A physiological investigation into methods of improving the post-capture survival of both the southern rock lobster, *Jasus edwardsii*, and the western rock lobster, *Panulirus cygnus*.

by

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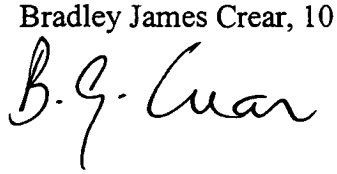
School of Aquaculture

This thesis contains no material which has been accepted for the award of any other degree or diploma in any tertiary institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text.

Bradley James Crear, September 1998

Bradley James Crear, 10 September 1998



ABSTRACT

The southern and western rock lobsters (*Jasus edwardsii* and *Panulirus cygnus*, respectively) form the basis of two of the major seafood export industries in Australia; between them earning over $500 M export dollars yearly. Although a major portion of the catch is exported as 'whole-cooked' or 'tailed' products, an increasing share of the catch is exported live. The majority of lobsters arrive at the processing sheds as live lobsters. However, a lack of basic physiological information has impeded advances in the design and management of transport and holding systems, often resulting in a deterioration of the physiological condition of some lobsters. Such physiological deterioration may result in the final product choice for the processors being limited, leading to a reduced value of the catch. The aim of this study was to develop an understanding of the physiology of lobsters, especially in relation to factors the lobsters may be subjected to during post-capture handling practices. This information could be used to redefine post-capture handling practices and holding system design and management.

Standard oxygen consumption of both species increased in response to increases in temperature and body weight. Activity had the greatest effect on oxygen consumption rates, causing an approximate 3-fold increase above standard rates. The increase in oxygen consumption due to activity decreased at temperatures approaching the upper and lower extremes of each species. After a period of activity and emersion oxygen consumption remained elevated for up to 8 hours. A marked diurnal rhythm was evident, with a 48% and 87% (*J. edwardsii* and *P. cygnus*, respectively) increase in oxygen consumption at night. This was largely related to increased activity at night. Feeding resulted in a substantial (greater than 2-fold in *P. cygnus*) and sustained (up to 48 hours) increase in oxygen consumption. Both species were essentially oxygen regulators, able to maintain standard rates of oxygen consumption down to around 30% water oxygen saturation. Below that oxygen level the lobsters became oxygen conformers. Activity resulted in an approximate doubling of the water oxygen level at which lobsters acted as oxygen conformers.

The total ammonia nitrogen (TAN) excretion rates of both species increased with increases in temperature and body weight. Activity had minor

influence on the TAN excretion rate. A diurnal rhythm was evident in *J. edwardsii* but not in *P. cygnus*. Feeding had a large affect on the TAN excretion rate, with an approximate 6-fold increase occurring in each species. The excretion rates remained high for over 24 hours post-prandial.

The effect of the dissolved oxygen level on recovery of *P. cygnus* from a period of activity/emersion was investigated. Based on the rate of recovery of various physiological parameters (oxygen consumption, haemolymph ammonia, lactate, glucose, and pH), the maintenance of water oxygen levels close to 100% saturation is recommended. Water oxygen levels less than 60% saturation slowed the rate of recovery. All lobsters recovering in water with oxygen levels less than 20% saturation died.

Carrying *P. cygnus* out of water imposes physiological disturbances to the lobsters. The severity of the disturbances increased when the relative humidity was lower and when wind was present. Spraying water over the lobsters prevents some of the physiological consequences of emersion, such as decreases in pH and haemolymph ammonia buildup, however it does not prevent haemolymph lactate increases. Therefore, lobsters still rely on anaerobic metabolism when emersed in sprays. There was no evidence that failure of lobsters to recover from a period of emersion was caused by gill damage.

A half hour period of emersion/handling at 23°C caused large physiological disturbances of *P. cygnus*. Halving the emersion/handling time did not decrease the extent of the physiological disturbances. Slow-chilling the lobsters to 11°C prior to emersion/handling, was an effective means of decreasing the physiological disturbances associated with emersion.

This study has developed our understanding of the physiological responses of the southern and western rock lobsters to factors affecting them during post-capture processes, and will allow the design and management of rock lobster holding facilities to be based on a sound scientific basis. It also represents a major contribution to knowledge on respiration and nitrogen metabolism of large decapod crustaceans.

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CHAPTER 1

General introduction

1.1 INTRODUCTION

The southern rock lobster, *Jasus edwardsii* (Hutton, 1875), and the western rock lobster, *Panulirus cygnus* (George, 1962) form the basis of two of the largest and most lucrative fisheries in Australia. South Australia (approx. 2500 tonnes annually), Tasmania (1500 tonnes) and Victoria (900 tonnes) all have major fisheries based on *J. edwardsii*, with some commercial fishing for the species occurring in southern Western Australia and southern New South Wales. The southern rock lobster fishery is worth around $150 million annually. *J. edwardsii* is also the target species for the major rock lobster fishery in New Zealand. *P. cygnus* is only caught commercially off the west coast of Western Australia. It supports the second largest rock lobster fishery in the world (Brown and Phillips, 1994) with an annual catch of between 9000 and 12000 tonnes, worth over $300 million.

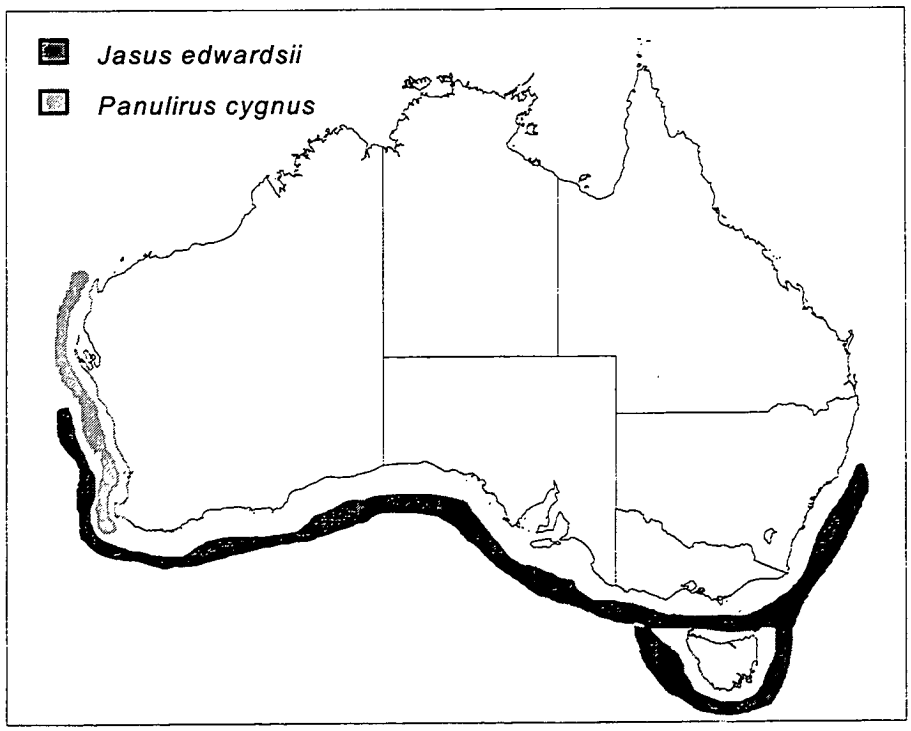

Fig 1.1: The distribution of *J. edwardsii* and *P. cygnus* in Australian waters.

J. edwardsii inhabits temperate waters and ranges from Coffs Harbour in northern New South Wales (30°18'S) to Geraldton in Western Australia (28°45'S)(Brown and Phillips, 1994), including Tasmania (Fig 1.1). It also inhabits

New Zealand waters. Research has shown that the Australian and New Zealand populations are the same stocks (Booth *et al.*, 1990). *J. edwardsii* is found in depths ranging from 1 to 200m, and in water temperatures ranging from 6°C in southern New Zealand (McKoy, 1985) to 23°C at the northern extremity of its range.

Mature adults usually move into the deeper offshore waters to breed. Females are fertilised externally by the male depositing a spermatophore on their sternal plates (MacDiarmid, 1988), between April and July (Brown and Phillips, 1994). The eggs are carried on the pleopods until hatching, which generally peaks in October. The larvae (called phyllosoma) develop at sea for between 9 and 24 months, before being carried back to the coast and settling out as pueruli. The puerulus is the transitional stage between the planktonic phyllosoma and the bottom-living juvenile (Kennedy, 1990). It resembles the adult form, but is completely clear and has enlarged pleopods for active swimming. Puerulus moult to the juvenile stage within two weeks after settling. Juveniles inhabit inshore waters and are generally found associated with reefs. Lobsters are foraging feeders and ontogentic changes in the diet of *J. edwardsii* have been found (Edmunds, 1995). Small juveniles feed predominantly on ophiuroids, isopods and bivalves, whereas older juveniles and adults feed predominantly on bivalves, crabs and other crustaceans, urchins and gastropods (Fielder, 1965; Edmunds, 1995). *J. edwardsii* can take anything from 5-11 years to reach legal size, with locality and sex significantly impacting on the time frame (Booth and Breen, 1994).

P. cygnus inhabits the tropical/temperate waters off the western coast of Western Australia and ranges from Northwest Cape (21°48'S) to Hamelin Harbour (34°30'S)(Holthuis, 1991) (Fig. 1.1). Adults and juveniles are found throughout the limestone and coral reefs, from shallow inshore areas to the edge of the continental shelf (at depths of around 80 metres). The water temperature ranges from 27°C at Northwest Cape to 16°C near Cape Leeuwin in the south (Grey, 1992).

Mature adults usually move into deeper waters to breed. Females are fertilised externally by the male depositing a spermatophore on their sternal plates, between July and December. The eggs are carried on the pleopods until hatching, which generally occurs from late October through to February (Brown and Phillips, 1994). The phyllosome larvae develop offshore over 9-12 months, before moving back towards the coast and settling as pueruli on the shallow limestone reefs (<40m). The pueruli moult to post-puerulus juveniles which are solitary in nature. As they

develop into juveniles they become more gregarious in nature (Jernakoff, 1990), eventually existing in social groups. They are basically opportunistic feeders, feeding on a wide variety of material, including seaweed, coralline algae, echinoderms, molluscs, crustaceans and polycheate worms (Joll and Phillips, 1984). The juveniles take 3-4 years to reach the minimum legal size for the fishery.

Both rock lobster fisheries have focussed on the live lobster trade in recent years. Almost 90% of the *J. edwardsii* catch and between 40 and 50% of the *P. cygnus* catch were exported live during the 1996/97 fishing season. In comparison during the 1991/92 season only 10% of the *P. cygnus* catch was exported live (Marec Pty Ltd., 1994). The methods required to maintain lobsters so they are suitable for live export are completely different to the methods used when the fisheries were predominantly reliant on products such as whole cooked and frozen tails. The care of live lobsters begins the moment they are taken from the traps, and the percentage of weak and dead lobsters that subsequently develops may be considerably diminished by giving the lobsters due care from this time on (Chaisson, 1932). Additionally, the delivery of good quality live lobsters to processors will help to ensure that any non-live products are also of premium quality. The aim of the industry should be for each lobster to be delivered to the processor in a condition which gives the processor maximum choice as to how to process (Harvie, 1993).

The holding and transport of live crustaceans has been practiced since early this century, although only a relatively small volume of literature has been published on the topic. Much of the research has been conducted on northern hemisphere species such as the American lobster, *Homarus americanus*, and the European lobster, *H. gammarus*. In 1932, Chaisson outlined and discussed many of the problems faced during the holding and shipment of *H. americanus*. During the 1950s, '60s and '70s research focused on the aerial transport of crustaceans, with the aim of increasing the survival rate of the animals (McLeese, 1958; McLeese and Wilder, 1964; McLeese, 1965; Witham, 1971). Recently, the increased commercial importance of marketing crustaceans alive has led to a significant amount of research being conducted on many European species (see Whyman *et al.*, 1985; Spicer *et al.*, 1990; Whiteley *et al.*, 1990; Beard and McGregor, 1991; Whiteley and Taylor, 1992; Schmitt and Uglow, 1997*a*). In Australia, the development of the live crustacean trade has also spawned research into methods for improving the live transport and

holding of several crustacean species (Varley and Greenaway, 1992; Morrissy *et al.*, 1992; Paterson, 1993*a*,*b*; Paterson *et al.*, 1994*a*).

In parallel with specific studies on live holding/transport, there has been a large number of studies investigating respiratory and acid-base responses to a wide variety of internal and environmental perturbations - including temperature, dissolved oxygen level, carbon dioxide level, emersion, exercise, salinity, and acid-base state of the water (see Waldron, 1991 for a review). These studies have developed an understanding of the biochemical and physiological changes crustacea undergo when exposed to various factors, including those which commonly occur during post-harvest procedures such as exposure to hypoxic conditions and handling stress.

Studies on the physiology and biochemistry of *J. edwardsii* and *P. cygnus*, particularly in relation to live holding and transport, have been limited. The physiology of *J. edwardsii* has not been studied in depth. Binns and Petersen (1969) studied the form and origin of nitrogen excretion. Waldron (1991) investigated the respiratory and acid-base physiology of lobsters subjected to handling and emersion. Waldron (1991) suggested that accurate knowledge of its response to environmental perturbations would be invaluable in ensuring higher survivorship and quality of exported animals.

The physiology of *P. cygnus* was extensively investigated during the 1970's (Dall, 1974*a*,*b*; Dall, 1975; Dall and Smith, 1978) however, these studies were not directly related to the physiological processes imposed during post-capture handling. Some studies relating to the handling of lobsters after capture (Anon., 1980*b*; Brown and Caputi, 1986) shed some light on the stress imposed on lobsters by the handling practices immediately post-capture. Recently studies directly related to the holding and transport of *P. cygnus* have been undertaken. Spanoghe (1997) studied the physiological and biochemical responses elicited by *P. cygnus* to the various post-capture processes. Tod and Spanoghe (1997) investigated the physiological effects of truck transport of lobsters, with the aim of developing improved onshore storage and transportation protocols. These studies have enabled the identification of protocols by which post-harvest handling techniques could be modified to reduce the occurrence of morbidity and mortality (Spanoghe, 1997).

It is apparent that the physiological and biochemical responses of both the southern and western rock lobster to the conditions imposed on them during post-

capture processes are very similar to those observed in many other species of subtidal crustaceans (Waldron, 1991; Spanoghe, 1997). For instance, emersion results in significant changes to the biochemistry and physiology of crustaceans (internal acidosis, hypoxia, hyperglycaemia, hypercapnia); thus, it should be avoided wherever possible. Many of the recommendations on post-capture handling made for other crustacean species (eg. Whyman *et al.*, 1985; Harvie, 1993) can be adopted, albeit cautiously, for either the *J. edwardsii* or *P. cygnus* fishery. For example, suggestions made by Chaisson (1932), such as the need for lobsters to be handled gently and for well designed holding crates/containers, have been highlighted as being important issues for the *J. edwardsii* industry (Harvie, 1993).

The ability of fishermen and processors to hold and transport live lobsters has improved significantly since the establishment of the live lobster trade. Improvements have generally been developed through empirical approaches (Evans and Spanoghe, 1993). However, obstacles still remain due to a lack of information regarding species-specific lobster physiology.

1.2 THE POST-CAPTURE PROCESS

The specific areas researched in this study are discussed below, but first it is necessary to develop a clear understanding of the processes a lobster may go through after capture. Lobsters are caught in baited pots (or traps) that are usually pulled at least once daily. The lobsters will be stressed because for the first time they have been subjected to air exposure, bright sunlight and handling. They are held for between a few hours and two weeks in tanks on board the fishing boat before being landed. There are two main methods of holding lobsters on boats, viz: (a) below deck tanks and (b) on deck storage facilities (usually fish bins). The ability of each particular on-board holding system to successfully hold lobsters will depend on factors such as water flow rate, stocking density and tank design.

After landing, the lobsters may be transported on trucks for a period of several hours before reaching the processing facilities. Truck transport methods are highly variable. The western rock lobster industry has made extensive use of controlled temperature water sprays in insulated trucks to transport lobsters from isolated sites (Tod and Spanoghe, 1997). Some companies rely on the use of

controlled temperature trucks, and others are transporting lobsters fully submerged in seawater tanks. Similar methods are used in the southern rock lobster industry. However, the use of trucks which have no insulating properties, or have open or partly enclosed carrying compartments is not uncommon. Such methods can cause additional stress to the lobsters as they may be subjected to wind and/or high temperatures.

The lobsters are usually held submerged in tanks at the processing facilities for 2-3 days. This period of time in tanks helps the lobsters to recover from the stresses of capture and transport, and allows them to purge their gut contents, thus improving their chances of surviving the export process. In an attempt to take advantage of seasonal price fluctuations, many processors are now holding significant quantities of lobsters for extended periods (several weeks/months) prior to export. The design of holding facilities is highly variable and ranges from sea cages (mostly used for *J. edwardsii* in South Australia) to high technology recirculating systems. By far the majority of lobsters are being held in tank/raceway systems using flow-through water. That is, water is being pumped from the ocean into the tanks and then exits the tanks and flows back into the ocean. These are quite simple systems but their success can be variable if the tank design, water flow and stocking density criteria are not met. Setting those criteria relies on a detailed understanding of the lobsters physiological requirements.

Lobsters may be subjected to many factors (poor water quality, emersion, temperature extremes, exposure to sunlight/wind, handling, overcrowding) during the post-capture process which may result in their health being compromised. For example, *P. cygnus* undergoes at least eight periods of handling between the time they are delivered to coastal depots until they leave the processing facility packed in export cartons (Spanoghe, 1997). Reducing the impact of any of these factors on lobster health would appear to be an obvious method of improving the overall quality of the lobsters.

1.3 OVERALL AIMS AND STRUCTURE OF THE THESIS

The quality of water provided in holding tanks for live lobsters has to be optimal in order to achieve minimal losses (Spanoghe, 1997). Specific information on which to base the design and maintenance of holding and transport systems are

limited. To optimise the quality of the water it is necessary to understand the physiological requirements of the lobsters. Techniques used to hold and transport live lobsters have mostly been developed on a trial and error basis without any knowledge of those physiological requirements. Therefore, scientifically based protocols for holding and transporting rock lobsters need to be established. This study focuses on obtaining information which will contribute to the further development of the live rock lobster industry, with the aim of increasing post-capture survival of both the western and southern rock lobsters.

Investigations on oxygen consumption and ammonia excretion of both lobster species are presented (Chapters 3, 4 and 5). Further investigations on optimising the dissolved oxygen levels, aerial transport and the relationship between temperature and stress were undertaken only on *P. cygnus* (Chapters 6, 7 and 8). Concentration of the research on *P. cygnus* has resulted from the greater need to establish handling protocols for that species. The exponential increase in the tonnage of *P. cygnus* being handled live has not been matched with the development of such protocols. In a review of the research and development needs of the Australian rock lobster industries, Phillips (1995) recommended that physiological studies which assisted post-capture handling of *P. cygnus* should be given the highest priority.

The general materials and methods are outlined in Chapter 2, however materials and methods specific to each Chapter are outlined in the Materials and Methods section of that Chapter. An overview of the results, and their implications to the industry, is given in the final chapter (Chapter 9).

1.4 STRESS

Much of this research focuses on lobsters which are stressed. It is necessary to define the word stress when it is used in the context of lobsters. Stress is a general term which indicates a change in biological condition beyond the normal range of resting conditions; the change challenges homeostasis and, as such, represents a threat to an animals well being (Barton and Iwama, 1991). Stress can be either readily reversible, a change that lies within the homeostatic capability of the lobsters, or it can be non-reversible, ultimately leading to death (Taylor *et al.*, 1997). Possible stressors for lobsters include, the capture process, handling, excessive activity associated with the escape response, crowding, poor water quality, emersion, and

exposure to sunlight and wind. Stress itself cannot be measured, and only the responses to stimuli can be quantitatively determined to reflect the degree or severity of stress experienced (Barton and Iwama, 1991). In crustaceans, many indicators of stress have been used; these include haemolymph lactate, pH, glucose, and ions (see Taylor *et al.*, 1997 for a discussion).

The time taken for both *J. edwardsii* and *P. cygnus* to recover homeostasis after a period of stress can be lengthy (>8 hours)(Waldron, 1991; Spanoghe, 1997), although the length of the recovery period varies depending on the stressor. Some of the primary aims of transport and holding systems are, to decrease the extent of the stress response (ie. try to limit it to readily reversible changes), and to minimise the period of time required to recover homeostasis after a stress response. Such approaches have been recognised as important steps to help alleviate the detrimental effects of stress in fish populations (Barton and Iwama, 1991).

1.5 OXYGEN CONSUMPTION

Spiny lobsters are aerobic animals which rely on the uptake of oxygen from water to drive their metabolic processes. In order to respire efficiently an animal must: (a) possess means of moving the respiratory medium across the gas exchange surfaces, (b) be able to take up oxygen from the medium, and (c) transport oxygen to the tissues and the carbon dioxide produced to the gas exchange surface (Waldron, 1991).

The gas exchange surfaces of spiny lobsters are gills, which are situated within the gill chambers on either side of the head under the carapace. The gills of lobsters are filamentous, consisting of simple filaments (trichobranchiate). The flow of water past the gills is generated by the beating of the scaphognathites, or gill bailers, which are the exopodites of the second maxilliped. The flow of water across the gills is countercurrent (Rogers, 1982; McMahon and Wilkens, 1983) although Rogers noted that because of the direction of haemolymph flow within the gill filament, countercurrent exchange will be less effective than in fish and in those crustaceans with lamellate gills.

The arrangement of the gills within the branchial chamber of *J. edwardsii* has been described by Rogers (1982)(Rogers described the gills of *J. novaehollandiae* which was concluded to be the same species as *J. edwardsii* by Booth *et al.* (1990)).

Understandably, Waldron (1991) found that *J. edwardsii* from New Zealand waters had the same gill arrangement as described by Rogers (1982). The gills of both the southern and western rock lobsters used in this study also had the same number, position and gross morphology. That is, there are 21 gills within each cavity and the length of the individual gills varies to fit within the shape of the cavity, but each tapers at the tip, with the distal filaments shorter than those at the base. Rogers (1982) also noted that some gill filaments in *J. edwardsii* had 2 septa, which divided the filaments into 3 channels instead of the usual 2. The same arrangement was also observed in *P. cygnus*. Rogers (1982) postulated that the middle channel acted as a pressure relief system.

Oxygen is one of the major water quality parameters determining the health of aquatic animals. It is essential that water flow and aeration are sufficient to provide adequate oxygen for the number of lobsters being held (Beard and McGregor, 1991). Low levels of oxygen in the water caused by coral spawnings have been postulated to be the cause of death of western rock lobsters held in cages at the Abrolhos Islands (Anon, 1993). Oxygen needs to be supplied to the animals at a rate that meets their metabolic requirements. However, there are many factors (both extrinsic and intrinsic) which affect the rate of oxygen consumption of crustaceans (Cockcroft and Wooldridge, 1985). Temperature, body weight, sex, activity, water oxygen tension, feeding, daily rhythms, moulting stage, and salinity have all been shown to affect oxygen consumption (Thomas, 1954; Halcrow and Boyd, 1967; Ansell, 1973; Rice and Armitage, 1974; Spoek, 1974; Laird and Haefner, 1976; Schembri, 1979; Bridges and Brand, 1980*a*; Penkoff and Thurberg, 1982; Taylor, 1981; Dall, 1986; Winkler, 1987; Houlihan *et al.*, 1990; Whiteley *et al.*, 1990). A full understanding of how oxygen consumption of *J. edwardsii* and *P. cygnus* is affected by such factors is essential to ensure that transport and holding systems are appropriately designed to minimise the impact of post-capture processes on the health of lobsters.

Studies on oxygen consumption of large decapod crustaceans have been limited and have centred on species such as the crab *Cancer magister* and the clawed lobsters *Homarus* spp. (McLeese and Watson, 1968; Spoek, 1974; McMahon *et al.*, 1979; Whiteley *et al.*, 1990). Considering their economic importance the palinurids, or spiny lobsters, have been poorly researched. Winget (1969) reported on the effects of dissolved oxygen levels, body weight, temperature and activity on oxygen

consumption of *Panulirus interruptus*. Buesa (1979) investigated the effects of body weight, dissolved oxygen levels, salinity and temperature on oxygen consumption by *P. argus* and the effect of body weight on oxygen consumption by *P. guttatus*. Zoutendyk (1989) studied the effects of temperature and body weight on oxygen consumption by *J. lalandii*.

Some information is available on oxygen consumption by *J. edwardsii* through the study by Waldron (1991). *J edwardsii* had a 25% higher oxygen consumption rate at 17°C than at 15°C, but the oxygen consumption rates at those temperatures were comparatively low compared to other crustaceans of similar size. When lobsters were exposed to air the oxygen consumption rate fell to approximately 2/3 of the resting rate in water. However, upon re-immersion the oxygen consumption rate was more than 2.5 times the resting rate and it remained elevated for more than 8 hours. *J. edwardsii* only had a limited ability to maintain its resting rate of oxygen consumption in the face of decreasing dissolved oxygen levels; below 50% saturation oxygen consumption by *J. edwardsii* became dependent on the ambient oxygen level. Waldron (1991) concluded that the lack of oxygen independence exhibited by *J. edwardsii* may prove limiting to its distribution. Waldrons' study provides a basis for understanding the oxygen requirements of *J. edwardsii* and will allow an intra-species comparison of lobsters separated by a considerable distance (approx. 2000 km). No previous studies on oxygen consumption by *P. cygnus* were found in the literature.

This study investigates the oxygen consumption response of *J. edwardsii* and *P. cygnus* to intrinsic and extrinsic factors which are commonly associated with post-capture processes - temperature, body weight, activity, feeding, handling, daily rhythm and dissolved oxygen level.

1.6 AMMONIA EXCRETION

A characteristic of aquatic crustacea is ammonotelism. Ammonia makes up 60 to 100% of the total excreted nitrogen in crustacea (Regnault, 1987). Ammonia excretion occurs either via active ion exchange (Na^+/NH_4^+) or by passive diffusion through the gill epithelium (Regnault, 1987). Ammonia can be toxic to crustaceans if allowed to accumulate to too high a concentration in the water (Tomasso, 1994), and even at low levels can inhibit growth (Chen and Lin, 1992). This is particularly

evident where other factors such as low dissolved oxygen levels, low salinity levels, or low pH also interact (Wajsbrot *et al.*, 1989; Chen and Lin, 1992; Russo and Thurston, 1991). No information is available on the toxic levels of ammonia for either *J. edwardsii* or *P. cygnus*; in fact, there are very limited ammonia toxicity data available for large decapod crustaceans. Cornick and Stewart (1977) found that the 30-day LC_{50} of adult *Homarus americanus* was 88 mg/l total ammonia nitrogen (TAN). Young-Lai *et al.* (1991) found that the 96-h LC_{50} of *H. americanus* increased with decreases in temperature, being 377 mg/l TAN at 5°C and 219 mg/l TAN at 20°C. The authors calculated that "safe" concentrations of ammonia for adult lobsters at 20°C was 21.9 mg/l TAN. Such levels are much higher than those recommended as safe for other aquatic species, such as fish, where levels of less than 0.5 mg/l TAN are recommended (Forteath *et al.*, 1993*b*). Even so, it is generally recommended that total ammonia levels of less than 0.5 mg/l be maintained in lobster holding tanks (Bunter, 1992; Harvie, 1993).

Ammonia accumulation in the water of flow through holding systems, can easily be prevented by ensuring that the water flow rates are high enough and that good water mixing prevails. However, where water re-use systems are in place ammonia can increase to significant levels. Trucks used to transport lobsters on land generally rely on cooled seawater sprays to maintain the health of the lobsters. Water is re-used and the concentrations of waste products can reach very high levels (25 mg/l Kris Carlberg, Geraldton Fishermens Co-op., pers. comm.). Land based recirculating holding systems are commonly used for holding southern rock lobsters, *J. edwardsii,* and are being increasingly used for holding western rock lobsters, *P. cygnus*. Recirculating systems rely on the establishment of bacteria in the biofilter to treat the water and decrease the concentrations of toxic nitrogenous substances. With poor biofilter design and/or management ammonia levels may rise and compromise the quality of the lobsters.

Empirical information on ammonia excretion would be helpful in determining the water flow rates or biological filter size necessary to maintain safe ammonia concentrations (Forsberg and Summerfelt, 1992). The rate of ammonia excretion can be influenced by many factors including temperature (Regnault, 1987; Chen and Lai, 1993; Chen and Kou, 1996), body weight (Needham, 1957; Carvalho and Phan, 1997), nutritional level (Dall and Smith, 1986; Regnault, 1987), diurnal rhythms (Dall and Smith, 1986), salinity (Chen and Nan, 1993; Chen and Lai, 1993), moult

stage (Regnault, 1979), and ambient ammonia concentration (Chen and Lin, 1995; Schmitt and Uglow, 1997*b*).

Very little information is available on the ammonia excretion rates of large decapod crustaceans. Wickins (1985) studied the effect of feeding on ammonia excretion by European lobsters, *Homarus gammarus*. Zoutendyk (1987) investigated the effect of feeding on ammonia and urea excretion by *Jasus lalandii*. In both of these studies the lobsters exhibited a significant increase in ammonia excretion after feeding. The form and origin of nitrogenous excretion by *J. edwardsii* was investigated by Binns and Peterson (1969). They determined that ammonia represented 72% of nitrogenous excretion and that very little of the ammonia was excreted via the urine. In crustaceans the major portion of ammonia is excreted via the gill epithelium (Regnault, 1987).

The importance of urea as a nitrogenous excretory product for crustaceans is being increasingly recognised (Quarmby, 1985). Urea was thought to comprise only a small percentage (1-5%) of the nitrogenous end-products of Crustacea (Regnault, 1987). However, more recent studies have shown that it may comprise almost ¼ of the nitrogen excreted by some species (Quarmby, 1985; Zoutendyk, 1987; Wajsbrot *et al.*, 1989). If all of the excreted urea was oxidised to ammonia then it would represent a significant increased nutrient load on the water quality in holding systems.

Knowledge of the ammonia excretion responses of *J. edwardsii* and *P. cygnus* to culture conditions are required to optimise the design and management of transport and holding systems. Therefore, the aim of this study is to determine the effect of several intrinsic and extrinsic factors on the ammonia excretion of *J. edwardsii* and *P. cygnus*, and to determine the endogenous urea excretion rate of both species.

1.7 DISSOLVED OXYGEN LEVEL AND RECOVERY FROM STRESS

The characteristic physiological responses of subtidal crustaceans to emersion/exercise is internal hypoxia, a mixed respiratory and metabolic acidosis, hyperglycaemia, a rapid depletion of energy pools in the muscle tissue, and an accumulation of metabolic waste products (Telford, 1968; Onnen and Zebe, 1983; Head and Baldwin, 1986; Vermeer, 1987; Whiteley *et al.*, 1990; Waldron, 1991; Regnault, 1994). A variety of processes take place during re-immersion - energy

pools (phosphagen and ATP) are recharged, anaerobic end-products are cleared from the tissues, and pH disturbances are corrected. An organism-level manifestation of all of the above, essentially cellular processes of recovery, is a period of supranormal oxygen consumption (the oxygen debt)(Ellington, 1983). Oxygen consumption (M_{O_2}) is normally elevated upon re-immersion, with M_{O_2} levels 3 to 10-fold greater than resting M_{O_2} levels (Booth *et al.*, 1982). Increased oxygen consumption is achieved by a number of factors including increased oxygen supply (via increased gill ventilation rate), increased oxygen transport away from the gills (increased heart rate and hence gill perfusion rate), and by an increase in oxygen binding to haemocyanin (McMahon and Wilkens, 1983). These changes also ensure the excretion of metabolic waste products, such as CO_2, is optimised.

During post-harvest handling *P. cygnus* may undergo a series of procedures during which the lobsters are emersed for periods of between 2 and 30 minutes. During the emersion periods they are often handled and the general response of the lobsters to handling is to try and escape, which is achieved by strong beats of the tail (tail flicks). Therefore, the post-harvest procedures generally result in disturbances to their physiology and biochemistry (ie. stress)(Spanoghe, 1997) and the development of an oxygen debt. It would appear beneficial to ensure complete recovery from each episode of stress so that the lobsters are in the best possible condition to handle any further periods of stress. When fish are not able to fully recover from an episode of stress the physiological responses to further episodes are generally cumulative (Pickering, 1992; Waring *et al.*, 1997). American lobsters, *Homarus gammarus*, which were deprived of the opportunity to recover after an episode of stress, were in a significantly worse state after a period of air travel than lobsters which had been allowed to recover (Whiteley and Taylor, 1992). Whiteley and Taylor suggested that the non-recovered lobsters could be more vulnerable to stressful situations occurring during air travel, since further reductions in haemolymph pH could prove fatal to individuals already suffering from a marked internal acidosis. The recovery from anaerobic metabolism must be sufficiently rapid and complete for the organism to cope with the next period of air exposure (Ellington, 1983). Unfortunately, time between episodes of stress is often limited during post-capture handling of *P. cygnus*, therefore optimising the speed of recovery becomes important.

The recovery from periods of exercise, handling, and emersion has been intensively studied in crustaceans (McDonald *et al.*, 1979; Onnen and Zebe, 1983; Head and Baldwin, 1986; Taylor and Whiteley, 1989; Waldron, 1991). However, the effect of the dissolved oxygen level of the water on the rate of recovery and the recovery response has not been evaluated. Minimum dissolved oxygen levels recommended in the literature for lobster holding systems vary from 40 to 80% saturation (Anon., 1980*a*; Beard and McGregor, 1991; Forteath *et al.*, 1993*a*; Boothroyd, 1994) although it is unsure where the values were derived from. Oxygen supersaturation has been suggested as possible tool to aid the recovery of lobsters (Forteath, 1995).

The dissolved oxygen level in the water can affect factors such as growth in lobsters. Chittleborough (1975) noted that the growth increments of *P. cygnus* was significantly affected at oxygen levels of 60-70% saturation. In *Jasus lalandii* there was a general decrease in growth and ingestion and an increase in intermoult period, with decreasing levels of oxygen saturation (Beyers *et al.*, 1994). It appears that even slightly decreased levels of oxygen saturation appear to have serious effects on the physiological processes of unstressed lobsters. Therefore, the aim of this study was to determine the effect of dissolved oxygen level on the recovery of lobsters, *P. cygnus*, after they were exposed to an episode of stress.

1.8 AERIAL TRANSPORT

Western rock lobsters are subjected to post-capture practices which may result in emersion for periods of up to 6 hours (transport by truck or carrier boat). Although western rock lobsters are able to handle up to 48 hours emersion under export conditions (Spanoghe, 1997), emersion is generally regarded as a stress which jeopardises the condition and/or life of crustaceans (Whyman *et al.*, 1985). For example, the mortality rate of *P. cygnus*, recovering after a period of emersion, increases in proportion to the amount of time they were emersed (Brown and Caputi, 1986).

Emersion also leads to weight loss in crustaceans (Herreid, 1969). Survival in air may be limited by the progressive loss of mass and an accompanying reduction in the volume of the haemolymph, which in turn is likely to limit the effectiveness of the circulation and oxygen delivery systems (Taylor *et al.*, 1987). However,

crustaceans may be able to tolerate substantial reductions in haemolymph volume because the relatively large volume and low pressures of the open circulation system (Taylor, 1982) render it less likely to failure following reductions in volume (Taylor *et al.*, 1987). Wind and low relative humidity (RH) can increase the rate of desiccation of crustaceans (Ahsanullah and Newell, 1977; Vermeer, 1987) and are factors which must be considered in the western rock lobster industry. Western rock lobsters are transported from the Abrolhos Islands to Geraldton, Western Australia, on carrier boats. Although lobsters on the carrier boats are protected from the wind as much as possible, a proportion are still exposed to some extent (pers. observation). Atmospheric RH around Geraldton can be as low as 34% during the months when the carrier boats are in operation (Steve Summers, Geraldton Meteorological Office, pers. comm.).

On board the carrier boats lobsters are held above deck in crates and seawater is continuously sprayed over them whilst they are in transit, which can be for a period of around 6 hours. Spray systems are also used in transport trucks which carry lobsters to Geraldton from depots up and down the coast, and are increasingly in use at short-term holding depots. Therefore, spray systems have evolved in the industry as a method which can decrease the effects of emersion on the health of the lobsters. The benefits of spray systems to emersed crustaceans has never been clearly demonstrated, however there is some evidence that they help in the excretion of carbon dioxide and may reduce reliance on anaerobiosis (Paterson *et al.*, 1994*b*).

Although the gills of the decapod crustaceans are covered with chitin, they are presumably still sensitive to desiccation (Grant and MacDonald, 1979) and must therefore be kept moist (Burnett, 1988). It has been suggested that gill damage caused by dehydration may contribute to the mortality of western rock lobsters (Anon, 1980*b*; Spanoghe, 1997). Morrissy *et al.* (1992) stated that actual desiccation of gill filaments causes irreversible membranous damage in crustaceans. There is no evidence to support either of these statements. The period of time that the American lobster, *H. americanus*, was able to survive out of water did not extend when it was kept wet with sea water sprays, suggesting that drying of the gills is not a cause of death in moist air (McLeese, 1965).

This study examines the affects of two environmental factors (humidity, wind) on the physical and physiological health of emersed lobsters as well as determining the benefits of using a seawater spray system to maintain the health of

the emersed lobsters. One of the pivotal aims was to examine the role gill damage played in reducing the ability of lobsters to recover from a period of emersion.

1.9 THE RELATIONSHIP BETWEEN TEMPERATURE AND STRESS

The live export of western rock lobsters, *Panulirus cygnus*, involves chilling the lobsters, and placing them into a packaging material (usually wood shavings or wood wool) in foam cartons, before they are air-freighted to the final destination. The packaging material serves to insulate the lobsters and helps to maintain a constant low temperature. It also acts as a shock absorber and thus minimises physical damage to the lobsters. Packaged in this manner lobsters are able to survive extended periods of emersion, with minimal losses occurring over a 30 hour export period. Similar methods are used worldwide to export lobsters (Richards-Rajadurai, 1989; Harvie, 1993; Kaleemur Rahman and Srikirishnadhas, 1994).

Chilling of the lobsters prior to packing is designed to achieve several objectives. Importantly lobsters are less active at cooler temperatures, therefore they are easier to handle and the effects of handling stress are minimised. Winkler (1987) outlined how handling increased the oxygen consumption rate of lobsters (*Homarus americanus*), principally by increasing their activity. The effect of handling on the oxygen consumption rate was minimised in the prawn, *Penaeus japonicus*, by decreasing the temperature (Paterson, 1993*a*). Lobsters require less oxygen to meet their metabolic requirements at colder temperatures. As crustaceans have a limited ability to uptake oxygen in air (Taylor and Whiteley, 1989), reducing their basal metabolic rate will aid in allowing their oxygen requirements to be satisfied by aerial oxygen consumption rates. The European lobster, *H. gammarus*, is able to supply most of its oxygen requirements when held in air at a temperature of 10°C: however, at higher temperatures its ability to supply its oxygen requirements is reduced, mainly because of the temperature dependent increase in oxygen consumption (Whiteley *et al.*, 1990). Finally, low transport temperatures should reduce the build up of metabolic waste products, such as CO_2 and ammonia, and decrease the reliance on anaerobic metabolism, and thus the accumulation of lactate, thereby reducing disturbances to the acid-base balance.

Two methods are used to chill crustaceans in preparation for live transport. The method most commonly used in lobster fisheries is called the slow-chill method.

This involves chilling the lobsters slowly over time from an ambient temperature to the desired temperature for export. The final temperature depends on the ambient temperature and, in some cases, on the time period of export (Kaleemur Rahman and Srikirishnadhas, 1994). In general, lobsters are acclimated to a system for at least 24 hours before chilling commences. They are chilled to the preferred temperature at a designated rate, and then held at that temperature for around 12 hours before packing commences. The other chilling method used, and the one most commonly used in the western rock lobster fishery, is termed the quick-chill (or dip-chill) method. This involves dipping the lobsters into chilled water for a period of time before packing. Generally lobsters are dipped into 11°C water for around 3 minutes (the time period can be size dependent with larger lobsters requiring more time) although there are many industry variations of this protocol. In the export of *J. edwardsii* dip-chilling is not recommended as the recovery time is short and lobsters are found to be very active or dead on reaching their destination (Anon, 1980*a*).

Observations at a *P. cygnus* processing shed showed that lobsters may be emersed for up to 30 minutes before being dip-chilled in preparation for packing into export cartons. During the emersion period the lobsters were very active (much tail-flicking occurred) and were subjected to handling. In some cases they were also exposed to sunlight and to high air temperatures. Such treatment would not ensure the lobsters are in the best possible physiological and biochemical condition to survive the transport period. This study examines the effect of those conditions on *P. cygnus* and investigates the use of alternative strategies which aim to minimise the physiological and biochemical disturbances imposed on lobsters during pre-export conditioning.

CHAPTER 2

General Materials and Methods

This Chapter outlines the General Materials and Methods used. Specific details applying only to a particular Chapter are in the Materials and Methods section of that Chapter, as are the statistical analyses used in each Chapter.

2.1 EXPERIMENTAL ANIMALS

Southern rock lobster, *Jasus edwardsii*.

Lobsters were obtained from commercial holding facilities and from the Tasmanian Department of Primary Industries, Taroona. They were maintained in 600 l recirculating seawater tanks for a minimum of 2 weeks prior to experimentation. Each tank had 14-16 concrete building blocks in it to serve as refuges for the lobsters. These were arranged so that the lobsters had shaded cover and so that multiple den openings were available (Lipcius and Cobb, 1994). Each tank was equipped with airstones to provide oxygen and ensure good mixing of the water. A maximum of thirty lobsters was kept in each tank. A trickle biofilter consisting of BioBalls and oyster shells was connected to each tank. To further cleanse the water a protein skimmer (or foam fractionator) was connected to each tank. The water temperature was maintained at 13 ± 1°C, pH 8.0-8.3 and salinity 35 ± 1‰. Only lobsters judged to be in intermoult were used for experiments. The moult index of Turnbull (1989) for *Panulirus ornatus* was used as a guide in determining the moult stage. Lobsters were fed twice weekly with either squid (*Nototodarus gouldii*) or blue mussels (*Mytilus edulis planulatus*). Lobsters were deprived of food for three days prior to experiments. Light was controlled to provide a 12-h light and 12-h dark photoperiod.

Western rock lobster, *Panulirus cygnus*.

Lobsters were obtained from commercial holding facilities (Geraldton Fishermen's Co-op and Batavia Coast Fisheries, Geraldton, Western Australia)

and were maintained in 600 l recirculating seawater tanks for a minimum of 2 weeks prior to experimentation. The water temperature was maintained at 23 ± 1°C, pH 8.0-8.3 and salinity 35 ± 1‰. Other details on the holding system are as described for *J. edwardsii*. Only lobsters judged to be in intermoult were used for experiments. The moult index of Turnbull (1989) for *P. ornatus* was used as a guide in determining the moult stage. Lobsters were fed twice weekly with either squid (*N. gouldii*) or blue mussels (*M. edulis planulatus*) but were deprived of food for three days prior to experiments. Light was controlled to provide a 12-h light and 12-h dark photoperiod.

2.2 MEASUREMENT OF OXYGEN CONSUMPTION

The respirometers (Appendix 1) used in the experiments were primarily used in the intermittent flow mode. In this system a measurement phase (closed system) is separated from a flushing phase (open-flow system) thus providing some of the advantages of closed respirometers (simplicity) and open-flow respirometers (stable water conditions). The periodicity of the whole cycle can be chosen so that during the closed measuring phase, the oxygen tension does not fall below a given level (Kaufmann *et al.*, 1989). The respirometers could be used in the closed mode when required.

Three respirometry chambers were made from PVC pipe. They had perspex dome shaped lids which were attached to the chamber by bolts. Rubber "o" rings were used to provide a seal. The total volume of each chamber was 18.3 l. A submerged powerhead pump (AquaClear - Powerhead 201) was used to ensure there were both good water mixing within each chamber and sufficient water flow past the membrane of each oxygen electrode. Dall (1986) outlined the inherent problems associated with measuring standard oxygen consumption where crustaceans are placed into smooth walled respirometers. Therefore, attachment points were built into the respirometers so that lobsters had a grasping surface and could remain quiescent; in the wild, lobsters normally remain immobile in caves and crevices during daylight hours (Lewis, 1981).

Water flow through the chamber was controlled by 24V AC solenoid valves (Burkert - Series 55135). The solenoids were operated via a datalogger (Datataker 50) which was programmed to a timing schedule that was suitable for

the particular sized lobster and water temperature being studied. For example, at 5°C it was necessary to stop water flow for 50 minutes before a significant decline in the water oxygen content was recorded. The normal cycle was 20 minutes closed (measuring) and 10 minutes open (re-oxygenating). Under normal circumstances the oxygen tension of the chambers did not fall below 80% of saturation at the end of the measuring period. Initial testing showed that resting lobsters are able to maintain a constant rate of oxygen consumption down to oxygen saturation levels of 40%. The chambers were submerged in a water bath which maintained the water temperature within 0.2°C of the designated temperature. Oxygen tensions were recorded with WTW (Wissenschaftlich-Technische Werkstatten) oxygen sensors (EO 96) and meters (OXI 96) connected to the datalogger. Calibration of the probes was carried out in water saturated air, within a calibration sleeve, following the manufacturers' instructions. The probe was calibrated at the appropriate temperature, although that is not necessary as the meter had a built in temperature compensation. It was found, however, that the response time was increased if the probe was calibrated at different temperatures to that of the water being measured. Because little drift was apparent, the calibration procedure was usually only carried out every three to four days. Calibration is a one point procedure therefore no zero % saturation point is required. However, occasionally the calibration of the probe was checked in oxygen deficient water (obtained via the addition of sodium metabisulphite - $Na_2S_2O_5$). Oxygen consumption (M_{O_2} - $mgO_2/g/h$) was determined from the equation:

$$Mo_2 = \frac{(Po_2 i - Po_2 f) * V * 60}{W * t}$$

where $P_{O_2}i$ is the initial oxygen tension in the respirometer (mg/l); $P_{O_2}f$ is the oxygen tension after the measuring period (mg/l); V is the volume of water in the respirometer adjusting for lobster volume (l); W is the weight of the lobster (g); and t is the time of the measuring period (minutes).

The system was thoroughly cleaned with sodium hypochlorite between runs to reduce bacterial oxygen consumption and tests with a blank chamber showed that there was no need to correct for respirometer oxygen consumption.

Lobsters were acclimated to the experimental chambers for 36 hours prior to the commencement of experiments.

Standard and active oxygen consumption

Standard oxygen consumption is defined as the minimum oxygen consumption for an unfed, resting fish (Fry, 1971). During the daytime lobsters usually remained motionless in the chambers unless disturbed by movement in the room. The standard oxygen consumption rate of a particular animal was determined when three identical and consecutive 20 minute measurements of oxygen consumption were recorded.

Active oxygen consumption was determined by taking lobsters from the respirometers and forcing them to be active (by handling) over a period of 5 minutes. The lobsters were replaced into the respirometers and the active oxygen consumption was measured, usually over a 15 minute period. This is essentially a measurement of post-exercise oxygen consumption. However, I think the results can be used as active rates and compared against other measurements of active rates for a number of reasons, as outlined below.

Active oxygen uptake should be maintained over a significant period, generally 1 hour (Brett, 1972). However, there was difficulty in forcing lobsters to be active for significant periods of time. A similar problem was also noted by Rutledge and Pritchard (1981) when estimating active oxygen uptake of *Pacifastacus leniusculus*. Active tail flicking of lobsters usually stopped after 5 minutes. Usual methods to induce activity, such as water stirrers (Innes, 1985), were not possible due to design of the respirometer. Another method was attempted using a system similar to that used by Dall (1986). The lobsters were tied into a bracket and the tail left hanging free. The idea was to induce active tail flicking for an extended period, however after a few flicks the lobsters usually stopped. Also it was not possible to handle lobsters and do oxygen consumption measurements at the same time.

Active oxygen consumption is basically a measure of the maximal level of oxygen consumption (Bennett, 1978). Lobsters will be repaying an accumulated oxygen debt after a short period of handling and air exposure. Therefore measuring the oxygen consumption during the period after handling and air

exposure should be a reasonably accurate approximation of active oxygen consumption. The time period used (15 minutes) is the same as used by Rutledge and Pritchard (1981) and significantly longer than used by Innes (1985)(2 minutes). Maximum oxygen consumption values of *J. edwardsii* were similar after exercise and handling in water and after a period of air exposure and handling (Waldron, 1991). The author argued that the maximum oxygen consumption rate measured was an accurate determination of the maximum oxygen consumption rate of *J. edwardsii*. In this study it was decided to use 5 minutes of air exposure and handling to determine active oxygen consumption as it was also indicative of post-capture processes the lobsters are subjected to.

Active rates are usually determined on animals which are fully acclimated to each experimental temperature (Rutledge and Pritchard, 1981). However, lobsters are undergoing a series of acute temperature fluctuations during post-capture processes. Therefore, data on the effect of acute temperature fluctuations would be more pertinent in this study. Vernberg (1983) also questioned the physiological/ecological value of undertaking temperature-metabolic studies where animals are subjected to constant temperature for various periods of time before oxygen consumption rates are determined.

The data were examined to determine if sex of the lobsters influenced oxygen consumption.

Temperature

The effects of acute temperature changes on the oxygen consumption of lobsters were investigated. Lobsters were acclimated to the respirometers at the holding temperature before the temperature was raised or lowered to the required temperature at a rate of 2°C per hour. Lobsters were kept at each temperature for 24 hours. Standard and active oxygen consumption rates were established as above.

Log_{10} transformed linear regressions of oxygen consumption versus temperature (T) were expressed by the general equation:

$$Log_{10} M_{O_2} = a + b\,T$$

where M_{O_2} = the weight-specific oxygen consumption (mg O_2/g/h); and T = the temperature (°C).

The aerobic scope for activity was calculated as the difference between standard and active oxygen consumption (Fry, 1947). Scope for activity (SFA) represents the amount of energy available to an organism through aerobic metabolism beyond that needed for maintenance (Fry, 1947). Aerobic expansibility is a measure of the ratio of the two oxygen consumption levels (Active/Standard). Q_{10} values were determined using the following equation:

$$Q_{10} = \left(\frac{M_2}{M_1}\right)^{10/T_2 - T_1}$$

where M_1 and M_2 are oxygen consumption at temperatures T_1 and T_2, respectively.

Lobster weight

Standard and active oxygen consumption of lobsters over a large body weight range (*J. edwardsii*, 186-2180 g; *P. cygnus*, 417-3000 g) were determined.

Log_{10} transformed linear regressions of the standard and active oxygen consumption versus weight (W) were expressed by the general equation:

$$\text{Log}_{10}\, M_{O_2} = a + b \log_{10} W$$

where M_{O_2} = total oxygen consumption (mg O_2/h), a = intercept on the Y-axis, b = the slope of the regression, and W = wet weight (g) of the lobster.

