International Symposium

on

Lobster Health Management

Proceedings

19-21 September, 1999
Adelaide

Convened by

AQUATIC SCIENCE RESEARCH UNIT,
CURTIN UNIVERSITY OF TECHNOLOGY

Editors

L.H. Evans & J.B. Jones

Sponsors

Fisheries Research and Development Corporation
South Australian Research and Development Institute
Muresk Institute of Agriculture,
Curtin University of Technology

TABLE OF CONTENTS

SYMPOSIUM AIM

The overall aim of the symposium is to provide a forum for information exchange between scientists and industry personnel on health management of lobsters. This information exchange will be achieved through the presentation of papers on topics of direct relevance to lobster health management and through panel discussions on issues raised by participants

ORGANISING COMMITTEE

Assoc. Prof. Louis Evans	- Symposium Convenor; Abstract Proceedings Editor
Dr Brian Jones	- Abstract Proceedings Editor, Committee Member
Dr Judith Handlinger	- Committee Member
Dr Ruth Reuter	- Committee Member
Dr John Norton	- Committee Member

KEY SPEAKERS

Professor Robert Bayer

Professor Bayer is the Director of the Lobster Institute, University of Maine, USA. Professor Bayer has had extensive experience in the investigation of health problems in clawed lobsters and works closely with industry in research aimed at improving industry practices.

Professor Richard Cawthorn

Professor Cawthorn is the Director of the Lobster Health Research Centre, Atlantic Veterinary College, University of Prince Edward Island, Canada. Professor Cawthorn leads a research group whose work is focussed on developing innovative approaches to health management in post-harvest lobsters and has published extensively on lobster disease conditions and health assessment.

Professor Kenneth Söderhäll

Professor Söderhäll, University of Uppsala, Sweden, leads an internationally recognised research team whose work deals mainly with crustacean immunity. Professor Söderhäll is a world authority in crustacean host defence mechanisms and has made a major contribution to the understanding of immune processes in crustaceans.

Lobster health and disease: basic concepts

Louis H. Evans

Aquatic Science Research Unit, Muresk Institute of Agriculture
Curtin University of Technology

HEALTH AND DISEASE CONCEPTS

The term 'health' describes a physiological state of an animal. A 'healthy' animal is one in which the physiological processes underpinning growth, maintenance, disease defense and reproduction are functioning normally. In this context the term 'normal' refers to a state which is appropriate and adequate for the animal at that point in its development. Simply put, the animal is in a state of 'ease'.

A 'diseased' animal, on the other hand, is in a state of 'dis-ease'. It is experiencing some form of injury or threat to its survival, which results in damage to body tissues and/or abnormal physiological function. The injury or threat may be in the form of a sudden environmental change e.g. exposing a fish to air or to a chemical toxin, or the invasion of body tissues by an infectious organism. These different causes of injury are collectively referred to as disease agents. Exposure to disease agents stimulates a whole suite of physiological processes – the host defense and immune processes – to counteract the threat and repair the damaged tissue.

Diseases are caused by many different biological, physical or chemical agents. Diseases of aquatic organisms are mostly due to exposure to pathogenic organisms such as viruses or bacteria, or to inadequate nutrition. Disease conditions can be mild, having few if any adverse effects on the animal, through to severe where the condition threatens survival. Whether or not a lobster will succumb to a disease is dependent on factors relating to the host, the environment and the invading organism. Decapod crustaceans exhibit a wide range of host defense reactions which are aimed at preventing tissue injury or infections (Smith & Chisolm 1992; Bachere *et al*. 1995; Evans *et al*. 2000a). The success of these defense reactions in preventing or overcoming the disease is strongly influenced by the existing health status of the host at the time of exposure. Health status is in turn affected by prior exposure to environmental stressors. Excessive stress responses to environmental stressors weaken lobsters and pre-dispose them to disease.

A healthy animal can withstand the challenge of a disease agent better than one in poor health, unless the agent has the capacity to cause disease regardless of health status. Some viruses and highly virulent bacteria, and some forms of nutrient deficiencies or chemical exposures, fall in the latter category.

