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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

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Abstract

Recent improved practices in the trawl fishery of Norway lobsters (*Nephrops norvegicus*) have made it possible to increase the proportion of these 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 the industry dealing with live-traded *Nephrops* to screen out the proportion of trawl-caught lobsters that nevertheless 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 (ATP) in the tail muscles, by the proportions of other nucleotides as expressed in the Adenylate Energy Charge (AEC), 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.

Key words: Norway lobster**,** *Nephrops norvegicus*, live transport, vigour, physiology, mortality

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. However, a number of trawl-caught animals fail to recover, and such animals do not survive subsequent live transportation, which may take 24-72h.

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 (Bernasconi and Uglow, 2008; 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).

In this study, we have validated a visual index based on the vigour displayed by trawl-caught *Nephrops*. Designing visual index-based protocols has proved to be a very useful approach for predicting mortality in other live-traded crustaceans (reviewed by Stoner, 2012). Therefore, the two aims of this study were to validate a vigour index that could be used routinely for screening live product for 'semi-dry' vivier transport for *Nephrops* by measuring a set of physiological condition-related parameters, and to determine if such an index is a good predictor of the subsequent survival of the animals following this type of transport.

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 (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 to 40 individuals per kilogram), which equates approximately to a size range of 27-37 mm carapace length. *Nephrops* were stored in tube-sets (Suppl. Fig 1) placed in aluminium tanks on board the vessels, which 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 h. Further details of handing 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. A set of animals from one haul was used for the physiological assessment of vigour, and a set of animals from a separate haul by the same vessel was used for the assessment of mortality.

2.2. Vigour index and Sampling procedure

On the day following capture *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

81 samples from the deep abdominal flexor muscle taken and immediately frozen in liquid
82 nitrogen and subsequently stored at -80 ˚C. These samples were used for biochemical
83 analysis.

84 ***2.3. Biochemical analysis***

85 Samples of frozen abdominal muscle (1 g) were weighed and homogenised on ice with
86 5 mL of chilled 0.6 M perchloric acid using an Ultra Turrax T25 homogeniser. The
87 homogenate was then centrifuged (Biofuge Fresco, Heraeus) at 16,000 *g* for 10 min at 4 ˚C
88 Muscle supernatants were used to determine ATP and its breakdown products, L-lactate and
89 arginine phosphate.

90 *2.3.1. Adenosine 5'-triphosphate and its breakdown products* - Nucleotide extracts were
91 prepared as described in Ryder (1985). Adenosine 5'-triphosphate (ATP) and its breakdown
92 products (adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), inosine 5'-
93 monophosphate (IMP), inosine (HxR) and hypoxanthine (Hx)) were analysed using a SP8800
94 ternary HPLC pump coupled to a PDA detector (Thermo Finnigan) set to monitor at 254 nm.
95 Separations were carried out as described in Albalat et al. (2009). Adenylate Energy Charge
96 or AEC was obtained according to Atkinson (1965) using the following formula

97
$$\frac{[ATP] + ½\,[ADP]}{[ATP] + [ADP] + [AMP]}$$
98

99

100 *2.3.2. L-lactate concentration* - L-lactate concentration was measured using the enzymatic
101 method described by Bergmeyer and Bernt (1974) and further modified by Hill et al. (1991).
102 Briefly, 50 µl of muscle homogenate supernatants were added to tubes containing 50 µl of
103 NAD^+ (50 mM), 0.85 ml of hydrazine buffer (0.6 M hydrazine hydrate, 5.6 mM EDTA, 1 M
104 glycine; pH 9.5) and 1 Unit of lactate dehydrogenase (LDH, Sigma) and incubated for 2 h at
105 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 from a separate haul 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 4x2), as per the standard company procedure. This was replicated 5 times to give 100 individuals from each of the 4 vigour groups. The boxes were then placed in refrigerated storage (6^{o}C) to simulate refrigerated transport to the customer, and were opened at 24 hour intervals for mortality assessment.

2.5. Data analysis

Data from physiological analyses are reported as mean values ± standard error of mean (SEM). Differences between groups were analysed by one-way 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 estimates were generated using the Kaplan-Meier analysis with 95% confidence intervals, using Prism 6. Survival estimates between different vigour categories were statistically compared by log-rank test.