Diurnal rhythm

Oxygen consumption of lobsters was recorded over a minimum of 48 hours to establish if a diurnal rhythm was present. This allowed the establishment of routine oxygen consumption, which is the oxygen consumption of fasting lobsters over 24 hours including that resulting from spontaneous activity (Becker

and Fishelson, 1986). Night-time oxygen consumption was calculated on all readings taken between 6PM and 6AM. Standard oxygen consumption was used as oxygen consumption during daylight hours as some disturbance (resulting in increased oxygen consumption) during the day was unavoidable. A video camera and infrared light (Javelin Electronics OS-45/IR-121N) were used to examine lobster activity during the night.

Handling and recovery

Lobsters were removed from the respirometer and emersed for 30 minutes. Continual disturbance (handling) for the first 5 minutes was followed by disturbance every 5 minutes. Lobsters showed a strong escape behaviour (tail flicking) during the initial period of disturbance. The response diminished as the emersion time increased and the tail flicking response was usually not evident after 30 minutes emersion. The lobsters were returned to the respirometers and their recovery monitored. A 30 minute period was selected as this is a typical maximum emersion time lobsters are subjected to during post-capture practices. For example, the period of time between when water is drained from a tank on a boat and when the lobsters are placed into a holding tank in the processing shed.

Feeding

The effect of feeding on oxygen consumption was determined by introducing a piece of squid (wet weight $\approx$ 3% of lobster wet weight) to each chamber. All lobsters used in the experiments were fed at the same time of day so that any effects of diurnal rhythm on oxygen consumption could be taken into account. Experiments where lobsters did not eat all of the squid were discontinued. To determine if an initial large increase in oxygen consumption in fed lobsters was due to increased activity, three lobsters (of each species) were subjected to the smell of feed by wafting squid in the water near the inlet to the respirometers. Their oxygen consumption rate was monitored for several hours after that treatment.

Dissolved oxygen level

The relationship between the dissolved oxygen level (P_{O_2}) and standard oxygen consumption was determined by closing the water flow off and following the response of settled lobsters to self-induced hypoxia. High dissolved oxygen levels were obtained by bubbling oxygen through the water. Oxygen consumption was averaged over each decade of change down to 40% saturation and each 5% saturation change below that.

The relationship between active oxygen consumption and P_{O_2} was determined by exposing emersed and handled (see above) lobsters to water with known oxygen levels. Oxygen consumption was measured over a 20 minute period after returning lobsters to the respirometers. Oxygen consumption was determined at six P_{O_2} levels (15, 35, 55, 75, 95, 115% saturation) and the dissolved oxygen levels were kept within 5% saturation of those designated levels. Dissolved oxygen levels were initially adjusted to the correct level by adding either oxygen or nitrogen to the water. After the animal was returned to the respirometer the oxygen level was continuously monitored. When the level fell (due to oxygen uptake by the lobster) to a 5% saturation value below the set level, oxygenated or aerated water was added (solenoid valve switched on). When the level reached 5% saturation above the set level, the flow of water into the respirometer was switched off. Therefore, a reading at 75% saturation represents the average oxygen consumption over the 70-80 % saturation range.

The critical oxygen level (P_c) was determined by calculating regression lines for the two distinctly different parts of the relationship between oxygen consumption and P_{O_2}, the horizontal high P_{O_2} segment and the sharply sloped low P_{O_2} segment. The critical P_{O_2} (P_c) was designated as the intersection point of the two lines (Cochran and Burnett, 1996).

2.3 MEASUREMENT OF AMMONIA EXCRETION

During the experimental periods the lobsters were kept individually in 40 litre plastic experimental chambers. Five chambers were used to hold lobsters, while a sixth was used as a control to test for background ammonia and urea

production or consumption. As in the oxygen consumption experiments the lobsters had attachment points in the chambers to grasp onto. This helps to ensure that activity during the daylight periods was minimised; activity can have a significant effect on metabolic rate (Dall and Smith, 1986). The volume of water in each chamber could be varied, and was regulated according to the requirements of each experiment, but generally was between 20 and 30 litres. Water was recirculated, with the water being treated with a biological filter. Water pH was maintained at 8.0-8.4 and salinity 32-36‰. Total ammonia nitrogen (TAN = NH_3 + NH_4^+) in the sump tank was maintained at less than 0.05 mg/l. The experimental system was in a temperature controlled room which maintained the temperature within 0.5°C of the experimental temperature. Light was controlled to provide a 12-h light and 12-h dark photoperiod. To ensure that dissolved oxygen levels in the water did not compromise the experiments, the containers were aerated. Volatilisation of ammonia from aerated tanks is negligible (Gerking, 1955; Forsberg and Summerfelt, 1992). Aeration also acted to mix the water thus ensuring the water samples were representative of the experimental chamber.

Water flow through each experimental chamber was able to be stopped, thus allowing an evaluation of the rate of ammonia excretion as its concentration increased within the chamber over time. Ammonia excretion rates were based on the difference between the concentrations of two consecutive (timed) samples (Schmitt and Uglow, 1997*a*). After a pre-determined period a large percentage (usually around 66%) of the water in the chambers was siphoned out and replaced with water of the same temperature from the sump tank. High ambient water ammonia concentrations can inhibit ammonia excretion (Needham, 1957; Regnault, 1987). Minimum concentrations have not been determined, however ammonia excretion of the shrimp *Crangon crangon* was not influenced by ammonia concentrations of the overlying water up to 1.2 mg/l (Regnault, 1986 in Regnault, 1987). Water changes were timed to ensure that maximum levels of less than 0.5 mg TAN/l were maintained. Ammonia excretion (TAN - mg TAN/g/h) was determined from the following equation:

$$TAN = \frac{(TAN_f - TAN_i) * Volume}{Weight * Time}$$

where TAN_f is the ammonia as nitrogen in the sample at the end of the measuring period in mg/l; TAN_i is the ammonia as nitrogen in the sample at the beginning of the sampling period in mg/l; Volume is the volume of water in the container in litres; Weight is the weight of the lobster in grams; and Time is the duration of the measuring period in hours. The effect of several intrinsic and extrinsic factors on ammonia excretion was determined. The effect of the factors (except for temperature) was investigated at 13°C for *J. edwardsii* and 23°C for *P. cygnus*. Lobsters were starved for 36 hours prior to undertaking experiments (except when determining the effect of feeding).

Temperature

The acute response of ammonia excretion to temperature was investigated. Lobsters were acclimated (13°C and 23°C, *J. edwardsii* and *P. cygnus* respectively) before the temperature was changed at a rate of approximately 1°C every hour to the other test temperatures. The mean weight (±SE) of *J. edwardsii* was 680±34 (n=11) and of *P. cygnus* was 440±10 (n=10). The lobsters were kept at each experimental temperature for 24 hours. Lobsters were left overnight before evaluating ammonia excretion during daylight hours. Q_{10} values were determined using the following equation:

$$Q_{10} = \left(\frac{AN_2}{AN_1}\right)^{10/T_2 - T_1}$$

where AN_1 and AN_2 are ammonia excretion rates at temperatures T_1 and T_2, respectively.

Log_{10} transformed linear regressions of the ammonia excretion versus temperature (T) were expressed by the general equation:

$$Log_{10}\, AN = a + b\, T$$

where T is the temperature in °C.

Body weight

The effect of body weight on ammonia excretion was measured over the weight ranges of 241-1625g for *J. edwardsii* and 400-3022 g for *P. cygnus*.

Log_{10} transformed linear regressions of the ammonia excretion versus weight (W) were expressed by the general equation:

$$Log_{10} AN = a + b \log_{10} W$$

where AN = the ammonia excretion (mg TAN/g/h), a = intercept on the Y-axis, b = the slope of the regression, and W = wet weight (g) of the lobster.

Diurnal rhythm

A diurnal rhythm of oxygen consumption was apparent for both species (see Chapters 3 and 4), therefore ammonia excretion was investigated to determine if a diurnal rhythm was present. Ammonia excretion was measured over the periods from 6AM to 6PM (light period) and from 6PM to 6AM (dark period).

Handling and recovery

The effect of emersion and handling over a 1/2 hour period was also investigated to see if increased rates of ammonia excretion were maintained for an extended period after the disturbance. Ammonia excretion was measured every hour for three hours prior to the disturbance period and then every hour for eight hours after returning the lobsters to the experimental chambers.

Feeding

The effect of feeding on ammonia excretion was investigated. Lobsters were fed squid (*Nototodarus gouldii* - approximately 3% of wet body weight) at 8AM and were allowed two hours to consume it. Any lobster which did not consume the squid within that time period was not included in the experiment.

Most lobsters consumed the squid within 15 minutes. The water was changed and water samples were taken after the 2 hour feeding period. Ammonia excretion was monitored for the next two to three days. For *J. edwardsii* water samples were taken every 2 hours for the first 14 hours and then four hourly for another 12 hours. Thereafter, samples were taken at 24-h intervals for two days. For *P. cygnus* water samples were taken every hour for 16 hours, four hourly for another 12 hours and 6 hourly for another 24 hours. A water change was carried out after 10 hours, after 24 hours and then every 24 hours. Endogenous ammonia excretion was determined on the day prior to the feeding experiments.

Urea

Duplicate water samples were taken during the diurnal rhythm study and these were used to determine the urea excretion rate of each species. A comparison was made between the level of ammonia and urea excretion of unfed lobsters.

Water sampling and analysis

Duplicate 15 ml water samples were taken at each sampling period and when these could not be analysed immediately, they were frozen at -15°C for a maximum of 1 week; a time period which is well within the recommended maximum storage time of 2 weeks (Parsons *et al.*, 1984). Ammonia was analysed by the phenol-hypochlorite method of Solarzano (1969) as adapted by Parsons *et al.*, (1984) and Frith (1993)(Appendix 3). In this method the ammonia reacts with phenol and hypochlorite in alkaline solution to form indophenol blue. Sodium nitroprusside is used to intensify the colour at room temperature. The intensity of the colour produced is proportional to the concentration of ammonia present and is measured spectrophotometrically (GBC UV/VIS 916). Urea was analysed by the urease method of McCarthy (1970) as modified by Carter and Brafield (1991)(Appendix 3). This method involves the enzymatic hydrolysis of urea, by urease, to carbon dioxide and ammonia (Price and Harrison, 1987). The liberated ammonia is assayed by the ammonia method outlined above. This method also measures ammonia present prior to hydrolysis. Therefore, the difference between

the ammonia concentration before and after urease treatment gives a calculation of the ammonia attributable to urea.

2.4 HAEMOLYMPH SAMPLING AND ANALYSIS

In view of the relationship between lobster body weight and oxygen consumption of *P. cygnus* (Chapter 4), a restricted weight range (367-515 g) was used in the experiments conducted in Chapters 6, 7 and 8. Lobsters were randomly selected from the holding tank. Randomisation was achieved by numbering the concrete blocks and using random number tables to select the block and therefore the lobster. To ensure the lobsters could be sampled as quickly as possible they were always caught by hand. This served to also minimise the escape behaviour (characterised by a tail flick response).

Haemolymph sampling

Prebranchial haemolymph was sampled (1 ml) from the infrabranchial sinus via an athrodial membrane at the base of a walking leg (usually the 3rd or 4th pair). The samples was withdrawn with an ice-chilled 1 ml syringe (Luer - Tuberculin) using a 21 gauge (Luer -21G*1½) needle. Care must be taken to minimize hemolymph air contact since changes in CO_2 equilibrium can alter pH values (Vermeer, 1986). Truchot (1975) reported the pH of crustacean blood exposed to air without mixing varies little from anaerobically obtained samples. However, haemolymph samples were obtained anaerobically to ensure minimum mixing with air. A small amount of haemolymph was taken into the syringe and expelled to displace the dead space in the syringe (Waldron, 1991). The haemolymph sample was then taken; samples were obtained within 20 seconds of lobster capture. The haemolymph was immediately placed into an ice-chilled 1 ml Eppendorf tube. Truchot (1975) reported the pH of crustacean blood exposed to air without mixing varies little from anaerobically obtained samples. Aliquots (150µl and 250µl) were pipetted into Eppendorf tubes containing either 150 µl of distilled water or 500 µl of perchloric acid (PCA). The addition of haemolymph to PCA causes the blood to deproteinise. The first tube (water diluted sample) was kept on ice for measurement of haemolymph ammonia and the second tube

(deproteinised sample) was snap-frozen in liquid nitrogen and stored at -86°C for later measurement of lactate and glucose. The haemolymph remaining in the original tube was used for testing pH (and osmolality when required).

Haemolymph analyses

Haemolymph pH was measured using a calomel electrode (Activon Semi-Micro AEP336) connected to a pH meter (WTW pH 323). The probe was calibrated in buffer solutions chilled to the same temperature as the haemolymph samples (0°C). Haemolymph pH at 0°C varies from in vivo pH at ambient temperatures, but this was an essential concession to retard clot formation (Vermeer, 1987). Lobster haemolymph clotted within 5 minutes if it remained at ambient temperature. Using the above method the pH of *P. cygnus* haemolymph was 0.49 units higher than the pH of haemolymph measured at ambient temperature (23°C)(Appendix 2). The haemolymph pH of *P. cygnus* at 23°C was 7.85, within the range of pHs (7.7 - 8.0) measured in other aquatic crustaceans (Wheatly and Henry, 1992), and very similar to that measured previously in *P. cygnus* (Spanoghe, 1997).

Haemolymph ammonia concentrations were measured using a Sigma test kit (No. 640) for urea nitrogen which is based on the phenol/hypochlorite method of Solorzano (1969)(Appendix 3). The absorption was measured at 640 nm with a GBC UV/VIS 916 spectrophotometer. Ammonium chloride standards (0 to 8 mg/l) and a distilled water blank were used. The addition of distilled water to the sample, in conjunction with keeping it ice-cold, prevented clotting of the haemolymph sample for over 3 hours. In comparison, ice-cold haemolymph remained unclotted for a maximum of only one hour. Dilution of the sample also ensured that the ammonia reading stayed within the range of the standards.

The deproteinised haemolymph samples were centrifuged at 8000 g for 3 minutes. The supernatant (600 µl was generally obtained) was neutralised with 3 mol/l KOH (6.4 µl per 600 µl). The samples were stored on ice for 15 minutes before centrifuging at 8000 g for 3 minutes so that the perchlorate precipitate could be removed. The supernatant (approx. 550 µl remaining) was either frozen (-86°C) for later analysis or analysed immediately for lactate and glucose.

Lactate concentrations were determined enzymatically using the Boehringer-Mannhein analysis kit (Cat. No. 139084) which does not suffer from the copper interference as reported for other lactate test procedures (Brian Paterson, QDPI, pers. comm.)(Appendix 3). The absorption was measured at 340 nm on a GBC UV/VIS 916 Spectrophotometer. Determinations were on 100 µl samples; where appropriate the samples were diluted to bring the sample values within range of the standard curve. Glucose concentrations were determined using a Sigma glucose test kit (No. 510), which is based on the glucose-oxidase method (Appendix 3). The absorption was measured at 450 nm on a GBC UV/VIS 916 spectrophotometer. All assays were run in duplicate.

CHAPTER 3

The effect of intrinsic and extrinsic factors on oxygen consumption by the southern rock lobster, *Jasus edwardsii*

3.1 INTRODUCTION

The southern rock lobster, *Jasus edwardsii*, is the basis of a $150 M fishing industry in southern Australia. Over the last 10-15 years the industry has focused increasingly on the live export of the lobsters with up to 90% of the catch now being exported. Oxygen is one of the most important water quality parameters determining the health of aquatic animals. A full understanding of the effect of intrinsic and extrinsic factors affecting oxygen consumption during post-capture processes is essential if the health of lobsters is to be optimised. This study investigates the oxygen consumption response of *J. edwardsii* to temperature, body weight, activity, feeding, handling, daylight/darkness and dissolved oxygen levels.

3.2 MATERIALS AND METHODS

General Materials and Methods used to determine oxygen consumption and the effect of the various intrinsic and extrinsic factors are outlined in Chapter 2, with the following species specific methods. All experiments (except for those examining the effect of temperature) were conducted at the acclimation temperature of 13°C.

Temperature

Twelve lobsters (635 g to 897 g) were used to investigate the effect of acute temperature changes on the oxygen consumption. Lobsters were acclimated to the respirometers at 13°C before the temperature was raised or lowered to the required temperature at a rate of 2°C per hour.

Lobster weight

Standard and active oxygen consumption of 47 lobsters ranging in size from 186 g to 2180 g were determined.

Diurnal rhythm

Oxygen consumption of 22 lobsters (380 g to 2140 g) was recorded over a minimum of 48 hours to establish if a diurnal rhythm was present.

Statistical analyses

Linear regressions were obtained by the least squares method and were tested for significance of regression by analysis of variance of the regression. Covariance analysis was used to test for differences of oxygen consumption with sex and activity, using lobster weight as the covariate. Students t-tests (paired where necessary) were used to evaluate differences in standard and active oxygen consumption rates at each experimental temperature. Paired student t-tests were used to evaluate when post-prandial and post-handling oxygen consumption had returned to standard levels. Where appropriate a Students t-test for samples with unequal variances was used. Paired t-tests were also used to evaluate if there was daily rhythm to oxygen consumption by comparing the average night-time rate to the standard rate. All analyses were performed on the SPSS statistical package with the α set at 0.05. All means are expressed as mean±SE.

3.3 RESULTS

3.3.1 Effect of temperature on oxygen consumption

Sex of the lobsters did not have a significant effect on either standard ($F=1.01$, $P=0.321$) or active ($F=0.02$, $P=0.880$) oxygen consumption. Therefore, the data for both sexes have been pooled. Standard and active oxygen consumption increased significantly ($F=139.58$, $P=0.001$; $F=22.46$, $P=0.018$ respectively) with increases in temperature (Fig. 3.1). Active oxygen consumption

was significantly higher ($P<0.01$) than standard oxygen consumption at each temperature. Standard oxygen consumption increased exponentially and is described by the equation:

$$\text{Log } M_{O2} = 0.047T - 2.25 \ (r^2=0.94)$$

Active oxygen consumption increased greatly between 5°C and 13°C. At 17°C and 21°C active oxygen consumption rates increased, but they were not significantly higher ($F=3.07$, $p=0.06$) than at 13°C. The response is described by the equation:

$$M_{O2} = -3.3*10^{-4}T^2 + 0.013T - 0.044 \ (r^2=0.89)$$

The quadratic model suggests a decline in oxygen consumption beyond 21°C, but more data points are required to confirm that presumption.

The aerobic scope for activity increases as temperature increases from 5°C, with a maximum SFA recorded at 13°C (Fig. 3.1). The increase in the scope for activity over that range was largely due to the increase in active oxygen consumption. At higher temperatures (17 and 21°C) the scope for activity decreases due to the decrease in the rate of increase of active M_{O_2}, associated with the exponential increase in standard M_{O_2}. Aerobic expansibility (Table 3.1) was highest at 9 and 13°C (2.79 and 3.00 respectively) and was lowest at the extremes of the temperature range, being 1.52 at 5°C and 1.68 at 21°C.

Temperature (°C)	Aerobic expansibility	Temperature range (°C)	Q_{10}	
			Standard M_{O_2}	Active M_{O_2}
5	1.52	5-9	4.3	19.4
9	2.79	9-13	3.0	3.6
13	3.00	13-17	2.6	1.1
17	2.13	17-21	2.3	1.3
21	1.68			
		Average 5-21	3.0	6.4

Table 3.1: The aerobic expansibility of the southern rock lobster *Jasus edwardsii* at each experimental temperature (n=12). The Q_{10} values of standard and active oxygen consumption for each temperature range are shown along with the average Q_{10} values over the whole temperature range.

* Aerobic expansibility = Active M_{O_2}/Standard M_{O_2}

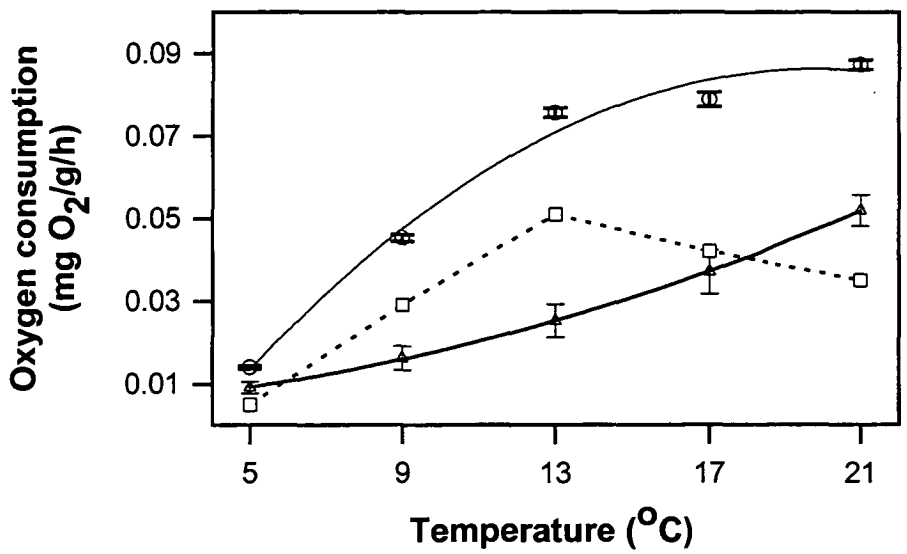

Figure 3.1: The effect of temperature on oxygen consumption (mean ± SE)(mg O_2/g/h) of the southern rock lobster, *Jasus edwardsii* (n = 12). Standard (Δ) and active (O) oxygen consumption rates both increased with temperature. The aerobic scope for activity (mg O_2/g/h) at each temperature is also shown (□).

The Q_{10} of standard oxygen consumption decreased as the temperature increased (Table 3.1) ranging from 4.3 ($Q_{10(5\text{-}9)}$) to 2.3 ($Q_{10(17\text{-}21)}$). The Q_{10} for the active lobsters showed a very different pattern. Between 5°C and 9°C active

oxygen consumption increased markedly which resulted in a $Q_{10(5-9)}$ of 19.4. Q_{10} values above 13°C are close to unity.

3.3.2 Effect of body weight on oxygen consumption

A log-log plot of total oxygen consumption (mg O_2/h) over wet body weight is shown in Fig. 3.2. Standard and active rates of total oxygen consumption (M_{O_2}, mg O_2/h) were positively correlated to the wet weight (W, g) of the lobsters. The regression equations describing the relationships are:

Standard oxygen consumption:

$\text{Log}_{10} M_{O_2} = 0.595 \log_{10} W - 0.396$ ($r^2 = 0.83$, $F = 215.9$, $p < 0.001$)

Active oxygen consumption:

$\text{Log}_{10} M_{O_2} = 0.690 \log_{10} W - 0.238$ ($r^2 = 0.77$, $F = 148.3$, $p < 0.001$)

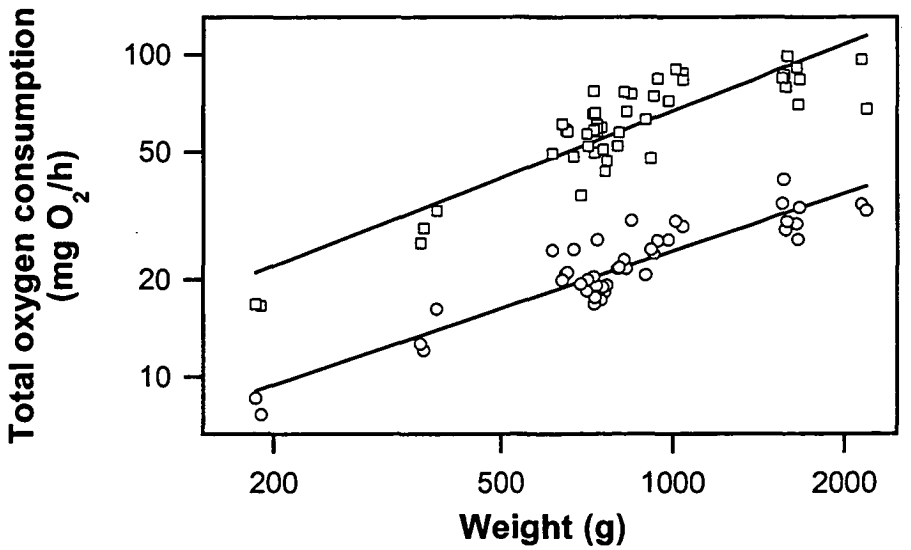

Figure 3.2: A log-log plot of total oxygen consumption (M_{O_2} - mg O_2/h) against body weight (g) of the southern rock lobster, *Jasus edwardsii*. Standard (O) and active (□) oxygen consumption rates over the weight range 186-2180 g are shown.

There was no significant difference between the slopes of the regressions for standard and active oxygen consumption (F=1.77, P=0.186), although there was a significant increase in oxygen consumption with activity (t=23.8, p<0.001). The weight-specific aerobic scope for activity decreased significantly (F=8.19, P=0.006) with weight (Fig. 3.3), and for a 700 g lobster was approximately 0.05 mg O_2/g/h. As indicated by the similarity between the *b* values, there was no significant difference (F=0.20 , P=0.65) in aerobic expansibility with weight. The mean aerobic expansibility was 2.72±0.08 (±SE) with a range between 2 and 4. In view of the relationship between body weight and oxygen consumption, a restricted weight range (600-900 g) was used in experiments where body weight was not a factor.

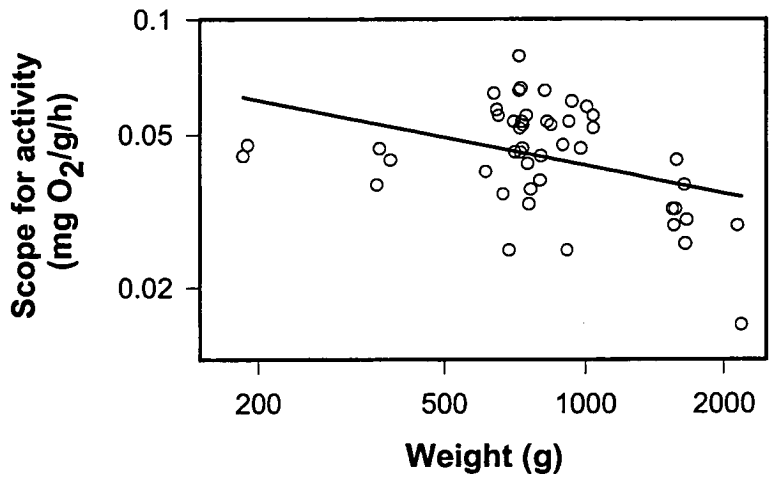

Figure 3.3: A log-log plot of weight-specific aerobic scope for activity (mg O_2/g/h) against body weight (g) of the southern rock lobster, *Jasus edwardsii.*

3.3.3 Effect of diurnal rhythm on oxygen consumption

Lobsters consumed significantly (t=7.916, P<0.001) more oxygen at night, with consumption up to four times the daytime rates being recorded. The oxygen consumption of an undisturbed 728 g lobster over a period of 48 hours is shown in Fig. 3.4. Average night-time consumption was 48.3±6.1% higher than standard

oxygen consumption. Using standard oxygen consumption as a measure of oxygen consumption during the entire 12 hour daylight period, and the recorded night-time rates, routine oxygen consumption was calculated to be 24.2% higher than the standard rate. In most night-time recordings two peaks of oxygen consumption were observed (Fig. 3.4); one lasting several hours immediately after the onset of darkness and the other for several hours prior to the lights coming back on. Infra-red video recordings established that periods of increased oxygen consumption correlated with periods of increased activity.

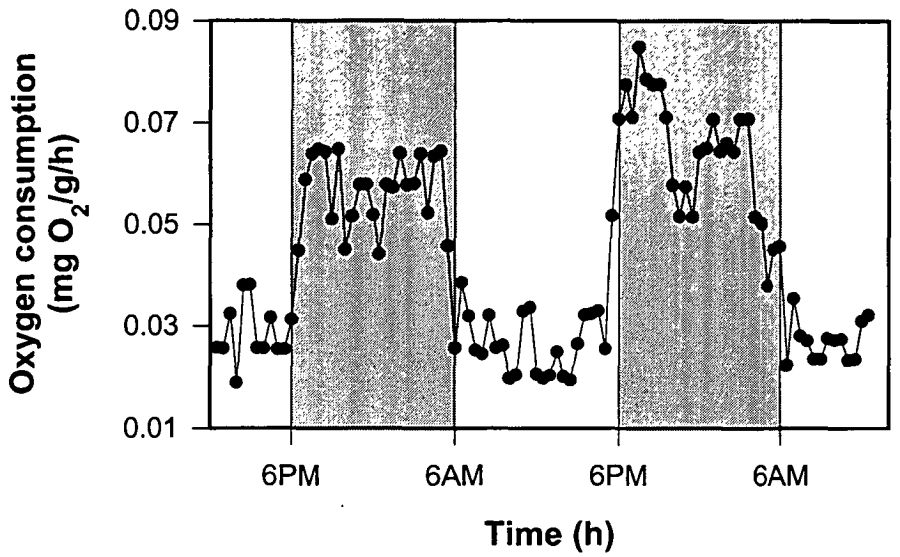

Figure 3.4: Oxygen consumption (mg O_2/g/h) of an undisturbed 728 g southern rock lobster (*Jasus edwardsii*) over a 48 h period. Each symbol represents oxygen consumption over a 20 minute measuring period. The lobster was in complete darkness between 6PM and 6AM. The line is drawn for ease of viewing.

3.3.4 Effect of emersion and handling on oxygen consumption

Handling and emersion caused a significant ($t=6.75$, $P<0.001$) increase in oxygen consumption upon re-immersion (Fig. 3.5). From the initial high level after re-immersion, oxygen consumption declined slowly until it was not significantly different ($t=1.67$, $P=0.13$) from the pre-emersion level at 4.5-5 hours.

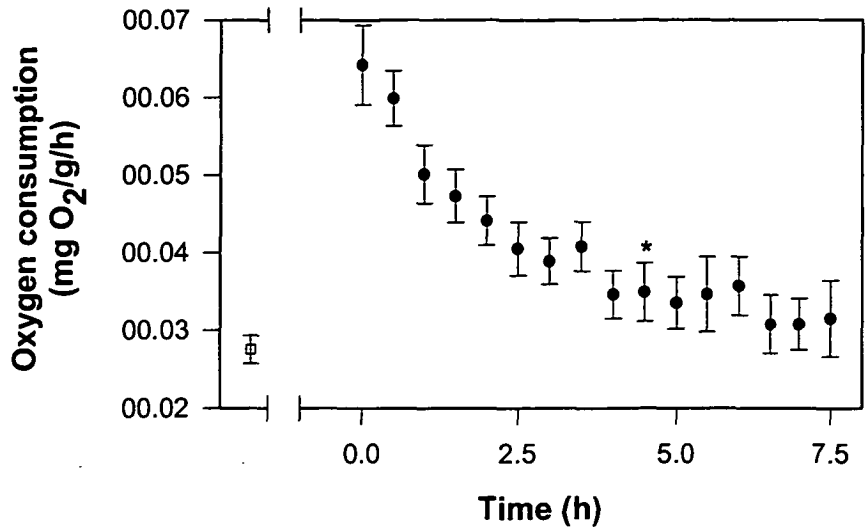

Figure 3.5: The effect of handling and emersion on oxygen consumption (mean ± SE)(mg O_2/g/h) of the southern rock lobster, *Jasus edwardsii* (n=10). Pre-handling (□) and recovery (●) oxygen consumption rates are shown. The break represents the 1/2 hour emersion and handling period. The asterisk indicates when oxygen consumption of recovering lobsters is not significantly different to the pre-handling level. Each reading represents the oxygen consumption rate measured over a 20 minute period after the time noted.

3.3.5 Effect of feeding on oxygen consumption

Oxygen consumption increased after feeding, reaching a maximum 10-13 hours post-prandial (Fig. 3.6). The maximum oxygen consumption was 1.72 times the pre-prandial level. From this maximum level, oxygen consumption slowly declined until it was not significantly different ($P<0.05$) from the pre-prandial level after 42 hours. The effect of diurnal rhythm on oxygen consumption during the post-prandial period did not appear to be strong, although it seems to become an influence on the second night after feeding. The influence of normal night-time activity may have prevented oxygen consumption from returning to standard M_{O_2} earlier than recorded. However, oxygen consumption during the daylight, one day after feeding was still 1.42 times the pre-prandial level.

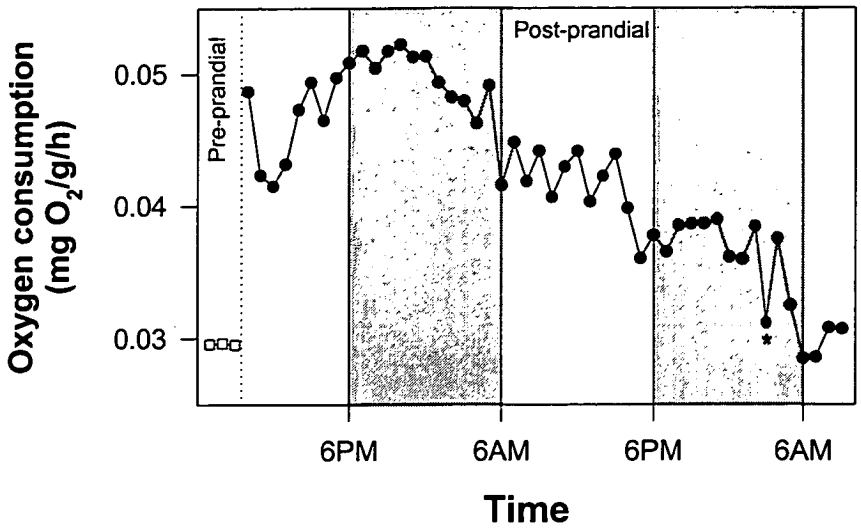

Figure 3.6: Oxygen consumption (mg O_2/g/h) of the southern rock lobster, *Jasus edwardsii,* over a 48 hour period (n=11). The lobsters were fed squid, *Nototodarus gouldii*, (3% of the lobsters body weight) at 9.00 AM on the first day. Pre-prandial (□) and post-prandial (●) oxygen consumption rates are shown. Each symbol represents the average oxygen consumption over 1 hour (ie. 2 measuring periods). For ease of viewing lines are drawn between succeeding data points and standard errors are not shown. The asterisk indicates when post-prandial oxygen is not significantly different to the pre-prandial level.

An initial large increase in M_{O_2} was observed in all lobsters used in feeding trials. The increase only lasted a maximum of 2 measuring periods (ie. 60 minutes)(Fig. 3.6) before returning to the relatively steady increase in M_{O_2} associated with feeding. It appeared to be associated with increased activity activated by the introduction of food. Lobsters subjected only to the smell of food in the water showed a very similar response but their M_{O_2} returned to standard rates within 1.5 hours.

3.3.6 Effect of the dissolved oxygen level on oxygen consumption

Settled lobsters were able to maintain a constant rate of M_{O_2} as the dissolved oxygen level of the water decreased (Fig. 3.7). Standard M_{O_2} was

maintained down to a critical oxygen level (P_c) of 36.7% saturation. Below P_c M_{O_2} decreased linearly with the dissolved oxygen level. M_{O_2} of active lobsters decreased with decreasing dissolved oxygen levels but the rate did not become significantly different until the dissolved oxygen level was 55% saturation. P_c for active lobsters was calculated to be 59.4% saturation. The aerobic scope for activity reduces with the dissolved oxygen level and is controlled by the active M_{O_2}. The scope at 55% saturation is 73% of that of the maximum, however at 35% saturation the scope is only 25% of the maximum aerobic scope.

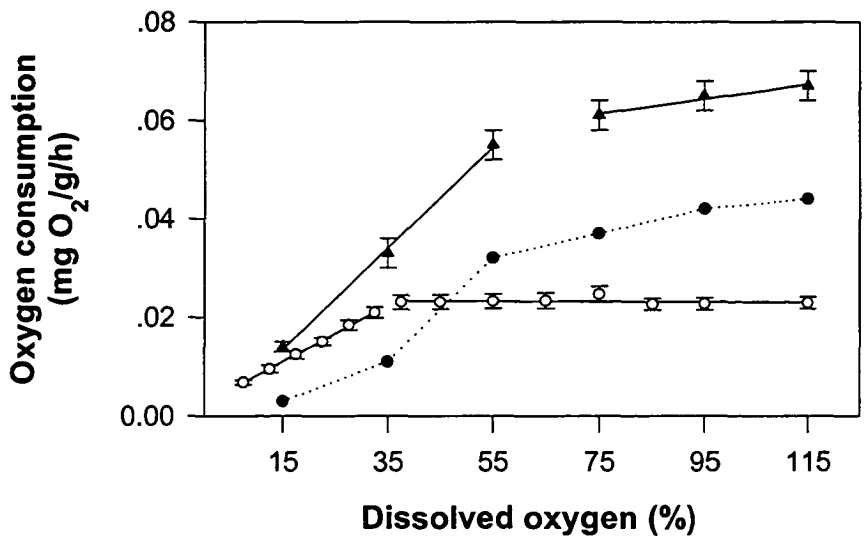

Figure 3.7: The relationship between dissolved oxygen level (%) and oxygen consumption (mean±SE)(mg O_2/g/h) of settled (O)(n=15) and active (▲)(n=12) southern rock lobsters, *Jasus edwardsii*. The aerobic scope for activity (mg O_2/g/h)(●) is also plotted as a function of the dissolved oxygen level.

3.4 DISCUSSION

3.4.1 Body weight

The dependence of oxygen consumption on body weight is well documented for most eumetazoans including crustaceans (Wolvekamp and

Waterman, 1960). Bridges and Brand (1980*a*) summarised the relationship for a series of decapod crustaceans and found scaling exponents (*b*) ranging from 0.286-0.877 for a temperature range of 8.5°C - 17.8°C. The *b* values obtained in this study for both the standard and active oxygen consumption rates (0.595 and 0.690 respectively) fall within this range and are close to 0.75: the *b* value evaluated for a wide variety of organisms (Hemmingsen, 1960). Bridges and Brand (1980*a*) noted that crustaceans in the large weight ranges tend towards a *b* value > 0.75 which suggests that oxygen consumption is more dependent on mass in larger crustaceans. However, the upper size range of crustaceans reported in Bridges and Brand (1980*a*) is limited (max. 770 g). Zoutendyk (1989) also obtained *b* values of 0.68 and 0.65 (at 8 and 10°C respectively) for *J. lalandii* ranging in weight from 20 g to 2500 g (at higher temperature *b* values of 0.8-0.9 were obtained). This suggests that oxygen consumption may be more dependent on surface area (*b* of around 0.67) in larger crustaceans, as hypothesised by Zeuthen (1953). Other factors such as temperature may have a modulating effect on the measured *b* value. More studies on the effect of body weight on oxygen consumption of large decapod crustaceans are required to help clarify the trends.

Standard rates of oxygen consumption vary widely with species even under a similar temperature regime (Table 3.2). Waldron (1991) obtained a lower value for *J. edwardsii* even though the study was carried out at a slightly higher temperature (15°C). As similar procedures were used for the studies the reason for the differences are uncertain but it may highlight intra-species variations of lobsters found at different locations (ie. Tasmania vs New Zealand). However, in common with Waldron (1991), this study found that oxygen consumption was lower than that reported for most other species at similar temperatures. *Panulirus cygnus* oxygen consumption rates were also low at similar temperatures (Table 3.2). However, animals from cold environments normally have oxygen consumption/temperature curves displaced to the left of those animals from warm environments (Vernberg, 1983) thus, *P. cygnus* would be expected to have comparatively low oxygen consumption rates at the lower temperatures. In some cases the extremely high rates obtained in other studies appear to be artefacts of experimental procedures (eg. insufficient acclimation time), as outlined by McMahon and Wilkens (1983) and Waldron (1991). In this study, the time taken for handled lobsters to return to standard rates of oxygen consumption are

protracted, even when the lobsters had been acclimated to the respirometry chamber before handling.

Species	Wet mass (g)	Temperature (°C)	M_{O_2} (mg/kg/h)	Reference
Jasus edwardsii	729	5	9	This study
		9	16	
		13	25	
		17	37	
		21	52	
	500	13	32	
J. edwardsii	300-730	15	20	Waldron, 1991
		17	27	
J. lalandii	500	8	27	Zoutendyk, 1989
		10	31	
		13	37	
		16	66	
		19	73	
Panulirus interruptus	200-600	13	49	Winget, 1969
		16	69	
		20	94	
P. cygnus	400-500	11	12	Chapter, 4
		15	20	
		19	31	
		23	46	
		27	67	
		31	96	
P. argus	417	27	106	Buesa, 1979
P. guttatus	157	27	119	Buesa, 1979
Homarus americanus	380-520	12	63	McLeese, 1964
		15	80	
		20	114	
		25	126	
H. americanus	≈ 500	5	43	McLeese and Watson, 1968
H. americanus	180-320	10	34	Penkoff and Thurberg, 1982
H. gammarus	675-680	15	22-27	Spoek, 1974
H. gammarus	230-600	10	11	Whiteley *et al.*, 1990
		15	31	
		20	44	
Cancer magister	700-1150	10	25-45	Johansen *et al.*, 1970 in Spoek, 1974
C. magister	551-960	8	31	McMahon *et al.*, 1979
Callinectes sapidus	200	20-28	87	Batterton and Cameron, 1978
Homarus vulgaris	220-510	15	33	Butler *et al.*, 1978

Table 3.2: Comparison of standard rates of oxygen consumption of the southern rock lobster, *Jasus edwardsii*, and the western rock lobster, *P. cygnus* (Chapter 4), with published values for some other large decapod crustaceans.

Scope for activity (SFA) represents the amount of energy available to an organism through aerobic metabolism beyond that needed for maintenance; for most metazoans this a good indication of their capacity for sustained work (Fry, 1947). When lobsters were active, either due to disturbance/handling/emersion or during normal night-time rhythms, there was a large increase in oxygen consumption. The increase in respiratory rates of active *C. maenas* was taken to be due to increased muscular activity (Wallace, 1972). The maximum aerobic expansibility of *J. edwardsii* (3.0) is lower than that of most fish, which are usually in the range of 3-7 (Jobling, 1994). The oxygen consumption increase and the weight-specific aerobic SFA for lobsters of 700 g ($\approx$ 0.05 mg O_2/g/h) are similar to that measured for other large decapod crustaceans (Spoek, 1974; McMahon *et al.*, 1979; Booth *et al.*, 1982; Waldron, 1991) and compares closely to values obtained for sluggish fish species (McMahon and Wilkens, 1983). *J. edwardsii* only has limited ability to carry out sustained aerobic work, as would be expected for a benthic, relatively inactive animal.

3.4.2 Temperature

The response to temperature of *J. edwardsii* was typical of that seen in many crustaceans (Cockcroft and Wooldridge, 1985, Dall, 1986), with standard oxygen consumption decreasing with decreases in temperature and increasing with increases in temperature (Vernberg, 1983). Active oxygen consumption also increased with temperature, however a maximal rate was attained at an intermediate, non-lethal temperature and it remained constant at higher temperatures, a response typical to that seen in many poikilotherms (Bennett, 1978). The freshwater crayfish, *Pacifastacus leniusculus*, exhibited a very similar response (Rutledge and Pritchard, 1981). The lowest temperature at which maximal oxygen consumption is attained is often the same as the preferred body temperature (Bennett, 1978). Maximum oxygen consumption by *J. edwardsii* was attained at 13°C which was the temperature of acclimation and a typical water temperature in their natural environment. Active oxygen consumption of *Panulirus interruptus* varied little with temperature over the range 16-20°C (Winget, 1969), suggesting a similar upper limit of M_{O_2} exists.

The question arises as to why active M_{O_2} does not increase at the higher temperatures. Active M_{O_2} of *J. edwardsii* at 13°C increased as the dissolved oxygen concentration became supersaturated. Although it was not a significant increase above that in normoxic water, it does add further evidence to the argument that delivery and diffusion systems limit active M_{O_2} (McMahon and Wilkens, 1983). The dissolved oxygen level decreases as water temperature increases, thus limiting the availability of oxygen. The decreased diffusion gradient may limit the uptake of oxygen. Environmental oxygen availability also appeared to limit the ability of sockeye salmon, *Oncorhynchus nerka*, to increase M_{O_2} at temperatures above 15°C (Brett, 1964). Also, oxygen uptake at the gills could be reduced as the oxygen affinity of haemocyanin decreases as temperature increases (Taylor, 1981).

In general Q_{10} values for standard M_{O_2} of crustaceans have been found to vary between 2 and 3; the values usually decrease with increasing temperature (Wolvekamp and Waterman, 1960). In some species a zone of temperature independence occurs, where standard M_{O_2} remains constant over a wide temperature range (Zoutendyk, 1989). This zone usually occurs at temperatures around the preferred temperature. No zone of independence was evident in the present study; it would be expected that a zone of temperature independence would occur in animals that are fully acclimated to the experimental temperatures. Studies of other large decapods have found Q_{10} values similar to *J. edwardsii* (3.0), eg. *J. lalandii* - $Q_{10(8\text{-}19)}$ = 2.5 (Zoutendyk, 1989), *Panulirus interruptus* - $Q_{10(13\text{-}20)}$ = 2.5 (Winget, 1969). In *J. edwardsii* the $Q_{10(5\text{-}9)}$ of 4.3 is high, but not unusual for crustacean species at the lower end of their temperature range, eg. *Penaeus monodon* - 3.6 (Liao and Murai, 1986), *P. esculentus* - 4.7 (Dall, 1986), and *P. californiensis* - 4.8 (Villareal and Rivera, 1993). In the crab, *C. sapidus*, there was also a large drop in M_{O_2} at low temperatures (Q_{10}=4.9)(Mauro and Mangum, 1982). The decreased M_{O_2} was associated with a sharp decrease in the heart rate, ventilation, and the intrinsic oxygen demand of the muscle. The authors suggested the crabs may go into metabolic "hibernation" because the high oxygen affinity of haemocyanin limits the ability of the tissues to use oxygen and they become hypoxic. Thus at 5°C *J. edwardsii* may have undergone a cold coma and

were reaching the extremes of their range of thermal tolerance; a point where the scope for activity is zero (Newell, 1979). Alternatively, the $Q_{10(10\text{-}15)}$ of *H. americanus* was 8.9 (Whiteley *et al.*, 1990) and the authors suggested that they were witnessing the respiratory responses to acute changes in temperature, as the lobsters were not acclimated to the lower temperature. The same basic methods used by Whiteley *et al.* (1990) to calculate the effect of temperature on M_{O_2} were used in the present study, indicating similar responses may have occurred.

Active Q_{10} values of close to 1.0 were measured at temperatures above 13°C. Similar values have been recorded as temperature increases above the "preferred" body temperature in many species of lower vertebrates (Bennett, 1978). The Q_{10} value for active oxygen consumption between 5 and 9°C was extremely high. An equivalent literature value could not be found although Q_{10}s of 8.9 and 7.7 were measured for non-temperature acclimated *H. gammarus* and *Penaeus japonicus*, respectively (Whiteley *et al.*, 1990; Paterson, 1993*a*). Lobsters remain very inactive when handled at 5°C. As activity is one of the major factors causing increases in oxygen consumption (Halcrow and Boyd, 1967; Newell, 1979), active M_{O_2} would not be expected to increase greatly at that temperature. The aerobic expansibility at 5°C was very small (1.52), but at 9°C when lobsters were much more active in response to handling, their aerobic expansibility increased substantially to 3.0. Therefore, the high active $Q_{10(5\text{-}9)}$ value appears to be due to the inability of lobsters to increase activity at the lower temperature. Q_{10} values are as much reflections of changed activity as of the temperature dependence of the metabolic reactions underlying the activity (Halcrow and Boyd, 1967).

The pattern of the SFA at different temperatures was similar to that seen in many fish species (Brett, 1964; Brett, 1972), and for the freshwater crayfish, *P. leniusculus* (Rutledge and Pritchard, 1981). SFA is generally highest at the preferred temperature decreasing at temperatures above and below that point. Active and standard M_{O_2} usually come together at the upper and lower lethal temperature of the species. Lower and upper lethal temperatures have not been determined for *J. edwardsii* but the results of this study show it is below 5°C and above 21°C for lobsters acclimated to 13°C. Below the "preferred" temperature active M_{O_2} decreased more steeply with temperature than standard M_{O_2}, causing

SFA to decrease as temperature decreased. Above the "preferred" temperature SFA decreased because active M_{O_2} remained constant while standard M_{O_2} continued to increase.

3.4.3 Emersion and handling

The extended time period taken to return to standard M_{O_2} after handling and emersion suggests that a large oxygen debt was incurred. The oxygen debt is due to two factors: (i) the increased activity of the lobsters caused by handling and emersion; oxygen consumption of *J. edwardsii* increases up to 3 times the settled level with activity. (ii) the decreased ability to uptake oxygen with emersion; emersed *J. edwardsii* have the ability to only take up about 1/2 of their settled oxygen requirements (Waldron, 1991). Therefore, the lobsters are only able to access approximately 1/6th of their oxygen requirements during the emersion period. Their metabolic requirements will probably be funded by anaerobic metabolism. Increases in M_{O_2} upon re-immersion are achieved by a rapid increase in oxygen supply to the gills (increased gill ventilation), an increase in oxygen transport away from the gills (increased cardiac output) and an increase in the oxygen gradient across the gills (increased participation of haemocyanin)(McMahon *et al.*, 1979; McMahon and Wilkens, 1983). Suggested uses for the excess oxygen include: (1) metabolising anaerobic end products; (2) re-establishing resting oxygen levels in body tissues; (3) replenishing high energy phosphate reserves; and (4) meeting energy costs associated with increased branchial chamber ventilation and haemolymph circulation (Herreid, 1980; Head and Baldwin, 1986). Large decapod crustaceans typically take around 8 hours to return to pre-exercise levels of oxygen consumption after a period of exercise and/or emersion (McMahon *et al.*, 1979; Waldron, 1991; Chapters 4 and 6). The slightly shorter timeperiod in this study may be a reflection of the low water temperature. Whiteley and Taylor (1990) found that lobsters, *H. gammarus*, took longer to recover from the effects of aerial exposure at 20°C compared to 10°C, and the timeperiod of recovery of *P. cygnus* increased as temperature increased (Chapter 4).

