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Aspects of the physiology of some crustacean species with particular reference to their live marketing.

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by

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To Mercedes

Thankyou for hearing me
Thankyou for loving me
Thankyou for seeing me
and for not leaving me
Thankyou for staying with me
Thankyou for not hurting me
You are gentle with me
Thanks for silence with me
Thankyou for holding me
and saying J could be
Thankyou for saying baby
Thankyou for holding me
Thankyou for helping me
Thankyou for breaking my heart
Thankyou for tearing me apart
Now J've a strong strong heart
Thankyou for breaking my heart

Sinnead O'Connor, 1994

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Note. Most of the information concerning the holding and transport practices of live *Nephrops norvegicus*, *Cancer pagurus* and *Homarus gammarus* used in this text were obtained directly from catchers, dealers and personnel involved in the trade.

Contents.

Chapter 1.

General introduction.

The live transport of aquatic animals is a common event in general aquaculture practices and also in the specialized trade of live lobsters and crabs, where the animals are transported alive from the fishing areas to the main market places. Such activities may involve long distance road or air journeys and their success depends on the delivery of a good quality product.

The maintenance and transport of live aquatic animals for commercial purposes are, nonetheless, very old practices. Excavations at the Roman settlement of *Cosa* (established in 273 BC on the coast of southern Tuscany) revealed the existance of two large fish tanks that were probably used to raise fry before their release into the adjacent lagoon or to hold live fish ready for the market (McCann 1979, Cunliffe 1988). According to Cunliffe (1988), most of the catch was used to produce a cooking additive called *Garum*, but it seems that a small percentage of the live fish were transported to Rome in especially constructed boats equiped with vivier wells.

Most of the techniques and procedures used in live transport are derived from undesigned "trial & error" attempts, by the personnel involved, and also a more scientific approach evolved in parallel with the development of intensive systems of aquaculture. Studies on the maintenance of shrimps and prawns under intensive conditions, such as the recent works of Wyban and Sweeney (1989) and Hopkings *et al.* (1994), provide a better understanding of the biological implications of keeping such animals in captivity at very high densities. The live transport in aquaculture is an inevitable procedure, as it is used to move postlarvae or juveniles between larviculture facilities and grow out tanks and also when new broodstock is brought in from the wild or from different premises. Such journeys may take anything from a few hours up to 1 or 2 days and may involve either local delivery or long distance air freight. Even though they have become very common, published details on such techniques are still limitted.

Singholka (1982) described an insulated cool-truck system to transport fish and crustaceans that could be easily fitted on a lorry, boat or train and which proved to be successful during trials with *Macrobrachium rosenbergii* at local fish farms in Thailand. Postlarvae and berried females of *Macrobrachium* may also be transported in inflated plastic bags (125-250 postlarvae *per* litre) which are normally filled with water (1/3) and pure oxygen (New and Singholka 1982). The authors enphasise that the bags must be kept at low temperature inside polystyrene boxes and, to minimize the occurrence of molting during transport, they must be filled with water from the stock tank and not with new water. *Penaeus japonicus* may be packed in moist, cool sawdust and transported for periods up to 24 h, and this reduces considerably the costs of the operation, as the extra weight caused by water

in most other transport systems is avoided (Spotts 1983). Studies on shipping procedures have also been made during laboratory simulations, where the effects of shipment time on water quality and survival of *M. rosenbergii* (Smith and Wannamaker 1983) and *P. setiferus* and *P. vannamei* (Robertson *et al.* 1987) were evaluated under different packing conditions.

Whatever the system or technique used, it seems imperative that the activity and general metabolism of the animals are depressed at such times and thus the speed at which water quality deteriorates is also reduced. During the transport of fishes alive, such reductions in activity and metabolism may be effected by the addition of anaesthetics into the water, but this procedure has not yet been tested for crustaceans on a commercial scale. The procedures described above were designed/tested for tropical species and water temperature reductions as a means to reduce metabolism is a common factor among them. In fact, as shown by Goodrick *et al.* (1993) and Paterson (1993), temperature can effectively improve survival and reduce the detrimental effects of handling during live transport of *P. japonicus*.

Another practice which requires the animals to be transported live is the North American trade of the blue crab *Callinectes sapidus*, which is marketed at premium prices immediately after molt, when their shells are still soft. Otwell and Webb (1977) tested several designs of containers during simulated road-transport of emersed crabs at different temperatures and relative humidity levels. The authors found that the transport of blue crabs out of water could be made successfully (4.2 % mortality rate after 24 h) at 10 °C in open baskets covered with moist burlap (80-95 % relative humidity).

A completely different approach to live transport of aquatic animals is that of the live trade of wild-caught crustaceans in Europe, as such animals will not have to grow or reproduce successfully in captivity. Hence, the animals are invariably submitted to rather more stressing conditions than those animals transported for aquaculture purposes. Transport is made on a much larger scale (up to 10 tonnes *per* load) and the crabs and lobsters are handled intensively and usually are transferred into several different vivier systems, under variable water conditions, between capture and final delivery point. In the UK, the market for live product is very limited and most of the UK catch is sold in Spain, France and Italy, which are countries with a tradition in seafood cuisine. As a result, animals caught in UK waters may have to endure a long journey when road-based transport is used. The main species traded in such activities are: the crabs *Cancer pagurus*, *Necora puber*, *Maja squinado* and *Carcinus maenas*, the lobsters *Homarus gammarus* and *Panulirus argus* and the prawn, or norway lobster, *Nephrops norvegicus*.

Essentially, the whole chain of events comprises the catchers who will sell the product to a dealer, who will store and eventually ship the animals to a major importer or exporter. The animals may have to wait for varibale periods of time before they are sorted, packed and the consignment is dispatched by surface or air freight to the mainland. It is not uncommon for the continental importer to be only an intermediate dealer and the animals may have to endure a further journey across Europe after being sold abroad.

On the catching vessel, the onboard vivier tanks may comprise anything from simple, large, plastic boxes with a hose pipe flushing seawater through them, to large, especially-designed, vivier wells. The onshore vivier tanks used are usually concrete structures and may be either supplied with seawater from a nearby supply (open systems) or with recirculated seawater. These latter systems are normally provided with biofilters and protein skimmers (closed systems). The vivier tank systems used on the lorries are made of a series of aluminium or fibre-glass tanks which are provided with aeration but biological or mechanic filters are not used.

The different species involved have specific fishing seasons, catching techniques and biological and physiological needs and thus will require the use of differentiated transport procedures and hardware.

The fishing and live transport practices of *Necora puber* was described by MacMullen (1983a, 1983b), Whyman *et al.* (1985) and Hosie (1993). The main fishery grounds for *Necora* are along the west coast of the UK but the species also occurs off the coasts of France and Spain. The crabs are fished with especially-designed fishing traps or, alternatively, with the traps used to fish *Nephrops*. These traps are baited and left immersed for periods up to 48 h at depths of *ca* 10 m. As this species is relatively small (*cf Cancer* or *Maja*) and very agressive, it is common practice to pack them thightly inside wooden crates which are covered and tied. Normally, the crabs are packed (8-9 Kg *per* box, 75-100 animals) immediately after they are caught and they may remain inside such containers until the final delivery. The containers are held in vivier systems onshore and the crabs may be exported by means of dry transport in crates (road/ferry), vivier lorry (biomass to water ratio of 1:2) or vivier vessels.

Several aspects involving the live transport of *Cancer pagurus* were studied by MacMullen *et al.* (1986), Uglow *et al.* (1986) and Hosie (1993). *Cancer* fisheries occurs widely in European waters and use baited traps or creels. Immediately after capture, the crabs may be kept emersed (periodically sprayed with seawater) or placed inside onboard vivier wells (longer trips). To reduce fighting and damage among the animals in the viviers and also to improve the safety

of the personnel which handle them, the clamping action of both claws is impaired by cutting the tendon of the flexor muscle in the propodite segment (Figure 2.2). This is the French version of a procedure known as "Nicking" which is normally executed onboard, immediately after the crabs are taken from the traps. On land, the most common way of transporting *Cancer* is with lorry vivier tanks which are supplied with aeration and cooling systems. The crabs may then be stored in dealers' premises to wait for the arrival of other loads or for delivery arrangements to be made or they may be transferred directly from the vivier wells in the boat to the smaller vivier tanks of the lorries. The animals are usually not restrained during such journeys and the vivier tanks are not provided with internal divisions. Biomass to water ratios used to transport *Cancer* are *ca* 1:1 or less and, although the water temperature is controlled, it may be influenced adversely by the external air temperature. Temperatures may also be dictated by the importer and this creates problems with loads that are split between dealers.

The procedures used to transport the other species of crabs and lobsters cited above are similar to the ones described here for *Cancer* and *Necora*. An exception, however, must be made for the handling and transport of live *Nephrops* which is fished extensively in North European waters, particularly along the Scottish coast. The fishery uses creels or trawls (the latter inflicts more physical damage than the former). Most of the catch is processed into a variety of different products which are sold mainly to France, Spain and Italy (Anonymous 1988) . A small, and relatively new, trade in live *Nephrops* (*cf* that of lobsters and crabs) has been developed and the consignments are usually taken to mainland Europe by air-freight.

Prawns used in the live trade must be creel-caught and the general onboard procedures are described later in Chapter 4 of this work. The prawns are usually subjected to handling practices similar to those described above for the other species and this may involve variable periods of emersion and the maintenance of prawns for variable periods of time in vivier tanks. The animals are normally transported without water but kept moist inside polystyrene boxes. Under such conditions, *Nephrops* are invariably dying when delivered and are sold as a 'fresh product'. More recently, however, a new method to transport live prawns has been used by Scotthish dealers from Harris (Hebrides). The animals are placed individually inside a series of PVC tubes which are kept immersed in vivier tanks (Anonymous 1995). The prawns may be maintained in such systems from the time they are caught until final delivery and this reduces the handling of the animals. Such systems may also avoid or reduce the exposure to other detrimental factors, such as mutual interaction and emersion, as the prawns may then be transported inside

vivier lorries. The negative points, however, are that the hardware is costly and can only be used with *Nephrops*. Additionally, the transport costs of the consignment will also depend on the weight of the water in the vivier and the hardware and whether a net profit is always attained with the use of this technique is not yet known. A reliable and widely-accepted system to transport live *Nephrops* (that could be kept alive for several days after delivery), such as those used to transport lobsters and crabs, remains to be developed.

Whatever the type of transport (surface or air-freight), purpose (aquaculture or marketing-related) and species used, the problems faced by the animals are very similar. They may be summarised as: excessive handling; emersion and desiccation; increased activity and mutual interaction; increased ambient levels of nitrogenous products; low ambient levels of dissolved oxygen. For two main reasons, the improvement of a determined transport activity depends on a thorough knowledge of the biology and physiology of the species used. Firstly, because most of the deterioration of water quality in the viviers, ponds and small containers used during live transport are caused by the animals themselves, as a result of their metabolism. Secondly, because the procedures used during live transport may have negative effects or even totally impair some essential physiological mechanisms, such as nitrogen excretion and oxygen consumption. A brief review of these two aspects of crustacean metabolism is, therefore, made in the following paragraphs.

The nitrogen excretion of decapod crustaceans may include variable amounts of urea, uric acid, aminoacids and nitrate, depending on a variety of environmental and physiological factors, but usually more than half of it is excreted as ammonia (Parry 1960, Sharma 1968, Spaargaren 1985, Wood *et al.* 1986, Regnault 1987). Due to its small molecular size and high solubility, ammonia can easily cross most biological membranes (see Kleiner 1981 and Campbell 1991) and, under normal conditions, can be rapidly flushed from the body to the external environment.

The metabolic pathways by which ammonia is formed in crustaceans have been reviewed by Schoffeniels and Gilles (1970), Claybrook (1983) and Regnault (1987). Ammonia is produced as the end-product of aminoacid and nucleic-acid catabolism by direct oxidation or deamination and transamination reactions. Amino-transferases, which catalyze the transamination of most aminoacids, have been found to occur in some species and glutamate dehydrogenase (GDH) may be commonly found in several species of crustaceans. The equilibrium for the GDH reaction strongly favours glutamate formation rather than ammonia release (Claybrook 1983) but this rule should not be generalized, as similar GDH activity levels were observed for either direction of the reaction in *Crangon crangon* by Regnault and Batrel (1987) and GDh activity has been found to affect ammonia

efflux rates in crustaceans (Batrel and Regnault 1985, King *et al.* 1985). Additionally, ammonia may also be released by the deamination of glutamine, serine, asparagine and adenylate.

Ammonia excretion in aquatic crustaceans and nearly all water-breathing animals occurs mainly across the gills, where it is released continually (Kormanik and Cameron 1981, Regnault 1987). Blood ammonia may be released to the external environment by simple diffusion of NH_3 or NH_4^+ and also by active transport of NH_4^+ (Evans and Cameron 1986, Kormanik and Cameron 1981, Kleiner 1981). NH_3 is a lipophilic molecule and may diffuse easily across most lipid membranes, in the same way as CO_2 and O_2. The diffusion of the larger NH_4^+ molecule may take place in between branchial cells, through the same routes used by other cations (Kormanik and Cameron 1981).

There are several items of evidence showing the presence of an active mechanism to remove ammonia from the blood of crustaceans, but the most obvious one is the excretion of ammonia against a gradient, as shown by Towle (1976) in *Callinectes sapidus*. A sodium input in exchange for ammonia output has been shown in *Callinectes sapidus* (Pressley *et al.* 1981), *Eriocheir sinensis* (Pequeux and Gilles 1981) and *Macrobrachium rosenbergii* (Armstrong *et al.* 1981). In marine and brackish water species, such Na^+/NH_4^+ coupled transport occurs mostly in the posterior pair of gills, which may have a much thicker epithelium covered by a thinner cuticle and a large number of mitochondria and are more specialized for ionic exchange (*cf* the anterior gills) (Gilles and Pequeux 1985, Lucu 1990). This mechanism is probably responsible for the increases observed in the ammonia excretion rates of several species of decapods during exposure to a lowered salinity, such as those found by Haberfield *et al.* (1975), Mangum *et al.* (1976), Spaargaren (1982) and Quarmby (1985). Other mechanisms that may be involved in the active transport of NH_4^+ accross the gills have been put forward, such as the basolateral Na^+/NH_4^+ exchange (Evans and Cameron 1986), but the evidences are still speculative.

Several biotic and abiotic factors may influence the nitrogen efflux rates of crustaceans. The alterations observed may be qualitative or quantitative, but very rarely do the animals shift from ammoniotelism to a different type of nitrogen efflux. The effects of temperature on the nitrogen efflux rates may vary according to the species and the temperature range considered (Regnault 1987). A direct relation, such as that observed by Needham (1957) on *Carcinus maenas* and Gerhardt (1980) on *Penaeus indicus* appears to be the general rule. Quarmby (1985), however, found that the relation between ammonia efflux rates and temperature in *Pandalus platycerus* was related to the stage of development of the

prawns, as adult ones showed a direct relation and juveniles an inverse relation. The responses of *Astacus astacus* nitrogen efflux rates to temperature are affected by the composition of the diet given to the animals (Kristianssen and Hessen 1992).

Nitrogen effluxes may also suffer qualitative and quantitative alterations in response to changes in the ionic and osmotic conditions of the medium (Sharma 1966, 1968, Haberfield *et al.* 1975, Mangum *et al.* 1976, Spaargaren 1982, Spaargaren *et al.* 1982, Chen *et al.* 1994). A reduction in water pH may increase the ammonia output of the fresh water prawns *Procambarus clarki* and *P. fallax* (Mauro and Moore 1987). Another environmental factor that may affect ammonia efflux rates of crustaceans is the concentration of ambient ammonia. Ammonia may be highly toxic and may also inhibit the excretion of nitrogen by crustaceans, as shown in *Carcinus maenas* by Needham (1957), *Nephrops norvegicus* by Hosie *et al.* (1991), *Cancer irroratus* by Kormanik and Evans (1984) and *Penaeus monodon* and *P. chinensis* by Chen *et al.* (1993, 1994). The animals are usually able to maintain normal efflux rates over a certain range of external ammonia concentration, as shown by Regnault (1986), and the reductions in ammonia effluxes usually occur only after external ammonia concentrations increase above a critical level.

Ammonia effluxes may also follow cyclic patterns that may be nycthemeral (day/night) (Marangos *et al.* 1990) or may be related to the moult cycle (Stern and Cohen 1982). *Penaeus japonicus* showed a higher rate of ammonia excretion at night than during day periods and these findings were probably related to the higher activity shown by this species at night (Marangos 1990). Increased efflux rates due to increased activity levels may also be found following handling and/or exposure to a changed environment (Armstrong *et al.* 1981) and probably reflect a general increase in metabolism at such times. Ammonia effluxes are also highly affected by the nutritional status of the animals, particularly by the amount of protein present in the diet which tends to be directly related to the nitrogen efflux rates (Nelson *et al.* 1979, Regnault 1983, Hewitt and Irving 1990, Yang 1993).

During periods of emersion, the absence of water at the gill surface may impair most of the functions of the branchial epithelium and ammonia efflux is no exception. The increases in blood ammonia levels during emersion, which are usually accompanied by supranormal efflux rates following re-immersion (Wood *et al.* 1986, Couper 1993, Regnault 1994), are clear evidence of this.

The crustacean respiratory systems (ventilation and gas exchange and transport) have been reviewed by Wolvekamp and Waterman (1960) and McMahon and Wilkens (1993). Oxygen uptake and carbon dioxyde excretion occurs mostly accross the gills by simple diffusion, which is enhanced by the presence of the

respiratory pigments. The exchange rate of both gases may be described by the Fick diffusion equation:

$$\dot{M} = \frac{K \times A}{E} \times \Delta P$$

Where $\dot{M}$ is the weight-specific amount of gas that moves across the membrane, K is the diffusion constant, A is the area of the gas exchange, E is the thickness of the gas exchange surface and ΔP is the partial pressure gradient for the gas across the surface.

The morphology, interlamellar spacing and size of the crustacen gills may be related to the natural habitat (Gray 1957) and also to the activity level and ionorregulatory capability of the species studied and, in terms of efficiency, the gills of the more advanced decapods are comparable to those of fishes (McMahon and Wilkens 1983). Oxygen uptake through the gills of crustaceans depends on the following processes: ventilation of the branchial chamber, which may be regulated by the scaphognathite pumping rate and/or by the stroke volume of individual beats (see Burggren and McMahon 1983); perfusion of the gills; oxygen transport by the circulatory system, which may be efficiently performed by the open circulatory system of the higher crustaceans, in terms of perfusion and control mechanisms (see Mangum 1983, McMahon and Burnett 1990 for a review); oxydation of different substrates by the tissues (Chang and O'Connor 1983).

All the above processes may be affected by a series of environmental and physiological factors but, among those that may occur during live transport procedures, the most important factors are: emersion and desiccation; exposure to hypoxia; increased activity; temperature alterations.

The respiration and general gas exchange of decapod crustaceans is highly limited during emersion and, according to deFur (1988), these may be caused by hypoventilation in air, a limited diffusion capability by the branchial tissue, a limited perfusion of the gills or the collapse and desiccation of the gill lamellae due to aerial exposure. Intertidal species, however, may show variable degrees of adaptation for oxygen uptake during emersion periods that are mostly absent in the fully aquatic species (Cameron 1981, Johnson and Uglow 1985, McMahon and Burggren 1988, al Wassia 1989). Such adaptations may involve the development of structures and morphological changes to prevent the colapse of the gill lamellae out of water (Cameron 1981, Johnson and Uglow 1985).

Whatever the degree of adaptation to emersion shown by aquatic and intertidal species, such air-exposure periods, and the associated reduction in oxygen

uptake, usually cause several alterations in the blood biochemistry composition and acid-base balance (Stott 1932, Kleinholz and Little 1949, Kleinholtz *et al.* 1950, Cameron 1978, Truchot 1979, Vermeer 1987, Burnett 1988, deFur 1988, Tyler-Jones and Taylor 1988, Taylor and Whiteley 1989, Schmitt and Santos 1993a among several others). The most common response observed is a switch to anaerobic pathways of energy production which leads to an increased utilization of carbohydrate and production of lactate and may also cause blood acidosis.

Oxygen consumption rates ($\dot{M}O_2$) may be dependent or independent of water oxygen tensions (P_{wO_2}) and this usually depends on the temperature, physiological state of the animals and to the dissolved oxygen range involved (Bridges and Brand 1980, Herreid 1980), but it appears to be basically related to the degree of adaptation to hypoxia of the species considered (see Vernberg 1983 for a review).

A reduction in $\dot{M}O_2$ caused by hypoxia was found in *Callinectes sapidus* by Batterton and Cameron (1978). A direct relation between $\dot{M}O_2$ and P_{wO_2} was also found to occur in fed and 3-day starved *Carcinus maenas* exposed to P_{wO_2} ranging from normoxia to 35 % saturation. A similar depression of $\dot{M}O_2$ during hypoxia was found in *Scylla serrata* by Davenport and Wong (1987) and this species was not able to exctract oxygen from seawater at mean P_{wO_2} below 4.2 torr. Most species, however, are oxyregulators ($\dot{M}O_2$ independent of P_{wO_2}) over a certain degree of hypoxia. *Cancer pagurus* was able to maintain steady $\dot{M}O_2$ down to a P_{wO_2} of 60 torr, below which it declined (Bradford and Taylor 1982). Similar results were found in *Homarus americanus* (McMahon and Wilkens 1975) and the squat lobsters *Munida rugosa* and *M. sarsi* (Zainal *et al.* 1992).

According to the reviews by Herreid (1980) and McMahon and Wilkens (1983), most crustacean species show an increase in oxygen uptake with increased locomotor activity and general disturbances. At such times, an increase in oxygen uptake may occur as a result of elevated ventilation, which may be effected by an increase in the scaphognathite beat rate and/or in the stroke volume of each beat (see Wilkens *et al.* 1984) or by removing oxygen from the inside of the gas exchange surface (increased gill perfusion) thus increasing the pressure gradient and favouring diffusion (McMahon and Wilkens 1983). However, the factor which may cause all these alterations and induce increased $\dot{M}O_2$ is the increased metabolic activity and oxygen uptake by the tissues, which are normally associated with supranormal locomotory activity.

Activity levels and metabolism may be affected by water temperature (lower temperatures will promote a general metabolic arrest) and a direct relation between water temperature and $\dot{M}O_2$ has been found to occur in several species of

crustaceans (Newell 1969, Nelson *et al.* 1977, Taylor 1981, Armitage and Wall 1982, Dalla-Via 1987, Kurmaly *et al.* 1989, Zanders and Rodriguez 1992).

Most of the conditions and procedures that may be used during live transport practices are stressing and produce detrimental effects on the animals. In a commercial situation, it is difficult to provide ideal conditions for all animals used and the knowledge of some of the biological and physiological responses to the transport practices and procedures (such as the ones studied in this work) may be used to identify which conditions are critical and should be totally avoided. Such an aproach is consistent with the HACCP (hazard analysis critical control point) approach to quality system analysis, increasingly being used by the fish industry.

This work analysed some of the physiological responses of *Macrobrachium rosenbergii* to temperature alterations and also monitored some of the commercial practices used to hold and transport *Homarus gammarus* and *Cancer pagurus*. The main objective of the present study was, however, centered on the laboratory simulation of the procedures used during the trade of the prawns *Nephrops norvegicus*. This is a relatively new trade in the live marketing of crustaceans and very little is known about the effects that the current techniques can have on the physiology of this species. Additionally, and based on the results obtained, a prototype method to hold and transport live *Nephrops* was developed.

Chapter 2.

General materials and methods.

2.1. Collection and maintenance of the experimental animals.

2.1.1. *Macrobrachium rosenbergii* (deMan).

Prawns were supplied in two separate lots by the Department of Aquaculture, Stirling University. The first group was reared at Stirling and transported by car inside plastic bags half filled with 15-20 l of fresh water. These bags were placed inside polystyrene boxes which provided a good protection from transporting procedures. The second group was packed in a similar way but brought to Hull by train (Four Star express service). Despite the transportation differences, with car-transported prawns receiving more care and train-transported prawns suffering some waiting time at both railway stations, the total transporting time in both situations was around 7 h and all prawns arrived at Hull University in good condition - with 100 % survival.

On arrival, the bags were taken from the polystyrene boxes and allowed to float in the holding aquaria for 2-3 h, as the prawns acclimatised to the new temperatures. Prawns were then transfered to the aquaria which contained brackish water (1-1.5 psu, 26-27 °C) under a 12/12 h photoperiod. Small prawns were kept together and building bricks and plastic netting were provided to avoid their excessive interaction. To avoid canibalism, large prawns were kept individually separated inside round cages made of plastic netting (5 mm mesh size). Aeration and biological filters (external canister filters) were provided and all prawns were fed *ad libitum* with pellets (see Appendix 2.1 for composition details).

All prawns were kept in the system for at least 15 days before the start of any experiments. *M. rosenbergii* were rather more active at dark than under normal light at the aquaria. Whenever experiments involved 24 h measurements, the prawns were acclimated for 10 days under an all light (low intensity) photoperiod. This procedure was designed to avoid light/dark effects on the metabolical rates measured.

2.1.2. *Nephrops norvegicus* (L.).

Nephrops were creel-caught off the north-west coast of Scotland, near the island of Skye (57° 38' N, 5° 51' W), at depths ranging from 150 to 250 m. Immediately after the prawns were taken from the creels their claws were imobilised with rubber bands (see Figure 2.1a) and they were kept inside perforated plastic containers covered with seawater-soaked hessian. Periodically, generous amounts of seawater were poured over these containers, keeping the prawns under highly

moist conditions - as the ideal situation of having an onboard flow-through vivier was not available. The prawns were kept under these conditions for an average of 5 h. Once ashore, the prawns were transfered into cages and kept immersed overnight in the sea.

Nephrops were transported to Hull University by car using the following methods: 1) Using an aquarium system in which the water was pumped over activated charcoal and was supplied with aeration and ice packs (Figure 2.1b). Water temperature oscillated between 6 and 8 °C. 2) The prawns were placed inside plastic bags with sea water and ice packs which were placed in polystyrene boxes. Water temperatures under these conditions were initially 6 °C and increased to 7.4, 7.8 and 8.0 °C in the different boxes by the end of the journey. 3) prawns were wrapped individually with wet paper tissue and surrounded by ice packs. At the end of the journey, temperatures inbetween the wrapped prawns were 2.4, 3.1, 3.5 and 6.6 °C in the different boxes (mean = 3.9 °C).

Method (1) proved to be the least stressful for the prawns, as all survived and were very active when taken from the transporting container (mortality = 0 %). Mortality was 3 out of 95 with method (2) (mortality = 3.16 %) and 2 out of 205 with method (3) (mortality = 0.98 %) and these latter two procedures proved to be valuable alternatives for transporting *Nephrops* when storage space is restricted.

On arrival at Hull, the prawns were transferred to seawater aquaria (12 °C, 34 psu) supplied with aeration and biological filters. Small lengths of plastic tubing (ϕ = 60-70 mm) and a 20-30 mm thick layer of fine sand were placed at the bottom of each aquarium to simulate the natural substratum and to provide substitute burrows for the prawns. The aquarium system was kept under low intensity light inside temperature controlled rooms at 75 % relative humidity. The prawns were fed twice weekly *ad libitum* with mussel flesh until 48 h before the experiments.

2.1.3. *Cancer pagurus* (L.).

Cancer used for laboratory experiments were purchased from local fishermen and delivered inside polystyrene boxes supplied with wood wool and ice. The crabs were then nicked according to the French technique and housed under identical water conditions as used to keep *Nephrops*.

The French nicking procedure is executed by cutting the tendon of the mobile upper segment of both claws, impairing their clamping action (Figure 2.2). Nicking is widely used in commercial practice to avoid the crabs fighting and causing mutual damage infliction during their live marketing. This procedure is

executed onboard the fishing boat immediatly after the crabs are taken from the creels.

These crabs were used to simulate live transport situations under laboratory conditions, hence a shorter acclimation time of 3 days and the nicking procedure was applied to them. These aimed to keep the physiological status of experimental crabs as similar as possible to those transported commercially.

2.1.4. *Homarus gammarus* (L.).

All experiments and data collection using *Homarus* were performed at the dealer's premises at Lairg or on the Orkneys islands, Scotland. Lobsters were either kept in tanks with recirculating seawater (Lairg facilities) or in concrete tanks where seawater from nearby was pumped through (Orkneys facilities). These tanks are used to keep lobsters for periods ranging from a few days to several months.

2.2. Collection and storage of samples.

2.2.1. Water samples.

In the laboratory, water samples collected for dissolved ammonia and total nitrogen analysis were stored in polypropilene microcentrifuge tubes (Eppendorfs) and immediately placed in the freezer at -20 °C. These tubes were not completely filled with water to avoid unsealing during freezing. Water samples for dissolved oxygen measurements were collected with 50 or 100 µl gas tight syringes (Hamilton) and immediately analysed.

Whenever possible, samples collected during field trips were also kept frozen using a portable 12 V/mains freezer (Engel, MRFT-525D-63), otherwhise they were kept cool using ice packs.

2.2.2. Haemolymph samples.

Haemolymph samples from *Cancer* and *Homarus* were collected from the sinuses at the base of the walking legs (4th or 5th pair of Peraepod) *via* a puncture of the arthrodial membrane between the coxopodit and the body wall with a Pasteur pipette. This proved to be the quickest way of collecting haemolymph samples from these crustaceans. Samples from *Nephrops* (never more than 500 µl) were

collected using 1 ml syringes with hypodermic needles - as Pasteur pipettes would have caused a greater damage to the animal.

After collection and pH determination, blood samples were immediately divided into aliquots according to the analysis required. Samples for glucose and lactate analysis were mixed separately with a 6 % solution of ice-cold perchloric acid (1:1, v/v); samples for dissolved ammonia analysis were diluted with NaCl solution (9 g l^{-1}) (1:19, v/v); the remainder was used for haemocyanin and protein determinations. All aliquots were frozen at -20 °C and analysed within 15 days.

Because of time and space limitations during field work, it was not possible to process the samples as described in the previous paragraph. Such samples were kept in Eppendorf tubes and placed directly into the portable freezer and were subdivided into aliquots in the laboratory prior to the assays. As freezing facilities were not available during onboard experiments, haemolymph samples collected were then kept cool using ice packs and polystyrene insulation.

Samples (20 µl) of pre and of post-branchial haemolymph for O_2 determinations were both taken from *Nephrops* within a maximum of 10 sec of the animals being picked up. Such samples were collected using 50 or 100 µl gas-tight syringes (Hamilton) and their oxygen contents immediately analysed. Pre-branchial haemolymph was obtained from the sinuses of the walking legs as described for the samples collected for biochemical analysis. Post-branchial haemolymph was obtained from the cardiac sinus *via* a puncture of the membrane underneath the posterior part of the cephalotorax.

2.3. Total ammonia (TA) determinations.

Haemolymph and water TA (NH_4^+ plus NH_3) in water and haemolymph samples were measured using a flow injection/ gas diffusion technique adapted from Clinch et al. (1988) and Hunter and Uglow (1993a) (Figure 2.3). This technique consists of a carrier stream of base NaOH (0.1 M) which is separated from a bromothymol blue solution (BTB) (0.5 g l^{-1}) by a gas-permeable membrane (PTFE). The BTB solution is used as an indicator within the 6.5-7.5 pH range.

Samples (0.5 ml) are injected into the carrier stream and all TA is converted to NH_3, which diffuses across the membrane and reacts with the BTB solution. The resulting colour change is detected by a photo-cell and the potential difference produced registered on a Talbot Instruments (Servoscribe 1s RE 543.20) graphic recorder. Solutions with known concentrations of $(NH_4)_2SO_4$, within the range expected for the samples, are used as standards. These solutions are prepared

before every analysis from a stock solution of $(NH_4)_2SO_4$ (0.5 mM) using fresh water, seawater or NaCl solution (9 g l^{-1}), depending on the origin of the samples. The peak hights of samples and standards are measured and a linear regression equation is obtained from which the results are calculated.

When seawater samples were run through the system, there was a consistent reduction in sensitivity which was detected as a progressive decrease in peak height produced by the standard solutions. Under these situations, linear drift correction factors (Willason and Johnson, 1986) were calculated and the samples corrected accordingly. These correction factors are obtained with the decay in peak hight observed between two standard solutions separated by a set number of samples A percentual reduction factor is then calculated for each individual peak registered. According to Hunter and Uglow (1993a) and Willason and Johnson (1986), such decreases in sesitivity were attributed to the following factors: reduction in gas permeability as the membrane became saturated with water; action of hydroxide on the membrane; reaction of hydroxide and carbonate ions producing precipitated salts that reduce membrane permeability.

2.4. Total nitrogen (TN) determinations.

A modified micro Kjeldahl technique based on Spaargaren (1982) and Yang (1993) was used to measure the TN levels in freshwater samples during experiments with *M. rosenbergii*. The Kjeldahl technique is based on the conversion of all nitrogen present in the samples into ammonia.

Freshwater samples (1 ml) and standard solutions with known concentrations of $(NH_4)_2SO_4$ (1ml) were placed inside test tubes (150 x 8 mm) along with 0.1ml of a digesting solution. This digesting solution was prepared with 96 % H_2SO_4 (200 ml), K_2SO_4 (134 g), $HgSO_4$ (2 g) and made up to 1000 ml with double distilled water. Test tubes were sealed with Parafilm (Sigma Chem. Corp.) to minimize evaporation during the heating process and placed in aluminium heating blocks for 12 h at 120 °C. Samples and standards were subsequently cooled to room temperature and their TA measured as described in item 2.3.

To ensure that the pH of the samples and standards were within the BTB indicating range, they were mixed with 200 µl of NaOH solution (2 M) and imediately injected into the NaOH carrier stream (1 M).

2.5. TA and TN efflux rate determinations.

The animals were placed individually in containers with a known amount of water and the TA and/or TN efflux rates were determined as the difference between the values obtained for two consecutive sampling times. All containers used were thouroughly cleaned and rinsed with a 10 % HCl solution to minimize bacterial action on the nitrogen excreted by the animals. This procedure was of particular importance during the experiments with *M. rosenbergii*, as the high temperatures used in such experiments may favour bacterial growth.

The water volume inside the containers varied according to the experiment and species used. Small volumes restrain movements and cause excessive build up of nitrogenous waste products, very large volumes impair detection of TA and TN effluxes. In most cases, animal:water ratios of 1:30 to 1:50 (w/v) were used and, considering the factors cited above, appeared to be appropriate.

2.6. Glucose determinations.

Deproteinized haemolymph samples for glucose determinations were taken from the freezer and immediately centrifuged for 5 min at 10000 rpm, 7200 x g.

Glucose levels from the resulting supernatant were analysed using colourimetric glucose-oxidase kits from Sigma Chem. Corp. (cat. no. 510). Samples (50 µl) were diluted with distilled water (1:19) and 100 µl of this solution added to the colour reagent (1:10) containing glucose oxidase, peroxidase and o-diasine, as supplied in the kits. Dilutions from the standard solution (100 mg 100 ml^{-1}) supplied with the kit were used to make a calibration curve. Blank (double distilled water) and standards were mixed with perchloric acid (1:1) and treated identically to the samples.

Samples, blank and standards were incubated at 37 °C in a water bath for 30 min to develop colour. Final colour intensity is proportional to the glucose concentration in the samples and was read at 450 nm in a LKB-Biochrom (Ultrospec 4050) spectrophotometer. The whole procedure is based on the following enzymatic reactions:

$$\text{Glucose} + 2H_2O + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + 2H_2O_2$$

$$H_2O_2 + \text{o-dianisidine (colorless)} \xrightarrow{\text{Peroxidase}} \text{Oxidised o-dianisidine (brown)}$$

2.7. Lactate determination.

Samples were defrosted and centrifuged as described for glucose determinations. Lactate levels were determined using two different kits. Initially, UV kits from Boehringher Mannheim (cat. no. 149993) were used but were later substituted for colourimetric kits from Sigma Chem. Corp. (cat. no. 735). This change was made because the Sigma kits offered a simpler and quicker analytical procedure.

In the first method used, 14 µl of supernatant were placed in a reagent flask with 1400 µl of a solution containing carbonate buffer (0.5 mol l^{-1}, pH 10), L-glutamate (63 mmol l^{-1}) and NAD (4.6 mmol l^{-1}). This mixture was separated in two aliquots of 0.7 ml. Into one of them (blank) was added 14 µl of ammonium sulphate (3.2 mol l^{-1}) and, into the second was added 14 µl of enzyme solution containing LDH (≥ 1632 U ml^{-1}) and GDH (≥ 102 U ml^{-1}). After 10-15 min of incubation, the absorbances of the aliquots were read at 340 nm and the results were obtained as 147.3 x Δabsorbance. The reactions envolved in this procedure are described as follows:

$$\text{L-lactate} + \text{NAD}^+ \overset{\text{LDH}}{\Leftrightarrow} \text{pyruvate} + \text{NADH} + \text{H}^+$$

$$\text{pyruvate} + \text{L-glutamate} \overset{\text{GPT}}{\Leftrightarrow} \text{L-alanine} + \alpha\text{-oxoglutarate}$$

In the second procedure used, 10 µl of supernatant or blank or standard solutions were pipetted into 1ml of a reagent containing lactate oxydase (400 U l^{-1}), peroxidase (2400 U l^{-1}), chromogen precursors and buffer (pH 7.2). Perchloric acid was added to blank (distilled water) (1:1) and to standard solutions (40 mg 100 ml^{-1}) (1:1). Samples, blank and standards were incubated for 10 min at 25 °C to develop colour and their absorbances read at 540 nm.

2.8. Haemocyanin and protein determinations.

Haemolymph samples for haemocyanin concentration estimations were diluted with distilled water (1:49, v/v) and their absorbances measured directly in a 10mm quartz cuvette at 335 nm against a distilled water blank. The haemocyanin concentrations were calculated using an extinction coeficcient (EmM - 1 cm) of

17.26 calculated from E1% - 1 cm = 2.83 (Nickerson and van Holde, 1971) on the basis of a functional subunit of 74000 (Antonini and Brunori, 1974).

Total protein concentrations were determined with Biuret reagent from Boehringer Mannheim (cat. no. 124281). The method consists of the formation of a coloured complex with cupric ions in alkaline medium. Samples (20 µl) or standard solution (6 g 100 ml^{-1}) (20 µl) or a blank (distilled water) (20 µl) were added to the biuret reagent (1 ml) provided with the kits. The tubes were incubated for 30 min at 25 °C to develop colour and readings were taken at 546 nm.

2.9. Dissolved oxygen (C_{aO_2}, C_{vO_2} and P_{wO_2}) determinations.

Determinations were made using a modified colouximetric technique (Peck and Uglow, 1990) (Fig 2.4). The system is based on a galvanic cell (A-120, Chandos Intercontinental) with an outer carbonaceous layer as a positive electrode and a cadmium coated nickel core as a negative electrode. The cell is impregnated with aqueous KOH and has an oxygen-free nitrogen gas carrier stream passing over it at a flow rate of 45 cc min^{-1} (Fisher controls, 1100).

The nitrogen gas passes through two pyrogallol ($C_6H_3(OH)_3$) oxygen traps and subsequently through a desorber, into which the samples are injected. In the desorber the dissolved oxygen present in sample is displaced by the N_2 gas. When the displaced oxygen passes through the fuel cell, it is converted to hydroxyl ions while the cadmium is oxidised to the hydroxide - according to the following reaction:

$$\tfrac{1}{2}O_2 + H_2O + 2e^- \rightarrow 2OH^-$$
$$Cd + 2OH^- - 2e^- \rightarrow Cd(OH)_2$$

Each atom of oxygen releases two electrons, producing a current which is proportional to the mass of oxygen present in the sample. The current produced is transformed into a potential difference using a resistor connected in parallel with the poles and registered on a graphic recorder (Talbot Instruments, Servoscribe 1s RE 543.20).

The pyrogallol solution is made by adding 0.5 g to 20 ml of distilled water and stiring in a pellet of NaOH until a light golden color develops. This colour gets darker when pyrogallol reacts with oxygen and may be used as an indicator as to when it should be replaced. Setup was made by adding 5ml of the pyrogallol solution into each of the oxygen traps and the system left running for *ca* 4 h prior

the start of measurements. This is made to completely deoxygenate the system and yeld a straight and stable baseline output.

When sea water samples (20 µl) were to be analysed, double distilled water (2 ml) was used in the desorber. When the bound oxygen of respiratory pigment was required to be analysed, the desorbing solution was uranyl acetate which effectively cleared the tightly-bound oxygen from the pigment. In the latter case the sample (20 µl) represented bound + dissolved oxygen.

Standards comprised a sample (20 µl) 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 (Appendix 2.2). Peak hight of standard was dependant on the dissolved oxygen and was taken as 100 % - base line was taken as 0 % and samples of unknown concentrations were calculated proportionately.

2.10. Weight specific oxygen consumption ($\dot{M}O_2$) determinations.

$\dot{M}O_2$ measurements were made using the respirometers shown in Figure 2.5. Seawater was passed through the respirometers (8-10) and driven into a sump, where aeration was provided. The water was then pumped into a header tank and back to the respirometers. The $\dot{M}O_2$ was determined as the ΔP_{wO_2} of two consecutive samples, taken after both inlet and outlet valves were shut. Samples were taken through a subaseal mounted on the lid and imediately analysed. One of the respirometers was kept without animals during all experimental procedures, as a means to monitor eventual bacterial influence on P_{wO_2} levels.

This closed $\dot{M}O_2$ measurement system was used in all experiments, as it provided a mean of measuring $\dot{M}O_2$ and TA efflux rates simultaneously.

2.11. Heart (HR) and scaphognathite (SR) rate determinations.

Heart rates and scaphognathite rates were measured according to a modified impedance technique (Dyer and Uglow, 1977). This technique is based on the transmission of a signal between two electrodes, one (small) atached to the animal and the other (large) immersed in the aquarium. Any alteration of the impedance between the two electrodes and very close to the small one, will be detected, filtered and amplified and will be registered by the system. Electrodes attached to the prawns were made using silver plated copper solid wires with KYNAR™ insulation

(ϕ O.25 mm) (RS cat. no. 357-261). Approximately 1mm of these wires was stripped at the end attached to the animal and the end of the external electrode was atached to a larger conductor. This ensured that only movements near the heart or scaphognathites of the prawns were registered. The number of beats registered in a pre-defined time interval were recorded in a microcomputer. The software used was developed by the Department of Electronic Engineering and allowed HR and HR to be automatically measured.

The carapace of the animals was carefully dried with tissue paper and the electrodes were affixed with cyanoacrylate glue. One electrode was placed near the heart and each scaphognathite electrode was hooked over the margin of the cephalotorax so that the cut cross-sectional area of the electrode was positioned near the scaphognathite. The animals were allowed to recover from the effects of handling and affixing electrodes before experiments were performed. According to Cumberlidge and Uglow (1977), the effects of handling and ataching electrodes are elevated HR and SR that will last for a few hours before steady levels are achieved.

