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# Scottish Nephrops Survey Phase III

Evaluation of measures for reducing bycatch and discards in a *Nephrops* fishery

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The Scottish Government Rural Affairs and the Environment: Marine Directorate European Fisheries Fund Project NC/3/19





# Contents

Preamble: Extracts from the application	. 111
Sustainable development of fisheries	Ш
Brief description of project	V
Project outline	VII
Project aims	VIII
Planned schedule of activities/work	IX
Scientific Report	1
Work Package 3.1: Bycatch composition and implementation of a self-assessment	
scheme in the North Minch Nephrops trawl fishery	1
Introduction	1
The North Minch <i>Nephrops</i> Fisheries	3
Objectives of Workpackage 3.1	6
Section 1 of Workpackage 3.1: Catch Composition and Key Species	8
Cod, Haddock and Whiting	10
Spurdog	13
Nephrops norvegicus	16
Methodology	17
Part A: Catch Composition	17
Study Area	24
Part B: Key Species	24
Cod, Haddock & Whiting	25
Spurdog	25
Nephrops	26
Data Analysis	26
Calculating the Number of <i>Nephrops</i>	27
Results	29
Species Composition and Broad Trends	29
Relationships Between Catches: Species Abundance	34
Relationships Between Catches: Biomass	38
Key Species	40
Cod	40
Haddock	44
Whiting	48
Spurdog	52
Age	55
Nephrops	57
Discussion	61

Section 2 of Workpackage 3.1: Self-Assessment of Bycatch & Discards	64
Introduction	64
Youngstrace	65
Self-assessment methodology	65
Feedback from fishermen	67
Validation of self-assessment methodology	68
Results	70
Discussion	76
Deliverables	77
Acknowledgements	78
References	78
Work Package 3.2: Identifying the most cost-effective way to obtain omega-3	
lipids and other nutraceutical compounds from Nephrops heads – a currently	
discarded portion of the catch	81
Introduction	81
Materials and methods	83
Capture and transport	83
Head paste and tissue sample preparation	84
Lipid extraction	84
Lipid class analysis	85
<sup>1</sup> H NMR spectroscopy	86
Fatty acid analysis	86
HPLC with Full Scan HRFTESIMS-Orbitrap	87
Results and Discussion	89
Norway lobster head paste samples from different fishing grounds	89
Norway lobster tissue samples from the Clyde area	91
Lipid class analysis	96
<sup>1</sup> H NMR spectroscopy of Norway lobster lipid extracts	98
Fatty acid analysis	104
Analysis of Norway lobster samples using HPLC with full scan HRFTESIMS	105
Conclusions	110
Deliverables	110
Acknowledgements	111
References	111

# Scottish Nephrops Survey Phase III

## Evaluation of measures for reducing bycatch and discards in a *Nephrops* fishery

### European Fisheries Fund Project NC/3/19

#### Preamble: Extracts from the application

#### Sustainable development of fisheries

One of the principal drivers behind the implementation of Phase III of the Scottish *Nephrops* Survey is to maximise sustainability of the *Nephrops* sector in Scotland. The *Nephrops* fishery is the most valuable in Scotland, with landings worth an estimated £89.3m in 2006. Most landings are made by demersal trawlers, from sites including the Minches and Firth of Clyde on the west coast. *Nephrops* trawling takes place on similar grounds as those inhabited by roundfish (e.g. cod, whiting and haddock), which, in the North Sea, are targeted by a large mixed-fishery fleet.

The required minimum mesh size is much smaller for *Nephrops* than for these roundfish species, and as a result juvenile cod, haddock and whiting as well as other at-risk species such as sharks and rays, are common bycatch species in the *Nephrops* fishery. However technical measures such as modified nets with separator panels, square mesh panels, square mesh codends or cutaway headlines are becoming available, which take a smaller bycatch of at-risk species without a concomitant reduction in the marketable catch of *Nephrops*.

The ability to demonstrate a reduction in bycatch, whether through measures such as avoiding grounds with sensitive species, or deploying more selective gears, is a necessary feature of an effective fishery management strategy, and the introduction of such technical measures may now be required by EU fisheries regulations. In order to implement such a strategy, an in-depth, independent scientific evaluation is needed of the current catch composition over time from commercial vessels operating in a representative *Nephrops* fishery. The effectiveness of any technical measures or other management strategies can then be gauged in relation to the current situation. The work package of Phase III of the Scottish *Nephrops* Survey will conduct such an initial evaluation in a representative *Nephrops* fishery, namely the North Minch fishery, landing to Young's Seafood Ltd. at Stornoway, thus providing essential information.

In order to provide important ongoing information about changes in the catch composition both over time, and following the introduction of any new technical measures, an effective self-assessment scheme for fishermen to record fishing activity, catch composition and the amounts of bycatch and discards is also required.

The fact that the majority of the fleet of *Nephrops* trawlers landing to Young's at Stornoway is already equipped with Youngstrace traceability systems provides a unique opportunity to proof this concept of a self-assessment recording procedure. The first Work Package of Phase III of the Scottish *Nephrops* Survey will exploit this advantage by conducting an initial validation of a self-assessment system for monitoring bycatch aboard trial vessels in the North Minch. It will then implement a working selfassessment system for monitoring bycatch aboard vessels across all Stornoway *Nephrops* trawl vessels landing to Young's.

The specific benefits of this work will be to obtain a complete data set of bycatch for two years from the North Minch fishery, and to identify potential management strategies from spatial / temporal trends in the data. The implementation of these measures will have the aim of conserving the fish populations in this fishing ground through a smaller bycatch of at-risk species, without reducing the marketable catch of *Nephrops*. In this way the project will contribute directly to the sustainability of this fishery.

The continued logging of bycatch composition in the North Minch fishery, particularly for at-risk species such as cod and spurdog, will directly assist in maintaining the MSC accreditation which is currently being approved for this fishery. Indeed, in the MSC Assessors' Report of that application, specific reference was made to the value of the work carried under Phase II of the Scottish *Nephrops* Survey, and a recommendation was made that the client, Young's Seafood Ltd, as holder of the chain of custody, should continue to work with the University of Glasgow in this area over the four year period following certification.

In fulfilment of another recommendation in the MSC Assessors' Report, the partnership of the University of Glasgow and Young's Seafood will also liaise with the Fisheries Research Service (FRS), who have an experienced gear selectivity team and are researching various new gear designs to minimise bycatch and reduce whitefish discards by allowing them to escape. As and when new technical measures in gear design are established by them, through ongoing projects within for example the Scottish Industry/Science Partnership (SISP), and these become available for use on commercial vessels, catches obtained with an approved design of modified net could be tested on trial vessels in the North Minch fishery against the existing bycatch data for at-risk species such as cod and spurdog. Comparative analyses with the previous data set could then be performed to evaluate these new technical measures for minimizing whitefish and elasmobranch discards in the North Minch Fishery. The experienced gear selectivity team at FRS have indicated their willingness to provide advice on these matters to the Scottish *Nephrops* Survey team.

The time-frame for evaluating any new technical measures in the North Minch fishery is dependent upon outside factors relating to their recommendation for use in west coast *Nephrops* fisheries, and this may fall outwith the period of Phase III of the Scottish *Nephrops* Survey. Moreover, such trials would involve additional costs. Therefore such comparative trials have not been included as an objective for Phase III of the Scottish *Nephrops* Survey. Rather, additional funds will be sought at an appropriate time in the future to conduct such trials, exploiting the sampling and logging procedures that will have been developed in Phase III.

# <u>B3</u> Please provide a brief description of the project (e.g. who is involved, what it is principally concerned with, what its main aims and objectives are).

Phase III will build on the considerable knowledge gained from Phases I & II of the project in order to meet to meet two main aims and a number of key objectives concerned directly with improving the sustainability of the fishery, and reducing the bycatch and discards. These objectives are well aligned with the strategy of the current EFF Scheme to which this application is made, but were not fully addressed in the earlier phases of the Scottish *Nephrops* Survey.

**The first aim** is to conduct an in-depth, independent scientific evaluation of the current catch composition over time from commercial vessels operating in a representative *Nephrops* fishery (the North Minch fishery, landing to Young's Seafood Ltd. at Stornoway), and to use these data to develop and implement an effective self-assessment scheme for fishermen to record fishing activity, catch composition and the amounts of bycatch and discards. This aim will be met through the following objectives:

- To record the catch composition obtained, both qualitatively (by recording the presence of all fish and invertebrate species) and quantitatively (from the weight of the major taxa).
- To record biometric data on key fish species (e.g. commercially valuable or threatened species) and to assess seasonal and temporal variation in the distribution of these species.
- If seasonal patterns are detected, to develop recommendations for best practice on the fishing grounds to avoid high levels of bycatch at certain times of year
- To use existing "YoungsTrace" traceability technology that is fitted to the majority of the fleet operating from Stornoway, together with communication with skippers and the data collected during the survey work in order to develop a self-assessment system for commercial skippers to record bycatch easily.
- To build spatio-temporal maps of catch and discard rates of sensitive species

**The second aim** is to identify the most cost-effective ways to recover various valuable nutrients and dietary supplements from the *Nephrops* heads, which are currently discarded from the catch, and to thereby commercialize them rather than waste them. In particular the work will focus on ways in which yields of Omega-3 lipids can be maximized. This aim will be met through the following objectives:

- To perform a proximate analysis of the 'head' of Nephrops, focusing on compounds that could be utilised as seafood products, additives or dietary supplements, or could yield other useful chemical products for recycling and use in other industries. In particular, analyses of lipid composition (Omega-3 lipids) will be performed, and a precautionary assessment will also be made of any potential toxins and contaminants.
- To investigate ways in which the yields of LC n-3 PUFA fatty acids can be maximised and the extractions optimised. This will be done by systematically comparing the lipid content and composition of heads obtained at different seasons, by comparing males and females and by examining the effects of variables such as the green sac (ovary) condition, the moult state and the health status of the animals, and other known threats to body condition such as those related to infection and post-capture stress.
- The yield of lipids in relation to the time after capture and storage will also be established, in order to estimate losses due to lipid oxidation. In these ways the most cost-effective ways for obtaining a maximum yield of LC n-3 PUFA fatty acids (Omega 3) from *Nephrops* heads will be identified.

**Delivery** of Phase III of the Scottish *Nephrops* Survey will be through two work packages (3.1 & 3.2), the outcomes of which will be a set of identifiable deliverables.

# Work Package 3.1 - Bycatch Composition and Implementation of a Self-Assessment Scheme in the North Minch Nephrops trawl fishery

The methodology will be similar to that used in the <u>Additional Survey Work</u> of Phase II, but will collect more extensive and detailed physical and biological data. Specifically this will include:

- GPS-derived vessel positions, vessel attributes, water temperature, air temperature, trawl duration, ground type, date and time, and tow speed for each trawl.
- Quantitative and qualitative analysis of the entire catch from each net under examination (which in some cases will involve a modified net, operating as a single rig or in a twin rig configuration with a conventional net). The wet weights of major taxonomic groups (roundfish, flatfish, sharks & rays and invertebrates) will be recorded, as well as a qualitative list of all species present in each haul.
- Length, weight and sex (where possible) of a subsample (approximately 100 individuals per trip) of key 'at-risk' species (particularly cod, haddock, whiting and spurdog) will be recorded.

- Sampling will take place over at least four days every quarter, from one or two vessels deemed typical of the Stornoway *Nephrops* fleet.
- Spatially-resolved data will be collected using GPS-derived positions from the quarterly sampling trips and analysed in conjunction with data collected by skippers within the proposed self-assessment scheme.
- A limited number of trawls will be undertaken in the Clyde sea area (by RV Aplysia) for reference and cross-comparison of catch composition

This scheme is based on existing technology used by Young's Seafood Ltd, who will take the lead in recruiting fishing vessels into the trials. Vessel and gear attributes will be recorded, and skippers will enter basic data on weights of major bycatch groups into the YoungTrace system. Each participating skipper will record bycatch for one trawl per month, to ensure that the system is practicable and will not reduce their working time unduly. The self-assessment system will be validated by periodic scientific sampling.

Work Package 3.2 - Identifying the most cost-effective way to obtain Omega-3 lipids and other nutraceutical compounds from *Nephrops* heads – a currently discarded portion of the catch.

The following methodologies will be adopted on samples of *Nephrops* heads collected from the Clyde Sea Area at regular intervals through the year, and also more occasionally from other grounds such as the North Minch, for comparison:

• Lipid composition and free fatty acid analysis will be performed using Gas Chromatography – Mass Spectrometry (GC-MS) on crushed *Nephrops* heads and also in the different tissues of the cephalothorax (digestive gland and gonad) collected at different times of the year.

#### B6 What is your project aiming to achieve and how will you measure its success?

Please provide details of expected benefits and targets as a result of completion of this project. Please include details of how the project will assist in achieving the Action Points in the <u>Strategic Framework for Scottish Aquaculture</u>, the aims of the <u>Scottish Food and Drink Policy</u>. And the Common Fisheries Policy

The expected benefits of the project in relation to the strategic aims of national and EU policies have been referred to in Sections B4 and B5. The success of the project will be measured against the planned deliverables.

#### Work Package 3.1

<u>Year 1:</u>

- 1. A validated self-assessment system for monitoring bycatch aboard trial vessels.
- 2. A complete data set of bycatch for one year from the North Minch fishery.

Year 2:

- 1. A working self-assessment system for monitoring bycatch aboard vessels implemented across all Stornoway *Nephrops* trawl vessels
- 2. A complete data set of bycatch for two years from the North Minch fishery
- **3.** The Identification of potential management strategies from spatial / temporal trends in the data

#### Work Package 3.2

<u>Year 1:</u>

- 1. A complete analysis of lipid content and composition of Nephrops heads
- 2. A preliminary analysis of LC n-3 PUFA lipid content (i.e. Omega 3) in relation to known variables (fishing grounds, season and gender)

#### Year 2:

1. The identification of the best fishing grounds and times of the year for a maximum yield of LC n-3 PUFA lipid (i.e. Omega 3) from *Nephrops* heads

# <u>C2</u> Within the stated timetable (12/01/2009 – 31/12/2010), please describe the planned schedule of activities/work.

<u>Work Package 3.1 - Bycatch Composition and Implementation of a Self-Assessment</u> <u>Scheme in the North Minch *Nephrops* trawl fishery</u>

#### <u>Time Scale</u>

<u>Year 1: 2009</u>

- Adapt the YoungsTrace system to provide comprehensive and practical logging of bycatch, and install this upgraded system on participating vessels for a trial period
- Collect catch composition data (quarterly)
- Collect biometric data on key species (quarterly)
- Compare scientific data to those from the self-assessment scheme
- Examine data set to identify evidence of spatial or temporal trends in bycatch rates which could be used to advise fisheries management in the area
- Modify the YoungsTrace system to accommodate skippers suggestions

#### Deliverables:

3.1.1 A validated self-assessment system for monitoring bycatch aboard trial vessels.

3.1.2 A complete data set of bycatch for one year from the North Minch fishery.

#### <u>Year 2: 2010</u>

- Implement the YoungsTrace self-assessment system across the Stornoway fleet
- Collect catch composition data every quarter
- Collect biometric data on key species every quarter
- Examine the scientific and self-assessment data for evidence of spatial or temporal trends in catches which could be used to advise fisheries management in the area
- Identify appropriate management measures for reducing the levels of bycatch, particularly the at-risk species cod and spurdog.

#### Deliverables:

- 3.1.3 A working self-assessment system for monitoring bycatch aboard vessels implemented across all Stornoway *Nephrops* trawl vessels
- 3.1.4 A complete data set of bycatch for two years from the North Minch fishery
- 3.1.5 The Identification of potential management strategies from spatial / temporal trends in the data

# Work Package 3.2 - Identifying the most cost-effective way to obtain omega-3 lipids and other nutraceutical compounds from *Nephrops* heads – a currently discarded portion of the catch.

#### <u>Time Scale</u>

#### <u>Year 1: 2008-2009</u>

- Characterise the crude extracts of whole heads supplied by the company for the following:
  - Total lipid content of pressed heads/claws obtained from tailed animals (mixed sizes and sexes) Composition of this lipid in terms of saturated/unsaturated forms, and amounts of LC n-3 PUFA (Omega 3)
- Identify LC n-3 PUFA lipid content (i.e. Omega 3) in relation to known variables:
  - Sample different grounds (Clyde and Stornoway) in different seasons (quarterly) to identify times of maximum yield.
  - From each sample look at males/females, female green sac condition, moult state and other indicators of health condition (eg. infection) to identify the richest source.
- Identify other compounds that could have a commercial interest as seafood products, food additives, dietary supplements or other "nutraceuticals", and detection of the presence of any relevant contaminants.

#### Deliverables:

- 3.2.1 A complete analysis of lipid content and composition of Nephrops heads
- 3.2.2 A preliminary analysis of LC n-3 PUFA lipid content (i.e. Omega 3) in relation to known variables (season and gender)

#### <u>Year 2: 2009-2010</u>

 Complete the analysis of Omega-3 lipids in relation to the known variables studied in order to identify the best grounds and times of the year for a maximum yield of these compounds

#### <u>Deliverables:</u>

3.2.3 The identification of the best fishing grounds and times of the year for a maximum yield of LC n-3 PUFA lipid (i.e. Omega 3) from *Nephrops* heads

The University of Glasgow will then be in a position to convert these laboratory techniques into industrial procedures for extraction and purification.

# Scottish Nephrops Survey Phase III

## Evaluation of measures for reducing bycatch and discards in a *Nephrops* fishery

European Fisheries Fund Project NC/3/19

**Scientific Report** 

## Work Package 3.1: Bycatch composition and implementation of a selfassessment scheme in the North Minch *Nephrops* trawl fishery

#### Introduction

Nephrops norvegicus (Linnaeus, 1758) is a marine decapod crustacean belonging to the family Nephropidae that lives in burrows constructed in areas of muddy sediment. It is the only recognised species of the genus (ITIS, http://www.itis.gov/), and can be found in the north-east Atlantic Ocean and Mediterranean Sea between depths of approximately 20m and 800m where there is suitable substrate (Bell *et al.*, 2006). The commercial value of *Nephrops* has grown steadily since the 1950s and it is now one of the most valuable in the UK with landings in 2007 worth approximately £104 million in Scotland alone (Keltz & Bailey, 2009). *Nephrops* is primarily captured by bottom-trawling, although a small proportion of landings are made using 'creels' or baited traps. The Scottish *Nephrops* trawl fisheries exist across several major fishing grounds, including the Farn Deeps and Fladden grounds in the North Sea where *Nephrops* is targeted as part of a mixed fishery which also lands roundfish such as cod, haddock and whiting, and the Clyde Sea Area and Minches off the west coast of Scotland where *Nephrops* is typically the only species targeted. The differences in how *Nephrops* is targeted in each area mean that the single-species fisheries are managed differently to

the mixed fisheries, and there are consequently differences both in how the fisheries operate and in the composition of their catches.

