Mariculture as a means to add value to the east coast rock lobster *Panulirus homarus rubellus* subsistence fishery: a physiological approach to define transport and growout protocols for wild caught juveniles.

Submitted in Fulfillment of the Requirements for the Degree of MASTER OF SCIENCE.

by
J.O.G. Kemp
June 2008

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

Firstly, I would like to thank my two supervisors. By facilitating my involvement in both this project and the Port Alfred Marine Research Laboratory, Prof. Pete Britz provided me with an amazing opportunity to pursue my interest in the science and technology of aquaculture. Your hands off approach to supervision allowed me the freedom to explore and your invaluable help, from critical comments on manuscripts to that uncanny ability to always find the funds to support this research, have allowed this thesis to come to fruition. Thank you for the chance to tinker! To my co-supervisor, Dr. Andy Cockcroft, your help in assisting this project to materialise in the first place, your on-going enthusiasm throughout its duration and the valuable critical comment on earlier manuscripts are greatly appreciated. Not to mention the trip up Bain's Kloof.

Secondly, this work would not have been possible without the help of a number of people, both in the field and at the lab. Dr. Horst Kaiser was always available to talk stats, experimental design and the untold intricacies of the peer review process. The lobster hunters; Guy Paulet, Mike Markavino, Ernst Thompson and Mike Grey were priceless in their ability to ferret my experimental animals from the reefs of Mdumbi. Brian Kemp, Ed de la Rey and Terry Longman provided the missing technical capabilities to help turn the blue prints of the respirometry system floating around in my head, into a reality in the lab. My fellow students at the Port Alfred Marine Lab, especially Alistair Green, were always there with perspective, a helping hand, a good laugh and a few cold ones when they were needed most. Faith, helped facilitate all things

organizational and put up with my endless inability to get the paperwork right. My friend the East Pier, was a calming constant in my life these last few years and provided both a ready supply of fresh mussels to keep the critters well fed and the looping barrels that kept me sane. I am extremely thank-full to you all for your help. I would also like to thank Meg and Alan, for helping me get this far and for their willingness during the editing process to add a few more hours to their balance sheet on the costs of genetic investment.

Finally, to my best friend, Georgina, to whom I was lucky enough to be married to during this study. Your support, love, friendship and ability to always see the lighter side when it eluded me is greatly appreciated.

The financial assistance from the Henderson Scholarship towards this research is hereby acknowledged. Options expressed and conclusions derived at, are those of the author and are not necessarily to be attributed to Rhodes University or the donor. Further financial assistance towards this research was obtained via the NRF Prestigious Scholarship Program and this contribution is hereby acknowledged.

ABSTRACT

In a context of declining capture fisheries and public pressure for greater access to marine resources, marine aquaculture is receiving increasing interest from the South African government as a means to increase the diversity of economic activities in coastal regions, thereby providing employment and reducing poverty. The east coast rock lobster *Panulirus homarus rubellus* is currently harvested by subsistence fisherman along the former Transkei coastline of south-east South Africa and presents a possible opportunity for ongrowing wild juvenile lobsters in culture facilities. Lack of compliance coupled with poor enforcement of the minimum size limit (65 mm carapace length) has resulted in the ongoing harvest of undersize size lobsters by subsistence fishers. Generally, fishers either consume these undersize lobsters or sell them to tourists for low prices. In line with international trends in rock lobster aquaculture, interest has subsequently arisen in the possibilities of ongrowing these undersize lobsters as a means of adding value to the *P. h rubellus* resource for subsistence fishers. The aim of this physiological study was to assess the biological feasibility of harvesting, transporting and culturing wild caught juvenile lobsters, thereby provide empirical data to inform the development of suitable transport and culture protocols. The experimental objectives were to asses the effect of temperature on growth and survival of *P. h. rubellus*, as well as the effects of a suite of extrinsic and intrinsic factors on ammonia excretion and oxygen consumption.

Juvenile lobsters were collected by hand from near-shore reefs (2-15 m depth) off Mdumbi in the former Transkei, Eastern Cape Province and transported by road (7 hours) to the Port Alfred Marine Research Laboratory where they were held in a recirculating

culture system. The effect of temperature over a range of 9.7 °C (18.9±0.7 to 28.6±1.5 °C) on the growth and survival of juvenile *P. h. rubellus* fed a diet of fresh mussel flesh was investigated. Specific growth rate (SGR) was significantly different between temperatures ($p = 0.01$), with the highest values recorded for the 24 °C and 28 °C treatments. There was no significant difference in moult increment (MI) between temperatures, however, intermoult period (IMP) differed significantly between temperatures ($p = 0.0015$) with mean IMP lowest at 24 °C, although not significantly different from the means of the 26 °C and 28 °C treatments. IMP was highest at 19 °C and 21 °C. Apparent feed intake was significantly different between treatments ($p < 0.0001$) and exhibited a strong positive correlation with increasing temperature. Food conversion ratio (FCR) differed significantly between temperatures ($p = 0.02$) with 24 °C exhibiting the most efficient FCR. The results for growth rate and food conversion efficiency suggested that 24 °C is optimal for the growout of juvenile *P. h. rubellus.*

In the second study, the effect of body weight, emersion, daily rhythm, feeding and ambient ammonia on the total ammonia nitrogen (TAN) excretion rate was investigated. Body weight ($n = 16$, range of 16.8 – 322 g) was positively correlated to daytime TAN excretion rate (mg h^{-1}). Re-immersion after one hour emersion in a moist environment was characterized by a significant increase in TAN excretion rate for the first hour compared to pre-immersion levels. The amount of TAN excreted during this period was as expected if basal TAN excretion rates were maintained during emersion. TAN excretion rates returned to pre-emersion levels by the end of the second hour. There was no evidence of a daily rhythm in TAN excretion rate for *P. h. rubellus*. TAN excretion

rates were elevated following feeding. An initial peak in TAN excretion rate after seven hours (7.58 times pre-feeding rate) was followed by a smaller peak after 13 hours (6.69 times pre-feeding rate). TAN excretion rate dropped to levels not significantly different from pre-feeding levels after 23 hours and consistently returned to pre-feeding levels after 42 hours. The TAN excretion rates of lobster exposed for two hours to an ambient TAN concentration of 1.02±0.10 mg l^{-1} and 2.3± 0.2 mg l^{-1} were not significantly different from TAN excretion rates recorded at low ambient water TAN prior to exposure. Exposure to an ambient TAN concentration of 4.45±0.78 mg l^{-1} had a significant effect on the TAN excretion rate, with pronounced ammonia uptake occurring for all animals at this concentration.

The third study investigated the effects of body weight, diurnal rhythm, feeding and emersion on lobster oxygen consumption. Body weight was positively correlated to both standard and active oxygen consumption (mg O_2 h^{-1}) while body weight was negatively correlated to mass-specific standard oxygen uptake (mg O_2 g^{-1} h^{-1}). Diurnal rhythm exhibited a strong effect on the lobsters oxygen consumption, with average night time values 67% greater than those recorded during the day. This was related to activity driven by intrinsic nocturnal foraging behaviour. Feeding resulted in a classic specific dynamic action (SDA) response, with postprandial oxygen consumption increasing to a peak before decreasing gradually to preprandial levels. Emersion resulted in a significant increase in oxygen consumption, with lobsters rapidly recovering to pre-emersion levels after four hours.

Results from these studies suggest that the capture, transport and culture of juvenile *P. h. rubellus* is biologically feasible. Empirical data generated were used to provide recommendations regarding species optimised transport and culture protocols. A purge time of 48 hours before transport is suggested to ensure that ammonia excretion and oxygen consumption are at basal levels. Furthermore, emersed transport for a period of one hour is characterised by rapid recovery upon re-immersion. In order to prevent the accumulation of stressors, it is suggested that consecutive periods of emersion are interjected with recovery periods (five hours) in water to allow the removal of accumulated ammonia and repayment of the oxygen debt incurred. The recorded ammonia rates indicate that a biological filter size of 4.8 m^3 is recommended for 1000 kg of fed lobsters in a culture situation, although this can be reduced considerably if lobsters are being held without feeding (0.72 m^3). A flow rate of 112 l kg^{-1} h^{-1} is required to meet the metabolic requirements of lobsters. Bottlenecks to the viable commercial culture of *P. h. rubellus*, and the ability of this practice to provide the socio-economic benefits that were envisioned, are discussed.

CHAPTER 1: General Introduction

1.1 Introduction

The South African government has been placed under increasing pressure to deal with past inequalities in access to land and natural resources and provide mechanisms for rural development. The country's Constitution stipulates that citizens have the right to not only a healthy environment (Section 24(a)), but also the right to benefit from natural resources for social and economic development. Historical imbalances in access to natural resources are a feature of many of South Africa's fisheries. Attempts to create equitable access to these resources have been confounded by the decline in the contribution of capture fisheries to food security and employment since the 1950's, driven by both over-exploitation and shifts in the distribution of some species (DEAT, 2007).

In response, marine aquaculture has recently been promoted by government as a means to increase the diversity of economic activity in coastal areas and in so doing address the principal challenges of poverty and unemployment. This thesis explores the biological feasibility of developing the east coast rock lobster *Panulirus homarus rubellus* for mariculture through the growout of wild caught juveniles, and thereby providing a means of adding value to a marine resource already harvested by rural subsistence fishers along the former Transkei coastline.

1.2 Biology and life history of P. h. rubellus

The procedures for developing a suitable culture environment and the associated culture techniques can be greatly enhanced by an understanding of the biology and life history of the candidate species. The East Coast rock lobster *Panulirus homarus rubellus* is one of three subspecies of the scalloped spiny lobster *P. homarus* (Linnaeus) and occurs in the south-western Indian Ocean along the south-east coasts of Madagascar and Southern Africa (Berry 1974; Smale 1978). Within the south-east African region, *P. h. rubellus* is distributed from Port Elizabeth in the south to at least Barra Falsa, Mozambique, in the north, with the greatest abundance centred along the Kwa-Zulu Natal coast (Fig. 1.1) (Heydorn 1969). It inhabits shallow-water reef environments (surfzone down to 20m depth) and its distribution and abundance appear to be correlated to the availability of its primary food organism, the brown mussel *Perna perna* (Berry 1971a).

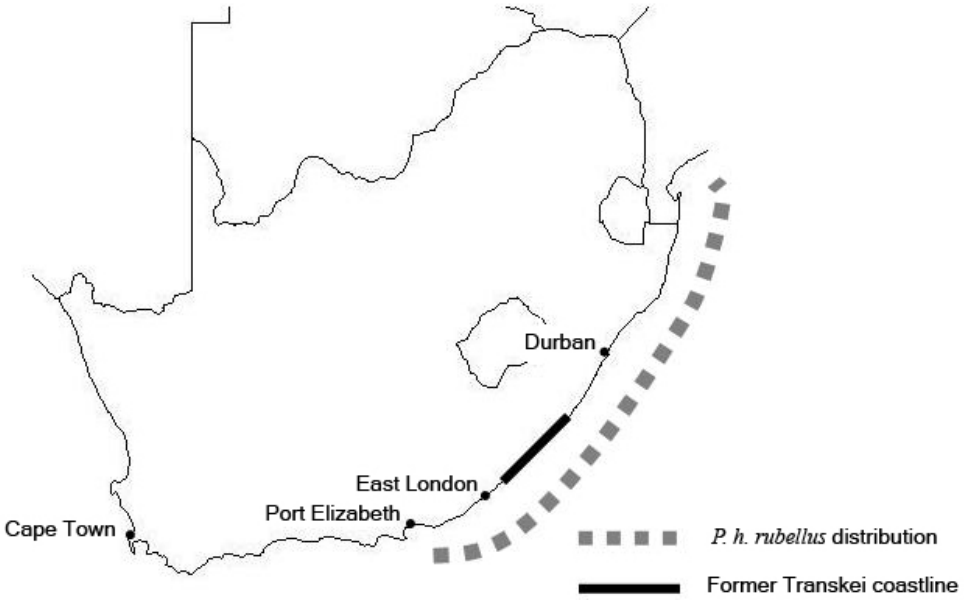


Figure 1.1: A distribution map of *P. h. rubellus* in South African waters depicting the stretch of coastline bordering the former Transkei homeland, Eastern Cape.

Most individuals are sexually mature at 55 mm carapace length with the breeding season occurring during the austral summer months (November to March). Large females may breed more than once a year (Berry 1971b). The females carry the eggs for between one and two months depending on water temperature, before they hatch as planktonic naupliosoma larvae (1-2 mm long). After developing offshore as (4-7 months) through a series of moults, the phyllosoma larvae return to the continental shelf and then metamorphose into the non-feeding puerulus stage, which actively swims towards the coast. Following settlement, the puerulus moults into a benthic juvenile stage which matures on nearshore reefs before mating and completing the lifecycle (Phillips & Mellville-Smith 2006)

Currently no direct commercial exploitation of *P. h. rubellus* exists in South African waters as it forms an important component of the intertidal subsistence fishery along the former Transkei coast and supports a large recreational lobster fishery in Kwa-Zulu Natal (~ 150 tons annually) (Fielding *et al.* 1994; Tomalin 1995; Cockcroft & Payne 1999). Along the former Transkei coastline, enforcement of the minimum legal size (MLS) of 65mm carapace length has proved problematic, and the removal of animals of sub-legal size for sale to tourists or personal consumption is therefore common among subsistence fishers (Fielding *et al.* 1994).

1.3 Historical background to exploitation and management

The harvesting of marine resources through small-scale fisheries provides a major source of food and income towards sustaining the livelihoods of millions of people globally (Kent 1997; Sowman 2006). The systematic neglect of this sector by fisheries managers in favour of commercial fisheries sectors, and the subsequent marginalization of subsistence fishers during the allocation of resources, has often resulted in over-harvesting and conflict with managers (Berkes 1990; Sowman 2006; Harris *et al.* 2006). A number of factors associated with South Africa's unique political history have further aggravated this phenomenon. The 'homeland system' enforced during the apartheid period (1948 – 1994) concentrated people in impoverished rural communities thereby increasing their reliance on natural resources (Harris *et al.* 2006). Furthermore, the introduction of discriminatory apartheid legislation during this period resulted in the

exclusion of "black" and "coloured"[1] fishers from access to commercial fisheries, ultimately leading to grossly unequal access to marine resources (Sowman 2006). Despite these restrictions subsistence activities continued illegally, and resulted in an informal poaching sector (Harris *et al.* 2006). Specifically along the east coast of South Africa, in waters bordering the former homelands, subsistence fishing continued under a complex regulatory arrangement of traditional communal systems as well as national and provincial laws (Hauck & Sowman 2003). Lobsters were informally harvested and sold to holiday makers by rural subsistence fishers in the Transkei for many decades with minimal management and control.

The rights of subsistence fishers were recognized in post-apartheid South Africa, but this was coupled with increased bureaucracy and control. Subsequent to South Africa's first democratic elections is 1994, a Fisheries Policy Development Committee (FPDC) was instituted in 1995. The FPDC, set up by the Minister of the Department of Environmental Affairs and Tourism to represent all interested parties, was tasked with developing a fisheries policy acceptable to all (Cochrane & Payne 1998). With the promulgation of the Marine Living Resources Act No. 18 (DEAT 1998) in 1998, subsistence fishers were for the first time recognized as a formal sector. This act defines a 'subsistence fisher' as "a natural person who regularly catches fish for personal consumption or for the consumption of his or her dependents, including one who engages from time to time in the local sale or barter of excess catch, but does not include a person who engages on a substantial scale in the sale of fish on a commercial basis". While formal recognition was

[1] "Black" and "coloured" were terms used during the Apartheid era to classify "non-white" people in terms of race.

seen as a positive step forward for subsistence fishers, no management systems existed for this sector and little information was available to inform the development of suitable management systems (Harris *et al.* 2006). In response, a Subsistence Fisheries Task Group (SFTG) was appointed in December 1998 to provide the national management agency, Marine and Coastal Management (MCM), with recommendations for the management of the subsistence sector. The SFTG's research focused on further defining this sector, determining functional areas and species suitable for the sector, developing management protocols and finally providing guidelines and mechanisms for the formation of a small-scale commercial sector as an alternative to subsistence fisheries where appropriate

The former Republic of Transkei, incorporated into the Eastern Cape Province in 1994, is located along the East coast of South Africa between the Kei and Mtamvuna rivers. Although the harvesting of shellfish by the local indigenous people of Transkei has occurred since prehistoric times (Siegfried *et al.* 1985), the intensity of the exploitation of marine resources has intensified in recent years (Robertson & Fielding 1997). This has been fueled by both the ongoing subsistence harvest of an increasing indigenous population as well as a growing awareness of the commercial potential of harvesting marine invertebrates for sale (Robertson & Fielding 1997). Commercially, by far the most important of these marine invertebrates is the east coast rock lobster *P. h. rubellus*, bringing returns in excess of half a million rand to local communities annually (Steyn *et al.* 2008). While (Heydorn 1969) reported lobster being collected for sale as early as the 1960's, an increase in visitors to the Transkei has driven an expansion of the fishery,

which supplies approximately 44 tons of lobster for sale annually (Robertson & Fielding 1997).

The preferred method of fishing (75% of fishers) is poling (Steyn *et al.* 2008). A mesh bag is attached to a long wooden pole via a length of rope. The bag is baited with shellfish (mainly limpets) and placed in the water at night until a lobster climbs onto it, at which point the bag and lobster are lifted from the water. The other method used is to free-dive for lobster, usually during the day. Harvesting restrictions that currently apply to the fishery include: (a) a minimum size limit of 65 mm carapace length (b) a closed season (1 November to the end of February inclusive) (c) a bag limit of eight (d) gear restrictions (no use of boats or artificial breathing apparatus) and (e) a prohibition on the collection of ovigerous and soft shell lobsters. Although *P. h. rubellus* was identified by the SFTG as one of the species recommended for small scale commercial use along the South and East coasts (Cockcroft *et al.* 2002), implementation of the SFTG's recommendations regarding the management and allocation of access rights for this species has received only fragmented attention by MCM (Sowman 2006).

In 2001, establishment of the MCM Sub-directorate Subsistence Fisheries Management (SFM) led to the appointment of an external agent and four extension officers in the Eastern Cape. They were tasked with facilitating the establishment of local co-management committees in order to identify *bona fide* subsistence fishers and provide a forum for engagement between MCM and fishers (Sowman 2006). Fishers continue to harvest lobster in terms of section 81(1) of the MLRA, under the so called "exemption

clause", or informally, essentially as illegal poachers (Sowman 2006). Over the period 2005 – 2008 a total of 7678 lobster exemptions (1858, 1858, 2325 and 1647 in 2005, 2006, 2007 and 2008 respectively) were allocated to subsistence fishers. Despite the delays associated with the allocation of subsistence fishing rights in the former Transkei, there is a growing interest in commercialisation of this fishery (anon 2006). It has been proposed that development of the subsistence sector into a small scale commercial sector would provide a means to improve the quality of life in this impoverished coastal region (Steyn *et al.* 2008). In order to facilitate this shift towards commercialization of the fishery, MCM began licensing buyers to purchase lobster caught by subsistence fishers. Licenses were issued to buyers in 2003, 2005, 2007 (n = 5) and 2008 (n = 3). Buyers were allocated stretches of coastline from which they could purchase the daily limit of 8 full-size lobster (> 65 mm CL) from subsistence fishers issued with exemptions by MCM. With the effective commercialization of this traditionally subsistence fishery, a new market for lobsters has been created over and above the seasonal market associated with local holiday makers and hotels. The increase in demand and improved prices associated with commercial buyers has fuelled a parallel increase in fishing effort. Although buyers are limited to buying size lobster, the harvest and illegal local sale of undersize lobsters continues. Furthermore, the development of a draft policy for the allocation of access rights for subsistence fishers remains ongoing. This process is currently being undertaken by an appointed task team, with the current draft being developed along the lines of the TURF model initially developed for marine invertebrate fisheries in Chile.

With the gazetting of the Policy for the Development of a Sustainable Marine Aquaculture Sector in South Africa (Notice 1109 of 2007)(DEAT 2007) by the Department of Environmental Affairs and Tourism (DEAT) in 2007, the South African government committed to supporting marine aquaculture development in South Africa. One of the policy strategies is to support research aimed at expanding the resource base, both through identifying and generating information about candidate species and through the development of improved or new farm technologies suitable for local conditions. The ongoing harvesting of undersize lobster in the Transkei (Fielding *et al.* 1994; Steyn *et al.* 2008), the difficulties associated with preventing this harvest through enforcement and the continuing drive to commercialize the resource led to interest in the ongrowing undersize lobster under culture conditions.

In 2006, MCM instituted a research initiative (MCM Provincial Research Project 046) to explore the biological and economic possibilities of ongrowing of lobster. The project had a two-tier approach. The first aimed at developing a suitable artificial diet for *P. h. rubellus*, which would then form the basis of a suite of production trials aimed at determining the economic feasibility of the practice. The second, of which the research presented in this thesis forms part, was to employ a physiological approach to determine suitable culture conditions and inform transport and holding protocols. The results from these two elements would ultimately combine to inform decisions within the national regulating body (MCM) as to whether to forge ahead with the inevitable legislative and institutional interventions that would be required. The current project also falls within the

context of a larger international trend in research aimed at the growout of wild harvested juvenile lobster (Phillips & Mellville-Smith 2006).