The detailed procedures used in experiments with *Macrobrachium* and *Nephrops* are described in the following two sections:

2.11.1. HR and SR determinations in *Nephrops norvegicus*.

Electrodes for HR measurements were placed through a small hole (ϕ = 0.3 mm) drilled through the anterior part of the prawns cephalothorax and, for SR measurements, as described in Figure 2.6a. The prawns were then placed inside the respirometers (Figure 2.5) and the electrode wires passed through the subaseal on the lid. The prawns were left to settle in the system for a minimum of 48 h before any experiment was performed.

2.11.2. HR and SR determinations in *Macrobrachium rosenbergii*.

Measurements were initially attempted with the prawns in 1.5 ‰ S water but the signal obtained was of a very poor quality. Increasing the conductivity by adding seawater considerably improved the signal and all measurements were then made in 5 psu medium.

Heart electrodes were fixed on the posterior end of the cephalotorax and the scaphognathites electrodes as shown in Figure 2.6b. After the electrodes were attached, the prawns were placed in the experimental aquaria and allowed to settle for at least 72 h before any experiment was performed.

a

b

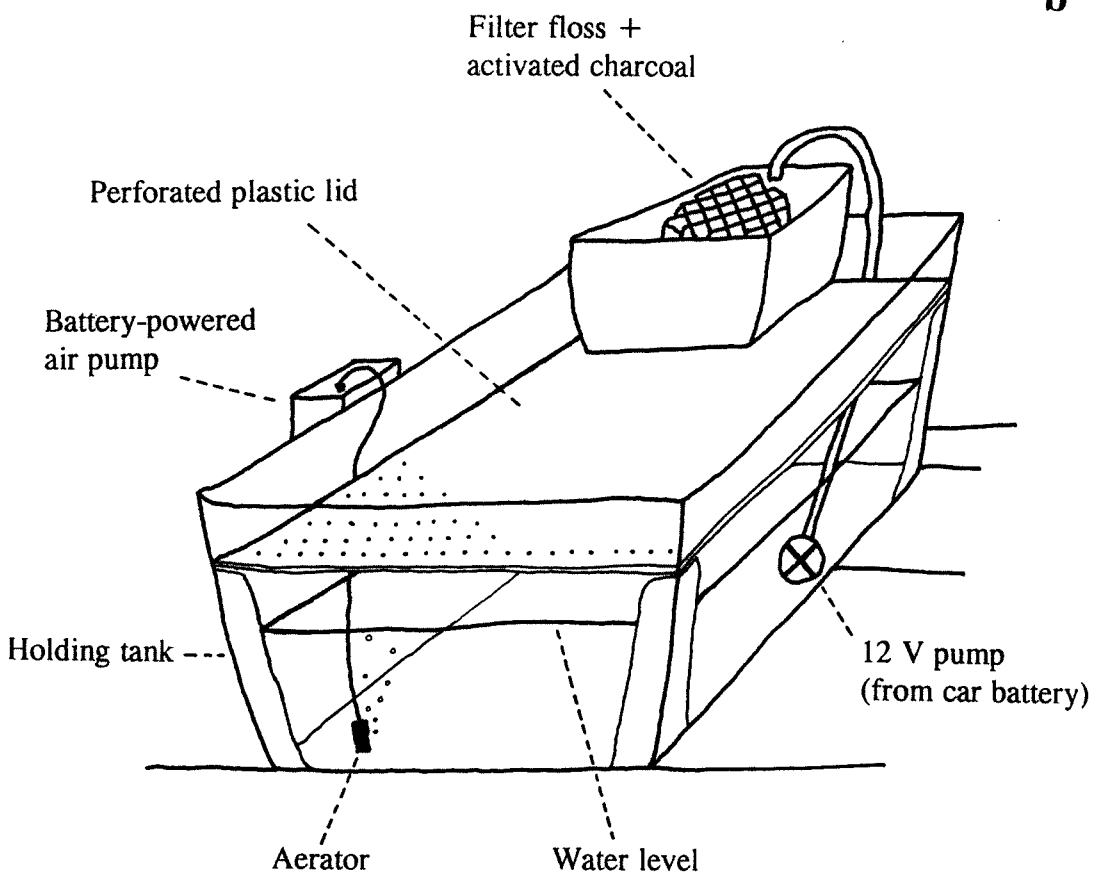


Figure 2.1. **a**- Claw from *Nephrops norvegicus* immobilized with rubber band to avoid fighting during transport.
b- Holding system used to transport *Nephrops norvegicus* in a car. Volume = 70 l.

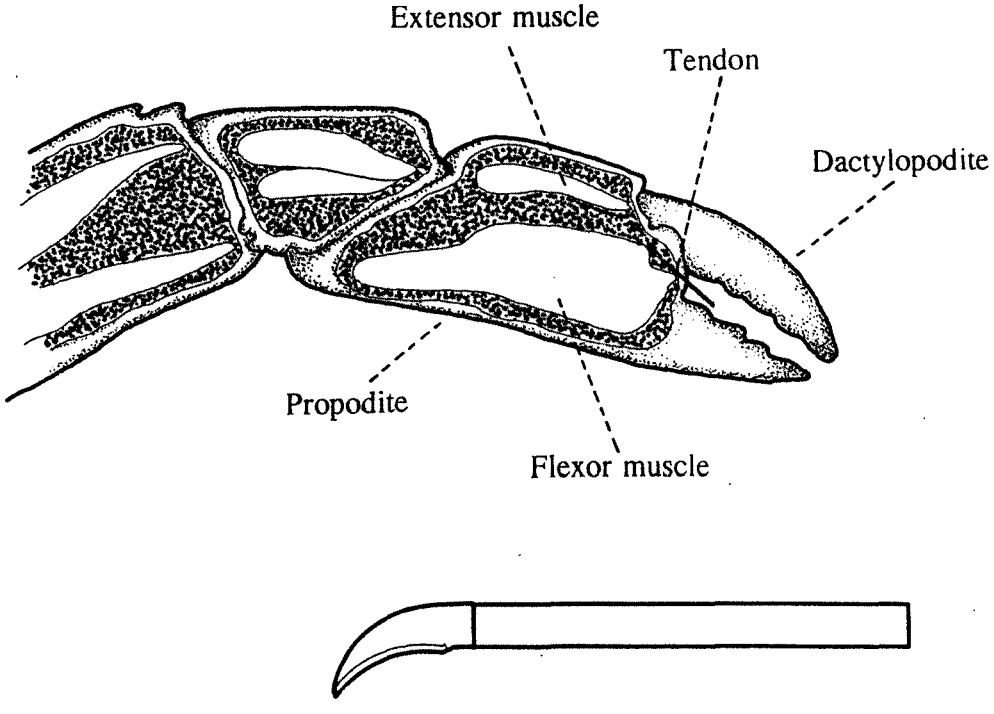


Figure 2.2. French nicking procedure used to impair the clamping action of the claws. The tendon of the flexor muscle of the dactylopodite is cut with a curved knife. Figure modified from Pearson (1908).

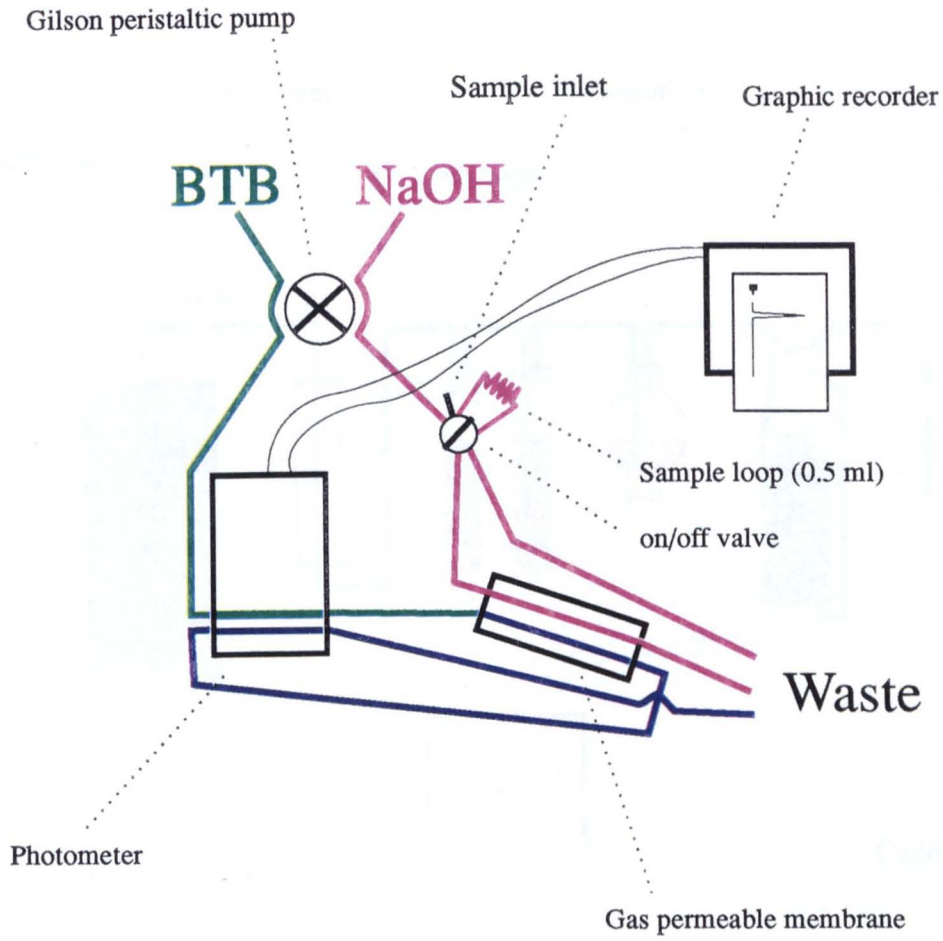


Figure 2.3. Flow-injection system used for dissolved ammonia determinations in water and haemolymph samples.

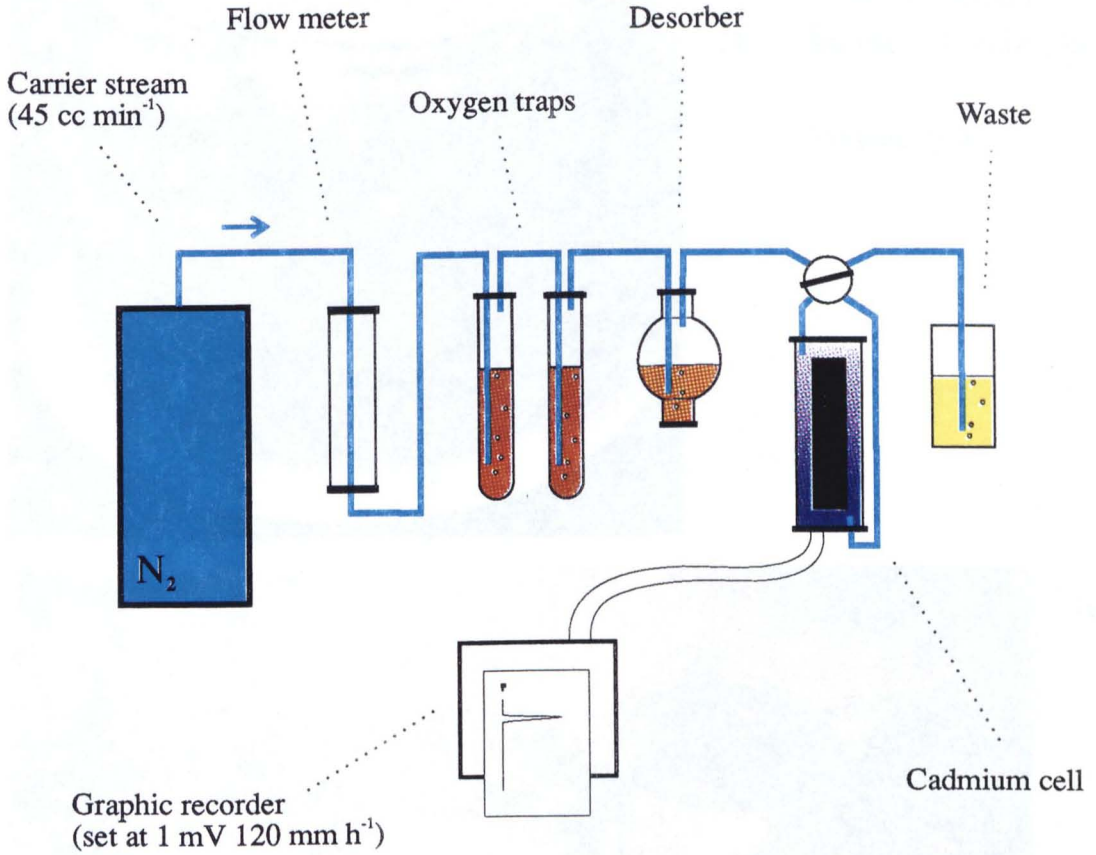
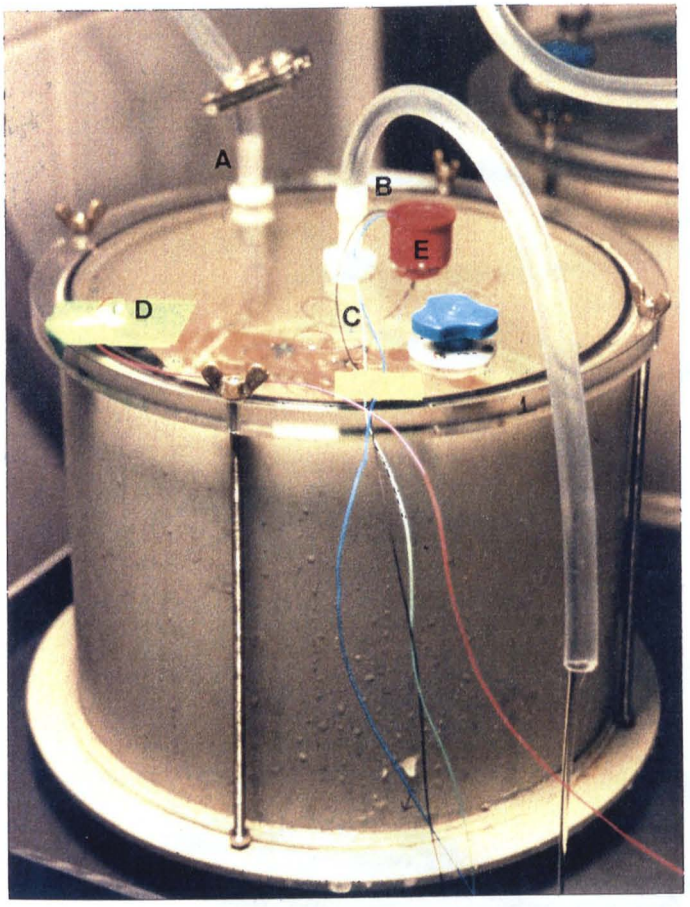


Figure 2.4. Colouximetric technique used for dissolved oxygen determinations.



a

A- Water inlet.
B- Water outlet.
C- Internal electrodes.
D- External electrode.
E- Sample collection place.

Volume = 4l.

b

Figure 2.5. Oxygen comsumption measurements.
a. Detailed view of respirometer.
b. System setup.

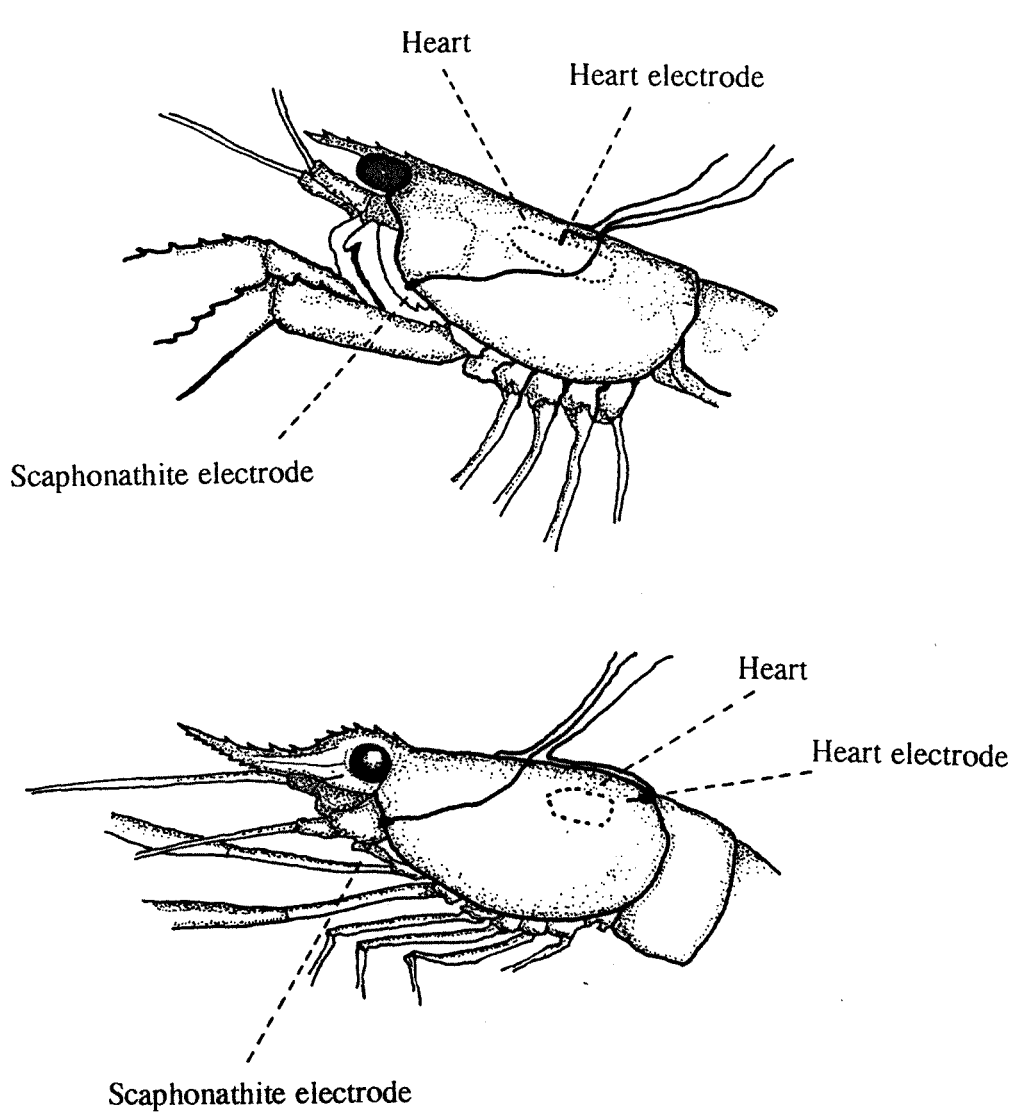


Figure 2.6. Attached electrodes for measurements of heart and scaphognathite rates.
a. *Nephrops norvegicus*.
b. *Macrobrachium rosenbergii*.

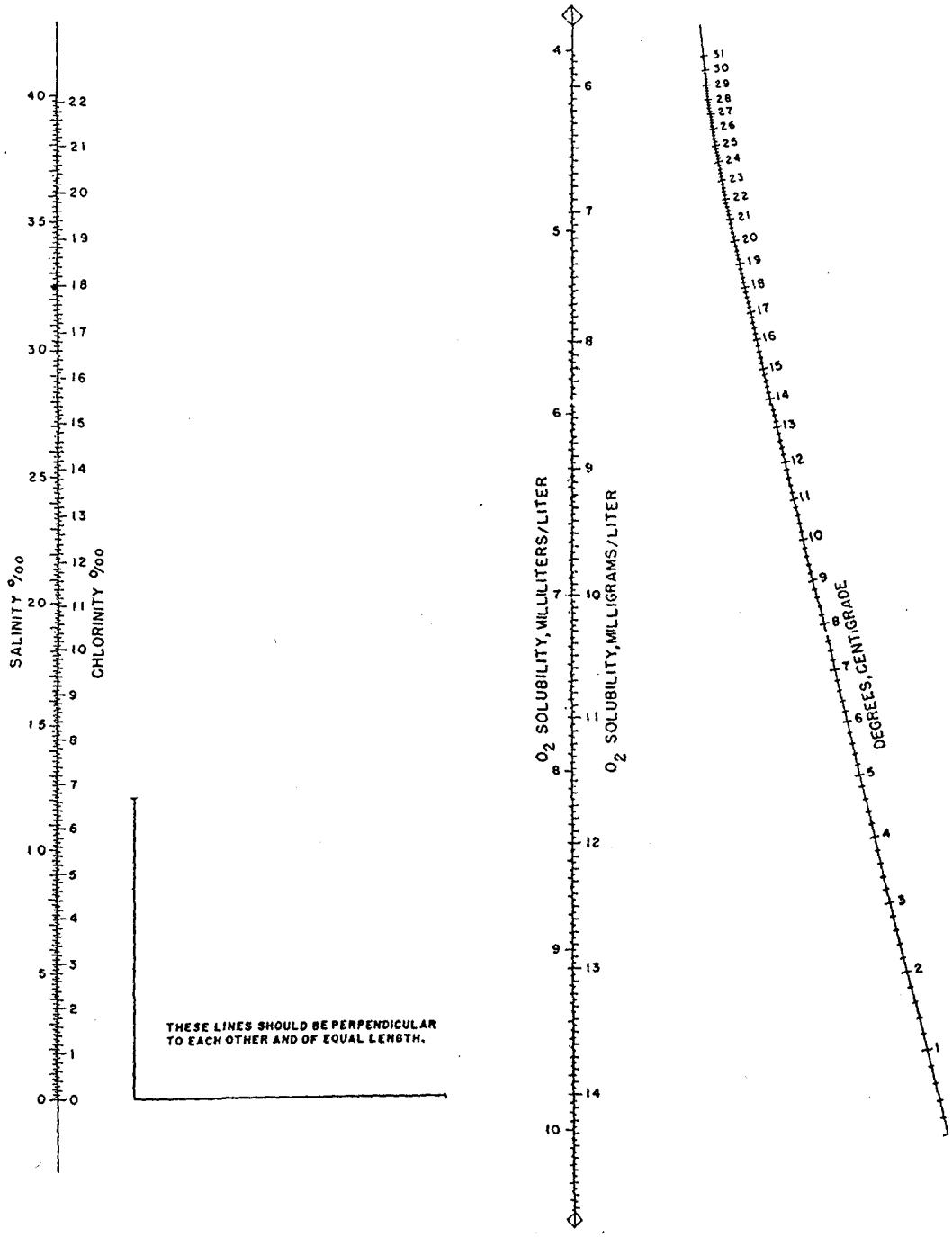
Diet ingredients	% dry weight
herring meal	17.0
dried bakers yeast	3.0
wheat meal	15.0
soya meal	20.0
rice bran	26.0
gelatin	4.0
agar	7.0
cod liver oil	2.5
vitamin premix	2.0
mineral premix	2.0
"fin stimm"	1.5

Vitamin premix composition	g/100g
Thiamine-HCl	0.1500
Riboflavin	0.5000
Pyroxidine-HCl	0.1500
Niacinamide	2.0000
Pantothenic acid	0.7500
Inositol	10.0000
Biotin	0.0150
Folic acid	0.0375
P-aminobenzoic acid	1.0000
Ascorbic acid	25.0000
Menadione (K3)	0.1000
β-carotene	0.1000
Cholecalciferol	0.0150
Cyanobalamine (B12)	0.0010
Choline chloride	20.0000
a-tocopherol acetate	1.0000
a-cellulose powder	39.1815

Mineral premix composition	g/100g
$CaHPO_4$	72.7760
$MgSO_4$	12.7500
NaCl	6.0000
KCl	5.0000
$FeSO_4$	2.5000
$ZnSO_4$	0.5550
$CuSO_4$	0.0785
$MnSO_4$	0.2538
$CoSO_4$	0.0478
$CaIO_3$	0.0295
$CrCl_3$	0.0128

Proximate analysis	% wet weight
Protein	37.8
Carbohydrate	33.5
Lipid	4.6
Crude fibre	4.4
Moisture	9.7
Ash	10.0

Appendix 2.1. Composition of *Macrobrachium rosenbergii* maintenance diet used in the experiments.



Appendix 2.2. Nomogram used to obtain the dissolved oxygen concentrations of standards used in P_{a0_2}, P_{v0_2} and P_{w0_2} determinations.

Chapter 3.

Effects of temperature change rate on nitrogen effluxes and heart and scaphognathite rates of *Macrobrachium rosenbergii* (DeMan).

3.1. Introduction

The bulk transportation of living post-larvae, juveniles or adults of various crustacean species is a commonplace aquaculture and marketing practice. Tropical prawns (*Macrobrachium* sp.) and shrimps (marine species) are transported on ice or in water in open containers for local deliveries but longer journeys usually require that they be in sealed plastic bags of fully-oxygenated water (usually packed within polystyrene boxes). The efficacy of such procedures is usually constrained by the inevitable reduction of dissolved oxygen levels and increases in dissolved carbon dioxide and ammonia in the shipping water, due to metabolism of the animals. Ammonia concentrations, developed during simulated transport experiments using *Macrobrachium rosenbergii* (Smith and Wannamaker,1983) and *Penaeus setiferus* (Robertson *et. al.*, 1987), reached 1000 μmol l^{-1} and 504 μmol l^{-1} respectively.

Supranormal dissolved ammonia levels have been widely reported to cause increased mortality and impaired growth rates in crustaceans (Wickins, 1976; Armstrong *et al.*, 1978; Provenzano, 1983; Chen and Lin, 1991; Chen and Lin,1992; Lin *et al.*, 1993). According to Campbell (1991), the general effect of ammonia is due to changes in pH that affect enzyme activity and, in most cases, toxicity is due to a combination of effects.

Lowering water temperature, to reduce animal activity and metabolism, is a technique used frequently for the live shipment of crustaceans (Singholka, 1982; Smith and Wannamaker, 1983; Robertson *et al*, 1987; Paterson, 1993). Paterson (1993) found that handling procedures enhanced oxygen consumption in *Penaeus japonicus* at 17 and 22°C but not at 12°C. This finding show that cooling may be an effective way to minimise handling effects on shrimps during packing. Water temperature changes are commonplace during the transport of live crustaceans but little is known of how such changes affect the nitrogen effluxes of these animals.

Temperature affects the heart and scaphognathite beat rates of crustaceans and such changes are normally associated with alterations in the transport of O_2 and CO_2 across the branchial tissue (Cameron and Mangum 1983 for a review). The excretion of ammonia also occurs mainly *via* the gills and a relation between ammonia efflux rates and cardio-ventilatory activities may exist. Furthermore, cardiac frequencies may be used as a means of measuring the metabolic activity and physiological adjustments of crustaceans to temperature alterations (Spaargaren and Achituv 1977, Burton et al. 1980).

This work deals with the nitrogen efflux of *Macrobrachium rosenbergii* when the animals are subjected to modest (4°C) temperature changes and also

evaluates the effects of gradual and sudden temperature alterations on the heart and scaphognathite rates of this species.

3.2. Materials and Methods.

Because temperatures around 18°C occur frequently during normal transport practices of *Macrobrachium* (Smith and Wannamaker, 1983), water temperatures in the present study were a minimum of 18 °C and, unless stated differently, did not exceed 26 °C. Our preliminary tests at 16°C indicated that the animals became inactive and were unable to maintain an upright posture at this temperature. New (1990) reported that temperatures below 14°C are generally lethal to *Macrobrachium.*

3.2.1. Total ammonia and total nitrogen efflux experiments.

Total ammonia (TA) and total nitrogen (TN) efflux rates were calculated based on the differences in concentrations between two consecutive samples that were separated by 6-7 h. Each animal was kept in a plastic container supplied with 800 ml of water sampled from the stock tank and all containers were kept in a water bath to minimize any undesigned temperature alteration. In all cases, the animals were allowed to settle for 2 h before the first water sample was taken. Three experiments were made without handling the animals or taking them from the water - thus eliminating these possible sources of sampling error. In all experiments, water temperature was taken from 26 °C → 22 °C → 18 °C → 22 °C → 26 °C.

Sudden (60 sec) temperature changes were made using a header tank that was connected with rubber tubing to the individual animal containers (Figure 3.1). New temperatures in these containers were achieved by flushing them with water from the header tank. Manipulation of appropriate valves (one controlling the tube from the header tank and another attached to each tube connected to the bottom of each individual container) allowed the rate of exchange and the volume in each container to be adjusted with precision. More gradual (5 h) changes were made by removing the small individual containers and the water bath to a temperature-controlled room at the required temperature. To avoid excessive ammonia build-up after new temperatures were achieved, the containers were flushed with clean water using the same header tank and tubing used in the previous experiment. Slow temperature changes (12 h), followed by a 72 h acclimation period, were made by manipulating the temperature of the room where the stock tank (*ca* 120 l) was kept. In this latter experiment, animals were starved for 24 h before water samples were collected. The animals were not kept all the time in the individual containers as the previous experiments but were transfered to them before every measurement of

efflux rate. All animals were again left to acclimated to these containers for two hours before the first sample was taken.

The effects of handling stress on *Macrobrachium* nitrogen efflux were examined either by handling the animals for *ca* 10 sec in one experiment or handling and emersing them for 6 min in another experiment. In each case, the ammonia efflux was monitored for the following 6h.

3.2.2. Heart (HR) and scaphognathite rates (SR) experiments.

All experiments were performed in a system consisting of stock tank (80 x 120 cm, bottom area) a header tank and an external water pump connected to a biofilter. Water was pumped from the stock tank into the header tank and, by gravity, taken back to the stock tank *via* a series of small tubes (Figure 3.2). The use of small tubes minimised water disturbances that could interfere with the HR and SR of the animals. After the electrodes were attached (see general material and methods), the animals were transfered to the stock tank and kept apart using plastic netting (Figure 3.2). Room temperature was maintained at 16 °C throughout and water temperatures were controlled using a thermostat placed in the header tank.

Sudden temperatures changes (2 min) were made by replacing the water of the header tank whilst the outlet valve was closed and then flushing it through the stock tank. The effects of the experimental procedures on the HR and SR of the prawns were evaluated by changing the water but without altering water temperature. HR and SR were registered during 8 min before and during immediately after temperature was altered and then 30, 60 ,120, 240 and 360 min after that. The system used to register HR and SR did not allow the measurement of more than one animal simultaneously and the 8 min interval used was composed of several small intervals (15 sec) alternating from prawn to prawn which resulted in a total measuring time for each prawn of 1 min. Two different experiments were made with sudden temperature alterations. In the first, water temperature was taken from 26 °C → 22 °C → 18 °C → 22 °C → 26 °C and in the second, water temperature was altered from 26 °C → 18 °C → 26 °C.

Gradual temperature alterations were made in the same system of tanks and were performed by switching the thermostat from 27 °C down to 18 °C and allowing water temperature to decrease naturally. HR, SR and water temperature were registered every hour. Twenty four hours after the start of the experiment, the thermostat was again switched to 27 °C and the procedure repeated. HR and SR of animals initially acclimated to 26 °C and then following 72 h of acclimation to 22 ° C and 18 °C were also measured .

3.3. Results.

3.3.1. TA and TN efflux rates.

During sudden temperature changes (Figure 3.3) initial TA and TN mean efflux rates were 0.25 ± 0.05 (SE) and 0.33 ± 0.5 (SE) μmol g^{-1} TA h^{-1}, respectively. They were not significantly changed ($P > 0.05$) when temperature was quickly reduced from 26°C to 18°C. However, when temperature was quickly brought back to 26°C, TA and TN efflux rates rose to *ca.* 3 x initial levels ($P < 0.05$). A completely different pattern was observed when gradual temperature alterations were imposed on the animals (Figure 3.4). TA and TN efflux rates decreased significantly ($P < 0.05$) from 0.28 ± 0.02 (SE) and 0.40 ± 0.07 (SE) μ mol g^{-1} TA h^{-1} at 26°C to 0.12 ± 0.02 (SE) and 0.20 ± 0.05 (SE) μmol g^{-1} TA h^{-1} at 18°C. Neither TA or TN were significantly different from initial values when 26° C was regained ($P > 0.05$). Initial TA and TN efflux rates of acclimated prawns (72 h) (Figure 3.5) were 0.69 ± 0.07 (SE) and 0.78 ± 0.09 (SE) μmol g^{-1} TA h^{-1}, levels which were significantly higher ($P < 0.05$) than the final rates. No significant differences ($P > 0.05$) were observed in TA and TN efflux rates at 26°C (initial levels), 22°C and 18°C. Q_{10} values for all experiments are shown in Table 3.1.

The calculated proportions of TA in the TN efflux rates (TA:TN ratio) at different temperatures are shown in Figure 3.6. During sudden changes, the TA:TN ratio at 22°C and 26°C (when temperature was increased) are significantly higher ($P < 0.05$) than those at the initial 26°C and 22°C intervals (ie. when temperature dropped). A trend of increased ratio of TA:TN as temperatures altered was found. During gradual temperature alterations, no significant difference ($P > 0.05$) in the TA:TN ratio was found, but a non-significant trend, similar to the one observed during sudden changes, was found. The TA:TN ratio of acclimated animals was not affected significantly ($P > 0.05$) by the temperature changes.

The TA efflux rate of *M. rosenbergii* during the first 30 min after handling (Figure 3.7) was higher ($P < 0.05$) than all subsequent rates. Similar results were found after the animals were handled and emersed for 6 min (Figure 3.7) ($P < 0.05$).

3.3.2. HR and SR.

The procedure used to alter temperature by changing the water did not interfere with the HR and SR of the animals, as such rates were not significantly changed ($P > 0.05$) following water replacement at the same temperature (Fig 3.8).

HR and SR during sudden temperature alterations from 26 °C → 22 °C → 18 °C → 22 °C → 26 °C are shown in Table 3.2. and Figure 3.9. HR decreased when the animals were exposed to 22 °C, as the values for the last two intervals at this temperature are significantly lower ($P<0.05$) than those observed before the change. SR values were variable and the differences measured during this interval were not found to be significantly different ($P>0.05$). When water temperature was taken to 18 °C, HR showed a further significant reduction ($P<0.05$) towards the end of this temperature interval and a significant but transient increase ($P<0.05$) of SR occurred immediately after temperature was altered. When the temperature was increased from 18 °C to 22 °C, an immediate increase in HR occurred ($P<0.05$) but SR remained relatively stable ($P>0.05$). When water temperature was brought back to 26 °C, HR and SR recovered initial values ($P>0.05$).

HR and SR values during sudden temperature alterations from 26 °C → 18 ° C → 26 °C are shown in Figure 3.10. Initial mean HR value was 139.74 ± 8.44 beats min^{-1} and decreased significantly ($P<0.05$) to 94.85 ± 5.89 beats min^{-1} immediately after the tempeerature change. HR then decreased significantly ($P<0.05$) once again, but more slowly, to a minimum of 64.34 ± 7.20 beats min^{-1} towards the end of this interval. Initial SR was 77.07 ± 13.67 beats min^{-1} and was not significantly altered ($P>0.05$) at 18 °C. When water temperature was then brought back to 26 °C, HR immediately increased significantly to 185.06 ± 16.62 beats min^{-1} and showed no significant ($P>0.05$) deviation from this rate during the remainder of the experiment. None of the mean HR values measured during this temperature interval were significantly different from initial values at 26 °C ($P>0.05$). When the temperature increased, SR immediately increased significantly from 48.47 ± 4.65 to 111.28 ± 15.27 beats min^{-1} but had then decreased significantly ($P<0.05$) to 67.56 ± 7.85 beats min^{-1} at the end of the experiment. The maximum value observed during this final 26 °C interval was 131.69 ± 14.28 beats min^{-1} which was significantly higher ($P<0.05$) than the initial value at 26 °C but the final SR value was not significantly different from the original 26 °C.

HR and SR during progressive (slow) reductions and increases in water temperature are shown in Figures 3.11 and 3.12. Initial HR was 159.31 ± 10.16 beats min^{-1} and decreased significantly (*ca* 55 %) to 71.35 beats min^{-1} ($P<0.05$) when the water temperature decreased from 27 °C to 18 °C. SR also showed a tendency to decrease during water cooling but the final rate measured was not significantly different from the original value ($P>0.05$). When the water was slowly heated back to 27 °C, HR increased again and reached initial levels

($P > 0.05$) after 5 h. SR again was not significantly altered ($P > 0.05$) but it showed an initial trend of rate increase.

When prawns were acclimated for at least 72 h to 18, 22 and 26 °C, a direct relationship between HR and water temperature was found and the values found at each temperature were significantly different from each other ($P < 0.05$) (Figure 3.13). SR values found at 18 °C and 22 °C were not significantly different ($P > 0.05$) but SR at 18 °C was significantly lower ($P < 0.05$) than the value for prawns acclimated to 26 °C. Q_{10} values calculated for HR and SR between 18 and 26 °C were 2.52 and 1.89, respectively.

3.4. Discussion

The effects of temperature on ammonia efflux rates are variable and depend on the species and temperature range (Regnault, 1987) and may vary with stage of development, as shown in *Pandalus platyceros* (Quarmby, 1985). A direct relationship between temperature (6°C and 18°C) and nitrogen efflux has been found in shore crab *Carcinus maenas* (Needham, 1957) and a similar result was obtained with the shrimp *Penaeus indicus* that was 48 h - acclimated to an increased temperature range (16 to 28°C) (Gerhardt, 1980). The responses of nitrogen efflux rates to temperature may also be influenced by diet and nutritional level, as found in *Astacus astacus* (Kristiansen and Hessen, 1992) and *Necora puber* (Couper 1993).

Undesigned effects of handling, aerial exposure and introduction to new aquaria were all avoided during temperature changes and the pattern variability found in the present studies was clearly related to the temporal differences in the temperature alterations.

M. rosenbergii showed two distinct responses to sudden temperature change; the one represented by very low Q_{10} values and the other by very high ones (Table 3.1). Hazel and Prosser (1974), reported that Q_{10} values considerably lower than 2-3, may signify instantaneous temperature compensation. Low Q_{10} values may also occur within the normal thermal regime of the animals' natural habitat (Vernberg and Vernberg, 1970). However, the very high Q_{10} values found when temperatures were increased suddenly, suggest that rapid compensation was not the case here.

Couper (1993) found that sudden reductions in water temperature (from 10 °C to 5 °C in 30 min) caused an initial increase in the ammonia efflux rates of *Necora puber*, before the rates decreased to levels lower than those of control animals (10 °C). In the present experiments, the pattern found during sudden cooling and warming could be reflecting a shock response of *Macrobrachium* to rapid temperature alterations. Increased ammonia effluxes were also found when *Macrobrachium rosenbergii* was subjected to other stressing situations. Armstrong *et al.* (1981) obtained extremely high ammonia efflux rates during the first hour after *M. rosenbergii* was caught with a net, handled and exposed to an alien environment (*cf* the levels found after a few hours of acclimation). Here, the data obtained after handling the animals and/or exposing them to brief periods of aerial exposure (Figure 3.7) show such treatments also evoke increased ammonia efflux rates.

Increased activity levels may be a shock response also and may evoke increased metabolism and nitrogen efflux rates. Visual observations made during the experiments revealed that sudden temperature changes resulted in increased

activity lasting only a few minutes. Considering that the efflux rate was measured over a 6-7 h period, the effects of such activity were probably too small to account for the efflux alterations found.

During sudden temperature drops, the failure to evoke a decreased nitrogen efflux rate is particularly important in view of the fact that, during live transport of tropical prawns and shrimps, deliberate temperature reductions may be given with the specific intention of lowering metabolic rate, hence ammonia efflux and consequent water contamination.

When the same temperature interval changes were made more gradually (Figure 3.4), the resulting Q_{10} values were of a magnitude similar to those given for a number of other crustacean species (Kinne, 1963; Taylor *et al.*, 1977; Zainal *et al.*, 1992). Very slow temperature changes (Figure 3.5), presumably reduced temperature change effects to levels where the animals could adjust and maintain almost constant nitrogen efflux rates throughout the experimental period. Similar results were obtained by Couper (1993) in *Necora puber* but not with *Cancer pagurus*.

Marine and freshwater crustaceans excrete their metabolic nitrogenous waste mainly as ammonia (60-100% of TN), which is formed from amino acid and nucleic acid catabolism (Parry, 1960; Claybrook, 1983; Regnault, 1987). According to Regnault (1987), the non-ammonia proportion of the total nitrogen excreted may be mainly urea, urate and/or amino acids. During shock responses, energy requirements and metabolism generally increase and may result in increased levels of circulating end- products. Under these circumstances it may be advantageous for the organism to remove such products in the quickest and energetically least expensive way. Ammonia can diffuse rapidly across biological membranes because of its small molecular size and high solubility (*cf* urea and uric acid) and it is metabolically inexpensive (Kormanik and Cameron, 1981; Schmidt-Nielsen, 1983). Therefore, the increased TA:TN ratio found during sudden temperature changes, which were less evident with more gradual temperature alterations and which were absent when some acclimation could occur between changes (Figure 3.6), may be a reflection of the shock response of the animal.

Heart rate and gill ventilatory activity of crustaceans are both affected by environmental variables, such as: temperature, salinity and P_{wO_2} (see deFur and Mangun 1979 and Cameron and Mangun 1983 for a review). Such measurements are also important in the study of stress caused by pollutants, as shown by Price and Uglow (1980). Heart and scaphognathite beat rates are usually linearly-related to temperature and this has been observed by Ahsanullah and Newell (1971) in *Carcinus maenas*, Florey and Kriebel (1974) in *Cancer magister* and *C. productus*,

Angersbach and Decker (1978) in *Astacus leptodactilus*, Villarreal (1990) in *Cherax tenuimanus*, among others. In the present experiments, such a response to temperature was found during sudden and slow temperature alterations (Figures 3.9-3.12) and also after a 72 h acclimation period to a changed temperature (Figure 3.13). Nakamura *et al.* (1994) also observed a direct relationship between HR and SR and temperature when exposing *Palinurus japonicus* to rapid cooling (*ca* 1 °C min^{-1}) and that such responses were affected by seasonal variations as cold-acclimated animals were less sensitive to cooling than warm-acclimated ones.

The responses of HR to temperature may vary according to whether the animals were allowed sufficient time to acclimate to the new temperatures, as shown by Burton *et al.* (1980) for *Callinectes sapidus*. When suddenly exposed to a changed temperature, the circulation of blood in the gills will affect the speed by which new internal temperatures are achieved. Spaargaren (1974), found that the half time necessary to equilibrate internal/external temperatures in *Carcinus maenas* is 4 times longer at low temperatures (*ca* 5 °C) than at high temperatures (*ca* 20 ° C). According to this author, the stroke volume of the heart was not affected by temperature and, therefore, heat transfer was found to be linearly related to heart rate. Considering these aspects and also the differences in heart rate observed when *Macrobrachium* was exposed to sudden reductions/increases in water temperatures (Figures 3.9 and 3.10), internal temperatures were probably achieved quicker during temperature increases than during temperature decreases. Temperature increases may then have caused a more intense shock to the animals than temperature decreases. The rate by which HR and SR changed immediately after water temperature was suddenly reduced from 26 to 18 °C was lower than when water temperature was subsequently increased back to 26 °C and this may be associated with such proposed difference in shock intensity.

