Fisheries Innovation Scotland

FIS007 - Post-catch survivability of discarded under-sized Norway lobsters (Nephrops norvegicus): Towards a regional and ecosystems-based approach

A REPORT COMMISSIONED BY FIS
AND PREPARED BY

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Published by: Fisheries Innovation Scotland (FIS)

This report is available at: http://www.fiscot.org.

Dissemination Statement

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Suggested Citation:

Albalat, A., McAdam, B., Fox, C. 2015. Post-catch survivability of discarded under-sized Norway lobsters (Nephrops norvegicus): Towards a regional and ecosystems-based approach. A study commissioned by Fisheries Innovation Scotland (FIS) http://www.fiscot.org/

Title: Post-catch survivability of discarded under-sized Norway lobsters (Nephrops norvegicus): Towards a regional and ecosystems-based approach

ISBN: 978-1-911123-06-4

First published: October 2015

Post-catch survivability of discarded under-sized Norway lobsters (*Nephrops norvegicus*): Towards a regional and ecosystems-based approach.

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Project Reference FIS07

Final Report submitted to Fisheries Innovation Scotland

August 2015

Project funded by

Fisheries Innovation Scotland

1. Title of the project: Post-catch survivability of discarded under-sized Norway lobsters (*Nephrops norvegicus*): Towards a regional and ecosystems-based approach.

2. Project Code: FIS07

3. Contractor's Representatives: Dr Amaya Albalat[1] and Dr Clive Fox[2]

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4. Date of commencement: 5th January 2015

5. Date of Completion: 31st August 2015

6. Total project costs: Total awarded £74,956

Total spent to date £70,566.01 (please note some charges still to be processed).

7. Objectives: The aim of this project was to collect new data on the survivability of discarded *Nephrops** caught by trawl representing commercial fishing operations on the West coast of Scotland. This was achieved by determining the survivability of discarded *Nephrops* from a physiological perspective by returning discards back at sea in tube-set perforated boxes and also by observing post-discard behavior using a seabed camera system.

**Please that throughout this report Norway lobster (Nephrops norvegicus) is referred as Nephrops or prawns.*

Executive Summary

- Discarding of *Nephrops* by a typical Clyde trawler operating a tubing fishery, and physiological recovery of discarded *Nephrops,* was studied in three trials in late winter (February), early spring (March) and summer (June) 2015. In addition, the behaviour of discarded *Nephrops* was studied in July 2015.
- From the three trials conducted (8 tows in total) around 30% of the catch (by numbers) was observed to be discarded because the animals were too small, were damaged, recently moulted or were visually infected with *Hematodium* spp..
- The 'discard' fraction of the catch was mainly composed of prawns which were larger than the minimum permitted landing size (MCS ≤20 mm).
- The composition of the discard fraction also varied seasonally. In winter and early spring (February and March trials) the discarded fraction contained more males and more visually infected animals than in early summer (June). In June, discarded animals were predominantly females of a bigger size than their counterpart discarded males, they were in a soft condition (indicating that are close to or have recently moulted) and contained mature and developed gonads.
- Taken data from all tows, 87% of the discarded *Nephrops* showed no observable damage after trawling.
- Overall, data from survival experiments indicated that 95% of discarded *Nephrops* were alive after 48 hour of recovery period in tube-sets.
- The use of tubes for recovery may have led to some additional physical damage of the prawns which might have affected around 5-6% of the test animals (averaged across all 8 tows).
- The physiological recovery potential of discarded *Nephrops* was high especially considering the biochemical condition of the animals shortly after being taken from the trawl (elevated L-lactate in the muscle, metabolically exhausted muscle with low AEC and activated PO activity in the haemolymph).
- However, post-discard survival for prawns infected with *Hematodium* spp. was lower (more than 50% were moribund or dead after 48 h).
- Similarly, damaged animals showed a lower post-discard survival than non-damaged ones indicating that damage is an important survivability factor (again for damaged animals 50% were moribund or dead after 48 h).
- Although absolute survival was not affected by higher air temperatures recorded in June, data from vigour index and physiological measures indicated that animals were more physiologically compromised in June than in March.

- Behavioural trials where post-discard *Nephrops* were observed at 30 m depth showed that animals subjected to air-exposure for < 60 mins recovered rapidly on re-immersion. They began to move around on the seabed within 2 minutes and appeared to exhibit normal behaviour in relation to interactions with each other.
- However, prawns exposed to air for around 90 mins took up to 10 mins to recover on the seabed. This time was sufficient to attract other scavengers and predators, such as crabs, which were observed attacking recovering *Nephrops*. At least in one instance escape attempts by the *Nephrops* were unsuccessful.
- The behaviour trials showed that discarded *Nephrops*, which are not physically damaged or exposed to long periods of air-exposure, recover rapidly on re-immersion and exhibit 'normal' movement and escape behaviours.
- Modelling the probability of death: If we were to consider damaged and infected animals as non-survivors, a stepwise regression was conducted in order to estimate the probability of death (95% confidence interval). Following this approach the probability of dying was around 29% in winter/early spring and 15% in early summer.
- Following this conservative approach if the fishermen were to land damaged discards the survival of discarded un-damaged animals was estimated at around 81% in February, and 78% in March and 94% in June (95% confidence interval). This strategy would improve the probability of survival even using this conservative approach.
- This work was carried out in a trawl commercial vessel that mainly targets the live '*Nephrops*' market, characterised by relatively short tow duration and therefore, data obtained in this project cannot be extrapolated to the whole *Nephrops* fleet but to similar vessels operating in the west coast and currently targeting the live maket.

Main Report –

1. Objectives and Primary Milestones

The aim of this project was to evaluate the survivability of discarded Norway lobsters, *Nephrops norvegicus* in commercial fishing operations on the West coast of Scotland[1]. Trials have been conducted using a typical trawler operating in the Firth of Clyde that mainly targets the live market ('tubing fishery').

This was achieved by using two different experimental approaches:

1. Firstly, the project determined the survivability of discarded *Nephrops* from a physiological perspective (this is the type of survival data used by ICES). To this end, discarded *Nephrops* were returned at sea in containers with individual compartments and their survival and condition monitored after 48 h. **Part 1 of the Report.**

   Milestones from Part 1 were:
   - To determine survivability of discarded *Nephrops* at the individual level during winter environmental conditions. This milestone was achieved by performing one survival recovery trial in February (2 tows) and another one March (3 tows).
   - Determine survivability of discarded under-size *Nephrops* at the individual level during early summer environmental conditions. This milestone was achieved by performing one survival recovery trial in June (3 tows).

2. Secondly, the project tested whether a seabed camera system could be used to observe the post-discard behaviour of the prawns and whether they attract predators if their escape responses are delayed by stress recovery. **Part 2 of the Report.**

   Milestones from Part 2 were:
   - Camera observations –To successfully deploy the SAMS camera system, to stock the camera arena with discarded under-sized *Nephrops* and to use the camera system to make time-lapse observations of the *Nephrops* behaviour and any interactions with predators under natural conditions (depth and sediment type on which *Nephrops* are normally found). This milestone was achieved by performing a trial in July.

[1] The Scottish west coast *Nephrops* fisheries include both smaller and larger trawlers which tend to operate in different areas, use different gears (single or twin-rig) and supply different markets. Further work is likely to be required to cover the full range of operations taking place on the west coast.

Part 1: Survivability of discarded *Nephrops* from a physiological perspective

1.1 Introduction

Scotland has traditionally been at the forefront of developing and implementing novel measures to manage its own fisheries. This approach is now needed in order to meet targets set by the reformed Common Fisheries Policy (CFP). The aim of this new CFP is the exploitation of living marine biological resources at levels which contribute to long-term sustainable environmental, economic and social conditions. Reinforced strategies within CFP include the introduction of measures to reduce discards. However, even with the implementation of new reduction measures, the reformed CPF has approved an obligation to land all catch, including discards from species which are subject to catch limits or quotas, except for some specific exemptions. One of the landing exemptions is for those species deemed to have a high post-catch survivability.

In the case of discarded under-sized Norway lobsters (*Nephrops norvegicus*) it is assumed by ICES that the survivability of this species post-discarding is 25%. However, this percentage is based on a single study conducted in 1975 by trawlers in the Bay of Biscay (1). Given the impact of the new landing obligation, the gear changes that have taken place in the last 40 years and the regional differences between different fishing areas we suggested that there was an urgent need to collect new scientific data to evaluate the survival potential of discarded *Nephrops* using regionally appropriate studies.

Norway lobster (*Nephrops norvegicus*) supports the most important shellfish fishery in the UK, representing an industry worth £86.7 million at first sale in 2011 (Barreto and Bailey, 2013). In general terms, the damage and mortality of discarded animals including *Nephrops* cannot be attributed to a single factor but rather to a number of interacting factors (Benoit et al., 2010). These include gear type, volume and composition of the catch, trawling time, handling practices on board and also environmental conditions such as water and air temperatures and changes in salinity (Benoit et al., 2010; Yergey et al., 2012).

In the case of *Nephrops*, mortality of trawled animals has been shown to increase with the extent of physical damage (Bergmann and Moore, 2001), which has been shown to increase with trawl duration (Milligan et al., 2009). Other factors such as post-harvest practices on-deck (aerial exposure), temperature and high light intensities have also been shown to have damaging consequences (Spicer et al., 1990). However, from a purely physiological perspective, other studies have demonstrated that *Nephrops* can recover relatively rapidly from the stress of trawling if post-harvest practices are optimised (Albalat et al., 2010).

The process of trawling *per se* has been shown to be extremely stressful for crustaceans in general, and in particular for the species studied in the present work, *N. norvegicus* (Albalat et al., 2009). Trawling produces an increase in muscle and haemolymph L-lactate (Harris and Andrews, 2005; Ridgway et al., 2006b; Albalat et al., 2009) due to the fact that during the trawling process energy requirements exceed the capacity of the aerobic metabolism of the animal, and therefore anaerobic metabolism is activated in order to maintain ATP levels. However, anaerobic metabolism is not an efficient metabolic route to maintain ATP levels and therefore, ATP eventually decreases while AMP increases to become the main nucleotide in the muscle (Mendes et al., 2001; Albalat et al., 2009). The balance between ATP, ADP and AMP can be summarised using the 'Adenylate Energy Charge' or AEC ratio. This ratio has been accepted as an important index to describe the energy status of the muscle.

On the other hand, phenoloxidase (PO) activities are commonly used to assess immune-competence and health condition in crustacean (Cheng and Chen, 2000; Coates et al., 2012). The proteins proPO and Haemocyanin (Hc) are present within crustacean haemolymph and hemocytes (immune cells) respectively (Coates and Nairn, 2014). During immune challenge and/or moulting, the inactive proPO is converted into an active enzyme, namely PO, and Hc is activated into Hc-d PO. Both proteins convert phenolic compounds into microbicidal pigments that can kill pathogens and aid clot formation (Cerenius and Söderhäll, 2004).

In this part 1 of the project we used a battery of visual indices and biochemical measures (muscle and haemolymph L-lactate, AEC values, total amount of protein and oxygenated haemocyanin concentrations in the haemolyph and active and total PO activities) to analyse not only the composition of the discarded fraction of the catch but also to biochemically assess the physiological and immunological condition of the discarded animals immediately after trawl and after an experimental recovery period of 48 h.

Visual indices measured to characterise the discarded fraction of the catch were: carapace length, sex, damage, vigour, moulting stage and visually infected animals with *Hematodinium* spp..

Biochemical measures analysed to characterise the physiological and immunological condition of the discarded animals were L-lactate in muscle and haemolymph (stress related; indicative of anaerobic metabolism), ATP and its breakdown products to be able to calculate the AEC ratio (stress related; indicative of energy status of the muscle), haemolymph total protein and oxy-haemocyanin (indicative of general animal condition) and active and total polyphenol activities (immune-related measures).

Another point which must be borne in mind in this Part 1 of the study animals were allowed to recover in a predatory-free enclosure. In order to more fully understand realistic post-discard survival in the sea it seems essential that the vulnerability of discarded *Nephrops* to predation risk is also examined. Behavioural results from this project are summarised in Part 2 of the report.

1.2 Materials and Method

Three trials trial were conducted during this project in the Firth of Clyde. In order to encompass a range of conditions, particularly changes in air temperature, one experiment was carried out in February, one in March and the last one in June. The commercial vessel Eilidh Anne GK2 was used. Fishing was conducted using a single-rig Harkess, rockhopper trawl with 85 mm mesh fitted with a Cod Recovery Zone Panel (standard commercial net and rig). The net does not have a tickler chain. Mesh size was checked for compliance the day before the first trial (11^{th} February 2015). On the days of the trials the skipper was asked to follow his normal fishing and catch handling practices. This vessel's operations are characterised by relatively short tows as the skipper concentrates on obtaining a high-quality catch the majority of which is 'tubed' for the live *Nephrops* market. In February, two tows were performed and in March and June, three tows were performed in each trip. Environmental and trawling coordinates and conditions were recorded for each tow. All trawls were carried out during daylight. Temperature and salinity profiles were recorded in February and March trials using a Valeport Castaway CTD at the trawl locations, when animals were returned to the sea and after the 48 h recovery period (Suppl. Table 1). Castaway CTD device was not working when June trial took place and therefore temperature readings were obtained from vessel's own devices while no salinity measures could be taken. Once the catch was on board, the skipper sorted the *Nephrops* according to four different commercial sizes: Large, medium, small and extra-small. Animals in the first three categories are normally 'tubed' and returned live to port. The skipper also has a buyer for the extra-small category prawns but these are not 'tubed'. Animals not fit for commercial purposes according to the skipper's criteria were placed in a basket as discards. From this discarded portion of the catch, 15 animals were immediately sampled to provide a biochemical baseline (time = 0; biochemistry data). Sex, carapace length (CL), damage index, vigour index, moulting stage, presence of mature gonads in the females (green gonads) and any obvious *Hematodinium* spp. symptoms were recorded for each individual prawn. Around 200 µl of haemolymph was then extracted from each prawn using 1 mL syringes coupled with 25G needles, 100 µl of each sample was then de-proteinased in 100 µl of perchloric Acid (PCA) 6 M, mixed and stored on dry ice. The rest of the haemolymph was also stored on dry ice in individually labelled Eppendorf tubes. Muscle samples were also collected from individual prawns and placed in liquid nitrogen. Thereafter, another 150 animals were randomly selected from the discard category and damage and vigour indexes scored as above. These animals were placed individually in identifiable tube-sets for the survival trial (baseline data; time = 0; survival trial) (Fig. 1). Air temperatures during sampling and sorting procedures were recorded as well as the time it took for the sampling to be completed. Tube-sets containing 150 discarded animals per each tow were closed in perforated plastic boxes (Fig.1) and returned to the sea suspended on a rope mooring. The boxes were placed at a depth below the near surface lower salinity layer (usually at around 30 m) (see results of the CTD casts). These animals were left to recover for 48 hours and after this time the boxes were collected. Sex, CL, damage index and vigour index were then recorded for all these animals (time = 48 h recovery; survival trial) and a sub-sample of 30 animals per tow were sampled for biochemical analysis as described above (time = 48 h recovery; biochemistry data). Again a CTD cast was performed before animals were collected and environmental conditions including aerial exposure time were recorded during sampling procedures.

Fig 1. A representative tube-set used for the survival trial, a tube-set box closed ready to be re-immersed for survival trial.

1.2.1 Damage Index

Damage was scored against a three-level index (no damage-0, slight damage-1, severe damage-2) introduced by Ridgway et al. (2006) and presented in Table 1 and Fig. 2. Injuries were not counted if there was evidence of tissue regeneration indicative of an old injury.

The damage index for each prawn in the discard trial was scored just after trawling and after the 48 h recovery period to assess if holding the animals in tube-sets in seawater had a detrimental effect on the physical condition of the animals (mainly due to handling of the animals in and out the tube-sets and the boxes in and out the sea).

Table 1. Damage index and criteria.

Damage Category	Criteria
0 (No damage)	No visible damage to external structure and no loss of limbs
1 (Minor damage)	Exhibit no more than two of: • Loss of two or fewer walking legs • Loss of not more than one claw • Soft tissue punctures or small puncture to the shell • Loss of the tip of the rostrum
2 (Major damage)	Exhibit at least one of: • Loss of more than two walking legs • Loss of both claws • Loss of an eye • Compressed or cracked body parts\segments • Major soft tissue punctures • Exhibit three or more criteria of category one animals

Fig 2. Photographs showing the different damage index categories, 1, 2, and 3 respectively in the dorsal and ventral view of the animal.

1.2.2 Vigour Index

Animals were classified according to five different vigour categories, namely A, B, C, moribund and dead according to the posture of the animal when held on air, antennae and claws position, tail tension and movement of walking legs (Table 2; Fig. 3). Similar vigour indexes are currently being used by the industry for the live trade of *Nephrops* and were adapted for this project.

Table 2. Characteristics of the different Vigour Index categories used in the trials.