Current management measures implemented in the single-species *Nephrops* fisheries in the UK include a Minimum Landing Size (MLS) of 20mm carapace length and minimum codend mesh sizes of 80mm (for single-rig gear), whereas in the mixed fisheries a minimum codend mesh size of 120 mm is enforced (ICES, 2005). Consequently, the capture of undersize roundfish is a much greater problem in the single-species fisheries as there is less opportunity for the fish to escape the gear (e.g. Briggs, 1985, Stratoudakis et al., 2001, Catchpole et al., 2007, Catchpole & Revill, 2008). As with all mobile-gear fisheries, the capture of bycatch animals and subsequent discarding is a significant problem in the Scottish *Nephrops* fisheries. However, several different meanings can be attributed to these terms (Kelleher, 2005), and some clarification is therefore required. For the purposes of this report, 'bycatch' will be defined as any non-target organism that is captured in the fishing gear and recovered onto the fishing vessel. It includes non-target animals that are subsequently sold commercially, but not animals that escape the gear before they can be recovered to the vessel. A 'discard' will refer to any animal that is caught in the gear, recovered onto the vessel and is then returned to the sea as waste. It includes commercially valuable species that cannot be landed (for example because they are below their legal minimum landing size (MLS) and landing them would exceed a vessel's quota restrictions) or because they are unwanted for economic reasons (as a result of 'high-grading' for example, or because there is no market for them (non-commercial species)). It does not include the cephalothoraces ('heads') of *Nephrops* that have been tailed, as this component of the catch is a natural by-product of processing.

In Scotland, previous studies have reported discard rates of 45-88% from the Firth of Clyde and North Sea (Fladden Ground) regions, but no data are available for the other major fishing grounds in the Farn Deeps or the Minches. Other work in the Celtic and

Irish Seas has shown relatively lower discard levels (55% and 30% respectively), but it should be noted that there have been various changes in the management of all these grounds since the studies were published and these trends may have changed in the intervening period. The practice of discarding any such organisms is highly wasteful however, as many species are unlikely to survive the stresses of the trawling process and will be returned to the sea either dead or dying (Chapman, 1981, Harris & Ulmestrand, 2004, Broadhurst et al., 2006). It also represents both a short- and longterm economic waste as commercially-targeted animals are lost from the immediate landings and if they die cannot be caught again in the future. It is also an ecological waste since there is a direct reduction of benthic productivity. While discards do provide a limited return of energy to the ecosystem, through providing an input of carrion to the food chain, Kaiser & Hiddink, 2007) estimate that the energy returned by discards in the North Sea accounts for only 15% of the productivity lost through removal of organisms during trawling. The ecological effects of fishing are undoubtedly complex, but minimising the amount of damage caused to organisms during fishing and the amount of material wasted through discarding should be a priority for future management.

#### The North Minch Nephrops Fisheries

The North Minch fisheries are managed under ICES Area IVa and Functional Unit (FU) 11 (Figure 1). Many of the Scottish vessels working in this area are based in the port of Stornoway on the Isle of Lewis, and include dredging, trawling and creeling vessels which predominantly target *Nephrops* and other shellfish in the north and south Minches to the east of the Outer Hebrides. *Nephrops* was the most valuable landed species in Stornoway in 2008, with a landings value of approximately £2.62 million (of a total value for all landed species of approximately £2.75 million). Commercial whitefish stocks in Area VIa as a whole are believed to be at extremely low levels (Keltz and Bailey, 2009), and unlike the mixed-fishery fleets in other areas, whitefish have a relatively low value to the fishermen working out of Stornoway, and so little bycatch is landed.



Figure 1: Map showing (a) ICES Areas in the west of Europe and (b) Functional Units within ICES division VIa (adapted from Keltz and Bailey, 2009).

Twelve of the trawl vessels operating out of Stornoway currently supply *Nephrops* to Young's Seafood Ltd. either as whole animals (largely for export) or 'tails' (largely for the domestic market), and are equipped with the 'YoungsTrace' traceability system, which has been designed to track each individual catch from the fishing vessel through the landing, processing and transportation stages and to the final consumer. It is this particular sector of the fleet that will be examined through the current project, and the specifications of these vessels are provided in Table 1. Thanks to the use of the 'YoungsTrace' traceability system and the results of an earlier pilot study carried out by Milligan *et al.* (2009) during 2007-2008, the trawlers using the system to target *Nephrops* in the north Minch were awarded Marine Stewardship Council (MSC) accreditation on 14<sup>th</sup> April 2009, the requirements of which define several of the major aims of this work.

Vessel Name	Year Built	Registration	Length (m)	GRT	Power (KW)	Gear Type	Codend mesh	SMP size
							size (mm)	(mm)
Comrade	1963	SY337	16.65	23.16	355	Single rig	70	90
Flowing Stream	1969	SY822	16.68	24.81	119	Single rig	70	90
Kaylana	1978	SY21	17	24.9	284	Twin rig	95	90
Laura Ann	1971	SY586	16.42	24.18	164	Single rig	70	90
Northern Star	1968	SY11	16.46	24.05	149	Single rig	70	90
Ocean Spirit	1979	SY21	13.1	23.6	134	Single rig	70	90
Sharon Rose*	1974	SY190	16.98	27.42	244	Twin rig	95	90
Wavecrest	1968	SY337	16.34	23.15	134	Single rig	70	90
Shiegra	1971	SY7	17.03	24.95	131	Single rig	70	90
True Vine	1974	KY7	15.24	23.43	171	Single rig	70	90
Lead Us	1972	SY144	15.51	24.37	274	Single rig	70	90
Faithful Friend	1970	FR615	18.26	unknown	235	Single rig	70	90

Table 1: List of trawler vessels supplying *Nephrops* to Young's Seafood in 2008

\*Sold in 2009 and replaced by the Silver Chord, SY101.

#### **Objectives of Workpackage 3.1**

The aims of this part of the project were to conduct an in-depth, independent scientific evaluation of the current catch composition over time from commercial vessels operating in a representative *Nephrops* fishery (the North Minch fishery, landing to Young's Seafood Ltd. at Stornoway), and to use these data to develop and implement an effective self-assessment scheme for fishermen to record fishing activity, catch composition and the amounts of bycatch and discards. It was intended that this work would provide useful data in improve the sustainability of the fishery in this region and provide evidence upon which future management measures could be made. These aims were to be met through the following objectives:

- To record the catch composition obtained, both qualitatively (by recording the presence of all fish and invertebrate species) and quantitatively (from the weight of the major taxa).
- To record biometric data on key fish species (e.g. commercially valuable or threatened species) and to assess seasonal and temporal variation in the distribution of these species.
- If seasonal patterns are detected, to develop recommendations for best practice on the fishing grounds to avoid high levels of bycatch at certain times of year
- To build spatio-temporal maps of catch and discard rates of sensitive species.
- To use existing "YoungsTrace" traceability technology that is fitted to the majority of the fleet operating from Stornoway, together with communication with skippers and the data collected during the survey work in order to develop a self-assessment system for commercial skippers to record bycatch easily.

To achieve these objectives, the following deliverables were defined under Work Package 3.1 of the project proposal:

- Quantitative and qualitative analysis of the entire catch from each net under examination (which in some cases will involve a modified net, operating as a single rig or in a twin rig configuration with a conventional net). The wet weights of major taxonomic groups (roundfish, flatfish, sharks & rays and invertebrates) will be recorded, as well as a qualitative list of all species present in each haul.
- Length, weight and sex (where possible) of a subsample (approximately 100 individuals per trip) of key 'at-risk' species (particularly cod, haddock, whiting and spurdog) will be recorded.
- GPS-derived vessel positions, vessel attributes, water temperature, air temperature, trawl duration, ground type, date and time, and tow speed for each trawl.
- Sampling will take place over at least four days every quarter, from one or two vessels deemed typical of the Stornoway *Nephrops* fleet.
- Spatially-resolved data will be collected using GPS-derived positions from the quarterly sampling trips and analysed in conjunction with data collected by skippers within the proposed self-assessment scheme.
- A limited number of trawls will be undertaken in the Clyde sea area (by RV Aplysia) for reference and cross-comparison of catch composition

This scheme was to be based on existing technology used by Young's Seafood Ltd, who were to take the lead in recruiting fishing vessels into the trials. For the self-assessment it was intended that vessel and gear attributes would be recorded using the YoungsTrace system already fitted to the vessels, and that skippers would enter basic data on the weights of major bycatch groups into the system. Each participating skipper was to record the bycatch from one trawl per month, to ensure that the system was practicable and would not reduce their working time unduly. This self-assessment system was to be validated by periodic scientific sampling.

#### Section 1 of Workpackage 3.1: Catch Composition and Key Species

The problem of bycatch and discards in the fishing industry has been well documented, although relatively few studies have examined the total bycatch composition of commercial Nephrops catches. Those studies that have been done have tended to focus only on the commercially important species within the bycatch (such as Atlantic cod, haddock and whiting) and have not generally included information on non-commercial fish species or the invertebrates. Similarly, the impact of trawling on benthic invertebrate communities has been well-studied, but little has been done in the context of bycatch from commercial catches. Additionally, these studies have tended to focus on the larger fishing grounds such as those in the North Sea, with some grounds (such as the Minches) receiving virtually no attention. The collection of complete bycatch data from several grounds would allow a greater understanding of both the health of the biological community surrounding a fishery, and how this is being affected by the fishery over time. It may also highlight particularly vulnerable species, and allow mechanisms to be put in place to mitigate the damage caused to those populations. One of the major aims of the present study was to quantify the bycatch 'community' in the North Minch Nephrops fishery in terms of abundance and biomass over the course of several months and to recommend future management actions that may be taken to reduce the levels of bycatch and subsequent discards in this fishery.

Bergmann *et al.* (2002) reported that invertebrates comprised at least 95% (including *Nephrops '*heads') of the discarded material from *Nephrops* trawlers in the north Clyde Sea Area. The effects of trawling on invertebrates in this area were found to vary considerably depending on the life history and morphology of individual animals, with organisms such as the brittlestar *Ophiura ophiura* being particularly vulnerable to physical damage, while more sturdy organisms such as the hermit crab, *Pagurus bernhardus* were less susceptible to damage (Bergmann *et al.*, 2001, Bergmann & Moore, 2001). Mortality rates of decapod crustaceans were also shown to increase with

the extent of physical damage (Bergmann and Moore, 2001) which has been shown to increase with increasing trawl duration (Bergmann *et al.*, 2001, Milligan *et al.*, 2009). It is unclear what the implications of such effects will be on the community structure within these grounds, and as fisheries management moves towards utilising ecosystem-based approaches there will be a need for more detailed studies of catch composition and total bycatch rates in other fishing grounds around Scotland and the UK.

Mobile gear fisheries are well-known for the damaging impact they can have on both the marine environment and on animals captured by the fishing gear, whether or not they are the target species. Mortality rates of trawl-caught animals are typically high due to the stresses to which they are exposed during the capture process (physical exhaustion, being crushed or damaged in the net, changes in ambient pressure and potential exposure to high temperatures and low salinity), while the catch is being processed on board the vessel (potentially high temperatures, long periods of emersion, crushing and physical damage from other organisms) and once they have been discarded from the vessel (predation from scavengers). Fish species that have a swim bladder are particularly vulnerable to changes in hydrostatic pressure during capture, and are also favoured prey items by scavenging seabirds (e.g. Berghahn & Rosner, 1992, Garthe et al., 1996). Organisms that can protect themselves from physical damage and mitigate the effects of such stresses (including shelled animals such as the large gastropod Buccinum undatum for example which often shows scarring on the shells from previous non-fatal contact with trawl gear; R.J. Atkinson, pers. comm.) may be less likely to die as a result of the trawling process, and may be expected to recover and survive if returned to the sea within a reasonable time frame. However, if the lifehistory strategy of a species makes it particularly vulnerable to fishing pressure, or if the species has already been subject to over-exploitation, then this additional discard mortality can have significant consequences for the future of that species.

#### Cod, Haddock and Whiting

In the UK Nephrops trawl fisheries, a wide variety of fish species are captured alongside *Nephrops* including several commercially important species (e.g. Briggs, 1985, Stratoudakis et al., 2001, Catchpole et al., 2005). Of particular interest are the Atlantic cod (Gadus morhua), whiting (Merlangius merlangus) and haddock (Melanogrammus aeglefinnus) as historically, stocks of these species supported extremely large and valuable fisheries around the UK. Subsequently, high fishing pressure and overexploitation have caused their decline, leading to the collapse of stocks in the west of Scotland. As a consequence, targeted fisheries are no longer allowed for any of these species in the west of Scotland, although there is a small total allowable catch (TAC) to allow some of the bycatch to be landed and sold. Landings data for the north-east Atlantic (total) and UK are shown for each of these species in Figure 1, which highlights the general decline in catches of these fish over the last 60 years. It should be noted that landings data do not necessarily represent the true state of a given fish stock since they are affected by a combination of factors, including quota restrictions and variations in fishing effort for example as well as by underlying changes in the stock densities. However they can give an overall impression of the long-term changes in a stock.

Within the Scottish *Nephrops* fishery, cod, haddock and whiting are either caught deliberately as alternative target species (e.g. in the mixed fisheries in the North Sea) or accidentally as bycatch (e.g. in the west of Scotland) and since they are demersal species and inhabit similar grounds to *Nephrops*, they can be captured using the same fishing gear. In the mixed fisheries, management regulations are in place which require a minimum codend mesh size of 120cm to be used where cod, haddock or whiting are a target species (ICES, 2005), while in the single-species *Nephrops* fisheries a minimum codend mesh size of 70mm is permitted (though a square mesh panel must be fitted to the topsheet in front of the codend) since whitefish are not targeted. Regardless of these measures however cod, haddock and whiting are still captured in the single-species fisheries and because of the smaller mesh size used, those animals caught are



Figure 1: Total landings of (a) cod, (b) haddock and (c) whiting made between 1950 and 2005 in the north-east Atlantic (total) and UK. Data are from the FAO Fisheries Dept., Fishery Information, Data and Statistics Unit.

often immature, undersize individuals which cannot legally be landed and must therefore be discarded at sea. Mortality rates for these species during the capture and discarding processes can be high (though also extremely variable depending on the strengths of different stressors and the experimental design; Chopin & Arimoto, 1995, Davis, 2002), meaning that a proportion of the discarded fish will simply be lost from the stock. Consequently they will not be alive to spawn and help replenish the stock, nor will they be available for capture by the fishery in the future at a time when they would be large enough to land.

To counter the high discard mortality rates, various management tools to reduce the capture rates (and presumably mortality) of these species through the use of modified fishing gears (e.g. Catchpole and Revill, 2008), effort reduction (e.g. the implementation of days-at-sea restrictions and quotas) and the use of spatial management (e.g. real-time closures in the North Sea) have been examined. The effectiveness of different management techniques in relation to bycatch reduction is not discussed further here. However at this stage it is necessary to consider the EU Cod Recovery Plan (CRP).

The CRP introduced on the 1<sup>st</sup> January 2009 (EC regulation 1342/2008) and was designed with the intention of reducing the total mortality of Atlantic cod throughout European waters, but the consequences of this plan to the viability of certain *Nephrops* fisheries may be extremely significant. Under this scheme, the fishing mortality rates of cod must be reduced by 25% in any geographic area where cod stocks fall below the minimum spawning biomass (MSB) level, or by 15% where they are above the MSB, but below the precautionary spawning biomass (PSB) level. In effect, this requires catches of cod (both bycatch and landed fish) to be monitored, and if mortality rates (i.e. capture rates) are too high, measures will be implemented to reduce the mortality within the particular fishing fleet. This will certainly affect *Nephrops* fleets which capture significant amounts of cod as bycatch, and is likely to lead to large reductions in permitted fishing

effort as a result. However, there are derogations which allow exemption from the CRP, including where:

- (a) "appropriate data on cod catches and discards are available to allow the Scientific, Technical and Economic Committee for Fisheries (STECF) to assess the percentage of cod catches made by each group of vessels concerned" or
- (b) "the percentage of cod catches as assessed by STECF does not exceed 1.5% of the total catches for each group of vessels concerned"

Detailed monitoring of the discards and bycatch in any EU member-state fishery may therefore have additional benefits to the fishing industry if it can provide evidence that a fishery is not having a negative impact on cod stocks beyond the 1.5% catch level imposed by the CRP. Such monitoring will form a key part of the present study, which will examine the catch rates and condition of cod, as well as haddock and whiting.

#### Spurdog

Populations of chondrichthyes (sharks and rays) are often highly vulnerable to the effects of fishing mortality due to their K-selected life history traits (including long life span, low fecundity, slow growth rates and low natural mortality rates for example), and sharks and rays are frequently taken as bycatch in a number of fisheries. One species of particular concern in the north-east Atlantic is the spurdog or spiny dogfish, *Squalus acanthias* Linnaeus, 1758.

*Squalus acanthias* is distributed throughout the temperate regions of the world's oceans, but has been subject to unregulated fishing pressure across much of its range in previous years and is now considered to be 'critically endangered' in the north-east Atlantic by the IUCN (Fordham *et al*, 2006). Landings data from the FAO show that catches of spurdog in the north-east Atlantic (total) and UK fisheries have been in

decline since the 1990s, as shown in Figure 2. It should be noted however that landings data alone are unsuitable for estimating the numbers of spurdog within a population due to their tendency to aggregate by age and sex (Compagno, 1984), so that it is possible to maintain high catches even from a reduced population.

In 2010, the total allowable catch (TAC) for this species was set at zero in all European areas (except for a small (10%) bycatch allowance) which is a reduction from a TAC of approximately 1000 tonnes in 2009 in ICES division VI (Council of the European Union, 2009).