In order to avoid disease outbreaks it is essential that stock health is optimised within the economic constraints of the production or holding system. However, while optimal stock health should be the aim of any processor or aquaculturist, this is difficult to achieve in practice. Under most rearing and holding conditions lobsters are exposed to various forms of environmental stressors, e.g. crowding and confinement, adverse water quality, handling and grading procedures, which have the potential to affect health. In well designed and maintained

holding systems the level of stress experienced by the lobsters does not significantly compromise performance or product quality. The successful manager is able to balance the trade-off between operational costs and stock health in such a way as to maximise financial returns from the operation. In poorly designed or maintained systems, on the other hand, stock health is compromised resulting in reduced production, poor product quality and reduced profits.

The impact of disease on lobster health is of minor importance in lobster postharvest handling as it is currently practiced in Australia but is of significance in overseas lobster industries where harvested stock are held for long periods in lobster pounds. The introduction of long term storage of harvested lobsters in Australian fisheries will inevitably lead to disease problems as will the culture of lobsters in hatcheries and grow-out facilities. Disease prevention through effective health management should therefore be the goal of any commercial operation based on lobster wild-stock harvest or aquaculture.

Stress and Disease

Lobster health is influenced by a range of factors, one of the most of important of which is stress. Stress responses are normal physiological reactions to changes in environmental conditions. These conditions include a wide range of factors such as water quality parameters (oxygen levels, pH, salinity, temperature, presence of toxins) physical factors (handling, injury, air exposure), behavioral interactions and nutrient availability. Exposure to these stressors leads to short and long term changes in cardiovascular and respiratory function, energy metabolism, fluid and ionic balance, acid-base balance and immunity (Selye 1973; Barton & Iwama 1991; Thompson *et al*., 1993; McDonald & Milligan 1997; Iwama *et al*. 1997; Hall & van Ham 1998). If the stressor is mild and of short duration the physiological disturbances are temporary. However, if the stressor is extreme, or if there is prolonged exposure even to a mild stressor, detrimental long-term effects can occur. These include reduced resistance to disease, reduced growth, impaired reproduction and reduced survival (Pickering & Pottinger, 1989; Lee & Wickins 1992; Iwama *et al*. 1997). In postharvest handling of lobsters both mild and extreme stressor exposure is likely to occur. In the latter case the physiology of the lobster may be so disturbed as to result in mortality. The sequence of events which occurs in a stress response is shown in Figure 1.

