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# Physiological stress in decapod crustaceans (*Munida rugosa* and *Liocarcinus depurator*) discarded in the Clyde *Nephrops* fishery

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**Abstract**

Crustacean discards experience stress during commercial fishing operations, due to increased exercise while in the trawl and aerial exposure during sorting of the catch. Physiological stress and recovery were assessed following trawling of two ecologically important decapod species, regularly discarded in the Clyde *Nephrops* fishery. Haemolymph samples taken from trawled swimming crabs, *Liocarcinus depurator*, and squat lobsters, *Munida rugosa*, had significantly higher concentrations of ammonia (0.308 and 0.519 mmol $l^{-1}$), D-glucose (0.14 and 0.097 mmol $l^{-1}$) and L-lactate (6.2 and 0.87 mmol $l^{-1}$) compared with controls, indicating an impairment of ammonia excretion and a switch to anaerobic metabolism. Concurrently, the haemolymph pH of trawled squat lobsters was low (7.47) compared with controls (7.75); however, the reverse trend was found in *L. depurator*. Initially elevated lactate (7.98 mmol $l^{-1}$) and glucose (0.73 mmol $l^{-1}$) concentrations of trawled and emersed (1 h) *L. depurator* were restored, 4 h after re-immersion along with pH (7.54). Crabs that had been emersed for 1 h had significantly higher concentrations of glucose (0.2 mmol $l^{-1}$) and lactate (5.14 mmol $l^{-1}$), and had more acidic blood (7.64) than *L. depurator* subject to 1 h of exercise, indicating that anoxia was the main cause of physiological stress. Crabs and squat lobsters lost 7% and 9% of their initial body wet weight following 1 h of emersion, although blood osmolarities did not change significantly. While all animals survived aerial exposure in our experiments, sorting of the catch on commercial boats

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takes up to 300 min, which could lead to mortality or sub-lethal chronic biochemical changes that could compromise fitness. 

*Keywords:* Discards; Haemolymph; Anoxia; Fishing impact; *Liocarcinus depurator*; *Munida rugosa*; Physiological stress; Trawling

## 1. Introduction

Commercial fishing has been estimated to produce 27 million t year$^{-1}$ of discards world-wide (Alverson et al., 1994). 'Discards' is a term used for non-target animals and undersized commercial species that are rejected from the catch. In recent years, the ecological effects of fishing have become a global environmental concern, resulting in a wealth of studies that were recently reviewed by Hall (1999).

The Norway lobster *Nephrops norvegicus* (L.), hereinafter referred to by genus alone, is one of the most valuable European shellfish resources, with official landings of around 60 000 t year$^{-1}$ world-wide, a third of which is landed in Scotland (Marrs et al., 2000). Wieczorek et al. (1999) estimated that the Clyde Sea *Nephrops* fishery generates 25 000 t year$^{-1}$ of discards. Up to 90% of Clyde Sea catches are discarded with swimming crabs *Liocarcinus depurator* (L.) and squat lobsters *Munida rugosa* (Fabricius), accounting for up to 51% and 59% of the total discard biomass, respectively (Bergmann et al., in press). Bergmann and Moore (2001) reported short-term mortalities of up to 25% in these decapods, and mortality continued for a period of 14 days after trawling. The capture process can be expected to cause stress and exhaustion as tows in the Clyde last for up to 280 min (Bergmann et al., in press). During this period, swimming crabs and squat lobsters are likely to endure physiological stress, as a result of increased swimming and tail-flip activity in attempts to escape. The seriousness of such trauma is increased by periods of on-deck exposure to temperature changes (Zainal et al., 1992), increased light intensity (Chapman et al., 2000) and air (Paterson and Spanoghe, 1997). Sorting times on Clyde Sea *Nephrops* trawlers range from 45 to 300 min (Bergmann, unpublished data). Vermeer (1987) ascribed chronic behavioural changes, such as reduced responsiveness to threatening stimuli and a diminished 'tail-flip' escape response in spiny lobsters, to neural damage caused by an increase in haemolymph lactate and ammonia following exposure to air. Similarly, Zainal et al. (1992) found that *M. rugosa* became torpid after periods of aerial exposure and elevated temperatures, which again can be expected to reduce survival owing to an increased susceptibility to predation.

A number of studies have shown that periods of hypoxia lead to physiological stress (e.g. Albert and Ellington, 1985; Vermeer, 1987; Schmitt and Uglow, 1997; Taylor and Waldron, 1997; Wileman et al., 1999), and Paterson and Spanoghe (1997) reviewed the usefulness of various haemolymph components as indicators of stress in decapod crustaceans. During anoxia, animals resort to anaerobic metabolism, leading to an increase in haemolymph L-lactate and D-glucose along with ammonia as its excretion through the gills becomes impaired (Vermeer, 1987; Paterson and Spanoghe, 1997).

