades were found to dominate the microflora: *Vibrio* spp. and *Photobacterium* spp., and within these two clades three species were most abundant. Within the *Vibrio* clade the *V. logei* were dominant and within the *Photobacteria*, *P. phosphoreum* and *P. profundum* were equally abundant.

Table 3.7 Identity, abundance and proportion of bacteria isolated from whole *N. norvegicus* held at 1 ± 1 ℃ for 8 days

Clade	Proportion [%]	Species	Proportion [%]
Shewanella sp.	- %	-	
Vibrio sp.	45 %	*V. logei*	90 %
		others	10 %
Photobacterium sp.	55 %	*P. phosphoreum*	55 %
		others	45 %
Pseudomonas sp.	- %	-	

Table 3.8 Identity, abundance and approximate numbers of bacteria isolated from the muscle of whole OGM-MAP-packed *N. norvegicus* held at 1 ± 1 ℃ for 8 days

Clade	cfu g^{-1}	Species	cfu g^{-1}
Shewanella sp.	0	-	
Vibrio sp.	8×10^6	*V. logei*	7×10^6
		others	1×10^6
Photobacterium sp.	10×10^6	*P. phosphoreum*	5×10^6
		others	5×10^6
Pseudomonas sp.	0	-	
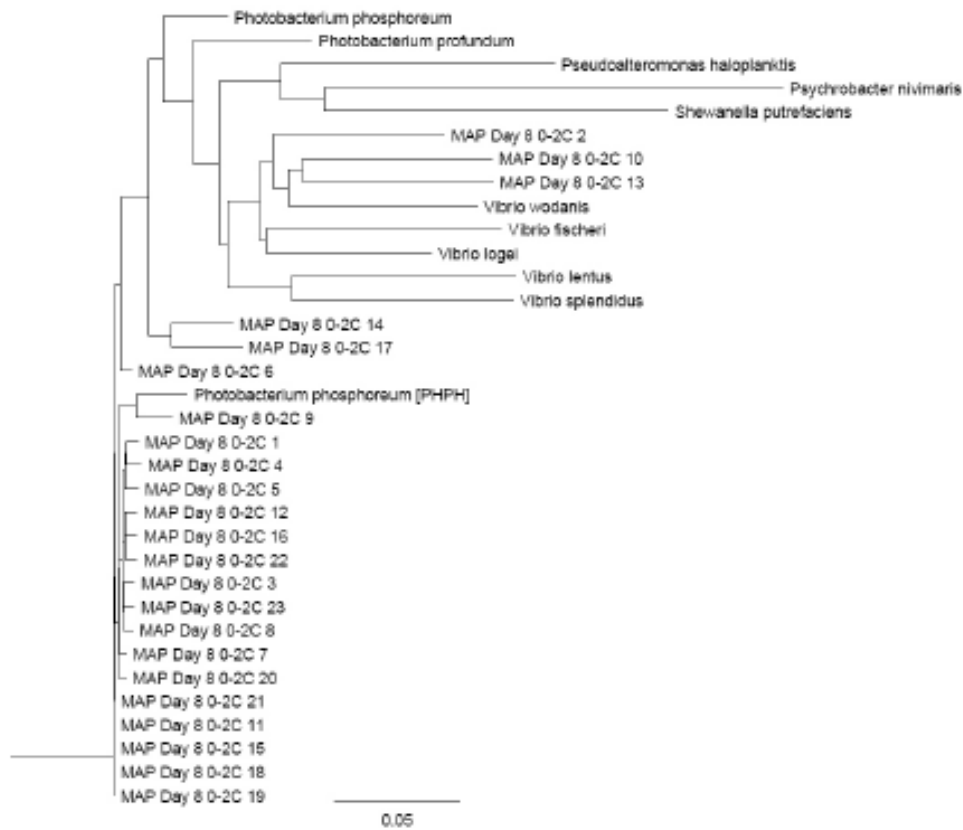
Figure 3.45 Phylogram of bacteria isolated from the muscle tissue of whole OGM-MAP-packed *N. norvegicus* stored at 1 ± 1 °C for 8 days

Comparison of the phylogenetic relationships was performed by alignment of approximately 710 nucleotides using partial 16S rRNA sequences. The alignment was done using the Clustal W method in Vectro NTI 10 *Advanced*. The phylogram was edited using FigTree 1.0. Type strains used for alignment were *Vibrio fischeri* (ATCC 7744, DSM 507 or NCMB 1281), *Vibrio logei* ATCC 29985, *Vibrio splendidus* (ATCC 33125 or NCMB 1), *Vibrio wodanis* (NCIMB 13582 or NVI 88/441T), *Vibrio lentus* (DSM 13759), *Photobacterium phosphoreum* (ATCC 11040), *Photobacterium profundum* (DSJ4 or JCM 10084), *Shewanella putrefaciens* (R1418), *Pseudoalteromonas haloplanktis* (ATTC 14393) and *Psychrobacter nivimaris* (88/2-7). The scale bar represents an evolutionary distance of 0.05 Knu (Knu = average number of nucleotide changes per sequence position).

Figure 3.46 Phylogram of bacteria isolated from the muscle tissue of whole *N. norvegicus* stored at 1 ± 1 ºC in air for 8 days

Comparison of the phylogenetic relationships was performed by alignment of approximately 710 nucleotides using partial 16S rRNA sequences. The alignment was done using the Clustal W method in Vectro NTI 10 *Advanced*. The phylogram was edited using FigTree 1.0. Type strains used for alignment were *Vibrio fischeri* (ATCC 7744, DSM 507 or NCMB 1281), *Vibrio logei* (ATCC 29985, *Vibrio splendidus* (ATCC 33125 or NCMB 1), *Vibrio wodanis* (NCIMB 13582 or NVI 88/441T), *Vibrio lentus* (DSM 13759), *Photobacterium phosphoreum* (ATCC 11040), *Photobacterium profundum* (DSJ4 or JCM 10084), *Shewanella putrefaciens* (R1418), *Pseudoalteromonas haloplanktis* (ATTC 14393) and *Psychrobacter nivimaris* (88/2-7). The scale bar represents an evolutionary distance of 0.05 Knu (Knu = average number of nucleotide changes per sequence position).

To further characterize the effect of the OGM on the quality of the *N. norvegicus,* K-

values and compounds produced mainly by bacteria were measured.

K-values (freshness indicator) increased in all the packs with storage time indicating

the loss of freshness with time (Figure 3.47a). This increase was somewhat more rapid

in packs stored in air compared to packs containing the OGM. However, differences were even clearer when looking at the hypoxanthine concentrations in the muscle (Figure 3.47 b). While levels above 0.5 µmol/g were found on day 5 in animals stored in air packs this compound was not detected to similar values for another 4 days in OGM packs (day 9 of storage).
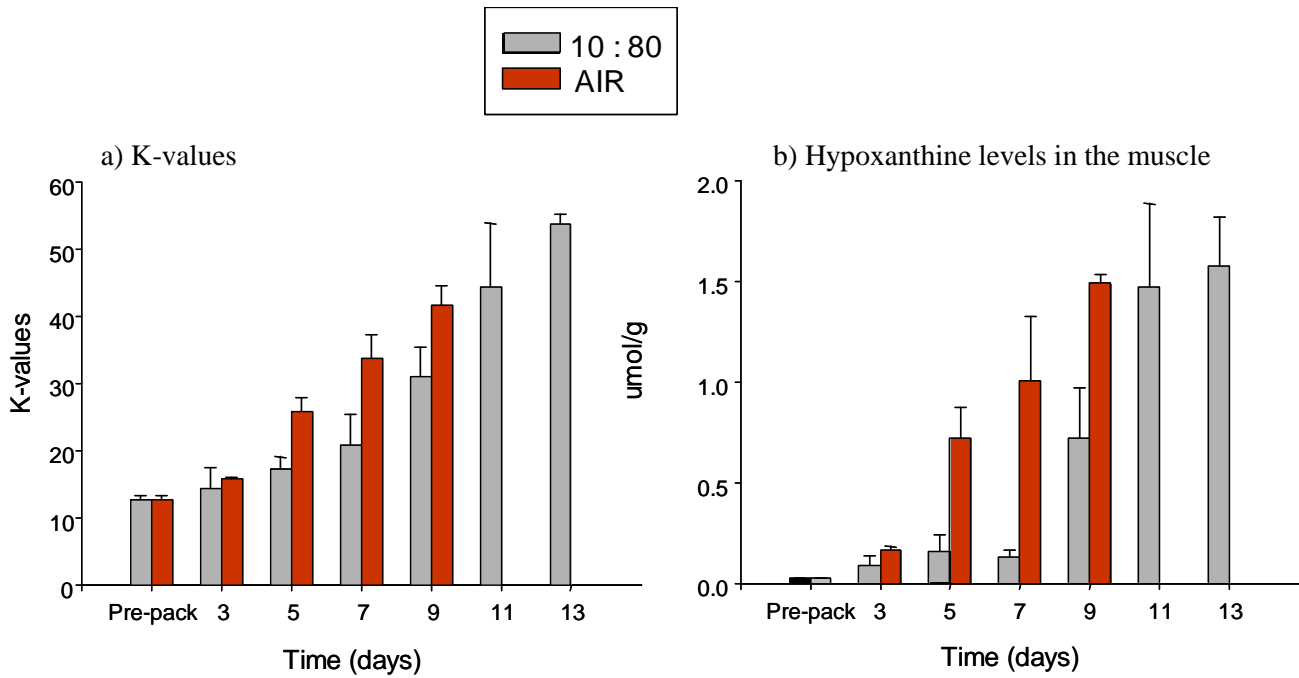
Figure 3.47 (a) K-values and (b) hypoxanthine concentrations in whole *N. norvegicus* during storage at 1 ± 1 ℃. Each point is the mean ± SEM of 3 independent measures per condition and storage time.

In this experiment, trimethylamine (TMA) levels were also measured in muscle samples from the different packs. Many bacteria are capable of producing TMA as they have the ability of using trimethylamine oxide (TMAO) as a terminal electron acceptor (Sikorski et al., 1990). This compound is the responsible for the fishy-ammoniacal smell typical in spoiled seafood products. Its suitability as a spoilage indicator in *N. norvegicus* was established in Phase I of the Scottsih *Nephrops* Survey. As shown in Figure 3.48 TMA concentrations increased more rapidly in samples from

air packs compared to samples from OGM packs. In fact, it took up to 6 days for the

product in OGM packs to reach the same TMA concentrations as in the air packs.
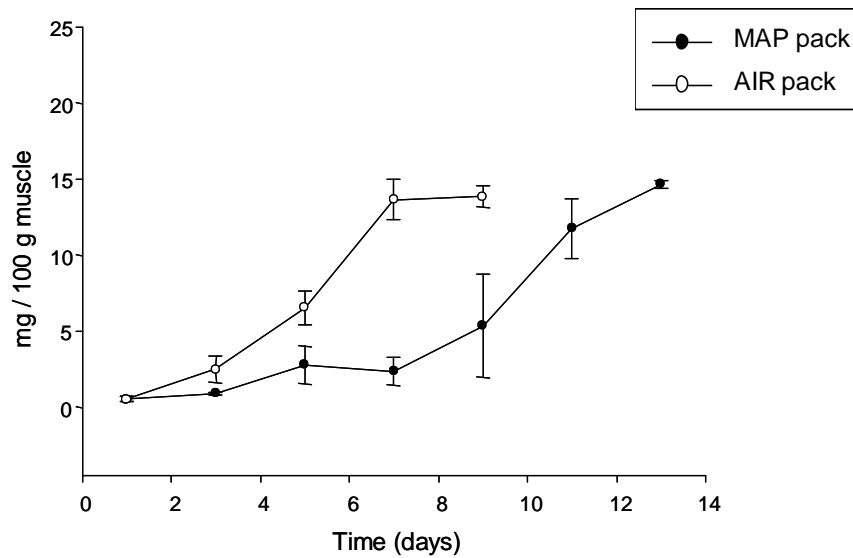
Figure 3.48 TMA concentrations in whole *N. norvegicus* during storage at 1 ± 1 ºC. Each
point is the mean ± SEM of 3 independent measures per condition and storage time.

Other important compounds as proof of spoilage in fish and shellfish are biogenic

amines. Although 7 different biogenic amines were analysed by HPLC only

putrescine, cadaverine and agmatine were found in significant amounts, and of these

agmatine was the predominant one. Agmatine is formed by decarboxylation of

arginine, which is the predominant free amino acid present in *N. norvegicus* muscle.

At the time that the air packs were rejected and no further analysis was performed

(day 9 of storage), the main difference between the product in packs containing the

OGM and air was the concentration of putrescine (Figure 3.49). This biogenic amine

was not detected in MAP packs up to day 9 of storage, while in air packs it increased

very rapidly on day 7. Therefore, putrescine could be a good indicator of an initial

decomposition of the samples, corresponding to the putrid smell that contributed to

the rejections of the air packs at this point. On the other hand, cadaverine and

agmatine increased in both packing conditions from days 7-9 and no large differences were found between OGM and air packs. Agmatine has been found to be a useful freshness index in other species such as squid. This is because agmatine can be detected in small amounts from an early time during storage and its concentration increases with storage time. In the case of *N. norvegicus* it would appear that this biogenic amine could potentially provide a good freshness index since it appears early during storage and thereafter increases with time. However, since no differences were found in agmantine concentrations between MAP and air packs, its suitability in MAP studies is uncertain.
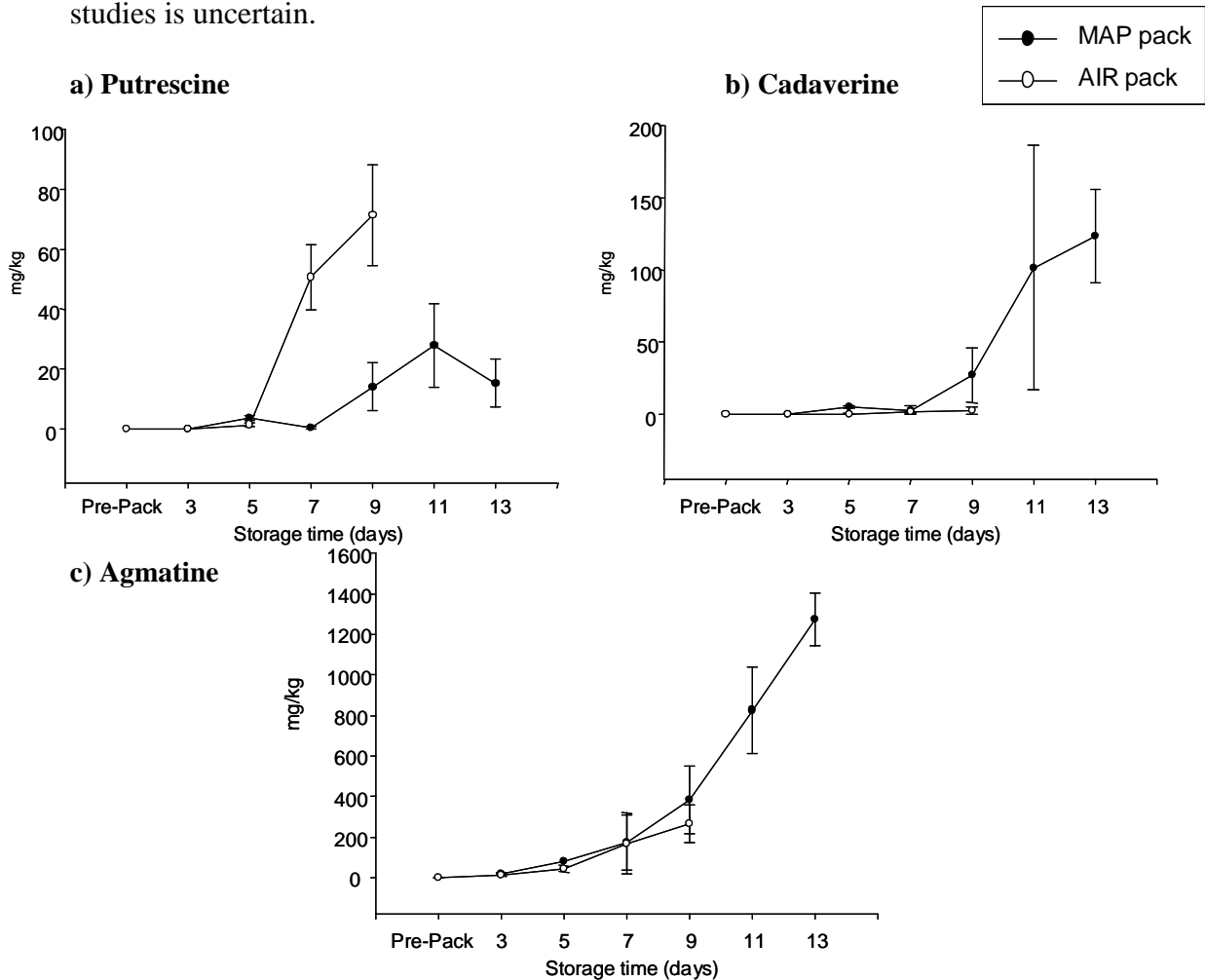
Figure 3.49 Biogenic amines concentrations in whole *N. norvegicus* during storage at 1 ± 1 °C. Each point is the mean ± SEM of 3 independent measures per condition and storage time.

Finally, samples from different storage times were assessed by a professional sensory panel trained to recognise different attributes in Norway lobsters. This analysis was performed at the Food Innovation Institute in Edinburgh. The full report form can be found in Annexe 2 of the present report. The trained panel performed the QDA (Quantitative Descriptive Analysis) method to profile each langoustine sample in terms of sensory attributes related to aroma, appearance, texture and flavour. For each of the 10 sensory attributes of this QDA test, a two-anchored linear scale (0-10) was used, in which the score of five is the mid-point. This scale is objective and has nothing to do with the subjective assessment ('like' or 'dislike') of a given panellist. However, the trained Panel was also asked to be subjective and score their degree of 'like or dislike' for 'overall liking' of each sample on a linear scale (0-10).

For this experiment samples were analysed on days:

- Pre-pack

- OGM (10:80) day 5

- Air day 5

- OGM (10:80) day 9

- Air day 9

- OGM (10:80) day 13

Table 3.9 Results from whole OGM-MAP-and air packed *N. norvegicus* held at 1 ± 1 ℃ for up to 13 days

Sample		Smell Character.	Smell Strength	Pink Patches	Inside Colour	Springiness	Firmness	Chewiness	Moistness	Flavour	Aftertaste	Overall Liking
Pre-pack	Mean	**5.22**	**6.38**	**6.07**	**6.45**	**5.32**	**5.46**	**4.72**	**5.85**	**6.09**	**6.04**	**5.47**
	St. D.	1.42	1.08	2.80	2.10	2.94	1.20	0.71	1.36	1.74	1.82	2.20
Air day 5	Mean	**5.73**	**6.39**	**3.28**	**6.51**	**4.91**	**5.39**	**4.31**	**5.52**	**6.47**	**6.48**	**6.15**
	St. D.	2.05	1.01	1.94	1.85	2.42	1.46	0.82	1.40	1.18	1.20	2.08
10:80 day 5	Mean	**5.52**	**6.25**	**6.24**	**5.68**	**6.30**	**6.64**	**5.71**	**5.35**	**6.31**	**6.17**	**5.89**
	St. D.	1.65	1.39	1.12	1.44	2.40	1.60	1.49	2.03	1.35	1.37	1.98
Air day 9	Mean	**5.92**	**7.01**	**5.73**	**5.64**	**5.96**	**6.90**	**5.75**	**5.29**	**6.15**	**5.99**	**5.79**
	St. D.	1.63	1.13	1.74	1.68	2.41	1.75	1.71	1.59	1.47	1.61	1.72
10:80 day 9	Mean	**5.03**	**6.85**	**7.24**	**5.30**	**6.43**	**7.22**	**6.32**	**4.85**	**5.58**	**5.53**	**5.55**
	St. D.	1.50	1.05	1.94	2.14	2.74	1.78	1.88	1.55	1.29	1.47	1.75
10:80 day 13	Mean	**5.02**	**6.77**	**6.58**	**5.58**	**5.80**	**6.95**	**6.60**	**4.76**	**5.35**	**5.28**	**5.14**
	St. D.	1.74	0.78	1.41	2.03	2.76	1.40	1.59	1.74	1.26	1.68	2.32
	F	8.715	2.357	4.311	3.211	0.596	3.466	5.559	0.741	4.309	5.090	4.291
	Sig.	5E-12	0.01	1E-05	6E-04	0.83	2E-04	2E-07	0.698	1E-05	8E-07	1E-05
ANOVA		***	*	***	**	NS	***	***	NS	***	***	***

The strongest smell was found in samples stored on air for 9 days, followed by samples stored on the OGM for 9 and 13 days (Table 3.9). Other attributes such as firmness also changed according to the treatment and samples seemed to get firmer with storage time, an effect even clearer in the packs containing the OGM. On the other hand, langoustines were sweeter on day 5 compared to pre-pack samples and afterwards sweetness decreased with storage time. When comparing the effect of the OGM to the pre-pack condition, the main changes even on day 13 are in the texture of the samples. Langoustines from OGM packs were described as firmer, chewier and with less of a sweet taste than the pre-pack samples (Figure 3.50). In general it seems that the positive effect of the OGM is lost from day 9 onwards. When asking the panel about their subjective opinion on the samples, the most liked samples were the ones on day 5 while the one they liked least was the 10:80 after 13 days of storage.

However, even at this point of long storage the panel did not disliked the samples (i.e.
the score of 'overall liking' for langoustines 10:80 on day 13 remained above 5).
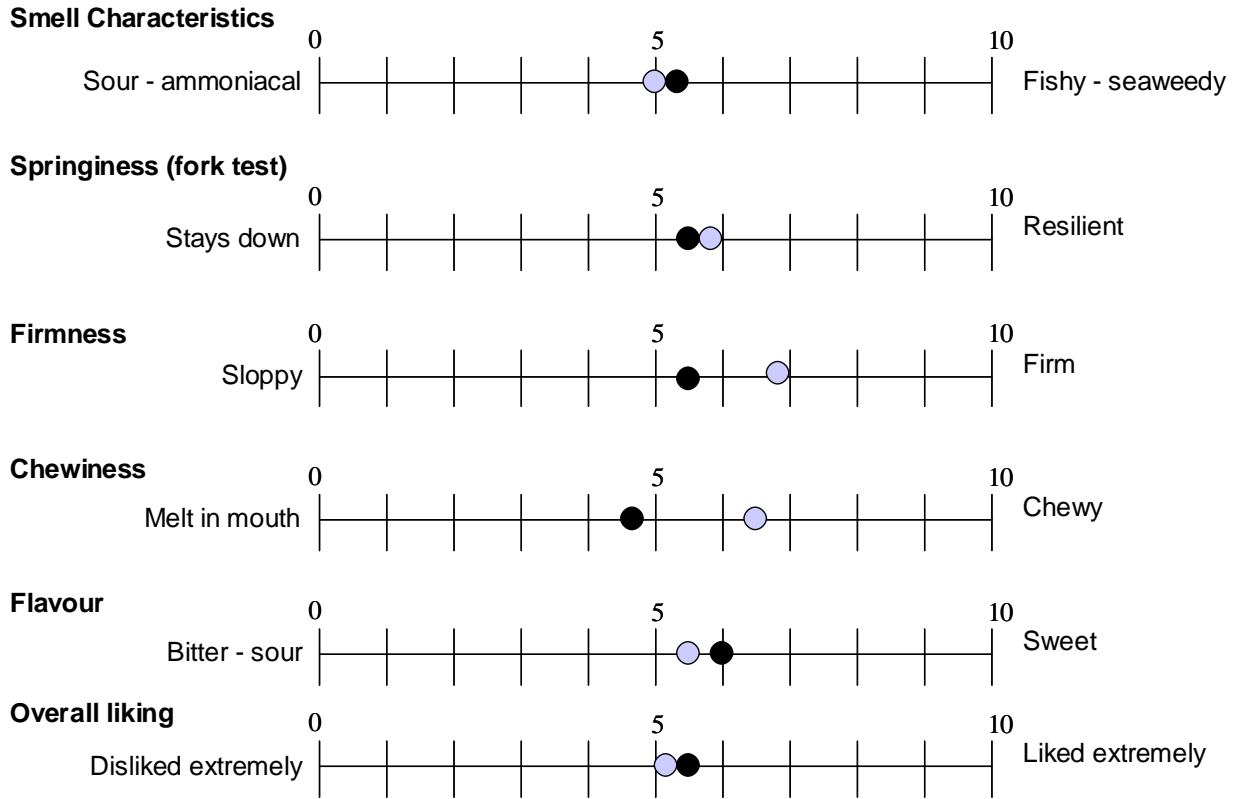
Figure 3.50 Sensory panel scoring from pre-pack to 10:80 on day 13 of storage at 1 ± 1 °C.

The effect of different temperature regimes using MAP

Storage using a stepped temperature regime

The effect of a stepped temperature regime on the OGM-MAP packs was measured (Figure 3.51). The temperature regime was as follows: 1 ± 1 ºC for the first 3 days, 3 ± 1 ºC for days 3 - 5 and 5 ± 1 ºC from day 5 onwards. As previously described, this simulated the temperatures along a typical commercial chain.
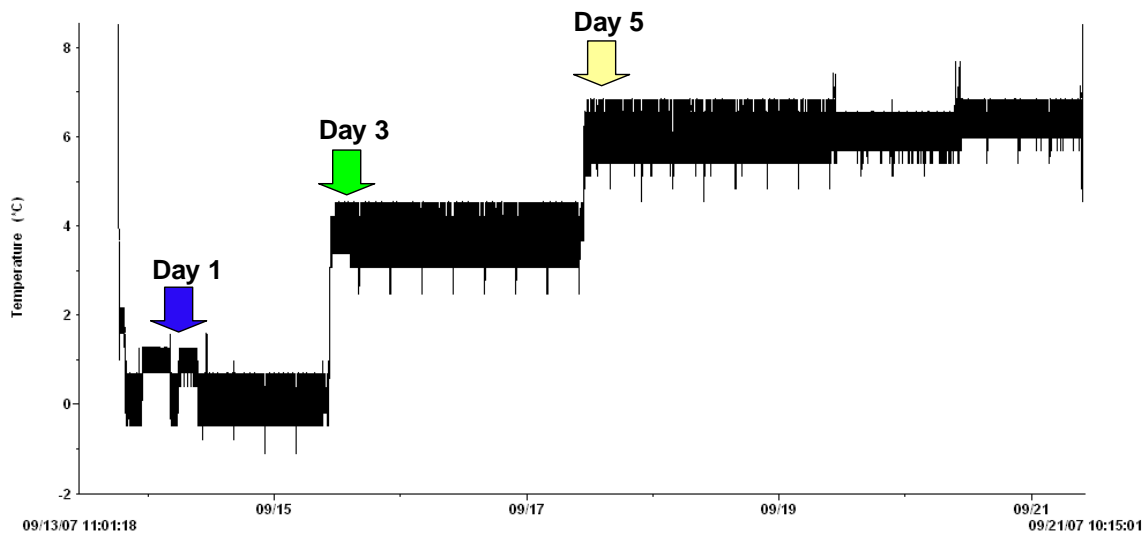
Figure 3.51 Temperature profiles in the packs stored for up to 9 days in the University of Glasgow following a stepped temperature regime trying to imitate a commercial situation.

This experiment was repeated twice, but on the second occasion there was a failure of the chill facility for pre-conditioning the product by iced storage. In this sense it simulates a real scenario, and the results are presented to show how important it is to keep to the recommended protocols at all times.

Results on Proper Storage using a stepped temperature regime

The initial total bacterial counts were found to be at 3.61 LOG_{10} cfu/g in the fresh samples. This count decreased to 2.77 LOG_{10} cfu/g during the 24 h of iced storage prior to MAP-packing. In the first 3 days of storage the OGM-MAP-packs and air control behaved similarly and reached levels of approximately 3.6 LOG_{10} cfu/g, but after the temperature was raised on day 3 there was a large increase in the total bacterial count of the air control so that the bacteria numbers reached 6.55 LOG_{10} cfu/g on day 5, but not in the OGM-MAP-packs. After the temperature was raised again on day 5, the total bacterial numbers in the OGM-MAP-packs became more similar to those in the air control (5.72 LOG_{10} cfu/g and 6.48 LOG_{10} cfu/g, respectively) and remained high (7.41 LOG_{10} cfu/g) until day 9 when the experiment was terminated due to sensory rejection of the OGM-MAP-packs.

The counts of the H_2S-producing bacteria and luminous bacteria showed similar trends to the total counts, with the same temperature-dependent pattern (Figure 3.52 (b) and (c)). *Pseudomonas* sp. counts stayed below the detection limit in the OGM-MAP-packs during the whole experiment, but in the air control they increased slowly, but continuously during storage to reach a level of 3.82 LOG_{10} cfu/g on day 9 (Figure 3.52 (d)).

(a) Total bacteria count

(b) H₂S-producing bacteria count

(c) Luminous bacteria count

(d) *Pseudomonas* sp. bacteria count
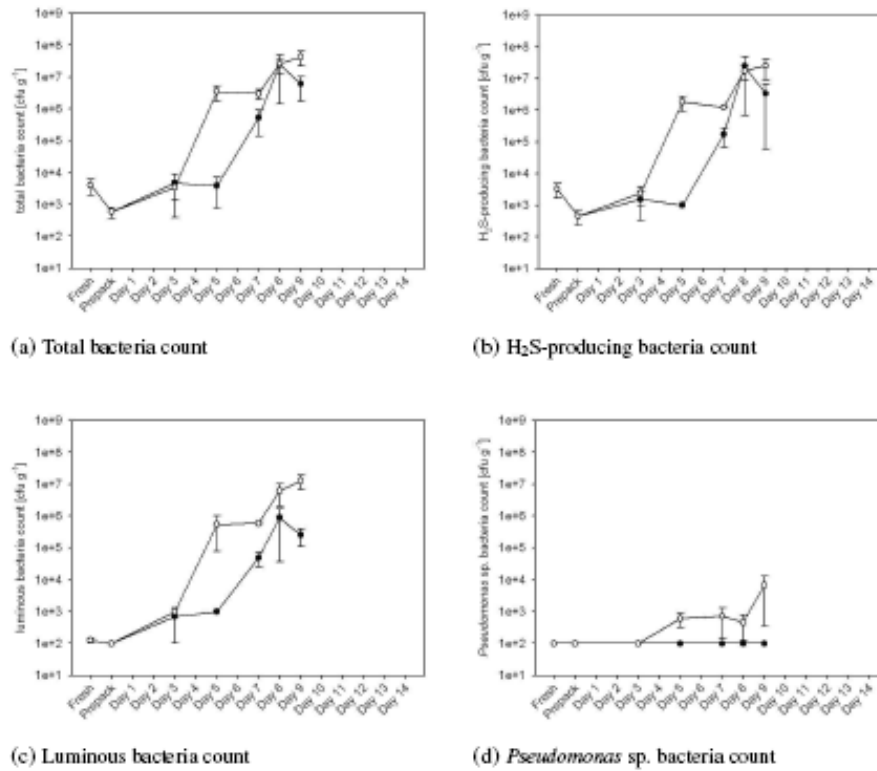
Figure 3.52 Various bacteria counts in whole *N. norvegicus*. Samples where stored following a stepping temperature protocol; (●) bacterial numbers in whole *N. norvegicus* packed in the OGM-MA; (○) bacterial numbers in whole *N. norvegicus* stored in air. Bacteria numbers are plotted on a logarithmic scale. Each point is the mean ± SEM of 3 x 5 samples.

Whole *N. norvegicus* packed in the OGM-MAP and stored following the 3-step-temperature regime were described as releasing fresh, hay-like, marine odour during the first 3 days of storage (score 0). On day 5, after the temperature was raised from 1 ± 1 ℃ to 3 ± 1 ℃, the lobsters scored in a region of 1 in the QIM [odour] scale. On day 7, after the final temperature increase on day 5, the packs did not change significantly, if compared with the results from day 5. On day 8, however, the MAP-packs were described as liberating old seaweed-like, musty and slightly ammonia-like odours and were rejected (score 2). The experiment was continued to day 9 when a score of 2.5 was given. By comparison, the air control packs, while they were scoring

low until day 3, scored above 2 on day 5 and were rejected, and by day 7 reached the

maximum score of 3 (Figure 3.53).
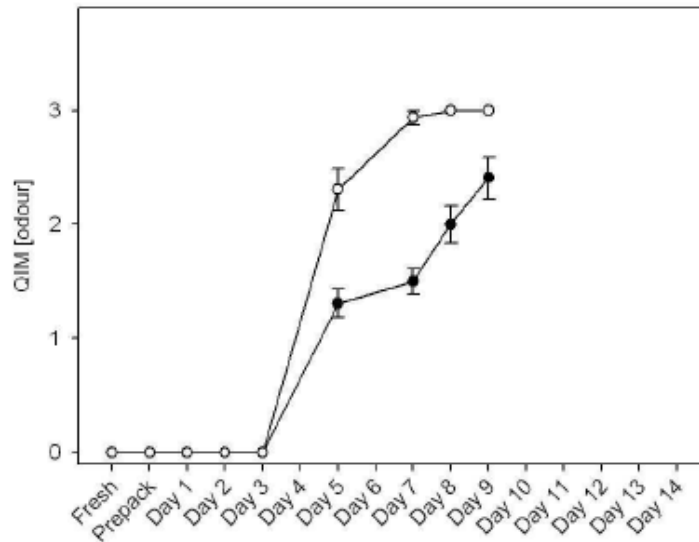
Figure 3.53 QIM scores in whole *N*. norvegicus. Samples where stored
following a stepping temperature protocol; (●) QIM in whole *N. norvegicus* packed in the
OGM-MA; (○) QIM in whole *N. norvegicus* stored in air. Bacteria numbers are plotted on a
logarithmic scale.

As shown in previous experiment, the K-value did not prove to be an effective

parameter to study the effect of MAP on whole fresh langoustines (Figure 3.54). In

this respect, no differences were found between packs containing the OGM and packs

containing air. This might be due to the fact that all packs were stored at exactly the

same temperature, and that the K-value is predominantly driven by temperature

changes that affect the biochemistry of autolytic enzymes. However, differences were

found in hypoxanthine concentrations, mainly produced by the action of bacteria.

Packs containing the OGM did not show hypoxanthine at considerable levels up to

day 7 of storage, while in air packs hypoxanthine was already present on day 5 of

storage.

a) K-values

b) Hypoxanthine in the muscle
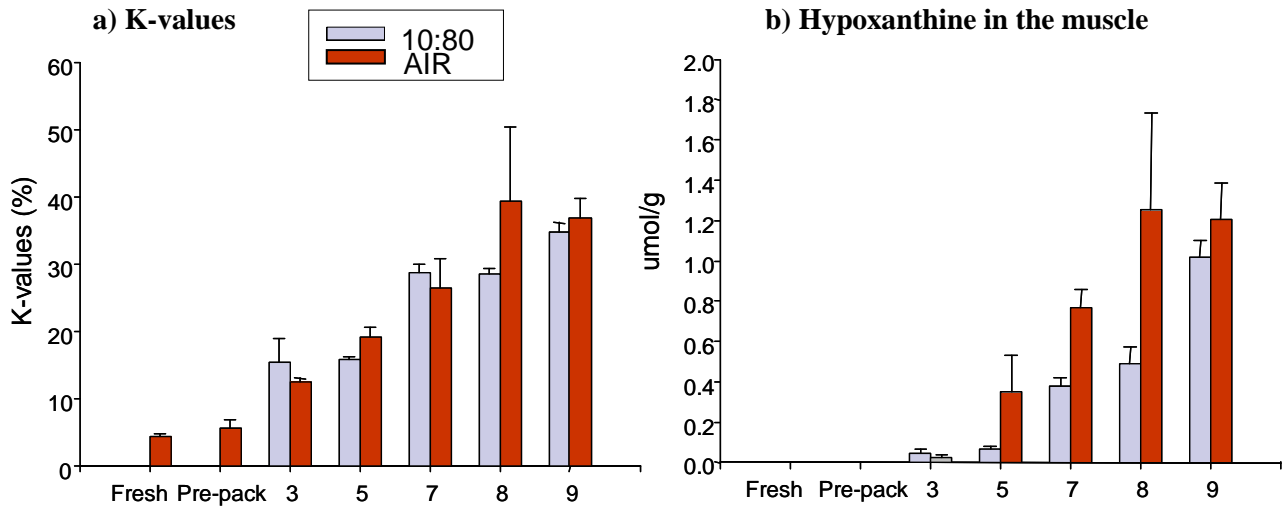
Figure 3.54 (a) K-values and (b) hypoxanthine concentrations in whole *N. norvegicus* during storage following a stepping temperature protocol. Each point is the mean ± SEM of 3 independent measures per condition and storage time.

