Purineolytic capacity response of *Nephrops norvegicus* to prolonged emersion: an ammonia detoxification process

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ABSTRACT: Under prolonged (>24 h) emersion, Norway lobster *Nephrops norvegicus* changed purineolytic pathways in response to increased levels of circulating ammonia. Haemolymph levels of urea and uric acid more than doubled during emersion, but xanthine oxidase was not detected, even after 72 h emersion. Xanthine dehydrogenase (XDH) was the only form of xanthine oxidoreductase detected, so the metabolic costs of producing uric acid would have been relatively small. Although ammonia, urea, uric acid and XDH all accumulated in the haemolymph during emersion, the relative proportions of ammonia and urea stayed the same at all times, which suggests the presence of enzymes from the uricolytic pathway (e.g. uricase and urease). Supranormal ammonia effluxes occurred almost immediately following re-immersion in amounts that indicate a high degree of hypoxia tolerance under emersion and that this is negatively related with the prevailing temperature. We conclude that *N. norvegicus* has purineolytic capacity and, under prolonged hypoxia, produces purines de novo as an ammonia-detoxification strategy that has probable survival value for a benthic burrowing species that may experience periods of severe hypoxia.

KEY WORDS: Emersion · Ammonia · Xanthine · Oxidoreductase · Dehydrogenase · Purineolytic · *Nephrops norvegicus*

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INTRODUCTION

Ammonia is the primary nitrogenous metabolic waste in aquatic crustaceans and the least metabolically expensive to produce since it is formed from catabolic reactions and requires no further processing before excretion (Kormanik & Cameron 1981). It is also very soluble in water and can be flushed easily from the gills. Emersion impairs gill functioning, and nitrogenous wastes produced under these conditions need to be transported or temporarily stored in a non-toxic form (Greenaway 1991). Although aquatic crustaceans are ammoniotelic, they are also capable of producing small amounts of other nitrogenous compounds (e.g. urea and uric acid, Claybrook 1983).

Haemolymph urate was found to increase in *Cancer pagurus* and other aquatic crustaceans under emersion (Regnault 1992, Durand & Regnault 1998) and under environmental hypoxia (Lallier et al. 1987, Lallier &

Truchot 1989, DeFur et al. 1990, Dykens 1991); such conditions occur during the capture and transportation phases of their commercial marketing. Purines (e.g. urate and uric acid) stored by crustaceans may originate, pre-formed, from the diet or from de novo synthesis from non-purine precursors (e.g. glycine, Linton & Greenaway 1997). The latter alternative is the only route that would allow detoxification of ammonia but was thought to be lacking in most crustaceans (Claybrook 1983). The evidence for the de novo purine synthetic capability in the land crabs *Birgus latro* (Greenaway & Morris 1989) and *Gecarcoidea natalis* (Linton & Greenaway 1997) prompted Linton & Greenaway (1998) to speculate that this may be a general crustacean ability.

Some nitrogenous metabolic wastes (e.g. ammonia, urate and trimethylamine) alter the oxygen affinity of haemocyanin (Taylor et al. 1985, Morris et al. 1986a,b, Taylor & Spicer 1987, Danford et al. 2002), but others

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(e.g. urea, nitrite and the purines adenine, guanine, inosine, adenosine, xanthine oxidoreductase) remain to be investigated. Although some organisms excrete purines directly, they usually have enzymes (e.g. xanthine oxidoreductase, XOR) that convert adenine or guanine to hypoxanthine or xanthine, and xanthine to uric acid by deamination (Fig. 1, Fried & Fried 1974). XOR is a flavoprotein that needs no cytochrome to mediate between it and oxygen; the product of the reaction is H_2O_2 , but since all cells contain catalase, the eventual outcome is that the substance is oxidated to product and H_2O (Harrison 1959). XOR has 2 forms: xanthine oxidase (XO) and xanthine dehydrogenase (XDH), both of which are molybdenum-containing enzymes (Stockert 2004).

