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Metabolic responses of *Nephrops norvegicus* to progressive hypoxia

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Abstract – This work studied some of the metabolic responses of *Nephrops norvegicus* to a progressive reduction in water oxygen tension (PwO_2) at 12 °C. Experiments were designed to simulate water quality conditions that may occur during the trade of live crustaceans. Oxygen consumption rates and ammonia efflux rates were found to be constant over a wide range of PwO_2 values (20.4-5.9 kPa). A similar result was found for the difference between post-branchial and pre-branchial oxygen concentrations (20.4-2.6 kPa), obtained from a separate experiment. Anaerobic pathways, however, were activated after PwO_2 reached 6.3 kPa, as blood lactate and glucose concentrations increased from 1.24 ± 0.08 and 1.17 ± 0.19 (T_0 values) to 10.55 ± 8.99 and 3.63 ± 0.89 mg · 100 mL^{-1} respectively. *N. norvegicus* was able to maintain blood pH levels at relatively constant values despite a drop in water pH levels and the accumulation of lactate observed at low PwO_2. Heart rates also remained stable during PwO_2 reductions, but scaphognathite beat rate increased considerably, probably as an attempt to maintain steady weight-specific oxygen consumption rates. *N. norvegicus* appeared to be well adapted to cope with progressive hypoxia as may occur during holding and transportation procedures. © Ifremer-Elsevier, Paris.

Hypoxia / metabolism / *Nephrops norvegicus*

Résumé – Métabolisme de *Nephrops norvegicus* lors d'une hypoxie progressive. Cette étude met en évidence les réponses de *Nephrops norvegicus* au niveau du métabolisme à une réduction progressive des pressions partielles de l'oxygène dans l'eau (PwO_2) à 12 °C. Des expérimentations ont été menées pour simuler les conditions qualitatives de l'eau susceptibles d'être rencontrées lors du transport de crustacés vivants. Les taux de consommation en oxygène et les taux de rejet en ammoniaque ont été constants sur une large gamme de PwO_2 de 20,4 à 5 kPa). Un résultat similaire a été trouvé pour la différence entre les concentrations en oxygène post et prébranchiales (de 20,4 et 2,6 kPa) obtenu à partir d'une expérimentation. Le transport en anaérobie, cependant étaient activé lorsque PwO_2 atteint 6,3 kPa, et est les concentrations en glucose et en lactate du sang augmentaient de 1,24 ± 0,08 et 1,17 ± 0,19 (valeurs de T_0) à 10,55 ± 8,99 et 3,63 ± 0,89 mg · 100 mL^{-1} respectivement. *N. norvegicus* est capable de maintenir un niveau de pH du sang à des valeurs relativement constantes en dépit de l'accumulation de lactate observée à faible pression partielle d'oxygène (PwO_2). Les battements cardiaques demeurent aussi stables durant les réductions de PwO_2 mais les battements des scaphognathites augmentant considérablement, probablement comme une tentative de maintenir équilibrés les taux de consommation en oxygène. *N. norvegicus* apparaît être bien adapté à affronter une hypoxie progressive comme cela peut être rencontré lors de la manipulation et du transport de crustacés. © Ifremer-Elsevier, Paris.

Hypoxie / métabolisme / *Nephrops norvegicus*

1. INTRODUCTION

Nephrops norvegicus is fished extensively along the Scottish coast and part of the catch is directed to long distance live-transport. Most dealers transport live prawns without water but kept moist and cool inside polystyrene boxes which are air-freighted to mainland Europe. These prawns are normally dying when delivered and are sold as a 'fresh' product, unlike lobsters and crabs which are usually transported immersed at all times and consequently may survive for several days after being delivered. Emersion seems to be the

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main deleterious factor in the transport of live *N. norvegicus* and must be avoided if a successful trade of live prawns is to be established [22].

