Targeting the live market: recovery of Norway lobsters *Nephrops norvegicus* (L.) from trawl-capture as assessed by stress-related parameters and nucleotide breakdown

Amaya Albalat, Simon Sinclair, John Laurie, Alan Taylor and Douglas Neil

Division of Ecology and Evolutionary Biology, Faculty of Biomedical and Life Sciences, University of Glasgow, G12 8QQ, Scotland, UK

*** Corresponding author: Tel. +44 (0)1413305969**

***Email address*: d.neil@bio.gla.ac.uk**

Abstract

The recovery potential of Norway lobsters *(Nephrops norvegicus*) held in on-board seawater tanks after trawl capture was assessed at two different times of the year (winter and summer). Survival recorded 24 h after trawl-capture was 84.83 ± 0.93 % in the winter compared to 75.35 ± 2.92 % in the summer. Stress-related parameters in the muscle (arginine phosphate, glycogen and L-lactate) and in the haemolymph (L-lactate) were measured, together with nucleotide breakdown products in the muscle (yielding the 'Adenylate Energy Charge' or AEC ratio). All parameters analysed were responsive to the stress of the trawl-capture and subsequently recovered towards resting values, but did so at different rates. The fact that some measures recovered at a faster rate than others should be taken into account when trying to develop an index of metabolic stress for this species. Animals trawled in the winter recovered to AEC values above 0.8 within 4 h of placing them in on-board seawater tanks, whereas animals trawled in the summer took 24 h to reach these values. Furthermore, at the end of the trials animals trawled in the summer presented significantly higher haemolymph L-lactate and lower muscle glycogen reserves than the animals trawled in the winter, suggesting a faster recovery in the winter compared to the summer. Finally, animals in the winter were better able to endure further stresses (an emersion of 1 h while animals were transported to the commercial handling facilities). Therefore, as a code of practice it is advised that trawled *N. norvegicus* directed to the live trade should be allowed to recover for at least 4-6 h in on-board tanks, and extra care should be taken especially in the summer, if further stresses such as additional emersion are to be applied within the first 24 h after capture.

Keywords: Crustacean, *Nephrops*, trawl-capture, recovery, temperature, AEC

Introduction

The fishery for the Norway lobster (*Nephrops norvegicus*) is currently the most valuable fishery in the UK (£104.3 M at first sale in 2007) (FRS, 2009). In recent years the demand for the supply of live animals to continental Europe has increased to such an extent that trawl-caught animals are increasingly being used to supply this live market, which has traditionally been supplied only by creel-caught animals.

Trawling has been shown to be extremely stressful for crustaceans in general, and in particular for the species studied in the present work, *N. norvegicus* (Albalat et al., 2009). The stressful effects of trawling are due to a sum of different stressors that are applied almost simultaneously, such as increased exercise due to the animals trying to escape from the net (Newland et al., 1992; Gornik at al., 2010), physical damage due to abrasion between animals and objects in the cod-end of the net and differences in pressure during hauling (ICES, 1994; Lancaster and Fridd, 2002; Milligan et al., 2009) and periods of oxygen deprivation as a result of emersion on deck (Ridgway et al., 2006a; Bernasconi and Uglow, 2008a; Gornik at al., 2010). This condition of metabolic stress in the animal is potentially reversible (Harris and Andrews, 2005; Uglow et al., 1986), but in some aspects is irreversible, being associated with an onset of necrosis in the muscle (Stentiford and Neil, 2000).

The stress responses of *N. norvegicus* to trawl-capture have been studied in detail by several authors. Trawling produces a depletion of muscle arginine phosphate and glycogen (Harris and Andrews, 2005; Albalat et al., 2009) together with an increase in muscle and haemolymph L-lactate (Harris and Andrews, 2005; Ridgway et al., 2006b; Albalat et al., 2009). These studies have shown that during the trawling process

energy requirements exceed the capacity of the aerobic metabolism of the animal, and therefore anaerobic metabolism is activated in order to maintain ATP levels. However, ATP concentrations have been shown to decrease significantly in newly trawl-caught animals, while AMP increases to become the main nucleotide in the muscle (Albalat et al., 2009; Mendes et al., 2001). In general, further loss of AMP and accumulation of IMP have only been reported in situations of extreme fatigue (Baldwin et al., 1999), while other ATP breakdown nucleotides such as HxR and Hx are only detected post-mortem, when ATP production has ceased (Gornik et al., 2010). The balance between ATP, ADP and AMP is described by the 'Adenylate Energy Charge' or AEC ratio (Atkinson, 1968) which has been recognised as an important index for describing the energy status of the living muscle cells (England and Baldwin, 1983; Maguire et al., 2002). In general terms, normal AEC values range from 0.87 to 0.94 (with the main nucleotide in the cell being high-energy ATP) in rested, un-stressed animals, while animals in irreversible physiological collapse are reported to have AEC values lower than 0.5 (with the main nucleotide being low-energy AMP) (Sylvestre and Le Gal, 1987). In this sense, Albalat et al. (2009) showed extremely low AEC values (around 0.3) in *N. norvegicus* just after trawling, implying that many of these animals may not survive or recover from such a trauma.

Although considerable work has been done to describe the metabolic state of *N. norvegicus* after capture, currently only limited information is available about the survival and recovery potential of trawled *N. norvegicus* that are destined for the live trade. So far recovery studies on trawled *N. norvegicus* have concentrated on the return of measures of glycemia and lactemia to normal levels, mainly in the haemolymph (Harris and Andrews, 2005; Ridgway et al., 2006b; Lund et al., 2009).

However, since these parameters tend to take a long time to return to normal values in crustaceans, a more immediate and sensitive measure is required to assess the recovery trajectory of *N. norvegicus* in the first 24 h after catch. In relation to this, in other crustaceans the return of ATP and high-energy phosphates occurs more rapidly than the removal of L-lactate and the restoration of glycogen stores (Onnen and Zebe, 1983). Therefore, measures of nucleotides could be used in recovery experiments as a more reliable method for assessing the efficacy of different methods of live transport (Paterson et al., 1997; Morris and Oliver, 1999) in order to minimise economic losses due to poor survival.

Practices used in the other live lobster fisheries, for example the one for the western rock lobster (*Panulirus cygnus*) involve placing the animals as soon as possible in on-board seawater tanks (Paterson and Spanoghe, 1997), and similar procedures are beginning to be adopted for *N. norvegicus*. Typical commercial practices supplying trawled *N. norvegicus* to the live market have included holding animals on deck in tube-sets or vertically subdivided containers with only shading or water spraying, or on ice in a cold room, resulting in very low survival rates, especially in the summer. Deployment of on-board seawater tanks on the deck of the fishing vessel could provide one means of increasing survival, and of preventing high mortalities, and a few commercial boats now have such facilities. However, the best operational practices to be used in conjunction with the use of such recovery tanks have yet to be established.

Therefore, the objectives of the present work were firstly to obtain a comprehensive understanding of the recovery potential of *N. norvegicus* after the stresses of trawling

and handling under commercial conditions, using a variety of parameters, and secondly to determine the time needed for recovery for this species when held in on-board seawater tanks at two different times of the year (winter and summer). To this end, several stress-related metabolites in the muscle (arginine phosphate, glycogen, L-lactate) and in the haemolymph (L-lactate) were measured together with muscle ATP and its breakdown products. Samples were taken immediately after trawling and over the subsequent 24 h recovery period that included a holding period of 6 h in on-board seawater tanks, a short overland transport out of water (1 h) and an overnight recovery in seawater tanks located in a commercial holding facility.

Material and Methods

Capture and holding

Norway lobsters (*Nephrops norvegicus*) were caught by otter trawl in the Clyde Sea area, Scotland, UK (55.35 N, 04.54 W; depth range 60-80 m). The vessel used in the trials was the M.V. *Eilidh Anne* (GK2), and the commercial trawl gear used was a single hopper trawl net with a cod end mesh size of 80 mm, towed at approximately 2 knots. To determine average survival values in the winter and summer, data were collected from landings of this boat during the months of December 2007 and August 2008 (13 trips in each month). To obtain animals for the stress measurements, a winter recovery trial was carried out on the 15th of December 2007, the trawl for which commenced at 08:00 h and lasted for 1 hour 45 minutes, with sorting and sampling of the animals lasting for 45 minutes. A summer recovery trial was carried out on the 21st of August 2008, the trawl for which commenced at 07:15h and lasted for 1 hour 30 minutes, and in this case, sorting and sampling of the animals lasted for 30 minutes. In both trials, hauling of the trawl net took approximately 15 min.