The life history characteristics of the spurdog, as with many other shark species, make it particularly vulnerable to fishing pressure. This is a long-lived species, with an estimated lifespan of approximately 50-75 years (Cailliet *et al.*, 1999) and one of the longest known gestation periods of any animal (up to 24 months) coupled with relatively low fecundity (1-15 pups born per cycle (Jones & Ugland, 2001), although up to 21 per female have been reported by Ellis & Keable, 2008) and long maturation time (approximately 9-11 years in the north-east Atlantic; Saunders & McFarlane, 1993). Additionally, female spurdog can reach a larger maximum total length (101-124cm) than males (89-100 cm) and females therefore tend to be preferentially targeted in areas where fisheries are permitted (Compagno, 1984). Since fecundity is directly correlated to the length of the female fish, a reduction in the largest individuals may have negative effects on the stability of the population as a whole (e.g. Henderson *et al.*, 2002).

It is unclear to what extent spurdog populations are able to compensate for declining numbers through variation in these life-history traits. Density-dependent variations in length- and age-at-maturity for example have been reported in several teleost fish stocks (e.g. Sharpe & Hendry, 2009) which appear to act as compensatory mechanisms to allow population recovery following decline. Whether such mechanisms



Figure 2: Total landings (tonnes) of *Squalus acanthias* made between 1950 and 2005 in the north-east Atlantic (total) and UK. Data are from the FAO Fisheries Dept., Fishery Information, Data and Statistics Unit.

can occur in spurdog populations is unclear, and certainly Henderson *et al.* (2002) found no evidence of changes to life-history traits as a result of fishing pressure.

#### Nephrops norvegicus

*Nephrops* is the target species for the fishery in the North Minch, and is highly valuable. However, populations of *Nephrops* have been shown to undergo seasonal cycles in moulting, emergence and breeding behaviour, with subsequent effects on the quality and value of catches made at different times of year (e.g. Stentiford *et al.*, 2001, Milligan *et al.*, 2009). Such trends have been well documented from the Clyde Sea Area in Scotland and the Mediterranean, but no reports have been published for the North Minch. Determining whether *Nephrops* in this region also show intra-annual variation, and if so, whether this has an effect on commercial catches and landings through the year may provide valuable data about fishing activity in this region, as well as providing additional data on the *Nephrops* population in the North Minch.

#### Methodology

#### Part A: Catch Composition

One of the main aims of this project was to determine the extent of discarding within the Stornoway *Nephrops* trawler fleet, and the total species composition of the catches. This was done over the course of a number of survey trips, which were carried out over four days every two months between December 2008 and December 2009 and over three days in March and June 2010.

The survey trawls of approximately two hours duration were carried out on board the MV '*Comrade*' SY337 (16.65m, 355kW), a single-rig vessel which used two different otter trawl nets while fishing commercially: a lighter 'disc' net (Fig. 4) and a heavier 'hopper' net (Fig. 5). In order to determine whether the net type had an effect on the overall catch composition, both nets were fished during each sampling trip. Care was taken to ensure that any effects caused by using different gear types could be distinguished from the effects of other factors during analysis of the data.

#### **Study Area**

The trawl sites for the initial monitoring work were chosen following discussion with the skipper, and were intended to be as representative of commercial fishing grounds in the area as possible. To this end, the precise locations of each tow were selected by the skipper to ensure data were collected about 'real' commercial catches. The GPS tracks for the tows made between December 2008 and December 2009 are shown in Figure 3.

Care was taken to ensure that the sampling regime was scientifically meaningful however, and would allow clear statistical analysis at the end of the survey. Two broad sampling areas were chosen for sampling within the North Minch, one to the south of Stornoway (south site) and one to the south-east (east site).



Figure 3: Maps of the study area: (a) The limits of the sampling area are highlighted by the red box and (b) Individual GPS tracks of each tow are shown and colour-coded by month: Red: December 2008; Blue: February 2009; Bright Green: April 2009; Yellow: June 2009; Pink: August 2009; Grey: October 2009; Dark Green: December 2009.

One or two trawls were made each day, and physical and environmental data were recorded to aid with subsequent analysis of the catches, which were:

- Trawl date and time,
- Trawl duration (minutes),
- Decimal GPS location (shot and haul points),
- Mean trawl depth (metres; average of start and end depths),
- Gear type (light or hopper),
- Tidal range (difference between maximum and minimum tidal heights on each day),
- Wind direction and speed.

Summary data for each trawl are displayed in Table 2. Trawls COM8, COM21 and COM33 were considered invalid due to fouling of the gear and have not been included in any analysis of catch composition.

Once each catch was recovered on board, the entire animal bycatch was sorted into major groups (roundfish, flatfish, invertebrates and elasmobranches) while the crew sorted the *Nephrops* according to their normal working practice (into graded whole *Nephrops* and tailed *Nephrops*). If a second trawl was made, it would be hauled and sorted in the same manner, and the catches from each haul stored separately until the vessel was back in the harbour. Weighing the catch at sea was not possible due to the motion of the vessel.

On return to the harbour, the major groups from each catch were weighed to the nearest 0.1kg and then sorted separately into individual species. All species were recorded and the numbers of individuals per species were counted. The weights of all roundfish and flatfish species were recorded separately, but the weights of the elasmobranchs were pooled into 'sharks' and 'rays & skate', while invertebrates were weighed according to phylum or sub-phylum (Cnidaria, Annelida, Mollusca, Crustacea, Echinodermata, and Ascideacea) due to the low mass of most species.



Figure 4: 'Light' trawl net used by MV Comrade.



Figure 5: 'Hopper' trawl net used by MV Comrade.

Trawl ID	Date	Vessel Name	Time Shot	Duration	Avg Depth	Gear Type	Site	GPS Start	GPS End
				(mins)	(m)				
COM1	08/12/2008	Comrade	13:48	145	90	Disc	South	58°07'N 6°18'W	58°07.48'N 6°18.11'W
COM2	09/12/2008	Comrade	09:52	123	82.5	Hopper	South	58°03.273'N 6°12.542'W	57°57.554'N 6°10.973'W
COM3	10/12/2008	Comrade	09:24	135	122.5	Hopper	South	58°04.172'N 6°19.294'W	57°59.900'N 6°16.323'W
COM4	10/12/2008	Comrade	12:04	138	115	Hopper	South	57°59.900'N 6°16.323'W	58°00.086'N 6°16.015'W
COM5	11/12/2008	Comrade	09:32	138	117.5	Hopper	South	58°04.411'N 6°19.211'W	58°00.160'N 6°16.342'W
COM6	11/12/2008	Comrade	12:05	130	120	Hopper	South	58°00.160'N 6°16.342'W	58°04.11'N 6°19°9'W
COM7	10/02/2009	Comrade	13:09	135	107.5	Disc	South	58°02.938'N 6°15.044'W	57°56.944'N 6°16.637'W
COM9	11/02/2009	Comrade	13:45	120	75	Disc	South	57°57.071'N 6°15.095'W	58°00.348'N 6°10.921'W
COM10	12/02/2009	Comrade	10:15	120	112.5	Disc	South	58°03.816'N 6°13.551'W	58°00.143'N 6°16.381'W
COM11	12/02/2009	Comrade	12:45	120	102.5	Hopper	South	58°00.131'N 6°16.376'W	58°06.255'N 6°14.811'W
COM12	13/02/2009	Comrade	10:18	164	115	Hopper	East	58°08.936'N 6°07.977'W	58°05.425'N 6°06.933'W
COM13	21/04/2009	Comrade	10:25	125	120	Hopper	South	58°02.616'N 06°15.538'W	57°57.247'N 6°16.258'W
COM14	21/04/2009	Comrade	13:30	130	140	Hopper	South	57°56.870'N 6°15.904'W	57°57.762'N 6°18.103'W
COM15	22/04/2009	Comrade	07:45	270	114.5	Hopper	East	58°06.857'N 6°08.082'W	58°04.285'N 6°17.517'W
COM16	23/04/2009	Comrade	10:15	135	120.5	Hopper	South	58°01.991'N 6°15.285'W	57°56.680'N 6°17.304'W
COM17	23/04/2009	Comrade	13:15	135	133.5	Hopper	South	57°56.980'N 6°17.381'W	58°02.595'N 6°15.186'W
COM18	24/04/2009	Comrade	09:45	120	117.5	Hopper	East	58°08.578'N 6°09.132'W	58°06.822'N 6°04.684'W
COM19	24/04/2009	Comrade	12:25	125	104.5	Hopper	East	58°06.554'N 6°04.795'W	58°02°595'N 6°15.186'W
COM20	16/06/2009	Comrade	10:06	139	107	Hopper	South	57°58.765'N 6°15.884'W	58°03.362'N 6°14.186'W
COM21	16/06/2009	Comrade	13:00	125	147.5	Hopper	South	57°59.818'N 6°19.334'W	58°02.639'N 6°19.562'W
COM22	17/06/2009	Comrade	09:25	125	123.5	Hopper	East	58°07.836'N 6°11.555'W	58°06.332'N 6°06.104'W
COM23	17/06/2009	Comrade	12:15	132	117	Hopper	East	58°06.441'N 6°05.593'W	58°06.310'N 6°10.308'W
COM24	18/06/2009	Comrade	09:45	120	93.5	Hopper	South	58°05.766'N 6°15.821'W	58°04.415'N 6°17.227'W
COM25	18/06/2009	Comrade	12:15	120	78.5	Hopper	South	58°04.595'N 6°17.096'W	58°05.412'N 6°20.576'W
COM26	19/06/2009	Comrade	09:20	120	104	Disc	East	58°08.371'N 6°12.852'W	58°05.086'N 6°05.831'W
COM27	19/06/2009	Comrade	11:30	155	112.5	Hopper	East	58°05.151'N 6°06.509'W	58°08.361'N 6°12.813'W
COM28	11/08/2009	Comrade	10:00	120	118.5	Disc	East	58°08.376'N 6°10.595'W	58°06.776'N 6°05.265'W
COM29	11/08/2009	Comrade	12:00	150	116	Hopper	East	58°07.274'N 6°04.461'W	58°06.615'N 6°09.162'W
COM30	12/08/2009	Comrade	10:10	120	129	Disc	South	58°03.093'N 6°15.043'W	57°58.640'N 6°16.357'W

Table 2: Summary data for each trawl

(cont. overleaf)

Trawl ID	Date	Vessel Name	Time Shot	Duration	Avg Depth	Gear Type	Site	GPS Start	GPS E'Nd
				(mins)	(m)				
COM31	12/08/2009	Comrade	13:00	120	110	Hopper	South	58°00.995'N 6°15.934'W	58°04.571'N 6°15.140'W
COM32	13/08/2009	Comrade	09:35	120	121	Hopper	East	58°08.494'N 6°12.893'W	58°07.315'N 6°05.461'W
COM33	13/08/2009	Comrade	11:55	120	117.5	Disc	East	58°07.7'N 6°05.3'W	58°07.7'N 6°11.5'W
COM34	14/08/2009	Comrade	09:50	120	104	Hopper	South	58°03.589'N 6°16.656'W	58°03.569'N 6°16.656'W
COM35	14/08/2009	Comrade	12:15	125	106	Disc	South	57°59°4'N 6°16°5'W	58°06.571'N 6°15.140'W
COM36	27/10/2009	Comrade	10:00	120	104	Disc	South	58°03.3'N 6°14.6'W	57°58.5'N 6°13.5'W
COM37	28/10/2009	Comrade	10:00	120	104	Disc	South	58°02.9'N 6°14.5'W	57°58.2'N 6°14.7'W
COM38	28/10/2009	Comrade	12:45	130	108.5	Hopper	South	57°58'N 6°14.7'W	58°02.8'N 6°14.8'W
COM39	29/10/2009	Comrade	10:00	120	107	Disc	East	58°08'N 6°04.6'W	58°02.7'N 6°08.4'W
COM40	29/10/2009	Comrade	12:45	125	87.5	Hopper	East	58°02.7'N 6°08.'W	58°03.1'N 6°06.8'W
COM41	30/10/2009	Comrade	10:00	150	98	Disc	South	58°02.4'N 6°14.'W	57°57.9'N 6°14.'W
COM42	30/10/2009	Comrade	12:30	90	88.5	Hopper	South	57°57.4'N 6°13.4'W	58'N 6°11.2'W
COM43	08/12/2009	Comrade	11:50	120	102	Disc	South	58°03.9'N 6°11.5'W	58°00.4'N 6°11.1'W
COM44	09/12/2009	Comrade	10:20	120	97.5	Disc	East	58°04.1'N 6°07. 'W	58°08.'N 6°00.'W
COM45	09/12/2009	Comrade	12:55	135	94	Hopper	East	58°07.7'N 6°00.'W	58°04.3'N 6°05.7'W
COM46	10/12/2009	Comrade	10:20	120	118.5	Disc	South	58°01.9'N 6°14.6'W	57°57.9'N 6°14.6'W
COM47	10/12/2009	Comrade	13:05	120	98.5	Hopper	South	57°57.2'N 6°14.6'W	58°01.2'N 6°11.1'W
COM48	11/12/2009	Comrade	10:20	150	93.5	Disc	Both	58°5.7'N 6°04.9'W	58°00.5'N 6°10.8'W
COM49	22/03/2010	Comrade	10:55	120	119.5	Disc	South	58°02.45'N 6°14.6'W	57°58.2N 6°15.9'W
COM50	22/03/2010	Comrade	13:30	120	100	Disc	South	57°58.1'N 6°14.3'W	
COM51	24/03/2010	Comrade	09:50	125	116	Disc	South	58°02.8'N 6°15.3W	57°57.7'N 6°16.0'W
COM52	24/03/2010	Comrade	12:30	125	149	Disc	South	57°57.5'N 6°16.0'W	57°59.6'N 6°18.0'W
COM53	25/03/2010	Comrade	08:20	100	106	Disc	South	58°04.0'N 6°15.9'W	58°00.0'N 6°15.8'W
COM54	25/03/2010	Comrade	10:30	110	117.5	Disc	South	58°00.0'N 6°16.1'W	58°04.5'N 6°13.2'W
COM55	15/06/2010	Comrade	10:15	120	100	Disc	South	58°02.3'N 6°14.0'W	57°58.1'N 6°14.8'W
COM56	15/06/2010	Comrade	12:45	118	104.5	Disc	South	57°58.0'N 6°14.4'W	58°03.0'N 6°14.3'W
COM57	16/06/2010	Comrade	10:10	120	105	Disc	South	58°03.0'N 6°14.5'W	57°54.7'N 6°19.4'W
COM58	16/06/2010	Comrade	15:45	120	106.5	Disc	South	57°58.4'N 6°14.4'W	58°03.2'N 6°14.6'W
COM59	17/06/2010	Comrade	09:50	125	104.5	Disc	South	58°03.4'N 6°14.6'W	57°58.8'N 6°14.4'W
COM60	17/06/2010	Comrade	12:25	80	95	Disc	South	57°58.8'N 6°14.3'W	58°04.8'N 6°14.9'W

Because pouts (*Trisopterus* sp.) were so abundant in some catches, it was occasionally not possible to count every individual. In such cases, at least three groups of 100 individuals were selected randomly from the total and weighed, and the average weight for 100 animals was then calculated. All Norway pout were then weighed, and the total weight was divided by the average value to give an estimate of the total number present in each catch.

#### Part B: Key Species

After the numbers and weights of each species had been recorded, all cod, haddock, whiting and spurdog were stored on ice and frozen. In addition, subsamples of approximately 100 pout and 100 hake were taken from one or two trawls per week where possible, as well as samples of approximately 100 whole *Nephrops* from each size grade (small, medium or large) and 200 *Nephrops* tails. The discarded *Nephrops* (which included undersize individuals and 'heads') were kept and weighed from at least two tows per week and a subsample was taken.

Samples of *Nephrops* from each grade were taken as randomly as possible. Baskets of sorted individuals (tails and whole grades) were washed using a standard fish washer before being drained into a basket. The washing served to mix the animals, which should have prevented the samples from being biased towards any particular section of the catch. The samples were then taken as randomly as possible by 'scooping' several animals at a time from the baskets by hand which should have avoided creating a size bias within each grade. Approximately 25kg of discarded *Nephrops* and *Nephrops* heads was retained as a subsample after the entire sample had been weighed. This portion of the catch was not washed prior to sampling, so the sample was made up from different baskets in an attempt to avoid biasing it towards one part of the catch.

All samples were stored on ice and frozen at -20°C by Young's Seafood Ltd. in Stornoway before being transported on ice to the university by haulier approximately one week
after capture. The samples were refrozen at -20°C on arrival at the university and stored until they were required.

## Cod, Haddock & Whiting

The samples of fish were allowed to defrost at room temperature for at least 24 hours before analysis. The total length (rounded down to the nearest 5mm) and total weight of each individual fish was recorded, as well as the sex and the weight of the viscera and of the gonads in cod, haddock and whiting. If the total length of an individual fish was less than 15cm, only the total length and weight were recorded. This was due to the very high prevalence of juvenile whiting and haddock in the catches in August and October 2009 and was necessary to reduce the amount of time taken by the analysis.

## Spurdog

The examination of the spurdog samples was carried out mainly by final year undergraduate student, Ms Ola Wands, as part of a final-year Honours project, to allow more thorough data collection and analysis to be carried out on this species. Samples of spurdog were allowed to defrost at room temperature for at least 24 hours before dissection, and the total length, total weight, sex, viscera weight, gonad weight and liver weight were recorded. The dorsal spines were also removed to allow the age of each individual to be estimated. The methodology for aging spurdog is well established, and involves counting the growth bands which appear on the dorsal spines, was according to the method of Holden and Meadows (1962). This technique was validated by Campana et al., 2006 using bomb dating which demonstrated that one calcified band on the spine corresponded to one year of growth. 'Double bands' were counted as a single year, and spines were not aged if the tip was noticeably worn, as such estimates would not be accurate. Spines were considered to be the same age whether the first band was black or white. It should be noted that this method is problematic if carried out by untrained personnel and can be subject to errors (Holden & Meadows, 1962). Expert confirmation of the ages may therefore be obtained if required.

25

## Nephrops

The samples of *Nephrops* were partially defrosted before analysis by running warm water over them to melt any ice and to separate individual animals; measures were taken from the partially frozen animals. The carapace length, tail width and sex of the whole *Nephrops* were then measured and recorded for each size class. The tail width and sex were recorded from the *Nephrops* tails, and the carapace length was extrapolated using the data from the whole animals.

