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# Stress response according to transport protocol in Norway lobster, *Nephrops norvegicus*

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**Abstract.—** The Norway lobster, *Nephrops norvegicus*, is a valuable and commonly exported European decapod crustacean, which experiences stress from point of capture and onward transport. Stressors such as air exposure duration (i.e. emersion period) and air temperature have been studied previously. We investigated whether mortality could be reduced by decreasing road vibrations during transport, and how physiological stress measurements were influenced in a transport simulation experiment, reflecting a typical short road journey along a supply chain. Baseline haemolymph samples were taken from lobsters sampled immediately after commercial capture using static traps (lobster pots). Individuals were emersed for one hour, either immobile or with continuous shaking; the latter to simulate conditions occurring during transport. Both treatments significantly increased Total Haemocyte Counts (THC) and serum glucose, lactate and ammonium concentrations compared to baseline animals. Individuals subjected to continuous shaking showed higher glucose and ammonium concentrations compared to individuals maintained immobile. We conclude that shaking appears to influence the physiological responses of *N. norvegicus* in addition to the effects of emersion alone, and the reduction of road vibrations (e.g. via simple cushioning) can reduce post-transport mortality.

***Key words***: *serum, THC, glucose, ammonium, lactate*

## ■ Introduction

In 2010, *ca.* 67,000 tonnes of *Nephrops norvegicus* (Norway lobster) were landed across Europe, the majority in the UK (*ca.* 58%, value *ca.* £100 million), Ireland and Scandinavia (both *ca.*12%, Ungfors *et al.*, 2013). Live *N. norvegicus* demand a higher price and can be sold fresh locally, or are impounded by shellfish merchants prior to onward transport and sale (Barrento *et al.*, 2008; Neil, 2012). Live and gravid lobsters are also required by restaurants, public aquariums, hatcheries and the research sector. Transport duration and the method in which they are held depends on capture location, value and crustacean species, although improvements in protocol could be initiated across supply chains to improve survival and condition (Barrento *et al.*, 2008; Neil, 2012).

Post-capture stressors include air exposure (emersion), handling and temperature variations, all of which impact respiratory, osmoregulatory and excretory organ systems. Research has been directed at improving survival and product quality through the identification and monitoring of physiological stress, physical damage, pathology and mortalities (reviewed by Stoner, 2012; Fotedar & Evans, 2011).

There are various methods to quantify physiological stress in crustaceans, but sampling of the haemolymph is a non-destructive method commonly used to quantify changes in glucose, lactate, ammonium, protein concentration and

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haemocyte counts (Stoner, 2012). Glucose is a traditional indicator of stress in decapods, and is mobilized (i.e. haemolymph concentration increases) as a result of an initial hormonal response to stress, to meet increased requirements for energy (Stoner, 2012) e.g. handling and emersion in *N. norvegicus* (Ridgway *et al.*, 2006a; Lund *et al.*, 2009). Increases in haemolymph lactate concentration are also closely allied with anaerobic respiration following handling emersion stress (Ridgway *et al.*, 2006a; Lund *et al.*, 2009; Stoner, 2012) and similarly, haemolymph ammonium concentration can also accumulate in emersed and physically stressed decapods (Stoner, 2012; Schmitt & Uglow, 1997). Protein concentration and haemocyte counts tend to decrease as a reaction to transport stress in decapods (Fotedar & Evans, 2011; Stoner, 2012) which may be allied to osmotic stress, hypoxia, temperature fluctuations, nutrition, infection or physical damage such as physical damage and bleeding.

There are commercial and ethical ramifications if the health and survival of *N. norvegicus* could be improved by reducing physical trauma during transportation. Emersion stress research has included controlled laboratory studies (Ridgway *et al.*, 2006a, b), simulation of commercial capture and handling protocols in the laboratory (e.g. Couillard *et al.*, 2015) and sampling of decapod crustaceans on supply chain routes (e.g. Basti *et al.*, 2010). The results indicate that physical handling at the point of capture is regarded as a stress factor (Hunter & Uglow, 1993; Jacklin & Combes, 2007); however there is less information investigating physical handling or shaking during transport. We undertook our study to investigate if mortality could be influenced by vibrations caused during road transportation, and secondly, the physiological response to shaking in a simulated transport experiment.