3.4.4 Diurnal rhythm

The increase in oxygen consumption and activity at night matches that observed in other subtidal species of decapods which typically show a diurnal rhythm in their behaviour patterns (Ansell, 1973; Naylor, 1988; Hammond and Naylor, 1977; Lipcius and Herrnkind, 1982; Du Preez, 1983; Dall, 1986). The routine M_{O_2} of 24% above standard M_{O_2} is comparable with the routine rate calculated by Dall (1986) for the prawn *Penaeus esculentus* of 8-12% above the standard rate. Such a small increase above standard rates is probably appropriate for benthic animals with limited activity (Dall, 1986). Carvalho and Phan (1997) suggested that the routine rate may be an underestimate of that found in the environment because the animal could not swim or develop normal behavioural patterns in the respirometers. *J. edwardsii* may have been able to exhibit more normal patterns in the respirometers because they could still freely walk, which is their prevalent means of locomotion in nature, therefore the calculated routine rate may be close to that found in the environment. The routine M_{O_2} of many fish species are typically 30-60% higher than standard M_{O_2} (Becker and Fishelson, 1986; Sims *et al.*, 1993). Even the largely sessile plaice, *Pleuronectes platessa*, has a routine M_{O_2} 30-45% above standard (Jobling, 1982). Zoutendyk (1991) did not observe any diurnal rhythms in oxygen consumption of the lobster *J. lalandii*, a species that would be expected to have similar activity patterns to *J. edwardsii.*

Where diurnal rhythms are present, light is the prime entraining factor (Naylor, 1988; Aréchiga and Rodríguez-Sosa, 1997). In the wild, *J. edwardsii* commences foraging just before dusk and continues through the night, ceasing at dawn (Fielder, 1965). Peak feeding activity occurs one to two hours after sunset (Lewis, 1981). The oxygen consumption response observed in the present study highlights another peak in M_{O_2} just prior to sunrise. Such secondary peaks are not unusual: Ansell (1973) observed two periods of increased oxygen consumption at night in *Cancer pagurus*, Kubo and Ishiwata (1964) observed a sunset and sunrise peak in activity by the Japanese spiny lobster, *P. japonicus* and Lipcius and Herrnkind (1982) also found that *P. argus* displayed secondary peaks. The second peak may be a consequence of the lobsters being unfed (therefore they are again on the search for food). Fielder (1965) found that the activity pattern of unfed *J.*

edwardsii remained at a much higher level through the hours of darkness, than does feeding activity. The author suggested that the fed lobsters had no need for further foraging after their initial feeding activity in the early hours of darkness. If the lobsters in the present study were unusually active due to being starved then the routine M_{O_2} would be somewhat lower than calculated above (24% above standard M_{O_2}). Alternatively, the second M_{O_2} peak may reflect the normal activity pattern of lobsters which may be generally returning to hides at that time of the night.

The night-time increase in oxygen consumption highlights the need to take diurnal changes in M_{O_2} into account when designing or interpreting studies of M_{O_2}. For example, when investigating the metabolic response to food it is imperative that the lobsters are fed at the same time of the day to ensure that the influence of diurnal rhythms can be accounted for. Also, many researchers have kept their study animals in the dark to ensure that the animals are not disturbed by movement in the room (eg. Batterton and Cameron, 1978). Such procedures may result in the calculation of elevated standard oxygen consumption rates due to the effect of light/darkness on activity.

3.4.5 Feeding

Post-prandial increases in oxygen consumption have been well studied in fish (see Jobling, 1981 for a review), however relatively few studies have been conducted on crustaceans. The general term for the response is specific dynamic action (SDA). The increase in oxygen consumption is associated with the extra energy produced for transportation of food in the alimentary tract, its digestion, absorption and post absorptive metabolic processes related to the ingested food (Hepher, 1988). Food elicited a strong locomotor response in *J. edwardsii*; the increased activity would probably account for the rapid rise in oxygen consumption after feeding. Similarly, a large increase in oxygen consumption immediately after feeding was also observed in *Penaeus monodon* (Du Preez *et al.*, 1992); the authors concluded that the initial rise was due to increased activity and feeding processes, whilst the later peak was due to the absorptive and digestive processes.

Many factors affect the size of the SDA (see Jobling, 1981), but in fish the general response is a peak level of between 2 and 3 times standard M_{O_2}, with the peak occurring within 12 hours post-prandial, and a duration of 24-36 hours. Therefore, *J. edwardsii* displayed a classic post-prandial increase in oxygen consumption. Similarly, oxygen consumption by the crab *Carcinus maenas* was 2.3-fold higher 3 h after a meal (2.6% wet weight to wet weight), and had returned to its previous value within 24 h (Houlihan *et al.*, 1990). Oxygen consumption by *Cancer pagurus* took 6-9 hours to reach maximum post-prandial levels (3.8-fold increase) and 24 hours to return close to pre-prandial levels (Ansell, 1973). Oxygen consumption of the American lobster, *H. americanus* almost doubled after feeding (McLeese, 1964). A SDA has also been clearly demonstrated for the land crabs, *Cardisoma guanhumi* and *Ocypode quadrata*, with peaks and durations of very similar magnitude to in the present study (Burggren *et al.*, 1993). In *J. edwardsii* the maximum increases in M_{O_2} after feeding was over 1/3 of the lobsters aerobic expansibility at 13°C. Thus, their aerobic scope for activity would appear to be severely reduced for an extended period after feeding.

3.4.6 Dissolved oxygen level

J. edwardsii were able to maintain its standard level of oxygen consumption down to a relatively low P_{O_2} (36.7%), below which M_{O_2} varied in proportion to water P_{O_2}. The dissolved oxygen tension where M_{O_2} becomes dependent is termed the critical oxygen level (P_c), and is used as the standard against which organisms are compared for hypoxic tolerance (Reiber, 1995). The P_c of *J. edwardsii* is similar to that evaluated for many other crustaceans living in well oxygenated environments. Values between 20 and 50% saturation are typical: *Homarus gammarus* 22% (Spoek, 1974), *H. americanus* 20-25% (McMahon and Wilkens, 1975), *Austropotamobius* sp. 25-32% (Wheatly and Taylor, 1981), *Penaeus esculentus* 25% (Dall, 1986), and *Carcinus maenas* 40-50% (Taylor, 1976, Truchot, 1975 in Morris and Taylor, 1985). Waldron (1991) obtained a P_c for *J. edwardsii* of 51%, a figure which was considerably higher than found in this study. The reason for the difference is unclear but the results of this study do not

support the view that a low degree of oxygen independence may limit the distribution of *J. edwardsii* (Waldron, 1991).

The critical oxygen tension for a given species is not constant (Reiber, 1995). The P_c of *J. edwardsii* increased by over 20% when the lobsters were active. Only a few studies have looked at the P_c of active crustaceans and it has generally been found that P_c is close to 100% saturation (*H. gammurus*, Spoek, 1974; *C. maenas*, Taylor, 1976; *Ebalia tuberosa*, Schembri, 1979; *Corystes cassivelaunaus* and *Galathea strigosa*, Bridges and Brand, 1980*a*; *P. esculentus*, Dall, 1986). Animals which are normally oxygen independent down to quite low P_{O_2} levels become oxygen dependent when active. However, the active P_c evaluated for *J. edwardsii* was much lower than seen in such studies. A similar result was obtained for *Heterosquilla tricarinata* (Innes, 1985). Similar methods were used in evaluating P_c in the two studies, with the ability of animals to uptake oxygen at specific P_{O_2} values being examined, rather than using the normal method of placing active animals into water and monitoring the depletion of oxygen. The purported lack of ability of crustaceans to remain oxygen independent when active may therefore be an artefact of experimental procedures, and requires further investigation.

H. tricarinata can maintain a reasonably high aerobic SFA over a wide range of dissolved oxygen levels, as would be expected of an animal which may experience prolonged periods of low environmental oxygen levels in its natural environment (Innes, 1985). The relatively low active P_c value of *J. edwardsii* means that it can also maintain a reasonably high aerobic SFA over a wide range of dissolved oxygen levels. However, the 27% reduction in aerobic SFA capability of *J. edwardsii* at 55% saturation may limit its ability to maintain physiological processes, such as oxygen consumption increases related to feeding. By way of illustration, when the closely related species *J. lalandii* was grown at various levels of dissolved oxygen, there was a general decrease in growth and ingestion and an increase in intermoult period, with decreasing levels of oxygen saturation (Beyers *et al.*, 1994). Such results would seem likely if the SFA response of *J. lalandii* to decreasing oxygen levels, was similar to *J. edwardsii.*

Conclusion: a clear understanding of factors affecting oxygen consumption by *J. edwardsii* has been developed. This information can assist in the development of procedures which ensure that the health of *J. edwardsii* is maximised through all processes of the fishery and export industry. The implications of these results for the southern rock lobster industry will be discussed in the General Discussion (Chapter 9).

CHAPTER 4

The effect of intrinsic and extrinsic factors on oxygen consumption by the western rock lobster, *Panulirus cygnus*

4.1 INTRODUCTION

The western rock lobster, *Panulirus cygnus*, is the basis of a $500 M fishing industry in Western Australia. Live export of lobster constituted 40-50% of the total catch during the 1996/97 fishing season. Maintaining rock lobsters in prime condition in holding tanks on board boats or in processing sheds requires the provision of high quality water. One of the major water quality parameters is oxygen. It is essential that water flow and aeration are sufficient to provide adequate oxygen for the number of lobsters being held (Beard and McGregor, 1991). Unfortunately very little information is available on the oxygen consumption rates of *P. cygnus* and its response to various extrinsic and intrinsic factors. The design of rock lobster holding tanks has developed in response to results obtained, rather than being designed to meet the lobsters biological requirements, based on sound scientific information. Therefore, this study determines the oxygen consumption response of *P. cygnus* to various extrinsic and intrinsic factors; temperature, body weight, feeding, handling, daylight/darkness and dissolved oxygen levels.

4.2 MATERIALS AND METHODS

General Materials and Methods used to determine oxygen consumption and the effect of the various intrinsic and extrinsic factors are outlined in Chapter 2 with the following species specific methods. All experiments (except for those examining the effect of temperature) were conducted at the acclimation temperature of 23°C.

Water temperature and lobster weight

The effect of acute temperature changes on the oxygen consumption of lobsters were investigated. Lobsters were acclimated to the respirometers at 23°C before the temperature was raised or lowered to the required temperature at a rate of 2°C per hour.

Oxygen consumption of lobsters under the following experimental procedures were examined:

(i) The effect of lobster weight and water temperature on standard oxygen consumption was determined with lobsters ranging in weight from 417 g to 3000 g. The lobsters were subjected to temperatures ranging from 11°C to 31°C (4°C steps).

(ii) The effect of lobster weight and activity on oxygen consumption at 23°C.

(iii) The effect of temperature and activity on the oxygen consumption of 400-500 g lobsters.

Diurnal rhythm

Oxygen consumption of 21 lobsters (417 g to 2900 g) was recorded over a minimum of 48 hours to establish if a diel rhythm was present.

Handling

This experiment was conducted at 15°C, 23°C and 31°C. The upper and lower temperatures were obtained by altering the temperature from 23°C at a rate of 2°C per hour. Lobsters were held at those temperatures for 24 hours prior to determining the response to handling.

Oxygen saturation level

This experiment was conducted at 15°C, 23°C and 31°C. The upper and lower temperatures were obtained by altering the temperature from 23°C at a rate of 2°C per hour. Lobsters were held at those temperatures for 24 hours prior to determining the response to the oxygen saturation level. The response of active lobsters at 23°C was also investigated.

Statistical analyses

Linear regressions were obtained by the least squares method and were tested for significance of regression by analysis of variance of the regression. Covariance analysis was used to test for differences of oxygen consumption with sex, activity and temperature, using lobster weight as the covariate. Students t-tests (paired where necessary) were used to evaluate differences in standard and active oxygen consumption rates at each experimental temperature. Paired student t-tests were used to evaluate when post-prandial and post-handling oxygen consumption had returned to standard levels. Where appropriate a Students t-test for samples with unequal variances was used. Paired t-tests were also used to evaluate if there was daily rhythm to oxygen consumption by comparing the average night-time rate to the standard rate. All analyses were performed on the SPSS statistical package with the α set at 0.05. All means are expressed as mean±SE.

4.3 RESULTS

4.3.1 The effect of temperature and body weight on oxygen consumption

Sex of the lobsters had no influence on oxygen consumption ($t=0.22$, $P=0.85$). Therefore the data from both sexes have been pooled. Log-log plots of total oxygen consumption (M_{O_2}) against body weight are shown in Fig. 4.1. Total oxygen consumption rates of *P. cygnus* increased significantly ($P<0.001$) with body weight at each of the experimental temperatures. The equations describing

the relationship at each temperature are shown in Table 4.1. Analysis of covariance showed that there were no significant differences (F=0.52, P=0.763) between the slopes (*b*) of the linear regressions (data for lobsters at 11°C were not included in this analysis due to a lack of data for intermediate weight lobsters at that temperature). The slope of the pooled regressions was 0.814. All intercepts (*a*) were significantly different (F=225, P<0.001) and increased significantly (F=194, P<0.001) with temperature (T). The relationship between the intercepts (*a*) and temperature was able to be described by the linear equation:

$$a = 0.051T - 2.075 \ (r^2 = 0.98) \qquad \text{Equation (1)}$$

By substituting the value of the pooled regression (0.814) and the value of *a* from Equation (1) into the general form of the regression of total oxygen consumption against body weight ($Log_{10} M_{O_2} = a + b \log_{10} W$), body weight and temperature can be related to oxygen consumption:

$$Log_{10}M = 0.814 \log_{10}W + 0.051T - 2.075$$

In view of the relationship between body weight and oxygen consumption, a restricted weight range (380-520 g) was used in experiments where body weight was not a factor.

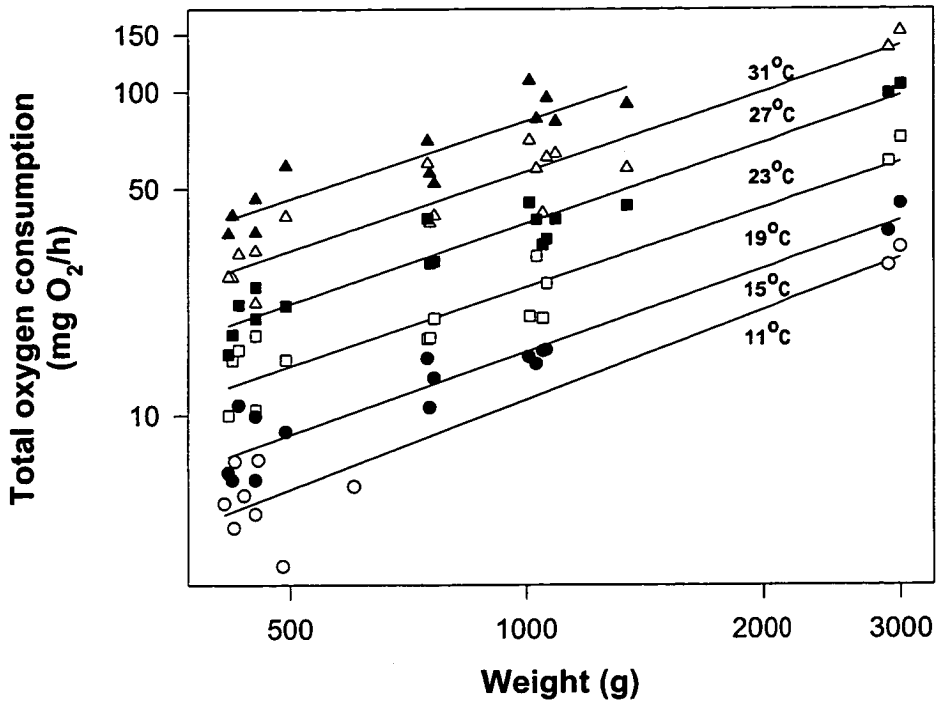

Figure 4.1: A log/log plot of total oxygen consumption (M_{O_2} - mg O_2/h) against wet body weight (g) of the western rock lobster, *Panulirus cygnus.* Standard oxygen consumption rates over the temperature range 11°C to 31°C and weight range 417 g to 3000 g were investigated.

Temperature (°C)	Linear regression equation	n	F	r^2
11	$Log_{10} M_{O_2} = -1.705 + 0.918 \log_{10} W$	10	69	0.90
15	$Log_{10} M_{O_2} = -1.355 + 0.850 \log_{10} W$	15	151	0.92
19	$Log_{10} M_{O_2} = -1.040 + 0.812 \log_{10} W$	15	101	0.89
23	$Log_{10} M_{O_2} = -0.900 + 0.830 \log_{10} W$	17	218	0.94
27	$Log_{10} M_{O_2} = -0.698 + 0.817 \log_{10} W$	17	121	0.89
31	$Log_{10} M_{O_2} = -0.504 + 0.805 \log_{10} W$	13	80	0.82

Table 4.1: Linear regression equations describing the relationship between total oxygen consumption (M_{O_2} - mg O_2/h) and body weight (W - g) at each of the experimental temperatures. The total number of lobsters (n), the F value for the ANOVA, and the r^2 of the equation for each temperature are also shown.

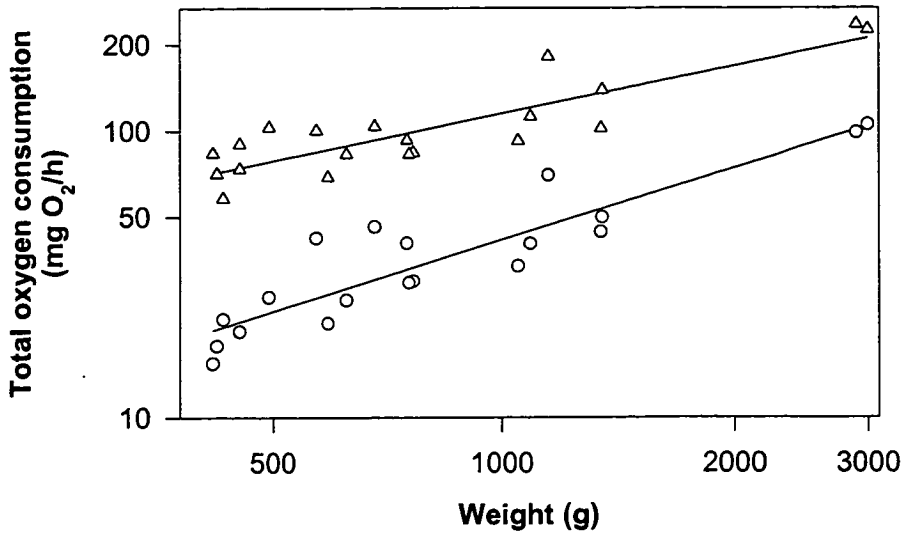

Figure 4.2: A log-log plot of total oxygen consumption (M_{O_2})(mg O_2/h) against body weight (g) of the western rock lobster, *Panulirus cygnus*. Standard (O) and active (Δ) rates of oxygen consumption at 23°C are shown.

4.3.2 The effect of activity and body weight on oxygen consumption

The relationship between standard and active rates of oxygen consumption was investigated over a range of body weights. Standard and active M_{O_2} (mg O_2/h) at 23°C increased significantly ($F=88.2$, $P<0.001$; $F=85.84$, $P<0.001$ respectively) with body weight (Fig. 4.2). The regression equations describing the relationships are:

Standard oxygen consumption:

$$\text{Log}_{10} M_{O_2} = 0.834 \log_{10} W - 0.881 \quad (r^2 = 0.831)$$

Active oxygen consumption:

$$\text{Log}_{10} M_{O_2} = 0.550 \log_{10} W + 0.411 \quad (r^2 = 0.755)$$

Activity caused a significant increase ($t=11.95$, $P<0.001$) in oxygen consumption. There was a significant difference ($F=6.05$, $P=0.019$) between the slopes of the regressions with the *b* value for active lobsters being much lower than for settled lobsters. This indicates that the aerobic expansibility decreased with weight (Fig. 4.3). Analysis of variance showed that the slope of the regression was significantly different ($F=6.20$, $P=0.023$) to zero and that the aerobic expansibility did decrease with weight. Larger lobsters had a decreased ability to increase their oxygen consumption with activity in comparison to smaller lobsters.

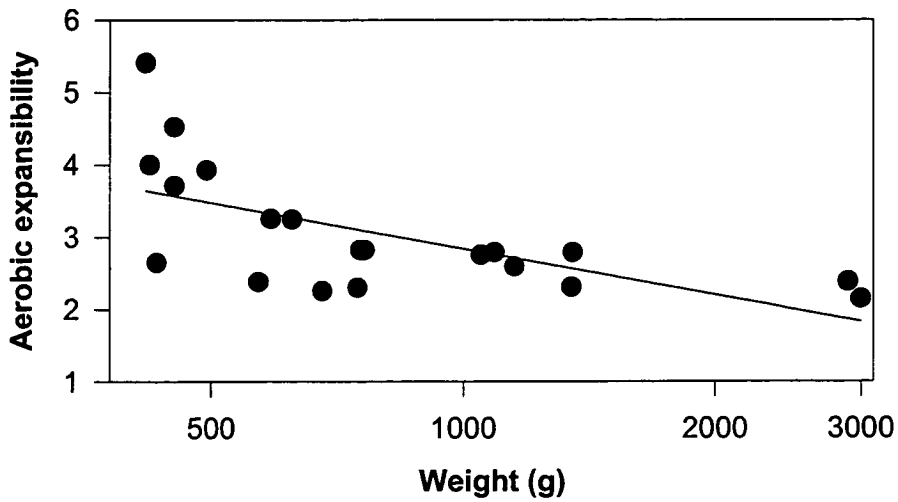

Figure 4.3: The aerobic expansibility of the western rock lobster *Panulirus cygnus* plotted against log weight. Aerobic expansibility = Active M_{O_2}/Standard M_{O_2}.

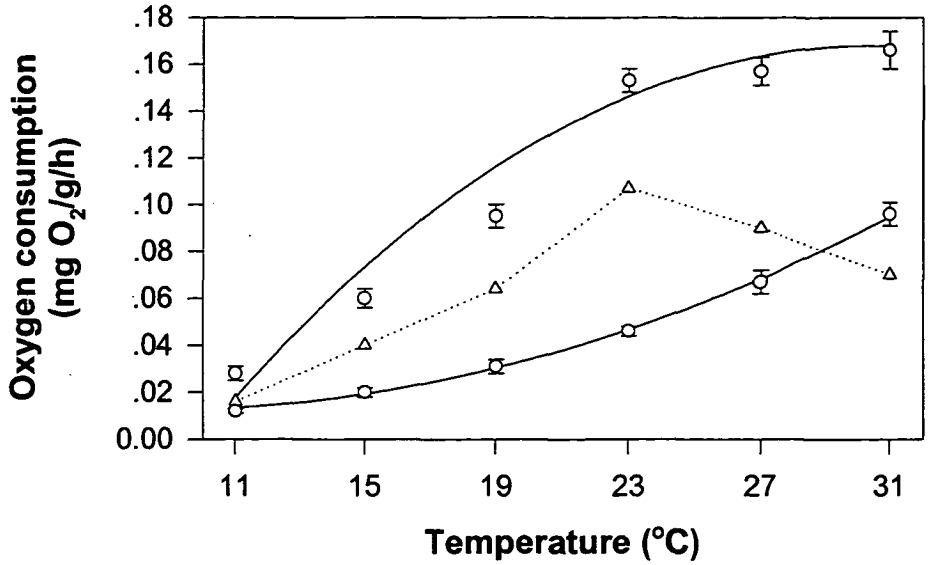

Figure 4.4: The effect of temperature on the standard ( ) and active (o) weight-specific oxygen consumption (mean±SE)(mg O_2/g/h) of the western rock lobster, *Panulirus cygnus* (n=12). The aerobic scope for activity (mg O_2/g/h) at each temperature is also shown (Δ).

4.3.3 The effect of activity and temperature on oxygen consumption

It has already been shown above that temperature has a significant effect on the standard M_{O_2} of *P. cygnus*. This relationship is further highlighted in Fig. 4.4 which plots the effect of temperature on standard and active M_{O_2} for 445.6±3.8 g (mean±SE) *P. cygnus*. There is a significant relationship between temperature and M_{O_2} for settled and active lobsters ($F=284.6$, $P<0.001$; $F=351.6$, $P<0.001$ respectively). The exponential relationship between standard M_{O_2} and temperature (T) can be described by the equation:

$$\text{Log}_{10} M_{O_2} = 0.045T - 2.38 \ (r^2=0.90)$$

At each experimental temperature the active M_{O_2} was significantly higher than the standard M_{O_2} ($P<0.001$ at all temperatures). Active M_{O_2} decreased greatly at temperatures below 23°C but at higher temperatures there was no significant

(F=1.68, P=0.19) increase in active M_{O_2}. The relationship was able to be described by the equation:

$$M_{O_2} = -4.02e^{-4} T^2 + 0.024 T - 0.201 \ (r^2=0.83)$$

The quadratic model suggests a decline in oxygen consumption beyond 31°C, but more data points are required to confirm that presumption.

The aerobic scope for activity decreased at temperatures above and below 23°C (Fig. 4.4). The decrease below 23°C is primarily due to the decrease in active M_{O_2}. Above 23°C the decrease is due to the active M_{O_2} remaining relatively constant and to the exponential increase in standard M_{O_2}. The aerobic expansibility was highest (3.33) at 23°C (Table 4.2). It was maintained at a similar level at 19 and 15°C (3.06 and 3.0 respectively) but decreased to 2.33 at 11°C. At higher temperatures the aerobic expansibility decreased until a level of 1.73 was obtained at 31°C.

The Q_{10} values for standard M_{O_2} decreased as the temperature increased, falling from 3.59 ($Q_{10(11\text{-}15)}$) to 2.46 ($Q_{10(27\text{-}31)}$) (Table 4.2). The Q_{10} values for active M_{O_2} also showed a general decrease as temperature increased but ranged from 6.72 (5-9°C) to 1.07 (23-27°C). The active Q_{10} values above 23°C were close to unity. The average Q_{10} values over the temperature range 11-31°C were very similar for both active and standard M_{O_2}.

Temperature (°C)	Aerobic expansibility*	Temperature range (°C)	Q_{10}	
			Standard M_{O_2}	Active M_{O_2}
11	2.33	11-15	3.59	6.72
15	3.00	15-19	2.99	3.15
19	3.06	19-23	2.68	3.29
23	3.33	23-27	2.56	1.07
27	2.34	27-31	2.46	1.15
31	1.73			
		Average (11-31)	2.86	3.08

Table 4.2: The aerobic expansibility of the western rock lobster, *Panulirus cygnus*, at each experimental temperature (refer Fig. 4.5). The Q_{10} values of standard and active oxygen consumption for each temperature range are shown along with the average Q_{10} values over the whole temperature range.

* Aerobic expansibility = Active M_{O_2}/Standard M_{O_2}.

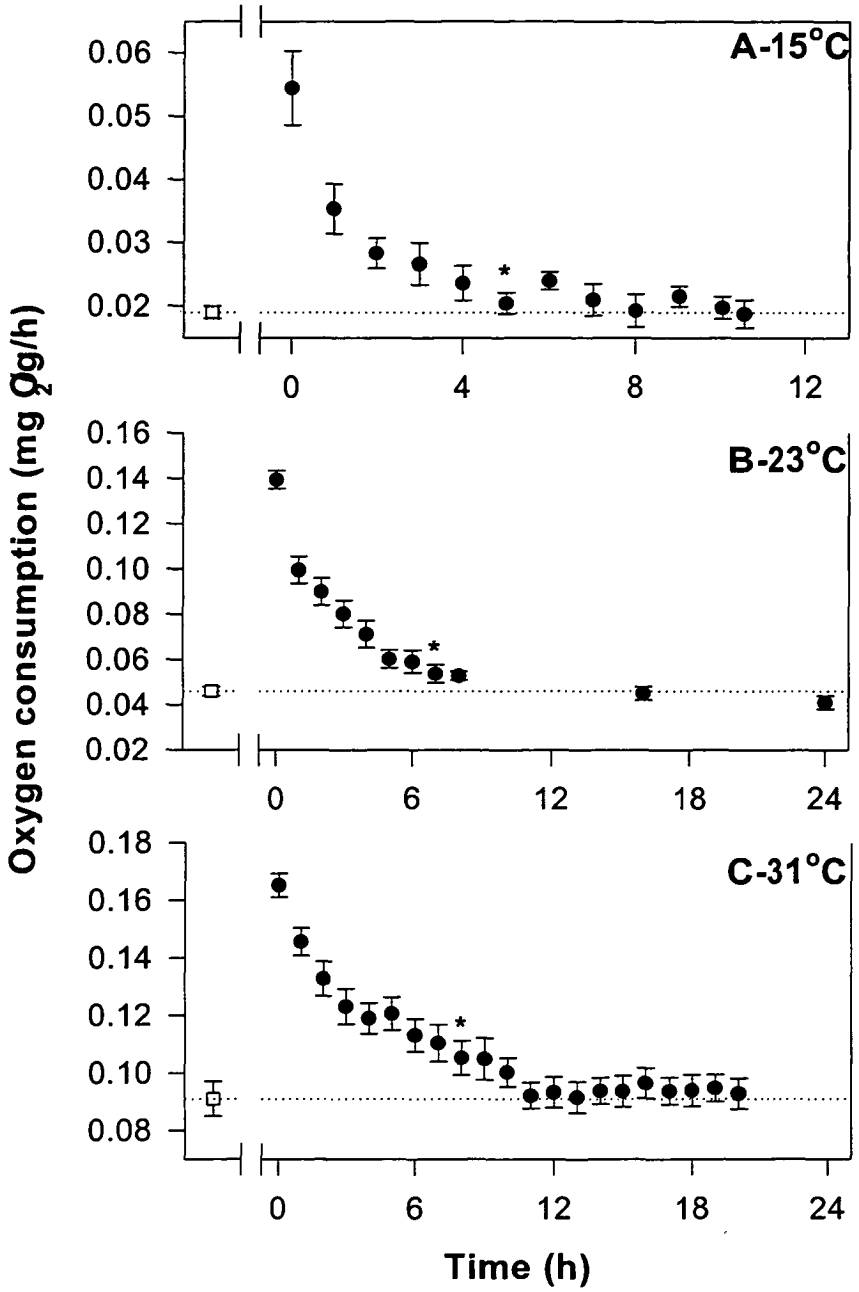

Figure 4.5: The effect of handling and emersion on oxygen consumption (●)(mean ±SE)(mg O_2/g/h) of the western rock lobster *Panulirus cygnus*. Pre-handling oxygen consumption (□) was quantified before lobsters were removed from the respirometers for ½ hour (indicated by the break). The response to emersion at different temperatures (A = 15°C; B = 23°C; C = 31°C) was investigated. The asterisks indicate the time when the oxygen consumption of recovering lobsters is not significantly different to the pre-handling level. Each reading represents the oxygen consumption rate measured over a 20 minute period after the time noted.

4.3.4 Effect of handling and emersion on oxygen consumption

Lobsters needed an extended period of time to return to their standard M_{O_2} after handling and emersion (Fig. 4.5). They required 5-5.5 hours to return to oxygen consumption levels that were not significantly different (t=0.92, P=0.37) to standard rates after handling and emersion at 15 °C. The time period increased to 7-7.5 hours (t=1.808, P=0.096) and 8-8.5 hours (t=1.62, P=0.125) at 23°C and 31°C respectively. The total amount of oxygen consumed above standard oxygen consumption during the recovery period was 0.074 mg O_2/g at 15°C, increasing to 0.277 mg O_2/g at 23°C and 0.302 mg O_2/g at 31°C. The oxygen consumed during recovery did not increase greatly at 31°C when compared to 23°C.

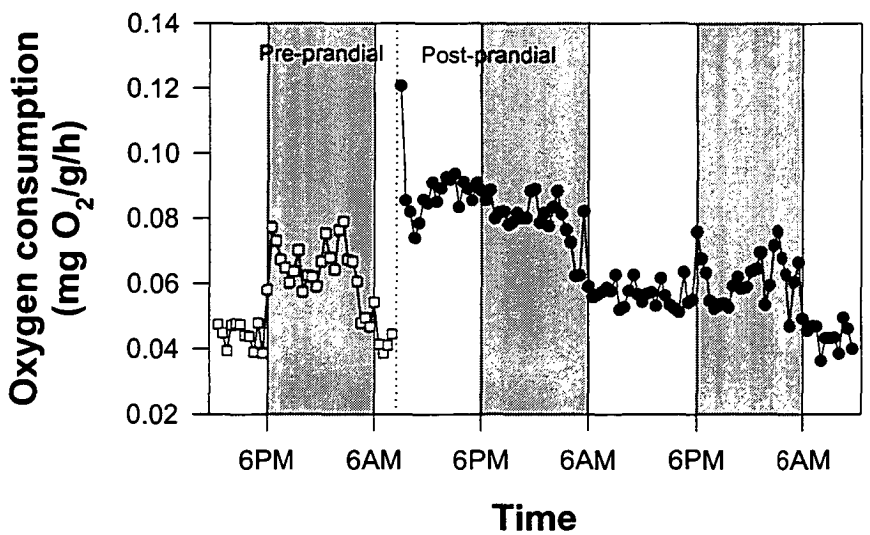

Figure 4.6: Oxygen consumption (mg O_2/g/h) of the western rock lobster, *Panulirus cygnus* (n=7), over a 72 hour period. The lobsters were fed squid, *Nototodarus gouldii*, (3% of the lobsters body weight) at 8.30 AM on the second day. Pre-prandial (□) and post-prandial (●) oxygen consumption rates are shown. The effect of daylight/darkness on oxygen consumption can be observed in the pre-prandial section. The lobsters were in complete darkness between 6PM and 6AM. Each symbol represents the average oxygen consumption over 30 minutes (ie. two measuring periods). For ease of viewing lines are drawn between succeeding data points and standard errors are not shown.

4.3.5 Effect of diurnal rhythm on oxygen consumption

Oxygen consumption increased significantly (t=5.154, P>0.001) at night (Fig. 4.6). The mean night-time increase was 87±18% (±SE) with a range from 4% to 338%. A large increase in M_{O_2} was generally observed in the measuring period immediately after the onset of darkness, with a return to standard M_{O_2} rates immediately after the lights came back on. Using the standard M_{O_2} rate as a measure of oxygen consumption during the entire 12 hour daylight period, and the recorded night-time rates, routine M_{O_2} was calculated to be 43.5% higher than the standard M_{O_2}. Infra-red recordings showed that increases in oxygen consumption were correlated to increases in activity of the lobsters.

4.3.6 Effect of feeding on oxygen consumption

An increase in oxygen consumption was observed post-prandially. However, the influence of a diurnal rhythm was still evident (Fig. 4.6). A large increase in M_{O_2} (up to 3 times standard M_{O_2}) was observed immediately after feeding which took three measuring periods (ie. 1.5 hours) to return to the relatively steady increase associated with feeding. The initial surge in M_{O_2} appeared to be due to increased activity associated with the procurement of the feed. Lobsters subjected only to the smell of food in the water showed a very similar response but their M_{O_2} returned to standard rates within 1.5 hours.

Oxygen consumption peaked 7 hours post-prandial at 0.094±0.008 mg O_2/g/h (mean±SE) and slowly declined after that time. The peak M_{O_2} was 2.19 times the standard M_{O_2}. M_{O_2} returned to pre-prandial levels 46 hours post-prandial (t=1.12, P=0.28) and may have returned earlier except for the influence of the diurnal rhythm.

The average night-time M_{O_2} prior to feeding was 0.064±0.002 mg O_2/g/h (mean±SE) which was 49% higher than the standard M_{O_2} of 0.043±0.001 mg O_2/g/h (mean±SE). The post-prandial oxygen consumption during the second night after feeding was 0.061±0.002 mg O_2/g/h (mean±SE). Both night-time readings were significantly higher (Pre t=9.42, P<0.001, Post t=8.97, P<0.001) than the standard M_{O_2} but were not significantly different (t=1.04, P=0.30) to each

other. Average M_{O_2} during the day-time on the day after feeding (0.057±0.001 mg O_2/g/h - mean±SE) was 33% higher than the standard M_{O_2} (significantly higher - t=9.32, P<0.001). Post-prandial increases in M_{O_2} still appeared to influence M_{O_2} 21-33 hours after feeding, but the M_{O_2} increases associated with normal diurnal activity become apparent during the second night after feeding (33+ hours).

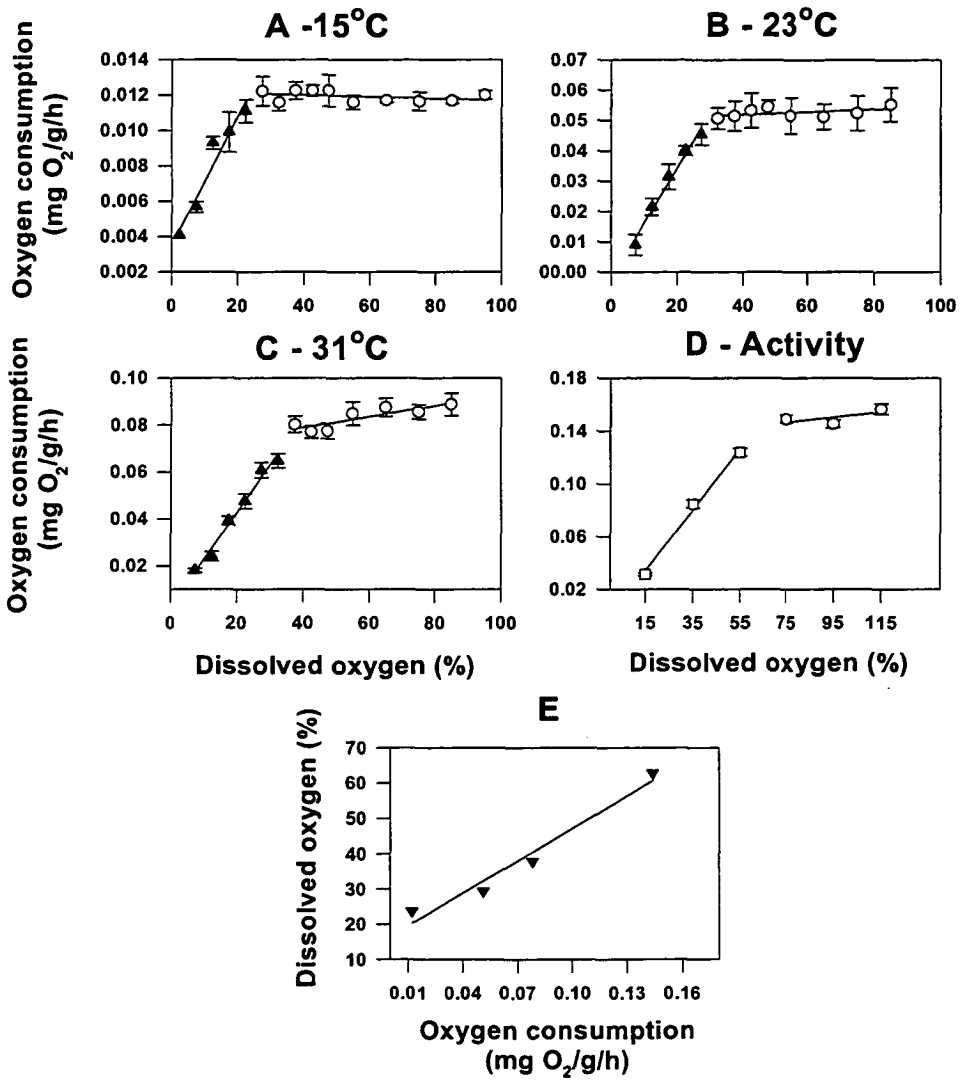

Figure 4.7: The relationship between dissolved oxygen level (%) and oxygen consumption (mg O_2/g/h) of *Panulirus cygnus* under various conditions. **A.** Oxygen consumption of settled lobsters (ie. standard oxygen consumption rates) at 15°C (n=6-9). **B.** Oxygen consumption of settled lobsters at 23°C (n=8). **C.** Oxygen consumption of settled lobsters at 31°C (n=8). **D.** Oxygen consumption of active lobsters at 23°C (n=9-12). **E.** Plot of the dissolved oxygen level at the critical oxygen tension (P_c) against oxygen consumption at P_c.

4.3.7 Effect of dissolved oxygen level on oxygen consumption

The critical oxygen tension (P_c) varied depending on the experimental condition the lobsters were maintained under (Fig. 4.7 A-D). P_c for settled lobsters increased as temperature increased, increasing from 23.7% dissolved oxygen saturation at 15°C to 37.9% at 31°C. P_c for the temperature of acclimation (23°C) was 29.4% saturation. The P_c for active lobsters at 23°C increased to 62.8% saturation. There was a significant (F=56.7, P=0.017) increase in P_c with increases in M_{O_2} (calculated from the point of intersection of the lines used to evaluate P_c)(Fig. 4.7E). The relationship could be described by the following linear equation:

$$P_c \text{ (\% saturation)} = 305.4\ M_{O_2}(\text{mg } O_2/\text{g/h}) + 16.66\ (r^2=0.97)$$

When the P_c values are expressed as mg/l, then P_c is close to 2 mg/l for each temperature (15°C - 1.93 mg/l; 23°C - 2.06 mg/l; 31°C - 2.26 mg/l).

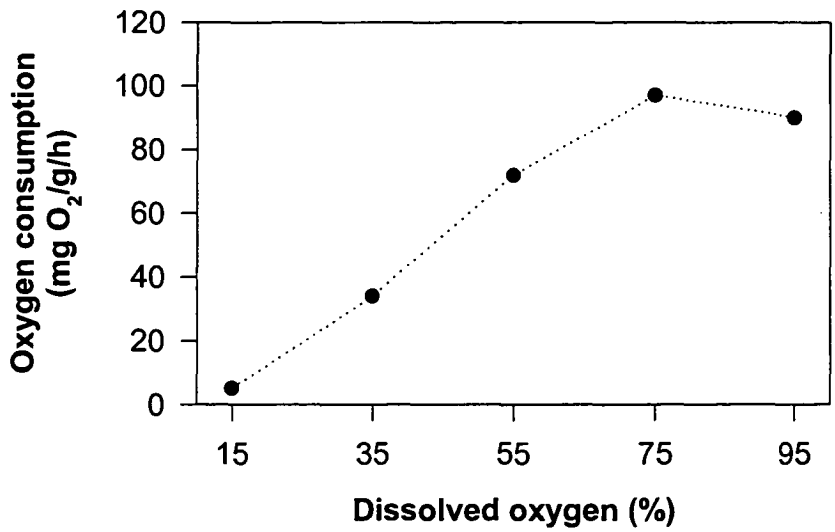

Figure 4.8: The aerobic scope for activity (mg O_2/g/h)(●) of the western rock lobster, *Panulirus cygnus*, at 23°C, plotted as a function of the dissolved oxygen level (calculated from data displayed in Fig. 4.7 B & D).

The aerobic scope for activity of lobsters at 23°C reduces as the dissolved oxygen level decreases (Fig. 4.8). The SFA is controlled by the response of active M_{O_2} to dissolved oxygen level, with the decrease in SFA at 55% saturation mirroring the decrease in active M_{O_2} at the same saturation. At 35% saturation the SFA had reduced to only 36% of the maximum SFA.

4.4 DISCUSSION

Unless referenced otherwise all discussion regarding *J. edwardsii* refers to the results outlined in Chapter 3.

4.4.1 Sex

As in the present study, no significant differences in oxygen consumption between sexes have been recorded in other studies (Laird and Haefner, 1976; Cockcroft and Wooldridge, 1985; Dall, 1986; Villarreal, 1990; Carvalho and Phan, 1997; Chapter 3, *J. edwardsii*). Sexual differences in oxygen consumption are not common in crustaceans (Laird and Haefner, 1976).

4.4.2 Body weight and activity

The slope of the pooled regression of oxygen consumption against body weight is within the range of 0.286-0.877 summarised by Bridges and Brand (1980*a*) for a series of decapod crustaceans. However, unlike *J. edwardsii*, the slope is >0.75; Bridges and Brand (1980*a*) noted that crustaceans in the large weight ranges tend towards high *b* values, which suggests that oxygen consumption is more dependent on body weight. In *J. edwardsii* oxygen consumption was more closely related to surface area. In *J. lalandii* the *b* value increased to 0.8-0.9 as temperature increased, suggesting that temperature may change the relationship. However, there was no decrease in *b* values as the temperature decreased in this study. The present studies of *P. cygnus* and *J. edwardsii*, provide no clearcut evidence about whether surface area dependency or body weight dependency determines the slope of the relationship between body

weight and oxygen consumption by large decapods. It may be that species specific differences are one of the largest factors determining the relationship.

One of the interesting features of the body weight and temperature response of oxygen consumption was the constancy of the slopes of the regressions at the various temperatures. Similar patterns have been observed by Dall (1986) for the prawn, *Penaeus esculentus* and by Carvalho and Phan (1997) for the seabob, *Xiphopenaeus kroyeri*. In this study the temperature range over which the constant relationship was maintained was 16°C, which suggests that *P. cygnus* can handle a wide temperature range; water temperatures in their natural habitat can vary from 16°C to 27°C (Gray, 1992). Van Donk and de Wilde (1981) found that the relationship between body weight and oxygen consumption broke down at temperatures outside of the temperature range within which *Crangon crangon* is usually found. The body weight/oxygen consumption relationship appears to be changing in *P. cygnus* held at 11°C, a temperature well below its natural range.

Activity resulted in a significantly higher oxygen consumption rate. Handling, emersion and exercise have all been shown to increase oxygen consumption of crustaceans (Booth *et al.*, 1982; Cockcroft and Wooldridge, 1985; Winkler, 1987; Patterson, 1993*a*; Carvalho and Phan, 1997), which is regarded as being an organism-level manifestation of all of the cellular processes that take place during recovery from a period of stress (Ellington, 1983). Processes include the recharging of energy pools (phosphagen and ATP), the clearing of anaerobic end-products from the tissues, and the correction of pH disturbances.

The mass exponent of oxygen consumption against body weight was smaller in active lobsters than resting lobsters, and is a reflection of the decreased aerobic expansibility of the larger lobsters. It is unclear why larger lobsters have a decreased aerobic expansibility in this study when there was no evidence of it in *J. edwardsii*. It may be species specific or it may be a result of the methods. The largest *P. cygnus* were about 1000 g heavier than the largest *J. edwardsii* and they did not easily fit into the respirometers. Space restrictions may have limited their ability to be fully active. However, as they were always handled and emersed out of the respirometers before oxygen consumption was measured over a short period after re-immersion they had extensive opportunities to be active.

4.4.3 Temperature and activity

Standard oxygen consumption of *P. cygnus* increased exponentially with temperature, as is the general response of poikilotherms to temperature (Wolvekamp and Waterman, 1960). Standard oxygen consumption rates of large decapods are summarised in Table 3.2 and highlight the paucity of information on oxygen consumption of large tropical and sub-tropical species. Even so, it is evident that *P. cygnus* has a much lower standard M_{O_2} than that measured for other species from similar temperature regimes. As for *J. edwardsii* this result has probably as much to do with the experimental procedures used in evaluating the rates, as it does to real differences between the species. Buesa (1979), for example, only allowed lobsters one hours adaptation to the respirometers prior to determining M_{O_2}.

The pattern of increase in standard M_{O_2} with temperature is similar to that recorded for *J. edwardsii*, with the Q_{10} being highest at the low temperatures and decreasing as the temperature increases. No zone of temperature independence was recorded and Q_{10} values were generally between 2 and 3 as recorded in many Crustacea (Wolvekamp and Waterman, 1960). The higher Q_{10} at low temperatures is probably a reflection of a "hibernation" response or a response to the acute temperature change as discussed in Chapter 3.

A reduction in standard oxygen consumption rates towards the upper temperature limit of a particular species has been recorded in Crustacea (Varo *et al.*, 1991). The standard oxygen consumption of *C. crangon* and *Muninda rugosa* declined at higher temperatures (Van Donk and de Wilde, 1981 and Zainal *et al.*, 1992 respectively). Oxygen consumption of *P. cygnus* continued to increase at temperatures up to 31°C suggesting that the upper limit has not yet been reached. Chittleborough (1975) found that juvenile *P. cygnus* were able to survive temperatures of 34°C, at least for an short duration.

The oxygen consumption response of active lobsters to temperature is similar to that observed for *J. edwardsii* and for the crayfish *Pacifastacus leniusculus* (Rutledge, 1981), with increases up to a maximum at an intermediate temperature and no further increases at higher temperatures. The lowest

temperature at which maximal oxygen consumption is attained is often the same as the preferred body temperature (Bennett, 1978), and in this study it was the temperature of acclimation. The mean annual temperature of inshore waters inhabited by juvenile lobsters is 20°C but optimum growth is attained at 25-26°C (Chittleborough, 1975), which suggests that 23°C is close to the preferred body temperature of *P. cygnus*. Also, the maximum scope for activity, which occurs at 23°C for *P. cygnus*, generally occurs at the preferred temperature of a species (Brett, 1956).

Oxygen consumption of active lobsters at 11°C probably reflects the general energy demand of the tissues, with lobsters displaying very little response to handling. Limitations within the somatic muscle fibres as to how much work can be performed could determine the active M_{O_2} by limiting demand at 11°C (Rutledge, 1981), resulting in the high $Q_{10(11\text{-}15)}$. Increased activity and extra demand by the muscle tissues for oxygen would drive an increase in oxygen consumption at 15°C. It would appear that the effect of temperature on thermochemical reactions is not the only factor controlling the increase in active oxygen consumption over the temperature range. At temperatures higher than 23°C active oxygen consumption does not increase. As discussed in Chapter 3 the limiting factor is probably the oxygen delivery and diffusion system.

Although the aerobic expansibility of *P. cygnus* is slightly higher than *J. edwardsii*, it is still at the low end of the spectrum of 3-7 evaluated for many fish species (Jobling, 1994). The aerobic scope for activity is slightly higher than that determined for other large decapod crustaceans (Spoek, 1974; McMahon *et al.*, 1979; Booth *et al.*, 1982; Waldron, 1991; Chapter 3 *J. edwardsii*), but that is a reflection of the higher standard oxygen consumption at the temperature of maximum aerobic scope of each species. The temperature range over which *P. cygnus* are able to maintain a high aerobic expansibility was much greater than in *J. edwardsii*. At 8°C below the "preferred" temperature the aerobic expansibility of *P. cygnus* had decreased by only ≈1/10, whereas in *J. edwardsii* it had decreased by almost a half. The ecological significance of such a phenomenon is unclear.

4.4.4 Handling and recovery

The recovery response was the same as recorded for many large decapod crustaceans (see Chapter 3 for discussion) and follows a typical Type V pattern; oxygen consumed during recovery exceeds the predicted oxygen deficit (Herreid, 1980). Such a pattern would be noted if the animals showed increased stress or physical activity in hypoxia (Herreid, 1980), as has occurred in this study.

