A NOVEL METHOD FOR THE STORAGE OF LIVE NEPHROPS NORVERGICUS (L.) DURING LIVE MARKETING

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Abstract

A system to hold and/or transport Nephrops for variable periods of time with very little physiological disturbances and also with a space/weight optimization was produced. Tests were performed at 14 and 6°C using cascade systems with trays that retained different amounts of water and where the prawns were held free and restrained. Tests were also made inside a polystyrene box under moist conditions at ca. 2°C. Water temperature had a marked influence on the metabolic rates of the prawns and this was evidenced by the higher temperature. Nephrops appear to be able to maintain aerobic metabolism independently of the amount of water that was retained at the bottom of the trays, provided that sufficient amounts of aerated seawater continuously reach the gills. Hyperglycaemia was observed, but was probably related to stress caused by the holding system and not to hypoxia. The prawns held inside polystyrene boxes showed anaerobiosis but they all survived the 12 h emersion period. Guidelines are made to design a system to hold Nephrops on a commercial scale.

The transport and marketing of live shellfish is a fast-growing international market. However, most of the procedures and techniques presently used in this trade were developed on a "trial-error" basis without a full understanding of the physiological and biological needs and characteristics of the different species used.

Whatever the type of transport (surface or air-freight) and species used, the problems faced by the animals are very similar. They may be summarized as: excessive handling; emersion and desiccation; increased activity and mutual interaction; increased ambient levels of nitrogenous products; low ambient levels of dissolved oxygen. The improvement of a determined transport activity depends on a thorough knowledge of the biological and physiological responses to the factors cited above and also on the use of well-planned procedures and systems.

The physiology of crustaceans subjected to commercial practices, or to simulations of such conditions, has been investigated for a number of different species (Spicer et al. 1990, Whitney and Taylor 1992, Paterson 1993) but the applied use of this type of information to design holding facilities and transport systems during fishing and commercial operations has received far less attention. Most of the studies of Nephrops related to its commercial exploitation have concerned other factors, such as: population dynamics, catch composition, fishing techniques and behavioral responses to the fishing gear used (see Atkinson and Naylor 1976, Briggs 1985, Bailey et al. 1986, Newland and Chapman 1989, Hill and White 1990, Cardador 1993, Stewart et al. 1993).

The present work aimed to produce a system whereby Nephrops could be held and transported, for variable periods of time, with minimum physiological disturbances and also with a consideration of the space/weight limitations which are of prime economic importance during such activities. All experiments were designed to minimize the effects of emersion which, according to Spicer et al. (1990), was shown to be harmful to Nephrops.

Materials and Methods

Nephrops norvegicus were creel-caught off the northwest coast of Scotland (57° 38' N, 5° 51' W) at depths of 150-250 m. Prawns with a mean weight of 28.5 ± 0.4 g SE (18.6 - 36.3 g) were brought to Hull in containers with aerated seawater. They were kept in running seawater (12°C, 34 psu) supplied with aeration and biological filters and under low intensity light. Small lengths of plastic tubing (ö 60-70 mm) and fine sand were placed at the bottom of the aquaria to provide substitute burrows and substratum for the prawns. The prawns were kept in this system for at least 60 days before experiments were made and they were fed twice weekly ad libitum with mussel flesh until 48 h before the experiments. All experiments were made inside temperature-controlled rooms at 12°C and 75% rel. humidity.

The experiments were performed using cascade systems in which a constant spray/flow of water was poured through a series of stacked trays containing prawns and also using a system in which prawns were kept emersed but under high humidity conditions and very low temperatures.

The cascade systems used are shown in Figure 1. Water from the header tank, the base of which was perforated to form a shower, poured through the trays into a sump and then was pumped back to the header tank. Three types of trays were used: 1) trays with a mesh base, hence without water retention (open trays); 2) trays with a perforated base but which retained a shallow layer of water of ca 2 mm depth where individual prawns were separated with plastic netting material (2 mm trays); 3) trays which retained a 15 mm deep layer of water and where the prawns were also individually separated (15 mm trays). The divisions that kept the prawns separated were 30 mm apart from each other and thus allowed little space for movements. All the cascade system were supplied with a plastic curtain surround to avoid water escaping and to minimize evaporation.