In the recovery trials, during on board sorting all of the *N. norvegicus* in the catch that showed signs of life (displaying limb movements) were collected and placed in a vertical position in tube-sets, each containing approximately 160 lobsters. The animals were selected at random by the skipper of the vessel from the size grade appropriate for the smallest of the commercial containers used. The selected animals were commercial grade 3 (30 to 40 individuals per kilogram), which equates approximately to a size range of 27 mm to 37 mm carapace length. For the purpose of the recovery trials one tube-set (160 animals) was filled on each day as above. This set was marked and placed in a tank. This marked set provided the animals for

subsequent sampling throughout the trial. In the winter recovery trial 32 % of the *N. norvegicus* caught were female and in the summer recovery trial 63 % were female. The tube sets of animals were held in purpose-built aluminium tanks located on the deck of the vessel and covered by the vessel's shelter deck. These tanks were supplied with seawater from a deck hose, which drew water continuously from ~3m depth below the hull. Surface water temperature, salinity and dissolved oxygen concentration (DO_2) were sampled periodically in these tanks, and the ambient temperature under the shelter deck adjacent to the tanks was also monitored. Data on bottom water temperature in the study area were obtained from the Clyde Environment and Fisheries Review conducted over the period 2006-2007 (Combes, 2007).

The tube-sets remained submerged in the on-board tanks for 6 h before they were transferred to a van that was refrigerated in the summer, to keep the indoor van temperature at 6-8 ˚C in both seasons where they were stacked in air for transport to the commercial handling facility (driving time ~1 h). Here 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 commercial ultraviolet sterilisers and chilled to a temperature of 8 ˚C.

Sampling procedure and storage of samples

Sampling commenced within 15 minutes of capture and also at 2, 3, 4 and 6 hours after placing the animals in the on-board tanks. Further samples were taken immediately after they had been transported by land to the commercial handling

facility and also on the following day. At each sampling time 15 animals taken from the marked tube-set were sacrificed.

Haemolymph samples were taken from the sinus at the base of the 5th pereiopod using a 25-gauge needle and a disposable syringe and placed on ice. Immediately afterwards, the animals were sacrificed and samples from the deep abdominal flexor muscle taken. The muscle samples were immediately frozen in liquid nitrogen and subsequently stored at -80 ˚C for later analysis.

Environmental parameters: air temperature (ºC), seawater temperature (ºC), salinity (PSS) and dissolved oxygen concentration (mg l^{-1}) in the on-board tanks were obtained using a handheld multi-parameter probe (Yellow Springs Instruments YSI 556).

After on-board sorting, survival of the whole live catch from both trials was assessed in the standard commercial manner by a system of 'pack-out'. This entails all live animals being packed into polystyrene boxes containing 4 Kg of live product (c.a.140 animals) for dispatch to customers, and all dead/dying animals being rejected. This generates a percentage of live versus dead animals by weight (Kg) from the whole live catch (after on-board sorting) on any given day. Mortality was assessed 24 h after sorting the animals on-board (i.e. after overnight recovery). In addition, survival data for a total of 13 landings of same boat in each of the months December 2007 and August 2008 were obtained in the same way.

Biochemical analysis

Samples of frozen abdominal muscle were weighed and homogenised on ice with 5 x volume (w/v) 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 and 500 µl of the clear supernatant was used to determine lactic acid concentration. Finally, the remaining supernatant was processed for the analysis of ATP and its breakdown products and arginine phosphate.

ATP and its breakdown products - Nucleotide extracts were prepared as described in Ryder (1985). ATP and its breakdown products 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). Standard curves were prepared from adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), inosine 5'-monophosphate (IMP), inosine (HxR) and hypoxanthine (Hx) all from Sigma Aldrich (Dorset, England, UK).

Adenylate Energy Charge or AEC was obtained according to Atkinson (1965) using the following formula,

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

Arginine phosphate - The concentration of arginine phosphate was determined according to the method of Viant et al. (2001). The same extracts prepared for nucleotide analysis were used for the determination of arginine phosphate. 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) .

Glycogen determination - Frozen muscle samples were freeze-dried for 24 h as described in Ridgway et al. (2006b). A sample of approximately 50 mg of dry muscle was added to 400 µl of 30 % KOH and boiled for 10 min. Afterwards, 700 µl of pure ethanol was added and the samples were left on ice for 2 h before being centrifuged at 14,000 rpm for 10 min. Pellets were resuspended with double distilled H_2O and 50 µl were incubated at 95-100 ºC for 10 min with 1 ml of anthrone reagent. The absorbance of the samples was measured in a spectrophotometer (Shimadzu, UV Mini 1240) at 600 nm and total glycogen (as glucosyl units) was determined according to Carrol et al. (1956) using a calibration curve constructed from standards of known concentrations of glucose (0.5-10.0 mM)..

L-lactate concentration - L-lactate concentration was measured in haemolymph and abdominal muscle homogenates. For the haemolymph samples, L-lactate was determined with a portable lactate analyser (Accutrend®, Roche Diagnostics, Basel, Switzerland) on board on the day of the experiments using freshly extracted samples. For the muscle, L-lactate was quantified in samples that had been frozen in liquid nitrogen, using the enzymatic method described by Bergmeyer and Bernt (1974) and further modified by Hill et al. (1991). Briefly, muscle homogenate supernatants as previously described were thawed and 50 µl were added to Eppendorf 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) at pH 9.5 and 1 unit of lactate dehydrogenase

(LDH, Sigma) and incubated for 2 h at 37 ˚C. The absorbance of a sample was measured at 340 nm on a spectrophotometer (Shimadzu, UV Mini 1240) and converted to a L-lactate concentration using a calibration curve constructed from standards of known concentrations of lactic acid (0.5-10.0 mM).

The accuracy of the portable lactate analyser for the determination of L-lactate in *Nephrops* haemolymph samples was confirmed by analysing a set of haemolymph samples using the enzymatic method and comparing these with the values obtained using the lactate analyser. In order to use the enzymatic method haemolymph was deproteinised using ice-cold perchloric acid 0.6 M (1/1) (v/v). It was found that there was a highly significant correlation ($r^2 = 0.960$) between the values for haemolymph lactate obtained using the two methods. As a result, the portable analyser was used throughout this study for the analysis of L-lactate in the haemolymph.

Statistical analysis

Data are reported as mean values ± standard error of mean (SEM). Differences between sampling times over the 24 h period studied (summer and winter trials separately) 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. The differences between winter and summer for each sampling point were analysed by independent samples t-test. In both cases, P-values lower than 0.05 were considered statistically significant.

Results

Environmental data and animal survival

The environmental parameters on the days of the winter and summer trials are shown in Table 1. Recorded air temperatures on-board were within the average ranges reported for the area studied (mean air temperatures in the West of Scotland are 5.5-7.0 °C in December and 14.0-16.0 °C in August) (www.metoffice.gov.uk). The seawater temperature in the on-board tanks was higher in the summer trial than the winter trial, reflecting the surface seawater temperatures at those times, while according to the Clyde Environment and Fisheries Review the average bottom water temperatures in the area studied are actually ~12 °C both in December and in August (Combes, 2007). Dissolved oxygen in the on-board seawater tanks remained at saturation point throughout in both trials, whereas salinity was lower in the summer trial compared to the winter trial, which could be related to rainfall in the days immediately preceding the summer trial.

In both seasons, sufficient animals were collected from a single trawl to conduct the recovery trial, and the time required to sort the catch was similar although it took 15 min less to sort the catch in the summer. According to the 'pack-out' procedure for estimating survival rates, there was a significant seasonal effect on mortality on the days of the trials (where animals were sampled for further analysis). This was consistent with the average values obtained from 13 other trips made by the same boat in the same two months using the same recovery protocol: the survival rate in the winter (84.83 ± 0.93 %) was significantly higher than in the summer (75.35 ± 2.92 %).

WINTER TRIAL

Nucleotides and AEC - The main nucleotide in muscle tissue immediately after trawl capture was AMP (Table 2 and Fig. 1). ATP recovered from a very low concentration at the point of capture (1.44 ± 0.38 μmol g^{-1}) to reach maximum concentrations (8.28 ± 0.38 μmol g^{-1}) after a holding period of 6 h in on-board seawater tanks. However, ATP rather than AMP became the main nucleotide within 4 h. After the subsequent transport by vehicle to the processing facility (animals transported out of water) the main nucleotide was still ATP. Although detectable levels of AMP were obtained, these values did not change significantly following the overnight recovery. IMP was detected in the muscle for up to 3 h post-capture, but was un-detectable after this time and throughout the remainder of the trial. The calculated AEC ratio was low after capture (0.31 ± 0.21), but within 4 h it had increased significantly (0.83 ± 0.04) (Fig. 2). The AEC value recorded following transportation to the processing facility was lower than that recorded after the holding period of 6 h in the on-board seawater tank, but this difference was not statistically significant.