## ■ Materials and Methods

### *Lobster procurement and road transport*

For experiment 1, four batches of intermoult berried (gravid) female *N. norvegicus* were caught by commercial fishermen using static traps (known variously as creels or lobster pots) although the source of each batch was unidentified. Lobsters were procured by Tarbert Shellfish Company Ltd, (Argyll and Bute, UK) between March–June 2013 and March–April 2014 ($n$ = 47, 56, 138, 144; total 385; EU size grade 2, 112–136 mm total length) and transported by a commercial vivier HGV (heavy goods vehicle), used to successfully transport live *N. norvegicus* across Europe. Lobsters were transported for an initial 9 hours by road (immersed individually in cells inside a "tube tray" matrix, henceforth referred to as matrix; *ca.* 30 cm × 30 cm × 20 cm; Fig. 1a, b) from Scotland to S. England. The matrix was transferred to a LGV (light goods vehicle) and further transported for an additional 2 hour period in emersed but humid, dark and cool conditions (*ca.* 8–10°C) from S. England to CSAR (Centre for Sustainable Aquatic Research, Swansea, UK). During emersed transport, for two initial batches the matrix was wrapped in damp paper inside a coolbox and restrained against the floor of the vehicle. For a further two batches, foam cushions were added between the coolbox and floor of the vehicle to reduce road vibrations.

### *Post transport husbandry*

On arrival at a pilot hatchery at CSAR, lobsters were placed individually in discrete pens with perforated walls and floors (*ca.* 8 cm × 15 cm × 20 cm) inside aerated shallow raceways (*ca.* 1300 cm × 650 cm × 30 cm, Fig. 1c). Water temperature was maintained steadily at 10–13°C with salinity at *ca.* 29 ppt. Water input (2 l/min) and aeration maintained dissolved oxygen above 80% saturation, and nitrogen parameters at acceptable concentrations (ammo-

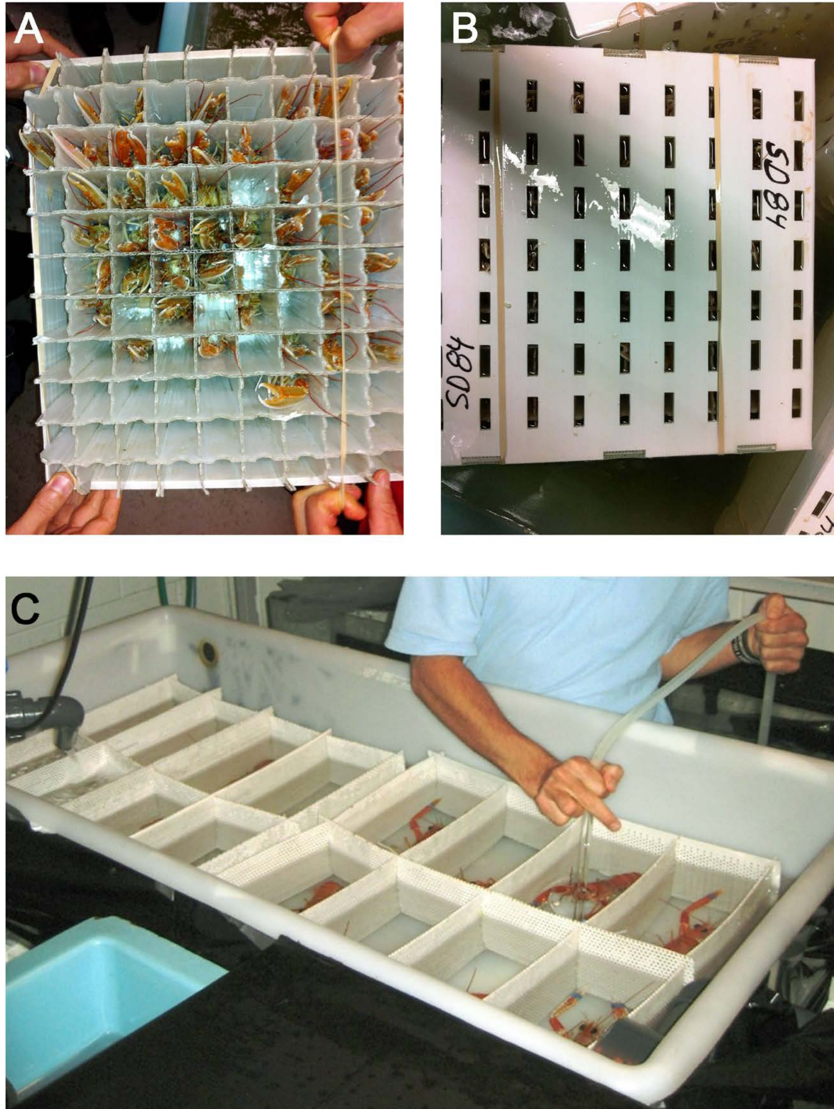


Fig. 1. Individual lobster cells. A. Example of tube tray matrix, used during transportation and simulated transport experiments. B. Matrix in use with a lid, to reduce incident light and maintain humidity. C. Shallow raceway containing discrete pens, used to maintain and monitor individual lobsters post-transportation, part of a pilot hatchery at CSAR.