The effects of maintaining Nephrops in these systems were studied initially at 6°C and at 14°C using two cascade systems at each of these temperatures. One of the systems contained ca 900 g of prawns in 51 of seawater (1:5.56, w/v) and three open trays and the other contained ca 1850 g of prawns in 101 of seawater (1:5.41, w/v) and three 2 mm trays and three 15 mm trays. Nephrops were taken from the stock tank and placed individually separated in the 2 and 15 mm trays and not separated in the open trays (groups of 10 prawns per tray in all cases). Haemolymph samples were collected from prawns in the stock tank (time 0) immediately before and then 12, 30 and 56 h after the prawns were transferred to the cascade system. All haemolymph samples (300 ìl) were collected through the arthrodial membrane of one of the pereiopods and the prawns were returned to the system. Water samples (1 ml) from the cascades were also collected at the above times and their ammonia contents determined. Measurements of blood pH were made immediately after collection using a JP PHM2 pHmeter and a Whatman protein-resistant microelectrode. The remainder of the sample was used for glucose, lactate, ammonia and protein determinations.

The use of 2 mm trays at 6°C offered the best results (see Discussion) and a second experiment was designed using 12 such trays but with an increased biomass/water ratio (ca 3840 g and 101 of water) (1:2.6, w/v) and also with an increased holding time. Water and blood samples were collected before (time 0) and 13, 29, 53 and 129 h following transference to the system. During this experiment, additional blood samples (20 ìl each) for total oxygen determinations were collected from the cardiac sinus (post-branchial blood) and through the arthrodial membrane of one of the pereiopods (pre-branchial blood). These samples were both collected using gas-tight syringes (Hamilton) within a space of 10 sec (max.) of the animals being picked up from the cascade system.

The experiments with emersed prawns were performed. Prawns were taken from the stock tank and placed inside a Polystyrene box between layers of sea water-soaked insulating wool and ice packs. The temperature of the system was measured periodically by placing a thermometer at different positions in the box. Blood samples were collected from prawns from the stock tank and from prawns following 1, 2, 4, 8 and 12 h of emersion and the ammonia, glucose, lactate and protein contents and pH of these samples were measured.

Total dissolved ammonia ($NH_4^+ + NH_3$) concentrations in the water and hemolymph were measured using a flow injection/gas diffusion technique adapted from Clinch et al. (1988) and Hunter and Uglow (1993). This technique comprises a carrier stream of NaOH (0.01 M) separated from an indicator solution (Bromothymol Blue, 0.05%) by a gas-permeable membrane (PTFE). All ammonia in the samples is converted to NH_3 which diffuses across the membrane and reacts with the indicator producing a Ph-dependent colour alteration that is detected by a photometer. The results are calculated based on a calibration curve produced with different concentrations of $(NH_4)_2SO_4$. Hemolymph samples were diluted (1:19) with saline solution (9 g l^{-1} NaCl) before analysis.

Haemolymph samples for glucose and lactate analysis were mixed with 6% ice-cold perchloric acid (1:1) immediately after collection. Such samples were centrifuged for 5 min at 10000 rpm 7200 x g and glucose and lactate levels were enzymatically determined with 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).

Hemolymph total oxygen (free + bound oxygen) was measured using a modified couloximetric technique by Peck and Uglow (1990). Samples were injected into a desorber (2ml of uranyl acetate as cleaving agent) from which the total oxygen is displaced by the N_2 stream and carried to the fuel cell (A-120, Chandos Int.). A gas flow of 45 cm^3 min^{-1} was used and the outputs were recorded on a graphic recorder (Talbot Instruments, Servoscribe 1S RE 543.20). Standards comprised a sample (20 il) of seawater fully saturated with air. The total dissolved oxygen content of such samples were obtained from a nomogram which utilized water temperature and salinity to provide the dissolved oxygen value. Peak height of standard was dependent on the dissolved oxygen and was taken as 100%-base line was taken as 0% and samples of unknown concentrations were calculated proportionately.

The homogeneity of variances of the groups were tested using the Levene test. All variables were analysed with oneway-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.

