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

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5 Amaya Albalat ^{a,1}, Simon Sinclair ^{a,2}, Douglas Neil ^{a*}

6 ^aInstitute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow G12 8QQ,
7 Scotland, United Kingdom

8 Present addresses:

9 ¹ Institute of Aquaculture, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, Scotland, United
10 Kingdom

11 ² Scotprime Seafoods Ltd., 11 Whitfield Drive, Ayr KA8 9RX, Scotland, United Kingdom

12 * Corresponding author at:

13 Institute of Biodiversity, Animal Health and Comparative Medicine, Graham Kerr Building, University of
14 Glasgow, Glasgow G12 8QQ, Scotland, United Kingdom

15 Email addresses: amaya.albalat@stir.ac.uk (A. Albalat), Douglas.Neil@glasgow.ac.uk (D.M. Neil)

16

17 **Abstract**

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

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

33 **1. Introduction**

34 Live crustaceans attract premium market prices, but for successful live transport
35 crustaceans must initially be in a good physical and physiological condition (reviewed by
36 Fotedar and Evans, 2011; Neil, 2012). This is determined by the nature of the capture process
37 used (Wilson et al., 2014) and also by the post-capture handling procedures (Milligan et al.,
38 2009; Raicevich et al., 2011; Leocádio et al., 2012; Lorenzon et al., 2013). Improved
39 practices in trawl fisheries, such as on-board recovery tanks, have made it possible to increase
40 the proportion of trawl-caught crustaceans that can be transported alive successfully.
41 However, a number of trawl-caught animals fail to recover, and such animals do not survive
42 subsequent live transportation, which may take 24-72h.

43 There have been several studies looking at the physiological condition of *Nephrops*
44 *norvegicus* (hereafter referred to by genus alone) and other shellfish species during live
45 transport (Bernasconi and Uglow, 2008; Lorenzon et al., 2007, 2008; 2013; Barrento et al.
46 2010, 2011) mainly by means of ‘wet’ vivier transport. Recently, an alternative to this ‘wet’
47 vivier transport is the transportation of live *Nephrops* in a ‘semi-dry’ state, packed in
48 polystyrene boxes and transported via standard refrigerated vehicles (Philp et al., 2015).

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

56 **2. Material and Methods**

57 ***2.1. Capture and Holding conditions***

58 *Nephrops* were caught by otter trawl in the Clyde Sea area, Scotland, UK (55.35 N,
59 04.54 W; depth range 60-80 m) in late May (spring time conditions). The vessel used was the
60 M.V. *Seren Y Don*, fitted with a single hopper trawl net with a cod-end nominal mesh size of
61 80 mm, towed at approximately 2 knots.

62 *Nephrops* of commercial grade 3 (30 to 40 individuals per kilogram), which equates
63 approximately to a size range of 27-37 mm carapace length. *Nephrops* were stored in tube-
64 sets (Suppl. Fig 1) placed in aluminium tanks on board the vessels, which were constantly
65 supplied (on an overflow basis) with running surface seawater via the vessels deck hose.
66 Seawater temperature at the time was around 15°C both at the bottom and the surface and
67 animals were left in these on-board recovery tanks for around 6 h. Further details of handling
68 procedures are given in Albalat et al. (2010).

69 *Nephrops* were landed at the port of Largs, Scotland and transferred to a refrigerated
70 van (6-8 °C), for transport to the company facility in Troon (45 min.). On arrival the tube sets
71 were placed in indoor tanks and were left undisturbed overnight. These tanks contained re-
72 circulated seawater that was filtered mechanically, sterilised using a commercial ultraviolet
73 steriliser (P10T-100W, Tropical Marine Centre, London, UK) and chilled to a temperature of
74 8 °C. A set of animals from one haul was used for the physiological assessment of vigour, and
75 a set of animals from a separate haul by the same vessel was used for the assessment of
76 mortality.