'Discarded' *Nephrops* were partially defrosted under warm running water, and then separated into 'heads' and whole animals. Each component was weighed to give an estimate of the mass of *Nephrops* discarded at sea from this vessel. The 'heads' were discarded without further analysis since the tails had already been measured from a separate sample, but the carapace length, tail width and sex of any whole animals was recorded.

## Data Analysis

Analysis of the abundance and biomass of bycatch species or groups was carried out using PRIMER 6 software (Clarke & Gorley, 2006). In order to ensure that trends were accurately identified and analysed, the numbers of each species in each haul and the weights of the major groups per haul were standardised by trawl duration prior to analysis, to give numbers and weights per haul per hour respectively. Multivariate analyses were then carried out on both transformed and untransformed data. The untransformed data were examined to determine the gross relationships between the 'real' catches, for which the analyses would give most weighting to the dominant species (including *Nephrops*, which is the most commercially significant species), while more subtle relationships arising as a result of the rarer species could be examined by transforming the data to down-weight the highly dominant species. Where comparisons between samples were examined, the abundance and biomass data were converted to a similarity matrix using the Bray-Curtis similarity index. The environmental data were normalised, then converted to a similarity matrix using Euclidean distance. The GPS positions were converted to a decimal scale before inclusion in the data set.

Multi-Dimensional Scaling (MDS) and cluster analysis were used to determine the relationships between the bycatch 'communities' from each haul, and BEST and ANOSIM analyses were used to determine the significance of environmental parameters or factors in explaining the differences in these communities. In general, 99 permutations were used for BEST and ANOSIM tests, and MDS analyses were restarted at least 100 times. In each case, significance was taken as p < 0.05.

Diversity indices were also calculated and compared between trawls.

## Calculating the Number of Nephrops

In order to have complete abundance data for the entire catch it was necessary to include the numbers of *Nephrops* present. However, since there were so many individuals in each trawl, the numbers had to be estimated from the biomass rather than counted directly.

Estimates were made by weighing several groups of 100 *Nephrops* from each of the 'discarded' (whole animals only), 'tails' and 'whole' categories, and determining the mean weight for each. In this case, animals in the 'whole' category were not graded by size (they were recorded as a 'mixed' grade), therefore allowing any estimates to be comparable to the original biomass measures. The approximate numbers of *Nephrops* in the total catch could then be calculated. Since the mean proportion of whole discarded *Nephrops* was calculated to be 0.21 of the total discarded component on average, this factor could be applied to give an estimate of the weight of the whole discarded animals

27

only and converted to an abundance estimate. The mean weight of 100 animals from each category is shown in Table 3.

Table 3: Mean weights of 100 whole Nephrops from the 'whole' and 'discarded' components, and the mean weights of 100 Nephrops tails. One standard deviation for each group is indicated.

Catch Component	Mean weight of 100 (kg)	Standard Deviation
Discarded	1.39	0.45
Tails	1.12	0.13
Whole	3.88	0.28

## **Condition Indices**

Condition indices were also calculated for the fish and spurdog samples where possible, using Fulton's Condition Index (K):

K = 
$$\frac{\text{Weight (g)}}{\text{Length (cm)}^3}$$
 x 100

Where the carcass weight (i.e. the weight of the body after the viscera are removed) was known, the Somatic Condition Factor (SCF) was calculated as follows:

SCF =  $\frac{\text{Carcass weight (g)}}{\text{Length (cm)}^3} \times 100$ 

This index is similar to Fulton's condition index, but does not take 'fullness' of the animal into account, and is potentially therefore a more accurate measure of the long-term condition of an individual. Both indices produce values close to one, and the higher the value, the better the condition of the animal is assumed to be.

These indices are suitable for animals that show an isometric growth pattern (weight increases as the cube of the length).

The Gonadal Somatic Index (GSI) was also calculated for cod, haddock and whiting samples from February 2009 onwards, to help verify the maturity status of the animals. The GSI was calculated as follows:

GSI = Total wet weight (g)

# Results

## **Species Composition and Broad Trends**

From nine survey trips comprising 57 valid trawls, a total of 85 species were recorded, including 24 species of roundfish, 9 species of flatfish, 8 species of elasmobranch, and 45 species of invertebrate (including *Nephrops*). A list of the species present is given in Table 4.

On average *Nephrops* was the most dominant species in the catches by both abundance (approx. 1045 captured per hour of trawling on average) and wet weight (approx. 36.3kg captured per hour of trawling on average). The bycatch was typically dominated by a few common species which were present in virtually all sampled catches. Table 5 shows the five most abundant bycatch species by number (per hour of trawling) while the dominant species by wet weight (per hour of trawling) are shown in Table 6. In each case, the values have been averaged across all tows and have been standardised by trawl duration. Table 4: List of species recorded from 57 trawls between December 2008 and June 2010

ROUNDFISH	INVERTEBRATES
Agonus cataphractus (Linnaeus, 1758)	Cnidaria
Chelidonichthys cuculus (Linnaeus, 1758)	Funiculina quadrangularis (Pallas, 1766)
Callionymus lyra Linnaeus 1758	Pennatula phosphorea Linnaeus, 1758
Capros aper (Linnaeus, 1758)	Actinauge richardi (Marion, 1882)
Clupea harengus Linnaeus 1758	Urticina sp.
Enchelyopus cimbrius (Linnaeus, 1766)	Adamsia carciniopados (Otto, 1823)
Gadus morhua Linnaeus 1758	Family Caryophylliidae
Gaidropsarus vulgaris (Cloquet, 1824)	Cyanea capillata (Linnaeus, 1758)
Lophius piscatorius Linnaeus, 1758	Cyanea lamarcki Péron and Lesueur, 1809
Melanogrammus aeglefinus (Linnaeus 1758)	Aurelia aurita (Linnaeus, 1758)
Merlangius merlangus (Linnaeus 1758)	Alcyonium digitatum Linnaeus, 1758
Merluccius merluccius (Linnaeus 1758)	Mollusca
Micromesistius poutassou (Risso, 1827)	Aequipecten opercularis (Linnaeus, 1758)
Molva molva (Linnaeus, 1758)	Arctica islandica (Linnaeus, 1767)
Phycis blennoides (Brünnich, 1768)	Loligo vulgaris Lamarck, 1798
Pollachius virens (Linnaeus, 1758)	Eledone cirrhosa Lamarck, 1798
Scomber scombrus Linnaeus, 1758	Family Sepiolidae
Trachurus trachurus (Linnaeus, 1758)	Order Nudibranchia: Species 1
Family Triglidae	Scaphander lignarius (Linnaeus, 1767)
Trisopterus spp.	Aporrhais pespelicanis Linnaeus, 1758
Zeus faber Linnaeus, 1758	Neptunea antiqua (Linnaeus, 1758)
Alosa alosa (Linnaeus, 1758)	Annelida
Conger conger (Linnaeus, 1758)	Aphrodita aculeata Linnaeus, 1761
Labrus bimaculatus Linnaeus, 1758	Crustacea
	Palinurus elphas (Fabricius, 1787)
FLATFISH	Munida rugosa Fabricius, 1775
Buglossidium luteum (Risso, 1810)	Pagurus prideaux Leach, 1815
Glyptocephalus cynoglossus (Linnaeus, 1758)	Pagurus bernhardus Linnaeus, 1758
Hippoglossoides platessoides (Fabricius 1790)	Cancer pagurus Linnaeus, 1758
Hippoglossus hippoglossus (Linnaeus, 1758)	Liocarcinus depurator (Linnaeus, 1758)
Lepidorhombus whiffiagonis (Walbaum, 1792)	Macropipus tuberculatus (Roux, 1830)
Limanda limanda (Linnaeus, 1758)	Goneplax rhomboides (Linnaeus, 1758)
Microstomus kitt (Walbaum, 1792)	Atelecyclus rotundatus (Olivi, 1792)
Pleuronectes platessa Linnaeus, 1758	Family Magidae
Scophthalmus rhombus (Linnaeus, 1758)	Crangon crangon (Linnaeus, 1758)
	Family Pandalidae
ELASMOBRANCHS	Pasiphaea sivado (Risso, 1816)
Dipturus oxyrinchus (Linnaeus, 1758)	Infra-order Caridea: Sp. 1
Galeus melastomus Rafinesque, 1810	Echinodermata
Leucoraja naevus (Müller & Henle, 1841)	Asterias rubens Linnaeus, 1758
Raja clavata Linnaeus, 1758	Luidia ciliaris (Philippi, 1837)
Raja brachyura Latont, 1873	Marthasterias glacialis (Linnaeus, 1758)
Kaja montagui Fowler, 1910	<i>Porania</i> sp.
Scyllorninus canicula (Linnaeus, 1758)	Sub-class Ophiuroidea
Squaius acanthias Linnaeus, 1758	Order Euryalida
	Parasticnopus tremuius (Gunnerus, 1767)
	Echinus Sp.
	Tupicata
	Lunicald
	Sup-phylum runicata

Species	Average number per hour	
	trawled	
Trisopterus spp.	212	
Pandalid shrimp	115	
Whiting	60	
Tall Sea Pen	21	
Actinauge richardi	18	

Table 5: The five most dominant species (by number) occurring in sample trawls and the average number captured in each trawl.

Table 6: The five most dominant species (by wet weight) occurring in sample trawls and the average biomass captured in each trawl.

	per hour trawled (kg)
Sharks	5.1
Trisopterus spp.	3.3
Whiting	2.7
Cnidaria	2.0
Crustacea	1.6
Sharks <i>Trisopterus</i> spp. Whiting Cnidaria Crustacea	per hour trawled (kg)   5.1   3.3   2.7   2.0   1.6

The mean proportion of each major group by wet weight is shown in Figure 6. Overall, the landed portion of the *Nephrops* catch comprised the largest component of the catches (mean = 55%), with non-target organisms accounting for 39% and discarded *Nephrops* for 6%. It should be noted that the weights of the discarded 'heads' was included in the weight of the tails since this is considered to be normal processing waste rather than bycatch or discard (A. Weetman, *pers. comm.*).

Figure 7 shows the mean catch weights for '*Nephrops'* (discards, heads and landed animals combined) and the bycatch groups by month. Overall, there were significant differences in the catch weights per month (ANOVA; p < 0.05), though *post hoc* analysis failed to determine where the differences existed. However, it is likely that they were influenced by the particularly small catches made during March 2010 which were generally smaller than those made during the rest of the period.



Figure 6: Mean overall catch composition from all trawls made from December 2008 to June 2010.

The weights of all bycatch groups captured per hour varied significantly with month (p < 0.01) with the exception of the invertebrates (p > 0.05). More roundfish were caught in December 2008, between August – December 2009 and in June 2010 than in February – June 2009 or March 2010 suggesting a general increase in catch weights of this group over the period. It is likely that low catches of roundfish in March 2010 are indicative of low total catches during that month. There appeared to be a decline in the weights of flatfish catches over the course of the survey until December 2009 with slight increases in biomass in March and June 2010. Catches of elasmobranchs were lower in June 2009 and March and June 2010, while a higher weight of *Nephrops* was captured per hour in December 2008, April and June 2009 than October 2009 or March 2010. Trends in *Nephrops* catches will be discussed in more detail later. Values for elasmobranchs and invertebrates were log-transformed to meet the assumptions of the ANOVA.



Figure 7: Mean proportion of each component of the catches by wet weight from each sampling trip. '*Nephrops*' represents the combined weights of both the landed *Nephrops* and the discarded animals and heads. Error bars show one standard deviation.

## **Relationships Between Catches: Species Abundance**

The abundance data were standardised (to account for differences in catch volume) and fourth-root transformed prior to analysis. An ANOSIM (ANalysis Of SIMilarity) test was carried out to determine whether any factors (such as sampling month, site (south or east), or gear type (hopper or disc net)) had a significant influence on the similarity between catches. This test showed a significant effect of both sampling month (global R = 0.827, p < 0.001) and site (global R = 0.395, p < 0.005).

SIMilarity PERcentages (SIMPER) analysis was carried out to determine which species contributed to the differences between the sites, but the results were not clear. At the south site, the 5 species contributing most to the similarity were *Nephrops*, pouts (*Trisopterus* spp.), whiting, lesser-spotted dogfish and hake (accounting for 39.8%) of the cumulative similarity. At the east site, the species were similar, with *Nephrops*, pandalid shrimp, pouts, hake and whiting accounting for 54.6% of the cumulative similarity within this region. Dissimilarity between the groups was only 30.5%, and no species accounted for more than 4.2% of the total, suggesting that the differences are quite subtle.

To visualise the relationships between the species abundance of catches, nonparametric 2D Multi-Dimensional Scaling (MDS) ordination was carried out, with the month of capture indicated in each case (Fig 8). These data generally show clustering by month, although the stress (simplistically, a measure of the error) of the 2D plot is relatively high (0.18). Better separation by month is apparent in the 3D plot, but this cannot be shown here.

BEST (BIOENV) analysis was carried out to determine whether any of the recorded environmental variables could explain the differences in composition between the catches, but none were found to be significant (p > 0.05). BVSTEP analysis did find that 20 species had a significant influence on the catch composition; these are shown in Table 7.

34

Table 7: Species identified as significant by BVSTEP analysis in explaining the relationships between catches (fourth-root transformed abundance data).

Haddock	Whiting	Four-Bearded Rockling	Plaice
Blue Whiting	Triglidae (Gurnards)	Horse Mackerel	Witch
Dab	Cuckoo Ray	Cuttlefish (Sepiolidae)	Hermit crab ( <i>Pagurus prideaux</i> )
Hermit crab	Brown shrimp	Pandalid shrimp	Glass shrimp
(Pagurus bernhardus)	(Crangon crangon)		(Pasiphaea sivado)
Brown crab	Squat lobster ( <i>Munida rugosa</i> )	Harbour crab	Sea Mouse

The effect each species has on the relationships between the catches can be displayed as a 2D 'bubbleplot', which is overlaid on the 2D MDS plot and in which the size of each 'bubble' is proportional to the abundance of the species in question. While this was not carried out for each of the 20 species indicated, examples are shown in Figure 9, using (transformed) abundance data from haddock, whiting and Pandalid shrimp.

Using Figure 8 as reference, it can be seen that there was a general increase in abundance of both haddock and whiting from left to right, which corresponds approximately with the month of capture, i.e. catches of both species increase over the survey period. Figure 9 (c) shows the opposite pattern for the pandalid shrimp, which appear to show a decline from the start of the survey through 2009, and are completely absent from catches taken in 2010.



# Abundance per hour

Figure 8: 2D MDS plot showing the relationships between the catches (fourth-root transformed abundance data). The sampling month is indicated for each catch (ANOSIM, Month: p < 0.001; Site: p < 0.003). Note the relatively high stress level of this plot.



Figure 9: Bubble plots of numbers of (a) *Nephrops,* (b) *Trisopterus* spp. and (c) Pandalidae superimposed over a 2D MDS ordination of untransformed abundance data (from Fig. 8).

# Relationships Between Catches: Biomass

The biomass data were standardised (to account for differences in catch volume) and fourth-root transformed prior to analysis. ANOSIM analysis showed that month and capture site were again the only significant factors to explain the similarities between the catches (Month: R = 0.652, p < 0.001; Site: R = 0.224, p < 0.03), and BEST (BIOENV) analysis again failed to find any significant effects of the environmental variables on the overall catch composition. Further testing (BVSTEP) found that 23 taxa had significant influences on the relationships between catches however, and these are shown in Table 8. A 2D MDS ordination of these data is shown in Figure 10.

Table 8: Species identified as significant by BVSTEP analysis in explaining the relationships between catches (fourth-root transformed biomass data).

Dragonet	Herring	Four-Bearded Rockling	Cod
Haddock	Whiting	Hake	Blue Whiting
Horse Mackerel	Pouts ( <i>Trisopterus</i> spp.)	Long-rough dab	Witch
Dab	Lemon Sole	Plaice	'Sharks'
Rays & Skate	Cnidaria	Mollusca	Annelida
Echinodermata	Ascideacea		



# Biomass per Hour

Figure 10: 2D MDS plot showing the relationships between the catches (fourth-root transformed abundance data). The sampling month is indicated for each catch (ANOSIM: p < 0.001).

## **Key Species**

## Cod

A total of 86 cod were collected and analysed (from a total of 101) from the 60 trawls between December 2008 and June 2010. Catches of cod were low throughout the sampling period, accounting for 0.8% of all catches made by wet weight. The percentage of cod captured per month (by wet weight) is shown in Figure 11 against a 1.5% limit which represents the derogation allowing exemption from quota restrictions under the EU Cod Recovery Plan. Although catches were variable, cod accounted for more than 1.5% of catches (by wet weight) only in December 2009 and March 2010.

Sampling month, survey site or types of gear used were not found to have any effect on the length of fish captured (p > 0.05) and length did not vary with sex (p > 0.05). The average length of cod was 32.5cm (MLS = 35cm) over the entire survey period, with undersized animals comprising 64% of the total. The length-frequency distribution of captured cod is shown in Figure 12.

The CI, SCF and GSI were found to vary significantly with length (p < 0.001), with larger cod generally having higher index values (Figure 13). The CI was found to slightly decrease with increasing GSI, though this was only just significant at 5% (p < 0.05). The CI and SCF also varied with month, and typically showed higher values in the autumn months (October: 1.12 and 1.03) than in the spring (April: 0.95 and 0.86). The highest CI value occurred in December 2008, though this was not mirrored in the SCF value.



Figure 11: Mean percentage catches of cod captured during each month of the study. The 1.5% limit (indicative of the derogation limit under the EU Cod Recovery Plan) is shown in red. Error bars show one standard deviation.



Figure 12: Length-frequency distribution of all Atlantic cod captured during the study period.



Figure 13: Mean CI and SCF values per month for Atlantic cod. Error bars show one standard deviation.

While GSI did not vary significantly with month (p > 0.05), it appeared that the highest index values were found in larger individuals which were over approximately 40-45cm in length. Linear regression showed the best fit to a quadratic curve ( $R^2 = 47.8$ , p < 0.001; Fig. 14). Piecewise regression may have been a more appropriate technique, but could not be conducted using the available software.