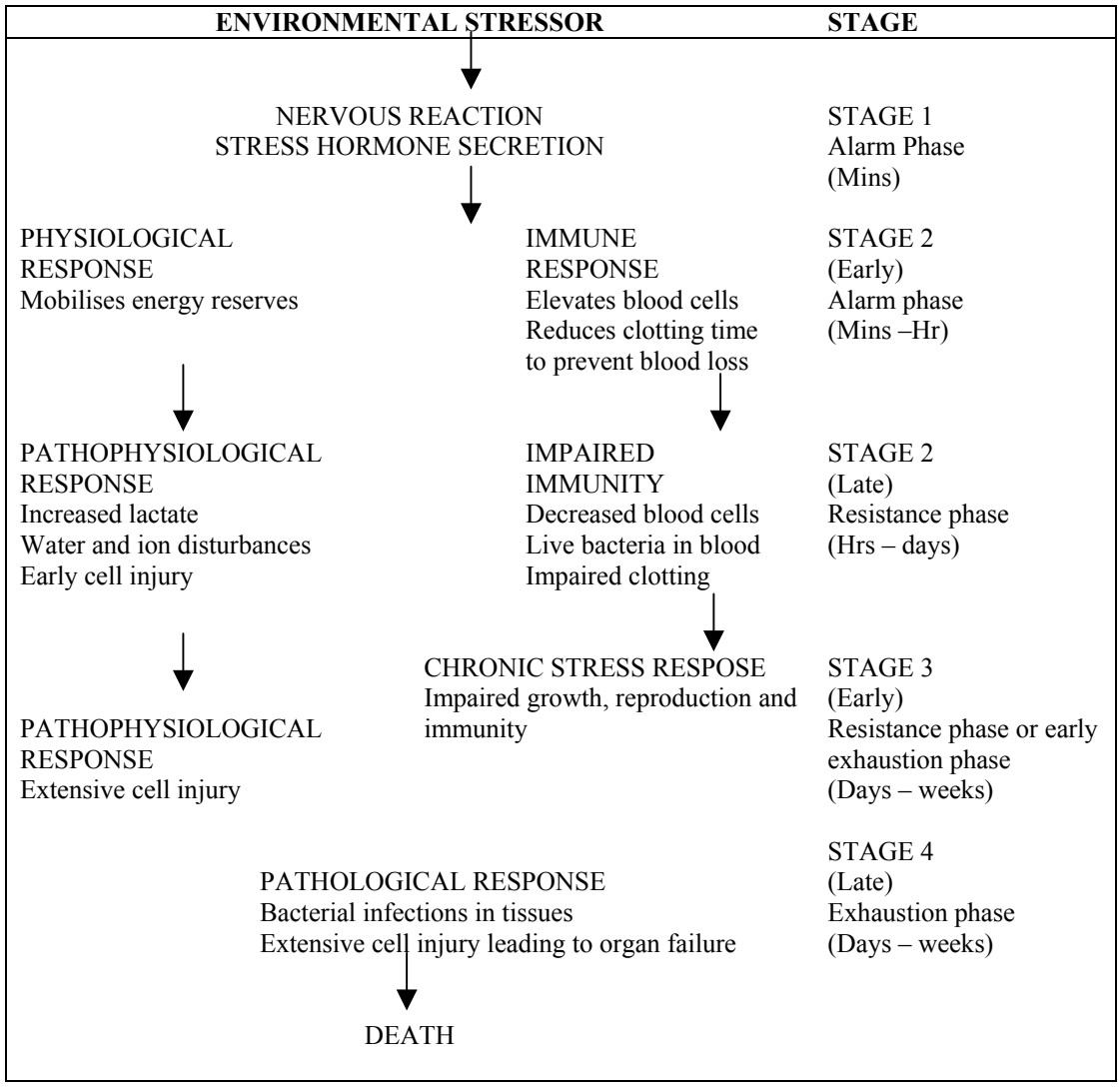


Figure 1 Classification of stages of stress response in lobsters

PHYSIOLOGICAL STRESS RESPONSES AND LIKELY CAUSES OF MORTALITY IN POSTHARVEST LOBSTERS

Lobsters are exposed to a range of harmful and stressful events during capture and postharvest handling and storage (Table I).

Table I Stress factors in lobster post harvest handling

Factor	Examples
Handling Stress	Winching up in pot Removal from pot Transfer to factory Packaging operations
Hypoxia Stress	On boat handling Transport to factory and to markets Exposure to low oxygen levels in tanks
Temperature Stress	Exposure to variations in environmental temperature on boat and during transport Dip treatment prior to packaging
Behavioural Stress	Limb autonomy from a variety of stimuli Crowding and aggression
Toxicity Stress	Exposure to high environmental ammonia Exposure to other dissolved toxins (e.g. copper, excreta)
Salinity Stress	Exposure to high and low salinity environments

Assuming that lobsters are relatively healthy when entering the trap, the development of weakness or poor health (morbidity) and/or death (mortality) must result from physical damage occurring during the processes of capture and post-capture processing or from physiological responses to post harvest stressors. These adverse physiological reactions occur through exposure of lobsters to environmental stressors that either alarm the lobsters, initiating an acute stress response, or cause a marked alteration in a physiological process such as oxygen uptake or ion regulation.

Four likely causes of morbidity and mortality in postharvest lobsters can be hypothesised:

- Cell injury and organ failure due to physiological disturbances - air exposure, rough handling and other stressors
- Opportunistic bacterial infections resulting from impaired immunity induced by above stressors
- Wounding - increased likelihood of bacterial infections
- Pre-existing disease conditions - weakens ability to resist stress

Stress reactions are likely to be a contributing factor to each of the above disease mechanisms.

Exposure to air, temperature extremes and physical processes leading to wounding or appendage loss are likely to the three main predisposing causes of mortality in postharvest

lobsters. These can lead to morbidity and mortality in lobsters by a number of different pathways. The actual cause of death is likely to be failure of vital organ function through cell injury caused by irreversible physiological dysfunction intracellular acidosis or opportunistic bacterial infections arising from either physical injury or from an impairment in the lobster's host defense responses (La Via & Hill, 1975; Wood *et al*. 1983; McDonald & Milligan, 1997). Stress responses induced by exposure to environmental stressors during capture and handling can lead to death through either of these pathways and are undoubtedly an important factor in the development of weakness or poor health in postharvest lobsters.

REFERENCES

Bachere, E., Mialhe, E., Noel, D., Buolo, V., Morvan, A.and Rodriguez, J. (1995) Knowledge and research prospects in marine mollusc and crustacean immunology. *Aquaculture*, **132**, 17-32.

