SMALL GRANT REPORT TO MASTS

Ref: SG287 Title: Population consequences and metabolic cost of being 'bolder' in Norway lobster (*Nephrops norvegicus*).

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Abstract
This study assessed (i) the existence of different degrees of boldness in the commercially exploited Norway Lobster *Nephrops norvegicus*, (ii) whether trawled animals differ in personality compared to those captured with creels and (iii) whether metabolic rate differs between *Nephrops* displaying bold and shy personalities. To this end, three personality tests were developed and applied to assess the degree of boldness, and the metabolic rates of selected specimens were tested in respiration chambers. We show for the first time that *Nephrops* display individual differences in the degree of boldness responses and that bolder responses are significantly higher in creeled animals compared to trawled ones. On the other hand, resting metabolic rates did not differ between the two personality types. This preliminary study is not yet comprehensive enough to allow assessing possible long-term population consequences of the two fishing methods. Collaborative follow-up studies are planned.

Background
The Norway lobster (*Nephrops norvegicus*) sustains one of the most important fisheries in the UK. *Nephrops* is a mud-burrowing decapod crustacean that is mainly caught by demersal trawl gear vessels, although a well-established inshore creel fishery is also present, especially in Scotland. *Nephrops* populations are known to undergo seasonal cycles of moulting and breeding, which have a very significant impact on the composition of males and females caught by trawl gear. Mature females emerge from their burrows to moult and mate during early summer after egg hatching has occurred and become more prevalent in the trawl catches. This female prevalence in trawl data generally lasts until late summer when they return to their burrows for the rest of the year (Milligan et al., 2009). The seasonal pattern in the behaviour of female *Nephrops* is important to understand from a fisheries perspective as the intrinsic behaviour of this species might protect females from being caught by trawling for most part of the year.
Data from inshore creel fishers targeting *Nephrops* is more scarce and difficult to obtain as vessels operate in a patchy fashion. Similar sex-seasonal patterns have been reported in creel fisheries as for trawling, however creeling could exert differential pressures upon

Nephrops, such as for instance a selective pressure on larger animals in the population (Adey 2007). Potential reasons behind the size-selectivity of creeling could be the fact that larger animals have been shown to spend more time per day foraging and therefore cover larger areas, which can lead to greater chance of capture (Chapman and Howard, 1979; Chapman et al., 1975). Furthermore, larger animals might deter smaller animals from entering the creels (Adey 2007). However, other factors such as personality rather than only size *per se* could additionally explain the selective pressure that creel fishing exerts on *Nephrops* populations in these more protected inshore fishing areas. This is based on the evidence that in other invertebrate species bold individuals are more curious and show higher risk-taking behaviours in a new environment as well as lower latency times to approach novel objects, whereas shy individuals are less willing to explore novelty and have higher latency times (Briffa et al., 2008; Mowles et al., 2012). Recent studies have also begun to observe links between these personality traits and underlying metabolic demands (Huntingford et al., 2010). This study aimed to identify personality traits in *Nephrops* and determine whether different capture methods – trawls and creels – attract animals with different degree of boldness. To this end, we developed and applied three personality tests to assess the degree of boldness in *Nephrops*.

Objectives

1- To establish boldness personality tests for *Nephrops norvegicus.*
2- To evaluate if bolder responses are more prevalent in creels compared to trawls.
3- To determine the metabolic cost associated with boldness.

Material and Methods

Experimental Set Up

Nephrops were caught in the Firth of Clyde, Scotland, UK. Trawled animals for the setting-up of the personality tests and final testing were kindly donated by 'Scotprime Ltd.' based in Troon and creeled *Nephrops* were purchased by a local shellfish trader 'Prentice Seafoods' in Tarbert, Scotland. Animals were transported to the University of Stirling campus by car in containers full of seawater with approximately 10 lobsters per container. On arrival sex, carapace length (CL, mm), damage and vigour were recorded. Damage index was based on classification used by Milligan *et al*, (2009) and vigour index as explained in Albalat *et al*, (2015). Vigour classifications were converted to numerical format for analysis (A=1, B=2, C=3).

Animals were individually identified using coloured bands placed on the propodus of the first periopod (cheliped). *Nephrops* were acclimatised for a week before any behavioural tests were carried out. Trials were carried out in a cold room where temperature was set at 11°C (±1). Water temperature in the holding tanks was 10.5°C (±0.5°C) and salinity between 35-37 PSS. The tanks contained numerous cuts of piping to provide shelter for the lobsters. Vigour and damage indices were recorded daily during the acclimatisation week and after that they were recorded every day that the animals were handled for testing.