Results

The survival rates during all the experiments and also during 3 days following transfer back to the stock tank was 100%. The transfer of the prawns to the cascade systems in which they were kept individually separated was accomplished by several tail flips and escape responses lasting ca 5-10 min. In the cascade system with open trays in which netting divisions were not provided, a similar behavior was found and tail flips were occasionally observed throughout the whole experimental time. When the prawns were placed in the polystyrene box these periods of supernormal activity lasted only a few seconds, as the prawns were restrained by the insulating wool used to keep them soaked.

The water TA levels and blood constituents of Nephrops held in the open tray cascade system at 14°C and 6°C are shown in Table 1. TA levels in the water increased progressively at both temperatures but the final TA levels found at 14°C were more than double those found at 6°C. Blood TA increased significantly ($P<0.05$) during the first 12 h in the 14°C system but, at the lower 6°C temperature, TA increase rates were much lower and significant increases were only found after 30 h. Blood protein concentrations were not significantly altered ($P>0.05$) while the prawns were maintained in this system at either temperature.

At 14°C, blood glucose levels increased significantly within 12 h following transfer to the cascade system and then remained stable for the remainder of the experiment. A different pattern was found at 6°C, when blood glucose remained constant ($P>0.05$) for 12 h but showed a significant increase ($P>0.05$) after 30 h. The mean concentration values observed at such times were the highest observed ($P<0.05$) among all the cascade systems used and were caused mainly by 3 individuals that showed glucose (also lactate) concentrations considerably higher than all others. It may be that such animals were placed in a position where less water was available to them and the effects caused by emersion prevailed. Lactate concentrations were not significantly altered ($P>0.05$) throughout the experimental time, except for the group referred to above (30 h, 6°C) which showed a higher individual variability and was found to be significantly higher than those from prawns sampled from the stock tank. A trend to decrease blood pH (acidosis) during the period in which the prawns were in the cascade occurred at both temperatures but such reductions were only significantly different after 30 h at 6°C.

When the prawns were held individually separated in the cascade system with 15 mm trays (Table 2), water TA also increased but at much lower rates than those found in the system with no retention. Water TA levels observed at 14°C were again much higher than those found at 6°C. Blood TA levels increased significantly ($P<0.05$) after the transfer to the 14°C system but were not significantly altered ($P>0.05$) when the prawns were kept at 6°C. Changes in blood protein were not found to be significant at either temperature ($P>0.05$). The haemolymph glucose concentrations measured 30 h after the prawns were transferred to the system at 14°C and 6°C were significantly higher ($P<0.05$) than those from prawns sampled from the stock tank. Haemolymph lactate levels were not significantly altered ($P>0.05$). Blood pH again showed a slight acidosis with time in the new systems but such differences were not found to be significant ($P>0.05$).

Water and blood parameters, measured on prawns held individually separated in 2 mm trays are shown in Table 3. Water TA concentrations are described above, as both trays (2 mm and 15 mm) were part of the same system. The pattern found for haemolymph TA concentrations when 2 mm trays were used was similar to that found for prawns held in 15 mm trays. At 14°C, blood TA increased significantly ($P<0.05$) 12 h after transfer to the system and then increased once again at 56 h.($P<0.05$). Blood glucose once again increased significantly ($P<0.05$) after 30 h in the cascade system and remained high at 56 h. Blood lactate concentrations were not significantly altered ($P> 0.05$). Blood pH showed a trend to decrease, but significantly lower values ($P<0.05$) were observed at 12 h and 56 h in the 6°C cascade system only.

The temperature had a clear effect on the nitrogen metabolism of the prawns, as all groups from all systems showed a significantly higher ($P<0.05$) mean blood TA value at 14°C than the 6°C.

The results obtained when Nephrops were held over 5 days at 6°C in a cascade system using 2 mm trays are shown in Table 4. Water TA increased with time and the increased rate was higher at the start than towards the end of the experiment. Haemolymph TA concentrations increased significantly ($P<0.05$) when the prawns were held in the cascade system and were already higher than control prawns (from the stock tank) at 13 h. Blood

C_aO_2, C_vO_2 and protein and hemocyanin concentrations were not significantly altered ($P<0.05$) throughout the whole period of maintenance in the cascade system. Haemolymph glucose concentrations increased significantly ($P<0.05$) 29 h after transfer to the system and a very high mean value was reached at 129 h and this was significantly higher ($P>0.05$) than all the mean values. Blood pH values were significantly lower than control prawns at 53 h but increased again at the end of the experiment. The mean values appeared to be less variable than those found in the previous experiments.