In a similar way, TMA concentrations in the muscle started to increase in packs containing air on day 5, while it took a further 2 days to increase in the OGM-packs (Figure 3.55). However, values of TMA on OGM-packs reached the same levels to packs containing air on day 8 of storage, indicating that a gain of 2-3 days is achieved in MAP.
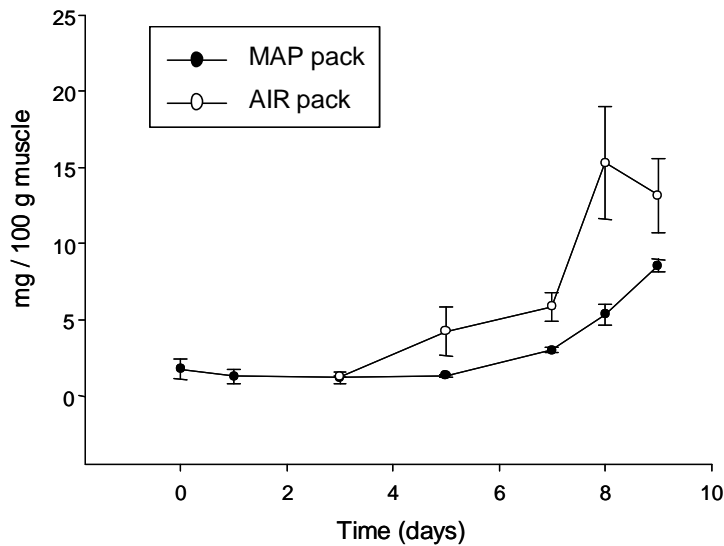
Figure 3.55 TMA concentrations in whole *N. norvegicus* during following a stepping temperature protocol. Each point is the mean ± SEM of 3 independent measures per condition and storage time.

Finally, biogenic amines were also measured in samples throughout the storage period studied (Figure 3.56). Similarly to the previous experiment, putrescine in air packs was higher than in the OGM packs. Absolute values of putrescine in air packs was higher in this experiment than in the previous experiment, indicating a negative impact on the quality due to increasing the temperature above 5 ℃ after day 5 of storage. On the contrary, both cadaverine and agmatine were low up to day 5 and increased considerably after this day independently of the gas treatment, in a similar way to that found in the previous experiment.
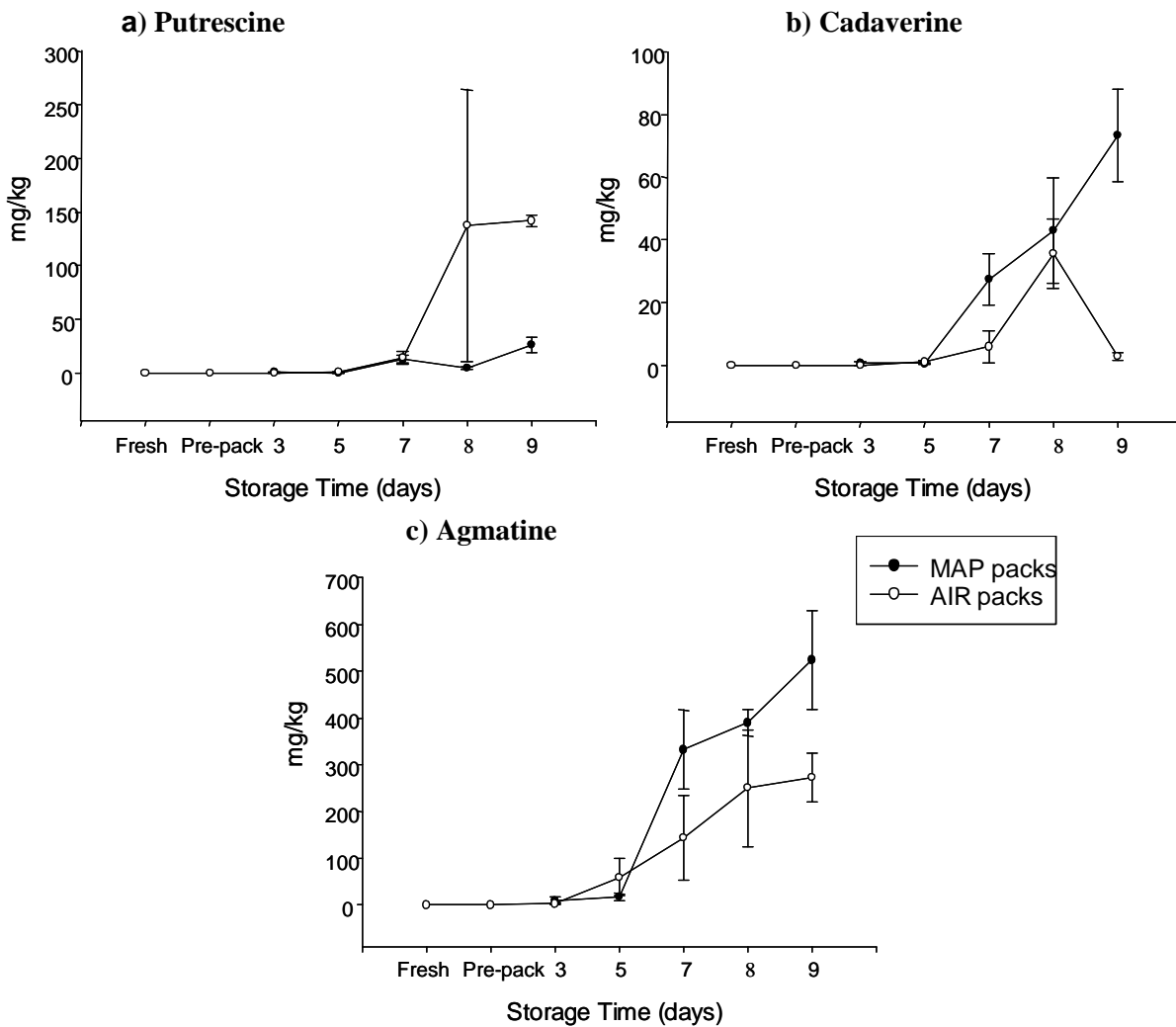
Figure 3.56 Biogenic amines concentrations in whole *N. norvegicus* during storage using a stepping temperature protocol. Each point is the mean ± SEM of 3 independent measures per condition and storage time.

Also as before, samples at different times of storage were evaluated by a professional sensory panel from the Food Innovation Institute. The full report form of these evaluations can be found in the Annexe 3 of the report.

The sample with the strongest smell was the air packs on day 9 of storage, followed by air packs on day 8 of storage (Table 3.10). No large differences were detected in the texture properties, although the tenderest samples were obtained in air packs stored for 5 and 7 days. In terms of overall liking, although some samples were liked more than others the differences were small. However, negative scores in overall liking were only obtained for air packs on days 8 and 9 and for the packs containing the OGM on day 9 of storage. In fact it would appear that packs containing the OGM scored better than the air packs from day 7 onwards, although sometimes the differences were very small. Examples of the scores obtained on day 8 are shown in Figure 3.57.

Table 3.10 Results from whole OGM-MAP-and air packed *N. norvegicus* held using a stepping temperature regime for up to 9 days

Sample		Smell Character.	Smell Strength	Pink Patches	Inside Colour	Springiness	Firmness	Chewiness	Moistness	Flavour	Aftertaste	Overall Liking
Pre-pack	Mean	**5.69**	**4.97**	**4.74**	**5.20**	**6.70**	**6.93**	**6.03**	**5.54**	**6.17**	**6.06**	**5.74**
	St. D.	0.80	1.28	1.95	2.41	2.23	1.48	1.09	1.00	0.97	1.16	1.62
10:80 day 5	Mean	**5.39**	**6.00**	**7.40**	**5.84**	**7.04**	**6.51**	**5.46**	**5.84**	**5.57**	**5.39**	**5.59**
	St. D.	1.69	1.32	1.70	1.32	1.81	1.21	2.32	0.79	1.55	1.48	1.27
Air day 5	Mean	**5.36**	**6.09**	**6.70**	**5.80**	**6.06**	**5.57**	**4.44**	**6.16**	**5.93**	**6.03**	**6.60**
	St. D.	0.90	1.10	1.56	1.03	1.34	1.26	1.03	1.25	1.00	1.16	1.36
10:80 day 7	Mean	**5.64**	**5.56**	**7.91**	**5.76**	**6.20**	**6.27**	**6.14**	**5.49**	**5.70**	**5.64**	**5.51**
	St. D.	1.68	0.97	1.56	1.06	1.79	1.47	1.05	1.22	1.18	1.03	1.03
Air day 7	Mean	**4.01**	**5.94**	**7.39**	**5.84**	**6.34**	**7.00**	**4.63**	**5.26**	**5.63**	**5.71**	**5.30**
	St. D.	1.63	1.97	2.23	1.52	2.66	1.67	1.51	0.85	0.94	1.32	1.79
10:80 day 8	Mean	**6.01**	**6.86**	**7.87**	**5.34**	**6.29**	**7.07**	**6.59**	**5.03**	**5.83**	**5.30**	**5.40**
	St. D.	0.98	1.66	1.16	0.47	1.70	1.39	1.70	1.53	0.91	1.76	1.54
Air day 8	Mean	**4.70**	**6.96**	**7.24**	**5.10**	**6.49**	**5.46**	**5.16**	**6.10**	**5.14**	**4.50**	**4.23**
	St. D.	2.12	0.94	1.63	1.98	1.37	1.07	1.11	0.71	1.40	2.04	1.73
10:80 day 9	Mean	**4.84**	**6.03**	**8.26**	**5.56**	**5.80**	**5.66**	**6.67**	**4.96**	**5.41**	**5.20**	**4.97**
	St. D.	2.23	1.57	1.56	1.20	1.28	1.43	1.52	1.76	0.79	1.19	2.43
Air day 9	Mean	**4.27**	**7.41**	**7.26**	**6.57**	**5.90**	**5.47**	**5.81**	**6.26**	**4.60**	**4.51**	**3.57**
	St. D.	1.31	1.58	1.68	1.49	1.56	1.21	0.92	0.74	1.79	1.99	2.61
ANOVA	F	1.000	3.140	5.550	0.560	0.240	1.980	3.140	1.040	1.510	1.070	1.480
	Sig.	0.454	0.002	0.000	0.852	0.994	0.043	0.002	0.419	0.146	0.401	0.158
		NS	**	***	NS	NS	*	**	NS	NS	NS	NS

Smell Characteristics

Sour - ammoniacal   Fishy - seaweedy

Springiness (fork test)

Stays down   Resilient

Firmness

Sloppy   Firm

Chewiness

Melt in mouth   Chewy

Flavour

Bitter - sour   Sweet

Overall Liking

Disliked extremely   Liked extremely
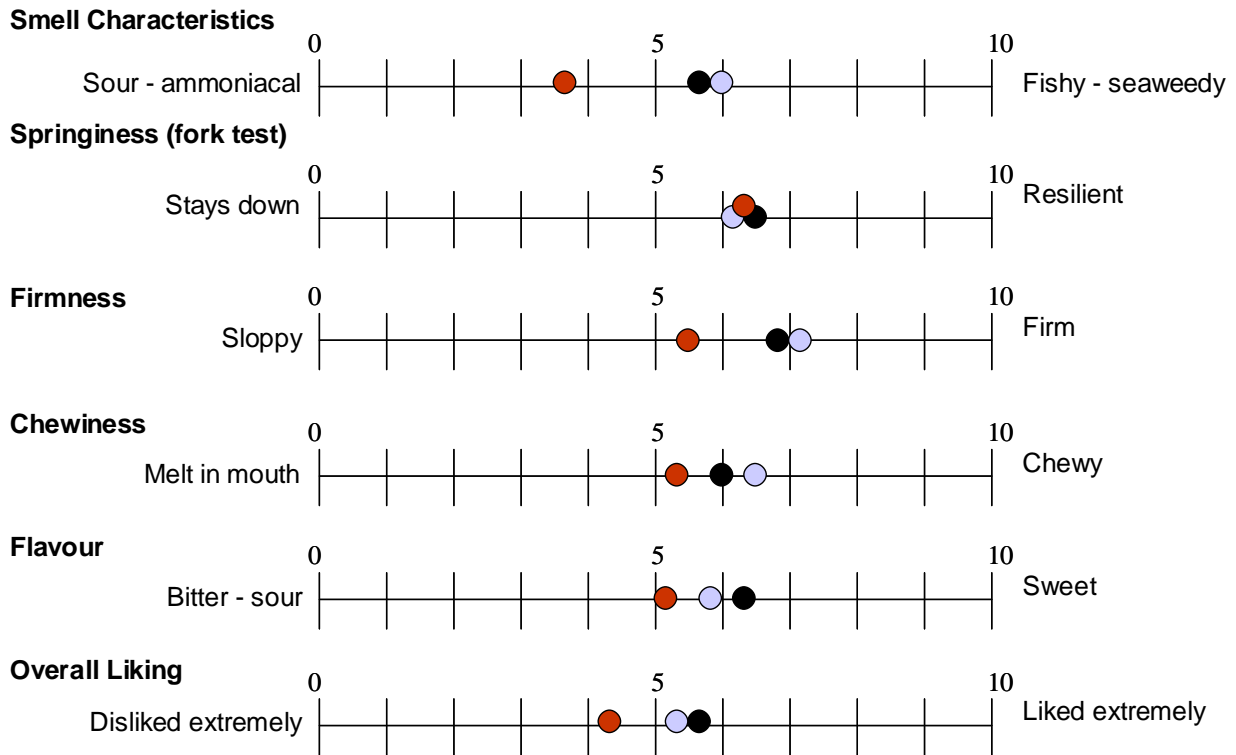
Figure 3.57 Sensory panel scoring from ● pre-pack, ○ 10:80 and ● air packs on day 8 of storage using a stepping temperature regime.

From this experiment samples at different times of storage were also kept frozen and sent to the industrial partner 'Young's Seafood Ltd.' for a sensory evaluation. The protocol used for shelf life assessment is detailed in Table 3.11 and the scheme used by the industrial partner to assess the samples is shown in Figure 3.58.

Table 3.11 Scoring system used by the industrial partner to do a sensory assessment of whole *N. norvegicus* on the present trial.

Rating Scale	Cooked odour	Cooked flavour
10	Weak sweet, milky	Saccharin
9	Clean and fresh	Slight sweet, mild shellfish
8.5	Clean Seaspray	Metallic, slight sweet, slight shellfish
8	Fresh seawater	Weak metallic, vanill, slight sweet
7.5		Slight weet, vanilla
7	Fresh	No sweetness
6.5	Slight shellfish	Neutral, very little flavour
6		Insipid
5.5	Slight ammonia, off odours	Slightly chalky, off flavours
5	Off odours, mousy	Chalky, off flavours
4	Off odours, strong mousy, musty	Strong off flavours, strong stale
3	Strong off odours, strong sour, faecal	Bitter, strong sour, faecal
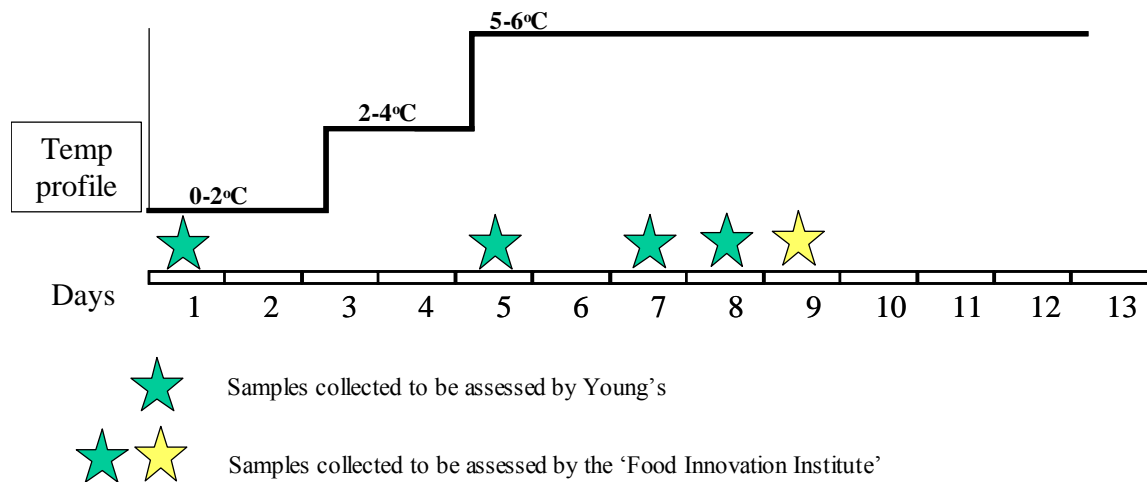
Figure 3.58 Schematic drawing showing the sensory sampling performed in the present MAP trial

From the sensory evaluation performed it would appear that both conditions, OGM and air packs would have the same shelf life of around 6 days (Table 3.12). These results were not in agreement with the results from the Food Innovation Institute, since it would appear that no gain in shelf extension is obtained with MAP technology. There are several possible explanations for this disparity in results that can be summarised as follows:

- The sensory evaluation performed by the industrial partner is much stricter that the evaluation performed by the 'Food Innovation Institute'

- Sample bags send to the industrial partner were not in the same condition as sample bags send frozen to the 'Food Innovation Institute'

To clarify if the sensory panel of the industrial partner could detect an extension of shelf life using the OGM a further experiment was performed in which technical staff from the company 'Young's Seafood Ltd.' perform both the MAP packing procedures and the subsequent sensory assessment of the product using their normal sensory evaluation methods. This trial also allowed the transfer of knowledge of the MAP

protocol from the University of Glasgow to the industrial partner, and is fully documented at the end of WP3.

Table 3.12 Sensory evaluation performed by the industrial partner of whole *N. norvegicus* on the present trial.

Treatment	Cooked aroma	Cooked flavour
Pre-pack	8.5	9
10:80 Day 5	8.5	9
Air Day 5	7	8
10:80 Day 7	6.5	4
Air Day 7	6	4
10:80 Day 8	6	3
Air Day 8	4	4

Comparison of the effects of different temperature regimes on the bacteria counts and the QIM in OGM-MAP-packed whole N. norvegicus

The effects of storage at either a constant temperature (1 ± 1 ℃) or at 1 ± 1 ℃, 3 ± 1 ℃, and 5 ± 1 ℃, were compared with the previously described 3-step-time-temperature regime (Figs. 3.59 (a) and (b)). Until day 5 both temperature regimes produced similar QIM and bacteria count results. However, when the last temperature step (from 3 ± 1 ℃ to 5 ± 1 ℃) was introduced, the packs diverged in terms of both measures, so that by day 8 the packs at 5 ± 1 ℃ had $> 10^6$ bacteria, and a QIM above the rejection limit. The same was true for the H_2S-producing bacteria and luminous bacteria.

(a) Total bacteria count   (b) QIM
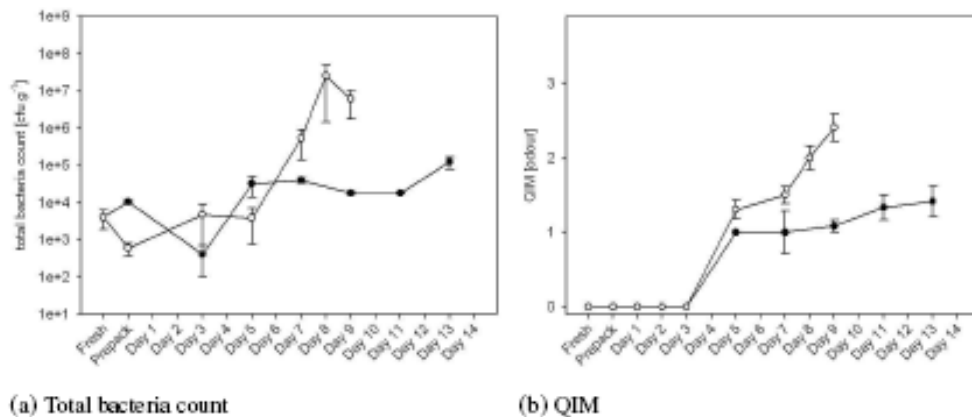
Figure 3.59 Various bacteria counts in whole *N. norvegicus* (a): (○) bacterial numbers in whole *N. norvegicus* packed in the OGM-MA. Samples where first stored at s1 ±C for the first 3 days, at s4 ±C for the following 2 days and finally at s6 ±C for the remainder of the storage time; (●) bacterial numbers in whole *N. norvegicus* packed in the OGM-MA. Samples where stored at 1 ± 1 ℃ during storage. Bacteria numbers are plotted on a logarithmic scale. Each point is the mean ± SEM of 3 x 5 samples. (b): (○) QIM results of whole, *N. norvegicus* stored using a stepping temperature protocol; (●) QIM results of whole *N. norvegicus* held at 1 ± 1 ℃. Each point is the mean ± SEM of 3 packs.

Similarly to the patterns observed in bacterial load and QIM, in this case K-values and hypoxanthine concentrations were similar in both experiments until day 5 of storage (Figure 3.60). However, when the last temperature step (from 3 ± 1 ℃ to 5 ± 1 ℃) was introduced, the packs diverged in terms of both measures, so that by day 7 the packs at 5 ± 1 ℃ had much higher K-value and hypoxanthine in the muscle compared to packs stored always at 1 ± 1 ℃. Eventually for both measures packs stored at constant low temperature reach same values as packs stored on stepping temperature regime on day 11 of storage.

a) K-values   **b) Hypoxanthine in the muscle**
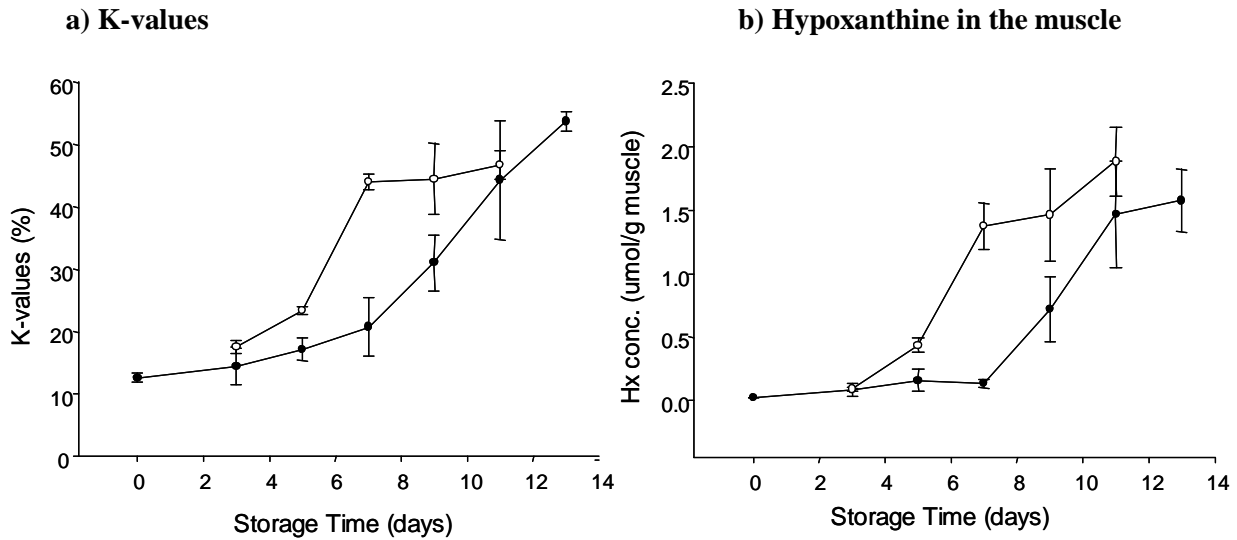
Figure 3.60 (a) K-values and (b) hypoxanthine concentrations in whole *N. norvegicus* during storage using different temperature protocols. (○) Whole *N. norvegicus* stored using a stepping temperature protocol; (●) whole *N. norvegicus* held at 1 ± 1 ºC. Each point is the mean ± SEM of 3 packs.

When comparing TMA concentrations in the muscle, differences between both temperature profiles are not so clear, probably due to a higher variability between samples. However, when looking at the trends of the curves it can be seen how in constant low temperature packs TMA starts to increase clearly on day 9 while in the stepping temperature packs it occurs on day 7 (Figure 3.61).
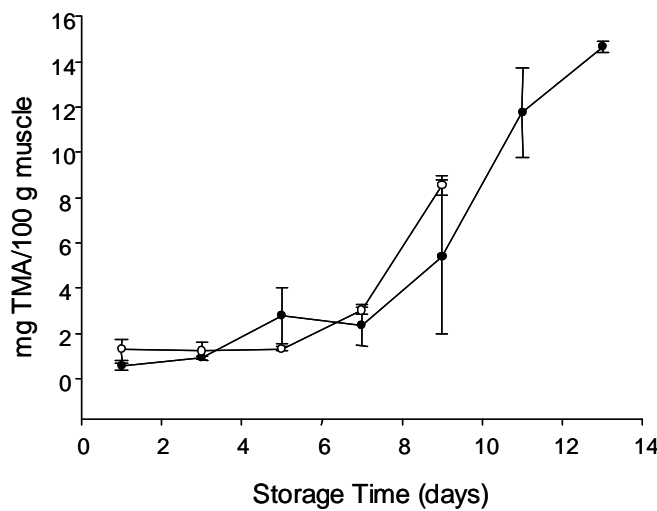
Figure 3.61 TMA concentrations in whole *N. norvegicus* during storage using different temperature protocols. (○) Whole *N. norvegicus* stored using a stepping temperature protocol; (●) whole *N. norvegicus* held at 1 ± 1 ºC. Each point is the mean ± SEM of 3 packs.

Therefore, when the proposed 3-step time-temperature regime was utilized the performance of the OGM-MAP was altered if compared to the constant low temperature storage regime. Interestingly, in the first 5 days of storage (while the temperature was in the range 1-4 ℃) the bacterial numbers, QIM, K-values and hypoxanthine concentrations were comparable with the results obtained with the OGM-MAP in the constant temperature trial, and no significant differences can be detected. However, once the temperature is stepped up to 5-6 ℃ the results diverge. Within 2 days of storage (day 7), the OGM packs from the elevated temperature group express significantly higher bacteria, QIM, K-values and hypoxanthine levels. Bacterial growth rates are greatly affected by temperature and even though the differences of 3 ℃ (from 1 to 4 ℃) and 2 ℃ (from 4 to 6 ℃) appear marginal, effects can be extensive. This is also known from other studies of seafood products. For example, an increase of 4 ℃ (from 4 to 8 ℃) reduced the shelf life of cod by more than 50 % (Reddy et al., 1999). It is reported that storage temperatures below 5 ℃ are beneficial and it is known that at storage temperatures above 5 ℃ the bacteriostatic effect of CO_2 becomes negligible (Cann, 2001).

Results on Improper Storage using a stepped temperature regime: what happens if the chill facility fails to work properly?

As discussed above, MAP technology does not work effectively if the temperature cannot be strictly controlled and low temperature (< 5 ℃) cannot be ensured. Such a failure occurred in one of trials conducted in the present study, which affected the pre-conditioning phase during the first 24 h after capture. The trial was nevertheless

continued, in order to evaluate the consequences of such lack of temperature control on the subsequent MAP packing. The fact that the chill facility was non-functional meant that:

- Langoustines stored in a fish box containing ice on top were not stored in a cold room at 5° C overnight (Figure 3.62) and that

- Packs after being prepared containing the OGM or air mix were not kept in a cold room until they were finally transported to the University of Glasgow.
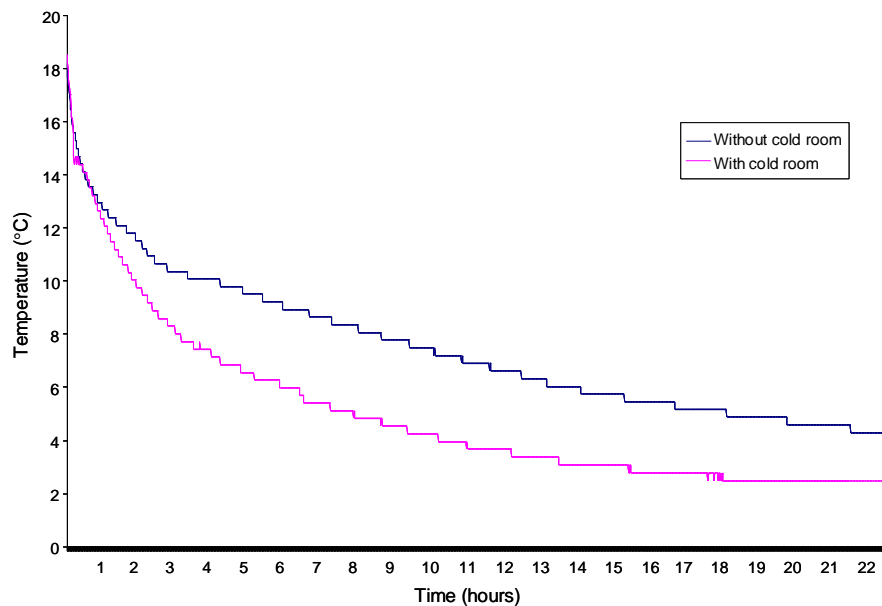
Figure 3.62 Temperature profiles in the middle of a fish box containing langoustines with ice on top and left in a working cold room 5-6 ºC (previous experiment) or not (present experiment) overnight.

In this trial the total bacteria load was similar in OGM and air packs up to day 3 of storage. On day 5, a clear increase was observed in bacteria numbers in both treatments (Figure 3.63). This situation was different from that in the previous experiment where the chill facility was operational. In that experiment, bacteria load in OGM packs was low on day 5 as well and did not increase until day 7 of storage. For instance, when the cold room was working bacteria on OGM packs on day 5 was

3.58 LOG$_{10}$ cfu/g while in this experiment where the cold room was not working the bacteria in OGM packs on day 5 was 5.64 LOG$_{10}$ cfu/g. This difference in total bacteria load indicates how crucial it is to maintain low temperatures throughout the whole process from catch to the consumer.
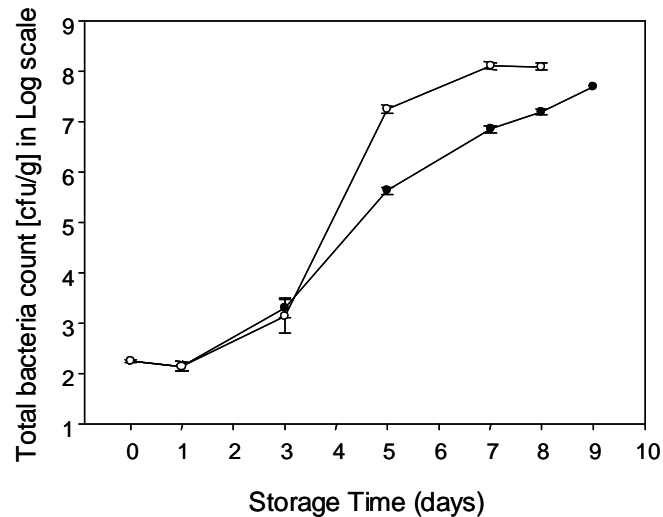
Figure 3.63 Total Bacteria counts in whole *N. norvegicus*. Samples where stored following a stepping temperature protocol; (●) bacterial numbers in whole *N. norvegicus* packed in the OGM-MA; (○) bacterial numbers in whole *N. norvegicus* stored in air. Bacteria numbers are plotted on a logarithmic scale. Each point is the mean ± SEM of 3 x 5 samples.

Similarly to the bacterial load results, QIM [odour] were affected by the fact that the cold room was not working and packs were left temperature un-controlled. QIM were low on day 3 but again increased considerably in both groups on day 5 (Figure 3.64). Although values were better in the OGM packs compared to air packs, absolute values were higher especially for OGM packs indicating that the overall quality was compromised by handling temperature.
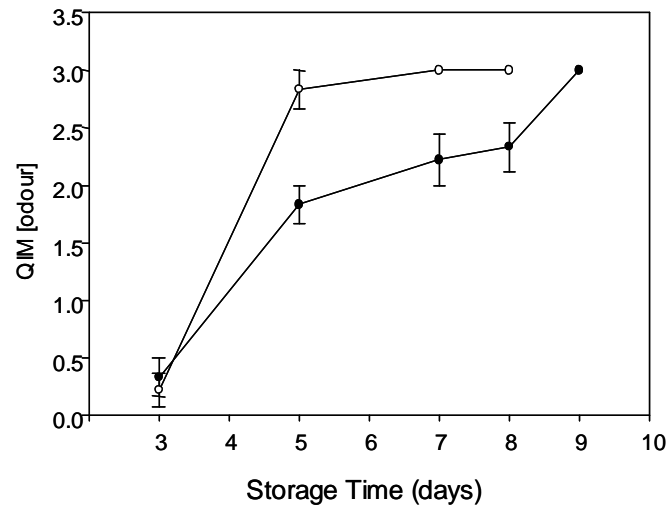
Figure 3.64 QIM [odour] in whole *N. norvegicus*. Samples where stored following a stepping temperature protocol; (●) bacterial numbers in whole N. norvegicus packed in the OGM-MA; (○) bacterial numbers in whole *N. norvegicus* stored in air. Each point is the mean ± SEM of 3 x 5 samples.

K-values were also measured as this parameter has shown previously to closely indicate temperature abuses in langoustine samples by our group. K-values in the OGM packs were lower than in air packs (Figure 3.65). However, more interesting observations can be drawn when comparing this experiment with the previous trial. K-value in OGM packs on day 7 was around 40 while in the previous experiment, where the cold room was working, was around 30. Likewise, hypoxanthine levels were higher in this experiment compared to the previous experiment for OGM and also air packs. Therefore, apart from comparing the effect of MAP it is important to understand the importance of absolute values that will determine the quality of the products.

a) K-values

| 10:80 |
| AIR |

b) Hypoxanthine in the muscle
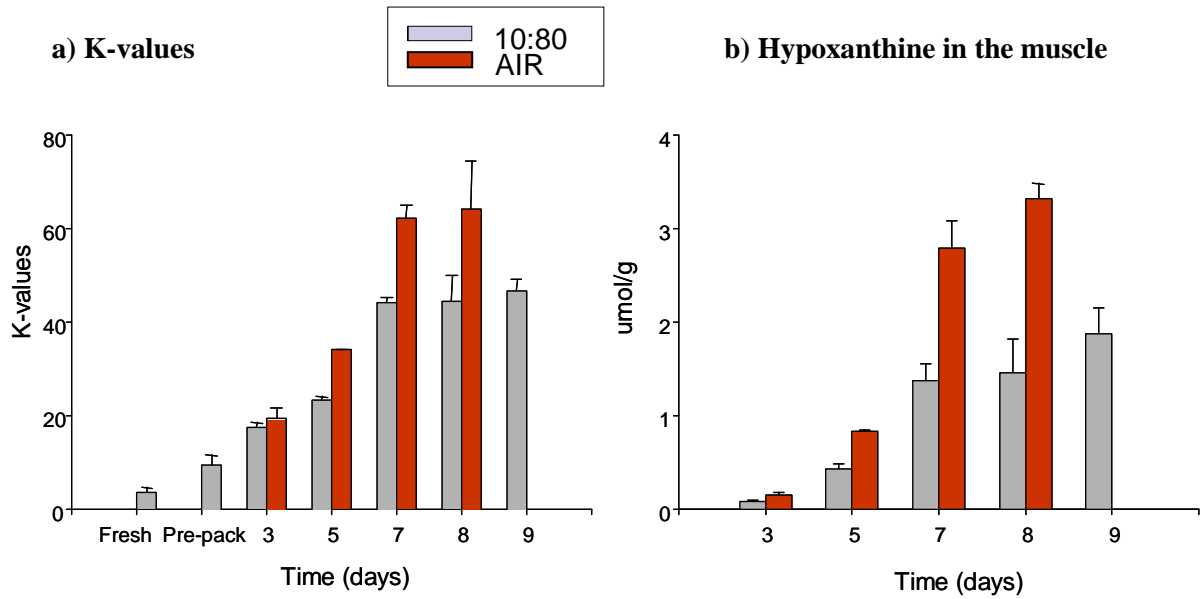
Figure 3.65 (a) K-values and (b) hypoxanthine concentrations in whole *N. norvegicus* during storage following a stepping temperature protocol. Each point is the mean ± SEM of 3 independent measures per condition and storage time.