Vigour A	Vigour B	Vigour C	Moribund	Dead
Animal displays defensive posture, and/or vigorous and possibly prolonged (>10 flips) tail flipping	Bouts of tail flipping are less frequent and vigorous, lasting for shorter periods (<10 flips approx). Animal less likely to adopt defensive posture	Bouts of tail flipping are infrequent and do not normally exceed 5 flips / bout (approx)	Tail flips are very infrequent and weak and are limited to 1 or 2 flips / bout	No limbs movement even if animal is stimulated
Antennae and claws are held high and may be 'waved' by the animal	Antennae and claws are raised but moved less vigorously	Antennae and claws are drooped but animal can raise them for short periods (a few seconds)	Antennae and claws are drooped and animal is unable to raise them	
Tail is held rigid up-right in an angle (when animal is not tail flipping)	Tail is held horizontally retains some tension	Tail is drooped and retains very little tension	Tail is limp	
Walking legs are strong and animal will often right itself	Walking legs are moved but animal normally cannot right itself	Walking legs are moved by the animal but it will not be able to right itself	Walking legs are moved very slowly if animal is stimulated	

Vigour A

Vigour B

Vigour C

Moribund

Fig 3. Photographs showing the different Vigour Index categories, A, B, C and moribund.

1.2.3 Visually infected *Nephrops* by *Hematodium* spp.

Animals were examined and classified according to their body colour (Field et al. 1992) as symptomatic of patent *Hematodinium* sp. infection, or as asymptomatic (which would include both truly uninfected and also sub-patently infected individuals). The validity of these visual criteria for indicating patent infection has recently been confirmed by correlating them with molecular methods for directly detecting parasites in the haemolymph (Beevers et al. 2012).

1.2.4 Moulting stage

The carapace hardness was taken as a simple measure of the moulting stage of individual *Nephrops*. Animals were classified as ‘soft’ is squeezing just behind the eyes gave a clear distortion. These soft animals include animals that could be at a late intermoult stage (i.e. calcium withdrawn from exoskeleton) or to have recently moulted. This methodology can be somewhat subjective but appropriate for the level of detail needed in the present study (Milligan et al., 2009).

1.2.5 Biochemistry Analysis

Haemolymph samples were analysed for L-lactate concentration (stress related, indicative of anaerobic metabolism), total amount of protein and oxygenated haemocyanin concentration (animal condition related measures) and also for active and total polyphenol oxidase (PO) activities (immune related measures). Muscle samples were analysed for L-lactate concentration and also nucleotide profiling, namely ATP, ADP, AMP, IMP, HxR and Hx. Nucleotide profiling in the muscle allowed the calculation of the Adenylate Energy Charge (AEC) which is a recognised indicator of the energy status of the muscle (how metabolically exhausted is the muscle). AEC was calculated according to the following formula:

$$\frac{[ATP] + \frac{1}{2}[ADP]}{[ATP] + [ADP] + [AMP]}$$

1.2.6 Analysis of L-lactate

L-Lactate concentration was measured in deproteinized haemolymph and abdominal muscle homogenates using the method described by Bergmeyer & Bernt (1974) and further modified by Hill et al. (1991). On the day of analysis samples were thawed and 50 µl were added to Eppendorf tubes containing 50 µl of NAD^+ (50 mM), 0.85 ml of hydrazine buffer (0.6 M hydrazine hydrate, 5.6 mM EDTA, 1 M glycine) at pH 9.5 and 1 unit of lactate dehydrogenase (LDH, Sigma) and incubated for 2 h at 37°C. The absorbance of a sample was measured at 340 nm on a spectrophotometer (Shimadzu, UV Mini 1240) and converted to an L-lactate concentration using a calibration curve constructed from standards of known concentrations of lactic acid (0.5-10.0 mM).

1.2.7 Analysis of ATP and its breakdown products

Nucleotide analysis was performed using High Performance Liquid Chromatography (HPLC) as firstly described by Ryder (1985) and modified by Albalat et al. (2009). The HPLC system consisted of a Spectra system P4000 pump coupled to a SN4000 autosampler and a UV1000 detector set at 254 nm. A Kinetex 5u C18 100A column 250 x 4.6 mm with an internal particle of 5 µm was used to conduct the separations. Standard curves were prepared from adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), inosine 5'-monophosphate (IMP), inosine (HxR) and hypoxanthine (Hx) in concentrations ranging from 0.1 to 1.0 mM (Fig. 4).

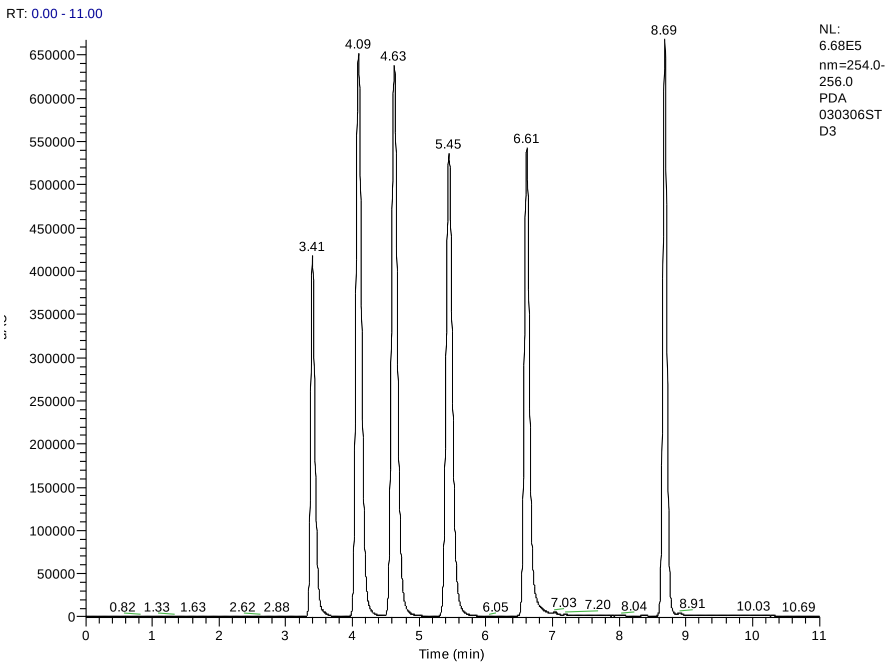

Fig 4. HPLC chromatogram showing the separation of ATP and its breakdown products. By order of elution the compounds are IMP, ATP, ADP, AMP, HxR and Hx.

1.2.8 Total protein and oxygenated-haemocyanin concentration in haemolymph

Haemolymph samples were thawed and centrifuged at 15,000g for 5 min at 4°C. The supernatant was removed and placed into a new tube and the free-cell supernatant was used in the four analyses described below. Haemolymph was then diluted 1:100 using 100mM Tris-HCl, pH 7.5 prior to analysis. UV absorbance measurements were carried out using an Ultrospec 2100 pro spectrophotometer. Protein concentrations were calculated using a standard A280 nm measurements (indicative of tryptophan residues) and the known value of 1.43 for 1 mg/ml *Nephrops* haemocyanin (Hc) in a quartz cuvette of 1 cm path-length (Coates and Nairn, 2013). The amount of oxygenated hemocyanin (Oxy-Hc) within the haemolymph was calculated using the same method as described previously by Coates et al. (2012). Briefly, absorbance measurements taken at 340 nm (indicative of typer-3 copper proteins) and the average molecular weight of a Hc subunit (72 kDa) were used to calculate the percentage of Hc with dioxygen bound, taking into account the absorption coefficient at this wave-length is 20,000 M^{-1} cm^{-1} (Coates and Nairn, 2013).

1.2.9 Active and Total Polyphenol Oxidase activities (POs)

The active PO enzyme within the haemolymph was measured by adding substrate (2 mM dopamine hydrochloride) directly to the diluted haemolymph (in 0.1 M Tris-HCl, pH 7.5) and recording an increase in absorbance at 475 nm, indicative of product (dopachrome) formation. Total (inducible) PO activity derived from the conversion of the inactive proPO and the latent Hc-derived PO were measured by pre-incubating haemolymph samples for 5 min (at room temperature) in the presence of 3.5 mM (0.1%) of the universal enzyme activator, sodium dodecyl-sulphate (SDS) (Coates et al., 2012), prior to the addition of substrate (2 Mm dopamine).

In both cases, samples were placed in a 96 well plate and monitored over a 10 min period (at 475 nm) using a Versamax microplate reader, maintaining a temperature of 25^{o}C. All conversions to units of activity per minute (U min^{-1}) were based on an absorption coefficient of 36,000 M^{-1} cm^{-1} for dopachrome at this wave-length.

1.2.10 Statistical Analysis

All the results obtained are represented as mean values +/- standard deviation (SD). The differences between groups were analysed using the analysis of variance (ANOVA). Homogeneity of variance was tested using the Levene test. A Post Hoc or multiple comparisons approach was then used to determine statistical differences between samples. *P*-values lower than 0.05 were considered statistically significant.

Survival proportions were analysed using logistic regression, with resulting logistic coefficients converted to proportions surviving the corresponding 95% confidence intervals.

1.3. <u>Results</u>

1.3.1 General Information on the trawls and environmental conditions

Tows and locations used for mooring the boxes (survival experiments) were carried out in the Firth of Clyde (Fig. 5).

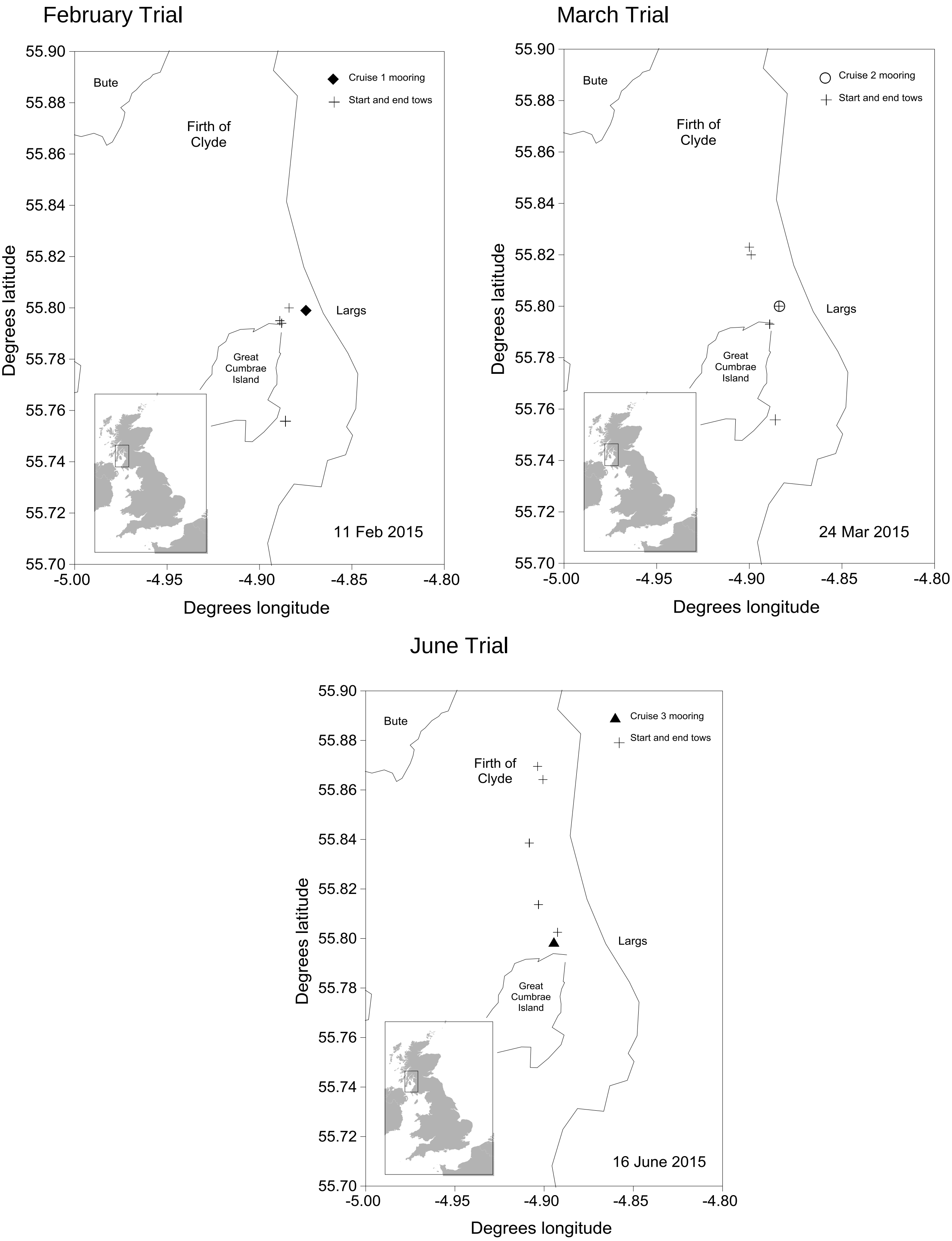

Fig 5. Map showing start and end locations of tows performed so far in the project and mooring locations used in survival experiments.

Detailed information on trawl characteristics and environmental conditions during sorting is presented in Table 3. Temperatures recorded in February and March were very similar (7-8.5°C) while air temperatures recorded in June were higher ranging from 12.5°C early in the morning to 15°C in the afternoon. Average sorting time was for this project was 83 ± 9 min. Note that sorting time in February and June was longer (between 95-103 min) compared to March were sorting time was around 60 min.

Table 3. General information and environmental conditions on the trawls performed in February, March and June trials.

	Feb-Tow 1	Feb-Tow 2	Mar-Tow 1	Mar-Tow 2	Mar-Tow 3	Jun-Tow 3	Jun-Tow 3	Jun-Tow 3
Date	11/02/15	11/02/15	24/03/15	24/03/15	24/03/15	16/06/15	16/06/15	16/06/15
Weather	Cloudy	Cloudy	Cloudy 6/8	Sunny-dry	Sunny-dry	Cloudy mist/calm	Sunny	Cloudy
Start time trawling	08:00	11:25	07:45	11:00	14:11	07:47	11:35	15:10
End time trawling	09:15	13:00	09:06	12:30	15:58	09:25	13:13	16:24
Trawl duration (min)	75	95	81	90	107	98	98	74
Location of trawl START	55° 45.4 4° 53.67	55° 48.0 4° 53.01	55° 47.94 4° 52.94	55° 48.09 4° 53.25	55° 49.21 4° 53.967	55° 48.82 4° 54.19	55° 48.15 4° 53.55	55° 48.11 4° 43.134
Location of trawl END	55° 47.67 4° 53.359	55° 47.63 4° 53.30	55° 47.71 4° 53.361	55° 47.73 4° 53.41	55° 49.38 4° 53.991	55° 52.17 4° 54.22	55° 51.85 4° 54.04	55° 50.31 4° 54.498
Start trawl depth (m)	35	55	60	80	66	86	84.5	63.4
End trawl depth (m)	60	60	80	60	60	84	87.8	96.3
Towing Speed (knots)	2.1	2.1	2.1	2.1	2.2	2	2.1	2
Air temp (°C)	7.1	7.4	7.6	8.5	8.4	12.5	14	15
Water Surface Temp (°C)	7.87	7.63	7.49	7.89	7.89	11.8	12	12
Water Bottom Temp (°C)	7.95	8.03	7.28	7.33	7.33	N.A.	N.A.	N.A.
Salinity water Surface (PSS)	32.60	32.20	31.97	31.72	31.72	N.A.	N.A.	N.A.
Salinity water Bottom (PSS)	32.87	33.17	33.1	32.93	32.93	N.A.	N.A.	N.A.
CTD Ref	11/02/15 CTD 1	11/02/15 CTD 2	24/03/15 CTD 1	24/03/15 CTD 2	24/03/15 CTD 2	N.A.	N.A.	N.A.
Time animals arrive on-board	09:20	13:08	09:18	12:41	16:11	09:38	13:27	16:35
Time animals back in the water	11:22	14:30	10:32	13:36	17:00	11:13	14:44	18:27
Sorting time (min)	122	84	74	55	49	95	77	112
Depth animals are put back to (m)	25	25	30	28	25	36	35	35
Location animals are but back to	55° 47.93 / 4° 52.487	55° 47.93 / 4° 52.487	55° 46.774 / 4° 53.677	55° 46.678 / 4° 53.690	55° 46.678 / 4° 53.690	55° 47.705 / 4° 53.616	55° 46.65 / 4° 53.62	55° 46.77 / 4° 53.57

1.3.2 Composition of the *Nephrops* catch

As mentioned in the methods, the skipper of the vessel was asked to follow his normal sorting practice as far as possible. The only difference was that normally 'discard' category animals would be sent over-board immediately on sorting whereas we had to retain them on board for a period of time for sampling. However, discussions with the skipper suggested that the total time taken to perform the baseline sampling and to place the 150 discard prawns in the recovery boxes was not very much longer than normally taken to complete catch sorting. The results obtained can therefore be regarded as the worst-case scenario, i.e. survival in reality should be even higher because most of the *Nephrops* would be discarded more rapidly under normal sorting. The numbers of animals in each size category are shown in Table 4. Animals classified as large, medium and small were all counted and measured while the size profiles of the extra-small and discarded grades are based on raising a sub-sample of measured animals by the total catch weight in that category.