1.4 Global trends in spiny lobster aquaculture

The majority of spiny lobsters that enter the world market originate from fisheries in Australia, New Zealand, South Africa, Cuba, Brazil, Mexico and the USA, with a total production of 74000 mt. Most of this high-value seafood resource is sold frozen, although the Japanese may pay up to \$100 kg^{-1} for live lobster (Wickens & Lee 2002). Owing to this demand, the majority of these fisheries are exploited or overexploited, leaving aquaculture as one of the few alternatives to expand production (Kittaka & Booth 2000). The major hurdle to the viable and large scale culture of spiny lobsters remains the difficulties associated with growing species through all larval stages (Kittaka & Booth 2000). Although a number of species have been cultured to settlement including *Jasus lalandii* (Kittaka 1988), *J. edwardsii*, *Panulirus japonicus*, *P. elephas* (Kittaka 2000) and *P. longipes* (Matsuda & Yamakawa 2000), the rate of survival has generally been very low and the rearing process plagued by bottlenecks in the production process, particularly with regard to larval nutrition and bacterial infections (Cox & Johnston 2003). To date there is no record of *P. homarus* being cultured to settlement, although it has been reared as far at stage VI larvae, when changes in feeding habits resulted in mortality (Radhakrishan & Vijayakumaran 1995). It is encouraging that despite these barriers, these early attempts have yielded larval periods for some species that are approximately half of those estimated for development in the wild (Phillips & Mellville-Smith 2006).

Given the difficulties associated with larval rearing (Kittaka, 2000; Cox & Johnston, 2003), the harvest and subsequent growout of wild caught pueruli and juveniles is considered to be the most feasible short-term option for lobster aquaculture (Crear *et al.,* 2000; Johnston *et al.,* 2006). Growout of wild harvested juvenile lobsters is an area of intense research interest (Phillips & Melville-Smith, 2006). It is currently employed in a number of countries including Taiwan, Singapore, India (Booth & Kittaka, 2000) and most notably in Vietnam where production in the cage-reared *P. ornatus* industry is estimated to exceed 3000 tonnes annually at an export value of US$90 M (Williams, 2007).

This form of partial culture presents a number of advantages as detailed by Hair *et al.* (2002): (1) Reduced juvenile propagation costs as hatcheries are not required (2) Socio-economic benefits associated with the collection and sale of juveniles allowing involvement of sectors not normally associated with aquaculture. (3) Increased productivity as harvested juveniles bypass the high juvenile mortality in the wild and can be ongrown for the market or used for stock enhancement. (4) Reduced risk of disease as wild harvested individuals are generally less prone to disease than their hatchery reared counterparts. The effects of removing pueruli from the wild has only been documented from one fishery, namely that for *P. cygnus* off Western Australia (Phillips *et al.* 2003). This study suggested that the impact of pueruli removal on the subsequent catch would be slight, unless excessively large numbers of individuals were removed, but even then this could be mitigated by a slight decrease in effort in the fishery. Furthermore, this study

confirmed that natural mortality in the first year after settlement was generally high, with predictions from models estimating a loss of 80-98%. Conflict with a wild fishery is possible with the removal of pueruli, especially during years of reduced settlement, however, if natural mortality is high, as suggested, then the removal of some pueruli for ongrowing could lead to greater overall production (Kittaka & Booth 2000).

The majority of research aimed at developing culture systems and protocols for ongrowing has centred on land-based systems, although the prospects of sea-cage farming have been explored (Jeffs & James, 2001; Simon & James, 2007). The successful growout of a number of species has already been achieved in the laboratory. Wild caught pueruli (carapace length (CL) of 8mm) of *P. cygnus* have been raised to market size (76 mm CL) in about 2.1 years with very high (>95%) survival (Phillips 1985). Growth and survival of juvenile (3.24 g) *P. ornatus* was unaffected by stocking density (14, 28 and 43 m^{-2}) with predicted final weights exceeding 1 kg in 18 months. In India, Vijayakumaran & Radhakrishan (1984) grew *P. homarus homarus* to 200 g in 5-6 months, although this growth was achieved after eyestalk ablation. Studies aimed at refining the growout process for a variety of species have been wide ranging. The effect of factors such as holding system type (Simon & James 2007), photoperiod (Crear *et al.* 2003), stocking density (James *et al.* 2001; Jones *et al.* 2001), shelter type (Crear *et al.* 2000; Johnston *et al.* 2006) and diet (see Williams, 2007 for a review) on growth and survival have been investigated. The majority of these studies have been conducted on Southern hemisphere species, specifically *P. cygnus*, *J. edwardsii*, *J. lalandii* and *P. ornatus*.

Studies aimed at defining optimal parameters for water quality have, to a large degree, been limited to determining the optimal temperature for growth of lobsters (Serfling & Ford 1975; Lellis & Russell 1990; Crear *et al.* 2000; Dubber *et al.* 2004). However, recent interest in providing specific data to improve the design of holding and transport systems associated with the live lobster trade has led to a suite of physiological studies. The effect of a variety of intrinsic and extrinsic factors (including temperature, body weight, activity, emersion, diel rhythm, feeding and dissolved oxygen level) on the oxygen consumption of both *P. cygnus* (Crear & Forteath 2001) and *J. edwardsii* (Crear & Forteath 2000) have been investigated. Perera *et al.* (2007) conducted similar experiments with *P. argus*, describing oxygen consumption in relation to body weight, temperature and feeding. This work on oxygen consumption was complemented by investigations into the effect of a similar suite of intrinsic and extrinsic factors on the ammonia excretion of *J. edwardsii* and *P. cygnus* (Crear & Forteath 2002). These studies resulted in practical recommendations to the live export industry regarding criteria for holding system design, such as flow rates (Crear & Forteath 2001) and biological filter requirement (Crear & Forteath 2002), many of which are directly applicable to prospective mariculture ventures.

1.5. Aims and objectives

The successful culture of *P. h. rubellus* will require the development of a cost-effective and nutritionally-adequate formulated diet (Williams 2007), optimisation of the design of growout tanks and rearing protocols, and the provision of optimal water quality for

growth and survival (Crear *et al.* 2000). The high-energy nature of the South African coastline, combined with a scarcity of protected bays and estuaries, necessitates the use of land-based systems for culture (Britz & Hecht 1990). Compared to ocean-based cage culture, providing optimal water quality within land based systems, especially when recirculating in nature, becomes an important consideration. A clear understanding of the basal physiology of the species is crucial to aid in the pre-emptive development of suitable post-harvest holding and growout systems. Furthermore, the theoretical supply chain associated with the harvest of lobster within the former Transkei can be divided essentially into three phases: (1) Capture by subsistence fishers followed by a period of emersed transport to a holding facility (2) Recovery in a local holding facility (3) Immersed transport to a central facility for growout. At certain periods in the process, lobsters will be exposed to potential stressors that, potentially, will challenge their homeostatic capability. This study therefore set out to determine the effect of a suite of extrinsic and intrinsic factors on the physiological response of lobsters by investigating:

(1) The effect of temperature on growth and survival (Chapter 2)
(2) The effect of body weight, diurnal rhythm, emersion, feeding and ambient ammonia levels on ammonia excretion (Chapter 3)
(3) The effect of body weight, activity, diurnal rhythm, emersion and feeding on oxygen consumption (Chapter 4)

Furthermore, based on the empirical data gathered in Chapters 2-4, recommendations regarding the design of transport and grow out protocols for *P. h. rubellus* are discussed further in Chapter 5. This is complemented by a discussion of some of the biological,

institutional and regulatory challenges regarding the harvest of early juveniles in the former Transkei.

CHAPTER 2: The effect of temperature on the growth, survival and food consumption of the east coast rock lobster *Panulirus homarus rubellus*.

2.1. Introduction

Water temperature is one of the most important environmental factors determining the growth rate of crustaceans (Hartnoll 1982). It has been shown to affect the growth of a number of spiny lobster species including *Jasus lalandii* (Dubber *et al.* 2004), *J. edwardsii* (Crear *et al.* 2000; Thomas *et al.* 2000), *P. argus* (Lellis & Russell 1990), *P. cygnus* (Phillips *et al.* 1977) and *P. interruptus* (Serfling & Ford 1975). While some studies have reported on the growth of *P. homarus* under captive conditions (Berry 1971b; Rahman *et al.* 1997; Kulmiye & Mavuti 2005), only one has investigated the effect of temperature on the growth of *P. h. rubellus* (Smale 1978).

It has been shown that crustaceans of the same species that have been collected from geographically different locations vary in their response to similar environmental conditions (Sastry & Vargo 1977). Furthermore, considerable variations in growth at the same temperature have been noted for other spiny lobster species, most notably *J. edwardsii* (Thomas *et al.* 2000). While Smale (1978) attempted to define the upper thermal limit of *P. h. rubellus*, the range of temperatures at which the lobsters were grown was limited to the upper portion of the range of temperatures experienced within its natural distribution. In addition, Smale's animals were sourced from the Kwa-Zulu Kwa-Zulu Natal coast that possesses higher ambient water temperatures than the former

Transkei coast to the South, which is the target area for ongrowing lobsters collected in this study.

The aim of this study was to determine the effect of water temperature (19 – 28 °C) on survival, growth, food intake and food utilization of juvenile *P. h. rubellus* collected from the former Transkei coastline to identify the most efficient temperature for culture.

2.2 Materials and Methods

2.2.1 Collection, transport and acclimation of experimental animals

Juvenile lobsters were collected by hand in the Mdumbi region of the Eastern Cape, South Africa, using free-diving and SCUBA to reach near-shore reefs at depths of 2-15 m. Following capture, lobsters where transferred to seawater filled 180 l PVC drums loosely lined with 8mm oyster mesh to purge for 48 hours. Water in the drums was oxygenated continually and exchanged twice daily. Lobsters were transported in these drums (seven hours by road) to the Port Alfred Marine Research Laboratory where they were placed in mesh baskets (0.8×0.5×0.5 m; 6 mm mesh) and held in ambient recirculating seawater. The lobsters were acclimated to captivity on a diet of crushed fresh mussel (*Mytilus galloprovincialis* and *Perna perna*) fed in excess three times a week.

2.2.2 Experimental system

Following acclimation for a period of four weeks, a total of 48 lobsters (40.4±9 mm carapace length (CL); 63.64±12.05 g) were selected from the holding cages and stocked into the experimental system. Pairs were randomly allocated to each of 24 fibreglass tanks (0.5×0.3×0.3 m; 45 l). The tanks were aerated with an air stone and each held two lidded oyster mesh baskets (0.2×0.15×0.3 m; 6 mm mesh) into which one lobster was placed. Shelter for lobsters was supplied in the form of a 10 cm length of PVC piping (63 mm diameter). Housing lobsters in individual cages not only prevented cannibalism and competition for food, but also allowed the exact time of moulting and growth of each individual to be accurately determined (Dubber *et al.* 2004). The tanks were housed in a constant-environment laboratory held at 18 °C and formed part of a biologically filtered partial re-circulating system (5500 l total volume; 1000 l filter volume; 10 % daily water exchange). Water temperature in each tank was controlled by chilling a shared supply sump tank to 18 °C, from where water was pumped directly to each individual tank in which submersible heaters heated the water to the desired temperature. A temperature range of 9.7 °C (18.9±0.7 to 28.6±1.5 °C) was attained using this method with treatments being divided into 5 temperature groupings, i.e. 19.2±0.3, 21.1±0.5, 24±0.4, 26.3±0.2 and 28.3±0.2°C, with means being significantly different (mean±SE; ANOVA, $F_{4,19}$=705.92, p=0.0000) (Table 2.1). A uniform flow rate to achieve one exchange h^{-1} was maintained across all treatments.

Table 2.1: Water temperatures (°C, mean ± SE) of tanks used to house juvenile *P. h. rubellus* during a 223 day growth trial.

Parameter	*Temperature Group (°C)*				
	19	***21***	***24***	***26***	***28***
Mean water temperature (°C) of group	19.2±0.3[a]	21.1±0.5[b]	24±0.4[c]	26.3±0.2[d]	28.3±0.2[e]
Mean water temperature (°C) of replicate tanks	18.9±0.7	21±0.7	24±0.9	26.2±1.1	28.6±1.5
	19.2±0.8	21±0.6	23.7±0.7	26.3±0.3	28.2±1
	19.2±0.8	21.6±0.7	23.8±0.7	26.3±0.8	28.4+1
	19.5±0.8	21±0.6	23.9±0.7	26.6±0.6	28.2±1.3
		21.4±0.5	23.5±0.6		28.2±0.9
			24.8±0.9		
Number of tanks	4	5	6	4	5
Number of lobster	8	10	12	8	10

Values with different superscripts are significantly different ($p < 0.05$).

2.2.3 Feeding

The experimental animals were fed in excess of their food uptake on a mixed diet of fresh, opened brown mussel *P. perna* and Mediterranean blue mussel *M. galloprovincialis* on day one (Monday), three and five of each week. Before being placed in the tank, mussels were opened, air dried with the shell side up on a mesh tray for 30 minutes and then weighed to the nearest 0.01 g (Denver Instruments MXX-612). Shell remnants and uneaten food (feed was always in excess on collection) were removed 24

hours after feeding on day two, four and six of each week and then dried and weighed as described previously. The lobsters were fed at 16h00 as *P. h. rubellus* is nocturnal with the greatest peak in feeding activity occurring during the initial stages of the dark photophase (Smale 1978). The feeding regime described ultimately resulted in lobsters remaining unfed on days two, four and six and, for logistical reasons, on day seven (Sunday). The seven day feeding regime was maintained throughout the trial, except on days 161-164, 179-180 and 191-194 when high seas prevented the collection of mussels.

2.2.4 Experimental procedure

Experimental animals were acclimated to the experimental system for four weeks. During the first two weeks the water temperature in each tank was gradually raised to the experimental temperature followed by a further two weeks of acclimation at the designated temperature. The growth trial then ran for a further 223 days. A 12L:12D cycle was maintained using overhead fluorescent lighting throughout the acclimation period and growth trial. Water temperatures and pH (7.9 – 8.1) were recorded on average two times per week (Hanna pH/Temp probe HI98128). Water quality was monitored by measuring the ammonium levels (Red Sea Ammonia Test Kit, NH_3 & NH_4^+ 0-1mg/l) of a random sample of five tanks every second week, with values remaining below 0.25 mg l^{-1} for the duration of the trial. Dissolved oxygen levels (Hanna Waterproof DO Meter HI9143) remained at or near saturation across all treatments.

Wet weight and carapace length (CL) of all animals was measured at the start of the trial and then at 37 day intervals following. After removing excess water, wet weight was

measured to the nearest 0.01 g using an electronic balance (Denver Instruments DXX-612) and CL was measured to the nearest 0.1 mm using vernier calipers. Moults and mortalities were recorded and removed each day. No lobsters moulted more than twice in the inter-weighing period. Dead lobsters were replaced with similar sized animals to maintain densities. Tanks were siphoned twice a week to remove waste and thoroughly cleaned at each weighing.

2.2.5 Data processing & calculations

Growth rates were calculated as specific growth rate (SGR) to overcome potential problems associated with exponential growth rates (Hopkins 1992; Crear *et al.* 2000; Johnston *et al.* 2006). SGR as a percentage of body weight per day (% BW day^{-1}), intermoult period (IMP), moult increment (MI) and condition factor (CF) were calculated for each lobster as follows:

SGR	=	(lnFinal weight – lnInitial weight) * 100 / number of days
IMP	=	Number of days between two consecutive moults.
MI (g or CL)	=	Weight or Carapace length (CL) at last weighing preceding moulting – weight or CL at first weighing following moulting.
CF	=	Wet mass / Carapace length

In cases where a lobster moulted more than twice during the trial, the mean IMP and MI values were used. The above method of calculating MI was possible as CL remained static during the IMP, and changes in lobster weight during the IMP were found to be negligible.

Apparent food intake (dry weight of mussel consumed per feeding as a percentage of lobster body weight) was calculated by subtracting the wet weight of uneaten mussel and shell from the wet weight of mussel and shell fed, taking into account the proportion of food lost into the water and the wet:dry mass ratio of mussel. The percentage of feed lost into the water was calculated at 19, 21, 24, 26 and 28 °C. Twenty mussels per temperature treatment were opened, air dried, weighed and placed in oyster mesh cages in aerated water and then re-weighed by the same procedure 24 hours later. Loss of mussel weight to water was negligible (<1%) at 19, 21, 24 and 26 °C and low (4.2±2.4 %) at 28 °C. In order to calculate the dry weight of mussel consumed, the wet:dry mass ratio of mussel (1.00 : 0.17) was determined by oven drying the flesh of a sub-sample of mussels (n = 50; 65.34±4.00 mm mantle length) for 24 hours at 60 °C. Apparent food intake (AFI) and food conversion ratios (FCR) were then calculated as below:

AFI = ((wet weight of mussel and shell (g) * percentage feed lost) - (wet weight of uneaten mussel and shell)) * 0.17 / lobster weight

FCR = Estimated dry weight mussel consumed (g) / Lobster wet weight increase (g)

Baskets in which mortalities occurred were excluded from calculations of total growth and food consumption (FCR, SGR, and final weight/CF). Data from completed moult cycles that occurred before mortality, or by replacement lobsters after mortality, were included in the calculations of IMP, MI and AFI.

2.2.6 Statistical analyses

Data were tested for homogeneity of variance and normal distribution of residuals using Levene's test and the Shapiro-Wilk W test respectively. When these assumptions were not met, a transformation (usually log-transformation) was performed. Parametric one-way ANOVA and Tukey's HSD *post-hoc* test were employed to test for differences in dependent variables between temperature groups where data were normally distributed and variances homogenous (significance level $p<0.05$). An analysis of covariance and Tukey's HSD *post hoc* test was used to test for differences in final CF and final weight using initial CF and initial weight as covariates respectively (significance level $p<0.05$). A non-parametric Kruskal-Wallis ANOVA by ranks test (α-error level: $p = 0.001$) was used when variances remained heterogeneous. Linear regression was employed to model the relationship between water temperature and AFI. All statistical tests were performed using Statistica 7.1 for Windows (StatSoft Inc., 2005).

2.3 Results

2.3.1 Growth and survival

Survival was good (>85%) with only three mortalities, one each at 19, 21 and 28°C, recorded during the trial period. All mortalities occurred during ecdysis. A further seven mortalities occurred due to heater failure (n = 4) or lobsters escaping their baskets (n = 3) but as these were the result of technical faults they were not included in the analysable data set. Specific growth rate (SGR) was significantly different between temperature treatments (ANOVA, $F_{4,16} = 4.46$; $p = 0.01$) being highest at 24 and 28 °C and lowest at 21°C (Table 2.2). SGR at 21°C was significantly lower than at 24 °C but not different from SGR at 19, 26 and 28 °C (Tukey's *post-hoc*). Although analysis of covariance showed final CF to be significantly different between temperatures (ANCOVA $F_{8,64} = 2.25$, $p = 0.03$), Tukey's *post hoc* test failed to show any significant differences, suggesting that the biological relevance of this effect is questionable. There was no significant difference in final weight between temperatures (ANCOVA, $F_{8,64} = 2.11$, $p = 0.05$).

Intermoult period (IMP) differed significantly between temperatures (ANOVA, $F_{4,19} = 6.67$; $p = 0.0015$) with mean IMP lowest at 24 °C although not significantly different from the means of the 26 and 28 °C treatments (Fig. 2.1). In contrast IMP was longest at 19 and 21 °C. There was no significant difference between the temperatures for MI in terms of either an increase in carapace length (ANOVA, $F_{4,19} = 0.79$; $p = 0.54$) or weight (Kruskall-Wallis ANOVA, $H = 1.49$, $p = 0.83$) (Table 2.2).

Table 2.2: Effect of temperature on the growth response, food intake and diet utilization of juvenile rock lobster, *P. h. rubellus*.

Parameter	Temperature (°C)				
	19.2±0.3	21.1±0.5	24±0.4	26.3±0.2	28.3±0.2
Initial weight (g)	62.52±4.60[a]	63.19±17.36[a]	61.55±7.94[a]	62.72±4.59[a]	68.25±6.74[a]
Final weight (g)	96.52±6.54[a]	93.45±19.46[a]	109.2±6.48[a]	99.04±11.06[a]	121±22.22[a]
Initial CF (CL/weight)	1.7±0.2[a]	1.6±0.3[a]	1.6±0.1[a]	1.7±0.1[a]	1.7±0.1[a]
Final CF (CL/weight)	2.1±0.1	2.1±0.3	2.3±0.1	2.2±0.2	2.5±0.3
IMP (days)	87±15[a]	96±20[ab]	60±13[c]	76±0[abc]	62±8[ac]
MI (g)	15.2±4.68[a]	13.28±4.85[a]	15.99±2.00[a]	14.46±3.43[a]	18.84±9.50[a]
MI (mm)	2.4±0.9[a]	2.6±0.4[a]	2.3±0.4[a]	2.3±0.5 [a]	3±0.8[a]
SGR total (%BW.day^{-1})	0.20±0.03[ab]	0.15±0.02[a]	0.26±0.04[b]	0.20±0.02[ab]	0.26±0.10[ab]
AFI (%BW.day^{-1})	1.4±0.2[a]	1.6±0.1[ab]	2±0.3[bc]	2.5±0.4[cd]	2.8±0.4[d]
FCR	2.5 ±0.3[ab]	3.7+0.6[a]	2.4±0.3[b]	3.4±0.5[ab]	3.6±2[ab]
Survival (%)	87.5	90	100	100	90

Values expressed as mean ± SE. Values in the same row with different superscripts are significantly different ($p < 0.05$). Mortalities are expressed as % survival per temperature. All mortalities occurred during moulting.