In order to avoid the problems caused by emersion, a small number of dealers have been experimenting with live transport of prawns in vivier lorries on their own or together with lobsters and crabs. Lobsters and crabs are transported in mobile systems for up to 48 h. Water oxygen levels under such circumstances may drop progressively and a situation of moderate to strong hypoxia may occur. Uglow et al. [28] have emphasised that low oxygen tensions may be a major problem during the transport of live *Cancer pagurus* in vivier lorries. Progressive reductions of water oxygen tensions down to 50 % saturation have been observed during the transport of live *Necora puber* [30]. These authors speculated that such values may drop considerably in the centre of the crates used to pack the crabs due to poor water circulation in this region.

Additionally, onboard vivier tanks are usually not provided with aeration systems and may be used to hold a large number of prawns. Increased levels of blood lactate in *N. norvegicus*, suggesting exposure to hypoxia, have been observed by R.F. Uglow under such situations. Also, *N. norvegicus* are normally held for variable periods of time in vivier tanks at the dealers' premises where a similar situation may occur. Vivier tanks are usually very basic structures where water circulation rates and aeration systems may not be able to supply all the oxygen required by the animals.

Exposure to hypoxia may affect several metabolical and physiological processes and, under such conditions, compensatory mechanisms to meet energy demand may be required. When PwO_2 falls to a critical level, oxidative metabolism may be supplemented by anaerobic metabolism.

Other adjustments to hypoxia are related to the cardio-ventilatory activity. Scaphognathite beat rate normally increases to compensate for the reduced PwO_2 but heart rate responses are usually more variable, depending on the species and experimental conditions used, and may not occur at all [5, 6, 12, 13, 20, 26, 17].

Other aspects which may cause stress during the storage and transport of live *N. norvegicus* (increased ambient ammonia and aerial exposure) have been recently investigated during laboratory simulations and real conditions [23, 24]. Such works have shown that the study of laboratory simulations, instead of isolated parameters under laboratory controlled conditions, may be a more adequate approach to investigate the physiological effects of live transport procedures. The present work studied some metabolic responses of *N. norvegicus* to a progressive reduction in PwO_2 and was designed to keep experimental conditions as close as possible to those which may occur in the trade.

2. MATERIAL AND METHODS

Nephrops norvegicus were creel caught off the west Scottish coast (57° 38' N, 5° 51' W) at depths of 150-250 m. Prawns weighing 18.4-36.5 g (mean = 28.8 ± 0.4 g SE) were transported to Hull University in containers with aerated seawater. They were kept in running seawater (12 °C, salinity 34) supplied with aeration and biological/mechanical filters and under low intensity light. The prawns were fed twice weekly with mussel flesh. Small lengths of plastic tubing (ϕ 60-70 mm) and a layer of 2-3 cm of fine sand were placed at the bottom of the aquarium to provide substitute burrows and substratum. All experiments were carried out inside temperature–controlled rooms at 12 °C and 75 % relative humidity. The prawns were kept in this system for at least 90 d before experiments started. A period of acclimation does not simulate real transport events but was required in order to standardise animal condition.

In all experiments the prawns were placed in a flow through system consisting of a header tank, a sump, a water pump and 10 respirometers (4 L each), each supplied with inlet and outlet valves and a Suba-seal. The seawater flowing through the respirometers (*c.* 1.2 $L.min^{-1}$) was taken to the sump where aeration and biological filtration was provided and then pumped back to the header tank. Progressive hypoxia was effected as a result of the oxygen consumption ($\dot{M}O_2$) of the prawns by closing the inlet and outlet valves and sealing each of the respirometers. Ambient CO_2 levels were not measured but a progressive increase must have occurred as a result of the metabolism of the prawns. Progressive hypoxia associated with progressive hypercapnia are relatively common events during live transport in vivier lorries. In all experiments, one of the respirometers was kept free of prawns but was otherwise subjected to the same experimental procedures and used as a control for bacterial action on the water oxygen tension (PwO_2). The effects of progressive hypoxia on *N. norvegicus* were studied in two separate experiments.