Figure 14: Linear regression showing total length (cm) against GSI values in cod. The fitted line follows a quadratic curve (p < 0.001).

#### Haddock

A total of 1372 haddock were examined from a total of 1551 captured between December 2008 and June 2010, and comprised an average of 1.6% of all catches by wet weight. The number of haddock captured each month was variable, with relatively few being captured between December 2008 and June 2009, and higher numbers between August 2009 and June 2010. The lengths of captured haddock also varied, with larger individuals being caught between December 2008 and June 2009 (median = 32.6cm) than during the rest of the study period (median = 16.45cm; Kruskal-Wallis: H = 962.95, p < 0.001), though there was a progressive increase in mean total length between August 2009 and June 2010. The total numbers and the mean total lengths of captured haddock are shown in Figure 15 and 16 respectively.

It appeared that gear type and site were significant factors affecting fish length, but because the data did not meet the assumptions of the GLM, the analyses were conducted using a Kruskal-Wallis test for individual months, and run separately for gear type and site differences. December 2008 and February 2009 had too few samples to analyse, and all trawls were made at the south site using disc nets in March and June 2010 so these data were not included. The results did not show consistent trends, but did suggest that site and gear type had an effect on the length of the haddock captured during October 2010, with slightly larger fish captured at the south site than the east site (hopper net: H = 11.83, df = 1, p < 0.001; disc net; H = 6.12, df = 1, p < 0.02). Statistically, there was also a difference between gear types at the south site (p < 0.005), but the median length for each net was 15.0cm, so it is unclear what the difference between the gears was. However, differences in gear type were also found at the south site in August 2009 and in December 2009 with the hopper net recovering larger haddock than the disc net in August (12cm and 11cm respectively; H = 14.97, df = 1, p < (0.001), but smaller ones in December (14.75cm and 16cm respectively; H = 6.13, df = 1, p < 0.02).



Figure 15: Total numbers of haddock captured during each month. Numbers are displayed on a logarithmic scale.



Figure 16: Mean total length (cm) of haddock captured per month. Error bars show one standard deviation.

The CI and SCF for haddock also varied significantly by month (p < 0.001) and showed a similar seasonal pattern to the Atlantic cod, with the lowest index values occurring earlier in the year (April 2009 and March 2010). The peaks were somewhat more variable and there was some discrepancy between the CI and SCF values. The peak CI values occurred in December 2008 and February 2009, with lower peaks occurring in June and October 2009, whereas the highest mean values for the SCF occurred in August 2009. These results are shown in figure 17. Neither index showed significant correlation with the length of the fish (p > 0.05).

The GSI showed significant variation with month (H = 44.29, df = 8, p < 0.001), however due to small sample sizes it was not possible to statistically determine where the differences existed. Figure 18 shows the GSI for haddock for each month, and there is an apparent peak in the values in February and April 2009, although the variance is also very high. A slight increase in the GSI occurred in March 2010, but it did not match the peak seen in 2009 (Fig. 18). To determine whether this was due to high numbers of small (and likely immature) animals, the analysis was repeated twice using 20cm and 25cm as the minimum lengths to be included in the data set. While both modifications caused slight increases in the GSI for March 2010, with the > 25cm group having a significantly higher GSI score than the other groups, the highest median value (0.007) was still far lower than the mean score from February 2009 (0.046).



Figure 17: Mean CI and SCF values per month for haddock. Error bars show one standard deviation.



Figure 18: Mean GSI per month for haddock. Error bars show one standard deviation.

## Whiting

5514 whiting were collected and analysed from a total of 7112 captured between December 2008 and June 2010, comprising 4.5% of catches on average (by wet weight). Similarly to the haddock, the numbers of captured whiting increased markedly during 2009, with the highest numbers being captured in August 2009 (Fig 19). This also corresponded to a decrease in the mean length of the fish (Fig. 20) suggesting that there was an influx of young animals to the population during this period. Despite an increase in total numbers in June however, the mean length does not decline until August suggesting that the June increase is caused by larger individuals. There was some evidence that the numbers of whiting were beginning to decline by June 2010.

The total length of the whiting in the catches varied significantly with month (H = 3560.75, df = 1, p < 0.001) as shown in Figure 20, and there was some evidence of variation with gear type and survey site as well. As for the haddock, the assumptions of a GLM could not be met, so each factor was investigated separately using the Kruskal-Wallis test. There were too few samples in December 2008 – April 2009 and all samples from March and June 2010 were made using disc nets at the south site so were also excluded.

Significant differences in the length of whiting captured between sites was found for all months with the exception of June 2009, although as with the haddock data, the results did not appear to be consistent. In general however, the fish captured by the disc net appeared to be smaller at the east site than at the south site (p < 0.05), whereas those captured by the hopper net generally appeared to be larger from the east site (p < 0.05) except in August 2009 when the reverse was true.

Significant differences between gear type were only found during October and December 2009, and the hopper net typically captured larger individuals than did the disc net, except in December at the south site.

48



Figure 19: Total frequency of whiting captured between December 2008 and June 2010 displayed on a logarithmic scale.



Figure 20: Mean length of whiting occurring in catches between December 2008 and June 2010. Error bars show one standard deviation.

The CI and SCF for whiting also varied significantly with month (p < 0.001) and showed a similar seasonal pattern to Atlantic cod and haddock, with the lowest index values occurring in April 2009. As for the haddock, the peak CI values occurred in December 2008 and February 2009, with little variation through the rest of the year. Values for SCF were also highest in December 2008 and February 2009, but showed a secondary peak in August 2009. These results are shown in Figure 21. SCF was significantly affected by the length of the fish, with larger fish generally being in better condition than smaller fish (p < 0.001), although there was a significant interaction with sampling month (p < 0.001) suggesting that the precise relationship between condition and length depends on when they were sampled. The model was a poor fit to the data however ( $R^2 = 20.3$ ) suggesting that other factors are more important in determining the SCF of the individual fish. The CI values did not meet the assumptions of the GLM and could not be analysed further.

The GSI showed significant variation with month (H = 159.97, df = 8, p < 0.001), with higher values in December 2008 and February 2009 than in the other months, and with a small secondary peak in March 2010 which was higher than the values recorded in October – December 2009 or June 2010. Figure 22 shows the mean GSI during each month.



Figure 21: Mean CI and SCF values for whiting captured between December 2008 and June 2010. Error bars show one standard error.



Figure 22: Mean GSI values for whiting captured between December 2008 and June 2010. Error bars show one standard error.

# Spurdog

A total of 100 spurdog were recovered from all trawls made between December 2008 and June 2010. The lengths and weights of the 6 animals captured in December 2008 were recorded on board the fishing vessel, but all other specimens were brought back to the University of Glasgow for more detailed examination. The numbers of spurdog captured during each survey trip are given in Table 9, and the length distributions for each month are shown in Figure 23.

Month	Sex	Number captured	Mean length (cm) (± 1 SD)
Dec 2008	M F	6 0	63.0 (± 18.5)
Feb 2009	M F	0 0	
Apr 2009	M F	0 0	
Jun 2009	M F	27 29	26.2 (± 2.4) 25.2 (± 3.1)
Aug 2009	M F	5 4	30.5 (± 4.0) 29.8 (± 5.1)
Oct 2009	M F	5 0	72.3 (± 3.1)
Dec 2009	M F	23 1	75.8 (± 3.5) 95.0
Mar 2010	M F	0 0	
Jun 2010	M F	0 0	

Table 9: Numbers and mean lengths of spurdog captured between December 2008 and March 2010





Individual spurdog were recorded as either immature or mature based on the relative length of the claspers in males or the presence of an enlarged or flaccid uterus in the females. All animals captured in June and August 2009 were immature while those captured in October and December 2009 were mature which correlated to the size of the individuals captured. Mature animals were found to be larger (mean total length = 75.9cm, p < 0.0001; mean total weight = 1465.1g, p < 0.0001) than immature animals (mean total length = 26.2cm, p < 0.0001; mean total weight = 51.7g, p < 0.0001).

Biometric measures (length, weight, anterior and posterior spine lengths) and the estimated age of each individual were strongly correlated (Figure 24), although there was evidence that the variability around the data increased in the larger (and older) individuals.



(b)





Figure 24: Morphometric relationships for spurdog: (a) Total length ( $log_{10}$  transformed) against total weight ( $log_{10}$  transformed),  $R^2 = 0.99$ ; (b) Total length (cm) against anterior ( $R^2 = 0.98$ ) and posterior ( $R^2 = 0.98$ ) spine lengths (mm); (c) Anterior against posterior spine length (mm),  $R^2 = 0.99$ .

## Age

Of the 100 total spurdog captured, 80 had spines which were considered suitable for aging. Of those 80 animals, the majority were young juveniles with estimated ages between 1 and 3 years, while the remainder were larger males with estimated ages of between 7 and 12 years. The estimated ages are shown in Figure 25 against the total length of each individual. Without expert guidance however, and with a low number of female samples in particular, it is difficult to assess how accurate these estimates are and the relatively high level of variability around each age point suggests that there is some degree of error in the data. Consequently, no attempt has been made at this stage to produce a growth curve.



Figure 25: Age-at-length estimates for 80 individual spurdog captured between December 2008 and June 2010.

## Nephrops

A total of 3408 discarded whole *Nephrops*, 3325 *Nephrops* tails and 2869 graded whole *Nephrops* were sampled and analysed between December 2008 and June 2010. To obtain an estimate of the original carapace length (CL) of the tailed *Nephrops*, the carapace length and tail width (TW) of the whole (discarded and graded) animals were plotted and the relationship between them established (Fig. 26). Since the CL : TW ratio was significantly different for male and female *Nephrops* (Mann-Whitney: W > 13000000, p < 0.0001), with females having a wider tail relative to their carapace length than males (Females 1.89 : 1; Males: 2.01 : 1), the equations were determined for each sex separately. These were found to be:

Females:  $CL = 0.62TW - 2.3918 (R^2 = 0.957)$ Males:  $CL = 0.52TW - 0.8572 (R^2 = 0.985)$ 



Figure 26: Correlation between carapace length and tail width in male (blue) and female (red) *Nephrops*. The trend lines are shown as a solid line for males, and dashed for females.

As expected, CL was found to vary with size class, with the smallest *Nephrops* being discarded, and the largest animals graded into the 'whole' size classes (small, medium or large) as shown in Figure 27. However some trends were apparent within each size grade over the course of the study period.

Overall, male *Nephrops* were found to be larger than females (median CL: males = 33.8mm, females = 27.5mm, Kruskal-Wallis: H = 1829.88, p < 0.001). Within the different size grades however, only small-medium (whole), tailed and discarded *Nephrops* showed differences in CL between the sexes, with males being larger in the small-medium (mean CL = 37.6mm) and tailed (median CL = 31.4mm) grades compared to females (mean CL = 36.7mm and median CL = 27.9mm respectively; p < 0.04). Conversely, discarded females were larger than discarded males overall (mean CL = 26.6mm and 26.0mm respectively; t = 5.83, p < 0.001).

There was an apparent seasonal change in the CL of female *Nephrops* being discarded, with larger animals being discarded in June 2009 and June 2010 than any other month (Kruskal-Wallis: p < 0.0001). Larger animals were also discarded in December 2009 and March 2010 than in December 2008 or in February, April or October 2009 (Kruskal-Wallis multiple comparisons: p < 0.001). The median CL for female discarded *Nephrops* was lowest in April 2008 (24.1mm) and greatest in June 2010 (28.5mm).

Similar patterns were found in discarded male *Nephrops*, although the patterns were less distinct. Again, larger *Nephrops* were discarded in June 2010 than any other month, with larger animals also discarded in June 2009 and March 2010 than in February, April, August or October 2009 (Kruskal-Wallis multiple comparisons: p < 0.0001), suggesting that there was some seasonal effect influencing the CL of the discarded male *Nephrops* as well. The median CL for male discarded *Nephrops* was lowest in April 2008 (23.6mm) and greatest in June 2010 (28.1mm). The variation in CL in discarded *Nephrops* is shown in Figure 28.

58



Figure 27: Boxplot of carapace lengths in each size category for male Nephrops showing the data range in each class. Red boxes show where significant differences occur. Outliers are not shown. p < 0.001



Figure 28: Chart showing discarded female (pink) and male (blue) CL during each survey month.

Figure 29 shows some notable differences in the sex ratio of the various size grades through the year. Peaks of female animals were present in all grades in June and August 2009, although it was most pronounced in the smallest animals (discarded) but less pronounced in the larger whole grade animals.



Figure 29: Sex ratio of *Nephrops* in each size grade: (a) Discards; (b) Tails; (c) Whole. Error bars show one standard deviation.
#### Discussion

The results show marked temporal variation in the composition of the catches over the course of this study, which is evident both in the overall abundance and biomass composition data, and also in the data for cod, spurdog haddock and whiting. This is similar to the results of other studies which have examined long-term trends in other fisheries. For example, Borges *et al.*, 2005 examined the discarding practice in the Irish demersal fishing fleet between 1993 and 2002, and found that the rate of discarding and the size of discarded fish was highly variable between years. In the Irish *Nephrops* fishery, they found particularly high discards of small haddock and whiting in 2001 and 2002 which were reported to be 'year 2' fish between approximately 10cm and 20cm in length, which corresponds to the lengths of those species discarded in the present study.

While the total area surveyed over the period was not particularly large, broad spatial differences were also recorded within the region between the 'south' and 'east' sites although the reason for this separation is unclear. In their study of the Clyde sea area Bergmann *et al.*, 2002 demonstrated that catch compositions could vary significantly over relatively small areas, as between the north and south basins. Those differences were believed to be the result of hydrodynamic and sedimentary differences between the two areas, but whether similar effects are occurring in the North Minch is not known.

However, despite the temporal and spatial variation in catch composition, the majority of the catches made were dominated by very similar species. In this study, these were found to be predominantly pouts (*Trisopterus* spp.), whiting, lesser-spotted dogfish Crustacea (such as pandalid shrimp), and Cnidaria (such as the tall sea pen and the 'golf ball anemone', *Actinauge richardii*). While the invertebrate bycatch has not been the subject of many studies, other investigations of the fish bycatch in *Nephops* fisheries in the Irish Sea, North Sea and Celtic Sea have also found whiting and pouts to be among

the dominant roundfish species (Briggs, 1985, Stratoudakis *et al.*, 2001, Bergmann *et al.*, 2002, Rochet *et al.*, 2002, Catchpole *et al.*, 2005).

The study by Bergmann *et al.* (2002) is the only one to describe the total catch composition including the invertebrates, but it records lower abundances of some species than were present in the North Minch. While separate grounds would not be expected to support the same communities, there were some differences of note, particularly the high abundance of the tall sea pen (*Funinculina quadrangularis*) in the North Minch compared to its absence in the Clyde sea area. This species occurs in the same mud habitats as *Nephrops* and is known to be susceptible to trawling disturbance due to its inability to withdraw into the sediment. Consequently, it is possible that this species could be used as an ecological indicator to indicate disturbance pressure between fishing grounds and 'pristine' areas, though far more data on the ecological requirements for this species and its natural distribution in Scotland are required.

The results for whiting and haddock suggest that a strong year class was recruited by each species in the Minches prior to this study, leading to high numbers of small, immature fish in the catches from August 2009 onwards. This is supported by the data which show a steady increase in the total length of the fish in this period, suggesting that they belong to a single year group which continued to grow over the study period. There was some evidence that the different fishing gears may have captured different sizes of fish relative to each other, but these trends were inconsistent and further study specifically designed to address this issue would be required to determine whether this is a real effect or not.

Catches of cod and spurdog were very low in virtually all catches, as might be expected considering the depleted state of these species in the west of Scotland. However, this made it difficult to determine whether there were any consistent trends in the data. Spurdog are known to aggregate by sex when mature or together as juveniles which

makes them susceptible to fishing pressure (Compagno, 1984), and the capture rates in this study reflect this, particularly for the juveniles. Therefore, despite apparent differences in the capture rate between the sites, it is unclear whether this result is meaningful at present, and further data on this population are required.

The *Nephrops* data appeared to show that they were handled relatively consistently by the fishermen, with clear differences in the size grades as would be expected. While, due to the sampling method, the samples of *Nephrops* taken in this study cannot be said to truly represent the underlying dynamics of population in the region, there were some interesting trends in the data. The sex ratio of *Nephrops* populations is known to vary over annual or biannual cycles in several regions, as females emerge from their burrows in the summer, moult and breed before returning to their burrows in the autumn (e.g. Thomas & Figueiredo, 1965, Bailey, 1984, Tuck *et al.*, 1997, Tuck *et al.*, 2000, Bell *et al.*, 2006).

The data from the present study are suggestive that *Nephrops* undergo similar patterns of emergence in the North Minch, with a peak emergence of females seen in June – August 2009 and in June 2010 particularly in the discarded and 'tailed' *Nephrops*. However, there was also an apparent trend related to the size of the individual animal, in that the greatest peak was seen in the discarded *Nephrops* and the lowest in the whole *Nephrops*. One possibility for this is that the quality of the *Nephrops* is lower in the summer months (potentially due to the moult condition; Milligan *et al.*, 2009) and that larger animals are being discarded. This is supported by the results of this study that show the largest animals being discarded in June 2009 and June 2010. However, it is also possible that larger females only choose to breed every second year rather than annually (see Bell *et al.*, 2006) and it may be this that is reflected in the results. Further investigations of the population structure would be required however before conclusions could be drawn.

# Section 2 of Workpackage 3.1: Self-Assessment of Bycatch & Discards Introduction

Long-term monitoring of fisheries catches requires a strong working relationship between fishermen, processors and scientists but can involve considerable monetary and time costs if vessels have to be chartered to carry out survey work or if an observer is placed on board. A self-assessment system which allows much of the survey work to be carried out directly by skippers and crew with minimal interference from scientific staff has the advantages of being relatively cheap and simple to implement and allows more vessels to be targeted than could necessarily be achieved using observers. For this study in particular, it would allow the entire fleet of ten vessels to be surveyed relatively easily, rather than continuing to focus on the single vessel used through Objective 1. Additionally, crews are free to work as normal with no extra people on board, and depending on the methods used, such a system need not significantly disrupt normal working practice.

Any self-assessment system must be reliable however as there is considerable risk of bias, particularly if fishermen are required to log data which may be detrimental to the fishery in the short-term such as high catch rates of a sensitive species for example. It is important therefore to include standardised checks to ensure that the system is not being abused and that the self-assessment records are an accurate reflection of the catches being made.