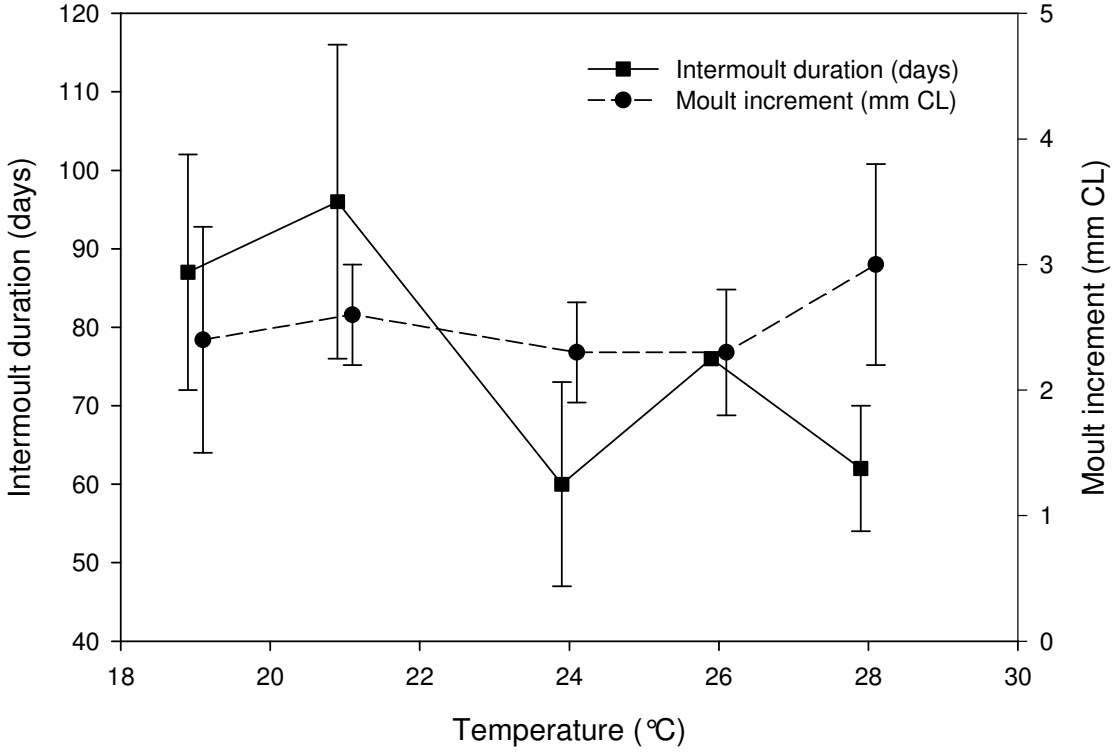


Figure 2.1 : Intermoult duration (■ - days) and moult increment (● - mm carapace length (CL) increase) for juvenile *P. h. rubellus* grown for 223 days at temperatures ranging from 18.9±0.7 to 28.6±1.5 °C.

2.3.2 Food intake

Differences in apparent food uptake (AFI) between temperature-treatments were highly significant (ANOVA $F_{4,19} = 18.44$; $p < 0.0001$) (Table 2) and AFI showed a positive correlation with increasing temperature ($y = -1.67 + 0.16x$; $r^2 = 0.81$) (Fig. 2.2). Feed conversion ratio (FCR) differed significantly between temperatures (Kruskall-Wallis

ANOVA, H = 11.34, p = 0.02). FCR at 24 °C was significantly lower than at 21 °C but not different from the 19, 26 and 28 °C treatments.

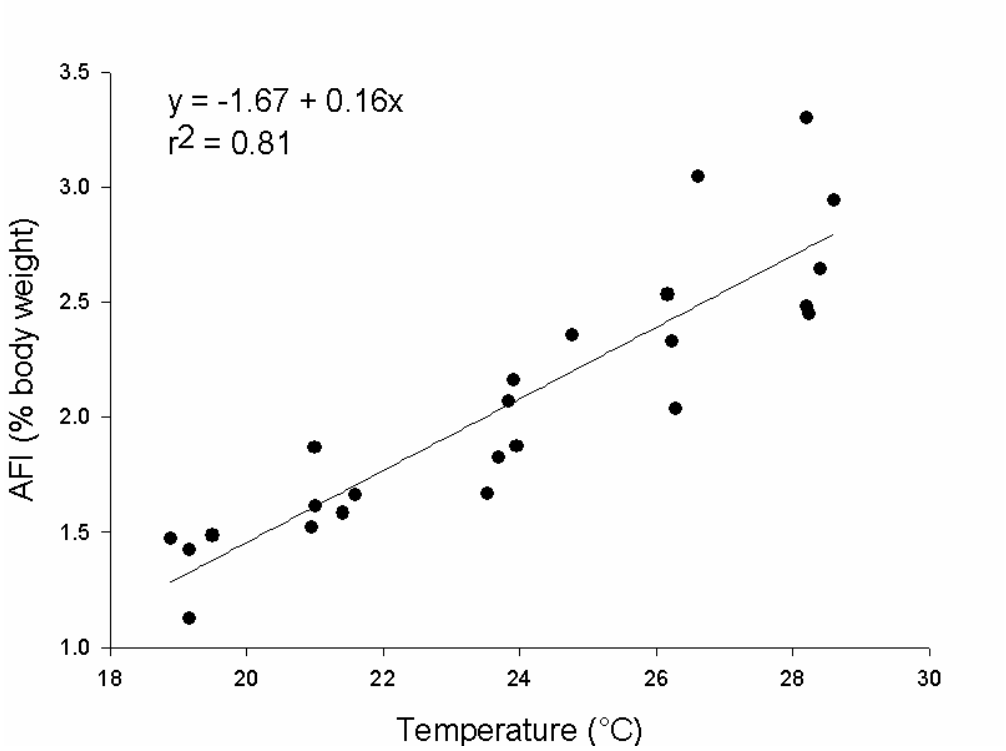


Figure 2.2: Apparent feed intake (AFI) as dry mussel consumed per feeding as a percentage of body weight for juvenile *P. h. rubellus* grown at temperatures ranging from 18.9±0.7 to 28.6±1.5 °C.

2.4 Discussion

Juvenile *P. h. rubellus* grown in this study exhibited good survival at temperatures ranging from 19 to 28 °C with temperature affecting a number of growth, food consumption and feed conversion parameters. The stepwise nature of growth associated with moulting is essentially a function of the moult increment, in terms of an increase in either weight or carapace length, and the moult frequency (Thomas *et al.* 2000). Values

obtained in this study for the moult increment of captive juvenile *P. h. rubellus* (2.3 – 3.0 mm CL) were similar to those obtained by Smale (1978) for *P. h. rubellus* (2.0 - 3.8 mm CL) and for *P. homarus* (2.3 – 3.4 mm CL) (Nair *et al.* 1981), but were larger than those obtained by Kulmiye & Mavuti (2005) for *P. h. homarus* (1.53 mm CL). Moult frequency in this study (60 – 96 days), particularly at the lower end of the range recorded, was similar to that obtained for equivalent-sized *P. h. homarus* (55-58 days for animals of 46-55 mm CL) (Kulmiye & Mavuti, 2005) but higher than values obtained by Smale (1978) for *P. h. rubellus* (40.8 – 59.6 days for animals of 40-49mm CL).

Hartnoll (1982) suggested that intermoult period (IMP) and moult increment both decrease with an increase in temperature. This has been observed for both *J. edwardsii* between 18 and 22 °C (Thomas *et al.* 2000) and *P. longipes* as temperature increased from 20 to 26 °C (Chittleborough 1975). While this study was unable to show that the temperature treatments exhibited a significant effect on the moult increment of animals, it did show a significant effect on the intermoult period. In the current study, IMP appears to be the main component of crustacean growth that underlies the differences in other growth performance criteria (Fig. 2.1). Lobsters that exhibited the lowest IMP (60±13 days and 62±8 days at 24 and 28 °C respectively) also performed best in respect of other growth criteria including final weight and SGR. The reduced performance of animals at 26 °C relative to 24 and 28 °C is not easily explained. Although, to all outward appearances, the lobsters in this treatment appeared healthy and were fed to excess as with the other treatments it is possible that undetected illness or nutritional stress may have impacted negatively on this treatment, resulting in a longer IMP. The relatively

large variation associated with SGR in the 28 °C treatment, coupled with a reduced sample size resulting from mortalities associated with technical faults (n = 4), also indicates the possibility of outlier-induced overestimation of SGR at 28 °C.

Smale (1978) also reported a consistent moult increment of approximately 3 mm CL increase at 24, 26 and 28 °C for *P. h. rubellus*, however at 30 °C the mean increment was reduced. In addition, the intermoult period was inversely related to temperature between 24 and 28 °C, with 28 °C having the shortest IMP for each size class, which appears to account for the marginally faster overall growth rate at 28 °C in that study. This is similar to findings by Serfling & Ford (1975) who observed that accelerated growth of *P. interruptus* (22 and 28°C) was associated with increased moulting rates rather than with an increase in the magnitude of the increment per moult. This pattern is also evident in *J. lalandii*, where increased growth at 15 °C relative to 10 °C was attributed to a shorter IMP (Hazell *et al.* 2001). The difference between IMP at 21 and 24 °C in this study suggest that temperatures below 24 °C affect IMP, and ultimately growth in *P. h. rubellus*.

Based on sea surface temperature charts for 26° S, compiled for the southern African region over a 10 year period (Christensen 1980), the geographical distribution of *P. h. rubellus*, as suggested by Heydorn (1969) (Port Elizabeth to Barra Falsa, Mozambique), would equate to a temperature range of 16 – 21 °C in winter and 20 – 26 °C in summer. However, while this temperature range is indicative of the entire geographical distribution of *P. h. rubellus*, the region of greatest lobster abundance, the Kwa-Zulu

Natal coast (Heydorn 1969), exhibits a thermal range towards the upper end of this span (19 – 21 °C in winter and 23 – 25 °C in summer). Furthermore, a decrease in abundance is evident when moving south from Kwa-Zulu Natal into the lower temperature range experienced by *P. h. rubellus*. Given the decrease in growth performance associated with temperatures below 24 °C in this study, temperature is likely to be one of the factors limiting the southern distribution of *P. h. rubellus*.

The growth rate of juvenile *P. h. rubellus* in the present study compares favourably with that of similar species currently being developed for aquaculture. The highest SGR obtained in this study, of 0.26 % day^{-1} at 24 and 28 °C, was slightly lower than the 0.28 – 0.33 % day^{-1} recorded for year-1 juveniles (38.7±0.28 mm CL) of the sub-tropical spiny lobster *P. cygnus* reported by Johnston *et al.* (2006), but substantially lower than the 0.52 % day^{-1} SGR recorded for mass-reared *P. homarus* (38±3.2 mm CL) sourced from the south-east coast of India (Rahman *et al.* 1997). The disparities in growth rates are likely the result of a number of factors. Feeding in the current study, although in excess, only occurred three times a week as opposed to daily in the other studies, and this may have limited growth. Furthermore, it has been suggested that lobsters cultured in isolation exhibit slower growth or lower survival than when kept in groups (Booth & Kittaka 2000), although Phillips *et al.* (1977) found no difference in the growth of *P. longipes cygnus* in isolation or in groups under any temperature regime. Growth rates tend to vary among species, with warm-water species growing faster than their cold-water counterparts (Booth & Kittaka 1994). The geographic separation between the studies is

also therefore likely to have played a role, with the warm water tropical *P. homarus* grown at 28±2 °C growing faster than the subtropical *P. h. rubellus* used in this trial.

Food consumption increased with an increase in water temperature. This is probably due to the increased metabolic activity associated with increased temperature (Wyban *et al.* 2000), and has also been noted for *P. argus* up to 30 °C (Lellis & Russell 1990) as well as *P. interruptus* (Serfling & Ford 1975). A similar pattern was shown by Smale (1978) for *P. h. rubellus*, where high-temperature groups had greater food consumption, except at 30 °C where decreased consumption was attributed to temperature stress. Despite similar growth at 24 and 28 °C, the effect of increased food consumption at the high temperatures is highlighted by the FCR at 28 °C, which is more that 30 % higher than for the 24°C treatment. Although growth was slower at 19 °C compared to 24 °C, the reduced food consumption at this temperature resulted in a similar FCR. Smale (1978) also recorded increased conversion efficiencies at lower temperature ranges (i.e. 24 and 26 °C) and suggested that this range was near optimal.

Aquaculturists must therefore make a compromise between water temperature, growth and food conversion (Lellis & Russell 1990). Increasing temperature up to 24 °C positively affected growth rate and FCR of juvenile *P. h. rubellus*, in comparison to those kept at 19 or 21 °C. However, the lack of a further increase in growth rate at temperatures above 24 °C, combined with the observed reduction in feed conversion efficiency suggest that culture at these elevated temperatures may prove both more costly and inefficient.

2.5 Conclusions

The current study indicates that good growth and feed conversion efficiency for juvenile *P. h. rubellus* is obtainable at 24°C. The adverse effect on growth of temperatures below 24 °C highlights the importance of site selection, or alternatively suggests the maintenance of temperatures above winter ambient levels, for successful culture to be realised. In addition, the communal nature of *P. h. rubellus*, its ready acceptance and consumption of formulated feeds, no captivity-related health problems and good survival all contribute to its suitability for culture. The design and management of systems that prevent cannibalism, as has also been observed in this species when held under high densities, has been highlighted as an important component in achieving successful mass culture in other species (Crear *et al.* 2000). Further research in this direction with *P. h. rubellus* is suggested with the recommendation that future trials be conducted at 24 °C.

CHAPTER 3: Ammonia excretion dynamics in the east coast rock lobster *Panulirus homarus rubellus*.

3.1 Introduction

Ammoniotelism is a characteristic of aquatic crustaceans (Schmitt & Uglow 1997), with the secondary excretory products being urea and amino acids (Cockcroft *et al.* 1988). Ammonia, the principal end-product of protein catabolism, can constitute 60-100 % of the total nitrogen excreted in crustacean (Regnault 1987; Radford *et al.* 2004; Kir *et al.* 2004). Although the ambient ammonia levels in the marine environment are generally very low (less than one µM), the accumulation of ammonia in artificial environments, both from the excretion of cultured animals and the mineralization of unconsumed food and faeces, can be toxic to crustaceans (Schmitt & Uglow 1997; Kir *et al.* 2004). Total ammonia nitrogen (TAN) in solution is composed of an un-ionised form (NH_3-N) and the ionised form (NH_4^+-N), with the proportions of each being dependent mainly on pH, but also on temperature, salinity and pressure (Whitfield 1974). The lipophilic nature of NH_3, combined with its tendency to diffuse readily across respiratory membranes, makes it the most toxic of the two forms to aquatic organisms (Chin & Chen 1987; Chen & Kou 1993). Data on ammonia toxicity for large decapod crustaceans is limited. A 30-day LC_{50} for adult *Homarus americanus* of 88 mg l^{-1} TAN was reported by Cornick & Stewart (1977). The 96-h LC_{50} for adult *H. americanus* increased with decreasing temperature, from 377 mg l^{-1} at 5°C to 219 mg l^{-1} at 20°C (Young-Lai *et al.* 1991). The authors recommended "safe" concentrations of 37.7 mg l-1 TAN and 21.9 mg l-1 TAN at 5°C

and 20°C respectively. Furthermore, sub-lethal concentrations of ambient ammonia can affect the growth (Chen & Kou 1992), ammonia excretion rate (Chen & Lin 1995), oxygen consumption rate (Chen & Lin 1995) and haemolymph ammonia levels (Chen & Kou 1993; Schmitt & Uglow 1997) in several species of crustaceans.

The culture process from wild capture to marketable lobster will involve holding lobster in a variety of water systems where ammonia can accumulate and potentially compromise the commercial value of the product (Crear & Forteath 2002). As a portion of the ambient ammonia present in a lobster culture/holding system will emanate from excretion by the cultured animals, an understanding of the ammonia excretion dynamics of the species is imperative to design suitable holding and culture systems and inform transport protocols. Ammonia excretion can be affected by extrinsic and intrinsic factors including temperature (Chen & Lai 1993), ambient ammonia concentration (Chen & Lin 1995; Schmitt & Uglow 1997), nutritional status (Dall & Smith 1986), daily rhythm (Dall & Smith 1986), body weight (Carvalho & Phan 1997; Crear & Forteath 2002), moult stage (Regnault 1979), diet (Perera *et al.* 2005), salinity (Chen & Lai 1993) and emersion (Crear & Forteath 2002). The aim of this study was to determine the effect of body weight, emersion, daily rhythm, feeding and ambient ammonia on the total ammonia nitrogen (TAN) excretion of the east coast rock lobster *P. h. rubellus*. Empirical information on TAN excretion rates in relation to these factors will assist in the design of suitable biological filters for holding and growout facilities, provide insight into key factors governing transport protocols such as recovery and purge times and provide target limits for water quality parameters.

3.2 Materials and Methods

3.2.1 Experimental animals

Lobster were collected, transported and acclimated to captivity as described in Chapter 2 (see 2.2 Materials and Methods - 2.2.1 Collection, transport and acclimation of experimental animals)

3.2.2 Experimental system and procedure

The methods used to determine the total ammonia nitrogen (TAN = NH_3-N + NH_4^+-N) excretion of individual lobster were based on those employed by Crear & Forteath (2002). Individual lobsters were housed in 25 l plastic chambers suspended in a water bath (1x0.6x0.3m) that formed part of a biologically filtered recirculating system. The system was housed in a temperature-controlled laboratory where water temperature was maintained using submersible heaters at 24 °C for all trials. Overhead fluorescent lighting provided a 12L:12D light regime and water pH and salinity were maintained at 7.7-7.9 and 33-36 mg l^{-1} respectively. Three chambers were used to determine TAN excretion rates. A fourth chamber served as a control to test for TAN production or consumption within the experimental chamber. Each chamber was supplied with an influent water supply from the filter sump with TAN below 0.03 mg l^{-1} and an outflow that emptied into the water bath. The chambers were aerated to prevent dissolved oxygen levels from being limiting and to provide adequate mixing of water, thereby ensuring water samples were representative of the experimental chamber. Dissolved oxygen levels were tested

intermittently and remained above 90 % saturation. Volatilisation of ammonia from aerated tanks is considered to be negligible (Forsberg & Summerfelt 1992; Crear & Forteath 2002) and this was supported by the stable TAN levels in the control chambers. Lobsters used for these experiments were considered to be in the intermoult stage. The moult index developed for *P. ornatus* by Turnbull (1989) was used as a guide to determine moult stage based on daily monitoring of the experimental lobsters both before and after the experiments. Animals that had moulted six days or less before the planned trial were excluded as well as those that moulted within 10 days after the trial. All lobster used for experiments had fully hardened exoskeletons.

The rate of TAN excretion was determined by stopping the flow of water to each chamber and measuring the increase of TAN in the chamber over time. TAN excretion rates (mg TAN g^{-1} h^{-1}) were based on the difference in TAN concentration in the chamber between two consecutive and timed samples (Schmitt & Uglow 1997) and were calculated as follows:

$$\text{TAN excretion rate} = ((TAN_{final}-TAN_{initial}) \times V) / (W \times T)$$

Where TAN_{final} is the TAN concentration in the sample as mg l^{-1} at the end of the measuring period; $TAN_{initial}$ is the TAN concentration in the sample as mg l^{-1} at the start of the measuring period; V is the volume of water in the experimental chamber in litres; W is the weight of the lobster in grams; and T is the duration of the measuring period.

The volume of water in the experimental chambers was maintained at 15 l for all experiments. Lobsters were purged for 48 hours prior to a trial and were acclimated overnight (18 h) to the experimental chambers under flow-through conditions, except preceding emersion trials when lobsters were purged for 72 hours and acclimated for 42 hours. During extended trials, the flow to the chamber was turned on at predetermined intervals for a period of 60 minutes to flush the chambers and prevent ammonia levels from exceeding 1.5 mg TAN l^{-1}, although levels generally remained well below 1 mg TAN l^{-1}.

Two 50 ml water samples were taken at each sampling event and frozen at -16 °C for up to seven days before analysis, well within the recommended maximum storage period of 2 weeks (Parsons *et al.* 1984). TAN was analysed on a 10 ml sub sample by the phenol-hypochlorite method of Solorzano (1969). A new calibration curve was determined for each batch of samples analysed.

3.2.3 Effect of body weight on TAN excretion

The effect of body weight on TAN excretion was determined during daylight hours using lobsters ranging in total body weight from 16.8 to 322g (n = 16). Water flow to the experimental chambers was stopped for a two hour period (10:00 – 12:00 h) during which water samples were taken at hourly intervals.

3.2.4 Effect of daily rhythm on TAN excretion

TAN excretion rates were measured for a five hour period during the light (10:00-15:00 h) and dark (19:00-24:00 h) photophase to test for the presence of a daily rhythm in TAN excretion. Lobsters (n = 12; 181.12 ± 22.89 g) were acclimated overnight before trials commenced. Water flow to the chambers was stopped at the start of the light and dark trial periods following which water samples were taken hourly. The chambers were returned to flow-through conditions between trials and overnight before the commencement of emersion trials on the same animals the following day.

3.2.5 *Effect of emersion on TAN excretion*

The effect of emersion on TAN excretion was determined by placing the lobster in a bucket without water with a closed lid for a period of one hour. Emersion in a humid environment for a period of one hour was considered representative of the transport conditions a lobster would undergo when being transported between its point of capture and a holding facility. TAN excretion rates were determined for one hour (10:00 – 11:00 h) prior to emersion and at one hour intervals for a further five hours (12:00 – 17:00 h) after the lobsters were returned to the experimental chamber.

3.2.6 Effect of feeding on TAN excretion

The effect of feeding on TAN excretion was determined after feeding (10:00 h) the lobsters with a quantity of squid (*Loligo vulgaris reynaudi*) equivalent to approximately 3 % of their body weights. The lobsters (n =12; 170.15 ± 23.79 g) were allowed 165 minutes to consume the food after which the chamber was flushed for 15 minutes. The first water sample was taken directly after flushing and then at one hour intervals

thereafter for the first 12 hours, at four hour intervals for another 24 hours, at six hour intervals for another 18 hours and finally once again after a further 16 hours. The chambers were flushed for a period of one hour at 5, 11, 32 and 51 hours after the first water sample was taken.