2.1. Experiment 1

N. norvegicus (groups of 9 individuals) were individually placed in the respirometers and, after an acclimation period of 12 h, the inlet and outlet valves were shut for 16 h. Water samples were then collected with a syringe and needle through the Suba-seal and pH, oxygen and total dissolved ammonia (TA) concentrations measured. The prawns were then removed from the respirometers and samples (20 µL) of pre- and post-branchial haemolymph were both collected within a space of 10 s (max.) of the animals being picked up and their oxygen contents immediately determined. Samples were collected using gas–tight syringes (Hamilton) from the sinus at the base of pereiopod IV or V (pre-branchial) or from the cardiac sinus (post branchial). Additional haemolymph samples (300 µL)

were collected via the pereiopod sinus for pH, lactate, glucose, protein and TA determinations. The whole procedure was repeated using different groups of prawns that remained in the sealed respirometers for 24, 32, 40, 48 and 56 h and also from prawns randomly sampled from the stock tank.

2.2. Experiment 2

Heart rate (HR), scaphognathite beat rate (SR), oxygen consumption and ammonia efflux rate were simultaneously measured during progressive reductions of PwO_2. The prawns were acclimated to the system until HR and SR were found to be stable (48 h) and the respirometers then sealed for 33 h, causing a gradual reduction in PwO_2. Immediately after, both valves were reopened and fully air-saturated seawater was flushed through the respirometers for 10 min. The clear Perspex lid of the respirometers was partially covered with black plastic to avoid visual disturbances that could affect the HR and SR of the prawns. Water samples were periodically collected via the Suba-seal and ammonia and oxygen contents and pH measured. At similar set intervals, the HR and SR of the prawns were recorded. Ammonia efflux and oxygen consumption rates were determined as the difference between the levels obtained for two consecutive timed samples.

Total dissolved ammonia ($NH_4^+ + NH_3$) concentrations in the water and hemolymph were measured using a flow injection/gas diffusion technique [4, 16]. These hemolymph samples were diluted (1:20) with a saline solution (9 $g \cdot L^{-1}$ NaCl) before analysis.

HR and SR were measured according to a modified impedance technique [8]. Electrodes attached to the prawns were made using silver-plated copper-solid wires with KYNAR™ insulation ($\phi = 0.25$ mm). Heart electrodes were introduced into the body through a small hole ($\phi = 0.25$ mm) drilled above the heart. Each scaphognathite electrode was hooked over the margin of the cephalothorax.

Hemolymph total oxygen (free + bound oxygen) concentrations and water oxygen tensions were measured using a modified couloximetric technique [21] Standards comprised a sample (20 µL) of seawater fully saturated with air. PwO_2 values were calculated based on the atmospheric pressure registered on the day that the experiments were performed.

Measurements of blood and water pH were made using a JP PHM2 pH meter and a Whatman protein-resistant microelectrode within 10 s of sample collection. Haemolymph samples for glucose and lactate analysis were mixed with 6 % ice-cold perchloric acid (1:1) immediately after collection. Glucose and lactate levels were determined enzymatically using glucose-oxidase kits from Sigma Chemic. Comp. (cat. no. 510) and UV kits from Boehringer Mannheim (cat. no. 149993), respectively. Total protein concentrations were determined with Biuret reagent from Boehringer Mannheim (cat. no. 124281).

The homogeneity of variances of the groups were tested using the Levene test. All variables were analysed by one way-ANOVA. When significant differences were detected the Tukey multiple range test was then applied to identify which groups were different from each other. All statistical analysis were performed at the 0.05 level of significance.

3. RESULTS

Bacterial action on PwO_2 levels was considered negligible as PwO_2 levels of the control respirometers (without prawns) remained constant throughout both experiments.