nium and nitrite below 0.02 mg/l and nitrate below 10 mg/l) with alkalinity 200 ppm $CaCO_3$ equivalent. Low ambient lighting (*ca*. 0.3 μmol/s/m$^2$) was used with a 12h:12h photoperiod. Lobsters were fed every other day with *ca*. 2 g of recently defrosted blue mussel (*Mytilus edulis*) or Atlantic prawn (*Pandalus borealis*) per lobster, with 24 hours allowed prior to cleaning the cells and outflow filter with a siphon. Mortalities were noted every day for up to 5 months, although mortality stabilized within 14 days after arrival.

***Simulated transport experimental design***

For experiment 2, intermoult berried (gravid) female *N. norvegicus* were caught by commercial fishermen using static traps from Gullmarsfjord, Sweden, and transferred (using a

covered, damp, polystyrene box, within 15 min of capture and emersion) to the Department of Biological and Environmental Sciences—Kristineberg for immediate use in experiments. Haemolymph (4–600 μl) was withdrawn, using a 1 ml syringe and 21 gauge needle, from a subsample of lobsters ($n=24$, mean Carapace Length, CL, 45.37 mm, range 33.6–51.8 mm) to yield baseline data, and was placed on ice. Remaining individuals were then split into two groups ($n=15$; mean CL 42.76 mm, range 38.4–49.3 mm; and CL 45.92 mm, range 37.0–51.8 mm) and individually placed vertically (head up) into discrete tube tray matrices (*ca.* 30 cm × 30 cm × 20 cm; Fig. 1a). One matrix was placed on a laboratory rocker (Labasco 3011, Gesallschaft für Labortechnick Gmbh, Burgwedel, Germany) at full intensity (*ca.* 50 RPM oscillatory motion, furthest extent of vertical travel *ca.* 20 mm, radius 30 cm; whilst we have no evidence that this identically replicates road transport movement, which likely includes $g$ forces from turning acceleration and deceleration, and vibrations which vary according to individual journeys, it does allow future replication). The other matrix was kept immobile, for comparison. Both tube tray matrices were covered with lids (Fig. 1b), and held at 15°C, emersed, and in darkness. Haemolymph was withdrawn, as previously described, from lobsters after 1 hour. The holding conditions and time period were designed to reflect the minimum time typically required for transport from the landing site to an end user (e.g. local wholesaler, shellfish merchant with impoundment facilities, aquarium). All lobsters were then placed in "flow through" aquaria (16°C, 32 PSU) and observed for 48 hours to confirm the survival for each treatment.

***Bleeding regime and assays***

We determined Total Haemocyte Counts (THC) as described by Powell & Rowley (2008). For serum chemistry assays, the haemolymph was left for 2 hours on ice to clot, centrifuged (10,000 × $g$; 5 min), and supernatant (serum) split into 50–100 μl fractions and stored at −20°C. Aliquots were defrosted within 6 days with samples for non-protein analysis deproteinised in 5% TCA, and appropriately diluted in 3% sodium chloride solution. Assays were performed in triplicate on 96 well flat bottom plates, calibrated against standard curves run on the same plate, using a spectrophotometer (iEMS, MTX Lab Systems, Inc, Vienna, USA). Serum protein, glucose and lactate concentration was determined using a BCA kit (Pierce & Warriner Ltd, Chester, UK), glucose oxidase kit (Amplex® Red, Molecular probes Inc, Eugene, USA) or lactate kit (Lactate Assay Kit, Sigma-Aldrich Company Ltd, Poole, UK) respectively, in accordance with the manufacturer's instructions. Ammonium was calculated (Indophenol method, after Bolz & Howel, 1978) using freshly prepared reagents and a standard range of ammonium chloride (0–1 μg/ml) measured at 635 nm.

***Statistical analysis***

Data were analysed using GraphPad-Prism (GraphPad Software, San Diego, USA). For experiment 1, percentage survival data is displayed for 14 days after transport; following stabilization of mortality at day 14, actual numbers of live and dead animals were compared between batches using Fisher's Exact test. For experiment 2, data are shown as average values (mean ± 1 SEM, $n=14$–24). Haemolymph and serum data were tested for normality (Kolmogorov–Smirnov test) and homogeneity of variances (Bartlett's test) and were subsequently analysed using ANOVA and Tukey post hoc tests.