3.2.7 Effect of ambient ammonia on TAN excretion

A total of 27 lobsters (110.69 ± 19.14 g) were used to investigate the effect of ambient water ammonia (mg TAN l^{-1}) on their TAN excretion. The TAN concentration of the water within the experimental chamber was increased by adding a pre-weighed amount of NH_4Cl (R grade) to each chamber and allowing 10 minutes for mixing. Three trials were conducted using mean ambient TAN concentrations of 1.02±0.10 (0.025 mg NH_3 l^{-1}), 2.3± 0.2 (0.059 mg NH_3 l^{-1}) and 4.45±0.78 (0.115 mg NH_3 l^{-1}) mg TAN l^{-1} respectively. For each trial, nine lobsters were randomly selected and placed into experimental chambers while a further chamber did not receive a lobster and served as a control. TAN excretion rates at low ambient water TAN (0.02±0.02 TAN mg l^{-1}) were initially measured initially for two hours (10:00 – 12:00 h) during the light photophase following which the chamber was flushed for 30 minutes. The ambient ammonia concentration in the chamber was then raised by the addition of NH_4Cl. Water samples were then taken at one hour intervals for two hours (12:15 – 14:15 h).

3.2.8 *Statistical analyses*

Linear regression, using log-log transformed data, was used to describe the relationship between lobster weight and total ammonia excretion rate. The Student's t-test was used to test for differences in daily rhythm (light photophase vs. dark photophase rates), feeding (pre-feeding vs. post feeding rates), emersion (pre-emersion (basal) vs. re-immersion rates) and ambient ammonia data. The Mann-Whitney U test was used to test for differences in the feeding and ambient ammonia data where Levene's test revealed unequal variances. All statistical tests were performed using Statistica 7.1 for Windows (Statsoft, 2005) at the 0.05 level of significance.

3.3 Results

Total TAN excretion (µg TAN h^{-1}) of *P. h. rubellus* increased significantly with an increase in body weight (*W*). This relationship is described by the equation $\log_{10}TAN = 0.68\log_{10}W - 0.316$ ($r^2 = 0.60$, $F = 20.78$, $p = 0.0004$) (Fig. 3.1). The relationship between mass-specific TAN excretion (µg TAN g^{-1} h^{-1}) and body weight (*W*) is described by the equation: $TAN = -0.0009\log_{10}W + 0.0034$ ($r^2 = 0.29$, $F = 5.74$, $p = 0.03$).

TAN excretion during the first hour following re-immersion was significantly higher than that recorded prior to emersion ($t = 3.65$, $p = 0.004$, $n = 12$). Approximately twice as much TAN was excreted during the first hour of re-immersion compared to pre-emersion excretion rates. TAN excretion returned to pre-emersion levels by the second hour after re-immersion (Fig. 3.2).

A diurnal rhythm in TAN excretion was not evident for *P. h. rubellus* with no significant difference between light photophase and dark photophase TAN excretion rates ($t = 0.16$, $p = 0.88$, $n = 12$).

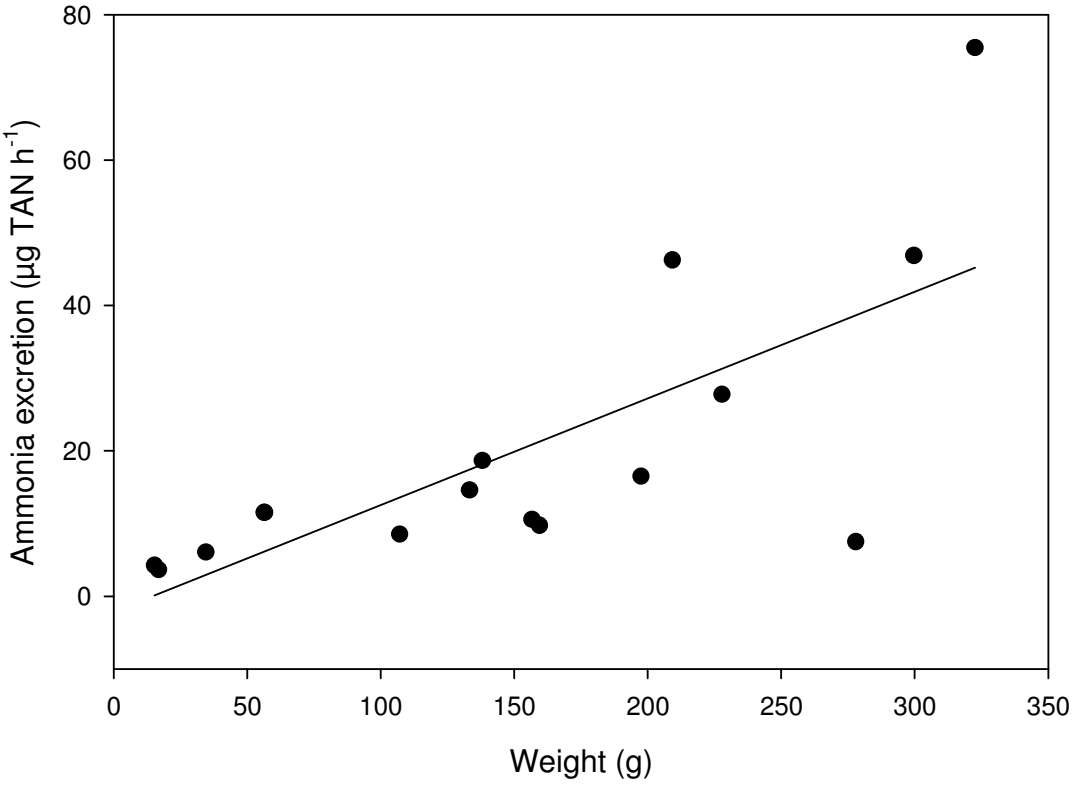


Figure 3.1: Total ammonia excretion (µg TAN h^{-1}) plotted against body weight (g) for *P. h. rubellus* over the body weight range of 16.8 - 322.6 g (n = 16). The relationship is described by the equation: $\log_{10}TAN = 0.68\log_{10}W - 0.316$ ($r^2 = 0.60$, $F = 20.78$, $p = 0.0004$).

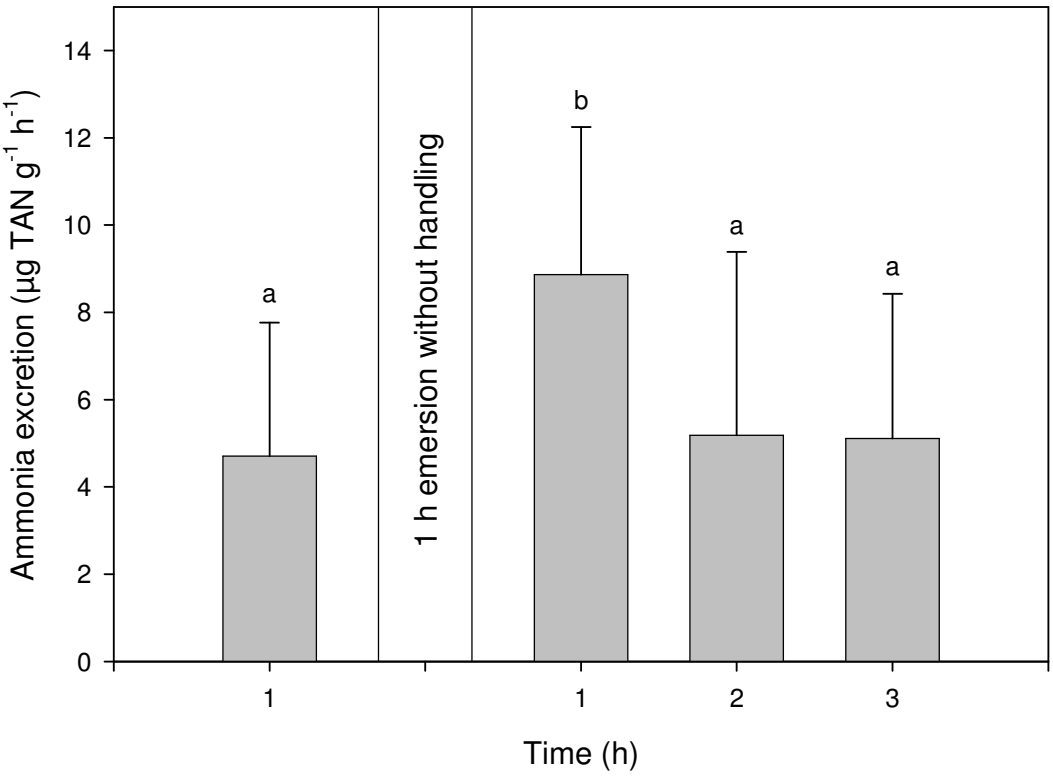


Figure 3.2 : TAN excretion (µg TAN g^{-1} h^{-1}) of *P. h. rubellus* (n = 12) 1 hour before and hourly following emersion for 1 hour in a moist environment without handling disturbance. Columns with differing letters are significantly different (t = 3.65, p = 0.004).

TAN excretion rates were elevated following feeding when compared to pre-feeding levels (Fig. 3.3). Two peaks in TAN excretion were evident following feeding, the first after seven hours (7.58 x pre-feeding rate) and a second smaller peak after 13 hours (6.69 x pre-feeding rate). TAN excretion similar to that of the pre-feeding level was first

recorded 23 hours after feeding and TAN excretion rates 42 hours after feeding were consistently and not significantly different from the pre-feeding rates.

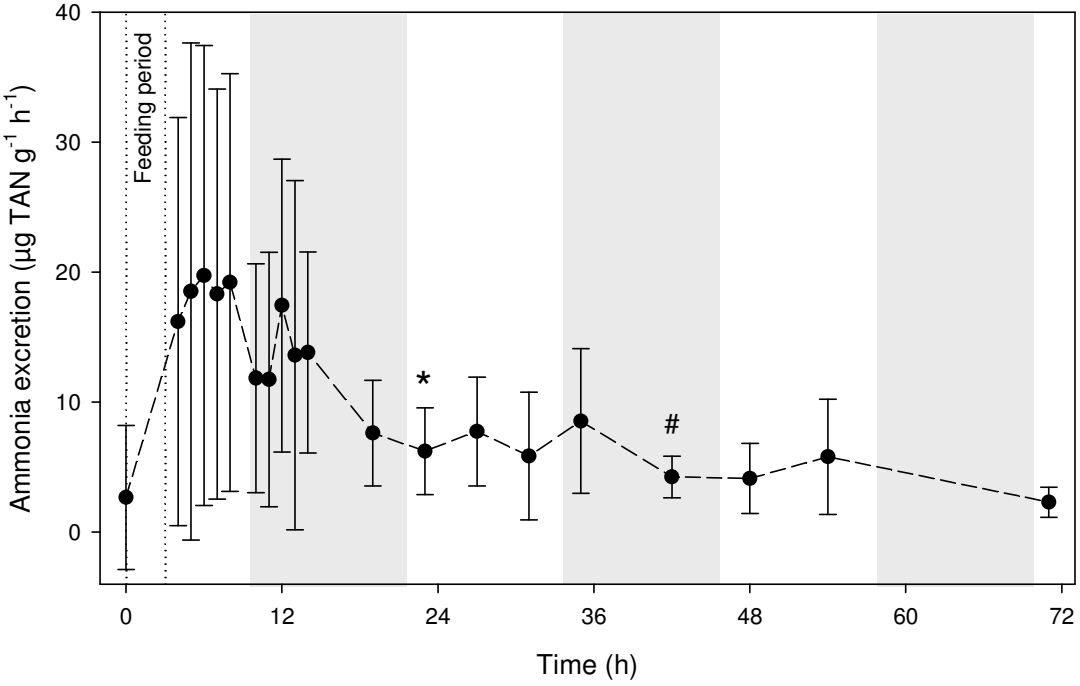


Figure 3.3: TAN excretion rate (µg TAN g^{-1} h^{-1}) of juvenile *P. h. rubellus* (n = 11) in response to being fed squid (*Loligo vulgaris reynaudi*) at 3% of body weight. Animals were allowed to feed for 3 hours prior to water sampling. Grey bands denote the dark photophase. An asterisks and # denote the time period when post-feeding TAN excretion was first and then consistently no longer significantly different from basal TAN excretion respectively.

Exposure to an ambient TAN concentration of 1.02±0.10 mg l^{-1} ($t = 0.54$, $p = 0.59$, $n = 9$) and 2.3± 0.2 mg l^{-1} (Mann-Whitney U Test, $Z = 1.46$, $p = 0.16$) had no significant effect

on the TAN excretion rate of lobster (Fig. 3.4). However, the response to ambient TAN of 2.3± 0.2 mg l^{-1} exhibited a large degree of variation compared to 1.02±0.10 mg l^{-1}, with ammonia uptake occurring in six of the nine animals. An ambient TAN concentration of 4.5±0.78 mg l^{-1} had a significant effect on TAN excretion, with pronounced ammonia uptake occurring for all animals at this concentration (Mann-Whitney U Test, Z = 3.58, p = 0.0003).

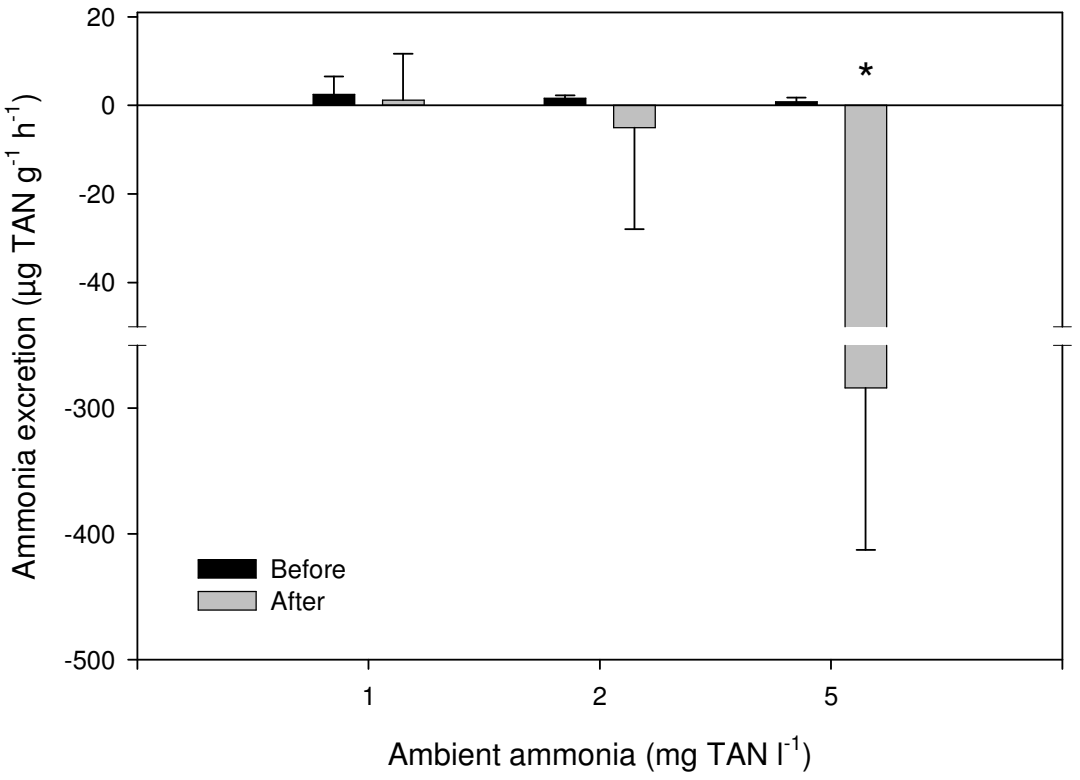


Figure 3.4 : Ammonia excretion (µg TAN g^{-1} h^{-1}) of *P. h. rubellus* juveniles at low ambient ammonia concentrations (0.02±0.02 mg l^{-1}) and after exposure for 120 minutes to ambient ammonia concentrations of 1.02±0.10, 2.3± 0.2 and 4.45±0.78 mg TAN l^{-1}.

An asterisks denotes TAN excretion rates that were significantly different ($p < 0.05$) after exposure.

3.4 Discussion

This study revealed that the TAN excretion rate of *P. h. rubellus* was affected by weight, emersion, feeding and ambient ammonia concentration, but there was no evidence of a daily rhythm. The physiology of the relation between body size and metabolic rate has been extensively examined in the literature for a range of species (Kleiber 1947; Clarke & Johnston 1999). The relationship between body weight and ammonia excretion in crustaceans exhibits a general trend of decreasing ammonia excretion per unit body weight with an increase in body weight (Crear & Forteath 2002). *P. h rubellus* conforms to this trend, with a weight exponent (*b*) of 0.68 for the log-log regression equation of total ammonia excretion versus body weight. A wide range of weight exponents have been calculated for decapod crustaceans such as 0.39 for *Carcinides maenas* (Needham 1957), 0.43 for *Jasus edwardsii* (Crear & Forteath 2002), 0.50 for *P. cygnus* (Crear & Forteath 2002), 0.75 for *Panaeus japonicus* (Marangos *et al.* 1990), 0.84 for *Macropetasma africanus* (Cockcroft & McLachlan 1987) and 0.88 for *Xiphopenaeus kroyeri* (Carvalho & Phan 1997). A similarly wide range (0.47 – 0.95) has also been reported for fish (Jobling 1994). It has been suggested that factors such as changes in the proportion of TAN excretion as a percentage of total nitrogenous excretion with body size (Crear & Forteath 2002) and/or limited size range of animals used in experiments (Carvalho & Phan 1997) could be responsible for such wide variations in *b* values.

The excretion of ammonia in crustaceans occurs at the gills, either passively through diffusion or actively against a concentration gradient via an exchange system involving NH_4^+ (Kormanik & Cameron 1981). The increase in ammonia excretion following re-immersion observed in this study suggests that these processes are inhibited by emersion, leading to the accumulation of ammonia in the haemolymph or other tissues. This ammonia is released rapidly following re-immersion as indicated in the present study by the return within an hour to pre-emersion ammonia excretion rates. The rapid return to base excretion levels following the accumulation of ammonia, usually within an hour, has also been observed in *P. cygnus* and *J. edwardsii* (Crear & Forteath 2002) following 30 minutes emersion with handling and in *Nephrops norvegicus*, following exposure to high ambient ammonia (Schmitt & Uglow 1997). Interestingly, although efflux rates had returned to control levels within 1 hour in *N. norvegicus*, above normal blood TAN levels were present for a further 4 hours. Furthermore, Crear & Forteath (2002) observed excess TAN excretion in both *P. cygnus* and *J. edwardsii* following re-immersion, where greater amounts of TAN were excreted than would have been expected given the basal excretion rate. In the light of elevated TAN excretion rates in *N. norvegicus,* following high activity during emersion (Schmitt & Uglow 1997), and in *X. kroyeri* due to handling (Carvalho & Phan 1997), these authors suggested that the excess TAN excreted was due to handling and activity during emersion. The lack of excess TAN excretion recorded in this study further supports this, given that the emersion process involved minimum handling and no activity was observed over the emersion period.

The double peak in TAN excretion rate following feeding observed for *P. h. rubellus* has been reported in a number of other large decapod crustaceans including *Homarus gammarus* (Wickens 1985), *J. lalandii* (Zoutendyk 1987), *P. cygnus* (Crear & Forteath 2002), *J. edwardsii* (Crear & Forteath 2002) and *H. americanus* (Hawkins *et al.* 1986). The magnitude of the peak (7.6 times preprandial levels with a duration of 23 h) is higher than that reported for most comparable studies with increase of 7.7 (10 h), 6.3 (26 h), 5.6 (30 h) and 4 (18 h) times for *J. lalandii*, *J. edwardsii*, *P. cygnus* and *H. americanus* respectively (Hawkins *et al.* 1986; Zoutendyk 1987; Crear & Forteath 2002). Hawkins *et al.* (1986) suggest that the peaks might be related to endogenous cycles influencing physical activity, digestive processes and hormonal secretions while Crear & Forteath (2002) highlight the possibility of the two peaks being related to different sources of dietary-derived TAN, with metabolically produced TAN being followed by excretory (faeces and urine) losses. The duration of the peak in TAN excretion has significant implications when considering the purge time required prior to transport. Given that TAN excretion first returned to preprandial levels after 23 hours, but only stabilised at those levels after 42 hours, a purge time of 24 – 48 hours is recommended for *P. h. rubellus* to minimise the build up of ammonia in transport chambers.

As noted above, ammonia excretion in crustaceans occurs via ion exchange of NH_4^+ for Na^+ or by passive efflux by during diffusion. Under normal conditions blood ammonia levels are much higher than ambient water concentrations. For example, blood ammonia concentrations in *Penaeus monodon* were approximately 7 mg l^{-1} when held in ammonia-free seawater (Chen & Kou 1993). Given this concentration gradient, ammonia diffuses

across the gill epithelium into the water. However, when crustaceans are exposed to ammonia-enriched media, where ambient ammonia levels exceed those in the blood, an influx of ammonia into the blood occurs with a concurrent decrease in ambient ammonia concentration (Schmitt & Uglow 1997).

Accumulation of ammonia in the blood at elevated levels of ambient ammonia levels has been noted for a number of decapod crustaceans, for example, Young-Lai *et al.* (1991) showed for adult *H. americanus* that increasing ambient ammonia levels were associated with a concomitant increase in blood ammonia. Similarly, haemolymph ammonia levels for *P. monodon* became a function of ambient ammonia-N levels when ambient levels exceeded 10 mg l^{-1}, suggesting a point at which the diffusion of ammonia from haemolymph to the water is reversed (Chen & Kou 1993). While no TAN uptake occurred in *P. h. rubellus* at an ambient TAN concentration of 1 mg TAN l^{-1} (0.025 mg NH_3 l^{-1}), uptake was observed at 2.3 mg TAN l^{-1} (0.059 mg NH_3l^{-1}) and 4.5 mg TAN l^{-1} (0.115 mg NH_3 l^{-1}). The level of TAN uptake observed at 4.5 mg TAN l^{-1} for *P. h. rubellus* in this study (283 µg TAN g^{-1} h^{-1}) is in the range of ~190 µg TAN g^{-1} h^{-1} (pH 7.5) to ~ 240 µg TAN g^{-1} h^{-1} (pH 8) presented for *Penaeus chinensis* exposed to an ambient TAN concentration of 5 mg TAN l^{-1} for 4 hours (Chen & Lin 1995). The partial uptake of TAN exhibited at 2.3 mg TAN l^{-1} in this study suggests that ambient TAN concentrations in this region represent the upper limit at which the diffusion gradient is reversed to allow influx and/or where active transport is no longer able to prevent TAN influx. Interestingly, ammonia uptake was already occurring at ambient TAN concentrations of 1mg l^{-1} for *P. chinensis*, although only at low pH levels (7 and 7.5)

(Chen & Lin 1995). This trend was also apparent for *P. monodon*, where haemolymph ammonia of shrimp exposed to a 50 mg l-1 ammonia-N solution was higher at pH 7.2 than at pH 8.2, suggesting that NH_4^+-N may play an important role in the elevation of haemolymph ammonia (Chen & Kou 1993). Furthermore, extended exposure (6 h) to high ambient TAN concentrations in *N. norvegicus* showed a sharp initial increase in blood TAN up to 2 hours followed by a decrease to 6 hours (Schmitt & Uglow 1997). This was attributed to an alternative regulatory mechanism, possibly involving the conversion of ammonia into nitrogenous compounds during exposure. The current study suggests that *P. h. rubellus* is able to prevent ammonia influx at ambient concentrations up to 1 mg TAN l^{-1}.