Arginine phosphate and glycogen concentrations in the muscle - Arginine phosphate was almost completely depleted after trawl capture (0.61 ± 0.31 μmol g^{-1}) (Table 2 and Fig. 3) but increased significantly after 4 h in the on-board seawater tanks and reached a maximum concentration (7.38 ± 0.3 μmol g^{-1}) after 6 h. A decrease in arginine phosphate concentrations was obtained after the transportation in air, although due to the inter-individual variability these changes were not statistically different. On the other hand, the concentration of glycogen in the muscle decreased slightly from its post-capture value at the 4 h recovery point, but after this time tended

to increase (with some fluctuations), so that its value was increased significantly by the following day (4.08 ± 0.70 mg g^{-1}) (Fig. 4).

L-lactate concentrations in the muscle and haemolymph - The concentration of L-lactate in the abdominal muscle immediately after capture was 30.26 ± 1.40 µmol g^{-1}. Although there was some variation in the concentration of L-lactate recorded after 2 h in the recovery tanks and after land transport in air, these were not significant. There was, however, a significant reduction in the L-lactate concentration following overnight recovery holding in land-based seawater tanks (Fig. 5a).

L-lactate in the haemolymph changed relatively little during the recovery period, although significantly higher values were obtained following the land transport in air, and then a significant reduction occurred following the overnight holding in land-based seawater tanks (Fig. 5b).

SUMMER TRIAL

Nucleotides and AEC- In the summer trial ATP became the main nucleotide after 3 h of placing the animals in on-board seawater tanks. This situation did not change even after the land transport of the animals to the processing facilities. Similarly, the AEC ratio was low after capture (0.24 ± 0.20) and increased significantly after 3 h. However, AEC values above 0.8 were only obtained after the overnight recovery period (0.91 ± 0.03) and IMP was detected throughout the sampling of this trial.

Arginine phosphate and glycogen concentrations in the muscle – Arginine phosphate was very low after capture (0.40 ± 0.10 µmol g^{-1}) and increased significantly 3 h after

placing the animals in on-board seawater tanks (3.73 ± 0.87 μmol g^{-1}). Similar to the pattern observed in AEC ratios, maximum concentrations of arginine phosphate in the summer trial were obtained only 24 h after capture (7.98 ± 0.79 μmol g^{-1}). Land transportation provoked a decrease in arginine phosphate which was not statistically significant. On the other hand, glycogen concentrations after capture were very low and no significant changes were obtained throughout the duration of the trial.

L-lactate concentrations in the muscle and haemolymph – Muscle L-lactate concentrations were highest after 2 h of placing the animals in the on-board tanks and after the land transport of the animals. These concentrations were significantly reduced after the overnight recovery period. Similarly, L-lactate in the haemolymph peaked at 2 h after placing the animals in the on-board tanks and after the land transport. However, except for these two sampling points L-lactate in the haemolymph did not change during the 24 h recovery period.

COMPARISON OF RATES OF CHANGE BETWEEN SEASONS

In the summer trial, although the recovery processes followed a similar pattern to those found in the winter, different rates and extents of change were nevertheless observed. Thus in the summer trial ATP concentrations initially increased more rapidly, but later they changed only slowly so that at 6 h their values (5.88 ± 0.65 μmol g^{-1}) were significantly lower than those obtained in the winter trial (8.28 ± 0.38 μmol g^{-1}) (independent t-test, $P<0.05$), and maximum concentrations of ATP in the summer were obtained only on the following day. The AEC values also reflected these seasonal differences in both their initial rates of change (significant difference was obtained at 3 h of placing the animals in on-board seawater tanks) and their later

extents of recovery, although in this case differences were not significant. On the other hand, in the summer IMP was detectable at low levels throughout the trial while in the winter IMP was not detected after 3 h of recovery and during the rest of the trial.

Other statistically significant differences in the summer compared to the winter were a lower muscle glycogen concentration after overnight recovery, higher muscle and haemolymph L-lactate concentrations after capture, after 2 h of placing the animals in the on-board tanks and after land transport, and a higher haemolymph L-lactate concentration after overnight recovery.

Discussion

Ability to recover

The present study has confirmed earlier findings that the trawling process is highly stressful. It brings the animals to an advanced state of exhaustion in which the main muscle nucleotide is AMP rather than ATP and the AEC ratio is extremely low (Mendes et al., 2002; Albalat et al., 2009). AEC values in other crustacean species exercised to exhaustion have been reported to be between 0.5 and 0.7 (Onnen and Zebe, 1983; Gäde, 1984; Maguire et al., 2002) and in general, it is accepted that animals with AEC values lower than 0.5 are in a state of physiological collapse and will therefore very rarely survive (Sylvestre and LeGal, 1987). However, we report for the first time that *N. norvegicus* has a considerable ability to recover from these extremely low AEC values (~0.3) observed after trawling if they are allowed to recover in tanks of flowing seawater on-board the trawl vessel. Moreover, the survivors recovered relatively rapidly, insofar as in the winter trial AEC values were

above 0.8 and the phosphagen concentrations had returned to their normal resting values within 4 h of placing the animals in on-board tanks.

This high capacity of *N. norvegicus* to reverse the nucleotide inter-conversions could be related to their life-style of occupying burrows in the sediment, habitats that can often become hypoxic (Rice and Chapman, 1971). The conversion of AMP to ATP, and hence the recovery of AEC, depend mainly on the presence of oxygen in the haemolymph. In this sense it has been reported that *N. norvegicus* like other burrowing decapods, are able to increase oxygen extraction efficiency and to hyperventilate in order to cope with hypoxic conditions (Atkinson and Taylor, 1988; Hagerman and Uglow, 1985). This ability to recover is also supported by the survival data. Thus, in the winter the survival rates after 24 h were around 85 %, a value that corresponds to those reported both by Ridgway et al. (2006b) and by Lund et al. (2009) for animals held in water following trawl capture in that season.

The effect of season

The first seasonal effect observed in the present study was a decrease in the survival of the whole live catch (after on-board sorting), assessed 24 h after recovery, in the summer compared to the winter. Secondly, as L-lactate is the major end-product of anaerobic metabolism in crustaceans (Spicer et al., 1990) results from the present study indicate that the extent of anaerobic metabolism was greater in the summer, as shown by higher muscle and haemolymph L-lactate concentrations after capture and after 2 h of placing the animals in on-board seawater tanks. These effects of season on survival and anaerobic metabolism could be due to several factors acting in combination in the summer, such as the higher water temperature and lower salinity at

the surface through which the animals were hauled and in which the animals were held in the on-board seawater tanks, the higher air temperature during the sorting and handling of the animals, and also the different sex ratio obtained in the catches (63 % of the catch were females in the summer compared to 32 % in the winter) that could have affected the energy demand of the animals during the recovery period (as many of those females are in a mature and reproductive stage).

A seasonal pattern of mortality was also obtained in the study of Lund et al. (2009). However, in our study absolute values of survival recorded 24 h after capture in the summer (84 %) were much higher than those obtained in the same season by Lund et al. (2009) (6 %), and more in line with those reported by Ridgway et al. (2006b) for *N. norvegicus* caught by trawl (75-90 %). A possible reason for the differences between these studies could be the temperatures at which the summer trials took place. In Lund et al. (2009) the seawater temperature difference that the animals faced during hauling was 9 °C (12 °C at bottom to 21 °C at surface) whereas in the present study it was only 3 °C (12 °C at bottom to 15 °C at surface). In addition the different air temperatures during sorting (18 °C in the Lund et al. (2009) study compared to 15 °C in the present study) may also have contributed to the different survival values obtained. Indeed it has been reported that the air temperature at which lobsters are being sorted, as well as the temperature and condition of the seawater in the on-board holding tanks, is influential for the survival and the recovery of lactemia and glycemia in the haemolymph (Lorenzon et al., 2007; Ridgway et al., 2006a; Paterson and Spanoghe, 1997).

On-board tanks should be supplied with well oxygenated seawater at a low temperature because disturbed lobsters have increased rates of oxygen demand (due to ATP demand) that are met by increased rates of ventilation (Crear and Forteath, 1997; Whiteley and Taylor, 1992; Taylor and Whiteley, 1989). At higher temperatures and during aerial exposure oxygen deprivation could be more severe, leading to lower survival rates as oxygen demand cannot be met. In agreement with this hypothesis, other studies that have focussed on the potential of this species to survive emersion-induced hypoxia have indicated the importance of the temperature at which the emersion takes place (Ridgway et al., 2006a; Bernasconi and Uglow, 2008b). In terms of disturbance of lactemia it is well known that in crustaceans, plasma CHH increases after certain stresses, provoking the mobilisation of glycogen that leads to L-lactate being accumulated. Thus, Lund et al. (2009) reported higher CHH levels in the summer compared to the winter after trawl capture and also 24 h after recovery. Correlations between L-lactate and CHH have also been previously reported in crustaceans (Santos and Keller, 1993). Although CHH titres were not measured in the present study, differences in this hormone could have explained why muscle and haemolymph L-lactate were higher after trawling in the summer compared to the winter. Interestingly, other authors have found greater increases of CHH and L-lactate at higher temperatures, and a longer time of recovery for lactemia has been described at higher temperatures in *N. norvegicus* (Ridgway et al., 2006a) and in the American lobster (*Homarus americanus*) (Lorenzon et al., 2007). In agreement with those studies, it was shown in the present work that, after overnight recovery, animals from the summer trial still had lower concentrations of muscle glycogen and higher levels of haemolymph L-lactate compared to the animals from the winter trial.