Behavioural Testing

Three behavioural tests were performed during the course of this study: A tapping test, consisting of holding the animal for a very short period of time in order to assess the fight or flight response of individual animals (Table 1); a novel object test, commonly used in behavioural studies to determine exploratory behaviour (Table 2); and a feeding test to provide an insight into an individual animal's response to a food item. The main objective of these behavioural tests was to find individual differences in behaviour between individuals that are a reflection of different personalities in *Nephrops*. These tests were then applied to the trawled *Nephrops* sourced at the end of January 2016 and the creeled *Nephrops* sourced in early March 2016. During these testing periods the tests were repeated twice (repeatability) with an interval of one week between each trial.

Table 1 – Different responses of the *Nephrops* during the tapping test. The "freeze" and "retreat" responses were interpreted as the shy responses, whilst "turn to face stimulant" and "no response" were extreme bold responses. The "tailflip" response was expected to be the standard response.

1	Freeze
2	Retreat
3	Walk forward
4	Tail flip
5	Turns to face stimulant
6	No response

Table 2 – Graded responses of the individuals from the novel object test. Touching the novel object was the boldest response and having no response and moving closer but not approaching the object were viewed as the neophobic shy responses.

1	Touches novel object
2	Approaches but does not touch novel object
3	Moves closer but does not approach novel object
4	No response

Respiration Rates in Nephrops norvegicus

Resting metabolic rate was measured with optical respirometry equipment available at Edinburgh Napier University. Once personality testing had concluded, the trawled and creel-caught *Nephrops* were transferred to four 120L tanks in the AquaLab of Edinburgh Napier University. The animals were acclimated to the new facility for two weeks in advance of the respirometry testing. Metabolic rate measurements then involved two steps: acclimation of the animals into the respirometry chamber (24 h) and the measuring of oxygen consumption in a closed system until oxygen saturation decreased to 80% (approximately 30 mins). After placing individual lobsters into a respirometry chamber, black felt was wrapped around the chamber (including the ends) to prevent *Nephrops* from reacting to or becoming stressed by any external movement in the lab. During acclimation to the respirometry chambers, the chambers were connected to a pump to provide fresh water to flow through them. There were two tanks set up for this experiment, holding five chambers each, four containing

Neprops and one blank control (Fig. 1) to measure respiration of bacteria present in the seawater (that later were subtracted from calculations). The water volume inside the *Nephrops* chambers varied between 1.36 and 1.41L, depending upon the size of the animals. Prior to measuring their respiration rates, the inflow and outflow valves of the respective chamber were closed. An optode was screwed onto a connector glued to the outside of the light transparent respirometry chamber, on top of an optical sensor spot glued to the inside of the chamber. The optode was connected to a Fibox 3 Trace Oxygen Meter (Presens, Germany), as well as a temperature sensor which was places in the tank holding the respirometry chambers to measure the water temperature whilst measuring respiration rates.

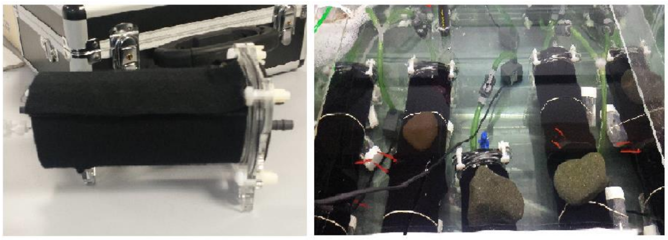

Figure 1 – Set up during respirometry experiment. Image on the left shows one respirometry chamber. Image on the right shows the tank set up during testing, four long chambers containing *Nephrops* and one blank in the centre.

Final concentrations were calculated using the following equation:
VO_2 = oxygen consumption rate (mg/g/h)
$[O_2]_1$ = oxygen concentration from lobster chamber ((initial $[O_2]$ – final $[O_2]$) * volume)
$[O_2]_2$ = oxygen concentration from blank chamber ((initial $[O_2]$ – final $[O_2]$) * blank volume)
W = weight of animal (g) and T = time (mins)

Results

Sex, Size and Animal Condition in Trawl and Creel-caught Nephrops norvegicus

As the aim of this study was to study personality differences between trawl and creel-caught Nephrops animals from similar size range were used. The mean carapace length (CL) of the trawl-caught *Nephrops* was 42.6 ± 0.7 mm (n=80) whilst that of the creel-caught lobsters was 44.2 mm ± 0.7) (n=22). A two-tailed Student's t-test showed that these sizes were not significantly different ($t_{(59)}$=-1.58, p>=0.05) (Fig 3).

Regarding the sex of the animals, from the trawl-caught lobsters 1 out of 80 sampled specimens was a female (1.25% of total catch) while 5 (22.7%) out of 22 creel-caught lobsters were females.

Mean vigour of trawled *Nephrops* was 1. 6 ± 0.1 compared to 1.8 ± 0.1 of creeled lobsters. The difference between the two was not significant. Increased levels of damage were documented in the trawl-caught *Nephrops*, 37% as opposed to 9% found in the creel-caught lobsters while mortality was also higher in the trawl-caught animals with 35% of *Nephrops* not surviving to the end of the behavioural testing period while only 13.6% mortality was recorded in the creel-caught sample.