Table 4. Composition of *Nephrops* catch (number) according to commercial grading: Large, medium, small, extra-small and discards.

	Feb-Tow 1	Feb-Tow 2	Mar-Tow 1	Mar-Tow 2	Mar-Tow 3	Jun-Tow 1	Jun-Tow 2	Jun-Tow 3		
Neprhops LARGE grade	28	30	14	7	21	2	4	0		
Neprhops MEDIUM grade	142	138	52	27	85	35	34	39		
Neprhops SMALL grade	336	374	157	91	170	184	320	251		
Neprhops EXTRA-SMALL grade	441	520	143	36	195	283	834	336		
Neprhops DISCARDED	233	768	177	73	268	259	286	201		
Total	1180	1830	543	234	739	763	1478	827		
									Average	**SD**
Discarded animals (%)	**19.7**	**42.0**	**32.6**	**31.2**	**36.3**	**33.9**	**19.3**	**24.3**	**29.9**	**8.1**

The total numbers caught on Trial 2 were lower than on Trial 1 and 3. This was expected by the skipper due to the exceptionally strong tides on the second trial (March trial) which normally reduce the catches of prawns. However, it is important to note that other west coast prawn fisheries delivering to different markets e.g. for processing, may have different discard profiles.

According to this data the rate of discarding, expressed as a proportion of total number of animals caught, averaged (± SD) of 29.9% ± 8.1. In terms of numbers the most abundant category caught was extra-small but these still have a market and would be landed as normal, at least by this skipper.

The sizes, measured as carapace lengths (CL), of the *Nephrops* in the different commercial categories are shown in Table 5. The average size of the discarded animals was 28.2 ± 1.0 mm, which was smaller than the extra-small category.

Table 5. CL (mm) of *Nephrops* according to commercial grading: Large, medium, small, extra-small and discarded according to current fishing practices in the fishing vessel used in the trials. Data represents the mean ± SD.

	Feb-Tow 1	Feb-Tow 2	Mar-Tow 1	Mar-Tow 2	Mar-Tow 3	Jun-Tow 1	Jun-Tow 2	Jun-Tow 3	**Average**
Large (mm)	50.2 ± 2.3	48.9 ± 2.5	49.0 ± 2.2	48.5 ± 2.7	50.1 ± 3.5	47.5 ± 1.5	50.7 ± 6.7		**49.3 ± 1.1**
Medium (mm)	44.9 ± 2.4	44.7 ± 2.3	43.5 ± 2.6	43.5 ± 2.1	43.9 ± 2.4	42.7 ± 2.8	45.1 ± 3.0	47.2 ± 4.1	**44.4 ± 1.4**
Small (mm)	38.1 ± 1.9	37.9 ± 2.6	36.2 ± 2.2	36.1 ± 2.3	37.8 ± 2.0	39.2 ± 2.4	38.3 ± 2.0	38.5 ± 2.5	**37.8 ± 1.1**
Extra Small (mm)	31.6 ± 1.7	30.9 ± 4.9	32.1 ± 2.1	32.9 ± 1.1	32.6 ± 1.9	32.8 ± 2.4	33.4 ± 1.7	33.6 ± 1.9	**32.5 ± 0.9**
Discards (mm)	26.9 ± 2.7	27.1 ± 2.6	28.1 ± 2.6	28.6 ± 2.8	27.9 ± 2.5	30.0 ± 1.9	28.9 ± 2.7	28.2 ± 2.5	**28.2 ± 1.0**

Therefore, according to the data collected in these trials the average CL of discards was above the official minimum conservation limit size (CL < 20 mm). In fact, discarded animals below the minimum conservation limit size comprised a very small proportion of the prawns being discarded (more detailed information shown in Fig. 8).

As this data was initially unexpected we compiled composition data from another project conducted in the University of Glasgow, led by Prof Douglas Neil and presented in the PhD thesis completed in 2012 by Dr Andrew Watts entitled 'Nutritional status and trophic dynamics of the Norway lobster *Nephrops norvegicus* (L.)'. In this thesis class size data was available monthly for a period of a year (Dec 08-Dec 09) in two locations in the Clyde Sea Area. This data is shown as Suppl. File 2 and is in broad agreement with data shown in these trials. Indeed, under-sized *Nephrops* (CL <20 mm) are only marginally present in trawls performed in the Firth of Clyde, presumably as a result of the mesh sizes used in the trawls.

The sex composition of these different commercial size classes is shown in Fig. 6. Two different trends can be detected. Firstly, males dominated the catch composition in large and medium categories in February, March and June and their percentage decreased with size category. Therefore, discards had the highest percentage of females in each trial. Furthermore, more females were present in the June tows (75%) compared to February (52%) and March (30%) and that is true not only for the discard fraction but for all commercial categories.

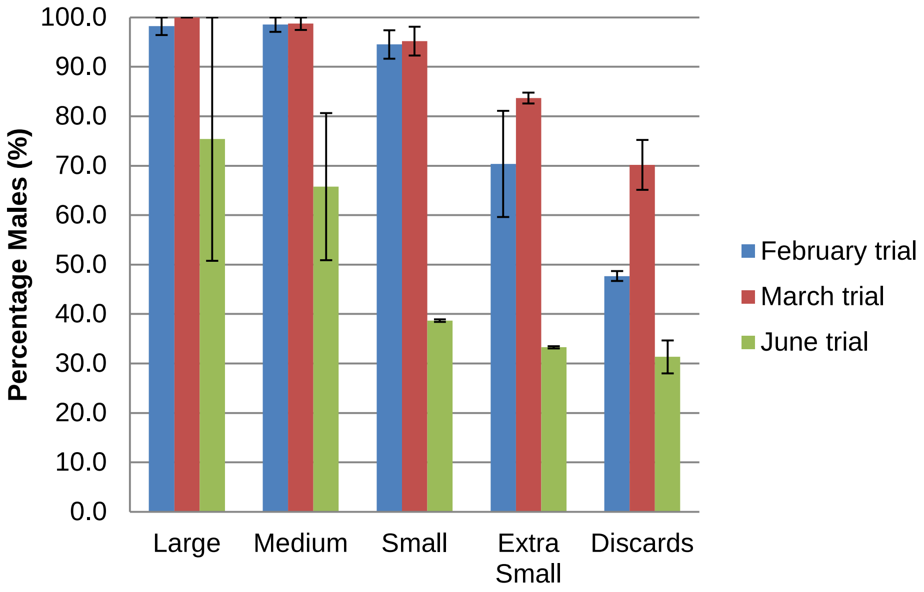

Fig 6. Percentage of males (%) obtained according to commercial grading: Large, medium, small, extra-small and discarded according to current fishing practices in the fishing vessel used in the trials.

1.3.3 Composition of discarded *Nephrops* used in the survival trials

One of the underlying objectives of this project has been to characterise the composition of the discards fraction using commercial fishing practices. As the skipper was asked not to change his practices it is believed that the discards obtained in this project are relevant to current fishing practices for boats operating in a similar manner in the Firth of Clyde. According to Table 5 discards are smaller and contain more females than the extra-small commercial size category used by the skipper (Fig. 6) (shown in previous section).

Furthermore, the CLs of the discards increased significantly with season ($p<0.05$). Therefore, in June discards were of a bigger size than those animals discarded in winter/early spring (Fig. 7). Larger animals were discarded in June because of other factors (see below).

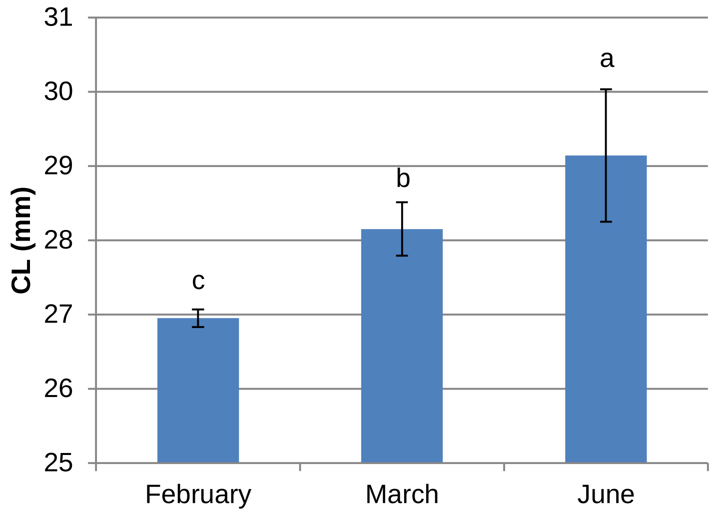

Fig 7. CL (mm) of discarded *Nephrops* in February, March and June trials. Values represent the mean ± SD. Different letters indicate significant differences (p<0.05).

Looking in more detail at the class size composition of the discards shows that the predominant size is in the range of 25-29.9 mm and that class size remains quite constant in the different trials. However, we can see how the proportion of 20-24.9 mm decreases seasonally as the proportion of 30-34.9 mm increases.

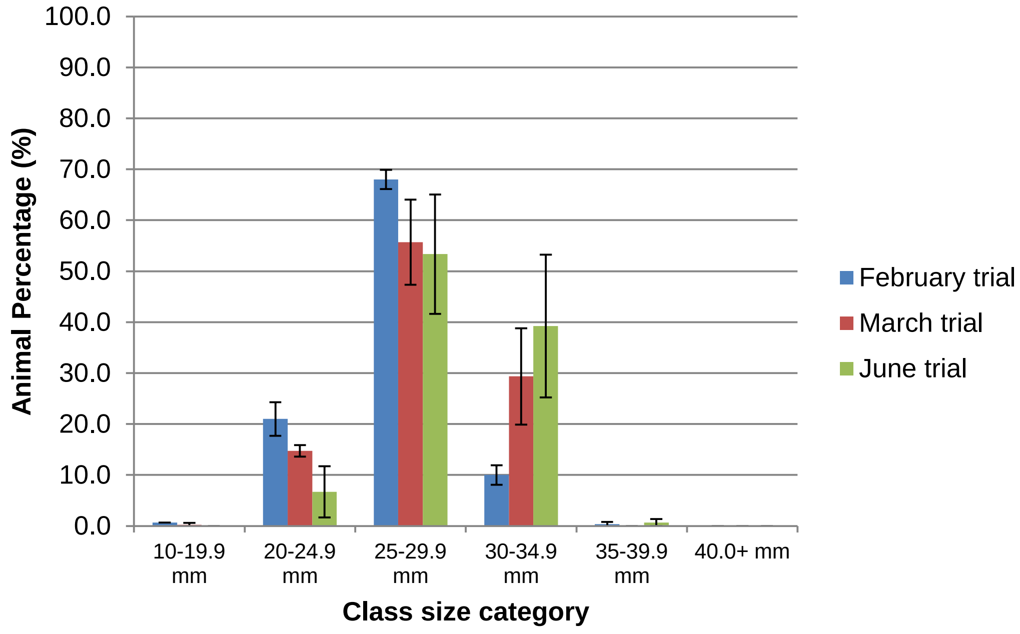

Fig 8. Average percentage of animals according to their class size from all discarded animals used in the survival trial. Data is mean ± S.D.

When looking at the CL between males and females on this discarded portion no pattern was observed for males, so discarded males are of similar size throughout the period studied. However, discarded females in June are in average bigger (29.3 mm) than females discarded in February (26.6 mm) and March (27.5 mm) as shown in Table 6 ($p<0.05$).

Table 6. CL (mm) according to the sex of the animals in the discarded portion of the catch. Data represents the mean ± S.D.

	Feb-Tow 1	Feb-Tow 2	Mar-Tow 1	Mar-Tow 2	Mar-Tow 3	Jun-Tow 1	Jun-Tow 2	Jun-Tow 3
CL Males (mm)	27.3 ± 2.5	27.5 ± 2.5	28.5 ± 2.2	29.0 ± 2.7	27.8 ± 2.5	29.7 ± 2.1	27.9 ± 2.8	27.8 ± 2.0
CL Females (mm)	26.5 ± 2.7	26.7 ± 2.5	27.3 ± 3.1	27.0 ± 2.6	28.1 ± 2.6	30.1 ± 1.7	29.3 ± 2.6	28.5 ± 2.6

These discarded bigger females in June were interestingly mainly soft (in fact, soft animals accounted for almost 40% of discards in June) and had mature gonads (commonly known as animals with ‘green heads’). Therefore, in June females emerge from their burrows to moult and mate and therein due to their soft carapace and ‘green heads’ appearance are discarded by the fishermen creating a clear seasonal change on the size and actual composition of the discard fraction (Fig. 9).

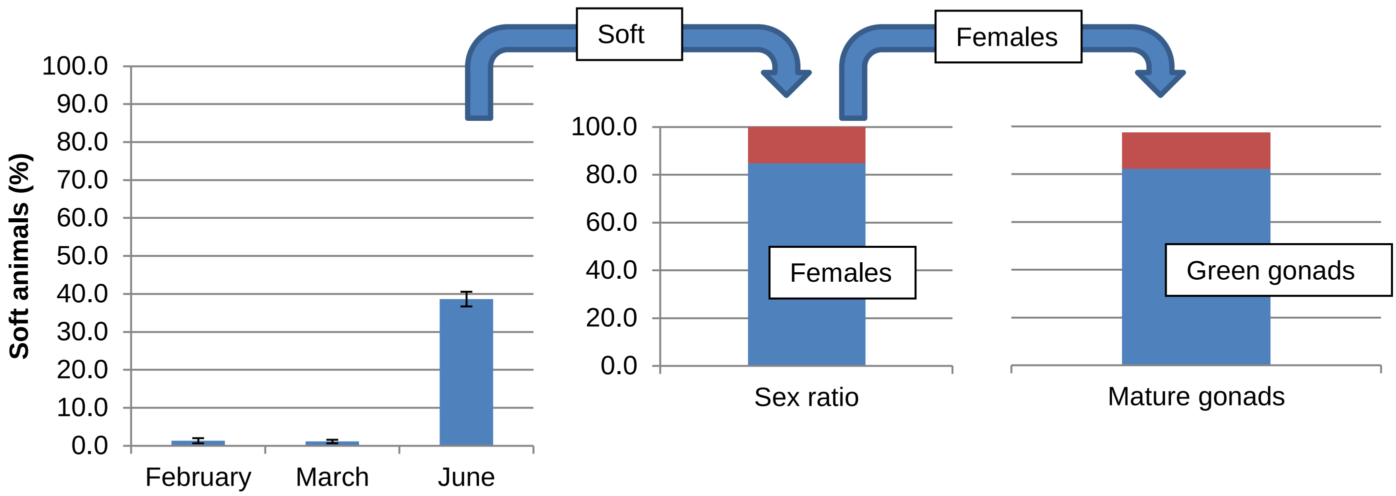

Fig 9. Percentage of animals classified as soft in the discard fraction. From the soft percentage next in the right shows the percentage of females/males and also from the soft females the percentage that showed mature gonads. Data is mean ± S.E.

Finally, the percentage of visually infected animals by *Hematodinium* spp. in the discards portion is shown in Table 7. The percentage of visually infected animals showed a seasonal pattern with visually infected animals been higher in February and March compared to June when almost no visually infected *Nephrops* were caught.

Table 7. Percentage (%) of visually infected by *Hematodinium* spp. and soft discarded *Nephrops* in the different trawls. Note the proportion of females on the soft categories and presence of developed green gonads.

	Feb-01	Feb-02	Mar-01	Mar-02	Mar-03	Jun-01	Jun-02	Jun-03
Visually infected	10.4	10.0	13.0	8.6	17.0	0.0	0.7	0.7
Soft animals	2.0	0. 7	0. 7	0. 7	2.0	35.3	42.0	38.7

In conclusion, in this study discards contained *Nephrops* that were not fit for commercial purposes, which included small *Nephrops* (smaller than the extra-small commercial category but mainly above MCL), recently moulted animals (predominantly soft females), damaged individuals, animals visually infected by *Hematodinium* spp. and other individuals that were deemed to be not fit for sale by the skipper.

1.3.4 Environmental Conditions on Survival Trials

CTD cast data are shown in Suppl. File 1 (please note CTD data available for February and March but not June due to CTD not working). Environmental conditions on the days animals were sampled after the recovery period are shown in Table 8. Air temperatures ranged from 6.5-7.5°C in February; between 6.4-9.7°C in March and between 11-12°C in June. These air temperatures are within average ranges reported in this area (Largs weather station 4°C February; 5.5°C March and 13.1°C in June). Furthermore, no significant changes were observed in the docking locations where animals were left for survival between time 0 and 48 h.

Table 8. General information and environmental conditions on the sampling days that animals from the survival trial were recovered on-board.