3. Results

3.1. Physiological Measures

The main nucleotide in the abdominal muscle of vigour categories A, B and C was ATP, while in animals of category D the main nucleotide was AMP (Fig.1A). Low concentrations of IMP and HxR were found in both category A (0.11 and 0.002 μmol g^{-1} respectively) and category B animals (0.28 and 0.048 μmol g^{-1}) with significantly higher concentrations of both these nucleotides found in both category C *Nephrops* (1.12 and 0.42 μmol g^{-1}) and category D (1.77 and 0.45 μmol g^{-1})noThe calculated AEC values (Fig. 1B) were not significantly different between category A, B and C. However, a significant decrease was obtained between categories A and B from category D.

On the other hand, concentrations of L-lactate in the abdominal muscle of category A and B animals were markedly lower than those of category C and D (Fig.1C) while no differences in arginine phosphate were obtained among the different categories (data not shown).

3.2. Mortality in simulated transport

Kaplan-Meier survival estimates showed a significant difference in survival probability according to the different vigour categories ($p<0.0001$) (Fig. 2). Category A had the lowest mortality, followed by category B then category C. All of the category D *Nephrops* were dead at the end of the first 24 hour period. After 48 hours the trend was the same, with lower mortality estimates in category A. After 72 hours the trend was still the same, although all of the category C *Nephrops* were dead at this time point.

4. Discussion

Survival studies on *Nephrops* have reported variable survival rates following capture, air exposure and transport procedures (Ulmestrand et al., 1998; Campos et al., 2015; Bergmann and Moore 2001; Philp et al., 2015), but if post-catch practices are optimised then relatively high survival rates can be achieved, even after trawling (Lund et al., 2009; Albalat

et al., 2010). The condition of such animals can nevertheless vary, especially with season (Lund et al., 2009), and for this reason visual methods to assess their condition are important (Stoner, 2012).

The results of this study show that it is possible to classify trawl-caught *Nephrops* into clearly defined vigour categories, reflecting the underlying physiological state of the animals. This is evidenced by a linear drop in ATP concentration between the categories from A through to D. Animals in category C had some features similar to those in categories A and B (e.g. AEC values), but other features indicative of a delay in returning to optimum energy conditions in the muscle (the presence of IMP and HxR, and elevated muscle L-lactate). This retarding or failure to regain physiological homeostasis in category C animals could be linked to the delayed but significant mortality observed in this group upon enduring subsequent stress, which could be seen as a general source of mortality (Stoner, 2012). This effect was even more pronounced in category D animals, which not only displayed significant amounts of IMP, HxR and muscle L-lactate but also a significantly lower AEC value at around 0.4. In the situation considered here (24 h after trawl-capture) it is unlikely that animals classified as D having AEC values of around 0.4 will have the ability to recover since values of less than 0.5 are widely considered to be the point of physiological collapse (Sylvestre and LeGal, 1987). This is further confirmed by the high mortality recorded within the first 24 hours of animals enduring subsequent semi-dry aerial exposure.

Although we have demonstrated that the vigour classification used in this study does reflect the differential physiological condition of trawl-caught *Nephrops*, in reality the utility of visual measures to assess crustacean species health and condition depends on establishing their link to subsequent mortality (Stoner, 2012). It is therefore significant that this study has found the physiologically-validated vigour index to be closely linked to mortality under a subsequent semi-dry vivier method of transport in a time-dependent manner.

The vigour-mortality correlation reported here could be relevant not only for the live transport of this and other closely-related lobster species, but also in cases where captured crustaceans are returned to sea as by-catch or discards, or are released for stock enhancement (Cook et al., 2003). In the case of *Nephrops* this topic has become current due to the introduction of the landing obligation as part of the reformed EU Common Fisheries Policy (CFP-EU regulation 1380/2013). From a policy perspective, being able to estimate or predict survival of post-catch discarded *Nephrops* using appropriate tools would be of benefit, since if survival is found to be high then discarded *Nephrops* could be returned to sea instead of being landed (Campos et al., 2015; Albalat et al., 2016; Méhault et al., 2016). Therefore, the capability of the presented vigour index to predict mortality, at least under stressful conditions like the ones used in this study (semi-dry transport) could prove to be a valuable tool not only for the industry but also for fisheries scientists and managers.