Trawled vs Creeled Nephrops Data

The non-parametric Mann-Whitney test was used to analyse the ordinal data collected from the tapping test. There was no significant difference between the responses of the trawled or creeled lobsters ($W_{(60)}$=767.5, p>=0.05). Interestingly, however, there were much more "no response" recorded in the creeled group at 15% as opposed to the 6.45% in the trawled group. A repeated measures ANOVA was used to analyse the effect that capture method had on the lobsters' responses from the three behavioural tests. Trawled and creeled lobsters showed no significant difference in their startle response durations from the tapping tests ($F_{(1,60)}$=0.05, p>=0.05). However, the number of times the creeled lobsters touched the novel object was significantly higher than that of the trawled lobsters ($F_{(1,60)}$=9.32, p<=0.01) and the latency time of the creeled lobsters to approach the novel object was significantly shorter than that of the trawled group ($F_{(1,59)}$=5.33, p<=0.05) (Fig. 2). A correlation was found between these two variables (r=-0.568, p<=0.001). In the feeding test, creeled *Nephrops* were again significantly quicker to feed ($F_{(1,59)}$=9.00, p<0.01) than the trawled ones (Fig. 3).

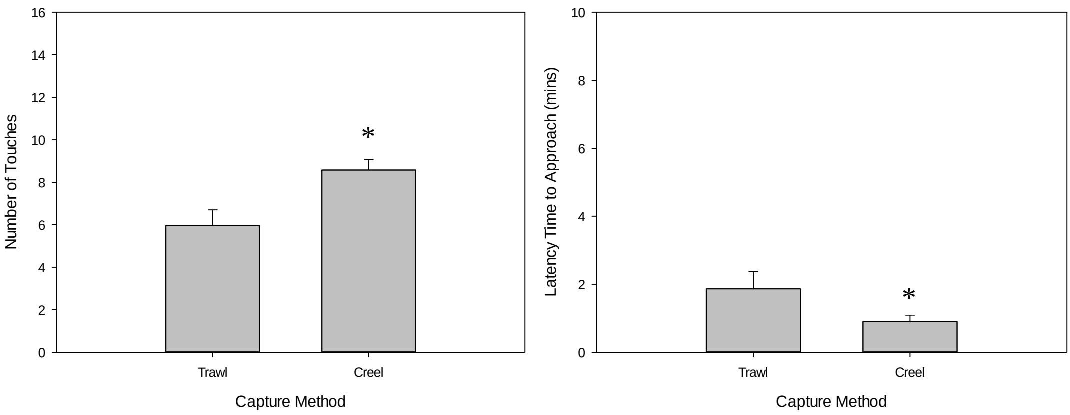

Figure 2 – Number of times *Nephrops* touched the novel object and latency time (mins) according to the capture method. Values represent mean ± SEM (n=62). (*) indicates significant differences between groups (p<0.05).

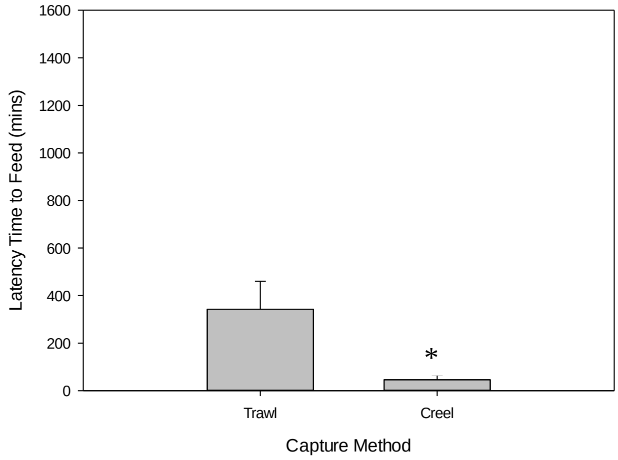

Figure 3 – Latency time (min) that it took for the *Nephrops* to eat. Values represent the mean ± SEM (n=62). (*) indicates significant differences between groups p<0.05.

Respirometry Analysis

Analysis of this data was carried out comparing the values from *Nephrops* showing bolder responses compared with individuals showing shier responses from both capture methods. No significant difference in resting metabolic rate was found between bold ($W_{(6)}$=22, p>=0.05) versus shy *Nephrops* ($W_{(4)}$=14, p>=0.05).

Conclusions, Implications and Further research

The shellfish industry is a growing trade leading to increased fishing pressures on commercially important species, including *Nephrops norvegicus*. Our behavioural data suggest that creels select for *Nephrops* with bolder traits than trawls and also attract more females. On the other hand, we observed no link between resting metabolic rate and animal personality. Whether or not this could have long-term population consequences cannot be concluded with current data.

In addition to the conducted research, this funding has allowed to establish a new collaboration between the academics involved from Universities of Edinburgh Napier and Stirling. The results are promising and given the synergy between the academics further collaborative work in this area is planned.

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