	Feb-Tow 1	**Feb-Tow 2**	**Mar-Tow 1**	**Mar-Tow 2**	**Mar-Tow 3**	**Jun-Tow 1**	**Jun-Tow 2**	**Jun-Tow 3**
Date	13/02/15	13/02/15	26/03/15	26/03/15	26/03/15	18/06/15	18/06/15	18/06/15
Air temp (°C)	6.5	7.5	6.4	8.3	9.7	12	11.5	11
Water temp Surface (°C)	7.51	7.51	7.47	7.47	7.47	11.5	11	11
Water temp Bottom (°C)	8.11	8.11	7.38	7.38	7.38	N.A.	N.A.	N.A.
Salinity water Surface (PSS)	32.61	32.61	31.32	31.32	31.32	N.A.	N.A.	N.A.
Salinity water Bottom (PSS)	33.24	33.24	32.88	32.88	32.88	N.A.	N.A.	N.A.
CTD number	13/02/15-CTD 1	13/02/15-CTD 1	26/03/15-CTD 1	26/03/15-CTD 1	26/03/15-CTD 1	N.A.	N.A.	N.A.
Time animals arrive on-board	10:09	10:07	09:45	13:04	13:04	09:51	11:30	14:25
Time start sampling	10:15	11:42	09:50	13:23	14:04	09:53	11:30	14:28
Time finish sampling	11:40	13:15	11:26	14:02	14:55	11:22	12:50	15:37
Total time aerial exposure (min)	91	188	101	58	111	89	80	69

1.3.5 Damage Index data after trawl and after recovery period

Taking the data from all the tows, 87.3% of the discarded *Nephrops* showed no observable external damage after trawling. This percentage decreased after the survival trial to 81.9% indicating that some animals were physically damaged (around 5-6%) during the handling process of putting the animals in and out the tube-sets and/or handling the boxes containing the tube-sets in and out the water (Fig. 10).

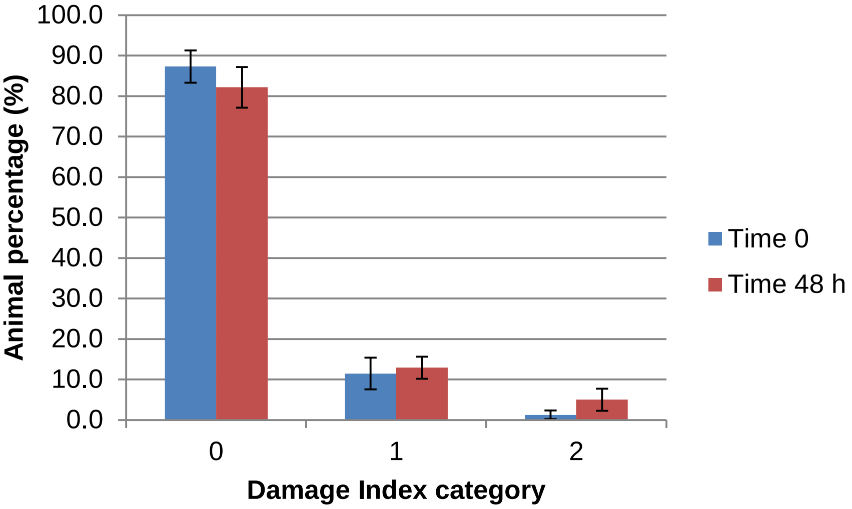

Fig 10. Average percentage of animals according to damage index from all discarded animals used in the survival trials. Data is mean ± S.D.

No seasonal effect was seen on the damage index, with values being quite low throughout (Table 9). Sex and size of the discarded animals did also not affect the damage index.

Table 9. Percentage (%) of discarded animals according to the damage index category for each survival trial.

Damage index

Time 0 h

	Feb-Tow 1	Feb-Tow 2	Mar-Tow 1	Mar-Tow 2	Mar-Tow 3	Jun-Tow 1	Jun-Tow 2	Jun-Tow 3
0	83.33	85.33	87.67	89.66	83.00	84.67	94.67	90.00
1	15.33	14.67	9.59	8.62	16.00	12.67	4.67	10.00
2	1.33	0.00	2.74	1.72	1.00	2.67	0.67	0.00

Time 48 h

	Feb-Tow 1	Feb-Tow 2	Mar-Tow 1	Mar-Tow 2	Mar-Tow 3	Jun-Tow 1	Jun-Tow 2	Jun-Tow 3
0	79.33	74.67	80.14	86.21	79.00	82.00	90.67	85.33
1	16.00	16.67	14.38	10.34	12.00	13.33	8.67	12.00
2	4.67	8.67	5.48	3.45	9.00	4.67	1.33	2.67

In June, soft animals showed a tendency to be more damaged, but even in this portion of the discards damage was quite low (Fig. 11).

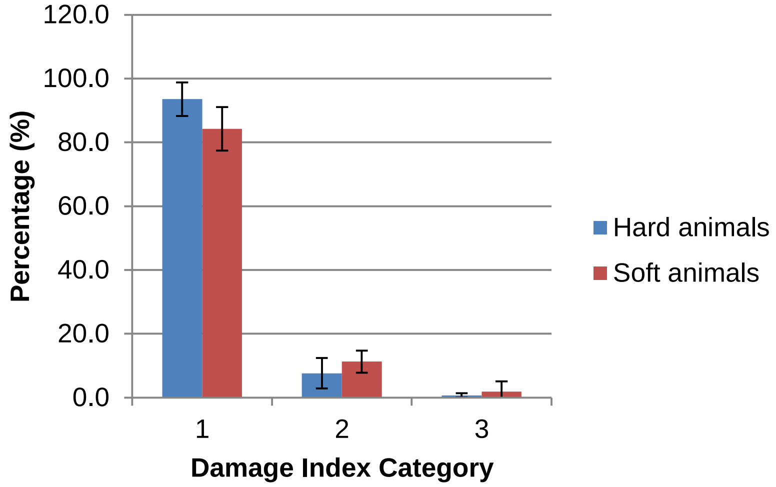

Fig 11. Average percentage of animals according to damage index from hard and soft discarded animals caught in the June trial. Data is mean ± S.D.

1.3.6 Survival and Vigour Index in *Nephrops* after 48 h of recovery period

Taking into consideration all the survival trials, 95% of discarded animals were alive after 48 h of recovery period in the tube-sets.

The recovery of discard *Nephrops* was therefore high, especially if we consider that after trawling (time 0) more than 50% of the discarded *Nephrops* were classified as vigour index C. Moribund and dead animals accounted for around 21% of the discards while around 27% of the discards showed a very active vigour after trawling (top categories A+B) (Fig.11).

After 48 h of recovery period at sea although 95% of discards were alive, when we classified them according to the vigour index animals classified as moribund + dead accounted for 12% of the animals in the recovery trials (Fig. 12).

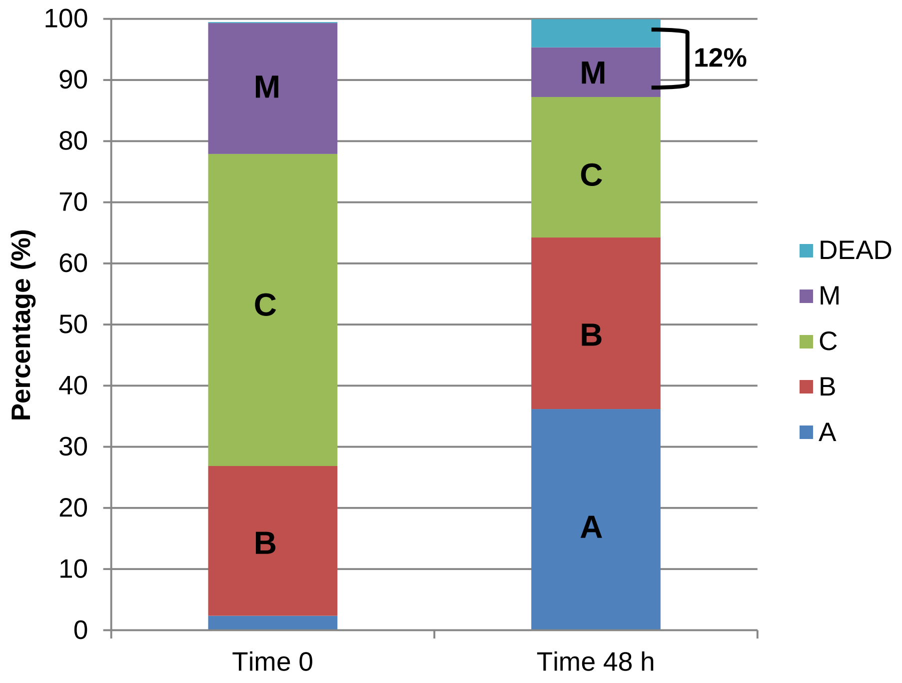

Fig 12. Proportion of animals classified according to the vigour index just after trawling (time 0) and 48 hours after recovery period at sea from all discarded animals used in the survival trials.

1.3.7 Vigour Index Seasonality effect

When looking at seasonality, data from vigour index at time 0 after trawling was quite variable between the different tows. The February trial in particular seemed to have a higher percentage of moribund animals compared with the March and June trials although there was a lot of variability between the two tows performed in February (data not shown). This could have been related to the relatively heavier catches in the winter trial although the obvious damage index data did not show such a strong pattern.

Interestingly, after 48 hour of recovery time the proportion of animals with a vigour A was higher in February/March compared to June regardless of their vigour at time 0 (Fig. 13).

This data would seem to indicate that although after trawling animals are in a similar vigour condition regardless of the time of the year their capability to fully recover their vigour is somewhat compromised in the summer months (June) with a lower proportion of animals having a vigour index A in June compared to February.

However, survival *per se* was not affected by season as the proportion of moribund + dead animals after 48 h was similar (around 10% in February; 16% in March and around 10% in June).

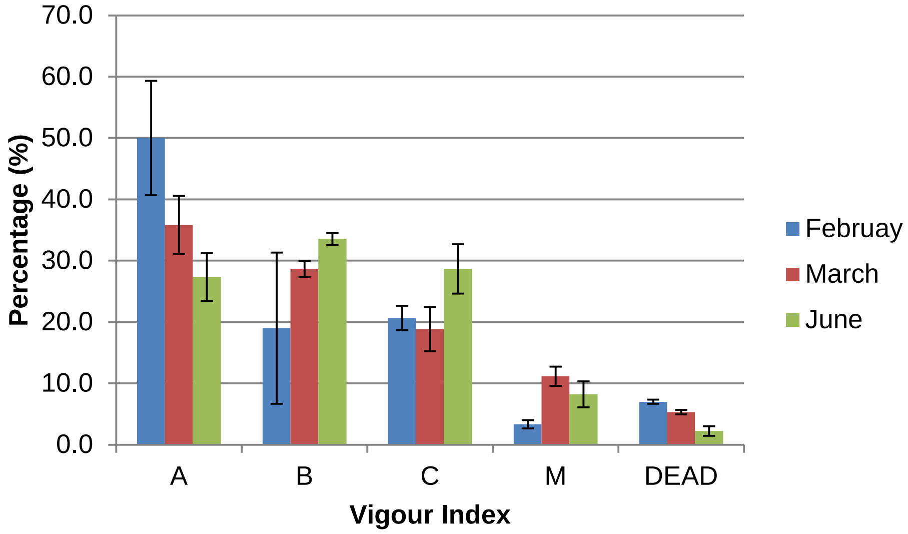

Fig 13. Proportion of animals classified according to the vigour index 48 hours after recovery period at sea from different seasonal trials.

1.3.8 Recovery potential according to initial vigour index and season

As shown in Fig. 14 the vigour index after trawl has an impact on the vigour that animals will display 48 h after the recovery period. So animals initially classified with a vigour B after trawl have good chances of improving their vigour condition to vigour A after 48 h. In fact less than 5% of them will become moribund or dead irrespectively of the time of the year.

Animals initially classified as displaying a vigour C after trawl also have good chances of recovery with only around 10-11% of them becoming moribund or dead also irrespectively of the time of the year. The recovery potential of moribund animals is somehow more compromised although still between 61-73% will have improved their vigour condition (vigour A+B+C) 48 h after the recovery period (please note that moribund animals accounted for around 20% of the discards at time 0).

Again plotting the vigour depending on the initial vigour index data indicates that season affects top vigour categories (higher in winter) but not the actual survival of the animals.

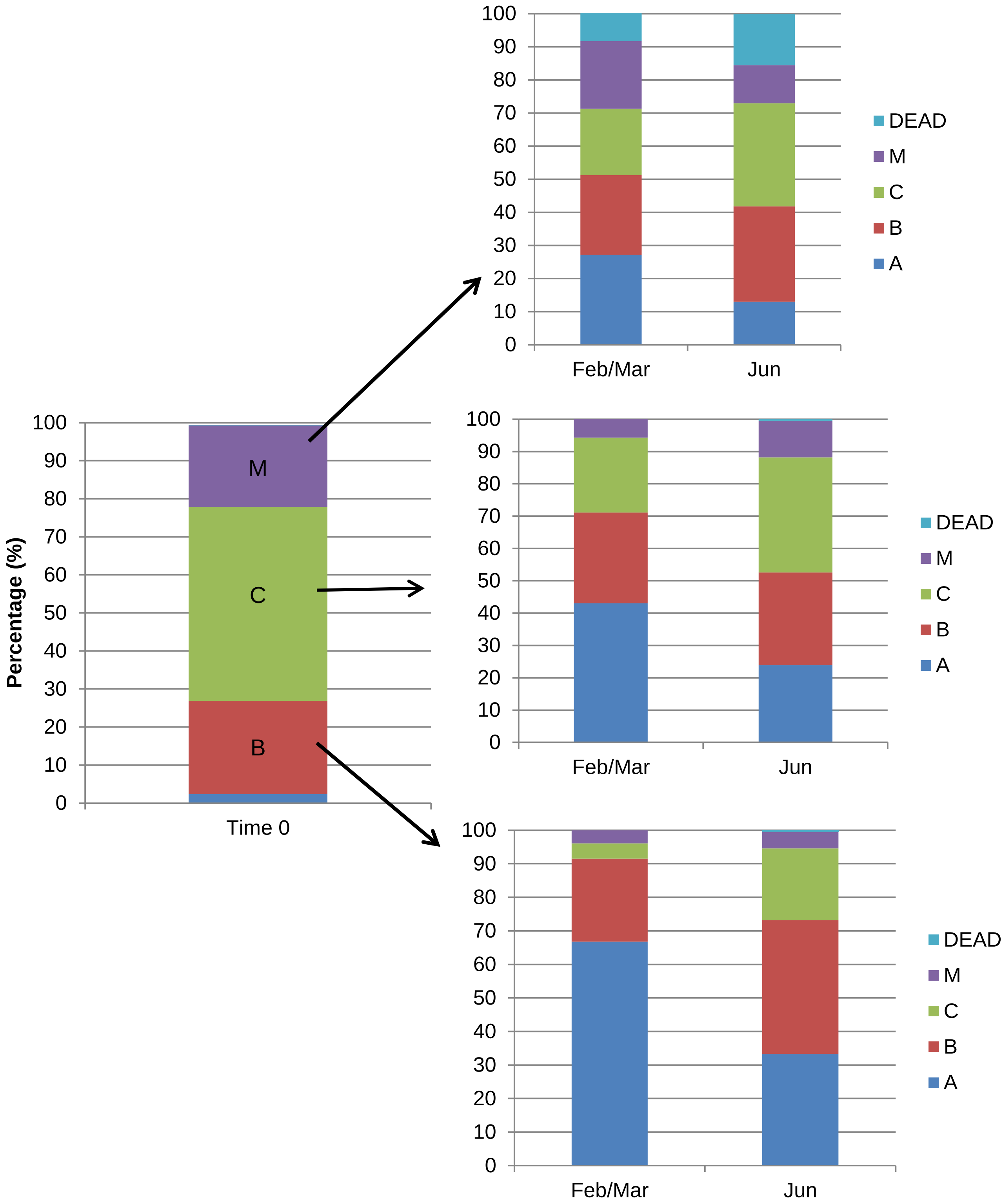

Fig 14. Proportion of animals initially classified with different vigour indices shown after recovery period at sea separating data from February/March separated from June data.

1.3.9 Survival and Vigour Index in visually infected *Nephrops* with *Hematodinium* spp. after 48 h of recovery period

Due to the fact that around 10-17% of the discarded animals were visually infected with *Hematodinium* spp. in February and March we also analysed the recovery on vigour of this subset of animals. As might be expected infected animals did not show as good recovery potential. Only around 8% of the visually infected animals were recorded as having a Vigour index A after the 48 h recovery period, together with animals classified as B the percentage was 20.4% (Fig. 15).