TMA concentrations were also determined in the different samples and results are plotted on Figure 3.66. This compound increased in both groups considerably on day 5 of storage. At this point concentrations of TMA in both treatments were above 10 mg/100 g of muscle. After this time, slightly lower concentrations were obtained in the OGM packs compared to air packs. However, absolute values of TMA in this experiment were higher to previous experiment which is in agreement with bacterial load and QIM results indicating a much poorer quality in this experiment regardless of the MAP treatment.
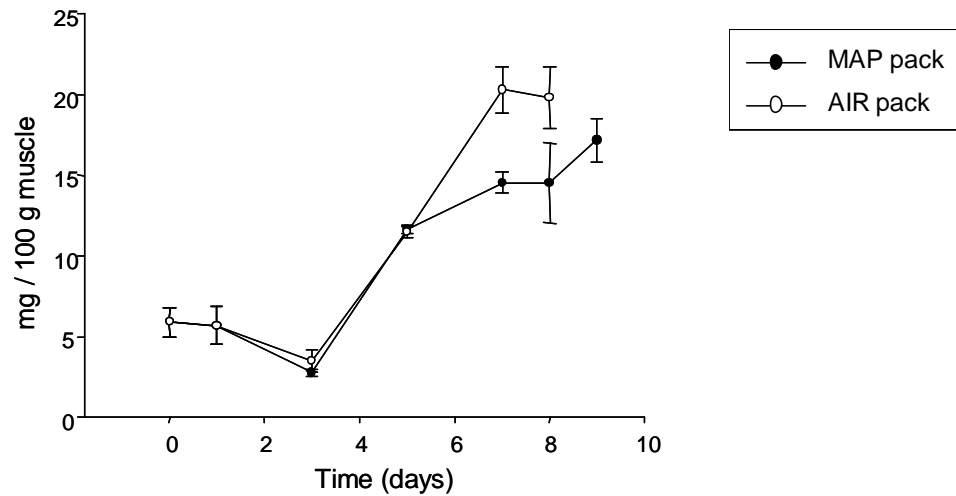
Figure 3.66 TMA concentrations in whole *N. norvegicus* during following a stepping temperature protocol. Each point is the mean ± SEM of 3 independent measures per condition and storage time.

As a final point, samples from this experiment were also tasted by the professional sensory panel (full report on Annexe 2). The fact that the cold room was not working at therefore temperature was not strictly maintain low throughout the whole process proved to have a major impact in the quality of the langoustines. This impact already described using laboratory techniques was supported by the panel that tasted the samples (Table 3.13). Smell characteristics were described in general as quite sour-ammoniacal especially at day 7 of storage and independently of the MAP treatment. The sample with the strongest smell was on packs with OGM on day 9 (note that no air packs on day 9 were offered to the tasting panel) while the one with the weakest smell was the 10:80 on day 5 of storage followed by pre-pack samples. These 2 samples were also described as having slightly sweet flavour while samples from both treatments on day 7 were bland or slightly bitter and 10:80 on day 9 and air on day 8 were perceived as sour or bitter flavour. According to the panel the most liked sample from this lot was the pre-pack followed by the 10:80 on day 5. For each storage time, packs containing the OGM scored better in overall liking compared to air packs.

Table 3.13 Results from whole OGM-MAP-and air packed *N. norvegicus* held using a stepping temperature regime for up to 9 days without a cold room

Sample		Smell Character.	Smell Strength	Pink Patches	Inside Colour	Springiness	Firmness	Chewiness	Moistness	Flavour	Aftertaste	Overall Liking
Pre-pack	Mean	5.22	6.38	6.07	6.45	5.32	5.46	4.72	5.85	6.09	6.04	5.47
	St. D.	1.42	1.08	2.80	2.10	2.94	1.20	0.71	1.36	1.74	1.82	2.20
10:80 day 5	Mean	4.69	5.94	6.44	5.11	6.48	6.88	5.97	5.18	5.45	4.74	4.64
	St. D.	2.12	1.98	1.89	1.93	1.94	1.71	1.89	1.88	1.58	1.98	2.80
10:80 day 7	Mean	2.83	6.74	6.86	5.17	6.64	7.97	7.74	4.77	4.52	4.43	3.94
	St. D.	1.68	2.19	1.95	1.93	2.25	1.47	1.47	1.88	2.22	2.31	2.94
Air day 7	Mean	3.13	7.51	6.19	4.32	6.10	6.31	5.85	5.07	4.84	4.44	3.52
	St. D.	2.28	1.83	1.73	1.59	2.81	1.24	0.94	1.73	1.85	1.81	2.34
10:80 day 8	Mean	2.69	7.18	7.25	4.94	6.17	7.03	6.66	4.39	5.29	4.47	3.48
	St. D.	1.72	1.61	1.64	1.19	1.86	1.36	1.26	1.62	1.72	2.18	2.16
Air day 8	Mean	2.75	7.60	6.35	3.44	6.36	6.59	5.88	5.36	3.76	3.19	2.72
	St. D.	1.88	1.81	2.21	1.77	2.27	1.76	1.95	1.56	2.12	2.11	2.55
10:80 day 9	Mean	2.06	8.04	7.14	4.81	6.36	7.58	7.19	4.89	3.73	3.26	2.53
	St. D.	1.76	1.66	1.70	1.89	2.41	1.48	1.83	2.27	2.02	2.05	2.76
	F	8.715	2.357	4.311	3.211	0.596	3.466	5.559	0.741	4.309	5.090	4.291
	Sig.	5E-12	0.01	1E-05	6E-04	0.83	2E-04	2E-07	0.698	1E-05	8E-07	1E-05
ANOVA		***	*	***	**	NS	***	***	NS	***	***	***

However, values in this experiment were different especially when compared to previous experiments. Smell characteristics scored lower than in previous experiment and taste was also described as more sour. On the other hand texture attributes scored in general slightly higher and the overall liking was affected negatively by these changes (Figure 3.67).
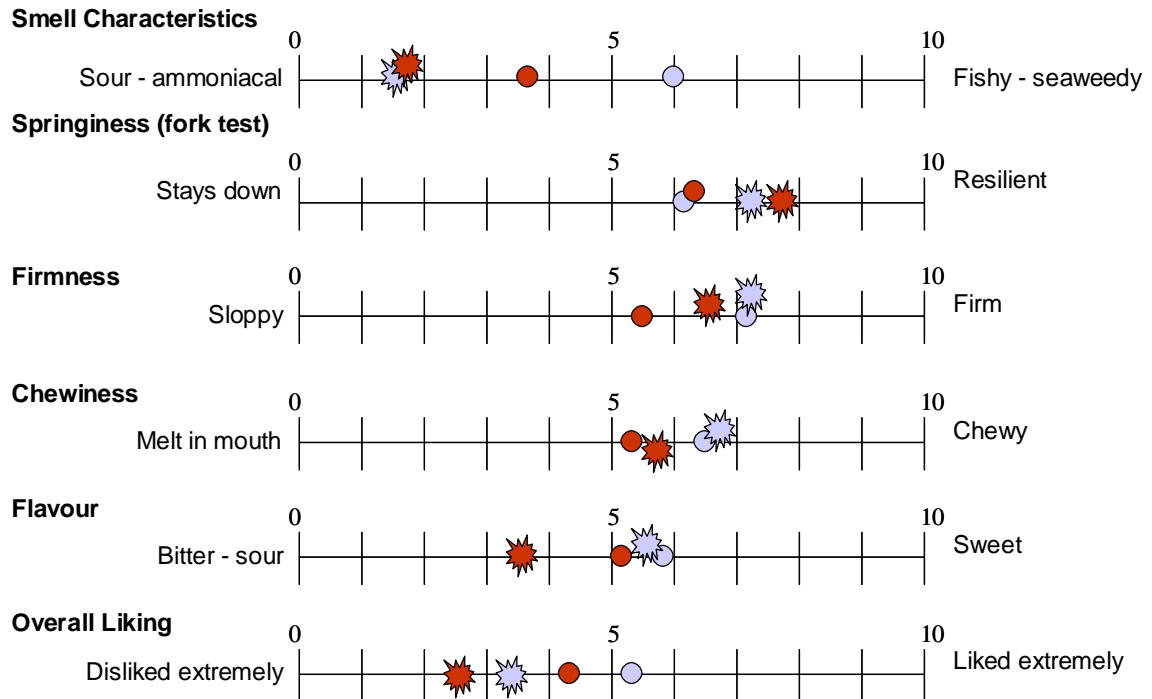
Figure 3.67 Sensory panel scoring from 10:80 and air packs on day 8 of storage using a stepping temperature regime with and without a cold room to ensure strictly low temperatures throughout the whole process. ○ 10:80 with cold room; ● Air with cold room; ✶ 10:80 without cold room and ✶ Air without cold room

Therefore, the conclusion of this trial is that if a low temperature cannot be ensured during post-capture handling, then the application of MAP technology cannot rescue spoiled samples.

Experiment to test the viability to pack more animals per pack using the OGM

Through discussions with the commercial partner, Young's Seafood Ltd., it was decided that one of the important variables in MAP packing was the number of animals per pack. In all the previous experiments 200 ± 20 g of whole langoustines were placed in each pack. This ratio of animals/pack had been adopted since this

amount of product would be easily marketable and it looked very appealing from a commercial point of view. However, another possibility would be to use the OGM to transport animals from UK to other European countries, such as Spain, Italy and France and then sell the langoustines as fresh taking them out from their MAP packs before retailing to the consumer.

To test this possibility packs of both 200 ± 20 g and 400 ± 20 g were prepared and tested, in order to determine if the OGM applied at this new ratio of product/gas would be still effective in extending the shelf-life of the langoustines. To test a higher product weight/gas ratio trays of greater strength were used in order to minimize the possibility of 'pack collapse'. The trays used for this experiment had a gauge of 800 μm material, an increase of 100 μm (14.3%) compared to the trays normally used that have a gauge of 700 μm material.

In this trial packs were stored at a constant temperature of 1 ± 1 ºC throughout storage in order to test over an extended period.

The first observation was that packs with 400 ± 20 g of langoustines did present 'pack collapse' (Figure 3.68) due to the absorption of CO_2 into the greater amount of animal tissues. The negative pressure in the pack induced considerable deformation, and the film was totally applied to the surfaces of the animals. The main problem of this is not the appearance of the pack (since consumers would not necessarily see it) but that contact of the film with the animals resulted in many cases in puncture of the film on one or more of the many sharp cuticular protuberances on the body and the claws. In

fact around 40 % of the packs containing 400 ± 20 g of langoustines had pierced

films, and had to be discarded.

Figure 3.68 Digital images showing the appearance of a 200 ± 20 g packs on the left and 400 ± 20 g packs on the right on day 5 of storage (note the negative pressure on both packs that caused pack collapse in the packs containing 400 ± 20 g of whole langoustines).

However, in terms of the effectiveness of the OGM in extending the shelf-life with

these two different packing ratios, no main differences were found. QIM scores

[odour] were very similar in both pack types (Fig. 3.69).
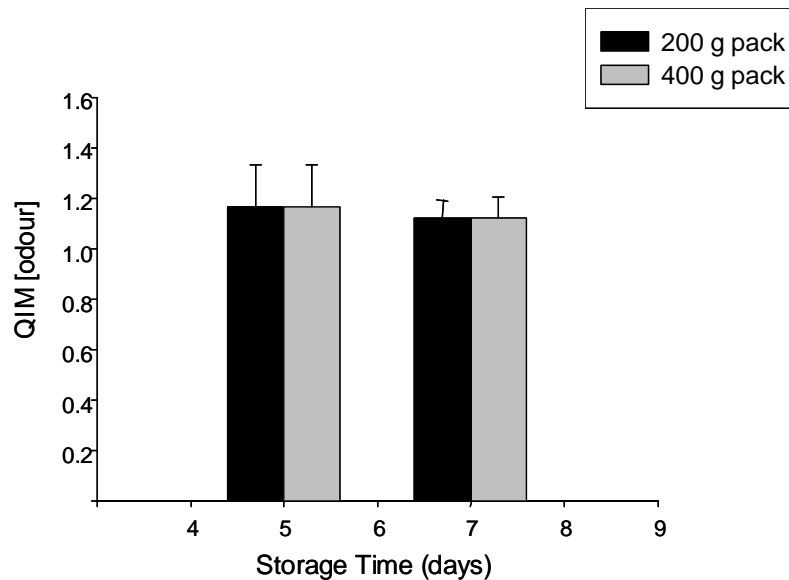
Figure 3.69 QIM [odour] scores in 200 ± 20 g packs and 400 ± 20 g packs on days 5 and 7 of storage at a constant temperature of 1 ± 1 ℃.

The total bacterial load was also similar in both pack conditions on days 5 and 7 of storage suggesting that this ratio of product/gas is still sufficient to provide the advantages of the OGM on the shelf-life extension of whole langoustines (Figure 3.70).
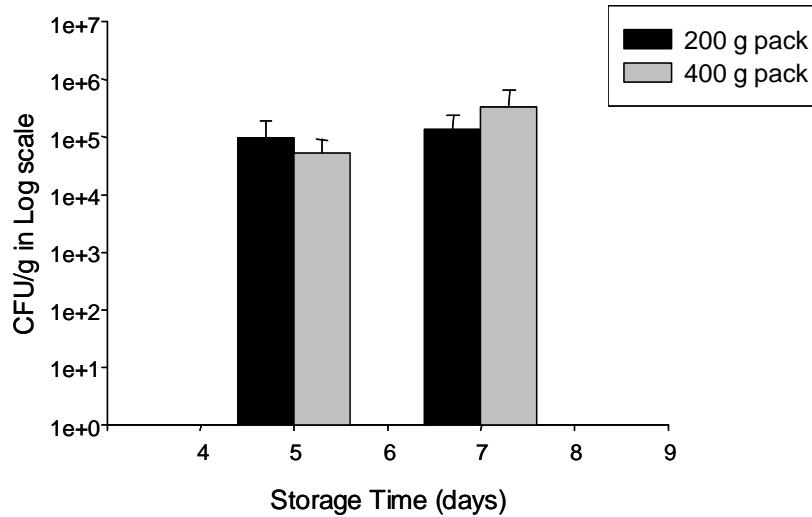
Figure 3.70 Total bacteria counts in 200 ± 20 g packs and 400 ± 20 g packs on days 5 and 7 of storage at a constant temperature of 1 ± 1 ºC.

Therefore, although this new higher ratio of product/gas seemed to work as well as a lower ratio, due to the occurrence of 'pack collapse' there would be considerable difficulties in transporting langoustines in that way to gain the benefits of MAP technology.

To avoid pack collapse it may be possible to use a sealing film of much greater thickness, although this may not cost-effective. Alternatively, measures could be introduced to prevent the animal tissues absorbing the CO_2 from the pack. One way to accomplish this is to re-pack the animals 24 h after they have been packed in the OGM, so that the tissues are by then partly or completely saturated with CO_2 and so will not absorb more form the second package.

In order to test if that would be a possible avoid 'pack collapse' in this way, packs containing 400 ± 20 g of langoustines were pack in the OGM as done in all previous experiments. However, 24 h later packs were opened and packed again containing the OGM. This protocol seemed to be very successful avoiding 'pack collapse' and no negative pressure was obtained in packs that had been re-packed in that way, even using a high ratio of product/gas.

Transfer of methodology and knowledge of the MAP technology to the
industrial partner 'Young's Seafood Ltd'- Phase IV

Once the OGM for MAP technology was identified and the effect of several parameters studied it was necessary to transfer the methodology to the industrial partner. To this end, a trial was arranged in which three technical staff from the company 'Young's Seafood Ltd.' came to the Marine Biological Station in Millport and performed a trial in conjunction with the scientific team from the University of Glasgow.

In November 2007, 3 members of the industrial partner and 1 member of the University of Glasgow joined the Research vessel 'Aplysia' from the UMBSM. Animals were collected by otter trawl from the Clyde Sea area and once on board the *N. norvegicus* were carefully washed with running seawater and transported to the Marine Biological Station in Millport. Animals were left overnight with ice on top in a cold room (5-6 ℃) until the following day.

Next day, animals were washed with running seawater and dipped in 2 % Melacide-SC20 for 15 min. Afterwards, animals were packed in the standard way using the OGM or air and transported on the same morning in a refrigerated truck to the facilities from the industrial partner in Grimbsy. Sensory assessment was carried out on days 1, 4, 5, 6, 7 and 8 of storage by the sensory panel from the industrial partner. Gas composition of the packs was checked and any punctured packs were immediately discarded from the analysis.

Furthermore, fillets from whitefish obtained from commercial vessels were transported from Grimsby to Millport on the day of the MAP packing and were packed in the OGM developed for *N. norvegicus,* and for comparison some fillets were packed on the MAP gas mix currently used by the industrial partner (containing 45 % of CO_2) and or air. Once packed, samples were transported back to Grimsby together with the samples from *N. norvegicus*. Finally, some samples from *N. norvegicus* were kept by the industrial partner and analysed for sulphite residue at the end of the trial.

The organoleptic shelf life assessment carried out by 'Young's Seafood Ltd.' on whole *N. norvegicus* and white fish consisted on the score of:

- Raw appearance

- Raw aroma

- Cooked whole appearance

- Cooked aroma

- Cooked texture

- Cooked flavour

The scoring was from 1-10 as follows:

❖ 10: Excellent

❖ 8: Very good

❖ 7: Good

❖ 6: Acceptable

❖ 5: Unacceptable

❖ 4: Poor

❖ 1: Inedible

Results on the organoleptic sensory evaluation performed by 'Young's Seafood Ltd.' on MAP- and air-packed *N. norvegicus* are shown in Table 3.14. Taking into consideration that a score of 5 represents an unacceptable product a safe shelf life of 6 days for *N. norvegicus* packed on air can be assigned, while the same animals packed on the OGM would have a shelf life of 8 days. Results from the industrial partner agree with the results obtained by the University of Glasgow, where a 2-day shelf life extension was determined when compared to product packed on air.

Table 3.14 Organoleptic shelf life assessment performed by 'Young's Seafood Ltd.' on MAP and air packed whole fresh *N. norvegicus*

LANGOUSTINES in MAP

Atribute/Day	1	4	5	6	7	8
Raw Apperance	10	9.5	8	8	6.5	7
Raw Aroma	10	8.25	7.75	7.75	6.75	6.75
Cook Apperance	10	9.5	8.5	7.5	6.25	6.5
Cook Aroma	8.75	9	8	6.75	6.5	6.5
Cook Text.	8	8	8.25	8	7	7
Cook Flavour	9	9.25	9.5	8	8	7.25

LANGOUSTINES in AIR

Atribute/Day	1	4	5	6	7	8
Raw Apperance	10	10	6.5	7.5	5.75	5
Raw Aroma	10	6.5	6	6.5	5.75	5.75
Cook Apperance	10	10	6.5	7	5.75	5.5
Cook Aroma	8.75	8	6.5	6.25	5.75	5.75
Cook Text.	8.25	8	7	6.5	6.5	6.8
Cook Flavour	9	8.5	8.75	7.25	6.5	6

On the other hand, sulphite residue levels were determined in the edible part of the *N. norvegicus* in the same samples on day 8 of storage. Samples were separated and frozen by the industrial partner and sent to CCFRA for the analysis of sulphite following the Monier-Williams methodology. Residue levels of less than 3 mg/Kg were obtained, confirming the results previously determined by the University of Glasgow (for more information see WP4).

On this trial fillets of whitefish obtained from commercial vessels were also packed using either 1) the OGM identified for the use in *N. norvegicus*, 2) the MAP gas mix currently used for this product by the industrial partner and 3) on air. Unexpectedly, since the OGM had been optimised for *N. norvegicus* but not for whitefish, results with the OGM 10:80 (O_2:CO_2) performed best (Table 3.15). Shelf life of whitefish fillets packed on air (score of 5 means unacceptable) was 4 days while shelf life using the current MAP mix from the industrial partner for this product was 5 days. However, using the OGM from the University of Glasgow optimised for *N. norvegicus* samples from day 8 were still acceptable.

Table 3.15 Organoleptic shelf life assessment performed by 'Young's Seafood Ltd.' on MAP and air packed whitefish fillets

Whitefish fillets in MAP 10:80

Atribute/Day	1	4	5	6	7	8
Raw Apperance	10	8	8	8	6	6.5
Raw Aroma	10	8	7.5	7.5	7	6.5
Cook Apperance	10	8	8	7	6.5	7
Cook Aroma	10	7.5	7.5	6.5	5.5	6
Cook Text.	10	8	7	6	7	6.5
Cook Flavour	10	8	8	6.5	7	6

Whitefish fillets in MAP currently used

Atribute/Day	1	4	5	6	7	8
Raw Apperance	10	8	8	6	6	6
Raw Aroma	10	8	7.5	6.5	6	6
Cook Apperance	10	8	8	6	6	6
Cook Aroma	10	7	7.5	5	5	5
Cook Text.	10	7.5	7.5	5	5	5
Cook Flavour	10	7.5	7.5	5	6	4

Whitefish fillets in air

Atribute/Day	1	4	5	6	7	8
Raw Apperance	10	8	8	7	6	6
Raw Aroma	10	8	7	7	6	6
Cook Apperance	10	7	8	5	6	6
Cook Aroma	10	6	5	5	5	5
Cook Text.	10	6	6	5	5	5
Cook Flavour	10	7	6	5	4	4

However, since this gas mix had not been tested before to be used in whitefish by the University of Glasgow more research should take place to evaluate its potential for whitefish fillets.

WP4. The effect of melanosis dipping on product quality

The main focus of this WP4 was to study the effects of anti-melanotic compounds on the quality of whole *N. norvegicus*. To this end, several experiments were conducted in order to answer the following questions:

- Do anti-melanotic compounds have any antibacterial action against spoilage bacteria, or have any impact on the quality of whole fresh *N. norvegicus*?

- What is the impact of the OGM-MAP on the blackening of *N. norvegicus*? Can MAP inhibit melanosis to the extent that no anti-melanotic is necessary?

- If anti-melanotic sulphite-based compounds are used (i.e. Melacide-SC20) what is the minimum effective concentration and what residue suphite levels are in the edible part of the animal?

Effect of anti-melanotic compounds on bacterial load and quality in whole N. norvegicus

In order to elucidate if commercially available anti-melanotic compounds have an antibacterial effect, and therefore a positive effect on the quality of whole *N. norvegicus,* the following experiment was performed.

Animals were caught by otter trawl in the Clyde Sea area using the RV 'Aplysia' from UMBSM. Once on board, animals were carefully cleaned with running seawater and the following treatments were applied:

- Un-dipped animals used as control, only washed with running seawater

- Animals dipped with a solution containing PrawnFresh™ (PF) from Xyrex® Ltd (Glasgow, UK) at the concentration of 1/1000 (v/v) for 15 min

- Animals dipped with a solution containing Freskor (FKR) from Hasenosa SA (Spain) at the concentration of 3 % (w/v) for 6 min

- Animals dipped with a solution containing Sodium Metabisulphite (NaMet) from Sigma-Aldrich (Dorset, UK) at a concentration of 2.5 % for 3 min

The concentrations and times of dipping were selected according to the recommended treatments by each manufacturer. Dipping took place in a 'dipping tank' containing seawater with the dissolved concentrations of anti-melanotic compounds. After treatment, animals were placed in plastic bags and stored on ice for up to 14 days. On days 3, 5, 8, 11 and 14 animals were sampled and the following analyses was performed:

- o Melanosis development was recorded using digital images and the Melanosis Score Index

- o Polyphenol oxidase activity was measured in the cephalothorax using a spectrophotometric method

- o Bacterial load was measured, including total viable counts, luminous bacteria, H_2S producing bacteria and *Pseudomonas* sp.

- o Muscle pH was measured

- o The K-value was derived, as a freshness indicator

Melanosis development or blackening took place as storage time progressed. The anti-melanotic compounds commercially available and tested in the present study were

successful in delaying melanosis development when whole *N. norvegicus* were stored

on ice (Fig. 4.1).
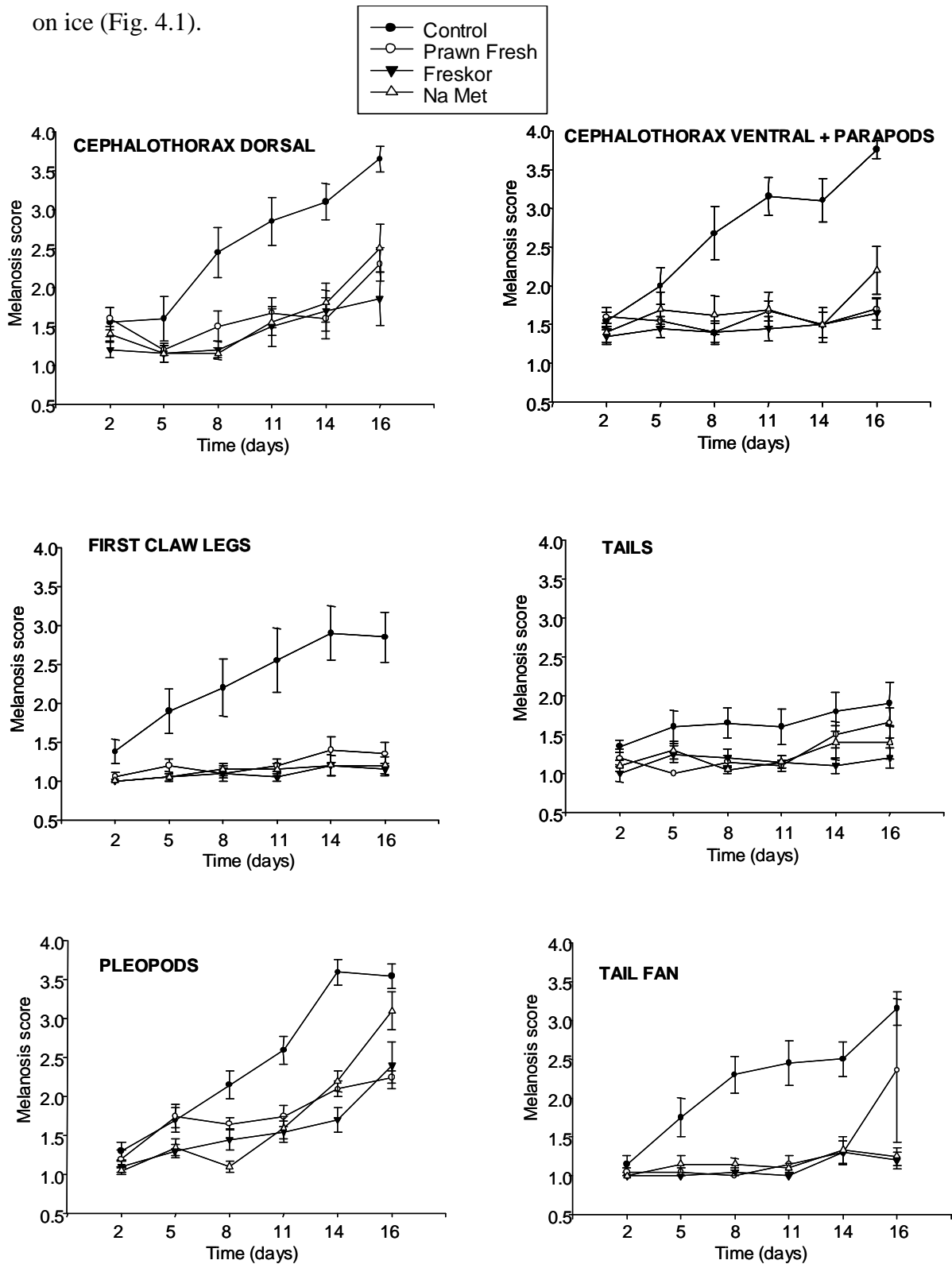
Figure 4.1 Melanosis scores in the different parts of the body in whole *N. norvegicus* un-dipped or dipped with PrawnFresh, Freskor or NaMet and stored on ice for up to 16 days.

Un-dipped animals developed melanosis more rapidly than dipped animals, and in some parts of the body melanosis had started from day 5 onwards. Blackening seemed to start in the dorsal and ventral cephalothorax first, then it seemed to spread onto the appendages and finally on the tails that were the parts least affected by melanosis. No large differences were found in the effectiveness of the different anti-melanotic products tested. However, to the eye it seemed that animals dipped with Freskor had a fresher and brighter look than animals treated with PrawnFresh and NaMet, an effect especially evident in the dorsal cephalothorax.

To further elucidate the anti-melanotic action of these compounds the polyphenol oxidase (PPO) activity was measured in the cephalothorax of the animals after the different treatments (Figure 4.2).

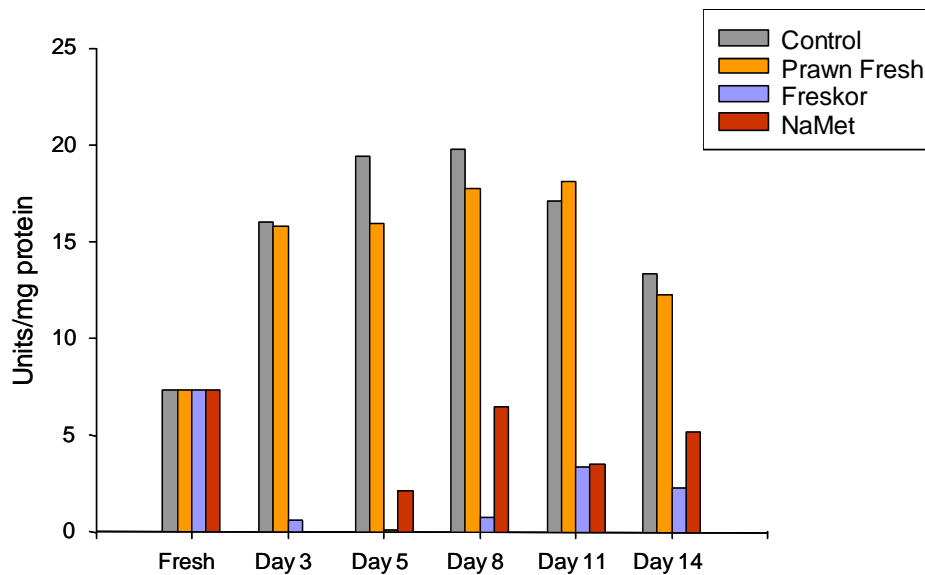**Figure 4.2** PPO activity in the cephalothorax of whole *N. norvegicus* un-dipped or dipped with PrawnFresh, Freskor or NaMet and stored on ice for up to 16 days.

Un-dipped animals had the highest PPO activity levels. These levels were surprisingly very similar to the PPO activities obtained in animals treated with PF. In both cases,

PPO activity increased up to day 8 of storage and started to decrease after this time. This decrease in PPO activity during the storage period could be related to PPO degradation by proteases, especially thiol proteases as described by Wang et al. (1994), a process that is associated with a final irreversible inactivation of the enzyme (Ramirez et al., 2003).

On the other hand, PPO activity was inhibited in animals treated with FKR and NaMet. In animals treated with FKR the enzymatic activity of PPO was almost totally suppressed up to days 8-11 of storage. In NaMet treated animals PPO re-appeared n day 5, and was thereafter constant at lower levels than in un-dipped animals.

PPO catalyzes the hydroxylation of o-monohydroxyphenols to o-dihydroxyphenols and the subsequent oxidation to quinones. The quinones are highly reactive and undergo non-enzymatic oxidation and polymeration to produce the coloured melanins responsible for the blackening in the shell (Soderhall and Cerenius, 1992).

FKR and NaMet are suphite-based compounds, which are strong reducing agents that take oxygen in competition to the enzyme PPO, thus inhibiting the formation of coloured melanins. Therefore, on the first days of storage the suphites from these 2 compounds could inhibit the PPO activity since they were able to interact with the formed quinones and reduce the orthoquinones back to the colorless di-phenols. Apart from containing sodium metabisulphite, FKR also contains citric acid, ascorbic acid, EDTA and sodium bicarbonate. Because PPO is dependent on copper ions for its activity, the presence of EDTA further inhibits the enzyme by binding to the copper ions. These extra additives may therefore explain why PPO was inhibited for a longer

period on animals treated with FKR compared to NaMet. However, as storage progressed sulphites were gradually consumed and the quinones could accumulate since not all PPO was inhibited.

On the other hand, the active ingredient in PF is 4-hexylresorcinol. The inhibitory effect of this compound has been attributed to a competitive inhibition due to its resemblance to the natural substrates for the enzyme (Diaz-Lopez et al., 2003). The fact that PF seemed not to affect the PPO activity when measured in the laboratory could be an artefact of the methodology used to measure the activity of this enzyme. Nevertheless, the effective dose of 4-hexylresorcinol differs depending on the species, physiological state and method of application.

Once the effectiveness of each compound on melanosis inhibition was established the next step was to evaluate the effect on these compounds on bacterial load. As shown in Figure 4.3 no main differences were obtained in total bacterial counts between the different treatments throughout the storage time tested.

Figure 4.3 Total bacteria counts in whole *N. norvegicus* un-dipped or dipped using different commercially available anti-melanotic compounds.

Pseudomonas sp. was also similar in the muscle of all the different treatment groups indicating that in the conditions of the present experiment the anti-melanotic compounds tested had no significant antibacterial effect.

Figure 4.4 *Pseudomonas* sp. counts in whole *N. norvegicus* un-dipped or dipped using different commercially available anti-melanotic compounds.

Muscle pH was also measured throughout the storage time tested. This parameter was proven to be an effective quality-age related parameter for *N. norvegicus* in Phase I of the Scottsih *Nephrops* Survey. Muscle pH increased with storage time linearly up to day 8 and then slowed down and no further increases were obtained between days 11 and 14 (pH at this point around 8.0-8.2) but again no differences were obtained according to different treatments (Fig. 4.5).
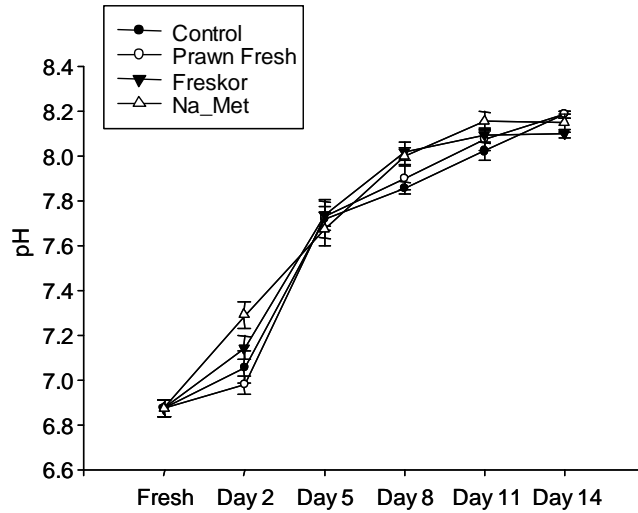
Figure 4.5 Muscle pH in whole *N. norvegicus* un-dipped or dipped using different commercially available anti-melanotic compounds.

K-values used as freshness indicator was also measured and results plotted in Figure 4.6. This parameter increases linearly with storage time and no differences were observed between the different treatments. In many fish species a K-value around 20 % has been set as a freshness limit, a value that was obtained in this experiment around day 5. However species differences are very important and therefore conclusions in this regard cannot be made at this point.
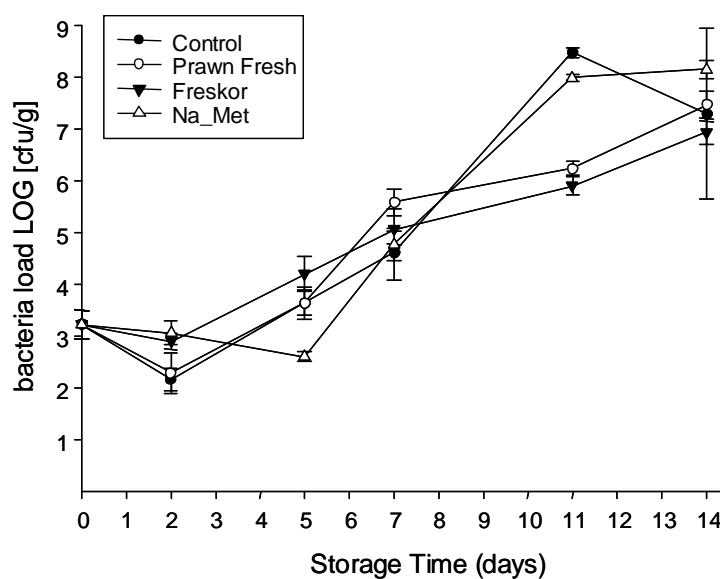

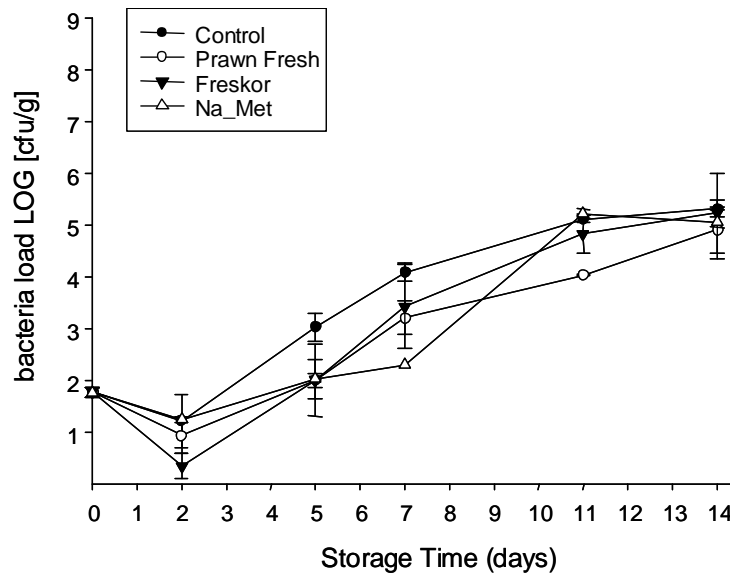**Figure 4.6** K-values in whole *N. norvegicus* un-dipped or dipped using different commercially available anti-melanotic compounds.