The duration of recovery and amount of oxygen consumed during recovery increased with temperature. Similarly, Whiteley and Taylor (1990) found that lobsters, *H. gammarus*, took longer to recover from the effects of aerial exposure at 20°C compared to 10°C. The duration of recovery at 23°C and 31°C was similar to that for other decapod crustaceans (Chapter 3). The amount of oxygen consumed during the recovery period may be a reflection of the reliance on anaerobic metabolism during emersion. Lactate concentrations in emersed *P. cygnus* increased in relation to increases in temperature (Chapter 8). As one of the suggested uses for the excess oxygen is for metabolising anaerobic end products (Herreid, 1980), then the extra oxygen consumption at 23°C and 31°C compared to at 15°C can be, at least partly, explained by the higher lactate concentrations after emersion. However, in *J. edwardsii* the calculated lactate portion of the oxygen debt accounted for only 5-20% of the total oxygen debt (Waldron, 1991). In the freshwater crayfish, *Cherax destructor*, half of the total oxygen debt was required for replenishing ATP and arginine phosphate reserves (alactic debt) in the tail muscle (Head and Baldwin, 1986) which are usually depleted by exercise (Head and Baldwin, 1986). The ability of *P. cygnus* to be active was severely reduced at 15°C suggesting that the alactic debt at that temperature may also have been considerably smaller than at the higher temperatures.

4.4.5 Diurnal rhythm

The increase in oxygen consumption of *P. cygnus* at night was associated with increased activity of the lobsters. Lobsters have been shown to move up to 700 metres during night-time foraging activity (Gray, 1992). As in *J. edwardsii* (Chapter 3) light again appears to be the main entraining factor, with oxygen consumption increasing immediately after the onset of darkness and decreasing

immediately the lights came back on. Tank studies showed that daily locomotor activity rhythms in individual *P. cygnus* are of a non-crepuscular nature, with peak activity occurring immediately after the onset of darkness; activity then remains at a lower level throughout the remainder of the night (Morgan, 1978). However, in tracking studies on reefs, foraging was found to be at its peak early in the evening, declined during the night and picked up again just before dawn, with minimal activity immediately after dawn, and little during the day (Jernakoff, 1987). In the present study several peaks in M_{O_2} occurred throughout the night. These appear to match the observations by Morgan (1978), which showed fluctuating rates of activity throughout the night.

The 87% increase in M_{O_2} of *P. cygnus* at night is much greater than recorded for *J. edwardsii* (48% increase), indicating that *P. cygnus* is a much more active animal than *J. edwardsii* during night-time activity. The aerobic expansibility of *P. cygnus* at 23°C is slightly higher than *J. edwardsii* at 13°C which may account for some of the increase. The routine M_{O_2} of *P. cygnus* (43.5% above standard) is within the range of routine rates (30-60% above standard) normally measured for fish (Becker and Fishelson, 1986; Sims *et al.*, 1993). The very large increases in night-time activity in this study emphasises the importance of accounting for diurnal changes when designing/interpreting M_{O_2} experiments in some species.

4.4.6 Feeding

The SDA response of *P. cygnus* was very similar to that of *J. edwardsii*. The peak M_{O_2} level obtained was slightly higher (2.19 compared to 1.72 times standard M_{O_2}) and comprises over 50% of the aerobic expansibility of *P. cygnus* at 23°C. As in *J. edwardsii* the aerobic scope for activity of *P. cygnus* is severely reduced for an extended period after feeding. Comparatively, the maximum increase in M_{O_2} by *C. maenas* after feeding was 2.3-fold above the resting rate, much less then the 5-14-fold increase found during activity (Houlihan *et al.*, 1990). The post-prandial peak in M_{O_2} was reached in approximately the same time by *P. cygnus* (7 hours) as by *J. edwardsii* (10-13 hours). The effect of diurnal

rhythm on M_{O_2} became evident the second night after feeding and indicates that SDA was not a strong modulator of M_{O_2} at that stage. Factors other than SDA, such as tidal or other biological rhythms, exerted a powerful influence on M_{O_2} in *Cancer pagurus* and *Maia squinado* (Aldrich, 1975). The results in the present study further highlight the need to ensure biological rhythms are accounted for when determining SDA in crustaceans.

The duration of SDA was 46 hours in *P. cygnus* and 42 hours in *J. edwardsii*, although the effect of the diurnal rhythm may have artificially increased the duration in both species. The average increases in M_{O_2} above standard M_{O_2} during daylight hours, the day after feeding, were also very similar (33% in *P. cygnus* and 42% in *J. edwardsii*). Both of these results indicate that there was very little difference between the species in the duration of SDA, when they were fed at 3% of their body weight. However, given the warmer experimental temperature in this study it would be expected that the duration of SDA in *P. cygnus* would be much less than in *J. edwardsii*. Decreased temperature has been shown to increase the duration of the SDA in fish (Jobling and Davies, 1980). Considerably more research on SDA, and the factors affecting it (temperature, ration size, body size), is required in order to develop a better understanding of the effects of feeding on oxygen consumption by large decapods.

4.4.7 Dissolved oxygen level

Many crustaceans are able to maintain M_{O_2} constant in the face of decreasing water oxygen tensions due to a hypoxia-induced gill hyperventilation, along with an increase in haemocyanin oxygen affinity and an improvement in the ability of the respiratory surfaces to transfer oxygen (Reiber, 1995). Below P_c they must reduce M_{O_2} and switch to anaerobic metabolism (Reiber, 1995) although there is considerable interspecific differences in the extent of anaerobiosis below P_c (Herreid, 1980). P_c varies with many intrinsic and extrinsic factors (Herreid, 1980) but is usually at its lowest in quiescent animals, well acclimated to their experimental conditions, and neither disturbed nor exposed to environmental stress (McMahon and Wilkens, 1983). *P. cygnus* was able to maintain standard

M_{O_2} rates down to 23.7% oxygen saturation at 15°C. Temperature increased the P_c level, with lobsters held at 31°C only able to maintain standard M_{O_2} rates down to 37.9% saturation. The P_c values are within the range typically evaluated for crustaceans living in well oxygenated environments (20-50%)(see Chapter 3.4). A decrease in oxygen-independence with increasing temperature has also been noted by Taylor *et al.* (1977) for *C. maenas* and by Bridges and Brand (1980*a*) for *Galathea strigosa*.

The P_c also increased with activity with a value of 62.8% saturation being obtained for active lobsters at 23°C. The active P_c of *P. cygnus* is well below the active P_c calculated for many crustaceans of close to 100% saturation, but is similar to that evaluated for *J. edwardsii* (Chapter 3). Pc is a variable parameter dependent on metabolic demands (Herreid, 1980), as highlighted in this study. In *P. cygnus* there is a linear relationship between M_{O_2} and P_c. The relationship allows the calculation of Pc when M_{O_2} of the lobsters is known, without resorting to hypoxia experiments.

At all temperatures the amount of oxygen in the water at the P_c was around 2 mg/l. This contrasts with the value for *J. edwardsii* which was calculated to be 3.11 mg/l. The lower P_c level is probably an adaptation to the low environmental oxygen levels the *P. cygnus* would find in a natural situation. The usual temperature range of *P. cygnus* is some 10°C above the usual temperature range for *J. edwardsii*, thus the amount of oxygen in fully saturated water is some 20% lower. Bridges and Brand (1980*a*) also found that crustaceans which are usually subjected to low environmental oxygen tensions had lower P_c levels. However, the P_c levels calculated for both species fall within the P_c levels calculated for large species of Macrura and Bracyura, which usually vary from 1-3 mg/l (Spoek, 1974). The P_c of active *J. edwardsii* was also slightly higher (5.03 mg/l) than the P_c for active *P. cygnus* (4.39 mg/l).

As in *J. edwardsii* the scope for activity decreases as the dissolved oxygen tension decreases below the active P_c. Therefore, *P. cygnus* are also able to maintain a high aerobic scope for activity down to reasonably low levels of dissolved oxygen tension (at least 70-80%). Chittleborough (1975) noted that the growth increments of *P. cygnus* were significantly effected at oxygen levels of 60-

70% saturation. The decreasing SFA may start to impact on physiological processes at that dissolved oxygen level.

Conclusion: the response of oxygen consumption by *P. cygnus* to various extrinsic and intrinsic factors has been evaluated. The implications of these results to the western rock lobster industry will be discussed in the General Discussion (Chapter 9).

CHAPTER 5

The effect of intrinsic and extrinsic factors on ammonia excretion by the southern rock lobster, *Jasus edwardsii*, and the western rock lobster, *Panulirus cygnus*.

5.1 INTRODUCTION

A characteristic of aquatic Crustacea is ammonotelism. Ammonia makes up 60% to 100% of the total excreted nitrogen in Crustacea (Regnault, 1987). Ammonia can be toxic to crustaceans if allowed to accumulate to too high a concentration (Tomasso, 1994), and even at low levels can inhibit growth (Chen and Lin, 1992). To optimise the design and management of transport and holding systems, the ammonia excretion responses of the culture animal to culture conditions are required. Therefore, this study investigates the effect of several intrinsic and extrinsic factors on ammonia excretion by the southern (*Jasus edwardsii*) and the western (*Panulirus cygnus*) rock lobsters, and investigates the endogenous urea excretion rate of both species.

5.2 MATERIALS AND METHODS

General Materials and Methods are outlined in Chapter 2. All experiments (except for those examining the effect of temperature) were conducted at 13°C for *J. edwardsii* and 23°C for *P. cygnus*.

Statistical analyses

Regressions were obtained by the least squares method and the significance of regression slopes, *b*, was tested by ANOVA. Students t-tests (paired) were used to test for differences in the diurnal rhythm (night-time Vs daytime rates), feeding (post-prandial Vs pre-prandial rates) and handling/emersion (re-immersion Vs endogenous rates) data. All analyses were performed on the SPSS statistical package with the α set at 0.05. All means are expressed as mean±SE.

5.3 RESULTS

5.3.1 The effect of temperature on ammonia excretion

Ammonia excretion (TAN - mg TAN/g/h) of *J. edwardsii* and *P. cygnus* increased significantly (F=143.2, P=0.001; F=302.2, P<0.001 respectively) with temperature (T - °C)(Fig. 5.1). The relationships were exponential and were able to be described by the following equations:

Jasus edwardsii

$$Log_{10} TAN = 0.041T - 3.57 \ (r^2=0.979)$$

Panulirus cygnus

$$Log_{10} TAN = 0.057T - 3.90 \ (r^2=0.987)$$

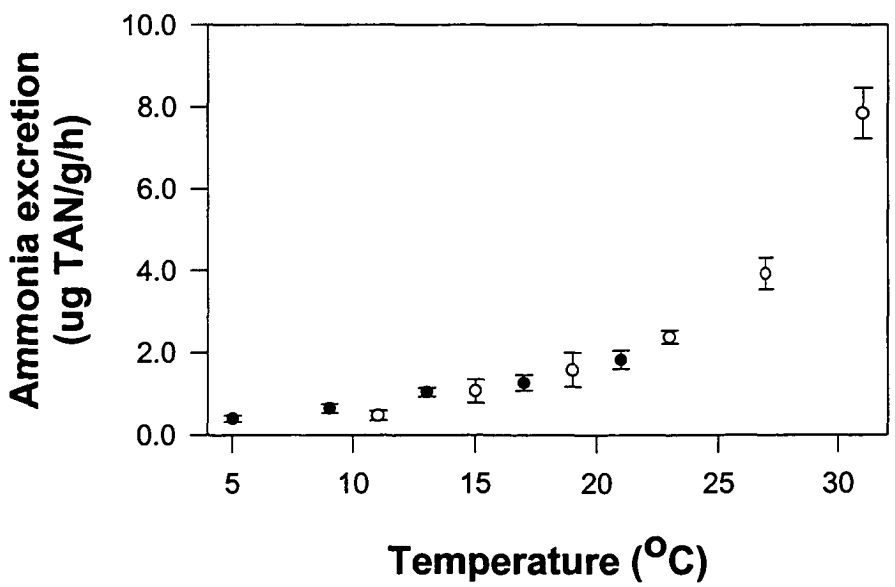

Figure 5.1: The effect of temperature on ammonia excretion (mean ± SE)(µg TAN/g/h) of the southern rock lobster, *Jasus edwardsii* (●)(n=11) and the western rock lobster, *Panulirus cygnus* (o)(n=10). Ammonia excretion increased exponentially with temperature.

Jasus edwardsii		*Panulirus cygnus*	
Temperature range	**Q_{10}**	**Temperature range**	**Q_{10}**
5-9	3.37	11-15	7.42
9-13	3.33	15-19	2.69
13-17	1.64	19-23	2.71
17-21	2.50	23-27	3.56
		27-31	5.60
5-21	**2.61**	**11-31**	**4.05**

Table 5.1: The Q_{10} values of ammonia excretion over each temperature range for both *Jasus edwardsii* and *Panulirus cygnus*. The average Q_{10} values over the whole temperature range are shown in bold.

The Q_{10} values for *J. edwardsii* were highest at the lower temperature ranges and lowest at the higher temperature ranges (Table 5.1). The $Q_{10(5\text{-}21)}$ value over the whole temperature range was 2.61. Q_{10} values for *P. cygnus* were very high at the extremes of the temperature range ($Q_{10(11\text{-}15)}$=7.42, $Q_{10(27\text{-}31)}$=5.60). These high values resulted in a $Q_{10(11\text{-}31)}$ value over the whole temperature range of 4.05.

5.3.2 The effect of body weight on ammonia excretion

A log-log plot of total ammonia excretion (TAN - mg TAN/h) over lobster body weight (W - g) is shown in Fig. 5.2. Ammonia excretion by both *J. edwardsii* and *P. cygnus* were positively correlated to body weight. The regression equations describing the relationship are:

Jasus edwardsii:

$\text{Log}_{10}\,\text{TAN} = 0.473\ \log_{10} W - 1.704$ ($r^2 = 0.42$, $F = 14.05$, $p = 0.001$)

Panulirus cygnus:

$\text{Log}_{10}\,\text{TAN} = 0.499\ \log_{10} W - 1.346$ ($r^2 = 0.69$, $F = 44.18$, $p < 0.001$)

In view of the relationships between body weight and ammonia excretion, restricted weight ranges (600-900 g, *J. edwardsii*; 380-520 g, *P. cygnus*) were used in experiments where body weight was not a factor.

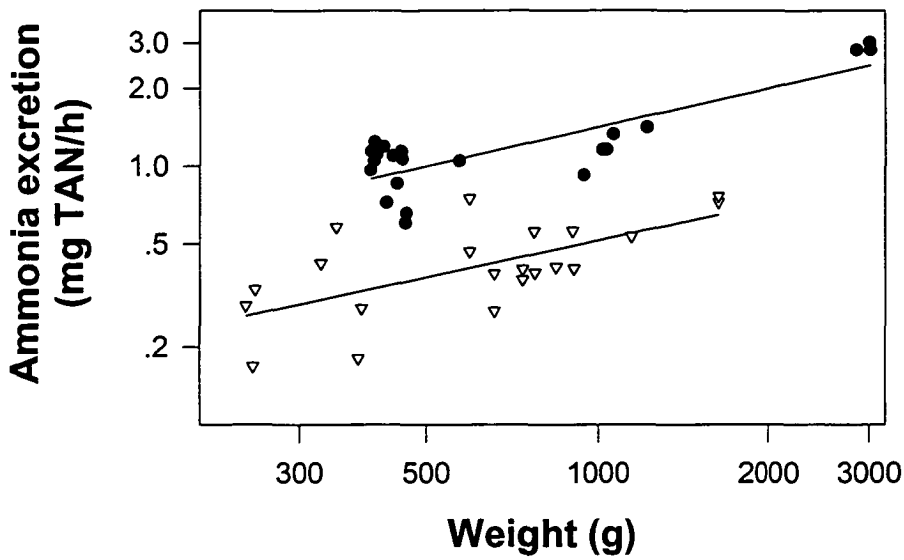

Figure 5.2: A log-log plot of total ammonia excretion (mg TAN/h) against lobster body weight (g) for *Jasus edwardsii* (▽)(n=21)and *Panulirus cygnus* (●)(n=22). Ammonia excretion was determined for lobsters over the weight ranges of 241-1625 g (*J. edwardsii*) and 400-3022 g (*P. cygnus*).

5.3.3 The effect of diurnal rhythm on ammonia excretion

A diurnal rhythm was evident for *J. edwardsii* with a significant (t=3.05, P=0.016, n=10) increase in ammonia excretion at night. Night-time ammonia excretion was 39.6% higher than daytime. There was no significant difference (t=1.30, P=0.22, n=10) between night-time and daytime ammonia excretion by *P. cygnus*.

5.3.4 The effect of handling and emersion on ammonia excretion

There was a significant (*J. edwardsii*, t=11.3, P<0.001; *P. cygnus*; t=6.7, P<0.001) increase in ammonia excretion after re-immersion following handling and emersion for both species (Fig. 5.3). However, the increase was only evident for the first hour after re-immersion; ammonia excretion was not significantly different (*J. edwardsii*, t=1.91, P=0.11; *P. cygnus*, t=0.255, P=0.80) to the pre-emersion levels by the second hour. Only ammonia excretion for the first three hours after re-immersion is shown here as it was constant for the remainder of the measurement period (5 hours).

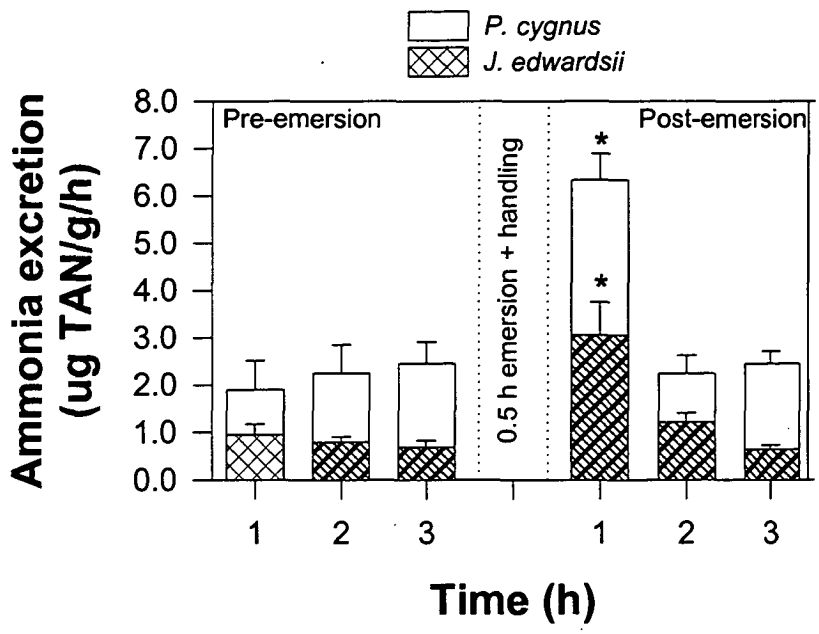

Fig. 5.3: Ammonia excretion (µg TAN/g/h) by *Jasus edwardsii* (n=6) and *Panulirus cygnus* (n=11) 3 hours before and after a ½ hour period of emersion and handling. The asterisks (*) denote significantly different values to the pre-emersion/handling values for each species.

5.3.5 The effect of feeding on ammonia excretion

Ammonia excretion increased after feeding in both species (Fig. 5.4 & 5.5). Ammonia excretion by *J. edwardsii* peaked twice over the 24 hour period after the commencement of feeding: peaks occurring 7 and 18 hours after feeding. Ammonia excretion declined after the second peak and was not significantly different (t=0.779, P=0.471) to the pre-prandial level 26 hours after feeding. Maximum ammonia excretion was 6.28 times pre-prandial levels.

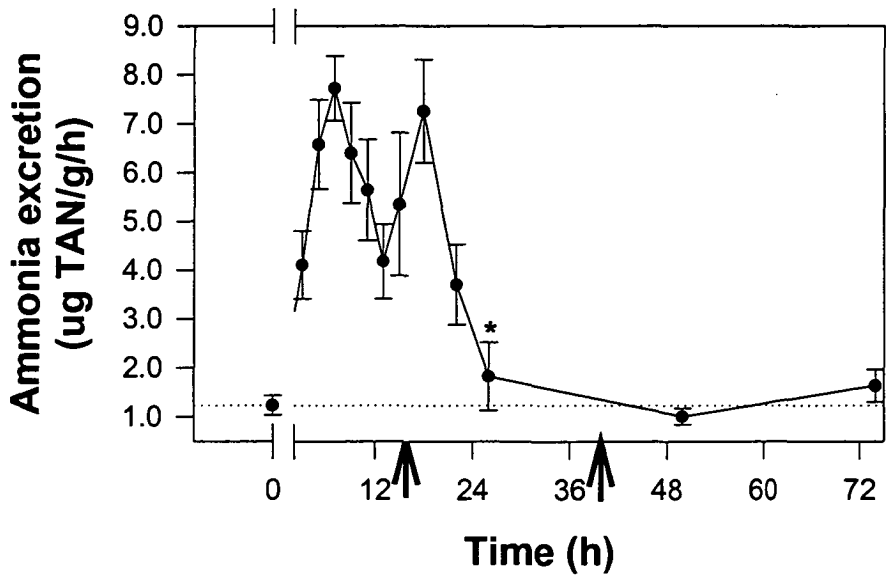

Figure 5.4: The ammonia excretion response to feeding of the southern rock lobster, *Jasus edwardsii* (n=5). The lobsters were fed at 8AM and allowed to feed for two hours (indicated by the break) before water sampling began. The dotted line indicates the pre-prandial level of ammonia excretion. The time period when post-prandial ammonia excretion becomes not significantly different to pre-prandial ammonia excretion is indicated by the asterisk (*). The arrows denote midnight.

Ammonia excretion by *P. cygnus* also showed a double peak over the 24 hour period after the commencement of feeding (Fig. 5.5). A large peak occurred after 8 hours (5.60 times the pre-prandial rate) and a second smaller peak (2.82 times the pre-prandial rate) after 15 hours. Ammonia excretion declined after the

second peak and was not significantly different (t=1.047, P=0.33) to pre-prandial levels after 30 hours.

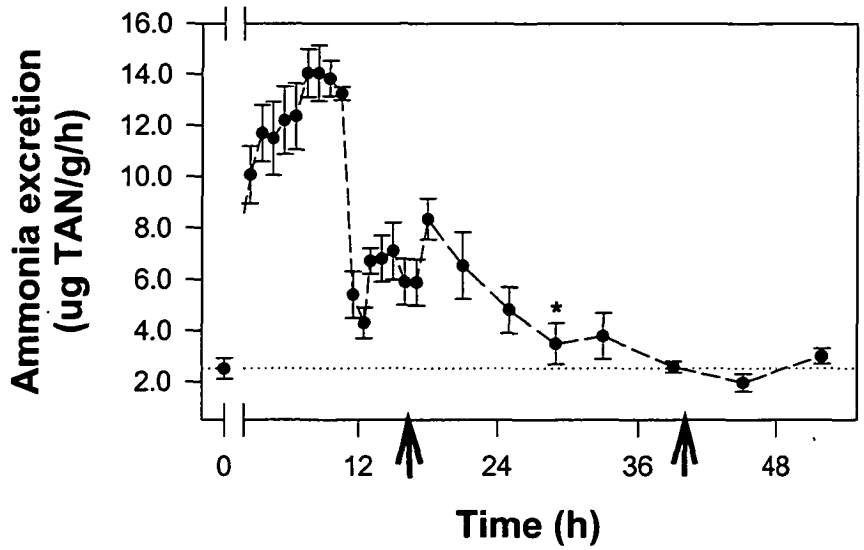

Figure 5.5: The ammonia excretion response to feeding of the western rock lobster, *Panulirus cygnus* (n=12). The lobsters were fed at 8AM and allowed to feed for two hours (indicated by the break) before water sampling began. The dotted line indicates the pre-prandial level of ammonia excretion. The time period when post-prandial ammonia excretion becomes not significantly different to pre-prandial ammonia excretion is indicated by the asterisk (*). The arrows denote midnight.

5.3.6 The relationship between ammonia excretion and urea excretion

Urea excretion was 21.25 ± 7.85% (mean±SE) of the total rate of ammonia and urea excretion of *J. edwardsii* and 17.42 ± 3.16% (mean±SE) of *P. cygnus*.

5.4 DISCUSSION

5.4.1 Temperature

Ammonia excretion by *J. edwardsii* and *P. cygnus* increases exponentially in response to increases in temperature. Temperature has long been recognised as one of the main exogenous factors affecting ammonia excretion by crustaceans. The relationship between ammonia excretion and temperature differs according to the species and the temperature range considered, although generally ammonia excretion increases as temperature increases (Regnault, 1987). Few observations have been reported for decapods (Regnault, 1987) and most of these have been for small species (Needham, 1957; Quarmby, 1985) or juveniles (Chen and Lai, 1993; Chen and Nan, 1993; Chen and Kou, 1996). No comparable information on the effect of temperature on the ammonia excretion of other large decapods was found in the literature.

The average Q_{10} of *J. edwardsii* falls within the normal range (2-3) for biological processes (Schmidt-Nielsen, 1990), although the Q_{10}'s are higher at lower temperatures. Similarly, the Q_{10}'s of oxygen consumption typically increase at lower temperatures (Chapters 3 and 4). However, the average Q_{10} of *P. cygnus* was high (4.05) with the Q_{10} values at the extremes of the temperature ranges being very high. The Q_{10} of *Carcinides maenas* was also very high (12.4) at the lower end of the temperature range (Needham, 1957). The author suggested it may reflect the general slowing of metabolic processes at low temperature. However, as outlined in Chapters 3 and 4 the high Q_{10} probably reflects the affect of low temperature on physical activity of the animals as well as on metabolic processes. Additionally, the composition of the nitrogenous excretory products may differ with temperature, resulting in Q_{10} values which are not truly reflective of the effect of temperature on the physiological process being studied. For example, the percentage of nitrogen excreted as ammonia, varied with temperature in the prawn, *Macrobrachium rosenbergii*, with concomitant changes in the level of amino acids and urea excreted (Chen and Kou, 1996). Quarmby (1985) also found that temperature varied the ratio of ammonia to urea excretion by *Pandalus platyceros* but the change was dependent on the development stage of the prawn.

5.4.2 Body weight

The relationship between body size and metabolic rate is a classic physiological subject extensively discussed in the literature (Carvalho and Phan, 1997). However, the relationship between body size and ammonia excretion in crustaceans has been poorly investigated. Nevertheless, studies have shown that ammonia excretion per unit weight decreases as body weight increases (Needham, 1957; Wajsbrot *et al.*, 1989; Marangos *et al.*, 1990; Carvalho and Phan, 1997). The value for the weight exponent, *b*, for total ammonia excretion would be expected to be around 0.75, the weight exponent of total oxygen consumption for a wide variety of organisms (Hemmingsen, 1960). However, weight exponents close to 0.5 were obtained for both *J. edwardsii* and *P. cygnus*. Weight exponents of 0.47-0.95 have been found in ammonia excretion experiments conducted for a range of fish species (Jobling, 1994) and a similar wide range of exponents have been calculated for decapod crustaceans. The weight exponent of *Xiphopenaeus kroyeri* was 0.88 (Carvalho and Phan, 1997), *Penaeus japonicus* 0.75 (Marangos *et al.*, 1990), *P. semisulcatus* 0.66 (Wajsbrot *et al.*, 1989), and *C. maenas* 0.39 (Needham, 1957). Table 5.2 summarises the ammonia excretion rates of decapod crustaceans over a wide range of body weights (0.08-800 g). A log-log plot of total ammonia excretion against body weight for the data is shown in Fig. 5.6. The weight exponent of the relationship was calculated to be 0.65 which is similar to that which relates the relationship between weight and oxygen consumption to body surface area (0.67). The relationship between ammonia excretion and body weight of decapod crustaceans is similar to that for oxygen consumption but studies on more species over a wide range of weights is required in order to clarify the subject. Factors such as the rate of ammonia excretion as a percentage of total nitrogenous excretion can change with body size (Quarmby, 1985), and would severely affect the ammonia excretion/body weight relationship.

Species	Body Weight (g)	TAN (µg TAN/g/h)	Reference
Penaeus brasiliensis	1.0	16.8	Romero, 1983
P. japonicus	42.8	6.3	Marangos *et al.*, 1990
	32.7	7.1	
	0.08	30.5	
P. japonicus	0.22	16.8	Chen and Lai, 1993
P. japonicus	5-7	428	Spaargaren *et al.*, 1982
P. esculentus	17.7	9.6	Dall and Smith, 1986
P. chinensis	0.32	19.2	Chen and Nan, 1993
P. chinensis	26.9	19.2	Chen *et al.*, 1993
P. indicus	5.0	43.75	Gerhardt, 1980
P. indicus	8.0	25.1	Wickins, 1976
P. aztecus	5.0	10.5	Wickins, 1976
P. monodon	1.6	32.9	Wickins, 1985
P. monodon	27.0	10.6	Wickins, 1985
P. semisulcatus	0.6	90.4	Wajsbrot *et al.*, 1989
	1.3	60.6	
	10.4	28.0	
	43.8	21.0	
Macropetasma africanus	1.0	55.2	Cockcroft and McLachlan, 1987
Crangon franciscorum	0.91	14.4	Nelson *et al.*, 1979
Palaemonetes varians	1.0	27.0	Snow and Williams, 1971
Xiphopenaeus kroyeri	1.0	31.2	Carvalho and Phan, 1997
	10.0	24	
Jasus edwardsii	680.0	2.2	This study
J. edwardsii	≈200.0	4.2	Binns and Peterson, 1969
Panulirus cygnus	440.0	2.6	This study
J. lalandii	≈800.0	1.1	Zoutendyk, 1987

Table 5.2: Routine endogenous weight-specific ammonia nitrogen (TAN) excretion (µg TAN/g/h) of decapod crustaceans at 25°C and salinity higher than 25‰ (after Carvalho and Phan, 1997, and Wajsbrot *et al.*, 1989). Q_{10} = 2 was used to convert ammonia excretion rates to 25°C.

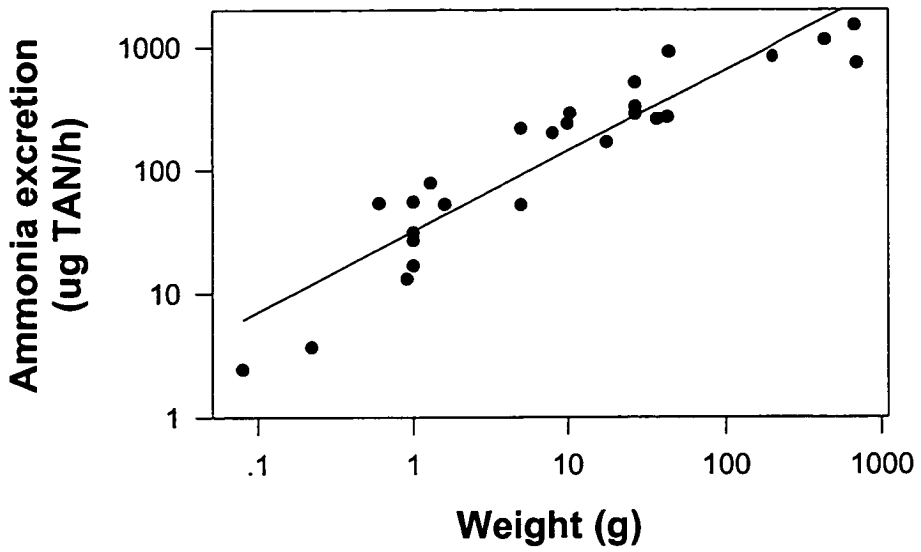

Figure 5.6: A log-log plot of total ammonia excretion against body weight of a variety of decapod crustaceans (see Table 5.2). The excretion rate of *P. japonicus* recorded by Spaargaren *et al.* (1982) was not included as it was unusually high. The relationship is exponential and described by the relationship:

Log_{10} TAN (ug TAN/h) = 0.65 log_{10} W (g) + 1.50 (r^2=0.86).

There have been few studies of ammonia excretion by large decapod crustaceans. The endogenous ammonia excretion rates of several species are compared in Table 5.3. Given the temperature and size differential the ammonia excretion rates are very similar. Ammonia excretion rates of large decapod crustaceans appear to be similar to that of fish in the same weight range. For example, the Japanese flounder, *Paralichthys olivaceus*, excreted 2.0 µg TAN/g/h for fish of around 500 g at 20°C (Kikuchi, 1995). The excretion rate of 900 g rainbow trout, *Oncorhynchus mykiss,* varied with temperature and rose from 1.3 to 3.6 µg TAN/g/h as temperature rose from 10 to 20°C (Jobling, 1994).

Species	Weight (g)	Temp. (°C)	Ammonia excretion (µg TAN/g/h)	Reference
Jasus edwardsii	680	13	1.04	This study
Panulirus cygnus	440	23	2.37	This study
Jasus lalandii	≈ 800	12	0.46*	Zoutendyk, 1987
Jasus edwardsii	≈ 200	14	2.0	Binns and Peterson, 1969

Table 5.3: Ammonia excretion of *Jasus edwardsii* and *Panulirus cygnus* compared with other large decapod crustaceans.
* this figure is based on the calculated moisture content of the lobsters being 71.3% (Chapter 7).

5.4.3 Diurnal rhythm

Lobsters exhibit activity rhythms, being more active at night than day, a phenomenon in common with many other subtidal crustaceans (Lipicus and Herrnkind, 1982). Light appears to be the main factor controlling activity patterns in *J. edwardsii* and *P. cygnus*; they usually remain inactive during daylight. Increased levels of oxygen consumption have been measured in conjunction with the increased night-time activity (Chapters 3 and 4). A night-time increase in ammonia excretion would therefore be expected. A daily pattern of ammonia excretion, with increases during the night, has been noted in several other crustacean species (Dall and Smith, 1986; Marangos *et al.*, 1990; Carvalho and Phan, 1997). The ammonia excretion pattern of *J. edwardsii* showed the expected response to daily rhythm and the increase at night was similar to the increase recorded for oxygen consumption (39.6% c/f. 48.3%). The lack of a night-time increase in ammonia excretion for *P. cygnus* is not easily explained and needs further investigation. However, the response of *J. edwardsii* confirms the existence of a rhythm associated with light, possibly controlled endogenously (Subrahmanyam, 1976 in Carvalho and Phan, 1997).

5.4.4 Handling and emersion

Ammonia is released to the external environment through the gills by diffusion and Na^+/NH_4^+ exchange across the epithelium (Kormanik and Cameron, 1981; Regnault, 1987). In the absence of water, such mechanisms may be greatly

impaired, and accumulation of ammonia in the haemolymph may occur (Regnault, 1994; Schmitt and Uglow, 1997*a*; Chapters 6, 7 and 8). The reduction of ammonia excretion by both *J. edwardsii* and *P. cygnus* to endogenous rates after 1 hour of re-immersion following a period of emersion and handling, indicates that the accumulated ammonia is released very quickly after re-immersion. The rapid decrease is probably a reflection of the higher ventilatory and circulatory activities resulting from emersion and handling, which would maintain a large gradient across the gills for ammonia excretion (Waldron, 1991). Similarly, the ammonia excretion rate of the crab, *Cancer pagurus*, had returned to its pre-emersion value within 1 hour of being re-immersed (Regnault, 1994). Most of the accumulated ammonia was excreted within 5 minutes of being re-immersed The ammonia excretion rate of the prawn, *Penaeus monodon*, the seabob, *Xiphopenaeus kroyeri*, and the prawn, *Nephrops norvegicus*, quickly attained a steady state after a short period of disturbance (Almendras, 1994*b*; Carvalho and Phan, 1997; Schmitt and Uglow, 1997*a* respectively). However, when *N. norvegicus* was emersed for 8 hours, it continued to excrete increased levels of ammonia even after 6 hours re-immersion. It is possible that some other nitrogenous metabolic end-products (eg. urate) are formed and stored during extended periods of emersion and that they are released slowly upon re-immersion (Schmitt and Uglow, 1997*a*).

If pre-immersion ammonia excretion rates were maintained during emersion then approximately 0.5 and 1.2 μg TAN/g would be expected to accumulate in the haemolymph of *J. edwardsii* and *P. cygnus*, respectively. In both species the amount of excess ammonia (above basal rates) excreted during the first hour after re-immersion was approximately 4 times greater than that calculated to accumulate in the haemolymph. As in the present study, Schmitt and Uglow (1997a) also measured excess levels of ammonia excretion after re-immersion and suggested it was caused by the large number of tail-flips and a high activity rate during emersion. Elevated ammonia excretion rates due to handling have been measured in *X. kroyeri* (Carvalho and Phan, 1997). Similarly, in the present study it is probable that the excess ammonia excreted upon re-immersion was due to activity and handling during emersion.

5.4.5 Feeding

The ammonia excretion response to feeding was similar to the oxygen consumption response to feeding (Chapters 3 and 4), with a rapid increase up to a peak, followed by a slow decline over time. The major difference in the responses was the double peak occurring in ammonia excretion. The size of the ammonia excretion peak and the duration was similar to that of other large decapods. Ammonia excretion by *J. lalandii* after feeding increased 7.7-fold (peaks at 4 and 8 hours post-prandial) and returned to pre-prandial values after 10 hours (Zoutendyk, 1987). For 300 g *Homarus americanus* the peak level after feeding was approximately 4 times the pre-prandial level (Wickins, 1985), although it could have been higher as the endogenous rate was not clearly established. Peaks occurred 6 and 12 hours after feeding and ammonia excretion was close to the pre-prandial levels after 18 hours. Peak values in fish after feeding vary from 4 to 10 times the endogenous excretion rate (Almendras, 1994*a*) and usually occur between 5 and 10 hours post-prandial before returning to pre-feeding levels within 24 hours. The length of time required for the rise and fall of ammonia excretion in fish is determined by the size of the meal, its composition and water temperature (Jobling, 1994). The results of this study confirm the need to maintain lobsters for a minimum of 36 hours before endogenous ammonia excretion rates can be accurately determined.

The double peak in ammonia excretion after feeding has been observed in other crustaceans (Wickins, 1985; Hawkins *et al.*, 1986; Zoutendyk, 1987). Hawkins *et al.* (1986) suggested that the peaks may be related to endogenous cycles influencing physical activity, digestive processes, hormonal secretions, or their combinations. In both *J. edwardsii* and *P. cygnus* the second peak (6-fold and 1.8-fold for *J. edwardsii* and *P. cygnus* respectively) occurred during the night which means the peak could be correlated to normal daily activity rhythms. However, the diurnal rhythm data indicate that there is little chance of peaks of such magnitude occurring due to increased activity. Also, no peak was observed during the second night after feeding in *P. cygnus*. Therefore, although endogenous cycles may be causing the peaks it does not appear to be related to a diurnal cycle of activity. Another possibility is that the two peaks represent

metabolically produced ammonia in the first instance and excretory (faeces and urine) losses in the second. Faeces was first noted 6 and 7 hours after feeding in *J. edwardsii* and *P. cygnus*, respectively. This fact does not preclude the above possibility as the time period when the majority of the faeces is excreted may be much later. Further work on these aspects of ammonia excretion in crustaceans is required.

5.4.6 Urea excretion

Urea has usually been reported to comprise 1-5% of the nitrogen excreted by crustaceans (Regnault, 1987). If ammonia excretion is taken to be 70% of nitrogen excreted then the ammonia:urea ratio would be a minimum of 14:1. Many of the studies that have determined both ammonia and urea excretion values have been with small crustaceans. In studies of large crustaceans, the ratio appears to be much smaller, indicating that a comparatively high percentage of the nitrogen is being excreted as urea. The ratio in this study was 3.7:1 for *J. edwardsii* and 4.7:1 for *P. cygnus*. The ratio for *Jasus lalandii* was 6.0:1 (Zoutendyk, 1987). The ammonia:urea ratio of *P. semisulcatus* decreased as body weight increased - 8.3:1 at 0.6 g to 2.7:1 at 43.8 g (Wajsbrot *et al.*, 1989). The ammonia:urea ratio of flounder, *P. olivaceus*, also decreased with body weight (Kikuchi, 1995). Quarmby (1985) highlighted the need to further investigate forms of nitrogen excretion other than ammonia. The apparent high rate of urea excretion of crustaceans with higher body weights requires additional investigation.

Conclusion: ammonia excretion of both *J. edwardsii* and *P. cygnus* is influenced by a number of exogenous and endogenous factors. As in fish, temperature and body weight were found to have large influences on the rate of ammonia excretion. However, feeding displayed the largest effect on the rate of ammonia excretion (at least in the short to medium term). Implications to the live holding of lobsters will be discussed in Chapter 9.

CHAPTER 6

Recovery from stress of the western rock lobster, *Panulirus cygnus*: the effect of dissolved oxygen level

6.1 INTRODUCTION

The characteristic physiological responses of subtidal crustaceans to emersion/exercise is internal hypoxia, a mixed respiratory and metabolic acidosis, hyperglycaemia, rapid depletion of energy pools in the muscle tissue, and accumulation of metabolic waste products (Telford, 1968; Onnen and Zebe, 1983; Head and Baldwin, 1986; Whiteley *et al.*, 1990; Waldron, 1991). A variety of processes take place during re-immersion - energy pools (phosphagen and ATP) are recharged, anaerobic end-products are cleared from the tissues, and pH disturbances are corrected.

As reduced dissolved oxygen levels can seriously effect the physiological processes of even unstressed lobsters, the aim of this study was to determine the effect of dissolved oxygen level on the recovery of the western rock lobster, *P. cygnus*, after it was exposed to an episode of emersion and handling.

6.2 MATERIALS AND METHODS

General Materials and Methods are as outlined in Chapter 2 with the following specific Materials and Methods.

The study was performed in two experimental series. The first series studied the oxygen consumption (M_{O_2}) of lobsters recovering from a period of stress. Lobsters were removed from the holding tank and emersed for 30 minutes. Continual disturbance (handling) for the first 5 minutes was followed by disturbance every 5 minutes. Lobsters showed a strong escape behaviour (tail flicking) during the initial period of disturbance. The response diminished as the emersion time increased and after 30 minutes emersion the lobsters were normally unresponsive to disturbance. Six to twelve lobsters were trialed at each of six oxygen levels (115, 95, 75, 55, 35, 15%). The dissolved oxygen in the water was controlled as outlined in Section 2.2. This resulted in the maintenance of dissolved

oxygen levels within 5% of the designated level. As a diurnal rhythm of oxygen consumption was evident in *P. cygnus* (Chapter 4) all experiments were commenced prior to 9 AM to ensure that none of the measurement periods fell during the night. Rates of M_{O_2} were calculated immediately after placing the disturbed lobsters in the respirometers (0 hours) and at 1, 2, 4, 6, 8, and 24 hours. Ten lobsters were used to determine the standard M_{O_2}.

The second series of experiments consisted of measuring the haemolymph parameters of lobsters over the same time period and under the same dissolved oxygen levels as above. Lobsters were removed from the holding tank and disturbed for 30 minutes before placing them into water of known oxygen level. Haemolymph samples were obtained immediately after the disturbance period, and after 1, 2, 4, 8 and 24 hours re-immersion. Dissolved oxygen was maintained within 5% of the designated level. Lobsters were only sampled once during each experimental run. They were replaced into the holding tank for a minimum of 48 hours before being subjected to another disturbance and recovery regime.

Twelve lobsters were used to determine the pre-disturbance haemolymph parameters (control). The haemolymph was sampled from 4 lobsters taken directly from the holding tank at 9 AM. Two further groups of 4 lobsters were tested 8 and 24 hours later, respectively.

Statistical analyses

The Students t-test was used to test for differences between pre-disturbance and post-disturbance values. Where appropriate a Students t-test for samples with unequal variances was used. The one-way ANOVA was used to test for differences between treatments at each time period. The Levene test was used to test for homogeneity of variance and where necessary an appropriate transformation was performed before further analysis. Comparisons of means following ANOVA was done using the Tukey-HSD test. Correlation analysis was used to measure the intensity of association of dissolved oxygen levels and lactate changes. All analyses were performed on the SPSS statistical package with the α set at 0.05. All means are expressed as mean±SE.

6.3 RESULTS

Survival was 100% in all treatments except for the 10-20% oxygen saturation. At 10-20% saturation no animals survived for more than 12 hours, so only results up to the 8 hour time period were obtained. There was no significant difference ($P>0.05$) between the control lobsters tested at 0, 8 and 24 hours. Therefore, the data were pooled for analyses. Also, there was no significant difference ($P<0.05$) between the treatment data at the 0 hour measuring period so the data were pooled.

In most treatments M_{O_2} was significantly higher ($P<0.05$) than resting M_{O_2} after re-immersion, with the M_{O_2} reducing slowly over time (Fig. 6.1). The M_{O_2} of lobsters recovered in 90-100% and 110-120% oxygen saturated water was not significantly different ($P>0.05$) to the control M_{O_2} after 8 hours re-immersion (Fig 6.1). The M_{O_2} of lobsters in the 70-80% treatment was not significantly different ($P>0.05$) to the controls after 24 hours. It was significantly higher ($P<0.05$) than the control M_{O_2} after 8 hours, however it was not significantly different ($P>0.05$) to lobsters in the 90-100% or 110-120% treatments (Table 6.1). The M_{O_2} of lobsters in the 50-60% treatment remained high ($P<0.05$) after 8 hours re-immersion, but was not significantly different ($P>0.05$) to the controls after 24 hours. Oxygen consumption of lobsters in 30-40% oxygen also remained high and was still significantly higher ($P<0.05$) than the control M_{O_2} after 24 hours re-immersion. The lobsters in this treatment maintained a constant state of M_{O_2} (at around 0.070 mg O_2/g/h) during the first 8 hours of recovery. Lobsters in the 10-20% oxygen treatment had significantly lower M_{O_2} ($P<0.05$) than the control M_{O_2} at each measurement period.

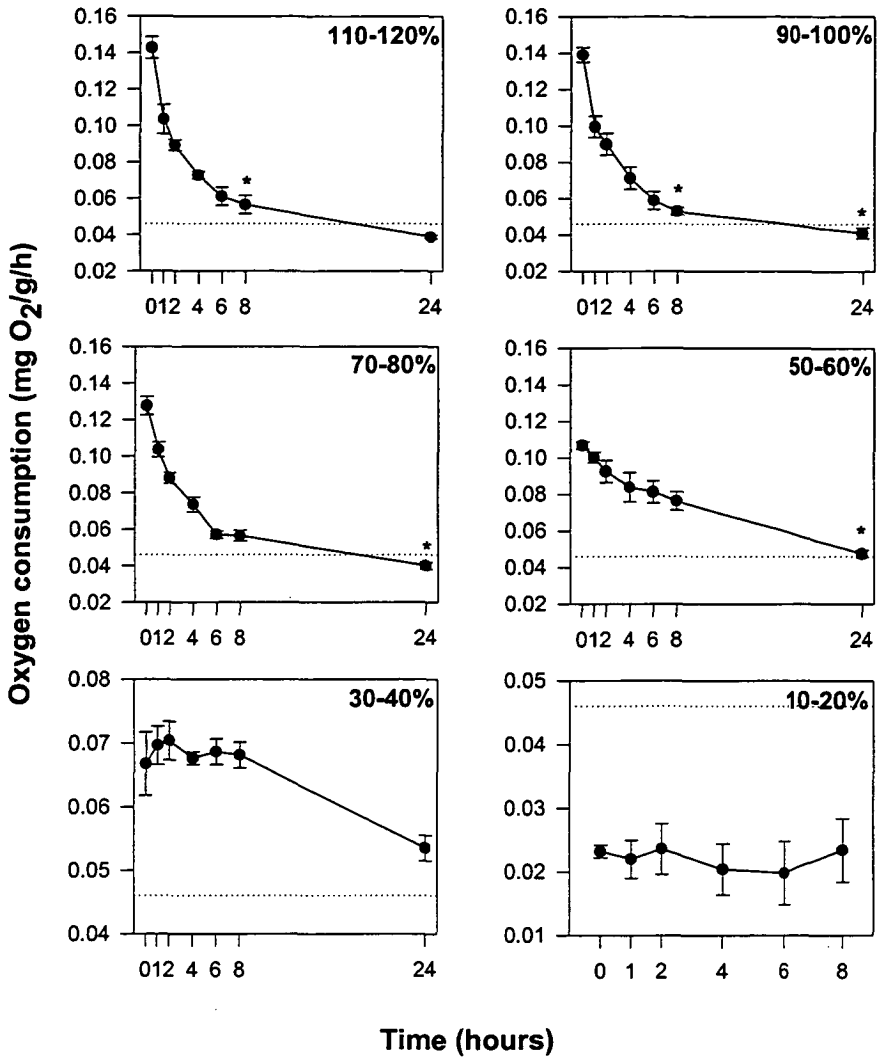

Figure 6.1: Oxygen consumption (mg O_2/g/h)(mean±SE) of the western rock lobster, *Panulirus cygnus*, over a 24-hour period during "recovery" from disturbance (n=6-12). The lobsters were disturbed (emersed and handled) for 30 minutes prior to re-immersion in water containing different concentrations of dissolved oxygen. The dissolved oxygen levels are shown in the upper right-hand corner of each graph. The dotted lines show the standard oxygen consumption of the lobsters (n=10). Values which are not significantly different to the standard oxygen consumption are indicated by an asterisk (*). The lines are drawn for ease of viewing.

Upon re-immersion, lobsters in the 110-120% and 90-100% oxygen saturation treatments, had significantly higher M_{O_2} ($P<0.05$) than lobsters in all other treatments (Table 6.1). At lower oxygen saturation M_{O_2} decreased significantly ($P<0.05$) with decreases in the dissolved oxygen level. Large

decreases in the M_{O_2} of lobsters in the 110-120%, 90-100% and 70-80% treatments during the first hour meant that their M_{O_2} was not significantly different to the 50-60% treatment after the first hour. After 6 hours recovery lobsters in the 50-60% treatment had a significantly higher M_{O_2} ($P<0.05$) than lobsters in the higher oxygen saturation treatments. After 24 hours recovery lobsters in 50-60% and 30-40% oxygen saturation had significantly higher M_{O_2} ($P<0.05$) than all other treatments. Lobsters in the 10-20% oxygen saturation had significantly lower M_{O_2} ($P<0.05$) than all other treatments at each time period.

Oxygen saturation (%)	Recovery time (hours)						
	0	1	2	4	6	8	24
110-120	a	a	a	ab	b	bc	b
90-100	a	a	a	ab	b	c	b
70-80	b	a	a	ab	b	bc	b
50-60	c	a	a	a	a	a	a
30-40	d	b	b	b	ab	ab	a
10-20	e	c	c	c	c	d	N/A

Table 6.1: The results of the ANOVAs comparing the oxygen consumption of the lobsters in each oxygen saturation treatment at a given measurement time during the 24 hour recovery period.
N/A - the lobsters in this treatment did not survive for 24 hours.

Lobsters recovered in water containing 70-80% dissolved oxygen or higher consumed the least amount of oxygen during the recovery period (Table 6.2). In comparison, lobsters in the 50-60% treatment consumed 1.3 times as much oxygen during the initial 8 hour recovery period and approximately twice as much oxygen in achieving full recovery (see Note b in Table 6.2). Lobsters in the 30-40% treatment consumed 0.75 times as much oxygen during the initial 8 hour recovery period, but had consumed 1.6 times as much oxygen after 24 hours even though they had still not achieved full recovery.