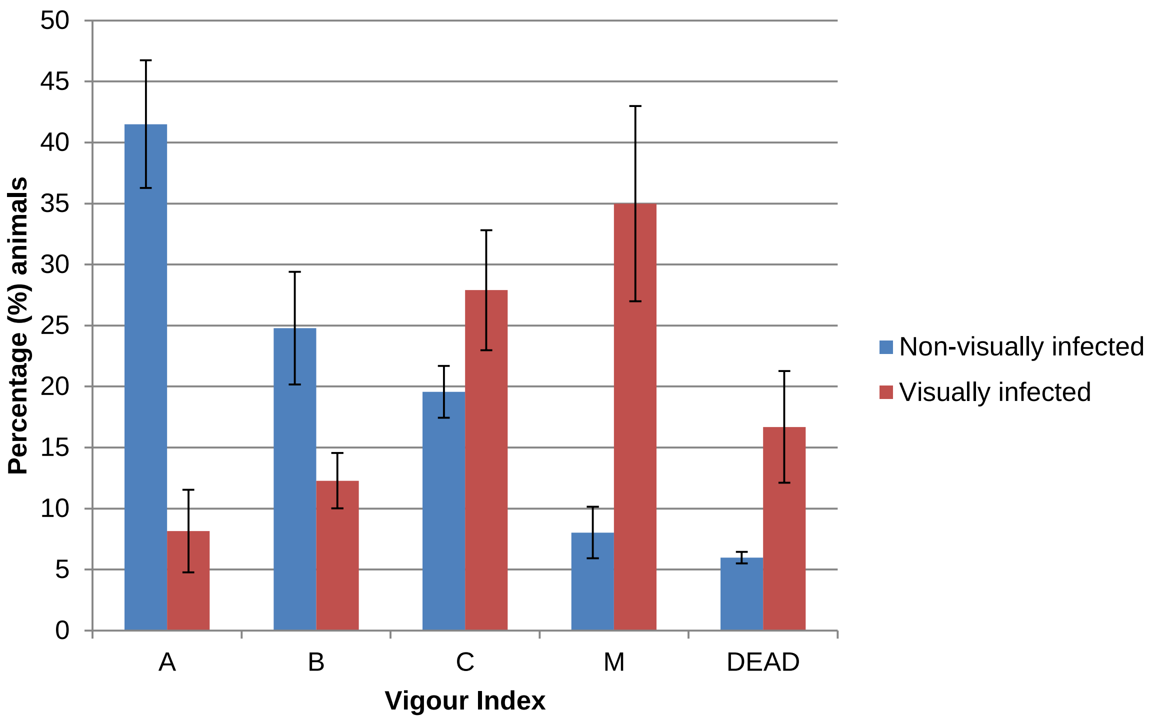

Fig 15. Non-visually and visually infected animals classified according to the vigour index 48 hours after recovery period at sea from all visually infected discarded animals used in the survival trials February and March.

Therefore, if for simplification we group vigour categories A+B+C as 'alive' then it becomes clear than more than 50% of the infected animals will be moribund or dead after the recovery period indicating poorer post-discard recovery for this portion of the catch (Fig. 16).

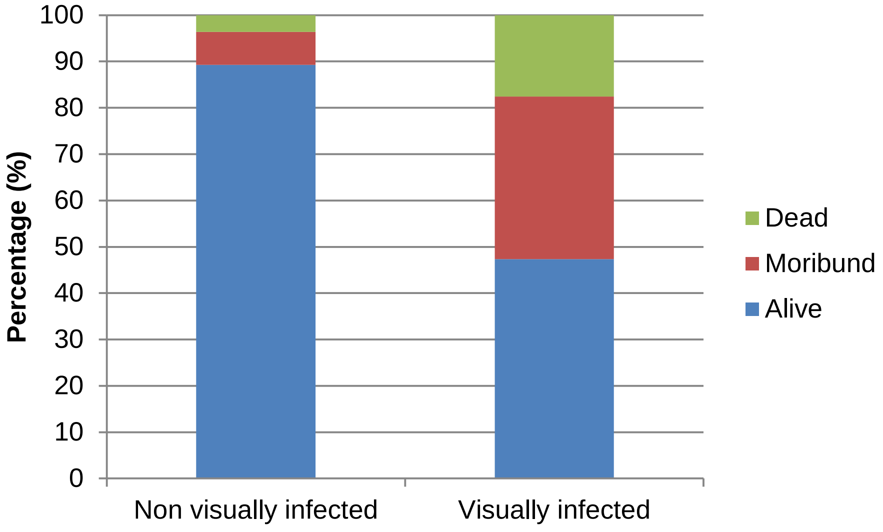

Fig 16. Simplified representation of Fig. 14 where vigour categories A+B+C have been merged as 'alive'.

1.3.10 Survival and Vigour Index in damaged animals after 48 h of recovery period

Although in this project the proportion of damaged discards was relatively low we have looked at the impact that being damaged has on the survival of discarded *Nephrops* after the 48 h recovery period. As shown in Fig. 17 as damage increases from non-damage (damage 0) to severely damaged (damage 2) the proportion of moribund and dead categories increases indicating that damage plays an important factor in the survivability of the discarded *Neprhops*. In this case and similarly to visually infected animals more than 50% of damage 2 animals are moribund or dead at 48 h of recovery period.

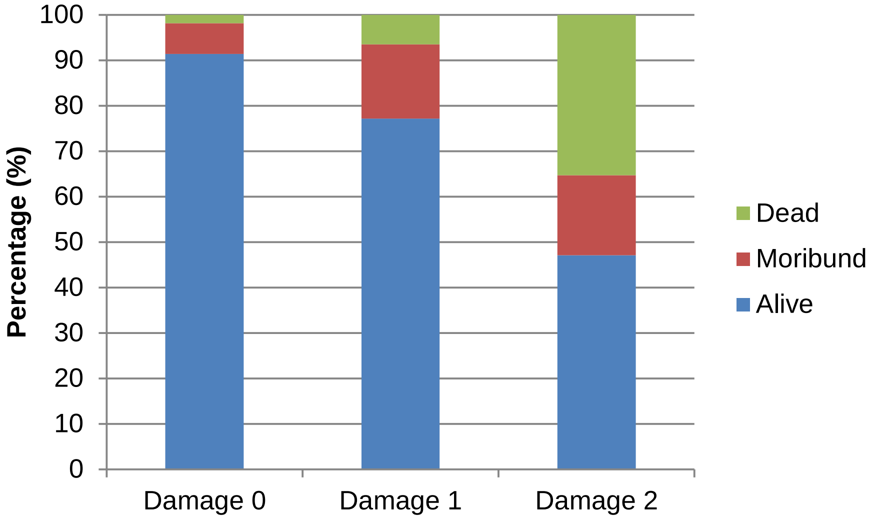

Fig 17. Proportion of animals according to the damage index in the alive, moribund and dead categories 48 hours after recovery period from all discarded animals used in the survival trials.

1.3.11 Biochemistry measures in Haemolymph and Muscle

1.3.11.1 L-lactate concentration in abdominal muscle and haemolymph

The concentration of L-lactate in the abdominal muscle immediately after trawl capture was 14.78 ± 0.32 µmol/g in March and 23.21 µmol/g in June. These values are considered relatively high for this species although lower than values previously reported in bigger *Nephrops* (32-37 mm CL) after being captured by trawl (Albalat et al., 2010). A significant reduction in L-lactate was observed for both seasons after a 48 h recovery period ($p<0.05$). A seasonal effect on the production of L-lactate in the muscle was also apparent ($p<0.05$). On the other hand, L-lactate concentrations in the haemolymph were much lower than those detected in the muscle and, although, a significant reduction was observed between after trawl and 48 h recovery, no seasonal effect was observed (Fig. 18).

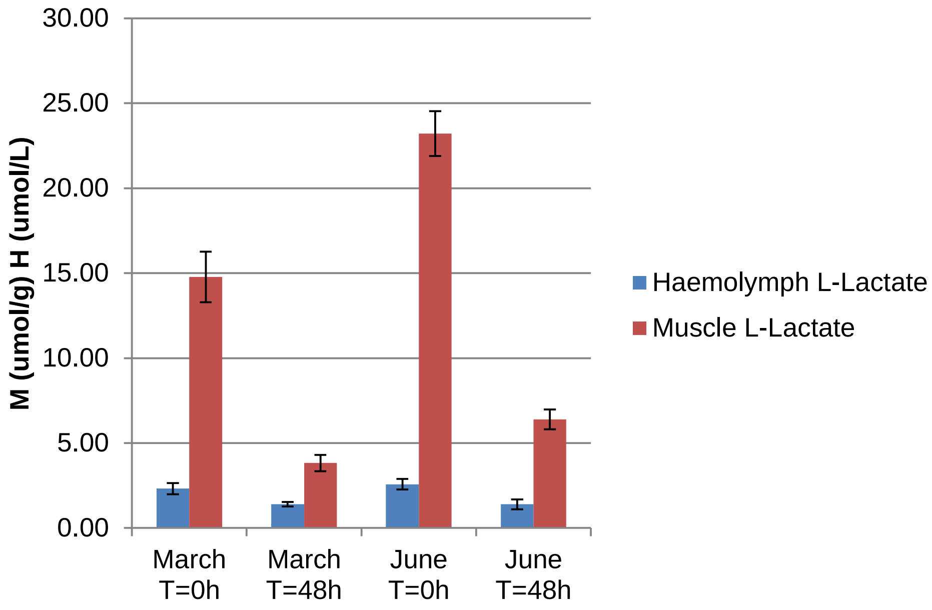

Fig 18. Abdominal muscle and haemolymph L-lactate concentrations in discarded Nephrops after capture by trawling and after a 48 h recovery period in tube-set boxes in March and June trials. Values represent the mean ± SEM of n=30 (for time zero values) and n=60 (for time 48 h recovery) for each trial.

1.3.11.2 Nucleotides and AEC values

The main nucleotide in the abdominal muscle immediately after capture was ATP in March and AMP in June. Animals at rest show ATP as the main nucleotide in muscle and only when animals are at advanced state of exhaustion is the main nucleotide AMP, instead of ATP. Therefore, the data indicated that discards in June were more physiologically exhausted from the trawl than discards in March. However, even animals analysed in March showed significant amounts of ADP and AMP accumulating in the muscle indicating that this tissue was in its way to exhaustion. In both seasons after a 48 recovery period ATP became the main nucleotide (Fig. 19).

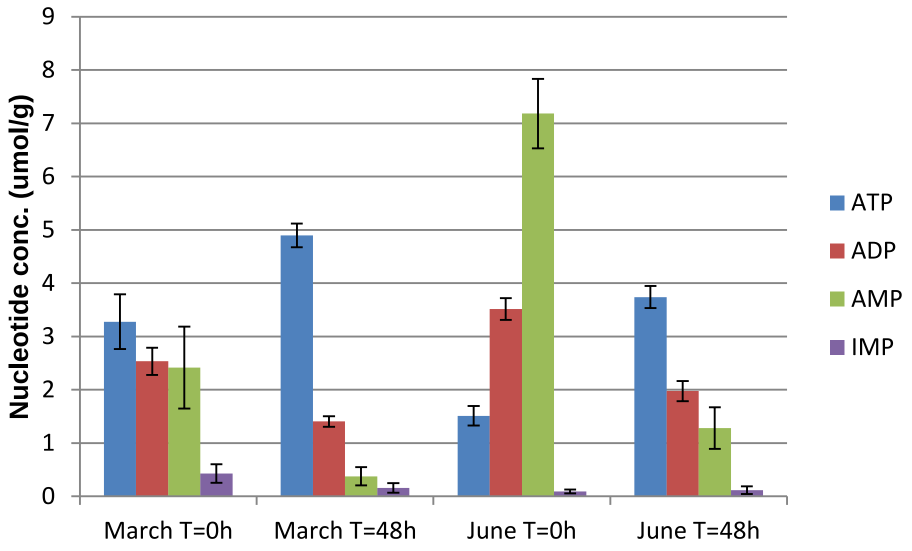

Fig 19. Nucleotide concentrations in abdominal muscle in discarded Nephrops after capture by trawling and after a 48 h recovery period in tube-set boxes in March and June trials. Values represent the mean ± SEM of n=15 (for time zero values) and n=40 (for time 48 h recovery) for each trial.

The balance between ATP and AMP is an important factor that can be represented as the 'Adenylate Energy Charge' or AEC ratio. This ratio indicates the energy status of the muscle. Lowest AEC values were obtained in June after trawling followed by March at the same time point. AEC values increased in both seasons after 48 h. However, even at this point AEC values in March (0.84) were higher than those obtained in June (0.75) (Fig. 20). For reference, AEC values in rested *Nephrops* have been reported as being > 0.8.

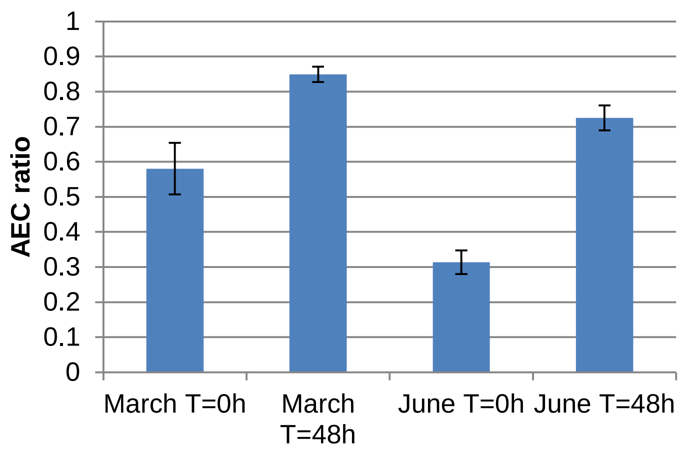

Fig 20. AEC ratios in abdominal muscle in discarded *Nephrops* after capture by trawling and after a 48 h recovery period in tube-set boxes in March and June trials. Values represent the mean ± SEM of n=15 (for time zero values) and n=40 (for time 48 h recovery) for each trial.

1.3.11.3 Total amount of protein (TAOP) and oxy-haemocyanin concentration

TAOP and oxy-haemocyanin were not affected by the trawling and recovery process. However, a clear seasonal effect was observed with discarded animals in June showing significantly higher concentrations of TAOP in the haemolymph. This increase in total protein observed in June could be related to the moulting process. During this key physiological process cryptocyanin increases in the haemolymph which may explain why protein concentrations were higher in June compared to March (Fig. 21).

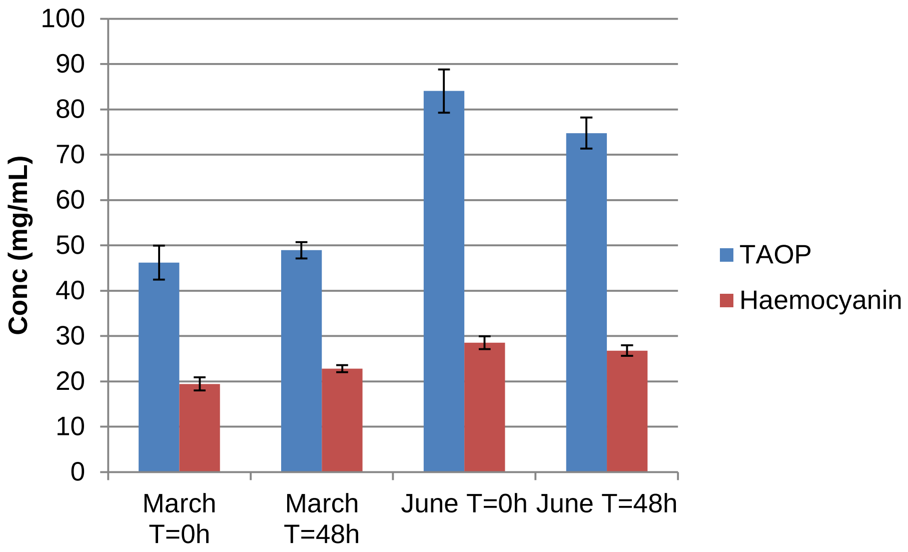

Fig 21. TAOP and haemocyanin in discarded *Nephrops* after capture by trawling and after a 48 h recovery period in tube-set boxes in March and June trials. Values represent the mean ± SEM of n=30-45 (for time zero values) and n=70 (for time 48 h recovery) for each trial.

1.3.11.4 Active polyphenol activity and Total polyphenol activity in haemolymph

PO activities are commonly used to assess immune-competence and health condition in crustacean species. The proteins proPO and Hc-PO are present within haemolymph and crustacean hemocytes (immune cells), respectively (Coates and Nairn, 2014). During immune challenge, the inactive proPO is converted into an active enzyme, namely PO, and Hc is activated into Hc-d PO. Both proteins convert phenolic compounds into microbicidal pigments that can kill pathogens and aid clot formation (Cerenius et al., 2010). In the present study, PO activity was higher at T0 and lower at T48 (indicative of recovery) in the March trial, but this effect could not be seen in samples from June trial. In fact, when the active PO activity was converted from units/min into % of active PO versus total PO, the same effect was seen in March with lower % of active PO after 48 h. In June, active PO values were higher after the recovery period indicating that the recovery was still in process in June (Fig. 22).