The object of the present study was to assess physiological stress and recovery following trawling of two ecologically important decapod species, regularly discarded in the Clyde Sea *Nephrops* fishery.

## 2. Materials and methods

The blood chemistry (viz. pH, L-lactic acid, D-glucose and ammonia concentrations) of trawled and creel-caught crustaceans was compared as an indicator of physiological stress in discarded decapod crustaceans. In a second experiment, recovery following trawling and aerial exposure was examined in swimming crabs. A third experiment distinguished between the effects of aerial exposure and prolonged exercise.

### *2.1. Physiological stress in trawled and creel-caught crustaceans*

In June 1999, RV Aora made two 2 h tows on a commercial *Nephrops* ground in Fintray Bay, Great Cumbrae Island (ca. 55°45′N 04°57′W) at 40–48-m depth and a speed of 2.8 knots, using a rock hopper otter-trawl with a 70 mm diamond-shaped mesh, reflecting local fishing practice. Records of the sea surface, bottom and air temperatures were made, as these factors may influence the parameters under investigation.

Within $<6$ min after hauling, 10 randomly collected male *L. depurator* and *M. rugosa* were transferred into shaded tubs of running seawater, and haemolymph samples (ca. 600 μl) were extracted, with a 1-ml syringe and a hypodermic needle (25 G), through the arthrodial membrane at the base of a pereiopod. This treatment allowed us to assess the physiological effects attributable to trawling, while providing a control for aerial exposure.

In order to mimic the physiological stress likely to occur during commercial fishing practice, blood samples were taken from a second treatment group of 20 individuals per species per haul. The sampling time per individual took up to 3 min, resulting in a time lapse of $<45$ min (aerial exposure) between the first and the last sample taken. During commercial fishing operations, a similar time-lapse is likely to occur, as some animals are discarded within a few minutes after hauling, while others are only returned to the sea after several hours.

Three days after trawling, blood samples were taken from intact control *M. rugosa* and *L. depurator* captured in *Nephrops* creels, baited with mackerel (2 day soak time) deployed in Fintray Bay. Creels were hauled on board one at a time, and haemolymph samples were taken, $<6$ min after hauling, to minimise aerial exposure.

All blood samples were stored in Eppendorf microtubes (1.5 ml) with liquid nitrogen immediately after withdrawal, to avoid clotting and further physiological reactions. On return to the laboratory, size and damage to individuals were recorded, and haemolymph samples were stored at $-20$°C until used. The effects of different types of storage on haemolymph chemistry were assessed, by comparing values for pH, L-lactate and D-glucose in pooled haemolymph samples subjected to flash-freezing in liquid nitrogen, storage at $-20$°C and direct treatment with PCA. No significant differences were found between these treatments.

The pH of haemolymph was measured using a pH meter (Russel, RL150) with a pH electrode (Russel, TR/CMAW711/TB), at the in situ temperature, on the date of collection, by defrosting samples in an adjusted cold water bath. After the removal of 100 μl haemolymph for the ammonia assay, the remainder was mixed with an equal volume of 0.6 M perchloric acid (PCA), to precipitate and inactivate proteins before centrifugation for 20 min at 10 000 *g*. The supernatant was neutralised with 2 M potassium bicarbonate and, after cooling on ice for 10 min, the precipitated potassium perchlorate was separated by centrifuging again. The supernatant was stored at −20°C prior to use.

The concentration of L-lactate in 50 μl of sample was determined by the enzymatic method of Gutmann and Wahlefeld (1974), as modified by Engel and Jones (1978). Levels of D-glucose were assessed using the glucose oxidase technique (Boehringer-Mannheim, Cat. No. 124028) in a microplate format as outlined by Webster (1996). Total dissolved ammonia concentrations were measured using the flow injection/gas diffusion method developed by Clinch et al. (1988), as modified by Hunter and Uglow (1993).

Where necessary, data were subject to $\log_{10}$-transformation and comparisons made using general linear models (GLM, MINITAB) and a Tukey–Kramer multiple comparison test. If data did not conform to a Gaussian distribution, a Kruskal–Wallis test was performed with subsequent pairwise Mann–Whitney *U*-tests. The significance criterion applied in all tests was $P < 0.05$.