The rate of recovery of ATP and hence AEC was also season-dependent. Initially, nucleotide inter-conversions appeared to be more rapid in the summer. In other crustacean species such as the greasyback shrimp (*Metapenaeus ensis*), arginine kinase, the enzyme responsible of the removal of the phosphate group from arginine phosphate to produce ATP, has been found to increase its activity with temperature (Wang et al., 2009). This fact could explain at least in part the initial faster inter-conversions in the summer. Moreover, seasonal differences in metabolic rates, swimming performance and the fact that during the summer *N. norvegicus* females leave their burrows to reproduce (and hence at that time represent a higher proportion of the catch) could all have affected the energy demand of the animals in a catch, and thus have had an impact in the recovery time-scale of ATP (Newland et al., 1988; Gerhardt and Baden, 1998; Milligan et al., 2009). However, it is important to notice that although the ATP concentration increased significantly more rapidly during the recovery period in the on-board tanks in the summer, values after 6 h of placing the animals in on-board seawater tanks were significantly lower in the summer than in the winter while AEC remained between 0.71-0.80. According to Ivanovici (1980), organisms living in optimal conditions have AEC values higher than 0.8, ratios that were reached only after 24 h recovery in the summer. In contrast, AEC values in the winter were above 0.8 from 4 h after the recovery period on-board, suggesting that in winter the animals were in a favourable condition from 4 h after trawl onwards. Another potential indicator of the more complete recovery in the winter was the disappearance of IMP at this time. Accumulation of IMP in the tissues has been regarded as a strong indicator of a compromised condition of crustaceans induced by extreme stressors (Chen et al., 1990; Paterson, 1993).

The effect of subsequent stresses

Practices in the fishery for the western rock lobster (*Panulirus cygnus*) involve placing the animals as soon as possible in on-board seawater tanks (Paterson and Spanoghe, 1997). Indeed, an adequate period of recovery in water has been found to be necessary after each stage of handling in the live transport of these lobsters, in order to avoid compounding the stress syndrome (Taylor et al., 1997). In accord with this, Whiteley and Taylor (1992) found that European lobsters (*Homarus gammarus*) deprived of the opportunity to recover after an episode of stress were in a significantly worse condition after a subsequent period of transport in air than lobsters that were left to recover fully before further transport.

In the present work, subsequent aerial exposure triggered another significant peak in haemolymph L-lactate in both seasons, suggesting that in *N. norvegicus* anaerobic metabolism was further activated even before the phosphagen reserves were totally depleted, in order to maintain ATP levels. However, the AEC values recorded in the winter did not decrease below 0.8 (in contrast to AEC values in the summer trial), and haemolymph L-lactate increased to a greater extent in the summer suggesting that animals coped better with the aerial exposure during the winter than during the summer.

Recovery rates of different measures and the possibility of defining an index of stress

All the studied parameters were affected by the process of trawling. However, some measures returned more quickly than others to values similar to those reported by other authors in rested *N. norvegicus* (Albalat et al., 2009; Ridgway et al., 2006b). During recovery, nucleotides and arginine phosphate concentrations recovered at a

faster rate than did measures related to anaerobic glycolysis (muscle glycogen, muscle L-lactate and haemolymph L-lactate). As mentioned earlier the conversion of AMP to ADP and ATP during recovery will depend on the presence of oxygen in the haemolymph, which would return relatively quickly to normal values since the animals hyperventilate when returned to seawater. This has been demonstrated in European lobsters both in the laboratory and after commercial transport out of water (Whiteley and Taylor, 1992; Taylor and Whiteley, 1989). On the other hand, haemolymph L-lactate takes much longer to recover in crustaceans as it is not excreted or readily mobilised, but is metabolised (Ellington, 1983). In many cases recovery following muscular activity is characterised by a further increase of L-lactate and a degradation of glycogen (Weinstein and Full, 1992). This phase of anaerobic energy production during recovery may serve as a means to restore muscle function as rapidly as possible by prioritising the conversion of AMP to ATP and replenishing the phosphagen reserves (Livingstone et al., 1981; Onnen and Zebe, 1983). In *N. norvegicus* a significant increase in the concentration of arginine phosphate was recorded at the same time as ATP became the main nucleotide, indicating a very rapid replenishment of phosphagen reserves, possibly to support any further muscle activity (England and Baldwin, 1983).

Muscle glycogen concentrations determined immediately after capture were similar to those reported in trawled *N. norvegicus* by Ridgway et al. (2006b) but were lower than the concentrations obtained in control (unstressed) or creeled *N. norvegicus* (Gornik et al., 2008; Albalat et al., 2009). Muscle glycogen concentrations increased significantly only at the end of the 24 h trial in the winter. A reciprocal time course for haemolymph L-lactate removal and glycogen recovery in the winter seemed to

indicate that muscle glycogen could be re-established at least in part via the oxidation of L-lactate into glycogen (Morris and Adamczewska, 2002). However, as other tissues (eg. hepatopancreas) were not analysed in this study conclusions about the fate of L-lactate should not be made and we can only note that the recovery of this end-product in *N. norvegicus* is slow, a feature that appears to be characteristic of decapod crustaceans (Gäde, 1984; Hill et al., 1991; Henry et al., 1994).

At this point it is relevant to consider which parameters could be most informative for assessing recovery in the live handling of this species. Physiological variables could be classified according to their sensitivity to a given stressor and to their rate of recovery. In terms of sensitivity, all measures were responsive to the trawling process and emersion of the animals in air. However, in terms of recovery some measures returned towards their rested values at a faster rate (ATP, AEC ratio, arginine phosphate) than others (muscle glycogen and haemolymph L-lactate). Therefore, research studies regarding the recovery of animals from a stressful situation and aimed at making recommendations for a code of practice should take into account that measures such as haemolymph L-lactate would not distinguish between an animal that is totally exhausted and one that is partially recovered but still engaged in repaying a metabolic energy debt. On the other hand, parameters such as the ATP concentration and the AEC could represent an earlier and more precise measure of the recovery state of the animals and give more accurate information on which commercial practices are more beneficial for a their rapid recovery.

Recommendations for a code of practice

From a fisheries point of view at least two conclusions can be drawn from the present work. Firstly, the amount of recovery time in on-board tanks is important to ensure that the animals have sufficient time to recover from the stress of capture and that they are better prepared physiologically to endure further stresses. Secondly, it seems that during winter the animals recover more completely within a period of ~ 6h, which would approximate to the post-capture holding period on a normal fishing trip by a 'day boat', and are thus more able to cope with a further stress such as aerial exposure during transport. For these reasons, it is recommended that trawled animals to be exported live should be selected from landings made early in the day, giving them more time to recover (at least 4 h) in the on-board tanks before further disturbance. On-board tanks should be kept with water fully aerated and covered from the sun to keep air and water temperatures as low as possible in the summer (with a target temperature of around 10 °C). Furthermore, it may be possible for animals to be transported to market sooner after capture in winter (without an overnight recovery) than in summer (when they need longer periods in holding tanks to fully recover from the stress of capture). However, by following these recommendations a significant proportion of the catch would be in a sufficiently good condition to be exported live even in the summer.

Acknowledgments

This work was supported by a grant from a Knowledge Transfer Partnership (KTP) Award (00095) to the University of Glasgow. The authors are thankful to the crew of the commercial vessel M.V. *Eilidh Anne* (GK2) and the industrial partner involved in the project.

Bibliography

Albalat, A., Gornik, S., Atkinson, R.J.A., Coombs, G.H., Neil, D.M. 2009. Effect of capture method on the physiology and nucleotide breakdown products in the Norway lobster (*Nephrops norvegicus*) Mar. Biol. Res. 5, 441-450.

Atkinson, R.J.A., Taylor, A.C. 1988. Physiological ecology of burrowing decapods. Symp. Zool. Soc. Lond. 59, 201-226.

Atkinson, D.E. 1965. Biological feedback control at molecular level. Science 150, 851-875.

Atkinson, D.E. 1968. The energy charge of the adenylate pool as regulatory parameter. Interaction with feedback modifiers. Biochemistry 7, 4030-4034.