Taken together, all these results it would appear that anti-melanotic treatments are necessary in order to avoid blackening development in *N. norvegicus*. This is very important when working with whole animals since melanosis in cephalothorax starts relatively early especially if low temperatures are not ensured at all times. However, none of the commercially available anti-melanotics had an effect on bacterial load, muscle pH and K-values. Therefore, using these products a shelf-life extension is obtained exclusively by their visual impact on the product, but not by an effect on the condition of the meat due to any anti-bacterial effect.

Impact of the OGM-MAP on the blackening of N. norvegicus

Studies presented by Chen et al. (1992 and 1993) showed an inhibition of the enzyme PPO by CO_2. This effect would be very beneficial if proven in *N. norvegicus* packed under MAP rich in CO_2. Since the OGM was high in CO_2 levels (80 %) it was decided to explore whether this level of CO_2 would on its own be sufficient to inhibit blackening in whole fresh *N. norvegicus*, without the need for additive treatments.

In order to test this hypothesis, animals were treated following the protocol established in WP3 for MAP products. Thus, after holding in a clod room overnight in a fish box containing ice on top, animals were washed the following morning and the following treatments applied:

- o Un-dipped animals (i.e. washed with running seawater only)

- o Animals treated with PF® at the concentration of 1/1000 (v/v) for 15 min (PF)

- o Animals treated with Melacide-SC20 at the concentration of 2 % (w/v) for 15 min (M-High)

- o Animals treated with Melacide-SC20 at the concentration of 1 % (w/v) for 5 min (M-Low)

Afterwards, animals were packed in the OGM and stored following the commercial protocol approved by the industrial partner 'Young's Seafood Ltd.'. This protocol consisted of storing the animals at 0-2 ºC for up to day 3 and then changing them to a temperature of 2-4 ºC for up to day 5 and finally storing them at a temperature of 5-6 ºC. In this case, packs were stored for up to 13 days and melanosis development was studied on days 3, 5, 8, 10 and 13. As in previous experiments, melanosis was scored

using digital images and the melanosis index score. Furthermore, sulphite residue

analysis was performed in the edible part of the animals (tail meat).

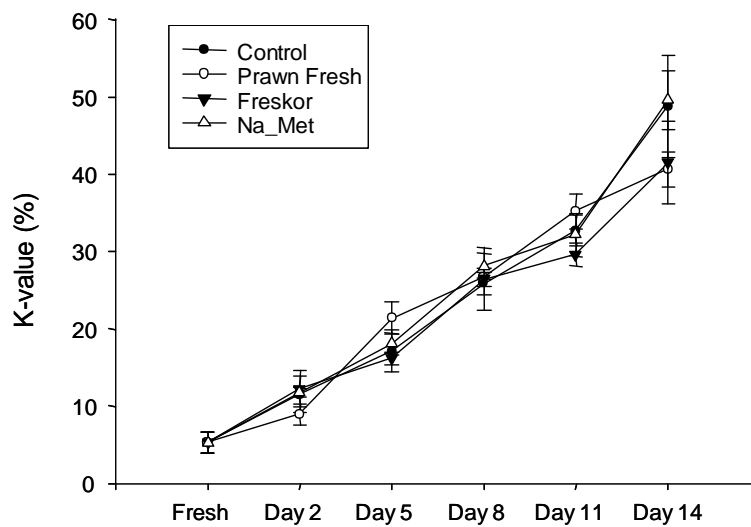**A) Day 5 of storage**   **B) Day 8 of storage**
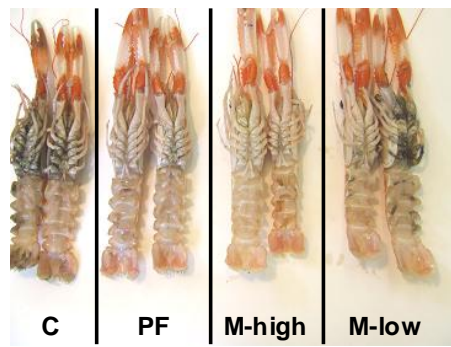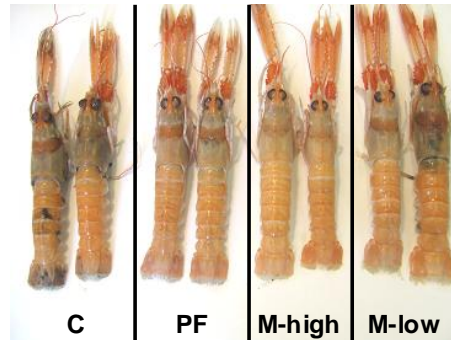
Figure 4.7 Digital images of whole *N. norvegicus* un-dipped or dipped using different commercially available anti-melanotic compounds together with MAP treatment. C) Un-dipped animals; M Low) melacide 1 % for 5 min; PF prawn-fresh; M High) melacide 2 % for 15 min

Results indicate that MAP treatment alone cannot inhibit melanosis effectively

enough to withdraw anti-melanotic additives under the storage conditions studied.
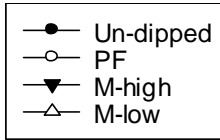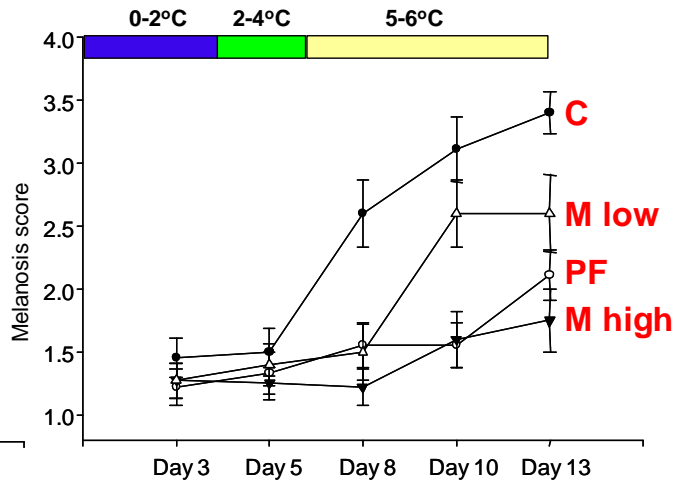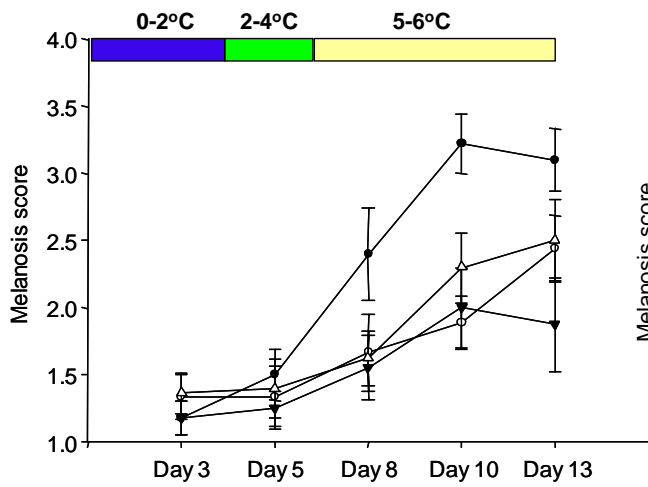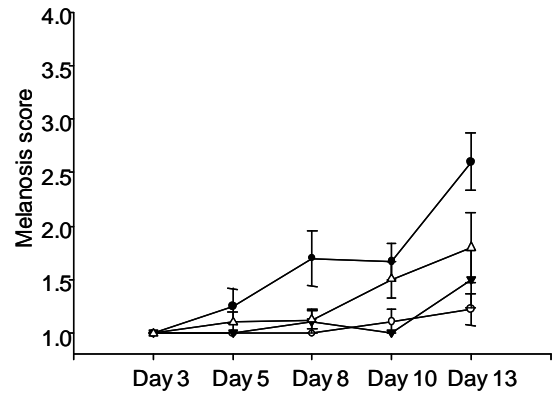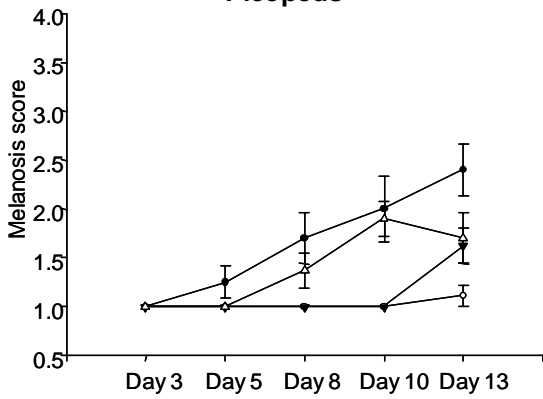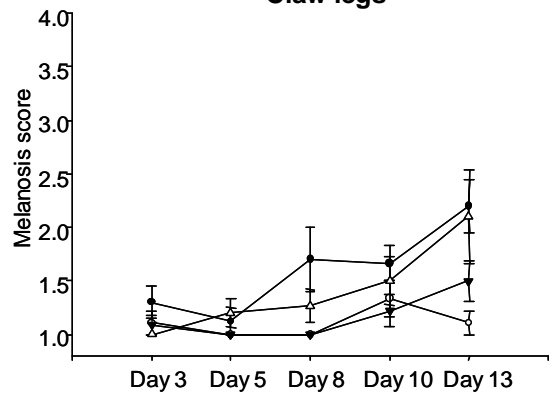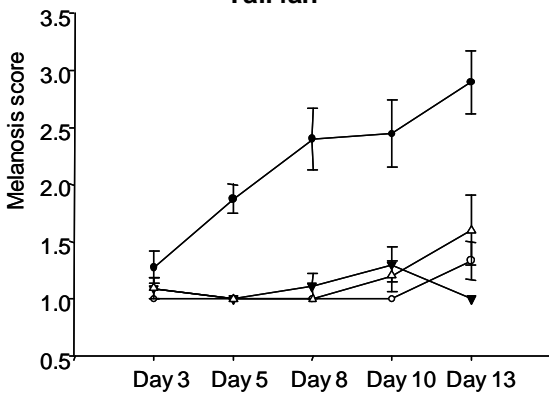
Figure 4.8 Melanosis Index Score of whole *N. norvegicus* un-dipped or dipped using different commercially available anti-melanotic compounds together with MAP treatment. C) Un-dipped animals; M Low) melacide 1 % for 5 min; PF prawn-fresh; M High) melacide 2 % for 15 min

Blackening started on day 5 of storage in un-dipped animals and it was very apparent on day 8 of storage (Figs. 4.7 and 4.8). When looking at the different anti-melanotics tested it would appear that PF and Mela-High were equally effective in retarding blackening development and therefore either of these two treatments could be used on whole fresh *N. norvegicus* stored under MAP conditions.

One of the issues raised by the industrial partner Young's Seafood Ltd. was to determine the lowest concentration of Melacide-SC20 needed in MAP products in order to effectively inhibit blackening development. This would have to main advantages, as it would:

- Reduce the amount of Melacide-SC20 used in the boats

- Reduce the residue levels of sulphite in the edible parts of the animal

To this end, in this experiment Melacide-SC20 was tested at a lower concentration (1 %) and at a shorter treatment time (5 min) to determine if it would be effective in inhibiting blackening development in MAP products. Results indicate that this concentration/treatment time was too low and some melanosis was detected on day 8 of storage.

Therefore from this experiment it was concluded that:

- MAP alone cannot inhibit melanosis development in the storage conditions studied

- PF 1/1000 for 15 min and Mela 2 % for 15 min were equally efficient in inhibiting melanosis while Mela 1 % for 5 min was too low to achieve the desired effect.

From these observations it was decided that more work was needed in order to find out the lowest effective concentration of Melacide-SC20 to be used in MAP products.

Optimization of the minimum concentration/treatment time of Melacide-SC20 to be used in MAP products

In order to find out the minimum effective concentration/treatment time combination of Melacide-SC20 to be used in MAP products, animals were treated following the protocol established in WP3 for MAP products. Thus, after holding overnight in a cold room in a fish box containing ice on top, animals were washed the following morning and the following treatments applied:

- o   Animals treated with Melacide-SC20 at the concentration of 1 % for 6 min
- o   Animals treated with Melacide-SC20 at the concentration of 1 % for 15 min
- o   Animals treated with Melacide-SC20 at the concentration of 1.5 % for 6 min
- o   Animals treated with Melacide-SC20 at the concentration of 1.5 % for 15 min
- o   Animals treated with Melacide-SC20 at the concentration of 2 % for 6 min
- o   Animals treated with Melacide-SC20 at the concentration of 2 % for 15 min

Afterwards, animals were packed in the OGM-MAP at stored using the stepping temperature protocol used in the previous experiment. Melanosis development was recorded using digital images and using the Melanosis Index Score.
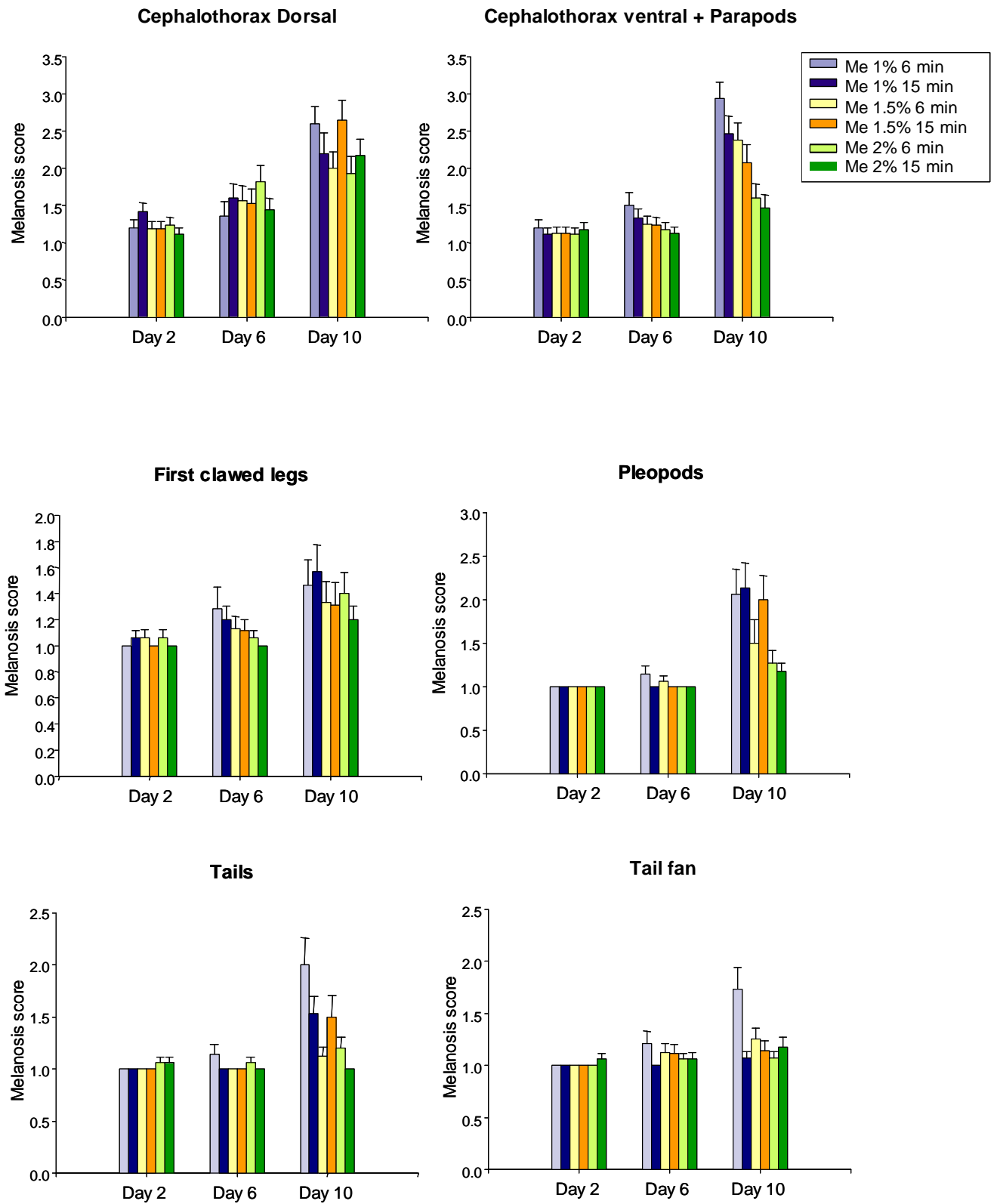
Figure 4.9 Melanosis Index Score of whole *N. norvegicus* dipped with melacide-SC20 at different concentration and treatment times together with MAP treatment.

The different effect of the decreasing concentrations/treatment times of Melacide-SC20 in MAP products was clearer as storage time progressed (Fig. 4.9). On day 2 of storage all the different concentration/treatment times gave a very similar inhibition of melanosis development. However, on day 10 of storage there is clearly a different degree of blackening inhibition due to the concentration and treatment time. The most effective combination was Melacide-SC20 at a concentration of 2 % for 15 and 6 min. A lower Melacide-SC20 concentration of 1.5 % was more unreliable even if applied for 15 min, and the concentration of 1 % independently of the treatment time was not effective, with melanosis being higher compared to the other 2 concentrations tested.

| M 1% | M 1% | M 1.5% | M 1.5% | M 2% | M 2% | M 1% | M 1% | M 1.5% | M 1.5% | M 2% | M 2% |
| 6 min | 15 min | 6 min | 15 min | 6 min | 15 min | 6 min | 15 min | 6 min | 15 min | 6 min | 15 min |
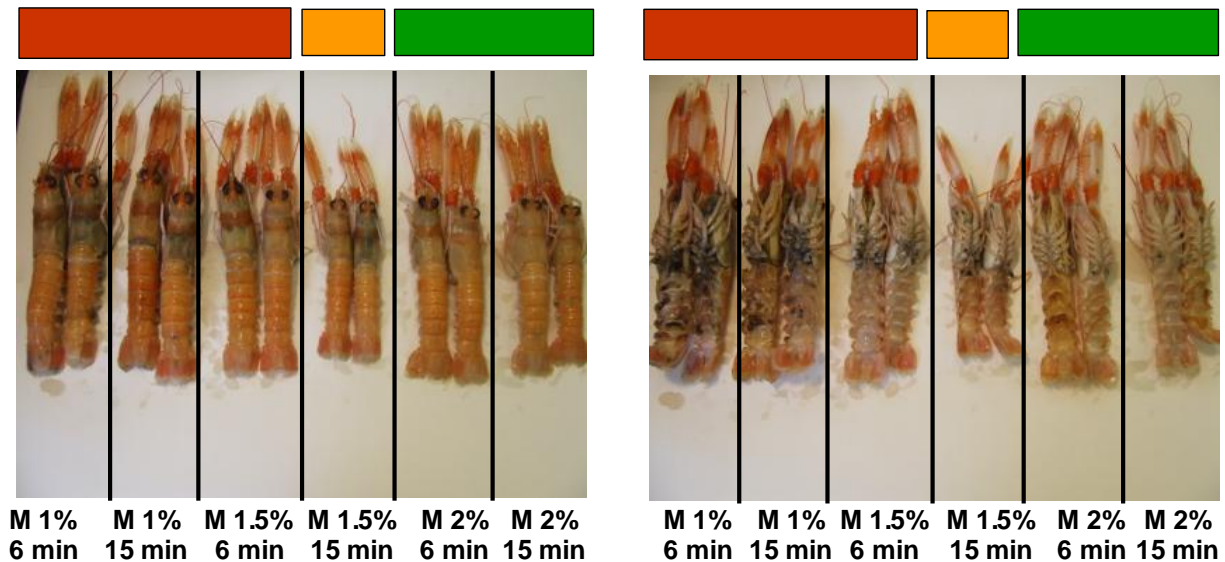
Figure 4.10 Digital images of whole *N. norvegicus* dipped with melacide-SC20 at different concentration and treatment times together with MAP treatment on day 10 of storage.

Since the concentration of Melacide-SC20 1.5 % proved to be unreliable and melanosis was present in some of the animals it could be concluded that the lowest effective concentration/treatment time combination was 2 % for 6 min.

The second important point of this experiment was to check the residue sulphite levels present in the edible parts of the treated animals. Sulphite analysis (TES-AC-094-

UKAS) UKAS accredited was performed by CCFRA Technology Limited in Camden, UK. The protocol measures sulphur dioxide (SO_2) using the Monier-Williams method. As shown in Table 3.1 in all cases the residue levels were lower than 10 mg/Kg. The detection limit of this assay depends on the actual weight of the sample. Given the amount of weight per condition we had available (100 g/condition) the detection limit in this experiment was 6 mg/Kg.

Table 4.1 Sulphur dioxide residue levels in the edible part of *N. norvegicus* dipped with melacide-SC20 at different concentration and treatment times together with MAP treatment on day 6 of storage.

	Sulphur dioxide residue in the un-cooked meat
Melacide 1 % 6 min	< 6 mg/kg
Melacide 1 % 15 min	< 6 mg/kg
Melacide 1.5 % 6 min	< 6 mg/kg
Melacide 1.5 % 15 min	9 mg/kg
Melacide 2 % 6 min	6 mg/kg
Melacide 2 % 15 min	7 mg/kg

The active ingredient in Melacide-SC20 is sodium hydrogen sulphate and its E number is E222. The fact that all samples contained a residue level lower than 10 mg/Kg was key finding. Following the European Parliament and Council Directive No. 95/2/EC it appears that if SO_2 content in the edible part is lower than 10 mg/kg it is considered not to be present and therefore it shall not be declared in the label of the product (Fig. 4.11).

EUROPEAN PARLIAMENT AND COUNCIL DIRECTIVE No 95/2/EC
of 20 February 1995
on food additives other than colours and sweeteners

(OJ No L 61, 18. 3. 1995, p. 1)

PART B

Sulphur dioxide and sulphites

E No	Name
E 220	Sulphur dioxide
E 221	Sodium sulphite
E 222	Sodium hydrogen sulphite
E 223	Sodium metabisulphite
E 224	Potassium metabisulphite
E 226	Calcium sulphite
E 227	Calcium hydrogen sulphite
E 228	Potassium hydrogen sulphite

Note

1. Maximum levels are expressed as SO_2 in mg/kg or mg/l as appropriate and relate to the total quantity, available from all sources.

2. An SO_2 content of not more than 10 mg/kg or 10 mg/l is not considered to be present.

Foodstuff	Maximum level (mg/kg or mg/l as appropriate). Expressed as SO_2
Burger meat with a minimum vegetable and/or cereal content of 4 %	450
Breakfast sausages	450
Longaniza fresca and *butifarra fresca*	450
Dried salted fish of the 'Gadidae' species	200
Crustaceans and cephalopods:	
— fresh, frozen and deep-frozen	150 [1]
— crustaceans, *panaeidae solenceridae, aristeidae* family:	
— up to 80 units	150 [1]
— between 80 and 120 units	200 [1]
— over 120 units	300 [1]
— cooked	50 [1]

Figure 4.11 Extract from the EU Directive 95/2/EC regarding the use of food additives (includes Melacide named E222) in Crustaceans.

In order to ensure that these sulphite residues were correct another experiment was carried out in which animals were dipped with Melacide-SC20 according to the following concentrations and treatment times:

- Animals dipped in Melacide-SC20 at the con. of 1.5 % for 15 min

- Animals dipped in Melacide-SC20 at the conc. of 2 % for 6 min

- Animal dipped in Melacide-SC20 at the conc. of 2 % for 15 min

- Animals dipped in Melacide-SC20 at the conc. of 2 % for 30 min

- Animals dipped in Melacide-SC20 at the conc. of 4 % for 6 min

- Animals dipped in Melacide-SC20 at the conc. of 4 % for 30 min

In this experiment 25 animals were used per condition and all the meat (edible part) from each condition (around 170 g) was sent on the following day in a frozen condition for analysis at the CCFRA. Given samples of these amounts the detection limit of SO_2 in this experiment was 3 mg/Kg.

Table 4.2 Sulphur dioxide residue levels in the edible part of *N. norvegicus* dipped with melacide-SC20 at different concentration and treatment times.

	Sulphur dioxide residue in the un-cooked meat
Melacide 1.5 % 15 min	< 3 mg/kg
Melacide 2 % 6 min	< 3 mg/kg
Melacide 2 % 15 min	< 3 mg/kg
Melacide 2 % 30 min	8 mg/kg
Melacide 4 % 6 min	4 mg/kg
Melacide 4 % 30 min	8 mg/kg

Again in none of the treatments tested did the sulphite levels exceed the residue concentration of 10 mg/Kg (Table 3.2). However, it appeared that treatment time was more relevant than the Melacide-SC20 concentration in determining any increase in sulphite residue. Therefore, short treatments times are recommended, to ensure the lowest residue levels.

To conclude this part it would appear that the treatment recommended would be Melacide-SC20 at 2 % for 6 min, which gives a good anti-melanotic effect and also very low sulphite residue levels (<3 mg/Kg).

One of the fundamental aims of this WP was to find ways to reduce melanosis development without the use of additives. Factors affecting blackening in shrimps and lobsters include the method of capture, rough handling of the catch and other 'traumatic' events. However, as observed by Barolo and Bark (1998) no correlation has been found between the initial PPO activity and black spot development during storage in *N. norvegicus*. For this reason mechanisms to inhibit melanosis should concentrate at the storage phase. So far from our results it would appear that during storage:

- Icing alone is not enough to inhibit melanosis in whole fresh *N. norvegicus*.

- MAP cannot inhibit melanosis development effectively, even given the fact that the OGM contains high levels of CO_2 and low levels of O_2

Another technology associated with the inhibition of PPO activity reported in the scientific literature is high hydrostatic pressure. High hydrostatic pressure has been tried as a PPO inhibitor in vegetables and also in some crustaceans (Montero et al., 2001). Results appear to be somewhat mixed, since some authors have reported slight enzymatic activation by high pressure (Asaka and Hayashi, 1991) while other authors have found total inactivation of PPO at very high pressures (Seyderhelm et al., 1996). Therefore, it would appear that inactivation depends on several factors such as the immersion medium, the amount of pressure, the temperature and the time, in addition to which the effect appears to be species-dependent (Montero et al., 2001).

Therefore, it was decided to examine whether high-pressure treatment could work as an alternative method for PPO inhibition in whole fresh *N. norvegicus*

High-pressure as an alternative treatment to inhibit blackening development in whole fresh N. norvegicus

To perform this experiment animals were caught by otter trawl using the RV 'Aplysia' from UMBSM as in previous experiments. Once on board, animals were carefully washed with running seawater and the following treatments were applied:

-   Un-dipped animals

-   Animals dipped with Melacide-SC20 at the conc. of 2 % for 15 min

Afterwards, animals were stored on ice and transported within 24 h to the 'Agri-Food and Biosciences Institute' in Belfast, Northern Ireland, which has facilities for applying high hydrostatic pressures to food products (Figure 4.12)

Figure 4.12 Image showing the high-pressure machine at the Northern Ireland Agri-Food and Biosciences Institute used in the high hydrostatic pressure experiment.

Pressure treatment was at 150, 300 and 500 MPa for 3 min at 20 ˚C using water as the pressure-transmitting medium. The machine used was a Stansted Foodlab 900 high-pressure isostat (Stansted Fluid Power Ltd, Stansted, UK). After pressure treatment animals were stored on ice and transported to the University of Glasgow within 10 h. Once at the facilities at Glasgow University animals were kept 1-2 ˚C for up to 21 days. Untreated controls followed the same protocol and were also transported in the same way, with the pressure treatment being the only variable different. Analysis of the samples took place on days 7, 14 and 21 of storage. Each time samples were taken for the analysis of melanosis development, bacterial load, K-value, hypoxanthine and TMA concentrations were also determined. Furthermore, on day 7 samples were assessed by an independent sensory panel in the 'Food Innovation Institute' in Edinburgh.

The first clear observation was that high hydrostatic pressure treatment activated melanosis development. In general, the effect was pressure-dependent, except on the dorsal cephalothorax where melanosis was highest at the intermediate pressures tested (150 and 300 MPa) (Figure 4.13).
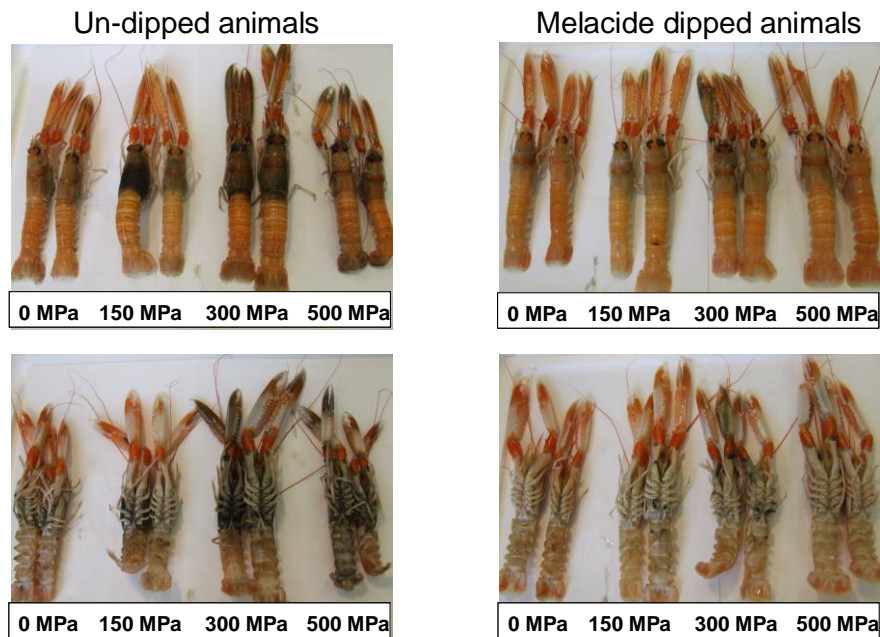
Figure 4.13 Digital images of whole *N. norvegicus* un-dipped or dipped with melacide-SC20 after high-pressure treatment on day 7 of storage.

However, Melacide-SC20 was able to inhibit the activation of melanosis produced by the high hydrostatic pressure treatment in all the parts of the body except the first clawed legs, where Melacide-SC20 was only able to arrest this activation effect at 150 MPa (Figure 4.14).
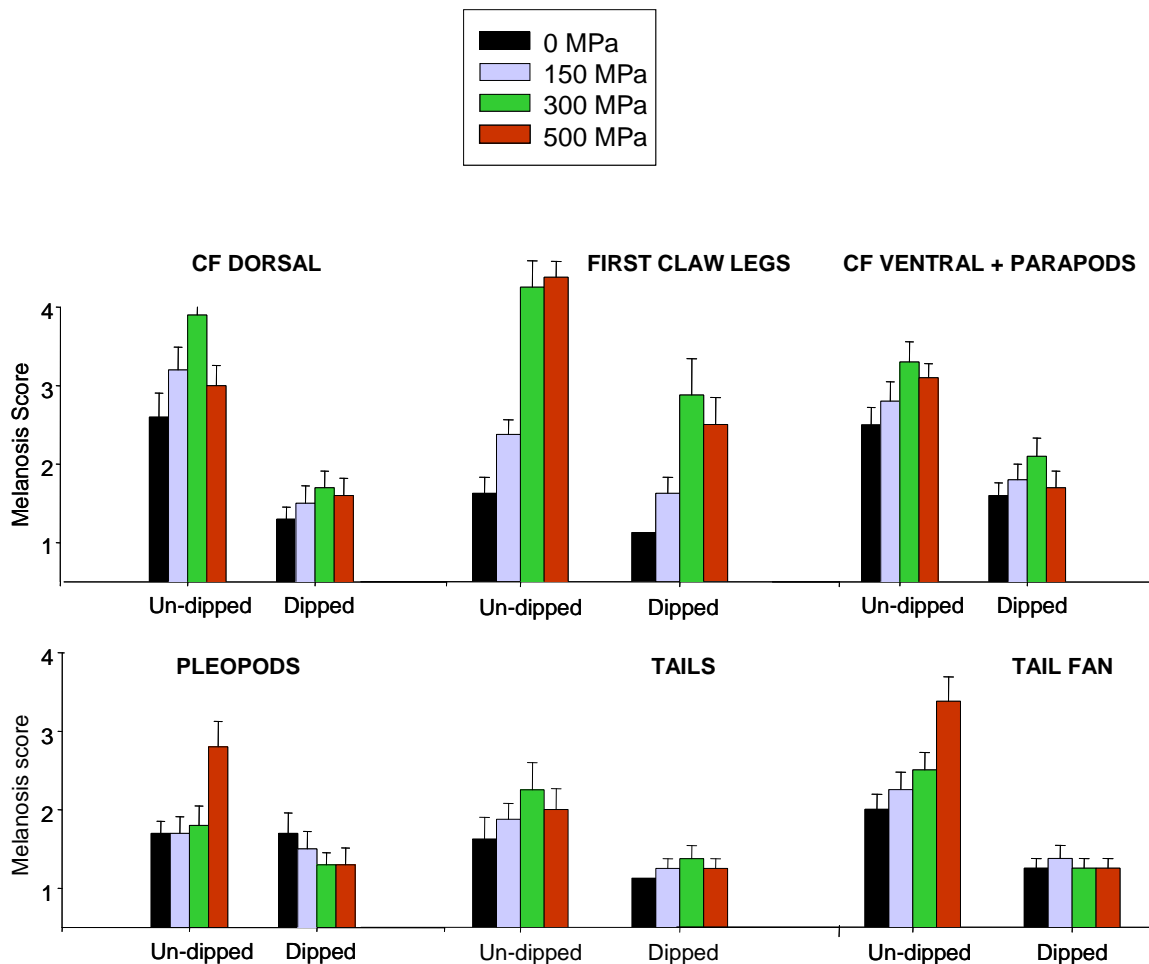
Figure 4.14 Melanosis Index Score of whole *N. norvegicus* un-dipped or dipped with melacide-SC20 after high-pressure treatment on day 7 of storage.

Therefore, it would appear that in fact high-pressure treatment does not inhibit the PPO enzyme but actually activates blackspot development in the conditions tested.

Other observations were that as pressure increased the meat of the animals presented a 'lightly cooked' appearance that was pressure dependent. A shucking effect has also been described in oysters and American lobster (*Homarus americanus*). However, in the conditions studied no shucking effect was observed in fresh or cooked samples (Figure 3.14).
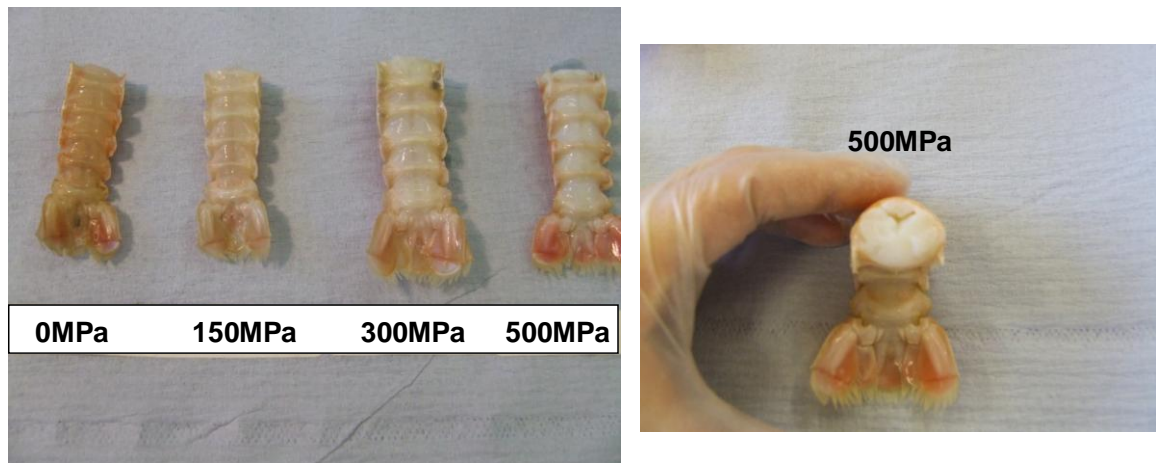
Figure 4.15 Visual appearance of whole *N. norvegicus* dipped with melacide-SC20 after high-pressure treatment, note the 'lightly cooked appearance' as pressure treatment increases and also no 'shucking effect' even at the highest pressure tested.

Apart from the possible effect of high hydrostatic pressure on melanosis, it was decided to assess its potential to extend shelf life. This methodology is currently used on a commercial basis in oysters to destroy pathogens and increase shelf life (Mermelstein, 2000).