Blood parameters of Nephrops taken from the stock tank and placed inside a polystyrene box at low temperatures (2 ± 0.6°C) are shown in Table 5. Blood pH decreased significantly ($P<0.05$) after 1 h then remained constant ($P>0.05$) until the end of the experiment. TA in the blood increased with emersion time but such increases were found to be significant (($P<0.05$) only after 12 h of emersion. Blood glucose concentrations increased after 1 h of emersion and continued to increase with emersion time, as the mean value found at the end of the experiment was significantly higher ($P>0.05$) than that found after 1 h of emersion. Lactate concentrations increased significantly ($P<0.05$) after 1 h of emersion and then remained stable until the end of the experiment. Protein levels were not significantly altered throughout the experimental time ($P>0.05$).

Discussion

The results obtained show that Nephrops can be partially emersed for a period of time which is long enough to include a few days of maintenance in the dealers' premises and also to include the road transport duration to reach the main markets in France and Spain. Similar cascade systems have been tested previously using other species of crustaceans. McLeese (1965) compared the effects of Homarus americanus under highly moist condition and in a spray/cascade system using screen-bottomed trays (without water retention) for several days. The author did not find any significant differences in the mortality rates of lobsters subjected to either conditions and concluded that cascading water above lobsters was not improving gas exchange across the gills (cf. lobsters held under moist conditions). Burnett et al. (1973) tested the use of a cascade system which had trays that retained 25 mm of water and found that such systems were considerably more efficient for maintaining Cancer magister than a simple spray system without any water retention. It must be stressed, however, that neither study considered the effects of these emersion periods on the blood chemistry or other metabolical aspects of the animals, which presumably would have provided a more accurate view of the efficacy of such systems.

Here, when Nephrops was held in cascade systems under three different conditions of water retention (Tables 1-3), the patterns of blood glucose and lactate concentration changes were found to be very similar. It appears that the amount of water retained on the bottom of the trays did not affect the respiratory capability of Nephrops, as far as the use of anaerobic pathways for energy production are concerned. This may be related to the fact that wet gills do not collapse and so reduce the surface area available for gas exchange. The collapse of the gill lamellae has been found to impair oxygen uptake and reduce survival and the morphological and behavioral adaptations to overcome this problem have been studied in several terrestrial and semiterrestrial species of crustaceans (Cameron 1981, Johnson and Uglow 1985, McMahon and Burggren 1988, Schmitt and Santos 1993b). The importance of aerated water on the oxygen uptake has been shown in species of intertidal crabs that can circulate small amounts of seawater over the carapace and increase its oxygen content (Hawkins and Jone 1982, Santos et al. 1987) Nephrops is a fully aquatic species and, as such, lacks most of the adaptations shown by those species that face periodic exposure to air. Nevertheless, it appears to be able to maintain aerobic metabolism out of water, provided that sufficient amounts of aerated seawater continuously reach the gill surface.

Such findings are corroborated by the fact that Nephrops was able to maintain C_aO_2 and C_vO_2 at constant levels, hence constant blood CO_2, for over 5 days in the 2 mm cascade system (Table 4). Hyperglycaemia, accompanied by increased levels of circulating lactate, are classic responses to emersion-related hypoxia in crustaceans. The use of anaerobic pathways requires considerably more glucose to produce the same amount of energy than when aerobic pathways are used and a reduction in the carbohydrate reserves may occur under such conditions. In addition to that, the animals will have to cope with the accumulation of lactate (end-product of such metabolic pathways). Such responses have also been observed in Nephrops subjected to emersion under various conditions (Spicer et al. 1990). Here, considering the evidence of steady blood oxygen concentrations and the low lactate levels found in all cascade systems, it is suggested that Nephrops was not under hypoxia. The hyperglycaemia found under such circumstances, clearly shown during the 5 days experiment (Table 4), was probably a response to other factors, such as the lack of space, interaction with other prawns and partial emersion which may have stressed the prawns. Homarus americanus also showed a similar hyperglycaemic response when submitted to various stress factors (Telford 1968, 1974).