Angersbach and Decker (1978) found that changes in scaphognathite beat rates altered P_{aO_2} of *Astacus leptodactylus*. Ammonia, like oxygen, enters and leaves the body mainly across the gills and a relationship between ammonia efflux rate and SR may be expected to exist. When prawns were subjected to sudden temperature changes from 26 °C to 18 °C to 26 °C (Figure 3.10), SR remained stable following the temperature reduction but increased above initial levels following alteration back to 26 °C. This pattern may be related to the maintenance of constant values of TA and TN efflux rates following a sudden temperature reduction and higher than initial values after temperature was brought back to 26 °C (Figure 3.3). Unfortunately, such measurements were not performed simultaneously due to experimental limitations.

The Q_{10} values observed for HR and SR, when the animals were acclimated for 72 h to the different temperatures, are in accordance with those generally found (2-2.5) for most physiological processes (Prosser 1991). The extremely high Q_{10} values (10.7) for HR in *Callinectes sapidus* (Burton et al. 1980) were attributed to a critical transition between a torpor situation at 10 °C and increased activity levels at 15 °C. Similar high Q_{10} values for HR (7.2-12.2) were also observed in *Ocypode saratan* between animals acclimated to 15 °C and 20 °C (Eshky et al. 1988) and, according to those authors, such values reflect that this species rarely experiences environmental temperatures < 20 °C. Temperatures used in these experiments were sufficiently low to effect a reduction in metabolism but were not so low as to put the animals in torpor caused by hypothermia.

The results obtained, in particular those related to TA and TN effluxes, are of some importance in the context of live marketing practices and in intensive aquaculture systems where high biomass:water ratios are commonplace. If sudden temperature changes occur in such situations, the dissolved ammonia concentration may increase considerably due to enhanced nitrogen effluxes. Such increases may be more rapid (hence dangerous) if the biological filtration systems are working near their limits or, as is generally the case in live transport procedures, are not used. It would seem expedient to minimize any temperature changes during the shipment of *Macrobrachium rosenbergii*. However, if temperature changes are required, they should be made gradually, avoiding either sudden changes or long acclimation times.

Table 3.1. *Macrobrachium rosenbergii*: Q_{10} values for total ammonia (TA) and total nitrogen (TN) efflux rates during sudden changes in temperature, gradual temperature changes and when acclimated for 72 h to each temperature.

Temperature change	Q10 (26 - 18 °C)		Q10 (18 - 26 °C)	
	TA	TN	TA	TN
Sudden	1.03	1.28	4.36	4.13
Gradual	2.87	2.43	3.36	2.00
Acclim. (72 h)	1.22	1.11	0.71	0.70

Table 3.2. *Macrobrachium rosenbergii*: Heart (HR) and scaphognathite rates (SR) of prawns submitted to a sequence of sudden (2 min) changes in water temperature. Values are mean and SE and n = 8 for each group. Time refers to the time elapsed after each new temperature was achieved.

Temperature (°C)	Time (min)	HR (beats min^{-1})	SR (beats min^{-1})
26	n/a	132.00 ± 9.36	77.81 ± 8.50
22	4	104.88 ± 8.84	69.19 ± 8.95
	34	109.88 ± 9.00	66.00 ± 14.10
	64	101.13 ± 8.68	53.19 ± 8.63
	124	95.87 ± 8.61	49.86 ± 7.56
	244	93.75 ± 8.34	64.92 ± 9.08
	364	92.50 ± 7.15	48.38 ± 5.39
18	4	105.25 ± 8.74	86.44 ± 8.55
	34	90.63 ± 3.06	97.50 ± 14.38
	64	86.50 ± 3.85	91.38 ± 17.05
	124	76.88 ± 5.66	60.94 ± 6.06
	244	70.25 ± 4.55	57.31 ± 9.19
	364	65.38 ± 2.55	60.63 ±8.32
22	4	102.63 ± 5.79	82.31 ± 12.65
	34	99.50 ± 7.84	62.19 ± 11.21
	64	104.63 ± 6.55	78.13 ± 13.88
	124	92.63 ± 2.96	53.19 ± 9.24
	244	105.00 ± 9.33	76.31 ± 14.66
	364	96.88 ± 5.71	62.69 ± 9.13
26	4	122.88 ± 8.59	115.00 ± 9.84
	34	111.75 ± 6.55	62.86 ± 14.33
	64	105.25 ± 9.84	75.88 ± 16.40
	124	123.75 ± 8.42	86.06 ± 10.30
	244	144.13 ± 5.58	91.71 ± 11.44
	364	134.38 ± 9.81	90.19 ± 10.51

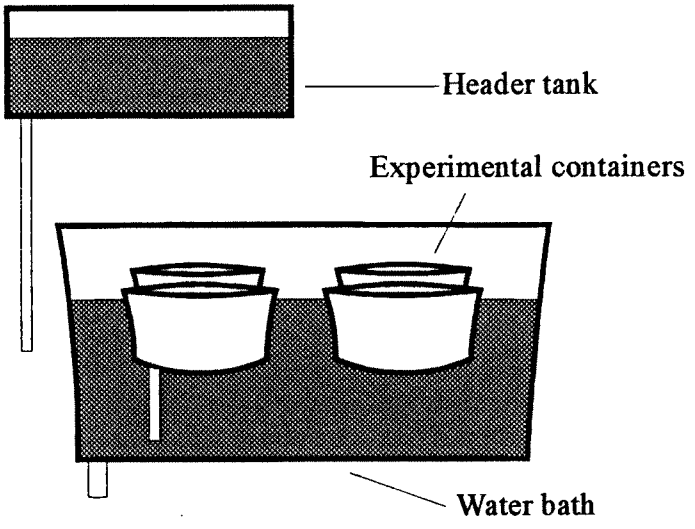


Figure 3.1. *Macrobrachium rosenbergii:* System used to effect sudden alterations in water temperature during measurements of total ammonia and total nitrogen efflux rates.

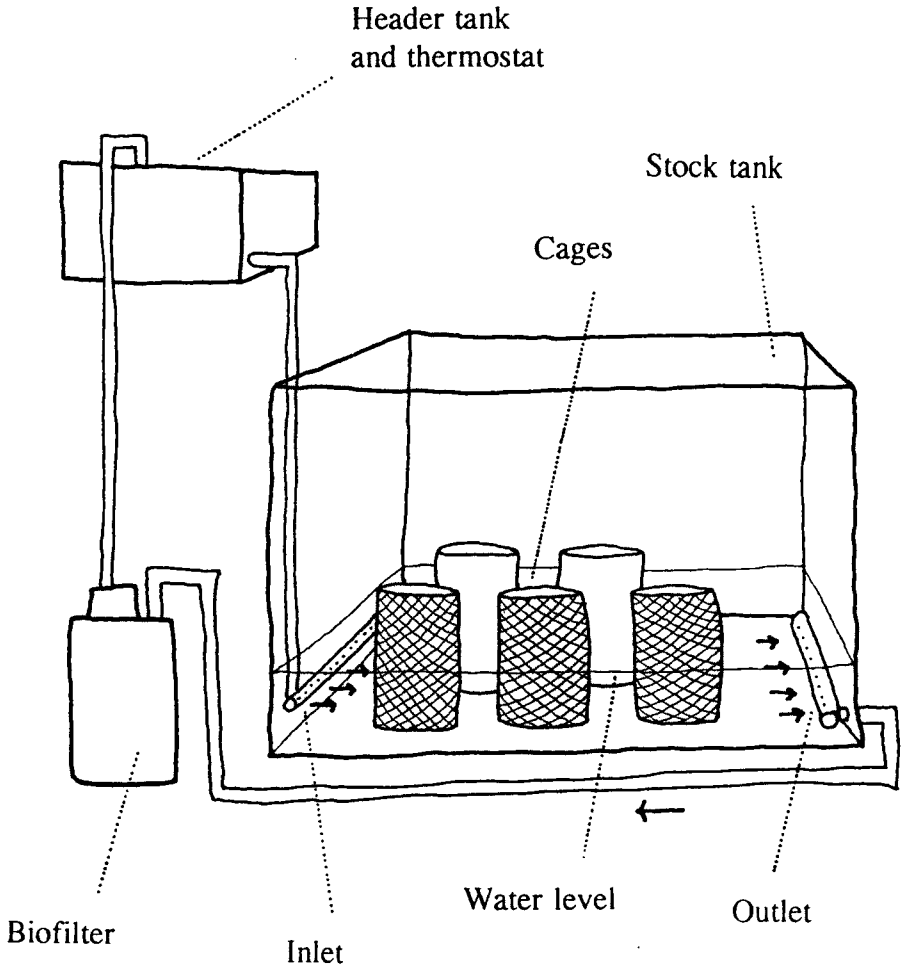


Figure 3.2. *Macrobrachium rosenbergii*: System used to effect water temperature alterations during heart and scaphognathite beat rate measurements.

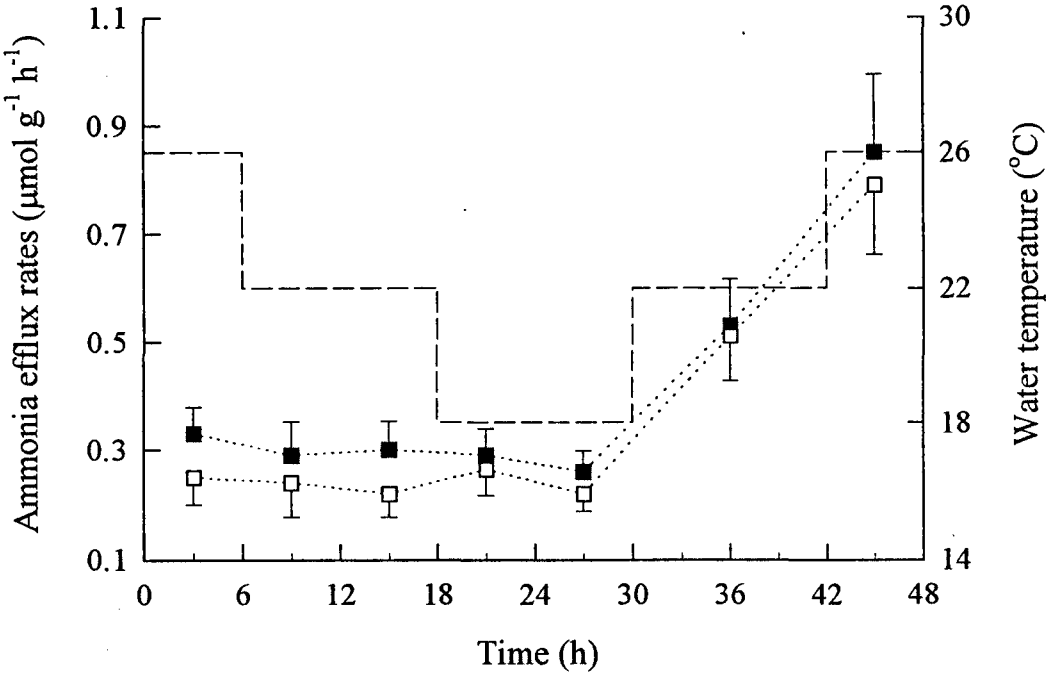


Figure 3.3. *Macrobrachium rosenbergii*: Total ammonia (□) and total nitrogen (■) efflux rates of prawns subjected to sudden water temperature alterations (---). Values are mean ± SE and n = 9 or 10.

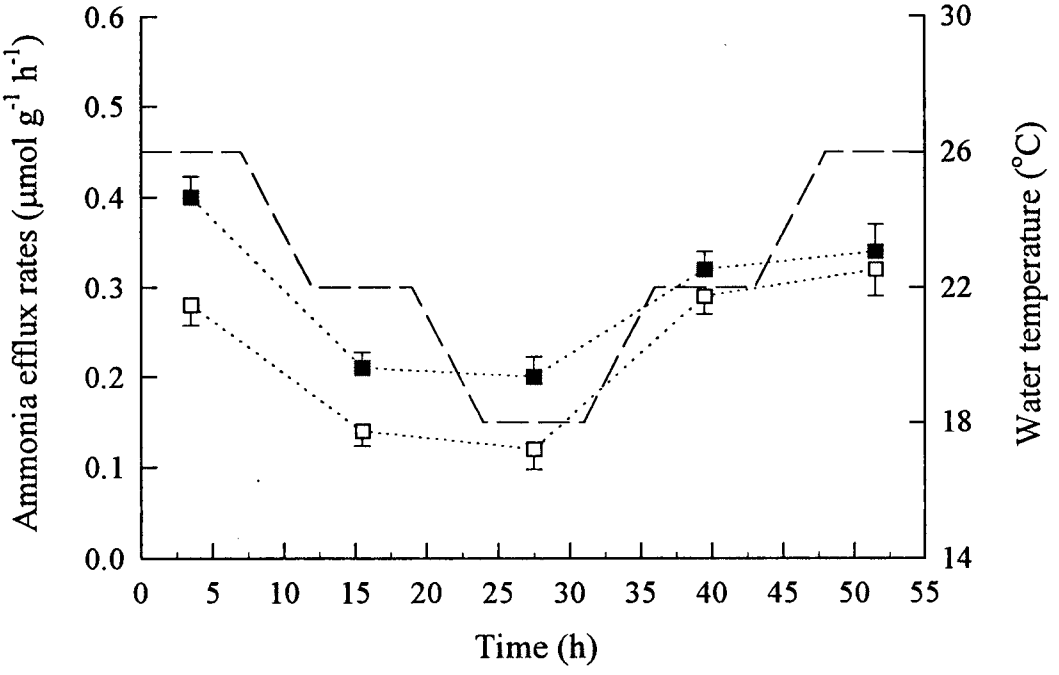


Figure 3.4. *Macrobrachium rosenbergii*: Total ammonia (□) and total nitrogen (■) efflux rates of prawns subjected to gradual water temperature alterations (---). Values are mean ± SE and n = 9 or 10.

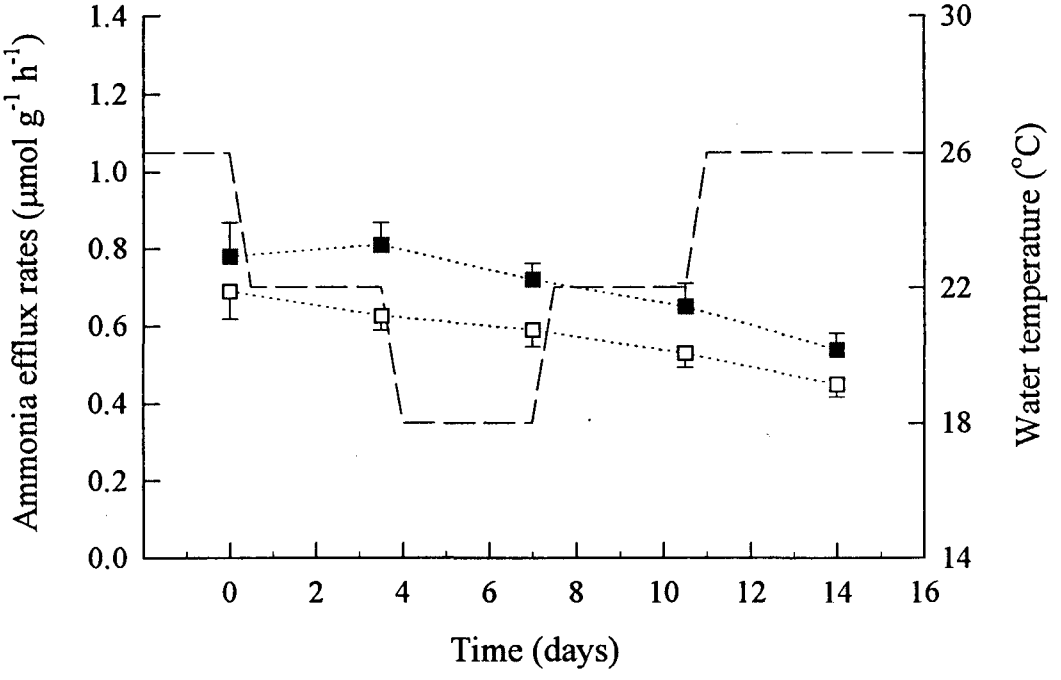


Figure 3.5. *Macrobrachium rosenbergii*: Total ammonia (□) and total nitrogen (■) efflux rates of prawns after acclimation for 72 hrs to each temperature (---). Values are mean ± SE and n = 9 or 10.

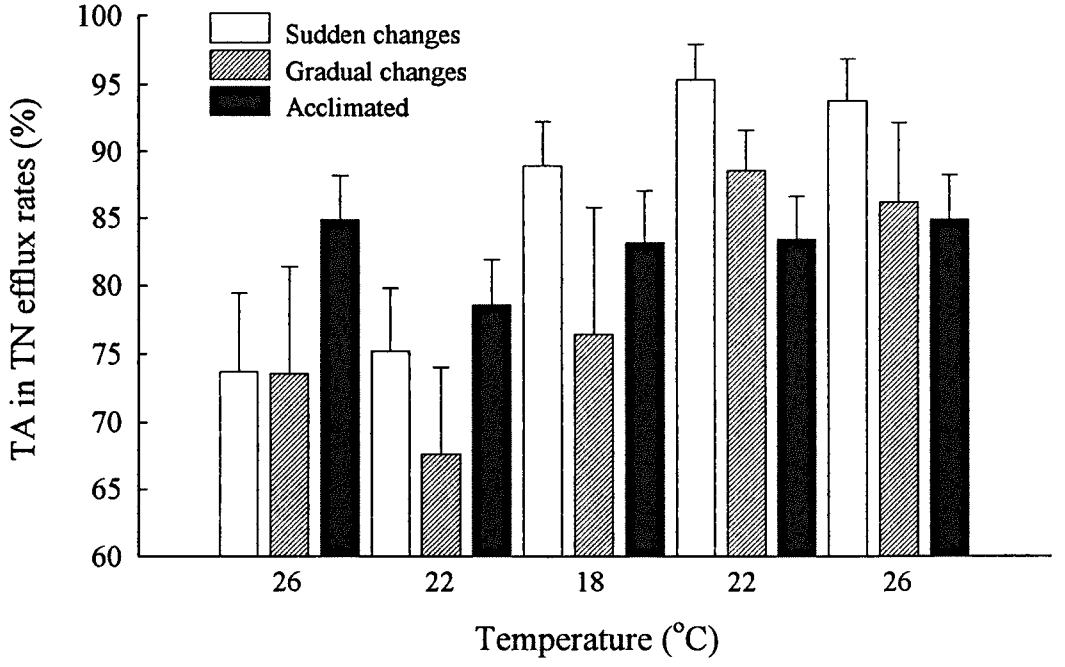


Figure 3.6. *Macrobrachium rosenbergii*: Proportion of total ammonia in the total nitrogen effluxes (TA:TN ratios) during each temperature interval of each experiment.

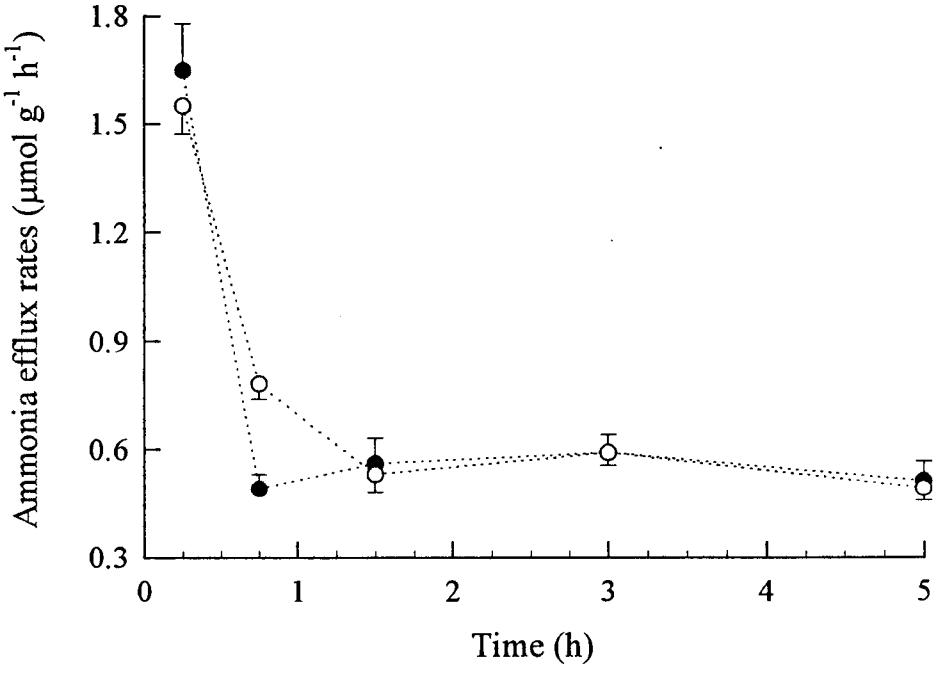


Figure 3.7. *Macrobrachium rosenbergii*: Total ammonia efflux rates immediately after handling (o) and after handling followed by a 6 min period of emersion (●). Values are mean ± SE and n = 9 or 10.

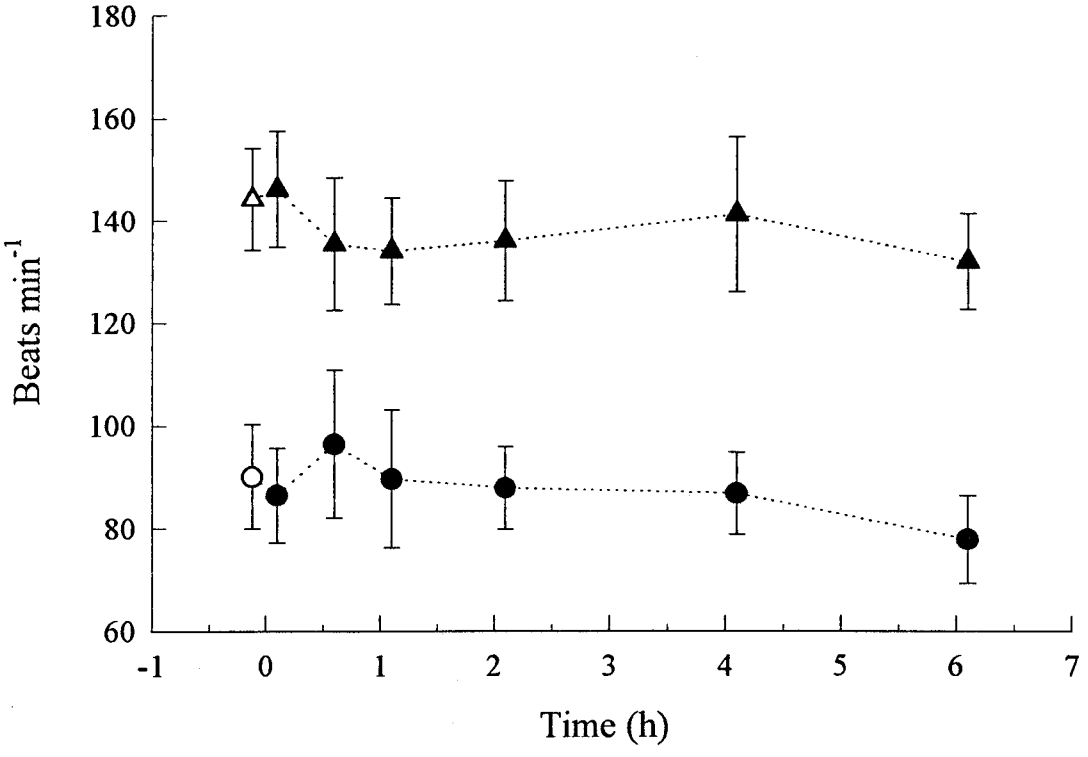


Figure 3.8. *Macrobrachium rosenbergii*: Control experiment. Heart (▲) and scaphognathite (●) rates of prawns subjected to water changes at a constant temperature (26 °C). Open symbols refer to values before water change. Values are mean ± SE and n = 8 for 8 min total recording time in each case.

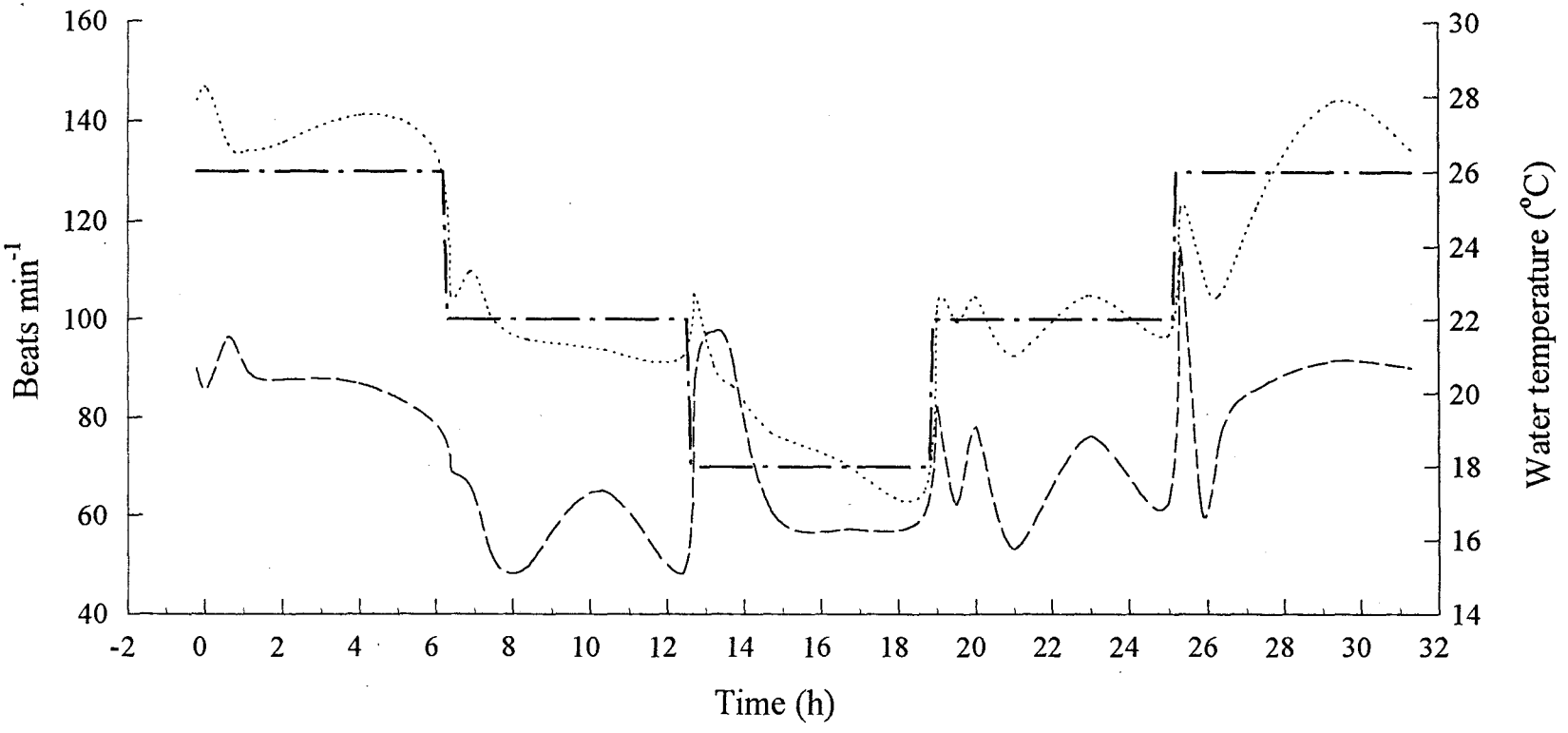


Figure 3.9. *Macrobrachium rosenbergii*: Heart (·····) and scaphognathite (---) rates of prawns subjected to sudden (2 min) alterations in water temperature (– · –). Curves were obtained using the values shown in Table 3.2.

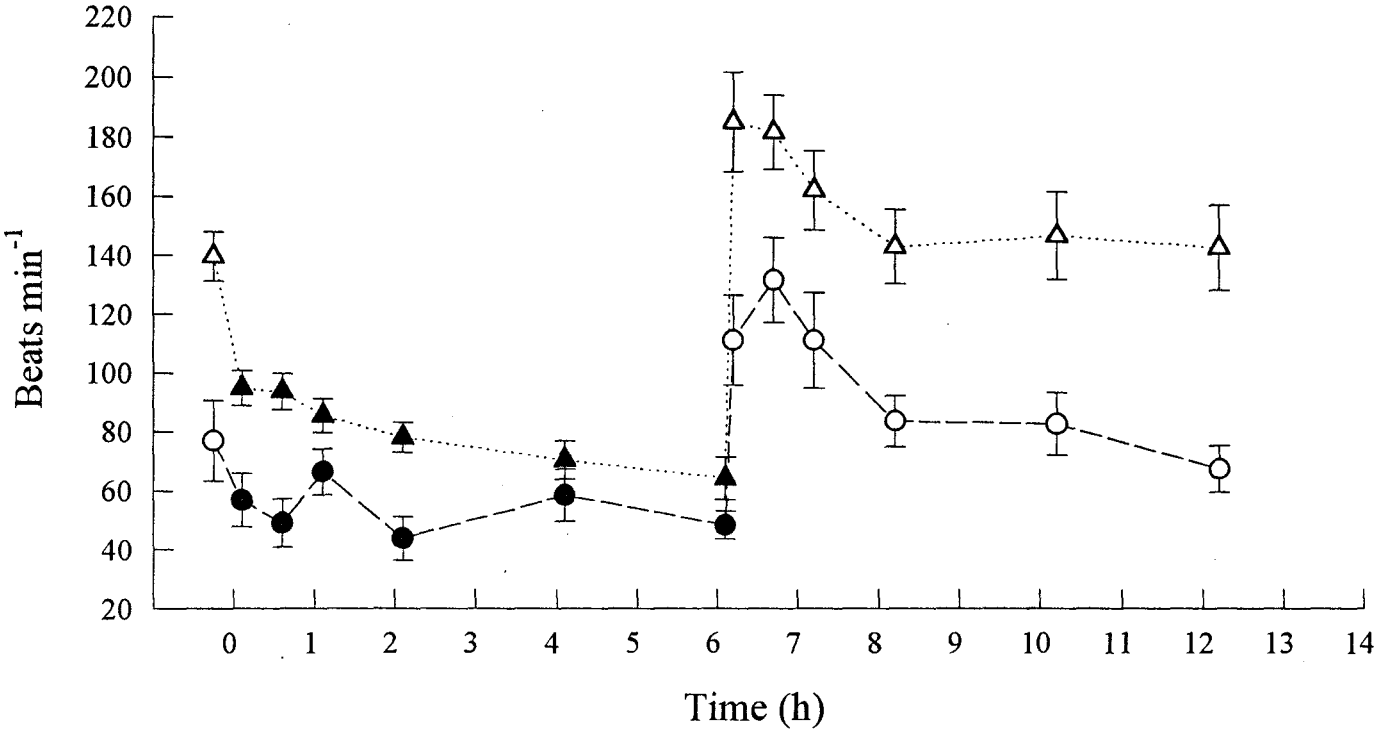


Figure 3.10. *Macrobrachium rosenbergii*: Heart (▲) and scaphognathite (●) rates during sudden (2 min) alterations in water temperature (26 → 18 → 26 °C). Dark symbols refer to prawns at 18 °C. Values are mean ± SE and n = 8 and refer to 8 min of recording in each case.

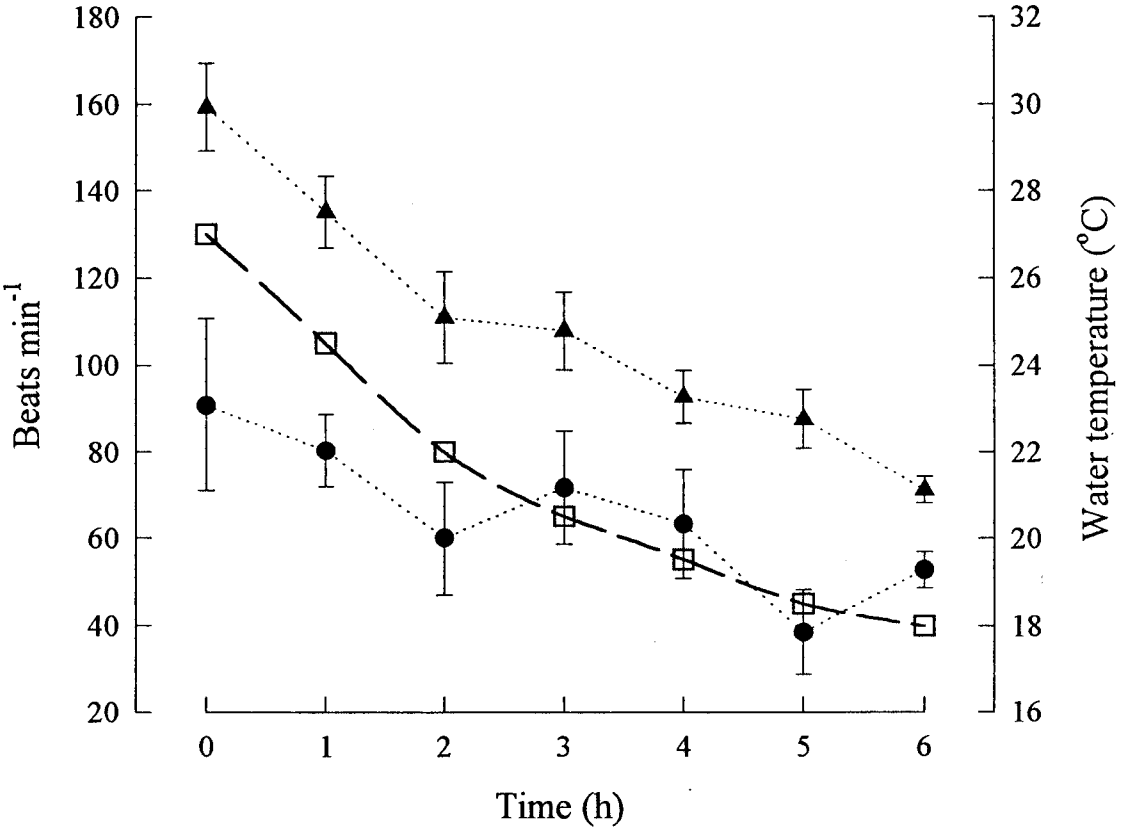


Figure 3.11. *Macrobrachium rosenbergii*: Heart (▲) and scaphognathite (●) rates of prawns subjected to gradual reductions in water temperature (□). Values are mean ± SE and n = 8 and refer to 8 min total recording time in each case.

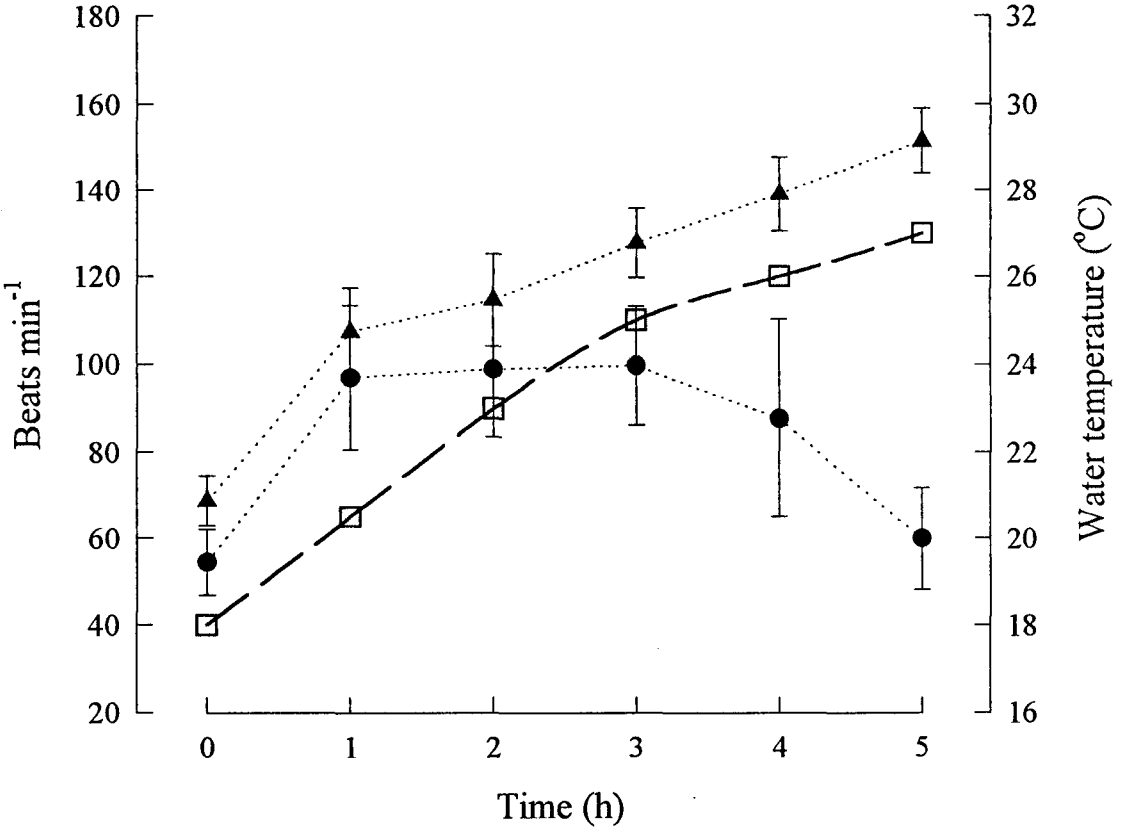


Figure 3.12. *Macrobrachium rosenbergii*: Heart (▲) and scaphognathite (●) rates of prawns subjected to gradual increases in water temperature (□). Values are mean ± SE and n = 8 and refer to 8 min total recording time in each case.

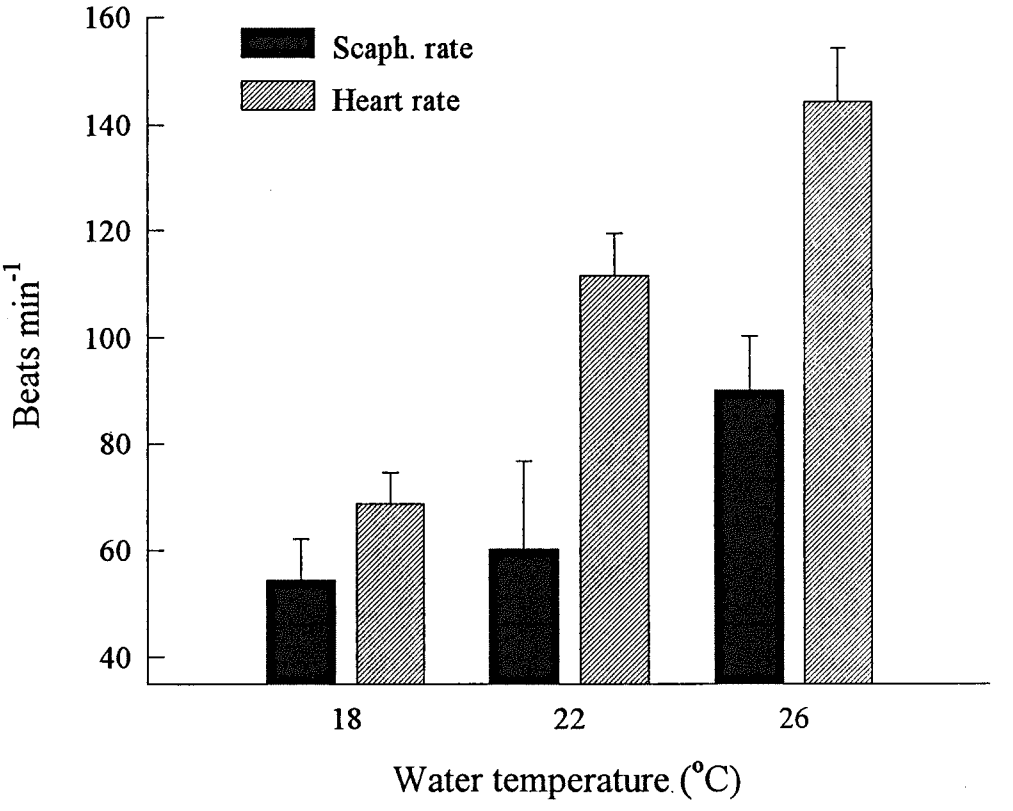


Figure 3.13. *Macrobrachium rosenbergii*: Heart and scaphognathite rates of prawns acclimated for 72 h to different water temperatures. Values are mean ± SE and n = 8 and refer to 8 min total recording time in each case.

Chapter 4.

Haemolymph constituent levels and ammonia efflux rates of *Nephrops norvegicus* (L.) during emersion.

4.1. Introduction

The effects of emersion on crustacean gas exchange and haemolymph chemistry have been extensively studied (Stott 1932, Kleinholz and Little 1949, Kleinholtz *et al.* 1950, Cameron 1978, Truchot 1979, Burnett 1988, deFur 1988, Tyler-Jones and Taylor 1988). Intertidal and semi-terrestrial species are well-known as having variable degrees of adaptations to improve oxygen uptake, thus reducing internal hypoxia, while emersed (Cameron 1981, Johnson and Uglow 1985, McMahon and Burggren 1988, al Wassia 1989). Fully aquatic species, however, are less well-adapted for emersion as gill functioning is usually impaired at such times. In addition to gas exchange, the release of circulating ammonia is mainly performed through the gills (Regnault 1987) but this aspect of crustacean gill function has not been extensively studied during emersion. Increased concentrations of blood ammonia and urate and branchial water ammonia were found in *Cancer pagurus* exposed to air and increased effluxes of ammonia following re-immersion have been found (Regnault 1992, 1994).

One of the present reasons for studying the responses of sublittoral crustaceans to emersion is the commercial importance that some species have when marketed alive. Whiteley and Taylor (1992) monitored the oxygen and acid-base metabolism during emersion of *Homarus gammarus* transported live to market and some effects of emersion on blood carbohydrate and lactate during simulated commercial procedures of *Nephrops norvegicus* have been analysed by Spicer *et al.* (1990).

Since the early 1950s, Scottish *Nephrops* fisheries have expanded considerably (Bailey et al. 1986) and 1992 landings (£ 31 m.) represent 50 % of the total value of all crustaceans landed in Scotland that year. A proportion of this market is directed to long-distance live transport and requires good quality prawns that are trap-caught, rather than trawled, to minimise damage. After the prawns are removed from the traps they may be transferred to onboard viviers (wells) or they may be kept emersed between layers of seawater-soaked hessian. Viviers eliminate emersion but keeping the animals emersed reduces handling procedures during unloading to the different retailers and may be the preferred method, as it uses less deck space.

The present experiments were designed to include actual and simulated events and to analyse their effects on some aspects of the blood physiology of *Nephrops*; including ammonia production and release. A better comprehension of such effects should improve the quality of delivered product and may assure better survival rates during transport procedures.