The empirical data presented in this chapter can be used to determine the required biological filter volume for a lobster recirculating system using the following formula:

$$\text{Biological filter volume} = (\textit{TAN excretion rate} \,/\, \textit{Nitrification rate}) \,/\, SSA_f$$

Where *TAN excretion rate* is the total TAN excreted (g) over 24 hours for the stock of lobster, *Nitrification rate* is the amount of TAN the biofilter can nitrify as g TAN m^{-2} day^{-1} and *SSAf* is the specific surface area of the filter medium as m^{-2} m^{-3}.

Using the above formula, the required biofilter volume for 1000 kg of *P. h. rubellus* at 24°C can be calculated. As lobsters will be fed during the growout period, the maximum postprandial TAN excretion rates are used. A 170g lobster produces ~20 ug TAN g^{-1} h^{-1},

therefore 1000 kg of 170 g lobsters will excrete 480 g TAN daily. Nitrification rates as high as 0.9 g TAN m^{-2} day^{-1} have been reported for seawater trickle biofilters (Nijhof 1994). As this rate can vary greatly depending on a variety of factors, including the hydraulic loading rate, water temperature, TAN loading rate, dissolved oxygen concentrations and pH (Eding *et al.* 2006), a conservative rate of 0.5 g TAN m^{-2} day^{-1} is assumed. Furthermore, the specific surface area of filter medium varies greatly between the various forms available. A compromise must be sought between specific surface area and void area in trickle filter media. While media with a high void area help prevent clogging, it does reduce the specific surface area of the media, thereby necessitating larger filters to attain the required nitrification rate (Eding *et al.* 2006). Filter media with a specific surface area of 200 m^{-2} m^{-3} are commonly used in trickle filters currently employed in aquaculture (Kamstra *et al.* 1998). Based on these values, the required biofilter volume for 1000 kg of postprandial lobsters at 24 °C is 4.8 m^3 (i.e [480/0.5]/200). If the re-circulating system was to be designed for holding purposes only, then the basal ammonia excretion rate (~3 ug TAN g^{-1} h^{-1}) could be employed. The required filter size would then be markedly less at 0.72 m^3 (i.e. [72/0.5]/200). Furthermore, Crear & Forteath (2002) highlight the contribution of TAN emanating from the oxidation of urea when calculating biofilters volume. For *P. cygnus* and *J. edwardsii*, this accounted for an additional 20% TAN present in a system.

3.5 Conclusions

Ammonia excretion in *P. h. rubellus* is influenced by a number of factors including body weight, emersion, feeding and ambient ammonia concentrations but shows no evidence of a daily rhythm. However, these factors however rarely influence lobsters independently under culture and transport conditions. Further research into the combined effect of these factors on lobster physiology will help to refine growout and transport protocols for this species.

CHAPTER 4: The effect of body size, diurnal rhythm, feeding and emersion on the oxygen consumption of the east coast rock lobster *Panulirus homarus rubellus*.

4.1. Introduction

Oxygen is one of the major parameters of water quality that affects the health of aquatic organisms (Crear 1998). Lobsters are primarily aerobic organisms and rely on the uptake of oxygen at the gills to supply their metabolic requirements. The filamentous gills are housed in the gill chambers located below the carapace within the lower lateral portion of the cephalothorax. Low concentrations of dissolved oxygen, even for brief periods, cause stress in aquatic organisms (Crear & Forteath 2001). Therefore, in a culture situation, it is imperative to ensure that the concentration of dissolved oxygen is sufficient to meet the metabolic needs of the animals being held and thereby maintain the animals in a healthy condition (Sugita & Deguchi 2000).

However, a number of extrinsic and intrinsic factors have been shown to affect the oxygen consumption rate of crustaceans (Cockcroft & Wooldridge 1985). Factors such as body weight, salinity, dissolved oxygen levels, temperature, diurnal rhythm, feeding, diet, handling and activity have all been shown to influence oxygen consumption in spiny lobsters (Buesa 1979; Crear & Forteath 2000; Crear & Forteath 2001; Perera *et al.* 2005; Perera *et al.* 2007). A sound physiological understanding of how these factors affect the oxygen consumption of lobsters is required if suitable holding and culture systems are to be developed and optimised for the species being cultured. Despite the ongoing

international interest in the growout of spiny lobster and the well developed live sale and transport infrastructure that already exists, physiological studies of this nature have only appeared recently and have been limited to a handful of species, most notably *P. cygnus* (Crear & Forteath 2001), *J. edwardsii* (Crear & Forteath 2000) and *P. argus* (Perera *et al.* 2007). No studies to date have investigated the oxygen consumption rates of *P. h. rubellus*.

The theoretical supply chain associated with harvesting *P. h. rubellus* from the wild and their subsequent growout, as discussed in Chapter 1, is likely to expose lobsters to a number of factors, including exposure to air and feeding, that will influence their oxygen consumption. Furthermore, intrinsic factors such body weight and diurnal rhythm can also affect oxygen consumption under culture conditions. This study investigates the oxygen consumption response of *P. h. rubellus* to body weight, photophase, feeding and emersion in order to gain empirical data for the development of optimised transport, holding and growout protocols.

4.2 Materials and Methods

4.2.1 Experimental animals

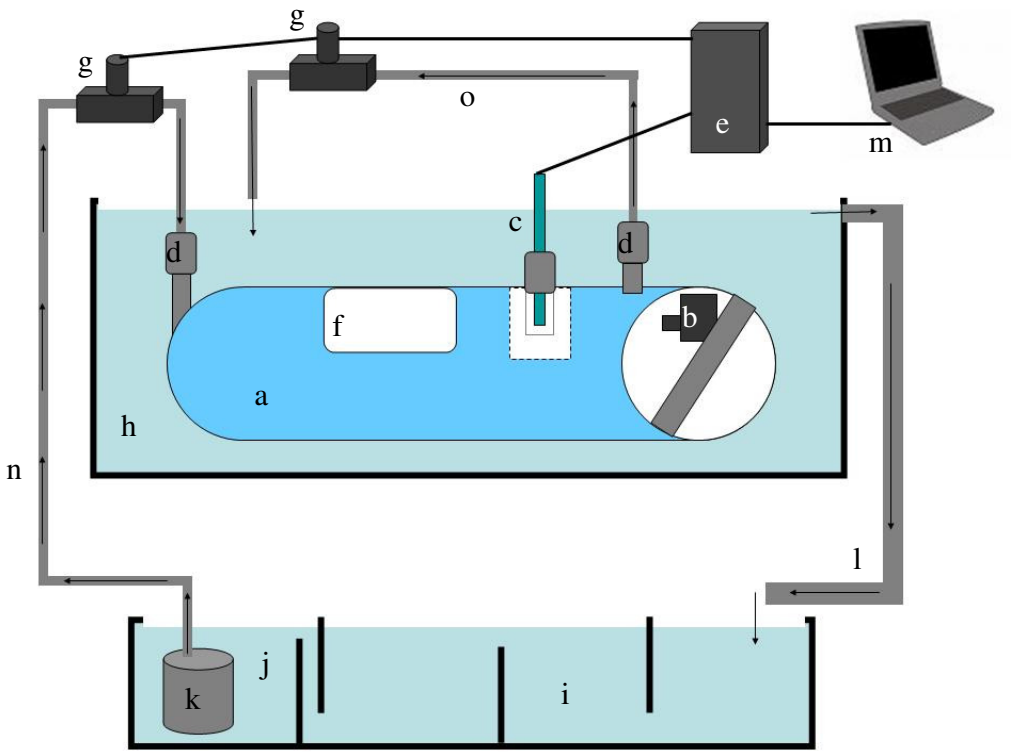
Lobsters were collected, transported and acclimated to captivity as described in Chapter 2 (see 2.2 Materials and Methods - 2.2.1 Collection, transport and acclimation of experimental animals)

4.2.2 Determination of oxygen consumption

The oxygen consumption of lobster was determined using intermittent flow respirometry. This form of respirometry retains some of the simplicity and low cost of closed respirometers without limiting the duration of the experiment due to oxygen depletion. Intermittent flow systems are characterized by a measurement phase (closed respirometer with no flow) and a flush phase (open respirometer with flow). The duration of the measurement phases is manipulated to allow sufficient time for a significant decrease in oxygen levels to occur, yet ensures that the concentration of dissolved oxygen is maintained at non-stressful levels. The flush phase allows fresh oxygenated water to enter the chamber and prevents the accumulation of waste products (Kaufmann *et al.* 1989).

Two intermittent flow respirometers arranged in parallel were used for the experiments (Fig. 4.1). The experimental chambers were constructed from 680 mm lengths of 160 mm diameter PVC piping (volume = 13.67 l). The piping was sealed at the inlet end and had a clear PVC screw-on lid on the outlet end that sealed to the chamber using a rubber O-ring. A clear window (250 mm x 200 mm) on the top surface of the chamber allowed observation of the lobsters during experiments. A submersible pump (Resun SP-850; flow rate = 350 l h^{-1}) was mounted in the lid of the respirometer to ensure both adequate mixing within the chamber and a sufficient flow of water past the oxygen probe. An overestimation of standard oxygen consumption of crustaceans, associated with excessive activity resulting from the use of smooth walled respirometers, has been suggested (Dall 1986). In order to negate this problem, the respirometer base was lined with oyster mesh to provide the lobsters with a suitable surface for attachment. The chambers were

submersed in a water bath that formed part of the experimental recirculating system. Water temperature in the system was maintained within 0.3 °C using submersible heaters.



KEY

a. respirometer chamber
b. submersible pump
c. oxygen probe
d. non-return valves
e. controller box housing solenoid relay and signal converter.
f. observation window
g. solenoid valves
h. water bath
i. biological filter
j. sump
k. supply pump
l. water bath overflow
m. laptop computer
n. respirometer inflow supply
o. respirometer outflow

Figure 4.1: Schematic diagram of the intermittent flow respirometry system. Two respirometers were used in parallel for experiments, although in the interests of clarity the diagram depicts only one of these. Diagram not drawn to scale.

Water flow to each respirometer was supplied by a submersible pump located in the bio-filter sump and controlled via a pair of solenoid valves (Hunter PGV 24V AC). The solenoid valves were located away from the experimental chambers and linked via lengths of 20 mm flexible piping. Non-return valves mounted on the chamber at the

inflow and outflow prevented water from the inflow and outflow lines from entering the chamber during the measurement phase and thereby compromising readings. The operation of the solenoid valves, and hence water flow through the chamber, was controlled via a relay system (ADAM™ Data Acquisition Module 4060) operated using custom control software developed using AzeoTech DAQFactory™ version 5.77. The software interface allowed the measurement period and re-oxygenation period to be set according to the size of lobster being tested.

Oxygen tension in the chambers was measured using TPS ED1 oxygen sensors and TPS microCHEM-DO2 meters. Analogue outputs from the oxygen meters were converted to digital format (ADAM™ Data Acquisition Module 4017) and transmitted to the control computer. Incoming oxygen tension data were logged every 30 seconds during the measurement phase. The probes were calibrated in oxygen saturated air according to the manufacturers' instructions. Span calibrations were carried out before each experiment while zero-point calibrations were conducted weekly. Both zero and span calibrations were carried out when a membrane was replaced.

The periodicity of the flow cycle was based on the size of the lobster being studied. A 10 min flush / 10 min closed cycle was used for the majority of trials, although smaller lobsters necessitated a longer measurement phase (20 min). This protocol ensured that the dissolved oxygen tension in the chambers generally remained above 80 % saturation. Experimental procedures, such as transferring the lobster into the chamber, can result in transient increases in oxygen consumption (Schurmann & Steffensen 1997). Furthermore,

postprandial increases in metabolism have been observed for lobster (Crear & Forteath 2000; Crear & Forteath 2001). Experimental animals used in the current study were therefore starved for 72 hours and acclimated to the respirometer chambers for 18 hours (overnight) and prior to conducting trials. Given the findings in Chapter 2, all trials were conducted at 24 °C.

Individual oxygen consumption (MO_2 as mg O_2 h^{-1}) and mass-specific oxygen consumption (VO_2 as mg O_2 g^{-1} h^{-1}) were determined using the following equation:

(i) $$MO_2 = [(PO_{2i} - PO_{2f}) V 60] / t$$

(ii) $$VO^2 = MO^2 / W$$

Where PO_{2i} is the initial oxygen concentration (mg l^{-1}) in the respirometer, PO_{2f} is the oxygen concentration in the respirometer at the end of the measuring phase, V is the volume (l) of the chamber adjusted for the lobster volume, W is the wet weight (g) of the lobster and t is duration of the measurement phase (min). The volume of the lobster was determined using displacement. The respirometer chambers were cleaned between trials with a 0.5% NaHCl solution to eliminate the effect of oxygen consumption by bacteria. Trials conducted using a blank chamber revealed that it is was not necessary to adjust for oxygen consumption due to microbial activity.

Fry (1971) defines standard oxygen consumption as the minimum oxygen consumption for an unfed, resting fish. In this study, standard oxygen consumption was determined

from quiescent lobster during daylight hours (11:00 – 15:00 h) once three consecutive and similar measurements had been achieved (Crear & Forteath 2000). When determining active oxygen consumption, activity should be maintained for an extended period. Difficulties associated with forcing lobster to remain active for significant periods of time have been highlighted in a number of studies (Rutledge & Pritchard 1981; Dall 1986; Crear 1998). As active oxygen consumption is in essence a measure of the maximal level of oxygen consumption (Bennett 1978), and lobsters will be repaying an oxygen debt after a period of emersion and handling, it has been argued that oxygen consumption measured after handling and air exposure is an valid estimation of active oxygen consumption (Waldron 1991; Crear & Forteath 2000). Therefore, active oxygen consumption in this study was determined over a 10 minute period following a five minute period of emersion and constant handling. The period of measurement (10 min) is less than the 15 minutes employed by Rutledge & Pritchard (1981), Crear & Forteath (2000) and Crear & Forteath (2001) but greater than the two minutes period used by Innes (1985).

4.2.3 Effect of body weight on oxygen consumption

The standard and active oxygen consumptions were determined for lobsters ranging from 52 – 541 g (n = 20). Log_{10} transformed linear regressions of individual oxygen consumption (MO_2) and mass-specific oxygen consumption (VO_2) versus weight (W) were expressed by the general equation: Log10 MO_2 or $VO_2 = a + b\log W$, where a is the intercept on the y-axis and b is the slope of the regression.

4.2.4 Effect of diurnal rhythm on oxygen consumption

Oxygen consumption of lobsters (n = 10, 221.15±30.77 g) was measured over a 48 hour period to investigate the effect of diurnal rhythm. Daytime oxygen consumption was calculated from readings taken during the light photophase (07:00 – 19:00 h) and night time oxygen consumption from reading taken during the dark photophase (19:00 – 07:00 h). This also allowed the routine oxygen consumption (i.e. the oxygen consumption of a fasting lobster over 24 hours including spontaneous movement) to be determined.

4.2.5 Effect of feeding on oxygen consumption

The effect of feeding on oxygen consumption was determined after feeding lobsters (n = 8, 229.83±27.8) with a quantity of squid (*Loligo vulgaris reynaudi*) equivalent to approximately 3 % of body weight All lobsters were fed at the same time of day (09:00 h) to control for any effects of diurnal rhythm on oxygen consumption. Data from lobsters that did not ingest the food within 1 hour of introduction into the respirometer were excluded from the analyses.

4.2.6 Effect of emersion on oxygen consumption and recovery

The oxygen consumption of lobsters (n = 7, 239.3±33.2 g) was measured over a one hour period (08:00 – 09:00 h) before they were removed from the respirometer and emersed for one hour. During emersion, lobsters were housed separately in lidded plastic buckets. Lobsters exhibited tail-flicking behaviour during removal from and return to the

respirometers, however, they generally remained quiescent between these events. Lobsters were returned to the respirometers after emersion and their recovery monitored. Emersion in a humid environment for a period of one hour was considered representative of the transport conditions a lobster would undergo when being transported between its point of capture and a holding facility.

4.2.7 Statistical analyses

Linear regressions describing the relationship between oxygen consumption and body weight were calculated by the least-squares method. An ANOVA was used to test the significance of the regression slope, *b*. The student's *t*-test was used to evaluate when postprandial and post-emersion oxygen consumption was not significantly different from standard levels. The Mann-Whitney U test was used to test for differences in the data on feeding and diurnal rhythm data where Levene's test revealed unequal variances. All statistical tests were performed using Statistica 7.1 for Windows (Statsoft 2005) at the p = 0.05 level of significance.

4.3 Results

4.3.1 Body weight

There was no significant effect of lobster sex on standard (MO_2 – SS 0.00, $F = 0.04$, p = 0.85; VO_2 – SS 0.003, $F = 0.22$, p = 0.65) or active (MO_2 – SS 0.009, $F = 1.1$, p = 0.31; VO_2 – SS 0.029, $F = 2.39$, p = 0.14) oxygen consumption. In light of this, data for both

sexes were pooled. The standard and active oxygen consumption rates (MO_2 as mg O_2 g^{-1} h^{-1}) of lobsters both increased with an increase in body weight (Fig. 4.2). These relationships are best described respectively by the following regression equations:

(i) $\text{Log}_{10}\text{Standard } MO_2 = -0.265 + 0.671\log_{10}W$

(MS = 0.82, F = 77.55 $r^2 = 0.82$, $p < 0.0001$)

(ii) $\text{Log}_{10}\text{Active } MO^2 = -0.492 + 0.923\log_{10}W$

(MS = 1.63, F = 184.26, $r^2 = 0.91$, $p < 0.0001$)

Standard mass specific oxygen consumption (VO_2 as mg O_2 g^{-1} h^{-1}) decreased with an increase in lobster weight (Fig. 4.3) This relationship is best described by the regression equation: $\text{Log}_{10}\text{Standard } VO^2 = -0.304 - 0.319\log_{10}W$ (MS = 0.185, F = 16.52, r^2 = 0.0.49, p = 0.0008). There was no linear relationship evident between active mass-specific oxygen consumption and lobster weight (p = 0.45).

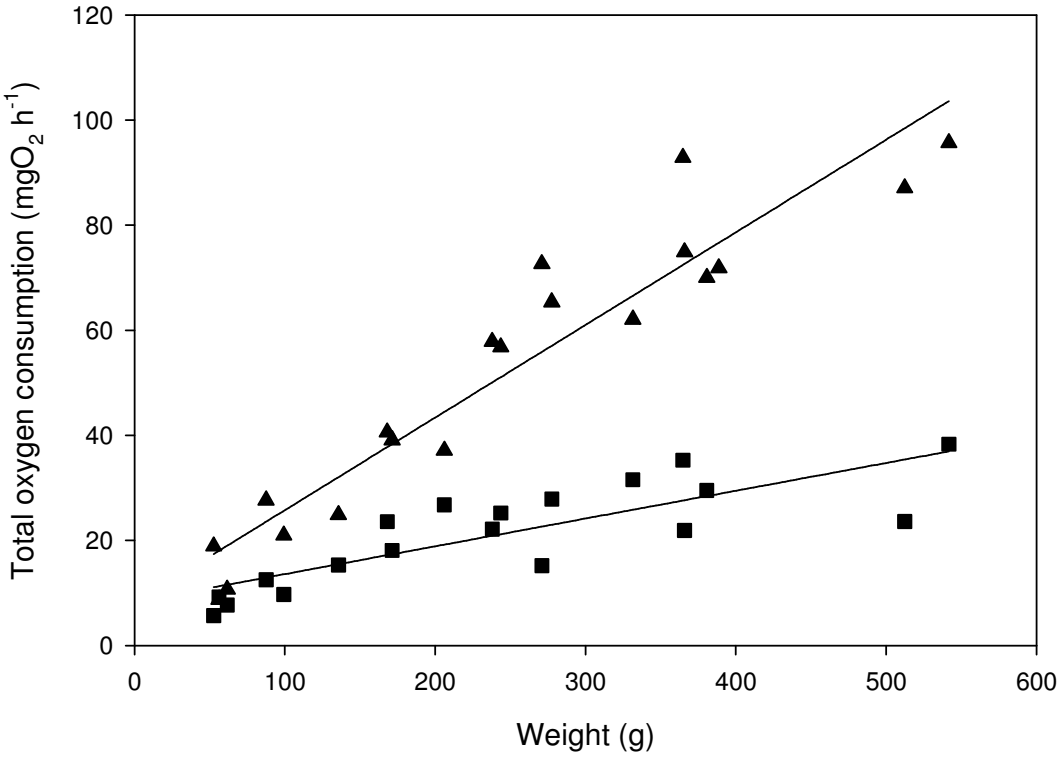


Figure 4.2: A plot of individual oxygen consumption (MO_2, mg O_2 h^{-1}) against body weight (g) of the east coast rock lobster *Panulirus homarus rubellus*. Standard (■) and active (▲) oxygen consumption rates are shown.

