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Short communication

Validation of a vigour index for trawl-caught Norway lobsters (*Nephrops norvegicus*) destined for the live market: Underlying links to both physiological condition and survivability

Amaya Albalat[1], Simon Sinclair[2], Douglas Neil*

Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, G12 8QQ, Scotland, United Kingdom

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ABSTRACT

Recent improved practices in the trawl fishery for Norway lobsters (*Nephrops norvegicus*) have made it possible to increase the proportion of trawl-caught lobsters that can be transported alive successfully. A major contributor to this has been the introduction of on-board seawater tanks, which allow for the recovery of animals immediately after they have been landed from the net. In this study, we have validated a vigour index that could be used both by fisheries scientists and by the industry dealing with live-traded *Nephrops*, allowing identification of the proportion of trawl-caught lobsters that fail to recover following capture and are not in a condition to survive live transportation. Results indicate that the process of visual selection into one of four possible vigour categories reflects with good accuracy the underlying physiological state of the animals, as assessed by the level of adenylate 5′ –triphosphate in the tail muscles, by the proportions of other nucleotides as expressed in the Adenylate Energy Charge, and by the amount of intra-muscular L-lactate present. The vigour index also correlates well with their subsequent survival potential in a semi-dry transport system.

1. Introduction

Live crustaceans attract premium market prices, but for successful live transport crustaceans must initially be in a good physical and physiological condition (reviewed by Fotedar and Evans, 2011; Neil, 2012). This is determined by the nature of the capture process used (Wilson et al., 2014) and also by the post-capture handling procedures (Milligan et al., 2009; Raicevich et al., 2011; Leocádio et al., 2012; Lorenzon et al., 2013). Improved practices in trawl fisheries, such as on-board recovery tanks, have made it possible to increase the proportion of trawl-caught crustaceans that can be transported alive successfully (Albalat et al., 2010). However, a number of trawl-caught animals fail to recover, and such animals do not survive subsequent live transportation, which may take 24–72 h.

There have been several studies looking at the physiological condition of *Nephrops norvegicus* (hereafter referred to by genus alone) and other shellfish species during live transport (Lorenzon et al., 2007, 2008, 2013; Barrento et al., 2010, 2011) mainly by means of 'wet' vivier transport. Recently, an alternative to this 'wet' vivier transport is the transportation of live *Nephrops* in a 'semi-dry' state, packed in polystyrene boxes and transported via standard refrigerated vehicles (Philp et al., 2015).

Designing visual index-based protocols based on behavioural metrics has proved to be a very useful approach for predicting mortality in various live-traded crustaceans (reviewed by Stoner, 2012). Several such visual vigour indices have been used in studies on *Nephrops* (Bernasconi and Uglow, 2008; Barrento et al., 2010, 2011), including an index we have developed (Albalat et al., 2016) that is currently being used by our industrial partner to screen live product for 'semi-dry' vivier transport. The aim of the present study was therefore to validate this vigour index by measuring a set of physiological condition-related parameters in animals from the different vigour categories, and also by determining if the index is a good predictor of the subsequent survival of the animals following transport.

* Corresponding author at: Institute of Biodiversity, Animal Health and Comparative Medicine, Graham Kerr Building, University of Glasgow, Glasgow, G12 8QQ, Scotland, United Kingdom.
E-mail addresses: amaya.albalat@stir.ac.uk (A. Albalat), douglas.neil@glasgow.ac.uk (D. Neil).
[1] Present address: Institute of Aquaculture, School of Natural Sciences, University of Stirling, Stirling, FK9 4LA, Scotland, United Kingdom.
[2] Present address: Scotprime Seafoods Ltd., 11 Whitfield Drive, Ayr, KA8 9RX, Scotland, United Kingdom.

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Table 1
Characteristics of the different vigour index categories of trawl-caught *Nephrops*, as used in the trials.

Category A	Category B	Category C	Category D	Dead
Animal displays a 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 a 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 limb 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 rigidly up at 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	

2. Material and methods

2.1. Capture and holding conditions

Nephrops were caught by otter trawl in the Clyde Sea area, Scotland, UK (55.35 N, 04.54 W; depth range 60–80 m) in late May (23/05/08; spring time conditions). The vessel used was the M.V. *Seren Y Don*, fitted with a single hopper trawl net with a cod-end nominal mesh size of 80 mm, towed at approximately 2 knots.