During the 5 days experiment, the prawns were not fed for a total of 7 days (including the 2 days of starvation before the start) and it seems that this period was not long enough to cause a detectable decrease in the haemocyanin concentrations, such as those found for Nephrops and Homarus gammarus during starvation or exposure to low quality diet (Hagerman 1983, Hagerman and Baden 1988, Baden et al. 1990).

Marine and freshwater crustaceans excrete their metabolic nitrogenous waste mainly as ammonia (60-100% of total nitrogen), which is normally excreted across the gills (Regnault, 1987). During emersion, the lack of water may highly impair the normal functions of the branchial epithelium, such as gas exchange, ionic regulation and ammonia efflux and blood ammonia levels may rise to very high levels. Here, blood TA concentrations were similar to the ambient ones in most of the situations studied, which shows that Nephrops TA effluxes were not restrained by the emersion periods imposed by any of the cascade systems tested. Otherwise, blood TA levels would be considerably higher than ambient ones.

TA accumulation in the blood and water was related to temperature (discussed below) and was higher in the cascade system without the plastic net divisions (Table 1), in comparison with the levels obtained when the prawns were restrained (Tables 2-4). Such differences in water and blood TA may have been the result of a general increase in activity and metabolism due to the mutual interaction and the number of tail flips shown by the unrestrained prawns.

Amongst the cascade systems tested, the main effect of temperature on the prawns is related to their ammonia production rates, which were clearly lower at 6°C than at 14°C (Tables 1-3). According to Regnault (1987) the effects of temperature on the nitrogen efflux rates of crustaceans may vary according to the species and temperatures used. However, as with most metabolic rates, a direct relation between nitrogen effluxes and temperature, such as those found by Needham (1967), Gerhardt (1980), Quarmby (1985), Kristianssen and Hessen (1992) and Couper (1993) seems to be the general rule.

Based on the blood and water levels of ammonia found, it appears that maintaining Nephrops in restrained systems at 6°C is the most adequate condition. Considering the absence of any significant differences between the respiratory responses and blood and lactate levels of prawns held in the different cascades, the one using 15 mm trays may be eliminated for transportation purposes, as the excess water will increase the overall weight of the system (cf 2 mm and open trays). The use of 2 mm trays, instead of open trays, may be the most safe option, as it ensures that all prawns have access to water in case they are not under direct reach of the spray/flux. Based on such evidences, the conditions used in the cascade system tested during the 5 days experiment seemed to be to most apropriate (2mm trays; restrained prawns; 6°C).

The experiment which involved the maintenance of prawns inside polystyrene boxes, was designed to evaluate the effects of very low temperatures (2°C) on the metabolism of emersed prawns. According to Whiteley and Taylor (1986) the rapid transfer of lobsters Homarus gammarus to 2°C water may induce autotomy of the limbs. Autotomy may occur as a reaction to stress or as an escape response, when one or more limbs are trapped (McVean 1983). In the present experiment, temperatures were reduced relatively quickly, from 12°C to 2°C in ca. 15 min, but not apparent loss of legs or claws, which could be related to the low temperatures used, was found to occur. The blood acidosis and lactate accumulation observed here (Table 5) were less intense than that shown in emersed Nephrops in previous experiments made at 12°C. These results show that the metabolism of Nephrops can be depressed at 2°C without any reduction to the quality of the prawns (intact and live animals) and that such prawns can fully recover after transfer back to the water. Nephrops is air-freighted live to Italy and Spain and may endure several hours of emersion due to loading/unloading and customs procedures. The delivered prawns, however, are usually moribund if not dead and are sold as fresh (D. McRae, pers. comm.). The specifications used in this experiments may be used to improve such procedures and deliver prawns of considerably higher quality that could be live in vivier systems for a few days after arrival.

The results obtained with the cascade systems show that Nephrops can be maintained in such systems for periods of at least 5 days and the reduced volume of water used (cf the volume of water of vivier tank systems) may represent an advantage, as the weight of consignment of live animals affect transport costs. Another advantage of this system is that the prawns may be selected and transferred to trays as soon as they are unloaded from the fishing boat and the trays may be transferred between dealers or into a mobile cascade system without handling the prawns, which will minimize aerial exposure and general damage to the animals.