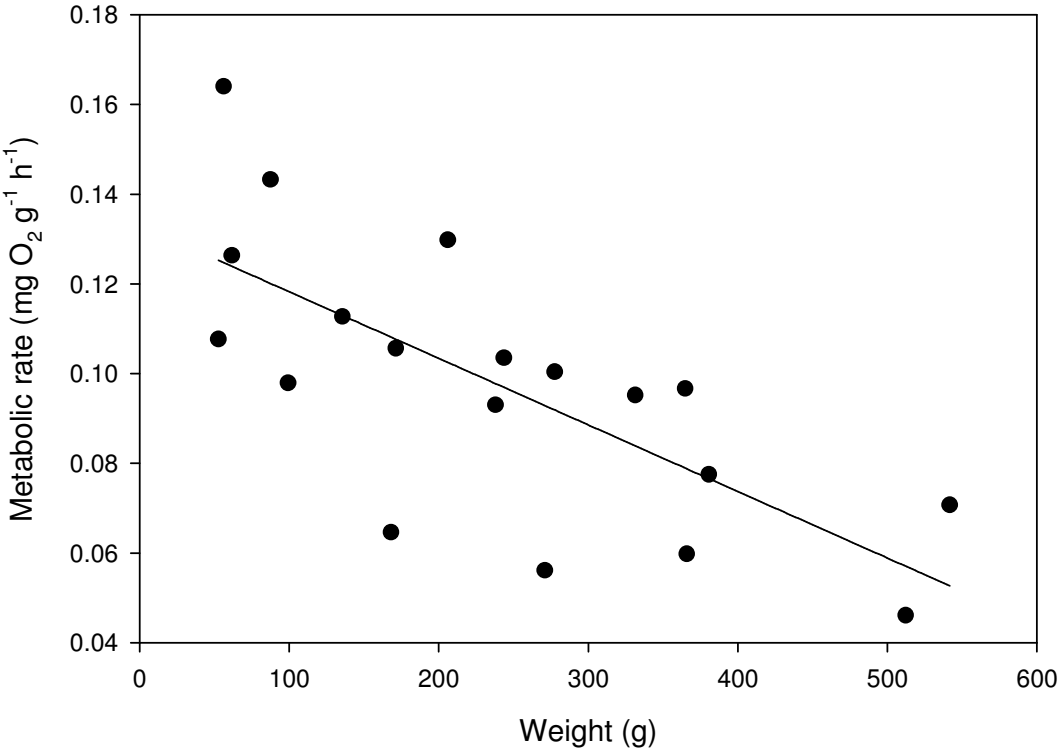


Figure 4.3: A plot of resting metabolic rate (VO_2, mg O_2 g^{-1} h^{-1}) against body weight for the east coast rock lobster *Panulirus homarus rubellus*.

4.3.2 Diurnal rhythm

Lobsters consumed significantly more oxygen during the dark photophase than during the light photophase (Mann-Whitney U Test, Z = 5.57, p < 0.0000). Average oxygen consumption at night was 67±11 % greater than day time consumption. Lobsters generally remained motionless in the respirometers during the day, while occasional night time observations generally found active lobsters. Due to the quiescent state of lobsters during daylight hours, average daytime oxygen consumption rates were considered a valid indication of standard oxygen consumption. The routine oxygen consumption was

therefore 26.5 % greater than standard oxygen consumption. The average oxygen consumption (VO_2 as mg O_2 g^{-1} h^{-1}) of lobsters (n = 10) over two consecutive days and nights was determined (Fig. 4.4).The onset of darkness is characterized by a rapid increase in oxygen consumption which gradually returns to daytime levels over the course of the dark photophase. There is some indication of a second but lower peak in oxygen consumption later in the night.

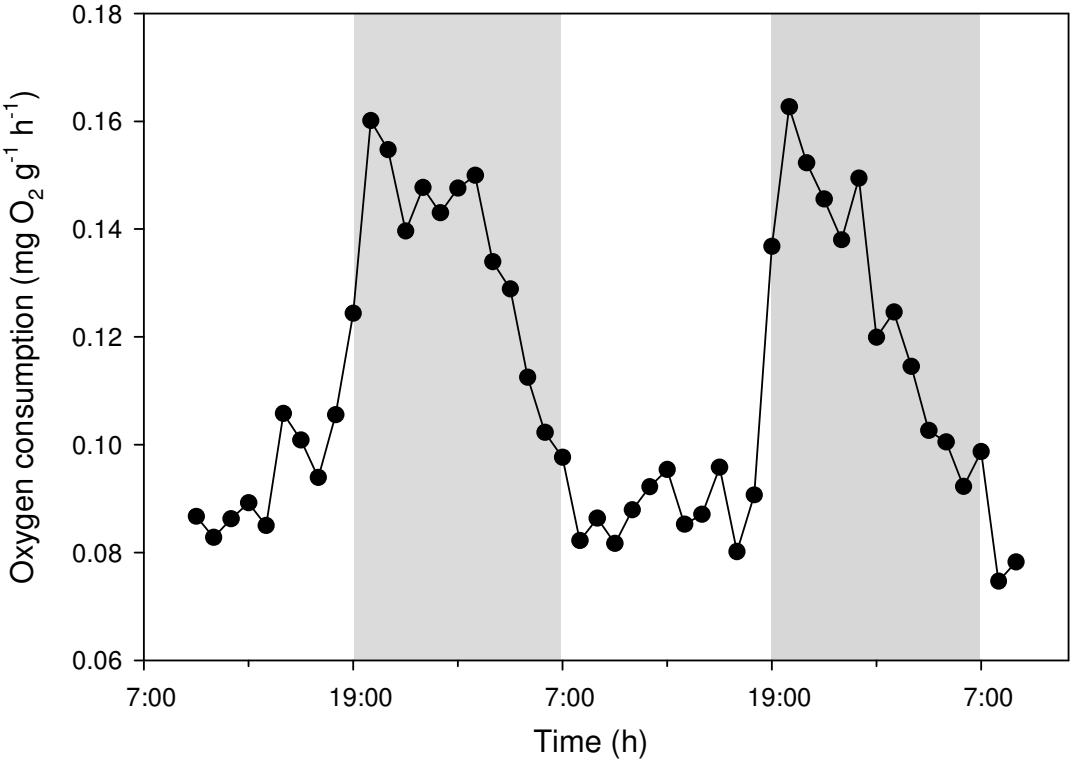


Figure 4.4: Oxygen consumption (mg O_2 g^{-1} h^{-1}) of the east coast rock lobster *Panulirus homarus rubellus* (n = 10, 221.15±30.77 g) over a 48 hour period. Grey bands denote the dark photophase. Each symbol represents the hourly average of oxygen consumption. In

the interests of clarity, lines link succeeding data points and standard errors are not shown.

4.3.3 Feeding

Following feeding, oxygen consumption increased to an initial daytime peak after 4-5 hours (Fig. 4.5). The maximum oxygen consumption rate at this initial peak was 1.84 times the preprandial level. Oxygen consumption dropped after this initial peak before increasing to a maximum postprandial oxygen consumption (2.02 times preprandial levels) after 10-11 h, at the onset of the dark photophase. Apart from the initial peak soon after feeding, the effect of diurnal rhythm on oxygen consumption remained strong, with well-defined nocturnal peaks following the onset of darkness and then gradually decreased to the reduced consumption associated with daylight hours. Oxygen consumption was not significantly different from preprandial levels 21 hours after feeding ($t = 1.64$, $p = 0.12$).

4.3.4 Emersion

A significant increase in oxygen consumption ($t = 6.78$, $p = 0.00003$) was observed after lobsters were returned to the respirometers following an hour of emersion in a moist environment. Oxygen consumption gradually decreased until four hours after re-immersion, when it was no longer significantly different ($t = 2.06$, $p = 0.06$) from pre-emersion levels (Fig. 4.6).

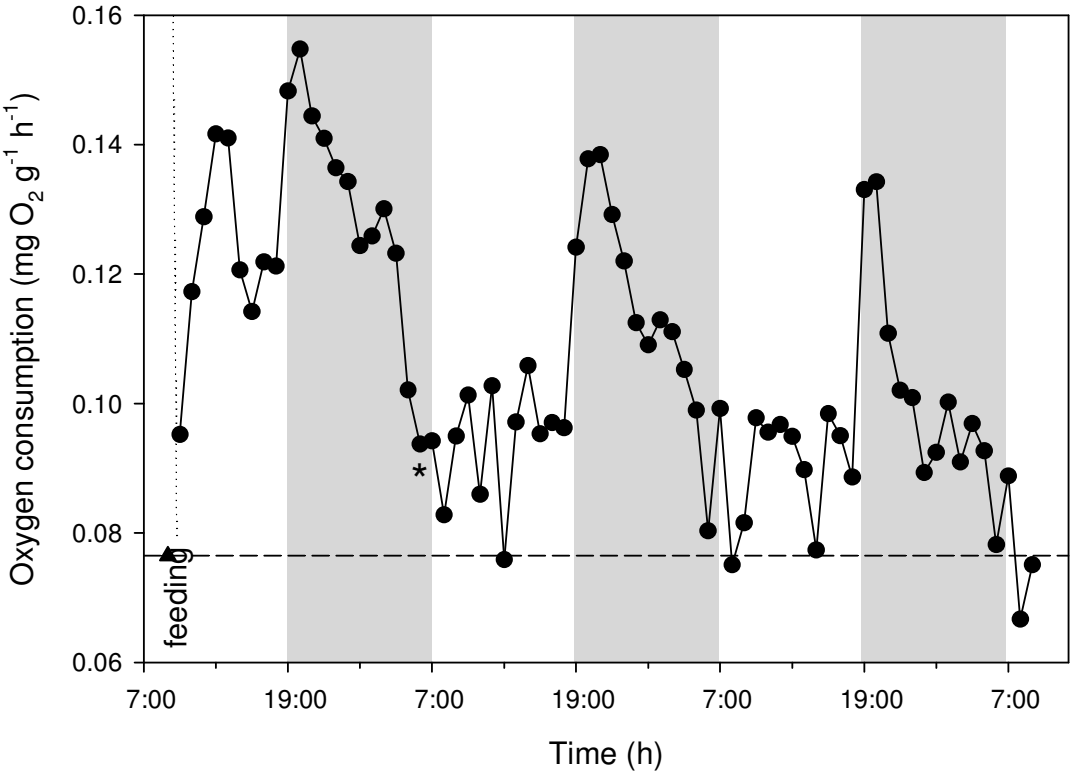


Figure 4.5: Oxygen consumption (mg O_2 g^{-1} h^{-1}) of the east coast rock lobster *Panulirus homarus rubellus* (n = 8, 229.83±27.8) over a 72 hour period after being fed with squid (*Loligo vulgaris reynaudi*) at approximately 3% body weight. Grey bands denote the dark photophase. Each symbol (▲ – preprandial; ● – postprandial) represents the hourly average of oxygen consumption. In the interests of clarity, lines link succeeding data points and standard errors are not shown. The asterisks denotes the time at which postprandial oxygen consumption is not significantly different from the preprandial rate.

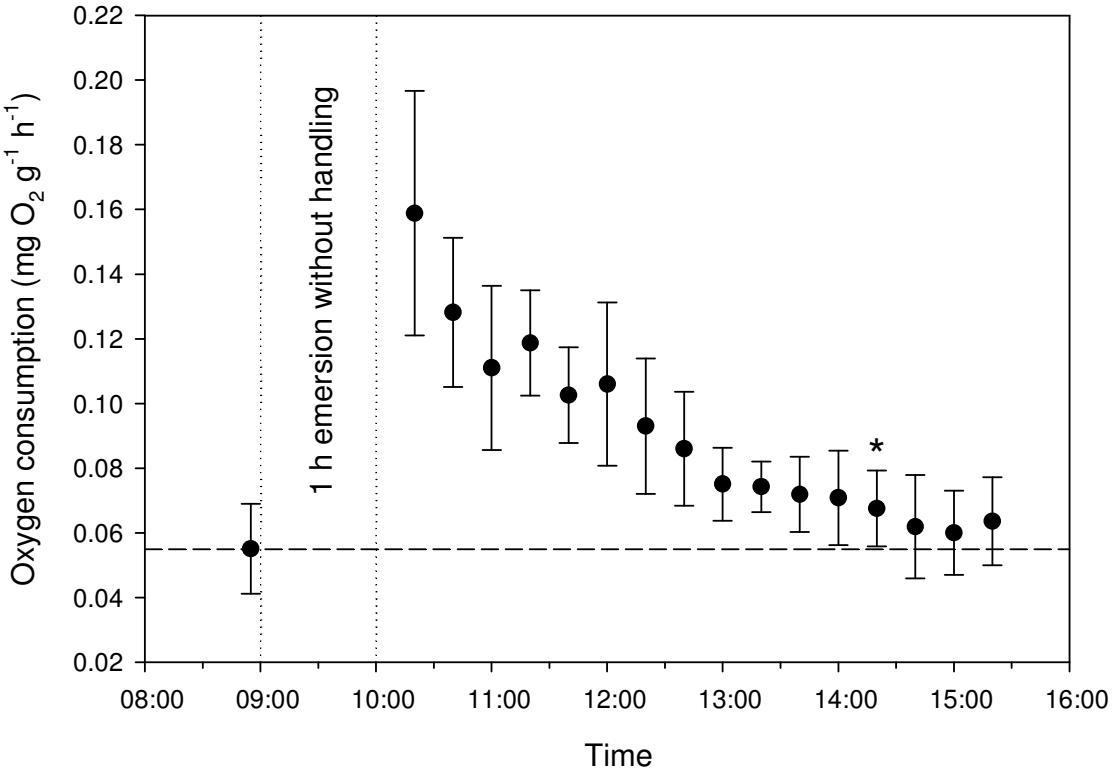


Figure 4.6: The response in oxygen consumption of the east coast rock lobster *Panulirus homarus rubellus* (n = 7, 239.3±33.2 g) to a one hour period of emersion in a moist environment. Each symbol represents the oxygen consumption rate over a 20 min period. The asterisks denotes the time at which post-emersion oxygen consumption is no longer significantly different from the pre-emersion rate.

4.4 Discussion

This study has demonstrated that body weight, activity, diurnal rhythm, feeding and emersion all have significant effects on the oxygen consumption of *Panulirus homarus rubellus*. Sex, however, did not influence oxygen consumption. This finding is in accordance with a range of decapod crustaceans including *Macropetasma africanus* (Cockcroft & Wooldridge 1985), *J. edwardsii* (Crear & Forteath 2000), *P. cygus* (Crear & Forteath 2001), *P. argus* (Perera *et al.* 2007) and *Xiphopenaeus kroyeri* (Carvalho & Phan 1997).

The allometric relationship between metabolic rate and body weight is a classic physiological subject that has been extensively discussed in the early literature (Kleiber 1947), yet remains poorly understood (Prosser 1973). The total oxygen consumption of *P. h. rubellus* exhibited an increase with increasing body weight, with the mass scaling exponents (*b*) obtained for standard (0.67) and active (0.92) oxygen consumption falling in the range documented for a range of decapod crustaceans. Bridges & Brand (1980) reported *b* values ranging from 0.286 to 0.877 for a suite of decapod crustaceans, although these values were obtained from studies conducted at low temperatures (8.5°C – 17.7°C). More specifically, in terms of panulirid lobsters, Crear & Forteath (2001) showed standard and active *b* values for *P. cygnus* of 0.83 and 0.55 respectively, while a study on *J. edwardsii* revealed *b* values of 0.595 (standard) and 0.690 (active) (Crear & Forteath 2000). Perera *et al.* (2007) studied the oxygen consumption-weight relationship

in *P. argus* where a *b* value of 0.754 was obtained, while Zoutendyk (1989) reported *b* values of 0.68 and 0.65 (8 °C and 10 °C respectively) for *J. lalandii*.

Interestingly, *b* values for *J. lalandii* were raised at higher temperatures (0.845, 0.777 and 0.910 at 13, 16 and 19 °C respectively) suggesting an effect of temperature on the mass scaling exponent in this species. This trend was, however, not evident in *P. cygnus*, where there was no significant difference between the slopes (*b*) of the linear regressions for temperatures from 15 to 31 °C (Crear & Forteath 2001). Bridges & Brand (1980) noted that crustaceans towards the upper spectrum of the weight range exhibited high *b* values ($b > 0.75$), suggesting that oxygen consumption showed a greater dependence on weight in large crustaceans. While this may to be the case for *P. cygnus* ($b = 0.83$), it appears that oxygen consumption in other species, such as *J. lalandii* and *J. edwardsii*, and in the current study on *P. h. rubellus* ($b = 0.67$), appear to conform to the concept of surface area dependency (*b* in the region of 0.67) (Cockcroft & Wooldridge 1985). Variations in reported *b*-values could be the result of the modulating effect of temperature (Crear & Forteath 2000), sample size and the size range of animals examined (White & Seymour 2005). Carvalho & Phan (1997) suggest that a range of at least one order of magnitude of the animal's wet weight should be used when investigating body size effects on metabolic rates.

The relationship between resting mass-specific oxygen consumption (mg O_2 g^{-1} h^{-1}) and body weight of *P. h. rubellus* exhibited a negative slope ($b = -0.32$) (Fig. 4.3). This conforms to the general physiological principle that mass-specific metabolic rates are

inversely proportional to body mass (Peters (1983) in Zoutendyk (1989)). This principle has been shown for a number of panulirid lobster species including *P. argus* (Perera *et al.* 2007), *J. edwardsii* (Crear & Forteath 2000), *J. lalandii* (Zoutendyk 1989) and *P. cygnus* (Crear & Forteath 2001). Mass-specific resting oxygen consumption values recorded for *P. h. rubellus* in the current study were in the range of 46 to 164 mg O_2 kg^{-1} h^{-1}. This range compared favorably with the values summarized for a selection of large crustaceans by Crear & Forteath (2000). However, the upper extent of the range recorded was notably higher than that reported for similar sub-tropical and tropical species such as *P. argus* (103 O_2 kg^{-1} h^{-1}) (Perera *et al.* 2007) and *P. cygnus* (96 O_2 kg^{-1} h^{-1}) (Crear & Forteath 2001). This result is possibly of a combination of the small size (52 g) of the animal that yielded the maximum value and some spontaneous activity when the reading was recorded. The effects of feeding regime, body weight, temperature and other environmental factors have also been suggested as explanations for inter-specific differences in metabolic rates (Perera *et al.* 2007).

The marked diurnal rhythm in the oxygen consumption of *P. h. rubellus* observed in this study is similar to that recorded for other sub-tidal panulirid lobsters. As with *J. edwardsii* (Crear & Forteath 2000) and *P. cygnus* (Crear & Forteath 2001), a large increase in oxygen consumption occurred directly after the initiation of the dark photophase. Furthermore, intermittent visual observations of *P. h. rubellus* in the respirometers revealed it to be generally quiescent during the day but active at night. This supports the suggestion that the nocturnal increase in oxygen consumption is linked to activity, with light being the predominant entraining factor (Crear & Forteath 2001). In

its natural environment, *P. h. rubellus* is considered to be a nocturnal species that shelters in rock crevices during the day and emerges at night to feed (Heydorn 1969; Berry 1971a).

Smale (1978) described the activity patterns of recently captured *P. h. rubellus* in the laboratory. Activity associated with feeding started immediately after sunset with the initial broad peak in activity decreasing towards midnight. A second smaller peak in activity occurred around midnight with activity decreasing towards dawn. There is some evidence of a second pre-dawn peak in oxygen consumption (i.e. activity) in the current study (Fig. 4.4), although it is not a marked as that described for *P. cygnus* (Crear & Forteath 2001) and *J. edwardsii* (Crear & Forteath 2000). The routine $\dot{V}O_2$ of 24.5% above standard $\dot{V}O_2$ recorded for *P. h. rubellus* is akin to the routine $\dot{V}O_2$ (24% above standard) reported for *J. lalandii* (Crear & Forteath 2000) but noticeably less than the routine $\dot{V}O_2$ (43.5% above standard) for *P. cygnus* (Crear & Forteath 2001). The large gap between routine $\dot{V}O_2$ and standard $\dot{V}O_2$ in *P. cygnus* was attributed to the large distances (up to 800 m), with the inherent metabolic costs, that this species travels when feeding. The low routine $\dot{V}O_2$ presented for *P. h. rubellus* in this study is as expected for a benthic organism that uses a low energy means of locomotion (walking) when foraging and remains motionless for a large portion of the day. It is also representative of the metabolic requirements in a culture situation where predators are non-existent. The routine $\dot{V}O_2$ is likely to be higher in the oceanic environment as it would also include oxygen consumption associated with the high energy tail-flick escape behavior.

Specific dynamic action (SDA) refers to the increased metabolic rate an animal experiences following ingestion of a meal (McCue 2006). SDA generally reflects the metabolic costs associated with preabsorptive, absorptive, and postabsorptive physiological processes (McCue 2006). These processes include the manipulation of food, absorption and storage of nutrients, deamination of amino acids and the increased synthesis of proteins and lipids associated with growth as well as excretory products (Whiteley *et al.* 2001). The SDA response of *P. h. rubellus* was characteristic of a range of other crustaceans (Whiteley *et al.* 2001). Feeding was followed by a steady rise in oxygen uptake which peaked before gradually returned to pre-feeding levels. However, the gradual decline was temporarily interrupted by a second peak in oxygen consumption associated with the onset of darkness, most likely associated with increased activity as opposed to the SDA response. This highlights the difficulties of characterizing the SDA response in animals that exhibit a strong diurnal influence on their base metabolism.