Barton, B. A. (1997) Stress in finfish: past, present and future – a historical perspective. In: *Fish Stress and Health in Aquaculture*. (Ed. by G. K. Iwama, A. D. Pickering, J. P. Sumpter, & C. B. Schreck), pp.1-33. Society for Experimental Biology, Seminar Series 62. Cambridge University Press, Cambridge.

Barton, B. A. & Iwama, G. K. (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Review of Fish Diseases,* **1**, 649-59.

Evans, L. H., Jones J. B. and Brock, J. A. (2000) Diseases of spiny lobsters. In: Spiny Lobsters.l Fisheries and Culture 2nd edn. (Ed. by B. F. Phillips & T. Kittaka). pp. 586-600. Blackwell Scientific Publications.

Hall, M. R. & Van Ham, E. H. (1998) The effects of different types of stress on blood glucose in the giant tiger prawn. *Penaeus monodon. Journal of the World Aquaculture Society,* **29**, 290-9.

Johnson, P. T. (1976) Bacterial infection in the blue crab, *Callinectes sapidus*: course of infection and histopathology. *Journal of Invertebrate Pathology*, **28**, 25-36.

Johnson, P. T., Stewart, J. E. & Arie, B. (1981) Histopathology *of Aerococcus viridans* var.*homari* infection (Gaffkemia) in the lobster, *Homarus americanus*, and a comparison with histological reactions to a Gram-negative species*, Psuedomonas perolens. Journal of Invertebrate Pathology*, **38**, 127-48.

LaVia, M. F. & Hill, R. B. Jr. (1975) *Principles of Pathobiology* 2nd edn. Oxford University Press, New York.

Lee, D.O'C. & Wickins, J. C. 1992. *Crustacean Farming*. Blackwell Scientific Publications, Oxford.

Pickering, A. D. & Pottinger, T. G. (1989) Stress responses and disease resistance in salmonid fish: effects of chronic elevation of plasma cortisol. *Fish Physiology and Biochemistry,* **7**, 253-8.

Selye, H. (1973) The evolution of the stress concept. *American Scientist,* **61**, 692-9.

Smith, V. J. & Chisholm, J. R. S. (1992) Non-cellular immunity in crustaceans. *Fish and Shellfish Immunology,* **2**, 1-31.

Thompson, I., White, A., Fletcher, T. C., Houlihan, D. F. & Secombe, C. J. (1993) The effect of stress on the immune response of Atlantic salmon (*Salmo salar L.*) fed different diets containing different amounts of vitamin C. *Aquaculture,* **114**, 1-18.

Wood, C. M., Turner, J.D. & Graham, M. S. (1983) Why do fish die after severe exercise? *Journal of Fish Biology,* **22**, 189-201.

Cellular response to injury in spiny lobsters

Brian Jones

Fisheries Western Australia, c/o Animal Health Laboratory,
3 Baron-Hay court, South Perth WA 6151 Australia

ABSTRACT

This paper presents a review of the cellular defense mechanisms of spiny lobsters. These mechanisms can be divided, for convenience, into three broad groupings: maintenance of exoskeleton integrity; foreign agent recognition, inactivation and elimination from the internal organs; and repair of damage by toxins. Cellular defense mechanisms are dependent on circulating haemocytes and phagocytes, fixed phagocytes and fibrocytes. The process or processes by which these cell types are generated and mature in the animal have not yet been adequately described for spiny lobsters. In addition, attention has only recently focused on the way in which cellular defence responses are influenced by environmental stress and by the nutritional and moult status of the lobster. These are areas of critical importance to animal husbandry and production in aquaculture. While rapid advances are being made in the understanding of humoral defense mechanisms of crustaceans there are still large gaps in our understanding of the cellular components of the system in spiny lobsters.

Key Words: review, encapsulation, haemocytes, lobster, Panuliridae, phagocytes.

I. INTRODUCTION

Understanding of the internal defense mechanisms of crustaceans began in the 1880's through the pioneering work of Metchnikoff on phagocytosis and the inflammatory process. Likewise, Cantacuzène, in a series of papers between 1912 and 1934 started investigation of the humoral defense responses. Work then languished until the 1960's (Sinderman 1971) when interest was renewed as research began on crustacean diseases of major economic significance, particularly Gaffkaemia in lobsters and fungal infections of freshwater crayfish.