### 2.2. Recovery experiment

In October 1999, RV Aora made a 2 h tow south of Little Cumbrae Island (ca. 55°41′N 04°57′W) at 74 m depth. Haemolymph samples from six randomly collected *L. depurator* were taken immediately after hauling, in order to assess physiological stress caused exclusively by capture in the trawl, while individuals for a second group were gathered and exposed to air. After 1 h, haemolymph was withdrawn from 10 crabs for the determination of physiological stress due to capture and aerial exposure, while the remainder of the animals were stored in shaded fish boxes (75 × 40 cm) supplied with running seawater (25 cm water depth), for the study of recovery after trawling and emersion. Haemolymph samples were taken from groups of 8–10 individuals at intervals of 0.5, 1, 2, 4, 6, 8, 12 and 24 h following aerial exposure. On return to the laboratory, all crabs were transferred to a communal outdoor holding tank (110 × 65 cm, water depth ca. 30 cm) supplied with running seawater at ambient temperature. Wet weight, carapace width, sex and damage of each *L. depurator* were recorded, and haemolymph samples were stored in liquid nitrogen immediately for further analysis (see above). The samples taken at different time intervals were compared using GLM and a Tukey–Kramer test.

### 2.3. Effects of exercise and aerial exposure

A third experiment distinguished between the effects of aerial exposure and exercise. In November 1999, a group of 14 *L. depurator* and 21 *M. rugosa* were caught in creels

at Fintray Bay, and subsequently held in separate holding tanks supplied with running seawater within $\pm 1$°C of the bottom temperature, on the date of collection. After 1 week, blood samples were taken from each individual, in order to assess the effects of tank maintenance and to gain a baseline for the effects of aerial exposure and exercise. One week later, a group of 10 tank-held male *L. depurator* were exposed to air for 1 h, prior to withdrawal of haemolymph. In order to mimic trawl-capture, a second group of crabs was transferred to a seawater sink ($60 \times 37$ cm), where they were periodically inverted and constantly kept in motion with a pond net for 1 h, before sampling their haemolymph. The samples were treated as outlined above, and data were compared by GLM (D-Glucose) or a Kruskal–Wallis test with subsequent pairwise Mann–Whitney *U*-test (pH, L-lactate).

### *2.4. Water loss after aerial exposure*

In March 2000, evaporative water loss was determined as percentage weight loss in tank-held *M. rugosa* and *L. depurator*, following 1 h of aerial exposure in individual wide-necked jars at 10°C. The animals were not blotted dry prior to weighing, as it was attempted to imitate commercial conditions. Haemolymph samples were also taken from these animals for the assessment of the blood osmolarity (freeze point depression, Roebling micro-osmometer type 12/12DR). Additionally, haemolymph was withdrawn from a group of control crustaceans that had been kept submerged. Differences in osmolarity were assessed using a two-sample *t*-test, and weights before and after aerial exposure were compared by a paired *t*-test (MINITAB).

## 3. Results

The concentrations of most blood constituents of *L. depurator* and *M. rugosa* examined in the present study were altered after trawling. All crustaceans survived aerial exposure.

### *3.1. Physiological stress in trawled and creel-caught crustaceans*

Table 1 shows the means of all haemolymph parameters examined in each treatment and species. The mean size and wet weight of animals in different treatment groups were similar. The temperatures recorded were 10°C at 45 m depth, 15°C at the sea surface and 16°C on deck (air).

The total ammonia concentrations were highest in *L. depurator* (GLM, $P < 0.001$, $F = 44.56$, $df = 2$) and *M. rugosa* (GLM, $P < 0.001$, $F = 10.1$, $df = 2$) subject to trawling and aerial exposure, with concentrations more than two times higher compared with controls and re-immersed *L. depurator*. By contrast, samples from control and immediately re-immersed trawled animals had similar ammonia concentrations.

Control *L. depurator* had the lowest D-glucose concentrations, followed by immediately re-immersed trawled crabs and emersed trawled crabs. All treatment groups were statistically different from each other (GLM, $P < 0.001$, $F = 33.95$, $df = 2$). While

Table 1
Mean ammonia, D-glucose, L-lactate concentration (mmol $l^{-1} \pm$SE) and pH in haemolymph samples from *L. depurator* and *M. rugosa* caught in creels and by trawling