77 ***2.2. Vigour index and Sampling procedure***

78 On the day following capture *Nephrops* were graded into one of four categories (A, B,
79 C and D) based upon the criteria outlined in Table 1 (also Suppl. Data - video recordings of
80 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

$$97 \frac{[\text{ATP}] + \frac{1}{2} [\text{ADP}]}{[\text{ATP}] + [\text{ADP}] + [\text{AMP}]}$$

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

106 1240) and converted to a L-lactate concentration using a calibration curve of lactic acid (0.5-
107 10.0 mM).

108 *2.3.3. Arginine phosphate* - The concentration of arginine phosphate was determined
109 according to the method of Viant et al. (2001). An Ultimate 3000 LCi Series HPLC system
110 (Dionex Corporation, Sunnyvale, USA) was used, fitted with a low-pressure gradient
111 quaternary analytical pump and coupled to a variable wavelength detector set at 205 nm.
112 Separation of arginine phosphate was achieved as described in Albalat et al. (2009).

113 ***2.4. Mortality in simulated transport***

114 In order to assess the survival of *Nephrops* from each vigour category, animals from a
115 separate haul were first graded into their appropriate vigour categories. These groups were
116 then packed separately into polystyrene boxes (n = 20/box) lined with newsprint dampened
117 with seawater and containing ice packs (Sorba-Freeze 4x2), as per the standard company
118 procedure. This was replicated 5 times to give 100 individuals from each of the 4 vigour
119 groups. The boxes were then placed in refrigerated storage (6°C) to simulate refrigerated
120 transport to the customer, and were opened at 24 hour intervals for mortality assessment.

121 ***2.5. Data analysis***

122 Data from physiological analyses are reported as mean values \pm standard error of mean
123 (SEM). Differences between groups were analysed by one-way analysis of variance
124 (ANOVA). Homogeneity of variance was tested using the Levene test. A Post Hoc or
125 multiple comparisons approach was then used to determine statistical differences between
126 samples. P-values lower than 0.05 were considered statistically significant.

127 Survival estimates were generated using the Kaplan-Meier analysis with 95%
128 confidence intervals, using Prism 6. Survival estimates between different vigour categories
129 were statistically compared by log-rank test.

130 **3. Results**

131 **3.1. Physiological Measures**

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

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

144 **3.2. Mortality in simulated transport**

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

151 **4. Discussion**

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

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

159 The results of this study show that it is possible to classify trawl-caught *Nephrops* into
160 clearly defined vigour categories, reflecting the underlying physiological state of the animals.
161 This is evidenced by a linear drop in ATP concentration between the categories from A
162 through to D. Animals in category C had some features similar to those in categories A and B
163 (e.g. AEC values), but other features indicative of a delay in returning to optimum energy
164 conditions in the muscle (the presence of IMP and HxR, and elevated muscle L-lactate). This
165 retarding or failure to regain physiological homeostasis in category C animals could be linked
166 to the delayed but significant mortality observed in this group upon enduring subsequent
167 stress, which could be seen as a general source of mortality (Stoner, 2012). This effect was
168 even more pronounced in category D animals, which not only displayed significant amounts
169 of IMP, HxR and muscle L-lactate but also a significantly lower AEC value at around 0.4. In
170 the situation considered here (24 h after trawl-capture) it is unlikely that animals classified as
171 D having AEC values of around 0.4 will have the ability to recover since values of less than
172 0.5 are widely considered to be the point of physiological collapse (Sylvestre and LeGal,
173 1987). This is further confirmed by the high mortality recorded within the first 24 hours of
174 animals enduring subsequent semi-dry aerial exposure.