Cellular defense mechanisms in spiny lobsters (Panuliridae) have seldom been studied directly, but inferences can be drawn from apparently similar mechanisms in other decapods (Tsing *et al.* 1989). These cellular defense mechanisms can be divided, for convenience, into three broad groupings: maintenance of exoskeleton integrity; foreign agent recognition, inactivation and elimination from the internal organs; and repair of damage by toxins. These systems are not mutually exclusive but share five basic processes: phagocytosis; haemocytosis; degranulation; coagulation (clotting); and encapsulation. Cellular defense mechanisms are particularly dependent on circulating haemocytes, fixed phagocytes and fibrocytes.

Maintenance of exoskeletal integrity

The chitinous exoskeleton of spiny lobsters is an effective barrier that prevents the entry of infectious agents as well as providing muscle anchorage and protecting underlying soft tissue. The first barrier presented by the exoskeleton against invasion is the very thin proteolipid epicuticular membrane or 'surface waxy layer' (Unestam 1973, Malloy, 1978, Fisher 1988). Beneath this layer is the calcified exocuticle. This is very difficult to penetrate, even for disease agents secreting extracellular chitinases. By contrast, the soft non-calcified endocuticle is easily penetrated by such agents (Unestam 1973). Maintenance of the epicuticle is dependent on diet (Fisher *et al.* 1976) and it is probable that penetration is also related to the nutritional status of the animal. Shell diseases are a characteristic of crustaceans held in captivity (Stewart 1993).

Rapid sealing of wounds to the exoskeleton is required to prevent loss of haemolymph and minimize opportunistic invasion. Reactions leading to wound repair in spiny lobsters consist of rapid haemocyte accumulation and aggregation at the wound site followed by intravascular clotting. Clotting is initiated by contact of hyalinocytes with seawater (Hose & Martin 1989). The clot results from direct conversion of a soluble fibrinogen (coagulogen) into crosslinked fibrin through the action of a coagulin released by haemocyte rupture (Fuller & Doolittle 1971a,b; Durliat & Vranckx 1981; Ghidalia *et al*. 1981; Hose *et al*. 1990; Aono & Mori 1996). This is followed by melanisation of the wound area to form a dense black membrane beneath which the new epidermis forms. Melanin is produced by the action of the enzyme polyphenoloxidase on melanin precursors (Unestam & Nylund 1972; Bauchau 1981) and has antimicrobial properties (Nyhlen & Unestam 1980; Söderhäll & Ajaxon 1982). The epidermis involutes into the wound utilizing the haemocyte network as basal support. New cuticle is formed by this epidermal layer and lies beneath the melanin membrane (Fontaine 1975). In association with the haemocyte response a dense network of collagen-like fibres forms. This fibrous tissue is not resorbed but remains as a scar (Fontaine & Lightner 1975).

Foreign agent recognition, inactivation and elimination

Foreign agent recognition, inactivation and elimination is effected through both cellular and humoral host defence responses. Immunorecognition is thought to be mediated through the prophenoloxidase system, a cascade of serine proteases and prophenoloxidase present in the haemocytes which is activated by the presence of non-self molecules and initiates melanization (Söderhäll & Smith 1986; Söderhäll *et al.* 1996). Subsequent host defence responses comprise cellular mechanisms together with humoral responses involving the actions of circulating antibacterial factors, lectins and other immunologically active molecules. Invertebrates do not exhibit acquired immunity (Roch 1999) although proteins, with domains belonging to the immunoglobulin superfamily, have been demonstrated (Lanz Mendoza & Faye 1996). Humoral responses will not be discussed further.

The relative importance of cellular and humoral host defence mechanisms in spiny lobsters has yet to be determined. It would seem, however, that circulating haemocytes play a central role in both through their involvement in immunorecognition and in the processes of inactivation and elimination.

Spiny lobster haemocytes

Spiny lobsters, as with most crustaceans, have at least three recognised blood cell types based on morphology and staining characteristics (Hearing & Vernick 1967; Hose *et al*. 1990; Jussila *et al*. 1998): granulocyte (large granule haemocyte; eosinophil); hyalinocyte; and semi-granulocyte (small granule haemocyte). Semi-granulocytes and granulocytes adhere readily to glass and plastic and emit long filopodia, assuming a stellate shape as described by Newman & Feng (1982) for haemocytes of *Cancer irroradiatus*, by Goldberg *et al*. (1984, 1986) for *Homarus*, and by Barracco & Amirante (1992) for *Squilla mantis*. Filopodia are apparently associated with surface adherence and the density and type of cytoplasmic granules in the haemocyte (Goldberg *et al*. 1986).