XDH is the rate-limiting step in the catabolism of purines, in which it catalyses the conversion of hypoxanthine to xanthine, and xanthine to uric acid. In these reactions, XDH utilises NAD^+ preferentially as the electron acceptor and produces NADH (Campbell 1995). Under certain conditions (e.g. metabolic stress to a tissue), XDH can be converted to its oxidase form XO, which utilises molecular oxygen as the electron acceptor (Hellsten et al. 1996) and thereby generates reactive oxygen species (ROS) in the formation of superoxide and hydrogen peroxide (Kayyali et al. 2001).

Urea is formed through the ornithine-urea cycle or uricolysis, and is thought to have a detoxification role when haemolymph ammonia is high (Spaargaren 1982), and urea and nitrite have been shown to increase in the haemolymph of *Penaeus monodon* (Chen et al. 1994) during exposure to high ambient ammonia. Urea increases the oxygen affinity of haemoglobin (Jokumsen & Weber 1980), and physiological factors that promote improved oxygen transport and aerobic respiration of emersed commercial crustaceans are of interest in the context of quality maintenance during transportation and merit further investigation.

The lack of confirmation of de novo purine synthesis in Norway lobster *Nephrops norvegicus* prompted this study, in which we focussed on the enzymatic step of XOR under emersion of this species. In particular, we examined the possibility that under prolonged (72 h) emersion, this step is used to synthesise purine de novo as a potential means of ammonia detoxification. There is evidence that *N. norvegicus* can survive extended periods of hypoxia, even anoxia (Hagerman & Pihl-

Baden 1988), and should de novo purine synthesis occur, then it would probably be an example of survival value being conferred in the natural habitat. Here, we attribute the ability of *N. norvegicus* to tolerate such an extended period of emersion to the chosen holding temperature ($5 \pm 1^\circ\text{C}$). We reasoned that this value is closer to that of the ambient temperature in the natural habitat, and it is less than temperatures we have seen used commonly in the export trade of this species. Thus the findings offer the potential of being of commercial value.

MATERIALS AND METHODS

Adult specimens of *Nephrops norvegicus* (L.) (mean weight 32.05 ± 1.01 g, $n = 42$) were obtained from Sutherland Fish and Game in Scotland. They were transported 'dry' in polystyrene boxes, which also contained bags of gel ice to minimise temperature fluctuations. Intermoult males, non-ovigerous and ovigerous females were used and were maintained in large, opaque, plastic tanks (1.5 m internal diameter) supplied with biologically filtered, recirculating, aerated seawater (salinity of 35 ± 1) at an ambient temperature of 5°C and a 12:12 h light:dark photoperiod. Ammonia levels were $<100 \mu\text{mol}$ total ammonia (TA) l^{-1} . All animals were allowed to acclimatise for 24 h (minimum) before being used for experiments.

Groups of $n = 12$ *Nephrops norvegicus* were emersed for periods of 24, 48 and 72 h, and a group of $n = 6$ animals was not emersed (control). Experimental emersion involved carefully removing animals from the water and packing them in polystyrene boxes lined with newspaper and containing a large gel ice pack. The boxes were stored for the appropriate emersion period in a temperature controlled room ($5 \pm 1^\circ\text{C}$). Following the emersion period, each box was unpacked and each animal was graded using an index of overt condition (Bernasconi & Uglow 2008). A sample group ($n = 6$ animals) was bled, and each of the other 6 was re-immersed into an individual acid-washed tank holding 1 l of seawater (salinity 35, known ammonia concentration of $<10 \mu\text{mol}$ TA l^{-1}). Samples of this water were collected in Eppendorf tubes (1.5 ml) after 0, 4, 10, 15, 30, 60 and 120 min and were frozen (-20°C) until analysis of TA.

A haemolymph sample (1.5 ml each) was collected from each of 6 ind. in the groups either prior to emersion (control) or at the end of the emersion period. Samples were also taken after 2 h of re-immersion. A separate group of animals ($n = 6$) was used for each sampling time, and no animal was sampled more than once. Haemolymph was collected using a sterile hypodermic needle (23 gauge) inserted through the arthro-

Fig. 1. Conversion of hypoxanthine to xanthine and xanthine to uric acid

dial membrane into the pre-branchial sinus at the base of the 4th or 5th pereopod (haemolymph). Each sample was divided into 3 and diluted 1:2 v/v with (1) de-ionised water (for TA analysis); (2) de-ionised water for XO analysis; and (3) 6% perchloric acid (as a de-proteiniser for analysis of uric acid and urea). All samples were then immediately frozen (-20°C). Analyses were carried out 2 d later.