These are laboratory tests and a cascade system to hold Nephrops on a commercial scale may be designed successfully based on the following guidelines:

- Low water and air temperature (6°C or less).
- A minimum layer of water of 2 mm on the bottom of the trays.
- Trays with internal divisions to individually separate the prawns.
- A mechanical filter placed on top of the cascade.
- Plastic covers at the side to avoid water loss and minimize evaporation.

The use of trays without internal divisions (or with removable ones) would allow the fishermen and dealers to use this system to hold and transport other crustaceans of different sizes, such as lobsters and crabs, together in the same system or in different loads. The biomass/water ratio could probably be taken to values ca. 1:1 (w/v) without compromising the efficiency of the system.

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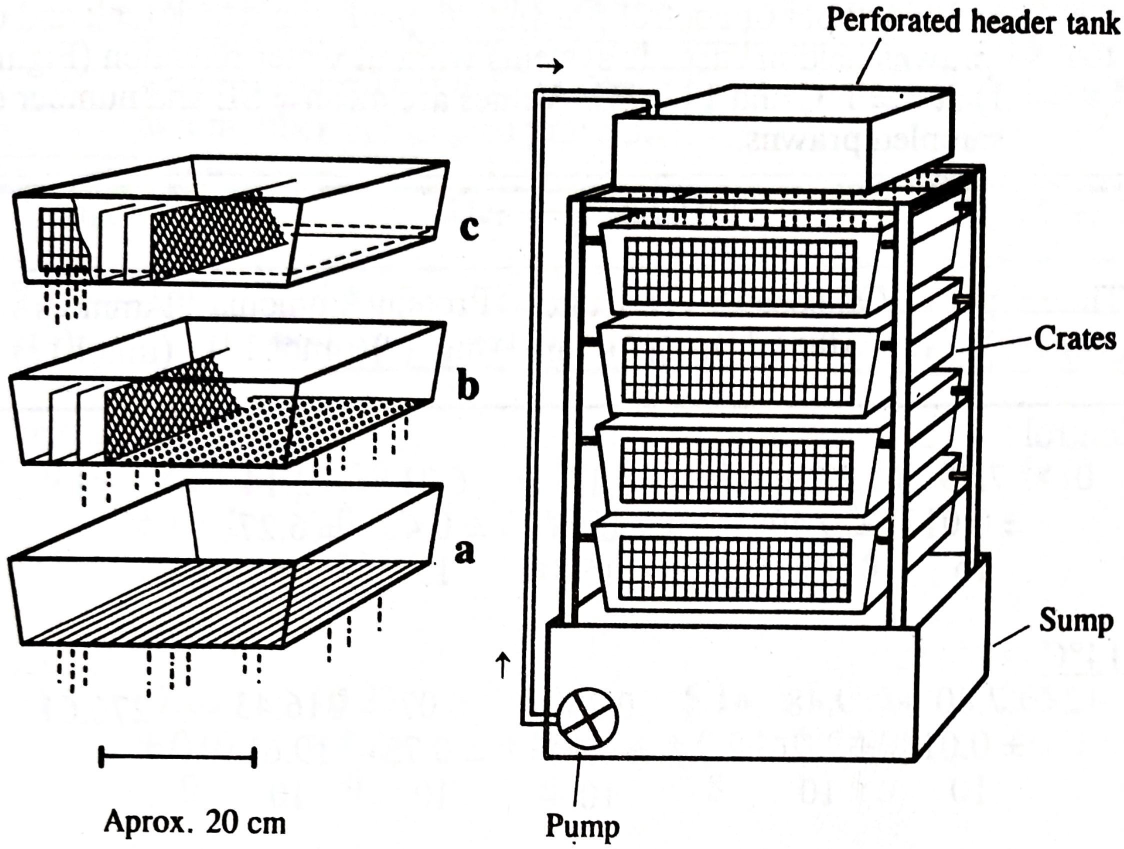

Figure 1. Nephrops norvegicus: Cascade and tray (32 x 18 cm, base area) system used to hold prawns during simulated transportation. a. Trays without water retention. b. Trays with water retention (ca. 2 mm, depth) and prawns individually separated. c. Trays with water retention (15 mm, depth) and prawns individually separated.