The system developed for use in the Stornoway fleet was based on existing 'YoungsTrace' technology designed by Young's Seafood Inc. and based on the scientific research carried out by the scientists from Glasgow University. The skippers and crew who would be carrying out the self-assessments were also consulted and their opinions were taken into consideration during the development stages.

# YoungsTrace

The YoungsTrace system, developed by Young's Seafood Ltd., is a traceability system which was originally designed to allow *Nephrops* trawl vessels to record when and where their catches were made by inputting their vessel's activity (e.g. trawling, hauling the gear, travelling) during the course of a fishing trip. In return for using the system, which provided valuable information on catch quality and which stocks were fished, Young's Seafood offered a higher price for any *Nephrops* catches landed while using it. Unlike the mandatory Vessel Monitoring Systems (VMS) that are a legal requirement on vessels greater than 12m and are always switched on (Neil Campbell, *pers. comm.*), the YoungsTrace system only recorded data following input from a member of a vessel's crew.

This original system was redesigned during 2010 to allow information on the bycatch to be recorded as well as catches of *Nephrops*, based on the methodologies developed during the 2007-08 pilot trial and in Section 1 of the present study. This has been initially limited to recording the total numbers of cod and spurdog in catches, but may be extended to include more detailed information on catches later in the study depending on its reception from the fishing fleet.

# Self-Assessment Methodology

The proposed self-assessment scheme was originally based on the methods developed and used during the scientific surveys, and required crews to sort one or two trawls per calendar month into five groups:

- 1. Nephrops (target catch, to be processed as usual)
- 2. Invertebrates
- 3. Roundfish
- 4. Flatfish
- 5. Sharks, Rays & Skate

Crews were asked to count and record the total numbers of cod and spurdog in the trawl, since these species were of particular importance. To assist with the sorting and ensure that it was done accurately, a photographic ID guide was produced showing the most common bycatch species for the area and the groups that they should be assigned to.

The number of baskets of each type were then recorded (to the nearest quarter basket), and could be converted into an approximate weight or proportion of the catch as appropriate and these data could then be compared to the data from the scientific trials. It was hoped that the two survey methods would complement each other, and produce a good overall picture of discarding practice and the bycatch composition within this fishery.

Finally, to ensure accuracy, skippers were asked to provide a random sample of approximately 20kg of bulk catch from one tow per month. This was then frozen and transported to the University of Glasgow for more detailed analysis of the species composition, weights and numbers.

Paper logbooks were distributed to the skippers in the fleet in March 2010 as a temporary measure while updates to the YoungsTrace system were being made. This required GPS, date and time information to be recorded manually to ensure that each catch could be traced.

Scientific trials of these methods were carried out in March and June 2010 to determine the level of accuracy that could be expected, and to establish how the self-assessment samples would compare to the more detailed scientific analysis of the catches carried out during Section 1.

## **Feedback from Fishermen**

In order to ensure that this system was practical for the fishermen involved to use and that logging the bycatch would not be excessively time-consuming, informal discussions were held with the fishermen to establish their views and to determine their views on the self-assessment methods after they had had an opportunity to trial them between March and June 2010. It was generally felt that sorting an entire catch required too much time, particularly during the summer months when skippers and crews were typically working exceptionally long hours anyway. As a result, no data were collected from the fleet before June 2010. The methodology was therefore adjusted so that crews were only required to record numbers of cod and spurdog and to provide a random catch sample once per month. Unfortunately however, even this has proved difficult to achieve and while some random samples have been received from Stornoway so far, they have been too small to compare with the previous work, and no logbooks have been received. This work is ongoing however, and it is hoped that these initial problems will have been overcome by the end of 2010.

# Validating the Self-Assessment Methodology

To assess whether the data obtained using the self-assessment system would be comparable to the scientific data collected during Objective 1, a trial was carried out during March and June 2010 on the *MV Comrade* to compare catches sorted by a) both scientists and crew and b) crew only. The crew were considered to be 'trained' in sorting methods as they had participated in previous surveys and were familiar with the procedures they were expected to use. Twelve tows were made, all at the 'south' sampling site (Fig. 30) to minimise spatial variability and followed the same methodology as used during Objective 1.



Figure 30: Maps of the study area showing (a) the limits of the sampling area (red box) and (b) detail of sampling area (tows made within red box).

As soon as each catch was recovered onto the vessel, a random sample was taken using a shovel to fill a fish basket with material directly from the hopper (prior to any sorting), and stored for analysis at the University of Glasgow. The remainder of the catch was sorted at sea, and the individual species were identified, counted and weighed once back in the harbour. This was done using the same procedures described in Objective 1 to allow these catches to be compared to tows made during 2009. Samples of key species (cod, spurdog, haddock and whiting) were collected as before. All 'discarded' material (including *Nephrops* heads and animals missed during sorting) was also kept and weighed, and a subsample was taken for analysis in Glasgow University. Each catch could therefore be divided into three components: the random sample taken from the hopper, the 'remainder' (i.e. the bulk of the catch which was not sampled) and the discards.

The size of each random sample, and the personnel used to sort each catch is shown in Table 10. It should be noted that very poor weather and small catches in March 2010 meant that there was not always enough material to collect a random sample. Catches improved in June 2010 (and were more typical of this vessel), allowing a more complete survey.

Date	Trawl ID	Sorted By	Random Sample
23/03/10	COM49	Crew & scientists	Not taken
23/03/10	COM50	Crew & scientists	Not taken
24/03/10	COM51	Crew only	¾ basket
24/03/10	COM52	Crew & scientists	¾ basket
25/03/10	COM53	Crew & scientists	¾ basket
25/03/10	COM54	Crew only	Not taken
15/06/10	COM55	Crew only	1/2 basket
15/06/10	COM56	Crew & scientists	¾ basket
16/06/10	COM57	Crew & scientists	¾ basket
16/06/10	COM58	Crew only	¾ basket
17/06/10	COM59	Crew & scientists	¾ basket
17/06/10	COM60	Crew only	¾ basket

Table 10: Data for each tow made in March and June 2010, showing the date, the personnel who sorted the catch and the size of the random sample taken (if any).

On return to the University of Glasgow, the 'discarded' material was separated into *Nephrops* heads and whole *Nephrops* as previously described and 'other species'. The 'other species' were animals that had been missed during the initial sorting and the identity and numbers of each species was recorded to allow the accuracy of the two groups of people to be compared. The random samples were analysed in the same way as the rest of the catch, and weights, numbers and the identity of each species was recorded. The compositions of the random sample and the rest of the catch were then compared in order to assess the feasibility of using random sampling as a possible self-assessment method. The data were then combined to allow the 2010 catches to be compared to those made in 2008-2009 using PRIMER.

#### Results

The catch data was interpreted using PRIMER 6 software, and was standardised and fourth-root transformed prior to analysis. MDS ordination and subsequent ANOSIM analysis showed significant differences in species composition between all three sections of the catch as shown in figure 31 (R = 0.715, p < 0.001). The month of capture also had a significant effect (R = 0.502, p < 0.001).

When the species composition random samples were compared to the remainder of each catch only, a significant difference was found between the catch sections (R = 0.439, p < 0.001) and between sample months (R = 0.688, p < 0.001) which can be seen in Figure 32.

The discarded sections were examined separately to determine how accurately each catch had been sorted by different personnel (i.e. whether one group was more likely to miss certain animals compared to the other). ANOSIM analysis showed that there was no significant difference between the discards whether they were sorted by the scientists and crew, or by crew alone (R = -0.375, p > 0.05) as shown in Figure 33. There was no significant effect of sampling month on these data either (R = -0.25, p > 0.05).



Figure 31: 2D MDS ordination showing the separation between the discarded (cyan), remainder (green) and random sample (blue) sections of each catch, and the sampling month is also indicated. ANOSIM: p < 0.002.



Figure 32: 2D MDS ordination showing the separation between the catch sections (labelled) and between sampling months. ANOSIM: p < 0.001. Note the relatively high stress of this plot.



Figure 33: 2D MDS ordination showing the separation between the catch sections (labelled) and between sampling months. ANOSIM: p > 0.05. SIMPER analysis was carried out to determine which species contributed most on average to the differences between the random and remainder sections of the catches. This test determined that 30 species contributed to 90% of the difference, with no single species contributing to more than 7% of the difference. Of the key species of interest, it was apparent that cod, whiting and haddock were more abundant on average in the remainder section than in the random samples, suggesting that this method of collecting a random sample leaves these species relatively under-sampled. By contrast, a higher average abundance of both *Nephrops* and pandalid shrimps were found in the random samples. The SIMPER data for these species is given in Table 11.

Species	Average Abundance		Average dissimilarity	Contribution (%)
	Remainder	Random		
Whiting	1.58	1.18	0.82	2.81
Haddock	1.48	1.10	0.81	2.75
Cod	0.51	0.15	0.63	2.15
Nephrops	2.70	2.90	0.43	1.47
Pandalid shrimp	0.97	1.06	0.83	2.82

Table 11: Average dissimilarity data for key species between the random and remainder sections of the catches.

However, when the proportional abundance of the major taxa within each catch section was examined, no differences were found between the remainder and random samples (p > 0.05) with the exception of the flatfish (H = 17.24, df = 2, p < 0.03) which were apparently undersampled in the random section. For all other groups (roundfish, elasmobranchs, invertebrates and *Nephrops*), there were significant differences between the discards and the other samples (p < 0.05), with fewer bycatch animals and more *Nephrops* being found in the discards. Similarly, the proportional wet weights of each major taxon only showed a significant difference in the weights of roundfish (H = 18.33, df = 2, p < 0.04), with a greater relative biomass occurring in the remainder than in either the random or discard samples. The proportional abundance and biomass for each major taxon are shown in figure 34.



Figure 34: Proportional (a) abundance and (b) biomass of the major taxa occurring in each section of the catch samples. Error bars show one standard deviation.

The discarded portions of each catch were dominated by *Nephrops* (94.7% by number and 92% by wet weight), with the remaining composition typically comprising small roundfish (such as *Trisopterus* spp.) and invertebrates (dominated by pandalid shrimp, *Munida rugosa* and *Funiculina quadrangularis*). Small long-rough dab were also occasionally recovered.

## Discussion

The results of this short trial show that a commercial fishing crew, once properly trained, are able to sort their catches into each of the major taxa described in section 1 as effectively as when a scientific team is working alongside. This is an encouraging result as it suggests that it may be possible to introduce wider self-assessment procedures in the future without loss of data quality (compared to a scientific assessment).

A number of challenges still remain however which would each need to be overcome before such a procedure could be introduced, including convincing the fishermen of the value of the work. This is likely to take time, but the procedures are now in place to ensure that the work continues across the fleet.

Small, random samples taken from a complete catch will not contain the full range of species present due, and some rarer species may be missed. However, the random samples have been shown to correspond well to the composition of the rest of the catch and showed similar distinction between the sampling months. As a result, this should be able to provide adequate baseline data on the overall catch compositions, while logbooks detailing the numbers of cod and spurdog captured at periodic intervals should provide suitable data on those species. Intermittent scientific observation will be required to ensure that the scheme operates as intended.

## Summary of main findings

The problems of bycatch and discarding in marine capture fisheries are a major concern for management, being economically wasteful and ecologically damaging practices. Although they are impossible to completely eliminate from fisheries that use indiscriminate gears (e.g. trawls), the bycatch composition of a fishery can provide valuable ecological data about the targeted ecosystem, particularly for species of commercial or conservation value. The aim of this study was to quantify the bycatch occurring in the Norway lobster (*Nephrops norvegicus*) trawl fishery operating in the North Minch (NW Scotland), which has not been previously studied.

Between December 2008 and June 2010, a total of 54 survey trawls were conducted aboard a commercial trawl vessel, and the abundance and biomass of all species present were recorded. Biometric data were also collected for species of interest (Atlantic cod, haddock, whiting and spurdog). A total of 81 taxa were recorded and the bycatch comprised an average of 39% of the catches by weight. Multivariate analysis of species composition showed significant effects of both sampling month and site, while analysis of individual species suggested that a year-class of juvenile whiting and haddock entered the fishery in summer 2009. Cod and spurdog were present in only very low numbers.

Based on this study, we suggest that the collection of commercial bycatch data on all taxa may be useful for advising fisheries management at an ecosystem level. This approach will also make it possible to identify potential indicator species and species of conservation importance.

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Work Package 3.2: Identifying the most cost-effective way to obtain omega-3 lipids and other nutraceutical compounds from *Nephrops* heads – a currently discarded portion of the catch

## Introduction

The Norway lobster (*Nephrops norvegicus*), a decapod crustacean also known as the Dublin bay prawn or langoustine, supports the most important shellfish fishery in the UK, representing an industry that was worth £59.7 million at first sale in 2008 (Curtis et al., 2010). In Scotland, Norway lobsters are caught largely by small trawling vessels that utilize smaller meshed codends (70 mm) than whitefish fisheries (Tuck et al., 1997; Stratoudakis et al., 2001). As well as landing large individuals that can be sold as fresh whole products, these trawls generate large quantities of smaller (and sometimes damaged) Norway lobsters. Advances in the processing sector have developed a market for this portion of the catch, whereby the tails (which contain the majority of edible meat) are detached from the 'heads' (cephalothorax) and processed to make 'scampi' (Bell et al., 2006). This practice has decreased the overall number of Norway lobsters discarded, but since the 'tailing' process is carried out on board the fishing vessels, the entire contents of the 'head' of these individuals are still discarded at sea as waste. This waste portion (hereafter referred to as head waste) includes the exoskeleton, claws, walking legs, digestive and reproductive organs, and eyestalks, which together account for at least 50% of the body weight of the animal (Phillips et al., 1980; Krishnapillai et al., 1999).

The treatment of crustacean wastes has become a more pressing issue in recent years for two primary reasons: 1) these wastes are highly perishable and create environmental pollution (Blanco et al., 2007; Arvanitoyannis and Kassaveti, 2008) and 2) they contain valuable compounds that could be used in other industries (Arvanitoyannis and Kassaveti, 2008; Limam et al., 2011). Analysis of waste products from other crustacean fisheries has uncovered economically valuable compounds within this material, such as chitin, carotenoids and polyunsaturated fatty acids (PUFAs) (Kurita, 2005; Sachindra et al., 2005; Ulven et al., 2011). As chitin is an effective fungicidal, antimicrobial, and antioxidative agent (Limam et al., 2011), it

has been used as a preservative in a variety of foods (Rong et al., 2010; Sanchez et al., 2011) and in biomedical applications (Moon et al., 2007). Carotenoids also exhibit antioxidant properties so are common additives to medications for a range of illnesses and to anti-ageing cosmetics (Hussein et al., 2006; Park et al., 2010). These pigments are also essential additives to fish feed for farmed species like salmon, as they provide the characteristic pink flesh coloration that the fish would normally acquire from their diet in the wild (Meyers, 1994; Rajasingh et al., 2007). Finally, oils from crustaceans such as krill have been found to exhibit the same positive health benefits as fish oils as they are similarly composed of PUFAs (Ulven et al., 2011).

The aim of this study was to determine the composition of the head waste produced in the Norway lobster fishery in order to determine if any commercially harvestable compounds are present that could be exploited. If so, these findings could lead to an alternative use for the large amount of waste produced in this industry. Geographic variation in body composition was also important to establish as Norway lobsters have a wide distribution, ranging as far north as Iceland and as far south as Mauritania, with fisheries throughout this range (Bell et al., 2006). In addition, yearly variation in the tissues of Norway lobsters occurs as individuals store lipids in their digestive glands (hepatopancreas) during summer for use during the winter months. Sexually mature females also develop enlarged gonads (ovaries) during the spring, potentially contributing large amounts of both lipid and yolk products to any head waste harvested over the summer months (Rosa and Nunes, 2002). These effects may be exacerbated by the fact that females are much more prevalent in trawl catches in the summer months compared to winter, due to a seasonal change in their behavior that makes them more available for capture (Chapman and Rice, 1971; Mente et al., 2009; Milligan et al., 2009).

In order to determine what economically valuable compounds could be extracted from the head waste product of Norway lobsters, its composition was examined not only by fatty acid analysis (GC-MS), as proposed in the original Workplan, but also by lipid class analysis (TLC-FID). In addition, a further series of a more extensive measures was made using a range of

sophisticated methods, namely, metabolomics (NMR spectroscopy and HPLC-HRFTESIMS), to identify other potential nutraceuticals such as antioxidants. Particular attention was paid the most appropriate season of the year for harvesting of these compounds and to possible geographic variations in composition. This work could contribute to the sustainability of this fishery by providing a new source of profitability from the existing level of exploitation.

## Materials and methods

#### Capture and transport

Norway lobsters (*Nephrops norvegicus*) were caught by otter trawl using 70 mm nets on two fishing grounds off the west coast of Scotland (the Clyde Sea area and the Minch, which are ~150 miles apart) between January 2009 and December 2009. In the Clyde Sea area, a transect in the Largs-Fairlie Channel (55' 51.351 N/4'54.424 W to 55'48.979 N/4'54.055 W) was trawled monthly by the research vessel RV Aplysia from the UMBSM research station. In the Minch, a transect off Stornoway (58'02.716 N/6'15.249 W to 57'57.195 N/6'15.742 W) was trawled bimonthly by the commercial fishing vessel MV Comrade III, operating out of the port of Stornoway.

Once the trawl nets were emptied onto the deck, Norway lobsters were separated from the catch. A random grab sample from the catch of individuals with a carapace length (CL) of less than 40 mm was then taken for use later in head waste studies. Lobsters with a CL greater than 40 mm would be sold whole, so were not used in this study (Krishnapillai et al., 1999). In the Clyde Sea area only, twenty males and twenty females were also selected for use in analyses of individual body organs (hepatopancreas and female gonad studies). Following sorting, the samples were washed, declawed, and placed on ice in insulated cool boxes for transport back to the University of Glasgow.

#### Head paste and tissue sample preparation

Approximately 70 animals were used to generate a head paste sample, equivalent to the commercial head waste product (Krishnapillai et al., 1999). The sex of each animal in this random sample was recorded and used to calculate the female sex ratio of the trawls in each month. The tails were then removed from the cephalothorax and the entire cephalothorax, including walking legs, eyes and all internal organs (including the hepatopancreas and female gonads) were blended together to create a fine paste using a knife-mill (Grindomix GM 200, Retsch, UK). The resulting head paste was placed in a container and frozen at -25°C.