In the case of whole *N. norvegicus* high hydrostatic pressure had a great impact on bacterial load. On day 7 of storage total viable counts decreased by 5 Log_{10} which means that un-treated samples had on average 10 000 000 cfu/g while samples treated with 500 MPa had 100 cfu/g. This decrease in bacterial load was pressure-dependent as shown in Figure 4.16. Interestingly, bacteria were not totally destroyed by the high pressure and eventually total viable counts also increased in the pressure-treated samples. However, it took another 14 days for the samples treated at 500 MPa to reach the bacterial load compared to un-treated samples.

A similar picture was obtained for H_2S producing bacteria, luminous bacteria and *Pseudomonas* sp. indicating that all different bacteria groups were affected negatively by the high hydrostatic pressure.
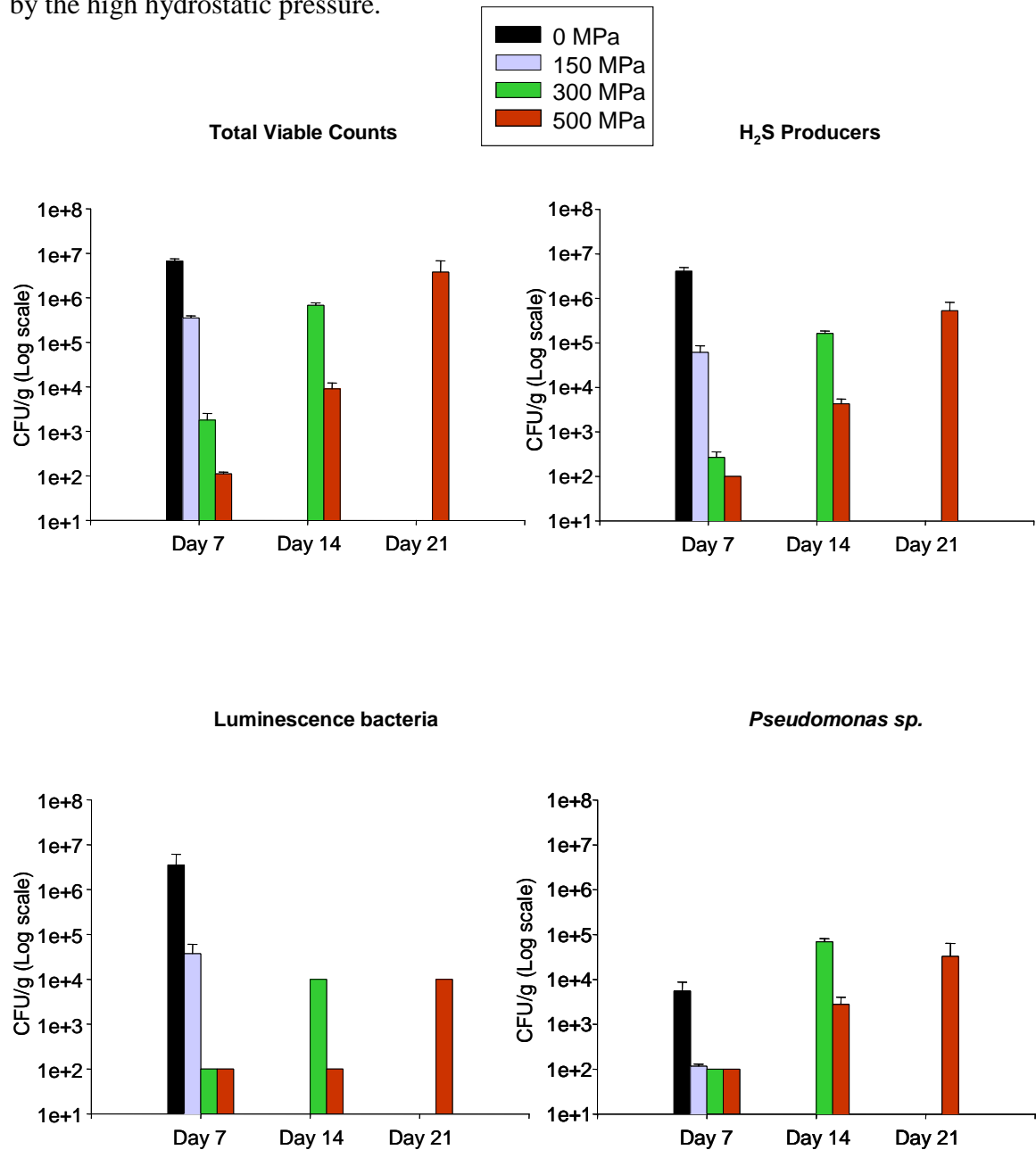
Figure 4.16 Bacterial load in whole *N. norvegicus* dipped with melacide-SC20 after high-pressure treatment at different storage times.

K-values used as a freshness indicator improved with high pressure treatment in a dose dependent manner on day 7 (Figure 4.17). K-value on un-treated samples on day 7 was 23.48 ± 6.24 while in samples treated with 500 MPa K-values were reduced by

more than half 10.99 ± 1.56. On day 14, K-values in un-treated and 150 MPa samples increased very quickly while samples treated at a higher pressure the increase in K-value was much slower.

Similarly to the pattern obtained in the K-value hypoxanthine concentration were lower in high pressure treated samples. Again on day 7 the pattern was pressure-dependent and then samples treated with higher pressures were more affected and hypoxanthine increase in a very slow manner (Figure 4.17).
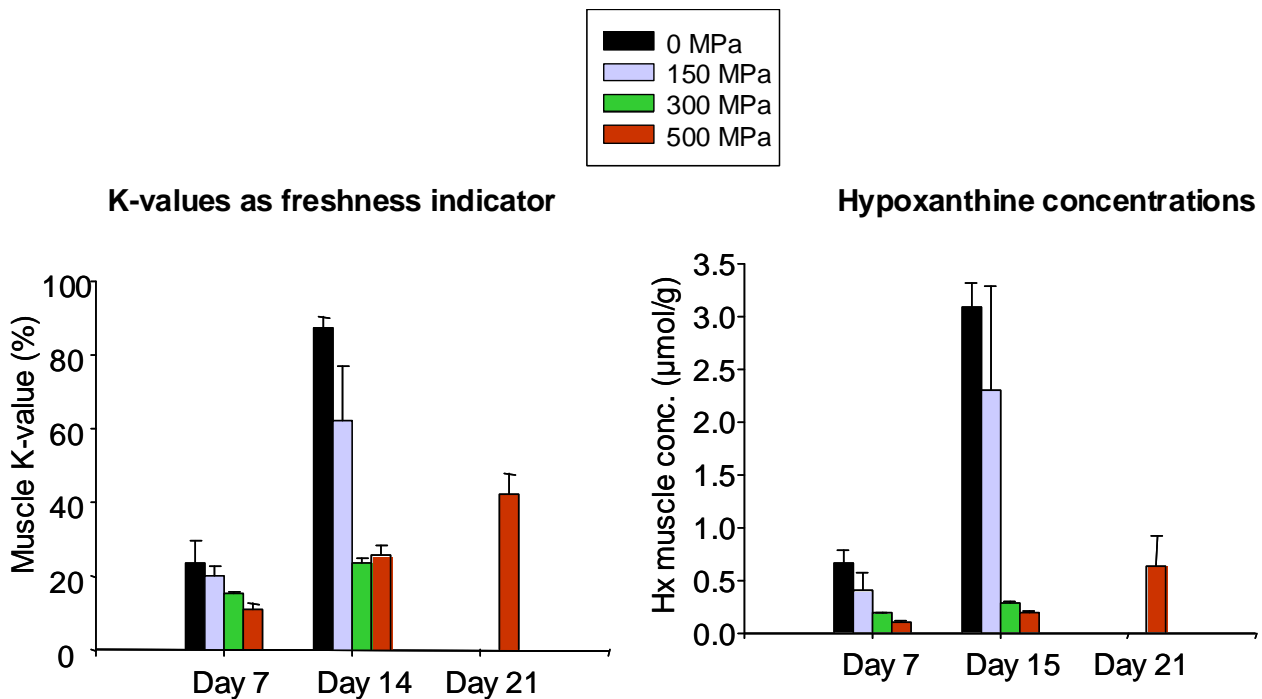
Figure 4.17 K-values and hypoxanthine concentrations in whole *N. norvegicus* dipped with melacide-SC20 after high-pressure treatment at different storage times.

Finally, TMA levels were also determined in the different samples at the different storage times. The response of TMA production was very similar to the hypoxanthine and K-value evolution (Figure 4.18). High pressure had a major impact on the production of TMA, which is mainly produced by spoiling bacteria.
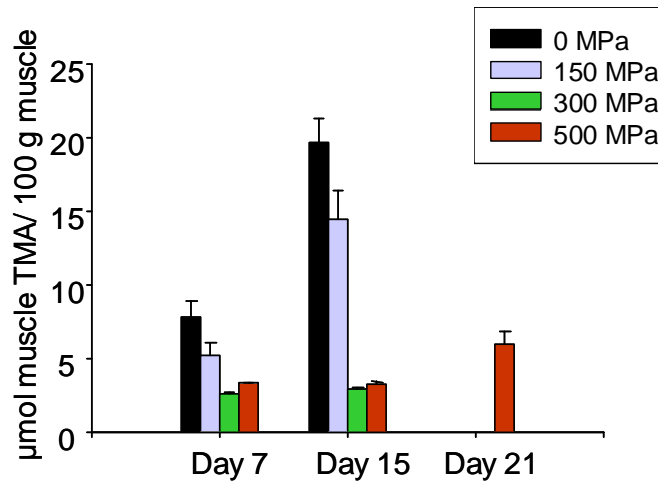
Figure 4.18 TMA concentrations in whole *N. norvegicus* dipped with melacide-SC20 after high-pressure treatment at different storage times.

Given the promising results obtained in terms of shelf life extension by high hydrostatic pressure treatment, samples on day 7 of storage were presented to the professional sensory panel in the 'Food Innovation Institute in Edinburgh'. Full details can be found in Annexe 4.

Looking at the 'overall liking' which is a subjective measure, the sample that was most liked was the one treated at a 150 MPa (Figure 4.19). On the other hand the least liked sample was the one treated at 500 MPa. The reason behind this result can be explained mainly by the changes in texture. Samples treated with the highest pressure tested (500 MPa) were described as firmer and very chewy. Probably the fact that they were very chewy had a negative impact on the overall liking, since this was the parameter that underwent the highest change.

The fact that the lower pressures tested (150 and 300 MPa) scored very similarly and that they were liked suggest that this technology could be very promising for whole

fresh *N. norvegicus*. However, more research is needed to optimize all the parameters involved in the high-pressure process (especially time and temperature). The results obtained suggest that this technology not only extends shelf life by inhibiting bacteria growth and destroying some autolytic enzymes, but also is also able to maintain flavour and texture in whole fresh *N. norvegicus*.
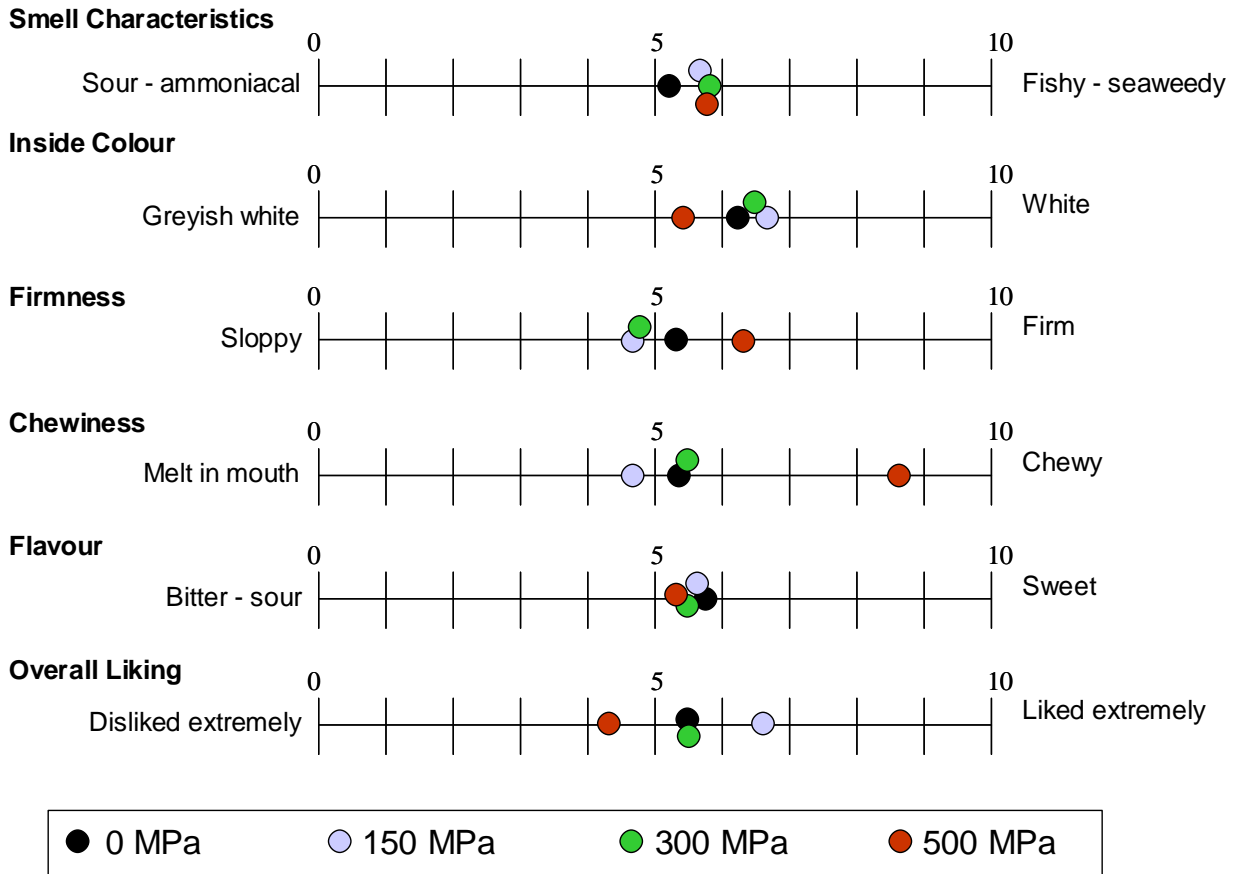
Figure 4.19 Sensory score performed by a professional sensory panel on whole cooked *N. norvegicus* dipped with melacide-SC20 after high-pressure treatment on day 7 of storage.

WP5 Calibration of assays for product quality against Sensory Evaluation

In this WP5 two main objectives were set:

1) The development of a Quality Index Measure (QIM) based on the existing QASAS scheme from Young's Seafood Ltd. and modified if necessary for whole fresh *N. norvegicus*

2) The establishment of a Sensory Evaluation protocol using a professional sensory panel specifically trained for cooked meat of *N. norvegicus,* so that this protocol can be used afterwards for sensory evaluations of samples from different experiments that will be also analysed in the laboratory.

Development of a Quality Index Measure (QIM) for whole fresh N. norvegicus

In collaboration with Young's Seafood Ltd, a QIM was developed for whole *N. norvegicus* stored on ice based on the existing QASAS scheme from the company. The QIM uses five different parameters: appearance of cephalothorax; appearance of claws; appearance of the upper-side of tail; appearance of the under-side of tail and odour. For each parameter scored a four demerit scores (from 0 - 3) are allocated. The detailed descriptions of the demerit quality scores are shown in Figure 5.1. The descriptions were taken initially from a previously published study on shrimp (Anonymous, 2002) and modified during an early trial phase. Usually, during the early development stage, 2 - 3 panelists analyzed a batch of 5 - 10 animals per sample point.

QIM scheme for Norway Lobster (*Nephrops Norvegicus*)

Attributes	criteria	index points
Appearance head	sharp contrast, carrot orange, pinkish, black eyes	0
	less shiny, bleached, slightly grey, creamy ends	1
	dull, dead, more grey, brownish, bleached eyes	2
	Black, dark grey, dull, grey eyes	3
Appearance claws	carrot orange, white, sharp contrast	0
	orange fading, bleached, dull	1
	light grey, slightly more algae green	2
	bleached , more algae green, creamy/yellowish	3
Uper site tail	Fresh orange, pinkish, white ends, some tail ends already brown	0
	bleached orange, tail ends more grayish and darker brown	1
	light brown over orange, creamy ends, bleached, brown tail ends.	2
	Distinct brown, green lines, black tail ends.	3
Under site tail	Transparent feet, pinkish, translucent meat	0
	discolored feet, milky meat	1
	Brown feet, yellowish meat	2
Odor	Fresh, hey, marine	0
	Les fresh, neutral	1
	Old seaweed, musty, slightly ammonia	2
	Sour, musty, ammonia	3
Total score		0 - 14

Figure 5.1 QIM scheme developed for whole *N.norvegicus*.

After the initial development was completed, the QIM was calibrated for iced storage using fresh whole *N. norvegicus*. The animals were caught as described in previous experiments, washed thoroughly and immediately stored on ice. Fresh animals were scored directly aboard the capture vessel, and subsequent scoring was carried out on a daily basis. Generally, animals were scored only once and then discarded to assure

that deterioration effects of a prolonged incubation at RT during scoring did not affect the outcome of the overall calibration. Twelve animals were scored per time point by 2 - 3 trained panelists. A scatter plot of the QIM scores shows that a high correlation was found between the QIM score and the storage time on ice and it can be seen that, in general, the quality deteriorates over time (Figure 5.2). Single analysis of sample attributes has previously shown that they are independent of each other (Neil et al., 2006). Around day 7, melanosis of the exoskeleton was severe and the odour was putrid and fishy. The product was rejected and rendered unfit for consumption. The shaded areas in Figure 5.2 show the rejection limits depending on either time or quality scores. If scores over 8 are assigned or if the sample is stored for longer than 6 - 7 days the shelf life has passed. The bracketed box (Figure 5.2 *) indicates the standard area of rejection during iced storage of whole *N. norvegicus*.
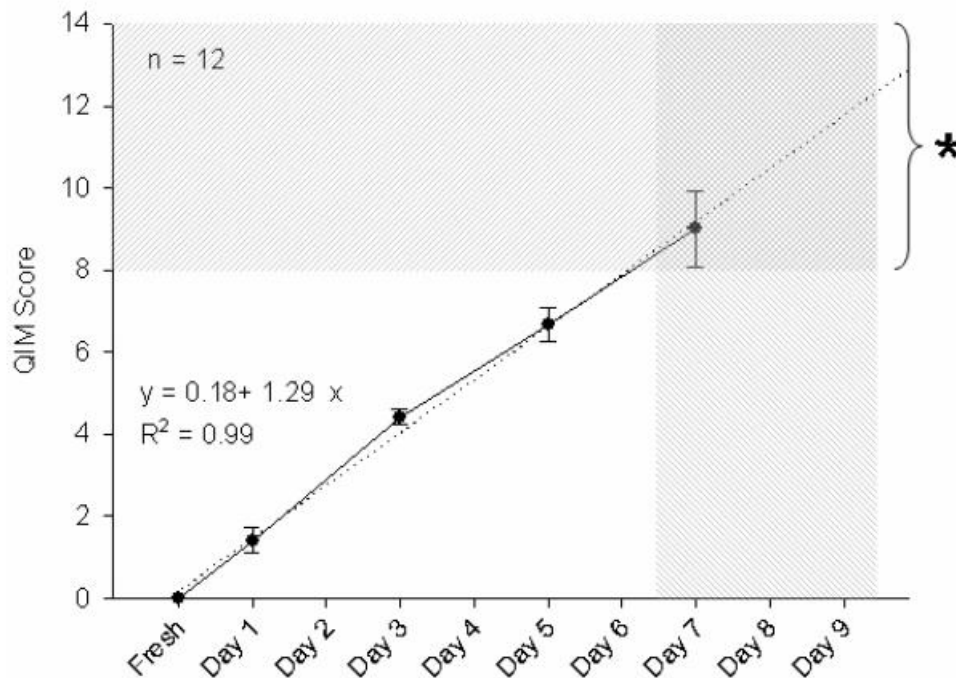
Figure 5.2 QIM calibration of whole *N. norvegicus* during storage on ice for up to 7 days. The shaded areas indicate either time or QIM score limits of rejection. The shaded areas indicate either time of QIM score limits of rejection. The asterisk flags the shaded box, which indicates the standard area of rejection for ice stored whole *N. norvegicus*, where both time and QIM overlap. Each point is the mean ± S.E.M of 12 samples.

However, in a commercial environment anti-melanotic compounds are used in order to inhibit blackening development. Therefore, the next step was to assess how the QIM would work under those conditions.

Effect of anti-melanotic treatment and a temperature-step on the QIM

In order to simulate commercial and retail procedures, whole *N. norvegicus* lobsters were treated with an anti-melanotic agent (Melacide-SC20) to prevent melanosis, and a temperature step to a higher storage temperature (from ice up to >6 ºC) from day 5 onwards was included. It was previously shown that differences in fish deterioration could be seen under different storage conditions (Nielsen et al., 1997). Therefore, in order to assess whether the anti-melanotic treatment and the temperature step might affect the QIM scheme, an additional experiment was carried out in which whole lobsters were treated with the anti-melanotic agent Melacide-SC20 at the concentration of 2 % for 15 min, were subsequently stored on ice for 5 days and were then transferred to >6 ºC for the remainder of the time. For each time point, between 2 and 3 trained panelists scored a set of 12 animals. The results clearly show that the QIM score was affected when animals were treated with Melacide-SC20 (Figure 5.3). The QIM results for the treated and differently stored product changed, if compared with the standard QIM result of whole *N. norvegicus*. The regression of the QIM score for the Melacide-SC20 treated lobsters was less steep and behaved differently from the standard. Although samples were still rejected because of their odour on day 7, the QIM score never reached the standard area of rejection previously established. However, the result for whole Melacide-SC20 treated lobsters still increased in a

linear manner, and thus gradual quality deterioration was indicated. At least one or more of the 5 previously chosen parameters of the QIM still gave reliable results, and the QIM was still able to distinguish between fresh and stored whole lobsters.
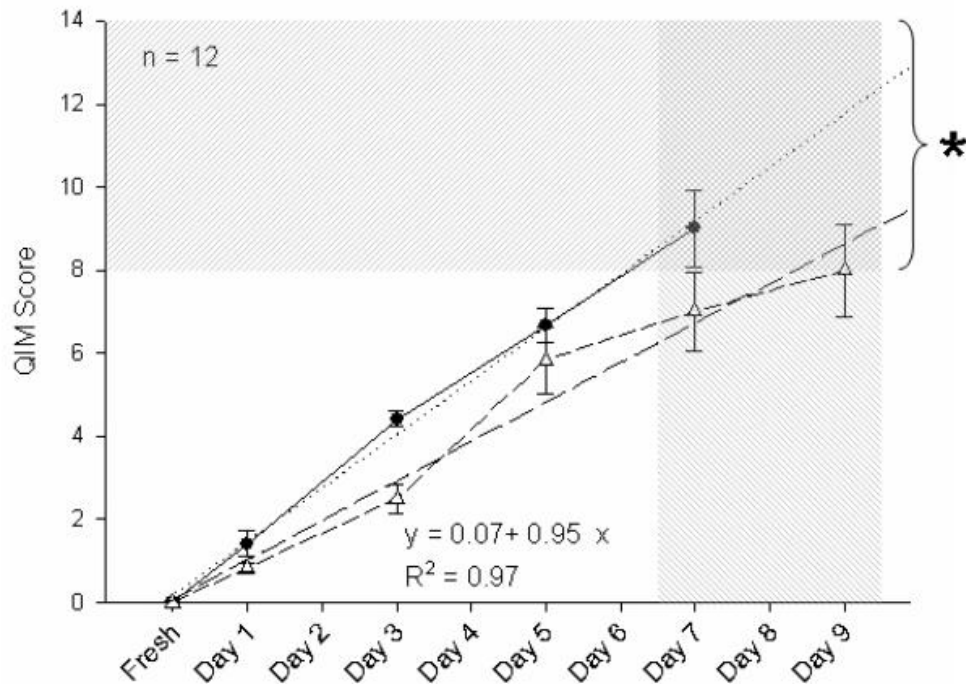
Figure 5.3 QIM calibration of whole *N. norvegicus* in un-dipped animals (•) or animals dipped with Melacide-SC20 (△) during storage on ice for 5 days and subsequent storage at around 6 °C. The asterisk flags the shaded box, which indicate either time or QIM score limits of rejection for ice stored whole *N. norvegicus*, where both time and QIM score limits overlap. Each point is the mean ± S.E.M

To clarify which of the parameters could be chosen to generate a revised QIM scheme for Melacide-SC20 treated lobsters or lobsters treated with a commercially available anti-melanotic agent, the data were disassembled into the single parameters and the results were plotted independently (Figure 5.4). If the parameters are analyzed in this way, it can be seen that within the Melacide-SC20 treated samples only two parameters, the cephalothorax appearance and the odour are truly linear in their behavior. These parameters were the main influences on the overall QIM score. All other parameters (first clawed legs, upper tail, underside tail) lost their linear

proportionality between day 5 and day 7. The quality attributes of the first clawed legs and the upper tail did not change from day 5 onwards and the quality attributes of the underside of the tail did not change from day 7 onwards. Furthermore, all parameters (except the odour) changed only slightly if compared with the maximum scale and never reached full scores within the time of analysis. It was shown before by Barbosa and Vaz-Pires (2004), that if the parameters in a QIM scheme do not change significantly over time, if they hardly reach high values or if they are irregular, the measure becomes meaningless and the parameter must be omitted from the QIM scheme. Therefore, 3 of the 5 parameters (claws, upper tail, underside tail) were removed from the revised scheme. Furthermore, since the changes in the odour were more significant than the changes in the quality and appearance of the cephalothorax region, it was decided to concentrate solely on the odour, when analyzing whole Melacide-SC20 treated *N. norvegicus* lobster in MAP packs.
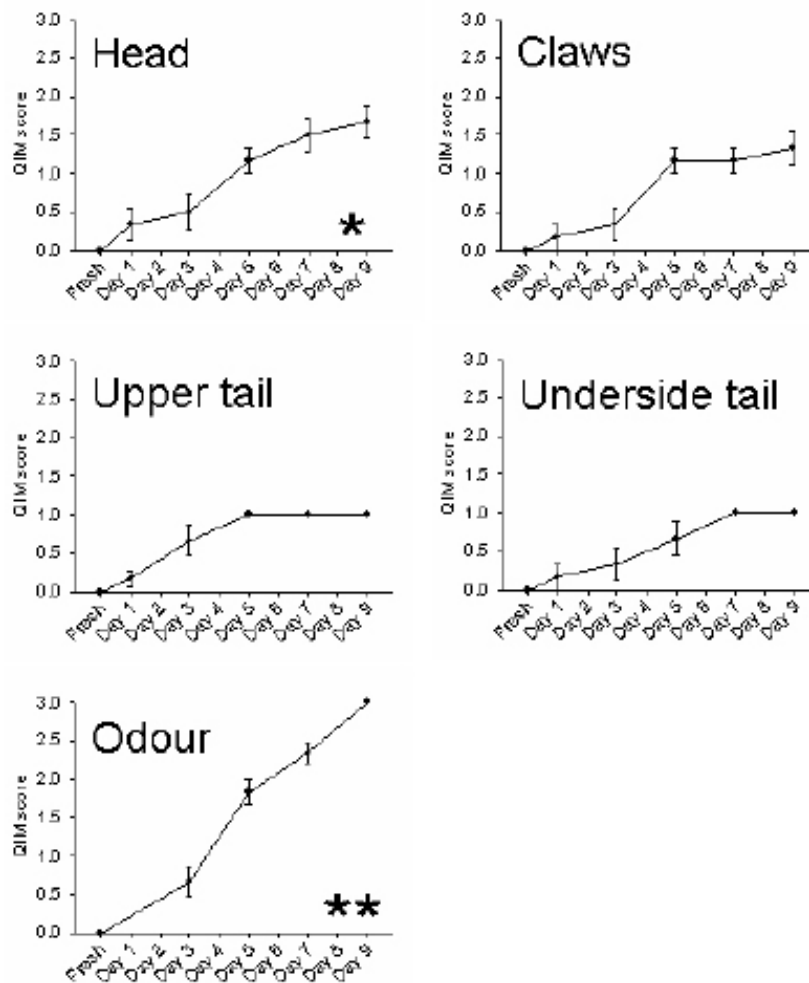
Figure 5.4 QIM scores of single parameters in whole *N. norvegicus* treated with Melacide-SC20 during storage on ice for 5 days and subsequent storage at around 6 °C. (*) and (**) indicate parameters, which behave linearly over time. (**) has a steeper slope than (*), and it is the only parameter that reaches a maximum score within the analysed time frame of 9 days. Each point is the mean ± S.E.M of 12 samples

In Figure 5.5 the QIM scores based on odour formation during storage on ice for 5 days and subsequent storage at around 6 °C show a good correlation between time in storage and odour development. The value rose continuously and reached the maximum of 3.0 points on day 9. However, if the shelf life established from the QIM of whole lobsters (7 days) was transferred to the odour scale of the revised QIM it was established that shelf life has passed when a limit of 2.0 scores on the revised scale was reached. At this score the quality parameters describe the odour of the sample as

'old seaweed-like', 'musty' and 'slightly ammoniacal'. The limit of a 2.0 score in the

odour was therefore set as the rejection point.
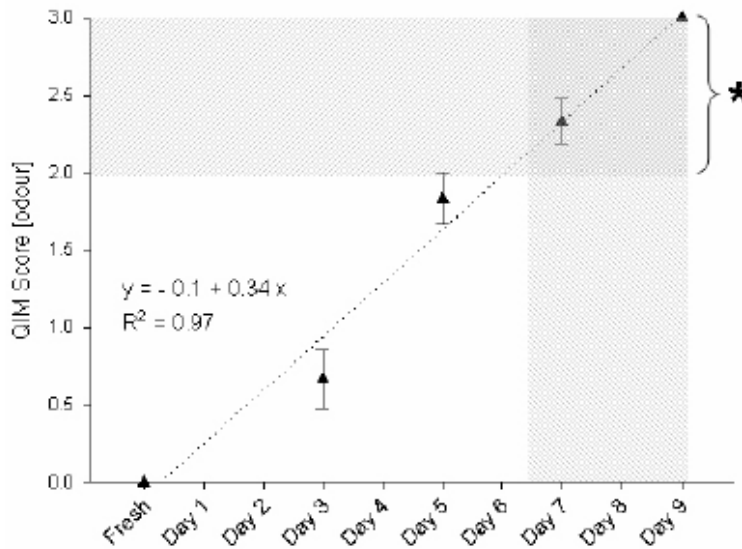
Figure 5.5 QIM scores for odour in whole *N. norvegicus* treated with Melacide-SC20 during
storage on ice for 5 days and subsequent storage at around 6 ºC. The shaded areas indicate
either time of QIM score limits of rejection. The asterisk flags the shaded box, which
indicates the standard area of rejection for ice stored whole *N. norvegicus*, where both time
and QIM overlap. Each point is the mean ± S.E.M of 12 samples

The fact that odour gave very good results as a QIM score attribute suggested the

potential to use available technologies that quickly measure complex odorous

matrices and are currently used in different industrial sectors. In this sense, it was

interesting to elucidate if systems such as the e-nose system supplied by 'Alpha

M.O.S.' of Toulouse, France could work as an objective analytical measure to classify

samples according to their sensory properties. To this end, samples packed in the

OGM or air and stored using the stepping temperature profile (see WP3) were sent to

the 'Alpha-M.O.S.' premises in France to be analysed using 2 different 'e-nose'

systems: The sensor array system αFOX 4000 and the Gas Chromatography system

Heracles (Flash e-nose).

To comply with the 'Confidentiality Agreement' with the industrial partner 'Young's Seafood Ltd.' the composition of the OGM was not revealed to the company performing the analysis (Alpha M.O.S.).

The preliminary results obtained indicate that e-nose technology has a great potential to be used as an objective measure to classify samples according to their sensory properties and therefore quality. The full report (Analysis Report 1455 from Alpha M.O.S.) containing all the results from this trial can be found in Annexe 5 .

Sensory Evaluation of cooked N. norvegicus using a professional sensory panel

Biochemical assays and instrumental methods for quality and freshness are objective tests performed in the laboratory. However, only when they are measured in relation to the sensory evaluation it is possible to properly interpret the laboratory results and set appropriate thresholds for acceptability based on such measures alone.

In order to achieve this objective a professional sensory panel was recruited by an appropriate food industry agency. In this particular case, the panel was recruited by the Food Innovation Institute in Edinburgh under the supervision of Dr. David Miskin. However, before the sensory evaluation could be performed it was necessary to train the panel and to identify the characteristics it would be important to focus on and score.

Training of a professional sensory panel to evaluate quality in N.norvegicus cooked meat

As a first approximation it was decided that the possible points to look at when doing a sensory evaluation of cooked meat of *N. norvegicus* could be:

1) Odour:

      Fresh, seaweed, slightly sulphurous characteristic shellfish smell

      None

      Slight ammonia

      Strong ammonia, sour

2) Flavour:

      Intensely sweet and metallic

      Sweet milky

      Loss of sweetness, neutral

      Sour, off

      Strong off flavour, bitter, very sour

3) Texture:

      Firm

      Slightly soft

      Soft

      Sloppy, little texture

Other possibilities: Springiness, juiciness, crumbliness, toughness, chewiness

In order to test if these properties would be adequate for the sensory evaluation of *N. norvegicus* samples treated in a different manner were obtained, analysed in the laboratory and also tested by the professional sensory panel.

To obtain samples treated in different ways, *N. norvegicus* were caught in April 2007 by otter trawl in the Clyde sea area using the Research vessel 'Aplysia' from UMBSM. Once on board animals were carefully washed with running seawater and the following treatments were performed:

- Animals frozen immediately

- Animals stored for 4 days on ice and then frozen

- Animals stored at 18 ºC for 24 h and then frozen

- Animals stored for 18 ºC for 8 h, then stored on ice for 16 h and finally frozen

These treatments were applied according to previous experiments (Phase I of the Scottsih *Nephrops* Survey) where a similar approach had been used and samples of different qualities were obtained. However, to confirm this hypothesis, samples from each group were analysed in the laboratory to obtain the: ATP and its breakdown products (hypoxanthine), K-values and biogenic amines.

K-values increased according to the ageing of the samples and temperature abuse magnitude. Also different amounts of hypoxanthine were found in the different treatments indicating than from a laboratory point of view the samples had different qualities and therefore were appropriate to be used in the training of the sensory panel (Figure 5.6).

a) K-values

b) Hypoxanthine concentrations
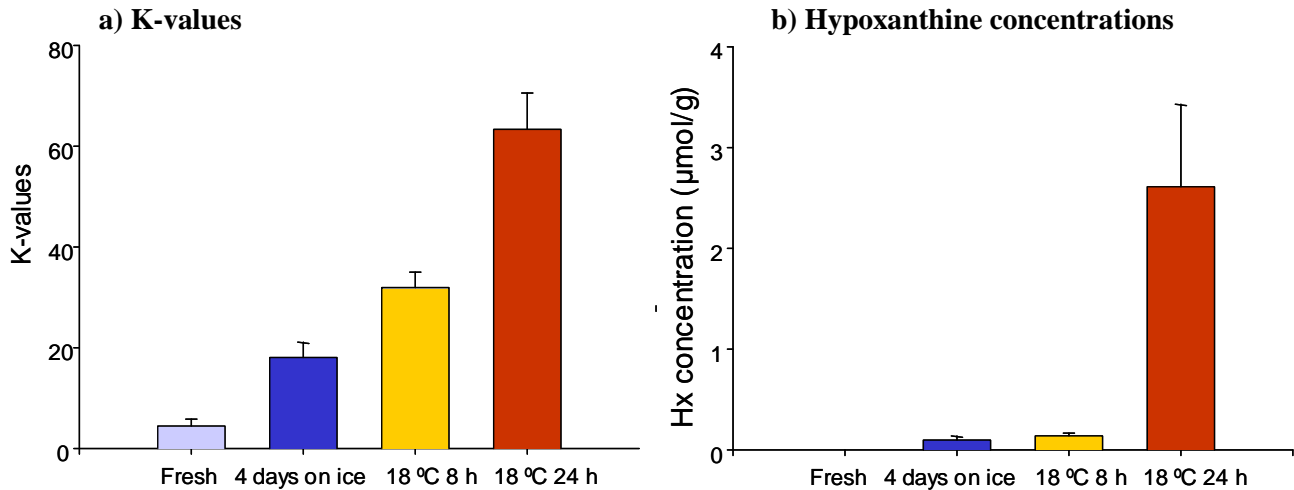
Figure 5.6 K-values and hypoxanthine concentrations in whole *N.norvegicus* stored at different conditions ready to be used for the training session of the sensory panel.