Table 1. Nephrops norvegicus: Changes to some of the blood constituents and blood pH of control prawns sampled from stock tank and of prawns held in cascade systems without water retention (Figure 1) at 6 ± 1°C and 14 ± °C. Values are mean ± SE and number of sampled prawns.

	Haemolymph					Water
Time (h)	pH	Glucose (mg 100 ml^{-1})	Lactate (mg 100 ml^{-1})	Protein (mg g)	Ammonia (µmol 1^{-1})	Ammonia (µmol 1^{-1})
Control						
0	7.79 ± 0.01 15	2.29 ± 0.40 15	0.17 ± 0.03 15	6.21 ± 0.40 15	82.14 ± 6.27 15	<1
14°C						
12	7.80 ± 0.01 10	9.48 ± 1.26 10	0.25 ± 0.04 10	6.07 ± 0.73 10	316.43 19.61 10	276.61
30	7.76 ± 0.03 10	9.80 ± 1.28 10	0.89 ± 0.38 10	6.47 ± 0.50 10	530.00 ± 26.49 10	467.26
56	7.76 ± 0.02	8.56 ± 2.79 9	0.37 ± 0.05 9	5.40 ± 0.44 9	750.00 ± 46.52 9	764.60
6°C						
12	7.73 ± 0.01 10	3.82 ± 0.88 10	0.23 ± 0.06 10	6.43 ± 0.53 10	117.86 ± 8.53 10	136.64
30	7.56 ± 0.02 10	20.05 ± 4.62 10	2.07 ± 0.97 10	6.50 ± 0.55 10	202.14 ± 10.32 10	263.64
56	7.59 ± 0.02 10	8.91 ± 2.41 10	0.89 ± 0.21 10	7.30 ± 0.53 10	277.14 ± 18.47 10	300.00

Table 2. Nephrops norvegicus: Changes to some of the blood constituents and blood pH of control prawns sampled from stock tank and of prawns held in cascade systems retaining a layer of 15 mm of water (Figure 1) at 6 ± 1°C and 14 ± °C. Values are mean ± SE and number of sampled prawns.

	Haemolymph					Water
Time (h)	pH	Glucose (mg 100 ml^{-1})	Lactate (mg 100 ml^{-1})	Protein (mg g^{-1})	Ammonia (µmol l^{-1})	Ammonia (µmol l^{-1})
Control						
0	7.79 ± 0.01 15	2.29 ± 0.40 15	0.17 ± 0.03 15	6.21 ± 0.40 15	82.14 ± 6.27 15	<1
14°C						
12	7.85 ± 0.02 9	10.23 ± 4.24 9	0.25 ± 0.04 9	5.18 ± 0.64 8	332.54 ± 14.09 9	265.55
30	7.72 ± 0.01 9	11.04 ± 2.45 9	0.45 ± 0.05 9	6.39 ± 0.60 9	486.51 ± 22.25 9	392.92
56	7.72 ± 0.02 10	11.78 ± 2.27 10	0.33 ± 0.06 10	5.01 ± 0.41 10	525.71 ± 23.82 10	419.47
6°C						
12	7.74 ± 0.01 10	5.53 ± 1.54 10	0.41 ± 0.05 10	5.99 ± 0.38 10	119.29 ± 7.30 10	72.73
30	7.74 ± 0.03 10	10.14 ± 1.25 10	0.32 ± 0.03 10	6.31 ± 0.45 10	108.21 ± 10.91 10	86.36
56	7.71 ± 0.03 10	11.72 ± 2.13 9	0.57 ± 0.12 10	5.95 ± 0.58 10	114.28 ± 10.32 10	113.36

Table 3. Nephrops norvegicus: Changes to some of the blood constituents and blood pH of control prawns sampled from stock tank and of prawns held in cascade systems (Figure 1) retaining a layer of ca. 2 mm of water at 6 ± 1°C and 14 ± °C. Values are mean ± SE and number of sampled prawns.