Haemolymph urea nitrogen and uric acid were measured using Sigma diagnostics kit no. 640-A and Thermo Electron diagnostics kit no. TR24321, respectively. Ammonia concentrations were assayed using a flow-injection gas diffusion system (Hunter & Uglow 1993).

Haemolymph for the XO assay (0.6 ml sample) was homogenised (50 strokes) using a precision bore, ground glass piston homogeniser, in 4 to 6 volumes of ice-cold 25 mmol l^{-1} Tris buffer (pH = 7.5) containing 1 mmol l^{-1} dithioerythritol (DTE), 6 mmol l^{-1} Na_2EDTA and 0.2 mmol l^{-1} phenylmethylsulphonyl fluoride (PMSF; proteinase inhibitor). Prior to use, 2 μl of PMSF stock (0.1 mol l^{-1}) were added for each 1 ml of buffer. The PMSF was dissolved in isopropanol at -20°C overnight. The homogenate was then centrifuged at $10\,000 \times g$ for 10 min, and the supernatant was used for the assays.

For the XO assays, endogenous uric acid was removed by incubating 120 μl of the homogenate with 1650 μl of assay buffer (100 mmol l^{-1} pH = 7.5 Tris buffer with 1 mmol l^{-1} DTE) and 30 μl of uricase (18 U ml^{-1} ; Boehringer Mannheim cat. no. 737 364) at 25°C for 10 min. The XO reaction was then initiated by adding 200 μl of 0.2 mmol l^{-1} xanthine substrate. XO activity was measured at 25°C by continuously monitoring the disappearance of xanthine at 270 nm for 10 min.

For the XDH assays, endogenous uric acid was removed from the homogenate as described for the XO assays. The XDH reaction was started by adding 200 μl of 0.2 mmol l^{-1} xanthine and 100 μl of 12 mmol l^{-1} NAD^+ . The reaction was then monitored continuously for 10 min by following the appearance of NADH at 340 nm.

Data are expressed as means $\pm$ SEM. Statistical comparisons between means were made using 1-way analysis of variance (ANOVA) and Levene's homogeneity of variances (SPSS for Windows 11.5). A probability level of $p < 0.05$ was used to assess significance in all measured parameters.

RESULTS

All animals survived the emersion and subsequent re-immersion periods to beyond 72 h, but all animals in the 48 h emersed group were in a weak condition following the initial emersion period.

The mean ammonia efflux of the immersed (control) group was $0.40 \pm 0.17 \mu\text{mol TA g}^{-1} \text{ h}^{-1}$ at 5°C . After emersion, the ammonia efflux increased but was not significantly different from that of the control group (Fig. 2).

The highest ammonia efflux rates in the 72 h emersion group following re-immersion occurred after 15 min. In contrast, efflux peaked in the 24 and 48 h emersed groups 30 and 60 min after re-immersion (0.89 ± 0.27 and $1.55 \pm 0.25 \mu\text{mol TA g}^{-1} \text{ h}^{-1}$, respectively). The 24 and 72 h emersed groups showed rates that increased gradually during the re-immersion period (with no significant inter-group difference: $p > 0.05$ in each case), and attained final values not significantly different from the initial rates. The mean efflux of the 48 h emersed group increased significantly ($F = 3.23$, $p < 0.05$) between 30 and 60 min re-immersion. The same group had a mean rate between 4 and 15 min post re-immersion that was not significantly different from the control value, but this increased over the remaining re-immersion period to give a final efflux that was significantly higher ($F = 2.94$, $p < 0.05$) than either the control or subsequent efflux rates.