| Treatment | Total ammonia | D-Glucose | L-Lactate | pH | Size (mm) | Wet weight (g) |
|---|---|---|---|---|---|---|
| *L. depurator* | | | | | | |
| Creel-caught **A**, $n = 43–47$ | 0.135 **C** ± 0.013 | 0.079 **BC** ± 0.0023 | 1.103 **BC** ± 0.13 | **7.99 BC** ± 0.013 | 52 ± 0.64 | 32 ± 1.23 |
| Trawled and re-immersed **B**, $n = 20–22$ | 0.138 **C** ± 0.014 | 0.117 **AC** ± 0.0071 | 2.426 **AC** ± 0.35 | 8.43 **AC** ± 0.075 | 54 ± 0.73 | 36 ± 1.62 |
| Trawled and emersed **C**, $n = 39–43$ | **0.308 AB** ± 0.016 | **0.140 AB** ± 0.0072 | **6.244 AB** ± 0.22 | 8.12 **AB** ± 0.048 | 54 ± 0.50 | 36 ± 0.92 |
| *M. rugosa* | | | | | | |
| Creel-caught **A**, $n = 46–49$ | 0.372 **C** ± 0.018 | 0.059 **C** ± 0.0009 | 0.099 **BC** ± 0.02 | 7.75 **BC** ± 0.018 | 30 ± 0.34 | 35 ± 1.34 |
| Trawled and re-immersed **B**, $n = 20–22$ | 0.415 **C** ± 0.032 | 0.065 **C** ± 0.0045 | **1.138 A** ± 0.21 | **7.42 A** ± 0.026 | 30 ± 0.50 | n.a. |
| Trawled and emersed **C**, $n = 40–42$ | **0.519 AB** ± 0.029 | **0.097 AB** ± 0.0051 | 0.870 **A** ± 0.08 | 7.47 **A** ± 0.018 | 30 ± 0.37 | n.a. |
| Tank-held, $n = 20–21$ | n.a. | 0.355 ± 0.0597 | 0.089 ± 0.01 | 7.92 ± 0.028 | 29 ± 0.57 | 37 ± 2.51 |

One group of crustaceans was immediately re-immersed after trawling (trawled and re-immersed **B**), while animals from a second group (trawled and emersed **C**) were sampled over a period of ca. 40 min (in air) following trawling. Blood of *M. rugosa* previously held in tanks was also analysed (tank-held), but not included in the statistical analysis. **A–C** indicate means significantly different from those of groups indicated in column 1 ($P < 0.05$) and highest values are highlighted by use of boldface.

Table 2
The effects of 1 h of aerial exposure or constant submerged exercise on *L. depurator* haemolymph pH, L-lactate and D-glucose concentrations (mmol $l^{-1} \pm$ SE)

| | *n* | D-Glucose | L-Lactate | pH |
|---|---|---|---|---|
| Tank-held **A** | 13–14 | 0.173 ± 0.0095 | 0.596 **B** ± 0.47 | 7.77 ± 0.057 |
| Aerial exposure **B** | 10 | 0.20 **C** ± 0.022 | 5.14 **AC** ± 0.63 | 7.64 **C** ± 0.044 |
| Exercise **C** | 10 | 0.15 **B** ± 0.013 | 1.48 **B** ± 0.85 | 7.89 **B** ± 0.053 |

**A**–**C** indicate means/medians significantly different from those of groups indicated in column 1 ($P < 0.05$).

glucose levels in control and immediately re-immersed trawled *M. rugosa* were similar, significantly higher concentrations were found in emersed trawled squat lobsters (GLM, $P < 0.001$, $F = 44.94$, $df = 2$). Crabs and squat lobsters held in tanks had much higher haemolymph glucose concentrations (Tables 1 and 2), exceeding control levels two-fold in crabs and six-fold in squat lobsters.

The lactate concentrations were significantly different in all *L. depurator* treatment groups (GLM, $P < 0.001$, $F = 102.4$, $df = 2$), with highest levels in emersed trawled crabs, followed by immediately re-immersed trawled animals and controls. The lactate levels in emersed trawled crabs were almost six times higher (6.2 mmol $l^{-1}$) compared with controls. By contrast, lactate concentrations were highest in immediately re-immersed trawled *M. rugosa*, although they were not statistically different from emersed trawled squat lobsters. The temperature of the sea-surface water used for re-immersion was 5°C higher than the bottom water. Creel-caught *M. rugosa* had significantly lower lactate concentrations (GLM, $P < 0.001$, $F = 107.18$, $df = 2$), with levels 11 and 9 times higher in emersed trawled and in immediately re-immersed trawled squat lobsters, respectively. Generally, *L. depurator* had higher lactate concentrations than *M. rugosa*.

The pH was lowest in haemolymph samples from creel-caught crabs, followed by emersed trawled crabs and immediately re-immersed trawled animals (GLM, $P < 0.001$, $F = 21.5$, $df = 2$). Repeated pH measurements of another batch of creel-caught *L. depurator* in November 1999 gave similar results (Mann–Whitney *U*-test, $P = 0.37$). By contrast, immediately re-immersed trawled and emersed trawled squat lobsters had the lowest pH, controls having a 0.3 unit higher blood pH (GLM, $P < 0.001$, $F = 82.54$, $df = 2$). The pH values of animals of the same treatment group varied greatly, e.g. from 7.24 to 7.90 in trawled squat lobsters.