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

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

193

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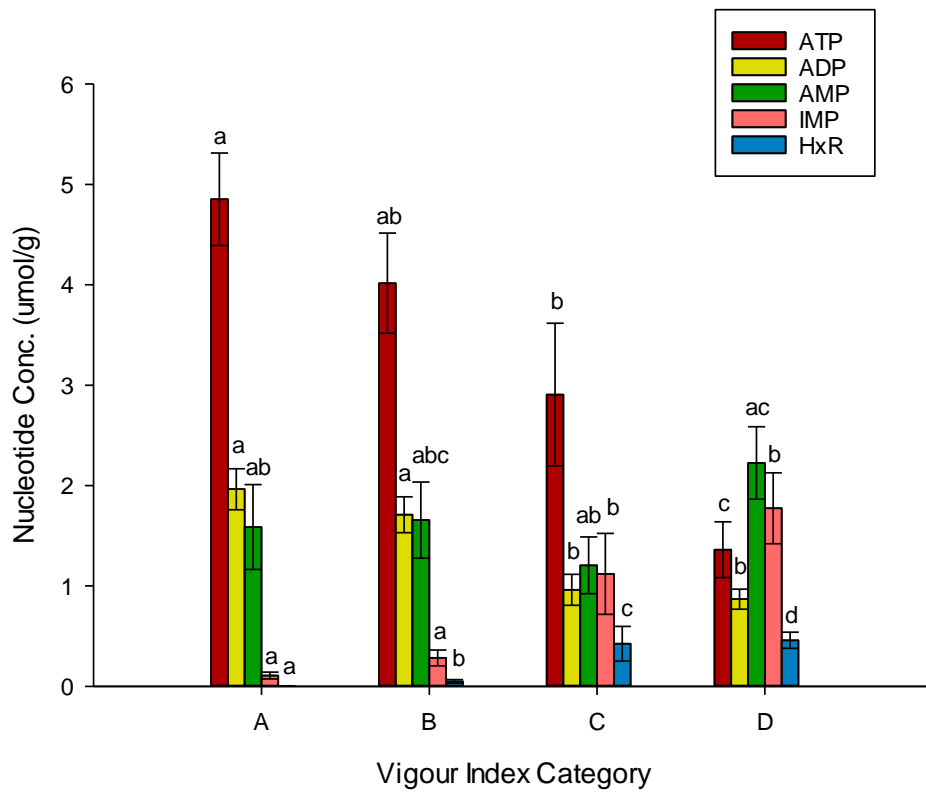
276 **Figure Legends**

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

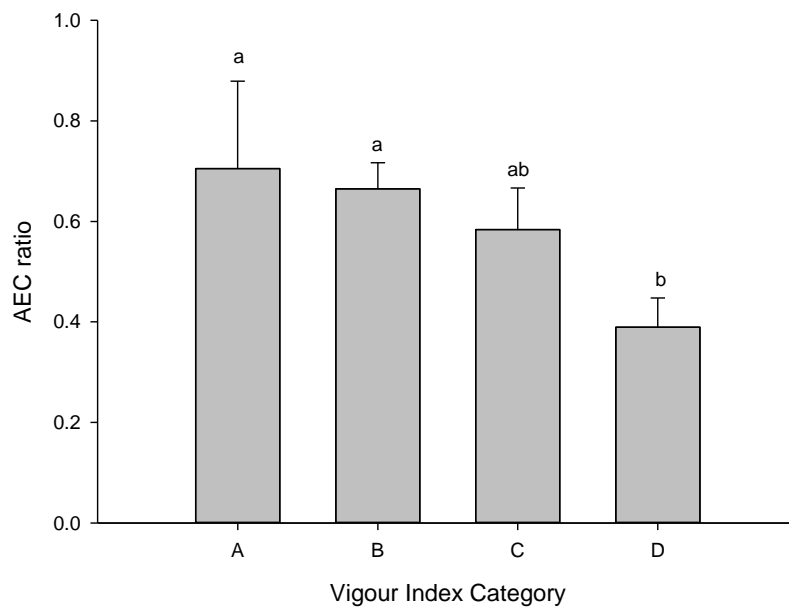
281
282 **Fig. 2.** Kaplan-Meier estimates of the survival of *Nephrops* from each of the four vigour
283 categories ($n = 100$ for each category) at consecutive sample points when using a semi-dry
284 transport system. Estimates of survival are shown as solid lines and 95% confidence intervals
285 as dashed lines.