Baldwin, J., Snow, R.J., Carey, M.F., Febbraio, MA. 1999. Muscle IMP accumulation during fatiguing submaximal exercise in endurance trained and untrained men. Am. J. Physiol. 277, 295-300.

Bergmeyer, H.U., Bernt, E. 1974. Determination of glucose with glucose oxidase and peroxidase. In: Bergmeyer, H.U. (Ed.), Methods of Enzymatic Analysis. Verlag Chemie-Academic Press, pp. 1205-1215.

Bernasconi, C.J., Uglow, R.F. 2008a. Effects of emersion and re-immersion on physiological and immunological variables in creel-caught and trawled Norway lobster *Nephrops norvegicus*. Dis. Aquat. Org. 81, 241-247.

Bernasconi, C.J., Uglow, R.F. 2008b. Effects of emersion-induced hypoxia on some haemolymph constituents on *Nephrops norvegicus*. Dis. Aquat. Org. 82, 135-143.

Carrol, N.V., Longley, R.W., Roe, J.D. 1956. The determination of glycogen in the liver and muscle by use of the anthrone reagent. J. Biol. Chem. 220, 583-593.

Chen, H.C., Moody, M.W., Jiang, S.T. 1990. Changes in biochemical and bacteriological quality of grass prawn during transportation by icing and oxygenation. J. Food Sci. 55, 670-673.

Combes, J. 2007. Clyde Environment and Fisheries Review. Available on-line at http://www.gla.ac.uk/marinestation/CFDP/review_report_frame.htm.

Crear, B.J., Forteath, G.N. 1997. Maintaining rock lobsters in prime condition on board lobsters boats – Part 1. Prof. Fisherman. March, 24-26.

Ellington, W.R., 1983. The recovery from aerobic metabolism in invertebrates. J.Exp. Zool., 228, 431-444.

England, W.R., Baldwin, J. 1983. Anaerobic energy metabolism in the tail musculature of the Australian yabby *Cherax destructor* (Crustacea, Decapoda, Parastacidae): role of phosphagens and anaerobic glycolysis during escape behaviour. Physiol. Zool. 56, 614-622.

FRS (2009) Fish and *Nephrops* Stock Information. Aberdeen: FRS Information Resources 45.

Gäde, G. 1984. Effects of oxygen deprivation during anoxia and muscular work on the energy metabolism of the crayfish, *Orconectes limosus*. Comp. Biochem. Physiol. 77A, 495-502.

Gerhardt, L., Baden, S.P. 1998. Gender- and oxygen-related irrigation behaviour of the decapod *Nephrops norvegicus*. Mar. Biol. 161, 553-558.

Gornik, S.G., Albalat, A., Atkinson, R.J.A., Coombs, G.H., Neil, D.M. 2008. Early post-mortem processes in the abdominal muscle of the Norway lobster (*Nephrops norvegicus*). Mar. Freshwater Behav. Physiol. 41, 241-256.

Gornik, S.G., Albalat, A., Atkinson, R.J.A., Coombs, G.H., Neil, D.M. 2010. The influence of defined ante-mortem stressors on the early post-mortem

biochemical processes in the abdominal muscle of the Norway lobster, *Nephrops norvegicus* (Linnaeus, 1758). Mar. Biol. Res. 6, 223-238.

Hagerman, L., Uglow, R.F. 1985. Effect of hypoxia on the respiratory and circulatory regulation of *Nephrops norvegicus*. Mar. Biol. 87, 273-278.

Harris, R.R., Andrews, M.B. 2005. Physiological changes in the Norway lobster *Nephrops norvegicus* (L.) escaping and discarded from commercial trawls on the West Coast of Scotland II. Disturbances in haemolymph respiratory gases, tissue metabolites and swimming performance after capture and during recovery. J. Exp. Mar. Biol. Ecol. 320, 195-210.

Henry, R.P., Booth, C.E., Lallier, F.H., Walsh, P.J. 1994. Post-exercise lactate production and metabolism in three species of aquatic and terrestrial decapod crustaceans. J. Exp. Biol. 186, 215-234.

Hill, A.D., Taylor, A.C, Strang, R.H.C. 1991. Physiological and metabolic responses of the shore crab *Carcinus maenas* (L.) during environmental recovery. J. Exp. Mar. Biol. Ecol. 150, 31-50.

ICES. 1994. Report of the sub-group on methodology of fish survival experiments. ICES C.M. 1994/B, 8, 46 pp.

Ivanovici, A.M. 1980. The adenylate energy charge in the estuarine mollusc, *Pyrazus ebeninus*. Laboratory studies of responses to salinity and temperature. Comp. Biochem. Physiol. 66A, 43-55.

Lancaster, J., Fridd, C.L.J. 2002. The fate of discarded juvenile brown shrimps (*Crangon crangon*) in the Solway Firth UK Fishery. Fish. Res. 58, 95-107.

Livingstone, D.R., De Zwaan, A., Thompson, R.J. 1981. Aerobic metabolism, octopine production and phosphoarginine as sources of energy in the phasic and catch adductor muscles of the giant scallop *Placopecten magellanicus* during

swimming and the subsequent recovery period. Comp. Biochem. Physiol. 70B, 35-44.

Lorenzon, S., Giulianini, P.G., Martinis, M., Ferrero, E.A. 2007. Stress effect of different temperatures and air exposure during transport on physiological profiles in the American lobster *Homarus americanus*. Comp. Biochem. Physiol. 147A, 94-102.

Lund, H.S., Wang, T., Chang, E.S., Pedersen, L.F., Taylor, E.W., Pedersen, P.B., McKenzie, D.J. 2009. Recovery by the Norway lobster *Nephrops norvegicus* (L.) from the physiological stresses of trawling: Influence of season and live-storage condition. J. Exp. Mar. Biol. Ecol. 373, 124-132.

Maguire, J., Jenkins, S., Burnell, G.M. 2002. The effects of repeated dredging and speed of tow on undersized scallops. Fish. Res. 58, 367-377.

Mendes, R., Quinta, R., Nunes, M.L. 2001. Changes in baseline levels of nucleotides during ice storage of fish and crustaceans from the Portuguese coast. Eur. Food Res. Technol. 212, 141-146.

Milligan, R.J., Albalat, A., Atkinson, R.J.A, Neil, D.M. 2009. The effects of trawling on the physical condition of the Norway lobster *Nephrops norvegicus* in relation to seasonal cycles in the Clyde Sea area. ICES J. Mar. Sci. 66, 488-494.

Morris, S., Adamczewska, A.M. 2002. Utilisation of glycogen, ATP and arginine phosphate in exercise and recovery in terrestrial red crabs, *Gecarcoidea natalis*. Comp. Biochem. Physiol. 133A, 813-825.

Morris, S., Oliver, S. 1999. Circulatory, respiratory and metabolic response to emersion and low temperature of *Jasus edwardsii*: simulation studies of commercial shipping methods. Comp. Biochem. Physiol. 122A, 299-308.

Newland, P.L., Chapman, C.J., Neil, D.M. 1988. Swimming performance and endurance of the Norway lobster *Nephrops norvegicus*. Mar. Biol. 98, 345-350.

Newland, P.L., Neil, D.M., Chapman, C.J. 1992. Escape swimming in the Norway lobster. J. Crustacean Biol. 12, 342-353.

Onnen, T., Zebe, E. 1983. Energy metabolism in the tail muscle of the shrimp *Crangon crangon* during work and subsequent recovery. Comp. Biochem. Physiol. 74, 833-838.

Paterson, B.D. 1993. The rise in inosine monophosphate and L-lactate concentrations in muscle of live penaeid prawns (*Penaeus japonicus*, *Penaeus monodon*) stressed by storage out of water. Comp. Biochem. . Physiol. 106A, 395-400.

Paterson, B.D., Spanoghe, P.T. 1997. Stress indicators in marine decapod crustaceans, with particular reference of western rock lobsters (*Panulirus cygnus*) during commercial handling. Mar. Freshwater Res. 48, 829-834.

Paterson, B.D., Grauf, S.G., Smith, R.A. 1997. Haemolymph chemistry of tropical rock lobsters (*Panulirus ornatus*) brought onto a mother ship from a catching dinghy in Torres Strait. 5th International Conference and Workshop on Lobster Biology and Management. Mar. Freshwater Res. 48, 835-838.

Rice, A.L., Chapman, C.J. 1971. Observations on the burrows and burrowing behaviour of two mud-dwelling decapod crustaceans, *Nephrops norvegicus* and *Goneplax rhomboides*. Mar. Biol. 10, 330-342.

Ridgway, I.D., Taylor, A.C., Atkinson, R.J.A., Stentiford, G.D., Chang, E.S., Chang, S.A., Neil, D.M. 2006a. Morbidity and mortality in Norway lobsters, *Nephrops norvegicus*: physiological, immunological and pathological effects of aerial exposure. J. Exp. Mar. Biol. Ecol. 328, 251-264.