	Haemolymph					Water
Time (h)	pH	Glucose (mg 100 ml^{-1})	Lactate (mg 100 ml^{-1})	Protein (mg g^{-1})	Ammonia (μmol 1^{-1})	Ammonia (μmol 1^{-1})
Control						
0	7.79 ± 0.01 15	2.29 ± 0.40 15	0.17 ± 0.03 15	6.21 ± 0.40 15	82.14 ± 6.27 15	<1
14°C						
12	7.80 ±0.01 10	6.16 ± 1.13 10	0.25 ± 0.03 10	5.67 ± 0.43 10	326.43 ± 15.06 10	265.55
30	7.76 ± 0.03 10	10.33 ± 1.19 10	0.27 ± 0.04 9	5.20 ± 0.46 10	430.00 ± 24.21 10	392.92
56	7.70 ± 0.01 10	11.86 ± 2.25 10	0.31 ± 0.05 10	6.47 ± .84 10	527.14 ± 29.47 10	419.47
6°C						
12	7.70 ± 0.02 10	5.25 ± 1.00 10	0.38 ± 0.04 10	6.83 ± 0.50 10	109.29 ± 8.11 10	72.73
30	7.71 ±0.02 10	13.15 ± 4.51 10	0.23 ± 0.04 10	5.95 ± 0.68 10	113.57 ± 5.16 10	86.36
56	7.70 ± 0.02 10	13.20 ± 3.73 10	0.30 ±0.04 10	6.59 ± 0.60 10	117.86 ± 13.18 10	113.36

Table 4. Nephrops norvegicus: Alterations in some blood constituents, oxygen concentrations and pH of prawns held in a cascade system retaining a thin layer of water (ca. 2 mm) during 129 h at 6 ± 1.2°C. Values are mean ± SE.

	Haemolymph						Water
Time (h)	pH	Glucose (mg 100 ml^{-1})	CaO_2 (ml l^{-1})	CvO_2 (ml l^{-1})	Protein (mg g^{-1})	Ammonia (µmol l^{-1})	Ammonia (µmol l^{-1})
0	7.82 ± 0.01 10	3.14 ± 0.53 10	16.75 ± 1.10 10	11.02 ± 0.92 10	7.50 ± 0.52 10	200.23 ± 13.40 10	<1
13	7.79 ± 0.01 10	5.67 ± 0.90 10	15.84 ± 1.33 10	12.53 ± 1.17 10	7.36 ± 0.70 10	269.56 ± 18.74 10	299.69
29	7.82 ± 0.02 10	13.05 ± 3.65 10	16.49 ± 1.11 10	13.49 ± 1.28 10	7.83 ± 0.38 10	447.11 ± 12.78 10	398.51
53	7.75 ± 0.01 9	15.76 ± 4.34 10	13.96 ± 1.46 10	10.15 ± 1.18 10	6.17 ± 0.59 10	604.57 ± 18.73 10	450.35
129	7.83 ± 0.02 10	55.07 ± 7.83 10	14.31 ± 0.75 10	11.76 ± 0.54 10	6.97 ± 0.38 10	681.68 ± 31.79 10	479.51

Table 5. Nephrops norvegicus: Alterations in some blood constituents and pH during emersion inside a polystyrene box. Prawns were placed in between layers of seawater-soaked insulating wool and ambient temperature under such conditions was 2 ± 0.6°C. Values are mean ± SE.

Time (h)	pH	Ammonia ($\mu mol\ l^{-1}$)	Glucose ($mg\ 100\ ml^{-1}$)	Lactate ($mg\ 100\ ml^{-1}$)	Protein ($mg\ g^{-1}$)
0	7.82 ± 0.01 10	200.23 ± 13.40 10	3.14 ±0.53 10	0.241 ± 0.05 10	7.50 ± 0.52 10
1	7.60 ± 0.04 10	245.586 ± 23.51 10	11.64 ± 2.16 10	7.37 ± 1.75 10	7.74 ± 0.68 10
2	7.55 ± 0.03 10	272.80 ± 17.40 10	19.44 ± 1.93 10	7.01 ± 1.76 10	7.02 ± 0.48 10
4	7.55 ± 0.03 10	350.56 ± 34.62 10	19.81 ± 3.46 10	7.25 ± 3.11 10	7.23 ± 0.56 10
8	7.59 ± 0.02 10	251.59 ± 49.83 10	25.14 ± 4.37 10	8.34 ± 6.20 10	6.55 ± 0.55 10
12	7.58 ± 0.04 9	494.41 ± 88.45 10	33.69 ± 6.37 9	13.26 ± 5.36 10	8.80 ± 0.79 10

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