3.1. Experiment 1

Results are shown in *table 1*. PwO_2 decreased from full saturation (*ca.* 20.4 kPa) down to 2.6 kPa after 56 h. Water pH values decreased 0.53 pH points during this time, probably as a result of CO_2 accumulation. Total ammonia (TA) increased from <1 to 37.8 ± 4.5 µmol $TA.L^{-1}$ during the same period. Pre- and post-branchial oxygen concentrations (CvO_2 and CaO_2, respectively) decreased progressively with decreasing PwO_2 but values were only significantly lower ($P < 0.05$) than initial values when PwO_2 was 8.9 kPa and 6.3 kPa, respectively. Blood glucose concentrations were stable for most of the experimental time and only increased significantly ($P < 0.05$) after PwO_2 reached very low levels (2.7 kPa). Blood lactate was the parameter showing the highest individual variability, as shown by the high standard error values found. Lactate started to increase when PwO_2 decreased to *ca.* 6.3 kPa but, because of such high variability, these increases were not significantly different ($P > 0.05$). Highly variable blood lactate levels were also found in *Eriocheir sinensis* subjected to hypoxic [10]. Blood pH remained relatively stable during PwO_2 reductions to *ca.* 6.3 kPa but then increased significantly ($P > 0.05$) with further reductions in PwO_2. The difference between CaO_2 and CvO_2 (oxygen extracted and used by the prawns) was not significantly altered ($P > 0.05$) by a reduction in PwO_2.

3.2. Experiment 2

PwO_2 decreased from 20.4 kPa to 5.9 kPa in 33 h and increased again to 13.9 kPa after the respirometers were flushed with normoxic water (*table II*). The water TA values measured during this period oscillated between <1 and 17.6 µmol $TA.L^{-1}$. Water pH dropped *ca.* 0.4 pH units and initial pH values were higher than the initial values for the previous experiment. The seawater used in the stock tank was periodically transported to the laboratory and stored in containers and the pH differences observed may have been related to the use of different batches of seawater in the stock tank. HR remained relatively stable throughout PwO_2

Table I. *Nephrops norvegicus*: Some water and blood parameters measured during progressive hypoxia. Prawns were placed in sealed respirometers and PwO_2 reductions were caused by oxygen consumption ($\dot{M}O_2$) (mean SE and n = 8 or 9 in each case); TA: total ammonia; ($NH_3 + NH_4^+$); CaO_2: arterial oxygen concentration; CvO_2: venous oxygen concentration.

	Water			Haemolymph						
Time (h)	PO_2 (kPa)	TA ($\mu mol \cdot L^{-1}$)	pH	CaO_2 ($ml \cdot L^{-1}$)	CvO_2 ($ml \cdot L^{-1}$)	ΔCO_2 ($ml \cdot L^{-1}$)	Glucose ($mg \cdot 100\ ml^{-1}$)	Lactate ($mg \cdot 100\ ml^{-1}$)	TA ($\mu mol \cdot L^{-1}$)	pH
0	20.4	< 1	7.67	12.36 ± 1.39	9.52 ± 1.00	2.84 ± 0.71	1.17 ± 0.19	1.24 ± 0.08	161.06 ± 10.10	7.78 ± 0.01
16	15.4 ± 0.3	12.29 ± 1.32	7.46 ± 0.01	10.60 ± 1.88	6.40 ± 1.04	4.20 ± 1.29	1.12 ± 0.20	1.51 ± 0.13	135.42 ± 11.48	7.75 ± 0.02
24	11.2 ± 0.5	20.99 ± 1.62	7.36 ± 0.02	8.55 ± 1.78	5.69 ± 1.45	2.86 ± 0.98	1.16 ± 0.16	1.28 ± 0.12	121.42 ± 16.52	7.77 ± 0.02
32	8.9 ± 0.9	21.77 ± 3.02	7.31 ± 0.02	7.58 ± 1.21	4.43 ± 0.50	* 3.15 ± 0.98	2.36 ± 0.40	1.01 ± 0.09	151.46 ± 18.06	7.78 ± 0.02
40	6.3 ± 1.2	27.46 ± 2.65	7.24 ± 0.02	6.18 ± 1.39	* 2.74 ± 0.54	* 3.44 ± 1.08	3.63 ± 0.89	10.55 ± 8.99	137.69 ± 14.26	7.78 ± 0.04
48	2.7 ± 0.8	35.14 ± 4.50	7.21 ± 0.02	4.34 ± 0.94	* 0.99 ± 0.24	* 3.35 ± 0.80	6.92 ± 1.75	* 11.96 ± 7.76	141.79 ± 14.66	7.87 ± 0.03 *
56	2.6 ± 0.6	37.77 ± 4.53	7.14 ± 0.02	4.12 ± 1.11	* 1.51 ± 0.68	* 2.61 ± 0.54	9.83 ± 2.19	* 19.48 ± 9.74	113.54 ± 8.03	7.87 ± 0.05 *