Ridgway, I.D., Taylor, A.C., Atkinson, R.J.A., Chang, E.S., Neil, D.M. 2006b. Impact of capture method and trawl duration on the health status of the Norway lobster, *Nephrops norvegicus*. J. Exp. Mar. Biol. Ecol. 339, 135-147.

Ryder, J.M. 1985. Determination of adenosine triphosphate and its breakdown products in fish muscle by high-performance liquid chromatography. J. Agr. Food Chem. 33, 678-680.

Santos, E.A., Keller, R. 1993. Effects of exposure to atmospheric air on blood glucose and lactate concentration in two crustacean species: A role of crustacean hyperglycaemic hormone (CHH). Comp. Biochem. Physiol. 106A, 343-347.

Spicer, I.J., Hill, A.D., Taylor, A.C., Strang, R.H. 1990. Effect of aerial exposure on concentrations of selected metabolites in the blood of the Norwegian lobster *Nephrops norvegicus* (Crustacea: Nephropidae). Mar. Biol. 105, 129-135.

Stentiford, G.D., Neil, D.M. 2000. A rapid onset, post-capture muscle necrosis in the Norway lobster, *Nephrops norvegicus* (L.), from the West coast of Scotland. J. Fish Dis. 23, 251-263.

Sylvestre, C., Le Gal, Y. 1987. In situ measurements of adenylate energy charge and assessment of pollution. Mar. Pollut. Bull. 18, 36-39.

Taylor, E.W., Whiteley, N.M. 1989. Oxygen transport and acid-base balance in the haemolymph of the lobster, *Homarus gammarus*, during aerial exposure and resubmersion. J. Exp. Biol. 144, 417-436.

Taylor, H.H., Paterson, B.D., Wong, R.J., Wells, R.M.G. 1997. Physiology and live transport of lobsters: report from a workshop. Mar. Freshwater Res. 48, 817-822.

Uglow, R.F., Hosie, D.A., Johnson, I.T., MacMullen, P.H. 1986. Live handling and transport of crustacean shellfish: an investigation on mortalities. Technical Report 280. Sea Fish Industry Authority, Edinburgh.

Viant, M.R., Rosenblum, E.S., Tjeerdema, R.S. 2001. Optimized method for the determination of phosphoarginine in abalone tissue by high-performance liquid chromatography. J. . Chromatogr. 765, 107-111.

Wang, J.S., Zheng, Z.L., Lei, J., Pan, J.C., Zou, G.L.. 2009. Cloning, expression, characterization and phylogenetic analysis of arginine kinase from greasyback shrimp (*Metapenaeus ensis*) Comp. Biochem. Physiol. 153B, 268–274.

Weinstein, R.B., Full, R.J. 1992. Intermittent exercise alters endurance in an eight-legged ectotherm. Am. J. Physiol. 262R, 852-859.

Whiteley, N.M., Taylor, W.W. 1992. Oxygen and acid-base disturbances in the haemolymph of the lobster *Homarus gammarus* during commercial transport and storage. J. Crust. Biol. 12, 19-30.

Figures

Fig. 1. Nucleotide profiles in the abdominal muscle of *N. norvegicus* after capture by trawling and over a 24 h recovery period, including a short period of land transport, in the A) winter and the B) summer trials. Values represent the mean ± SEM of fifteen specimens. Nucleotide values that are significantly different with time are represented by different letters ($P<0.05$).

Fig. 2. AEC ratios in the abdominal muscle of *N. norvegicus* after capture by trawling and over a 24 h recovery period, including a short period of land transport, in the winter and in the summer. Values represent the mean ± SEM of fifteen specimens. For each trial (summer or winter separately) values that are significantly different are represented by different letters, whereas significant differences between season for each time interval are represented by an asterisk ($P<0.05$).

Fig. 3. Arginine phosphate concentrations in the abdominal muscle of *N. norvegicus* after capture by trawling and over a 24 h recovery period, including a short period of land transport, in the winter and in the summer. Values represent the mean ± SEM of fifteen specimens. For each trial (summer or winter separately) values that are significantly different are represented by different letters, whereas significant differences between season for each time interval are represented by an asterisk ($P<0.05$).

Fig. 4. Glycogen concentrations in the abdominal muscle of *N. norvegicus* after capture by trawling and over a 24 h recovery period, including a short period of land transport, in the winter and in the summer. Values represent the mean ± SEM of

fifteen specimens. For each trial (summer or winter separately) values that are significantly different are represented by different letters, whereas significant differences between season for each time interval are represented by an asterisk ($P<0.05$).

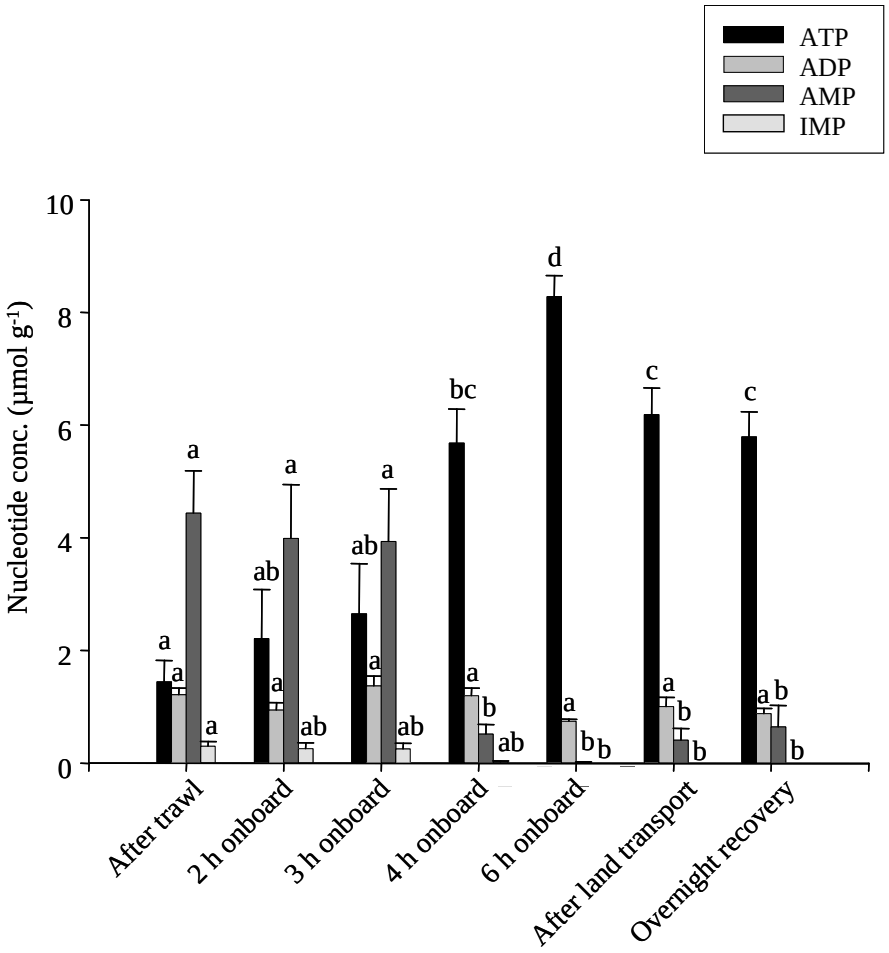
Fig. 5. a) Abdominal muscle L-lactate and b) haemolymph L-lactate concentrations in *N. norvegicus* after capture by trawling and over a 24 h recovery period, including a short period of land transport, in the winter and in the summer. Values represent the mean ± SEM of fifteen specimens. For each trial (summer or winter separately) values that are significantly different are represented by different letters, whereas significant differences between season for each time interval are represented by an asterisk ($P<0.05$).