### 3.2. Recovery experiment

Figs. 1 and 2 illustrate the physiological recovery process in *L. depurator* following trawling and emersion. The temperature recorded at 74 m depth and at the sea surface was 13°C, and 14°C on deck (air). At the end of the emersion period, L-lactate levels increased to nearly 8 mmol $l^{-1}$ and decreased following re-immersion, reaching significantly lower concentrations after 4 h (GLM, $P < 0.001$, $F = 35.53$, $df = 9$) and stabilising thereafter. Values similar to those from tank-held crabs were reached between 4 and 6 h (Fig. 1 and Table 2).

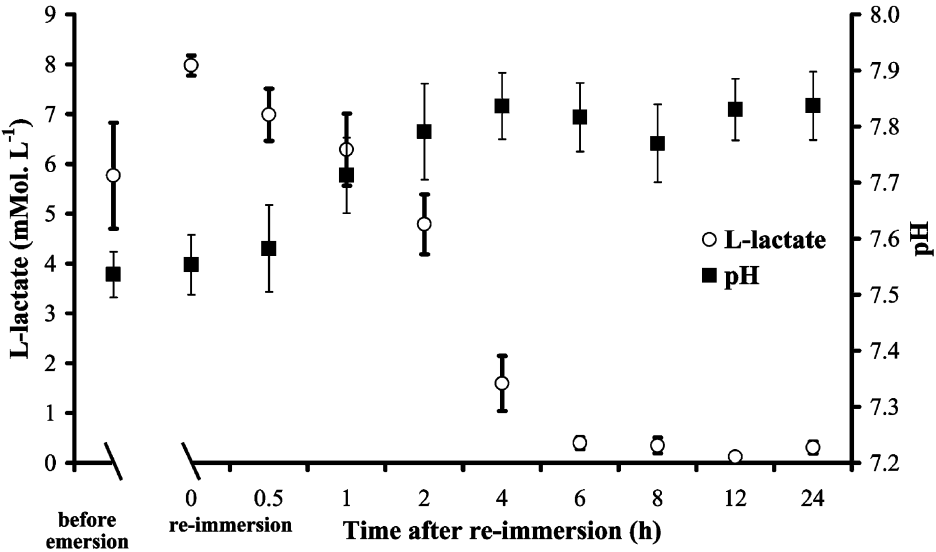


Fig. 1. Changes in the concentration of haemolymph L-lactate and pH in *L. depurator* over 24 h following trawling and 1 h of emersion.

The haemolymph pH followed a similar pattern. After being low initially (7.54), the pH increased steadily to 7.84 over the 24 h monitoring period (Fig. 1). Although a GLM

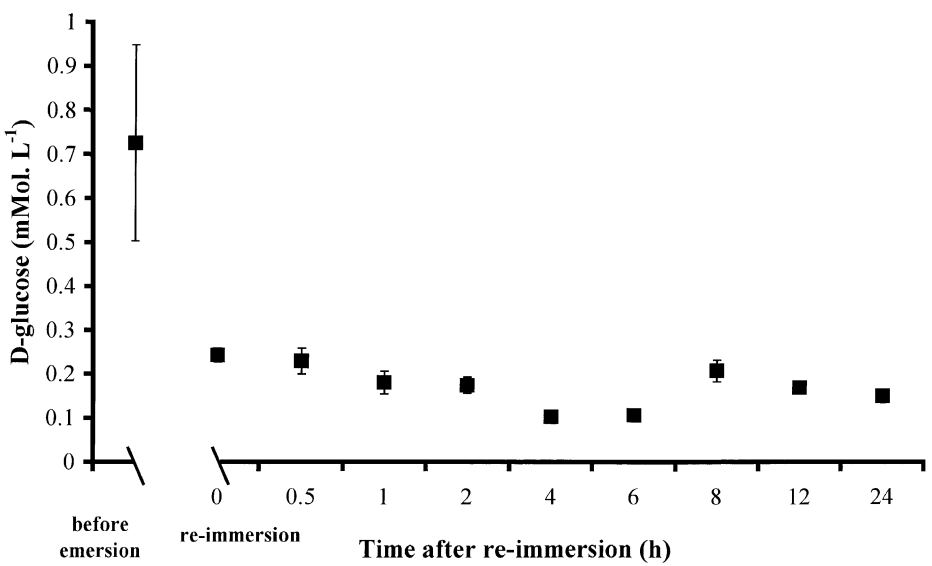


Fig. 2. Changes in the concentration of haemolymph D-glucose in *L. depurator* over 24 h following trawling and 1 h of emersion.

analysis indicated highly significant overall differences ($P < 0.001$, $F = 3.87$, $df = 9$) a Tukey–Kramer test only revealed statistical differences between samples taken at the end of 1 h emersion compared with those taken 4, 12 and 24 h following re-immersion. A Spearman's rank correlation test revealed a strong negative correlation between pH and lactate ($r = -0.510$, $P < 0.001$).