## ■ Results

***Post transport mortality***

For lobsters transported to CSAR, post-transport mortality was low (batch 3; less than 5% after 14 days) or zero (batch 4) when transport-

ed with cushioning and reduced road vibration, whilst mortality events ceased within 3 days of arrival (Fig. 2). In contrast, both batches without cushioning exhibited higher mortality (batches 1 and 2, 10 to 25% respectively). For these lobsters, mortality started within 1 day of arrival, and continued for an extended period, with final mortality events up to 13 days post transport. The first 14 days' data are shown only, by which time post-transport mortality had ceased in all batches and subsequently remained zero or negligible for 3–5 months. By day 14, the total proportion of dead lobsters in batch 1 was significantly greater than batch 2, or cushioned batches 3 or 4 (Fisher's Exact, $P < 0.01$–0.001) whilst the proportion of dead lobsters in batch 2 was significantly greater than cushioned batch 4 (Fisher's Exact, $P < 0.05$).

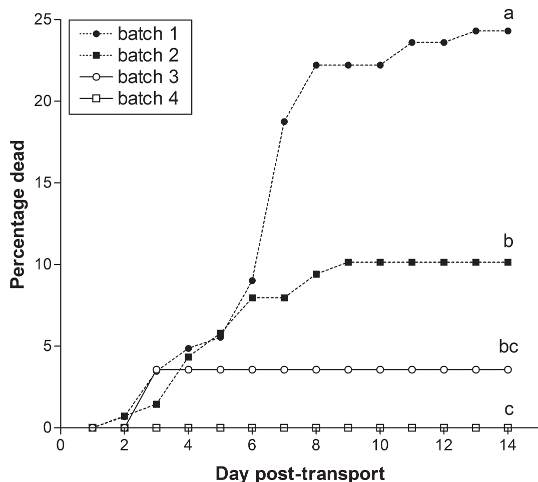


Fig. 2. *Nephrops norvegicus*. Post-transport mortality of adult gravid females, up to 14 days after arrival in CSAR, following four discrete transportation events. Unbroken or broken lines denote transportation with ($n = 47$–56) and without ($n = 138$–144) cushioning respectively. Different letters at day 14 denote significant difference between numbers of live and dead lobsters ($P \leq 0.05$).

### *Simulated transport*

Of the 54 individuals used no mortalities occurred during the 48 hour experimental period. However, haemolymph and serum chemistry data differed between baseline samples and those emersed for 1 hour, either immobile or with shaking (Table 1). Serum glucose, ammonium and lactate concentrations increased after emersion, particularly in the group subjected to shaking. Ammonium concentration in baseline and immobile lobsters were significantly lower than in individuals subjected to shaking (ANOVA and Tukey post hoc tests, $P < 0.01$–0.001). Serum glucose concentrations were all significantly different from each other ($P < 0.05$–0.001) and increased successively from baseline, immobile lobsters and those subjected to shaking. Serum lactate concentration also increased significantly between baseline, and both immobile and shaken lobsters (ANOVA and Tukey post hoc tests, $P < 0.001$), although high variation obscured any difference between the two emersed groups. Compared to baseline values, THC counts increased significantly following emersion in the shaken group (ANOVA and Tukey post hoc tests, $P < 0.001$). In contrast, serum protein measurements were not

Table 1. Haemolymph changes in adult gravid female *Nephrops norvegicus* between baseline lobsters shortly after removal from water, and after 1 hour of emersion (immobile or shaken). Different superscript letters across rows denote significant difference between treatments ($P \leq 0.05$). $n = 14$–24. Degrees of freedom for all parameters: 2 (treatment), 51 (residual) and 53 (total).