Table 1. Environmental parameters in the on-board tanks on the days of the trials and survival data on the days of the trials and also on 13 different trips of the same boat per season, following the same recovery protocol (survival average). The asterisk indicates a significant difference between seasons ($P<0.05$)

	Winter trial	Summer trial
Air temperature (°C)	5.5 - 7.5	14 - 17
Seawater temperature (°C)	10 ± 0.3	15 ± 0.2
Salinity (PSS)	30.5	28
DO_2% (mg L^{-1})	100 (8.8)	100 (7.9)
Survival (%) day of trials	87	74
Survival (%) average	84.83 ± 0.93	75.35 ± 2.92*

Fig. 1.

a)


ATP
ADP
AMP
IMP
Nucleotide conc. (µmol g-1)
0
2
4
6
8
10
a
a
a
a
ab
a
ab
a
ab
a
ab
bc
a
b
ab
d
a
b
b
c
a
b
b
c
a
b
b
After trawl
2 h onboard
3 h onboard
4 h onboard
6 h onboard
After land transport
Overnight recovery

b)

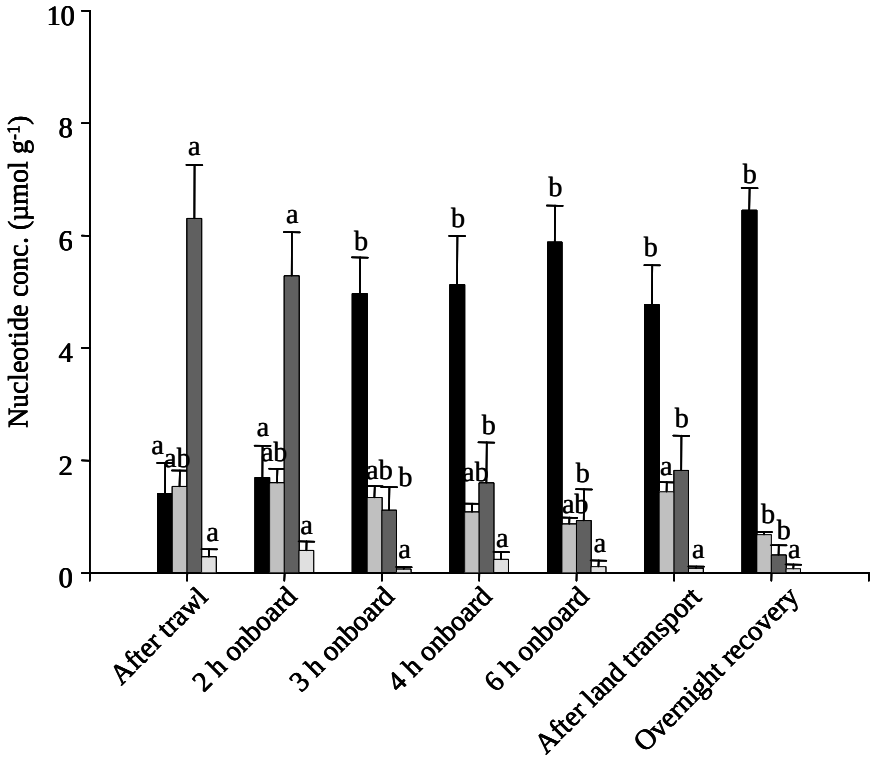

Nucleotide conc. (µmol g-1)
0
2
4
6
8
10
a
ab
a
a
ab
a
b
ab
b
a
b
ab
b
a
b
ab
b
a
b
b
a
b
b
b
a
b
b
After trawl
2 h onboard
3 h onboard
4 h onboard
6 h onboard
After land transport
Overnight recovery

Fig. 2.

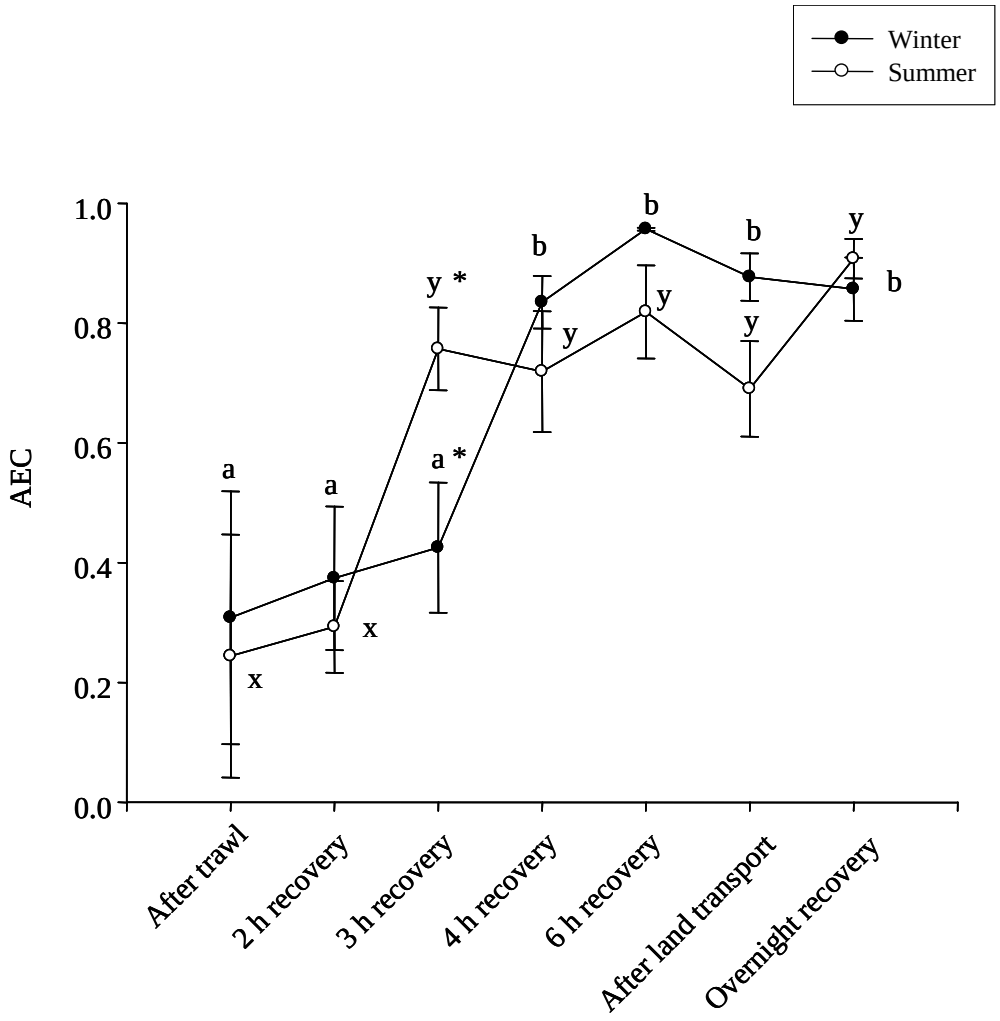

Winter
Summer
AEC
1.0
0.8
0.6
0.4
0.2
0.0
a
a
a *
b
b
b
y *
y
y
y
b
x
x
After trawl
2 h recovery
3 h recovery
4 h recovery
6 h recovery
After land transport
Overnight recovery

Fig. 3.

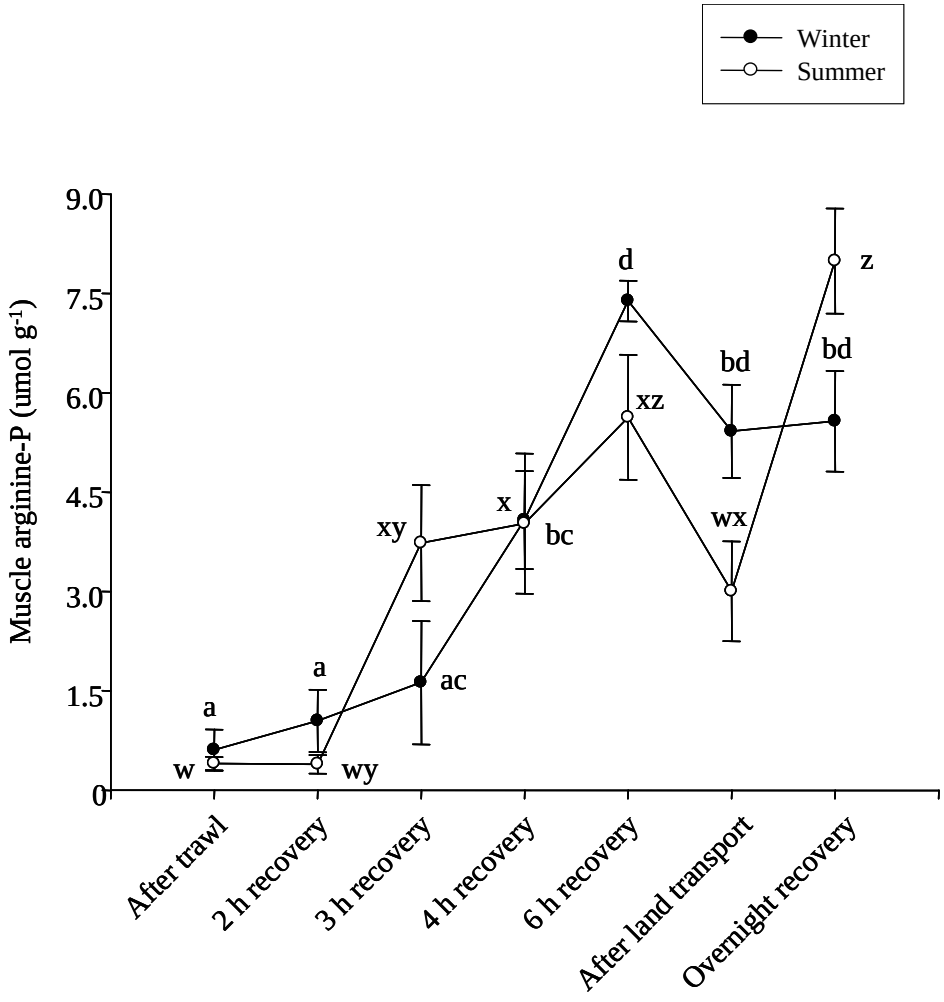

Winter
Summer
Muscle arginine-P (umol g-1)
9.0
7.5
6.0
4.5
3.0
1.5
0
a
w
a
wy
xy
ac
x
bc
d
xz
bd
wx
z
bd
After trawl
2 h recovery
3 h recovery
4 h recovery
6 h recovery
After land transport
Overnight recovery

Fig. 4.