Time	Dissolved oxygen saturation (%)					
	10-20	30-40	50-60	70-80	90-100	110-120
8 hours	N/A	0.184	0.334	0.251[c]	0.253	0.266
24 hours	N/A	0.425[a]	0.593[b]	N/A	N/A	N/A

Table 6.2: The total amount of oxygen consumed (mg O_2/g) above standard oxygen consumption during the recovery period.
N/A not applicable to this time period at that particular dissolved oxygen saturation

[a] Oxygen consumption was still significantly higher than standard after 24 hours so the total oxygen consumed during recovery would be slightly higher than this value.

[b] Oxygen consumption may have returned to standard prior to the 24 hour period so this value may be an overestimation.

[c] Although the oxygen consumption was still significantly higher than standard after 8 hours it was not significantly different to either the 110-120% or 90-100% rate. Therefore, the total level of M_{O_2} was only calculated up to the 8 hour mark.

Lobster haemolymph pH decreased significantly ($P<0.05$) during the 30 minute disturbance period, from control levels of 8.36±0.01 to 7.66±0.03. The changes noted in the first hour of re-immersion showed two distinct patterns: (a) at oxygen levels of 70-80% and higher the pH remained at the low level measured after the emersion period, whilst (b) at lower oxygen levels the pH increased markedly (Fig. 6.2, Table 6.3).

The pH of lobsters in 90-100% oxygen saturation increased rapidly after the initial hour and was not significantly different to the controls after 4 hours. The pH remained at that level for the remainder of the experiment. Lobsters in 110-120% oxygen saturation showed a similar response, however there was a slight overshoot (not significant) of the pH after 4 hours. The pH then reduced until it was significantly lower ($P<0.05$) than the control and all other treatments after 24 hours. The pH of the 70-80% treatment recovered more slowly than either the 90-100% or 110-120% oxygen saturation so that after 4 hours the pH was still significantly lower ($P<0.05$) than normal pH. After 8 hours recovery the pH had increased to a level which was significantly higher ($P<0.05$) than the controls, however by 24 hours the pH had returned to the controls ($P>0.05$).

The pH of lobsters in the 50-60% and 30-40% treatments increased rapidly after re-immersion. The pH overshot the control levels in both treatments (50-60% - by 4 hours; 30-40% - by 8 hours). After 24 hours re-immersion, the pH of both

treatments was not significantly different (P>0.05) to normal, although the pH of the 30-40% treatment remained high.

The pH of lobsters in the 10-20% treatment also increased rapidly during the first hour of re-immersion. After 4 hours re-immersion it was still significantly lower (P<0.05) than the control, however after 8 hours it was significantly higher (P<0.05).

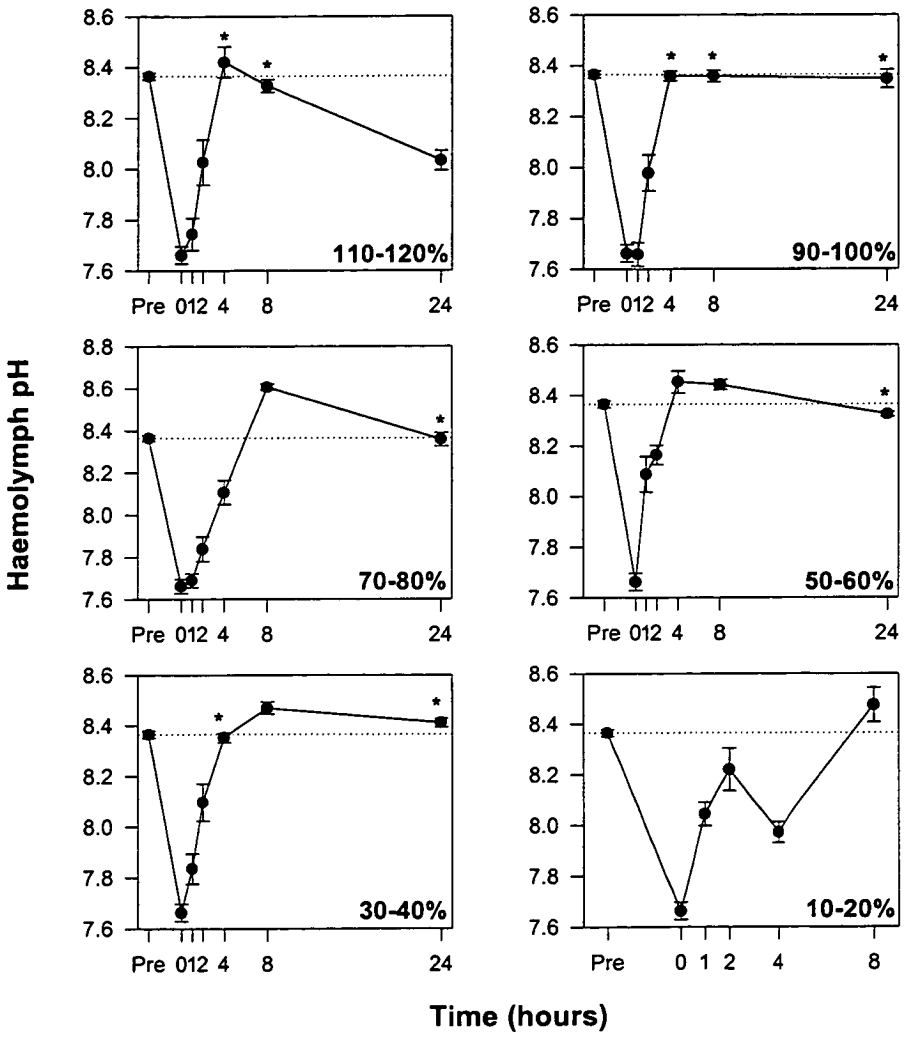

Figure 6.2: Haemolymph pH (mean±SE) of the western rock lobster, *Panulirus cygnus*, over a 24-hour period during "recovery" from disturbance (n=6-12). The lobsters were disturbed (emersed and handled) for 30 minutes prior to re-immersion in water containing different concentrations of dissolved oxygen. The dissolved oxygen levels are shown in the lower right-hand corner of each graph. The dotted lines show the pre-disturbance haemolymph pH of the lobsters (n=12). Values which are not significantly different to the pre-disturbance concentration are indicated by an asterisk (*). The lines are drawn for ease of viewing.

Oxygen saturation (%)	Recovery time (hours)					
	0	1	2	4	8	24
110-120	a	bc	abc	a	c	b
90-100	a	c	bc	a	c	a
70-80	a	bc	c	b	a	a
50-60	a	a	ab	a	b	a
30-40	a	b	ab	a	b	a
10-20	a	a	a	b	b	N/A

Table 6.3: The results of the ANOVAs comparing the haemolymph pH of the lobsters in each oxygen saturation treatment at a given measurement time during the 24 hour recovery period.
N/A - the lobsters in this treatment did not survive for 24 hours.

Haemolymph ammonia increased significantly ($P<0.05$) during the 30 minute disturbance period, from control levels of 4.53±0.45 mg/l to 5.85±0.26 mg/l. Ammonia concentrations decreased after re-immersion and were either not significantly different ($P>0.05$) to, or were significantly lower ($P<0.05$) than the controls after 1 hour. The concentration in most treatments remained similar to control levels during the remainder of the re-immersion period. However, after 8 hours re-immersion the ammonia concentration of lobsters in the 50-60%, 90-100% and 110-120% treatments was significantly higher than the controls and all other treatments (Fig. 6.3, Table 6.4).

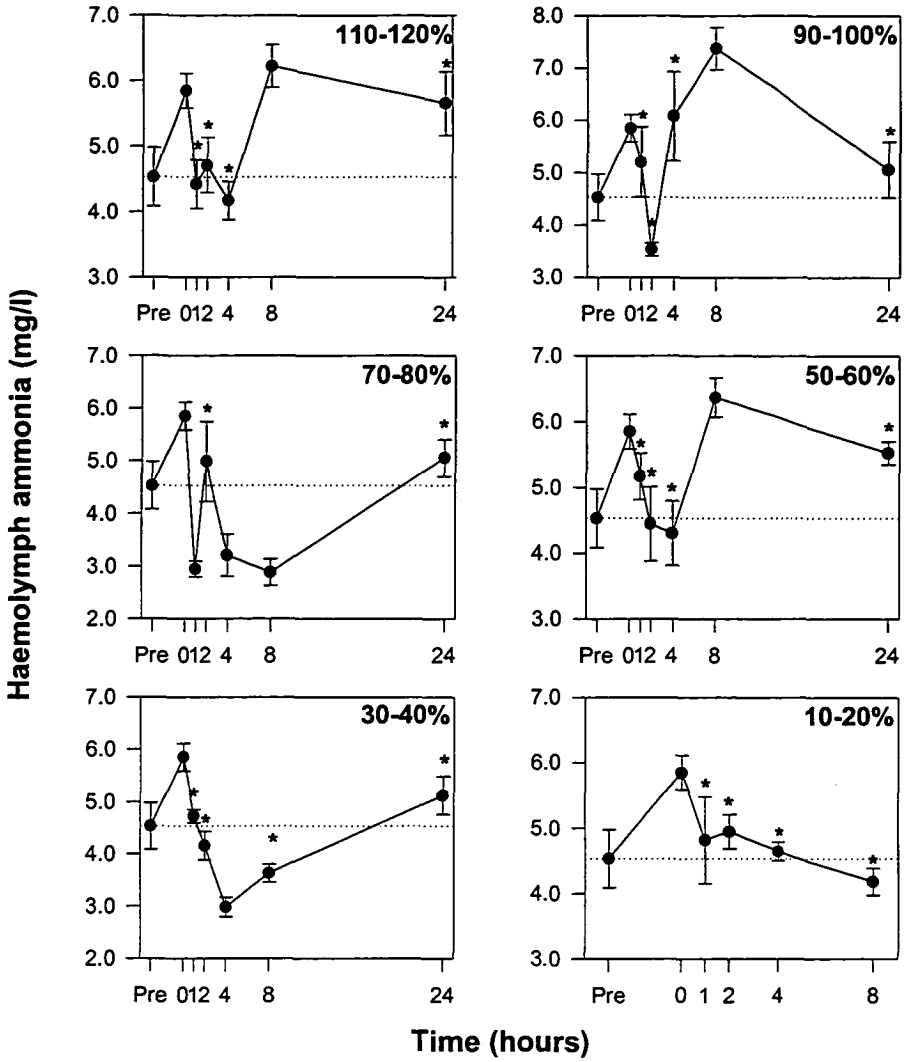

Figure 6.3: Haemolymph ammonia concentration (mg/l)(mean±SE) of the western rock lobster, *Panulirus cygnus*, over a 24-hour period during "recovery" from disturbance (n=6-12). The lobsters were disturbed (emersed and handled) for 30 minutes prior to re-immersion in water containing different concentrations of dissolved oxygen. The dissolved oxygen levels are shown in the upper right-hand corner of each graph. The dotted lines show the pre-disturbance haemolymph ammonia concentration of the lobsters (n=12). Values which are not significantly different to the pre-disturbance concentration are indicated by an asterisk (*). The lines are drawn for ease of viewing.

Oxygen saturation (%)	Recovery time (hours)					
	0	1	2	4	8	24
110-120	a	a	a	bc	b	a
90-100	a	a	a	a	a	a
70-80	a	b	a	c	d	a
50-60	a	a	a	bc	b	a
30-40	a	a	a	c	cd	a
10-20	a	a	a	ab	c	N/A

Table 6.4: The results of the ANOVAs comparing the haemolymph ammonia concentration (mg/l) of the lobsters in each oxygen saturation treatment at each measurement time during the 24 hour recovery period.
N/A - the lobsters in this treatment did not survive for 24 hours.

Haemolymph lactate increased significantly ($P<0.05$) during the 30 minute disturbance period (Fig. 6.4), from a resting level of 0.05±0.02 mmol/l to 2.44±0.37 mmol/l. The lactate concentration increased further during the first hour of re-immersion with the largest increases occurring in at the lower dissolved oxygen saturations (Table 6.5; Fig. 6.5). In the 110-120%, 90-100%, and 70-80% treatments the lactate concentration then decreased until it was not significantly different to the control after 8 hours re-immersion. In the 50-60% treatment the lactate concentration was significantly higher ($P<0.05$) than the control after 8 hours, however it was not significantly different ($P>0.05$) to any of the above treatments (Table 6.5). Lobsters re-immersed into 30-40% and 10-20% oxygen saturation had very large increases in lactate during the first hour. After 2 hours re-immersion they had significantly higher lactate ($P<0.05$) than all other treatments. The lactate concentration of lobsters in the 30-40% treatment decreased slowly but it remained significantly higher ($P<0.05$) than the control after 24 hours. The lactate concentration of lobsters in the 10-20% treatment remained high during the 8 hours of measurements and was significantly higher ($P<0.05$) than all other treatments after 4 hours.

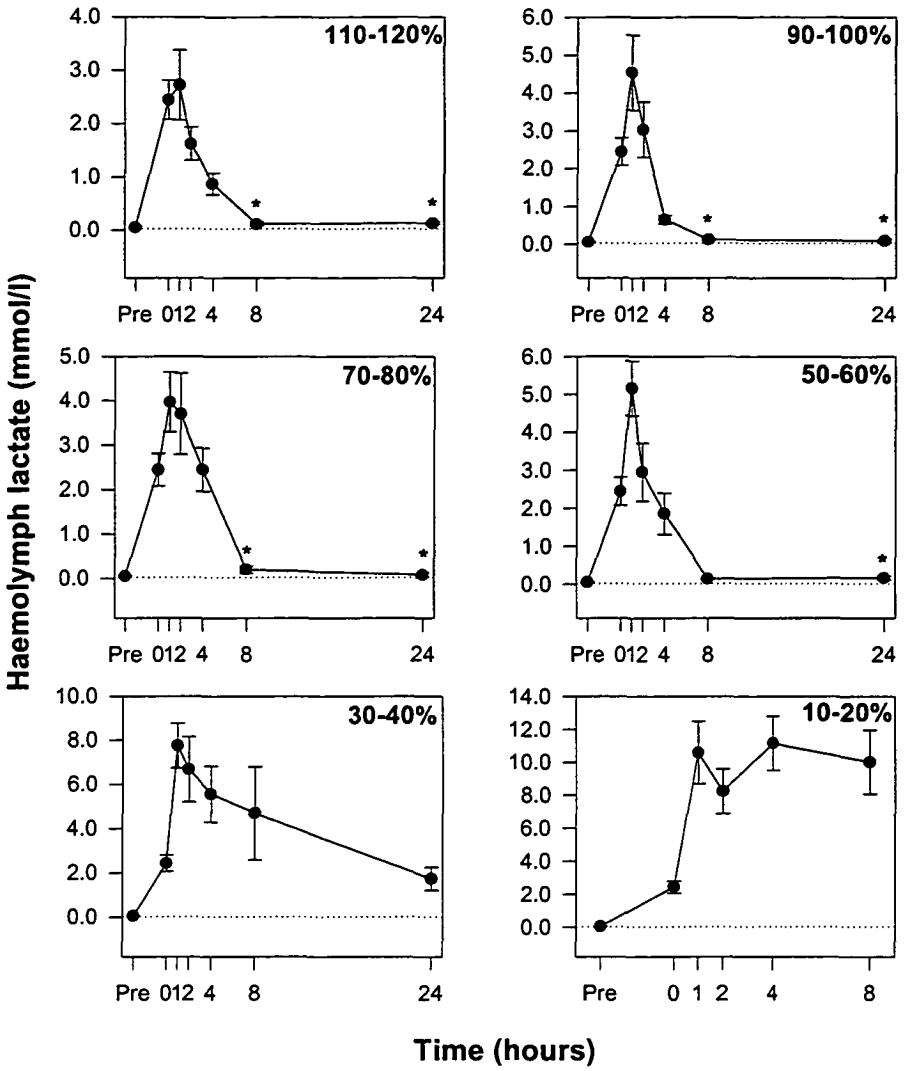

Figure 6.4: Haemolymph lactate concentration (mmol/l)(mean±SE) of the western rock lobster, *Panulirus cygnus*, over a 24-hour period during "recovery" from disturbance (n=6-12). The lobsters were disturbed (emersed and handled) for 30 minutes prior to re-immersion in water containing different concentrations of dissolved oxygen. The dissolved oxygen levels are shown in the upper right-hand corner of each graph. The dotted lines show the pre-disturbance haemolymph lactate concentration of the lobsters (n=12). Values which are not significantly different to pre-disturbance concentration are indicated by an asterisk (*). The lines are drawn for ease of viewing.

Oxygen saturation (%)	Recovery time (hours)					
	0	1	2	4	8	24
110-120	a	c	b	d	c	b
90-100	a	c	b	d	c	b
70-80	a	c	b	c	c	b
50-60	a	bc	b	cd	c	b
30-40	a	ab	a	b	b	a
10-20	a	a	a	a	a	N/A

Table 6.5: The results of the ANOVAs comparing the haemolymph lactate concentration (mmol/l) of the lobsters in each oxygen saturation treatment at a given measurement time during the 24 hour recovery period.
N/A - the lobsters in this treatment did not survive for 24 hours.

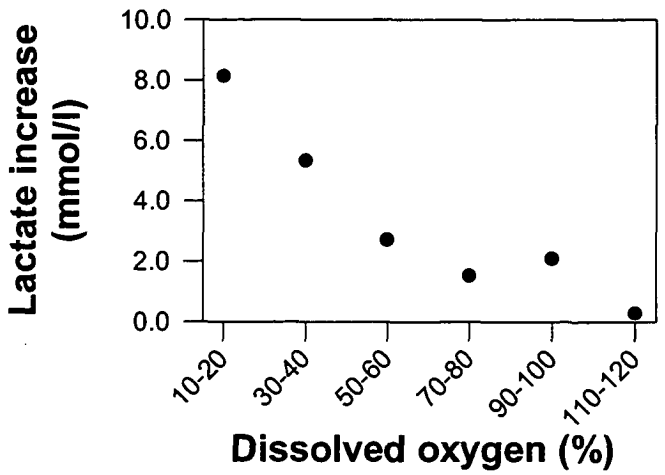

Figure 6.5: Increase in haemolymph lactate (mmol/l)(●) during the first hour of recovery after re-immersion of lobsters, *P. cygnus*, in water at the different dissolved oxygen levels.

The increase in haemolymph lactate during the first hour after re-immersion was correlated (P=0.005) with the water oxygen level (Fig. 6.5). However, the rate of lactate removal between the first and fourth hours of re-immersion showed no correlation (P=0.28) with oxygen level (Table 6.6).

Oxygen level (%)	10-20	30-40	50-60	70-80	90-100	110-120
Recovery (mmol/l/h)	-0.19	0.73	1.10	0.51	1.30	0.62

Table 6.6: The rate of recovery (mmol/l/h) of *P. cygnus* from the first to the fourth hour of re-immersion at the different oxygen levels.

Haemolymph glucose increased during the 30 minutes disturbance period from 0.35±0.06 mmol/l to 0.44±0.06 mmol/l, but it was not a significant increase ($P>0.05$) (Fig. 6.6). After 1 hours re-immersion the glucose concentration was significantly higher ($P<0.05$) than the controls in all treatments. The largest increases were measured in the low oxygen saturation treatments (Table 6.7). In general, the high glucose concentrations were maintained for between 2 and 4 hours before they decreased; most were not significantly different ($P>0.05$) to the controls after 8 hours re-immersion. Lobsters in the 110-120% and 50-60% treatments still had significantly higher concentrations ($P<0.05$) than the controls after 8 hours re-immersion, however the concentrations were not significantly different to those in all other treatments except for the 10-20% treatment.

The glucose concentrations of lobsters in the 10-20% treatment was significantly higher ($P<0.05$) than in all other treatments after 4 hours re-immersion (Table 6.6). After 8 hours re-immersion the concentration had decreased until it was significantly lower ($P<0.05$) than all other treatments except for the 30-40% treatment.

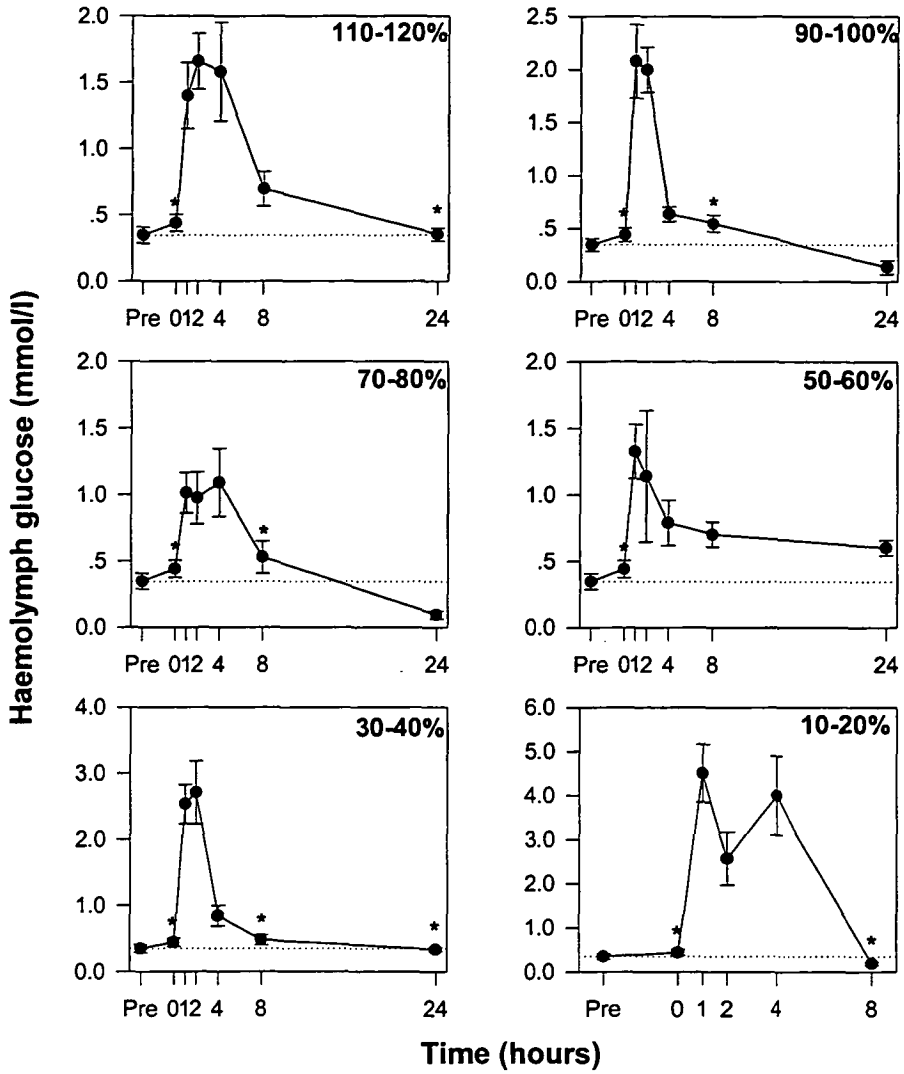

Figure 6.6: Haemolymph glucose concentrations (mmol/l)(mean±SE) of the western rock lobster, *Panulirus cygnus*, over a 24-hour period during "recovery" from disturbance (n=6-12). The lobsters were disturbed (emersed and handled) for 30 minutes prior to re-immersion in water containing different concentrations of dissolved oxygen. The dissolved oxygen levels are shown in the upper right-hand corner of each graph. The dotted lines show the pre-disturbance glucose concentration of the lobsters (n=12). Values which are not significantly different to the pre-disturbance concentration are indicated by an asterisk (*). The lines are drawn for ease of viewing.

Oxygen saturation (%)	Recovery time (hours)					
	0	1	2	4	8	24
110-120	a	cd	bcd	b	a	b
90-100	a	bc	abc	b	a	c
70-80	a	d	d	b	a	c
50-60	a	cd	cd	b	a	a
30-40	a	b	a	b	ab	b
10-20	a	a	ab	a	b	N/A

Table 6.7: The results of the ANOVAs comparing the haemolymph glucose concentration (mmol/l) of the lobsters in each oxygen saturation treatment at a given measurement time during the 24 hour recovery period.

$^{N/A}$ - the lobsters in this treatment did not survive for 24 hours.

6.4 DISCUSSION

Disturbance induced a decrease in the haemolymph pH, and increases in the haemolymph lactate, glucose and ammonia concentrations of *P. cygnus*. Similar responses have been observed in other crustaceans (Vermeer, 1987; deFur *et al.*, 1988; Santos and Keller, 1993; Zou *et al.*, 1996). Recovery from these physiological disturbances, although essentially showing similar patterns to other species (McDonald *et al.*, 1979; Waldron, 1991), was influenced by the dissolved oxygen level of the re-immersion water.

The aerobic response of *P. cygnus* during recovery follows a typical Type V pattern. That is, the oxygen consumed during recovery exceeds the predicted aerobic oxygen deficit (Herreid, 1980). Such a pattern would be noted if the animals showed increased stress or physical activity in hypoxia (Herreid, 1980), as has occurred in this study. If it is assumed that the emersed lobsters can take up approximately 50% of the oxygen that they are able to take up in water (Whiteley and Taylor, 1990; Waldron, 1991) and that they were fully active over the 30 minute period of disturbance, then the maximum oxygen deficit would be approximately 0.06 mg O_2/g. In fact, this is less than 1/4 of the oxygen debt incurred at high dissolved oxygen levels.

Increases in M_{O_2} after re-immersion are achieved by a rapid increase in branchial water flow and cardiac output, increases in both the O_2 diffusion gradient and the rate of diffusion of O_2 across the gill epithelium, and by greater

participation of haemocyanin in oxygen delivery (McMahon *et al.*, 1979; Booth *et al.*, 1982; Waldron, 1991). Suggested uses for the excess oxygen include: (1) metabolising anaerobic end products; (2) re-establishing resting oxygen levels in body tissues; (3) replenishing high energy phosphate reserves; and (4) meeting energy costs associated with increased branchial chamber ventilation and haemolymph circulation (Herreid, 1980; Head and Baldwin, 1986). In *Jasus edwardsii* the calculated lactate portion of the oxygen debt was only 5-20% of the total debt (Waldron, 1991). However, that calculation was based on the haemolymph lactate concentration, and does not take into account the possibility that intracellular lactate concentrations may be many times higher than the haemolymph concentration, as seen in other crustaceans (Phillips *et al.*, 1978; Greenaway *et al.*, 1992). Re-calculation based on a predicted whole body lactate concentration means the lactate portion may constitute 50% of the total oxygen debt (Waldron, 1991). Hill *et al.* (1991*a*) also showed that only part of the increased oxygen uptake associated with recovery from anoxia is concerned with the removal of lactate. In the freshwater crayfish, *Cherax destructor*, half of the total oxygen debt was required for replenishing ATP and arginine phosphate reserves (alactic debt) in the tail muscle (Head and Baldwin, 1986). The energy reserves in the shrimp, *Crangon crangon,* and the crayfish, *C. destructor*, were severely depleted after short periods of exercise (Onnen and Zebe, 1983; Head and Baldwin, 1986). The level of exercise induced in the present study suggests that a large amount of the increased oxygen debt would be used in the repayment of the accumulated alactic debt as the energy reserves would be severely depleted.

The recovery period at high dissolved oxygen levels was similar to that observed for other crustaceans (McMahon *et al.*, 1979; Waldron, 1991). However, the recovery period increased as the oxygen level decreased, indicating that the lobsters were either (a) not accessing sufficient oxygen to repay the debt as quickly (as discussed previously) or (b) were increasing the size of the debt due to the processes involved in repaying the debt or both. Thus:

(a) The ability of lobsters to uptake oxygen upon re-immersion was dependent on the dissolved oxygen level. The critical oxygen tension (P_c) for active *P. cygnus* was calculated to be 62.8% saturation (Chapter 4). Calculation

of P_c from the data in this study gives a value of 63.1% saturation, which is close to the above value. The aerobic scope for activity of *P. cygnus* increased as oxygen levels increased up to P_c (Chapter 4). In this study, the M_{O_2} increased with increases of dissolved oxygen above P_c which means the aerobic scope for activity would also continue to increase. The amount of oxygen available to the lobsters above normal maintenance requirements (ie. aerobic scope for activity), increases as the dissolved oxygen level in the water increases. Lobsters with a large aerobic scope for activity should be able to increase the speed of repayment of oxygen debts when they do occur. Lobsters in the 30-40% treatment were very limited in the amount of oxygen they were able to extract from the water (≈50% of maximum M_{O_2}); total oxygen usage over the first 8 hours of re-immersion is ≈25% lower than in the higher oxygen treatments. Therefore, access to oxygen is a major problem with these lobsters and would explain (at least partly) the slow recovery rates. Similarly, in response to an injection of lactate *C. maenas* increased M_{O_2}, but the response was smaller, and lasted longer, under hypoxic conditions compared with normoxic conditions (De Wachter *et al.*, 1997). The authors suggested the response was due to the larger aerobic scope at the higher oxygen level. In crustaceans, it appears that active oxygen consumption is limited by the delivery and diffusion systems (McMahon and Wilkens, 1983), especially as the oxygen saturation level decreases (Rutledge, 1981). The results here also indicate that the movement of oxygen from the water to the haemolymph was diffusion limited and that an increased diffusion gradient allowed an increase in oxygen uptake.

(b) The total amount of oxygen consumed in the 50-60% treatment during the first 8 hours of re-immersion was some 30% higher than the treatments with higher oxygen levels. Although the oxygen consumed was high they still had not paid off the oxygen debt, which indicates that there were increased energetic costs associated with recovery at that oxygen level. Thus, the energetic costs of obtaining oxygen also appear to play a part in extending the time period of recovery.

The question arises as to why the total oxygen debt is greater at lower dissolved oxygen levels. The increased oxygen debt could be due to one or a combination of reasons. These include: (a) increased cost of branchial chamber ventilation and haemolymph circulation; (b) increased activity of the lobsters; (c) increased reliance on anaerobic respiration, together with production of lactate and associated energetic costs of resynthesising the substrate:

(a) Branchial chamber ventilation costs 30% of total M_{O_2} in resting crabs, *C. maenas* and it is expected that the cost would increase with activity (Wilkens *et al.*, 1984). The diffusion of oxygen from the external medium to the haemolymph via an oxygen gradient would be minimal at the lower oxygen levels. To optimise the uptake of oxygen, lobsters would need to continuously renew the branchial chamber water. In *J. edwardsii* the ventilation frequency increased to near maximum levels in unstressed lobsters when the oxygen level was around 50% saturation (Waldron, 1991). As *P. cygnus* continues high rates of oxygen consumption for extended periods during recovery at the lower oxygen levels then the energetic cost of obtaining that oxygen could be high. Energy used for branchial chamber ventilation would limit the aerobic capability of repaying the oxygen debt.

(b) Lobsters re-immersed in low oxygen water may expend energy as they try to find more oxygenated areas. Newman and Pollock (1971) noted that *J. lalandii* actively try to avoid water with low oxygen levels. However, no increased activity was noticed in this study when lobsters were re-immersed in water containing low oxygen levels.

(c) The energetic costs of gluconeogenesis, if it is occurring (see discussion below), would further add to the oxygen cost of recovery. If glucose is resynthesised from lactate via a procedure such as the Cori cycle then approximately 1/6th of the energy able to be produced from glucose would be used during the process (Stryer, 1988). With 18 or 19 times as much

substrate being used to produce the same amount of energy anaerobically as aerobically (Schmidt-Nielsen, 1990) the energetic cost of resynthesising the substrate would be significant.

The pH response of crustaceans to the combination of stressors used in this study (emersion, exercise and handling) have not often been investigated. Spanoghe (1997) recorded a similar large change (0.7 pH units) in *P. cygnus* after one hour of emersion and handling. In other studies, crustaceans have usually been subjected to only one of the above stressors but similar haemolymph pH falls have been recorded (McMahon *et al.*, 1979; Booth *et al.*, 1982; Vermeer, 1987; Regnault, 1992; Paterson *et al.*, 1994*a*). A pH change of that magnitude must be considered a large physiological perturbation (Vermeer, 1987). However, *P. cygnus* exposed to air for 6 hours recorded a pH change of close to 1.0 unit with 100% survival upon re-immersion (see Chapter 7). The pH change observed in this study appears to be well within the range of pH disturbances which *P. cygnus* is able to endure without causing severe disruptions to their physiology. The time period taken to return to normal pH values after re-immersion was similar to that measured in other crustaceans (McDonald *et al.*, 1979; Waldron, 1991; Whiteley and Taylor, 1992).

Recovery of acid-base status was complicated by the appearance of high concentrations of lactate in the haemolymph on re-immersion. McDonald *et al.* (1979) suggested that the post-exercise depression (ie. 1 hour post re-immersion) of haemolymph pH was due to increases in lactate, however, the pH in *P. cygnus* only remained low after re-immersion at the higher dissolved oxygen levels. The increase in haemolymph lactate was lowest in these lobsters. The high M_{O_2} of lobsters held at the higher oxygen levels may result in CO_2 levels remaining elevated in the haemolymph, thus helping to maintain a low pH during the initial stages of recovery. Elimination of accumulated CO_2 is usually rapid, presumably due to the high initial concentration gradient across the gills, the relatively high capacity coefficient for CO_2 in water (Dejours, 1981) and the occurrence of hyperventilation (Taylor and Whiteley, 1989). However, haemolymph CO_2 partial pressure remained significantly elevated for 2 hours during re-immersion after a period of emersion and exercise in *J. edwardsii* (Waldron, 1991). After the initial

period of recovery the observed increases in haemolymph pH at the higher oxygen levels are probably due to two reasons. Firstly, there were substantial decreases in M_{O_2}, and hence decreases in the production of CO_2. Secondly, there is probably a continuing rapid elimination of CO_2.

Lobsters at low oxygen levels (50-60%, 30-40%, 10-20%) had large increases in lactate during the first hour of re-immersion, however their haemolymph pH showed significant increases over that time period. Taylor and Wheatly (1981) noted that the potential acidosis which the increase in lactate represents was overridden by a respiratory alkalosis due to the washout of CO_2 during the period of hyperventilation. In *Nephrops norvegicus* the haemolymph pH also increased following re-immersion despite high lactate levels still being present (Schmitt and Uglow, 1997*a*). The authors concluded that CO_2 accumulation was mainly responsible for the emersion-induced acidosis. In this study, oxygen consumption upon re-immersion decreased with the oxygen level, meaning less CO_2 would have been produced. Also, the high ventilation and perfusion activities would promote the excretion of CO_2 across the gills. These two factors combined may have resulted in the large pH increase in lobsters after 1 hour of re-immersion into poorly oxygenated water.

The low pH of lobsters after 24 hours recovery in 110-120% oxygen saturation indicates a respiratory acidosis is occurring. Ventilation rates typically return to pre-stress levels within 24 hours (McMahon *et al.*, 1979; Waldron, 1991). The large oxygen diffusion gradient occurring due to the high environmental dissolved oxygen level would favour a decrease in the rate of ventilation. In all aquatic animals that have been studied, environmental hyperoxia results in a decrease in breathing (Sinha and Dejours, 1980) and leads to new steady states in metabolic CO_2 excretion, with modified internal CO_2 partial pressures (P_{CO_2})(Truchot, 1993). Thus, acid-base disturbances of respiratory origin are induced, resulting in increased P_{CO_2} and decreased pH (hypercapnic acidosis)(Truchot, 1993). However, the effect of hyperoxia on crustacean haemolymph pH have normally been investigated with very high dissolved oxygen levels (400% Dejours and Armand, 1980; 400% Sinha and Dejours, 1980; 400%, Massabuau *et al.*, 1984). The effect of concentrations just above saturation has not been studied, although Dejours and Armand (1980) found that there was a

pH decrease of approximately 0.1 units in *Astacus leptodactylus* when it was subjected to 200% saturation. The results in this study tend to suggest that *P. cygnus* reacts by reducing their ventilatory drive with a concomitant change in acid-base balance. The pH change of 0.3 units was large compared to the pH change in *A. leptodactylus* of 0.13 units (Sinha and Dejours, 1980) or in *Cancer irroratus* of 0.15 units (Wheatly, 1987).

A haemolymph alkalosis was measured during the recovery period in lobsters subjected to water which was not fully oxygen saturated (ie. 70-80% oxygen saturation and lower). Crustaceans generally hyperventilate in response to hypoxia, leading to hypocapnic alkalosis due to an increase in the rate of excretion of CO_2 (Hagerman and Uglow, 1985; Truchot, 1993). The pH of *A. leptodactylus* increased by 0.16 units when they were exposed to oxygen levels of 30% saturation (Sinha and Dejours, 1980). Similar overshoots in pH have been noted in other studies of crustaceans undergoing recovery (Truchot, 1975; Whiteley and Taylor, 1992; Spanoghe, 1997), which indicates that the oxygen levels in the recovery tanks may have been lower than optimal.

Respiratory alkalosis causes an increase in haemocyanin oxygen affinity (Bohr shift)(Morris and Taylor, 1985; Reiber, 1995) which would enhance the uptake of oxygen at the gills. In *Orconectes rusticus* the increase in haemocyanin oxygen affinity due to the Bohr shift allowed 60% post-branchial saturation as opposed to 20% saturation under normoxic conditions (Wilkes and McMahon, 1981*a,b*). Lactate also serves to increase the oxygen affinity of haemocyanin (Booth *et al.*, 1982; Graham *et al.*, 1983; Morris *et al.*, 1986; Greenaway *et al.*, 1992); lactate appears to exert a direct allosteric effect on the oxygen binding site of haemocyanin (Graham *et al.*, 1983). The increase in haemocyanin oxygen affinity plays a major role in increasing the rate of diffusion of oxygen across the gills by removing oxygen from solution, thereby aiding maintenance of M_{O_2} (Reiber, 1995) although it does not appear to function in conserving the oxygen venous reserve at the tissues (Graham *et al.*, 1983). During the early stages of recovery, the acidosis will initially reduce oxygen affinity, but the associated production of lactate will at least partially compensate for such an effect (Morris and Taylor, 1985). In *A. pallipes* increases in oxygen affinity due to Ca^{2+}, lactate and HCO_3^- may have even increased the affinity above that found in resting

crayfish under normoxia (Morris *et al.*, 1986). Thus, oxygen delivery to the tissues during periods of low oxygen availability appear to be optimised due to the physiological changes occurring during the recovery period.

Resting levels of haemolymph lactate (0.05 mmol/l) were very low but are similar to those measured in some other crustacean species (0.14 mmol/l, Waldron, 1991; 0.14 mmol/l, Paterson *et al.*, 1994*a*; 0.09 mmol/l, De Wachter *et al.*, 1997). However, the resting lactate levels of *P. cygnus* in a study by Spanoghe (1997) varied from 0.5 to 2.5 mmol/l. The differences in levels in *P. cygnus* between the two studies may indicate differences in the state of the lobsters in the respective holding systems. The lobsters in this study were at low density in a recirculating system, with individual hides, and were acclimated to the holding system for at least two weeks prior to experiments. The lobsters in Spanoghe's study were generally at high density, usually subject to disturbance during day to day activities at the holding depot (Spanoghe, 1997), in a flow through system, with communal hides, and lobsters were regarded as being rested after 24-72 hours in the system. There have been few reports on the haemolymph lactate levels of crustaceans which have been emersed and exercised. However, in *J. edwardsii* after a short period of exercise followed by 1 hour of emersion the lactate levels increased by $\approx$ 1.0 mmol/l (Waldron, 1991) and in *P. cygnus* the lactate increased by 2 mmol/l after 40 minutes of emersion and disturbance (Spanoghe, 1997); increases which were similar to this study.

Lactate is the main end-product of anaerobic metabolism in decapod crustaceans (Gäde, 1983; Gäde, 1984; Hill *et al.*, 1991*a*). The increase in haemolymph lactate concentration of *P. cygnus* indicates that the species was unable to maintain an adequate supply of oxygen to the tissues during the period of disturbance and needed to rely, at least partially, on anaerobic metabolism to supply its energy requirements (Spicer *et al.*, 1990). The rise in lactate concentration after re-immersion has also been noted in other crustaceans subjected to periods of exercise and/or emersion (McDonald *et al.*, 1979; Taylor and Wheatly, 1981; Whiteley and Taylor, 1992). Increased haemolymph lactate concentrations after re-immersion may be due to the release of lactate previously stored in the tissues during the disturbance period, as suggested by Taylor and Wheatly (1981) and Waldron (1991). In *C. destructor* it appears that a steady state

between tail muscle and haemolymph lactate pools is reached quite rapidly (Head and Baldwin, 1986), hence lactate release may not fully explain the increased levels. Another possible explanation is that lactate production may have increased on re-immersion due to a high energy demand requiring a contribution from both aerobic and anaerobic metabolism (Grieshaber, 1978; Head and Baldwin, 1986; Gruschczyk and Kamp, 1990; Whiteley and Taylor, 1992). Onnen and Zebe (1983) suggested that the use of anaerobic metabolism during the recovery process may ensure that the muscle function is restored as soon as possible. They concluded that aerobic processes could not provide the energy necessary for a rapid restitution of recovering muscles either because the supply of oxygen by the haemolymph is limited or the muscles lack sufficient capacity for aerobic energy production. In this study, the relative increase in the lactate concentration during the first hour was dependent on the dissolved oxygen level in the water. When oxygen could not fully fuel the aerobic portion of the energy requirements of recovery, the shortfall was made up via anaerobic metabolism, with the concomitant increase in haemolymph lactate. This suggests that the observed increase in haemolymph lactate is probably due to the continued use of anaerobic energy sources after re-immersion, rather than the release of sequestered lactate. During recovery in the 110-120% treatment, metabolism appears to be mainly aerobic as shown by the absence of further accumulation of lactate.

The increase in haemolymph lactate levels during the first hour of re-immersion is correlated to the water oxygen level. The rate of lactate elimination over the 3 hours from 1 to 4 hours re-immersion is similar to the rates of removal in some other crustaceans (Taylor and Spicer, 1987; Paterson, 1994*a*; Spanoghe, 1997). The fact that the rate is similar at all oxygen levels (except 10-20%) indicates that the removal of lactate from the haemolymph is not dependent on the oxygen level, and that the timeperiod of removal is controlled by the initial rise in lactate during the first hour of re-immersion. However, the rate of lactate removal in *C. maenas* appeared to be faster under normoxia than under hypoxia (De Wachter *et al.*, 1997). Crustaceans generally lack the ability to rapidly remove lactate (see discussion below). The continued high level of lactate in the 10-20% treatment could be due to a lack of oxygen to help metabolise the lactate or due to the continued production of lactate during the recovery process. As that

concentration of oxygen is well below the P_c value for *P. cygnus* it is highly likely that anaerobic metabolism would be utilised to at least partially fund the metabolic requirements. The scallop, *Chlamys opercularis*, when exposed to air after a period of exercise (ie. anaerobic recovery), also maintained a high octopine (the anaerobic end-product) level (Grieshaber, 1978). However, when it was recovered in oxygen saturated seawater the octopine concentration initially increased and then quickly decreased; a similar response to lactate in *P. cygnus* when it was recovered in oxygen saturated water.

There appears to be considerable interspecific differences in the processes used by crustaceans to remove lactate. For example, Hervant *et al.* (1995) outlined the differences in the ability of two amphipods to excrete and resynthesis the lactate during hypoxia and recovery. Removal of the lactate during recovery is achieved by one or a combination of three processes - (a) excretion of lactate into the external medium (b) oxidisation of lactate for energy once normoxic conditions return, or (c) conversion of lactate back into storage products such as glycogen (gluconeogenesis) at the cost of increased oxygen utilisation once the aerobic state is restored (Herreid, 1980). In general decapods do not appear to excrete lactate (Phillips *et al.*, 1977; Bridges and Brand, 1980*b*; Hill *et al.*, 1991b), however some studies have found evidence of lactate excretion (Zebe, 1982; Head and Baldwin, 1986; Hervant *et al.*, 1995). Oxidation of the lactate and/or gluconeogenesis appear to be the main processes involved in lactate removal in crustaceans (Phillips *et al.*, 1977; Gäde *et al.*, 1986; Hill *et al.*, 1991*b*; Hervant *et al.*, 1995).

The slow rate of lactate removal from the haemolymph indicates that *P. cygnus*, like other crustaceans, lack the means for rapid removal of lactate (McDonald *et al.*, 1979; Booth *et al.*, 1982; Ellington, 1983; Albert and Ellington, 1985; Lowery and Tate, 1986). The rate of clearance has been correlated with the oxygen characteristics of the environment exploited by the species; those species which are more likely to encounter hypoxia in their natural environment are physiologically better adapted for rapidly removing the accumulated lactate when aerobic conditions return (Bridges and Brand, 1980*b*). As spiny lobsters live subtidally, generally in well oxygenated environments, throughout their life cycle

there has been no selective pressure to evolve behavioural, anatomical, or physiological adaptations to aerial exposure (Vermeer, 1987) or hypoxia.

In crustaceans, the timecourse for the elimination of haemolymph lactate is generally much slower than the recovery time for M_{O_2} (McDonald *et al.*, 1979; Booth *et al.*, 1982). In this study both haemolymph lactate and M_{O_2} returned to normal levels after 8 hours at the higher dissolved oxygen levels. At 50-60% oxygen saturation lactate returned to resting levels quicker than M_{O_2}, whereas at 30-40% saturation neither lactate nor M_{O_2} returned to resting levels after 24 hours. Lactate elimination and increased oxygen consumption appear to be more tightly coupled in this species than in other crustacean species studied.

The haemolymph ammonia concentration of *P. cygnus* increased over the disturbance period, as has been noted in several other crustacean species (Waldron, 1991; Regnault, 1994; Schmitt and Uglow, 1997*a*). The ammonia concentration in the control lobsters (4.53 mg/l) was similar to that measured in other decapod crustaceans (7.2 mg/l, Vermeer, 1987; 5.7 mg/l, Young-Lai *et al.*, 1991; 4.0 mg/l, Regnault, 1994; 5.4 mg/l, Spanoghe, 1997; 2.0-3.0 mg/l, Schmitt and Uglow, 1997*b*). Ammonia is released to the external environment through the gills by diffusional movement and Na^+/NH_4^+ exchange across the epithelium (Kormanik and Cameron, 1981; Regnault, 1987). In the absence of water, such mechanisms may be greatly impaired, and the accumulation of ammonia in the haemolymph may occur (Schmitt and Uglow, 1997*a*). As observed in this study, ammonia clearance from the haemolymph occurs very rapidly upon re-immersion (Regnault, 1994). In *P. cygnus* this is also indicated by the rapid decrease in the rate of excretion of ammonia into the external water after a period of emersion (Chapter 5). The rapid decrease is probably a reflection of the higher ventilatory and circulatory activities which would maintain a large gradient across the gills for ammonia excretion (Waldron, 1991). The reason for the increase in ammonia concentration in several treatments after around 8 hours of recovery is unclear.

Haemolymph glucose concentrations of control lobsters are similar to those measured in other studies; 0.4-0.5 mmol/l for *Homarus americanus* (Telford 1968); 0.2-0.3 mmol/l for *Nephrops norvegicus* (Spicer *et al.*, 1990; Schmitt and Uglow, 1997*a*); 0.4-0.5 mmol/l for *Carcinus maenas* (Santos and Keller, 1993); 0.2-0.4 mmol/l for *P. cygnus* (Tod and Spanoghe, 1997). The maximum levels of

haemolymph glucose measured in this study (1.0 to 4.5 mmol/l) also covered the range of maximum levels measured by those researchers. As has been noted in other studies (Onnen and Zebe, 1983; Gruschczyk and Kamp, 1990; Tod and Spanoghe, 1997), there was a marked hyperglycaemia in the haemolymph of *P. cygnus* one hour after re-immersion. In this study, the hyperglycaemia was more severe in lobsters subjected to a low concentrations of dissolved oxygen, suggesting that more energy substrate was required because the lobsters were performing increased levels of anaerobic metabolism. Glucose may appear due to the mobilisation of energy stores as a source of fuel for anaerobic metabolism (Spicer *et al.*, 1990). When aerobic mechanisms of energy production are impaired, in order to provide a given amount of energy, more glucose must undergo anaerobic glycolysis (Storey and Storey, 1990), as anaerobic glycolysis produces only about 1/20th of the energy produced via aerobic glycolysis (Eckert *et al.*, 1988). During recovery in this study, anaerobic glycolysis (as indicated by lactate concentration), increases as oxygen saturation decreases. Therefore, the observed increases in glucose concentration would be expected.

The time period of recovery from hyperglycaemia has not been well studied, but it was similar to that for *P. cygnus* in Spanoghe's (1997) study, and to that recorded for *Palaemon serratus* and *P. elegans* after a period of emersion (Taylor and Spicer, 1987). However, in the shrimp *C. crangon*, haemolymph glucose levels had not returned to pre-exercise levels after 10 hours of recovery (Onnen and Zebe, 1983). Also, in the freshwater crab, *Eriocheir sinensis*, glucose concentrations had not returned to pre-anoxic conditions after 12 hours of normoxic exposure (Zou *et al.*, 1996).

The haemolymph glucose concentration in the 10-20% treatment remained high after 4 hours re-immersion, and then decreased rapidly so that after 8 hours re-immersion the concentration 0.19±0.04 mmol/l. Due to the high lactate concentration it would appear that the lobsters energy requirements were still very high at that time, as they were providing much of their energy anaerobically. The sudden decrease in glucose levels suggests that the lobsters were running out of energy supplies to fund their requirements. Death of the lobsters occurred after approximately 12 hours re-immersion. In *Callianassa californiensis* marked hyperglycaemia through the first 13 hours of anoxic exposure was followed by a

slow decrease in haemolymph glucose coinciding with the decrease in glycogen reserves (Hawkins, 1970 in Taylor and Spicer, 1987). Under completely anoxic conditions, energy production in *P. elegans* can only occur using anaerobic pathways which appear unable to meet the energy demands of the prawns for long periods, so that survival under those conditions was limited (Taylor and Spicer, 1987). The haemolymph glucose concentration of *Liocarcinus puber* was very low after 24 hours emersion and almost 80% of the animals had died by that time (Johnson and Uglow, 1985). Therefore, death of the lobsters in this study may have been due to the loss of energy substrate. The reason for the low haemolymph glucose concentrations in the 70-80% and 90-100% oxygen saturation treatments after 24 hours re-immersion is unclear.

Conclusion: the duration and the effectiveness of the recovery process are of great functional importance. Recovery from anaerobic metabolism should be sufficiently rapid and complete for the organism to cope with further periods of stress. In the case of muscles powering escape responses, this process of recovery must be sufficient to allow the organism to evade predators (Ellington, 1983). Using speed of recovery as the criteria for evaluating the effectiveness of the oxygen levels, the results indicate that oxygen levels of 90-100% or 110-120% saturation optimise recovery.

Oxygen has a considerable effect on the recovery response of *P. cygnus*. Physiological responses such as respiratory alkalosis appear to occur only when oxygen levels are less than optimal. There is little doubt that in other crustaceans similar effects would occur. However, most authors discuss water as being "oxygenated" or "well aerated", and the actual oxygen saturation level is rarely reported in the literature. This study highlights the importance of accounting for factors such as oxygen saturation if comparisons between species or between studies of the same species are to be valid. The results of this study, in relation to the maintenance of western rock lobsters in holding systems will be discussed in Chapter 9.