The mean haemolymph TA of the immersed (control) group was $271.9 \pm 47.9 \mu\text{mol l}^{-1}$. Circulating ammonia accumulated linearly in all groups during emersion, but final concentrations were not significantly higher ($p > 0.05$) than immersed (control) values in any but the 72 h emersed group (Fig. 3). Emersion for 72 h also resulted in a significantly higher haemolymph ammonia concentration ($F = 8.24$, $p < 0.05$) than the 24 h emersion group, but not the 48 h group, although the 24 and 48 h emersion groups were statistically identical. During the 2 h re-immersion period, haemolymph ammonia decreased in all groups, but this was significant only in the 72 h group ($t = 2.6$, $p < 0.05$).

Fig. 2. *Nephrops norvegicus*. Total ammonia (TA) efflux of the control group (no emersion, ●), 24 h emersed group (○), 48 h emersed group (▼) and 72 h emersed group (△). Data are mean $\pm$ SEM

Fig. 3. *Nephrops norvegicus*. Haemolymph ammonia concentrations after 0 (Control, black bar), 24, 48 or 72 h emersion (dark grey bars) and after a 2 h re-immersion period (light grey bars). Values are the mean of $n = 6$ in each case with SEM

The mean control group haemolymph uric acid concentration was $0.04 \pm 0.01 \mu\text{mol ml}^{-1}$. After 24, 48 and 72 h emersion, uric acid concentrations showed a direct linear relationship with emersion time (0.09 ± 0.03 , 0.10 ± 0.02 and $0.11 \pm 0.02 \mu\text{mol ml}^{-1}$, respectively; Fig. 4), and all were significantly higher ($F = 4.73$, $p < 0.05$) than the control value. Although uric acid decreased in all groups during the re-immersion period, their concentrations remained significantly higher ($F = 3.93$, $p < 0.05$) than the control values after 2 h.

Fig. 4. *Nephrops norvegicus*. Haemolymph uric acid concentrations after 0 (Control, black bar), 24, 48 or 72 h emersion (dark grey bars) and after a 2 h re-immersion period (light grey bars). Values are the mean of $n = 6$ in each case with SEM

The mean control group haemolymph urea concentration was $0.30 \pm 0.03 \mu\text{mol ml}^{-1}$ (Fig. 5), and all groups showed significantly higher values ($F = 2.58$, $p < 0.05$) after 72 h emersion. The re-immersion period resulted in the urea concentrations of all groups returning to levels not significantly different from the control values. The urea concentration in the 72 h emersed group decreased significantly ($t = 1.78$, $p < 0.05$) during the re-immersion period.

XDH was the only form of XOR detected within the haemolymph of *Nephrops norvegicus* (Fig. 6). Despite a decrease in the XDH activity from the mean control value ($0.53 \pm 0.11 \text{ pmol ml}^{-1} \text{ min}^{-1}$), there were no significant differences between the control and the subsequent emersion and re-immersion concentrations ($F = 1.55$, $p > 0.05$ in all cases). After an initial decrease in XDH activity over 24 and 48 h emersion, XDH in the haemolymph increased slightly after 72 h.

The proportions of the absolute amounts of ammonia, uric acid and urea in the haemolymph are given in Fig. 7 and expressed in relative (%) terms (Table 1). Although XDH was found in all groups, the amounts present were so small they cannot be shown in Fig. 7. Observation shows that, with the exception of 24 h re-immersion and 48 h emersed groups, the percentage of ammonia and urea present in the haemolymph of all animals was similar (Table 1), and these 2 compounds accounted for the majority of nitrogenous waste during this study. Fig. 7 and Table 1 show the variability of each of the constituents in absolute and relative terms, respectively, and reveal that the relative proportion of each constituent remained fairly stable.