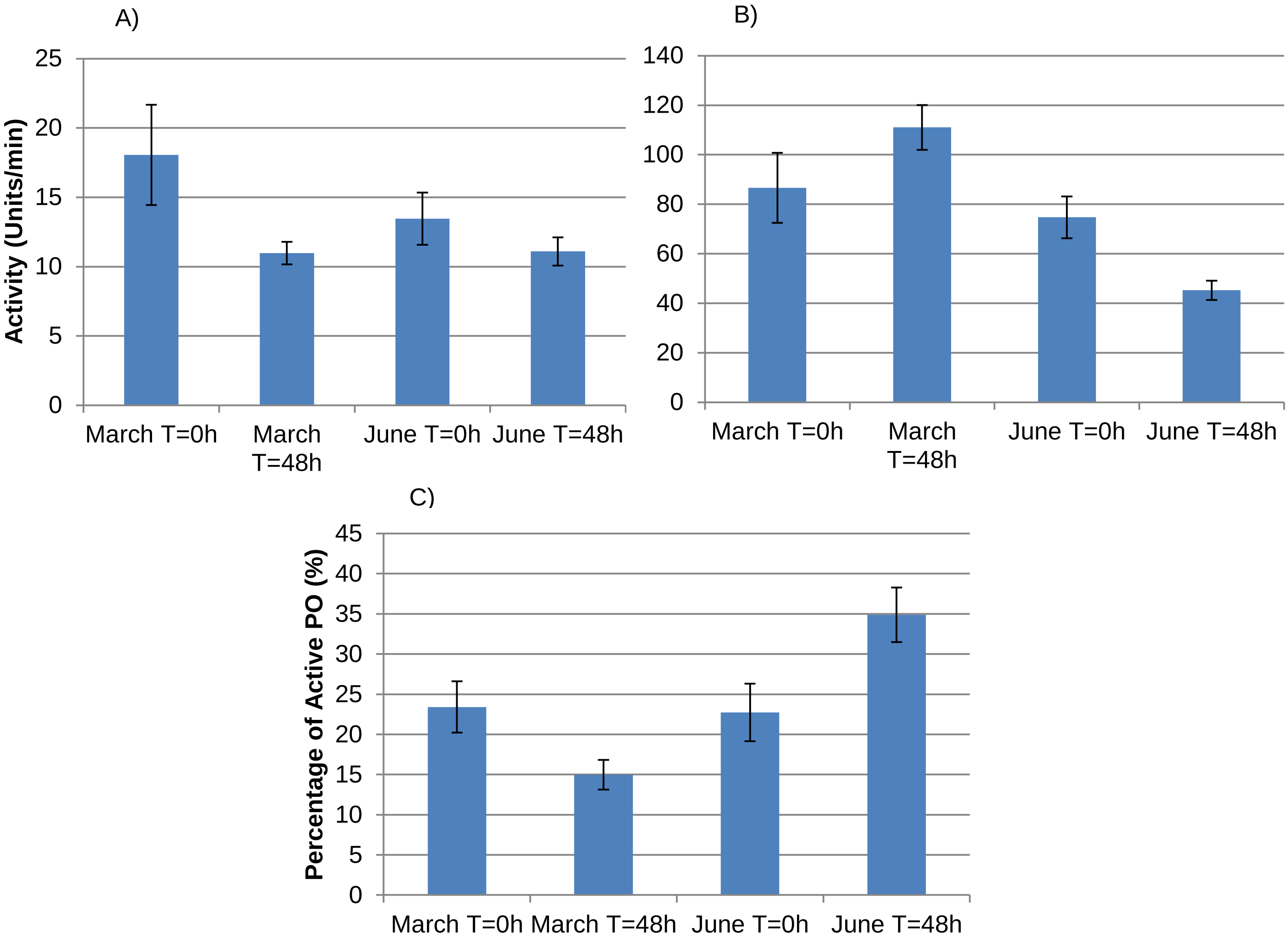

Fig 22. A) Active PO; B) Total PO and C) % active PO/total PO in discarded *Nephrops* after capture by trawling and after a 48 h recovery period in tube-set boxes in March and June trials. Values represent the mean ± SEM of n=30-45 (for time zero values) and n=70 (for time 48 h recovery) for each trial.

1.3.12 Modelling the probability of survival/death

Survival was analysed using a logistic regression with trawl date, carapace length and sex as covariates. Only trawl date was found to be significant from the other two. Results indicate that 95% confidence intervals (CIs) for the probability of dying were 4-10% in February, 3-8% in March and 1-4% in June.

This analysis was repeated, but treating damaged and infected discarded *Nephrops* as non-survivors (worst case scenario). Again only trawl date was significant and 95% CIs for probability of dying were 22-32% February, 26-36% March and 12-18% June.

To consider the effect of only discarding undamaged *Nephrops*, the second analysis was repeated on the subset of discards that were undamaged when trawled. This yielded 95% CIs for non-survival of: February 12-21%, March 17-27% and June 4-8%, representing an improvement in survival (over the set of undamaged plus damaged discards) of approximately 10%.

To consider the effect of only discarding non-visually infected *Nephrops*, the second analysis was repeated on the subset of discards that were uninfected when trawled. This yielded 95% CIs for non-survival of: February 18-28%, March 16-25% and June 11-18%, representing an improvement in survival (over the set of uninfected plus infected discards) of approximately 4%.

Therefore, using a logistic regression approach to estimate a more conservative figure of survival that takes into account that damaged and infected animals are effectively non-survivors indicates that even in this scenario if fishermen were to land only damaged animals then undamaged discards (including infected and moribund) would have a probability to survive of 82-79% in February, 83-73% in March and 96-92% in June.

1.4. <u>Discussion</u>

Data collected in this study has shown that the commercial vessel used in the trials was discarding approximately around 30% of the *Nephrops* catch. The commercial vessel's operations was characterised by relatively short tows as the skipper mainly targets the live *Nephrops* market. Therefore, the levels of discarding from this study cannot be generalised as this level is likely to vary between different fisheries and also vary depending on the market being supplied.

The discard fraction was composed of animals that had a CL smaller than the extra-small commercial category but bigger than the minimum conservation landing size (MCS), which for the Clyde fishery is 20 mm. In fact, almost no animals below the MCS were trawled in the Clyde (less than 1% of the discarded *Nephrops*). This is an important point as data indicates that under-sized discards (<20 mm) are not being trawled at least in the area studied. This data is in agreement with other studies (PhD thesis from Andrew Watts, University of Glasgow and Milligan et al. 2009) in the Clyde Sea Area.

In general, discards contained more females than any of the commercial categories throughout the year. Furthermore, a clear seasonal effect in the composition of the discards was apparent with more females being present in June (75%) compared to February (52%) and March (30%). These females discarded in June were bigger than their counterpart males and also were soft and had mature gonads being visible through the cephalothorax area (commonly referred to as 'green heads'). Therefore, as in June females emerge from their burrows to moult and mate due to their soft carapace and 'green heads' appearance they are discarded by the fishermen creating a seasonal change on the discards composition. Peak female prevalence in the catches has been reported in the Clyde Sea Area from May to June in agreement with our observations (Milligan et al., 2009). Another seasonal effect observed on the composition of the discards was the discarding of visually infected *Nephrops* by *Hematodinium* spp.. The percentage of visually infected animals was higher in February/March compared to June when almost no visually infected animals were actually discarded. The seasonality of *Hematodinium* spp. infection in the Firth of Clyde has been studied in detail. Data indicates that although a sub-patent infection is highly prevalent throughout the year, the patent infection is seasonal appearing predominantly in spring (Beevers et al., 2012). Interestingly, the fisherman involved in this study was able to identify and discard animals with a patent infection as shown by the discarding pattern of visually infected animals that were picked up in February and March. Therefore, discarding patterns should not be inferred based on studies conducted only during one time of the year.

Finally, damaged animals were also present in the discarded fraction of the catch (around 13%) although in general the percentage of damaged discards was relatively low in this study possibly due to the relatively low duration of the tows (between 1-2h).

Therefore, discarded animals in the present study were composed of animals with a CL smaller than the extra-small commercial category but bigger than the MCS, soft females with developed gonads (especially present in June), visually infected animals with *Hematodinium* spp (especially present in February/March), damaged animals and any other animals that the skipper consider not to be in a marketable form.

Taking into consideration all survival trials around 95% of discarded animals were alive after 48 h of recovery period in tube-sets. However, if we take into account moribund animals then the percentage of moribund and dead animals after the recovery period accounted for 21% of the discards. This recovery can be considered high especially if we take into consideration the physiological condition of the discards at time zero. After trawl, discarded animals had increased L-lactate levels in the muscle and haemolymph, low AEC values (indicative of muscle exhaustion) and increased PO activity (activated immune response). Similar results have been previously reported indicating that indeed trawling produces an activation of anaerobic metabolism in captured *Nephrops* in order to maintain (although not effectively) energy levels (Albalat et al., 2009). This initial physiological condition was more compromised in June compared to March (higher muscle L-lactate and lower AEC values in June) suggesting that animals were more exhausted in June. However, after the recovery period not only the vigour index improved in most of the discards but also their physiological condition. Interestingly, a seasonal effect was observed where the recovery to top vigour categories and biochemical indices was lower in June compared to the March trial. However, survival *per se* was not affected by season indicating that animals were able to cope with the extra physiological demands present during early summer. This seasonal effect could be attributed to the actual temperatures that animals faced during on-board handling (temperatures in March were around 8°C compared to 15°C in June) and/or also could be attributed to the condition of the animals at this time of the year (soft females near to moulting or recently moulted in June).

From the survival trials it also became apparent that visually infected and damaged animals had fewer chances to survival after the recovery period. For both conditions, the percentage of moribund and dead animals after 48 h of recovery was higher than 50%. These results could be expected given the physiological demands that both infection and damage can have on the condition of the animals. Indeed *Hematodinium* infection has been reported to place a heavy metabolic load on the host lobster, with reported reduced glucose in the haemolymph and significant lower levels of pancreatic glycogen (Stentiford et al., 2001).

Taking the data from all recovery trials survival was then analysed using a logistical regression with trawl date, CL and sex and covariates. From this analysis it became apparent that season was significant from the other two and therein a probability of dying was calculated according to the trawl date. Following this approach with the data gathered the probability of dying (95% CI) was 4-10% in February, 3-8% in March and 1-4% in June.

However, given the fact that visually infected and damaged animals had fewer chances of survival the analysis was repeated considering visually infected and damaged animals as non-survivors. Using this conservative approach (worst case scenario from a physiological perspective) the probability of dying were 22-32% in February, 26-36% in March and 12-18% in June. Given the weight that damage has on the probability of surviving we also calculated the probability of dying if fishermen were to land all damaged animals and were to return at sea only un-damaged discards. In this case, the probability of dying was 12-21% in

February, 17-27% in March and 4-8% in June. Therefore, from this logistical regression analysis it would appear that landing damaged animals (or therein reducing damaged animals due to the trawling and on-board practices) would have a positive effect on the survival of this portion of the catch.

Part 2: Behaviour of post-trawl discarded *Nephrops*

2.1 Summary

We undertook a series of behavioural observations on discarded prawns in the Firth of Clyde in order to obtain information on their fate under natural conditions. This section of work within the overall project was developmental because prior to the study there were many unknown factors such as: how rapidly the prawns would recover; how rapidly the prawns would move away from the landing site; what the visibility in the study site would be; whether currents would simply move animals out of the study site and how long animals could be practically observed for. Placing prawns within cages on the seabed would obviously solve the second issue and has been used as an approach in some survival studies. However such cages, even if not entirely surrounding the study site, will prevent predators reaching the prawns. We therefore opted to try studies in which the prawns were placed on an un-obstructed area of seabed. We constructed a camera rig cabled to an attendant research vessel allowing real-time video observations to be recorded at up to 50 m depth. Because visibility was likely to be limited we designed a system where the camera would be relatively close to the seabed (< 1 m). However, this means that each camera had a limited field of view so multiple cameras were installed to try and cover a larger area of seabed. A second practical problem was how to place animals under the camera rig. It had originally been planned to use an ROV for this but logistical issues meant that the ROV was not available on the dates when the trials had to be conducted. Instead the SAMS scientific diving team were employed to carry discarded animals down to the camera. As the study was conducted in water depths of 28-29 m dive time was restricted to about 5 minutes. Also each diver required a period of desaturation following the dive meaning that actual time underwater was constrained.

The camera rig was successfully deployed from the SAMS research vessel 'Calanus' on 29th July 2015 off Largs in the Firth of Clyde and video of the seabed captured. The study was conducted in collaboration with the same fishing vessel and skipper as parts 1 and 2 of this project. In total, five deployments were made beginning around 09:00. The skipper followed his normal fishing practice with tows being between 1.5 and 2 h duration. At the end of each tow the normal sorting procedure was followed excepting that a set of small prawns was placed in a separate tube-tray. The fishing vessel then steamed over to the research vessel and the set of small prawns transferred across. Ten animals then selected at random from the tray and their size and condition recorded before they were placed in a perforated plastic

container and taken down to the camera rig by diver. On reaching the rig the prawns were placed under the cameras. The diver stayed at the rig for some minutes making visual observations. Compared with the situation where under-sized prawns would be immediately discarded, this procedure caused some delay in animals being returned to the water but this was no more than 45 minutes and is compared with the time taken for normal commercial sorting in this fishery.

In the main deployments, where the delay in returning animals to the sea was as short as possible, nearly all animals (estimated at ~38 out of 40) recovered very rapidly. The exposure to seawater during the descent seemed to act as a stimulant from the more torpid state (generally vigour index A or B) observed on the research vessel. On being placed on the seabed, prawns exhibited almost immediate righting action and began moving around. The majority of the animals dispersed out of the camera view within a few minutes and seemed to exhibit normal behaviour. The only potential predators observed in the area were shore crabs (*Carcinus maenas*) and other predators, such as fish, did not seem to be attracted to the study site. In the initial test deployment the animals had been held on the research vessel in air for around 90 mins while equipment was being setup. For commercial sorting in this particular fishery this would probably represent the upper limit of time animals would be exposed to air before being returned to the sea. These prawns took several minutes to recover and several interactions with crabs were observed; at least one of these animals would probably have failed to survive.

These results suggest that small prawns caught in short duration tows in the Clyde tubing fishery and returned to sea within 45 mins of the net being hauled are in a good enough condition to recover rapidly on being returned to the seabed. Discarded prawns were able to recover to the extent that they began to move away from the discard site within a few minutes and seemed to exhibit normal interactions with each other (e.g. some defensive posturing followed by evasive movements). They should therefore be able to evade, or defend themselves from predators, at least to the same extent as un-trawled animals exposed on the seabed surface in the same area. However, when poorer condition animals were returned to the seabed, recovery took several minutes. Some of these prawns remained in an un-righted defenceless position which was a long enough to attract the attention of predators, such as crabs. These results suggest that if prawns are held in air for extended periods before being returned to the sea, a good proportion of these animals will probably not recover quickly enough to escape predation.

It is important to note that these results should not be extrapolated to other *Nephrops* fisheries with different fishing, sorting and catch handling practices. Similarly, in other locations the abundance and types of potential predators is likely to be different.

Although successful, the design of the camera rig could be improved for future use. Illumination was a particular problem as the present arrangement using mainly the LEDs built into the cameras tended to cause excessive glare from particles in the water. In the present study it also proved impossible in the time available to correctly align all the cameras so that a larger area of seabed was being consistently imaged. Ideally the rig should be fully set up and the camera positions adjusted and locked before transport to the field site. Preliminary pilot work at proposed study sites is recommended because prior knowledge is needed on the likely visibility as this affects the height that the camera layer needs to be set at. Despite these problems the camera rig did record useful data providing insight into the immediate fate of discarded *Nephrops* from this particular fishery in the Firth of Clyde. Although recovery of animals in this study was so rapid that it could be observed by diver, dive times at these depths are limited to around five minutes. The camera rig therefore has the potential to be used to make much longer observations on the fate of discarded animals, particularly those in poorer condition, or studies at greater depths. If studies were to be conducted in depths exceeding 30 m, an alternate method for placing study animals within the camera arena would need to be developed. Studies in water exceeding 50 m depth would require alternate cameras and/or an ROV to be used. Retention of animals within the relatively limited footprint of the camera arena (1 m^2) does remain an issue, especially when one does not wish to exclude potential predators.

2.1 Introduction

The immediate survival of discarded organisms can be broken down into several stages (Fig 23). If water column predators, such as cod, are in the area then discarded prawns in both good and poor condition will be susceptible to predation during their descent to the seabed. However, this descent is likely to be relatively rapid so the time period they are exposed to this source of predation should be short. Once they reach the seabed they may recover rapidly (indeed they may have recovered during the descent) and, assuming they have been discarded over suitable habitat, they should exhibit normal behaviour. Because prawns do move naturally around on the surface of the seabed as part of their normal behaviour, their overall survival rate should be similar to that of animals which have not been caught and discarded. On the other hand, if the prawns are in poorer condition, they will remain on the seabed in an inert state for some time. During this period they will be unable to defend themselves or take evasive action and so may be subject to increased rates of mortality. If they are in a defenceless position for some time this may also attract predators, probably via chemical cues in the water.