Biogenic amines also differed between the different groups. In fresh frozen samples no biogenic amines were detected, while on the other hand in samples left at 18 ºC for 24 h putrescine and agmatine were very high and to a lesser extent cadaverine and spermidine were also detected (Table 5.1).

Table 5.1 Biogenic amines concentrations in whole *N.norvegicus* stored at different conditions ready to be used for the training session of the sensory panel.

	Fresh	4 days on ice	18 C 8 h	18 C 24 h
TYRAMINE	n.d	n.d	n.d	n.d
PUTRESCINE	n.d	n.d	n.d	59.57 ± 25.58
CADAVERINE	n.d	0.46 ± 0.18	0.59 ± 0.06	2.23 ± 1.30
HISTAMINE	n.d	n.d	n.d	n.d
AGMATINE	n.d	n.d	2.92 ± 2.36	78.47 ± 6.48
SPERMIDINE	n.d	0.36 ± 0.22	0.50 ± 0.40	0.35 ± 0.18
TRYPTAMINE	n.d	0.24 ± 0.16	0.56 ± 0.20	n.d

As these samples showed clear differences in the biochemical parameters measured in the laboratory, samples were transported frozen to the Food Innovation Institute to be used as a training material to determine which parameters should be scored in further experiments.

Once the session with the panel was performed the following parameters were chosen to be scored. For each of the 10 sensory attributes chosen a two-anchored linear scale (0-10) was used, in which the score of 5 is the mid-point (Figure 5.7). This scale is objective and dose not relate to whether a given panellist's like or dislike of a sample. The sensory attributes chosen were:

Aroma: smell characteristics and smell strength

Appearance: Pink patches on top and inside colour

Texture: Springiness, firmness, chewiness and moistness

Flavour: Flavour and aftertaste

At the end of the scoring the trained panellist were then asked to score the degree of like or dislike, called the 'overall liking', of each sample on a linear scale (0-10). On this scale, a score of 0 means 'extremely disliked' and score of 10 means 'extremely liked'. Therefore, scores below 5 indicate a disliked sample.

Smell Characteristics

Sour - ammoniacal   ⊢──┼──┼──┼──┼──┼──┼──┼──┼──┼──┤   Fishy-seaweedy
(0 ··· 5 ··· 10)

Smell Strength

Weak   ⊢──┼──┼──┼──┼──┼──┼──┼──┼──┼──┤   Strong
(0 ··· 5 ··· 10)

Pink patches on top

No pink   ⊢──┼──┼──┼──┼──┼──┼──┼──┼──┼──┤   Covered in pink
(0 ··· 5 ··· 10)

Inside colour

Greyish white   ⊢──┼──┼──┼──┼──┼──┼──┼──┼──┼──┤   White
(0 ··· 5 ··· 10)

Springiness (fork test)

Stays down   ⊢──┼──┼──┼──┼──┼──┼──┼──┼──┼──┤   Resilient
(0 ··· 5 ··· 10)

Firmness

Sloppy   ⊢──┼──┼──┼──┼──┼──┼──┼──┼──┼──┤   Firm
(0 ··· 5 ··· 10)

Chewiness

Melt in mouth   ⊢──┼──┼──┼──┼──┼──┼──┼──┼──┼──┤   Chewy
(0 ··· 5 ··· 10)

Moistness

Dry   ⊢──┼──┼──┼──┼──┼──┼──┼──┼──┼──┤   Moist
(0 ··· 5 ··· 10)

Flavour

Bitter - sour   ⊢──┼──┼──┼──┼──┼──┼──┼──┼──┼──┤   Sweet
(0 ··· 5 ··· 10)

Aftertaste

Bitter   ⊢──┼──┼──┼──┼──┼──┼──┼──┼──┼──┤   Sweet
(0 ··· 5 ··· 10)

Figure 5.7 Sensory attributes decided to be used in sensory scoring of whole *N.norvegicus* after the training session of the sensory panel.

Once the panel was trained and a sensory scoring system was established, the following experiments were scored:

- Experiment of tails on ice for up to 11 days: Results will be presented in this WP5

- Samples infected with *Hematodinium* sp.: Results will be presented in WP6

- Samples from MAP containing the OGM or air and stored for different periods of time and using the constant low temperature protocol or the stepping temperature profile: Results are presented in WP3

- Samples treated with high hydrostatic pressure technology: Results are presented in WP4

Furthermore, a validation trial was conducted in which a set of samples from a MAP experiment were analysed in the laboratory and sensory assessed by the Food Innovation Institute in Edinburgh and by Young's Seafood Ltd. Ltd in Grimsby, UK.

Full reports of the work carried out by the professional sensory panel can be found in the Annexe-reports 1-4. However, the main results will be discussed as appropriate throughout this report.

Sensory Evaluation of tails stored at 0 ºC for up to 11 days

The first trial conducted using the trained professional sensory panel was on tails of *N. norvegicus* stored at 0 ºC. To this end, animals were caught by otter trawl in June 2007 using the Research vessel 'Aplysia' from UMBSM. Once on board, animals were carefully washed with running seawater and immediately tailed. Tails were stored in plastic bags on ice and transported at the facilities in the University of Glasgow. Tails were stored in an incubator set at 0 ºC and temperature profile was scored to ensure that the incubator had been working properly at all times (Figure 5.8).
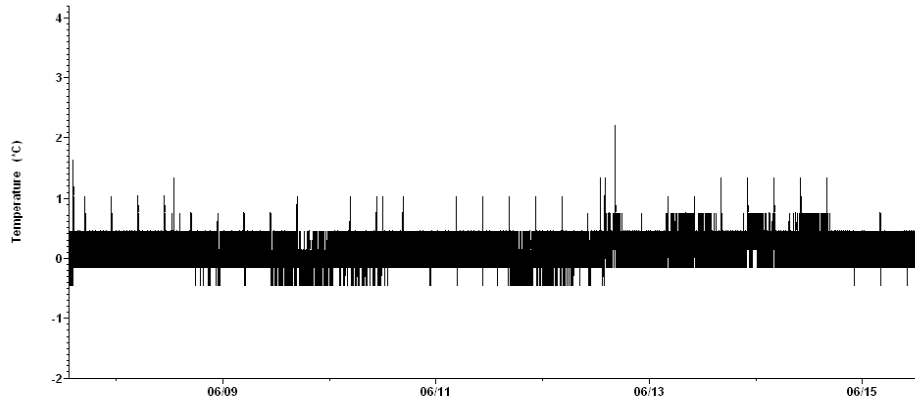
Figure 5.8 Temperature profile in the incubator containing the tails stored at 0 ℃ for 11 days.

On days 0, 1, 3, 5, 7 and 11 samples were analysed for total bacterial load, pH in the tail muscle and ATP and its breakdown products to calculate the K-value.

Bacterial load increased with storage time more or less linearly from day 2 to day 11 (Figure 5.9). On day 11, bacterial load was 4.75 ± 0.03 cfu/g on a Log_{10} scale.
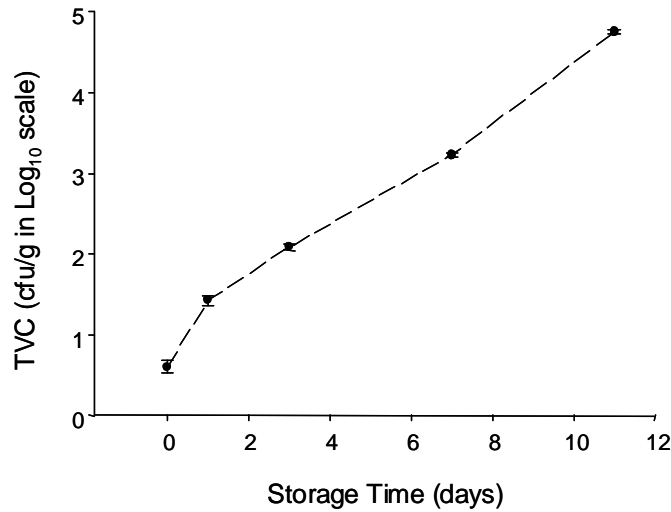
Figure 5.9 Bacterial load using the rapid plates method in the tails of *N.norvegicus* stored at 0 ℃ for 11 days.

Similarly, muscle pH increased in a linear manner up to day 11 were values were 7.96 ± 0.03. Maximum pH values have been observed in previous experiments to be between 8.00 and 8.20 suggesting that values on day 11 were very close to its plateau.
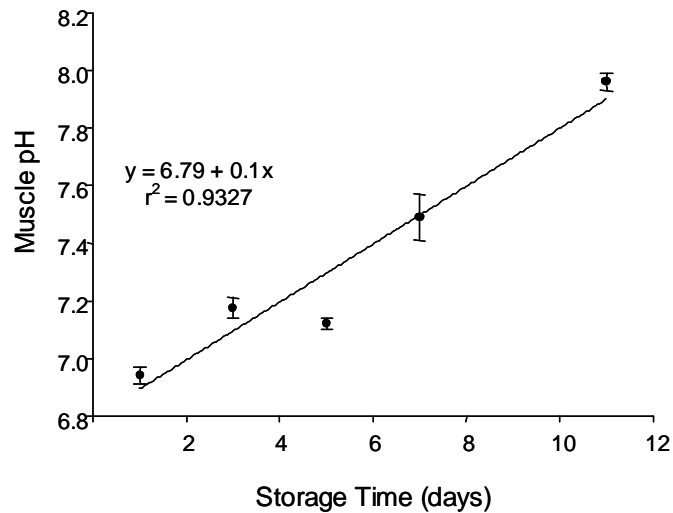
Figure 5.10 Muscle pH in the tails of *N.norvegicus* stored at 0 ºC for 11 days.

ATP and its breakdown products were measured as storage time progressed. Some of these compounds are very relevant from a quality point of view, as demonstrated in Phase I of the Scottsih *Nephrops* Survey. In this sense IMP has been shown to give a 'nice meaty' taste in fish products while hypoxanthine has been shown to give a 'bitter-off' taste. In this experiment highest IMP concentrations were recorded on day 3 and decreased afterwards while hypoxanthine increased slowly during storage time and considerable amounts were found on day 11 (0.75 µmol/g) (Figure 5.11).
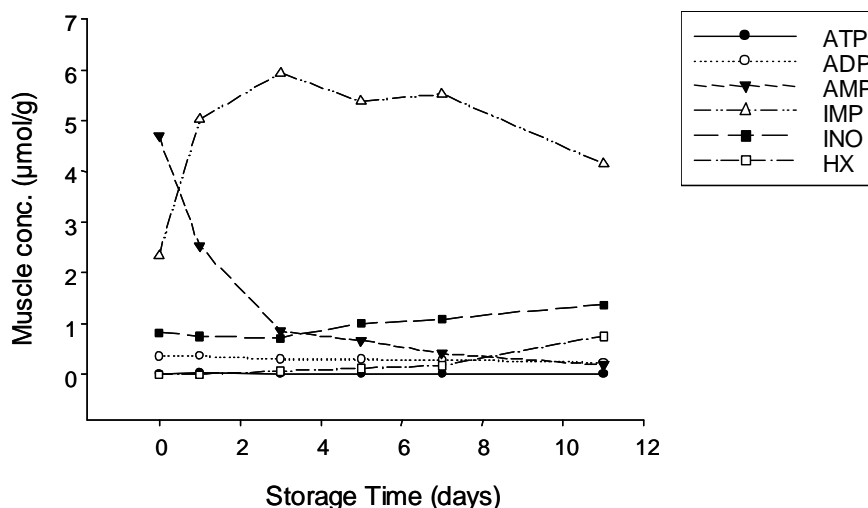
Figure 5.11 ATP and its breakdown products measured in the tail muscle of *N.norvegicus* stored at 0 ℃ for 11 days.

According to these values it was possible to calculate the K-values as storage time progressed. As shown in Figure 5.12, K-values did not increase significantly until day 3. However, after this time K-values increased and maximum values were obtained in day 11 (33.36 ± 5.88). As discussed earlier in this report, K-value is an important measure as it is used in many fish species to set a limit for freshness. This limit in the K-value is highly dependent on the species but as a general rule in many seafood product 20 % of K-value is regarded as a freshness limit. In our case K-value around 20 would be obtained around day 7-8 suggesting that this time could be the limit if the product has been maintained on ice all the time. These results would be in agreement with the observations obtained using the QIM (rejection limit using QIM [odour] was around day 6-7).

Finally, frozen samples were sent to the 'Food Innovation Institute' and they were thawed, cooked and assessed by the trained professional sensory panel. Complete data sets can be found in Annexe 1. However, some of the data are summarised in Table 5.2. The samples with the strongest smell were from day 11. In respect to changes in texture as storage time progressed, samples were firmer, chewier and drier. On the

other hand, no big differences were found in flavour and aftertaste but the lowest scores were recorded on day 11. Interestingly, the samples most liked were from day 3 of storage. These samples had:

- Fishy/seaweedy smell without having a strong smell

- Half covered with pick patches on top

- Texture in the middle range

- Slightly sweet flavour, not bland with slightly sweet aftertaste

Results indicate that 11 days was too long for keeping the samples at 0 °C, while up to 7 days it was acceptable. Interestingly, samples from day 3 of storage scored better than fresh samples from day 0. These results suggest two different possibilities. First, that some 'maturation' process in the meat occurs, and it is received positively by the panel or secondly that costumers in general are not used to having very fresh products on their table and therefore they prefer samples that are fresh but not straight from the sea.

Table 5.2 Sensory attributes and scores in *N.norvegicus* tails scored at 0 ºC for up to 11 days.

Sample		Smell Character.	Smell Strength	Pink Patches	Inside Colour	Springiness	Firmness	Chewiness	Moistness	Flavour	Aftertaste	Overall Liking
Z-OIF	Mean	**7.19**	**6.54**	**4.65**	**6.29**	**6.67**	**7.18**	**7.03**	**5.93**	**6.63**	**6.53**	**6.27**
	St. D.	1.04	1.23	2.32	1.51	1.44	1.06	1.31	1.87	1.28	1.40	1.67
Z-OID1	Mean	**6.67**	**6.08**	**2.43**	**5.87**	**4.58**	**6.54**	**5.62**	**6.35**	**6.69**	**6.17**	**6.65**
	St. D.	1.29	1.40	1.02	1.67	1.56	1.76	1.97	1.31	1.04	0.90	1.55
Z-OID3	Mean	**6.23**	**6.19**	**4.18**	**5.29**	**5.07**	**6.63**	**6.14**	**6.89**	**6.43**	**6.14**	**6.72**
	St. D.	1.63	1.81	2.10	1.45	1.99	1.73	1.89	1.32	1.43	1.48	1.47
Z-OID5	Mean	**5.64**	**6.04**	**5.41**	**5.59**	**5.04**	**6.74**	**6.86**	**6.17**	**6.18**	**5.62**	**6.29**
	St. D.	1.49	1.21	1.73	0.77	1.77	1.48	1.56	1.59	1.18	0.99	1.74
Z-OID7	Mean	**6.14**	**6.80**	**5.84**	**5.91**	**5.14**	**6.04**	**5.81**	**6.44**	**6.16**	**5.79**	**6.47**
	St. D.	1.32	1.38	1.38	1.52	1.54	1.23	1.40	1.44	1.51	1.17	2.18
Z-OID10	Mean	**5.13**	**7.03**	**5.76**	**4.77**	**6.11**	**7.52**	**7.12**	**6.00**	**5.51**	**5.48**	**5.40**
	St. D.	2.46	1.14	1.41	0.68	1.93	1.64	1.46	1.19	1.12	1.38	1.72
	F	3.007	2.034	11.343	1.694	3.428	3.270	3.203	1.228	2.011	1.280	2.997
	Sig.	0.001	0.029	0.000	0.081	0.000	0.001	0.001	0.274	0.032	0.242	0.001
ANOVA		**	*	***	NS	***	**	**	NS	*	NS	**

WP6. Variation in product quality with location and supplier

As mentioned in the Introduction, one of the objectives in this work package was to assess whether or not the occurrence of the parasite *Hematodinium* could be monitored in commercial samples from different fishing grounds. The Youngstrace traceability system provides the necessary Global Positioning System (GPS) tracking systems on-board vessels to monitor their position when trawling. Moreover the boxes of tailed product from the catch are individually barcode labelled before landing. If samples were taken from these labelled landings it would offer the investigator a chance to accurately place every trawl transect from that vessel with date and time of day. Commercial samples are however subject to some degree of selection, and landings do not represent what is available in the net. In the Clyde Sea area fishermen are known to discard patently diseased *Hematodinium* infected *N. norvegicus* but the degree to which other types and conditions of animal are selected or discarded is unclear.

Given the novel opportunity to gather GPS traceability on commercial catches and considering the commercial practices of the fishers, the three aims of the current study were as follows. The fist was to develop a protocol where tails selected from a commercial catch could be sexed and given a carapace length (C/L) (the standard measure of lobster size). Secondly, to evaluate the selectivity of the post harvest commercial processing and sorting, i.e. what size and sex composition will a landed commercial sample compared to research trawls? The third aim was to select a robust diagnostic procedure for *Hematodinium*, using the tail alone, which has sensitivity greater than using patent disease to classify infected animals. A combination of

monthly research cruises using simulated commercial trawls in the North Clyde Sea area (to generate unbiased representative catch compositions and disease prevalence) and commercially landed product from vessels supplying Young's Seafood Ltd. were used.

Acquisition of commercially landed N. norvegicus tails

N. norvegicus tails were made available from Young's processing plant based in Annan, Scotland. All tails entering the processing plant were landed at the port of Trion in Ayrshire, North Clyde Sea area Scotland. Two sample dates were chosen, spring (April) and summer (July) 2007. Because little data exists on disease prevalence in the Clyde Sea area from other than research cruises, previous data and experience on patent prevalence and sub-patent prevalence in the North Clyde Sea area dictated the decision to sample on these dates. Spring sampling (n=200; two vessels) meant evaluating the discarding of patently-diseased animals and summer sampling (n=600; six vessels) meant sampling at a point where there is little to no patent disease. Tails were randomly sampled directly from the fish box (chilled tails on ice). Tails were transported on dry ice to the University of Glasgow and frozen at -20$^{\text{o}}$ C.

Measuring commercial tail damage and generation of carapace length (C/L) data

Carapace length (C/L) data for tailed material were generated allometrically from research trawls, where whole animals were available in large numbers. The relationship between C/L and tail width T/W on the 2$^{\text{nd}}$ and 3$^{\text{rd}}$ segments was tested for use as a means to generate C/L from a tail alone. From the November and

December 2006 research trawls, C/L and T/W were measured from a total of 250 animals (3rd abdominal segment) and 100 animals from October 2007 (2nd abdominal segment). In its application for commercial tails, only animals from the summer commercial sample were measured and by the 2nd abdominal segment. Any damage to segment structure (artefact of tailing), sufficient to preclude a width measurement was recorded. If no width measurement was available the tail was not sized.

Sexing the commercial lobster tails

Only animals from the summer sample were sexed. Sexing the tails was done using the 1st and 2nd pleopods and the male appendix masculina. The presence or absence of pleopod sets 1 and 2 was recorded. Male *N. norvegicus* have heavily calcified first pleopods (a copulatory appendage) easily distinguishable from the female. If the first set of pleopods was missing (artefact of tailing), the appendix masculina (also a copulatory appendage seen only on males) on the second male pleopods was used. If both the 1st and 2nd pleopods were missing the tail was not sexed.

Research trawls

Simulated commercial *N. norvegicus* trawls were conducted aboard the RV 'Aora' from the University of London Marine Biological Station, Millport (UMBSM). Trawls were conducted monthly between September 2006 and July 2007 (excluding October) and again in October 2007. Trawl depths were between 80 and 100m. A standard commercial net with a 70mm mesh size was towed south of Little Cumbrae Island in the North Clyde Sea area, on a standard transect for 1.5 Hours. Towing period was always between 0830 and 1100 hrs. One hundred *N. norvegicus* were

selected at random from the trawl, sexed, C/L measured and bled. Haemolymph was drawn from the haemal sinus at the base of the 5th pereiopod using 2ml syringes and 26guage needles. The haemolymph was mixed 1:1 with marine anticoagulant and frozen at -20 ° C for diagnostic use.

Haemolymph enzyme linked Immunosorbent assay (ELISA)

Prevalence of *Hematodinium* sp. infection in the simulated commercial trawls was investigated using an ELISA. All 100 animals from the December to July research trawls were tested. The procedure was similar to that described by Small *et al*. (2002), with some minor modifications. The haemolymph and anticoagulant mixtures were diluted 1:500 and used to coat duplicate wells of a high-binding ELISA plate (COSTAR) at 50µl per well for 0.5 hrs. After washing 5 times with PBS with 0.05% Tween-20 (PBST) the plates were blocked with 10% low-fat milk powder in PBST (Marvel) for 45 minutes and washed a further 5 times with PBST. Anti-*Hematodinium* sp. antibody (Field and Appleton, 1996) was diluted 1:5000 with the blocking solution and coated at 50µl per well for 0.5 hrs. The plate was subsequently washed 5 times with PBST and goat antibody against rabbit IgG conjugated with horseradish peroxidase (SIGMA) was diluted in blocking solution at 1:10000 and coated at 50µl for 0.5 hrs. After 5 washes with PBST, 100µl of Tetramethlybenzidine ultra slow ELISA substrate for horseradish peroxidase (SIGMA) was added at 100µl per well and the colour was allowed to develop for 5 minutes. Colour intensity was read at a wavelength of 560nm using Biolinx software (version 2.1) a spectrophotometer (). Positive and negative controls were run in duplicate on each plate. Positive control was the haemolymph from patently diseased Nephrops and negatives were haemolymph from animals in captivity for a year without showing any sign of

disease. Blank wells that received no homogenate were run in parallel with test samples and on every plate.

Assessing patent disease using cuticle colour change and pleopod scores

N. norvegicus with cuticle colour change associated with patent *Hematodinium* infection were distinguishable in the catch. A white milky haemolymph (due to large numbers of parasites in the haemolymph) was also visible on more advanced patent animals and aided a diagnosis. ELISA positive pleopods from December 2006 and January and February 2007 were scored on the five-point pleopod scale developed by Field & Appleton (1996). This procedure scores the aggregation of parasites and haemocytes in the pleopod blade and is a more sensitive measure of patent disease since it detects infections prior to the patent cuticle colour change.

Development of an immunoassay system for N. norvegicus tails

The ELISA procedure described above was used as the basis for *Hematodinium* sp. diagnosis in commercial tails. This is a rapid high-throughput assay, a feature directly applicable to screening large sample numbers and one successfully applied to research trawls. It was decided that the diagnostic material available (the tail) should give the same minimum diagnostic sensitivity (5×10^4 parasites / ml) when compared to the haemolymph ELISA. Specifically, any haemolymph positive should be positive by assaying tail material. Haemolymph ELISA positive animals from the December 2006, January and February 2007 trawls were used as positive control material. The

pleopods from these haemolymph ELISA positive animals had the parasite aggregation in their pleopods scored by the method of Field et al (1992) (see above) and photographed using a using a Minivid microscope camera (Minivid California, USA) and an Olympus dissecting microscope.

Haemolymph ELISA positive animals were tailed as in a commercial operation and frozen at -20 C to represent commercial storage. Samples were defrosted before use. The diagnostic materials chosen were the tail muscle and the 2nd pleopods. Tail muscle was dissected out from the middle of the tail and homogenised in phosphate buffered saline with Tween-20 (PBST), pH 7.8 (SIGMA) at a ratio 1:10 w: v. Two of the 2nd segment pleopods were also homogenised in 200 ul of PBST. Homogenates were then spun for 10 minutes at 5000 x g and their supernatants were stored at -20 C for use in the ELISA. Homogenate was diluted 1:125 with PBS and serially diluted to 1:000. These dilutions were run induplicate in the ELISA procedure described above. The adapted ELISA was used on both spring samples (2 x 100) and 3 from 6 of the summer samples (3 boats; 3 x 100) using the 2nd or 3rd pleopods.

Research trawl disease prevalence and catch composition

Hematodinium prevalence showed distinct temporal variation over the 10 month period where a diagnosis was made. Infected *N. norvegicus* were present in every month and total *Hematodinium* ELISA prevalence peaked in March 2007, also the month where patent disease began (Table 6.1). Patent disease was also highest in March 2007 (n=18) but not all the ELISA positive lobsters showed patent signs of disease. The difference between total ELISA and patent prevalence was 4 (sub-patent) animals which had not yet developed patent disease. All ELISA positive animals

showed patent disease in April 2007 (n=13) but this figure dropped sharply after this month. Patent animals were observed until June 2007. There was over a 10 fold increase in total prevalence from December 2006 (2%) through to March 2007 (21%) and conversely, this was matched by the drop in prevalence from the 21% peak to 3% by July 2007.

Sex specific prevalence varied markedly between months and between sexes in research trawls over the sample period. Prevalence's for both sexes were low (<6%) until the peak prevalence in March when 52.3% of females compared to 13.8% of males were infected. In April 2007, the percentage of females infected was lower (37%) but was nearly 4 times higher than that of males (4.1%). The influence that female prevalence had on total prevalence however was not as high as for males because until May 2007, the females represented a much lower proportion of the catch (see Table 6.1).

Over the 10 months disease investigation 71 infected animals from research trawls were diagnosed by ELISA. There was no significant difference between the size of male and female infected animals from the research trawls. However, the infected animals were in a narrow size range of between 19 and 37mm C/L. The ELISA positive animals from December, January and February showed varying pleopod scores from 0 to 2.

Table 6.1. Catch composition data for commercial trawls in the North CLYDE SEA AREA showing sex ratios, total disease prevalence, ELISA prevalence, cuticle colour method prevalence, and sex specific disease prevalence.

	%Male	%Female	Tot Prev %	ELISA Prev	Colour Prev	% Male infected	% Fem infected
Sep	64	36					
Nov	76	24					
Dec	88	12	2	2	0	2	0
Jan	80	20	3	3	0	2	5
Feb	71	29	10	10	0	9	10.3
Mar	72	28	21	21	18	13.8	52.3
Apr	73	27	13	13	13	4.1	37
May	39	61	10	10	4	15.3	6.5
Jun	44	56	8	8	5	9	7.1
Jul	50	50	3	3	0	2	4

Methods to generate carapace length, sex, moult stage and catch composition data for commercial tails

Six hundred tails from July 2007 were analysed for biometric data. Damage to tails was most apparent as broken sternal ribs on the ventral abdomen. This damage precluded T/W and therefore C/L data from 1-10 % of the tails from the range of boats tested. For sexing, the first set of pleopods was present on between 90 and 99 % of tails but the second set did not drop below 94 % which could be used to sex the tail. Of the sexed tails, apparent sex ratios were skewed to a female bias above that of the research trawl. The proportion of female tails varied between 55 and 71.2 % with research data showing 50 % for July (Table 6.2). A sub-sample from each of the first 3 boats in the summer sample was checked for moult stage by low-power microscopy. Accurate and time efficient moult staging was not feasible due to increased pleopod opacity, mechanical damage and melanosis.

Table 6.2. Data for percentage of first pleopods present (PL1), second pleopods present (PL2), tail width data taken (T/W), catch composition (% females and males) and successful ELISA tests. If the second pleopods were present the tail could always be sexed. A successful ELISA test was applied to every tail in the summer sample. July 2007.

Boat	PL1%	PL2%	T/W%	%sexed	%fem	%male	ELISA%
1	90	98	91	98	55.1	44.9	100
2	99	99	99	99	68.6	31.4	100
3	92	98	98	98	66	34	100
4	96	100	99	100	63	37	100
5	90	94	96	94	71.2	28.8	100
6	95	97	95	97	62	38	100

Size distributions of commercially landed material

Because of the higher incidence of small females in the commercial samples, the data for C/L generated allometrically showed a small sized bias (Figure 6.1). Excluding outliers, the range of C/Ls (generated allometrically) was between 22.5 and 34.8mm for commercial tails compared to a range of 20 – 45 mm C/L for a random sample of research trawl animals for July.
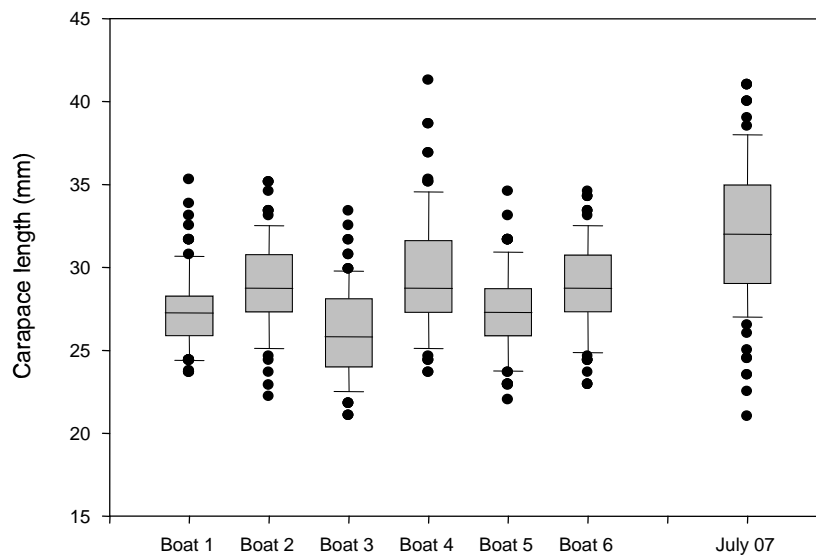

Figure 6.1. Box plot showing size distributions of commercial boats samples 1 – 6 and the July research trawl size data. Black dot data points represent outliers, the box represents the middle 50% spread of the data and the T-bars represent the full quartile range. The median line runs across the box.

Immunoassay application for commercial tails

The ELISA applied to pleopod homogenates gave positive results for all animals that also gave positive haemolymph reactions. These pleopods showed a score of between 0 and 3 for parasite aggregation. The assay was applied to both boats from the spring sample, and boats 1 – 3 from the summer sample.

ELISA assay results for the research vessel indicated a disease prevalence of 21 % in the spring (March data in Table 6.1) (RSP in Fig. 6.2). However, of these 18 % were also identifiable as infected by visible inspection, according to their body colour (filled red column in Fig. 6.2). In contrast, ELISA assay results for the commercial vessels indicated spring prevalences of only 3 and 5 % (boats 1 and 2 respectively), yielding a mean value of 4.5 % (CSP in Fig. 6.2). One possible explanation for this lack of correspondence between the prevalences reported from the research vessel and the commercial vessels is that the visibly infected animals were being discarded to a large extent from the commercial catch during sorting on board (open red column in Fig 6.2).

In the summer there were no visible infections from any vessels, but from the research vessel again 3 % of animals were infected according to the ELISA assay (July data in Table 6.1) (RSM in Fig. 6.2), and the summer samples from boats 1, 2 and 3 gave prevalences of 4, 3 and 6 % respectively, yielding a mean of 4.3 % (CSM in Fig. 6.2). At this time there was no basis for visually identifying infected animals, and this might explain the close correspondence of the prevalences reported from the research vessel and the commercial vessels at this time.
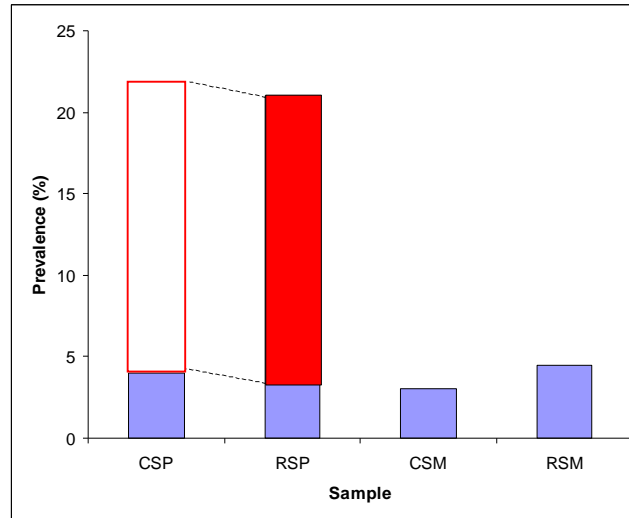
Figure 6.2. Prevalences for commercial samples in spring (CSP; mean) and summer (CSM; mean) and the research trawls in spring (RSP) and summer (RSM). The filled red column on RSP represents visibly infected animals. The open red column on CSP represents a projection of the degree to which visibly infected animals may have been removed. Patent infections were not observed in summer samples.

Therefore, the following conclusions can be drawn from this part of the work:

- The data from the commercial samples from 2007 represent the first investigation of disease using commercial *N. norvegicus* tails. Given the history of research sampling in the North Clyde Sea area, comparative (non-biased) results on the life history and disease prevalence in *N. norvegicus* stocks are available to explain commercial sample structure.

- The size difference between research and commercial catches may be due to smaller animal selectivity for tailed product. Sex bias in commercial samples is explained by the same selectivity, as females represent smaller size classes in the catch for large periods of the year. *N. norvegicus* C/L was successfully generated allometrically from over 91 % of all tails. Damage was sufficient to preclude T/W measurements for up to 10 % of commercial tails but ELISA reactions were 100% available.

- Secondly, *Hematodinium* prevalence from commercial catches in the spring of 2007 (3 and 5 %) did not match the research trawl prevalence which was 21 % by ELISA. However, 18 % of these animals exhibited cuticle colour change and could have identified and discarded from the commercial catches. The remaining 3 % were not colour positive and would have been landed. Summer commercial prevalences were in accordance with research prevalence data.

- There were no anomalous commercial datasets from this investigation. By reviewing the life-history strategies of the host lobster, parasite patency and theoretical commercial filtering (on research data), commercial landings show expected values for disease prevalence. The successful use of an antibody based assay (ELISA) for commercial material represents 'proof of concept' for a Lateral-Flow assay designed for *Hematodinium* screening. However, colour change associated with patent disease, if it leads to them being rejected during sorting of the catch, will bias the samples from late winter, spring and early summer, and if they are used for diagnosis will give artificially low prevalence values. Application of commercial sample screening must therefore take into account fishing practices and in the North Clyde Sea area, and this should exclude times of year when visually infected (and hence unmarketable) *N. norvegicus* are discarded. *Hematodinium* sp. and *Hematodinium*-like infections have been shown to affect a variety of commercially important crustacean species such as the snow crab (*Chionoecetes opilio*) and the blue crab (*Callinectes sapidus*). There, the parasite affects its host in such ways that the animals become non-marketable, leading to a huge economical loss within the industry. One of the reasons for the non-marketability is the fact

that the meat tastes bitter; therefore the condition is also termed 'bitter crab disease'.

In the Norway lobster (*Nephrops norvegicus*), *Hematodinium* sp. infection has been well reported, and changes in the biochemistry, physiology and behaviour of lobster due to the presence of the parasite have been described. However, there is little information about how the parasite affects the quality of the meat's organoleptical properties, and if the bitter taste described in crabs is also present in Norway lobster.

In Phase I of the Scottsih *Nephrops* Survey the effects of *Hematodinium* sp. infection on the quality of *N. norvegicus* tails were analysed. The main conclusions were that the parasite was affecting the breakdown of ATP in such a way that parasitised tails had higher K-values, indicating that the autolytic phase was quicker in these animals compared to un-parasitised animals. However, when looking at bacterial load and TMA concentrations no differences were found between parasitised and non-parasitised individuals suggesting that any changes in quality were because of the presence of the parasite and not because of the presence of more bacteria in the parasitised group.

In this Phase II, to gain a further insight into the effect of the parasite on the quality of the muscle a sensory evaluation was carried out to uncover if changes could be detected when the meat is cooked and if the meat flavour becomes bitter as in the case of crabs infected with *Hematodinium* sp.

To this end, *N. norvegicus* were caught by otter trawl in the Clyde Sea area by the research vessel 'Aora' from UMBSM and classified once on board as heavily-

parasitised and non-parasitised using the colour body method. Once separated, both groups were stored and transported on ice to the facilities in the University of Glasgow. On arrival, samples from both groups were directly frozen at −20 ℃ while another set of samples from each group were stored on ice for 4 days. After this time samples were frozen at −20 ℃ and send on dry ice to the 'Food Innovation Institute' in Edinburgh to perform a sensory evaluation (Full details of the Sensory Evaluation can be found in Annexe 1).

After catch, parasitized animals had a neutral smell, were slightly less firm, less chewy and they were described as bland in flavour and after taste compared to non-parasitised animals (Figure 6.3). The overall liking of parasitised samples was already negative, even just after catch when the samples should be regarded as 'fresh'. Therefore, results indicate that the sensory panel could find considerable differences between samples from parasitised and samples from non-parasitised animals. The fact that these changes were detected even just after catch points to the importance of rejecting these animals and discarding them from the catch as soon as possible.

Figure 6.3 Sensory evaluations performed by a professional sensory panel on whole *N. norvegicus* heavily parasitized or non-parasitised after catch.