CHAPTER 7

Carrying lobsters (*Panulirus cygnus*) out of water - the effect of environmental factors on health of lobsters

7.1 INTRODUCTION

Western rock lobsters (*Panulirus cygnus*) are subjected to post-capture practices which result in emersion. Subtidal crustaceans subjected to emersion suffer internal hypoxia, a mixed respiratory and metabolic acidosis, hyperglycaemia, and an accumulation of metabolic waste products (Telford, 1968; Whiteley and Taylor, 1990; Regnault, 1994). Western rock lobsters are able to handle up to 48 hours emersion under export conditions (Spanoghe, 1997). Emersion, however, must be regarded as a stress which jeopardises the condition and/or life of crustaceans (Whyman *et al.*, 1985). For example, the mortality rate of re-immersed *P. cygnus* increased in proportion to the amount of time they were emersed (Brown and Caputi, 1986). It has been suggested that gill damage caused by dehydration may contribute to the mortality of re-immersed western rock lobsters (Anon, 1980*b*; Spanoghe, 1997). Systems which spray seawater over the lobsters when they are emersed, have evolved as a means of decreasing the effects of emersion on the health of the lobsters.

This study examined the affects of two environmental factors (humidity, wind) on the physical and physiological health of emersed lobsters, as well as determining the benefits of using a seawater spray system to maintain the health of the emersed lobsters. One of the pivotal aims was to examine the role gill damage played in reducing the ability of lobsters to recover from a period of emersion.

7.2 MATERIALS AND METHODS

General Materials and Methods are as outlined in Chapter 2. Lobsters weighing between 367 and 515 grams, of both sexes, were used in this series of experiments.

The condition of the lobsters was assessed on a quantitative measure of lobster response to handling as devised by Tod (1995) for *P. cygnus* (Table 7.1). Twelve lobsters were used for each experiment. To allow enough time for the

sampling and testing procedures to be completed lots of 3 lobsters were selected at one hour intervals. Body markings were used to identify the lobsters through the experimental stages.

The lobsters were weighed to 0.1 g (Mettler - Toledo PB3001) after excess water was removed from the gill chamber, and the animal was dried. The lobsters were held around the carapace in a head down position and moved gently through a downward arc six times (Vermeer, 1987). External water was wiped off with a towel. After weighing they were placed into mesh boxes (20 mm mesh), which were then set up according to each treatment as outlined below:

Treatment 1: Held at 23°C in 40% relative humidity (RH) with dim light measuring 0 $\mu E\ m^{-2}\ sec^{-1}$.

Treatment 2: As in Treatment 1 but with a fan blowing wind into the mesh boxes at the rate of 2-4 km/h.

Treatment 3: Held at 23°C in 92% RH with dim light measuring 0 $\mu E\ m^{-2}\ sec^{-1}$.

Treatment 4: As in Treatment 3 but with a fan blowing wind into the mesh boxes at the rate of 2-4 km/h.

Treatment 5: As in Treatment 3 but with water being sprayed over the lobsters at 30 l/h. A constant stream of water rather than a fine mist was maintained.

Tests on board a lobster carrier boat transporting lobsters from the Abrolhos Islands to Geraldton in Western Australia had shown that a wind speed of the above magnitude was appropriate. The windspeed in exposed sections of the boat was 16 km/hour but due to the nature of the carrying system (wind breaks and sheer density of lobsters) much of that wind did not penetrate to the lobsters. Wind speeds varying between 0 and 6 km/hour were measured within the lobster crates. Wind speed was measured with a Davis "Wind Wizard" Wind Speed Indicator.

The 40% RH treatment was obtained by running the experiment in an air-conditioned room. The 92% RH treatment was obtained by running the experiment in a room containing large quantities of water in aquaria. Both of these systems maintained very constant humidities over the course of the experiment. The RH and light intensity were measured with a LI-COR Steady State Porometer LI 1600

(Vaisala HUMICAP and LI-190s-1 Quantum Sensor, respectively) at the beginning and end of each experimental treatment. The RH at Geraldton during April and May averages 47% with readings as low as 34% (Steve Summers, Geraldton Meteorological Office, pers. comm.,).

Six hours after exposing the lobsters to the treatments the lobsters were sampled for condition, haemolymph, weight (after removing excess water from the branchial chamber and drying in the case of Treatment 5), and oxygen consumption. After measuring oxygen consumption rates the lobsters were re-immersed into oxygen saturated water for 24 hours before sampling again for condition, haemolymph and weight. The oxygen consumption rates of lobsters in Treatment 2 were also tested at that time period. Survival was recorded.

Condition	Lobster behaviour
0	Dead, no discernible response
1	No tail response, but will respond to eye squeeze, bailer moving in gill chamber
2	Weak tail response, tail dislocated and sagging away from thorax, incapable of holding tail in normal position
3	Moderately active lobster, tail response and position normal but lacking capacity for several rapid flicks, legs may or may not be extended, antennal movement generally not strong
4	Active lobster, strong tail flick response with gradual weakening, legs extended, strong antennal movement
5	Extremely active, tail arched back past horizontal line with thorax, antennae swept back, legs extended and stiff, often numerous spontaneous rapid tail flicks with little discernible weakening

Table 7.1: Criteria used for determining the condition of lobsters during the experiment (after Tod, 1995).

Four lobsters were used as controls, and tested immediately for haemolymph parameters. A further six control lobsters were treated as per the experimental lobsters but they were placed in a holding box in the main tank. The lobsters were sampled initially (condition and weight), replaced into water and sampled 6 hours later (condition, weight, haemolymph) and replaced into water for re-sampling 24 hours later (condition, weight, haemolymph). Only lobsters which had been caught at the first attempt (and could therefore be sampled within 20 seconds of first disturbance) were used as controls because tail flips caused a depression of haemolymph pH (unpub. data), as also noted for *Panulirus argus* (Vermeer, 1987).

Oxygen consumption

Oxygen consumption was determined by the depletion method as outlined in Chapter 2. This consisted of placing the lobsters into closed respirometers and measuring the amount of oxygen used by them over a 10-15 minute interval (the time period was dependant on the rate of oxygen uptake). The level of oxygen in the respirometers did not fall below 80% saturation.

Haemolymph

Haemolymph samples were used for measuring pH, lactate, glucose and ammonia as outlined in Chapter 2.

Haemolymph osmolality

Haemolymph osmolality was measured with an Advanced Instruments MiniOsmometer using 10µl samples of the haemolymph used to determine pH. The samples were kept on ice until the osmolality was analysed (always completed within 1 hour).

Haemolymph ions

The haemolymph used to determine pH and osmolality was frozen at -86°C for later determination of haemolymph ions (calcium, magnesium, potassium and sodium) by Atomic Absorption Spectrophotometry (Spectra AA 300 Varian). The haemolymph samples were prepared for analysis by adding 1 ml of 1000 ppm strontium solution to 100 µl of each sample. Each sample was then further diluted to 10 ml using a 1% nitric acid solution.

Body water content and water loss

The body water content of four lobsters was calculated by weighing them before and after they were freeze dried (Dynavac Freeze Drier FD3) for 4 days. The

rate of water loss during the experiments was calculated from the change in body weight (Herreid, 1969).

Gill histology

The podobranch and anterior arthrobranch gills of the third pereiopod were used for gill histology. Gill tissues were processed in an automatic tissue processor (Tissue Tek II) for 24 hours before being embedded in wax. They were sectioned at 5 μm and stained with haemotoxylin and eosin in a staining machine (Shandon Linistain GLX). Morphological changes to the gills were examined under light microscope.

Statistical analyses

Analysis of variance (ANOVA) was used to test for differences between treatments. Separate ANOVAs were carried out on the 6 hour emersion data and the 24 hour re-immersion data. A Students t-test was used to test for differences between the initial and 6 hour controls, and to test for differences between the control and treatment oxygen consumption rates. Where there were no significant differences between them, the data were pooled for the ANOVA analyses. The Levene test was used to test for homogeneity of variance and where necessary an appropriate transformation (usually logarithmic) was performed before further analysis. Where transforms failed to correct heterogeneity of variance, data were still analysed, however α was set at 0.01 for these analyses. Comparisons of means following ANOVA was done using the Tukey-HSD test.

Paired t-tests were used to determine significant differences in weight at the 6-h emersion and the 24-h re-immersion time compared to the initial weight. A Mann-Whitney non-parametric test was used to test for significant differences in condition. All analyses were performed on the SPSS statistical package with α set at 0.05 except for the situation described above. All means are expressed as mean±SE.

7.3 RESULTS

Decreases in haemolymph parameters, such as pH, with repeated sampling have been noted (Waldron, 1991). However, the haemolymph parameters of the control lobsters after the 24 hour re-immersion period were not significantly different to the parameters at the end of the emersion period. It was concluded that the sampling procedure itself did not influence haemolymph parameters in the experimental animals.

7.3.1 Body weight changes and survival

The body water content of the lobsters was 71.3±0.9% (n=4). Wind increased the desiccation rate of lobsters (Table 7.2). The rate of water loss of lobsters held in wind at low RH was over 4 times greater than that of lobsters with no wind at low RH (Table 7.3). Lobsters which had been emersed in low RH and wind (Treatment 2) were significantly lighter ($P<0.001$) and had lost 13.35% of the initial weight over the 6 hour period. Lobsters which had been kept in a humid environment with wind (Treatment 4) had also lost a significant ($P<0.001$) amount of weight but had only suffered a 6.24% weight loss. Lobsters in Treatment 1 (low RH, no wind) also lost a significant ($P<0.001$) amount of weight (3.14%). Lobsters placed under a spray (Treatment 5) gained a significant ($P=0.025$) amount of weight (0.46%) during the emersion period. The weight of lobsters in all treatments were not significantly different from the initial weight after being re-immersed for 24 hours

Treatment *condition*	Initial Wgt (g)	6 h Wgt (g)	% difference to initial weight	24 h Wgt(g)	% difference to initial weight
Control	434.4 (5.8, n=6)	433.5 (5.6, n=6)	-0.19 (0.19)	433.3 (5.7, n=6)	-0.25 (0.24)
Treatment 1 *Low RH*	440.4 (10.0, n=12)	426.4[b] (9.3, n=12)	-3.14 (0.3)	433.6 (17.5, n=6)	-0.03 (0.16)
Treatment 2 *Low RH, wind*	426.0 (5.5, n=10)	369.2[b] (6.6, n=10)	-13.35 (0.76)	431.5 (9.4, n=5)	-0.26 (0.42)
Treatment 3 *High RH*	412.5 (6.9, n=12)	411.1 (7.2, n=12)	-0.33 (0.21)	413 (8.3, n=8)	-0.35 (0.23)
Treatment 4 *High RH, wind*	427.8 (4.3, n=12)	401.0[b] (3.7, n=12)	-6.24 (0.41)	426.2 (4.5, n=6)	-0.18 (0.28)
Treatment 5 *High RH, spray*	428.5 (12.8, n=9)	430.5[a] (12.9, n=9)	0.46 (0.18)	429.1 (12.8, n=6)	0.14 (0.11)

Table 7.2: Weights and percentage weight changes (mean ± SE) of lobsters, *Panulirus cygnus,* subjected to various treatments during a 6 hour period of emersion and a 24 hour re-immersion period. The 6 hour and 24 hour weights were compared to the initial weight within each treatment. The number of lobsters (n) at each time period is also shown in brackets. Letters denote significantly different results (a = >0.01<0.05; b = <0.001).

Survival was 100% in all groups apart from the lobsters in Treatment 2. Two lobsters died during the 6 hour emersion period and another four during the 24 hour re-immersion period. Thus, there was a 50% mortality. An analysis of the Treatment 2 results reveals that lobsters which survived had an average weight loss of 11.88% (SE=0.53, n=5) with none of the lobsters having a weight loss greater than 13.75% during the initial emersion period. Of the lobsters that died within the 24 h re-immersion period, the average weight loss was 15.57% (SE=0.95, n=4) with no lobsters having a weight loss less than 14.05% during the initial emersion period. The two lobsters that died during the 6 hour emersion period had weight losses of 17.37% and 22.15% at the time of weighing.

	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Rate of water loss (%/h)	0.52	2.20 2.60[1]	N/A	1.04	-0.08

Table 7.3: The rate of water loss or gain (%/h) of lobsters, *Panulirus cygnus*, during 6 hours of emersion under various experimental treatment. It is assumed that the rate of water loss was constant over the emersion period.

N/A There was negligible water loss in the lobsters in those treatments.

[1] The rate of water loss for lobsters which died during the re-immersion period.

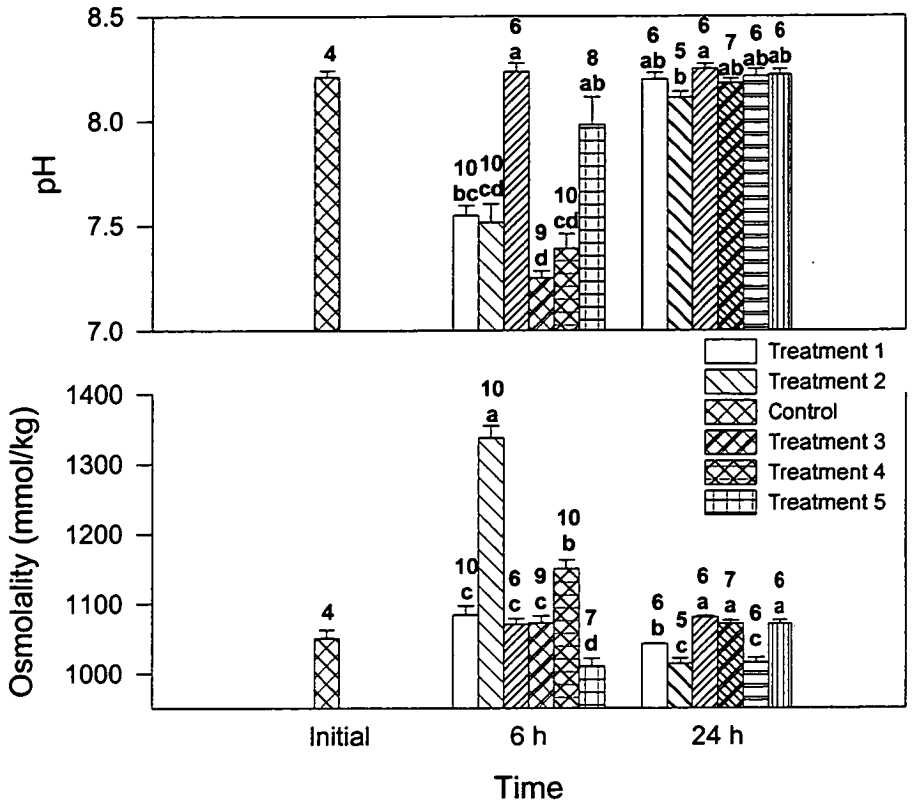

Figure 7.1: The effect of various experimental treatments on the haemolymph pH (mean±SE)(A) and osmolality (mmol/kg)(mean±SE)(B) of *Panulirus cygnus* after 6 hours under various experimental treatments, followed by 24 hours re-immersion in normoxic water. The number of lobsters tested is shown above each bar. Different letters denote significantly different results.

7.3.2 Haemolymph pH

Haemolymph pH (Fig. 7.1) of all treatments except for Treatment 5 (spray) was significantly lower ($P<0.001$) than the control after 6 hours emersion. All treatments except Treatment 2 (low RH, wind) recovered during the re-immersion period. However, the pH of lobsters in Treatment 2 (low RH, wind) was not significantly different ($P>0.05$) to any of the other Treatments after 24 hours re-immersion. In Treatment 5 (spray), two lobsters moved position and were not sitting directly under the spray. Although they were being kept wet, it was the result of

peripheral spray and splashing. The haemolymph pH of these 2 lobsters were 7.71 and 7.29; these results were not used in the calculations and no other haemolymph parameters were obtained from the two animals.

7.3.3 Osmolality

The haemolymph osmolality of control lobsters was 1062.1 mmol/kg (Fig. 7.1). Haemolymph osmolality of lobsters emersed in wind increased significantly ($P<0.0001$) to 1149.7 mmol/kg and 1337.0 mmol/kg, for lobsters held at high and low RH respectively. The haemolymph osmolality of lobsters held in the spray decreased significantly ($P<0.001$) to a value of 1010.0 mmol/kg. Animals in Treatment 1 (low RH, no wind) had a weight loss of 3.14% however their osmolality did not increase significantly over the 6 hour emersion period. After 24 hours re-immersion lobsters which had been emersed in wind (Treatments 2 and 4) had significantly lower ($P<0.001$) osmolality than all other treatments. The osmolality of the water in the holding tank was 1079.0 ± 8.2 mmol/kg (n=4). There was no significant difference ($P>0.05$) between the haemolymph osmolality of the controls and the osmolality of the water in the holding tank.

Treatment *conditions*	Haemolymph osmolality change (%)	Weight change (%)	Possible change in haemolymph volume (%)	Possible change in water content volume (%)
Treatment 2 *low RH, wind*	+25.88	-13.35	-70.26	-18.72
Treatment 4 *high RH, wind*	+8.25	-6.24	-32.84	-8.75
Treatment 5 *spray*	-4.5	+0.46	+2.42	+0.65

Table 7.4: Percentage change in haemolymph osmolality and body weight of *Panulirus cygnus* over a 6 hour emersion period. The comparison is restricted to treatments which resulted in a significant change in both haemolymph osmolality and body weight (ie. Treatments 2, 4 & 5). The results are compared to the theoretical changes in haemolymph volume and water body content if the haemolymph volume was taken to compose 19% of wet body weight (Dall, 1974*b*) and body water content 71.3% of wet body weight.

An evaluation of the percentage change in body weight compared to the percentage change in osmolality is shown is Table 7.4. The relative change in haemolymph osmolality was greater than the relative change in body weight, whether there was an increase or decrease in osmolality. Calculations for Treatments 2 and 4, where lobster weight decreased and osmolality increased, show that if the change of body weight was only restricted to the haemolymph then the resulting change in haemolymph volume would be much higher than the change in osmolality recorded. However, if the change in body weight is calculated as a percentage of total body water then the body weight changes are very similar to the osmolality changes. In contrast, in Treatment 5 where lobsters increased in weight and decreased in osmolality, similar comparisons suggest that the change in body weight is more closely related to a possible change in haemolymph volume than to a change in body water content.

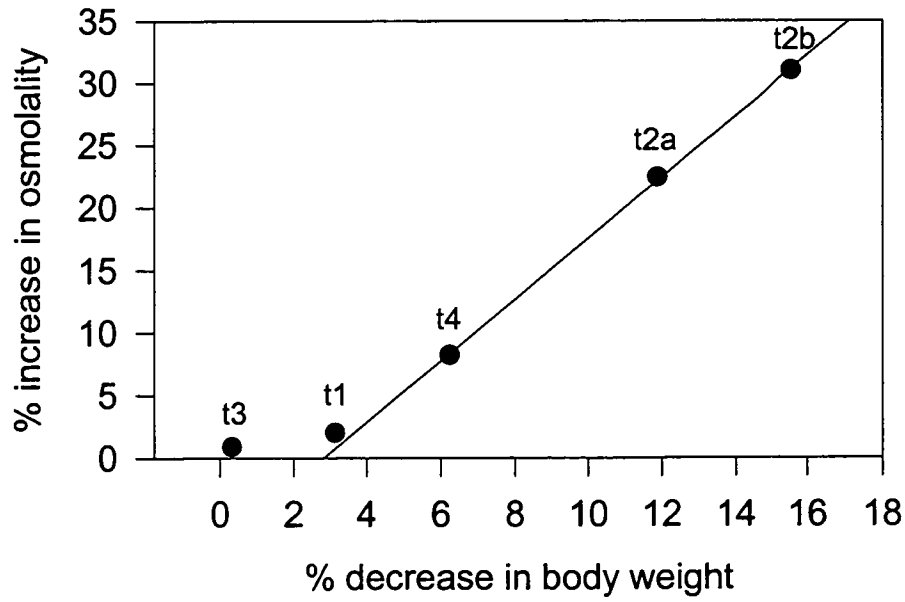

Fig. 7.2: The increase in osmolality of *P. cygnus* haemolymph (expressed as % of control value) plotted against the decrease in body weight (expressed as % of initial weight) for each of the four treatments (t) which resulted in decreased body weight after the emersion period. The data for Treatment 2 (low RH, wind) were broken down into lobsters which survived the 24 h re-immersion period (t2a) and lobsters which died (t2b).

Haemolymph osmolality increased little until approximately 3% of the body weight had been lost, showing a substantial and linear increase above that point (Fig. 7.2).

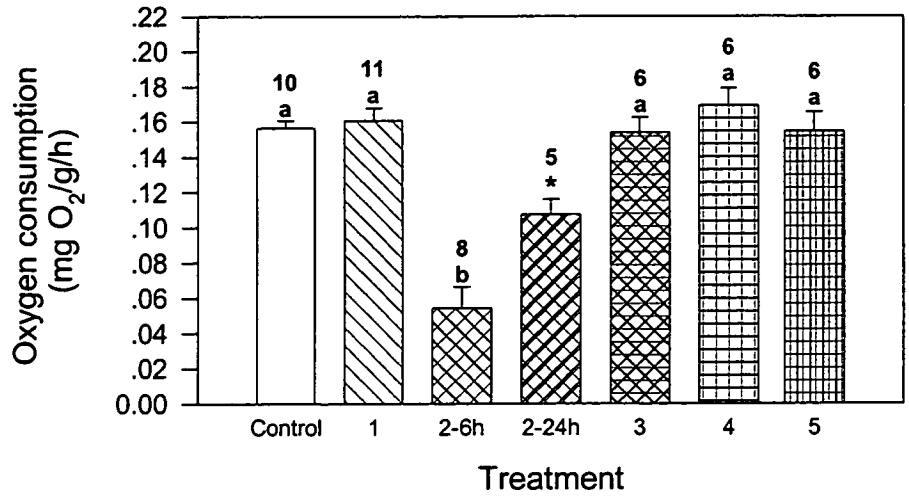

Figure 7.3: The oxygen consumption rates (mg O_2/g/h)(mean±SE) of *Panulirus cygnus* after 6 hours emersion exposure to various experimental treatments. Lobsters exposed to Treatment 2 (low RH, wind) were tested again after 24 hours re-immersion. The number of lobsters tested is shown above each bar. Different letters denote significantly different results.

- the oxygen consumption of Treatment 2 lobsters after the 24 hour recovery period was still significantly lower than the control (Students t-test).

7.3.4 Oxygen consumption

Lobsters exposed to low RH and wind (Treatment 2) during the 6 hour emersion period had significantly lower ($P<0.001$) oxygen consumption rates upon re-immersion (Fig. 7.3). After re-immersion in oxygen saturated water for 24 hours their oxygen consumption rate was still significantly lower ($P<0.001$) than the controls, although it was significantly higher ($P=0.04$) than the rate after the emersion period. The oxygen consumption rates of lobsters in other treatments did not differ significantly from the control. The pooled rate of oxygen consumption of lobsters recovering from emersion (0.160mg O_2/g/h - excluding Treatment 2) was not

significantly different (P=0.11) from the maximum rate of oxygen consumption of lobsters at 23°C (0.153 mg O_2/g/h - Chapter 4).

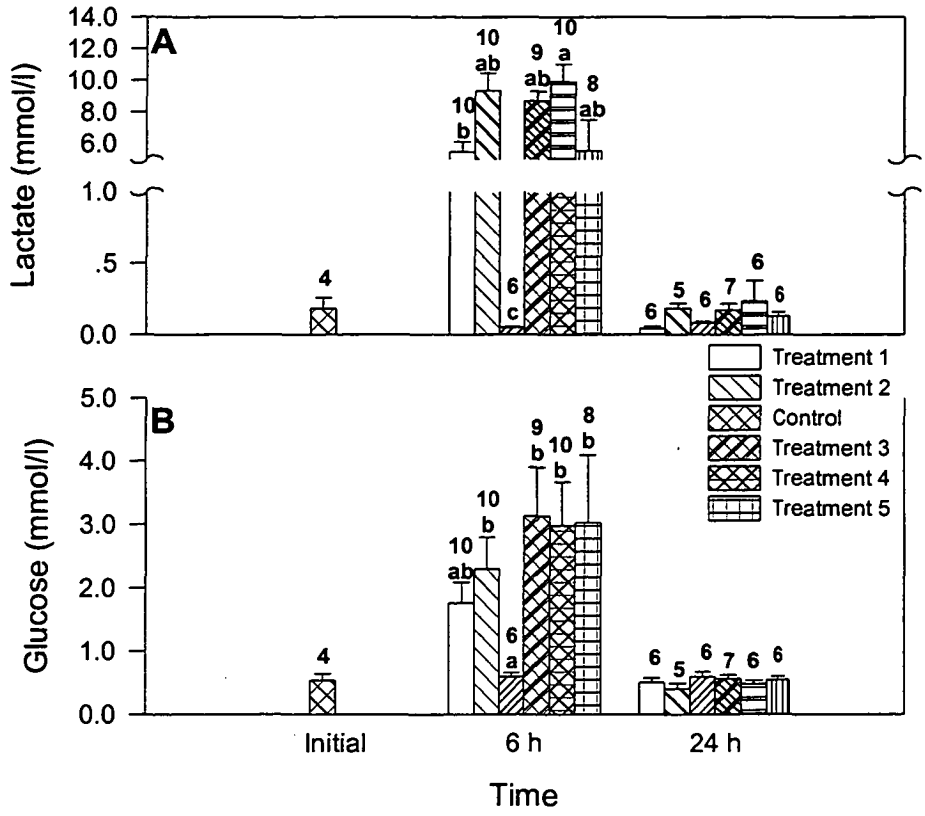

Figure 7.4: Haemolymph lactate (A) and glucose (B) concentrations (mmol/l)(mean±SE) of *Panulirus cygnus* exposed to 6 hours emersion under various experimental treatments, followed by 24 hours re-immersion in normoxic water. The number of lobsters tested is shown above each bar. Different letters denote significantly different results. There was no significant difference in either parameter after 24 hours re-immersion.

7.3.5 Lactate and glucose

Haemolymph lactate concentrations increased significantly (P<0.03) in all treatments after 6 hours emersion (Fig. 7.4). Haemolymph glucose increased in all treatments during emersion although it was not significant (P>0.05) in Treatment 1 (low RH/no wind). After 24 hours re-immersion both parameters had returned to control concentrations in all treatments.

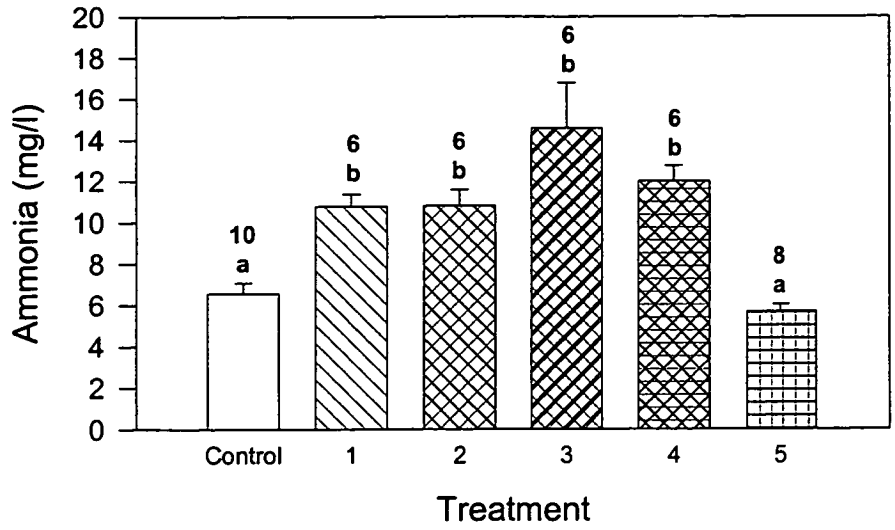

Figure 7.5: The ammonia (mg/l)(mean±SE) concentration in the haemolymph of *Panulirus cygnus* after 6 hours emersion to the experimental treatments. The number of lobsters tested is shown above each bar. Different letters denote significantly different results.

7.3.6 Ammonia

Haemolymph ammonia concentrations (Fig. 7.5) increased significantly ($P<0.02$) in all treatments during the 6 hour emersion period, apart from lobsters under the spray (Treatment 5).

Time	Control	Treatment 1 Low RH	Treatment 2 Low RH, wind	Treatment 3 High RH	Treatment 4 High RH, wind	Treatment 5 Spray
Initial	5(6)	5(12)	5(12)	5(12)	5(12)	5(12)
6-hour	5(6)	3.3*(12)	0.7*(12)	3.4*(12)	2.5*(12)	4.5*(11)
24-hour	5(6)	4.6*(6)	2.6*(5)	5(12)	3.5*(10)	5(11)

Table 7.5: The condition of lobsters, *Panulirus cygnus*, before and after 6 hours emersion under various experimental treatments. The condition of the lobsters after 24 hours re-immersion in normoxic water is also shown. The number of lobsters tested is shown in brackets. Lobsters which were significantly different to their initial condition are denoted by an asterisk (*).

7.3.7 Condition

Initially all lobsters were assessed as being in excellent condition and the control lobsters remained so throughout the trial period (Table 7.5). The condition of all of the treatment lobsters was significantly lower ($P<0.05$) after the 6-hour emersion period. Lobsters exposed to wind were in the poorest condition, with Treatment 2 lobsters (low RH, wind) showing no response to handling at all. The lobsters in the spray treatment were still in good condition after emersion although they could not sustain their tail flicking response for an extended period. After re-immersion for 24 hours the condition of lobsters in Treatments 1, 2 & 4 were still significantly lower ($P<0.01$) than the controls. Treatment 2 lobsters remained limited in basic defence strategies such as antennal movement.

7.3.8 Haemolymph ions

Haemolymph sodium increased in all treatments after 6 hours emersion (Fig. 7.6), except for Treatment 5 (spray), although only significant increases were recorded for Treatments 2 and 3 (Fig. 7.6). There was no clear correlation between the changes in $[Na^+]$ compared to changes in osmolality (Table 7.5). In Treatment 2 they were closely correlated but in Treatment 3 the $[Na^+]$ increased by 20% in contrast to osmolality which did not change. The $[Na^+]$ decreased in Treatment 5 and was significantly lower than all other treatments, but not the control. After 24 hours re-immersion it had returned to control levels in all treatments.

Magnesium concentrations increased significantly ($P<0.05$) in all treatments except for Treatment 5 (spray)(Fig. 7.6). The % increases in magnesium were much greater than expected based on changes in haemolymph osmolality (Table 7.5). After 24 hours re-immersion the $[Mg^{++}]$ had still not returned to control levels in Treatment 2.

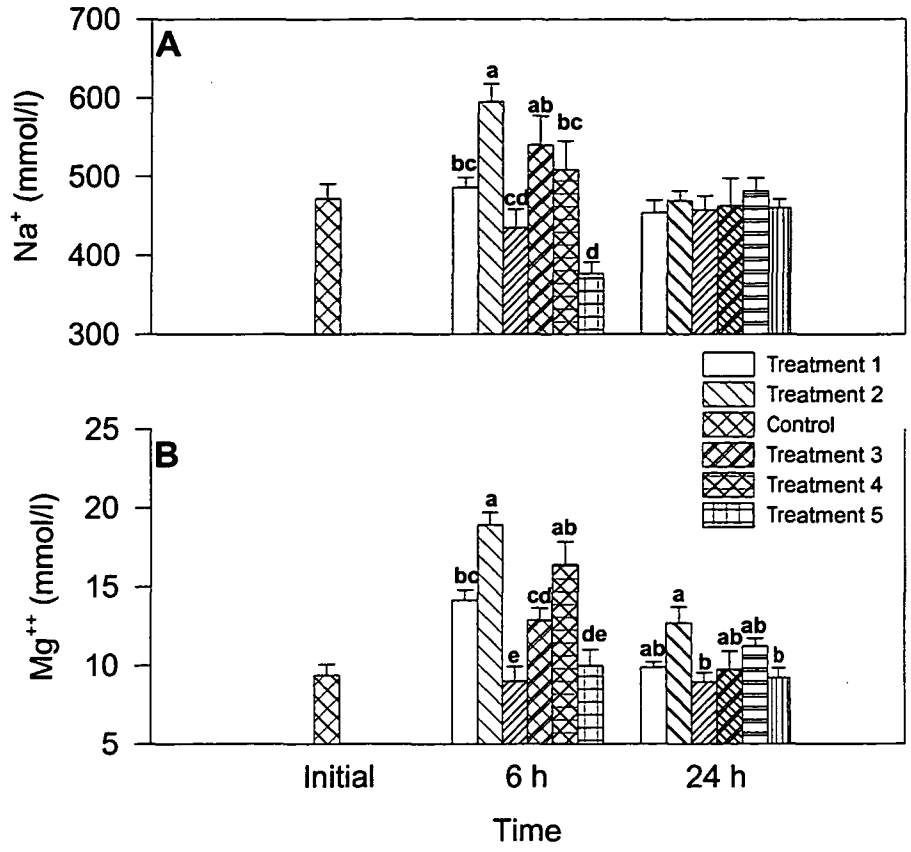

Figure 7.6: Haemolymph sodium (A) and magnesium (B) concentrations (mmol/l)(mean±SE) of *Panulirus cygnus* exposed to 6 hours emersion under various experimental treatments, followed by 24 hours re-immersion in normoxic water. The number of lobsters tested at each point is the same as in Figure 7.4. Different letters denote significantly different results. There is no significant difference between the Na^+ values after 24 hour re-immersion.

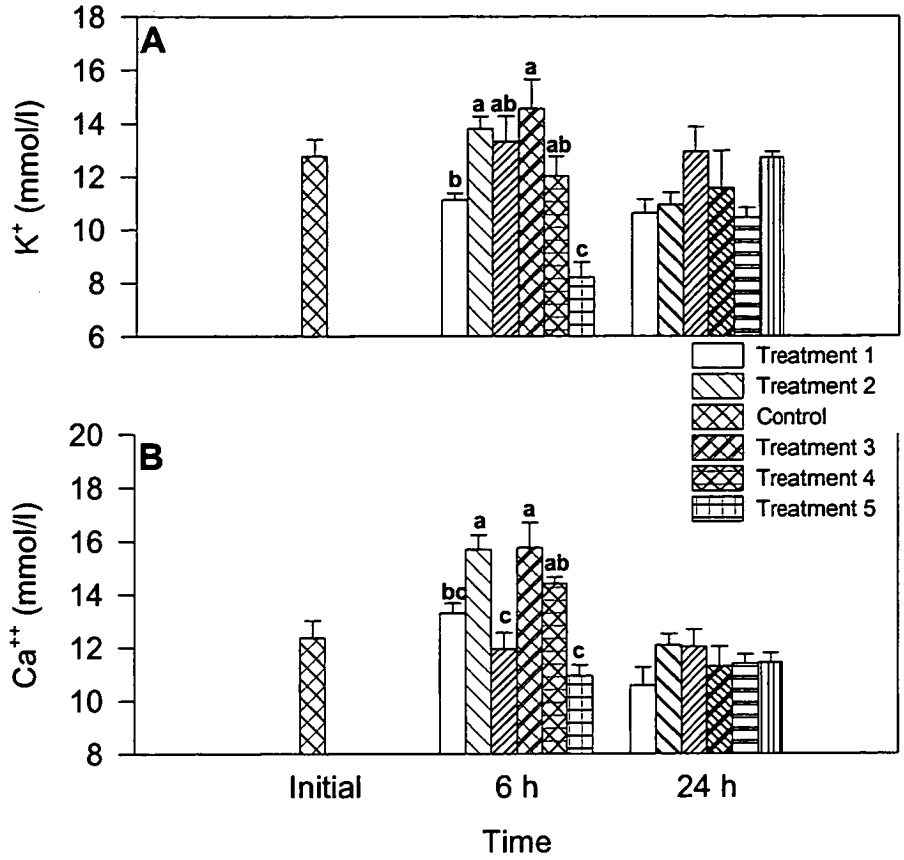

Figure 7.7: Haemolymph potassium (A) and calcium (B) concentrations (mmol/l)(mean±SE) of *Panulirus cygnus* exposed to 6 hours emersion under various experimental treatments, followed by 24 hours re-immersion in normoxic water. The number of lobsters tested at each point is the same as shown in Figure 7.4. Different letters denote significantly different results. There is no significant difference between the K^+ and Ca^{++} values after 24 hour re-immersion.

	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Osmolality	-----	25.88	-----	8.25	-4.50
Sodium	-----	32.30	20.13	-----	-----
Magnesium	54.50	107.00	40.64	79.68	-----
Potassium	-----	-----	-----	-----	-37.2
Calcium	-----	29.59	30.13	19.01	-----

Table 7.6: Proportional changes in osmolality, $[Na^+]$, $[Mg^{++}]$, $[K^+]$ and $[Ca^{++}]$ of the haemolymph of the western rock lobster, *P. cygnus*, after 6 hours of emersion under various treatments. The results are expressed as a percentage of the control levels.

Haemolymph potassium decreased significantly in Treatment 5 (spray)(Fig. 7.7) and the % change was much larger than predicted on the basis of osmolality change (Table 7.6). The calcium concentration increased significantly ($P<0.05$) in Treatments 2, 3 and 4. Although there appeared to be some correlation between the % increases and the osmolality changes in Treatments 2 and 4, there was no osmolality increase in Treatment 3 but the [Ca^{++}] increased by 30% (Table 7.5). After 24 hours re-immersion both the [K^{+}] and [Ca^{++}] returned to control concentrations in all Treatments.

7.3.9 Gill structure and histology

There was no obvious gross morphological changes in the gills of lobsters which had been severely dehydrated (Treatment 2) compared to lobsters which had been emersed but did not lose body weight (Treatment 3). In dehydrated lobsters superficial water was still present around the gill filaments. The gills were clumped (Fig. 7.8) resulting in the exposure of large areas of gill surface to air. Gill clumping was also evident in lobsters in Treatment 3, which had been emersed in high RH, so it appears as if clumping was specifically the result of emersion, rather than other factors such as desiccation.

Gill filaments of severely dehydrated lobsters (Treatment 2) had a wrinkled appearance compared to the gill filaments from an immersed lobster (Fig. 7.9 A and B). Gills from lobsters in all other treatments did not appear morphologically different to the immersed lobsters gills.

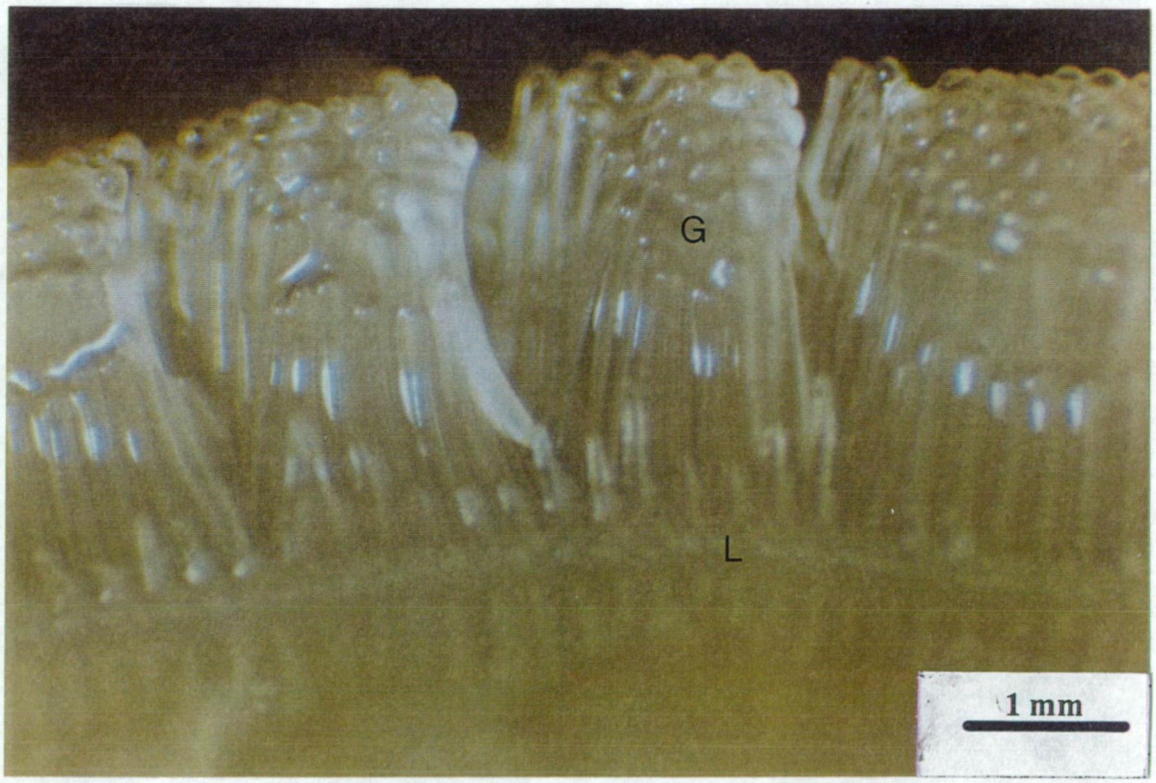

Figure 7.8: A photo of the mid-section of a gill of a western rock lobster, *Panulirus cygnus*, which had been exposed to 6 hours emersion in low RH and wind (Treatment 2). Clumping of the gill filaments is evident, resulting in the exposure of large areas of the gill surface to air. Residual branchial water is also visible. This lobster survived the emersion period despite losing 11.3% of its body weight. *G*, gill filaments; *L*, lateral surface of the central gill axis.

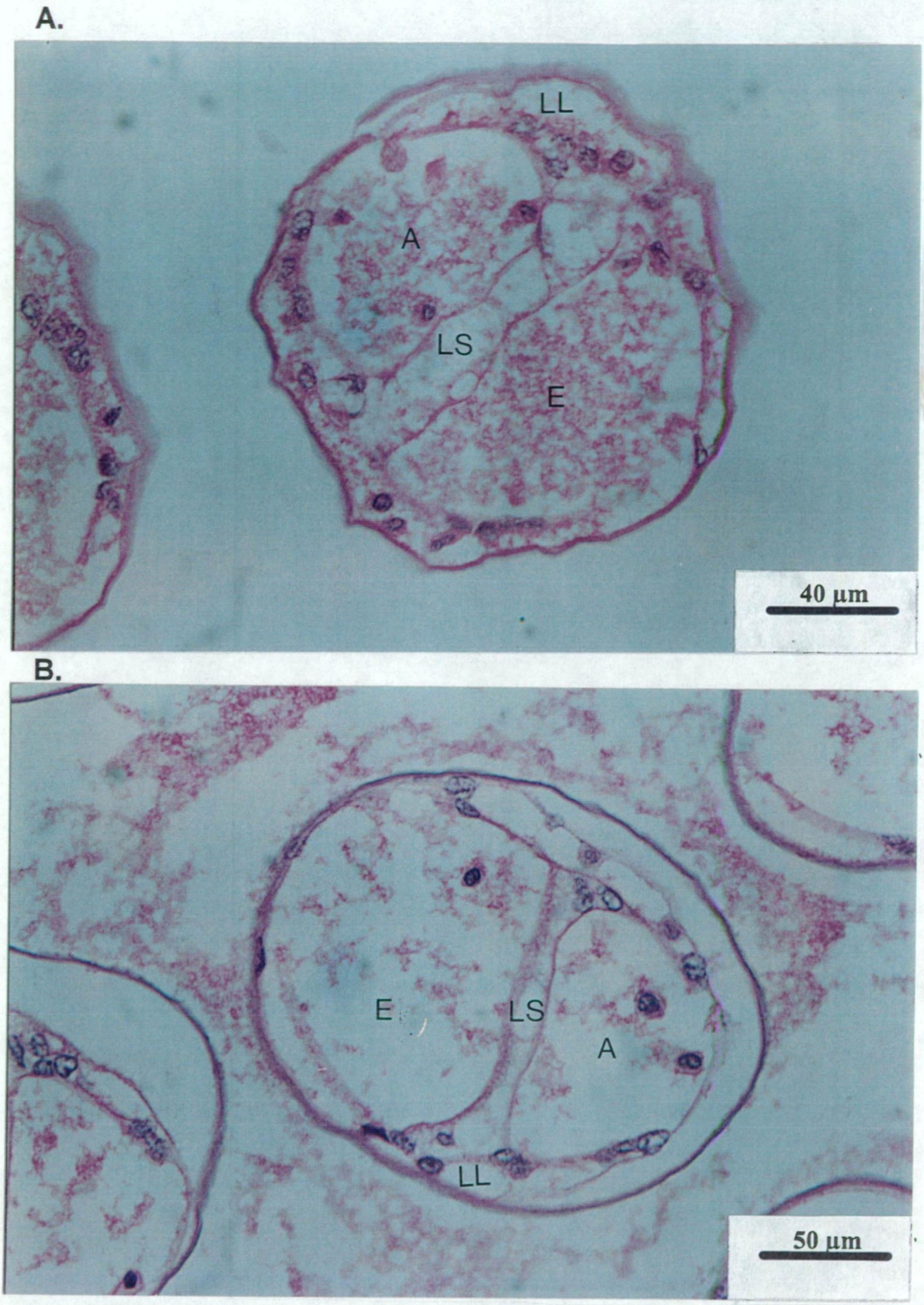

Figure 7.9: A transverse section of a gill filament of a lobster which had been severely dehydrated (A)(Treatment 2) compared to that from an immersed lobster (B). Note the wrinkled appearance of the exterior surface of the gill filament of the dehydrated lobster. *A*, afferent channel; *E*, efferent channel; *LL*, lateral lacuna; *LS*, longitudinal septum.

7.4 DISCUSSION

Spraying seawater over the lobsters during emersion reduced the physical, physiological and biochemical disturbances associated with holding crustaceans in air for extended periods. When subtidal crustaceans are emersed they usually suffer from a combined respiratory and metabolic acidosis, resulting from the failure of the gills to maintain gaseous exchange in air (Taylor and Innes, 1988). An acidosis was not apparent in lobsters sprayed with seawater even though high levels of lactate were measured after the emersion period. The respiratory component of the acidosis appears to have been almost completely dispersed due to the use of sprays. CO_2 accumulation has been shown to be mainly responsible for emersion-induced acidosis in other crustaceans (Taylor and Wheatly, 1981; Schmitt and Uglow, 1997*a*). Lobsters in the other treatments showed typical responses to emersion and their condition was adversely affected when wind and/or low relative humidity were introduced as factors, probably because of the increased desiccation rate.

7.4.1 Water loss/gain

The rate of loss of water from crustaceans is influenced by many factors including body size and shape, initial water content, humidity, habitat preference and temperature (Herreid, 1969; Young, 1978). The maximum rate of water loss in this study was close to that measured in other subtidal crustaceans such as *P. argus* (Vermeer, 1987) and *Callinectes sapidus* (Herreid, 1969) held under similar environmental conditions. Subtidal crustaceans suffer 3-5 times the rate of water loss of crustaceans from semiterrestrial or terrestrial environments (Herreid, 1969). It has been proposed that the greater gill surface area of subtidal crustaceans increases the rate of water loss (Ahsanullah and Newell, 1977), however other factors such as increased integument permeability also appear to play a part (Herreid, 1969). Lobsters held in low RH suffered much greater rates of water loss than lobsters held at high RH. Similarly, decreases in RH resulted in significant increases in the rate of water loss from two species of crabs (Ahsanullah and Newell, 1977). However, wind was the major factor determining the rate of water loss in *P. cygnus*; lobsters in wind at low RH had 4 times the weight loss of lobsters not subjected to wind. Vermeer (1987) noted that wind speed increased the rate of water loss in *P. argus*.

Water may be lost in the following ways during emersion: (a) by discharging water from the gill chamber (ii) by faecal losses, (c) by urination, (d) by evaporation from the gills and gill chamber and (e) by other integumentary losses (Herreid, 1969). Methods (d) and (e) are generally regarded as the most likely routes of water loss. Although urine excretion can comprise up to 10% body weight/day, it generally decreases with emersion and does not contribute substantially to weight loss (Tyler-Jones and Taylor, 1986). Herried (1969) suggested that the integument may be the major route of water loss because dead crabs lost water at the same rate as live crabs. Other studies have also observed similar rates of water loss in dead and live crabs (Ahsanullah and Newell, 1977; Innes *et al.*, 1986) suggesting that water loss is a passive process. However, Ahsanullah and Newell (1977) proposed that it occurred principally via the gills and gill chamber. As 90% of the total external surface of crustaceans is contained in the gills (Cameron and Mangum, 1983) they are likely to be one of the major sites of water loss.

Significant loss of body water causes death in crustaceans. In this study no lobsters survived the loss of more than 14% of their body weight and no lobsters died if they lost less than 14% of their body weight. Prawns (*Penaeus japonicus*) died after approximately 16% of their initial body weight was lost (Samet *et al.*, 1996) whilst the subtidal crabs, *Menippe mercanaria* and *Arenaeus cribarius*, died after losing 12 and 14% of their body weight, respectively (Herreid, 1969). It appears that subtidal crustaceans can handle the loss of approximately 12-16% of their initial body weight before death occurs, a figure much lower than for most intertidal or terrestrial crustaceans (Jones and Greenwood, 1982; Innes *et al.*, 1986). The rate of loss of the water would also play a significant part in determining the amount of body water able to be lost before death. Death occurred in *Leocarcinus puber* after the loss of only approximately 5% of the body weight (Johnson and Uglow, 1985), however the rate of loss was slow and other physiological disturbances over the emersion period, such as increasing levels of lactate, were probably the cause of death rather than weight loss.

Water loss could occur either from the haemolymph or from the whole body or from a combination of both. In the freshwater crayfish, *Austropotamobius pallipes*, a 10% decrease in body weight was associated with a 75% reduction in haemolymph volume, as well as a slight decrease in muscle water content (Taylor *et al.*, 1987). The authors proposed that during dehydration *A. pallipes* protects the

intracellular fluid volume whilst preferentially losing water from the haemolymph and the extra-cellular fluid volume. The extent of the calculated reduction in haemolymph volume was far greater than the measured increase in haemolymph osmolality in *P. cygnus*. Some of the weight loss used in the calculations was probably due to evaporation of surface water, with approximately 3% weight loss occurring before haemolymph osmolality was significantly affected. Taking into account that 3% and recalculating the data in Table 7.3 still means that the calculated haemolymph volume change was approximately double the haemolymph osmolality change. The concentration effects of the haemolymph may have promoted the movement of water to the blood from the tissues (Johnson and Uglow, 1985) and negated the possible rise in haemolymph osmolality. The other possibility is that the movement of blood ions from the haemolymph to other body tissues or vice versa may have contributed to the observed changes. This possibility will be discussed below.