Fig. 5. *Nephrops norvegicus*. Haemolymph urea concentrations after 0 (Control, black bar), 24, 48 or 72 h emersion (dark grey bars) and after a 2 h re-immersion period (light grey bars). Values are the mean of $n = 6$ in each case with SEM

Fig. 6. *Nephrops norvegicus*. Haemolymph xanthine dehydrogenase activity after 0 (Control, black bar), 24, 48 or 72 h emersion (dark grey bars) and after a 2 h re-immersion period (light grey bars). Values are the mean of $n = 6$ in each case with SEM

Fig. 7. *Nephrops norvegicus*. Absolute proportions of ammonia, uric acid and urea in the haemolymph. Em: Emersion, Re-im: re-immersion

Table 1. *Nephrops norvegicus*. Relative percentage of constituents investigated in the haemolymph during emersion and re-immersion period

5°C		Ammonia%	Uric acid %	Urea %
0 h		44	7	49
24 h	Emersed	39	9	52
	Re-immersed	27	13.5	59
48 h	Emersed	35	9.7	55
	Re-immersed	44.6	12	43
72 h	Emersed	47	7	46
	Re-immersed	42	10	48

DISCUSSION

During emersion, the production of ammonia as the primary end product of nitrogen metabolism may continue, but ammonia may accumulate in the haemolymph to toxic levels and branchial exchange may not be possible. Marine crustaceans reduce their rate of nitrogen metabolism considerably, but increases in haemolymph ammonia levels generally occur in marine decapods subjected to both short-term emersion (DeFur & McMahon 1984, Vermeer 1987, Bergmann et al. 2001) and to longer periods of emersion (Regnault 1992, Schmitt & Uglow 1997, Durand & Regnault 1998, Bernasconi & Uglow 2008), even though metabolic depression is a strategy that may reduce the rate of ammonia production in some species (Regnault 1992).

Reduced metabolic rates result in low soluble uric acid body burdens in the gastropod *Littorina saxatilis* (Oliv) (Smith & Smith 1998). Some emersed, subtidal decapods decrease their respiratory and metabolic activities to approximately 30% of their resting level (Burnett & McMahon 1987, Regnault 1994, Durand & Regnault 1998, Morris & Oliver 1999), and a reduction in aerobic metabolism has been shown to occur during prolonged emersion in *Cancer pagurus* and *Necora puber*, with both species reducing their rate of ammonia excretion by 95% (Durand & Regnault 1998). Ammoniogenesis in *Cardisoma carnifex* appeared to cease during a 192 h period of emersion (Wood et al. 1986) but was not the case for *Nephrops norvegicus* in our study, as ammonia gradually accumulated in the haemolymph during prolonged emersion. The tolerance of an increased haemolymph ammonia level from 271.9 ± 47.9 to $742.9 \pm 149.4 \mu\text{mol TA l}^{-1}$ over 72 h of emersion confirms findings by Hagerman & Pihl-Baden (1988), who found that this burrowing species is able to survive periods of days or even weeks of eutrophication-induced severe hypoxia. This indicates that haemolymph regulatory mechanisms continue to operate during emersion despite the considerable variability that occurred in absolute amounts of each constituent.

Uricotelism is more energy-consuming than ammonotelism, but often the advantage of producing uric acid is that its insolubility results in its precipitation in the excretory organ and being voided with minimal loss of water (Potts 1967, Greenaway 1991). The actual end-product of nitrogen metabolism (ammonia, urea or uric acid) is a function of the toxicity of ammonia and the water available for its excretion (Smith & Smith 1998). Uric acid is formed through the degradation of nucleic acids (Claybrook 1983, Regnault 1987) and is usually stored in epidermal and gut cells. It has been found to occur in the robber crab *Birgus latro* (Green-

away & Morris 1989), and is stored in spongy connective tissue cells in the gecarcinid land crab *Gecarcoidea natalis* (Linton & Greenaway 1997).

Haemolymph uric acid levels between 0.01 and 0.1 $\mu\text{mol ml}^{-1}$ were found in *Carcinus maenas* (Binns 1969), 0.08 $\mu\text{mol ml}^{-1}$ in *Cancer pagurus* (Regnault 1992), 0.2 $\mu\text{mol ml}^{-1}$ in *Panulirus japonicus* (Huang & Chen 2001) and 0.12 $\mu\text{mol ml}^{-1}$ in *Penaeus monodon* (Cheng & Chen 2002). These values are similar to those found in haemolymph of the animals in the present study. Haemolymph uric acid levels increased linearly with emersion duration from concentrations of $0.04 \pm 0.01 \mu\text{mol ml}^{-1}$ (control group) to 0.09 ± 0.03 , 0.10 ± 0.02 and $0.11 \pm 0.01 \mu\text{mol ml}^{-1}$ over 24, 48 and 72 h emersion, respectively (Fig. 4). The increase over 72 h emersion of $0.07 \mu\text{mol ml}^{-1}$ ($70.0 \mu\text{mol l}^{-1}$) thus prevented 280 μmol of ammonia accumulating in the haemolymph (1 μmol of uric acid liberates 4 μmol of ammonia). This suggests that *Nephrops norvegicus* can respond to prolonged emersion by storing accumulated nitrogen in a comparatively non-toxic form for subsequent excretion as ammonia after re-immersion.