4.2. Materials and Methods

The effects of emersion on haemolymph chemistry of the prawns were studied under two different situations. In one experiment, high relative humidity (HH) emersion, the prawns were placed between layers of seawater-soaked hessian and then placed inside plastic containers with a perforated base. Every 20 min, seawater was poured through the containers, simulating a common procedure used to keep prawns alive and in good condition during fishing operations. In a second experiment, low relative humidity (LH) emersion, the animals were taken from the water and placed individually in dry, rectangular plastic containers. In both experiments the prawns remained out of water for 8 h. Haemolymph samples were collected from different groups of 8-10 prawns before and after 2, 4 and 8 h of emersion and again after 2 h following re-immersion.

Samples of pre-branchial and of post branchial haemolymph were both collected within a space of 10 sec (max.) of the animals being picked up from their aquaria. An additional blood sample (300-500 µl) was taken for lactate, glucose, pH and total ammonia determinations.

In separate experiments, prawns were exposed to air for 8 h under the same conditions as the two previous experiments and their subsequent ammonia efflux were monitored for a period of 6 h following their re-immersion. After the emersion periods, each prawn was placed in a rectangular plastic container with 1.5 l of seawater drawn from the stock tank. Water samples (1.5 ml) were collected at times 0, 0.5, 1, 2, 4 and 6 h after re-immersion and the total ammonia efflux rates of the prawns determined.

In addition, the effects of emersion on blood glucose, lactate and ammonia concentrations of *Nephrops* were monitored onboard a Scottish fishing vessel during normal capture activities (12 h journeys using baited creels). Prawns were taken from the creels and temporarily placed in a container with sea water. After the completion of emptying 1 fleet of traps (*ca* 60-80 traps) (Figure 4.1), the prawns were separated by size and placed in perforated plastic trays with seawater-soaked hessian (Figure 4.2), as described for the laboratory experiment. Normally, 200-300 prawns were placed together between layers of hessian and the trays were flushed with sea water every 20-30 min. The whole procedure (collection from the creels, re-baiting, size selection and replacing the fleet in the water) took 30-40 min to be accomplished. During such periods, the prawns were understandably roughly handled due to the limited time to perform all operations.

Periodically, groups of prawns from the catch were randomly marked with colour-coded rubber bands (on the claw) and the marking time registered. At the

end of the trip, haemolymph samples from all marked prawns were collected. On landing, a further group of prawns, in perforated cages, were re-immersed in the sea and haemolymph samples were collected 14 h later. During such procedures, air and water temperatures were 8.3 ± 0.3 °C and 7.5 ± 0.1 °C, respectively.

4.3. Results.

Levels of all blood constituents of *Nephrops* examined during these experiments were altered and all prawns survived the emersion periods. After the prawns were exposed to air several tail-flips were observed. Exact frequencies of these were not measured, but the number of flips during LH emersion was clearly higher than during HH emersion.

Total ammonia (TA) in the blood increased significantly ($P<0.05$) from 96.04 ± 8.75 μmol l^{-1} (immersed prawns) to 293.16 ± 12.87 μmol l^{-1} within the first 2 h of LH emersion and then to 667.11 ± 74.25 μmol l^{-1} after 8 h (Figure 4.3.) ($P<0.05$). After 2 h of re-immersion TA levels decreased but were still higher than initial levels of immersed prawns ($P<0.05$). During HH emersion, TA accumulated in the blood but at considerably lower rates than during LH (Figure 4.3). Blood TA levels after 8 h of emersion (328.18 ± 85.10 μmol l^{-1}) were significantly higher ($P<0.05$) than that of control immersed prawns (137.87 ± 15.11). However, most of this increase occurred within the first 2 h of emersion. Initial levels were regained after 2 h of re-immersion ($P>0.05$).

Ca_{O_2} and C_{vO_2} of *Nephrops* during LH and HH emersion are shown in Figure 4.4 and 4.5. Under LH conditions, both variables decreased sharply ($P<0.05$) during the first 2 h of emersion, from 9.84 ± 1.32 to 0.89 ± 0.15 ml l^{-1} (Ca_{O_2}) and from 5.31 ± 0.79 to 0.55 ± 0.07 ml l^{-1} (C_{vO_2}). Values remained constant throughout the emersion period and increased again when the prawns were re-immersed. C_{vO_2} after re-immersion was significantly higher ($P<0.05$) than initial levels. Ca_{O_2} was also higher than initial levels but this difference escaped significance at the 5 % level. A similar pattern was found during HH emersion when Ca_{O_2} decreased from 10.68 ± 1.55 to 1.44 ± 0.36 ml l^{-1} and C_{vO_2} decreased from 7.46 ± 1.52 to 0.90 ± 0.18. Initial Ca_{O_2} and C_{vO_2} were regained during the 2 h of re-immersion.

Blood pH decreased significantly ($P< 0.05$) from 7.77 ± 0.04 to 7.30 ± 0.05 during the first 2 h of LH emersion and remained relatively constant throughout the remaining emersion period (Fig 4.6.). After re-immersion, blood pH levels increased significantly ($P<0.05$) but were still significantly lower ($P<0.05$) than initial values. During HH emersion, a similar reduction in blood pH was observed (Fig 4.6.). Values decreased from 7.70 ± 0.05 to 7.35 ± 0.08 after 2 h of emersion and increased again when the prawns were re-immersed ($P<0.05$). Unlike LH emersion, significant differences between initial and final blood pH values were not found ($P>0.05$).

Blood glucose concentrations increased significantly ($P<0.05$) within 2 h of LH or HH emersion and remained constant until the experiments ended (Fig 4.7). Maximum concentrations observed were 29.13 ± 4.43 mg 100 ml^{-1} at 4h of LH emersion and 30.31 ± 4.94 mg 100 ml^{-1} at 8 h of HH emersion. Lactate concentrations increased steadily ($P<0.05$) from 1.05 ± 0.10 to 112.96 ± 1.63 mg 100 ml^{-1} during 8 h of LH emersion and remained significantly higher ($P<0.05$) than initial values after re-immersion (Fig 4.8). During HH emersion also, blood lactate concentrations showed a significant increase ($P<0.05$) but at lower rates of accumulation than during LH emersion. The maximum mean value found was 69.16 ± 12.39 mg 100 ml^{-1} after 8 h of emersion and, following re-immersion, remained significantly higher ($P<0.05$) than those of control prawns.

TA efflux rates following re-immersion are shown in Figure 4.9. Immediately after the prawns were placed in the experimental containers, TA effluxes of all groups were significantly higher ($P<0.05$) than that of control group after 15 min of re-immersion. TA efflux rates of LH and HH groups decreased throughout re-immersion time but were still higher than control group ($P<0.05$).

The results obtained during fishing operations are shown in Table 4.1. The general pattern found during onboard emersion was similar to the patterns found during laboratory based LH and HH emersion periods. Blood ammonia increased during emersion and after 3.5 h was significantly higher ($P<0.05$) than initial values. Glucose concentrations increased significantly ($P<0.05$) within the first 2 h of emersion and then remained relatively constant. Blood lactate concentrations increased during emersion and values obtained after 2 h were significantly higher ($P<0.05$) than initial values. All variables measured returned to initial values after 14 h of re-immersion ($P<0.05$).

4.4. Discussion.

Marine and freshwater crustaceans excrete nitrogen mainly as ammonia, which is formed from aminoacid and nucleic acid metabolism (Parry 1960, Claybrook 1983, Regnault 1987). Ammonia is released to the external environment through the gills by diffusional movement and $Na^+/NH4^+$ exchange across the epithelium (Kormanik and Cameron 1981, Evans and Cameron 1986). In the absence of water, such mechanisms may be greatly impaired and accumulation of ammonia in the haemolymph, as observed in the present work, may occur. The slower rate of haemolymph ammonia level increase during emersion shown by the HH group (*cf* the LH group) (Figure 4.3.) may have been because of lower production and/or excretion rates. It is not known whether there was a complete cessation of ammonia excretion during LH emersion but it appears that the HH emersion conditions allowed some ammonia excretion to take place.

The blood ammonia increases found in both the emersed groups were considerably less than values calculated on the basis of a continued immersed production rate coupled with a cessation of excretion (Table 4.2). This discrepancy may be due to: 1) lowered ammonia production; 2) some ammonia efflux; 3) a switch in the biochemical pathways of nitrogen metabolism; 4) storage of ammonia elsewhere than in the blood. The ammonia efflux rates following emersion was higher than that excreted by control prawns (Figure 4.9.) and the calculated absolute amounts excreted (Table 4.3) were considerably higher than those accumulated in the haemolymph during emersion. Assuming that immediate post-emersion ammonia effluxes are related to metabolism during emersion, the discrepancy between observed and predicted blood ammonia levels for emersed prawns may only have been caused by options 3 and/or 4, above.

Regnault (1994) also found a level of post re-immersion ammonia efflux in *Cancer pagurus* not explicable by the accumulation of ammonia and urate in the blood or ammonia in the gill chamber water reservoir and the involvement of some other fluid store was suggested. Some 80 % of this excess ammonia was released within 5 min of *C. pagurus* being reimmersed and this supports the contention that ammonia had been stored. The present results reflect a different situation as no such large ammonia efflux occurred rapidly on re-immersion. In view of the mobility and pottentially-detrimental effects of ammonia (Campbell 1991), it is possible that some other nitrogenous metabolic end-products were formed and stored during emersion of *Nephrops*.

An increased GDH-mediated glutamate production in *C. pagurus* muscle was found by Regnault (1992), but only after 12 h of emersion. Glutamate formation

from ammonia requires ATP and may not be a suitable option during emersion, when energy production is very expensive as it may occur anaerobically. During emersion-induced impairment of $\dot{M}O_2$ and nitrogen efflux, the production of urate instead of ammonia would be advantageous as urate is less toxic than ammonia and, according to Lallier and Truchot (1989) and Truchot and Lallier (1992), it enhances haemocyanin-O_2 affinity. Increased blood urate values have been found in *Carcinus maenas* under hypoxia (Lallier *et al.* 1987) and emersed *C. pagurus* (Regnault 1992).

The amount of ammonia excreted over 6 h following LH emersion was higher than that accumulated in the haemolymph and also *ca* twice the predictable values if ammonia production was maintained throughout (Tables 4.2 and 4.3). This increase may have been caused by an increased metabolism following anaerobiosis or by the large number of tail-flips and general escape responses made during emersion. Under normoxia, extended periods of tail-flipping are supported by glycolitic processes (Newland *et al.* 1992) and may thus evoke increased lactate levels in the haemolymph and flexor muscles.

Keeping *Nephrops* periodically flushed with seawater apparently does little to improve the gas exchanges of emersed prawns as they probably lack the structures reported by Johnson and Uglow (1985) and Cameron (1981) to prevent the collapse of the gill lamellae out of water. The LH and HH groups showed significant decreases in blood total O_2 and pH and increases in circulating lactate and glucose levels, indicating severe hypoxia during emersion. After 2 h of emersion the LH group showed a Ca_{O_2} and Cv_{O_2} reduction of *ca.* 90 % (*cf.* a *ca.* 87 % reduction for the HH group) and the corresponding Ca_{O_2} - Cv_{O_2} values 11 and 5 fold reductions, respectively. Similar reductions in blood oxygen content and Ca_{O_2} - Cv_{O_2} were found in *Homarus gammarus* during emersion. Under such conditions, circulatory alterations would have been needed to maintain tissue oxygen supplies at normal immersed rates. According to the Fick equation, such compensation may be achieved by increasing blood flow to the tissues (Mangum 1983), but increased heart rates during emersion are normally not observed and the increase to stroke volume needed to effect the necessary cardiac output seems very unlikely.

According to deFur (1988), the limitation to crustacean branchial gas exchanges during emersion may be due to hypoventilation, diffusion limitations or collapsed gills. Such events cause CO_2 accumulation which, with increased lactate levels, may result in a blood acidosis, as found in the present studies. Blood pH increased rapidly when prawns were re-immersed and normal pH levels were maintained in the HH group. As blood lactate concentrations were high at such times, it appears that CO_2 accumulation was mainly responsible for the emersion

induced acidosis. Whiteley and Taylor (1992) found that, after 14 h of emersion during marketing, *Homarus gammarus* regained normal CO_2 tensions an pH values after 1 and 3 h respectively following re-immersion but that blood lactate levels remained high over the following 24 h.

Spicer at al. (1990) showed that the hyperglycemic response of *Nephrops* to emersion was different when the prawns were kept on ice (0 °C) than when kept cool (10 °C). Under the former conditions, hyperglycemia developed steadily whereas at 10 °C the response was transient and reached high concentrations. Transient hyperglycemia was found to occur with this species during hypoxia (Smullen and Bentley 1993). In addition to hypoxia, the hyperglycemia may have been caused by handling stresses in the present studies as such responses have been shown to occur in *Homarus americanus* (Telford 1968, 1974). According to Hochachka and Somero (1984) and Storey and Storey (1990), when C_{aO_2} falls below a critical level, anaerobic ATP production may be replaced by a strong depression of metabolism, which will ration the use of carbohydrates and maximise survival time. However, reduced activity and metabolic rate will also repress some glucose utilization which may induce hyperglycemia as suggested by Sheer and Sheer (1951), Santos *et al.* (1988) and Schmitt and Santos (1993a). A metabolic depression did not occur here during LH and HH emersion and the energy requirements at these times were satisfied *via* anaerobic pathways, a strategy which suggests that *Nephrops norvegicus* is poorly suited to cope with emersion-induced hypoxia.

Here, lactate accumulation was found to be related to the prevailing humidity. The blood lactate levels found during HH emersion were lower than those during LH emersion, which may have been caused by lactate removal. According to Ellington (1983), this could have been by excretion, oxidation and gluconeogenesis. Crustaceans do not seem to excrete lactate (Bridges and Brand 1980b) and oxydation and gluconeogenesis under tissue hypoxia seems unlikely. Under both emersion conditions the activity of the prawns increased but the increase was greater under LH emersion. Tail flips may cause functional anaerobiosis in *Nephrops* (Newland *et al.* 1992) and Gäde (1984) found that excercised *Orconectes limosus* showed a direct relationship between anaerobic ATP production and tail flip frequency. Phillips *et al.* (1977) showed that the lactate production in the tail muscle of *Homarus gammarus* during excercise diffused into the haemolymph. A similar happening in the present experiments could have caused the higher levels of blood lactate in the LH emersed group of prawns.

After 2 h of re-immersion following HH and LH emersion, normal pre-emersion blood lactate concentrations were not regained. According to Ellington

(1983), the time taken to remove lactate from crustacean haemolymph is generally protracted, whereas animals that regularly experience hypoxia in their natural habitat may be more adapted to a rapid recovery. Taylor and Spicer (1987) suggested that, following hypoxia, the rock pool species *Palaemon elegans* is better adapted to remove lactate than the essentially subtidal shrimp *Palaemon serratus*. Blood lactate disappears more rapidly from burrowing species (*eg. Nephrops*), than in other crustaceans (Bridges and Brand 1980b). Normal blood lactate levels took 24 h to recover following 12 h of anoxia in *Orconectes limosus* (Gäde 1984) and following 14 h of emersion during transport of marketed *Homarus gammarus* (Whiteley and Taylor 1992). However, lactate disappearance by oxidation and/or gluconeogenesis may be masked by a diffusion into the haemolymph of lactate from other tissues, resulting in the steady post re-immersion levels found in the present experiments.

During onboard emersion experiments, the prawns were handled exclusively by fishermen, except when marking with rubber bands or collecting blood samples. In addition to the stress caused by the emersion period itself, the prawns experienced the stresses of being removed from the creels, thrown into trays with a considerably higher density of prawns than those used in the laboratory experiments. Such practices, however, did not appear to cause any differences between the patterns obtained for blood ammonia, glucose or lactate concentrations obtained from onboard and laboratory experiments. It seems, on the basis of blood chemistry, that the stress imposed by emersion exceed those caused by the onboard handling practices during normal fishing operations. It must be noted, however, that only uninjured prawns were sampled in all experiments and several damaged prawns were found among the catch. The combined effects of injuring and emersion on the blood chemistry and general quality of the catch are presently unknown.

The procedure of keeping *Nephrops* between layers of seawater-soaked hessian, together with periodical irrigation with seawater onboard appears to have little effect on the maintenance of the intrinsic quality of the catch. The use of a system that will keep the prawns at least partially-immersed in running seawater is likely to improve their nitrogen excretion and reduce the occurrence of anaerobiosis and is to be strongly recommended.

Table 4.1. *Nephrops norvegicus*: Haemolymph levels of ammonia, glucose and lactate (mean ± SE and n = 9 or 10) during emersion immediately following collection from traps during normal fishing operations.

	Haemolymph levels		
Emersion time (h)	Ammonia (μmol l^{-1})	Glucose (mg 100 ml^{-1})	Lactate (mg 100 ml^{-1})
0 (from creel)	213.60 ± 21.35	1.43 ± 0.29	1.40 ± 0.44
2	254.57 ± 22.63	22.73 ± 5.22	30.96 ± 3.81
3.5	381.33 ± 35.81	19.61 ± 2.04	36.29 ± 4.62
5	388.50 ± 22.76	14.98 ± 1.92	44.15 ± 6.95
7	544.67 ± 47.25	20.18 ± 3.01	71.87 ± 9.48
8 emersion + 16 re-imm.	153.33 ± 7.75	3.30 ± 0.70	0.61 ± 0.14

Table 4.2. *Nephrops norvegicus*: Blood ammonia content (μmol prawn^{-1}) of emersed prawns kept under low humidity (LH) and (HH) conditions. Blood volumes were taken as being 30 % of fresh weight. Predicted values were calculated on the assumption of zero excretion and on ammonia production rate of 0.204 μmol g^{-1} h^{-1} = mean efflux rate of immersed (control) prawns. all values are presented as mean ± SE for n = 8 or 9 prawns.

Emersion time (h)	0	2	4	8
LH experiment				
Ammonia content.	0.83 ± 0.09	2.28 ± 0.19	3.15 ± 0.34	5.36 ± 0.92
Ammonia accumulated above T_{0h}.	_	1.45 ± 0.19	2.33 ± 0.34	4.54 ± 0.92
Predicted values	_	11.40 ± 0.72	23.94 ± 1.02	43.75 ± 4.32
Predicted values above T_{0h}.	_	10.58 ± 0.72	23.11 ± 1.02	42.94 ± 4.32
HH experiment				
Ammonia content.	1.08 ± 0.10	2.51 ± 0.44	2.58 ± 0.93	2.62 ± 0.61
Ammonia accumulated above T_{0h}.	_	1.43 ± 0.44	1.50 ± 0.93	1.54 ± 0.61
Predicted values	_	12.83 ± 1.14	23.24 ± 1.27	47.11 ± 3.47
Predicted values above T_{0h}.	_	11.75 ± 1.14	22.16 ± 1.27	46.03 ± 3.27

Table 4.3. *Nephrops norvegicus*: Ammonia excreted per prawn following 8 h of emersion under control conditions and under low humidity (LH) and high humidity (HH) conditions. All values are given as mean ± SE for n = 8 or 9 prawns.

Re-immersion time (h)	0.5	1	2	4	6
Control					
Ammonia excreted.	7.68 ± 1.73	3.87 ± 0.68	6.56 ± 0.95	10.80 ± 1.84	11.17 ± 2.03
Accumulated ammonia excreted.	7.68 ± 1.73	11.55 ± 1.97	18.11 ± 2.65	28.90 ± 3.88	40.07 ± 5.60
After LH emersion					
Ammonia excreted.	15.97 ± 5.28	27.06 ± 3.79	28.56 ± 1.85	38.83 ± 3.27	29.53 ± 4.74
Accumulated ammonia excreted.	15.97 ± 5.28	43.03 ± 2.65	71.59 ± 3.67	110.42 ± 4.90	139.95 ± 7.88
Accumulated ammonia excreted in excess of control.	8.29 ± 5.28	31.48 ± 2.65	53.48 ± 3.67	81.52 ± 4.90	99.88 ± 7.88
After HH emersion					
Ammonia excreted.	11.82 ± 3.06	8.12 ± 1.21	17.37 ± 2.78	26.83 ± 5.82	24.38 ± 5.82
Accumulated ammonia excreted.	11.82 ± 3.06	19.94 ± 3.91	37.31 ± 6.16	64.14 ± 11.80	88.52 ± 17.28
Accumulated ammonia excreted in excess of control.	4.14 ± 3.06	8.39 ± 3.91	19.20 ± 6.16	35.24 ± 11.80	48.45 ± 17.28

Figure 4.1. Fishing procedures of *Nephrops norvegicus*. Taking prawns from the creels.

Figure 4.2. Fishing procedures of *Nephrops norvegicus*. Selecting prawns by size.

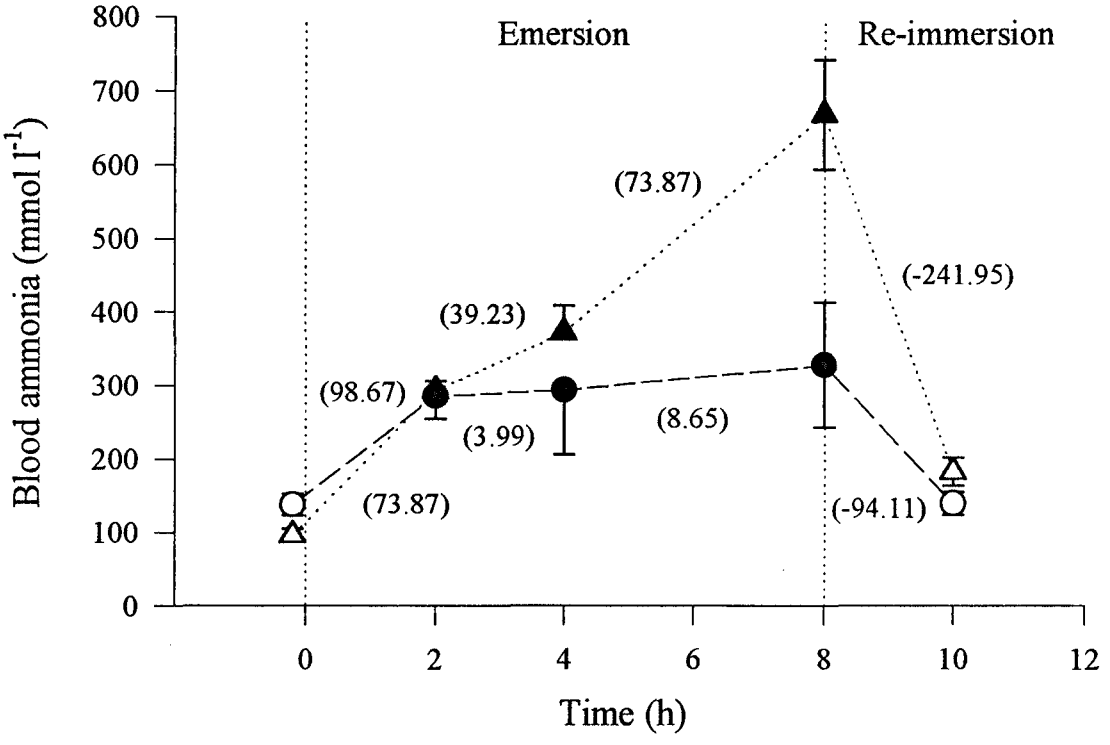


Figure 4.3. *Nephrops norvegicus*: Blood ammonia concentration (mean ± SE and n = 8-10) during low (▲) and high humidity (●) periods of emersion and subsequent re-immersion (open symbols refer to immersed prawns). Values in brackets are ammonia accumulation rates (μmol l^{-1} h^{-1}) of each interval.

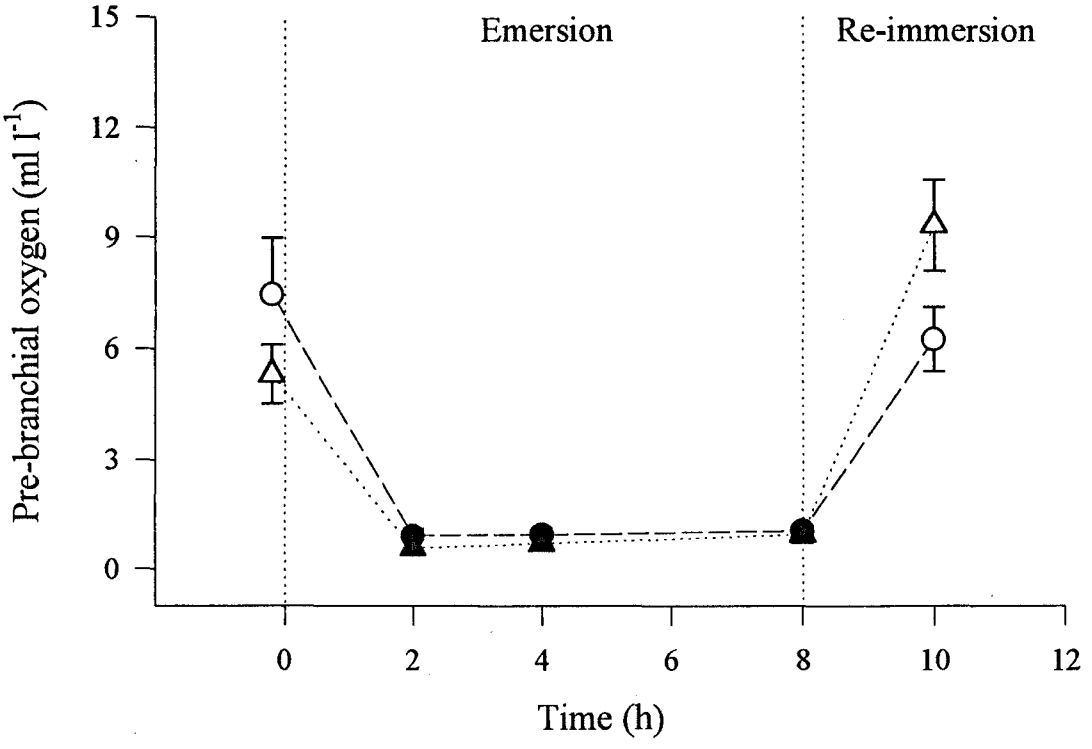


Figure 4.4. *Nephrops norvegicus*: Blood pre-branchial O_2 concentrations (mean ± SE and n= 8-10) during low (▲) and high humidity (●) periods of emersion and subsequent re-immersion (open symbols refer to immersed prawns).

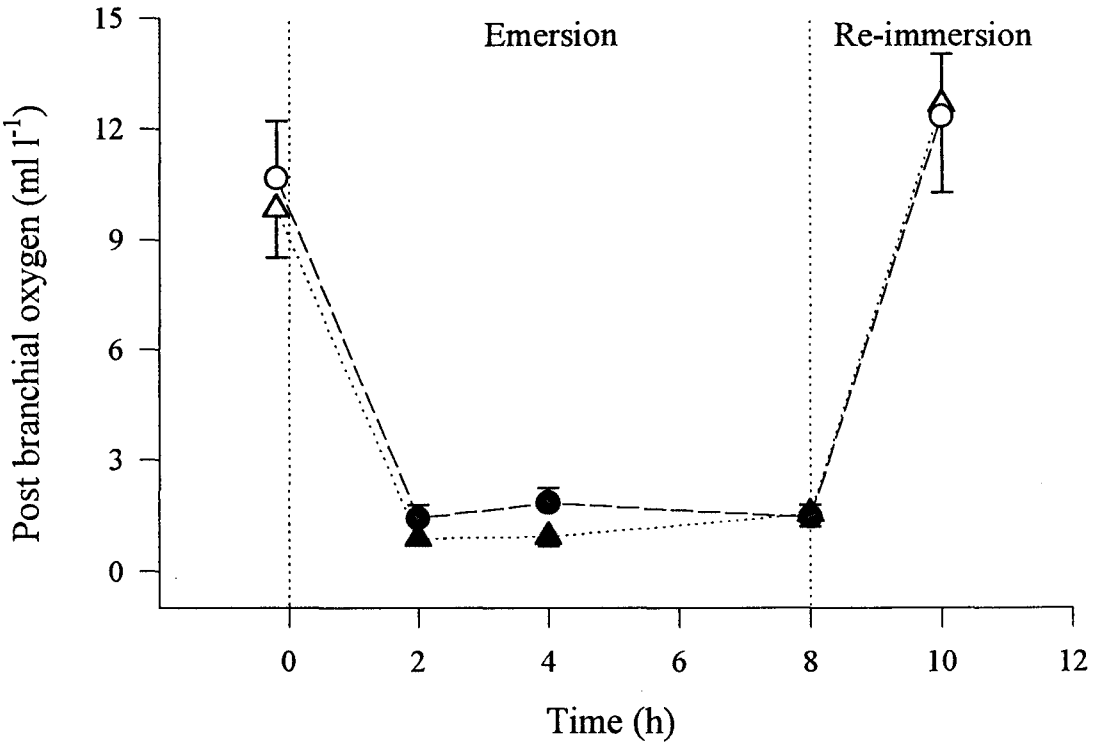


Figure 4.5. *Nephrops norvegicus*: Blood post branchial O_2 concentrations (mean ± SE and n= 8-10) during low (▲) and high humidity (●) periods of emersion and subsequent re-immersion (open symbols refer to immersed prawns).

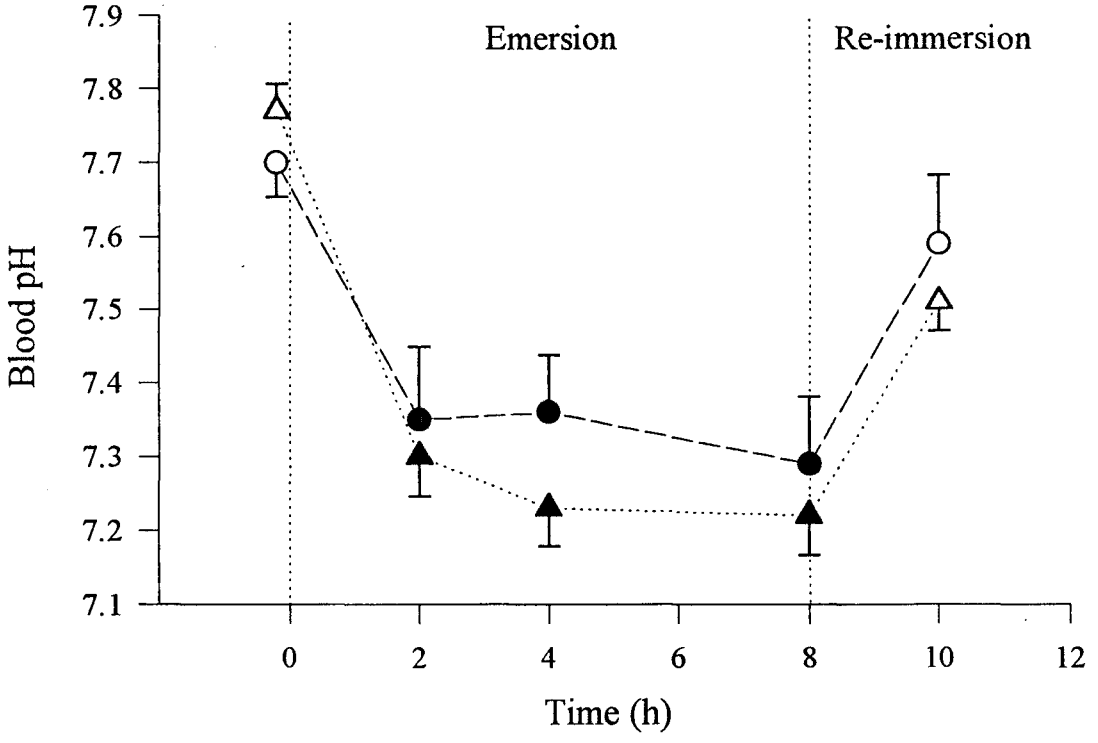


Figure 4.6. *Nephrops norvegicus*: Blood pH (mean ± SE and n= 8-10) during low (▲) and high humidity (●) periods of emersion and subsequent re-immersion (open symbols refer to immersed prawns).

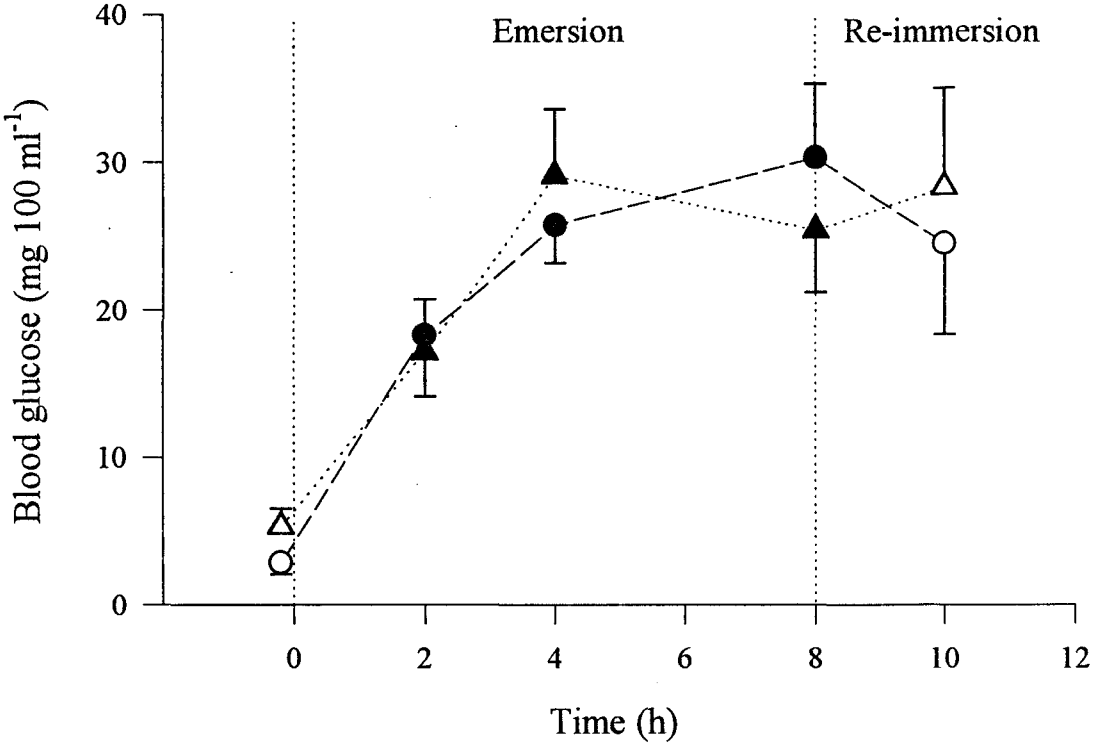


Figure 4.7. *Nephrops norvegicus*: Blood glucose concentrations (mean ± SE and n= 8-10) during low (▲) and high humidity (●) periods of emersion and subsequent re-immersion (open symbols refer to immersed prawns).

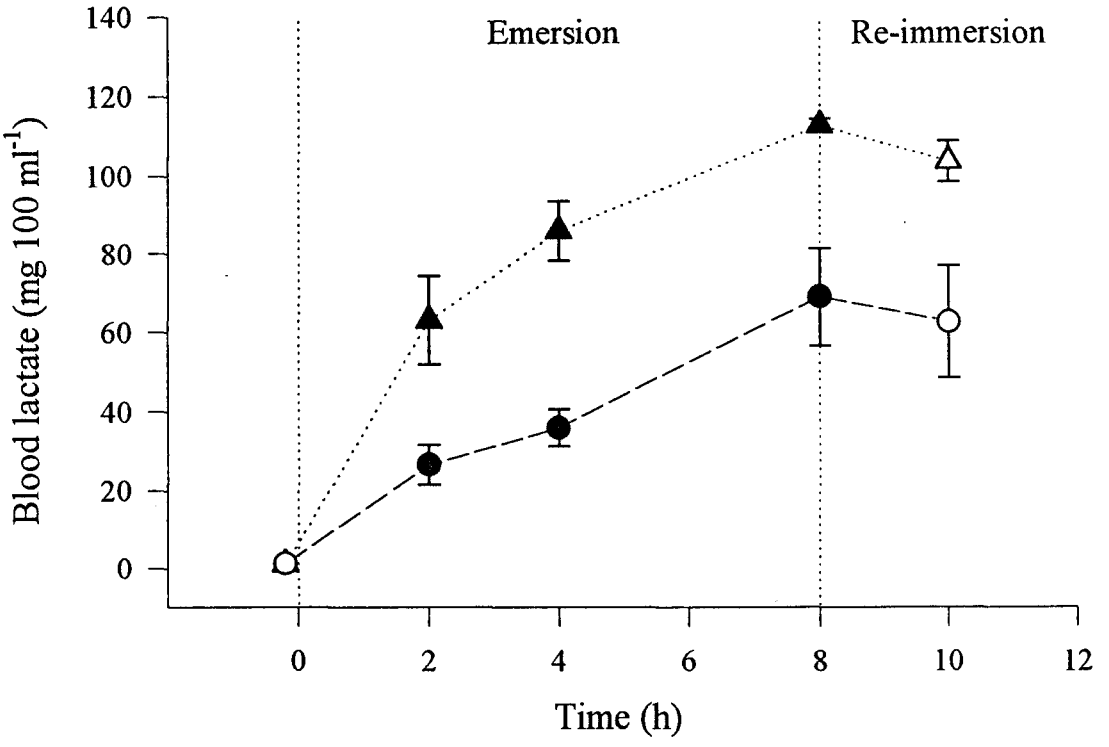


Figure 4.8. *Nephrops norvegicus*: Blood lactate concentrations (mean ± SE and n= 8-10) during low (▲) and high humidity (●) periods of emersion and subsequent re-immersion (open symbols refer to immersed prawns).

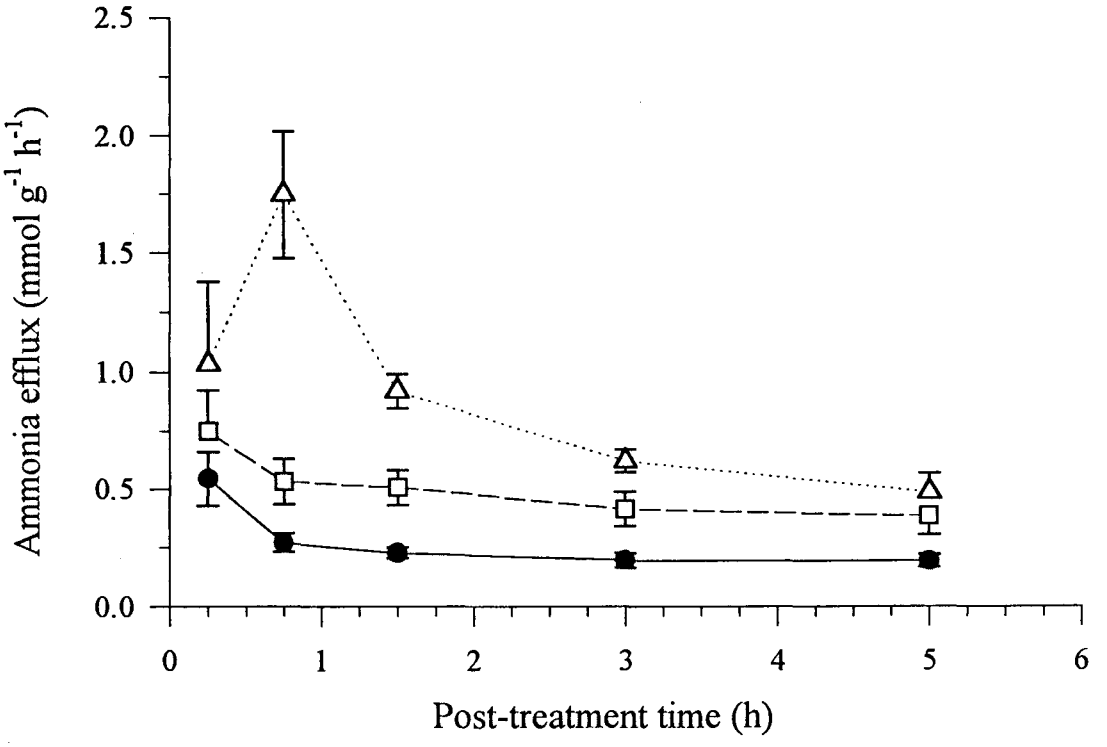


Figure 4.9. *Nephrops norvegicus*: Ammonia efflux (mean ± SE and n= 8 or 9) following 8 h of dry (Δ) or humid (□) periods of emersion and control prawns kept immersed throughout (●).

Chapter 5.

Effects of ambient ammonia levels on blood ammonia, ammonia excretion and heart and scaphognathite rates of *Nephrops norvegicus* (L.).

5.1. Introduction.

Ammoniotelism is a characteristic of aquatic crustaceans. Ammonia is normally formed during various catabolic reactions and is easily excreted across the gills by diffusional movement and/or ionic exchange mechanisms without any further processing (Kormanik and Cameron 1981, Evans and Cameron 1986, Regnault 1987). Amounts and rates of ammonia production and excretion may be influenced by nutritional status, moult stage, activity levels, salinity and temperature (Needham 1957, Regnault 1987, Hunter and Uglow 1993b) and the comprehension of such factors is important in predicting and controlling ambient ammonia levels in aquaculture. Ambient ammonia levels in the sea and freshwater environments are usually very low and the ammonia excreted by the animals under such circumstances rarely imposes a problem to them. The same may not be true when the animals are held in artificial environments, such as aquaculture or the vivier tanks used during live marketing and transport, when ammonia levels may ocasionally rise and compromise the quality of the livestock.

According to Campbell (1991) most of the toxic effects of ammonia are related to pH changes that affect enzyme activity and such effects are known to have caused increased mortality and lowered growth rates in several species of crustaceans (Wickins 1976, Armstrong *et al.* 1978, Provenzano 1983, Chen and Lin 1991, 1992, Chen *et al.* 1990, Lin *et al.* 1993). Toxicity may be affected by environmental and physiological factors. Chen and Lin (1991) found that LC_{50} values for ammonia in *Penaeus penicillatus* was *ca.* 80 % lower at 25 psu than at 34 psu and, according to Wajsbrot (1990), *P. semisulcatus* is twice as sensitive to ammonia at 27 % DO than at 96 % DO saturation and mortality rates caused by ammonia were affected by moult stage. Our understanding of the physiological mechanisms used to cope with progressive or sudden increases in ambient ammonia are also very important but, to date, these aspects have been far less investigated.

Nephrops norvegicus is fished extensively in north European waters and a very small part of the catch is transported and marketed alive or fresh (see General Introduction). During such situations, the animals may be held in poorly-designed vivier tank systems in which water quality control is inefficient or absent and ammonia may accumulate. Freshly-caught *Nephrops* may also be put into systems which already contain high levels of ammonia and the effects of such treatments are unknown. These experiments were designed to evaluate the responses of *Nephrops* to increased dissolved seawater ammonia concentrations.

5.2. Materials and methods.

All experiments were made inside temperature-controlled rooms at 12 °C and 75 % rel. humidity. Total ammonia (TA) concentrations in the stock tank were < 1 μmol l^{-1} and increased TA concentrations were obtained by adding NH_4Cl (Anal-R grade) to seawater. All TA values of ammonia-spiked seawater refer to approximate values. Water samples were taken from all the solutions used and their TA contents did not differ more than 1 % of calculated values.