Apart from offering the sensory panel 'fresh' samples, 4-day-old samples were also evaluated using the same protocol. In this way it was possible to evaluate how post-mortem progression is affected by the parasite and measured by the tasting panel. As shown in Figure 6.4, samples from heavily-parasitised animals scored poorly in all the attributes scored. Smell was more sour-ammoniacal and texture was softer and sloppier. However, flavour and after taste was described as bland but not bitter, suggesting that the effect of *Hematodinium* sp. is different from what has previously been described for other crustaceans. However, the overall liking for samples from

parasitised animals was less than the mid point 5 and therefore the sensory changes

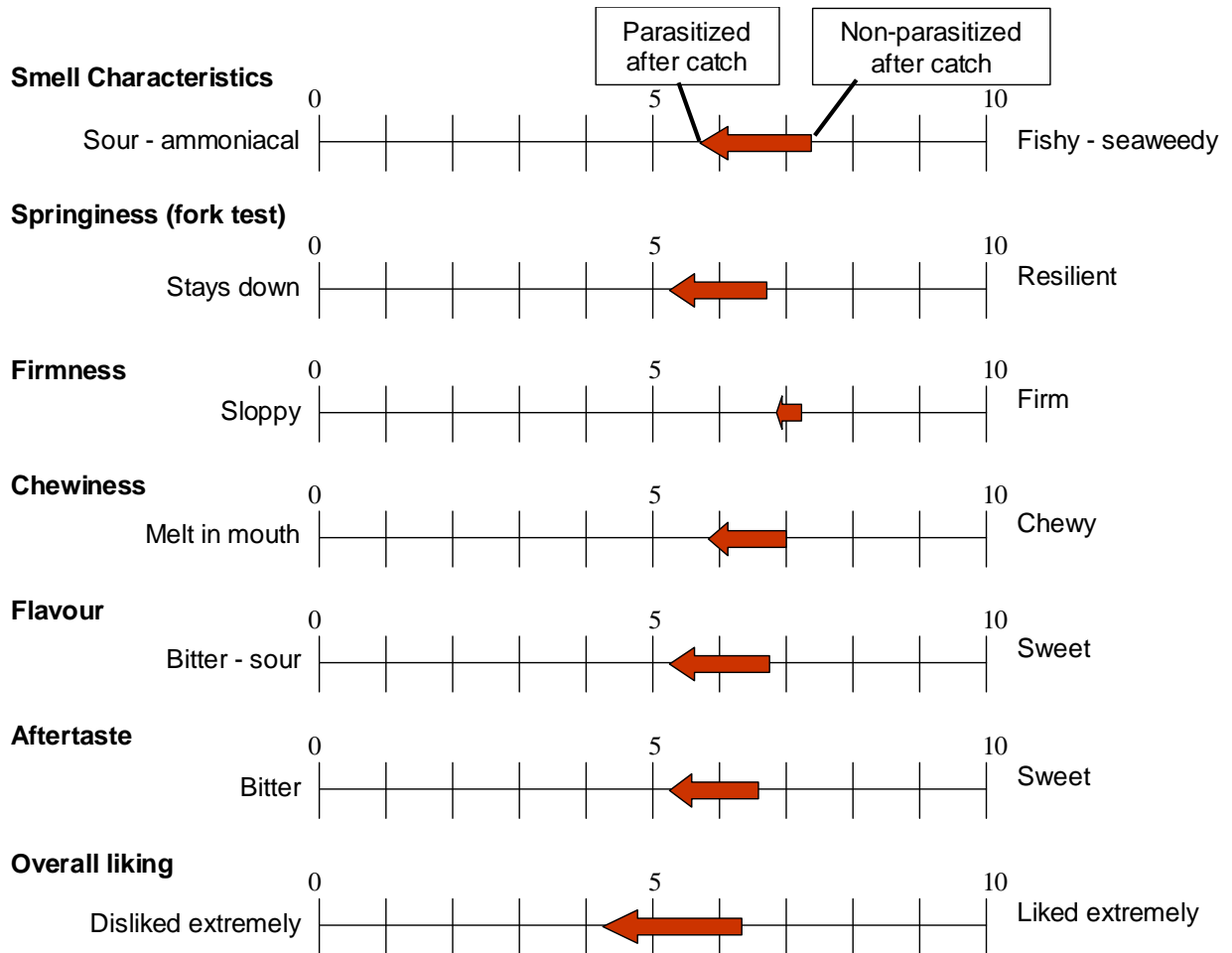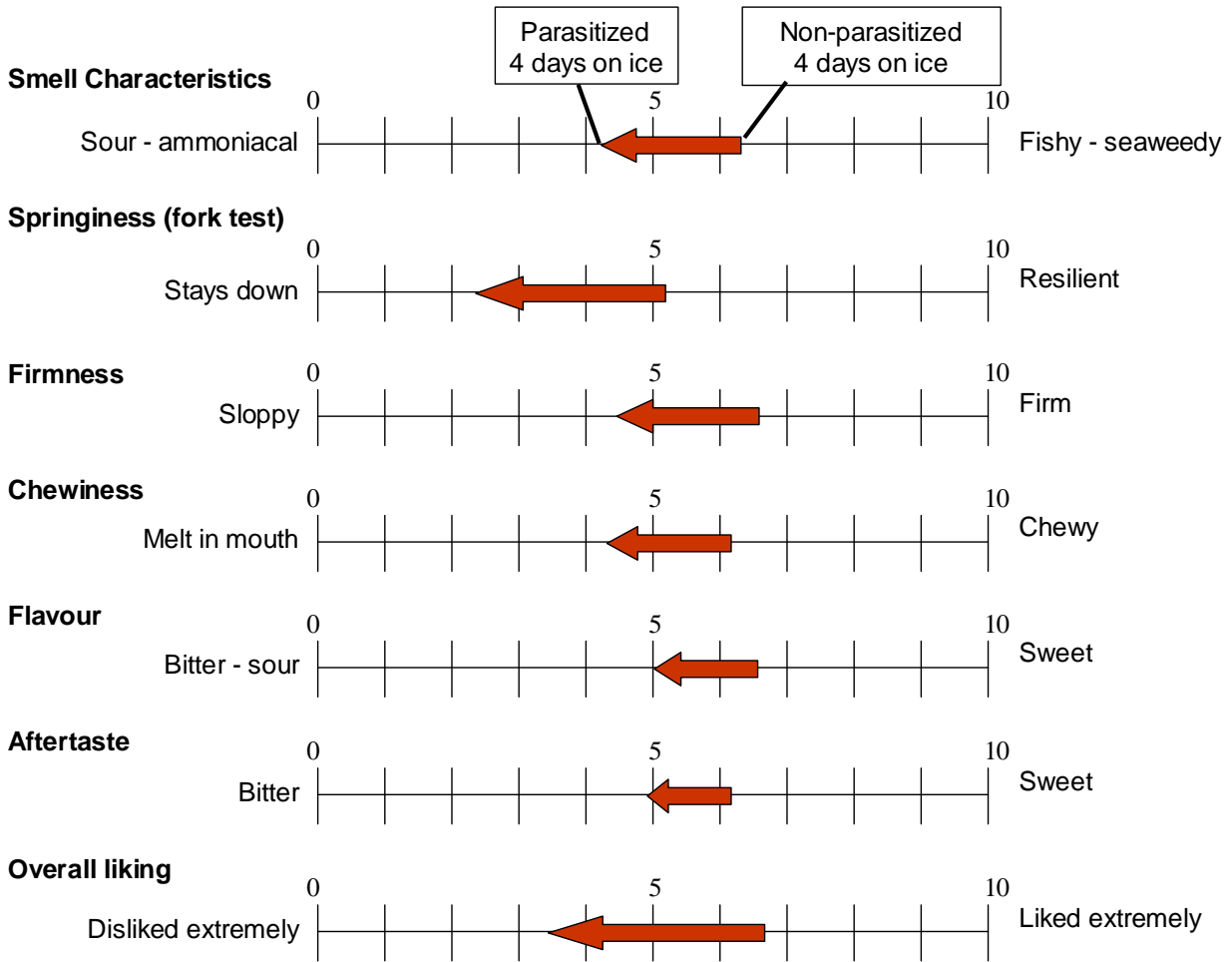produced by the parasite were scored as negative by the panel.

Figure 6.4 Sensory evaluations performed by a professional sensory panel on whole *N. norvegicus* heavily parasitized or non-parasitised 4 days after being stored on ice.

Additional Survey Work – Bycatch and discard assessment in *Nephrops* trawls

As an extension to Work Package 7 from Phase I and Work Package 6 from Phase II of the Scottsih *Nephrops* Survey, the additional task was undertaken to carry out initial surveys of the catch composition of the *Nephrops* trawls in catches from commercial vessels in the North Minch, and log these in the 'Yougsrace' traceability system.

Introduction

The capture of non-target organisms (bycatch) is a major problem in the fishing industry, as most of the bycatch will often be discarded by the fisherman as waste if it cannot be landed, or has no commercial value. Levels of discarded animals are currently estimated at 7.3 million tons worldwide each year (Kelleher, 2005). While this appears to represent a reduction in previous estimates of global discards (Alverson *et al.,* 1994), shrimp fisheries continue to discard the highest amount of material of any fishery (62.3% of total catches). The demersal trawl fisheries for *Nephrops norvegicus* (Linnaeus, 1758; hereafter referred to by genus alone) specifically discards approximately 42.7% of all catches by weight on average (Kelleher, 2003).

The consequences of capturing large proportions of unwanted organisms have been well documented, and have both economic and ecological effects (Hall *et al.*, 2000). Many species that are captured in demersal trawl gears do not survive the process, and are therefore returned to the sea dead or dying (Broadhurst *et al.,* 2006). In fisheries

which enforce a minimum landing size (MLS), discards often also contain undersize individuals belonging to the target species which may be immature or juvenile animals.

The *Nephrops* fishery is the most valuable in Scotland, with landings worth an estimated £89.3m in 2006 (FRS, 2008). Most landings are made by demersal trawlers, from sites including the Farn Deeps and Fladden Ground in the North Sea, and the Minches and Firth of Clyde on the west coast (FRS, 2008). Current management of the west coast stocks includes a MLS of 20mm carapace length (ICES, 2005) and implementation of minimum mesh sizes (70mm for single-rig gear, 80mm for twin-rig gear) (Bergmann *et al.,* 2002). Those fishing vessels that land *Nephrops* tails rather than (or in addition to) whole *Nephrops* will have increased levels of discard, as the cephalothorax or 'head' of *Nephrops* is separated from the tails and discarded at sea.

The *Nephrops* fleet fish similar grounds as those fished for roundfish (e.g. cod, whiting and haddock), and these species are targeted by a large mixed-fishery fleet in the North Sea (Rätz *et al.,* 2007). However, the required minimum mesh size is much smaller for *Nephrops* than for these roundfish species (120mm; ICES, 2005b) and juvenile cod, haddock and whiting are common bycatch species in the *Nephrops* fishery (e.g. Catchpole *et al.*, 2007, Catchpole and Revill, 2007, Stratoudakis *et al.,* 2001, Briggs, 1985).

Various strategies have been proposed to reduce the bycatch of roundfish in *Nephrops* trawls, including a number of gear modifications (e.g. separator panels, square mesh panels, square mesh codends, cutaway headlines). For a modification to be successful

and more likely to be adopted by commercial fishers, it must effectively reduce the unwanted bycatch species without simultaneously reducing the numbers of target animals (and therefore the profitability) of the catch (e.g. Burridge and Robins, 2000).

Different species are known to behave in stereotypical ways when they encounter trawl gear. Haddock and whiting for example, will be herded and swim in front of the trawl net approximately 1m above the seabed for a period of time before tiring, and rising in the water column (Main and Sangster, 1981). By contrast, *Nephrops* never rise more than 1m above the seabed, and do not display any herding behaviour (Main and Sangster, 1985).

The cutaway headline net design is one that specifically takes advantage of this behaviour in haddock and whiting. One recent design of a coverless trawl net, commissioned and tested by SeaFish (Arkley and Dunlin, 2003), reported approximately 70% reductions in the quantities of haddock and whiting, and a minor loss of 4.5% *Nephrops*. Trials in the North Sea demonstrated a reduction in catches of haddock and whiting by 50%, with no change in the *Nephrops* catch (Revill *et al*., 2006). This net was not size-selective for haddock or whiting, and reduced catches across the entire size range. While it would therefore not be a suitable design for a mixed fishery, it may be beneficial to a single-species *Nephrops* fishery, such as those on the west coast of Scotland.

Aims

These trials aim to quantitatively and qualitatively compare the performance of a new design of trawl gear in reducing the bycatch levels in the Scottish fishery for *Nephrops norvegicus* (hereafter referred to as the *Nephrops* fishery) compared to current standard gear. The modified gear was designed with a cut-away headline, theoretically allowing certain fish species a better opportunity to escape upwards away from the mouth of the trawl gear. Previous studies suggest that whiting (*Merlangius merlangus*) and haddock (*Melanogrammus aeglefinnus*) would benefit most from such a design.

Study Area

Over the three trials, a total of 15 trawls were carried out in the North Minch to the east of the Isle of Lewis (Outer Hebrides). The study area is shown in Figure 1. All tows were carried out between mean depths of 102m and 130m. The *Nephrops* stock in this area is believed to be stable or increasing (ICES, 2005).
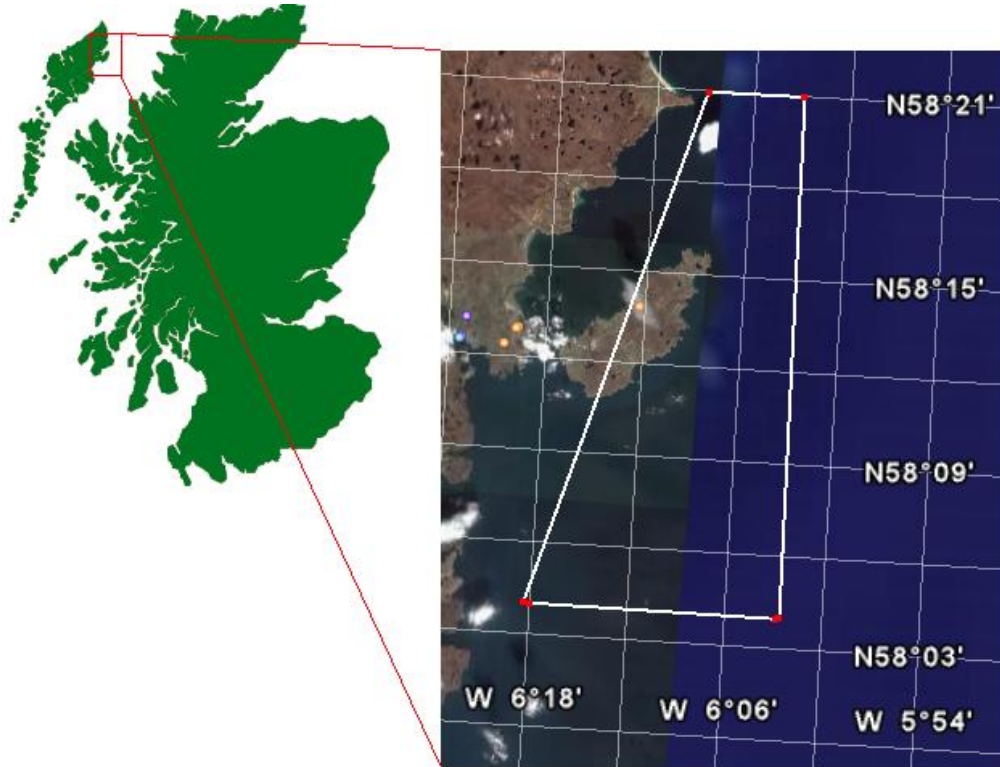
Figure 1: Map showing the approximate area in which the trawls were made through the
course of the preliminary trials (red and white boxes). Map from Google Earth.

Methods

The trawl trials were carried out from the MFV *Sharon Rose* (17m, 54tons, 244kW), a
twin-rig trawler based in Stornoway on the Isle of Lewis. The vessel was equipped
with the 'Youngs Trace' system, which monitors when and where the vessel is
towing, sorting and traveling.

The *Sharon Rose* was fitted with a standard 80mm mesh net and a 'modified'
(coverless) net (Fig. 2). Being a twin-rigger, it was possible to fish with both nets
simultaneously, which provides better data for analysis. So far, three trial sessions
have been carried out using this setup, and are described below. Information on each
tow is given in Table 1.

Trial 1

The first trial was carried out from 3rd – 7th September 2007. A single trawl was carried out each day, and the duration varied between two and four hours. The standard net was rigged on the port side of the vessel, and the modified net on the starboard side for the duration of the trial.

Trial 2

The second trial was carried out on 10th and 11th December 2007. Three trawls were made, each lasting two hours before the weather deteriorated and the trial had to be cancelled. The standard net was installed on the starboard side of the vessel and the modified net on the port side for these tows.

Trial 3

The third trial took place from 17th – 20th March 2008. Six 2-hour trawls and one 3-hour trawl were carried out, and the nets were alternated between each side of the vessel each day (Table 1).
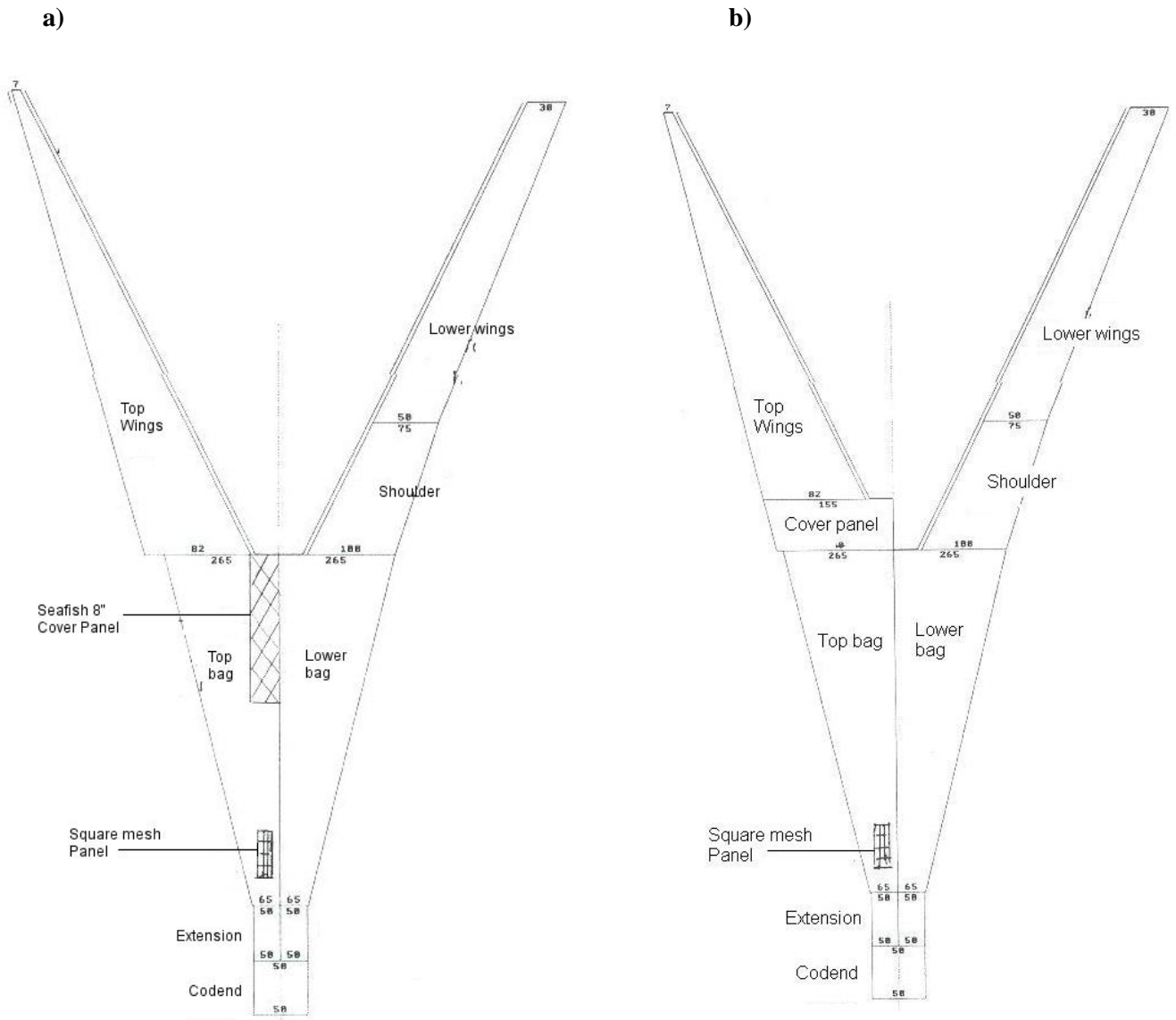
a)   b)

Figure 2: Diagrams showing the nets used in the three trials. (a) Standard net; (b) Modified, coverless net.

Table 1: Description of each trawl and gear used.

| | | | Side of Vessel | |
Date	Trawl No.	Trawl duration	Normal gear	Modified gear
03/09/2007	Sep 1	4 hours	Starboard	Port
04/09/2007	Sep 2	4 hours	Starboard	Port
05/09/2007	Sep 3	2 hours	Starboard	Port
06/09/2007	Sep 4	3.5 hours	Starboard	Port
07/09/2007	Sep 5	2 hours	Starboard	Port
10/12/2007	Dec 6	2 hours	Port	Starboard
10/12/2007	Dec 7	2 hours	Port	Starboard
11/12/2007	Dec 8	2 hours	Port	Starboard
17/03/2008	Mar 9	2 hours	Port	Starboard
18/03/2008	Mar 10	2 hours	Starboard	Port
18/03/2008	Mar 11	2 hours	Starboard	Port
19/03/2008	Mar 12	2 hours	Port	Starboard
19/03/2008	Mar 13	3 hours	Port	Starboard
20/03/2008	Mar 14	2 hours	Port	Starboard
20/03/2008	Mar 15	2 hours	Port	Starboard

Once the tows were completed, the catches from each net were brought on board and examined separately. The entire catch from each net was initially sorted into major groups, which were:

- *Nephrops* tails

- Whole *Nephrops*

- *Nephrops* heads (first trial only; discarded)

- Commercial species of roundfish

- Non-commercial species of roundfish

- Flatfish

- Elasmobranchs (sharks and rays)

- Invertebrates

Within these groups, the total weight of each species present was recorded. The weights of individual commercial fish species were also recorded. During the first

trial, the lengths of a sub-sample of the commercial roundfish and flatfish were also recorded, but there was not enough time to do this during the second or third trials.

Data analysis

Weights are presented as means and standard errors by net type and by group or species, as appropriate. Error bars are also shown on each graph where appropriate and indicate the degree of variability within a sample.

When comparing two or more results, statistical analysis is required to confirm whether there is a real difference between them or not. This involves assessing the differences in average values between groups in relation to the amount of variability within groups. Two groups are considered 'significantly different' if there is less than a 5% probability that the observed difference between samples could have arisen if there was, in reality, no difference between the sampled groups. This probability is shown by the 'p' value below each figure. If $p < 0.05$, the results are considered to be statistically significant and indicative of a real difference between groups.

All analyses have been performed using the statistical program Minitab 15.

Results

Based on the data from all three trials, the mean weight of total catch did not differ significantly between net types (Fig. 2; $p > 0.05$).
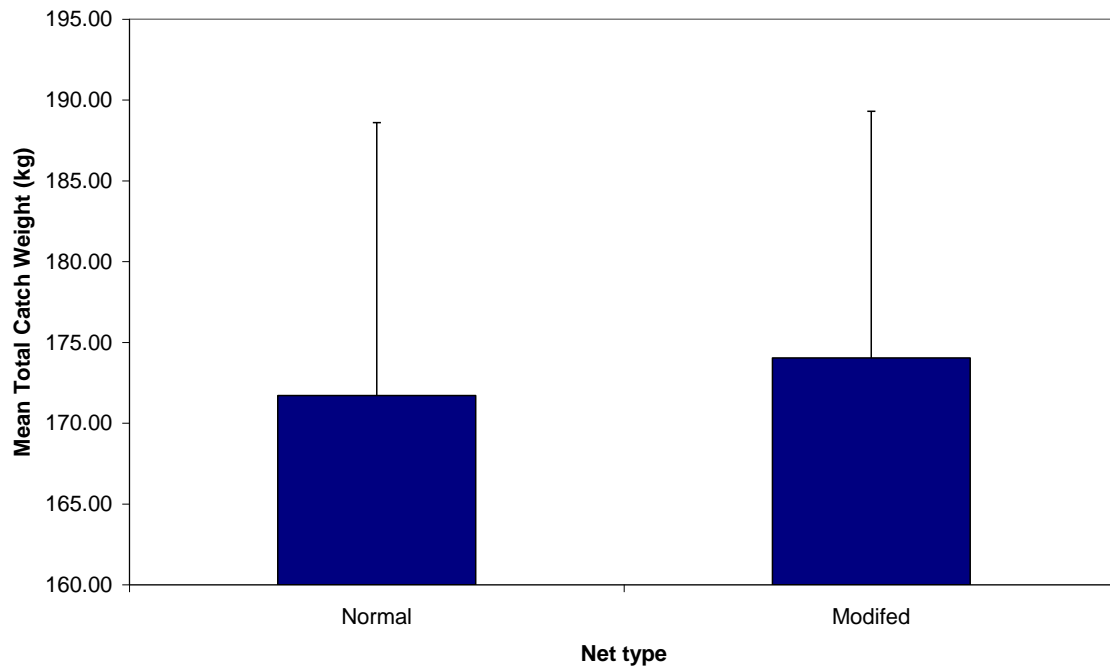
Figure 2: Mean weight of the total catches from each net. Paired t-test: $p > 0.05$. Error bars show one standard error.

After the September trial, the catches from the standard gear appeared to be heavier. However, since the nets were not swapped from one side to the other during this trial, it was unclear whether this was an effect of the differences in net design or simply a bias in the catch efficiency of the *Sharon Rose*. This is a common source of error on paired trawlers, so the nets were swapped to the opposite sides of the boat for the December trial. When the results were aggregated for the first two trials, a significant difference was apparent between the sides of the vessel ($p < 0.05$), with the starboard side net appearing to catch consistently more than the port side net (Fig. 3). Since the March trial, this trend has disappeared, and there is no significant difference between the nets. However, since this is potentially a large source of error, the nets will continue to be swapped throughout subsequent trials to ensure the best data are collected for statistical analysis.
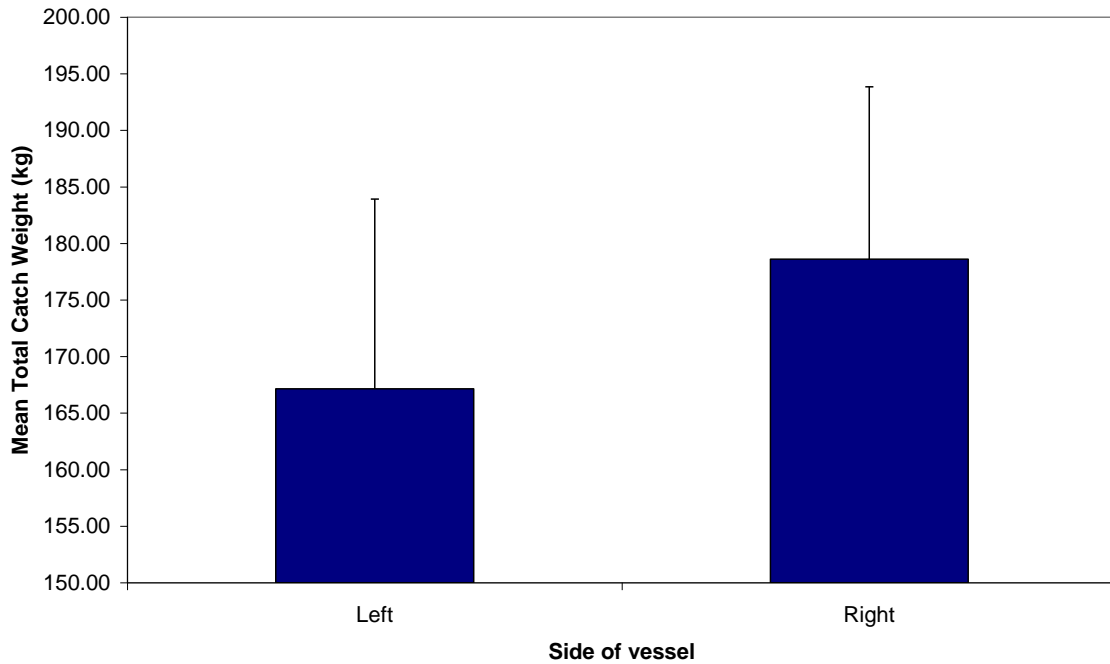
Figure 3: Mean weight of the total catches from each side of the *Sharon Rose*.
Paired t-test: $p > 0.05$. Error bars show one standard error.

Nephrops **tails**

There was no significant difference in the weights of *Nephrops* tails caught between either the different nets or sides of the boat. This was true both for each individual trial, and for the aggregated data.

Catch composition: overall

The overall catch composition is shown in Figure 4. Trawl data for the two nets in all three trials are shown.   The 'discard' portion contains all non-target (i.e. non-*Nephrops*) animals, and accounts for 46% of all material recovered during trawling with the normal net, and 44% for the modified net. Within the *Nephrops* portion, the '*Nephrops* heads' would also be discarded, bringing the total discarded material to approximately 73% for both the normal and modified nets (green areas).   The remaining 27% of the catch from the nets would be landed and sold (orange areas).

Normal net   **Modified net**
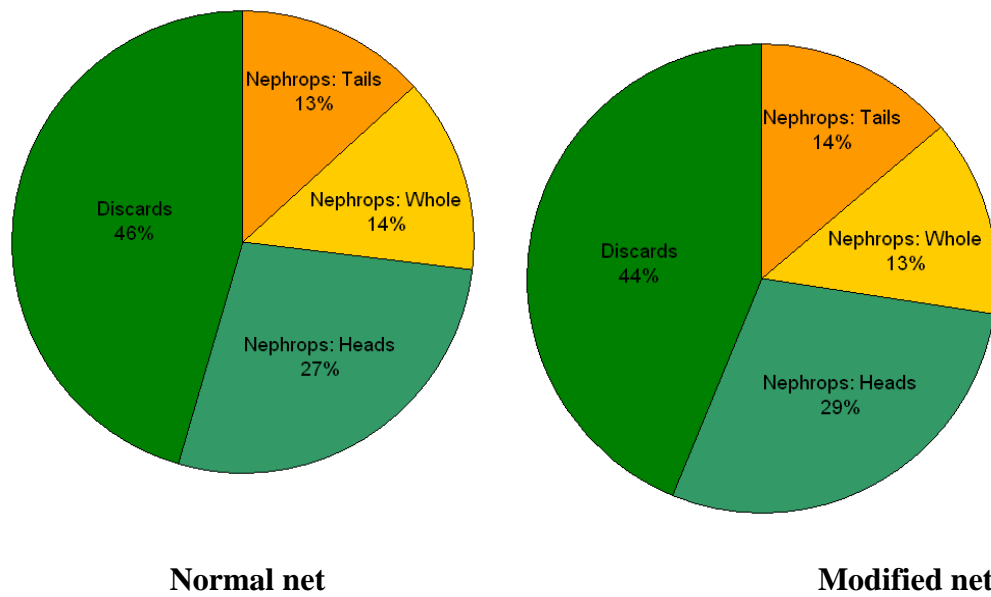
Figure 4: Percentage of catch components by weight for both nets based on data from all trials. The green area shows the material that is discarded; the orange area is material that would be sold. There is no significant difference in the weights of *Nephrops* (tails, heads or whole animals) between the two nets.

Mean *Nephrops* weights

The mean weight of *Nephrops* caught did not differ significantly between the nets (Fig. 5).

Figure 5: Mean weights of the different parts of the *Nephrops* catch.
Paired t-test: p > 0.05. Error bars show one standard error.

Standardising the bycatch results

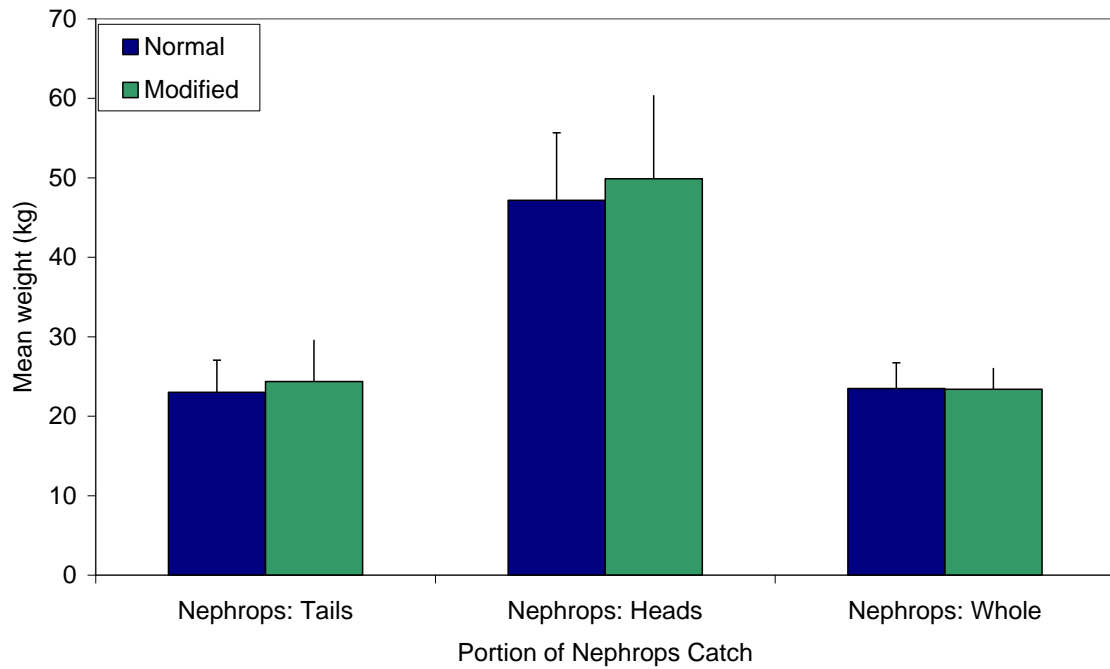Since the total weight of the catches depended on the side of the boat each net had been attached to, a straightforward comparison of the bycatch weights taken by each would be misleading. Therefore, the bycatch weights have been standardised with respect to the weight of *Nephrops* tails (since this is the largest part of the targeted catch). All standardised results are thus expressed as kilograms of bycatch per kilogram of *Nephrops* tails (kg kg^{-1}).

Bycatch

There were no significant differences between the normal and modified nets in the standardised weights of any of the main bycatch groups (Fig. 6). The pattern of differences among bycatch groups has changed over the course of the three trials. After the initial trial in September, there appeared to be a difference in the 'roundfish' group. This disappeared following the December trial and a difference in the 'sharks and rays' appeared instead. The aggregated data for all three trials shows that this difference has also now disappeared, and none of the main groups show any significant difference.
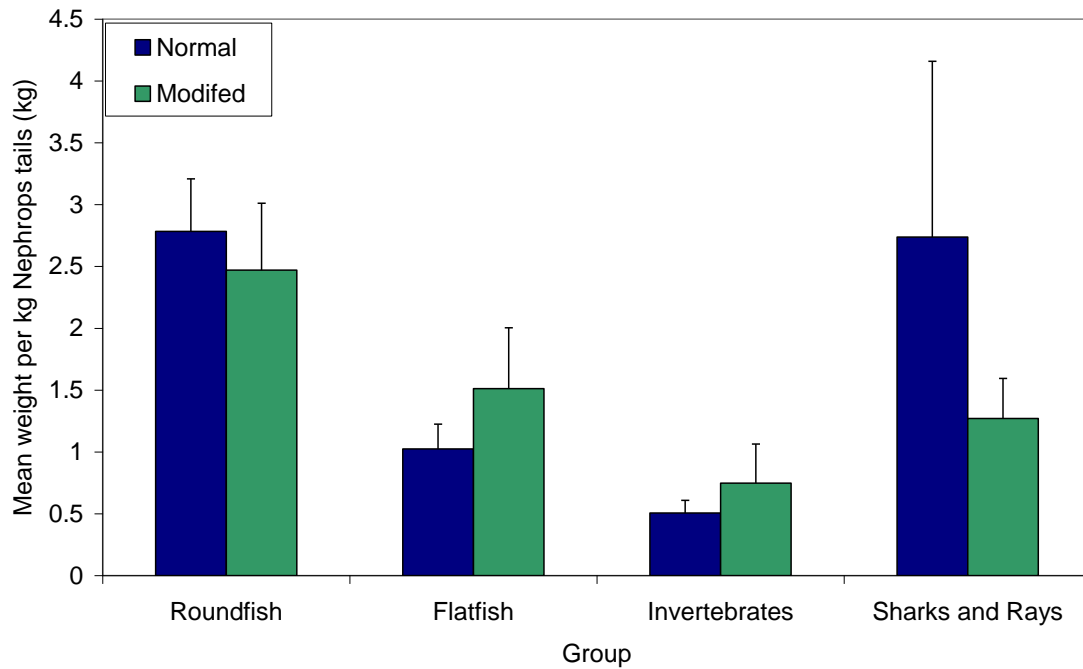
Figure 6: Mean standardised weights of each major bycatch group from each net. Wilcoxon's matched pairs test: p > 0.05 for all groups. Error bars show one standard error.