* Significantly different than T_0 ($P<0.05$).

Table II. *Nephrops norvegicus*: Some water parameters, cardio-ventilatory rates and weight-specific oxygen consumption ($\dot{M}O_2$) measured during progressive hypoxia. Prawns were placed in sealed respirometers and PwO_2 reductions were caused by $\dot{M}O_2$ (mean SE and n = 8 or 9 in each case); TA: Total ammonia ($NH_3 + NH_4^+$); HR and SR: heart rate and scaphognathite rate, respectively.

Time (h)	PwO_2 (kPa)	Water TA ($\mu mol \cdot L^{-1}$)	Water pH	HR ($beats \cdot min^{-1}$)	SR ($beats \cdot min^{-1}$)	Time interval (h)	$\dot{M}O_2$ ($\mu L \cdot g^{-1} \cdot h^{-1}$)	TA efflux ($\mu mol \cdot g^{-1} \cdot h^{-1}$)
0	20.4	< 1	7.85	21 ± 5	37 ± 9	0-6	13.6 ± 1.0	0.05 ± 0.01
6	18.3 ± 0.1	3.40 ± 0.41	7.81 ± 0.01	19 ± 2	35 ± 5	6-12	18.8 ± 2.6	0.09 ± 0.02
12	15.2 ± 0.5	7.97 ± 1.18	7.72 ± 0.01	22 ± 1	42 ± 8	12-18	17.0 ± 1.4	0.07 ± 0.01
18	12.7 ± 0.5	11.08 ± 1.58	7.63 ± 0.01	19 ± 2	37 ± 7	18-24	17.4 ± 1.4	0.06 ± 0.01
24	9.9 ± 0.5	13.82 ± 1.80	7.52 ± 0.01	19 ± 1	47 ± 8	24-30	19.0 ± 4.7	0.04 ± 0.01
30	7.2 ± 0.7	16.20 ± 2.31	7.49 ± 0.01	17 ± 2	81 * ± 7	30-33	16.5 ± 3.0	0.06 ± 0.02
33	5.9 ± 0.6	17.56 ± 2.49	7.47 ± 0.01	24 ± 3	115 * ± 11			
33[a]	13.9 ± 0.3	9.47 ± 1.43	7.69 ± 0.01	32 ± 3	* 108 * ± 11	33-36.5	19.7 ± 3.7	0.03 ± 0.02
36.5	12.2 ± 0.3	10.16 ± 1.58	7.63 ± 0.02	19 ± 3	45 ± 9			

[a] Immediately after seawater was flushed through the system for 15 min.
* Significantly different than T_0 ($P<0.05$).

reductions but a trend to increase immediately after normoxic seawater was flushed through was observed, as HR was significantly higher than the values observed at 5.9 kPa. SR started to increase after PwO_2 reached 7.2 kPa ($P<0.05$), remained higher than initial values following the water change but returned to initial values 3.5 h after that $\dot{M}O_2$ and TA efflux rates were not significantly altered throughout any PwO PwO_2 alterations.