The effects of ambient ammonia on blood ammonia concentrations of *Nephrops* were studied in two separate experiments. In the first, 120 prawns from the stock tank were transferred (20 animals each) to 6 plastic containers (35 x 45 cm, base) with 30 l of ammonia-spiked seawater (2000 μmol l^{-1}). Blood samples were collected from animals from the stock tank and from 60 animals (10 each time) at 10, 30, 60, 120, 240 and 360 min following transfer to the plastic containers. The remaining 60 animals in the plastic containers were returned to the stock tank and the sampling procedure repeated once again. In the second experiment, 20 prawns were placed in each of 5 plastic containers each provided with a different concentration of ammonia-spiked seawater (0, 500, 1000, 2000 or 4000 μmol l^{-1}). Haemolymph samples (200 μl) were collected *via* the pereiopod sinus using disposable syringes and needles. Initial samples were taken from 10 prawns of each concentration after 9 h exposure. The remaining 10 animals at each concentration were placed individually in rectangular plastic containers each supplied with 1.5 l of seawater from the stock tank and their TA efflux rates were then monitored. Water samples (1.5 ml each) were collected from each of the individual experimental containers at times 0, 15, 30, 60, 120, 310 and at 400 min. Final haemolymph samples were then collected from these animals. Calculated TA efflux rates were based on the difference between the concentrations obtained for two consecutive (timed) samples.

Heart (HR) and scaphognathite rates (SR) were measured in a flow-through system consisting of a header tank, a sump, a water pump and the respirometers described in the general materials and methods (Figure 2.5a). The total volume of the system was 122 l in the first experiment and 130 l in the second experiment and the flow rate through each individual container was 1.9 l min^{-1}. Prawns were individually placed in the small containers and the electrode wires passed through a Suba-seal placed on the lid. Water TA concentrations were altered without handling or interfering with the prawns at any time.

The effects of ambient ammonia on *Nephrops* HR and SR were analysed in two different situations. In one experiment water TA concentrations were increased

from control conditions to 2000 μmol l^{-1} by adding NH_4Cl into the sump and header tank and allowing the system to recirculate. Twenty four hours after that, the water TA concentration was lowered by replacing the water in the sump and header tank with clean seawater whilst the containers were closed. Then the small containers were allowed to flush out of the system for *ca.* 10 min with clean seawater from the header tank. TA concentrations in the system were thus reduced to 15 μmol l^{-1}. HR and SR were monitored during 9 h following each alteration of water TA levels (increase and decrease).

In a second experiment, *Nephrops* were placed in the flow-through system and ambient TA concentration was progressively increased over a period of 6 h. Such increases were performed by adding pre-weighed amounts of NH_4Cl into the sump every 20 min and HR and SR were registered every hour. After 6 h TA levels were lowered to 70 μmol l^{-1} using the same procedure described above for the previous experiment and HR and SR were measured one hour later.

5.3. Results.

All animals used in these experiments survived exposure to increased ambient ammonia concentrations. Figure 5.1 shows the results obtained for blood ammonia concentrations and blood pH following transfer of *Nephrops* from normal seawater (< 1 μmol TA l^{-1}) to enriched (2000 μmol TA l^{-1}) seawater. Blood TA levels increased significantly ($P<0.05$) more than 7-fold within 10 min of transfer and maximum values were measured at 2 h following transfer. Blood TA levels then decreased significantly ($P<0.05$) over the following 4 h but were still significantly ($P<0.05$) higher than the original values. Blood pH dropped significantly ($P<0.05$) 0.15 pH units to a minimum value measured after the first hour following transfer but progressively regained original pH values during the remaining 5 h of exposure (Figure 5.1). After 6 h of exposure to enriched medium, the *Nephrops* were transferred back to clean seawater which, after 10 min, resulted in a significant drop ($P<0.05$) in mean blood TA levels from 807.31 ± 34.55 to 392.18 ± 27.70 μmol TA l^{-1}. Blood TA values then remained relatively stable but had dropped significantly ($P<0.05$) once again after 4 h (Figure 5.1.). The blood pH remained unchanged during the first 10 min in clean seawater but then rapidly dropped ($P<0.05$) over the following 20 min and reached minimum values 2 h following transfer to clean seawater. For the remainder of the measuring period, the blood pH rose steadily and, after 6 h, values were not significantly different ($P>0.05$) from the original values measured.

Table 5.1 summarises the data obtained on blood TA and glucose levels and pH values when groups of prawns were transferred from clean seawater to media enriched with various levels of ammonia. Blood TA levels increased significantly ($P<0.05$) in all groups after 9 h exposure. When returned to clean seawater, mean blood ammonia levels dropped and although original values were not regained in the 5 h recovery period, the values attained were not signicantly different ($P>0.05$) from those of the control group which was subjected to similar handling procedures.

Blood pH increased significantly ($P<0.05$) in the groups transferred to 500, 1000 and 2000 μmol TA l^{-1} but, after return to clean seawater, only the blood pH of the 2000 μmol TA l^{-1}-treated group dropped significantly ($P<0.05$) (Table 5.1). When exposed to 4000 μmol TA l^{-1} seawater, then blood pH dropped to a value significantly lower than the control group and increased significantly when returned to clean seawater ($P<0.05$ in each case).

Hyperglycemia occurred in all groups when originally removed from the stock tank and placed in the experimental containers under ammonia-enriched conditions. When transferred to the smaller containers containing clean seawater,

the blood glucose levels dropped to values not significantly different from original values ($P<0.05$ in all cases).

TA efflux rates immediately after transfer from 500, 1000, 2000 and 4000 μ mol TA l^{-1} to <1 μmol TA l^{-1} were respectively 1.65 ± 0.11, 1.98 ± 0.13, 3.14 ± 0.38 and 6.46 ± 0.51 μmol g h^{-1} and all these values were significantly higher ($P<0.05$) than that of the control group (0.53 ± 0.14 μmol g h^{-1}) (Figure 5.2.). TA efflux rates of all experimental groups subsequently decreased and, apart from the 4000 μmol TA l^{-1}-treated group, the values of TA excreted between 0.5 and 1 h after transference were not significantly different ($P>0.05$) than those of the control group. TA excreted by prawns transfered from 4000 μmol TA l^{-1} reached control values between 1 and 2 h after transference ($P>0.05$).

The HR and SR of prawns submitted to progressive increases in water TA concentrations are shown in Figure 5.3. Inital HR and SR were respectively 43.40 ± 7.02 and 88.17 ± 8.92 beats min^{-1} and they increased significantly ($P<0.05$) to 83.27 ± 5.84 and 171.99 ± 23.90 beats min^{-1} when water TA reached 4000 μmol TA l^{-1}. After that, following the water TA concentration reduction to 70 μmol TA l^{-1}, HR and SR still remained significantly higher ($P<0.05$) than initial values. The HR and SR of prawns transferred from <1 to 2000 μmol TA l^{-1} and back to <1 μ mol TA l^{-1} are shown in Figure 5.4. Initial HR and SR were respectively 44.67 ± 8.73 and 96.15 ± 18.49 beats min^{-1} and such values were not significantly altered ($P>0.05$) throughout the experimental time, despite an aparent trend for rates to increase initially.

5.4. Discussion.

By virtue of its small molecular size and high solubility, ammonia can diffuse rapidly across biological membranes (Schmidt-Nielsen 1983) and when crustaceans are exposed to media with dissolved ammonia levels greater than those occurring in the blood, an influx-induced elevation of blood ammonia levels may occur. Chen and Kou (1993) measured ammonia influx in *Penaeus monodon* exposed to various ambient ammonia levels and showed that influx rates were directly related to water pH. In these studies, a 9 h exposure period to various ammonia-enriched media induced elevated blood TA levels but, in all cases, blood TA levels failed to equilibrate with ambient levels. In all the media with TA concentrations higher than 500 μmol TA l^{-1} the prawns were able to maintain internal TA values at *ca* 30 % of the ambient levels (Table 5.1). This suggests strongly that regulatory mechanisms were operating to remove and/or transform the accumulating blood ammonia. Further evidence of a regulatory system is provided by the data summarized in Figure 5.1, which show that, on exposure to ammonia-enriched medium (2000 μmol TA l^{-1}) the blood ammonia rose from 5.6 to 59 % of the ambient levels after 2 h but dropped to 40 % ambient TA levels after 6 h.

When prawns were returned to clean seawater after exposure to TA-enriched media, their recovery was rapid but not immediate, with TA efflux rates reaching those of control prawns within 1 h (Figure 5.2). Most of the ammonia accumulated in the blood had apparently disappeared within 10 min of the recovery period and blood TA levels then remained constant until decreasing further after 4 h. This period of stable, supranormal blood TA levels in normal medium may reflect a period when accumulated ammonia was being removed from other tissues into the haemolymph or when other forms of nitrogen were being reconverted to ammonia.

Ammonia may be removed from the haemolymph by excretion or by storage in some other tissue. Excretion of ammonia against a concentration gradient has been shown to occur and is clear evidence of the presence of ionic exchange systems involving NH_4^+ (Kormanik and Cameron 1981). Active transport of ammonia across the gills of water breathing animals may take place via basolateral or apical Na^+/NH_4^+ exchange (Evans and Cameron 1986). If such mechanisms were responsible for maintaining low internal TA levels, then an increased energy demand may be needed to cope with the requirements of intense ionic exchange. In the present studies, however, blood glucose levels did not appear affected by exposure to ammonia enriched media. The hyperglycaemic response found occurred also in the control prawns subjected to similar handling procedures.

Furthermore, Chen *et al.* (1993) found that ammonia effluxes were less than influxes when *Penaeus chinensis* were exposed to *ca.* 700 and 1400 μmol TA l^{-1} and thus demonstrating that active transport mechanisms could not match the inward diffusion from the external environment at such concentrations.

The TA content of the blood of each prawn exposed to an enriched medium was calculated on the basis of blood volume = 30 % fresh body weight (Table 5.1) and the amount of TA excreted by each animal following return to clean seawater was also calculated (Table 5.2). These data reveal that the amount excreted during recovery exceeded by far that accumulated in the blood during exposure. The values shown in Table 5.1. refer only to TA accumulated in the haemolymph but, even if TA accumulated equally among all tissues, internal TA accumulated would still be lower than that excreted during recovery. This is an indication that ammonia was either stored elsewhere or, more likely, was transformed to some other nitrogenous during exposure.

Regnault (1994) raised the possibility that emersed crabs may store ammonia in some fluid compartment or in the acidic gut fluids. A further possibility could be the amination of ammonia as a detoxification process. Ammonia may be used to produce glutamate (Claybrook 1983) and the reversible reaction (ammonia formation and glutamate synthesis) is controlled by the activity of the enzyme GDH (Batrel and Regnault 1985, King *et al.* 1985, Regnault 1987, Regnault and Batrel 1987). An increased GDH activity towards glutamate synthesis was found in emersed *Cancer pagurus* which, presumably, were unable to excrete ammonia in the absence of gill chamber ventilation (Regnault 1992). Some or all of these mechanisms may have occurred in the present experiments as a means of reducing the effects of ammonia influx but, further research on GDH activity on *Nephrops* is necessary to elucidate this aspect.

If no regulatory mechanism existed and internal and external ammonia levels were in equilibrium, the theoretical values of the excess blood ammonia content of the animals exposed to the media mentioned above over that of the control group would be 7.68, 16.74, 40.65 and 90.28 μmol prawn l^{-1}. These theoretical values for the 500 and 1000 μmol TA l^{-1} groups are still less than the quantities of ammonia excreted during recovery. It would appear that, at these exposure levels, ammonia was removed from the haemolymph creating a gradient by which more ammonia diffused in. At the higher concentrations it may be that the stored or transformed ammonia accumulated under exposure was less rapidly reconverted to ammonia during recovery.

Blood pH may be affected by a variety of environmental and physiological factors and branchial excretion of acid-base equivalents is one of the mechanisms

used to regulate pH (see Truchot 1983, Wheatly and Henry 1992, Truchot 1994 for a review). Sudden influxes of ammonia may affect this balance as NH_3 entering the body may cause an alkalosis as it may capture H^+ to form NH_4^+. This did not happened in the present experiments and the small, transient decreases in blood pH shown in Figure 5.1 may have been the result of metabolic and/or respiratory acidosis at such times.

Cardiac and ventilatory beat activity changes have been measured in association with physiological responses of crustaceans to altered environmental conditions in a number of studies. Temperature have been shown to have a direct effect on such rates and the temperature coefficients (Q_{10}) values obtained generally aproach those which are considered to tipify physiological processes (2-3) (deFur and Mangum 1979, Spaargaren and Achituv 1977). Perfect acclimation to a changed temperature may also occur, as shown in *Calinectes sapidus* (Burton *et al.* 1980). Salinity-induced rate changes have also been found but such responses appear to be variable (deFur and Mangum 1979) and may be influenced by the rate in which the alteration is effected (Dyer and Uglow 1980). Ambient $P_{W}O_2$ may affect $P_{a}O_2$ and $P_{V}O_2$ and compensatory adjustments of the circulatory and ventilatory systems have been described (Coyer 1977, Hagerman and Uglow 1985, Taylor *et al.* 1973, Uglow 1973, Wilkens *et al.* 1984). The majority of species studied have shown a bradycardia or unchanged heart rates accompanied by increased scaphognathite rates until a critical $P_{W}O_2$ is achieved and increased hypoxia beyond this levels results in pronounced bit retardations. Such adjustments are, presumably, related to the oxygen carrying performances of the blood and, ultimately, to the oxygen consumption of the animals.

As shown by Angersbach and Decker (1978) on *Astacus leleptodactilus*, an increase in scaphognathite beat rate can increase $P_{a}O_2$. Ambient ammonia concentrations may also be expected to impact on cardio-ventilatory performances as ammonia too may enter or exit the blood *via* the branchial tissue. Kwee (1993) found that increases in ambient ammonia (*ca* 0-100 μmol TA l^{-1}) resulted in elevated HR and SR increases, but this author worked with progressive increases in water TA and at a much lower range of concentrations than the ones used here. In the present studies, both the HR and SR were ultimately altered by water TA concentrations but only at concentrations of 2400 μmol TA l^{-1} and above (Figure 5.3). Even when organ rates were monitored immediately after an increase in ambient TA to 2000 μmol TA l^{-1}, changes in HR and SR were not apparent (Figure 5.4). *Nephrops* is a burrowing species found principally associated with fine deposits which indicate a general lack of water currents and such species may be expected to be tolerant of low $P_{W}O_2$ and high TA concentrations in their burrows.

This may be why only very high TA concentrations affected HR and SR and such responses have little or no ecological relevance as such TA levels are unlikely to occur naturally. However, *Nephrops* is a species of considerable commercial importance and is frequently marketed alive and very high TA levels may be found if the prawns are held in poorly-designed systems or at high densities. The elevated HR and SR levels measured at high TA levels possibly reflect an altered metabolism caused by the toxic effects of ammonia. The maintenance of elevated organ beat rates 1 h after ambient TA was brought back to normal levels reinforces this view as excess blood ammonia is comparatively rapidly flushed from the blood. Besides, elevated organ rates are just likely to enhance influxes in high ambient TA media as effluxes in low ambient TA media.

These data on cardio-ventilatory responses to very high dissolved ammonia levels are preliminary and further work on ammonia fluxes in association with the activity of such organs need to be made to evaluate their physiological relevance.

Table 5.1. *Nephrops norvegicus*: Blood ammonia and glucose concentrations and pH of prawns exposed for 9 h in 18 l containers to various concentrations of ammonia and 5 h after subsequence transfer to clean seawater (< 1 µmol TA l^{-1}) in 1.5 l containers. Control prawns were kept in low ammonia seawater at all times. The absolute blood TA levels refer to the accumulated values above blood TA of control prawns in 18 l. Values are mean ± SE of n = 9 or 10 in each case.

Experimental condition.	Glucose (mg 100 ml^{-1})	pH	TA (µmol l^{-1})	TA_{in}/TA_{out} (%)	TA (µmol $prawn^{-1}$)
Control prawns from stock tank.	2.23 ± 0.44	7.69 ± 0.01	91.26 ± 5.63		
Control prawns in 18 l containers.	7.39 ± 1.59	7.82 ± 0.03	104.92 ± 13.01		
Control prawns in 1.5 l containers.	3.28 ± 0.74	7.84 ± 0.03	174.59 ± 16.95		
500 µmol l^{-1}	6.45 ± 1.51	7.86 ± 0.02	284.70 ± 28.66	56.94	1.31 ± 0.21
500 to < 1 µmol l^{-1}	4.74 ± 1.41	7.83 ± 0.02	203.11 ± 16.06		
1000 µmol l^{-1}	9.64 ± 1.57	7.89 ± 0.01	336.81 ± 12.93	33.68	1.69 ± 0.09
1000 to < 1 µmol l^{-1}	3.52 ± 0.77	7.82 ± 0.02	253.04 ± 27.10		
2000 µmol l^{-1}	6.03 ± 1.88	7.81 ± 0.01	606.38 ± 33.04	30.00	3.66 ± 0.24
2000 to < 1 µmol l^{-1}	2.31 ± 0.44	7.72 ± 0.02	164.87 ± 12.62		
4000 µmol l^{-1}	12.76 ± 3.15	7.63 ± 0.03	1456.18 ± 57.55	36.40	9.86 ± 0.42
4000 to < 1 µmol l^{-1}	4.78 ± 1.26	7.77 ± 0.03	255.41 ± 33.09		

TA_{in}/TA_{out} = relation between haemolymph and external TA concentrations.

Table 5.2. *Nephrops norvegicus:* Absolute values of excreted ammonia (μmol prawn^{-1}) in excess of that excreted by control following 9 h exposure to various ammonia concentrations and subsequent transfer to clean seawater (< 1 μmol l^{-1}). Values are given as mean ± SE of n = 9 or 10.

Time after transfer (h)	Exposure concentration			
	500 μmol l^{-1}	1000 μmol l^{-1}	2000 μmol l^{-1}	4000 μmol l^{-1}
0.25	7.89 ± 0.86	9.53 ± 1.04	15.43 ± 2.18	38.29 ± 3.27
0.5	11.79 ± 1.23	14.43 ± 1.75	21.31 ± 2.36	51.86 ± 4.06
1	14.43 ± 1.81	17.27 ± 2.35	27.79 ± 3.55	62.53 ± 5.47
2	19.70 ± 3.18	21.48 ± 4.37	34.26 ± 5.69	73.44 ± 6.50
3.5	27.32 ± 4.72	29.99 ± 5.63	36.14 ± 8.34	87.38 ± 9.70
5	30.91 ± 5.61	34.56 ± 23.29	32.41 ± 10.06	86.38 ± 10.17

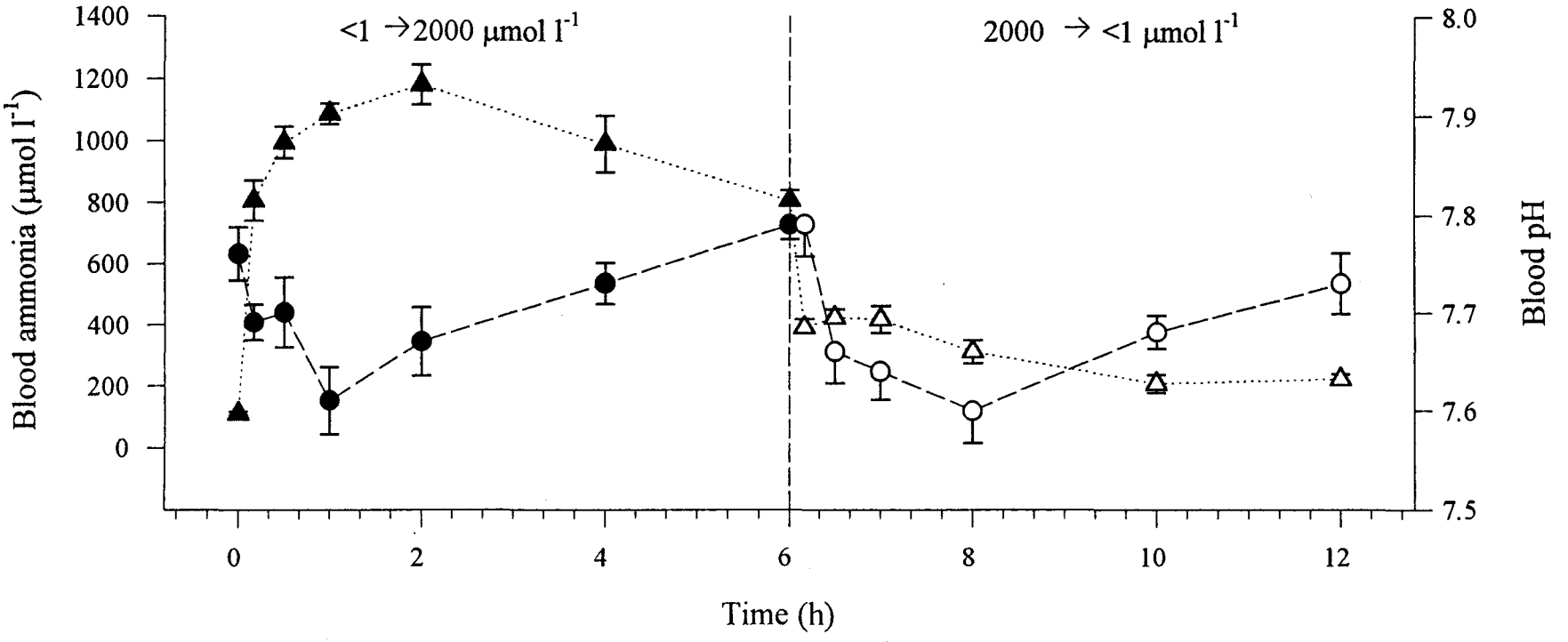


Figure 5.1. *Nephrops norvegicus*: Haemolymph total ammonia (TA) concentrations (▲) and pH (●) following exposure to ammonia-enriched (2000 µmol TA l^{-1}) seawater (closed symbols) and following return to clean (< 1 µmol TA l^{-1}) seawater (open symbols). Values are given as mean ± SE for n = 9 or 10 in each case.

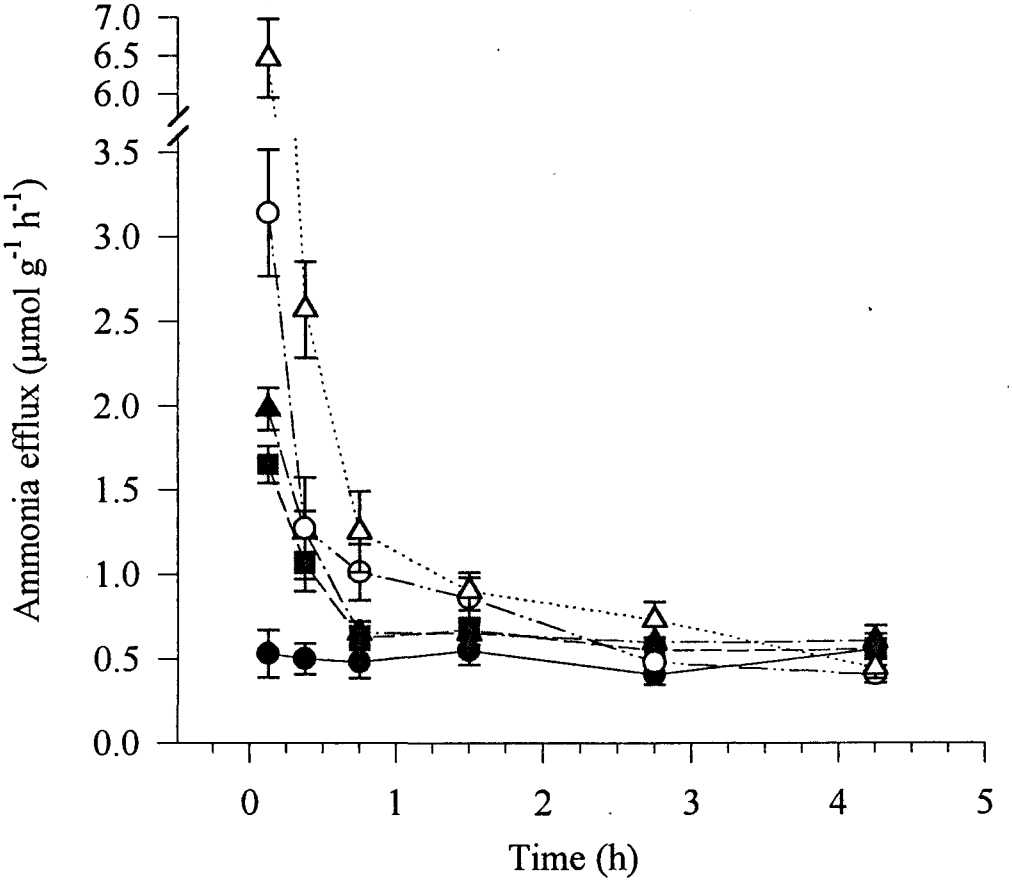


Figure 5.2. *Nephrops norvegicus*: Weight-specific total ammonia (TA) efflux rates following 9 h exposure to seawater containing 500 (■), 1000 (▲), 2000 (o) and 4000 (Δ) µmol TA l^{-1} and following return to clean (< 1 µmol TA l^{-1}) seawater. Control prawns (●) were kept in low TA seawater at all times. Values are given as mean ± SE for n = 9 or 10 in each case.

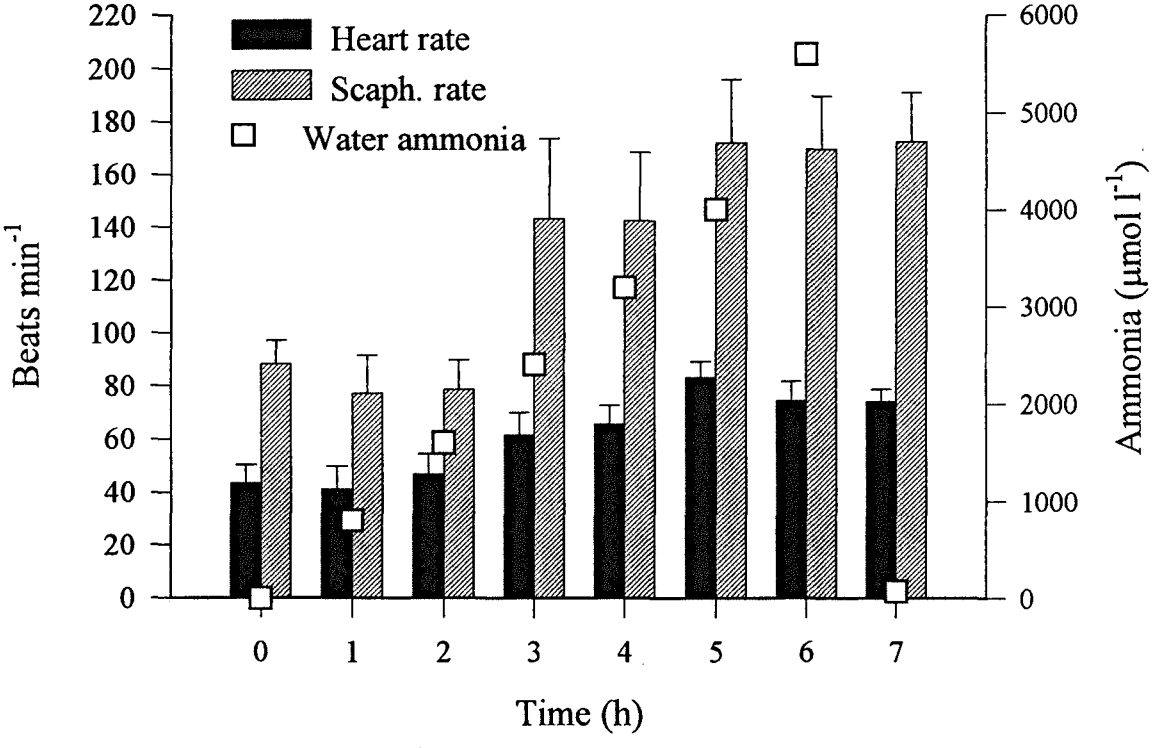


Figure 5.3. *Nephrops norvegicus*: Mean heart and scaphognathite rates during progressive increase of ambient ammonia levels and after return to low ammonia concentration seawater. Values are given as mean ± SE for n = 8 in each case.

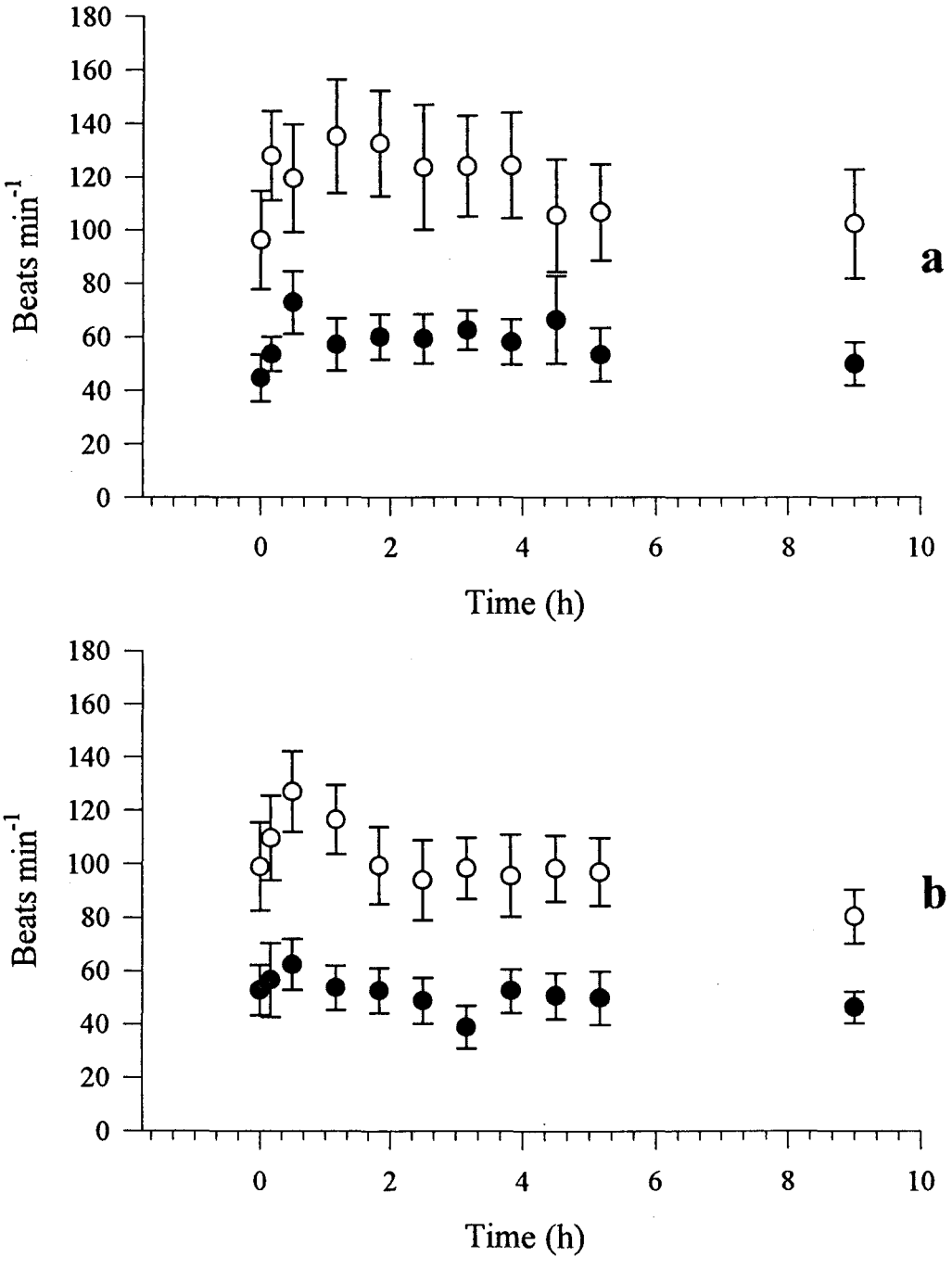


Figure 5.4. *Nephrops norvegicus*: Mean heart (●) and scaphognathite (o) rates following exposure to ammonia-enriched (2000 μmol TA l^{-1}) seawater (**a**) and following return to low ammonia seawater (**b**). Values are given as mean ± SE for n = 8 in each case.

Chapter 6.

Some effects of progressive hypoxia on *Nephrops norvegicus*

6.1. Introduction.

During commercial practices of marketing live crustacean shellfish there are a number of situations when ambient oxygen may decrease and critical levels of hypoxia occur. At present, the procedures used in the trade of live, creel-caught, Scottish *Nephrops norvegicus* involve the maintenance of prawns, for variable periods of time, in vivier tanks at the dealers' premises (M. MacDonald, D. MacCrae, pers. comm.). Such procedures are used to enable dealers to build up sufficient numbers of prawns to make a consignment economically viable to be transported. The water filtration and circulation systems of these tanks are usually poorly-designed and, due to a high biomass/water ratio, a progressive reduction in dissolved oxygen may occur in them. Additionaly, such systems are not specially designed to hold *Nephrops* and are also used to hold other species of crustaceans such as: *Necora puber*, *Homarus gammarus*, *Cancer pagurus* and *Panulirus argus*, depending on which are available at the time. This may cause increased activity and oxygen consumption rates in the system due to excessive mutual interaction and this may exacerbate the hypoxia-related problems.

Exposure to hypoxia may affect several metabolical and physiological processes and, under such conditions, compensatory mechanisms to maintain the energy supply to the tissues are required. The respiratory adjustments to ambient hypoxia usually include alterations in the cardio-ventilatory activity and modulation of blood oxygen affinity. Scaphognathite beat rate normally increases to compensate for the reduced P_{wO_2} but heart rate responses are usually more variable, depending on the species and experimental conditions used, and may not occur at all (Uglow 1973, Coyer 1976, deFur and Mangum 1979, Hagerman 1982, McMahon and Wilkes 1983, Hagerman and Uglow 1985, Taylor and Spicer 1991). Despite being relatively low (Mangum 1983), the oxygen affinity of crustacean haemocyanins was found to increase with increased blood urate, lactate and CO_2 concentrations and pH values (Truchot 1975, Graham et al. 1983, Lallier and Truchot 1989, Burnett 1992, Truchot and Lallier 1992) and this may enhance oxygen extraction during a situation of hypoxia (Lallier and Truchot 1989).

Another type of adjustment to hypoxia is related to the metabolic pathways used for energy production. When P_{wO_2} falls to a critical level, the energy supply to the tissues may be obtained partially or totally by using anaerobic pathways, resulting in increased concentrations of blood glucose and lactate. Such alterations may cause a reduction in the reserves of carbohydrate, as found in *Nephrops* by Baden *et al.* (1994). These are classic response to hypoxia which have been found in several species submitted to various experimental conditions.

The survival rate of *Homarus americanus* to hypoxia is higher in lobsters previously acclimated to a reduced P_{wO_2} than in those lobsters acclimated to normoxic water (McLeese 1956). In a similar way, it may be expected that the responses of animals submitted to progressive reductions in P_{wO_2} will differ from those shown by animals submitted to sudden P_{wO_2} reductions. Some effects of hypoxia on *Nephrops* blood chemistry, cardio-ventilatory rates and some other physiological aspects have been studied previously under different experimental conditions (Hagerman and Uglow 1985, Hagerman et al. 1990, Hosie et al. 1991, Smullen and Bentley 1993). The present study, however, aimed to analyse the effects of progressive reductions in P_{wO_2} by simulating in the laboratory a situation that may occur during the normal trading practices of *Nephrops norvegicus*.

6.2. Material and methods.

These experiments were performed using the respirometers shown in the General Material and Methods section (Figure 2.5). The system used consisted of 10 respirometers which were supplied with seawater from a header tank. The seawater flowing through the respirometers (*ca* 1.2 l min^{-1}) was taken to a sump where aeration and biological filtration was provided and then pumped back to the header tank. Such procedures were designed to maintain optimum water quality while the prawns were acclimating to these new conditions. In all experiments, one of the respirometers was kept without prawn but was otherwise subjected to the same experimental procedures and used as a control for bacterial action on P_{wO_2}. The effects of progressive hypoxia on various aspects of *Nephrops* physiology were evaluated in two sets of experiments. P_{wO_2} reductions were effected in sealed respirometers as a result of the $\dot{M}O_2$ of the prawns and other substances such as ammonia and CO_2 were also expected to accumulate.

In a first set of experiments, 9 prawns were placed in the system described above and, after an acclimation period of 12 h, the inlet and outlet valves were shut for 16 h. After that, water samples were collected for ammonia, oxygen and pH determinations. The prawns were then removed from the respirometers and samples of pre and post branchial haemolymph were immediately collected using gas tight syringes, as described in General Material and Methods, and their oxygen content determined. After the collection of these samples, additional haemolymph samples (300 µl) were collected through the arthrodial membrane of one of the pereiopods for lactate, glucose, protein and ammonia determinations and for pH measurements. 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.

The second experiment dealt with simultaneous heart (HR) and scaphognathite (SR) beat rate measurements, oxygen consumption and ammonia efflux rate measurements. Electrodes were attached to the prawns according to General Material and Methods (Figure 2.6a) and the prawns placed in the respirometers which were with running seawater. After the period of acclimation (48 h), the inlet and outlet valves were shut and the respirometers remained sealed for 33 h, causing a gradual reduction in P_{wO_2}. Immediately after that, both valves were reopened and fully air-saturated seawater was flushed through the respirometers for 10 min. The lid of the respirometers was made of clear Perspex which was partially covered with black plastic to avoid any undesigned interferences on the HR and SR of the prawns. Water samples were periodically collected *via* the

Subaseal fitted into the lid of each respirometer and their 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 samples.

6.3. Results.

There were no differences between the peak height of samples collected from the control respirometer and those taken from the aerated seawater from the sump during all experiments and, therefore, bacterial action under such circumstances was considered negligible.

The results obtained are shown in two ways. In Table 6.1 and 6.2, the results are shown as mean values for each of the sampling times and in Figures 6.1 to 6.7 the individual results are shown, as a function of oxygen tensions in the water or oxygen concentrations in the blood. Due to the individual variability in oxygen consumption during the experimental period, the P_{wO_2} reduction rates were also variable. The presentation of individual values aimed to illustrate better the pattern obtained in the experiments which was not allways clearly shown when the data were grouped as mean values as a function of time.

In Table 6.1, P_{wO_2} decreased from full saturation (*ca* 155 torr) down to 19 torr 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 37.77 ± 4.53 µmol TA l^{-1} during the same period. C_{aO_2} and C_{vO_2} decreased progressively with decreasing P_{wO_2} but values were only significantly lower ($P<0.05$) than initial values when P_{wO_2} was 46.99 torr and 66.98 torr, respectively. Blood glucose concentrations were stable for most of the experimental time and only increased significantly ($P<0.05$) after P_{wO_2} reached very low levels (20.20 torr). Blood lactate was the parameter showing the highest individual variability, as shown by the high standard error values found. Lactate started to increase when P_{wO_2} decreased to *ca* 47 torr but, because of such high variability, these increases were not significantly different ($P>0.05$). Blood pH remained relatively stable during P_{wO_2} reductions to *ca* 47 torr but then increased significantly ($P>0.05$) with further reductions in P_{wO_2}. The difference between C_{aO_2} and C_{vO_2} (oxygen extracted and used by the prawns) was not significantly altered by a reduction in P_{wO_2}.

When the individual results are plotted as a function of oxygen, it is possible to observe that blood glucose was unaffected until P_{wO_2} decreased to 17 torr or when C_{aO_2} and C_{vO_2} reached 4.2 ml l^{-1} and 1.6 ml l^{-1}, respectively (Figure 6.1). This is similar to the pattern shown for blood glucose in Table 6.1, but the same is not true of blood lactate concentrations. In Figure 6.2, it is clear that blood lactate remained stable until P_{wO_2} decreased to 16 torr and the point where lactate starts to show increases seems to be even more precisely determined by C_{vO_2} (0.5 ml l^{-1}). Blood glucose started to increase before the lactate and this aspect is also shown in

Figure 6.3 where high values of blood lactate were found only when associated with increased concentrations of blood glucose. Water pH was found to be linearly related to P_{wO_2} ($r^2 = 0.948$) and individual values of blood pH show acidosis at very low P_{wO_2} (Figure 6.4). C_{aO_2} and C_{vO_2} are plotted as a function of P_{wO_2} in Figure 6.5.

During the second set of experiments, P_{wO_2} decreased from 153 torr to 44 torr in 33 h and increased again to 104 torr after the respirometers were flushed with normoxic water (Table 6.2). The TA values measured during this period oscillated between <1 and 17.5 μmol TA l^{-1}. Water pH dropped significantly ($P<0.05$) *ca* 0.40 pH points 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 P_{wO_2} 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 44 torr. SR started to increase after P_{wO_2} reached 54 torr ($P<0.05$), 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 P_{wO_2} alterations.

The individual values of HR and SR during P_{wO_2} reductions are shown in Figures 6.6 and 6.7. HR shows a large individual variability and the lack of P_{wO_2} dependence is evident. SR was also variable but the trend to accelerate with hypoxia is shown.

6.4. Discussion.

The ability to withstand hypoxia is highly variable interspecifically amongst crustaceans and the metabolic and physiological responses shown may relate to the natural habitat of the different species. Burrowing species, such as *Nephrops*, that live in muddy substrata (Figueiredo and Thomas 1967) are more likely to be exposed to reduced levels of P_{wO_2} and may, therefore, be more adapted to face hypoxia than those species normally living in well-aerated water (see Vernberg 1983 for a review). They may be oxyregulators, with a P_{cr} of oxygen below which $\dot{M}O_2$ is depressed, or they may be oxyconformers. *Callinectes sapidus* maintained normoxic levels of O_2 extraction efficiency (53 %) throughout stepwise reductions of P_{wO_2} (minimum of 24 torr) and this resulted in reduced rates of $\dot{M}O_2$ during hypoxia (Batterton and Cameron 1978). Similar results were found with the mud crab *Scylla serrata*, which was found to be an oxyconformer and totally unable to extract oxygen from seawater at tensions below 4.2 torr (Davenport and Wong 1987). A different type of response was found here with *Nephrops*, which may be exposed to seasonal periods of hypoxia (< 30 % saturation) (Hagerman and Baden 1988, Baden *et al.* 1990). In these experiments, $\dot{M}O_2$ rates were found to be constant over a P_{wO_2} range from fully-saturated seawater to 44.26 torr (Table 6.2) and this shows that *Nephrops* were able to extract oxygen from the water at these levels of P_{wO_2}. The values obtained for C_{aO_2} - C_{vO_2} agree with such results, as they were also maintained at relatively constant levels over a wide range of P_{wO_2} (155-19 torr). Lallier and Truchot (1989), found that *Carcinus maenas* ΔP_{O_2} (P_{aO_2} -P_{vO_2}) was significantly reduced imediately after exposure to hypoxia and then increased slightly with exposure time, probably due to the modulation of hemocyanin oxygen affinity.