7.4.2 Survival

Death of crustaceans as the result of weight loss is probably due to increases in the viscosity of the haemolymph causing problems with circulation and oxygen delivery (Taylor *et al.*, 1987; Samet *et al.*, 1996). In prawns, a low haemolymph viscosity helps to reduce tissue and gill resistance and ensure the circulatory system remains efficient (Anderson, 1989).

7.4.3 Osmolality

The relationship between increase in osmolality and decrease in body weight suggests that the weight loss in treatments which lost little weight (less than 3.5 % of body weight) was due to water loss from the exterior of the lobsters rather than from haemolymph. This weight loss component could be either superficial water adhering to the lobsters or water in the branchial chambers (Morrissy *et al.*, 1992). The authors suggest that the water lost from the branchial chambers is presumably lost by seepage due to gravity and that it occurs very rapidly (within two hours). The procedures used in this study meant that most of the branchial water was initially removed, thus restricting it as a source of the initial weight loss component. The increase in

osmolality may have been delayed as water from tissues was sequestered to maintain a constant osmolality during the initial stages of dehydration.

Lobsters held in the spray increased in weight. There is evidence that this situation occurs in commercially held lobsters; after a period in a seawater spray swelling of lobsters has been reported (W. Hosking, Geraldton Fishermens Co-op; R. Bailey, Batavia Coast Fisheries, pers. comm.). The increase in weight could only occur due to an increase in the water content of the lobsters and only if they were actively or passively taking up water whilst emersed. The decrease in blood osmolality is another indication that water uptake is occurring. The change in osmolality is closely correlated to the calculated change in haemolymph volume of lobsters held under the spray, which suggests that the water was being taken-up into the haemolymph. Being totally subtidal crustaceans, *P. cygnus* would be expected to be an osmoconformer; the controls in this study had the same osmolality as seawater. Dall (1974*a*) found that *P. cygnus* was an osmoconformer and was able to maintain its osmoconformity over the salinity range 25-45‰. Osmoconformity is one of the most direct means of minimising the diffusive movements of ions and water between the haemolymph and the external medium (Mantel and Farmer, 1983). Water is taken up via passive osmosis through the body surface, generally via absorption through the gills (Mantel and Farmer, 1983). In some instances, such as just prior to the moult, water may also be taken up via the gut (Dall and Smith, 1978; Mykles, 1980), however it is unlikely that this occurred in this study as it is doubtful that water could have entered the gut under the experimental conditions. It also appears that the onset of drinking is triggered by a significant reduction in haemolymph volume in dehydrating crabs (Greco *et al.*, 1986); reduction of haemolymph volume did not occur in the lobsters which gained weight during emersion. Drinking accounted for only a minor portion of the uptake of water required to balance estimated urine production in *P. argus* (Malley, 1977).

As discussed below it appears that *P. cygnus* was able to move water into the branchial chamber when emersed under the water spray, thereby providing a pathway for water uptake. Water uptake would normally occur at a rate which would match water loss, and in aquatic crustaceans water loss would occur via urine production and excretion (Mantel and Farmer, 1983). The rate of urine production in crustaceans varies between species from about 1% body weight (BW)/day to about 10% BW/day (Mantel and Farmer, 1983; Tyler-Jones and Taylor, 1986). The spiny lobster, *Jasus*

edwardsii, had a urine production rate of 4.8% BW/day (Binns and Peterson, 1969). If the same urine production rate was assumed for *P. cygnus*, then over the 6 hour emersion period 1.2% BW of urine would be excreted. However, during emersion the urine excretion rate was significantly reduced in the freshwater crayfish, *A. pallipes*, and over a 24 hour period it was only about half of the value recorded when the crayfish were immersed (Tyler-Jones and Taylor, 1986). If a similar situation occurred in *P. cygnus* then the lobsters may not have been able to excrete urine at a sufficient rate to equal the passive inflow of water. Therefore, lobsters appear to be able to uptake water but not excrete it at a sufficient rate when emersed under seawater sprays, resulting in increased body weight and decreased haemolymph osmolality.

There is evidence that crustacean urine flow rates can be controlled and adjusted to the minimum compatible with osmoregulatory requirements (Tyler-Jones and Taylor, 1986). Changes in the rate of urine flow could be produced by decreased osmolality of haemolymph, by changes in internal volume or pressure, or by chemical mediation operating in direct response to the external medium (Mantel and Farmer, 1983). Precise control of haemolymph volume implies a mechanism for sensing changes in this parameter (Greco *et al.*, 1986). It has been suggested that stretch receptors on muscles in the posterior cephalothorax of the subtidal crab, *C. borealis*, might serve as sensors for changed haemolymph volume, resulting in decreasing urine flow with decreases in haemolymph volume (Greco *et al.*, 1986). However, it appears that urine flow is decreased in *P. cygnus* even though there is no decrease in haemolymph volume. Either different mechanisms are in control in *P. cygnus* or the mechanism changes in response to emersion.

After 24 hours of re-immersion lobsters which had increased in osmolality during the emersion period had osmolality values which were lower than the controls. This may indicate a slight "overshoot" mechanism was being used, similar to outlined by Dall (1974*a*), resulting in a slight hypo-osmolality which had not been corrected as yet.

7.4.4 Branchial chamber water stores

Crustaceans retain some water in the branchial chamber when they are emersed. Terrestrial crustaceans have the ability to retain the water in the chamber for an extended period (McMahon and Wilkens, 1983), whereas in subtidal crustaceans the water usually drains away within the first few hours (Taylor and Innes, 1988; Morrissy *et al.*, 1992). In this study, most of the branchial water was removed in the initial experimental procedure. It is generally believed that gills must be kept moist during emersion to ensure that oxygen uptake is optimised (McMahon and Wilkens, 1983), however no data are available to support this view (deFur, 1988). Varley and Greenaway (1992) argued that during emersion oxygen uptake is impaired because water trapped between the gill lamellae rapidly becomes depleted of oxygen. Although, deFur and McMahon (1984) suggested that clumping of the gill filaments leads to a reduction in the effective surface area for gas exchange, which could restrict adequate ventilation and perfusion of the gills, a few studies have indicated that gill clumping increases oxygen uptake as more gill surface area is exposed (Taylor and Wheatly, 1981; Innes *et al.*, 1986; deFur, 1988; Varley and Greenaway, 1992). Oxygen uptake could also be perfusion limited during emersion due to bradycardia (deFur and McMahon, 1984) and increased resistance to haemolymph flow through the gill lamellae (deFur *et al.*, 1988). Waldron (1991) noted an increase in heart rate of *J. edwardsii* as emersion time increased and suggested that it was due to the drying out of the gills and hence a decrease in the external pressure on the gills. Increased heart rate will increase gill perfusion and in the study by Waldron (1991) it was associated with a slight increase in oxygen uptake with emersion time. The internal hypoxia associated with emersion often leads to anaerobic metabolism and consequently a progressive accumulation of lactate (Taylor and Innes, 1988). The haemolymph lactate and glucose of lobsters held under the spray was similar to that measured in lobsters in the other treatments, suggesting that oxygen uptake was not improved in lobsters held under a spray.

Aquatic crustaceans, in general, have trouble excreting carbon dioxide while in air (Samet *et al.*, 1996). In terms of the evolution of air-breathing, branchial gas exchange is not an effective means of aerial oxygen transfer, but as long as the animal can maintain a reservoir of water in contact with the gills it is an effective means of maintaining a low haemolymph CO_2 content (Taylor and Innes, 1988).

Haemolymph CO_2 accumulation appears to be mainly responsible for emersion-induced acidosis (Taylor and Wheatly, 1981; Schmitt and Uglow, 1997*a*), therefore having a suitable method to dispose of CO_2 would be an important step in decreasing the effect of emersion on acid-base disturbances. Intertidal crustaceans have made use of the method by retaining branchial water for an extended time after emersion. Although it does appear to eventually drain (Burnett and McMahon, 1987; Taylor and Innes, 1988) water is used as a CO_2 sink in several species while available (Burnett and McMahon, 1987). The intertidal red rock crab, *Cancer productus*, has the ability to access interstitial seawater to ensure there is always a branchial water store during emersion (deFur *et al.*, 1983). The role of the seawater sprays in offsetting the physiological disturbance in emersed subtidal crustaceans is not clear. In emersed spanner crabs, *Ranina ranina*, the effect of sprays was to decrease the extent of the acidosis compared to crabs held in humid air, probably by increasing CO_2 excretion (Paterson *et al.*, 1994*b*). Periodical wetting of the stone crab, *Menippe mercenaria*, during emersion improved survival (Simonson and Hochberg, 1986). However, mud crabs, *Scylla serrata,* exposed to sprays did not have reduced haemolymph CO_2 levels (Varley and Greenaway, 1992). Maintenance of a normal haemolymph pH in *P. cygnus* when they were held in spray systems, indicates that CO_2 excretion is occurring. Water was present in the branchial chamber after the emersion period which suggests the lobsters managed to take-up water into the gill chamber. Due to the position of the lobsters and the direction of the spray it would have been impossible for the water to enter the chambers via gravitation or via the flow of the water. In intertidal species the uptake of interstitial water is accomplished by the production of a substantial vacuum in the branchial chamber, which draws water into the branchial chamber via setae (Wolcott, 1976; deFur and McMahon, 1984; Thompson *et al.*, 1989). Whether a similar mechanism is able to be used by subtidal crustaceans is unknown. Alternatively, Regnault (1994) proposed that *C. pagurus* could possibly collect seawater with the setae of its appendages and convey it to the branchial chambers. Whatever mechanism is used by *P. cygnus*, it appears that it does have some ability to move water into the branchial chamber.

Lobsters not subjected to the spray would not have had access to any branchial water stores because of the experimental procedures. However, the effect of removing the branchial water stores on the acid-base disturbances of those lobsters would be expected to be minimal, due to the limited ability of subtidal crustaceans to

retain branchial water stores (Taylor and Innes, 1988; Morrissy *et al.*, 1992). Lobsters without the branchial water route for preventing the accumulation of CO_2 need to rely on an internal source to buffer the increase in CO_2. The major source of calcium carbonate accounting for the compensation of emersion acidosis is probably the shell (Henry *et al.*, 1981). This is indirectly indicated by reports showing that the calcium concentration increases in the haemolymph during the compensatory phase (Truchot, 1990). Lobsters in this study, in all treatments other than the spray treatment, showed increases in the haemolymph calcium concentrations, which indicates the mobilisation of carbonate buffers (this finding will be discussed below).

7.4.5 Haemolymph pH

Respiratory acidosis appears to be responsible for most of the pH decrease observed during the emersion period. Lobsters in the water spray maintained a high pH despite increases in haemolymph lactate of similar magnitude to the other treatments. Although metabolic acidosis can occur, respiratory acidosis is the most typical response of emersed aquatic animals (Truchot, 1990). It has been shown to be the cause of pH decreases in decapod crustaceans (Whiteley and Taylor, 1990; Schmitt and Uglow, 1997*a*) and was the suggested cause of pH decreases observed in *P. cygnus* (Chapter 6). The capacity of sprays to reduce a pH decrease was also found by Paterson *et al.* (1994*b*) for *R. ranina*, although the effect was not as strong. However, the periodic wetting method used by Schmitt and Uglow (1997*a*) did not prevent a decrease in pH associated with emersion. The pH decreases in the present study of 0.8 to 1.0 units are some of the largest decreases recorded for crustaceans (c/f. Booth *et al.*, 1982; Vermeer, 1987; Schmitt and Uglow, 1997*a*) and indicate significant physiological disturbances. Metabolic processes require the blood and tissues to remain within a certain physiological range of pH (Paterson *et al.*, 1993); acidosis is commonly postulated as being a cause of death in crustaceans (Taylor and Whiteley, 1989; Whiteley and Taylor, 1990). However, Whiteley and Taylor (1992) suggested that acid-base status could be symptoms of the deteriorating condition of the animals rather than the causal agent. In this study most lobsters had recovered after 24 hours re-immersion. Lobsters which died during re-immersion had a mean pH decrease of 0.8 units during emersion, which is at the lower end of the decreases

measured. Haemolymph pH does not appear to be a reliable indicator of the ability of lobsters to recover from a physiological disturbance.

7.4.6 Lactate

It is quite obvious from the large increases in haemolymph lactate observed in all treatments, that emersed lobsters were unable to supply all of their energy requirements via aerobic metabolism. The calculated decrease in haemolymph volume due to desiccation could not account for the 50-100 times increases in haemolymph lactate measured. Although there is a fair degree of interspecies variation in lactate accumulation in subtidal crustaceans (Table 7.7), in general there is a large increase within a relatively short period of emersion. Spicer *et al.* (1990) postulated that there was a general relationship between the rate of accumulation of lactate and the ability to tolerate emersion. Intertidal species such as *C. maenas* and *Scylla serrata*, do not accumulate lactate or accumulate it at very slow rates during emersion (Johnson and Uglow, 1985; Varley and Greenaway, 1992). Such fast rates of lactate accumulation would therefore suggest that *P. cygnus,* like many other subtidal crustaceans, have limited ability to handle emersion. Seawater sprays reduced the rate of accumulation of lactate in *Ranina ranina* (Paterson *et al.*, 1994*b*) and periodic wetting reduced the rate in *Nephrops norvegicus* (Schmitt and Uglow, 1997*a*), but it did not effect the rate of accumulation in *P. cygnus*. Schmitt and Uglow suggested that the decreased rate in their study was due to the decreased activity of the periodically wetted lobsters, however the spray system did not appear to affect the level of activity of *P. cygnus*. Spray systems would not be expected to reduce the rate of anaerobic glycolysis in emersed crustaceans (Paterson *et al.*, 1994*b*), therefore the results of this study are not surprising.

Although the elimination of lactate is generally slow in crustaceans (Bridges and Brand, 1980*b*), it would be expected to be completed during the 24 hour re-immersion period as it only took 8 hours for the haemolymph lactate of *P. cygnus* to return to control levels after a period of stress (Chapter 6).

Species	[lactate]	Conditions	Reference
Liocarcinus puber	6.1	8 hours at 15°C	Johnson and Uglow, 1985
Panulirus argus	5.5	2 hours at 22-30°C	Vermeer, 1987
Nephrops norvegicus	10	9 hours at 10°C	Spicer *et al.*, 1990
Jasus edwardsii	7	8 hours at 17°C	Waldron, 1991
Ranina ranina	15	3 hours at 25°C	Paterson *et al.*, 1994*a*
R. ranina	3	3 hours at 19°C	Paterson *et al.*, 1994*b*
Cancer pagurus	4.5	8 hours at 16-18°C	Regnault, 1994
N. norvegicus	12	8 hours at 12°C	Schmitt and Uglow, 1997*a*
Panulirus cygnus	6-10	6 hours at 23°C	This study

Table 7.7: Haemolymph lactate concentrations (mmol/l) of subtidal decapod crustaceans after a period of emersion.

7.4.7 Glucose

Hyperglycaemia has been shown to occur as a result of handling stress (Telford, 1968), and has been observed in *P. cygnus* as the result of such stress (Dall, 1974*b*). It has also been associated with crustaceans subjected to anoxia or emersion induced asphyxiation (Johnson and Uglow, 1985; Taylor and Spicer, 1987). Haemolymph glucose is produced during the mobilisation of energy stores in anaerobic metabolism (Spicer *et al.*, 1990). Anaerobic metabolism requires an increased supply of substrate due to the low levels of energy produced compared to aerobic metabolism (Eckert *et al.*, 1988). The effect on *P. cygnus* of handling and emersion for 30 minutes was to increase glucose concentrations up to twice that of controls (Chapters 6 and 8). Haemolymph glucose in emersed subtidal crustaceans tends to increase steadily over time, with levels of 1.3 mmol/l reached after 4 hours in *Liocarcinus puber* (Johnson and Uglow, 1985), 1.5 mmol/l after 18 hours in *Nephrops norvegicus* (Spicer *et al.*, 1990), 1.5-2.5 mmol/l after 6-8 hours in *P. cygnus* (Spanoghe, 1997), and 1.6 mmol/l after 8 hours in *N. norvegicus* (Schmitt and Uglow, 1997*a*). The absolute levels in this study after 6 hours emersion of 2-3.5 mmol/l were comparatively high but probably reflect the elevated temperature and resultant, higher metabolic rate. The high glucose concentration in lobsters subjected to spray again suggests that lobsters in the spray did not maintain oxygen uptake and they relied on anaerobic processes for much of their energy requirements. Glucose concentrations decreased to control levels within 24 hours as would be expected based on the time period of recovery recorded in Chapter 6 and by Spanoghe (1997).

7.4.8 Ammonia

There was no increase in the haemolymph ammonia concentrations of lobsters in the spray treatment. Ammonia is released to the external environment via the gills by diffusion and by Na^+/NH_4^+ exchange across the epithelium (Kormanik and Cameron, 1981; Regnault, 1987). In the absence of water, such mechanisms may be impaired, and the accumulation of ammonia in the haemolymph may occur (Schmitt and Uglow, 1997*a*). Ammonia is able to be excreted into the branchial water of *Cancer pagurus* during emersion, although only in small amounts (Regnault, 1994); ammonia still accumulated in the haemolymph (Regnault, 1992). There was no water being sprayed over the crabs during that study, and branchial water had no chance of being renewed. Therefore, the branchial water may have become saturated with ammonia and the resulting concentration barrier may have limited the excretion of ammonia from the haemolymph. Although the excretion of ammonia against a concentration gradient has been shown to occur in crustaceans (Kormanik and Cameron, 1981), it generally cannot match the inward diffusion of ammonia from environments with high ammonia concentrations (Young-Lai *et al.*, 1991; Chen *et al.*, 1993). In this study the use of a spray system may have resulted in the continuous renewal of branchial water, thus permitting the removal of ammonia from the haemolymph during the emersion period. This is in direct contrast to the other treatments where haemolymph ammonia increased significantly during the emersion period indicating that ammonia was unable to be excreted.

The concentration of ammonia in the haemolymph after the emersion period (10-14 mg/l) was similar to that in *P. argus* after only 2 hours of emersion at ≈ 26°C (Vermeer, 1987) and in *C. pagurus* after 18 hours of emersion at ≈ 17°C (Regnault, 1992). Although there are considerable interspecific differences in haemolymph ammonia concentrations (Florkin, 1960) much of the variation in the rate of ammonia accumulation observed in the three species can probably be explained by the environmental conditions in each of the studies (ie. temperature variations).

The effect of the high ammonia concentration on *P. cygnus* is unknown, but crustaceans, in general, appear to have a high tolerance to high haemolymph ammonia levels. The American lobster, *H. americanus*, was able to tolerate environmental ammonia concentrations of 100 mg/l, which equate to blood ammonia concentrations of ≈ 35 mg/l (Young-Lai *et al.*, 1991), significantly higher than

recorded in this study. The land crab, *Cardisoma* carnifex, was able to tolerate haemolymph ammonia levels of ≈ 100 mg/l during dehydration (Wood *et al.*, 1986). Nitrogenous end products also appear to have a negligible role in blood acid-base balance (Regnault, 1992).

7.4.9 Oxygen consumption

The significant decrease in haemolymph pH and increases in haemolymph lactate and glucose indicate that the lobsters were suffering from hypoxia during emersion. Upon re-immersion, oxygen consumption by lobsters in all treatments (except for low RH/wind) was the same as the active rate of *P. cygnus* at 23°C (Chapter 4). This suggests they were servicing a significant "oxygen debt" (Herreid, 1980) during the initial stages of re-immersion. However, lobsters exposed to low RH/wind had limited ability to uptake oxygen after re-immersion or after 24 hours of recovery. Although the lobsters were not able to uptake maximum rates of oxygen, they did not show any reliance on anaerobic metabolism 24 hours after re-immersion. The decreased ability to uptake oxygen may have resulted from:

(a) a limited supply of oxygen to the gills - although not recorded, the scaphognathite rate 'appeared' to be much slower than normally observed in lobsters actively uptaking oxygen, which indicates that the supply of oxygen to the gills may have been reduced. Decreased pumping rate would probably be associated with the poor condition of the lobsters.

(b) limited diffusion of oxygen across the gills - it has been suggested that gill damage caused by dehydration contributes to the documented mortality in emersed *P. cygnus* (Anon., 1980*b*). Such damage would act to limit the diffusion of oxygen across the gills. There was no evidence that damage to the gills had occurred in this study. The gills of severely dehydrated lobsters were wrinkled, probably because the volume of haemolymph flowing through them was severely reduced. However, the gills were still reasonably moist after the emersion period, even when the lobsters were close to death. Other soft tissue parts of the lobsters had a similar wrinkled appearance after the emersion period but were very dry to touch. Gills surfaces appear to retain

some moisture even when lobsters are very desiccated. Additional gill functions, such as ionic exchange with the medium, which appear to have been maintained after re-immersion, also indicate that gill damage may not have occurred.

(c) limited perfusion of the gills with haemolymph - as mentioned previously, death of crustaceans due to weight loss is probably the result of increases in the viscosity of the haemolymph causing problems with circulation and oxygen delivery (Taylor *et al.*, 1987; Samet *et al.*, 1996). Upon re-immersion the high haemolymph osmolality could have limited the ability of lobsters to perfuse the gills with haemolymph. However, this possibility would not explain the oxygen consumption being reduced after 24 hours re-immersion, when the haemolymph osmolality was low.

The above lobsters were in very poor condition after the emersion period and continued to die during the re-immersion period. Those which survived were still in poor condition 24 hours after re-immersion. Vermeer (1987) found that the escape behaviour (tailflick response) of *P. argus* was impaired 24 hours after a 2 hour period of emersion. Tailflicking is a basic reflex required by lobsters as it is an important survival tool. Any treatment which affects the basic tail flick response may also affect integrated nervous system functions such as feeding, locomotion, and social and sexual behaviour (Vermeer, 1987). The moult increments of lobsters (*P. cygnus*) which had been subjected to emersion, decreased in accordance with the time period of emersion (Brown and Caputi, 1986). Nervous tissue of subtidal osmoconformers appears particularly sensitive to fluctuations in osmotic and/or ionic concentrations of body fluids (Treherne, 1980).

Lobsters needed to be in very poor condition before there was a reduction in the ability to uptake oxygen. Some lobsters (high RH/wind and low RH/no wind treatments) could uptake oxygen at maximal rates upon re-immersion yet still had not recovered full condition 24 hours later, which suggests that emersion was creating problems unassociated with the respiration process. Vermeer (1987) suggested that nervous system damage induced by hypoxia, acidosis, and perhaps osmotic imbalances is likely the cause of the behavioural aberrations in immersed spiny

lobsters after a period of exposure. Lobsters showing a reduced ability to uptake oxygen may have suffered from such nervous system damage.

7.4.10 Haemolymph ions

The haemolymph ion concentrations were very similar to concentrations measured in *P. cygnus* by Dall (1974*a*). Regulation of the haemolymph ion concentrations appears to occur to a similar extent in *P. cygnus* as in most subtidal crustaceans: sodium and calcium have slightly elevated concentrations compared to the external medium, potassium has the same or slightly elevated concentration and magnesium has between 20 and 80% of the concentration (Mantel and Farmer, 1983). In active crustaceans, magnesium is usually maintained at concentrations less than 50% of that found in the medium (Mantel and Farmer, 1983). Magnesium concentrations of *P. cygnus* in this study were about 25% of normal seawater concentrations.

Lobsters emersed in spray had [K^+] levels far lower than predicted due to the dilution effect of decreased haemolymph osmolality. The potassium ions may have been shifted into other tissues or excreted. Burnett and McMahon (1987) found that base (eg., Na^+, Mg^{++}, Ca^{++} and K^+) was excreted into the branchial water stores of three littoral crab species when they were emersed and suggested that it was an important method of removing CO_2 from the haemolymph. However, the process only appears available to species which are osmoregulators and thus have branchial ion pumps (Burnett and McMahon, 1987). Branchial ion-exchange mechanisms may be responsible for maintaining haemolymph acid-base status (Burnett, 1988). Such mechanisms are not generally available to emersed subtidal crustaceans, but emersion in a water spray may create the possibility for these mechanisms to occur. The large (but not significant) decrease in Na^+ concentration in these lobsters also suggests that base excretion may be occurring.

Magnesium concentration increases were large and were independent of the degree of dehydration in all non-spray treatments. Magnesium has been used for decades as a narcotising agent in the immobilisation of marine invertebrates (Morritt and Spicer, 1993). It is an anaesthetic that depresses neuromuscular transmission by competing with Ca^{++} for binding sites (Sartoris and Pörtner, 1997*b*). For crustaceans to achieve a high level of activity, [Mg^{++}] is usually held at low levels in the

haemolymph, which requires active regulation (Sartoris and Pörtner, 1997*a*). In decapod crustaceans, Mg^{++} is excreted via the antennal gland (Cornell, 1979). There are several potential benefits to the lobsters for actively increasing [Mg^{++}]. Elevated [Mg^{++}] may be an adaptation in crustaceans allowing them to undergo prolonged periods of inactivity or to recover from environmental or metabolic stress (Sartoris and Pörtner, 1997*a*). Also, a high concentration of Mg^{++} increases the affinity of haemocyanin for oxygen (Mangum, 1983) and could therefore improve oxygen uptake in emersed lobsters. Magnesium is found at high concentrations intracellularly (Morritt and Spicer, 1993), which is the most likely source for the extra Mg^{++} found in emersed lobsters. Lobsters in the spray treatment were in the best condition after the emersion period, displaying high levels of activity when handled. This finding correlates well with the low haemolymph [Mg^{++}] measured in these lobsters. After 24 hours re-immersion lobsters which had been subjected to low RH/wind were in the worst condition, and maintained the highest [Mg^{++}]. These results further highlight the clear relationship between haemolymph [Mg^{++}] and the level of activity in crustaceans (Morritt and Spicer, 1993).

Calcium concentration increases also occurred in all non-spray treatments. It is unclear whether the increases in [Ca^{++}] are due to physiological processes or osmolality changes associated with desiccation. However, when lobsters were subjected to high RH/no wind there was no osmolality increase which could account for the observed increase in [Ca^{++}]. Similar [Ca^{++}] increases have been noted in various subtidal crustaceans subjected to emersion (deFur *et al.*, 1980; Taylor and Whiteley, 1989; Waldron, 1991), and have been used as an indication of the mobilisation of bicarbonate from the calcified exoskeleton to counteract respiratory acidosis (Truchot, 1990). Spanoghe (1997) observed an increase in the [Ca^{++}] of emersed *P. cygnus* and also suggested that a compensation process was occurring. In the present study, the observed acidosis after 6 hours of emersion was only slightly lower than that occurring after 30 minutes of emersion (Chapters 6 and 8), indicating that some mechanism is being used to arrest the acidosis. Lobsters in the spray treatment did not suffer from acidosis or [Ca^{++}] increase. Cameron (1985) found that external seawater was a much greater source of acidosis compensation than carapace carbonate in the blue crab, *Callinectes sapidus*. Similarly, when emersed *P. cygnus* was able to access an external seawater source it did not need to rely on the

mobilisation of bicarbonate from the calcified exoskeleton to counteract a respiratory acidosis.

Haemolymph ions had, in general, returned to control levels after 24 hours re-immersion, indicating that ionic exchange mechanisms were functioning correctly.

Conclusions: the benefits of spraying water over emersed crustaceans has not been clearly demonstrated in the literature (Paterson *et al.*, 1994*b*). In this study, there was considerable improvement in the condition of the lobsters in the spray treatment. Acidosis, which is a common feature of subtidal crustaceans exposed to air, was abated, and ammonia buildup and desiccation were prevented. These factors are considered to be major determinants of the health of emersed crustaceans and the prevention of such physiological changes would probably ensure that the lobsters would, at least in the short term, maintain their condition. However, the spray did not prevent an increase in lactate or glucose and it resulted in the uptake of water, a decrease in osmolality and significant changes in haemolymph ions. The short term changes in these factors may not be health threatening, however continuous emersion in a spray may increase their importance. Thus, although sprays would appear to be "common sense" (Paterson *et al.*, 1994*b*) they may be creating "secondary" physiological changes which are causing health problems. McLeese (1965) found that seawater sprays did not improve survival of *H. americanus* during an experiment run over an extended time. Survival may have been compromised as the result of "secondary" physiological changes. The benefits and problems associated with holding lobsters in spray systems for extended periods need to be further addressed.

Another reason for the observed differences between studies (McLeese, 1965; Varley and Greenaway, 1992; Paterson *et al.*, 1994*b*; Schmitt and Uglow, 1997*a*) in determining the usefulness of sprays may relate to the amount of water actually passing over the crustaceans (Paterson *et al.*, 1994*b*). This point was clearly demonstrated during this study when two lobsters in the spray treatment moved out of the spray system. Although kept damp, they did not have the full impact of the spray and the pHs recorded indicate that these two

lobsters did not benefit. It appears for a spray system to be effective the water needs to impact directly on the lobsters.

The implications of these results for the western rock lobster industry will be discussed in Chapter 9.

CHAPTER 8

The use of time and temperature to control emersion and handling stress in the western rock lobster, *Panulirus cygnus*

8.1 INTRODUCTION

The live export of western rock lobsters, *Panulirus cygnus*, involves chilling the lobsters, and placing them into a packaging material (usually wood shavings or wood wool) in foam cartons, before they are air-freighted to the final destination. Chilling of the lobsters prior to packing is designed to achieve several objectives: lobster activity decreases, oxygen consumption decreases, and physiological disturbances associated with emersion decrease. Two methods are used to chill crustaceans in preparation for live transport - the slow-chill method and the quick-(or dip) chill method, which is the one most commonly used in the western rock lobster industry.

Observations at a *P. cygnus* processing shed showed that lobsters may be emersed for up to 30 minutes before being dip-chilled in preparation for packing into export cartons. During the emersion period the lobsters were very active (much tail-flicking occurred) and were subjected to handling. In some cases they were also exposed to sunlight and to high air temperatures. Such treatment would not ensure the lobsters are in the best possible physiological and biochemical condition to survive the transport period. This study examines the effect of pre-packing conditions on lobsters and investigates the use of alternative strategies which aim to minimise the physiological and biochemical disturbances imposed on lobsters during pre-export conditioning.

8.2 MATERIALS AND METHODS

General Materials and Methods are as outlined in Chapter 2. Lobsters weighing between 383 and 506 grams, of both sexes, were used in this series of experiments. All lobsters were starved for 36 hours prior to being used in an experiment. The lobsters were subjected to three experiments:

(i) The effect of a three minute dip in 12°C on blood parameters was tested on lobsters immediately after moving them from the holding tank at 23°C and after a 30 minute period of stress (emersion and handling). Six control lobsters (haemolymph sampled without subjecting the lobsters to stress) were sampled.

(ii) The effect of a 15 minute and a 30 minute stress on blood parameters of 6 lobsters. Six control lobsters (haemolymph sampled without subjecting the lobsters to stress) were sampled.

(iii) The effect of a 30 minute stress at 6 different temperatures (11, 15, 19, 23, 27, and 31°C) on the blood parameters of 6-11 lobsters. The lobsters were placed into a chilling tank at 23°C and the temperature was increased or decreased to the appropriate temperature at a rate of 2°C per hour. The lobsters were left overnight at the test temperature before testing the following morning. Six control lobsters (haemolymph sampled without subjecting the lobsters to stress) were sampled at each temperature.

Statistical analyses

The Students t-test was used to test for differences between the control lobsters and the treatment lobsters. Where appropriate a Students t-test for samples with unequal variances was used. One-way ANOVAs were used to test for differences where multiple treatments were used (eg. between the control lobsters at each temperature or the effect of emersion time on haemolymph parameters). The Levene test was used to test for homogeneity of variance and where necessary an appropriate transformation was performed before further analysis. Comparisons of means following ANOVA was done using the Tukey-HSD test. Differences were considered significant if $P<0.05$.

Linear regressions were obtained by the least squares method and were tested for significance of regression by analysis of variance of the regression. All

analyses were performed on the SPSS statistical package with the α set at 0.05. All means are expressed as mean±SE.

8.3 RESULTS

After 30 minutes of stress and 3 minutes dip-chilling the lobsters had significantly higher ($P<0.05$) haemolymph lactate and glucose concentrations and significantly lower ($P<0.001$) haemolymph pH than the controls(Fig. 8.1). Most of the changes in these blood parameters occurred during the 30 minute stress period although all blood parameters continued to digress further from the control levels during the 3 minute dip-chilling period. Removing lobsters from a 23°C holding tank and placing them directly into a 12°C dip-chilling tank for 3 minutes did not result in any significant changes in the blood parameters (Fig. 8.2).

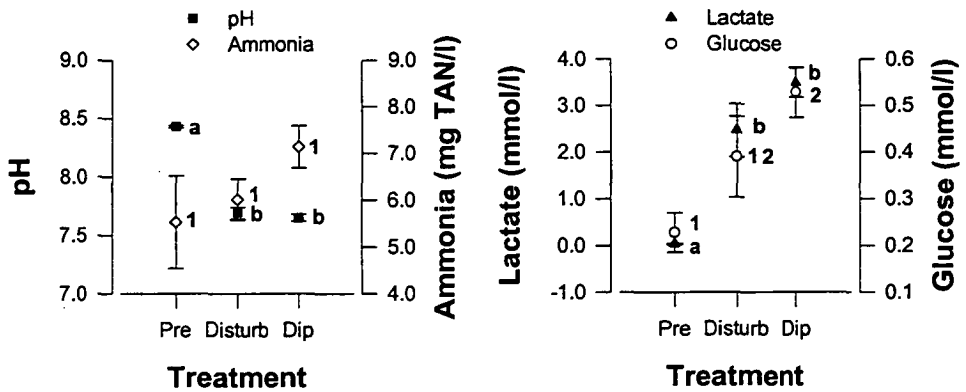

Figure 8.1: The effect of stress followed by 3 minutes dip-chill (dip) in 12°C water on the haemolymph pH, and haemolymph ammonia, lactate and glucose concentrations of the western rock lobster, *Panulirus cygnus* (n=6). The lobsters were in 23°C water prior the 1/2 hour emersion period.

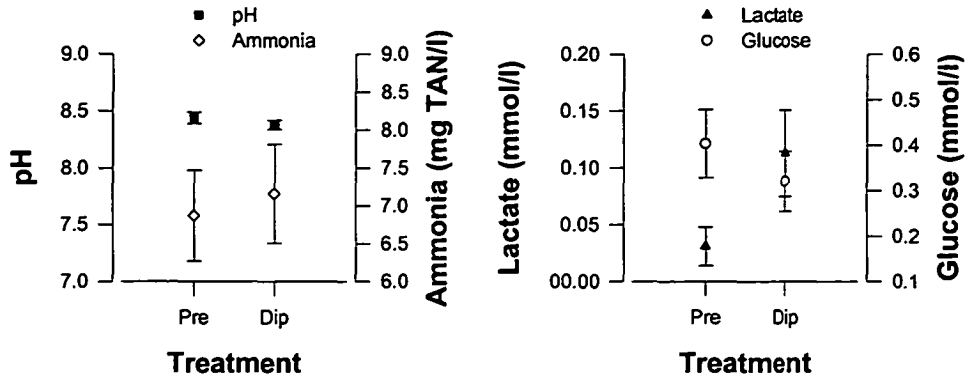

Figure 8.2: Haemolymph pH and haemolymph ammonia, lactate and glucose concentrations of the western rock lobster, *Panulirus cygnus*, before and after a 3 minute dip-chill (Dip) in 12°C water (n=6). The lobsters were transferred from a 23°C holding tank directly into the chilled water.

After 15 minutes stress the haemolymph pH and haemolymph lactate concentration were significantly different ($P<0.05$) to the control levels (Fig. 8.3). After 30 minutes stress all of the blood parameters were significantly different ($P<0.05$) to control values. However, none of the 30 minute blood parameters had changed significantly from the 15 minute stress values. Haemolymph ammonia and glucose concentrations increased steadily over the 30 minute emersion period, while pH and lactate showed the greatest changes in the first 15 minute emersion period.

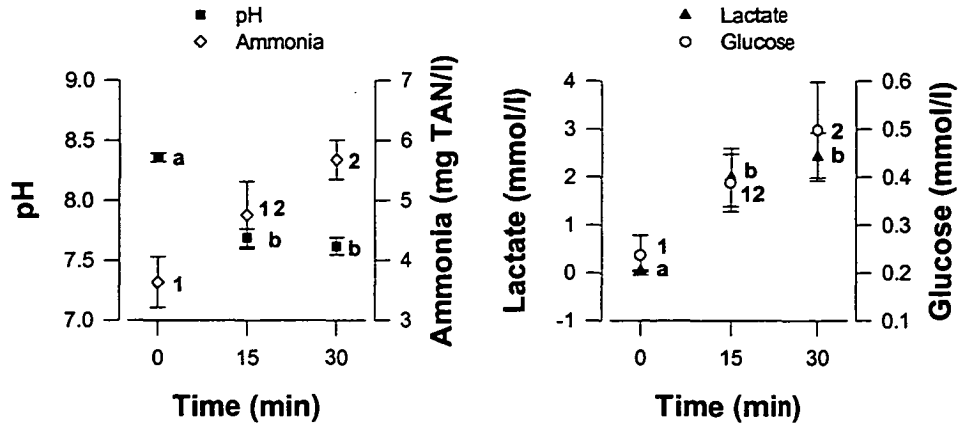

Figure 8.3: The effect of 15 and 30 minutes emersion and handling on the pH, and ammonia, lactate, and glucose concentrations of the haemolymph of the western rock lobster, *Panulirus cygnus* (n=6).

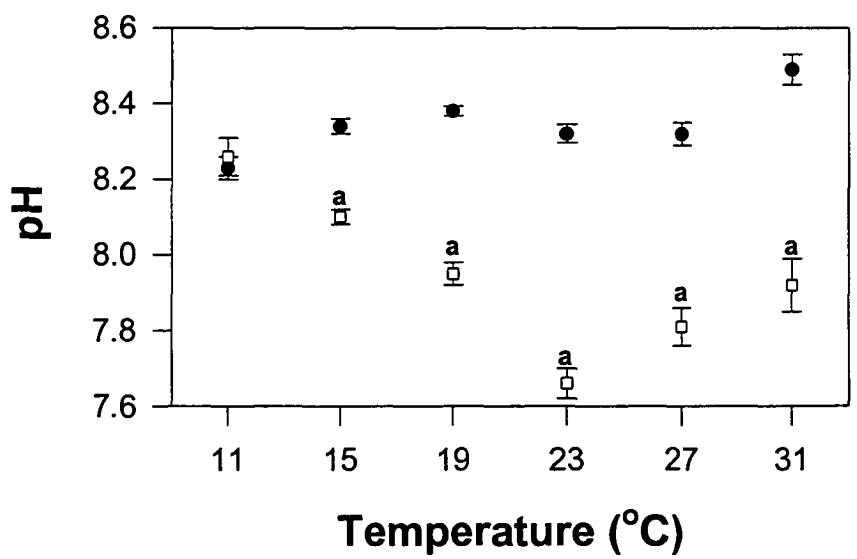

Figure 8.4: The effect of emersion and handling at various temperatures (slow-chill) on haemolymph pH of the western rock lobster, *Panulirus cygnus* (n=6-11). Control (●), Post-stress (□). The letters denote significantly lower pH values for the stressed lobsters compared to the control lobsters.

The haemolymph pH of lobsters subjected to 30 minutes stress after a period of slow-chilling, was significantly ($P<0.001$) lower than that of the control lobsters at all temperatures except for 11°C (Fig. 8.4). The maximum decrease in pH occurred at 23°C; the magnitude of the change reduced at higher and lower temperatures. The pH at 23°C was significantly lower ($P<0.05$) than all other pH values after the stress period. The pH of lobsters prior to the stress period was significantly lower ($P<0.05$) at 11°C and significantly higher ($P<0.05$) at 31°C.

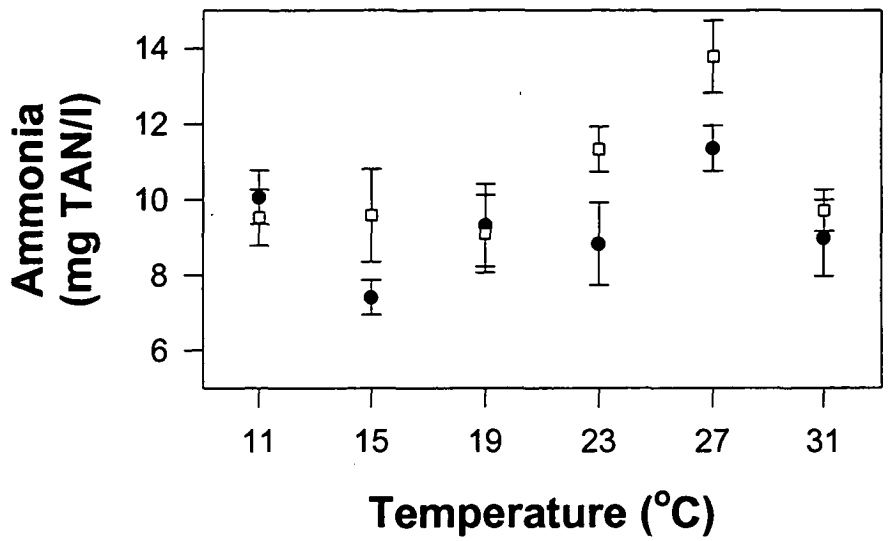

Figure 8.5: The effect of emersion and handling at various temperatures (slow-chill) on haemolymph ammonia of the western rock lobster, *Panulirus cygnus* (n=6-11). Control (●), Post-stress (□).

Haemolymph ammonia concentrations after 30 minutes stress were not significantly different ($P>0.05$) to the control concentrations in any of the treatments (Fig. 8.5).

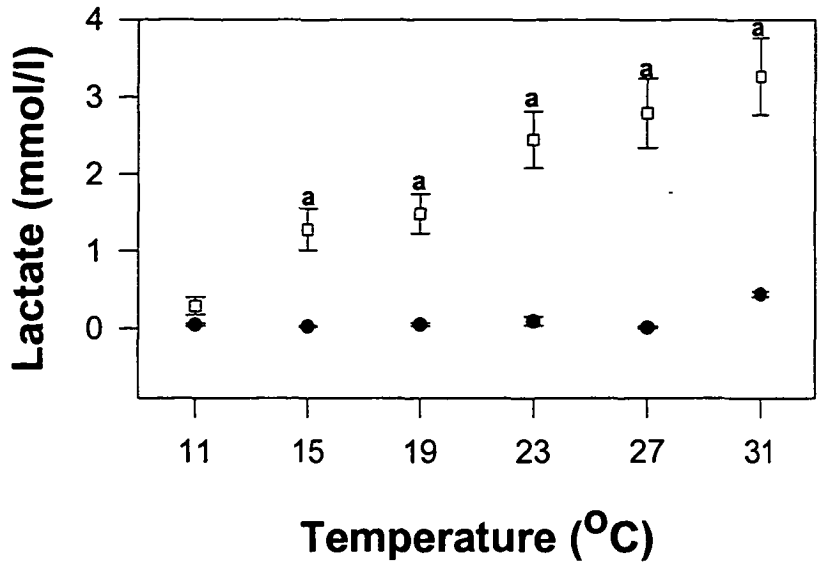

Figure 8.6: The effect of a period of stress at various temperatures (slow-chill) on haemolymph lactate of the western rock lobster, *Panulirus cygnus* (n=6-11). Control, (●) Post-stress (□). The letters denote significantly lower pH values for the stressed lobsters compared to the control lobsters.

Haemolymph lactate of the lobsters increased significantly ($P<0.01$) after a period of stress at all temperatures except for at 11°C (Fig. 8.6). There was a significant ($F=137.2$, $P<0.001$) correlation between temperature and lactate concentration after stress. The relationship could be described by the following equation:

Lactate (mmol/l) = 0.145 Temperature (°C) - 1.14 ($r^2=0.97$)

The lactate concentration of the control lobsters was significantly higher ($P<0.001$) at 31°C than at all other temperatures.

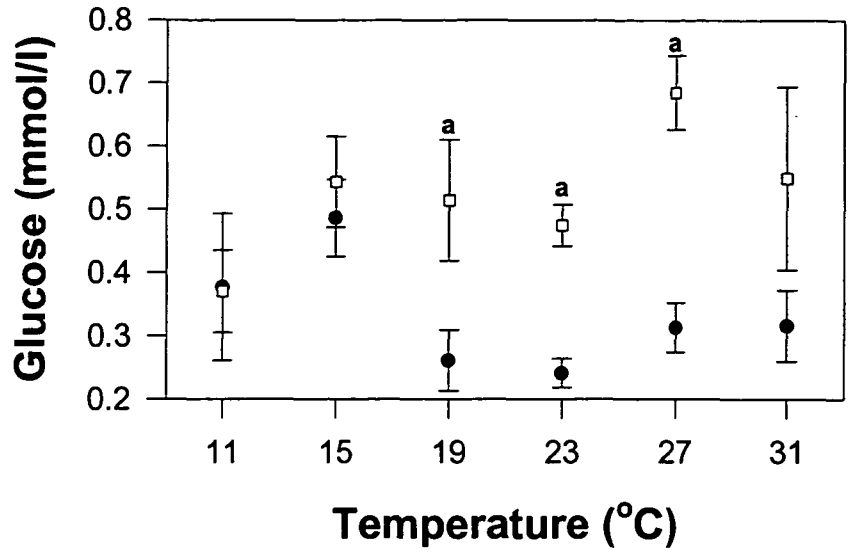

Figure 8.7: The effect of a period of stress at various temperatures (slow-chill) on haemolymph glucose of the western rock lobster, *Panulirus cygnus* (n=6-11). Control, (●) Post-stress (□). The letters denote significantly lower pH values for the stressed lobsters compared to the control lobsters.

Haemolymph glucose was significantly higher ($P<0.05$) after a period of stress at 19, 23 and 27°C (Fig. 8.7).

8.4 DISCUSSION

Emersion and exercise in crustaceans are generally reflected by internal hypoxia (deFur *et al.*, 1988; Varley and Greenaway, 1992), a pronounced respiratory and metabolic acidosis due to a rise in haemolymph CO_2 and lactate (Truchot, 1975; Taylor and Wheatly, 1981), hyperglycaemia (Telford, 1968; Santos and Keller, 1993) and a build-up of metabolic by-products such as ammonia (Schmitt and Uglow, 1997*a*). The simulated live export procedure in this study elicited similar responses in *P. cygnus* and indicated that they were in a stressed condition after being dip-chilled. Most of the physiological disturbances

occurred during the initial 30 minute emersion period prior to dip-chilling. Therefore, the use of alternative strategies to minimise the disturbances to the lobsters physiology and biochemistry would appear necessary.

Reducing the time period over which the lobsters are emersed prior to dip-chilling is a logical method of decreasing the stress on the lobsters. However, halving the emersion time to 15 minutes did little to reduce the physiological and biochemical disturbances. Disturbances to the physiology and biochemistry occur rapidly in response to disturbance in crustaceans. In the blue crab, *Callinectes sapidus,* large haemolymph pH and lactate disturbances were observed after 2 minutes of exercise (Booth *et al.*, 1982). Lactate in the tail muscle of *Crangon crangon* increased significantly after only 10 seconds of exercise (Onnen and Zebe, 1983). Also, Vermeer (1987) noted a very quick decrease in the pH of the lobsters, *P. argus*, which escaped initial capture and performed tail-flicks and Truchot (1973) found that blood pH dropped rapidly 1-3 minutes after emersion. It was apparent in the present study that the control lobsters needed to be caught quickly and haemolymph samples taken immediately (within 20 seconds) to ensure pH and lactate levels were representative of resting lobsters. If the lobsters escaped the initial attempt at capture and performed 2 or 3 tail-flicks in their escape response then lactate levels generally measured between 0.5 and 1.0 mmol/l and the pH decreased by approximately 0.3 units (Crear, unpub. data). It appears that if the effects of emersion time on the physiology of *P. cygnus* are to be minimised then emersion time needs to be severely reduced. In the present study, the removal of lobsters from the holding tank and placement directly in the dip-chilling tank greatly decreased the extent of the physiological disturbances imposed on the lobsters.

The quick changes in haemolymph parameters suggests that (a) energy requirements are, at least partially, satisfied by anaerobic metabolism, and (b) there is a very quick release of lactate from the muscle tissue into the haemolymph. The use of anaerobic metabolism to satisfy the energy requirements during the escape response (rapid tail-flicking) or during the initial recovery period has been demonstrated in various crustaceans (Booth *et al.*, 1982; Onnen and Zebe, 1983; Head and Baldwin, 1986). There appears to be considerable interspecific differences in the fate of lactate produced in the muscle tissue. In *C.*

crangon very little of the lactate produced was secreted into the haemolymph (Onnen and Zebe, 1983). In *Leptograpsus variegatus* haemolymph lactate was considerably lower than total body lactate after a period of exercise (Greenaway *et al.*, 1992). However, in *Cherax destructor* a steady state between tail muscle and haemolymph lactate pools is reached quite rapidly (Head and Baldwin, 1986). There also appears to be a close correlation between the muscle lactate and haemolymph lactate levels in *P. cygnus* (Spanoghe, 1997). This study indicates that lactate is released from the tissues into the haemolymph rapidly in *P. cygnus*.

Lobsters recover quite quickly from handling stress (≈ 8 hours - Chapter 6) so the experimental protocol used to test the effectiveness of temperature (slow-chill method) at reducing emersion and handling stress, should have ensured that they were in a non-stressed condition, at least in terms of handling stress. However, stress created due to the temperature change is unknown. Complete acclimation to a change in temperature in decapod crustaceans may take weeks (McLeese, 1956; Rutledge, 1981). The responses to acute changes in temperature described in this study exclude any adaptive physiological changes that may have occurred following temperature acclimation.

Temperature affects the physiology of the lobsters even before they are emersed. The haemolymph pH and lactate increased in rested lobsters held at 31°C. The pH of crustaceans generally decreases in response to increases in temperature; it is postulated that the changes ensure the maintenance of a constant relative alkalinity (Howell *et al.*, 1973). The metabolic rate of *P. cygnus* increases with temperature (Chapter 4), whilst the availability of oxygen in the water decreases due to the temperature dependent decrease in the capacity of water to dissolve oxygen. Therefore, to supply sufficient oxygen to meet requirements, increased ventilation volumes are necessary. In *Homarus gammarus* the gill ventilation rate increased between 10 and 20°C to match the increase in M_{O_2} (Whiteley *et al.*, 1990). In *Muninda rugosa*, both the gill ventilation rate and the heart rate (and hence gill perfusion rate) increased as the temperature increased (Zainal *et al.*, 1992). Such increases in the gill ventilation and perfusion rates would assist the excretion of CO_2 across the gills, thus driving increases in haemolymph pH. Observations of the lobsters showed them to be very restless when held at 31°C. The aerobic scope for activity of *P. cygnus* is limited at that

temperature (Chapter 4) which probably means that there is a greater reliance on anaerobic metabolism to fund energy requirements, leading to the higher levels of lactate measured in resting lobsters at 31°C. The increased pH and lactate levels would both increase the oxygen affinity of haemocyanin (Taylor, 1981; Greenaway *et al.*, 1992), resulting in an increased uptake of oxygen at the gills. These changes would help to alleviate the decreased oxygen affinity of haemocyanin caused by the high temperature (Truchot, 1975) and help to maintain oxygen levels in the lobsters.