| | Baseline ($T_0$) | | Emersed immobile ($T_{+1}$) | | Emersed and shaken ($T_{+1}$) | | F | Overall P value |
|---|---|---|---|---|---|---|---|---|
| | Mean ± SEM | *Range* | Mean ± SEM | *Range* | Mean ± SEM | *Range* | | |
| Serum glucose (μg/ml) | $48.64 \pm 7.71^a$ | *12.65–187.40* | $94.97 \pm 13.62^b$ | *9.61–165.40* | $147.80 \pm 14.87^c$ | *94.29–271.50* | 19.73 | $< 0.001$ |
| Serum ammonium (μg/ml) | $7.22 \pm 0.32^a$ | *4.54–10.81* | $8.64 \pm 0.85^a$ | *4.66–17.42* | $12.25 \pm 1.1^b$ | *6.22–19.51* | 13.54 | $< 0.001$ |
| Serum lactate (μg/ml) | $0.50 \pm 0.06^a$ | *0.12–1.27* | $6.34 \pm 1.30^b$ | *1.46–17.54* | $9.25 \pm 1.26^b$ | *2.65–17.52* | 28.65 | $< 0.001$ |
| Serum protein (mg/ml) | $535.21 \pm 34.76$ | *69.17–780.15* | $546.15 \pm 21.18$ | *439.90–735.20* | $477.82 \pm 39.78$ | *195.21–892.93* | 0.98 | 0.3806 |
| THC (cells/ml × $10^7$) | $1.45 \pm 0.08^a$ | *0.85–2.11* | $1.83 \pm 0.16^{ab}$ | *0.54–2.86* | $2.32 \pm 0.21^b$ | *0.65–3.45* | 9.98 | 0.002 |

significantly different between the baseline and either of the emersed treatments.

## Discussion

Decapod crustaceans require immersion for aerobic respiration and ammonotelic excretion. Ridgway *et al.* (2006a) observed an increase in glucose and hypoglycaemic hormone (CHH) in lobsters emersed for periods under 4 hours. Hyperglycaemia in decapod crustaceans is a primary adaptive marker for stress, as CHH releases glucose from glycogen reserves used as a substrate during anaerobic respiration. (Ridgway *et al.*, 2006a, b; Stoner 2012). The elevated glucose we observed in shaken compared to immobile groups may be explained by more rapid removal of adherent water droplets inside the cephalothorax and gills. Lund *et al.* (2009) also found lower haemolymph oxygen content and partial pressure in *N. norvegicus* stored vertically compared to individuals maintained horizontally, suggesting that the former may impair gill ventilation or perfusion. These combined factors could have led to a faster onset of anaerobic metabolism, and therefore increased glucose and lactate production, and for both emersed groups.

Emersed *N. norvegicus* also show increased serum ammonium concentrations during dry transport, particularly in low humidity, windy or sunny conditions (Jacklin, 1996; Schmitt & Uglow, 1997). The brown shrimp *Crangon crangon* also show increased ammonium concentration and excretion rate within minutes of capture and handling (Hunter & Uglow, 1993), likely due to increased heart rate and ventilation. These data correlate with our observation of increased serum ammonium in Norway lobsters following emersion, and particularly with additional shaking.

Stress induced modulation of THC in decapod crustaceans is variable, and may be species and methodology specific (Fotedar & Evans, 2011). For example (Ridgway *et al.*, 2006a) recorded a decrease in THC in *N. norvegicus* over long emersion periods due to a cellular immune response to increased circulating bacteria. In contrast, emersion may increase the number of cells/ml haemolymph due to dehydration, whilst elevated THC has been recorded in crustaceans exposed to physical disturbance (Fotedar & Evans, 2011) so stress type may initiate different immune responses. Our results indicate a significant increase in THC in lobsters following air exposure and with shaking, compared to baseline values.

The stress response of decapod crustaceans has an initial adaptive phase (Stoner, 2012) defined as avoidance behavior with concurrent hormone mediated release of glucose, in order to promote homeostasis. During our experiment, glucose clearly increased in Norway lobsters from baseline values, and between immobile and shaken lobsters maintained in air. Should initial adaptive responses fail, a secondary maladaptive phase becomes apparent, described as a loss of homeostasis which results in changes in serum metabolites (which includes increased ammonia, lactate and altered immune function; Stoner, 2012). It is not possible to confirm the observed physiological changes as a mechanistic explanation for improved survival of cushioned lobsters post-transport. We observed no obvious physical trauma e.g. autotomy, exoskeleton damage and bleeding following transportation. Mortalities occurred several days after arrival in aquaria, before survival stabilised, and it is probable that mortalities were related to physiological stress in the days immediately following transportation (Stoner, 2012). In the second experiment, we observed significant physiological changes in both adaptive and maladaptive responses in shaken lobsters compared to those that remained stationary, indicating that shaking influences *N. norvegicus* physiology in addition to the effects of emersion alone.

Whilst immersed transportation of individual *N. norvegicus* in a well maintained vivier sys-

tem is employed by commercial shellfish merchants, this equipment is not available to everyone, whilst the cost of transporting water may not be economically viable. If emersed transportation is necessary, simple reduction of road vibrations (e.g. via cushioning) is a straightforward and cost effective addition to transport protocols. Alongside maintaining cool, humid and dark conditions, we suggest this is a reasonable approach to promote post transport survival following short road journeys.

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