The $\dot{M}O_2$ values found in the present experiments are similar to those measured for *Nephrops* by Hagerman and Uglow (1985) and they show that *Nephrops* is able to maintain energy supplies using aerobic pathways at P_{wO_2} = *ca* 44 torr. *Munida rugosa* and *M. sarsi*, both species that may inhabit the same type of area as *Nephrops*, also showed independent $\dot{M}O_2$ rates over a wide range of P_{wO_2} and had a P_{cr} which was found to be 40-55 torr (Zainal *et al.* 1992). It is emphasised that *Nephrops* were carefully-handled and kept individually-separated at all times during the present experiments. When held at commercial facilities, the increased activity and stress that accompany normal marketing procedures will change such aspects and functional anaerobiosis, as described by Gäde (1984), may prevail.

Anaerobic responses, such as hyperglycaemia and increased concentrations of blood lactate have been found in *Nephrops* exposed to hypoxia (Hagerman *et al.* 1990) and these authors observed such responses but only when the prawns were submitted to very low P_{wO_2} (16 torr). Smullen and Bentley (1993) also found transient increases in blood glucose during exposure of *Nephrops* to reduced P_{wO_2}. Forgue *et al.* (1992) found increased blood lactate concentrations in *Eriocheir sinensis* below *ca* 10 % O_2 saturation but the $\dot{M}O_2$ was still constant even at this P_{wO_2}. This finding does not agree with the general understanding that anaerobic pathways are switched on only after aerobic pathways are impaired. In the present experiments, anaerobic pathways were activated only when the prawns were subjected to 16-17 torr (Figures 6.1 and 6.2). Hyperglycaemia, however, started at higher blood O_2 concentrations than did lactate and the point where lactate concentrations started to accumulate appears to be better related to P_{vO_2} than to P_{aO_2} or P_{wO_2}. Taylor and Spicer (1987) observed hyperglycaemia in *Palaemon elegans* and *P. serratus* exposed to 5, 10 and 30 torr but lactate increases were found only at 5 and 10 torr. Such ranges of P_{wO_2}, P_{vO_2} and P_{aO_2} where hyperglycaemia occurs but where lactate concentrations remain low may indicate a preparatory period, where the animals have detected hypoxia and mobilized glucose stores, but where the use of anaerobic pathways is still not required.

Ammonia efflux rates of *Nephrops* have been shown to increase linearly with increases in ambient oxygen (Hagerman et al. 1990). Similar results were shown for *Carcinus maenas* (Regnault and Aldrich 1988), which they argue indicates either that ammonia was accumulating in the blood or that other nitrogenous end-products, such as urate, were formed under such conditions. Urate was found to accumulate in the blood of *C. maenas* during hypoxia (Lallier et al. 1987) and this was found to increase the hemocyanin-O_2 affinity and play an important respiration role during hypoxia (Lallier and Truchot 1989). However, Dykens (1991) found that urate increases in *C. maenas* exposed to hypoxia were caused by a failure of uricase which was probably caused by the reduced P_{wO_2}, as uricase molecules remained active throughout hypoxia but showed a low affinity for oxygen. In the present experiments, ammonia effluxes were not altered during P_{wO_2} reductions and it may be that the results described above concerning ammonia effluxes during hypoxia were just reflecting a depressed metabolism under this situation. A metabolic arrest may be a strategy to cope with hypoxia as it may avoid or minimize the use of anaerobic pathways (Hochachka and Somero 1984, Storey and Storey 1990). Lowery and Tate (1986) observed that *Callinectes sapidus* was more active immediately after exposure to hypoxic water (*cf* normoxia) but then activity levels fell considerably to a point were the crabs did not react to mechanical stimulation.

The present results show that ammonia effluxes and $\dot{M}O_2$ were not altered during hypoxia, reflecting that normal metabolism (normoxic levels) was probably not depressed at such times.

P_{wO_2} was found to be directly related to water pH values and this probably reflects an accumulation of CO_2 in the water as a result of metabolic activity. Such hypercapnic conditions may cause blood acidosis which may be gradually compensated (Truchot 1983, 1994 for a review). Assuming that *Nephrops* was exposed to supranormal P_{wCO_2} above normal values, the prawns were able to compensate for such increases as blood pH values were not reduced throughout the experimental period. As summarized by Wheatly and Henry (1992), the pH of the extracellular fluid may be regulated by the following mechanisms: 1) buffering of protons with inorganic/organic buffers; 2) respiratory control of P_{CO_2}; 3) buffering with $CaCO_3$ from the carapace; 4) acid-base exchange with the intracellular fluid and/or the external medium. According to these authors, the main haemolymph buffer is haemocyanin which does not seem to be involved in acid-base regulation and strategies 2 and 3 are used mainly by terrestrial and semiterrestrial species. In the present experiments, an uptake of HCO_3^- to buffer the acidic effects of hypercapnia would also contribute to the pH reductions found in the water. Truchot (1979) found an increased infflux of base in *Carcinus maenas* exposed to hypercapnia. A similar compensatory mechanism was also found in *Callinectes sapidus* by Cameron (1978).

The ventilatory responses to CO_2 have received little attention in aquatic crustaceans and are controversial. According to Truchot (1994), the depression of gill water flow rate under hyperoxia, which causes hypercapnia, is evidence of the lack of ventilatory responses to increased P_{CO_2}. The pattern presently found for HR and SR of *Nephrops* exposed to progressive hypoxia, and a supposed progressive increase in P_{wCO_2}, was similar to that observed by other authors in crustaceans subjected to hypoxic but normocapnic conditions.

When *Nephrops* were subjected to progressive hypoxia down to 44 torr, HR was not altered but SR increased considerably. The same pattern was found in *Palaemon elegans* and *P. serratus* that showed hyperventilation during hypoxia (30 % sat.) but heart rates (HR) were not different than those of shrimps at normoxia (Taylor and Spicer 1991). A similar result was previously observed for *Nephrops* by Hagerman and Uglow (1985). An increased scaphognathite beat rate (SR) was also found in *Orconectes rusticus* when P_{wO_2} decreased to levels below 100 torr and HR remained stable but decreased when P_{wO_2} was < 40 torr (McMahon and Wilkes 1983). Such increases in SR usually aim to compensate for the reduced oxygen tensions in the water so maintaining a constant supply of O_2 to the gills. Burggren

and McMahon (1983) investigated the pumping efficacy of the scaphognathite of *Orconectes virilis* during hypoxia and found that ventilatory adjustments may also occur by active and/or passive adjustments in the stroke volume of each beat. According to these authors, each scaphognathite beat generates subambient pressure in the branchial cavity and, following each propulsion stroke, the scaphognathite channel is fully open and reflux of water may occur. Passive control may happen because the slower the beat rate the longer the channel will remain open and this can reduce considerably the stroke volume until a situation whereby pumping may be virtually ineffective. Active control was shown when different stroke volumes were observed at the same SR but at different P_{wO_2}. Hence, the increased SR found in the present experiments will be ensuring that passive reductions in stroke volume are absent during hypoxia but, whether active increases occur in *Nephrops* under these circumstances remain to be investigated.

The open circulatory system of crustaceans has been regarded as a low performance system without the means to effect adequate tissue perfusion and fine adjustments. However, according to the review by McMahon and Burnett (1990), such systems are highly efficient and are capable of a degree of regulation similar to those of vertebrate closed systems. These authors also stated that stroke volume may actually be the most important factor controlling cardiac output in the crustacean open circulatory system. McMahon and Wilkens (1975) suggested that an increase in cardiac output occurs during hypoxic exposure in *Homarus americanus* (based on Fick calculations) and this response was also found by Jorgenson et al. (1982) in *Cancer magister* exposed to similar conditions. According to Dejours (1981), oxygen consumption may be described by the following relation:

$\dot{M}O_2 = Vb\ (P_{aO_2} - P_{vO_2})\ (\alpha O_2)$,

where Vb is cardiac output and αO_2 is the solubility coefficient for oxygen. As $\dot{M}O_2$ and the difference between post and pre-branchial blood oxygen were not altered during the present experiment, cardiac output was maintained constant during hypoxia (down to *ca* 44 torr).

Nephrops norvegicus thus appears to be well-adapted to face progressive hypoxic exposure accompanied by increased CO_2 concentrations as may occur in burrows. It is noteworthy, however, that the animals used in these experiments were unstressed, intact animals. The effects of hypoxia on animals previously subjected to emersion and possibly with damage to the fine branchial tissue or on animals subjected to a series of hypoxic exposures (conditions likely to be observed during commercial practices) may differ from those presently found and remain unknown.

Table 6.1. *Nephrops norvegicus*: Some water and blood parameters (mean ± SE) measured during progressive hypoxia. Prawns were placed in sealed respirometers and P_{wO_2} reductions were caused by $\dot{M}O_2$ (n = 8 or 9 animals in each case).

	Water			Haemolymph					
Time (h)	P_{O_2} (torr)	TA (μmol l^{-1})	pH	C_{aO_2} (ml l^{-1})	C_{vO_2} (ml l^{-1})	Glucose (mg 100 ml^{-1})	Lactate (mg 100 ml^{-1})	TA (μmol l^{-1})	pH
0	155	< 1	7.67	12.36 ± 1.39	9.52 ± 1.00	1.17 ± 0.19	1.24 ± 0.08	161.06 ± 10.10	7.78 ± 0.01
16	115.90 ± 2.27	12.29 ± 1.32	7.46 ± 0.01	10.60 ± 1.88	6.40 ± 1.04	1.12 ± 0.20	1.51 ± 0.13	135.42 ± 11.48	7.75 ± 0.02
24	83.93 ± 3.75	20.99 ± 1.62	7.36 ± 0.02	8.55 ± 1.78	5.69 ± 1.45	1.16 ± 0.16	1.28 ± 0.12	121.42 ± 16.52	7.77 ± 0.02
32	66.98 ± 6.62	21.77 ± 3.02	7.31 ± 0.02	7.58 ± 1.21	4.43 ± 0.50	2.36 ± 0.40	1.01 ± 0.09	151.46 ± 18.06	7.78 ± 0.02
40	46.99 ± 9.18	27.46 ± 2.65	7.24 ± 0.02	6.18 ± 1.39	2.74 ± 0.54	3.63 ± 0.89	10.55 ± 8.99	137.69 ± 14.26	7.78 ± 0.04
48	20.20 ± 5.69	35.14 ± 4.50	7.21 ± 0.02	4.34 ± 0.94	0.99 ± 0.24	6.92 ± 1.75	11.96 ± 7.76	141.79 ± 14.66	7.87 ± 0.03
56	19.10 ± 4.70	37.77 ±4.53	7.14 ± 0.02	4.12 ± 1.11	1.51 ± 0.68	9.83 ± 2.19	19.48 ± 9.74	113.54 ± 8.03	7.87 ± 0.05

Table 6.2. *Nephrops norvegicus*: Some water parameters, cardio-ventilatory rates and $\dot{M}O_2$ (mean ± SE) measured during progressive hypoxia. Prawns were placed in sealed respirometers and P_{wO_2} reductions were caused by $\dot{M}O_2$ (n = 8 or 9 in each case).

Time (h)	P_{wO_2} (torr)	Water TA (μmol l^{-1})	Water pH	HR (beats min^{-1})	SR (beats min^{-1})	Time interval (h)	$\dot{M}O_2$ (μl l^{-1})	TA efflux (μmol g^{-1} l^{-1})
0	152.65	< 1	7.85	21.43 ± 5.46	37.49 ± 8.59	0-6	13.55 ± 0.97	0.05 ± 0.01
6	137.05 ± 0.51	3.40 ± 0.41	7.81 ± 0.01	19.08 ± 2.40	35.40 ± 5.29	6-12	18.77 ± 2.61	0.09 ± 0.02
12	114.10 ± 3.59	7.97 ± 1.18	7.72 ± 0.01	22.18 ± 1.41	41.80 ± 7.83	12-18	17.02 ± 1.38	0.07 ± 0.01
18	95.52 ± 3.98	11.08 ± 1.58	7.63 ± 0.01	18.92 ± 2.26	37.05 ± 7.42	18-24	17.44 ± 1.41	0.06 ± 0.01
24	74.13 ± 3.87	13.82 ± 1.80	7.52 ± 0.01	18.63 ± 1.39	46.94 ± 8.24	24-30	18.97 ± 4.68	0.04 ± 0.01
30	53.67 ± 5.38	16.20 ± 2.31	7.49 ± 0.01	16.79 ± 1.71	81.06 ± 7.41	30-33	16.47 ± 3.02	0.06 ± 0.02
33	44.26 ± 4.69	17.56 ± 2.49	7.47 ± 0.01	23.88 ± 3.16	114.91 ± 11.35			
33*	104.28 ± 2.55	9.47 ± 1.43	7.69 ± 0.01	31.93 ± 2.89	107.61 ± 11.30	33-36.5	19.73 ± 3.70	0.03 ± 0.02
36.5	91.29 ± 1.87	10.16 ± 1.58	7.63 ± 0.02	19.38 ± 2.73	44.59 ± 9.14			

* Immediately after seawater was flushed through the system for 15 min.

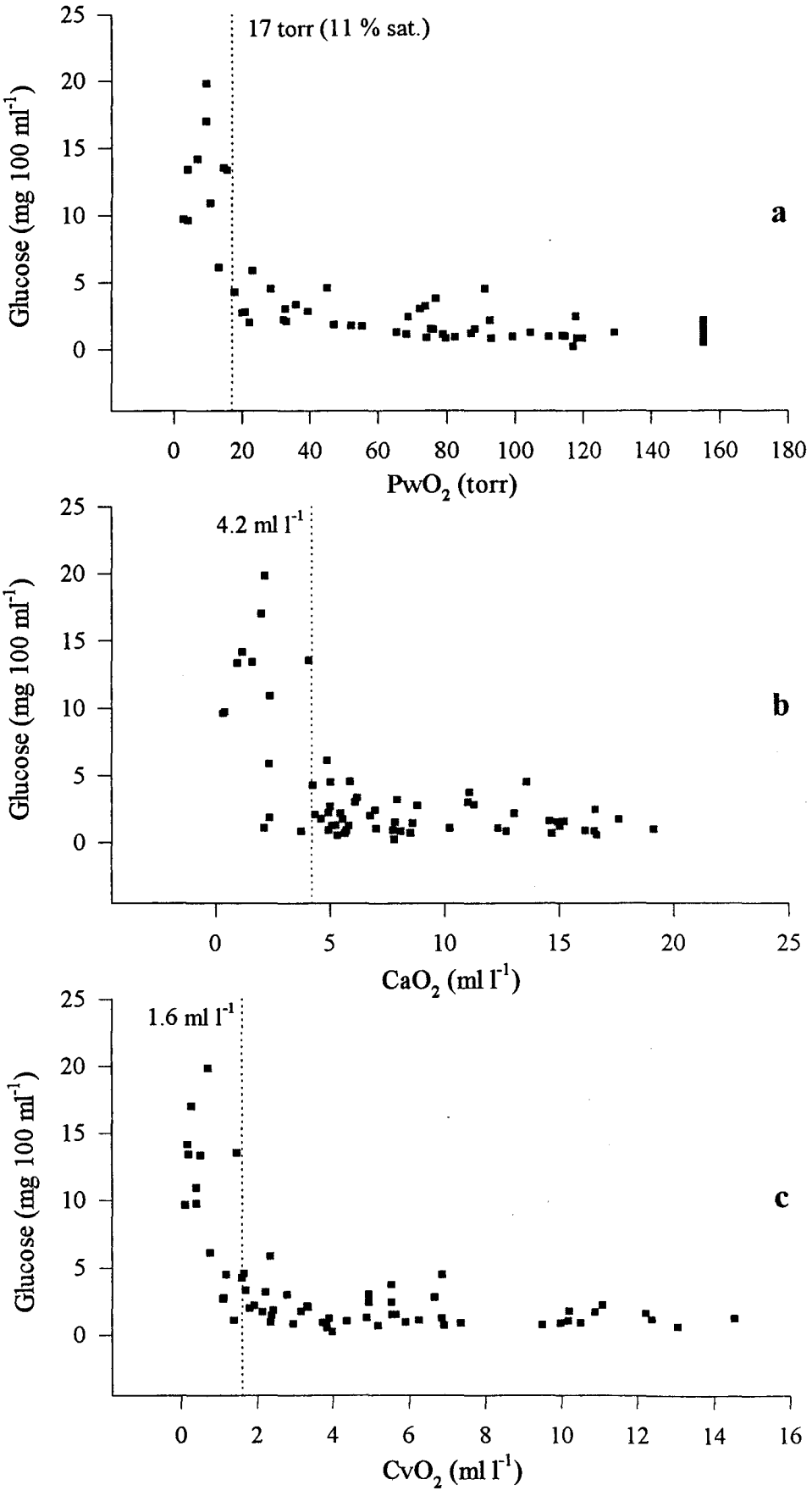


Figure 6.1. *Nephrops norvegicus*: Blood glucose concentrations at different tensions of: **a.** medium oxygen; **b.** Post branchial blood oxygen (C_{aO_2}); **c.** Pre-branchial blood oxygen (C_{vO_2}). Hypoxia was achieved gradually.

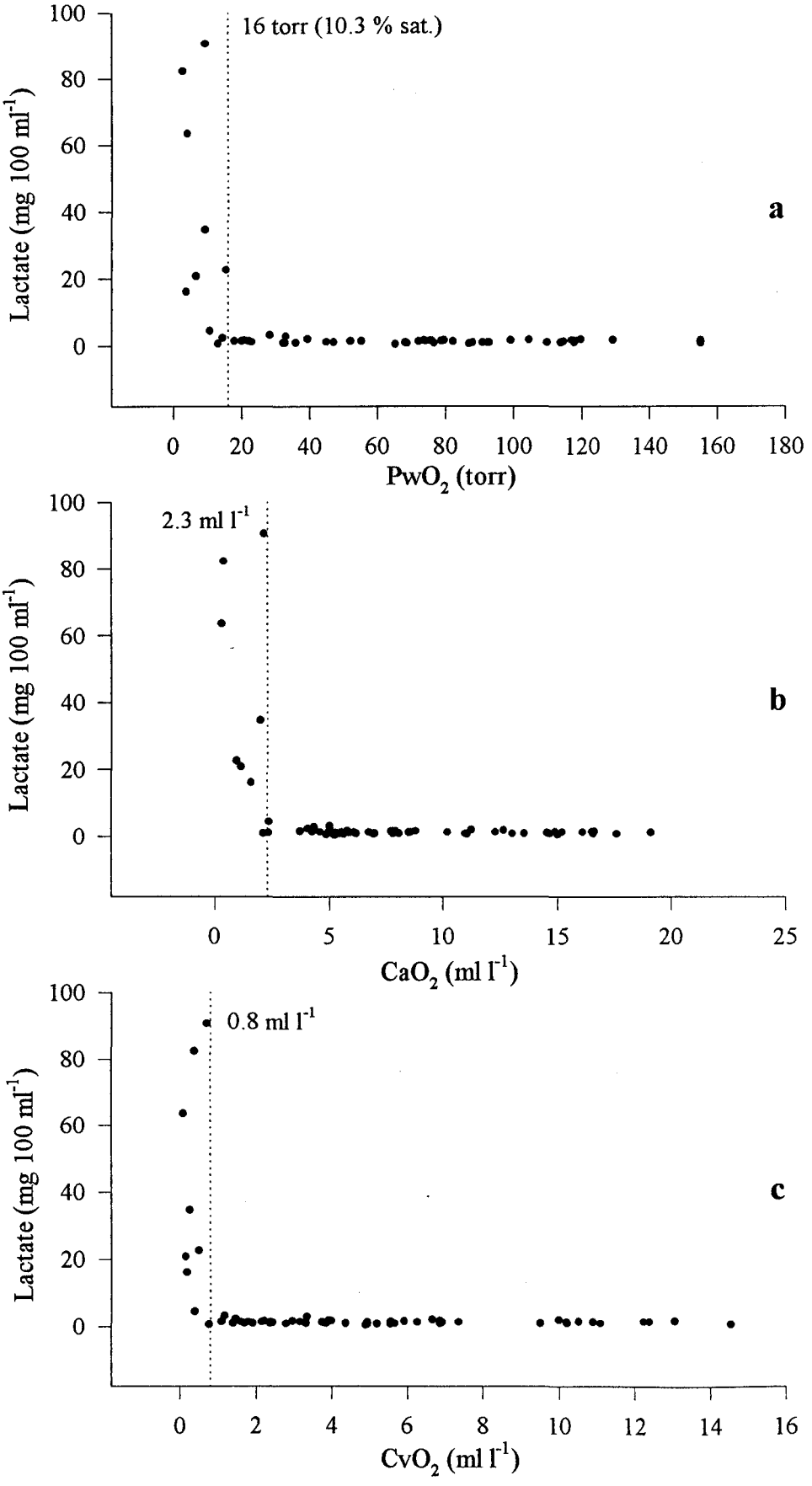


Figure 6.2. *Nephrops norvegicus*: Blood lactate concentrations at different tensions of: **a.** medium oxygen; **b.** Post branchial blood oxygen (C_{aO_2}); **c.** Pre-branchial blood oxygen (C_{vO_2}).. Hypoxia was achieved gradually.

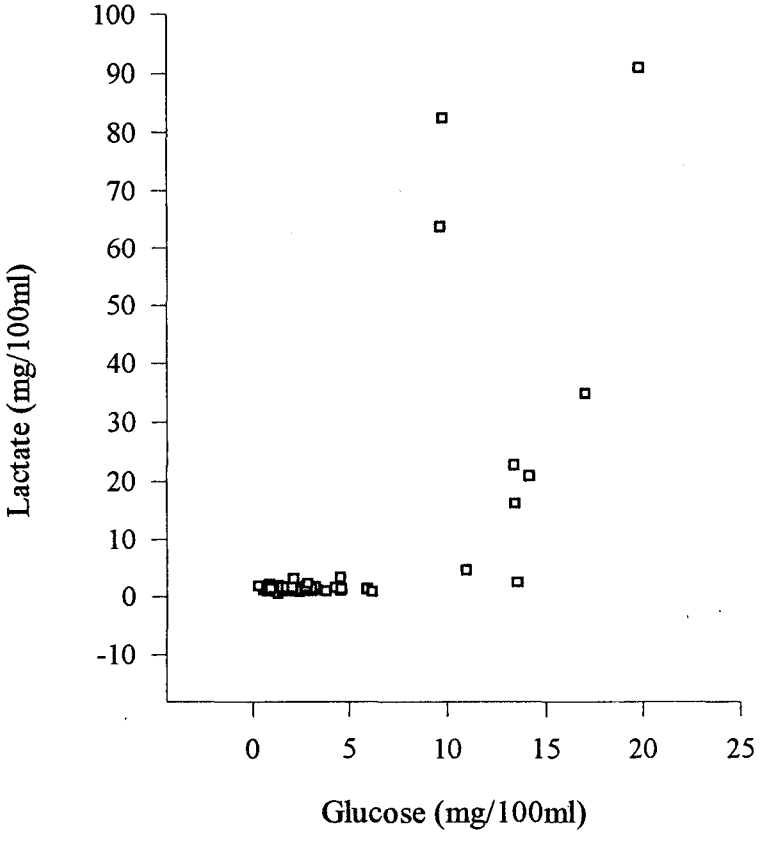


Figure 6.3. *Nephrops norvegicus*: Relationship between blood glucose and blood lactate concentrations of prawns exposed to progressive hypoxia.

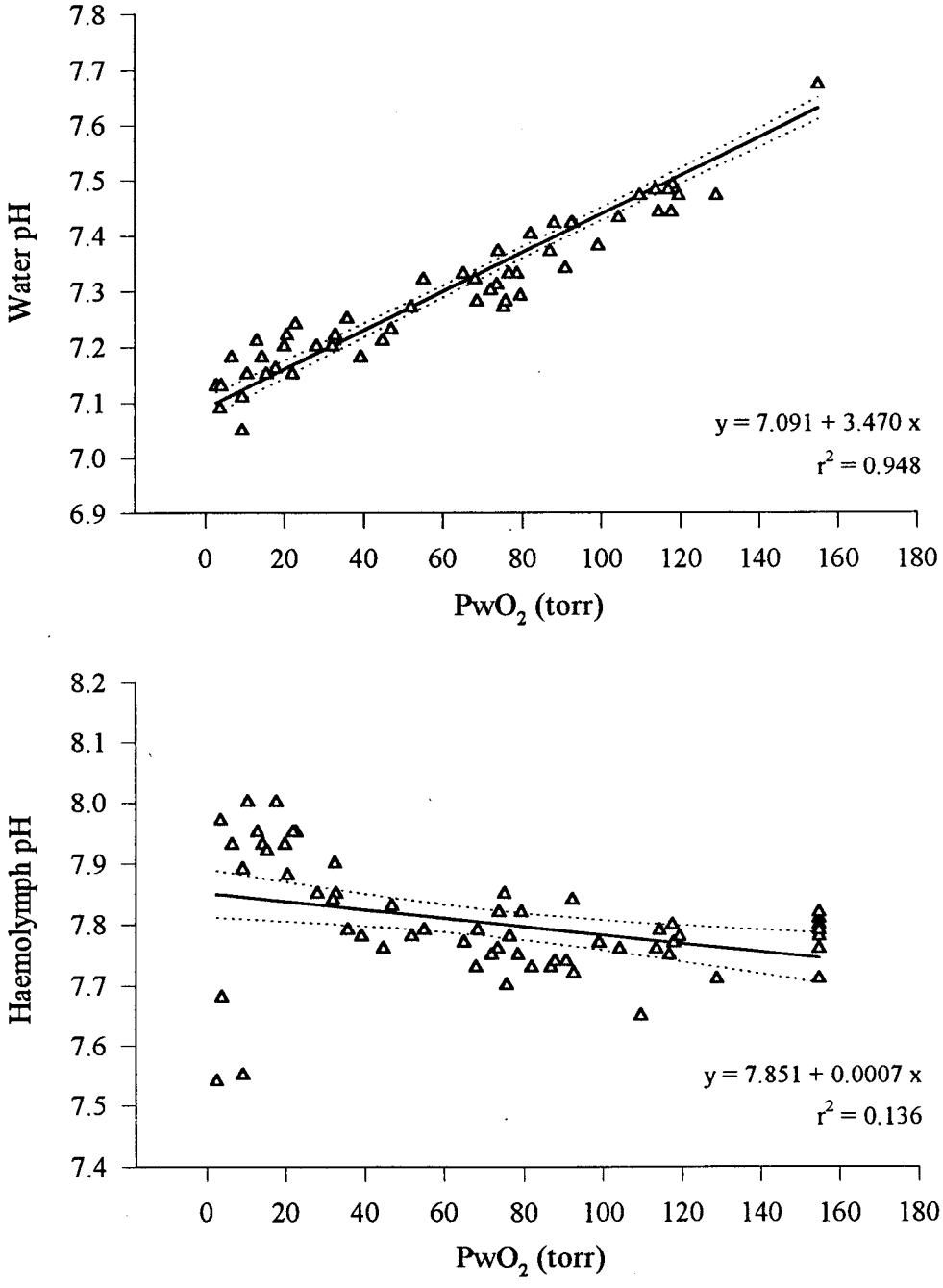


Figure 6.4. *Nephrops norvegicus*: Haemolymph and water pH at different P_{wO_2}. Hypoxia was achieved gradually.

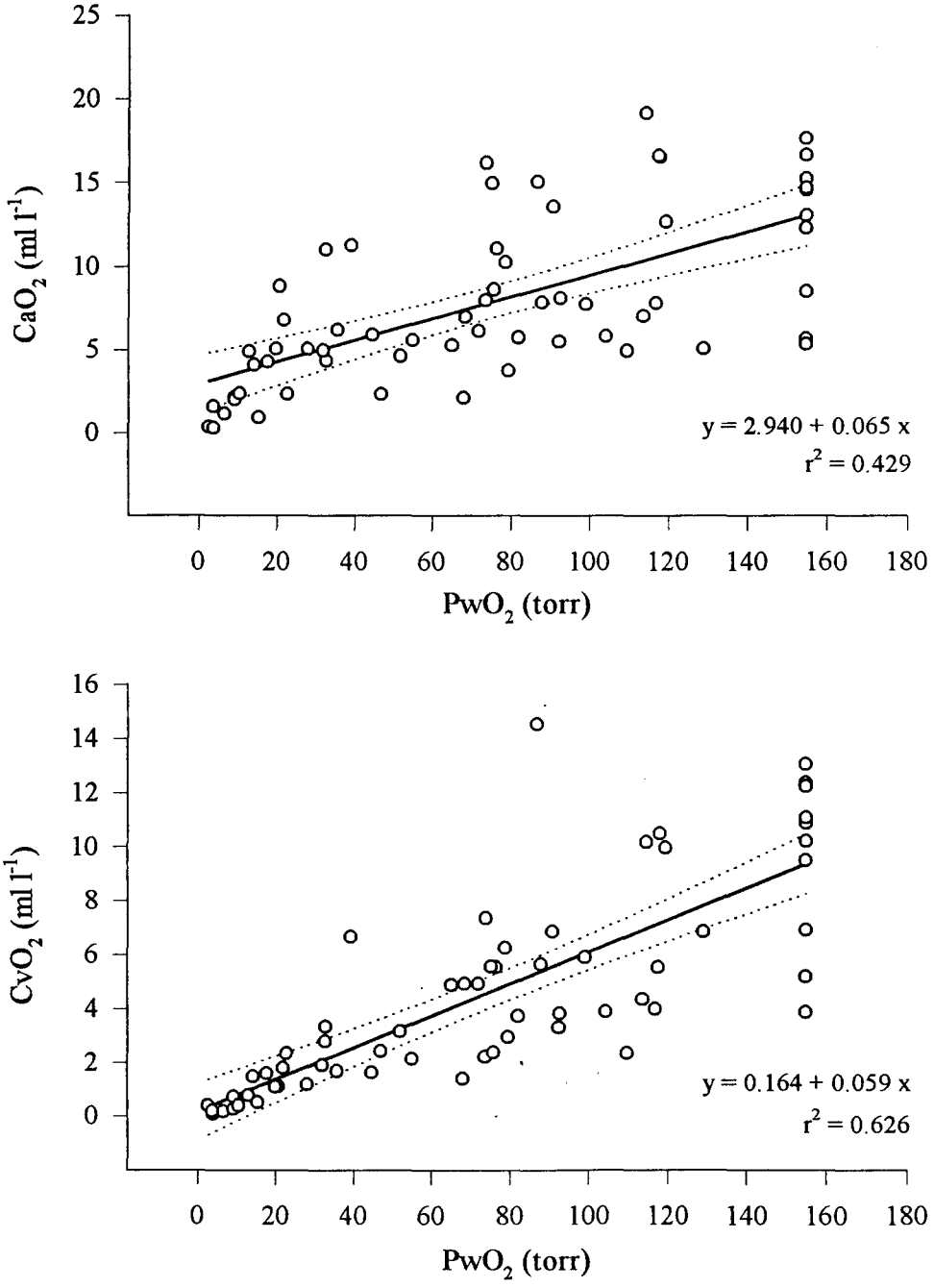


Figure 6.5. *Nephrops norvegicus*: Blood post (C_{aO_2}) and pre branchial oxygen concentrations (C_{vO_2}) at different tensions of water oxygen (P_{wO_2}). Hypoxia was achieved gradually.

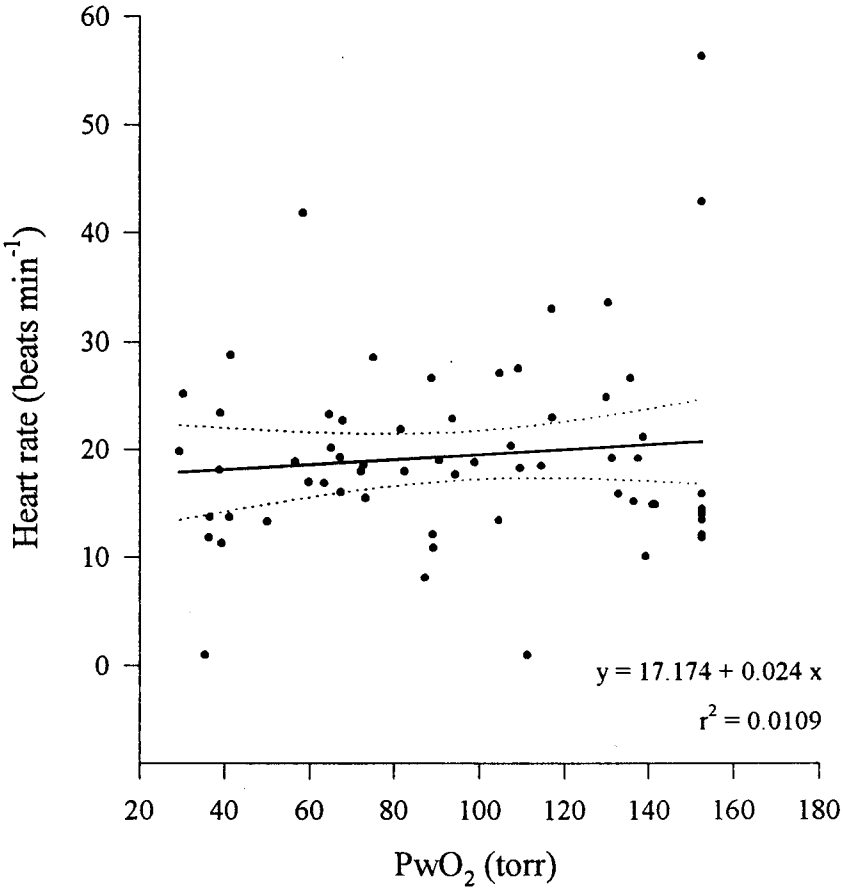


Figure 6.6. *Nephrops norvegicus*: Heart rates of prawns at different P_{wO_2}. Hypoxia was achieved gradually.

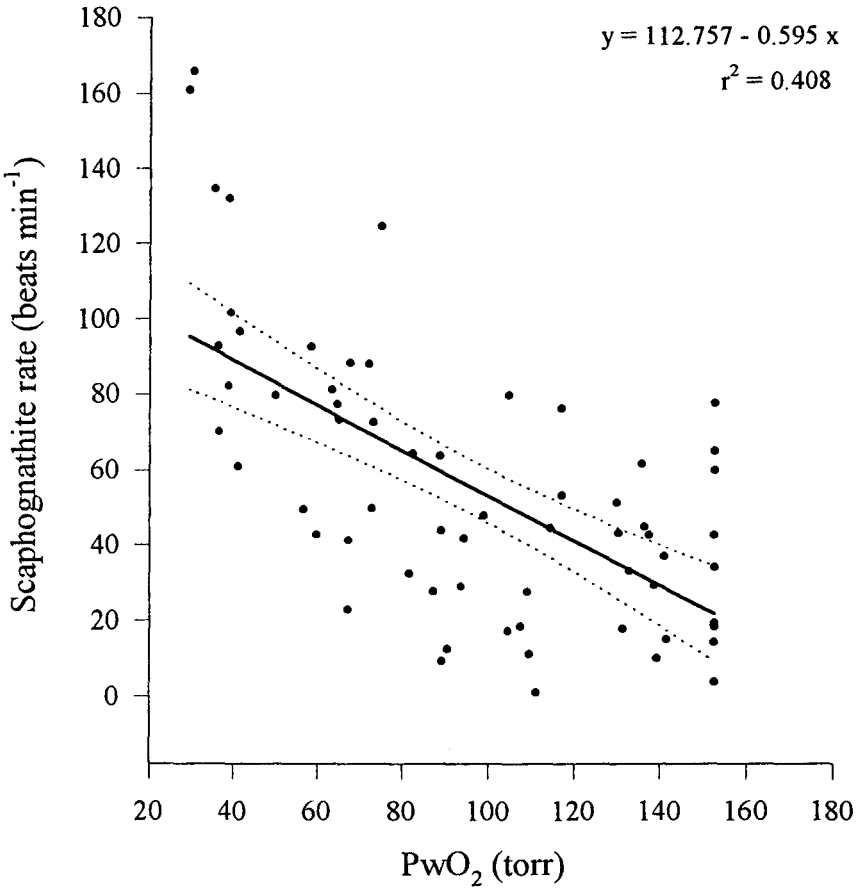


Figure 6.7. *Nephrops norvegicus*: Scaphognathite rates of prawns at different P_{wO_2}. Hypoxia was achieved gradually.

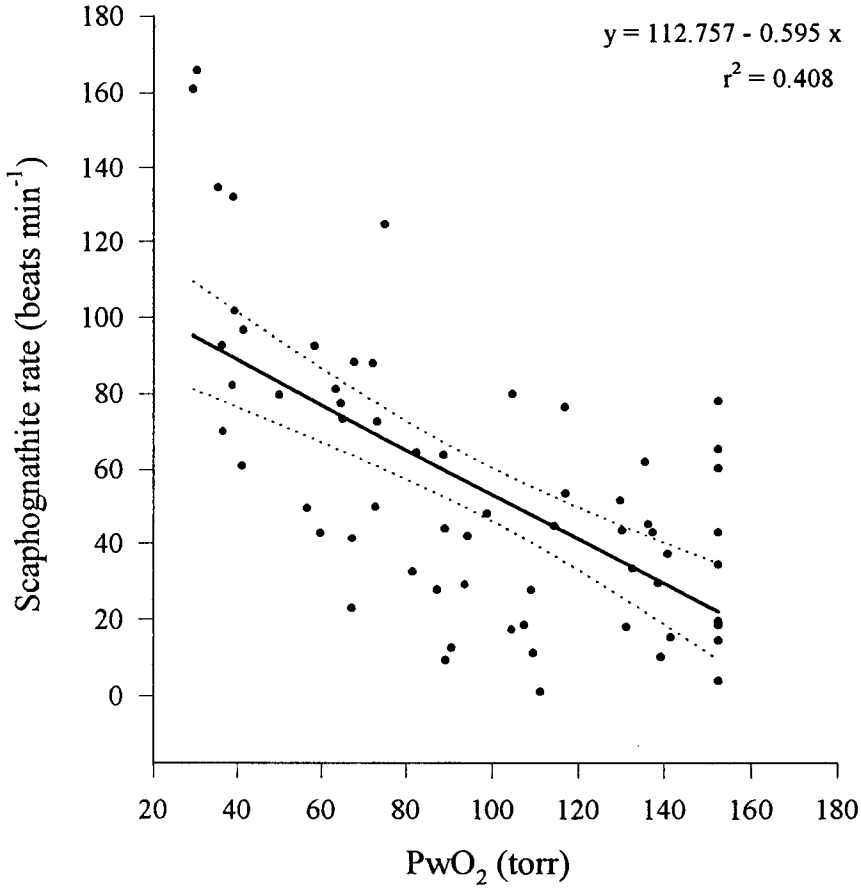


Figure 6.7. *Nephrops norvegicus*: Scaphognathite rates of prawns at different P_{wO_2}. Hypoxia was achieved gradually.

Chapter 7.

A novel method for the storage of live *Nephrops norvegicus* (L.) during live marketing.

7.1. Introduction.

Three main factors that may have detrimental effects on *Nephrops* during commercial operations (emersion; increased concentrations of ambient ammonia; progressive hypoxia) were studied in Chapters 4, 5 and 6 of this thesis. *Nephrops* showed great ability to adapt and compensate for high levels of ambient ammonia, as mechanisms to remove and/or store ammonia as a different compound were found to occur. The respiratory, ventilatory and general metabolic responses of *Nephrops* to hypoxic exposure have also shown that this species is well-suited to cope with such environmental circumstances. The fact that *Nephrops* is able to maintain normal (normoxic conditions) aerobic metabolism at a very reduced P_{wO_2} is of prime importance as, otherwise, the use of anaerobic pathways may cause a rapid depletion of the carbohydrate reserves (Lehninger, 1984) and may result in weak prawns which are less able to survive such conditions. Provided that such conditions (high ambient ammonia and low P_{wO_2}) do not exceed certain limits (see chapters 5 and 6 for critical levels) they should not impose a problem during handling and holding procedures. The same does not seem to be true for air exposure, as the prawns were highly succeptible to the emersion periods used in the experiments described in Chapter 4, even under very high relative humidity.

The physiology of crustaceans subjected to commercial practices, or to simulations of such conditions, has been investigated for different species (Spicer *et al.* 1990, Whiteley 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 behavioural responses to the fishing gear used (Atkinson and Naylor 1976, Briggs 1985, Bailey et al. 1986, Newland and Chapman 1989, Hill and White 1990, Cardador 1993, Stewart et al. 1993).

Most of the procedures and techniques presently used in the trade of live shellfish were developed on a "trial-error" basis without a full understanding of the physiological and biological needs and characteristics of the different species used. 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, amongst the stressors examined in the previous sections of this thesis, was shown to be the most harmful for *Nephrops*.

7.2. Materials and methods.

These 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 emersed systems in which prawns were kept in high humidity conditions and very low temperatures. All experiments and sample collection procedures were made inside temperature controlled rooms.

The cascade systems used are shown in Figure 7.1. Water from the header tank, the base of which was perforated to form a shower, poured through the trays into a sump and then 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 animal movements. All the cascade systems 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 5 l of seawater (1:5.56, w/v) and three open trays and the other contained *ca* 1850 g of prawns in 10 l 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 transfered to the cascade systems. All haemolymph samples (300 µl) were collected through the arthrodial membrane of one of the pereiopods and the prawns were returned to the system. Water samples (1 ml) were also collected at the above times and their ammonia contents determined. Blood pH was measured immediately after sample collection and 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 10 l 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 μ l 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) (see Gen. Mat. and Meth. section 2.2.2.).

The experiments with emersed prawns were performed as shown in Figure 7.2. 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 *Nephrops* 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.

7.3. 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 accompanied 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 behaviour 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 supranormal 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 7.1. 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 tempeerature.

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 7.2), 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 transfered 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 7.3. 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.

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 at 6 °C.

The results obtained when *Nephrops* were held for over 5 days at 6 °C in a cascade system using 2 mm trays are shown in Table 7.4. 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 other mean values. Blood pH values were significantly lower than control prawns at 53 h but incresed 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 7.5. Blood

pH decreased significantly ($P<0.05$) after 1 h and 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 ($P>0.05$).

7.4. 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 maintaining *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 accross 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 studies 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 7.1-7.3), 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 behavioural 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 Jones 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 surfaces.

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 ΔC_{O_2}, for over 5 days in the 2 mm cascade system (Table 7.4). Hyperglycaemia, accompanied by increased levels of circulating lactate, are classic responses to emersion-related hypoxia in crustaceans. Such responses have also been observed in *Nephrops* subjected to emersion under various conditions (Spicer *et al.* 1990, Chapter 4 of the present work). 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 7.4), 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).

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. 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 7.1), in comparision with the levels obtained when the prawns were restrained (Table 7.2-7.4). 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 7.1-7.3). 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 (1957), 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 such evidences, the conditions used in the cascade system tested during the 5 days experiment seemed to be to most apropriate (2 mm trays; restrained prawns; 6 °C).