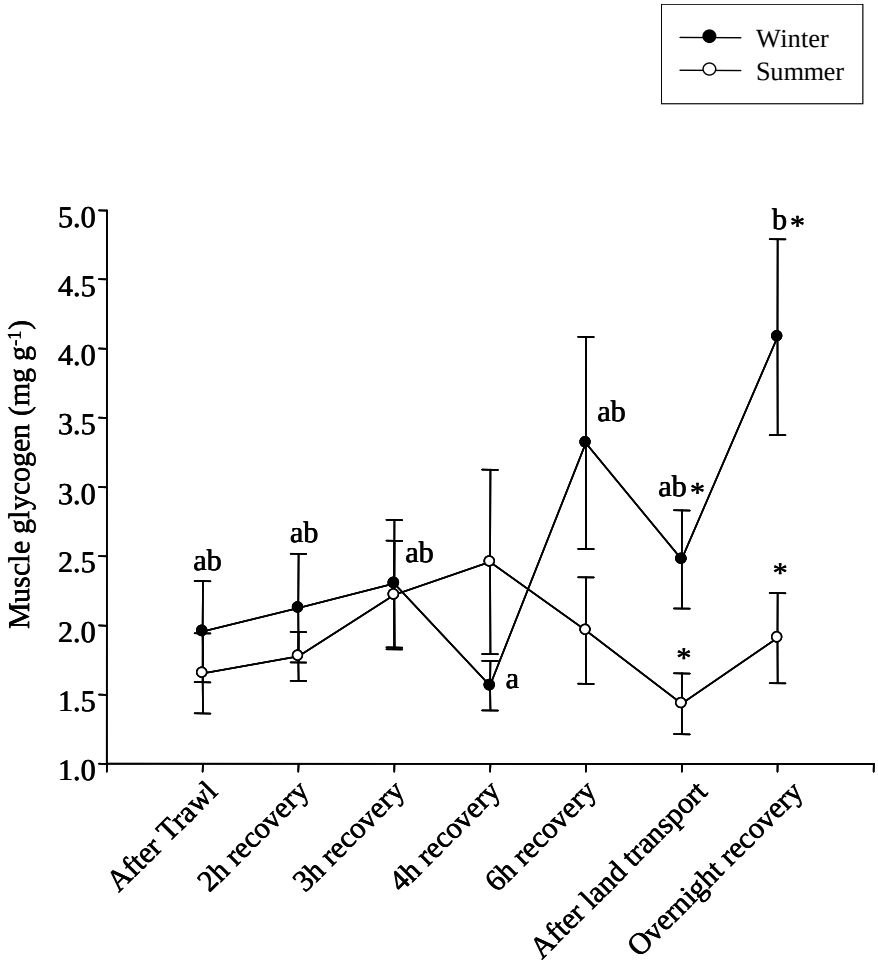
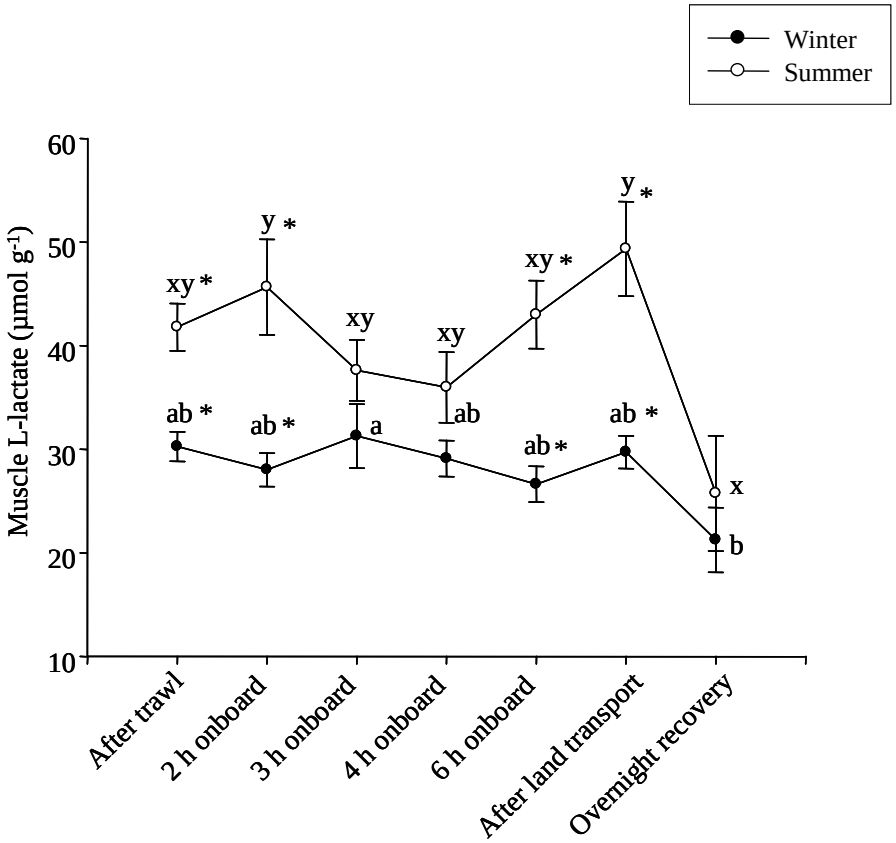
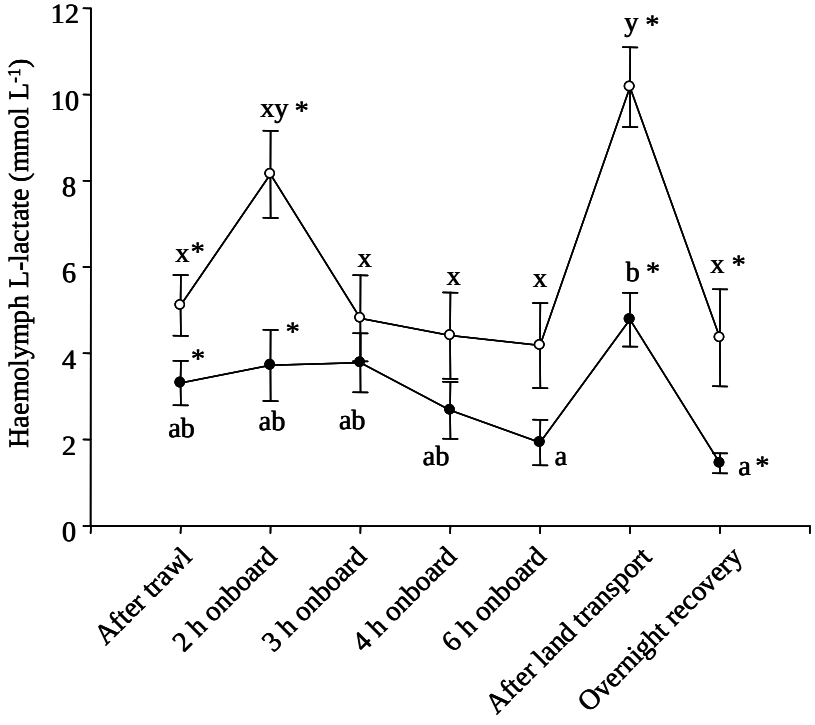

Winter
Summer
Muscle glycogen (mg g-1)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ab
ab
ab
a
ab
ab*
b*
*
*
After Trawl
2h recovery
3h recovery
4h recovery
6h recovery
After land transport
Overnight recovery

Fig. 5.

a)


Winter
Summer
Muscle L-lactate (µmol g-1)
60
50
40
30
20
10
xy *
y *
xy
xy
xy *
y *
ab *
ab *
a
ab
ab *
ab *
x
b
After trawl
2 h onboard
3 h onboard
4 h onboard
6 h onboard
After land transport
Overnight recovery

b)


Haemolymph L-lactate (mmol L-1)
12
10
8
6
4
2
0
x*
xy *
x
x
x
y *
b *
x *
*
*
ab
ab
ab
ab
a
a *
After trawl
2 h onboard
3 h onboard
4 h onboard
6 h onboard
After land transport
Overnight recovery