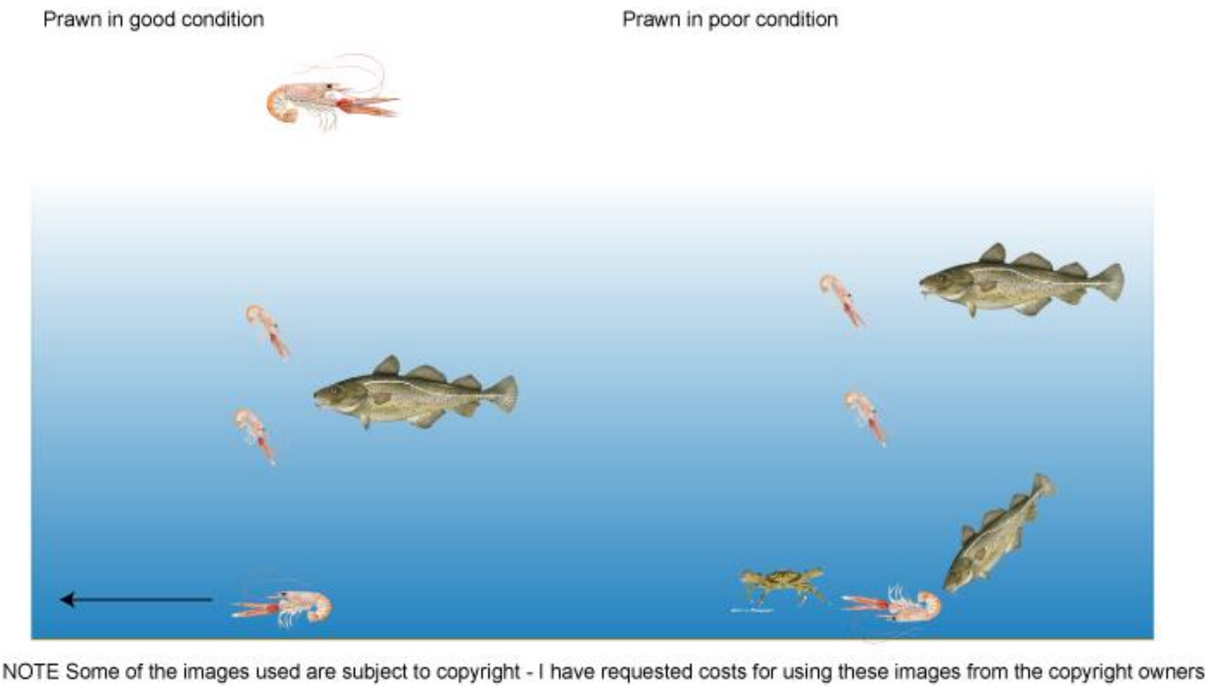

Fig 23. Potential behavioural interactions of discarded *Nephrops*

Most studies conducted to date on the fate of post-discarded *Nephrops* have been undertaken by allowing post-trawled animals to recover either in tubes (as in this study) or in cages (Harris and Andrews, 2005). Although this approach will provide information on whether the prawns have recovered physiologically they are protected from predation during recovery. If recovery actually takes some time, then the actual mortality rates experienced by un-protected recovering animals on the seabed are likely to be higher than estimated from recovery trials where the animals are protected. The rates of mortality experienced by post-trawled discarded prawns should therefore be related to (i) the length of time taken to regain normal predator avoidance behaviour (ii) the abundance of potential predators in the immediate area.

The aim of the present study was to collect information on the recovery rates of post-trawled discarded *Nephrops* under natural conditions. This is challenging because methods need to be applied which both allow observations over potentially extended time-periods but which also interfere with natural behaviour as little as possible.

2.3 Materials and method

At the start of this study we had little information on how rapidly discarded prawns might recover under natural conditions. Various observation options were considered including use of divers or ROVs. The study was likely to take place in water around 30 m depth and at this depth submersion time for divers is limited around 5 minutes. Using an ROV to make observations could potentially allow longer observation periods but a trial suggested it would be difficult to hover the ROV close to the seabed without raising a cloud of sediment. We therefore chose to trial a fixed camera frame which could be placed on an area of seabed and allow potentially long video recordings to be made. A 1 m^3 frame was constructed incorporating a height adjustable camera layer (Fig 2**Error! Reference source not found.**4). Because the visibility in areas such as the Clyde was likely to be low, we designed the frame so that the camera layer could be adjusted to be close to the seabed (< 1 m). This then led to the problem that most underwater cameras will have a limited field of view at these observation distances. Therefore six, 50 m submersible 12V cameras (VN37CSHR-W36, Visionhitech Co., Ltd, Korea) were mounted in the camera layer in a grid layout so that they would cover a larger area of seabed. The cameras were connected to a multi-channel Digital Video Recorder (Hybrid 16 DVR, Huviron Ltd., Korea) on-board the research vessel which would be anchored next to the rig. These cameras include built-in LED illumination and are powered by 12V DC supplies and only draw a few mA of power, thus minimising any electrical hazard had higher supply voltages had been required.

A pilot visit was undertaken to the Firth of Clyde and a number of potential study sites inspected using a small ROV (Deep Trekker (DTG2). A site with suitable depth and sediment was identified and where the visibility and currents seemed acceptable to allow video recording (see accompanying video clips). Because the rig would be deployed for at least 12 hours, along with an anchored research vessel, it was also necessary to identify a location where operations would not present a navigation hazard to other vessels.

The SAMS research vessel 'Calanus' travelled to the Clyde and deployed the camera rig on 29th July 2015 off Largs (55.805°N 004.882°W). The position of the camera rig had to be moved slightly before the last deployment because of a strengthening north-westerly wind. The final deployment took place in slightly shallower water (25 m) at 55.807°N 004.884°W. Air temperatures were recorded several times throughout the day using an electronic thermometer and three CTD casts were made using a Valeport Castaway (Valeport Ltd., Totnes, Devon, UK).

Prawns were obtained from the same fishing vessel and skipper as in parts 1 and 2 of this project. In total, five deployments were made beginning around 09:00. The skipper followed his normal fishing practice with tows being between 1.5 and 2 h duration (Table 12). The normal sorting procedure was followed at the end of each tow excepting that a set of small prawns was placed in a separate tube-tray. The fishing vessel then steamed over to the research vessel and the set of small prawns was transferred across. Ten animals were then selected at random from the tray and their size and condition recorded before they were placed in a perforated plastic container and taken down to the camera rig by diver. On reaching the rig the prawns were placed under the cameras. The diver stayed at the rig for some minutes making visual observations. The delays in animals being returned to the water were around 30 to 60 mins after the end of the trawl tow. Discussions with the skipper indicated that this would not be atypical of how long it would take to sort a catch. There was a longer delay (around 90 mins) for the first deployment, which was treated as a test-run. Air exposure of 90 mins before discarding would probably represent a worse-case scenario for this particular fishery.

Fig 24. The camera rig deployed at the study site at around 30 m depth. The mesh on top of the frame is to protect the cameras from the lifting strops if they were to drop down.

Recorded video was transferred from the DVR as MP4 files and edited in Solveig Multimedia video splitter. Individual video streams were merged into a composite montage using a custom Matlab script. A summary of the video clips resulting from this study is given in Table 13.

2.4 Results

Recordings were successfully captured from the camera rig (Fig. 25**Fig**). Unfortunately in the time available it proved impossible to optimise the alignment of the cameras so that the area of seabed imaged was less than the planned 1 m^2. This was due to the fields of view changing once the cameras were underwater caused by refraction so that although the cameras had been aligned on deck, they were no longer aligned underwater. Ideally the whole rig would need to have been tested, all the cameras aligned and locked in position while in shallow water prior to the field study but, because of time constraints, this proved impossible. Nevertheless, the results showed that with some additional work the system would be capable of monitoring around 1 m^2 of seabed over extended periods of time. Problems with illumination were also encountered but this could be improved in future by replacing the in-camera LED illumination with oblique infra-red lighting.

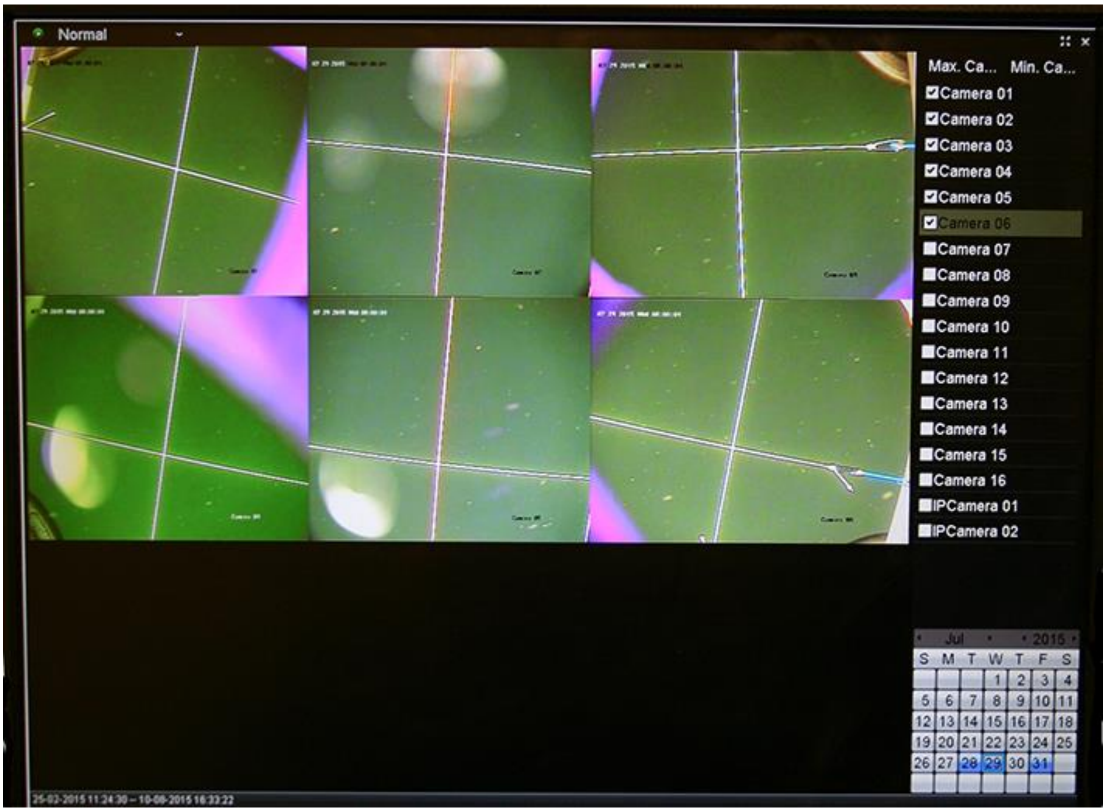

Fig 25. DVR monitor showing images from the six cameras – up to 16 cameras could potentially be included

Table 12: Deployment details (times UTC)

		Prawn	1	2	3	4	5	6	7	8	9	10
Deployment 1	Trawl began	06:00										
	Trawl tow ended	07:30										
	Prawns transferred to Calanus	07:45										
	Prawns placed under camera	09:09										
	Post-trawl treatment	~90 mins in air										
	Air temperature (^oC)	9.5										
	Comments	Test dive (sizes of animals not recorded)										
Deployment 2	Trawl began	07:45										
	Trawl tow ended	09:45										
	Prawns transferred to Calanus	10:00										
	Prawns placed under camera	10:50										
	Post-trawl treatment	~ 60 mins in air										
	Air temperature (^oC)	16.5										
	Size (mm)		28	30	30	28	28	30	30	30	31	29
	Vigour		B	A/B	A	A/B	B	B	B	A/B	B	B
	Comments	Spare animals were sunk in tube-tray at 20 m and also used in deployment 3										
Deployment 3	Trawl began	As above										
	Trawl tow ended	As above										
	Prawns transferred to Calanus	As above										
	Prawns placed under camera	11:20										
	Post-trawl treatment	~ 30 mins air, 65 mins recovery at 20 m										
	Air temperature (^oC)	17.0										
	Size (mm)		25	27	28	28	29	27	28	28	31	32
	Vigour		B	B	B	A	A/B	C	B/C	B	B	B
	Comments											
Deployment 4	Trawl began	10:30										
	Trawl tow ended	12:30										
	Prawns transferred to Calanus	12:43										
	Prawns placed under camera	13:04										
	Post-trawl treatment	~ 30 min air										
	Air temperature (^oC)	17.3										
	Size (mm)		28	26	25	28	26	26	29	26	27	27
	Vigour		A	B/C	B/C	B	B	A/B	B	B	B	B

Table 12: Deployment details (times UTC)

		Prawn	1	2	3	4	5	6	7	8	9	10
Deployment 5	Trawl began	13:00										
	Trawl tow ended	14:40										
	Prawns transferred to Calanus	15:00										
	Prawns placed under camera	15:30										
	Post-trawl treatment	~ 50 mins air										
	Air temperature (^{o}C)	17.3										
	Size (mm)		25	25	25	25	25	28	26	29	26	22
	Vigour		B/C	B/C*	B/C	B/C	B/C	B/C	B/C	B/C	B/C	B/C
	Comments	Deployment used to diver observations and to take flash stills photographs, video not analysed further										

*This prawn was in damage category 1 (loss of claw), all others were in category 0 (no obvious visible damage)

Table 13: Summary of video clips accompanying this report. Note that it was not possible in the time available to get the cameras aligned optimally so that there is partial overlap in the images recorded in each camera and the total area of seabed imaged is less than the 1 m^2 which was planned.

Filename	Timestamp (hh:min:sec)	Time since release (min:sec)	Summary main events
MillportSitePilotSurvey			Quite high current flow near seabed, poor visibility
LargsSitePilotSurvey			Lower current flow near seabed (video shot at similar time to Millport site), seabed generally visible, reasonably flat mud with occasional epifauna
Montage_Deploy1	09:09:23	00:00	Prawns released into arena
	09:09:44	00:21	2 prawns show almost immediate recovery
	09:09:48	00:25	Diver withdraws from site
	09:10:11	00:48	Prawn at top of group exhibits a flick-tail escape response, another prawn begins to recover and move around
	09:10:53	01:30	Another prawn recovers and moves away; 5 unrecovered animals remain
	09:12:00	02:37	Four more prawns begin to show signs of recovery, limb movement, attempts at righting
	09:14:53	05:30	All prawns except one showing some signs of recovery although three animals have still failed to fully right themselves.
	09:16:23	07:00	All prawns except two have either left the arena or are moving within the arena
	09:18:13	08:50	Prawn at top left interacts with another recovering animals and exhibits a flick-tail escape
	09:18:53	09:30	All prawns righted, all except one moving around, squat lobster enters arena and one prawn evades
	09:19:33	10:10	Prawn nips squat lobster which moves away, one prawn still only partially righted (camera 5)
	09:20:07	10:44	Squat lobster attacks partially righted prawn
Montage_Deploy1 con/td	09:20:36	11:13	First crab arrives and chases off squat lobster
	09:20:39	11:16	Crab attacks recovered prawn but prawn exhibits escape response

	09:21:13	11:50	Crab attacks damaged prawn which exhibits a flick-tail response but then fails to right itself (moving from camera 5 to 6)
	09:21:31	12:08	Second crab then moves in to attack a separate prawn (camera 5)
	09:21:37	12:14	Both crabs then attack the prawn
	09:21:53	12:30	Prawn is dragged out of camera arena by one crab and second crab resumes searching
Montage_Deploy2	10:49:54	00:00	Prawns placed in camera arena
	10:50:01	00:07	Eight or nine prawns seem to recover almost immediately and begin to disperse
	10:50:08	00:14	Remaining prawns right themselves and begin moving around
	10:51:01	01:07	All animals appear to be moving around within, or have exited the arena, rate of tidal flow picking up compared with earlier deployment
	10:51:44	01:50	Single prawn re-enters arena, moving around on seabed
	10:51:50	01:56	Second prawn re-enters after exhibiting tail-flick escape response from some unseen stimulus
	10:53:05	03:11	End
Montage_Deploy3	11:20:54	00:00	Tide had picked up and diver reported that the prawns were all active but dispersed very rapidly, video of a single animal walking within the arena
	11:21:35	00:39	End
Montage_Deploy4	13:04:35	00:00	Prawns placed in arena, camera 3 not working
	13:04:40	00:05	Four animals show good recovery and move away
	13:04:45	00:10	All animals have righted themselves and are beginning to recover
	13:05:02	00:27	Three animals remain within arena
	13:05:44	01:09	Four prawns continue moving around within arena
	13:06:31	01:56	Two prawns show interaction then move apart
	13:06:49	02:14	
	13:10:00	05:25	End

During the study the air temperature rose from 9.5°C in the early morning to around 17°C by 10:00. CTD casts made at the observation sites showed a layer of lower salinity water reaching down to around 5 m depth (Fig 26). Water temperatures were warmer at the surface (11.5 to 13°C) dropping to around 10.7°C below 15 to 20 m depth.