The mean D-glucose concentration immediately after trawling (0.725 mmol $l^{-1}$) was significantly higher than all other samples (GLM, $df = 9$, $F = 9.27$, $P < 0.001$). Afterwards, glucose levels stabilised around 0.1–0.25 mmol $l^{-1}$ with a slight increase after 8 h (Fig. 2), although this was statistically not significant. These glucose concentrations were similar to those obtained from tank-held *L. depurator* (Table 2), but higher than those from controls (Table 1).

### 3.3. Effects of exercise and aerial exposure

Table 2 shows that haemolymph concentrations of D-glucose and L-lactate were significantly higher, and pH is significantly lower, in *L. depurator* exposed to air for 1 h compared with those subject to 1 h of exercise ($P < 0.05$). Most exercised crabs did not reach exhaustion. They were still able to right themselves at the end of the experiment, although this took longer than at the beginning. Tank-held crabs had the lowest lactate concentrations, but levels of glucose and pH were intermediate between the two other treatment groups. A Pearson correlation test (MINITAB) revealed a strong negative correlation between lactate and pH ($r = -0.921$, $P < 0.001$). While lactate concentrations of emersed crabs were similar to those from the emersed trawled animals of Experiment 1 (Table 1), those exposed to extended exercise had lower lactate concentrations compared with the immediately re-immersed trawled crabs of Experiment 1 (Table 1).

### 3.4. Water loss after aerial exposure

The mean wet weights of *L. depurator* and *M. rugosa* were significantly lower after 1 h of emersion ($P < 0.001$) (Table 3). Crabs and squat lobsters lost up to 7% and 9% of their body weight, respectively. A Pearson correlation test revealed a positive correlation between body size and weight loss for both *L. depurator* ($r = +0.625$, $P < 0.005$) and *M. rugosa* ($r = +0.814$, $P < 0.001$). The mean haemolymph osmolarity of crabs that

Table 3
Mean weight loss and osmolarity (mOsmol kg $H_2O^{-1}$) of *L. depurator* and *M. rugosa* after 1 h of emersion

| | *n* | Mean wet weight (g) ± 95% CI | | Mean osmolarity (mOsmol kg $H_2O^{-1}$) ± 95% CI | |
|---|---|---|---|---|---|
| | | Immersed | Emersed | Immersed | Emersed |
| *L. depurator* | 20 | 27.39 ± 2.98 | 26.00 ± 2.84*** | 1054 ± 44.9 | 1004.8 ± 25.5 |
| *M. rugosa* | 22 | 27.11 ± 5.74 | 25.55 ± 5.53*** | 963.0 ± 36.3 | 1002.0 ± 33.9 |

*** $P < 0.001$ indicates significant differences.

were exposed to air for 1 h was lower compared with animals that were kept immersed, although this was not statistically significant ($P = 0.054$) (Table 3). Haemolymph samples taken from squat lobsters subject to aerial exposure and those from constantly immersed *M. rugosa* had similar osmolarities (Table 3).

## 4. Discussion

The present study has shown for the first time how discarding practices affect non-target crustaceans at a physiological level. During sorting of the catch, non-target invertebrates spend up to 300 min on deck before being returned to the sea. We have shown that a trawl of 2 h duration, followed by 1 h of emersion, causes considerable stress to crustaceans, as indicated by changes in pH and an accumulation of ammonia, D-glucose and L-lactate in the haemolymph.

Marine decapods excrete ammonia produced during nitrogen catabolism by diffusion through the gills and $Na^+/NH_4^+$ exchange across the epithelium (Hagerman et al., 1990; Schmitt and Uglow, 1997). If not eliminated rapidly, ammonia accumulates and may reach toxic levels (Durand et al., 1999). During periods of emersion, such excretion is impaired, resulting in a significant increase of haemolymph ammonia (Vermeer, 1987; Hagerman et al., 1990), as was observed in the two sublittoral decapods *M. rugosa* and *L. depurator* subject to trawling and aerial exposure in the present study. The fact that controls and immediately re-immersed trawled crustaceans had similar ammonia concentrations implies that emersion was chiefly responsible for the accumulation of ammonia observed in trawled animals. Generally, ammonia concentrations were similar to those reported for *Nephrops* (Hagerman et al., 1990). Schmitt and Uglow (1997) and Wileman et al. (1999) have shown that ammonia efflux rates in *Nephrops* increase considerably following re-immersion, reaching control levels after 24 h. However, Vermeer (1987) ascribed chronic behavioural changes in the spiny lobster *Panulirus argus* to alterations in haemolymph constituents, such as ammonia, lactate and pH. Although these alterations may not be directly lethal, they could increase the susceptibility to predation, hence lowering the chances of survival in situ after discarding.