Hepatopancreas and female gonad tissues were extracted from the 20 male and 20 female Norway lobsters collected in the Clyde Sea area trawls. Each individual was weighed (without claws) to determine the body weight. For both male and female Norway lobster samples, the hepatopancreas tissue was collected and weighed. The hepatopancreas tissue was pooled together by sex and frozen at -25°C. In the female samples, gonad tissue was collected and the weight of the gonad was recorded. The gonad tissue from all of the female samples collected was pooled and frozen at -25°C. From these data, the hepatosomatic index (HSI) and the gonadosomatic index (GSI) were calculated using the following formulas:

> HSI = hepatopancreas wet weight / body wet weight x 100 GSI = gonad wet weight / body wet weight x 100

#### Lipid extraction

In order to extract lipids from the Norway lobster tissues sampled, the Folch extraction method was used (Folch et al., 1957). Prior to extraction, head paste, hepatopancreas, and female gonad samples were freeze-dried, using an Edwards Freeze Dryer Modulyo cooled to - 40°C and attached to an Edwards 2 Stage #5 pump to create a vacuum. Extracted lipids were then resuspended in HPLC chloroform:HPLC methanol (2:1) with BHT (0.01%, w/v) to a final concentration of 10 mg mL<sup>-1</sup> and frozen at -25°C. Three replicate lipid extractions were performed on each pooled head paste and tissue sample. The percentages of lipid from the three replicates were averaged in order to reduce variability from individual extractions. The

average total lipid content (in grams) of individual tissues (hepatopancreas and female gonads) was determined using the following formula:

Total Lipid Content = Mean Weight of the Tissue x (Percentage of Lipid/100)

Geographic and temporal variation in the HSI, GSI, percentage of lipid, and total lipid content of the tissues were assessed using generalized linear models (GLMs) in the statistical software R version 2.15.0 (R Development Core Team, 2012). GLMs were also performed to determine if any gender effects were occurring in the HSI, percentage lipid of the hepatopancreas, and total lipid content of the hepatopancreas.

## Lipid class analysis

The lipid class composition of extracts was identified using a thin layer chromatography-flame ionization detector system (TLC-FID). Silica gel Chromarods SIII were activated prior to spotting by passing them through the flame in the FID system. Then, lipid extracts were spotted on the Chromarods and allowed to air dry for 5 minutes. The spotted chromarods were scanned at 3.1 mm s<sup>-1</sup>, with 0.95 kg cm<sup>-2</sup> of hydrogen pressure and air flow at a rate of 2000 ml min<sup>-1</sup> within the FID. The plates were then placed in a CaCl<sub>2</sub> humidity chamber (5 min) after which they were left in the solvent tank vapor (10 min). Classes were identified using the T Datascan software (Bioscan Inc., Tokyo, Japan). The latroscan analyzer quantified the lipid class composition of the lipid extracts in terms of polar and neutral lipids. The solvent hexane-diethyl ether-formic acid (85:15:0.04) was used for separating neutral lipids while chloroform-methanol-water (75:35:3.5) was used for separating polar lipids. Differences in lipid class composition between the tissue types and the sexes were assessed using a similarity percentage (SIMPER) analysis in the software Primer 6.

# <sup>1</sup>H NMR spectroscopy

Head paste, hepatopancreas, and female gonad lipid extractions and methanol/water layers were analyzed using <sup>1</sup>H NMR Spectroscopy. Each lipid extraction sample was resuspended in deuterated chloroform (CDCl<sub>3</sub>) and NMR spectra were measured using a Jeol ECX400 NMR spectrometer at 400 MHz using a 1D sequence with pre-saturation of the water resonance at ambient temperature. In addition, selected samples were run using a 2D NMR method, <sup>1</sup>H-<sup>1</sup>H homonuclear correlation spectroscopy (COSY) along with total correlation spectroscopy (TOCSY) in order to confirm the spin systems of each of the components as well as to gain a better understanding of the low concentration compounds found in the spectra.

The 1D NMR spectra were processed using MNova version 6.2.1. Then, for principal component analysis, data were imported into Alice 2, where compounds were identified based on the location of peaks' chemical shift and the peak integration. Temporal and geographic variations in individual compounds were analyzed using GLMs (as described above).

## Fatty acid analysis

Fatty acid methyl esters (FAMEs) were derived from head paste lipid extracts from the Clyde Sea area and the Minch in February, April, August, and October, using thin layer chromatography (TLC). TLC was carried out using acid catalyzed transmethylation for a 16-hour period at 50°C. As both neutral and polar lipids were present in the extracts, 1 mL of toluene and 2 mL of 1% sulphuric acid (v/v) in methanol were used. All solvents were HPLC grade. In addition, a 17:0 free fatty acid standard was added to the TL. The resulting FAMEs were then purified by thin layer chromatography on 20x20 plates. Plates were chromatographed in isohaxane:diethyl ether:acetic acid (90:10:1, v:v) up to 1 - 1.5 cm from the top of the plate and allowed to air dry. To visualize the FAMEs, plates were sprayed with 1% (w/v) iodine in chloroform. The FAME bands were then scraped from the TLC plate. 1ml iso-hexane: diethyl ether (1:1 v/v) with BHT and 9ml iso-hexane: diethyl ether (1:1 v/v) without BHT was added to the impregnated silica, and the tubes were vortexed and centrifuged. The solvent was then removed into clean tubes (not disturbing the silica) and evaporated to dryness again. A volume of 1 ml of iso-hexane was added to the sample, which was stored in a brown GC bottle under OFN and stored at  $-20^{\circ}$ C. The samples were processed by gas chromatography at (GC) the Institute of Aquaculture, University of Stirling, UK. The fatty acids in each sample were separated and quantified in a GC (Fisons GC-8160, Thermo Scientific, Milan, Italy) equipped with a 30 mm x 0.32 mm i.d. x 0.25 µm ZB-wax column (Phenomenex, Cheshire, UK), fitted with 'on column' injection and flame ionization detection. Hydrogen was used as the carrier gas. The initial oven thermal gradient was at 40°C min<sup>-1</sup> from 50°C - 150°C, followed by a gradient of 2°C min<sup>-1</sup> to a final temperature of 230°C. Individual FAMEs were identified by comparison with known standards (Supelco 37 FAME mix; Sigma-Aldrich Ltd., Poole, UK) and published data (Tocher & Harvie, 1988). Data were collected and processed using Chromcard for Windows Version 1.19 (Thermoquest Italia SpA, Milan, Italy). Additional peaks were identified by mass spectrometry on two samples. The area of a given peak (%) was then converted to a percentage of known peaks (ca. 95% of all peaks). Peaks were identified in the order that they passed through the GC, and then the data were sorted into the actual biological groupings for statistical analysis. Geographic and temporal variation in the concentrations of economically valuable fatty acids were determined using GLMs (as described above).

#### HPLC with Full Scan HRFTESIMS-Orbitrap

Ten microliter lipid extracts, methanol/water layers, and solvent blanks (solvent used to resuspend lipid extracts) were separated using an ACE Helic C18 HPLC column with an HPLC pump and a PDA detector. A binary solvent system was used, in which mobile phase A consisted of water with 0.1% formic acid ( $CH_2O_2$ ) and mobile phase B consisted of acetonitrile ( $CH_3CN$ ) with 0.1% formic acid ( $CH_2O_2$ ). The column was eluted with a 40-minute gradient of 5% - 100% acetonitrile with 0.1% formic acid at a flow rate of 400 µL min<sup>-1</sup>. The sample tray was held at 25°C.

This HPLC system was coupled to a high-resolution Fourier transform electrospray ionization mass spectrometer (HRFTESIMS) Orbitrap (Exactive). The spray voltage was set to 4.5 kV for positive ionization mode and 3.0 kV for negative ionization mode, with all other conditions held

constant in both positive and negative modes. The heated capillary was set to 275°C. The sheath gas flow was set to 60 units while the auxiliary gas flow was set to 25 units. Internal lock mass calibration was performed during the run.

Raw LC-MS data files were processed and compared using the differential expression analysis software Sieve version 1.2.1. Sieve aligned the spectra from all of the samples according to the retention time and measured the mass to charge (*m/z*) ratio of each of the components, allowing the peaks and their associated intensities for specified compounds to be assessed simultaneously in all samples. In addition, Sieve sorted the components into a numbered list of frame IDs based on their prevalence in each of the samples. Low numerical frame IDs were assigned to components with highest intensity. The chromatogram associated with each individual compound was examined visually in order to determine which frame ID numbers represented noise and could therefore be discarded from further analysis. In addition, any frame IDs that were expressed in the solvent blank samples were removed from further analysis so that only peaks associated with the organic extract were analyzed. This edited list was then compared by Sieve with the KEGG (Kyoto Encyclopedia of Genes and Genomes) PATHWAY database. This is a public, online database that can identify compounds detected in metabolomics studies, based on the molecular weight, and categorizes the components into

In order to determine the similarity between sample compositions, hierarchical cluster analysis (HCA) was conducted with uncertainty assessed using multiscale bootstrap resampling (1000 samples) to calculate an approximately unbiased (AU) p-value for each cluster. The analysis was plotted using dendrograms, with AU p-values marked in red. Clusters with a significant AU p-value (p < 0.05) were denoted with red rectangles. This analysis was performed in the statistical software R version 2.12.1 using the pvclust package (Suzuki and Shimodaira, 2006).

#### **Results and Discussion**

#### Norway lobster head paste samples from different fishing grounds

The percentage of lipid in the head pastes in trawls from the Clyde Sea area and the Minch exhibited similar trends, with concentrations peaking in both locations in the summer months (Figure 35A). No significant geographic difference in percentage of lipid in the head paste was found between the Minch and the Clyde Sea area ( $F_{1,37} = 1.357$ , p = 0.252). Seasonal variation was significant, indicating that the head pastes in the summer months contain a significantly higher percentage of lipid than the winter months ( $F_{11,27} = 3.878$ , p = 0.002). These results are highly similar to the lipid content of krill oil, a highly valuable substance; studies show that krill oil varies seasonally from 12-50% lipid based on dry weight (Tou et al., 2007).

The proportion of female Norway lobsters in the Clyde Sea area trawls peaked in the May (Figure 35B), a result consistent with a previous study on this fishing ground (Milligan et al., 2009). In the Minch, a similar trend in trawl composition was found, though the proportion of females caught in trawls peaked in June, likely due to the bimonthly sampling schedule in this fishing ground (Figure 35B). In each site, the maximum percentage of lipid was two months later than the peak in the female sex ratio in trawls, indicating that the trawl sex ratio was not the sole determinant of the head paste lipid percentage.



Figure 35: A. The percentage of lipid in the head pastes of trawled Norway lobsters in the Clyde Sea area and the Minch in 2009. Values are the mean +/- standard error. B. The percentage of female Norway lobsters in the random sample taken from the trawls in the Clyde Sea area and the Minch in 2009.

#### Norway lobster tissue samples from the Clyde area

To further investigate the processes influencing the amount of lipid in the head paste, the percentages of lipid in the main lipid storage organs, the hepatopancreas and female gonad, were determined for male and female Norway lobsters from one of the fishing grounds, the Clyde Sea area, in 2009. The lipid percentages in the hepatopancreas samples varied significantly throughout the year ( $F_{1,36} = 28.763$ , p < 0.001), ranging from 25% to 59% (Figure 36A). Concentrations decreased from January until May, then increased through the summer months (no tissue samples were available for extraction from October and November 2009). Variation in lipid percentage between the sexes was also found to be significant ( $F_{1,36} = 8.637$ , p = 0.006), likely due to the overall higher lipid percentage in female hepatopancreas samples in every month sampled except June 2009 (Figure 36A).

A trend in temporal variation of the hepatosomatic index (HSI) for both males and females was found, though not significant ( $F_{8,25} = 2.038$ , p = 0.083). This result is likely due to the elevated HSI values in males from August to November 2009 (Figure 36B). Despite this difference in trend between the sexes, no significant variation was found between male and female HSI values ( $F_{1,32} = 1.2778$ , p = 0.2667). HSI values were not available, however, in October. The values and trends found for the HSI and percentage of lipid in the hepatopancreas are comparable to a previous study of organ indices in female Norway lobsters, though previous studies have not compared these indices between male and female Norway lobsters to the best of our knowledge (Rosa and Nunes, 2002).



Figure 36: A. The percentage of lipid in the hepatopancreases of male and female Norway lobsters from the Clyde Sea area. Values are mean percentages of lipid +/- the standard error.
B. Hepatosomatic Index (HSI) for the male and female Norway lobsters from the Clyde Sea area.
Values are the mean HSI. Information on standard error was not available for these data.

The female gonads showed quite different trends to those found in the hepatopancreas. The percentage lipid in the female gonads throughout the year showed no significant variation ( $F_{1,16}$  = 0.0176, p = 0.896), ranging from 17% to 26% (though no lipid percentage data were available from October and November 2009) (Figure 37A). However, the gonadosomatic index (GSI) exhibited greater changes through the year, peaking in July at 8% and reaching its minimum in December at 1% (Figure 37B). No GSI data were available from October 2009. However, this variation was not significant ( $F_{1,15}$  = 0.7908, p = 0.388). This increase in GSI over the summer months agrees with previous findings for Norway lobster females off the coast of Portugal; however, concurrent increases in the concentration of lipids in the ovaries were also reported (Rosa and Nunes, 2002). This difference could be accounted for by the geographic differences in the studies; as warmer climates are found in Portugal, females likely emerge from their burrows earlier in the year to feed and potentially can achieve higher concentrations of lipids in their gonads than their Scottish counterparts (Bell et al., 2006). The female body concentration of lipid will, therefore, peak in summer months, as they maximize storage of lipids in their hepatopancreas and maintain high percentages of lipid in a larger gonad.



Figure 37: A. The percentage of lipid in the gonads in female Norway lobsters from the Clyde Sea area. Values are mean percentages of lipid +/- standard error. B. Gonadosomatic index for Norway lobster females from the Clyde Sea area in 2009. Values are means of the GSI of all specimens used in the sample. Data to calculate standard error were not available.

Although the nature of the seasonal trends in the hepatopancreas and female gonad were different, trends in the total lipid content of the organs throughout the year were highly similar, with peak lipid content occurring in all organs in July and August 2009 (Figure 38). Total body weight for females was also significantly higher in summer ( $F_{8,8} = 6.3322$ , p < 0.001), though total body weight in males did not vary significantly throughout the year ( $F_{8,8} = 1.1859$ , p = 0.408). No significant differences were found in total lipid content between male and female hepatopancreas samples ( $F_{1,30} = 0.001$ , p = 0.9223). Total lipid content in the hepatopancreas samples varied from 0.186 grams per organ in May to 0.763 grams per organ in August. Temporal variation in total lipid content in the hepatopancreas samples was significant ( $F_{7,24} = 8.8672$ , p<0.001), indicating that there is a significantly higher quantity of lipid in these organs in the summer months.



Figure 38: Total lipid content (g) of male and female Norway lobster organs from the Clyde Sea area, based on the mean organ weights of the 20 individuals sampled.

Females have a greater total lipid content than males due to the presence of two lipid-rich organs, the gonad and the hepatopancreas (Figure 38). Both the total lipid content in the female gonad ( $F_{7,8}$  = 45.722, p<0.001) and the sum of the total lipid content in the female organs ( $F_{7,8}$  = 13.887, p<0.001) exhibited significant temporal variation, indicating that the quantity of lipids is highest in females in the summer months. As more females with maturing gonads and lipid-rich hepatopancreases appear in the catch through the summer, these trends help explain the seasonal co-variation found between the head paste percentage of lipid and the female sex ratio (Figure 35). In addition, as total lipid content in both sexes was maximized in July and August 2009, the delay in the peak head paste lipid percentage from the peak female sex ratio can be attributed to the increased lipid storage in these organs through the summer months.

#### Lipid class analysis

Lipid class composition of male and female hepatopancreases, female gonads, and head paste, as determined by TLC-FID, is presented in Table 12. In all analyzed tissues, neutral lipids accounted for the majority of lipids present (66.7% to 87.2%) while polar lipids comprised the minority (12.8% to 33.3%).

SIMPER analysis indicated that free fatty acids (FFA) drove differences between the head paste samples and hepatopancreas/female gonad samples (accounting for 23.55% and 32.80% of dissimilarity in relation to hepatopancreas and female gonad samples respectively). Differences in hepatopancreas composition also existed between males and females, with phosphatidylinositol (PI), FFA, and phosphatidylserine (PS) accounting for the majority of dissimilarity found (42.13%, 28.09%, and 17.45% respectively).

The lipid class composition in the Norway lobster was highly similar to compositions previously established in krill. Krill oil and its associated products have become a valuable industry (Tou et al., 2007). In both crustaceans, the neutral lipid triacylglycerols (TAG) represented the most abundant component in both sexes. In addition, phosphatidylcholine (PC) was the major
phospholipid, with the highest concentration found in the female gonad in both the Norway lobster and krill. Cholesterols were also found in highest concentrations in the abdominal tissue and stomach in krill, which would explain the higher levels of sterols in the Norway lobster head paste analysis as compared to the hepatopancreas samples (Albessard et al., 2001). These similarities in the lipid class composition indicate that the Norway lobster oil could potentially be as profitable as krill oil.

Table 12: Lipid class composition of male and female hepatopancreas, female gonads, and head paste from *Nephrops norvegicus*, as determined by thin layer chromatography-flame ionization detector system (TLC-FID). The values are expressed as percentages of the total lipids (%).