The pH of rested lobsters decreased at 11°C, which contrasts to that measured in various crustaceans where decreased temperature caused increases in pH (Truchot, 1983). The M_{O_2} of *P. cygnus* at 11°C is approximately 30% of the M_{O_2} at 23°C. This fact, together with the temperature dependent increase in oxygen availability at that temperature, should allow a significant reduction in the gill ventilation and gill perfusion rates. In *M. rugosa* both the heart rate and ventilation rate decreased as the temperature decreased (Zainal *et al.*, 1992). Thus, in *P. cygnus* held at low temperatures, excretion of CO_2 may not be optimised, and its concentration may increase in the haemolymph accounting for the decreased pH.

The physical response to handling at the various temperatures differed widely. At 19 and 23°C the lobsters could maintain the tail-flick response for an extended period. After about 3-5 minutes (> 50 tail-flicks) they were generally exhausted but could recover enough so that when they were handled some time later (5-10 minutes) could perform another extended period of tail-flicking. At 27 and 31°C the lobsters responded strongly to handling but were exhausted within a couple of minutes (<30 tail-flicks). They did not appear to recover from the initial exhaustion and further handling could generally elicit only a poor tail-flick response. At 15°C the lobsters were able to show quite an extended response to handling although the tail-flicks were not strong. At 11°C the lobsters only responded very weakly to handling (very weak tail-flicks if they did occur) and basically appeared to be in a state of immobilisation.

The physiological response of *P. cygnus* to emersion varied with the temperature. Chilling the lobsters to 11°C resulted in only minor physiological disturbances after the 30 minute period of stress. Although there was a slight

increase in lactate, pH remained high, which indicates that there was very little increase in the level of CO_2 in the haemolymph. The M_{O_2} of resting or active lobsters at 11°C is low (30% and 20% of resting and active M_{O_2} of lobsters at 23°C, respectively - Chapter 4) so the rate of CO_2 production over the stress period would be low. In lobsters held at 15, 19 and 23°C the physiological disturbances increased as the temperature increased. The level of CO_2 in the haemolymph of *H. gammarus* increased as the emersion temperature increased (Whiteley and Taylor, 1990; Whiteley *et al.*, 1990) probably due to the temperature dependent increase in aerial oxygen uptake (Thomas, 1954). Increased activity at the higher temperatures could also be influencing the physiological disturbances. The capacity of lobsters to be active at lower temperatures is limited. Similarly, there was no increase in the haemolymph lactate of the velvet swimming crab, *Liocarcinus puber*, when it was exposed to hypoxia at 10°C, although there was a significant increase at 13°C (Whyman *et al.*, 1985). The Q_{10} of active *P. cygnus* is 4.4 over the temperature range 11-23°C (Chapter 4). This value is well above the Q_{10} values of 2 to 3 normally expected in thermochemical reactions (Eckert *et al.*, 1988). Therefore, increased activity and extra demand by the muscle tissues for energy, as well as the direct effects of temperature on energy requirements and oxygen uptake capacity, probably account for increases in the physiological disturbances over the 11-23°C temperature range.

At 27 and 31°C the effect of the period of stress on acid-base balance decreased and the pH of the lobsters was higher than that measured at 23°C. This is despite the fact that haemolymph lactate levels are higher at these temperatures. Lactate decreases the haemolymph pH in crustaceans (McDonald *et al.*, 1979) but it does not appear to be the main component controlling the pH of *P. cygnus*. When *P. cygnus* are re-immersed after a period of emersion, the pH increases significantly even though lactate levels remain high, indicating that the excretion of CO_2 is crucial in determining the pH (Chapter 6). The relatively high pH in this study after emersion at 27 and 31°C indicates that the level of CO_2 in the haemolymph decreased at these temperatures. There are a number of factors which could explain the result.

(a) The ability to uptake oxygen at the higher temperatures may have decreased. The aerial oxygen consumption of *Petrolisthes eriomerus* decreased significantly at 30°C compared to lower temperatures although it had risen as the temperature increased from 10°C to 25°C (Stillman and Somero, 1996). Waldron (1991) noted a progressive decrease in the scaphognathite rate of *J. edwardsii* after a period of exercise during emersion, and suggested that it may have been related to the breakdown of acid-base regulation or to excess lactate accumulation. Lobsters which had been exercised during emersion could not maintain the same rate of oxygen consumption as lobsters which had not been exercised (Waldron, 1991). In the present study, the lobsters became exhausted much faster at the higher temperatures and did not appear to recover from the initial exhaustion during the stress period. Their ability to maintain a high gill ventilation rate may have been limited, resulting in a reduced oxygen uptake. Also, oxygen uptake at the gills would be reduced due to the high temperature and low pH, which decrease the oxygen affinity of haemocyanin (Taylor, 1981; Greenaway *et al.*, 1992). Part of the reduction in oxygen affinity would be offset by the high level of lactate which serves to increase the affinity (Booth *et al.*, 1982). Additionally, in subtidal crustaceans such as *Cancer productus,* cardiac output, and hence the gill perfusion rate, is considerably decreased in air (deFur and McMahon, 1984). Exhaustion at 27 and 31°C may further serve to decrease the cardiac output, thus limiting the rate of oxygen uptake at the gills.

(b) Oxygen demand by the lobsters may have been reduced. Although the general activity of the lobsters decreases after the initial period of escape behaviour, this appears to be due to exhaustion, rather than being an attempt to reduce oxygen demand. The very high lactate levels after the period of stress at 27 and 31°C also indicate that the lobsters are maintaining high energy requirements.

(c) Buffering of the acidosis may have been achieved at the high temperatures at a faster rate than at 23°C. In decapod crustaceans the major source of

bicarbonate used to buffer the blood pH is probably the calcified exoskeleton (deFur and McMahon, 1984; Truchot, 1990). The period of time this buffering system works over is extended, with only partial compensation for the acidosis occurring over 24 hours in *Carcinus maenas* (Truchot, 1975) and *Austropotamobius pallipes* (Taylor and Wheatly, 1981). In *P. cygnus*, some recovery of haemolymph pH occurred after 27 hours of emersion (Spanoghe, 1997). However, a continuous decline in pH was observed in *P. cygnus* during a 60 minute period of emersion (Spanoghe, 1997). Therefore, compensation would not be expected to have had a significant impact on the acidosis during the 30 minutes period of stress in this study.

Glucose probably increases as the result of the mobilisation of energy stores as a source of fuel for anaerobic metabolism (Spicer *et al.*, 1990), and it is well known that aerial exposure evokes a hyperglycaemic response in crustaceans (Johnson and Uglow, 1985). Norwegian lobsters, *Nephrops norvegicus*, kept on ice (0°C) had a much reduced glucose increase than lobsters maintained at 10°C (Spicer *et al.*, 1990). The authors suggested it may be due to the reduced activity and handling stress of lobsters maintained at the lower temperature. Similarly, in the present study, increases in haemolymph glucose levels were not observed at the lower temperatures, which indicates that the reduced activity of the lobsters may have reduced the requirement for additional energy during the period of stress.

Conclusion: it has been recognised for many years that the ability of lobsters to handle live transport is affected by their condition prior to the transport period (Chaisson, 1932). Whiteley and Taylor (1992) suggested that lobsters in poor condition will be more vulnerable to stressful situations occurring during transit, since further reductions in haemolymph pH could prove fatal to individuals already suffering a marked internal acidosis. Evans and Spanoghe (1993) suggested that post-harvest procedures which reduce haemolymph lactate levels of lobsters should prolong survival during transport. The results of this study indicate that the dip-chill method

effectively minimises the physiological disturbances imposed on *P. cygnus* only when the lobsters are taken directly from the holding tank and placed in the dip tank. The slow-chill method was very effective at reducing the physiological perturbations of handling when the lobsters were chilled down to 11°C. The implications to industry of the results of this study will be discussed in the General Discussion (Chapter 9).

CHAPTER 9

General discussion, conclusions and future studies

This study has examined in detail the effect of extrinsic and intrinsic factors on the rates of oxygen consumption and ammonia excretion of both the southern rock lobster, *J. edwardsii*, and the western rock lobster, *P. cygnus*. Further studies focussed on *P. cygnus* and examined the effect of temperature on the extent of physiological disturbances imposed by emersion and activity, as well as the level of oxygen required in the water to optimise recovery from those physiological disturbances. Finally, how the physiological disturbances caused by emersion were influenced by relative humidity and exposure to wind were investigated, along with the physiological benefits of using seawater sprays during emersion. The major findings in each area are discussed below, with emphasis on their application to the rock lobster fishing and processing industry.

9.1 OXYGEN CONSUMPTION (Chapters 3 and 4)

9.1.1 Temperature

Oxygen consumption of lobsters increases with water temperature, which is in direct contrast to the capacity of oxygen to dissolve in water. For example, at 21°C, resting *J. edwardsii* requires 137% more oxygen than at 13°C, however there is 16% lower capacity of water for oxygen at the higher temperature. Thus, a 183% higher water flow rate must be maintained at 21°C than at 13°C to ensure the water oxygen level is maintained at an adequate level (>80% saturated). Other problems, such as lobsters being more active and aggressive, and thus harder to handle at higher temperatures, mean that maintaining the water temperature at the lower end of the range for the particular species would be beneficial when holding lobsters.

Future studies: In this study the effect of acute temperature changes on oxygen consumption was investigated. However, crustaceans can take an extended period to acclimate fully to a new temperature regime.

Determining the oxygen consumption rates of lobsters as they acclimate to different temperatures would be of interest.

9.1.2 Body weight

Total oxygen consumption increases with body weight, however on a per weight basis, larger lobsters consume less oxygen than smaller lobsters. Lobster weight is commonly used as a means of separating lobsters at holding facilities. Based on oxygen requirements large lobsters can be maintained at a higher stocking weight than smaller lobsters. For example, a tank which could maintain 1000 kg of 450 g *P. cygnus* would be able to maintain 1289 kg of 2000 g *P. cygnus*. Where space limitations exist stock management procedures should ensure that larger lobsters are stocked at a higher weight density then smaller lobsters.

9.1.3 Diurnal rhythm

The oxygen consumption rate of lobsters increases during the night due to increases in the level of activity. On the other hand, the level of oxygen in water can decrease overnight as plants consume oxygen during darkness. This natural decrease in the dissolved oxygen concentration, coupled with an increase in oxygen consumption of the culture organism may decrease the dissolved oxygen concentration to levels at which the cultured organism may be stressed or even die (Du Preez *et al.*, 1992). Management practices should ensure that oxygen does not become limiting overnight.

9.1.4 Activity/handling/emersion

Lobsters can display maximum oxygen consumption rates even during routine activity, such as that associated with darkness. Once the activity ceases, for example, when the lights come on, oxygen consumption quickly returns to standard rates. However, the high oxygen consumption rates associated with excessive activity, such as that resulting from emersion and/or handling, are maintained for an extended period. High levels of oxygen need to be supplied to the animals over that

period. As lobsters will go through many post-capture processes which result in emersion, handling, and/or increased activity, then for much of the post-capture period they would have high oxygen consumption rates. To ensure that the supply of oxygen is not limited at any time during the post-capture process it needs to be delivered at a rate which would satisfy the lobsters active rate of oxygen consumption. Special attention should be given to minimising the periods of emersion/disturbance/handling that lobsters are subjected to.

9.1.5 Feeding

As baited pots are used to capture lobsters, there is every possibility that lobsters will have eaten just prior to being brought on board fishing boats. Only 12-15% of *J. lalandii* delivered to the processing plants had completely empty guts, indicating that the most of the animals had recently eaten (van Wyk *et al.*, 1986). It is therefore likely that the post-capture oxygen consumption rate of lobsters will be significantly higher than standard, due to the effects of specific dynamic action. This does not even account for the effects on oxygen consumption of increased activity, handling and/or emersion. The higher oxygen consumption rate associated with feeding would result in a lowering of the scope for activity of lobsters. Thus, there would be a slower repayment of any oxygen debts accumulated during the post-prandial period and a decreased portion of the energy budget would be available to cope with stressors imposed during the post-prandial period. American lobsters, *Homarus americanus*, held out of the water after being fed, survived for a considerably shorter period than lobsters which had been starved (McLeese, 1965). The higher oxygen consumption rate of the fed lobsters would certainly have contributed to the result. This further highlights the need to minimise the post-capture periods of emersion/disturbance/handling that lobsters are subjected to.

If lobsters are to be fed whilst they are being held then the holding facility managers need to ensure that sufficient water oxygen is supplied to meet the high rate of oxygen consumption associated with feeding.

Future studies: determine how the high oxygen consumption rate during the post-prandial period influences the extent of physiological disturbances caused by emersion/activity during that period.

9.1.6 Dissolved oxygen level

The high critical oxygen tension of active lobsters means that the oxygen tension of the water needs to be maintained at a high level to ensure that oxygen supply is not limiting the ability of lobsters to uptake oxygen. Measurements on board lobster boats showed that in certain situations the lobsters are being maintained in water where the oxygen tension is well below the critical oxygen tension. Such a situation would retard the rate of recovery of the lobsters from post-capture physiological disturbances, increasing the likelihood that lobsters will be subjected to further periods of disturbance before they have completely recovered from the original disturbance.

9.1.7 Species specific differences/similarities

In general, the oxygen consumption response of *J. edwardsii* and *P. cygnus* to the extrinsic and intrinsic factors examined in this study is similar. However, some species-specific differences were found. These include: (a) the standard M_{O_2} of *P. cygnus* is almost twice as high as *J. edwardsii* at each species "preferred" temperature. This is probably mostly a reflection of the higher "preferred" temperature of *P. cygnus*. (b) The aerobic expansibility of each species is similar which means that the aerobic scope for activity is twice as great for *P. cygnus*. (c) A high aerobic scope for activity of *P. cygnus* was able to be maintained over a significantly wider temperature range than for *J. edwardsii*. (d) The rate of oxygen consumption at night (ie. the increase above standard) of *P. cygnus* was almost twice that of *J. edwardsii*. The above differences probably indicate that *P. cygnus* is a more active animal than *J. edwardsii*. The higher oxygen consumption rate of *P. cygnus* needs to be taken into account when designing holding systems. However, in general, practices which seek to maintain the health of lobsters during post-capture processes do not need to be significantly different for each species.

9.1.8 Summary

Such a thorough understanding of how extrinsic and intrinsic factors affect oxygen consumption has not been evaluated for any other species of large decapod crustacean. Activity has the largest influence on oxygen consumption rate; holding systems which are designed to supply sufficient oxygen to satisfy the active rate of oxygen consumption will guarantee that environmental oxygen availability does not compromise the ability of lobsters to survive and maintain health in the post-capture environment. An industry application of some of the above information on *J. edwardsii* is shown in Appendix 4.

9.2 AMMONIA EXCRETION (Chapter 5)

Increasingly lobsters are held in systems which re-use the water (re-circulating systems). The successful design and operation of suitable water treatment units depends upon knowledge of the cultured animals' feeding behaviour, excretion patterns and tolerance to recycled water (Wickins, 1985). Ammonia is one of the most common pollutants found in intensively managed aquaculture systems, and can be toxic to crustaceans (Colt and Armstrong, 1981). In re-circulating systems ammonia is removed by the actions of bacteria living in a biological filter; if the filter cannot handle the ammonia load then ammonia can accumulate to dangerous levels. Feeding has the greatest influence on the ammonia excretion rate of *J. edwardsii* and *P. cygnus*. Live lobster holding systems are unique, in that lobsters are usually not fed during the time they are held. Therefore, during the period lobsters are held most would only be excreting endogenous levels of ammonia. This is especially true for the southern rock lobster where fishermen are at sea for up to ten days prior to their catch being transferred to live holding systems. If animals are delivered to the holding system soon after capture, then the use of a purging tank prior to placement in the recirculating system will ensure that overloading of the biological filter will be minimised, as ammonia excretion rates take less than 30 hours to return to endogenous levels after feeding.

The results show the similarity the ammonia excretion response of the two species to the various extrinsic and intrinsic factors examined in this study. As for oxygen consumption, the ammonia excretion of *P. cygnus* was generally twice as high as that of *J. edwardsii* under any particular factor. Eg. the ammonia excretion rate at the "preferred" temperature of each species.

The data for the ammonia excretion rate of *J. edwardsii* has been used to calculate the biological filter unit required to handle the load from 1000 kg of lobsters (Appendix 5).

Future studies: the determination of toxic levels of ammonia to lobsters and the effect of ambient ammonia concentration on oxygen consumption of lobsters.

9.3 RECOVERY (Chapter 6)

The maintenance of oxygen levels close to saturation have been shown to increase the ability of lobsters to rapidly recover from physiological disturbances. It is imperative that holding systems should be designed so that oxygen levels do not fall below 70-80% saturation at any time. This is relatively simple and inexpensive to achieve. The physiological problems associated with holding disturbed lobsters in water with low levels of oxygen were apparent, with slow recovery rates, and in the extreme case, death. Similar low oxygen levels are frequently measured in industry situations.

Future studies: determine the effect of further episodes of activity/emersion/handling during the recovery period on the extent of physiological disturbance and on the timecourse of recovery.

9.4 AERIAL TRANSPORT (Chapter 7)

The results of this study highlight that the best method of holding lobsters during transportation is to leave them fully immersed in top quality water. Tod and Spanoghe (1997) came to a similar conclusion when they investigated truck transport

of *P. cygnus*. Similarly, Schmitt and Uglow (1997*a*) suggested that the use of a system which kept *Nephrops norvegicus* at least partially immersed in running seawater was likely to improve its nitrogen excretion and reduce the occurrence of anaerobiosis and was to be strongly recommended. However, keeping lobsters immersed is not always practical from an industry perspective, therefore carrying them emersed but with seawater sprays appears to be a reasonably good alternative, and is a method which has been extensively used within the western rock lobster industry. Spray systems provide many benefits to *P. cygnus*, compared to being emersed without them. Although they serve to prevent the occurrence of some physiological changes associated with emersion, and decrease the rate at which some other physiological parameters decline, the lobsters still suffer physiological disturbances, which may be detrimental over an extended period of emersion.

It was obvious that the seawater spray had to impact directly on the lobsters to be beneficial. Some lobsters which had moved out of the direct path of the spray (still subject to splashing) showed physiological changes similar to lobsters which had been emersed without seawater sprays. Lobsters being transported on carrier boats from the Abrolhos Islands to Geraldton in Western Australia, are generally carried on the decks with seawater sprays. Although huge volumes of seawater are sprayed over the lobsters much of the water is channelled so that it not actually impacting in the lobsters. Channelling occurs partly due to the design of the basket lids, which commonly do not have enough void space, and partly due to the shear numbers of lobsters within each basket. The baskets are stacked on top of one another resulting in only the top one or two baskets of lobsters actually receiving large amounts of spray. To ensure the sprays are reaching the optimum numbers of lobsters, the density of lobsters needs to be decreased, and the design of the basket lids needs to be such that it does not impede the flow of water.

Future studies: the physiological effect of the longterm emersion of lobsters under spray systems. The effect of activity/disturbance whilst the lobsters are emersed.

9.5 TEMPERATURE AND STRESS (Chapter 8)

The capture and preparation process immediately prior to the chilling and packing steps induces an extraordinary amount of stress on the lobsters, making it questionable whether the animals would be in optimal physiological condition to endure being exported (Spanoghe, 1997). It has been shown in this study that the dip-chill method of preparing lobsters can effectively minimise the physiological disturbances imposed on *P. cygnus* prior to export, but only when the lobsters are placed directly in the dip tank from the holding tank. The requirement of getting lobsters out of a storage tank, sorted and weighed, and into the chill tank would preclude the process occurring quickly. Even a 15 minute period of emersion and handling results in large physiological disturbances. Furthermore, lobsters at ambient temperature are very active and difficult to handle, meaning the process of sorting and weighing can be difficult.

The benefit of cooling prawns (*Penaeus japonicus*) before preparing them for live export appears to be to allow them to be handled more conveniently due to a physiological shock (Paterson, 1993*a*). Similarly, *P. cygnus* was very easy to handle after a period of chilling, meaning the chances of lobsters being physically damaged during the handling process would be minimised. Additionally, the use of a slow-chilling decreased the extent of the physiological disturbances imposed on the lobsters by a period of emersion and handling, thus they were in the best possible physiological and biochemical condition to survive the transport period. In this study, slow-chilling lobsters to 11°C minimised the physiological disturbances resulting from emersion and handling, however the optimum temperature of chilling would probably change depending on the ambient temperature.

The possible physiological benefits brought about by alternative methods of pre-transport preparation, such as slow chilling, need to be weighed up against the economic costs of executing the processes. The benefits of slow-chilling may be minor compared to other factors causing major physiological disturbances during the export process. It must be noted that the export of live western rock lobsters is normally carried out successfully, with minimal losses, using the present dip-chill method of preparing the lobsters. This study outlines some possible problem areas of

the present dip-chilling practice and highlights methods which could be used to overcome those problems if they are found to become industry concerns.

Future studies: determine if the physiological benefits of slow chilling are able to be sustained throughout the export process.

9.6 CONCLUSION

This study has developed our understanding of the physiological responses of the southern and western rock lobsters to factors affecting them during post-capture processes, and will allow the design and management of rock lobster holding facilities to be based on a sound scientific basis. It also represents a major contribution to knowledge on respiration and nitrogen metabolism of large decapod crustaceans.

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APPENDIX 1

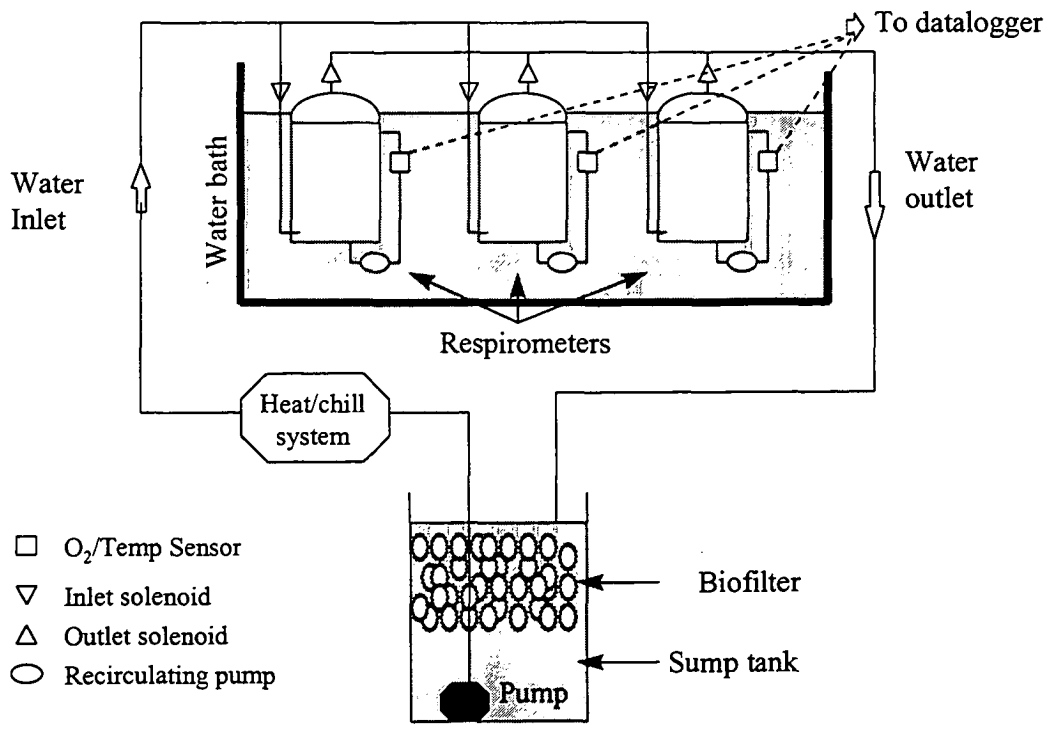
The respirometer system

Three respirometers were setup in parallel. The respirometers were primarily used in the intermittent flow mode however they could be used in the closed mode when required. The total volume of each chamber was 18.3 l. A submerged powerhead pump was used to ensure there was both good water mixing within each chamber and sufficient water flow past the membrane of the oxygen electrode.

Oxygen consumption (M_{O_2}) was determined from the equation:

$$Mo_2(mgO_2 / g / h) = \frac{(Po_2 i - Po_2 f) * V * 60}{W * t}$$

where $P_{O_2}i$ is the initial oxygen tension in the respirometer (mg/l); $P_{O_2}f$ is the oxygen tension after the measuring period (mg/l); V is the volume of water in the respirometer adjusting for lobster volume (l); W is the weight of the lobster (g); and t is the time of the measuring period (minutes).



APPENDIX 2

Haemolymph pH measurements

Comparison of the measurement of haemolymph pH of *P. cygnus* at 0°C to measuring it at ambient temperature (23°C)(Table 1). Lobsters were held at 23°C prior to taking the haemolymph samples. A 2 ml haemolymph sample was taken from each lobster: 1 ml was placed into an ice-cold eppindorf tube and 1 ml was placed into an eppindorf tube at 23°C. The chilled tube was placed into an ice-cold bath and the other tube was maintained at 23°C. The pH of the haemolymph at 23°C was measured immediately (with the pH electrode calibrated at 23°C). The pH of the chilled haemolymph was measured after the pH electrode was calibrated in ice-cold buffers.

Temperature	0°C	Ambient (23°C)
pH	8.38	7.79
	8.30	7.84
	8.31	7.60
	8.34	7.87
	8.41	8.00
	8.37	7.91
	8.24	7.84
	8.33	7.90
Average (± SE)	8.34 ± 0.02	7.85 ± 0.04

Table 1: The haemolymph pH of *P. cygnus* measured at 0°C compared to measurement at ambient temperature (23°C).

APPENDIX 3

Biochemical methodology

The procedures used for the analysis of haemolymph lactate, glucose and ammonia and water ammonia and urea are outlined below.

1. LACTATE ANALYSIS

Lactate concentrations were determined enzymatically using the Boehringer-Mannhein analysis kit (Cat. No. 139084). L-lactic acid is oxidised by nicotinamide-adenine dinucleotide (NAD) in the presence of L-lactate dehydrogenase (L-LDH) to pyruvate. The equilibrium of this reaction lies almost completely on the side of L-lactate. However, by trapping pyruvate in a subsequent reaction catalysed by the enzyme glutamate-pyruvate transaminase (GPT) in the presence of L-glutamate, the equilibrium can be displaced in favour of pyruvate and NADH. The amount of NADH formed in the above reaction is stoichiometric to the amount of L-lactic acid. The increase in NADH is determined spectrophotometrically as absorbance at 340 nm. The absorbance measured is compared against a calibration curve to determine the concentration of lactate present in the samples. All samples are run in duplicate.

REAGENTS

1. 0.6 M perchloric acid (PCA) - 50.8 ml of 70% PCA in 1litre deionised water
2. 3 M KOH
3. Reagents from Boehringer Mannheim test kit (No 139084) (Solution 1) Glycylglycine buffer, (Solution 2) NADH, (Solution 3) GPT suspension, and (Solution 4) LDH solution. Use the solutions to make the following two solutions (make to volume depending on the number of samples to be done).

   Solution A - 1000 ul buffer (**Sol. 1 - bring to 25°C before use**)+ 200 ul NADH (**Sol. 2**)+ 20 ul GPT (**Sol. 3**)+ 600 ul DDW

Solution B - 20 ul LDH (**Sol. 4**) + 400 ul DDW

4. Standards (using supplied standard solution)

Standard	Dilution	Conc (mmol/l)
1	1:19	0.111
2	1:9	0.222
3	1:5	0.37
4	1;3	0.555
5	1:1	1.11
6	no dilution	2.22

PROCEDURE

Extraction

(a) Add 250 µl haemolymph to 500 µl 0.6 M PCA, Vortex in Eppendorf. Can freeze (liquid nitrogen then at -86°C) at this time if haven't got time to do the runs.

(b) Sit on ice for 10 minutes.

(c) Centrifuge at 8000g for 3 minutes - stand on ice.

(d) Neutralise supernatant with 6.4 ul of 3 M KOH to 150 ul of supernatant.

(e) Vortex thoroughly and sit on ice for 15 minutes.

(f) Centrifuge at 8000g for 3 minutes and separate the supernatant (this settles out the precipitated perchlorate which interferes with the reading).

Assay

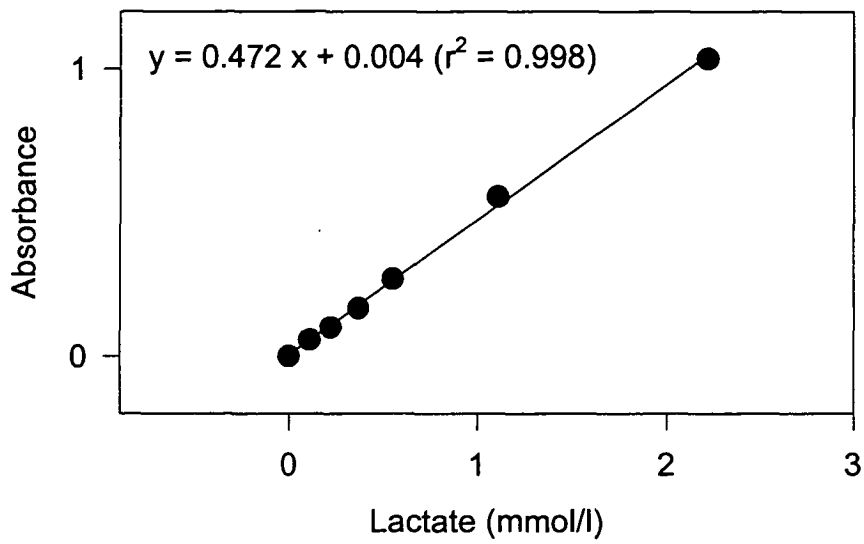
1. Add 900 ul of Solution A.
2. Add 100 ul of blank, standard or test solution.
3. Read at 340nm after 5 min (reading at this time was found to be unnecesary).
4. Add 200 ul of Solution B.
5. Read at 340 nm after 30 min.

The samples may need to be diluted to fall within the range of the standard curve.

CALIBRATION CURVE

A new calibration curve was made up for each sample run. A typical calibration curve is shown in Figure 1.

Figure 1: Lactate calibration curve obtained from standards



CALCULATIONS

Average blanks and subtract this value from all readings. Calculate the sample lactate concentration using the calibration curve. Multiply the result by 3 (for dilution with PCA) and by 156.4/150 (for addition of KOH). All samples are run in duplicate.

2. GLUCOSE ANALYSIS

Glucose concentrations were determined enzymatically using a Sigma glucose test kit (No. 510), which is based on the glucose-oxidase method. The sample is added to a mixture containing glucose-oxidase, peroxidase and o-

dianisidine. The final colour intensity is proportional to the glucose concentration. The absorption was measured at 450 nm on a GBC UV/VIS 916 spectrophotometer.

REAGENTS

1. Enzyme solution - add contents of 1 capsule of PGO enzymes (Sigma No. 510-6) to 100 ml distilled water in an amber bottle.
2. Colour reagent solution - reconstitute 1 vial of o-Diansidine Dihydrochloride (Sigma No. 510-50) with 20 ml distilled water.
3. Combined enzyme-colour reagent solution - combine 100 ml of enzyme solution with 1.6 ml of colour reagent solution.
4. Standards (using supplied standard solution)

Standard	Dilution	Conc (mmol/l)
1	1:99	0.0556
2	3:97	0.1668
3	6:94	0.3336
4	10:90	0.556

PROCDURE

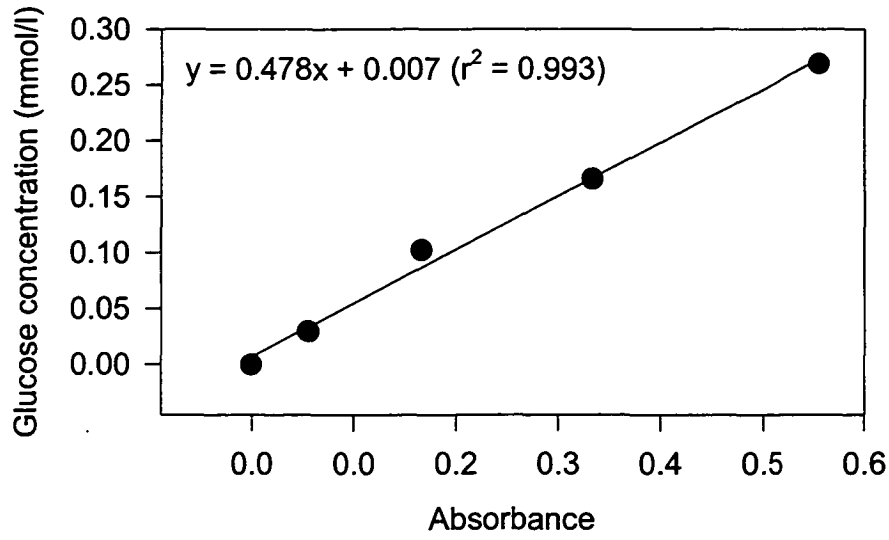
The supernatant obtained from the extraction procedure outlined in the lactate procedure (see above) is tested for glucose.

1. Add 100 ul of blank, standard and sample solution
2. Add 2 ml of combined enzyme-color reagent solution
3. Incubate at 37°C for 30 mins or at room temperature (18-26°C) for 45 minutes. (Avoid exposure to bright light)
4. Read at 425-475 nm using blank as a reference

CALIBRATION CURVE

A new calibration curve was made up for each sample run. A typical calibration curve is shown in Figure 2.

Figure 2: Glucose calibration curve obtained from standards



CALCULATIONS

Average blanks and subtract this value from all readings. Calculate the sample glucose concentration using the calibration curve. Multiply the result by 3 (for dilution with PCA) and by 156.4/150 (for addition of KOH).

3. WATER AMMONIA ANALYSIS

Ammonia was analysed by the phenol-hypochlorite method of Solarzano (1969). The following methods were adapted from Parsons *et al.* (1984) and Frith *et al.* (1993). Ammonia reacts with phenol and hypochlorite in alkaline solution to form indophenol blue. Sodium nitroprusside is used to intensify the colour at room temperature. The intensity of the colour produced is proportional to the concentration of ammonia present and is measured spectrophotometrically as absorbance. The absorbance measured is compared against a calibration curve to determine the concentration of ammonia present in the samples.

REAGENTS

(1) Phenol solution: 20g of phenol in 200ml of 95% ethanol.

(2) Sodium nitroprusside solution: Dissolve 1.0 g sodium nitroprusside in 200 ml of deionised water. Store in an amber bottle in the refrigerator. The solution is stable for at least one month.

(3) Alkaline reagent: Dissolve 100g tri-sodium citrate and 5 g NaOH in 500 mL deionised water.

(4) Sodium hypochlorite solution: Commercially available hypochlorite which should be about 1.5N. Keep in the dark.

(5) Oxidising solution: Mix a 4:1 ratio of solution of Reagent 3 and Reagent 4. This solution should be made up fresh before use and is stable for less than one day.

(6) Standards: To prepare a 100 mg/l as N standard (stock solution), add 0.0382 g reagent grade NH_4Cl to 50 ml distilled water in a 100 ml volumetric flask. Stir to dissolve and dilute to volume with distilled water. Prepare a 10 mg/l standard by pipetting 10 ml of the stock solution into a 100ml standard flask and making the solution up to mark with deionised NaCl (3.5%) solution. Prepare 0.3, 0.6, 1.0 and 2.0 mg/l standards by pipetting 1.5, 3, 5 and 10 ml of the 10 mg/l standard into 50 ml standard flasks and making up to the mark with deionised NaCl solution. A blank solution is comprised of the deionised NaCl solution. Use 0.0, 0.3, 0.6. 1.0 and 2.0 mg/l standards to create the calibration curve.

SAMPLES

Duplicate 15 ml water samples were taken at each sampling period and when these could not be analysed immediately, they were frozen at -15°C for a

maximum of 1 week; a time period which is well within the recommended maximum storage time of 2 weeks (Parsons *et al.*, 1984).

PROCEDURE

Take 15 ml of each standard and sample and add to the test tubes. To each of the standards and samples add the following.

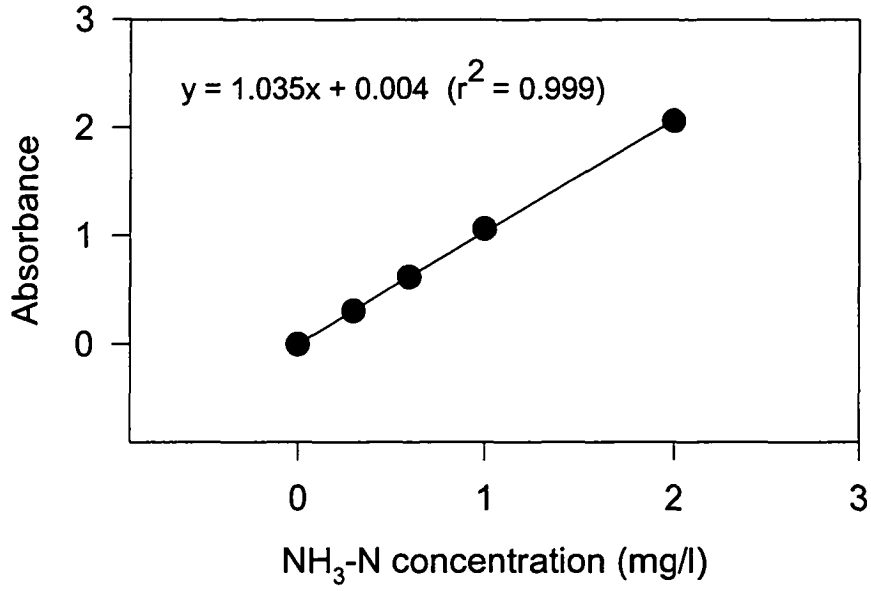
(a) 0.6 ml of phenol solution
(b) 0.6 ml of sodium nitroprusside solution
(c) 2.0 ml of oxidising solution

Mix the tube after each addition. Cover the tops of each tube and keep them in the dark for one hour at room temperature. The colour is stable for approx. 24 hours after the reaction period. At the end of this time measure the absorbance against a blank at 640 nm, using a 1 cm cuvette. Calculate the sample ammonia concentration using the calibration curve.

CALIBRATION CURVE

A new calibration curve was made up for each sample run. A typical calibration curve is shown in Figure 3.

Figure 3: Calibration curve obtained from standards



CALCULATIONS

Calculate the sample ammonia concentration using the calibration curve. This procedure estimates the total ammonia concentration. This is comprised of ionised (NH^+_4 -ammonium) and unionised (NH_3) ammonia. The proportion of unionised ammonia present depends on the pH and temperature of the water at the time of sampling. A table is available that allows you to calculate the level of unionised ammonia present in the sample.

RANGE AND PRECISION

Frith *et al.* (1993) recommends a range of 0.005-1.0 mg/l although concentrations of up to 2 mg/l still maintained a good calibration curve. Samples with higher concentrations were diluted to less than 2 mg/l. Frith *et al.* (1993) suggests that the precision (as relative standard deviation) is about 2.5% at 50 µg/l.

4. HAEMOLYMPH AMMONIA ANANYSIS

Ammonia needs to be determined on haemolymph samples that are not de-proteinised. Haemolymph ammonia concentrations were measured using a Sigma test kit (No. 640) for urea nitrogen which is based on the phenol/hypochlorite method of Solorzano (1969)(as outlined above). The addition of distilled water to the sample, in conjunction with keeping it ice-cold, prevented clotting of the haemolymph sample for over 3 hours. In comparison, ice-cold haemolymph remained unclotted for a maximum of only one hour. Dilution of the sample also ensured that the ammonia reading stayed within the range of the standards. The absorption was measured at 640 nm with a GBC UV/VIS 916 spectrophotometer.

REAGENTS

(1) Phenol nitroprusside solution
(2) Alkaline hypochlorite solution (Sodium hypochlorite 0.2%)
(3) Standards - make up 1, 2, 4 and 8 ug/ml standard with NH_4Cl (as outlined above in Water Ammonia Analysis).

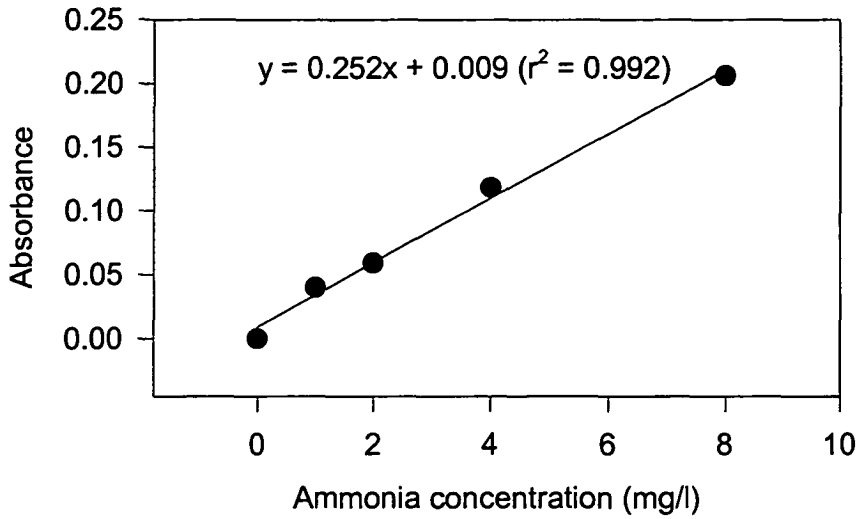
PROCEDURE

1. Add 1 ml phenol nitroprusside solution to each blank, standard and sample (mix the tube).
2. Add 1 ml alkaline hypochlorite solution (mix the tube).
3. Allow tubes to develop colour at room temperature for 20-30 minutes and measure at 640 nm (colour is stable for 1 hour).

CALIBRATION CURVE

A new calibration curve was made up for each sample run. A typical calibration curve is shown in Figure 4.

Figure 4: Haemolymph ammonia calibration curve for standards



CALCULATIONS

Calculate the sample ammonia concentration using the calibration curve. Multiply the result by two to allow for the dilution of ammonia by water.

5. WATER UREA ANALYSIS

Urea was analysed by the urease method of McCarthy (1970) as modified by Carter and Brafield (1991). This method involves the enzymatic hydrolysis of urea, by urease, to carbon dioxide and ammonia (Price and Harrison, 1987). The liberated ammonia is assayed by the ammonia method outlined above. This method also measures ammonia present prior to hydrolysis. Therefore, the difference between the ammonia concentration before and after urease treatment gives a calculation of the ammonia attributable to urea.

REAGENTS

1. Citrate buffer: 0.5M sodium citrate (to pH 7). 14.705 g in 100ml of distilled water.

2. Urease: 0.18 g/100 ml 0.5 M citrate buffer (100 IU/ml).
3. Other reagents for calculation of ammonia as outlined above

PROCEDURE

(a) Add 1 ml of buffered urease per 10 ml of sample
(b) Gently shake tube
(c) Incubate at 50°C for 50 minutes
(d) Let cool to room temperature
(e) Measure the ammonia concentration as outlined above. Use standards that have urease added and follow the same procedure as for the samples.

CALCULATIONS

The nitrogen present due to urea is obtained by subtracting the nitrogen present due to ammonia from the total nitrogen present due ammonia and urea.

6. REFERENCES

Frith, M., Forteath, N. and Wee, L., 1993. Appendix II. Chemical determination of selected water quality parameters. In: P. Hart and D. O'Sullivan (Editors), Recirculation systems: Design, construction and management. Aquaculture Sourcebook, Tasmania. pp. 111-128.

Parsons, T.R, Maita, Y. and Lali, C.M., 1984. A manual of chemical and biological methods for seawater analysis. Permagon Press, Oxford. 173 pp

Solorzano, L., 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr., 14:799-801.

APPENDIX 4

Oxygen consumption - application of the results to industry

The following is an abbreviated section of an article which appeared in Austasia Aquaculture (Crear, 1997). Some additional parts have also been added. It demonstrates how the information obtained on *J. edwardsii* oxygen consumption can be used in industry.

Temperature

The equation describing standard rates of oxygen consumption of *J. edwardsii* (Section 3.3) is able to be used to calculate the amount of oxygen required at any particular temperature (Table 1). The equation is:

Log M_{O2} (mg O_2/kg/h) = 0.047T - 2.25 (where T is temperature)

Temperature (°C)	M_{O2} (mg O_2/g/h)
5	0.010
9	0.015
13	0.023
17	0.035
21	0.055

Table 1: The oxygen requirements (mg O_2/g/h) of *J. edwardsii* at various temperatures (°C).

Oxygen consumption increases as the temperature of the water increases. Many holding systems are recirculating systems. Each operator has a preferential temperature but most systems are maintained between 9 and 13°C. Lobsters maintained at 13°C will require 50% more oxygen than lobsters maintained at 9°C. Some systems are flow-through and the temperature of the water can become considerably higher than 13°C. Lobsters consume over 50% more oxygen at 17°C than at 13°C.

The amount of oxygen able to be dissolved in water varies with the temperature: as the temperature increases the amount of oxygen decreases. When water has as much oxygen present as it is normally able to contain (that is, it is in

equilibrium with atmospheric oxygen), the water is defined as being 100% oxygen saturated. At 9°C fully saturated seawater contains 9.2 mg/l of oxygen whereas at 17°C it contains only 7.8 mg/l. Therefore, an increase in temperature causes both an increase in the oxygen requirements of lobsters and a decrease in the availability of oxygen. Cooler water has obvious advantages regarding supplying oxygen to the lobsters. Also, lobsters are less active and aggressive at cooler temperatures making them easier to handle.

Weight

Lobsters are usually graded, stored and exported on weight basis. Weight has a large influence on the requirements of lobsters for oxygen. Larger lobsters consume more oxygen; a 2000 g lobster requires over twice the amount that a 500 g lobster requires (Table 2). However, on a weight basis they consume less. Table 1 shows that 100 kg of 2000 g lobsters consume only 3/5th of the oxygen that 100 kg of 500 g lobsters consume. Therefore, you can maintain a higher total weight of larger lobsters in a tank compared to smaller lobsters.

Weight (g)	**Total oxygen cons. (mg/h)**	**Total oxygen cons. (mg/100kg/h)**
500	16.6	3320
2000	38.1	1905

Table 2: Oxygen consumption of 500 g and 2000 g lobsters on a per lobster and a per weight basis.

Handling

Handling and air exposure of lobsters are unavoidable parts of the fishing and processing procedure. The usual response of lobsters to such activities is to adopt an escape behaviour which is highlighted by the tail-flicking response. The increased activity causes an increase in oxygen consumption; lobsters will consume almost 200% more oxygen during periods of activity then when they are inactive. They can recover quickly from short periods of activity, however if there is prolonged activity and air exposure, it can take over 7 hours to return to normal consumption levels. It is important that sufficient oxygen is available to cover

periods of increased requirements. More importantly, practices need to be adopted which minimise the amount of handling and air exposure lobsters are subjected to.

Water oxygen level

The level of oxygen present in the water is a major factor in determining the ability of lobsters to use that oxygen. Lobsters are able to maintain their consumption rates as the oxygen level decreases via several physical and biochemical mechanisms. To do that efficiently there must be a certain level of oxygen present in the water. Our research has shown that there needs to be greater than 60% oxygen saturation to ensure that oxygen itself is not limiting the amount of oxygen being consumed by the lobsters.

Lobsters are able to survive much longer in air than in stagnant water. They have some ability to uptake oxygen from air, although this constitutes only about a third to a half of their requirements under normal conditions. Therefore, if they are exposed to air they will build up an oxygen debt but they are able to survive. However, once the oxygen is depleted in a tank of water then the lobsters aren't able to access any oxygen at all. If 100 kg of 700 g lobsters are being held in a static, un-aerated 1000 l tank at 13°C they would deplete the oxygen reserves in just over 3 hours. Therefore, alarms need to be present to provide a warning in the case of a electrical/mechanical failure of the pumping/aeration system. If alarms are not fitted then the tank should be self-draining to ensure the lobsters do not remain in stagnant water.

Information on lobsters oxygen requirements will assist in the design and management of holding systems, both on board boats and in processing sheds. Water flow rate requirements based on our information is outlined in Table 3. To ensure oxygen levels are nearly always adequate aeration should also be provided. Aeration is an easier and cheaper option compared to increasing water flow rates It has the added advantage of ensuring good water mixing which ensures that there are no dead spots into which weak lobsters can get shunted.

	9°C	**13°C**	**17°C**	**13°C + activity**
Water flow	435	735	1186	2000

(litres/100kg/hr)				

Table 3: The water flow requirements of 100 kg of 700 g lobsters at various temperatures when a minimum oxygen level of 60% is maintained. The calculations assume that there is no aeration and that the incoming water is 100% saturated. The requirement of active lobsters at 13°C is also shown.

Reference

Crear, B.J., 1997. Oxygen - an important requirement for holding live southern rock lobsters. Austasia Aquaculture, March/April 1997, pp. 69-70.

APPENDIX 5

Calculations on biological filter size

Lobsters are generally not fed when they are held in recirculating systems. Therefore, the endogenous rate of ammonia excretion can be used to calculate the necessary volume of a biological unit.

The total ammonia nitrogen excretion (TAN) rate of 500 g *J. edwardsii* at 13°C is 1 μg/g/h. Therefore, 1000 kg of lobster will excrete 24 g of TAN per day. The specific nitrification surface area (SSA) refers to the total exposed surface area of the substrate in the filter or the area on which the bacteria can grow. The SSA is calculated by the following formula:

SSA = ammonia production rate / nitrification rate

The daily ammonia oxidation rate of a well conditioned submerged filter at 20°C is 0.55 g TAN/m^2/day (Kikuchi *et al.*, 1994). At 13°C the rate would be expected to be considerably lower than that. Therefore, a rate of 0.40 g TAN/m^2/day is presumed.

The SSA based on the above data is calculated to be 60 m^2 (ie. 24/0.04). Now the volume of substrate required to give the SSA can be calculated.

Required biofilter volume = SSA / Specific surface area of filter medium

It is assumed that the specific surface area of the filter medium is 200 m^2/m^3. Therefore, the required biofilter volume is 0.3 m^3 (ie. 60/200). However, this calculation does not take into account the contribution of urea to the ammonia nitrogen. If it is assumed that all of the urea was oxidised to ammonia nitrogen then there would be approximately 20% more TAN in the system. Thus the biofilter would need to be 0.36 m^3 to be able to nitrify all of the TAN.

If the lobsters were to fed in such a system the biofilter would be far too small to handle the TAN load because of the large increase in TAN excretion associated with feeding.

REFERENCES

Kikuchi, K., Honda, H. and Kiyono, M., 1994. Ammonia oxidation in marine biological filters with plastic filter media. Fish. Sci., 60:133-136.