Hyperglycaemia is induced by a release of crustacean hyperglycaemic hormone (CHH) (Kallen et al., 1990; Webster, 1996; Lorenzon et al., 1997) in response to (emersion) stress leading to an increased glycogen utilisation, and has been reported for a number of crustaceans (e.g. Johnson and Uglow, 1985; Taylor and Spicer, 1987; Hagerman et al., 1990; Wileman et al., 1999; Schmitt and Uglow, 1997). Similarly, in the present study, high glucose levels were found in both *L. depurator* trawl treatments, while controls had significantly lower glucose levels. This implies that each of the two treatments (aerial exposure and trawling) induced a mobilisation of glucose. Crabs in the sorting pounds were often actively engaged in feeding, fighting and locomotion, presumably necessitating the mobilisation of glucose (M. Bergmann, personal observation).

In the recovery experiment, glucose levels were high immediately after trawling and decreased following emersion and re-immersion, indicating that the initially mobilised glucose had subsequently been metabolised. This was mirrored by a simultaneous

increase of lactate (Figs. 1 and 2). Similarly, Wileman et al. (1999) reported no further increase of glucose in *Nephrops* discards following 1 h of emersion. Glucose concentrations after 24 h remained elevated compared with those from creel-caught controls, but were in the same range as tank-held animals. This implies an experimental artefact attributable to tank-maintenance conditions. Aerial exposure resulted in significantly higher glucose concentrations compared with the batch given extended exercise, although glucose levels of tank-held animals were somewhat in-between, suggesting that crabs kept in communal tanks were subject to increased locomotory activity. Glucose concentration may depend on a number of factors, such as nutritional state, moult stage (Chang, 1995), and time of day (Kallen et al., 1990), rendering it a less suitable indicator of stress (J. Spicer, personal communication). Hill et al. (1991) found a significant decrease in *Carcinus maenas* tissue glycogen following periods of anoxia and recovery; however, glucose concentrations showed little change, indicating that glucose may not provide an accurate indicator of hyperglycaemia. Lorenzon et al. (1997) recommended the use of the more sophisticated CHH radioimmuno-assay for the assessment of environmental stress in crustaceans.

Glucose concentrations were generally lower in samples from the more quiescent crustacean *M. rugosa* (Zainal et al., 1992). Emersed, trawled squat lobsters had significantly higher levels of glucose compared with controls or immediately re-immersed trawled animals, indicating that aerial exposure was mainly responsible for the glucose build-up observed in this species. Interestingly, Lorenzon et al. (1997) reported higher glucose levels (0.62 mmol $l^{-1}$) in control *M. rugosa* from the Adriatic Sea, possibly due to local and seasonal differences. Wileman et al. (1999) found glucose levels almost an order of magnitude higher in *Nephrops* discards compared with *L. depurator* and *M. rugosa*, and a three fold increase compared with creel-caught *Nephrops*.

Increased haemolymph lactate concentrations following periods of exercise or emersion indicate a switch to anaerobic metabolism, and have been reported for a range of marine crustaceans (e.g. Albert and Ellington, 1985; Johnson and Uglow, 1985; Taylor and Spicer, 1987; Vermeer, 1987; Forster et al., 1989; Hill et al., 1991; Zainal et al., 1992; Schmitt and Uglow, 1997; Wileman et al., 1999). Similarly, the lactate concentrations of trawled and immediately re-immersed *L. depurator* in our study were significantly higher compared with controls, but lower than those of trawled and emersed crabs, implying that while aerial exposure led to the main build-up of lactate, increased locomotory activity during the trawling process also contributed to an accumulation of lactate in accord with other studies (Forster et al., 1989; Field et al., 1991; Wileman et al., 1999). The fact that we found higher haemolymph lactate concentrations in emersed crabs than in exercised individuals further supports this argument. The two-fold higher lactate concentrations in creel-caught *L. depurator* compared with tank-held crabs could be a result of agonistic encounters with conspecifics or competitors over the bait in the creels. Sneddon et al. (1999) and Huntingford et al. (1995) observed increases in lactate after fighting in crabs *C. maenas* and *Necora puber*. The accumulation of lactate results in an oxygen debt, which is repaid by an increase in the rate of oxygen consumption during the recovery phase (Paterson and Spanoghe, 1997). In the present study, lactate concentrations appeared to approach those of tank-held crabs 4 h after re-immersion.

Similar recovery times have been reported for rock lobsters *Jasus edwardsii* (Taylor and Waldron, 1997). However, most studies employed longer emersion times, making a direct comparison with present results difficult.