Nephrops of commercial grade 3 (30–40 individuals per kilogram), which equates approximately to a size range of 27–37 mm carapace length, were stored in tube-sets (Suppl. Fig. S1) placed in aluminium tanks on board the vessel (160 *Nephrops*/tube set box). On board tanks were constantly supplied (on an overflow basis) with running surface seawater via the vessels deck hose. Seawater temperature at the time was around 15 °C both at the bottom and the surface and animals were left in these on-board recovery tanks for around 6–8 h (for all animals used in this study). Further details of handling procedures are given in Albalat et al. (2010).

Nephrops were landed at the port of Largs, Scotland and transferred to a refrigerated van (6–8 °C), for transport to the company facility in Troon (45 min). On arrival the tube sets were placed in indoor tanks and were left undisturbed overnight. These tanks contained re-circulated seawater that was filtered mechanically, sterilised using a commercial ultraviolet steriliser (P10T–100W, Tropical Marine Centre, London, UK) and chilled to a temperature of 8 °C. Approximately 24 h after capture a set of animals from one haul was used for the physiological assessment of vigour (Sections 2.2 and 2.3), and a set of animals from a separate haul by the same vessel was used for the assessment of mortality (section 2.4). At this point animals were classified according to their vigour index.

2.2. Vigour index and sampling procedure

Nephrops were graded into one of four categories (A, B, C and D) based upon the criteria outlined in Table 1 (also Suppl. Data – video recordings of tail flipping). Immediately afterwards, ten animals from each category were sacrificed and samples from the deep abdominal flexor muscle taken and immediately frozen in liquid nitrogen and subsequently stored at −80 °C. These samples were used for biochemical analysis.

2.3. Biochemical analysis

Samples of frozen abdominal muscle (1 g) were weighed and homogenised on ice with 5 ml of chilled 0.6 M perchloric acid using an Ultra Turrax T25 homogeniser. The homogenate was then centrifuged (Biofuge Fresco, Heraeus) at 16,000*g* for 10 min at 4 °C. Muscle supernatants were used to determine ATP and its breakdown products, L-lactate and arginine phosphate.

2.3.1. Adenosine 5′-triphosphate and its breakdown products

Nucleotide extracts were prepared as described in Ryder (1985). Adenosine 5′-triphosphate (ATP) and its breakdown products (adenosine 5′-diphosphate (ADP), adenosine 5′-monophosphate (AMP), inosine 5′-monophosphate (IMP), inosine (HxR) and hypoxanthine (Hx)) were analysed using a SP8800 ternary HPLC pump coupled to a PDA detector (Thermo Finnigan) set to monitor at 254 nm. Separations were carried out as described in Albalat et al. (2009). The Adenylate Energy Charge or AEC, which is a recognised index for describing the energy status of the living muscle (Atkinson, 1965), was obtained according using the following formula:

$$\frac{[ATP] + {}^{1}\!/\!_{2}[ADP]}{[ATP] + [ADP] + [AMP]}$$

2.3.2. L-lactate concentration

L-lactate concentration was measured using the enzymatic method described by Bergmeyer and Bernt (1974) and further modified by Hill et al. (1991). Briefly, 50 μl of muscle homogenate supernatants were added to 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; pH 9.5) and 1 unit of lactate dehydrogenase (LDH, Sigma) and incubated for 2 h at 37 °C. Absorbance was measured at 340 nm on a spectrophotometer (Shimadzu, UV Mini 1240) and converted to a L-lactate concentration using a calibration curve of lactic acid (0.5–10.0 mM).

2.3.3. Arginine phosphate

The concentration of arginine phosphate was determined according to the method of Viant et al. (2001). An Ultimate 3000 LCi Series HPLC system (Dionex Corporation, Sunnyvale, USA) was used, fitted with a low-pressure gradient quaternary analytical pump and coupled to a variable wavelength detector set at 205 nm. Separation of arginine phosphate was achieved as described in Albalat et al. (2009).

2.4. Mortality in simulated transport

In order to assess the survival of *Nephrops* from each vigour category, animals were first graded into their appropriate vigour categories. These groups were then packed separately into polystyrene boxes (n = 20/box) lined with newsprint dampened with seawater and containing ice packs (Sorba-Freeze 4 × 2), as per the standard company procedure. For each vigour category 20