After the initial catabolism of urate, the resulting product (most likely urea) is probably transported via the haemolymph to the gills where urease catabolises it to ammonia. Many crustaceans show periodically high levels of urea in their haemolymph (e.g. up to 28 mmol l^{-1} in *Holthuisana transversa*, Greenaway 1991) and excrete some urea, although it is generally a minor component of total nitrogen output. Urea formation was found to be as high as 0.5 to 10.77 $\mu\text{mol ml}^{-1}$ in the haemolymph of terrestrial crabs (Greenaway & Nakamura 1991, DeVries et al. 1994, Taylor & Greenaway 1994, Linton & Greenaway 1995), and similar concentrations of urea were found in the haemolymph of *Nephrops norvegicus* during this study. Concentrations reached levels of $0.7 \pm 0.1 \mu\text{mol ml}^{-1}$ after a 72 h emersion period, which was an increase of $0.4 \mu\text{mol ml}^{-1}$ ($410.0 \mu\text{mol l}^{-1}$) in comparison to the control concentrations ($0.3 \pm 0.03 \mu\text{mol ml}^{-1}$). In this instance, an increase of 410.0 μmol urea in the haemolymph prevented 820.0 μmol of ammonia from accumulating (1 μmol urea liberates 2 μmol ammonia). Therefore, urea may function as a temporary detoxification mechanism for ammonia during emersion, as suggested by Spaargaren (1982).

Urea-N excretion did not increase in *Penaeus paulensis* when exposed to high levels of ambient ammonia (Schmitt & Santos 1998), but did increase in *P. japonicus* and *P. monodon* when exposed to ambient ammonia in the range of 0.04 to 51.46 mg l^{-1} ammonia-N (3.2 to 3673.2 $\mu\text{mol TA l}^{-1}$; Chen & Cheng 1993). When the haemolymph NH_4^+ concentration in *Carcinus maenas* became $>0.28 \text{ mmol l}^{-1}$, urea increased rapidly in the haemolymph, whereas at $<0.2 \text{ mmol l}^{-1}$, urea was not

detected (Spaargaren 1982). This is paralleled in the present findings in which control group haemolymph ammonia was $271.9 \pm 47.9 \mu\text{mol TA l}^{-1}$ or 0.27 mmol l^{-1} . As ammonia increased under emersion, the haemolymph urea increased linearly in the 24, 48 and 72 h groups. This suggests the presence of the enzymes uricase, allantoinase, allantoicase and urease from the uricolytic pathway. These enzymes which aid in the degradation of uric acid to urea would account for the increasing concentration of urea in the haemolymph of *Nephrops norvegicus* during emersion.

Crustaceans are believed to lack a complete urea cycle, and synthesis of urea is possible only by degradation of uric acid in the uricolytic pathway or by the catabolism of the essential amino acid arginine (Hartenstein 1970, Claybrook 1983, Regnault 1987). The enzymes involved in uricolysis are uricase, allantoinase and allantoicase, which complete the breakdown of uric acid. Uricase and XDH activity has been detected in several species of aquatic crustacean including *Carcinus maenas* (Dyken 1991), freshwater crayfish *Orconectes rusticus* (Sharma & Neveu 1971), land crab *Gecarcinus natalis* (Linton & Greenaway 1998) and Kuruma shrimp *Marsupenaeus japonicus* (Cheng et al. 2004), indicating that they are capable of producing uric acid and urea via the purine nucleotide and uricolytic pathways. Uricase activity was not measured during this investigation, but XDH activity was detected, and the presence of small quantities of uric acid and significantly larger concentrations of urea indicate that uricase activity is probably present in *Nephrops norvegicus* and raises the possibility that *N. norvegicus* is also capable of utilising the purine nucleotide and uricolytic pathways.