Roundfish

Although there was no overall difference in the standardised weights of individual roundfish species between the nets, there was a significant difference in the weights of whiting captured by each net (Fig. 7). A difference in the weights of whiting was recorded following the December trial. There was a significant difference in the 'others' group (containing non-commercial species such as John Dory and dragonets for example) following both the September and December trials, but not in the March trial.
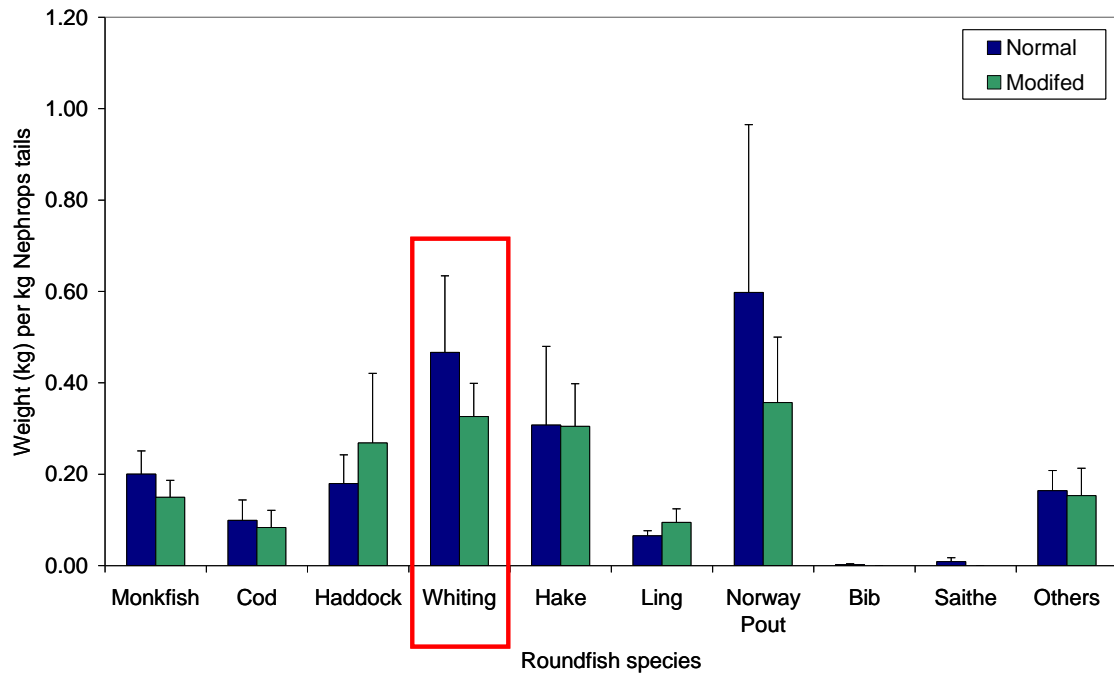
Figure 7: Mean standardised weights of individual roundfish species from each net. The red box highlights the significant difference. Wilcoxon's matched pairs: whiting: $p = 0.02$. Error bars show one standard error.

Flatfish

There were no significant differences between the nets in the standardised weights of the flatfish species (Fig. 8). This is the same result that was found following the September and December trials.
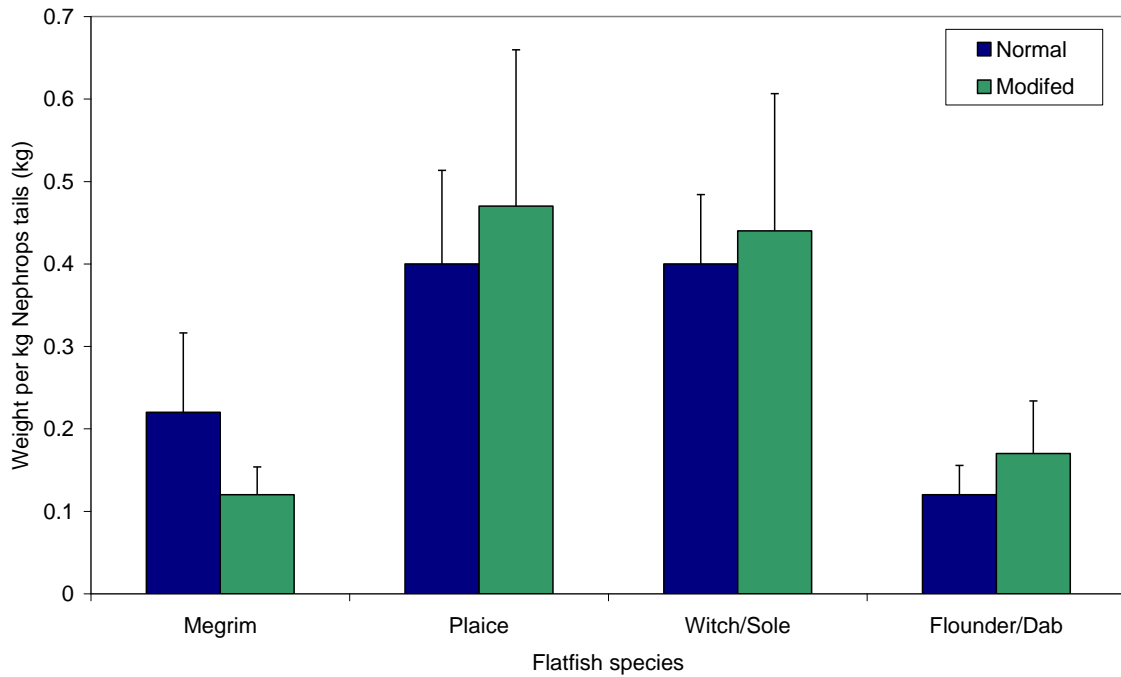
Figure 8: Mean normalised weights of individual flatfish species from each net. Wilcoxon's Matched pairs: p > 0.05 for all groups. Error bars show one standard error.

Invertebrates

There were no significant differences in the standardised weights of invertebrate groups between the nets (Fig. 9). This is the same result that was found following the September and December trials.
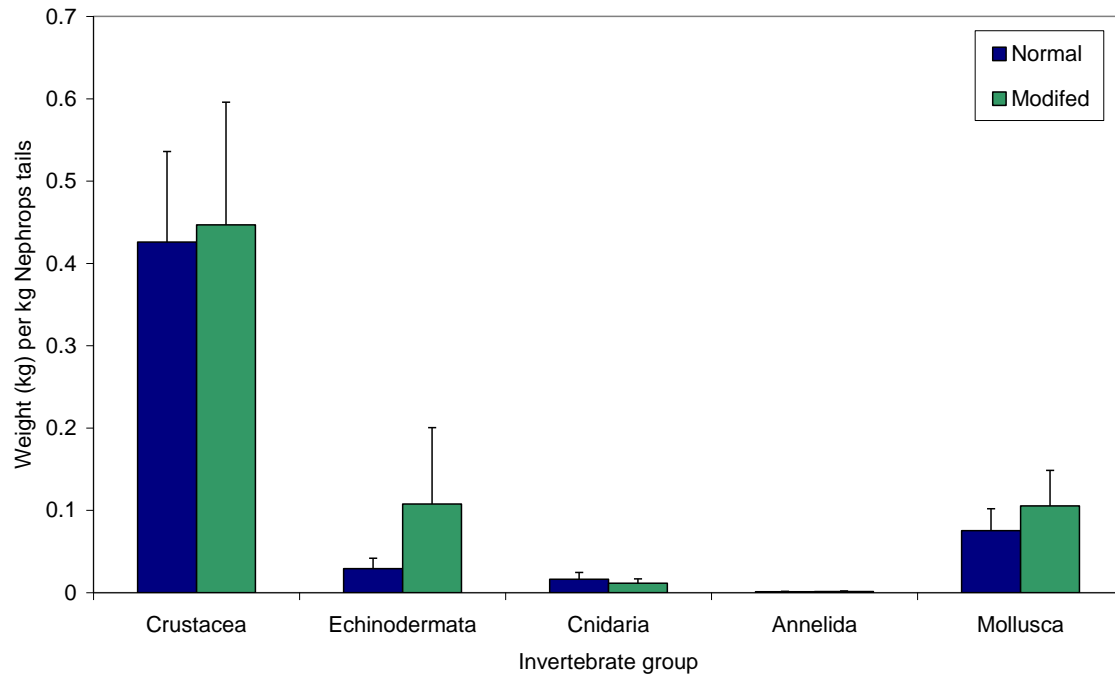
**Figure 9:** Mean normalised weights of invertebrate groups from each net. Wilcoxon's Matched Pairs: $p > 0.05$ for all groups. Error bars show one standard error.

Species composition

From these 3 trials, 86 species were recorded, including 7 species of commercial roundfish, 21 species of non-commercial roundfish, 7 species of flatfish, 9 species of sharks and rays, and 41 species of invertebrate. The complete species list is given in Appendix A.

A number of sensitive or threatened species were present in these catches, particularly including the long-nosed skate (*Dipturus oxyrinchus*; IUCN red-list status: near threatened) and spurdog (*Squalus acanthias*; IUCN red-list status: vulnerable). The

numbers of spurdog captured in December was much greater than in September or March. Stocks of this species are depleted, and ICES have recommended a ban on targeted fisheries (ICES, 2007). If large numbers of spurdog are vulnerable to trawling at certain times of year or in certain areas, there could be a significant detrimental impact on their populations. The spurdog is known to form single-sex aggregations of individuals of similar age (ICES, 2006), so it could be possible to remove a large part of a year-class quite quickly through trawling. Should further studies substantiate this concern, it would be desirable to investigate methods for reducing the numbers of spurdog caught as bycatch in the *Nephrops* fishery.

Conclusions and Recommendations

These results should be viewed cautiously until a larger study can be carried out, as it is possible that seasonal trends could have a large impact on the final results.

The present findings indicate that the catch rate and catch composition of the two nets was very similar, but that the modified net reduces the bycatch of whiting, and may reduce the catch of sharks and rays at certain times of year, without a concomitant reduction in the marketable catch. Whiting is one species that might be expected to escape capture by a net with a cut-back headline (Catchpole and Revill, 2007). These differences between the nets have become apparent as data have been accumulated from several trials. It is possible that this reflects seasonal changes, or differences in the fishing ground and the weather in different trials, but this should be resolved by increasing the number of samples over a longer-term study.

A left-right bias in the catch efficiency of the nets was recorded in the December trial. While this bias was not evident in the March trial, it would be prudent to continue swapping the nets in subsequent trials to avoid confounding the comparison of net types, and to allow proper analysis of the results.

The data gathered from this preliminary trial provides an indication of the level of bycatch and discard rates in the Stornoway area. However, to progress, a larger systematic study is required that will examine bycatch and discard rates in a range of vessels (both single- and twin-rig trawlers), over different fishing grounds (e.g. the Minches and the Clyde Sea Area) and throughout the year.

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Appendix A

Major Group	Latin name	Common Name
Invertebrates	*Adamsia carciniopados*	
Invertebrates	*Aequipecten opercularis*	Queen scallop
Invertebrates	*Alcyonium digitatum*	Dead man's fingers
Invertebrates	*Aphrodita aculeata*	Sea mouse
Invertebrates	*Arctica* sp.	
Invertebrates	*Asterias rubens*	Common starfish
Invertebrates	*Astropecten irregularis*	Burrowing starfish
Invertebrates	*Brissopsis lyrifera*	Heart urchin
Invertebrates	*Cancer pagurus*	Edible (Brown) crab
Invertebrates	*Crangon crangon*	Brown shrimp
Invertebrates	*Cyanea capillata*	Lion's mane jellyfish
Invertebrates	*Echinus esculentus*	Edible sea urchin
Invertebrates	*Eledone cirrhosa*	Curled octopus
Invertebrates	*Funiculina quadrangularis*	Tall sea pen
Invertebrates	*Goneplax rhomboides*	Square crab
Invertebrates	*Henricia oculata*	Bloody henry (starfish)
Invertebrates	*Inachus* sp.	
Invertebrates	*Liocarcinus depurator*	Harbour crab
Invertebrates	*Loligo forbesii*	Common squid
Invertebrates	*Luidia ciliaris*	7 armed starfish
Invertebrates	*Marthasterias glacialis*	Spiny starfish
Invertebrates	*Munida rugosa*	Squat lobster
Invertebrates	*Nephrops norvegicus*	Norway lobster
Invertebrates	*Neptunea antiqua*	
Invertebrates	*Pagurus bernhardus*	Hermit crab
Invertebrates	*Pagurus prideaux*	Hermit crab
Invertebrates	*Pandalus borealis*	Pink shrimp
Invertebrates	*Porania* sp	Cushion star
Invertebrates	*Sepia officinalis*	Common cuttlefish
Invertebrates	*Sepiola atlantica*	Little cuttlefish
Invertebrates	Spider crab A	
Invertebrates	*Stichastrella rosea*	
Invertebrates	Unknown anemone 1	
Invertebrates	Unknown anemone 2	
Invertebrates	Unknown anemone 3	
Invertebrates	Unknown gastropod 1	
Invertebrates	Unknown gastropod 2	
Invertebrates	Unknown gastropod 3	
Invertebrates	Unknown hydroid	
Invertebrates	Unknown ophiuroid	
Invertebrates	Unknown white shrimp	
Commercial roundfish	*Gadus morhua*	Atlantic cod
Commercial roundfish	*Lophius budegassa*	Black monkfish
Commercial roundfish	*Lophius piscatorius*	Monkfish
Commercial roundfish	*Melanogrammus aeglefinus*	Haddock
Commercial roundfish	*Merlangius merlangus*	Whiting

Commercial roundfish	*Merluccius merluccius*	Hake
Commercial roundfish	*Molva molva*	Ling
Flatfish	Dab \ Flounder	
Flatfish	*Lepidorhombus whiffiagonis*	Mekrim
Flatfish	*Pleuronectes platessa*	Plaice
Flatfish	*Scophthalmus rhombus*	Brill
Flatfish	Witch \ Sole	
Non-commercial roundfish	*Aspitrigla cuculus*	Red gurnard
Non-commercial roundfish	*Callionymus lyra*	Dragonet
Non-commercial roundfish	*Capros aper*	Boarfish
Non-commercial roundfish	*Clupea harengus*	Herring
Non-commercial roundfish	*Cyclopterus lumpus*	Lumpsucker
Non-commercial roundfish	*Gaidropsarus vulgaris*	3-beard rockling
Non-commercial roundfish	*Labrus mixtus*	Cuckoo wrasse
Non-commercial roundfish	*Myoxocephalus scorpius*	Scorpionfish
Non-commercial roundfish	*Pholis gunnellus*	Butterfish
Non-commercial roundfish	*Phycis blennoides*	Greater forkbeard
Non-commercial roundfish	*Pollachius virens*	Saithe
Non-commercial roundfish	*Rhinonemus cimbrius*	4-beard rockling
Non-commercial roundfish	*Rockling*	
Non-commercial roundfish	*Scomber scombrus*	Mackerel
Non-commercial roundfish	*Syngnathus sp.*	Pipefish
Non-commercial roundfish	*Trachurus trachurus*	Horse mackerel
Non-commercial roundfish	*Trigla lucerna*	Tub gurnard
Non-commercial roundfish	*Trisopterus esmarkii*	Norway pout
Non-commercial roundfish	*Trisopterus luscus*	Bib
Non-commercial roundfish	Unknown sp. 1	
Non-commercial roundfish	*Zeus faber*	John Dory
Sharks and Rays	*Dipturus oxyrinchus*	Long-nosed skate
Sharks and Rays	*Galeus melastomus*	Black-mouthed dogfish
Sharks and Rays	*Leucoraja naevus*	Cuckoo ray
Sharks and Rays	*Raja brachyura*	Blonde ray
Sharks and Rays	*Raja montagui*	Spotted ray
Sharks and Rays	*Scyliorhinus canicula*	Lesser-spotted dogfish
Sharks and Rays	*Squalus acanthius*	Spurdog
Sharks and Rays	Unknown ray 1	

DELIVERABLES from the Phase II of the Scottsih *Nephrops* Survey Research conducted by the Partnership between Youngs'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. A list of recommendations based on scientific data to the industrial partner is outlined at the end of this section.

OBJECTIVES

- To identify the main factors which affect the product quality of whole *Nephrops norvegicus* (langoustines), in comparison to tailed product, and to determine the most effective handling and packing methods for prolonging the shelf life of this whole product.

  o *The main differences between handling whole langoustines rather than tails have been identified.*

  o *The following factors that affect the product quality of whole langoustines have been evaluated:*

    ▪ *Physical damage according to trawl duration*

    ▪ *The impact of necrotic animals in the overall quality of the catch*

    ▪ *Effective handling: the effect of anti-melanotic additives and other alternatives to inhibit melanosis development*

    ▪ *The effectiveness of different storing conditions*

    ▪ *Packing methods for prolonging shelf life: Modified Atmosphere Packing (MAP)*

- To establish objective benchmarks for the quality of both tailed and whole fresh products, by calibrating the developed biochemical and instrumental assays against sensory evaluations performed by an independent, professional sensory panel.

  o *A sensory scoring system has been developed for whole fresh langoustines un-treated or treated with anti-melanotic products*

  o *A professional sensory panel has been trained for whole fresh langoustines and results from the sensory data have been calibrated against the developed biochemical methods.*

- To identify the key features of the population biology and ecology of *N. norvegicus* that impact upon the body condition of the animals and their post-capture quality, and to determine how these vary with location, fishing practices and transport.

  o *The tailed landings composition from eight boats in the Clyde Sea showed very few to no soft tail or egg-bearing (berried) female tails. Both are undesirable and lower the quality of the product. The tailed landings data is in accordance with research data on lobster life history strategies. This indicates a high degree of selectivity by fishermen to mange lobster body condition in lndings.*

Scope of project

Phase II of the Scottish *Nephrops* Survey was achieved through a series of 6 workpackages (WP), that used the suite of methodologies developed in Phase I of the project for assessing product quality, freshness and spoilage rates. These were applied to different products and procedures.

WP1. Post-capture changes in the quality of whole langoustines

In order to exploit the expanding market opportunities for whole langoustines, supplied as a chilled fresh product, it is essential to understand the processes that underlie quality loss in the whole animal, compared to the tailed product. Using measures for quality, freshness and spoilage developed in Phase I of the Scottish Nephrops *Survey, the following factors will be assessed immediately on capture by research vessel, and for up to 7 days thereafter while the product is held on ice or at different ambient temperatures.*

- The extent to which degradation occurs due to autolysis by enzymes from the organs in the 'head'

  o *Experiments have been carried out in order to compare the differences in post-mortem degradation between tail muscles from tails alone or from the tails of whole langoustines. Results indicate that autolytic enzymes from the head do not affect the biochemistry of the muscle from the tail (i.e. the main edible part).*

- The production of any undesirable breakdown products (eg. Histamine)

  o *A methodology to measure seven biogenic amines, including histamine, has been established using HPLC. Histamine did not appear to be a main concern in* N. norvegicus, *even in spoiled samples. The main undesirable biogenic amines of bacterial origin found in* N. norvegicus *were agmatine and, to a less extent, putrescine and cadaverine.*

- The effect of this autolysis on the texture and flavour of the tail meat

  o *Flavour-enhancing products such as IMP, and a bitter-off flavour compound (hypoxanthine) were analysed in whole langoustines stored at 0-1 ºC for up to 7 days. IMP concentrations were highest on day 3 of*

storage and Hx increased slowly throughout the storage. Comparisons were made with tail meat from tails alone. IMP in tail meat from tails alone was highest on day 1 of storage at the same temperature, indicating that the autolytic phase in whole langoustines is slower compared to tails. However, after day 3 no differences were found between the two products.

- The time course of post-mortem changes, including changes in tissue pH and the onset of rigor mortis

  o *Changes in muscle pH were studied in muscle tail from whole langoustines and compared to tails alone. Muscle pH increased with storage time up to 8.0-8.2, when a plateau was reached. No differences were found between the two types of products.*

  o *According to visual observations the onset of rigor mortis was absent in whole animals, similarly to the case with isolated tails.*

- The time course and extent of bacterial spoilage.

  o *Total bacteria counts were analysed in tail meat from whole langoustines and compared to tails alone. No differences were found in bacterial load, indicating that the time course and the extent of bacterial spoilage in whole langoustines is very similar to those in tails.*

Leading to a better understanding on the key quality issues to be addressed when working with whole animals instead of tails.

WP2. Factors that affect the product quality of whole langoustines

- The effect of trawl duration on the quality of whole langoustines – is there any advantage in very short trawls for supplying this product in a better condition?

  - *Physical damage in whole langoustines was recorded (using the damage index from Phase I of the Scottish Nephrops Survey) according to trawl duration. Physical damage especially in 'hard' animals increased significantly with trawling time. Since this portion of the catch would be the most appropriate to be sold as whole fresh langoustines, short trawls (60 min) are recommended in order to minimise physical damage.*

- The effects of capture damage on quality loss, including the occurrence of tail muscle necrosis.

  - *The biochemistry of necrotic animals was studied in terms of quality loss. Live necrotic animals had higher levels of muscle L-lactate indicating that these animals are more stressed than non-necrotic animals. From analyses of the nucleotides, although ATP was the main nucleotide, IMP and INO were also present suggesting that part of the muscle is in a condition equivalent to the post-mortem stage. K-values were higher in necrotic animals indicating a significant quality loss of the catch if necrotic animals are a significant part of the catch. Optimised capture and handling protocols established in Phase I and Phase II of the Scottish* Nephrops *Survey should be applied in order to minimise the percentage of necrotic animals in the catch.*

- The effectiveness of icing procedures in maintaining product freshness

- o *Cooling rates of boxes full of tails or full of whole langoustines were determined. No differences were found in the cooling rates of these two products, indicating that studies carried out with tails in Phase I of the Scottish Nephrops Survey would also apply for whole langoustines.*

- o *Leaving a fish box full of whole langoustines in a cold room was not an effective cooling procedure to ensure high quality. Icing on top of the box proved to be an effective additional treatment, and cooling of the animals in the middle of a fish box was then obtained within 3-4 hours.*

- Comparison of product quality from different supply ports and vessels in relation to post-capture handling procedures, icing methods and transportation conditions.

  - o *Comparison of product quality from different vessels was carried out and compared with the results from the research vessel used by the University of Glasgow. The blood (Hematodinium sp.) parasite was found to have a low prevalence in commercially landed samples compared to research trawl data.*

Leading to recommendations for the most appropriate methods for capturing, storing and handling langoustines for the whole fresh market.

WP3. Establishing the effectiveness of gas flushing for prolonging shelf-life

- Determine the effectiveness of different $CO_2/O_2/N_2$ gas mixtures as inhibitors of the growth and activity of the known specific spoilage organisms for *Nephrops* when grown in culture

- Test the effect of different gas mixtures on reducing the rate of spoilage of *Nephrops* meat under controlled conditions

  - *A full range of different $CO_2/O_2/N_2$ gas mixtures was tested under controlled conditions. In a first cycle, the rate of spoilage was measured as QIM progression and bacterial load. Gas mixtures containing more than 50 % of CO_2 and less than 50 % of O_2 gave the most effective results.*

  - *In a second cycle a more detailed range of $CO_2/O_2/N_2$ gas mixtures was tested under controlled conditions. Results indicated that bacterial growth is significantly reduced when the gas mix contains high levels of CO_2 (60-100 %). On the other hand, O_2 concentrations of 0-15 % had an inhibitory effect on melanosis development when compared to animals packed on air.*

- Establish the optimal gas mixtures for maintaining quality and freshness of whole langoustines.

  - *The optimal gas mix (OGM) for whole langoustines was identified. The OGM is formed by 10 % of O_2, 80 % of CO_2 and 10 % of N_2. Under controlled conditions this OGM caused a significant reduction in bacterial load, hypoxanthine and TMA concentrations and was safe from a biogenic amine point of view. Melanosis was also inhibited compared to animals packed in air.*

- Using product obtained directly from the research vessel, prepare modified atmosphere packs (MAP) of whole langoustines using the optimal gas mixes.

- o *MAP packs of whole langoustines were packed using the OGM and the effects of several parameters were studied. The effects studied and the main conclusions obtained were:*
  - ▪ *De-gutting the animals: De-gutting the animals does not have any inhibitory effect on bacterial growth in the tail muscle. Therefore de-gutting the animals is not recommended in product to be packed in MAP.*
  - ▪ *Packing fresh or 1 day old animals: Similar values were obtained when packing fresh and 1 day old animals, indicating that it is not detrimental to pack 24 h after catch if during that time animals are stored in a cold room with ice on top.*

- Using the full suite of tests, measure the quality and freshness of the MAP product at different times, and determine the maximum shelf life that can be obtained.
  - o *Maximum shelf life was determined after using the full suite of tests. Using a stepping temperature protocol imitating what would happen in a commercial operation, the shelf life in air packs was 5 days and in the OGM packs was 7-8 days.*
  - o *This shelf life of MAP packs could be further extended if lower temperatures were maintained. In this sense, when leaving the packs at a constant temperature of 1-2 ºC, the shelf life of product in packs containing the OGM was between 11-13 days.*

- Monitor MAP packed product for the production of any undesirable breakdown products (eg. Histamine).

- o *Samples from whole langoustines packed in the OGM were analysed for any breakdown products such as hypoxanthine, TMA and biogenic amines (including histamine). In general, undesirable breakdown products produced by the action of SSO were reduced in MAP packs containing the OGM for up to day 7-8.*

- Determine whether a professional sensory panel can detect any differences in the sensory properties MAP packed product that represent loss of flavour

  - o *Samples from different MAP experiments were assessed by a professional sensory panel. The main changes due to storage time and spoilage progression were an increase in smell, slight loss of flavour and also changes in texture properties. MAP packs containing the OGM scored better than air packs as storage time progressed.*

- Test the effectiveness of different chill temperatures in combination with MAP packaging on maintaining product quality.

  - o *The effectiveness of the OGM was very dependent on the temperature of storage.*

  - o *The effect of different temperature regimes on spoilage rates was assessed in pack containing the OGM or air. Above 5 ºC the optimal gas mix started losing its anti-bacterial action, and therefore care should be taken that the temperature is strictly controlled throughout the process.*

- Compare the shelf-life of MAP packed product based on whole langoustines obtained from the normal supply chain with that of animals obtained directly from the research vessel.

  o *An experiment to transfer the protocol and knowledge of the MAP*

   protocol used to extend the shelf life in whole langoustines was carried

   out under the supervision of the industrial partner Young's Seafoods

   Ltd. The results obtained by the industrial partner on the shelf life of

   the MAP products were the same as those obtained by the University of

   Glasgow (between 7-8 days).

Leading to the identification of an optimal gas mix for MAP packs to be used in whole fresh *Nephrops* that maintain quality for the longest period of time, thus increasing their shelf-life.

WP4. The effect of melanosis dipping on product quality

- Do the various dip treatments for preventing melanosis of the shell of whole langoustines have and check any effect on the quality of flavour of the meat

  o *Various commercially available anti-melanotic additives were tested on whole fresh langoustines stored at 0-1 ºC for up to 13 days. No changes in the flavour of the meat (IMP and hypoxanthine) were obtained between the different treatments compared to un-treated animals.*

- Analyse if melanosis dip treatments have an antibacterial action against spoilage bacteria

  o *No differences were found in total viable counts (representing total bacterial load) and* Pseudomonas *sp. between un-treated and animals treated with different anti-melanotic additives. Therefore, any shelf life*

extension obtained by anti-melanotic treatment is due to the impact of these compounds on the external appearance of the langoustines, but not because of any improvement in the quality of the meat.

- Study if icing alone, if adequately applied can provide effective suppression of melanosis over the required shelf-life of the product

  - *Icing alone did not provide an effective suppression of melanosis over the required shelf life. Therefore, anti-melanotic treatments are necessary for whole animals required for this duration.*

  - *Another methodology tested to reduce the development of melanosis was high hydrostatic pressure. This technology inhibited bacterial growth very effectively in a pressure-dependent manner. A pressure of 150 MPa was effective in inhibiting bacteria growth without producing any changes in flavour and texture, showing the potential of this technology in shelf life extension for this product. However, melanosis was activated at all the pressures studied.*

- Compare if melanosis development is affected by MAP packing

  - *Melanosis was partially reduced by the OGM due to its high content of CO_2 together with a low level of O_2. However, anti-melanotic treatments are needed since the step temperature profile (after day 5 temp. is increased above 5 ºC) used commercially activates melanosis to such an extent that the OGM is not able to inhibit the enzyme (PPO) involved in producing melanin.*

Leading to a deeper understanding on the action of various shell anti-melanotic treatments in *Nephrops* and their effects on quality. Furthermore, other

possibilities (icing, MAP and high hydrostatic pressures), instead of additives, to suppress or delay melanosis development have been tested and recommendations have been made.

WP5. Calibration of assays for product quality against Sensory Evaluation

The biochemical assays and instrumental methods for quality and freshness become fully valid only when they are measured directly in relation to the sensory evaluation of flavour and texture of the meat. Without such cross-calibration it is not possible to properly interpret the values obtained form objective tests, or to stipulate appropriate thresholds for acceptability based on such measures alone.

- A professional sensory panel will be recruited by an appropriate food industry agency and will be trained in evaluating langoustine product

  o *A professional sensory panel from the Food Innovation Institute, Edinburgh was recruited and trained using samples of whole langoustines treated in different ways to obtain samples with different qualities.*

  o *The trained panel (10-12 people in each session) performed a Quantitative Descriptive Analysis (QDA) to profile each langoustine sample in terms of sensory attributes.*

- A sensory scoring system, based on the existing QASAS scheme, will be established

  o *For fresh un-cooked samples a sensory scoring system (QIM) was developed and validated for whole langoustines stored at 0-1 ºC.*

  o *However, the QIM did not perform well when animals were treated with anti-melanotic additives, such as Melacide-SC20. For this reason the QIM*

developed was adapted for use when the product to be tested has been dip with anti-melanotic treatments.

- Whole langoustines and tailed product will be obtained under standard conditions using the research vessel, and will be subjected to controlled temperature abuse or held for defined periods of shelf-time.

  - *Langoustines and tailed product were stored at 0-1 ºC and held for up to 11 days. Samples were taken at different storage times.*

- Duplicate samples will be prepared for simultaneous testing using the established laboratory procedures, and for evaluation by a trained professional sensory panel.

  - *Samples from different trials were analysed in the laboratory and were also evaluated by the professional sensory panel. Various experiments conditions were tested: langoustines kept on at 0-1 ºC, langoustines from MAP experiments (3 different trials) and langoustines infected with* Hematodinium *sp.*

- The sensory evaluations will be repeated with a range of defined product types and qualities, until a consensus is obtained for the sensory evaluation

  - *A range of defined product types and qualities have been evaluated by the sensory panel and a consensus was obtained in identifying acceptable from non-acceptable samples*

- The laboratory measures will then be correlated with the sensory measures, and appropriate thresholds will be established.

  - *Results from the sensory evaluations were correlated with laboratory measures, and appropriate thresholds were established*

- Validation trials will be made both by laboratory measures and by sensory panel evaluations on product obtained form commercial supplies, to establish conformity.

- o *Samples treated with the OGM were evaluated by a sensory panel from the industrial partner and also by the sensory panel of the Food Innovation Institute. Their results were compared and conformity was established.*

- Achieving conformity will validate the quality indices and thresholds based on the laboratory measures.

Leading to the establishment of a sensory scoring assay to be used in un-cooked *Nephrops* tail meat, called QIM and also the establishment of a trained professional sensory panel for cooked *Nephrops* tail meat. Thresholds for acceptable product based on biochemical and sensory evaluations have been set.

WP6. Variation in product quality with location and supplier

- Establish methods for determining the sex, body size and moult state of animals from either whole langoustines of from the tailed product delivered to Young's Seafood Ltd. premises.

  - o *Using a combination of research trawls (North Clyde Sea area) and commercial samples from the same location delivered to the Annan factory of Young's seafood Ltd., methods for establishing the size (carapace length) and sex from commercially landed tails were developed and tested. It has thus become possible to determine size and sex from tails alone. However, it is not possible to derive moult stage from commercial tails.*

- Bring into use the immunological methods developed in Phase I of the Scottish *Nephrops* Survey for testing the level of infection by the blood

parasite *Hematodinium* sp. in *N. norvegicus*, and demonstrate that these methods can be applied successfully to either whole langoustines or to tailed product arriving at Young's Seafood Ltd. premises.

      o *Infected animals from research trawls, which showed infection levels from covert (not visible by eye or by light microscopy) to patent (cuticle colour change which are discarded by fishermen) were taken through the commercial process (tailing). All levels of infection detected by immunoassay on blood samples were also detectable from pleopod and tail muscle homogenates. The assay was applied to samples obtained in the spring and summer of 2007, and showed that disease prevalences recorded were in accordance with expected ranges. Also it has been demonstrated that a sample of a single pleopod removed from either a tail or whole animal is sufficient for a successful diagnosis.*

- Using these procedures in conjunction with data from the 'Youngstrace' traceability systems on supplying vessels, determine the seasonal variations in catch composition, body condition and infection prevalence in the different sea areas that supply Young's Seafood Ltd.

      o *Seasonal variation in catch composition resulting from altered burrow emergence patterns of lobsters was reflected in the size distributions of the tails entering Annan factory. Infection prevalence was within expected values for the Clyde Sea area, although commercial samples taken at the peak of infection (spring) from the same location reflect a certain level of discarding of visually infected animals during sorting of the catch.*

- Determine the natural variation in the flavour and texture of tail meat in relation to these seasonal factors

  - *Samples of whole langoustines patently infected with the parasite* Hematodinium *sp. were evaluated by a professional sensory panel. Results indicate that infection alters some of the sensory attributes to such an extent that samples are strongly rejected by the panel.*

- By comparing product fished by different supply vessels from the same locations, identify any differences in product quality that may be attributable to differences in post-capture handling and transport procedures.

  - *Soft and berried tails were virtually absent from all tail samples in spring and summer 2007. Damage to ventral cuticle structure was limited to <10%. Melanosis was evident in a high percentage of tails from the spring samples, but not for those taken in the summer, indicating a possible post-capture temperature abuse. All data on quality of the tails were based on the visual examination of individual tails.*

In conjunction with data from the YoungsTrace traceability systems on supplying vessels, recommendations for best fishing practices and effort to catch animals in good body condition and with minimal infection levels, at given seasons and locations have been given.

On the basis of these findings, the following **List of recommendations based on scientific data** is made to Young's Seafoods Ltd.:

- When working with whole animals it is important to reduce trawling time to ~60 min in order to increase the amount of un-damaged animals that can be sold as whole langoustines.

- Reduce stress during the handling of the catch to minimise the proportion of necrotic animals, since these can affect the quality of the catch.

- Melanosis develops in whole langoustines, even if iced properly all times. Therefore it is important to wash the animals carefully and treat them with anti-melanotic additives before they enter the supply chain.

- During storage, it is important to not only to leave the whole langoustines in a cold room but also to ice the box on top, which will speed considerably the cooling rate in the middle of the box

- By packing whole langoustines in the OGM identified by the University of Glasgow it is possible to reduce the spoilage rate and extend the shelf life.

- When working with MAP it is important to control the temperature throughout the whole process, since MAP cannot recover spoiled product and it loses its effectiveness when packs are stored above 5 ºC.

- Determining levels of *Hematodinium* infection throughout the year is important since this parasite has been shown to affect the quality and the shelf life of the langoustines.

The final recommendation to Young's Seafoods Ltd. would be that with the data obtained in the Phases I and II of the Scottish *Nephrops* Survey it is possible for them to achieve greater control over the '**chain of custody**' by mounting key information

about each catch into the Youngstrace system. In this way the industrial partner could have all the information necessary to determine the expected shelf life of the landed products, and decide the best ways to commercialise them.

Additional Survey Work – Bycatch and discard assessment in *Nephrops* trawls

As an extension to Work Package 7 from Phase I and Work Package 6 from Phase II, of the Scottish *Nephrops* Survey the additional task was undertaken to carry out initial surveys of the catch composition of the *Nephrops* trawls in catches from commercial vessels in the North Minch, and log these in the YougsTrace traceability system.

These initial surveys provided information on the catch composition from a commercial vessel operating in the North Minch fishery. Valuable data on at-risk and indicator species (including Atlantic cod, spurdog and the tall seapen) were obtained. These data have also been used to form the basis of a trial self-assessment system, using the vessel-based Youngstrace traceability system. These demonstrations also contributed significantly to this fishery recently obtaining Marine Stewardship Council (MSC) accreditation.

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