Elevated lactate values were also recorded for the two *M. rugosa* trawl treatments, indicating that tail-flipping exercise during trawling could have been the main factor responsible for the accumulation of lactate. Although Zainal et al. (1992) reported absolute lactate concentrations twice as high as in our study (2.2 mmol $l^{-1}$, after 1 h emersion), the difference in lactate concentrations between control and emersed crabs was only half that of our study. This could be due to different temperatures and experimental practice. In comparison, *Nephrops* discards showed both higher absolute lactate levels and a 13-fold increase (to 11.9 mmol $l^{-1}$) compared with creel-caught controls (Wileman et al., 1999). Together with the higher glucose levels, this indicates that *Nephrops* is either more active during trawling or more vulnerable to emersion stress.

Zainal et al. (1992) reported a recovery period of 11 h, following 4 h of aerial exposure for *M. rugosa*; a realistic time-span, as sorting on Clyde trawlers can last for up to 300 min (Bergmann and Moore, 2001). Lactate clearance took 25 h in the closely related squat lobster *Galathea strigosa* (Bridges and Brand, 1980), although aerial exposure was 4 h longer than in our study. After such periods of emersion on deck, discarded squat lobsters often become torpid (Zainal et al., 1992) and can be expected to be at high risk of predation. Although the concentrations of lactate, ammonia or $H^+$ ions that are lethal to *M. rugosa* and *L. depurator* are hitherto unknown, Taylor and Spicer (1987) reported tissue concentrations of 16.7 and 9.6 μmol $g^{-1}$ in freshly dead glass shrimps (*Palaemon elegans* and *P. serratus*) after anoxia. The mean lactate concentration of emersed trawled *L. depurator* from the present study (6.2 mmol $l^{-1}$) could easily be exceeded and could achieve the above levels during longer periods of deck exposure, and could have been a cause of short-term discard mortalities observed by Bergmann and Moore (2001).

Several authors have reported a low pH in crustacean haemolymph as a result of respiratory and metabolic acidosis due to an increase of lactate and bicarbonate-carbonic acid during anaerobic metabolism (e.g. Vermeer, 1987; Hill et al., 1991; Taylor and Waldron, 1997; Wileman et al., 1999). Accordingly, the blood pH of control *M. rugosa* was ca. 0.3 units higher compared with the two trawl treatment groups corresponding with high levels of lactate in these treatments. Zainal et al. (1992) reported a similar pH range for the same species.

Surprisingly, the haemolymph of creel-caught *L. depurator* was more acidic than that of trawled animals. This contrasts with the low lactate levels found in the same treatment group. A repeat of the experiment yielded similarly low haemolymph pH values, so that experimental error can be excluded, but the reason for the low blood pH remains unexplained. Within 4 h after trawling and re-immersion, the pH had increased by 0.3 units and approached similar values to those obtained from tank-held animals, indicating recovery. This corresponds well with the significant reduction of haemolymph lactate recorded after 4 h. Similarly, the lower pH (by 0.25 units) following 1 h of emersion compared with exercised crabs was mirrored by significantly higher concentrations of lactate, implying that the low pH was a result of metabolic acidosis.

The 7% and 9% reduction of body mass in *L. depurator* and *M. rugosa* after 1 h of emersion was probably due to evaporation of a residual water film on the body surface, but some water loss may also have occurred through the gills and the integument in the absence of waxy cuticles in marine crustaceans (Herreid, 1969). Evaporation depends on air circulation, temperature and humidity, so that our results can only be regarded as instantaneous. Johnson and Uglow (1985) reported lower weight losses of 1.36% and 0.75%, respectively in the brachyurans *C. maenas* and *N. puber*, and Vermeer (1987) found a weight loss of 2.4% $h^{-1}$ in spiny lobsters *P. argus*. The higher weight loss observed in our study was probably due to experimental differences, since crustaceans had not been blotted dry prior to weighing. Although reported for other decapods (Johnson and Uglow, 1985), there was no significant increase in haemolymph osmolarity following 1 h of emersion, indicating that water was chiefly lost from the integument surface rather than from body fluids.

Being truly sublittoral species, *L. depurator* and *M. rugosa* are unlikely to experience emersion stress in their natural environment and are probably less adapted to anoxia than other species, e.g. *C. maenas*. Brown and Caputi (1985) reported significantly lower moult increments until the second moult in *P. cygnus* that had been exposed to air for 1 h. This implies that while not being lethal, physiological emersion stress could impose longer term metabolic costs to discarded crustaceans. Physiological changes during longer periods of anoxia may well cause mortality, as has been reported for *M. rugosa* (Zainal et al., 1992), *P. elegans* and *P. serratus* (Taylor and Spicer, 1987) and *P. cygnus* (Brown and Caputi, 1985). In addition, torpid decapods returned to the sea may be prone to predation, and physiological changes could cause neural damage resulting in deviant escape behaviour, as shown in spiny lobsters *P. argus* (Vermeer, 1987). A reduction of sorting times in commercial practice could be achieved by shorter tow durations and could be expected to benefit both non-target crustaceans and undersized *Nephrops* discards.

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