The time taken to reach the maximum postprandial oxygen uptake in this study (1.84 times preprandial levels after 4-5 hours) is similar to that reported for *P. cygnus* (2.19 times preprandial levels after 7 hours) but less than *J. edwardsii* (1.72 times preprandial levels after 10-13 hours (Crear & Forteath 2000); 1.6 times preprandial levels after 18 hours (Radford *et al.* 2004). Maximum oxygen consumption of the tropical lobster *P. argus*, fed both natural foods and formulated diets, occurred between 1 and 4 hours after feeding (Perera *et al.* 2007). It is known that as habitat temperature decreases the SDA duration increases (Whiteley *et al.* 2001). Provided the same holds true for the time to peak oxygen uptake as proposed by Perera *et al.* (2007), then the results obtained for *P. h.*

rubellus further support this argument, with the time to peak O_2 uptake described above increasing as one moves from tropical (*P. argus*) through sub-tropical (*P. h. rubellus* & *P. cygnus*) to temperate (*J. edwardsii*) species. The duration of the SDA response in *P. h. rubellus* (21 hours) was notably less than both *P. cygnus* (46 hours) and *J. edwardsii* (42 hours), however, it was suggested in both those studies that the effect of diurnal rhythm may have artificially extended the duration.

Lobsters in the current study were able to recover from a period of emersion (i.e. return to pre-emersion oxygen consumption levels) within 4 hours. This recovery period is towards the lower end of the range of that recorded for other large decapod crustaceans. It took *J. ewardsii* 4.5-5 hours to return to pre-emersion levels (Crear & Forteath 2000) while recovery in *P. cygnus* was related to temperature, ranging from five hours at 15 °C to eight hours at 31 °C (Crear & Forteath 2001). During the recovery period, *P. h. rubellus* consumed 3.2 times the oxygen debt incurred during emersion. The additional oxygen consumed is likely related to the switch to anaerobic respiration during emersion, which results in the creation of anaerobic end products that require metabolism (Crear & Forteath 2001). Raised levels of anaerobic end products such as lactate have been observed in large decapod crustaceans during exposure to air (Crear 1998; Morris & Oliver 1999).

The oxygen consumption rates described in this chapter allow the determination of suitable flow rates for *P. h. rubellus* using the following formula (Perera *et al.* 2007):

Flow rate = (*Biomass* * VO_2) / (*Solubility* O_2 – *Minimum* O_2)

Where *Biomass* is the mass of lobsters in the tank in kg, VO_2 is the oxygen consumption rate of fed lobsters in mg O_2 kg^{-1} h^{-1}, *Solubility* O_2 is the solubility of oxygen in water at 24 °C and 35 ‰ salinity in mg l^{-1}, and *Minimum* O_2 is the recommended minimum dissolved oxygen concentration in mg l^{-1}.

The oxygen consumption rate of the experimental lobsters facilitates calculation of dissolved oxygen and associated water flow rate to maintain live lobsters in culture systems. The greatest oxygen consumption in this study was recorded during the nocturnal peak, with values in the region of 160 mg O_2 kg^{-1} h^{-1}, therefore this value is recommended to ensure that oxygen is not limiting at any point during the diurnal cycle. Oxygen solubility at 24 °C and 35 ‰ salinity is 7 mg l^{-1}. Although resting lobsters have been shown to maintain constant oxygen consumption rates down to 40 % saturation (Crear 1998), a minimum recommended dissolved oxygen level of 70 % (4.9 mg l^{-1}) (Perera *et al.* 2007) was used. Therefore the flow rate for 1000 kg of 200-250 g lobsters was calculated at 112 l h^{-1} kg^{-1}. This flow rate is applicable for both holding and culture systems as the SDA response did not cause an increase in oxygen consumption over and above the intrinsic effect of diurnal rhythm.

4.5 Conclusions

This study shows that oxygen consumption in *P. h. rubellus* was influenced by body weight, activity, diurnal rhythm, feeding and emersion. Sex of the lobsters did not influence their oxygen uptake. The ability of lobsters to recover rapidly (within four hours) from a period of emersion is a valuable trait and suggests that it is feasible to successfully transport wild caught individuals to a holding facility. It also highlights the importance of allowing sufficient time for recovery before further transport to prevent the accumulation of stressors over time. Furthermore, the duration of the SDA response is directly indicative of a suitable purge time after last feeding before transport. By ensuring that oxygen consumption is at standard levels before transport, the potential oxygen debt incurred during aerial transport is minimised thereby ensuring minimum recovery times. Of particular interest is the significant effect of activity on oxygen consumption, especially due to diurnal fluctuations that result from nocturnal foraging behaviour. Culture systems should be capable of maintaining dissolved oxygen at levels that provide for the metabolic requirements of lobsters during these periods of peak demand during their diurnal cycle.

CHAPTER 5: General discussion and recommendations

This physiological study was conceived with the purpose of providing empirical data for the development of species-optimised transport, holding and growout protocols for the east coast rock lobster *P. h. rubellus*. Given the limitations discussed in Chapter 1, land-based culture systems offer the most viable option for the development of this species for culture. Unlike ocean-based cage culture, the provision of optimal water quality in these land-based systems becomes an important consideration, especially if recirculating systems are employed. However, the use of partial recirculating or full recirculating systems does provide a valuable opportunity to manipulate key water quality parameters towards optimal levels. This is particularly true of water temperature, one of the key determinants of growth in crustaceans (Hartnoll 1982).

The investigation into the effect of water temperature on growth, survival and food consumption of juvenile lobsters, described in Chapter 2, provides a valuable insight into the advantages of being able to regulate the water temperature in a culture system. The decrease in specific growth rate (SGR) at temperatures below 24 °C highlights the negative effects of low temperatures on the growth of *P. h. rubellus*. Water temperature not only had a significant effect on growth criteria, but also on food consumption by lobsters during the trial. With feed typically a major cost in aquaculture (Radford *et al.* 2004), it is important to optimise its use in a culture environment. Lobsters in this study showed a strong positive correlation between water temperature and food consumption. Feed conversion into animal wet weight was most efficient at 24 °C, and decreased at

higher temperatures (26 and 28 °C). This result, seen together with the associated fast growth rate, indicate that 24 °C is close to the optimal temperature for the culture of *P. h. rubellus.*

The high survival rate and lack of captivity-related health problems observed in this study are a positive indication of the suitability of lobsters to survive extended periods under culture conditions. The growth and feeding responses observed provided an organism level indication of the physiological processes occurring within the lobsters. Having determined the optimal temperature for these processes from a culture perspective, the subsequent physiological work investigating the dynamics of oxygen consumption and ammonia production, and the associated effects on water quality, provide valuable insights for the development of lobster culture systems and protocols.

Ammonia is the principal end product of protein catabolism in crustaceans, comprising 60-100 % of the total nitrogen excreted (Radford *et al.* 2004; Kir *et al.* 2004). Accumulation of ammonia in culture systems can prove toxic to crustaceans (Schmitt & Uglow 1997) and even at sublethal concentrations can affect growth (Chen & Kou 1992). In Chapter 2, the effect of a selection of extrinsic and intrinsic factors on the ammonia excretion rate of *P. h. rubellus* was explored. This was undertaken to provide recommendations for developing culture systems and protocols that are capable of maintaining suitable water quality and reducing stress. Feeding caused a significant increase in total ammonia nitrogen (TAN) excretion rates, which took 42 hours to return consistently to preprandial levels. The results indicate that a rapid accumulation of

ammonia is most likely to occur when lobsters are being transported from a holding facility to a centralized growout system. Under these conditions large numbers of lobsters are typically held in a small body of water with no form of biological filtration or replacement water flow. In order to reduce the amount of ammonia produced by lobsters under these conditions it is imperative that lobsters are starved for at least 42 hours prior to transport to negate the influence of postprandial increases in TAN excretion.

Similarly, a significant increase in TAN excretion was noted after emersion, however the return to basal levels was rapid, within one hour of re-immersion. Given this finding, it is suggested that lobsters be allowed to purge accumulated ammonia between periods of emersion to prevent its cumulative effect. Ammonia uptake was observed in *P. h. rubellus* at ambient TAN concentrations between 2 and 5 mg TAN l^{-1}. This suggests that the lobsters' active transport mechanisms were unable to maintain a homeostatic balance of internal TAN concentration under these conditions. It has been suggested that ammonia entering the haemolymph of crustaceans is rapidly converted to the ionized form (NH_4^+). This leads to the release of hydroxyl ions and a concomitant increase in blood pH, which significantly affects enzyme-catalysed reactions and membrane stability (Campbell 1973). Ammonia uptake was not evident at an ambient TAN concentration of 1 mg l^{-1}, suggesting that TAN levels in culture systems should be maintained at or below this level. The required biofilter volume for 1000 kg of postprandial lobsters at 24 °C was calculated at 4.8 m^3. The required filter size would be markedly less (0.72 m^3) if the re-circulating system was to be designed for holding purposes only, as the basal ammonia excretion rate (~3 ug TAN g^{-1} h^{-1}) could be employed. Furthermore, as highlighted by

Crear & Forteath (2002), the TAN emanating from the oxidation of urea can account for a further 20% TAN in the system.

The provision of sufficient dissolved oxygen to meet the metabolic needs of the animals being held in a culture or holding system is crucial if the health of the animals is to be maintained, and maximum growth and survival attained (Sugita & Deguchi 2000). In Chapter 4, the influence of both extrinsic and intrinsic factors on the oxygen consumption rate of *P. h. rubellus* were explored. Diurnal rhythm had a highly significant effect on oxygen consumption, with night-time levels being 67% higher than those recorded during the day. This is a classic example of an intrinsic driver of oxygen consumption, in that the effect of nocturnal foraging activity was retained in a culture system, despite food being introduced during the day and lobsters being observed feeding during daylight hours. Feeding also resulted in increased oxygen consumption. The specific dynamic action (SDA) followed a classic pattern of a postprandial increase in oxygen consumption that gradually decreased to preprandial levels. The duration of the response (21 h) suggests that lobsters should be purged for a period at least 21 hours (~ one day) before transport (either emersed or immersed). This will ensure that the metabolic requirement for oxygen is at basal levels resulting in reduced demand for oxygen during immersed transport in a low volume / high density scenario and a reduced oxygen debt during emersed transport. However, given the postprandial ammonia excretion data presented in Chapter 3, a purge time of 48 hours will ensure both oxygen consumption and ammonia excretion rates are at basal levels. Interestingly, the duration and magnitude of SDA in *J. edwarsdsii* was significantly less for lobsters fed in the morning compared to those fed at night (Radford

et al. 2004). Further research into this phenomenon is required for *P. h. rubellus* if the energy content of feed, and therefore possibly growth, is to be optimized. Furthermore, the required flow rate for 1000 kg of 200-250 g lobsters was calculated at 112 l h^{-1} kg^{-1}. This flow rate is applicable for both holding and culture systems as the SDA response did not cause an increase in oxygen consumption over and above the intrinsic effect of diurnal rhythm.

In summary, during this study *P. h. rubellus* exhibited a number of characteristics that support the feasibility of wild harvesting juveniles for ongrowing purposes. These include:

1) Lobsters acclimated well to a recirculating culture system and exhibited similar growth rates to other species of panulirid lobsters currently being investigated for their aquaculture potential.
2) There were no outward indications of disease while in captivity.
3) Lobsters recovered rapidly from a period of emersion suggesting that the successful aerial transport of lobsters from the point capture to a holding facility is possible.

While it appears biologically feasible to harvest, transport, and culture wild caught *P. h rubellus*, a number of substantial bottlenecks need to be negotiated before viable commercial culture of this species can occur and any socio-economic benefits be derived from the practice. Firstly, a reliable supply of early juveniles cannot be assumed, as puerulus settlement can vary both spatially and temporally. If the TURF based model of

the allocation of subsistence fishing rights (see Chapter 1) is implemented, the potential exists for spatial shifts in puerulus settlement which may leave subsistence fishers without a resource and mariculturists without a product. In addition, current knowledge regarding the *P. h. rubellus* stock along the former Transkei coastline is limited and current harvest models have been aimed at determining the allowable harvest of legal sized animals (Fielding 2005). Research needs to be conducted into the availability and potential harvest of early juveniles that can be obtained from the nearshore area, as well as the sustainability of this practice. This information will prove crucial in determining the economic feasibility of ongrowing juveniles and will be required if the regulatory barriers to allowing the harvest of undersize lobsters are to be overcome.

Any move to allow the harvest of juvenile lobsters needs to be weighed against the total harvest from the resource. Available evidence suggests that fishing effort is increasing rapidly as a result of regulations promoting the commercial sale of lobsters caught by subsistence fishers. Previously, only local sale of lobsters was allowed to holiday makers and hotels and a relatively low price prevailed. The permitting of commercial buyers has resulted in a dramatic increase in price and fishing effort which is of concern to resource managers. Recent management recommendations to Marine and Coastal Management for the lobster fishery in the Transkei include:

1) That the minimum size limit of 65 mm carapace length should be maintained.
2) That a management strategy involving the harvesting communities is implemented, to ensure compliance and help monitor catches, and

3) That regulations regarding gear limitations should be maintained, to ensure the deeper water stock remains un-fished (Fielding 2005).

Given these recommendations, obtaining permits to harvest undersized lobsters in the current regulatory environment is unlikely. The sustainability of harvesting a portion of newly-recruited lobsters would have to be shown. Given the results of studies on other species where mortality in the first year is high (80 – 98 %) (Phillips *et al.* 2003), this off-take may prove sustainable. The growout of lobsters also provides opportunities for enhancement of the natural population. Release of older juveniles following a period of culture may provide a means of mitigating the negative effects of harvesting juveniles on recruitment into the adult fishery. Other alternatives such as the interchangeable quota system employed in New Zealand, where adult quotas can be exchanged for the right to harvest an increased number of pueruli and early juveniles (Booth & Kittaka 2000), should be explored. Once again, the need for a dedicated research effort to understand these processes, as well as the political will power to initiate and fund that research, is required.

In conclusion, while the data presented in this thesis suggests that it is biologically feasible to catch, transport and grow wild caught juvenile *P. h rubellus*, considerable research effort is still required on a number of fronts. The development of a suitable artificial diet and culture protocols, as well as extended production trials, will be required if the economic feasibility of lobster growout is to be determined. Furthermore, a number of sustainability, regulatory and institutional barriers will have to be overcome if the mariculture of *P. h rubellus* is to be successful as a tool to increase the diversity of

economic activities along the former Transkei coastline and in so doing address the challenges of poverty and unemployment. In the event that these barriers are not overcome, this thesis does provide valuable information towards the development of optimal holding and transport systems for marketing live lobsters.

REFERENCES

Anon. 2006. Transkei lobsters. *The Oracle - Newsletter of the Oceanographic Research Institute* **44**, 2.

Bennett, A. F. 1978. Activity metabolism of the lower vertebrates. *Annual Review of Physiology* **400**, 447-469.

Berkes, F. 1990. Native subsistence fisheries: a synthesis of harvest studies in Canada. *Arctic* **43**, 35-42.

Berry, P. F. 1971a. The spiny lobsters (Palinuridae) of the east coast of southern Africa: Distribution and ecological notes. *Oceanographic Research Institute - Investigational Report* **27**, 1-23.

Berry, M. 1971b. The biology of the spiny lobster *Panulirus homarus* (Linnaeus) off the east coast of southern Africa. *Oceanographic Research Institute - Investigational Report*, **28**, 1-71.

Berry, B. F. 1974. A revision of the *Panulirus homarus* group of spiny lobsters (Decapoda, Palinuridae). *Crustaceana* **27**, 31-42.

Booth, J. D. & Kittaka, J. 1994. Grow-out of juvenile spiny lobster. In: Phillips, B. F., Cobb, J.S. & Kittaka, J. (Eds.), Spiny Lobster Management. Fishing News Books. Oxford, United Kingdom, pp. 424-445.

Booth, J. D. & Kittaka, J. 2000. Spiny lobster growout. In: B. F. Phillips & J. Kittaka (Eds.), Spiny Lobsters: Fisheries and Culture. Blackwell Science, Oxford. pp. 556-585.

Bridges, C. R. & Brand, A. R. 1980. Oxygen consumption and oxygen-independence in marine crustaceans. *Marine Ecology Progress Series* **2**, 131-141.

Britz, P. J. & Hecht, T. 1990. Aquaculture in South Africa; history, status and prospects. The Aquaculture Association of South Africa, NMB Printers, Pretoria, 80 pp.

Buesa, R. J. 1979. Oxygen consumption of two tropical spiny lobsters, *Panulirus argus* (Latreille) and *P. guttatus* (Latrielle) (Decapoda, Paluniridae). *Crustaceana* **36**, 99-107.

Campbell, J. W. 1973. Nitrogen Excretion. In: Progresser, C. L. (Ed.), Comparative Animal Physiology. W.B. Saunders, Philadelphia, pp. 279-316.

Carvalho, P. S. M. & Phan, V. N. 1997. Oxygen consumption and ammonia excretion of *Xiphopenaeus kroyeri* Heller (Penaeidae) in relation to mass, temperature and experimental procedure. Shrimp oxygen consumption and ammonia excretion. *Journal of Experimental Marine Biology and Ecology* **209**, 143-156.

Chen, J. C. & Kou, Y. Z. 1992. Effects of ammonia on growth and molting of *Penaeus japonicus* juveniles. *Aquaculture* **104**, 249-260.

Chen, J. C. & Kou, Y. Z. 1993. Accumulation of ammonia in the haemolymph of *Penaeus monodon* exposed to ambient ammonia. *Aquaculture* **109**, 177-185.

Chen, J. C. & Lai, S. H. 1993. Effects of temperature and salinity on oxygen consumption and ammonia excretion-N excretion of juvenile *Penaeus japonicus*. *Journal of Experimental Marine Biology and Ecology* **165**, 161-170.

Chen, J. C. & Lin, C. Y. 1995. Responses of oxygen consumption, Ammonia-N excretion and Urea-N excretion of *Penaeus chinensis* exposed to ambient ammonia at different salinity and pH levels. *Aquaculture* **136**, 243-255.

Chin, T. S. & Chen, J. C. 1987. Acute toxicity of ammonia to larvae of the tiger prawn, *Penaeus monodon*. *Aquaculture* **66**, 247-253.

Chittleborough, R. G. 1975. Environmental factors affecting growth and survival of juvenile western rock lobster *Panulirus longipes* (Milne-Edwards). *Australian Journal of Marine and Freshwater Research* **26**, 177-196.

Christensen, M. S. 1980. Sea-surface temperature charts for southern Africa, south of 26°S. *South African Journal of Science* **76**, 541-546.

Clarke, A. & Johnston, N. M. 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology* **68**, 893-905.

Cochrane, K. L. & Payne, A. I. L. 1998. People, purses and power: developing fisheries policy for the new South Africa. In: Pitcher, T.J., Hart, P.J.B. & Pauly, D. (Eds.), Reinventing Fisheries Management. Kluwer Academic Publishers, London.

Cockcroft, A. C. & Wooldridge, T. 1985. The effects of mass, temperature and molting on the respiration of *Macropetasma africanus* Bals (Decapoda: Penaeoidea). *Comparative Biochemistry and Physiology* A**8**, 143-148.

Cockcroft, A. C. & McLachlan, A. 1987. Nitrogen regeneration by the surf zone panaeid prawn *Macropetasma africanus*. *Marine Biology* **96**, 343-348.

Cockcroft, A.C. & Payne, A.I.L. 1999. A cautious fisheries management policy in South Africa: the fisheries for rock lobster. *Marine Policy* **23**, 587-600.

Cockcroft, A. C., Webb, P. & Wooldridge, T. 1988. Nitrogen regeneration by two surf-zone mysids, *Mesopodopsis slabberi* and *Gastrosaccus psammodytes*. *Marine Biology* **99**, 75-82.

Cockcroft, A. C., Sauer, W. H. H., Branch, G. M., Clark, B. M., Dye, A. H. & Russell, E. 2002. Assessment of resource availability and sustainability for subsistence fishers in South Africa, with a review of resource management procedures. *South African Journal of Marine Science* **24**, 489-501.

Cornick, J. W. & Stewart, J. E. 1977. Survival of American lobsters (*Homarus americanus*) stored in a recirculating refrigerated seawater system. *Journal of the Fisheries Research Board of Canada* **34**, 688-692.

Cox, S. L. & Johnston, D. J. 2003. Feeding biology of spiny lobster larvae and implications for culture. *Reviews in Fisheries Science* **11**, 89-106.

Crear, B. J. 1998. 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*. PhD, University of Tasmania.

Crear, B. J. & Forteath, G. N. R. 2000. The effect of extrinsic and intrinsic factors on the oxygen consumption by the southern rock lobster, *Jasus edwardsii*. *Journal of Experimental Marine Biology and Ecology* **252**, 129-147.

Crear, B. J. & Forteath, G. N. R. 2001. Flow rate requirements for captive western rock lobsters (*Panulirus cygnus*): effects of body weight, temperature, activity, emersion, daily rhythm, feeding and oxygen tension on oxygen consumption. *Marine and Freshwater Research* **52**, 763-771.

Crear, B. J. & Forteath, G. N. R. 2002. Feeding has the largest effect on the ammonia excretion rate of the southern rock lobster, *Jasus edwardsii*, and the western rock lobster, *Panulirus cygnus*. *Aquacultural Engineering* **26**, 239-250.

Crear, B. J., Thomas, C. W., Hart, P. R. & Carter, C. G. 2000. Growth of juvenile southern rock lobsters, *Jasus edwardsii*, is influenced by diet and temperature, whilst survival is influenced by diet and tank environment. *Aquaculture* **190**, 169-182.

Crear, B. J., Hart, P. R. & Thomas, C. W. 2003. The effect of photoperiod on growth and survival of the southern rock lobster, *Jasus edwardsii*. *Aquaculture Research* **34**, 439-444.

Dall, W. 1986. Estimation of routine metabolic rate in a panaeid prawn, *Penaeus esculentus* Haswell. *Journal of Experimental Marine Biology and Ecology* **96**, 7-74.

Dall, W. & Smith, D. M. 1986. Oxygen consumption and ammonia-N excretion in fed and starved tiger prawns, *Panaeus esculentus* Haswell. *Aquaculture* **55**, 23-33.