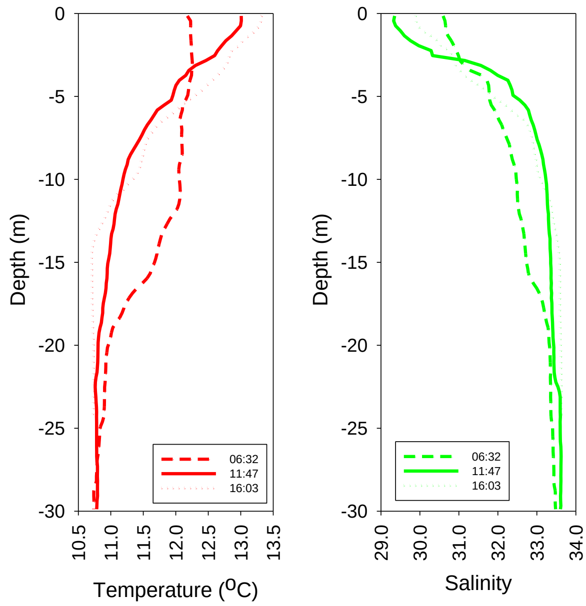

Fig 26. CTD water column profiles from the camera deployment locations

Virtually no under-sized prawns were caught by the commercial vessel during the day so animals in the smallest size class (22 – 32 mm C.L.) were used for the observation trials. All test animals, except one, were in damage category 0 i.e. no visible signs of damage. Prawns caught in the morning were all in vigour index A or B after transfer to the Calanus. The vigour indices of prawns caught in the afternoon seemed slightly lower (mainly B/C). This was probably a result of the higher air temperatures they were exposed to during sorting on the fishing vessel.

In the main deployments (deployments 2 through 5), the delay in returning animals to the sea was kept relatively short (30-45 mins air exposure) and nearly all animals (estimated at ~38 out of 40) recovered very rapidly. The exposure to seawater during their descent seemed to act as a stimulant and most animals were active shortly after being released from the plastic container. As mentioned above the vigour of animals in the last deployment was slightly lower (mainly B/C), yet, apart from one animal, they also recovered rapidly. On being placed on the seabed, prawns exhibited almost immediate righting action and began moving around (Table 1313). The majority of the animals dispersed out of the camera view within a few minutes and seemed to exhibit normal behaviour. The main potential predators observed in the area were crabs (*Carcinus maenas*) and other predators, such as fish, did not seem

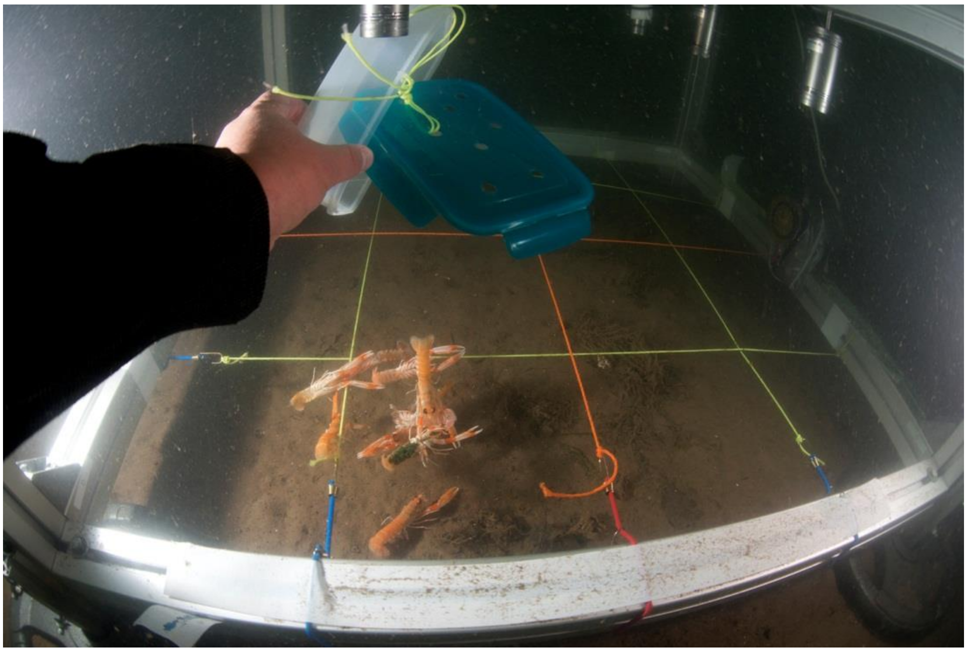

Fig 27. Underwater still showing prawns being released by diver under into the camera arena (Deployment 5).

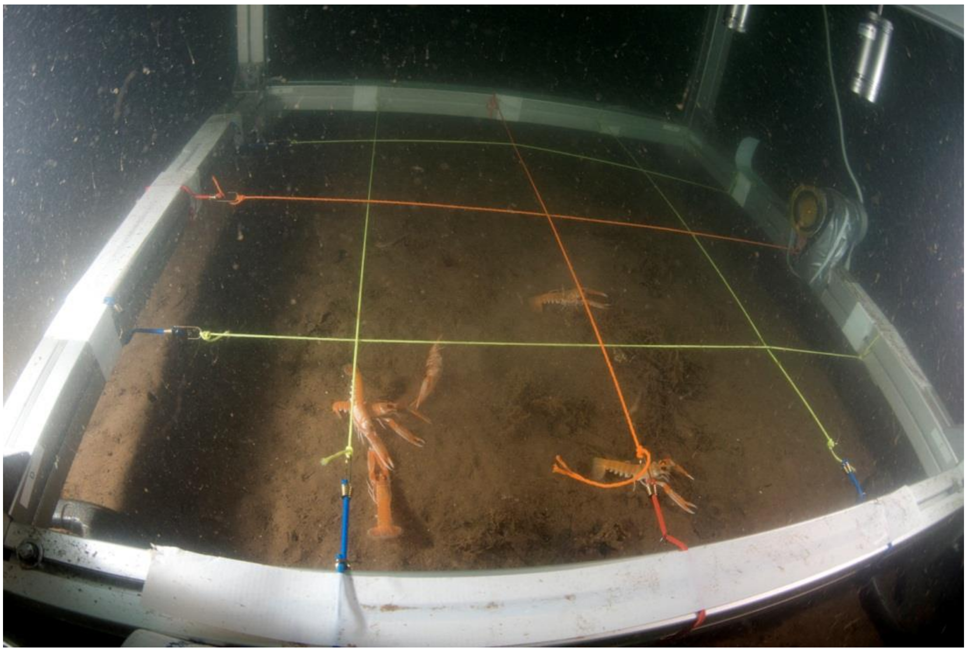

Fig 28. Underwater still taken a few minutes after release of prawns. Note that there is a gap beneath the edges of the frame so that both prawns and potential predators were free to move in and out of the arena. Since ten animals were released into the arena, four had already moved out of the arena by the time the photograph was taken (Deployment 5).

to be abundant around, nor attracted, to the study site. In the initial test deployment the animals had been held on the research vessel for around 90 mins while the equipment and dive team were being prepared. This provided an interesting comparison to the main deployment results. These prawns did not recover so rapidly on being placed on the seabed, although eight out of ten did recover eventually. Several interactions with crabs were observed and at least one animal would probably have failed to survive (the prawn in question being attacked by two crabs and eventually being dragged out of the arena by one of the predators - Table 13).
Despite the problems with the camera system, useful video footage was captured showing the behaviour of post-trawled prawns as they were placed on the seabed under reasonably natural conditions (Figs. 27 and 28). Table 13 summarises the video clips which accompany this report.

2.5 Discussion and conclusions

The results suggest that small prawns caught in short duration trawl tows in the Clyde tubing fishery are in a good enough condition to recover rapidly on being returned to the sea. Discarded prawns which had been in air for up to 60 mins were able to recover within a few minutes and begin to move away from the study site. They should therefore be able to evade, or defend themselves from predators, at least to the same extent as un-trawled animals on the seabed surface in the same area. However, when poorer condition animals, which had been held in air for around 90 mins, were returned to the seabed, recovery took noticeably longer. Some of these prawns remained in an un-righted defenceless position for several minutes which was a long enough to attract the attention of predators, such as crabs.

However, all the animals used in the behaviour trial were either un-damaged or at most had minor physical damage. Furthermore, they were all hard-shelled, did not have obvious *Hematodium* infection and were mostly in vigour condition A/B before re-immersion. The recovery trials in Part 1 of this report showed that soft-shelled, damaged or infected *Nephrops* have lower survival rates over 48 h in protected enclosures. It is also likely that such animals might not recover so rapidly on re-immersion and thus might also suffer higher rates of predation shortly after discarding.

In descending to the seabed prawns must also cope with a change in water temperature and salinity. In the Clyde, particularly in some inshore areas, freshwater run-off leads to a warmer, lower salinity layer near the surface. When prawns are kept for any extended period in such water they will begin to suffer stress (Ian Wightman pers. com.). Although we were not able to track the fate of prawns freely released at the sea-surface, we might assume that because the amount of time spent in the upper few meters of the water column is probably rather small after discarding, brief exposure to this warmer, less saline water, should not cause much ill effect.

Overall these results suggest that if prawns are held in air for extended periods (90 mins) before being returned to the sea, a proportion of these animals may not recover quickly enough to escape the attention of predators. In the present study, crabs were attracted to the discarded prawns within about 10 mins. Such relatively rapid predation events will not be apparent in recovery experiment data where the animals are protected from predators in enclosures. Such physiological trials are clearly important but should be supplemented with behavioural studies, as conducted here. Similar results might have been obtained in aquarium tanks but it is generally impractical to transport post-trawled animals to suitable research aquaria in the short periods of time required. Tank-based experiments could also be set up on the research vessel although it would probably be difficult to replicate the conditions on the actual seabed (levels of illumination, abundance of predators, currents etc.) all of which might affect behavioural results.

The camera system proved to be a cheap and reasonably effective solution to making underwater video recordings of animal behaviour on the seabed in depths of 30 m. In particular, it allowed longer recording periods than could be achieved by divers operating at these depths (Deployment 1). However, the design of the camera rig could certainly be improved for future use. Despite the use of multiple cameras, a relatively small area of seabed (around 1 m^2) can be imaged. When visibility to the seabed is very low, for example due to sediment being raised by currents, even this coverage is hard to achieve. Further exploration of using wider angle cameras, or cameras with tilt and pan capability might help with this although in sites with low visibility, effective imaging will always be challenging. Illumination was also a particular issue as the present arrangement, relying mainly on the LEDs built into the cameras, tended to cause excessive glare back from particles in the water. Further testing of oblique illumination should overcome this problem. In the present study it also proved impossible, in the time available, to align all the cameras optimally. Ideally the rig should be fully set up in shallow water and the camera positions adjusted and locked before being transported to the field site. Preliminary pilot work at proposed study sites is also essential because prior knowledge on the likely visibility is required to allow the height of the camera layer to be set during pre-deployment. Relatively sheltered sites with low tidal currents are also required for such studies from a logistical perspective and the safety of other shipping around the study site must also be considered.

Despite these problems the approach generated useful video data providing insight into the immediate fate of discarded *Nephrops* from this particular fishery in the Firth of Clyde. Although recovery of animals in the main deployments in this study was so rapid that it could mostly have been recorded by diver, dive times at these depths are limited to around five minutes. The camera rig proved especially useful in recording the fate of discarded animals in poorer condition (deployment 1) where recovery took in excess of 5 mins. The camera rig could also prove very useful if it proves necessary to conduct similar studies at sites deeper than 30 m. However, in this case an alternate method for placing study animals within the camera arena would probably need to be developed because scientific diving is limited to one dive per day beyond 30 m depth. Retention of animals within the relatively limited footprint of the camera rig (1 m^2) remains an issue although potentially test animals could be tethered if the main objective were to assess likely responses to predators.

5. The extent to which the objective were met

The objectives of this project were met as shown in the executive summary and information contained in this report.

The first objective was to determine the survivability of discarded *Nephrops* from a physiological perspective. This was accomplished by performing three independent experiments. Please note that in the proposal we projected to do two experiments but we have been able to deliver an extra trial. Furthermore, we have also been able to do extra biochemical measures that were not included in the proposal. With all the information collected we have been able to give not only an absolute survival measure but also we have determined the physiological condition of the discards taking into consideration seasonal effect and the impact of damage, vigour, moulting and infectious status.

Secondly, the project tested whether a seabed camera system could be used to observe the post-discard behaviour of the prawns and whether they attract predators if their escape responses are delayed by stress recovery. In this project we have successfully deployed the SAMS camera system and collected informative observations on the behaviour of discarded *Nephrops* and any interactions with predators under natural conditions. All the videos recorded are available to Fisheries Innovation Scotland through a CD.

6. Details of possible future research

We have already discussed the fact that the results from this study should not be extrapolated without care to other *Nephrops* fisheries. In particular, post-discard survival rates will be affected by the amount of damage and stress the *Nephrops* experience during capture – factors which will be affected by tow durations, size of catch, amount of by-catch as well as sorting time and how they are handled on board the fishing vessel. The behaviour work highlighted the importance of the local predator field when *Nephrops* are discarded, particularly if *Nephrops* are not in optimal condition and take some time to recover. The local predator field is also expected to vary with discarding location. In the Clyde the most common predators/scavengers appeared to be crabs and squat lobsters whereas in other areas, fish might be more common.

The levels of discarding are also likely to vary between different fisheries. *Nephrops* discarded in the Clyde fishery were nearly all above the minimum conservation size limit and were discarded either because they were too small to market, or for other reasons e.g.

damaged, soft-shelled or diseased. However, it is possible that in other grounds size composition would be different and in those cases the discarding of under-sized *Nephrops* would have to be evaluated (for example other parts of the Clyde including Aisla Craigh where smaller size animals have been reported-ICES 1998). Discarding patterns would likely need to be established for individual fisheries because they will vary, depending on the market being supplied. In some fisheries discarding may even be a relatively minor issue and therefore intensive research might not be justified. There was also clearly a seasonal pattern in the Clyde fishery discards with smaller animals being more common at certain times of year but more soft-shelled female *Nephrops* being discarded at other times. Discarding patterns should not therefore be inferred based on studies only conducted during one part of the year.

The behaviour studies we conducted, although limited in extent, clearly demonstrated that discarded *Nephrops* are unlikely to survive long if they do not recover rather rapidly. The physiological studies also showed that metabolic stress indicators were still detectable, even after 48 h recovery. Further work may be required to understand whether these levels of physiological stress significantly impair the ability of animals to escape predators. Given the short time period in which animals must recover to avoid predation (< 10 mins in this study), further work on the levels of physiological stress and muscle energy reserves after short recovery periods may be needed. Moreover, testing recovery allowing for longer observations than 48 h would be relevant. In this study, survival trials were conducted for 48 h as this time has been shown to be sufficient for stress-related physiological parameters to recover (Albalat et al., 2010) while avoiding a negative impact of keeping the animals in an artificial set up such as the tube-sets. Investigating alternative methods for holding and assessing survival of discarded *Nephrops* over a longer time period of 48 h would indeed be very informative.

The camera system developed proved to be a relatively cheap approach for making observations of the behaviour of post-discard animals under reasonably natural conditions at the sort of depths (~29 m) animals are being discarded at in this fishery. However, the design of the camera rig could be considerably improved with a bit more time. In particular, ensuring camera alignment before deployment to the field, using the full 9 camera array and improving the illumination system are recommended.

Logistically the Clyde was an ideal location for conducting this study due to close proximity of relatively shallow (~30 m depth) sheltered water etc. If it is deemed necessary to undertake similar work in more offshore locations e.g. the Minches, then careful consideration to logistical aspects, especially for any underwater behaviour work would be needed.

Given the lower survival rates for *Hematodium* infected *Nephrops* noted in this study, consideration should be given as to whether discarding of infected animals should continue as this may only serve to amplify infection levels.

It is hoped that the results generated by this project will also help inform future decisions regarding whether continued discarding of *Nephrops* should continue in this fishery once the landing obligation comes into effect. If discarding of *Nephrops* is permitted to continue, a potential out-reach product based on this project could be a short set of guidelines for fishers on discarding *Nephrops* in the Clyde tubing trawl fishery (and possibly any other *Nephrops* fisheries with similar characteristics), in terms of maximising the survival chances for animals to be discarded - recommended handling, maximum time of air exposure, approach to diseased and/or damaged *Nephrops* etc.

7. List of publications arising out of the project

Due to the confidentially agreements in place no publication have been available to the public, fishing or academic communities. Once Fisheries Innovation Scotland agrees to make this data public authors are planning to:

- Summarise the results from this project in a publication that will be submitted to a Fisheries Journal.
- Organise a meeting with Clyde Fisheries Association to do the knowledge transfer from this project.
- Authors will be presenting this data also in the Marine Alliance for Science and Technology for Scotland Annual Meeting in October 2015.

Acknowledgments

The authors wish to acknowledge financial funding from the Scottish Fisheries Innovation Centre. The authors would like to acknowledge the enthusiastic co-operation of Ian Wightman, the skipper of the Eilidh Anne trawler; the skipper and crew of the SAMS research vessel ‘Calanus’ and the NERC Scientific Dive team (based at SAMS) without whom this work would not have been possible. The authors are also grateful to Mr Arnaud Collard, MSc student in Aquatic Pathobiology, Institute of Aquaculture, University of Stirling for his laboratory work during the project and Dr Christopher Coates, Swansea University for his guidance and expertise in the Phenoloxidase and haemocyanin analyses.

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