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Figure Legends

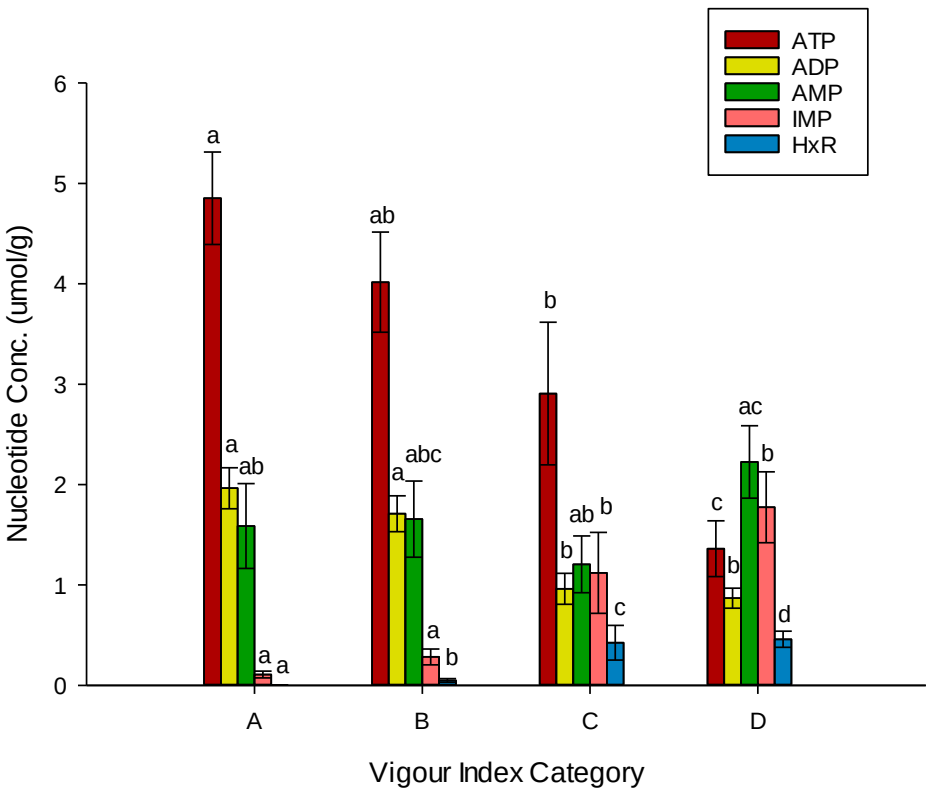
Fig. 1. A) Nucleotide profiles in the abdominal muscle of *Nephrops* from each of the four vigour categories after an overnight recovery period in seawater tanks; B) corresponding AEC values; C) Muscle L-lactate values. Values represent the mean ± SEM of ten specimens. Values that are significantly different with time are represented by different letters ($P<0.05$).

Fig. 2. Kaplan-Meier estimates of the survival of *Nephrops* from each of the four vigour categories (n = 100 for each category) at consecutive sample points when using a semi-dry transport system. Estimates of survival are shown as solid lines and 95% confidence intervals as dashed lines.

Suppl. Fig. 1. Image of a representative tube-set box used in the trials

Fig. 1

A)