The responses of *Nephrops* to emersion under different conditions were studied in Chapter 4 of this thesis. The present 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 a 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 no apparent loss of legs or claws, which could be related to the low temperatures used, was found to occur . The blood acidosis found after the first hour of emersion (Table 7.5) was less intense than that shown by emersed prawns in Chapter 4 (Figure 4.6). Anaerobiosis did occur but the accumulation of lactate was considerably lower than that of prawns emersed at 12 °C in Chapter 4 (Figure 4.8). These results show that the metabolism of *Nephrops* can be generally depressed at 2 °C without any reduction to the quality of the prawns (intact and live animals). *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.) and the specifications used in this experiments may be used to improve such procedures.

The results obtained with the cascade systems show that *Nephrops* can be maintained successfully 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 consignments of live animals affects transport costs. Another advantage of this system is that the prawns may be selected and transfered to the trays as soon as they are unloaded from the fishing boat and the trays may be transfered between dealers or into a mobile cascade

system without handling the prawns, which will minimise aerial exposure and general damage to the animals. These are preliminary 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.

Table 7.1. *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 with no water retention (Figure 7.1.) at 6 ± 1 °C and 14 ± 1 °C. Values are mean ± SE and number of sampled prawns.

Time (h)	Haemolymph					Water
	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	7.80 ± 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 9	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 7.2. *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 7.1) at 6 ± 1 °C and 14 ± 1 °C. Values are mean ± SE and number of sampled prawns.

Time (h)	Haemolymph					Water
	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 7.3. *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 7.1.) retaining a layer of *ca.* 2 mm of water at 6 ± 1 °C and 14 ± 1 °C. Values are mean ± SE and number of sampled prawns.

Time (h)	Haemolymph					Water
	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	7.80 ± 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 ± 0.84 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 7.4. *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.

Time (h)	Haemolymph						Water
	pH	Glucose (mg 100 ml^{-1})	C_{aO_2} (ml l^{-1})	C_{vO_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 7.5. *Nephrops norvegicus*: Alterations to some blood constituents and pH during emersion inside a polystyrene box. Prawns were placed in between layers of seawater-soaked insulating wool (Figure 7.2.) and ambient temperature under such conditions was 2 ± 0.6 °C. Values are mean ± SE.

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

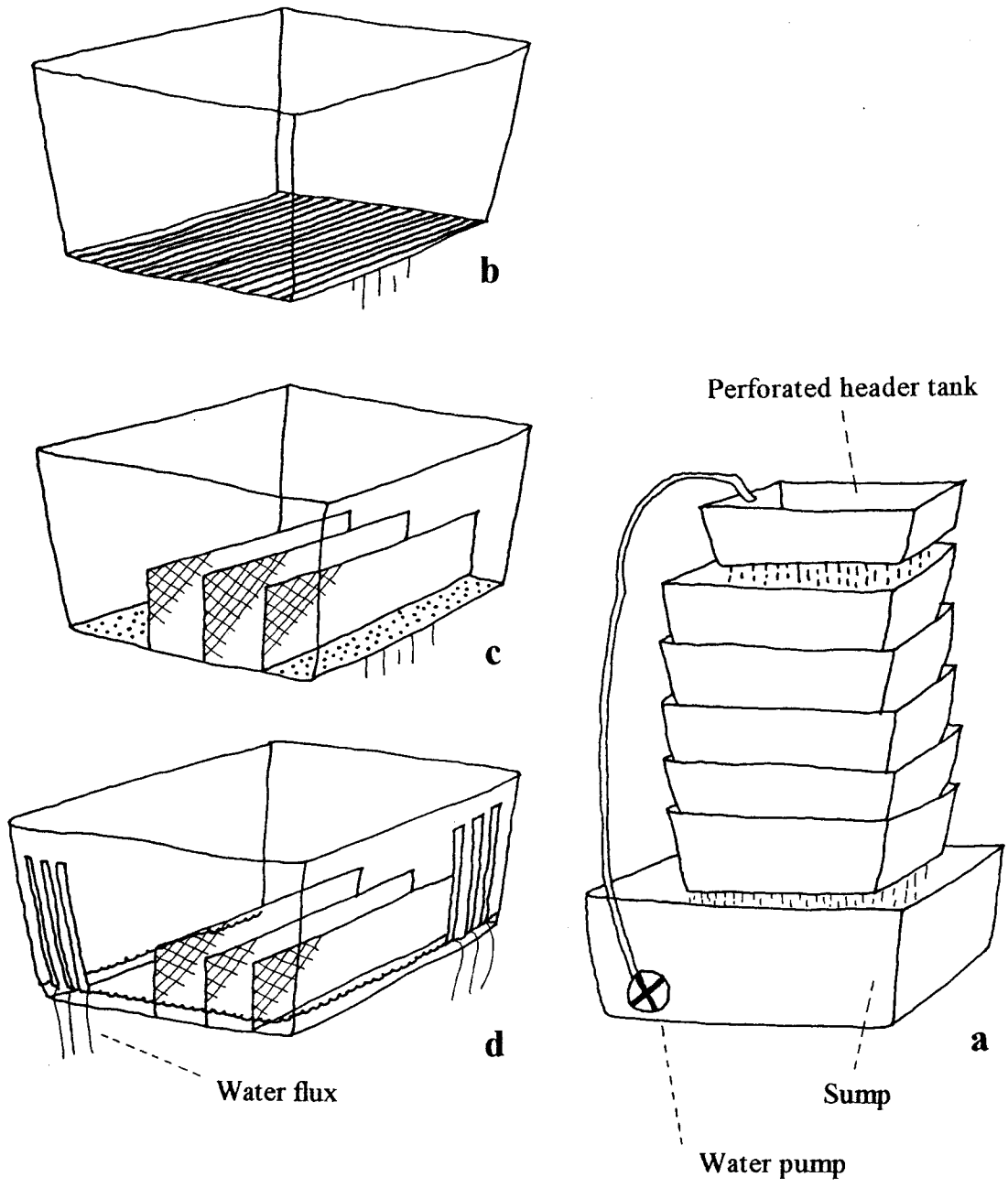


Figure 7.1. *Nephrops norvegicus*: **a.** Cascade and tray (32 x 18 cm, base area) systems used to hold prawns during simulated transportation. **b.** Without water retention. **c.** Water retention (*ca.* 2 mm, depth) and prawns individually separated. **d.** Water retention (15 mm, depth) and prawns individually separated.

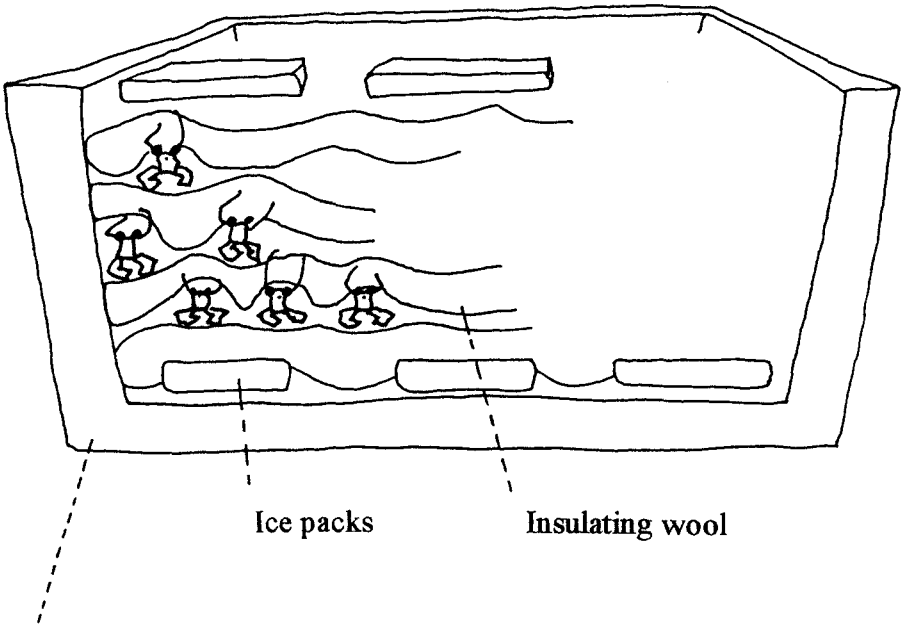


Figure 7.2. *Nephrops norvegicus*: System used to simulate a 12 h transport of emersed prawns. Temperature in between the layers was 2 ± 0.6 °C.

Chapter 8.

Case studies involving monitoring of live transport practices.

8.1. Introduction.

The techniques and procedures currently used by the fish trade to hold and transport live crustaceans were evolved mostly as a result of experimentation which has not included a proper, systematic study of the biological requirements of the species involved. On some occasions, the results obtained may be cost-effective and excellent but, at other times, high mortality rates occur and the reasons for these are usually unclear. The collection of data and field observations of such procedures, coupled with experiments under laboratory-controlled conditions, may help to improve livestock-holding practices and enhance the chances of delivering a suitable product. Unfortunately, such data are very scarce, as there is a general fear amongst the dealers that "valuable" information may be passed to potential competitors and also there has been no appreciation of the problem or the means to tackle it. Additionally, laboratory experiments are unable to simulate accurately all the marketing conditions that occur during commercial practices and may only be used properly in two ways: 1) to improve the knowledge on the biology and physiology of the species used 2) to answer specific questions that may arise from problems which occur during live marketing.

Whyman *et al.* (1985) studied the conditions under which *Necora puber* were held and transported from the UK to Spain. The work carried out by these authors included a literature review of the subject, visits to several of the major exporters and importers, laboratory experiments that simulated some of the conditions observed during transport and also a preliminary study of alternative methods of transport of *Necora*. Several guidelines to improve the procedures used by dealers emerged from this work but, as stressed by the authors, it was difficult to establish a formula that could offer a perfectly safe method to transport *Necora*. In addition to this work, the handling and transport of live *Cancer pagurus* was investigated in two separate studies (Uglow et al. 1986, MacMullen et al. 1986). The authors supervised a few consignments that transported crabs to continental markets and, in addition to the parameters studied by Whyman *et al.* (1985), they also studied the physical damage which occurred and which usually reduces the survival chances of individual crabs. Significant differences were observed among the different consignments, which emphasised that conclusions and remarks should be based on a long term study and/or based on several independent studies.

Hosie (1993) produced a detailed description of the main procedures used in the live trade of *Cancer pagurus* and *Necora puber* and included the monitoring of some long-distance journeys to Spanish dealers. The present work aimed to investigate some of the problems which were occurring regularly and to find

possible explanations in terms of the physiology and biology of the species used. The studies may be divided in two distinct sections. Firstly, several aspects of a journey from Ireland that delivered two separate consignments of *Cancer pagurus* to France were monitored and such studies were later complemented with laboratory analysis and experiments. Secondly, two visits (separated by 12 months) were made to facilities used to hold *Homarus gammarus* prior to consignment and blood/water samples were collected and experiments made *in situ*.

It is hoped that these studies may contribute to the present knowledge of such aspects of the Trade and, associated with other observations, lead to the improvement of the handling of crustaceans in the live trade.

8.2. Case study 1: Transport of *Cancer pagurus* from Downings, Ireland to Roscoff and Douarnenez, France.

This work was carried out as part of an investigation of the mortalities that occurred during the transport of live Irish brown crabs to the French market. These occasional mortalities induced the importing dealers to refuse payment for crabs that died within a 24 h period after their arrival. This investigation also aimed to assess the handling procedures and water quality of the vivier tanks used by the importing French dealers.

8.2.1. Procedures used to monitor the journey.

These data were collected during two journeys from Downings, Ireland to Roscoff and Douarnenez, France in July 1994. The route, the locations where samples were collected and the journey time between the different sampling locations are shown in Figure 8.1. The lorries used in these trips contained 18-20 vivier tanks which were isolated from each other (without water exchange) and were supplied with aeration and kept under relatively constant temperature with the air-cooling system of the lorries. The crabs were placed in the vivier at an approximate biomass/water ratio of 1:1. Each lorry carried 8-10 t of crabs.

The procedure used to supervise these trips involved the collection of vivier-tank water and crab haemolymph samples and also to produce a photographic survey of the unloading practices and of the facilities available to hold the livestock. At different points along the route, water samples (*ca* 1 ml each) were collected from each of the vivier tanks from the lorry and the vivier water temperatures measured. At the same time, a haemolymph samples was taken from 1 crab from each of the tanks by perforating the arthrodial membrane of one of the legs with a glass Pasteur pipette and collecting the blood in an Eppendorf tube. All samples (water + haemolymph) were immediately placed in a 12 volts portable freezer (-20 °C) and later transferred to the freezer (-20 °C) in the laboratory. Haemolymph and water samples were also collected at different situations (see Tables 8.1-8.2) following arrival at the premises of the dealer.

8.2.2. Materials and methods used during laboratory experiments.

During both consignments, the water temperature in the vivier tanks oscillated around 17 °C and such high temperatures may have been responsible for the very high levels of total ammonia (TA) found to accumulate in the vivier tanks

during the journey. The experiments made in the laboratory were designed to evaluate the effects of low temperature on the ammonia production of *Cancer pagurus* during holding in conditions similar to those used in the trips. All crabs used were nicked, according to the procedure described in General Materials and Methods (Section 2.1.3, Figure 2.2), and acclimated for 24 h before the start of the experiments.

Cancer pagurus were held for 48 h under three different conditions: 1) immersed in containers with seawater at 17 C, 2) immersed in containers with seawater at 5 C and 3) emersed in a cascade system at 5 C. The biomass to water ratio was approximately 1:1.2 in all situations. The cascade system consisted of a series of stacked plastic trays supplied with an overhead tank and a sump. Seawater was continuously pumped from the sump into the header tank and through the trays containing the crabs. The water ammonia concentrations in all the systems were monitored throughout and, at the end of the 48 h period, haemolymph samples from the crabs were collected. At this time, further groups of crabs, one from each of the systems, were transferred to individual containers (20 x 35 cm base) with 8 l of low ammonia seawater (< 1 μmol l^{-1}) and their TA effluxes measured for 12 h following the transfer.

8.2.3. Results and discussion.

A great deal of the information related to the normal procedures that are generally used in this and in other journeys were kindly supplied by the drivers and are described along the text. Both consignments started to be monitored at Larne, when the crabs had already been intensively handled and were on the road for some time. A brief description of the normal practices (D. A. Meakin pers. comm.) that were used before the sampling and monitoring started may, therefore, contribute to the analysis of the results obtained.

Onboard procedures are largely limited by time and the handling of the crabs will obviously be affected by that. *Cancer* are taken from the pots, nicked (see Figure 2.2) and put in the onboard vivier well. At such times, the crabs may be strongly attached to the pot and dislodging them may cause damage to legs and claws. Arriving onshore, the vivier well is almost completely drained and the crabs are placed in boxes, weighed and transferred into the viviers on the lorry that may take several hours. These procedures will result in variable periods of emersion and handling and, to remove the crabs from the vessel, personnel usually have to step on the animals. Additionally, the lorry may have to wait for a load coming from a different vessel or from a different port and these will result in extended time of

maintenance in the viviers, which are not provided with filters and water may not be replaced.

The vivier systems used in both lorries were found to be adequate but some improvement could be made with relatively small alterations. *Cancer* were placed in the tanks which were *ca* 120 cm deep and were not provided with internal divisions. The placement of horizontal trays, sectioning the tank, may be an alternative to reduce the mutual interaction and activity levels inside the vivier. The aeration was very strong and some of the crabs were virtually carried around by the bubbles produced. The use of aeration systems that produce smaller air bubbles would be more gentle with the animals and also increase the area/volume ratio of the air pumped through. A comprehensive review on the design and management of aeration systems was made by Colt and Orwicz (1991). The control of water temperature would be more efficiently performed if the temperature of the air pumped by the aeration system was better controlled.

The vivier tanks in the lorries were loaded with seawater which, supposedly, had a very low TA level. Five hours following the departure of the consignment, TA concentrations were already very high and increased progressively during all the journey (Tables 8.1 and 8.2). The fact that one consignment showed water TA levels *ca* double the other reflects the variability that may occur between loads. Whatever the rate was, such increases were caused by the ammonia produced by the animals, which may be excreted by simple diffusion of NH_3 or NH_4 or active transport of NH_4 (see General Introduction). It may be that the initial higher rates of TA increase (*cf* rates towards the end of the journey) were caused by the period of emersion and increased handling that accompanied the unloading/loading procedures at the start. Ammonia is excreted mainly through the gills and, when the crabs are out of water, this aspect of gill functioning may be impaired and blood ammonia levels may rise. Following re-immersion, the ammonia accumulated whilst out of water will be quickly excreted and, considering a blood to water ratio of 0.3:1 (taking blood volume as 30 % of fresh body-weight) this can have a large impact on water TA concentrations. A burst in ammonia efflux rates immediately after re-immersion was observed by Regnault (1994) in *Cancer pagurus*.

Ammonia is a very toxic compound and its effects on the organism may cause death, which results mainly from disturbances in the acid-base balance that may impair enzyme activity (Wickins 1976, Armstrong *et al.* 1978, Provenzano 1983, Chen *et al.* 1990, Campbell 1991, Chen and Lin 1991, Chen and Lin 1992, Lin *et al.* 1993). In the present study, TA concentrations in the blood were always lower than that in the vivier-tank water and this shows that *Cancer* was able to use active mechanisms to excrete ammonia at such times, as water TA levels increased

progressively during all the journey. The ability to maintain blood TA at lower levels than water TA has been shown for other species (Chen and Kou 1993, Chen et al. 1993, Chapter 5 of this study) and *Calinectes sapidus* was also found to excrete ammonia against a gradient (Towle 1976). All values of blood TA decreased back to normal levels after the animals were unloaded to the vivier tanks at the French dealers.

Blood protein levels and haemocyanin were not significantly altered during the journey. The values found here were similar to those previously measured in *Cancer* during transport practices (Uglow *et al.* 1986). Glucose and lactate concentrations in the blood, however, showed a different pattern. The high levels of blood lactate observed at the start of the journey probably reflects the initial period of emersion during the unloading/loading procedures described above. Such values decreased progressively throughout the journey in both consignments, which shows that the crabs were able to maintain energy requirements mainly using aerobic pathways throughout the journey. In crustaceans, the recovery of lactate levels following a period of emersion or hypoxia usually takes several hours (Bridges and Brand 1980b, Gäde 1984, Whiteley and Taylor 1992).

When the load taken to Roscoff arrived, the drivers were told to drain all the viviers and wait for the start of the unloading procedures. However, due to a delay in dealing with another customer, such unloading procedures took over 2 h to start and the crabs were visibly less active and weaker than before that. Samples taken before and after that emersion period show a significant increase in blood lactate concentrations (Table 8.1). Such increases were associated with a significant increase ($P < 0.05$) in blood glucose levels. This mobilisation of carbohydrate stores at the end of a long and stressing journey, when the crabs are supposedly much more fragile than under normal situations, may be one explanation for the differences in mortality rates that may be found on crabs submitted to the same treatment all the way. It is noteworthy that the mortality rate of this load was 8.90 %, which was slightly higher than that of the load taken to Douarnenez (6.76 %).

When the consignments arrive in France, all procedures of handling and unloading the crabs are dictated by the French dealers and may, therefore, vary in terms of quality and speed. In both cases observed, unloading took place after the vivier-tanks were drained and this was clearly an additional factor of stress in Roscoff, as the lorry was open and the crabs were exposed to air temperatures of *ca* 32 °C during such times (Figure 8.2). Additionally, drained viviers retain a small amount of water containing decomposing material, such as legs and claws. The crabs at the base of the tanks will spend several hours immersed in that low quality water and also suffer the pressure of the whole load of crabs upon them, as the

buoyancy provided by water is not available anymore. Mortality among those crabs was clearly higher then those left emersed, and a screened-bottom tray, or similar, placed a few centimetres above the bottom of the vivier could be used to avoid that.

The crabs were individually taken from the viviers and placed in stack-and-nest boxes where they were weighed (Figure 8.3). During this procedure, some of the boxes could be overfilled with crabs, or not used in the stack position, and the animals from the lower boxes may have to support the weight of several filled boxes on top, as shown in Figure 8.4. The crabs were also unloaded inside plastic baskets, which produced even worse results as the crabs protrude their legs through the holes of the baskets and the placement of another basket on top normally caused the loss of such appendices (Figure 8.5). It seems clear that the containers used in these activities need to be redesigned.

The quality of the vivier-systems used to keep the animals after their arrival were found to be generally good (Figures 8.6 and 8.7) and, following 24 h in these systems, all crabs appeared to be in good condition (Table 8.1 and 8.2). Water TA was found to be < 15 μ mol l^{-1} in all the viviers where crabs from Ireland were placed. However, the viviers seemed to be badly prepared to cope with failures of the system or unexpected and rapid deterioration of water quality and some viviers in poor conditions were found (Figure 8.8). In some situations, when it was necessary to transfer crabs into a different vivier system or when they were sold to a new dealer, the procedures were again suitable for criticism as the crabs were thrown into containers and the personnel involved may virtually walk on top of the animals (Figure 8.9).

The increased TA levels in the holding water proved to be the major problem during the journey, as the crabs did not seem to be lacking oxygen. In crustaceans, the excretion of nitrogenous compounds is usually directly related to ambient temperature (see Newland 1957 and Regnault 1987 for a review) and external TA levels may depend on water temperature. The oxygen consumption of active (1 h after handling) and settled (6 h after handling) *Cancer pagurus* is reduced when ambient temperature is reduced (Uglow *et al.*. 1986) and similar results were found for ammonia efflux rates in this species (Couper 1993). These changes are a result of a general depression of metabolism at lower temperatures. The present experiments showed that it is possible to reduce considerably the amount of TA that accumulates during transport if lower temperatures are used. Blood constituents and water TA concentrations during a 48 h simulation of live transport of *Cancer pagurus* at two temperatures are shown in Tables 8.3 and 8.4. TA accumulated in the blood at 5 °C was *ca* half those values measured in crabs held at 17 °C. Lower levels of blood glucose were also observed at the lower

temperature for crabs maintained immersed. The rates by which TA accumulated in the water were also much smaller at 5 °C than at 17 °C.

TA efflux rates of crabs following 48 h in the cascade system and immersed under simulated transport conditions are shown in Figure 8.10. The crabs showed higher efflux rates immediately after transfer to the experimental containers with low ammonia water (*cf* efflux rates 2 h after transfer). Initial efflux rates (within the first 15 min) of crabs previously maintained at 17 °C were higher than those previously maintained at 5 °C. Such differences, however, were very small and are not likely to impact on the water quality of the vivier at the destination.

The temperature used to transport the crabs from Ireland to France was determined by the import dealers and intended to coincide with the temperatures that they would be using in their vivier tanks at the time of arrival. Such procedures are understandable, as they were used to avoid a thermal shock caused by transference to higher water temperatures. However, most of the journey could have been made at *ca* 5 or 6 °C and, when approaching the delivery place, the cooler in the lorry could be adjusted as to cause a gradual increase in water temperature. Such a temperature control of large volumes of water would, however, require an aeration system capable to effect a tight control of air temperature.

8.3. Case study 2: Maintenance of *Homarus gammarus* (L.) in vivier tanks.

The lobster *Homarus gammarus* is caught using baited traps or creels and is landed in several ports around Britain. *Homarus* is one of the species that reach the highest prices in the market, in comparison with other species that are traded live, such as *Necora puber*, *Cancer pagurus* and *Carcinus maenas*. Such prices peak at certain times of the year, such as Christmas and Easter, and some dealers will stock lobsters in vivier systems for weeks or even months so that they can profit from such seasonal price increases. The data presented here were collected from premises at Lairg and in the Orkney Islands (Scotland) during two occasions 12 months apart. The first visit was made at the request of the dealers as abnormal lobster mortality rates were occurring at the premises and whilst in transit to the continent. The second visit was made to verify the results obtained by using the guidelines and suggestions for improving the handling and holding procedures that were made at the time of the first visit.

The facilities at Lairg consisted of three vivier tanks with a closed system of water recirculation where all lobsters were kept free (ie. they carpeted the tank). The facilities in the Orkneys were located near the sea and comprised 16 rectangular vivier tanks (Figure 8.11). Water was continuously pumped from the sea into the different viviers and then taken by gravity back to the sea. *Homarus* were maintained free or kept separate in cages that were stacked inside the vivier.

8.3.1. Materials and methods.

During the first visit, samples of seawater (1 ml) were collected from each vivier tank from near the water inlet, the middle position and near the water outlet. The TA concentrations of these samples were measured. Information related to the contents of each tank, such as: species, biomass and holding procedure (free or restrained in cages) was also collected. Additionally, blood samples were taken from some lobsters from each of the vivier tanks at Lairg and also from some of the tanks in the Orkneys. Haemolymph samples from the lobsters were collected and stored as detailed in the previous section for *Cancer pagurus*, and their TA, glucose, lactate, protein and haemocyanin concentrations determined.

During the second visit, the sampling procedures were repeated and TA efflux rates of free and restrained lobsters were also measured. Lobsters were taken from the holding tank and placed individually in plastic containers (35 x 65 cm base size) with 18 l of water taken from the vivier tank. The TA efflux rates were calculated on the basis of the differences in TA concentrations obtained for two

consecutive samples elapsed by 4 h. The first water sample was taken 2 h after the lobsters were placed in the plastic containers.

8.3.2. Results and discussion.

The water TA concentrations obtained for the different viviers during the first visit to the Orkneys are shown in Table 8.5. The conditions of the vivier system in the Orkneys were found to be satisfactory, without any apparent reasons for high mortality rates. Ammonia levels in the water and blood (Tables 8.5 and 8.7) were found to be low and they were independent of the biomass held in the vivier at the time. Water TA values per tonne of lobster were found to be higher in the tanks holding free lobsters than those holding restrained lobsters. This aspect may be related to the, obviously low, activity levels of the latter.

All the blood parameters measured appeared to be normal (Table 8.7). Blood haemocyanin concentrations were lower than those found by Hagerman (1983) for lobsters fed on a variety of diets but were similar to those of starved lobsters. It may be that the diet given to the animals was not adequate, as far as the haemocyanin levels are concerned but, for the for reasons discussed below, it was advised to avoid or minimize the feeding procedures.

Some of the lobsters (free and restrained) were in that system for over 4 months and were fed regularly with fish flesh. It was possible to observe the feeding procedures and it was evident that it was being overdone (Figure 8.12). Should the pumping systems fail, water quality would probably deteriorate very quickly under such conditions. It was also observed that poor quality lobsters (weak, missing legs and claws) were kept together with intact, active ones. It was advised that a constant inspection of the animals should be made and poor lobsters should be immediately removed and kept separately.

The system was found to be satisfactory, as previously said, but not very well prepared for eventual failures. The high mortality rates that were observed from time to time appeared to be related to a combination of different factors such as the following. The high biomass that was held at some of the viviers would cause a rapid deterioration in water quality if, by any reason, the pumping system breaks down. Adding to that, the effects of uneaten food and perhaps dead or moribund animals in the tank and, if such failures occur overnight, the consequences may be disastrous. Among the recommendations made during this visit were:

- The use of a minimum of three water pumps independently connected.
- The use of a biofilter for each of the viviers.

- Alarm systems that indicate electric failures.
- Keep feeding to a minimum.
- Avoid keeping lobsters for periods longer than 6 weeks.
- Keep poor quality lobsters separated.
- Once the lobsters are selected by size they should be kept restrained.

The holding systems visited at Lairg were a good example of inappropriate practices. The results obtained are shown in Tables 8.6 and 8.7. The water TA levels and blood TA concentrations of lobsters from viviers 2 and 3 are relatively low as they had been only recently (36 h) put in those systems. Vivier 1 was a closed system (the same water was recirculated) and had a mechanical filter but was not provided with biological filters. Viviers 2 and 3 were provided with biological filters which were not well designed, as water was circulating through only *ca* 10 % of the filtering material. Blood TA levels of lobsters from vivier 1 were found to be well above normal levels (values observed at the Orkneys) and did not appear to be suited for a long journey in vivier tanks.

Table 8.8 shows the water TA values obtained for the different tanks in Orkneys during the second visit. Water quality was again found to be good and this reflected in the low TA values measured. The filter systems suggested were not added to the viviers but, instead, a much smaller number of lobsters per tank was being used, which would reduce the speed of water deterioration in case of failure.

Lobsters that were crippled or weak were kept separated in a different vivier tank. Such lobsters were characterised by having much lower blood protein and haemocyanin concentrations than those healthy lobsters that were kept free or restrained (Table 8.9). Free lobsters showed higher blood glucose levels than all others and this may be related to the also higher levels of activity and interaction shown by them.

Mortality rates were considerably reduced following the implementations made at the time of the first visit. However, should the filter systems be improved with biofilters and additional water pumps, the biomass could be increased and the systems could then work safely at their full capacity.

Table 8.1. *Cancer pagurus*: Water ammonia and some haemolymph constituents of samples collected during transport of animals from Downings, Ireland to Roscoff, France. Values are mean ± SE and n = 18 - 20 in all cases.

Sample	Journey time (h)	Water ammonia (μmol l^{-1})	Blood ammonia (μmol l^{-1})	Glucose (mg 100 ml^{-1})	Lactate (mg 100 ml^{-1})	Protein (g 100 ml^{-1})	Hemocyanin (mmol l^{-1})
Larne	5	1526.9 ± 66.1	566.6 ± 35.8	12.60 ± 2.26	39.57 ± 7.74	6.07 ± 0.53	0.56 ± 0.07
Lymm	19	3093.0 ± 199.8	641.0 ± 65.7	20.09 ± 2.67	10.26 ± 2.03	6.80 ± 0.73	0.60 ± 0.06
Poole	26	4179.1 ± 150.9	878.1 ± 42.3	12.09 ± 1.27	7.28 ± 3.48	5.61 ± 0.33	0.64 ± 0.06
Roscoff - arrival	41	4369.4 ± 141.8	1091.2 ± 70.8	18.01 ± 1.06	9.65 ± 4.64	6.67 ± 0.46	0.71 ± 0.07
Roscoff - 2.5 h emersed.	-	-	1158.7 ± 112.8	32.10 ± 3.55	30.31 ± 2.26	6.14 ± 0.40	0.67 ± 0.05
Roscoff - viviers 24h after arrival.	-	-	266.5 ± 21.0	12.72 ± 1.16	2.64 ± 0.49	6.07 ± 0.34	0.64 ± 0.05

Table 8.2. *Cancer pagurus*: Water ammonia and some haemolymph constituents of samples collected during transport of animals from Downings, Ireland to Douarnenez, France. Values are mean ± SE and n = 18 - 20 in all cases.

Sample	Journey time (h)	Water ammonia (μmol l^{-1})	Blood ammonia (μmol l^{-1})	Glucose (mg 100 ml^{-1})	Lactate (mg 100 ml^{-1})	Protein (g 100 ml^{-1})	Hemocyanin (mmol l^{-1})
Larne	5	871.74 ± 45.6	375.6 ± 21.6	9.57 ± 1.56	32.23 ± 6.29	6.20 ± 0.40	0.72 ± 0.06
Lymm	19	2209.5 ± 45.5	391.6 ± 65.7	12.57 ± 1.58	23.06 ± 8.12	6.08 ± 0.40	0.61 ± 0.08
Poole	26	2758.7 ± 67.5	472.5 ± 42.3	17.18 ± 1.62	9.48 ± 3.22	6.93 ± 0.53	0.80 ± 0.06
Douarnenez - arrival	43	3868.2 ± 99.6	939.9 ± 49.4	11.18 ± 1.40	20.90 ± 7.08	6.14 ± 0.46	0.64 ± 0.06
Douarnenez - viviers 2h later.	-	-	249.8 ± 20.9	12.31 ± 1.05	12.16 ± 6.02	6.27 ± 0.33	0.68 ± 0.05
Douarnenez - viviers 24h later.	-	-	206.9 ± 16.5	9.58 ± 1.47	9.72 ± 3.91	5.68 ± 0.41	0.64 ± 0.07

Table 8.3. *Cancer pagurus*: Haemolymph constituents (mean ± SE and number of sampled crabs) after 48 h of holding in aerated seawater at 17 ± 1.2 and 5 ± 0.8 °C and in a cascade system at 5 ± 1.4 °C without water retention.

Experimental conditions.	Ammonia (μmol l^{-1})	Glucose (mg 100 ml^{-1})	Lactate (mg 100 ml^{-1})	Protein (g 100 ml^{-1})
17 °C	957.23	10.03	2.35	7.27
	± 45.67	± 2.71	± 0.25	± 0.64
	6	6	6	6
5 °C	490.57	3.29	2.17	7.23
	± 36.35	± 0.89	± 0.10	± 0.96
	6	6	6	6
Cascade 5 °C	344.65	16.92	3.55	6.98
	± 43.66	± 8.47	± 0.68	± 0.78
	9	9	9	9

Table 8.4. *Cancer pagurus*: Water ammonia concentrations (μmol l^{-1}) during holding in aerated seawater at 17 ± 1.2 and 5 ± 0.8 °C and in a cascade system at 5 ± 1.4 °C without water retention (1:1.2 water to biomass ratio).

Immersed crabs			Cascade system	
Time (h)	17 °C	5 °C	Time (h)	5 °C
0	< 1	< 1	0	< 1
6	583.78	100.00	6	161.87
17	1475.68	200.00	18	460.43
24	1767.57	257.14	25	582.73
30	2205.41	328.57	30	607.81
40	2740.54	457.14	34	611.51
48	2890.60	542.86	44	820.14
			48	935.25

Table 8.5. *Homarus gammarus*: Water ammonia levels (µmol l^{-1}) near the water inlet (TA 1), middle position (TA 2) and water outlet (TA 3) of viviers at the Orkney Islands (Scotland) during visit 1.

Vivier	TA 1	TA 2	TA 3	TA 3 tonne^{-1}*	Holding conditions
1	< 1	1.31	5.49	7.84	700 kg *Homarus* - free
2	< 1	2.38	2.38	1.14	2086 kg *Homarus* - restrained
3	< 1	< 1	3.03	9.71	312 kg *Homarus* - free
4	< 1	2.12	3.87	2.01	1920 Kg *Homarus* - restrained
5	< 1	< 1	1.53	-	*Pecten* + 434 kg *Homarus* free
6	< 1	< 1	3.93	2.14	1828 Kg *Homarus* - restrained
7	1.08	3.61	5.23	2.74	1907 Kg *Homarus* - restrained
8	1.34	4.19	5.36	18.48	290 Kg *Homarus* - free
9	< 1	< 1	< 1	-	390 Kg *Panulirus* - free
10	< 1	< 1	< 1	-	380 Kg *Panulirus* - free
11	< 1	4.71	8.60	10.75	800 Kg *Homarus* - free
12	< 1	0.56	6.66	-	unknown weight Hom*arus* - free
13	< 1	< 1	1.47	-	350 Kg *Panulirus* - free
14	< 1	6.27	6.27	3.62	1728 Kg *Homarus* - restrained
15	< 1	< 1	< 1	-	390 Kg *Panulirus* - free
16	< 1	3.93	7.43	8.94	831 Kg *Homarus* - free

* Ammonia levels per tonne in tanks containing *Homarus gammarus*.

Tabel 8.6. *Homarus gammarus*: Water ammonia levels (µmol l^{-1}) near the water inlet (TA 1), middle position (TA 2) and water outlet (TA 3) of viviers at Lairg (Sutherland, Scotland) during visit 1. Biomass values were not available.

Vivier	TA 1	TA 2	TA 3	Holding conditions
1	595.08	579.54	588.85	Mechanic filter. Cloudy and foammy water.
2	34.03	41.49	57.64	Biological filter and protein skimmer.
3	27.20	26.27	29.68	Biological filter.

Tabela 8.7. *Homarus gammarus*: Haemolymph constituents (Mean and SE) of lobsters from the holding facilities at the Orkney Islands and at Lairg during visit 1.

Vivier	TA (μmol l^{-1})	Glucose (mg 100 ml^{-1})	Lactate (mg 100 ml^{-1})	Protein (g 100 ml^{-1})	Hemocyanin (mM)
Orkneys 1	278.60	3.99	1.21	5.13	0.366
	± 41.11	± 1.59	± 0.11	± 0.39	± 0.056
	8	8	8	6	4
Orkneys 4	225.79	2.33	1.12	5.44	0.276
	± 29.88	± 0.91	± 0.13	± 0.72	± 0.040
	8	8	8	4	6
Orkneys 6	239.25	2.11	1.78	5.13	0.248
	± 46.99	± 0.98	± 0.18	± 0.67	± 0.047
	8	8	7	8	8
Lairg 1	679.87	6.66	0.76	6.62	0.367
	± 29.55	± 2.10	± 0.13	± 0.61	± 0.040
	8	8	8	8	8
Lairg 2	183.94	4.68	1.20	4.85	0.237
	± 28.59	± 0.79	± 0.33	± 0.63	± 0.041
	8	8	7	8	8
Lairg 3	222.90	8.81	1.30	6.57	0.275
	± 30.28	± 2.71	± 0.24	± 0.83	± 0.048
	8	8	7	8	8

Table 8.8. *Homarus gammarus*: Water ammonia levels (μmol l^{-1}) of viviers at the Orkney Islands (Scotland) during visit 2.

Vivier	TA	TA ton^{-1}	Weight (Kg)	Holding conditions.
1	8.00	-	-	Pecten (40.70 Kg)
2	7.82	28.09	278.40	Free (cripples or berried)
3	9.91	38.43	257.90	Free
4	11.58	45.54	254.30	Free (poor)
5	10.33	25.04	412.50	Restrained
6	12.03	31.71	379.40	Free (poor or berried)
7	4.21	-	-	Not available
8	12.08	-	-	Not available
9	5.71	15.96	357.80	Restrained
10	6.92	15.91	435.00	Restrained
11	7.22	15.43	467.80	Restrained
12	3.31	9.07	365.00	Restrained
13	4.63	19.29	240.00	Restrained
14	6.61	13.07	505.90	Restrained
15	5.95	14.54	409.20	Restrained

Table 8.9. *Homarus gammarus*: Haemolymph constituents (Mean and SE and n = 9 or 10) of lobsters from the holding facilities at the Orkney Islands (Scotland) during visit 2.

Holding conditions	Glucose (mg 100 ml^{-1})	Lactate (mg 100 ml^{-1})	TA (μmol l^{-1})	Protein (g 100 ml^{-1})	Hemocyanin (mM)
Restrained.	2.04 ± 0.65	0.97 ± 0.10	215.94 ± 16.19	4.53 ± 0.33	0.49 ± 0.06
Free.	9.35 ± 2.96	2.48 ± 0.71	298.29 ± 27.82	6.15 ± 0.36	0.69 ± 0.05
Poor quality (free).	0.94 ± 0.36	1.05 ± 0.12	257.91 ± 31.84	2.31 ± 0.38	0.14 ± 0.04

Figure 8.1. *Cancer pagurus*: Transport of crabs from Downings, Ireland to Roscoff and Douarnenez, France (July - 1994). Journey time, route and sampling locations are shown.

Figure 8.2. Unloading *Cancer pagurus* transported from Ireland to Roscoff, France. The tanks in the lorry are drained and the whole procedure may take up to 3 h to be accomplished.

Figure 8.3. Unloading *Cancer pagurus* transported from Ireland to Roscoff, France.

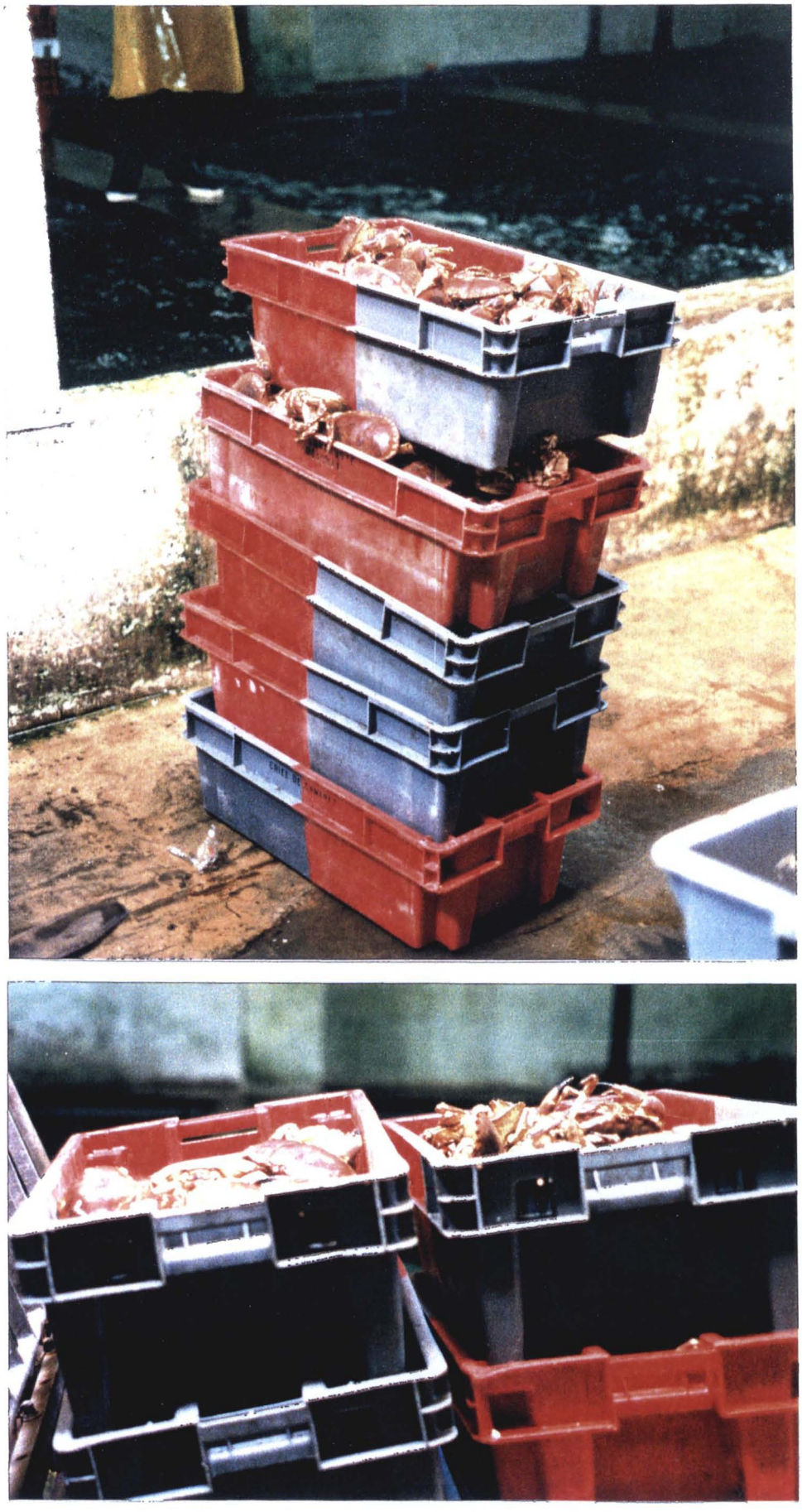


Figure 8.4. Unloading *Cancer pagurus*. Crabs in the lower boxes support the weight of the upper boxes.

Figure 8.5. Unloading *Cancer pagurus*. Crabs usually insert their legs through the holes of the baskets and damage is caused by the weighth of the baskets on the top.