Degradation of purine nucleotides (adenine and guanine) results in the production of adenosine monophosphate (AMP), inosine monophosphate (IMP), guanosine monophosphate (GMP), xanthosine monophosphate (XMP), adenosine, inosine, guanosine, xanthosine, hypoxanthine and xanthine prior to the formation of uric acid (Cheng et al. 2004). IMP is formed in response to stress, and reduces the AMP concentration while ATP production is impaired. Delayed accumulation of L-lactate and IMP during simulated commercial transport of the prawns *Penaeus japonicus* and *P. monodon* prompted suggestions that they were initially able to obtain adequate O_2 uptake (Paterson 1993).

Tolerance to anoxic environments may in part be due to the ability to withstand re-oxygenation rather than the anoxia itself, and this is where the relative activities of XDH and XO may play a role (Dyken & Shick 1988). Kayyali et al. (2001) demonstrated that XDH/XO are phosphoproteins and that acute hypoxia can greatly increase XDH/XO through post-

translational modifications and that phosphorylation of the XDH/XO protein correlates with acute enzyme activation. Activities of XDH and XO, which oxidise hypoxanthine and xanthine to uric acid, have been observed in the hepatopancreas of several species of aquatic crab (Dyken 1991, Lallier & Walsh 1991) and a land crab (Linton & Greenaway 1998, Dillaman et al. 1999).

In the haemolymph of *Nephrops norvegicus*, XDH was the only form of XOR present (Fig. 6). This is consistent with the majority of other crustaceans studied in which the dehydrogenase form of XOR was also dominant. In *Carcinus maenas*, Dyken (1991) found only traces of XDH activity in heart, gill and muscle tissues, and no XO activity was found in *C. maenas* even after 24 h of hypoxia, indicating not only that the XDH in this species is resistant to XO conversion, but also that urate production by XDH could proceed during hypoxia providing that substrate and NAD⁺ were available. However, Dyken (1991) found only traces of XDH activity in the hepatopancreas of 17.4 nmol urate g⁻¹ min⁻¹, a value similar to that in *Callinectes sapidus* (17.5 nmol g⁻¹ min⁻¹; Lallier & Walsh 1991). The increase in XDH after 72 h reflected the observations found in previous work for other *N. norvegicus* haemolymph constituents following prolonged emersion (Bernasconi & Uglow 2008).

It is well-established that the XDH form of XOR is predominant *in vivo* (Brass 1995, Fredericks et al. 1995). The presence of XDH as the source of XOR means that the cost of synthesising uric acid de novo is relatively small. The de novo synthesis of inosine monophosphate costs 7 to 9 ATP per molecule (Campbell 1995). Conversion to uric acid using XDH produces 2 NADH per molecule of uric acid generated, and their oxidation yields 6 ATP (Campbell 1995). Thus the overall cost of synthesis of uric acid using XDH is only 1 to 3 ATP molecule⁻¹, whereas urea synthesis costs 4 ATP molecule⁻¹ (reviewed by Linton & Greenaway 1998).

During prolonged emersion, it becomes imperative for aquatic crustaceans to store nitrogenous wastes in some form less toxic than ammonia. Urate, uric acid and urea are the likely candidates for such a role, as they are less or non-toxic, and in the case of urate and uric acid, are stored as solids and so conserve essential water, and help regulate the osmolarity of internal cells. Urea is stored in the haemolymph, from where it is transported to the gills for excretion upon re-immersion. From this investigation, we conclude that the ability of *Nephrops norvegicus* to withstand prolonged emersion, and the isolation of both XDH, uric acid and urea, suggests that this animal has purineolytic capacity and therefore the ability to convert purines de novo when exposed to air.

Acknowledgements. This study was partly funded by a contract from the Irish Fisheries Board. We thank Sutherland Fish and Game, Scotland, for supplying the trap-caught specimens.

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