ATP
ADP
AMP
IMP
HxR
Nucleotide Conc. (umol/g)
0
1
2
3
4
5
6
a
a
ab
a
a
ab
a
abc
a
b
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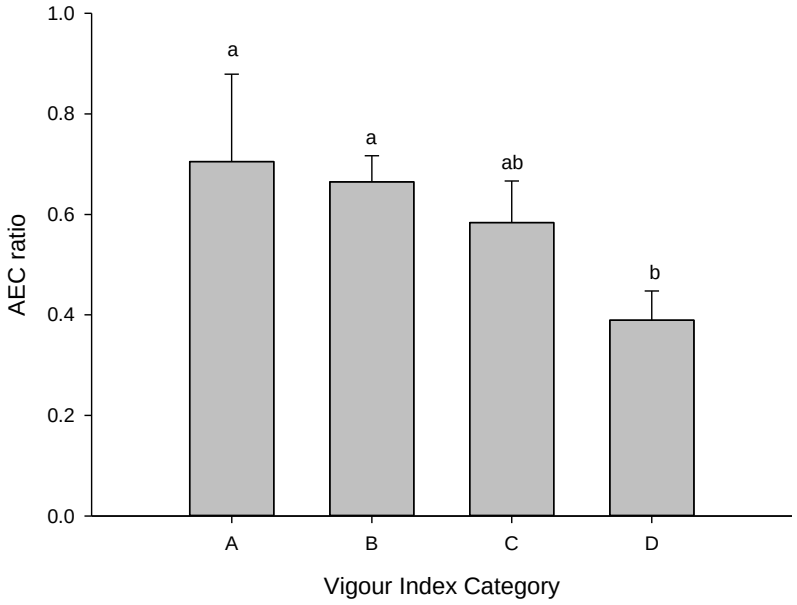
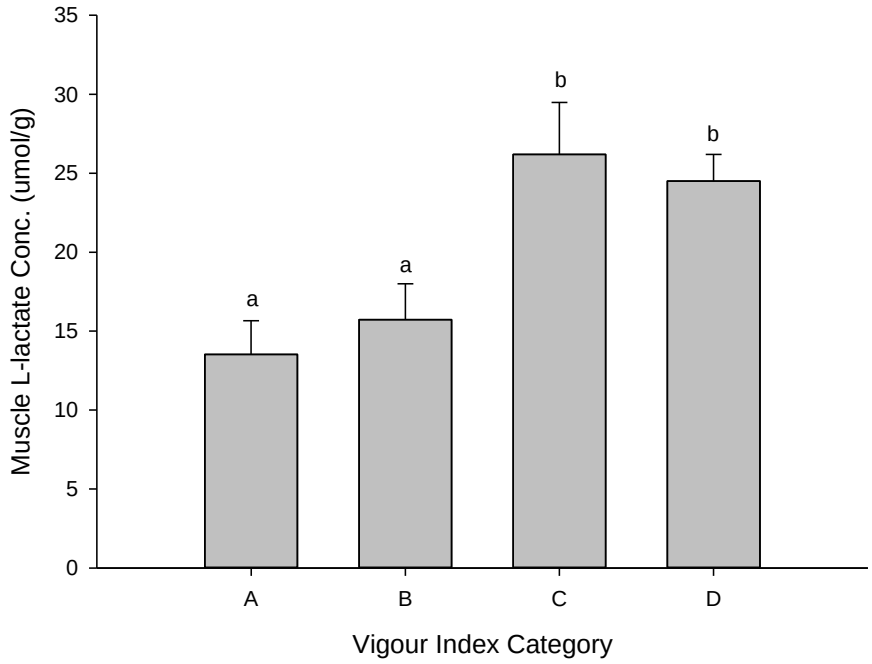
ab
b
c
c
b
ac
b
d
A
B
C
D
Vigour Index Category

B)

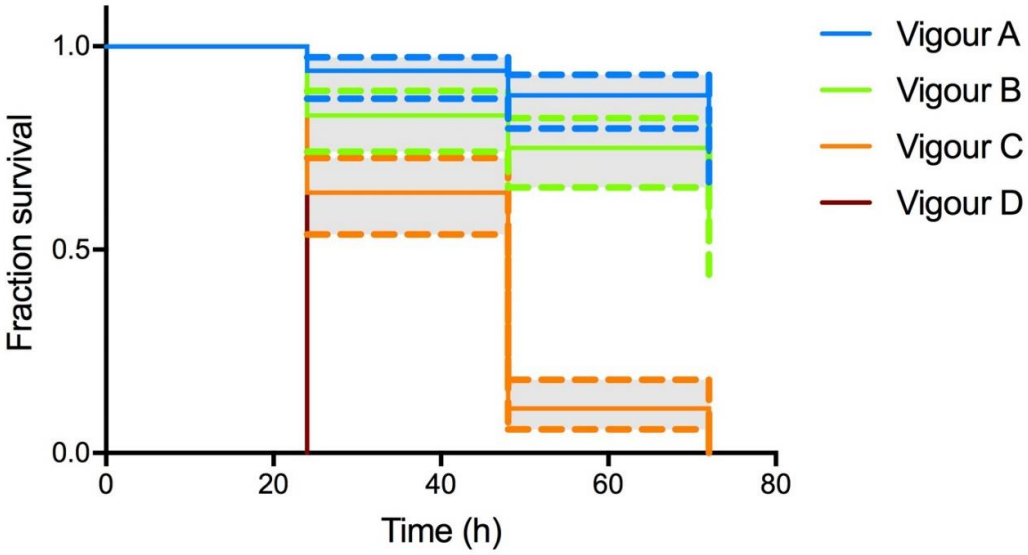

AEC ratio
0.0
0.2
0.4
0.6
0.8
1.0
a
a
ab
b
A
B
C
D
Vigour Index Category

319 C)


35
30
25
20
15
10
5
0
Muscle L-lactate Conc. (umol/g)
a
a
b
b
A
B
C
D
Vigour Index Category

320

321 **Fig. 2**


Fraction survival
1.0
0.5
0.0
0
20
40
60
80
Time (h)
Vigour A
Vigour B
Vigour C
Vigour D

322

Suppl. Fig. 1