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287 **Suppl. Fig. 1.** Image of a representative tube-set box used in the trials
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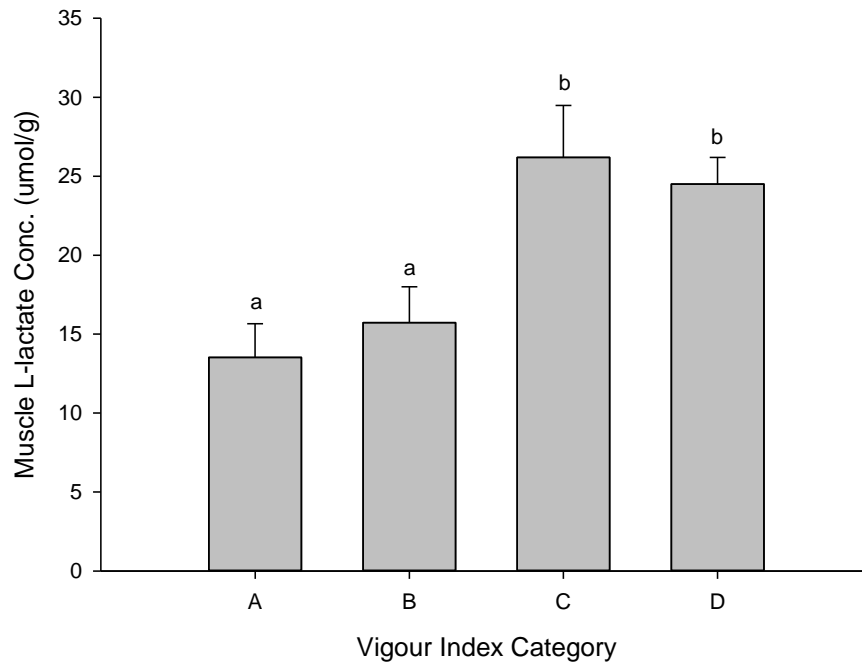
289 **Fig. 1**
290 A)



316 B)

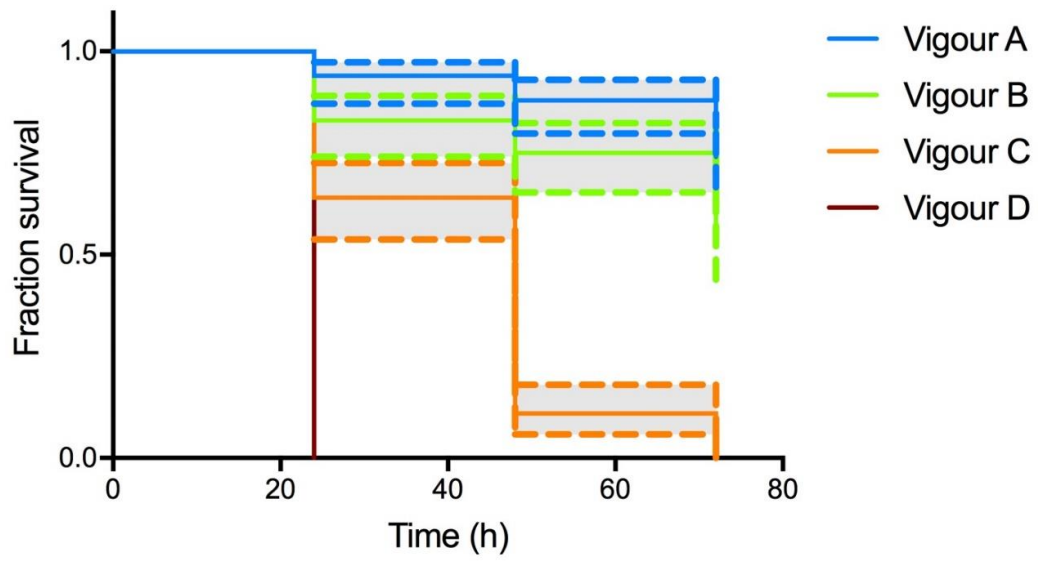


319 C)



320

321 **Fig. 2**



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323 **Suppl. Fig. 1**
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