Figure 8.6. Holding facilities of a French dealer at Roscoff, Britanny.

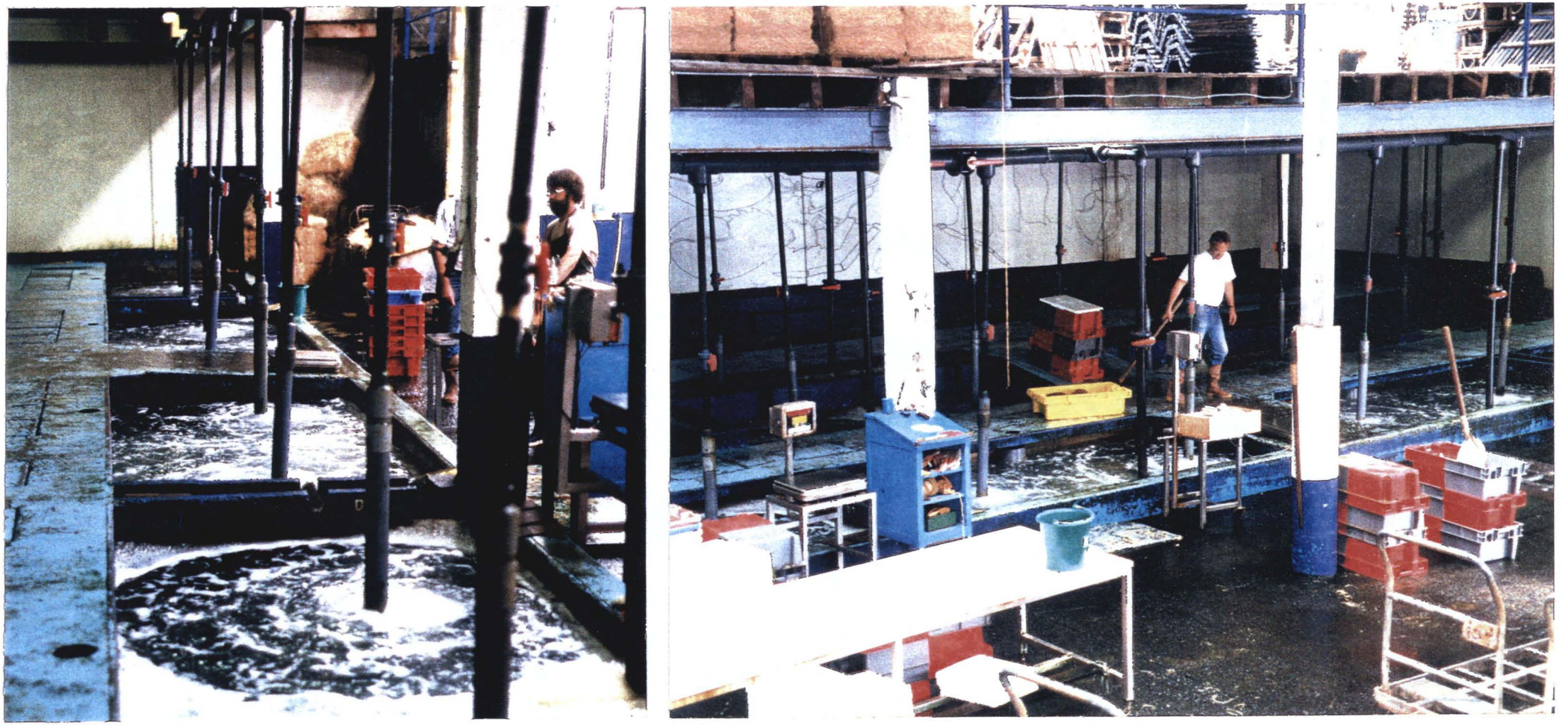


Figure 8.7. Holding facilities of a French dealer at Douardenez, Britanny.

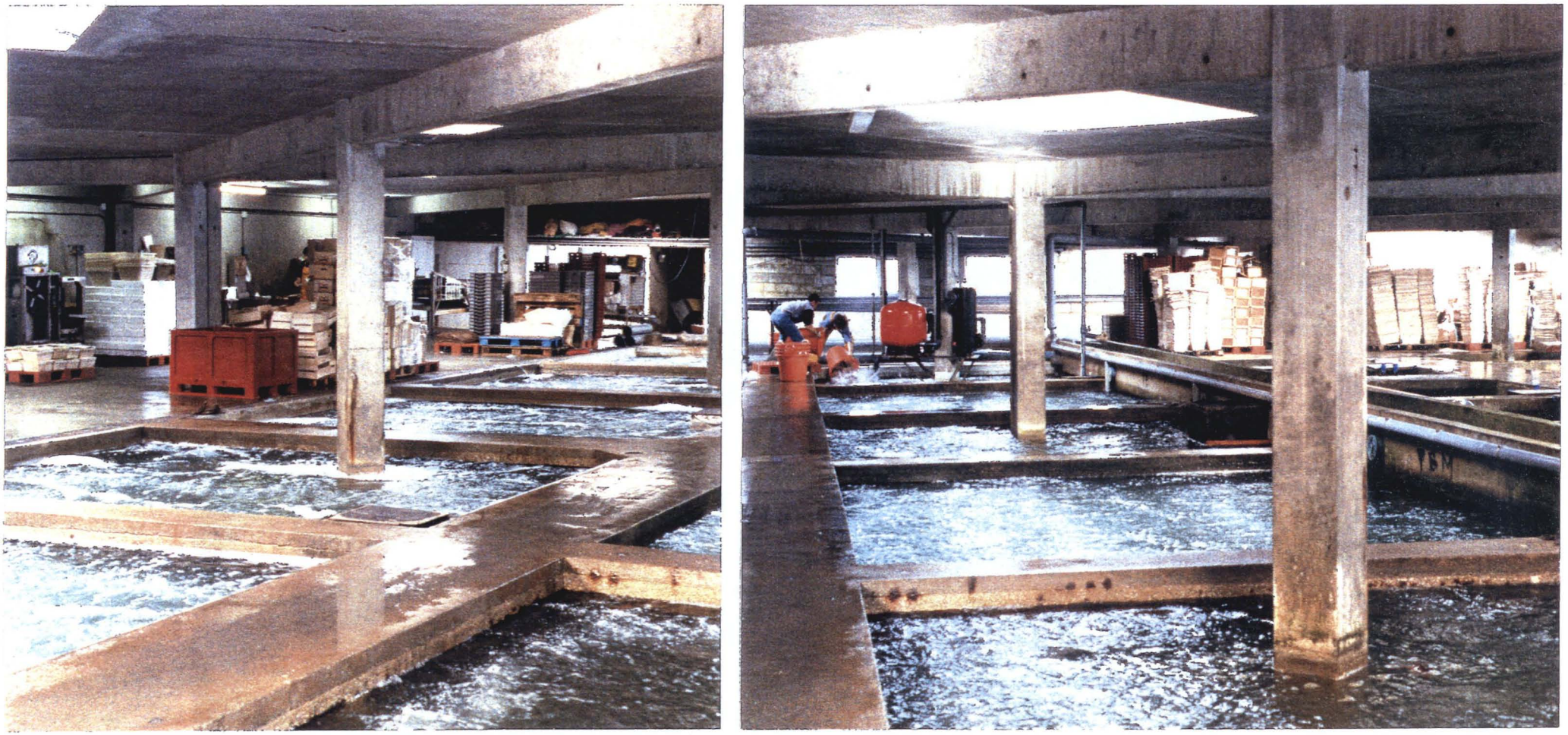


Figure 8.8. Holding facilities of a French dealer at Roscoff, Britanny.

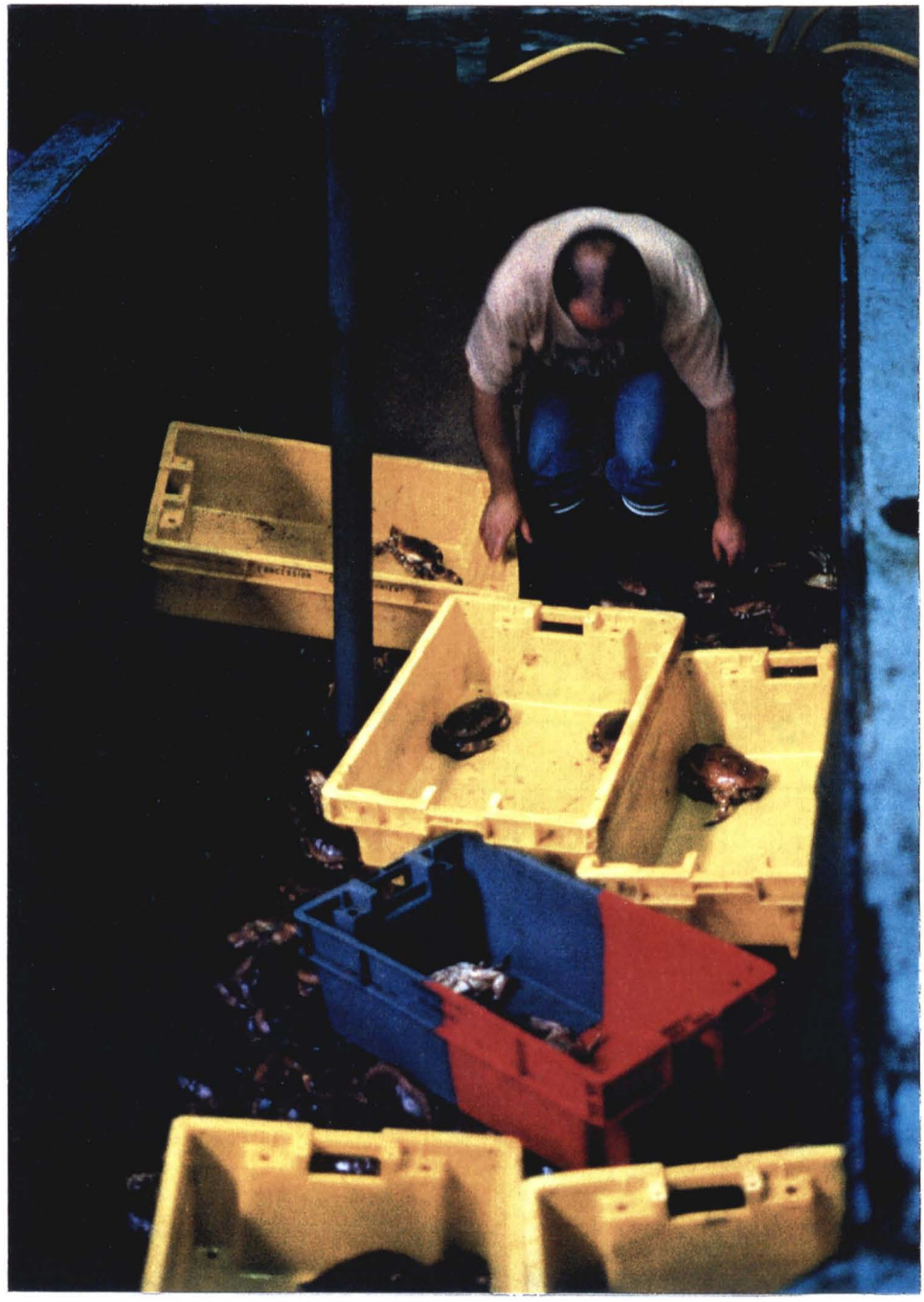


Figure 8.9. Selecting *Cancer pagurus* from the holding tank at Douardenez, France.

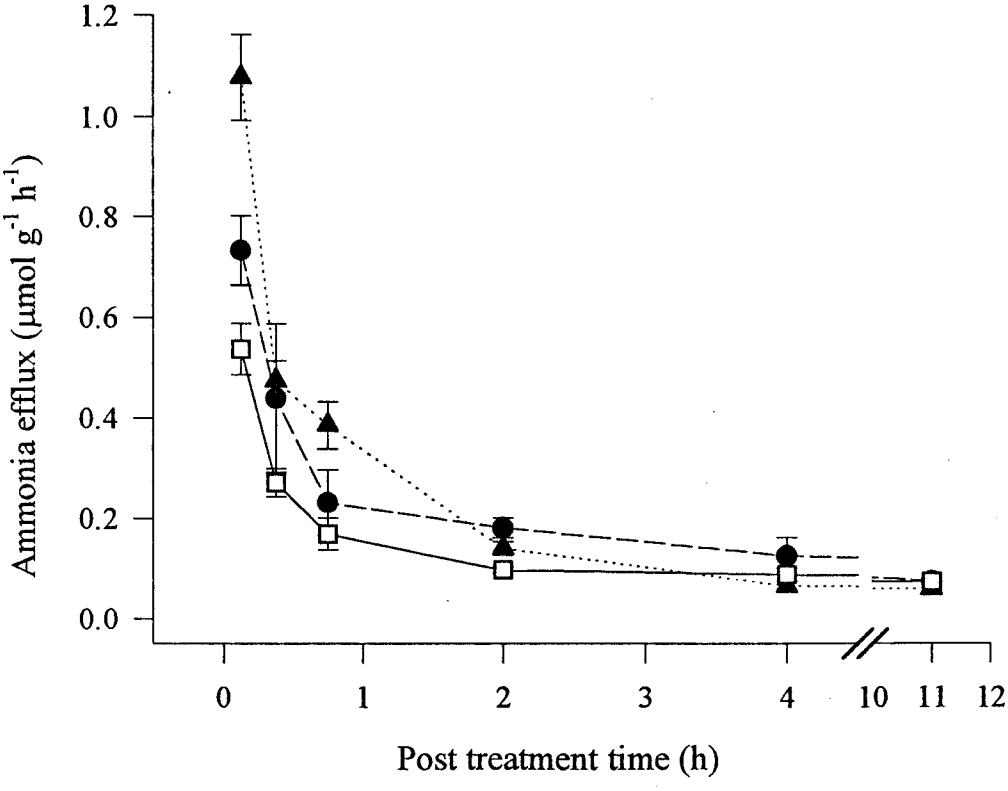


Figure 8.10. *Cancer pagurus*: Ammonia effluxes of crabs kept for 48 h in a cascade system at 6 °C (□) and of crabs held for 48 h immersed in seawater at 17 (▲) and 6 °C (●).

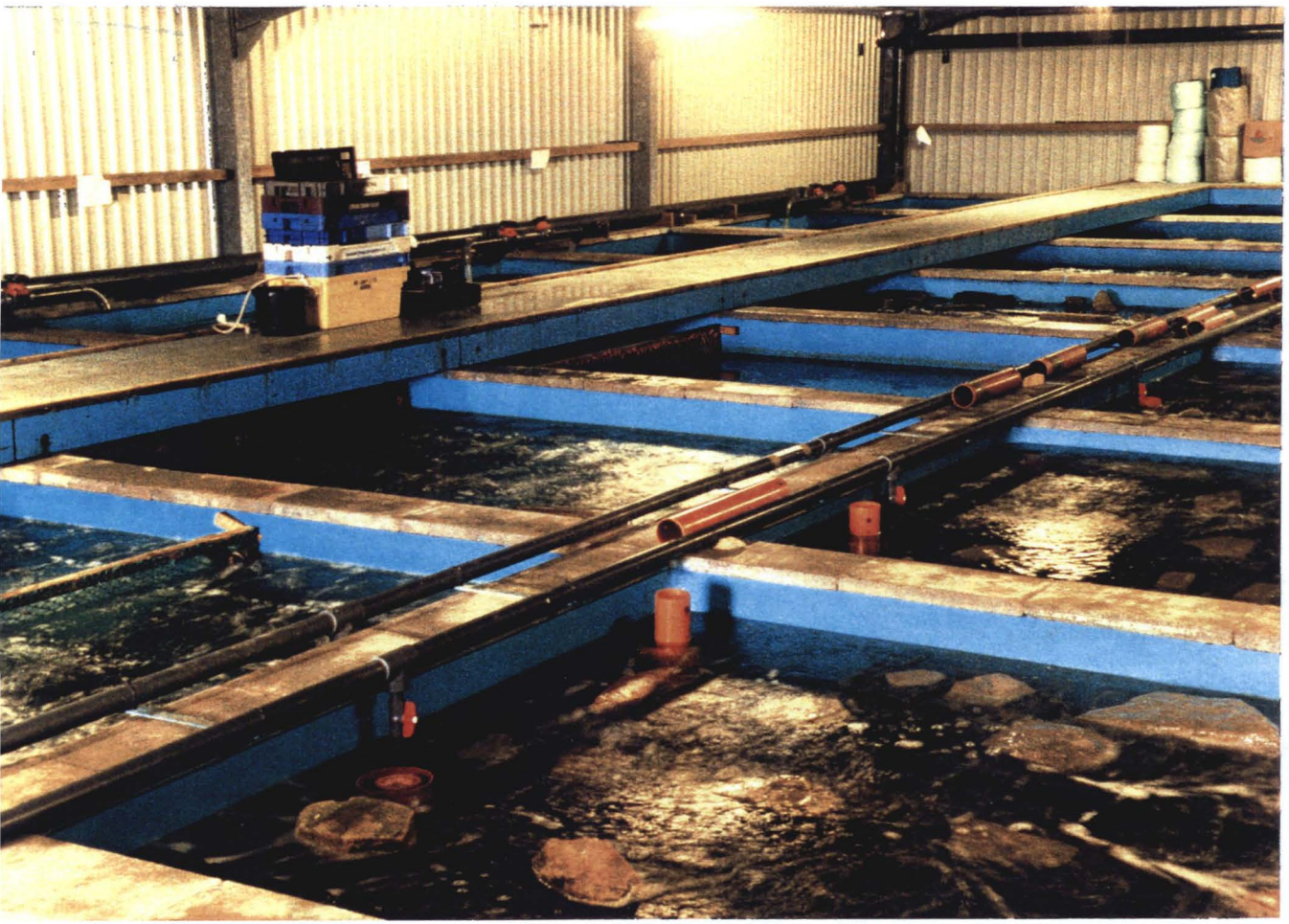


Figure 8.11. *Homarus gammarus* holding facilities at the Orkney Islands, UK. Lobsters are held free or restrained in plastic trays.

Figure 8.12. Feeding of *Homarus gammarus* in viviers at the Orkney Islands, UK.

Chapter 9.

General discussion and conclusions.

9.1. General considerations.

This work provides information on the main factors that may affect the physiology of crustaceans during live transport. All the species used in this study are either intensively transported alive or, like *Nephrops norvegicus*, are strong candidates for the live trade. A large body of information is available in the literature concerning the stress factors that were studied here, but very little work has been done specifically on the way that these factors can affect the animals during live-transport. These may only be achieved with experiments involving tightly-focused laboratory simulations or during monitoring of real transport operations. Some information on this subject was produced by Whyman *et al.* (1985), MacMullen *et al.* (1986), Uglow *et al.* (1986) and Hosie (1993) on *Necora puber*, *Cancer pagurus* and *Carcinus maenas*. Additional works have also been made on species that are transported live for aquaculture purposes, such as *Macrobrachium rosenbergii*, *Penaeus japonicus*, *P. setiferus*, *P. japonicus* and *P. vannamei* (Singholka 1982, Smith and Wannamaker 1983, Robertson *et al.* 1987, Paterson 1993).

One of the main points to emerge from the present work is related to the effects of temperature on the nitrogen efflux of *Macrobrachium rosenbergii*. The results obtained here show that the use of temperature reductions as a tool to effect a general reduction in the metabolism must be viewed with care and must consider the procedures used to produce such alterations. It is not known, however, whether the patterns found here for nitrogen release will be the same for other metabolic processes (*eg* oxygen consumption and osmoregulation), but the detrimental effects that increased levels of ambient ammonia may have on the organism (see General Introduction and Armstrong 1978 and Campbell 1991) are, nevertheless, a good motive for caution on the use of temperature alterations prior to transportation.

The main objective of this study was to evaluate the responses of *Nephrops* to some of the factors that may cause stress during the transport of animals alive. The results obtained in Chapters 4, 5, 6 and 7 show that *Nephrops* is well suited to cope with the adverse environment that normally occurs during live transport practices and it should be considered as a strong candidate for this type of trade, in a similar way to the more established trades of *Cancer pagurus* and *Homarus gammarus*.

A detailed description of two consignments that transported *Cancer* from Ireland to France was made and, in addition to the observations made on the holding practices of *Homarus gammarus* in the Orkney Islands, provides additional

information on the procedures and practices used in the live trade of crustaceans and their repercussion on the physiology of the animals.

Most of the physiological responses observed in the present work resulted from alterations in water temperature, emersion and desiccation, exposure to hypoxic conditions or increased levels of ambient ammonia. The main findings related to each of these items are considered in the following paragraphs.

9.2. Effects of temperature.

Temperature can alter several physiological mechanisms of crustaceans, including most metabolic processes, and this is usually reflected in their oxygen consumption rates. Short term changes may effect large alterations in $\dot{M}O_2$, but such changes may be compensated for if the animals are allowed time to acclimate (see Taylor 1981 for a review). The cardiac and ventilatory frequencies and the nitrogen efflux rates of crustaceans are also directly related to the metabolic activity of the animals and may also be affected by ambient temperature (Needham 1957, Ashanullah and Newell 1971, DeFur and Mangum 1978, Gerhardt 1980, Cameron and Mangum 1983, Quarmby 1985, among others). Here, the influence of water temperature on physiological processes were found to occur in *Macrobrachium rosenbergii*, *Nephrops norvegicus* and *Cancer pagurus*.

The effects of temperature on nitrogen effluxes may be influenced by factors such as the nutritional status of the animals tested, stage of development and temperature range used (Quarmby 1985, Regnault 1987, Kristianssen and Hessen 1992, Couper 1993). In *Macrobrachium*, the responses of nitrogen effluxes to temperature, described in Chapter 3, were dependent upon temperature alteration rate. Sudden alterations (4 °C min^{-1}) appeared to promote effluxes and also to increase the proportion of ammonia in the total nitrogen (TA:TN) excreted by the animals. These were shown by the lack of a reduction in efflux rates during water temperature reductions from 26 °C to 18°C and the subsequent *ca* 3-fold initial values increase when temperature was brought back to 26 °C. This response may be a result of stress caused by the shock of sudden alterations in a similar way to those responses to stress found here, after the animals were handled out of water, and those found in *Macrobrachium* by Armstrong *et al.* (1981), following handling and transfer to a new environment.

During more gradual alterations in water temperature (4 °C in 5 h), which supposedly eliminated the shock effect, a direct relation between water temperature and nitrogen effluxes was observed (18-26 °C). When the animals were allowed to settle in each of the temperatures tested for a period of 72 h, they were able to

adjust and maintain nearly constant efflux rates during temperature reductions and increases. The increases in the TA:TN ratio found during sudden alterations were less evident during more gradual alterations and did not occur when the animals acclimated for 72 h between changes.

Heart (HR) and scaphognathite rates (SR) were also altered by temperature. During sudden reductions from 26 °C to 18 °C the rate by which HR and SR decreased was lower than that found during a subsequent increase from 18 °C to 26 °C. According to Spaargaren (1974), heat transfer between the internal and external environment is directly related to the heart rate of the animal and therefore it appears that internal temperatures in *Macrobrachium* submitted to the temperature alterations described above were altered more rapidly during temperature increases than during temperature decreases. SR rates may also be involved in the maintenance of constant values of TA and TN efflux rates of prawns subjected to sudden temperature alterations (see Discussion in Chapter 3 of this work). SR may alter the post branchial oxygen levels of *Astacus leptodactylus* (Angersbach and Decker 1978) and a similar influence of SR on the transference of ammonia across the gills may have been produced here on blood ammonia. Progressive temperature reductions and increases alter the HR and SR of the animals at a more regular rate then when sudden temperature changes are effected and this is reflected on the absence of peaks in the activity of these organs during the former situation.

Temperature also affected the metabolism of *Nephrops norvegicus*, as shown by the experiments described in Chapter 7. A direct relation between temperature and ammonia formation/release, found during some of the experiments with *Macrobrachium*, was also found for *Nephrops*. In Chapter 7, this was clearly shown by the differences obtained for ammonia accumulation rates between cascade systems maintained at 6 °C and those maintained at 14 °C. Among the variables tested during such experiments, blood and water ammonia were found to be the ones that were mostly affected by such differences in water temperature.

During the monitoring of the transport of *Cancer pagurus* from Ireland to France (Chapter 8), ambient temperature affected the animals under two different conditions. Firstly, the crabs were subjected to emersion under very high air temperature (above 30 °C) upon their arrival and this had a clear detrimental effect on them, as mortality was higher among the crabs that were held longest under such conditions. Secondly, ammonia production was again found to be affected by temperature. The laboratory experiments showed that the accumulation of ammonia that occurred during the transport of crabs at 17 °C (from clean seawater to *ca* 4000 $\mu mol\ l^{-1}$) could have been greatly reduced if the consignment was held at 5 °C (*ca* 5.3 fold reduction).

All these results show that temperature can have a considerable influence in the formation/release of ammonia in the species studied (whether they are from warm or cold water origin) and that this may ultimately reflect on the rates of ammonia accumulation in the water where the animals are held (vivier tanks, ponds and containers). Considering that water quality at such times is a factor of prime importance, these results stress the importance of a tight and well-planned control of water temperature during transport activities. Even when temperature alterations are effected as part of the transport procedures, the results obtained with *Macrobrachium* show that the alterations have to be carefully studied in order to be effective.

9.3. Effects of hypoxia and emersion.

The main result of exposing aquatic animals to hypoxic conditions or periods of emersion is that oxygen uptake by the gills and oxygen delivery to the tissues may become impaired. The responses of crustaceans during oxygen deprivation may involve utilisation of anaerobic pathways, modifications in the ventilatory rates and cardiac output, increases in haemocyanin oxygen affinity and may also generate behavioural adaptations such as a decrease in the activity levels (see Introduction in Chapter 6). Perhaps the most studied aspects among those cited above are the metabolism of carbohydrate and the use of anaerobic pathways. Once aerobic metabolism begins to fall, energy production may proceed anaerobically and this has been shown in several species of crustaceans (Gäde 1983, Johnson and Uglow 1985, Lowery and Tate 1986, Taylor and Spicer 1987, Hagerman *et al.* 1990, among several others). If the energy requirements of the animals are not depressed at such times (reduced activity levels), the shift to anaerobic pathways may cause a rapid depletion of carbohydrate reserves and these may reduce survival chances during hypoxia and emersion.

Here, *Nephrops* appeared to be well-suited to face a situation of progressive hypoxia in the water, as the use of anaerobic pathways (shown by increased levels of circulating glucose and lactate) only started to occur at very low P_{wO_2} (16-17 torr). The prawns were able to maintain a steady oxygen consumption rate, hence normal rates of aerobic metabolism, over a wide range of oxygen concentrations and these was reflected in the maintenance of a constant difference between C_{aO_2} and C_{vO_2}. The constant $\dot{M}O_2$ and ammonia efflux rates found, indicate that metabolism was not depressed at such times. The maintenance of constant $\dot{M}O_2$ probably derived from the increase observed in the SR during hypoxia. It is noteworthy that during maintenance in viviers under supranormal biomass levels,

which is commonplace during live transport practices, activity levels may differ from those of laboratory-maintained animals and the point where anaerobiosis starts to occur may have to be adjusted to compensate for such conditions. Increased locomotory activity and general disturbances may increase oxygen consumption rates (Herreid 1980, McMahon and Wilkens 1983).

Tissue hypoxia may also be caused by periods of emersion, when water loss and desiccation may impair the use of the branchial epithelium for gas exchange. Such effects of emersion were evidenced by the results obtained in Chapter 4. Surprisingly, the procedure of keeping *Nephrops* under very wet conditions, similar to those used by the fishermen during normal fishing operations, proved to be of little effect in reducing the consequences of emersion, as far as gas exchange and the use of anaerobic pathways are concerned.

In both experimental conditions, high humidity (HH) and low humidity (LH), blood oxygen levels and pH decreased sharply and blood glucose and lactate levels increased during emersion. The blood acidosis that was found in these experiments, which was absent when hypoxia occurred in the water, was probably a result of the inability to excrete CO_2 during emersion. Metabolic arrest did not occur at such times and LH emersion appeared to have induced an increase in the activity levels of the prawns.

It appears that *Nephrops* is poorly-adapted to extract oxygen out of water even when the gills are kept moist (HH emersion). However, when aerated seawater was poured over the carapace of the prawns and probably supplied the branchial tissue with a flux of water (cascade systems in Chapter 7), the anaerobic responses found during HH and LH emersion and during exposure to hypoxia did not occur. The blood of these prawns was oxygenated at all times and increased lactate concentrations were not found.

The effects of emersion were also evident during the transport of live *Cancer* to France. The high concentrations of circulating lactate associated with the apparently poor state of the crabs during the unloading procedures at Roscoff reflect the detrimental effects that emersion can have on crabs after a long and stressing journey.

Besides oxygen uptake, another physiological aspect that may be affected by emersion is the production and release of nitrogen compounds, mainly ammonia. Under normal conditions, ammonia can be easily transferred across most biological membranes and it is continuously excreted mainly across the gills of crustaceans (Kleiner 1981, Kormanik and Cameron 1981, Regnault 1987, Campbell 1991). During emersion the efficiency of such mechanisms may be reduced and ammonia may accumulate in the blood, as found in *Cancer pagurus* by Regnault (1992).

In the present experiments with *Nephrops*, blood ammonia concentrations increased during HH and LH periods of emersion. The accumulated blood ammonia values, however, were smaller than the predicted ones and also smaller than the calculated absolute amounts that were excreted following re-immersion (see Chapter 4). These results show that *Nephrops* possesses mechanisms to minimise the build up of excessive (potentially toxic) levels of circulating ammonia. It seems unlikely that ammonia was stored elsewhere than the blood (due to its toxicity) and it appears that the formation of ammonia as a metabolic-end product was replaced by the formation of a different nitrogenous compound and/or circulating ammonia was converted into something else. Chapters 4 and 5 of this work discuss the mechanisms that may be involved in the control of blood ammonia levels in *Nephrops*, as the responses that occurred during emersion and during exposure to supranormal ambient ammonia were found to be similar.

It is clear that emersion can highly affect the physiology of the animals by promoting internal hypoxia and increased levels of nitrogenous-end products. It may be difficult to eliminate the existence of periods of emersion during normal live transport operations, but the impact of these periods on the animals may be greatly reduced. This may be done by avoiding emersion after the animals have been exposed to other stressing factors, such as those that occur during a long journey, and by emersing the animals at very low temperature, such as those used in *Nephrops* during the tests performed in Chapter 7.

The effects of emersion may also be avoided or minimised with the use of spray/cascade systems that will keep the animals partially immersed or will at least provide a constant supply of aerated water (Burnett *et al.* 1973, Chapter 7). The maintenance of crustaceans emersed under moist conditions may also reduce the effects and/or improve survival (*cf* unprotected emersion) (Otwell and Webb 1977, Paterson 1993) but may still affect the blood physiology of the animals, as shown in Chapter 4, and this will have to be considered if the animals are required to be kept alive following transport.

9.4. Effects of increased ambient ammonia.

Ammonia may cause alterations to the acid-base balance and enzyme activity of aquatic animals (Campbell 1991) and the resulting toxicity may cause reduced growth rates and mortality (Wickins 1976, Armstrong *et al.* 1978, Provenzano 1983, Chen *et al.* 1990, Chen and Lin 1991, 1992, Lin *et al.* 1993). According to Campbell (1991), the general toxicity of ammonia remains a relatively vague concept and, in most cases, toxicity results from a combination of several effects at

the organellar and/or cellular level which may vary from tissue to tissue and from species to species. Ammonia is a very small molecule and may diffuse easily across most lipid membranes (Kormanik and Cameron 1981, Schmidt-Nielsen 1983). When aquatic animals are exposed to external ammonia levels which are higher than those in the blood, an influx-induced increase in blood ammonia may occur, and these has been found by Chen and Kou (1993) in *Penaeus monodon* and also in Chapter 5 (*Nephrops norvegicus*) and Chapter 8 (*Cancer pagurus*) of this work.

Nephrops was able to maintain blood ammonia at concentrations lower than those of the external environment (ambient ammonia levels ranging from 500 to 4000 μmol l^{-1}). The amount of ammonia excreted during recovery in clean seawater exceeded by far that amount calculated to have accumulated in the haemolymph during exposure to high ambient levels. In a similar way to the blood ammonia increases caused by emersion, such differences indicate that ammonia was converted into something else during such times. It is noteworthy that such mechanisms would reduce the build up of high internal levels of ammonia, but will not eliminate the existing gradient between internal and external environments and, therefore, the influx of ammonia may proceed.

Increased levels of ambient ammonia had little effect on HR and SR of *Nephrops*, which increased significantly only when external ammonia concentrations reached 4000 μmol l^{-1}. All prawns survived the experimental increases in water ammonia and it appears that *Nephrops* is well-suited to cope with supranormal levels of ambient ammonia.

Very high levels of ammonia in the water were observed during the live transport of *Cancer* (Chapter 8). The crabs were also able to maintain blood ammonia concentrations at levels lower than the external ones and were also able to excrete ammonia against a gradient (evidence of active transport mechanisms).

The levels of ammonia in the water may be controlled with the use of biofilters or with partial and periodical replacements of the water from the vivier tanks and containers. The efficiency of biofilters for the treatment of large amounts of water will depend upon careful design and constant supervision by technical staff. During live transport practices these may not be possible nor may be the replacement of water from viviers and containers. Under such circumstances, the level of ambient ammonia may be controlled effectively by reductions in water temperature, as shown in Chapter 8 of this work.

9.5. Further work.

The following topics of research may provide further evidence and additional information on the main findings of this work.

1. The study of oxygen consumption rates of *Macrobrachium rosenbergii* during sudden temperature alterations.

2. Additional studies on the nitrogen metabolism of *Nephrops norvegicus* during emersion and exposure to increased ambient ammonia concentrations.

3. The study of physiological aspects of *Nephrops* during successive exposures to stress factors such as emersion, hypoxia and high ambient ammonia concentrations.

4. Commercial-scale testings of a cascade system using the specifications of Chapter 7.

Summary.

The success of practices for the live transport of crustaceans is highly dependent upon a thorough knowledge of the biological and physiological requirements of the species used. The principal aim of this work was to simulate in the laboratory some of the conditions likely to be observed during such commercial practices and to evaluate their effects on some species of commercial value.

Chapter 3 studied the effects of temperature change rates (ranging from 18-26 °C) on the ammonia and total nitrogen effluxes and heart and scaphognathite beat rates of *Macrobrachium rosenbergii*. Sudden temperature drops (4 °C min^{-1}) failed to evoke a reduction in the nitrogen efflux of the animals, but subsequent increases of similar magnitude were characterised by very high Q_{10} values and rates *ca* 3 fold that of acclimated animals at the same temperature. More gradual changes (4 °C in 5 h) caused a transient reduction in the nitrogen effluxes and 72 h of acclimation to a changed temperature eliminated the effects of the changes on the efflux rates. The proportion of ammonia in the total nitrogen excretion appears to be a reflection of the intensity of the shock caused by temperature alterations. Heart and scaphognathite rates showed that internal temperatures were probably achieved quicker during sudden temperature increases than during reductions. Temperatures used in these experiments were sufficiently low to effect a reduction in metabolism without causing a situation of torpor. Temperature reductions are commonly used to depress the metabolism of *Macrobrachium rosenbergii* during live transport and these results show that the effectiveness of such techniques may depend on temperature change rate.

Chapter 4 comprises a series of experiments that were designed to evaluate the effects of high (HH) and low relative humidity (LH) on the blood physiology of *Nephrops norvegicus*, including ammonia production and release. The increases in blood ammonia levels observed during emersion occurred at much lower rates when prawns were at HH conditions. Ammonia efflux rates after re-immersion were higher than those found for control animals and the absolute amounts of ammonia excreted at such times were considerably higher than those calculated to have accumulated in the blood during emersion. Possible explanations for such differences are discussed. Blood pre- and post-branchial oxygen concentrations decreased rapidly to *ca* 10 % pre emersion values within 2 h of emersion at HH and LH conditions. Emersion-induced tissue hypoxia was evidenced by increased blood glucose and lactate concentrations. Haemolymph sampling during real fishing

operations produced similar results to those obtained in laboratory. Despite the fact that they may survive air-exposure, the ability of *Nephrops* to cope with emersion periods is very poor and appears to be little affected by high humidity conditions.

Chapter 5 evaluated the effects of increased levels of ambient ammonia on the cardio-ventilatory performance and on blood ammonia and ammonia efflux rates of *Nephrops norvegicus*. In all the concentrations used, the prawns were able to maintain blood ammonia at levels lower than the external ones. After the prawns were transferred back to low ammonia seawater, the absolute amounts of ammonia excreted were considerably higher than those calculated to have accumulated in the blood. Possible mechanisms involved in the removal and/or transformation of the accumulating blood ammonia are suggested. Heart and scaphognathite rates were not altered when the prawns were subjected to sudden alterations in ambient ammonia (<1 μmol l^{-1} to 2000 μmol l^{-1}) and, when the changes were made more gradually, both rates showed increases only at 4000 μmol l^{-1}. *Nephrops* seem to be well-dapted to cope with the high levels of ambient ammonia that are likely to occur during the trade of live animals.

Chapter 6 studied some of the metabolic responses of *Nephrops norvegicus* to a progressive reduction in ambient oxygen tensions (P_{wO_2}). Oxygen consumption rates and ammonia efflux rates were found to be constant over a wide range of P_{wO_2} values (153 down to 44 torr) and a similar result was found for the difference between post- and pre-branchial oxygen tensions (155 down to 19 torr). These findings show that normal metabolism (normoxic levels) was probably not depressed and that *Nephrops* are able to maintain aerobic metabolism at such times. Anaerobic pathways were activated only after P_{wO_2} reached 16-17 torr (shown by increased blood glucose and lactate concentrations). *Nephrops* 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 P_{wO_2}. Heart rates also remained stable during P_{wO_2} reductions, but scpahognathite beat rate increased considerably, which probably resulted in the steady weight-specific oxygen consumption rates observed. *Nephrops* appeared to be well-adapted to cope with progressive hypoxia, as it may occur in their natural burrows or during commercial maintenance in vivier ponds.

Chapter 7 aimed to produce a system whereby *Nephrops* could be transported and/or held for variable periods of time with very little physiological disturbances and also with a space/weight optimization. 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 or 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 rates of ammonia accumulation in the cascade systems at 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 in the polystyrene box showed anaerobiosis, but at a lower intensity than that found for prawns in Chapter 4 of this work. Guidelines were made to design a system to hold *Nephrops* on a commercial scale.

Chapter 8 is divided in two sections: one that monitored two consignments of *Cancer pagurus* from Ireland to France, which were complemented by laboratory experiments, and another that monitored *Homarus gammarus* holding facilities in Scotland. A description of the procedures used to hold *Cancer* during such journeys was made. Haemolymph and water ammonia concentrations during the journey increased progressively and, at the end of the journey, the levels were *ca* 1000 and 4000 μmol l^{-1}, respectively. Alterations in blood lactate and glucose occurred and were found to be related to emersion periods during loading/unloading procedures that could be easily minimized or avoided. The containers and baskets used to unload the animals were found to be causing great damage to the animals and need to be redesigned. Several detrimental handling procedures were observed at the end of the journey, when the animals are more vulnerable. Laboratory experiments showed that the accumulation of ammonia during the journey could be greatly reduced with reductions in water temperature. In Scotland, the vivier tanks and water used to hold *Homarus* were found to be mostly in good condition and the lobsters generally healthy. The mortality observed by the dealers was probably related to mishandling of the system and of the lobsters. A series of recomendations were made, which were concerned to: improvements of the holding system; feeding of the animals; handling and maintenance procedures.

Summary of recommendations.

Holding and transport of *Macrobrachium rosenbergii*.

★ Sudden temperature alterations should be avoided as they may cause supranormal rates of nitrogen efflux and may also increase the proportion of ammonia in the total nitrogen excreted.
★ A metabolic depression may be successfully made using gradual (5h) temperature drops. Such reductions in the metabolic rates will minimize water quality deterioration.
★ A water temperature of 18 °C is sufficiently low to effect a reduction in metabolism but is not so low as to put the animals in torpor caused by hypothermia.

Holding and transport of live *Nephrops norvegicus*.

★ On the basis of blood chemistry, the stress imposed by emersion seems to exceed the stress caused by the onboard handling practices that occur during normal fishing operations.
★ The procedure of keeping *Nephrops* between layers of seawater-soaked hessian, together with periodical irrigation appears to have little effect on the maintenance of the intrinsic quality of the catch.
★ The use of a system that will keep the prawns at least partially-immersed in running seawater is likely to improve their nitrogen excretion and reduce the occurrence of anaerobiosis and is to be strongly recommended.
★ *Nephrops* is well-adapted to cope with the increased levels of ambient ammonia that may occur during trade practices and this seems to be a factor of comparatively minor importance in the success of live holding and transport procedures.
★ It must be emphasised that exposure to high ambient ammonia levels in combination with exposure to other stressing factors (*eg* emersion, hypoxia) should be avoided.
★ This species is also well-adapted to face progressive hypoxic exposure accompanied by increased CO_2 levels as may occur in burrows.
★ 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.

★ Trays without water retention and internal divisions may not be ideal but are acceptable, as they could then be used to hold species of different sizes and/or shapes (*eg* crabs and lobsters).

Holding and transport of *Cancer pagurus*.

★ Minimize the duration of emersion periods - mainly during the summer months when high air temperatures prevail.

★ Immediately after the crabs were loaded in the lorry, water ammonia levels increased at rates much higher than those observed during the rest of the journey. This can be reduced with a water change following loading.

★ Transport should be made at lower temperatures (6 - 7 °C) than those currently used. This will reduce the accumulation of ambient ammonia and the oxygen consumption rates of the crabs.

★ The placement of a screened-bottom tray, slightly raised from the bottom of the vivier-tanks would avoid exposure of crabs to the low quality water that is normally retained on the bottm of the tanks after they are drained.

★ The containers used to unload the animals should be redesigned (see Discussion, Chapter 8), as they were found to inflict damage on the animals.

Long term holding of *Homarus gammarus*.

★ A minimum of three water pumps should be used and connected independently and the viviers should also be provided with biofilters.

★ Feeding should be kept to a minimum and it is not advisable to maintain the lobsters in the systems for periods longer than 6 weeks.

★ Poor quality lobsters should be kept separated and once they are selected by size they should be restrained to avoid excessive mutual interaction.

Symbols and abreviations.

BTB - Bromothymol blue.

C_{aO_2} - Total oxygen (bound + free) in post branchial haemolymph.

C_{vO_2} - Total oxygen (bound + free) in pre branchial haemolymph.

HH - High relative humidity.

HR - Heart beat rate.

LH - Low relative humidity.

$\dot{M}_{O_2}$ - Weight specific oxygen consumption.

P_{cr} - Critical pressure.

PTFE - Polytetrafluoroethylene

P_{wCO_2} - Pressure of water carbon dioxyde.

P_{wO_2} - Pressure of water oxygen.

SR - Scaphognathite beat rate.

TA - Total ammonia ($NH_3 + NH_4^+$).

TN - Total nitrogen.

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