Lipid Class	Hepatopancreas (Male)	Hepatopancreas (Female)	Gonad (Female)	Head Paste
Dolog Linida				
Polar Lipius				0.6
Lysophosphatidylcholine	0	0	0	0.6
Sphingomyelin	0	0	0.2	0.6
Phosphatidylcholine	6.8	6.6	21.1	10.4
Phosphatidylserine	0.7	1.4	0	1.1
Phosphatidylinositol	0.7	0	0	1.3
Cardiolipin/	0	0	0	0
Phosphatidylglycerol				
Phosphatidylethanolamine	4.7	4.8	11.9	8
Total	12.9	12.8	33.3	22
Neutral Lipids				
Sterols	4.1	4.6	10.7	11.4
Free Fatty Acids	1.6	0.5	0	12.6
Triacylglycerides	81.4	82.2	66.7	78
Sterol Esters	0	0	0	0
Total	87.1	87.2	66.7	78

# <sup>1</sup>H NMR spectroscopy of Norway lobster lipid extracts

One-dimensional <sup>1</sup>H NMR spectra of head paste lipid extracts from Clyde Sea area Norway lobsters in winter and summer are shown in Figure 39. The spectra detected the primary metabolites chitin, FFAs, triglycerides, low molecular weight polysaccharides, and the pigment astaxanthin. Expanded spectra are shown in Figure 40, illustrating the peaks associated with the pigment astaxanthin. The two-dimensional COSY and TOCSY NMR spectroscopy methods confirmed the presence of these primary metabolites, while no additional secondary metabolites were detected.



Figure 39: <sup>1</sup>H NMR spectra from the head pastes derived from Norway lobsters (mixed sexes) trawled from the Clyde Sea area in (A) February 2009 and (B) August 2009. These representative spectra illustrate the similarity in major compound composition throughout the year, including (1) triglycerides, (2) free fatty acids, (3) chitin, (4) low molecular weight polysaccharides, and (5) astaxanthin. The chloroform reference peak is also indicated (6).



ppm 6.35 6.30 6.25 6.20 6.15 6.10 6.05 6.00 5.95 5.90 5.85 5.80 5.75 5.70 5.65 5.60 5.55 5.50

Figure 40: Expanded <sup>1</sup>H NMR spectra from the head pastes derived from Norway lobsters (mixed sexes) trawled from the Clyde Sea area in (A) February 2009 and (B) August 2009, which illustrate the presence of astaxanthin within the samples.

Major compound composition in the Norway lobster head paste and tissues throughout 2009, as determined through <sup>1</sup>H NMR spectroscopy, is presented in Table 13. These relative percentages were measured through the integrals depicted on their respective <sup>1</sup>H NMR spectra. The signals at 4.15 and 4.25 ppm were assigned to the oxygenated methylene units of the glyceride moiety; these were used as the reference integral for two hydrogens each, in order to relatively quantify the presence of the other major detectable metabolites. Of these major compounds, chitin, the pigment astaxanthin and FFAs represent economically valuable compounds for potential extraction from the waste product. The FFA composition is examined further below.

Table 13: Major compound composition (in terms of percentage extracted lipid) in head paste samples from the Clyde Sea area and the Minch as well as male (M) and female (F) hepatopancreas and female gonad samples from the Clyde Sea area, as determined using <sup>1</sup>H NMR spectroscopy.

	Chitin	Triglycerides	Fatty Acids	Astaxanthin	Polysaccharides
Clyde Head Paste					
January	53.40	16.27	25.75	0.10	4.48
February	55.14	12.39	28.18	0.34	3.96
April	57.81	13.46	25.01	0.21	3.51
July	58.62	15.28	21.48	0.03	4.59
August	57.96	13.58	26.03	0.03	2.40
October	57.91	15.06	23.85	0.09	3.10
Minch Head Paste					
February	58.85	15.39	19.75	0.35	5.66
August	58.82	14.89	23.24	0.08	2.97
Clyde Hepatopancreas					
February (M)	52.47	14.74	32.20	0.00	0.59
August (M)	60.83	16.05	21.19	0.00	1.93
February (F)	52.90	18.43	27.19	0.00	1.47
August (F)	52.11	17.61	29.23	0.00	1.06
Clyde Gonads (F)					
February	59.90	11.72	21.77	0.18	6.44
August	55.97	11.82	25.73	0.05	6.42

The head paste composition found in the Minch samples in February and August 2009 corroborate the findings from the Clyde Sea area, as the ratios of each compound are comparable during those months (Table 14). In addition, analysis of the hepatopancreas and female gonads indicates that the presence of astaxanthin in the female gonad tissue most likely dictates the astaxanthin levels found in the complete head paste, since no astaxanthin was detected in the hepatopancreas samples of either males or females (Table 13).

	Clyde			Minch				
Fatty Acid	February	April	August	October	February	April	August	October 123
14:0	38.90	55.76	121.58	116.96	49.36	47.56	235.23	229.52
anteiso 15:0	10.23	9.12	18.90	21.29	14.70	13.49	33.00	31.65
iso 15:0	6.25	6.57	10.36	12.00	8.23	9.00	13.40	12.19
15:0	26.26	24.52	43.62	41.75	22.07	21.79	50.17	37.36
iso 16:0	13.71	12.72	20.81	21.95	14.83	15.56	20.49	17.56
10:0 mtning 17:0	404.10	481.81	900.37 59.06	930.03	499.85	485.18	11/8.00	900.40
iso 17:0	32.69	32.40	49.16	48.82	31.06	34 30	41.09	32.55
iso 18:0	23.04	20.63	40.62	42.99	28.50	28.25	43.19	30.98
18:0	151.36	152.64	295.22	281.60	150.77	154.29	276.99	197.87
anteiso 19:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
150 19:0	18.85	15.16	25.26	24.25	9.17	8.81	0.00	0.00
19:0	8.71	8.21	14.90	14.71	9.13	10.03	17.17	15.21
20:0	14.51	13.77	11.99	25.23	20.41	14.24	29.46	16.89
22.0	5.17	0.00	0.00	24.38	20.25	25.00	0.00	0.00
Total	836 36	884 49	1660.85	1675 36	913.03	898 99	2029.06	1568 19
saturated	000.00	001.15	1000.00	10/0.00	222.05	0,0.,,,	2020.00	1000.10
16:1n-9	8.80	8.69	20.08	18.25	11.19	10.35	25.14	24.27
<b>16:1n-</b> 7	204.46	262.76	552.92	425.19	188.31	178.84	576.79	484.44
17:1	28.31	25.38	43.80	42.41	32.32	34.16	55.71	54.47
18:1n-9	540.35	527.34	983.62	892.13	542.22	530.58	1318.93	1360.25
18:1n-/	300.92	308.92	22,26	200.40	2/0.16	2/4.38	499.26	400.88
20:1n-11	208 70	178.16	341 38	27.95	90.89	74.22	163 25	113 20
20:1n-9	137.96	121.83	198.36	212.88	173.97	148.06	654 54	954.00
20:1n-7	208.70	178.16	341.38	284.97	90.89	74.22	163.25	113.20
22:1n-11	37.65	48.75	37.71	62.96	161.65	128.71	731.95	1209.58
22:1n-9	21.71	27.64	37.26	60.91	27.78	25.35	110.09	98.99
22:1n-7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24:1n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	1000.48	1040.95	5087.08	2807.54	1094.87	1485.04	4557.00	5002.05
18.2n-6	26.13	27.69	63 61	61 40	8 00	34 58	93.25	90.38
18:3n-6	6.03	6.05	10.63	0.00	3.64	3.56	13.95	7.94
20:2n-6	44.84	45.20	74.33	68.63	48.05	53.18	85.39	73.15
20:3n-6	6.61	5.04	10.36	10.69	4.76	5.01	12.74	7.49
20:4n-6	146.09	149.85	284.05	232.94	119.98	169.94	214.52	147.76
22:4n-6	25.90	24.09	58.06	54.49	28.28	29.85	47.40	28.86
22:Dn-6	22.02	21.30	32.89	31.48	15.91	16.54	34.89	23.38
18:3n-3	8.62	9.26	17 72	23 10	14 92	3 47	64 57	61 97
18:4n-3	9.96	25.72	63.15	49.40	13.89	13.82	86.05	79.19
20:3n-3	6.61	7.29	11.72	12.58	11.06	11.48	34.66	28.41
20:4n-3	10.00	14.54	30.71	30.17	14.52	16.82	62.13	62.75
20:5n-3	382.74	551.43	1170.63	895.83	342.54	437.81	973.72	736.44
22:5n-3	63.95	65.55	155.20	135.87	57.49	58.19	148.85	120.58
22:0n-3	309.59	381.23	033.24	579.96	409.16	202.18	1080.93	1031.74
16-2	2.66	0.74	2082.57	17.20.90	2 10	1040.77	16.02	2121.08
16:3	0.00	7 73	17.45	12.00	0.00	0.00	0.00	0.00
16:4	0.00	6.96	14.63	12.08	0.00	0.00	0.00	0.00
Total	3.66	24.42	55.06	41.84	3.19	4.17	16.83	19.35
Total PUFA	1072.75	1358.67	2671.37	2228.36	1095.40	1363.60	2969.89	2519.39
16:0 DMA	15.23	17.99	24.90	23.92	26.84	28.53	33.11	32.33
18:0 DMA	13.71	13.29	17.54	17.51	22.84	23.47	28.35	30.54
Total DMA	3.25	1.20	12 /2	8.22	8.90	63 62	8.70 70.22	5.57
22:0 NMUD	0.51	12.00	10.54	23.51	35.74	24.10	57.45	53.02
22.0 INIVIID	3.31	12.00	12.04	20.01	55.74	24.10	57.45	55.02

Table 14: Fatty acid concentrations in head paste samples from the Clyde Sea area and the Minch throughout 2009 (mg 100g<sup>-1</sup>lipid).

Percentages of astaxanthin and chitin in Clyde Sea area head pastes (as calculated using the <sup>1</sup>H NMR ratio found in lipid extracts and the original lipid percentage value found during the extraction process) varied throughout the year. Astaxanthin levels were highest in February (0.023%) and reached a minimum in August (0.003%) in the head paste (Figure 41A). However, using a GLM, temporal variation in astaxanthin was not significant ( $F_{1,4} = 1.451$ , p = 0.295). These percentages are comparable to other crustacean waste products. Crawfish waste contains approximately 0.0153% carotenoids while shrimp offal yields between 0.0119% and 0.0153% carotenoids; of these carotenoids, 40.3% to 92.2% can be astaxanthin (Meyers and Bligh, 1981; Shahidi and Synowiecki, 1991; Sachindra et al., 2005). However, there is an inevitable loss of a proportion of astaxanthin and other carotenoids during the extraction process, though this could be minimized by tailoring the extraction methods to the Norway lobster as was done in other crustacean waste industries (Shahidi and Synowieki, 1991).

Levels of chitin in the head paste increased significantly ( $F_{1,4} = 8.693$ , p = 0.042) between January (3.133%) and July (6.331%) (Figure 41B). Concentrations of chitin in the lipid extracts from the head paste were lower than previous studies in crawfish and shrimp waste products, in which chitin represented 17 to 32% of the crude dry weight; however, these previous studies assessed the chitin content in terms of the crustacean shell exclusively (Shahidi and Synowiecki, 1991). Further analyses of the individual parts of the Norway lobster waste product may, therefore, yield more efficient processing techniques for these valuable compounds.

In order to ensure that no important compounds were being missed, one methanol/water layer sample, from the Clyde Sea head paste in July, was analyzed using <sup>1</sup>H and COSY NMR spectroscopy. The amino acid tryptophan was identified in the spectrum through the multiplicities and the coupling constant that would be expected of spin systems in its structure. Tryptophan is an essential amino acid in protein metabolism, which is usually attained from a balanced diet. However, in recent years, studies suggest that it also plays a role in other human neurological functions such mood, sleep, and appetite. It has therefore become a common

additive to medications and nutritional supplements for treatment of various neurological ailments, and could therefore potentially be exploited as well (Heine et al., 1995).



Figure 41: Percentages of (A) astaxanthin and (B) chitin in head paste samples from the Clyde Sea area throughout 2009, as calculated by multiplying the ratio of each component found using <sup>1</sup>H NMR spectroscopy by the percentage lipid found in the head paste.

### Fatty acid analysis

The fatty acid composition of head pastes from both Clyde Sea area and the Minch throughout 2009 are presented in Table 14. The n-3 PUFAs exhibited their highest concentrations in summer in the Clyde Sea area while, in the Minch they continued to increase through October. Using a GLM, month accounts for a significant amount of variation in n-3 PUFAs in the head paste ( $F_{3,4} = 13.144$ , p = 0.015) while site does not ( $F_{1,6} = 0.1118$ , p = 0.750) (Figure 42A). The majority of n-3 PUFAs found in the head paste was the economically valuable docosahexaenoic acid (DHA) (Figure 42B) and eicosapentaenoic acid (EPA) (Figure 42C). DHA did not exhibit significant temporal variation ( $F_{3,4} = 1.8997$ , p = 0.271), whereas EPA were significantly higher in the summer months ( $F_{3,4} = 7.5943$ , p = 0.040). Available n-6 PUFAs peaked in the summer as well, though this trend was not quite significant ( $F_{3,4} = 6.1987$ , p = 0.056, Figure 42D). No



Figure 42: The amounts in the head paste (g kg<sup>-1</sup>) of (A) all n-3 polyunsaturated fatty acids (PUFAs), (B) the n-3 PUFAs docosahexaenoic acid (DHA), (C) the n-3 PUFA eicosapentaenoic acid (EPA) and (D) all of the n-6 PUFAs.

These results indicate that although levels of some of the economically valuable FFAs are available in high levels throughout the year, the highest overall concentrations of PUFAs will occur in the summer months.

Although the total level of PUFAs in the Norway lobster is slightly lower than in krill, the percentage of EPA in the head paste is comparable to previously measured levels in krill. In addition, the concentration of EPA measured in this study is as much as triple the levels found in salmonid fish in previous studies (Tou et al., 2007).

#### Analysis of Norway lobster samples using HPLC with full scan HRFTESIMS

Lipid extracted from the head paste and tissues were also analyzed using a HPLC-HRFTESIMS orbitrap, in order to determine if any economically valuable minor compounds could be detected. In the positive ionization mode, 11,078 compounds were detected, while in the negative mode, 3,991 compounds were detected. From the approximately 11,000 compounds detected in the positive ionization mode, 725 compounds were determined to be true peaks, while the remainder was discarded as noise or due to their presence in the solvent used to preserve the lipid extracts. From the approximately 4,000 compounds detected by the negative ionization mode, 468 compounds were used in analysis while the rest were also discarded as noise or for their presence in the solvent.

The molecular weight of each compound was determined by the mass spectrometer (MS) and compared with the database KEGG PATHWAY by Sieve version 1.2.1 in order to determine its identity. From the total of 1,193 compounds accepted as true peaks from both the positive and negative ionization modes, only 60 compounds were identified from the KEGG database, though none of these were economically valuable in terms of their chemical composition.

Correlations in the relative composition of the samples were determined using hierarchical cluster analysis (HCA, Euclidean distance, clustered using Ward's method) with p-values calculated by multiscale bootstrap resampling to determine if temporal or geographic variation

existed in the composition of either the complete head paste or the individual tissues. In the lipid extracts from the Clyde Sea area head pastes obtained throughout 2009, two statistically significant clusters were found. The first included February and April (p = 0.05) and the second included January, July, August, and October 2009 (p = 0.01) (Figure 43A), indicating that overall head paste composition varies significantly with season. No significant geographic differences between the Minch and the Clyde Sea area were found in the summer, with samples from February and August in the Minch clustering with those from August 2009 in the Clyde Sea area area. However, the head paste lipid extract from the February in the Clyde Sea area clustered separately from the others, indicating some difference in that month (Figure 43B).

In the tissue samples, the composition of lipid extracts from the hepatopancreas in males and females indicated that significant differences existed temporally, consistent with what was found in the head paste. However, no significant differences were found by sex (Figure 43C). Unlike the hepatopancreas samples, the gonad composition of females from the Clyde Sea area showed no significant variation between February and August, though the GSI previously illustrated the increased total lipid content attributed to the larger gonad organ in the summer months. Therefore, females are likely the stronger drivers of the temporal variation exhibited in the head paste through all of the analyses, as they contribute two lipid rich organs (hepatopancreas and gonad) to the head paste in the summer months (Rosa and Nunes, 2002).



Figure 43A: Hierarchical cluster analysis dendrogram showing the significance of cluster patterns in head paste samples from winter and summer in the Minch throughout 2009. The numbers at the top left of the cluster indicates the AU p-value. Rectangles are placed around clusters with significant p-values (p<0.05).



Figure 43B: Hierarchical cluster analysis dendrogram showing the significance of cluster patterns in head paste samples from winter and summer in the Minch throughout 2009. The numbers at the top left of the clusters indicate the AU p-value. Rectangles are placed around clusters with significant p-values (p<0.05).



Figure 43C: Hierarchical cluster analysis dendrogram showing the significance of cluster patterns in male and female hepatopancreas samples from winter and summer in the Clyde Sea area throughout 2009. The numbers at the top left of the clusters indicate the AU p-value. Rectangles are placed around clusters with significant p-values (p<0.05).

#### Conclusions

In conclusion, using various analytical methods that extended beyond the scope of research proposed in the original Workplan, a number of potentially valuable compounds have been detected in the Norway lobster head waste, particularly chitin, astaxanthin and PUFAs. The amounts of these vary with season, often by large amounts. In summer months, however, levels of all of the important compounds found were comparable to previous studies in other crustacean wastes. The biological basis for these variations is a combination of seasonal differences in the sex ratios of the catches and seasonal changes in the size of the female gonad and in the composition of the hepatopancreas. An understanding of all these variations is important in developing a cost-effective harvesting strategy that maximizes yields of useful compounds from the Norway lobster fishery head waste.

#### Summary of main findings

The Norway lobster (*Nephrops norvegicus*) supports the most important shellfish fishery in the UK. Norway lobsters are sold either whole or as 'tails-only' for the scampi trade. However, in the 'tailing' process, the contents of the 'head' (cephalothorax) are discarded as waste. The aim of this study was to determine if any commercially harvestable compounds are present in the head waste product, which could provide an alternative source of income for the fishery and increase its sustainability. The head waste composition was determined using lipid class analysis (TLC-FID), fatty acid analysis (GC-MS), and metabolomics (NMR spectroscopy and HPLC-HRFTESIMS). Norway lobster oil, chitin, polyunsaturated fatty acids (PUFAs), and the carotenoid pigment astaxanthin were detected and could represent potential marketable products from this waste. However, as these compounds are found in highest quantities in the summer months, the most effective financial strategy may be to retain the 'heads' through the summer only.

Following these findings, the University of Glasgow is in a position to convert these laboratory techniques into industrial procedures for extraction and purification.

110

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