4. DISCUSSION

The ability to withstand hypoxia is highly variable amongst crustaceans. Burrowing species, such as *N. norvegicus* [9] are usually more adapted to face hypoxia than those species normally living in well-aerated water [29]. *N. norvegicus* may be exposed to seasonal periods of hypoxia below 30 % of saturation [1, 14]. Baden et al. [2], collected *N. norvegicus* from

waters with oxygen concentrations between 12 and 20 % saturation.

Here, MO_2 rates are similar to those measured previously for *Nephrops* [13] and were found to be constant over a PwO_2 ranging from fully-saturated seawater to 5.9 kPa (*table II*). The values obtained for CO_2 (CaO_2 - CvO_2) agree with such results, as they were also maintained at relatively constant levels over an even wider range of PwO_2 (20.4 - 2.6 kPa). *Munida rugosa* and *M. sarsi*, both species that may inhabit the same type of area as *N. norvegicus*, also showed independent MO_2 rates down to 5.3 kPa of hypoxia [31]. The relation between MO_2, arterial and venous O_2 concentrations and cardiac output is discussed later in this section.

The increased levels of blood lactate concentrations found for prawns at PwO_2 of 6.3 kPa were not significantly different than those found for prawns at normoxia ($p > 0.05$), due to high individual variability, but showed that at least some of the prawns were using anaerobic pathways at this level of moderate to strong hypoxia. Blood glucose concentrations showed a similar pattern. The increased utilisation of carbohydrates, which accompanies anaerobiosis, may lead to a depletion of the glycogen reserves stored in the muscle and hepatopancreas of *N. norvegicus* [2]. Similar anaerobic responses have been previously observed in *N. norvegicus* subjected to hypoxia but only at PwO_2 of 2.1 kPa (16 torr) and below [15, 25].

When held at commercial facilities, the increased activity and stress that are usually associated with normal marketing procedures will alter such aspects and functional anaerobiosis, as defined by Gäde [11], may prevail and increase considerably the utilisation of carbohydrate.

PwO_2 was found to be directly related to water pH values but this is more likely to be a reflection of the accumulation of CO_2 in the water as a result of the metabolic activity. Assuming that *N. norvegicus* were exposed to supranormal PwO_2, the prawns were able to compensate for such increases as blood pH values were not altered throughout the experimental period.

When *N. norvegicus* were subjected to progressive hypoxia down to 5.9 kPa, HR remained relatively stable but SR increased considerably. Such increases in SR usually aim to compensate for the reduced oxygen concentrations in the water so maintaining a constant supply of O_2 to the gills. The ventilatory adjustments to hypoxia may also occur by alterations in the stroke volume of each beat, as found for *Orconectes virilis* [3].

Another adjustments to low PwO_2 may be an increase in the supply of haemolymph to the gills [19]. An increase in cardiac output was observed during hypoxic exposure in *Homarus americanus* (based on Fick calculations) [18]. A similar response has been found in *Cancer magister* which showed that the increases in cardiac output observed during progressive hypoxia were due to increases in the stroke volume [17]. Here, *N. norvegicus* was able to face progressive hypoxia down to 5.9 kPa without altering HR (*table II*) or the stroke volume, as cardiac output (stroke volume × heart rate) remained constant. This is shown by the following equation of Dejours [7]:

$$MO_2 = Vb\ (PaO_2 - PvO_2)\ (\alpha O_2),$$

where Vb is cardiac output and O_2 is the solubility coefficient for oxygen.

As MO_2 and the difference between post and prebranchial blood oxygen were not altered in the present experiments, cardiac output was maintained constant during hypoxia.

N. norvegicus thus appears to be well adapted to face progressive hypoxia as may occur in the trade of live crustaceans, provided that such hypoxia is kept at moderate levels. *N. norvegicus* is also well adapted to face periods of increased ambient ammonia concentrations but poorly adapted to cope with emersion, see other results obtained by Schmitt and Uglow [23, 24]. Hence, the maintenance of prawns in vivier tanks followed by transport in vivier lorries is a valid alternative to the air-freight of emersed prawns in polystyrene boxes, as it may deliver more lively and active individuals which will command higher prices at the retail market.

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