Department of Environmental Affairs and Tourism (DEAT). 1998. Marine Living Resources Act, Act 18 of 1998.

Department of Environmental Affairs and Tourism (DEAT). 2007. Policy for the Development of a Sustainable Marine Aquaculture Sector in South Africa, General Notice 1109 of 2007, Marine Living Resources Act (18/1998)

Dubber, G. G., Branch, G. M. & Atkinson, L. J. 2004. The effects of temperature and diet on the survival, growth and food uptake of aquarium-held postpueruli of the rock lobster *Jasus lalandii*. *Aquaculture* **240** 249-266.

Eding, E. H., Kamstra, A., Verreth, J. A. J., Huisman, E. A. & Klapwijk, A. 2006. Design and operation of nitrifying trickling filters in recirculating aquaculture: A review. *Aquacultural Engineering* **34**, 234-260.

Fielding, P. J. 2005. Abundance and distribution of East Coast rock lobster (*Panulirus homarus*) along the Wild Coast, Eastern Cape - Towards an estimate of the number of subsistence permits that could be issued in the sub-regions to ensure sustainable harvesting. Report for Marine and Coastal Management, Fieldwork - Resource and Environmental Consulting, 1-9.

Fielding, P. J., Robertson, W. D., Dye, A. H., Tomalin, B. J., van der Elst, R. P., Beckley, L. E., Mann, B. Q., Birnie, S., Schleyer, M. & Lasiak, T. A. 1994. Transkei coastal fisheries resources. *Oceanographic Research Institute - Special Publication* **3**, 1-175.

Forsberg, J. A. & Summerfelt, R. C. 1992. Effect of temperature on diel ammonia excretion of fingerling walleye. *Aquaculture* **102**, 115-126.

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

Fry, F. E. J. 1971. The effect of environmental factors on the physiology of fish. In: Hoar, W.S. & Randall, D.J. (Eds.), Fish Physiology - Vol 1. Academic Press, New York, pp. 1-98.

Hair, C., J. Bell & P. Doherty. 2002. The use of wild-caught juveniles in coastal aquaculture and its application to coral reef fishes. In: Stickney, R.R. and McVey, J.P. (Eds.), Responsible Marine Aquaculture. CABI Publishing, Oxon, UK, 391 pp.

Harris, J. M., Branch, G. M., Clark, B. M. & Sibiya, S. C. 2006. Redressing access inequities and implementing formal management systems for marine and estuarine subsistence fisheries in South Africa. In: Castilla, J.C. & McClanahan, T.R. (Eds.), Fisheries Management: Progress toward Sustainability, Wiley-Blackwell, pp. 112-138.

Hartnoll, R. G. 1982. Growth. In: Bliss, D.E. (Ed.), The Biology of Crustacea. Academic Press, pp. 111-196.

Hauck, M. & Sowman, M. 2003. Waves of Change: Coastal Fisheries and Co-Management in South Africa. University of Cape Town Press, Cape Town.

Hawkins, C. M., Castell, J. D. & Leroyer, V. 1986. Patterns and rates of ammonia excretion by juvenile American lobsters, *Homarus americanus*, fed casein- and crab-based diets. *Canadian Journal of Fisheries and Aquatic Science* **43**, 1290-1294.

Hazell, R. W. A., Cockcroft, A. C., Mayfield, S. & Noffke, M. 2001. Factors influencing the growth rate of juvenile rock lobsters, *Jasus lalandii*. *Marine and Freshwater Research* **52**, 1367-1373.

Heydorn, A. E. F. 1969. Notes on the biology of *Panulirus homarus* and on length/weight relationships of *Jasus lalandii*. *Division of Sea Fisheries - Investigational Report* **69**.

Hopkins, K. D. 1992. Reporting fish growth: A review of the basics. *Journal of the World Aquaculture Society* **23**, 173-179.

Innes, I. J. 1985. Aerobic scope for activity of the borrowing mantis shrimp *Heterosquilla tricarinata* at low environmental oxygen tensions. *Comparative Biochemistry and Physiology* A**81**, 827-832.

James, P. J., Tong, L. J. & Paewai, M. P. 2001. Effects of stocking density and shelter on growth and mortality of early juvenile *Jasus edwardsii* held in captivity. *Marine and Freshwater Research* **52**, 1413-1417.

Jeffs, A. G. & James, P. J. 2001. Sea cage culture of the spiny lobster *Jasus edwardsii* in New Zealand. *Marine and Freshwater Research* **52**, 1419-1424.

Jobling, M. 1994. Fish Bioenergetics. Chapman and Hall, London.

Johnston, D., Melville-Smith, R., Hendriks, B., Maguire, G. B. & Phillips, B. 2006. Stocking density and shelter type for the optimal growth and survival of western rock lobster *Panulirus cygnus* (George). *Aquaculture* **260**, 114-127.

Jones, C. M., Linton, L., Horton, D. & Bowman, W. 2001. Effect of density on growth and survival of ornate rock lobster, *Palinurus ornatus* (Fabricius, 1798), in a flow-through raceway system. *Marine and Freshwater Research* **52**, 1425-1429.

Kamstra, A., Van Der Heul, J. W. & Nijhof, M. 1998. Performance and optimisation of trickling filters on eel farms. *Aquacultural Engineering* **17**, 175-192.

Kaufmann, R., Forstner, H. & Wieser, W. 1989. Respirometry - methods and approaches. In: Bridges, C.R. & Butler, P.J. (Eds.), Techniques in Comparative Respiratory Physiology. Cambridge University Press, Cambridge, pp. 49-76.

Kent, G. 1997. Fisheries, food security, and the poor. *Food Policy* **22**, 393-404.

Kir, M., Kumlu, M. & Eroldogan, O. T. 2004. Effects of temperature on acute toxicity of ammonia to *Penaeus semisulcatus* juveniles. *Aquaculture* **241**, 479-489.

Kittaka, J. 1988. Culture of the palinurid *Jasus lalandii* from egg stage to puerulus. *Nippon Suisan Gakkaishi* **54**, 87-93.

Kittaka, J. 2000. Culture of larval spiny lobsters. In: Phillips, B.F. & Kittaka, J. (Eds.), Spiny Lobsters: Fisheries and Culture. Blackwell Science, Oxford, pp. 508-532.

Kittaka, J. & Booth, J. D. 2000. Prospectus for aquaculture. In: Phillips, B.F. & Kittaka, J. (Eds.), Spiny Lobsters: Fisheries and Culture. Blackwell Science, Oxford, pp. 465-473.

Kleiber, M. 1947. Body size and metabolic rate. *Physiology Reviews* **27**, 511-541.

Kormanik, G. A. & Cameron, J. N. 1981. Ammonia excretion in animals that breathe water: A review. *Marine Biology Letters* **2**, 11-23.

Kulmiye, A. J. & Mavuti, K. M. 2005. Growth and moulting of captive *Panulirus homarus homarus* in Kenya, western Indian Ocean. *New Zealand Journal of Marine and Freshwater Research* **39**, 539-549.

Lellis, W. A. & Russell, J. A. 1990. Effect of temperature on survival, growth and feed intake of postlarval spiny lobsters, *Panulirus argus*. *Aquaculture* **90**, 1-9.

Marangos, C., Alliot, E., Brogren, C. & Ceccaldi, H. J. 1990. Nycthermal variations of ammonia excretion in *Panaeus japonicus* (Crustacea, Decapoda, Panaeidae). *Aquaculture* **84**, 383-391.

Matsuda, H. & Yamakawa, T. 2000. The complete development and morphological changes of larval *Panulirus longipes* (Decapoda, Panuliridae) under laboratory conditions. *Fishery Bulletin* **66**, 278-293.

McCue, M. D. 2006. Specific dynamic action: A century of investigation. *Comparative Biochemistry and Physiology* A**144**, 381-394.

Morris, S. & Oliver, S. 1999. Circulatory, respiratory and metabolic response to emersion and low temperature of *Jasus edwardsii*: simulation studies of commercial shipping methods. *Comparative Biochemistry and Physiology* A**122**, 299-308.

Nair, R. V., Soundararajan, R. & Nandakumar, G. 1981. Observations on growth and moulting of spiny lobsters *Panulirus homarus* (Linnaeus), *P. ornatus* (Fabricius) and *P. penicillatus* (Olivier) in captivity. *Indian Journal of Fisheries* **28**, 25-35.

Needham, A. E. 1957. Factors affecting nitrogen-excretion in *Carcinides maenas* (Pennant). *Journal of Physiology and Comparative Oecology* **4**, 209-239.

Nijhof, M. 1994. The application of commercially exploited recirculating systems, concepts in the culture of Turbot (*Scophthalmus maximus* L.). In: Remmerswaal, R.A.M. & Lavens, P. (Eds.), Turbot Culture: Problems and Prospects. Proceedings of the Satellite Workshop of World Aquaculture'93. European Aquaculture Special publication No. 22. Oostende, Belgium, pp. 273-282.

Parsons, T. R., Maita, Y. & Lali, C. M. 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford.

Perera, E., Fraga, I., Carrillo, O., Diaz-Iglesias, E., Cruz, R., Baez, M. & Galich, G. S. 2005. Evaluation of practical diets for the Caribbean spiny lobster *Panulirus argus* (Latreille, 1804): effects of protein sources on substrate metabolism and digestive proteases. *Aquaculture* **244**, 251-262.

Perera, E., Diaz-Iglesias, E., Fraga, I., Carrillo, O. & Galich, G. S. 2007. Effect of body weight, temperature and feeding on the metabolic rate in the spiny lobster *Panulirus argus* (Latreille, 1804). *Aquaculture* **265**, 261-270.

Peters, R. H. 1983. The Ecological Implications of Body Size. Cambridge University Press, Cambridge.

Phillips, B. F. 1985. Aquaculture potential for rock lobsters in Australia. *Australian Fisheries* **44**, 2-7.

Phillips, B. F. & Mellville-Smith, R. 2006. *Panulirus* Species. In: Phillips, B.F. (Ed.), Lobsters: Biology, Management, Aquaculture and Fisheries, Wiley-Blackwell, pp. 359-384.

Phillips, B. F., Campbell, N. A. & Rea, W. A. 1977. Laboratory growth of early juveniles of early juveniles of the western rock lobster *Panulirus longipes cygnus*. *Marine Biology* **39**, 31-39.

Phillips, B. F., Mellville-Smith, R. & Cheng, Y. W. 2003. Estimating the effects of removing *Palulirus cygnus* pueruli on the fishery stock. *Fisheries Research* **65**, 89-101.

Prosser, C. 1973. Oxygen: respiration and metabolism. In: Ladd Prosser, C. (Ed.) Comparative Animal Physiology. Environmental Physiology - Vol. 1.

Radford, C. A., Marsden, I. D. & Davison, W. 2004. Temporal variation in the specific dynamic action of juvenile New Zealand rock lobsters, *Jasus edwardsii*. *Comparative Biochemistry and Physiology* A**139**, 1-9.

Radhakrishan, E. V. & Vijayakumaran, M. 1995. Early larval development of the spiny lobster *Panulirus homarus* (Linnaeus, 1758) reared in the Laboratory. *Crustaceana* **68**, 151-159.

Rahman, M. D. K., Srikrishnadhas, B. & Anandasekaran, A. S. M. 1997. Growth performance of spiny lobster *Panulirus homarus* (Linnaeus) under mass rearing. *Journal of Aquaculture in the Tropics* **12**, 243-253.

Regnault, M. 1979. Ammonia excretion of the sand shrimp *Crangon crangon* (L.) during the moult cycle. *Journal of Experimental Biology* **133**, 199-204.

Regnault, M. 1987. Nitrogen excretion in marine and fresh-water Crustacea. *Biological Review* **62**, 1-24.

Robertson, W. D. & Fielding, P. J. 1997. Patterns and economics of resource utilisation and tourism along the Transkei coast. *Oceanographic Research Institute - Special Publication* **4**, 5-104.

Rutledge, P. S. & Pritchard, A. W. 1981. Scope for activity in the crayfish *Pacificus leniusculus*. *American Journal of Physiology* **240**, 87-92.

Sastry, A. N. & Vargo, S. L. 1977. Variation in the physiological response of crustacean larvae to temperature. In: Vernberg, F.J., Calabrese, J., Thurberg, F.P. & Vernberg, W.B. (Eds.), Physiological Response of Marine Biota to Pollutants. Academic Press, New York, pp. 401-424.

Schmitt, A. S. C. & Uglow, R. F. 1997. Effects of ambient ammonia levels on blood ammonia, ammonia excretion and heart and scaphognathite rate of *Nephrops norvegius*. *Marine Biology* **127**, 411-418.

Schurmann, H. & Steffensen, J. F. 1997. Effects of temperature, hypoxia and activity on the metabolism of juvenile Atlantic cod. *Journal of Fish Biology* **50**, 1166-1180.

Serfling, S. A. & Ford, R. F. 1975. Laboratory culture of juvenile stages of the California spiny lobster *Panulirus interruptus* (Randall) at elevated temperatures. *Aquaculture* **6**, 377-387.

Siegfried, W. R., Hockey, P. A. R. & Crowe, T. T. 1985. Exploitation and conservation of brown mussel stocks by coastal people of Transkei. *Environmental Conservation* **12**, 303-307.

Simon, C. J. & James, P. J. 2007. The effect of different holding systems and diets on the performance of spiny lobster juveniles, *Jasus edwardsii* (Hutton, 1875). *Aquaculture* **266**, 166-178.

Smale, M. J. 1978. Migration, growth and feeding in the Natal rock lobster *Panulirus homarus* (Linnaeus). *Oceanographic Research Institute - Investigational Report* **47**.

Solorzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnology and Oceanography* **14**, 799-801.

Sowman, M. 2006. Subsistence and small-scale fisheries in South Africa: A ten-year review. *Marine Policy* **30**, 60-73.

StatSoft, Inc. 2005. STATISTICA (data analysis software system), version 7.1. www.statsoft.com.

Steyn, E., Fielding, P. J. & Schleyer, M. H. 2008. An assessment of the artisinal fishery of east coast rock lobster, *Panulirus homarus* (Linnaeus) in Transkei. Manuscript in preparation.

Sugita, H. & Deguchi, Y. 2000. Shipping. In: Phillips, B.F. & Kittaka, J. (Eds.), Spiny Lobster: Fisheries and Culture. Blackwell Science, Oxford, UK, pp. 633-640.

Thomas, C. W., Crear, B. J. & Hart, P. R. 2000. The effect of elevated temperature on survival, growth, feeding and metabolic activity of the southern rock lobster, *Jasus edwardsii*. *Aquaculture* **185**, 73-84.

Tomalin, B. J. 1995. Invertebrates harvested in KwaZulu-Natal: Their ecology, fishery and management. *Oceanographic Research Institute - Information Booklet* **1**, 1-22.

Vijayakumaran, M. & Radhakrishan, E. V. 1984. Effect of eyestalk ablation in the spiny lobster *Panulirus homarus* (Linnaeus): 2. On food intake and conversion. *Indian Journal of Fisheries* **31**, 148-155.

Waldron, F. M. 1991. Respiratory and acid-base physiology of the New Zealand rock lobster, *Jasus edwardsii* (Hutton). PhD University of Canterbury, Christchurch, New Zealand.

White, C. R. & Seymour, R. S. 2005. Sample size and mass range effects on the allometric exponent of basal metabolic rate. *Comparative Biochemistry and Physiology* A**128**, 74-78.

Whiteley, N. M., Robertson, R. F., Meagor, J., El Haj, A. J. & Taylor, E. W. 2001. Protein synthesis and specific dynamic action in crustaceans: effects of temperature. *Comparative Biochemistry and Physiology* A**128**, 593-604.

Whitfield, M. 1974. The hydrolysis of ammonium ions in sea water. *Journal of the Marine Biology Association UK* **54**, 565-580.

Wickens, J. F. 1985. Ammonia production and oxidation during the culture of marine prawns and lobsters in laboratory recirculation systems. *Aquacultural Engineering* **4**, 155-174.

Wickens, J. F. & Lee, D. O. 2002. Crustacean Farming: Ranching and Culture. Blackwell Science, Oxford, U.K.

Williams, K. C. 2007. Nutritional requirements and feeds development for post-larval spiny lobster: A review. *Aquaculture* **263**, 1-14.

Wyban, J., Walsh, W. A. & Godin, D. M. 2000. Temperature effects on growth, feeding rate and food conversion of the Pacific white shrimp (*Panaeus vannamei*). *Aquaculture* **138**, 267-279.

Young-Lai, W. W., Charmantier-Daures, M. & Charmantier, G. 1991. Effect of ammonia on survival and osmoregulation in different life stages of the lobster *Homarus americanus*. *Marine Biology* **110**, 293-300.

Zoutendyk, P. 1987. Nitrogen excretion by the Cape Rock lobster *Jasus lalandii* and its possible contribution to the inshore Benguella system. *South African Journal of Marine Science* **5**, 565-574.

Zoutendyk, P. 1989. Oxygen consumption by the Cape rock lobster *Jasus lalandii*. *South African Journal of Marine Science* **8**, 219-230.

APPENDIX 1: Determination of water ammonia.

Water ammonia concentration was analysed using the phenol-hypochlorite method of Solorzano (1969) with the following methods being adapted from Parsons *et al.* (1984), Frith *et al.* (1993) and Crear (1998). Indophenol blue is produced by the reaction of ammonia with phenol and hypochlorite in an alkaline solution. This colour is intensified by the addition of sodium nitroprusside at room temperature before being measured spectrophotometrically as absorbance. The intensity of the colour is proportional to the concentration of ammonia and the absorbance measured is compared to a calibration curve to ascertain the concentration of ammonia in the sample.

1.1 Samples

Duplicate 50 ml samples were collected in PVC specimen bottles at each sampling period and frozen immediately at -16°C. Samples were analysed within one week of collection in order to remain within the 2 week maximum storage time suggested by (Parsons *et al.* 1984). Samples were defrosted and mixed by shaking before extracting a 10ml sub-sample for analysis.

1.2 Preparation of reagents

1.2.1 Phenol solution: Dissolve 20g of phenol in 200ml of 95% ethanol.

1.2.2 Sodium nitroprusside solution: Dissolve 1.0g sodium nitroprusside in 200ml of deionised water. Store refrigerated in an amber bottle; solution stable for 1 month.

1.2.3 Alkaline reagent: Dissolve 100g tri-sodium citrate and 5g NaOH in 500 ml deionised water.

1.2.4 Sodium hypochlorite solution: Commercially available hypochlorite ("JIK" brand, non-perfumed) which should be 1.5N.

1.2.5 Oxidising solution: Mix a solution reagent 1.2.3 and reagent 1.2.4 at a 4:1 ratio. This solution is stable for less than a day and was mixed fresh for each sample run.

1.3 Preparation of standards

A 100 mg l^{-1} as N standard (stock solution) was prepared by adding 0.0382 g of dried reagent grade NH_4Cl to 50 ml distilled water in a 100 ml volumetric flask. The flask was swirled to dissolve the NH_4Cl and diluted to volume with distilled water. A 10mg l^{-1} standard was prepared by pipetting 10 ml of stock solution into a 100 ml volumetric flask and making the solution up to 100 ml with deionised NaCl (3.5%) solution. Standard solutions of 0.3, 0.6, 1.0 and 2.0 mg l^{-1} were prepared by pipetting 1.5, 3.0, 5.0 and 10 ml of the 10 mg l^{-1} standard solution into 50 ml volumetric flasks and making up to 50 ml

volume with deionised NaCl (3.5%) solution. The calibration curve was created from 0.3, 0.6, 1.0 and 2.0 mg l^{-1} standards and a blank solution (0.0 mg l^{-1}) comprised of the NaCl solution.

1.4 Sample analyses

10 ml samples were pipetted from the original 50 ml water samples and the standard solutions into test tubes. The following procedure was used for the addition of reagents:

1. Add 0.4 ml of phenol solution.
2. Mix.
3. Add 0.4 ml of sodium nitroprusside solution.
4. Mix.
5. Add 1.35 ml of oxidizing solution.
6. Mix.
7. Cover the tubes and allow to incubate in the dark for at least 1 hour at room temperature.
8. Measure the absorbance over 1cm against a blank at 640 nm.
9. Calculate the sample ammonia using the calibration curve.

A new calibration curve was determined at each sample run (see Figure 1 for a typical curve). The calibration curve allows the calculation of total ammonia nitrogen (TAN). However, it must be noted that ammonia in solution (TAN) is comprised of both ionized

(NH_4^+) and unionized (NH_3) forms, with the proportion of the unionized ammonia being dependant on pH and temperature at the time of sampling.

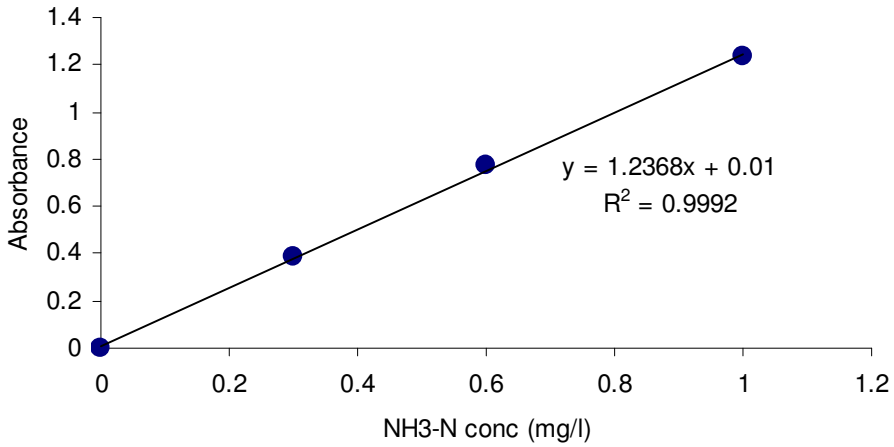


Figure 1: A typical calibration curve obtained from the standard solutions.