It has been generally accepted, but not proved, that the three types represent different developmental stages of one cell line, with the granulocyte being the terminal stage (Bodammer 1978; Mix & Sparks 1980; Jussila *et al*. 1998). It should be noted, however, that Cornick & Stewart (1978) described four cell types in *Homarus americanus*, based on cell histochemistry and morphology. They described two hyalinocye types and two granulocytes, one eosinophilic and one chromophobic. Williams & Lutz (1975) presented evidence for five types in *Carcinus maenas*, based on cell histochemistry. They divided granulocytes into two classes based on whether the granules stained for glycogen or not. Barracco & Amirante (1992) found two subgroups of semi-granulocytes in *Squilla mantis* (Stomatopoda) which were ultrastructurally distinct. Johnston *et al.* (1973) reported two haemocyte cell lines in *Carcinus maenas*, those they called *alpha* cells have a strongly basophilic nucleus and form a single developmental series from small ribosome-rich non-granular cells to large carbohydrate laden haemocytes. They describe *beta* cells as large with a moderately acidophilic nucleus when stained with Wright's stain and there is a uniform and intense acidic granulation of the cytoplasm, and they are rarely seen intact in blood samples. Hose & Martin (1989) found that hyalinocytes initiate coagulation and they lyse in the presence of bacterial toxins and seawater, while granulocytes and semigranulocytes are involved with phagocytosis and encapsulation. If there is only one cell line, and that has yet to be established, then the changes in morphology and histochemistry as the cell matures need to be much more rigorously defined.

The location and function of the haematopoetic tissue is not well understood. In *Nephrops norvegicus* it is believed to be a thin sheet of tissue, on the dorsal and lateral surfaces of the cardiac stomach, and probably on the floor of the cephalic cavity, that shows a marked seasonal cycle of activity (Field & Appleton 1995). The location in prawns and shrimps is better known (Bell & Lightner 1988) and haematopoetic tissue has been found to occur in the same locations in spiny lobsters (pers. obs.). However, it would seem that the ultrastructure, cytochemistry and activity of haematopoietic tissue in spiny lobsters is a neglected field.

Cellular defense mechanisms - foreign agents

Inflammation has been studied in considerable detail in penaeid shrimp, and the process appears to be identical in spiny lobsters (Martin *et al*. 2000). Injection of shrimp with carmine (a neutral contaminant) is followed by accumulation of carmine in the dorsal abdominal artery, ventral abdominal vein, heart and gills. By 30 h post-injection carmine is only visible in the gills, heart and injection site (Fontaine & Lightner 1974). Histologically

the carmine forms tightly packed extracellular masses, at the injection site, which are infiltrated and phagositised by haemocytes. Circulating carmine particles are then trapped by fixed phagocytes lining the blood vessels and in sinusoids of the gill filaments. These particles finally accumulate in the distal gill filaments and heart. Brown melanised nodules consisting of necrotic haemocytes containing phagositised carmine develop in the periopods and as cysts in the connective tissues of the gill cover by a process of filtration rather than through the action of fixed phagocytes and are subsequently shed at moulting (Martin *et al*. 2000). Carmine containing haemocytes also migrate through the midgut epithelium and into the lumen of the antennal gland. Smith & Ratcliff (1980a,b) studied clearance of foreign agents from gills of the crab *Carcinus maenas*. They found that there were two mechanisms in operation: aggregation of haemocytes into 12-25 μm diameter clumps of 5 to 50 haemocytes containing trapped bacteria; and the formation of elongate, diffuse networks of phagocytic haemocytes in the gill blood sinuses.

Aggregation of semi-granulocytes and granulocytes is accomplished by a combination of binding by pseudopodia and humoral factors. This occurs in response to foreign agents such as *Vibrio* sp. (Johnson 1976; Newman & Feng 1982) and is the precursor to encapsulation for foreign agents too large to phagocytise. Aggregation is often accompanied by extensive pre-mortem clotting of plasma and, in severe cases, the aggregation and plasma clotting can obstruct haemolymph leading to massive focal necrosis (Johnson 1976).

Phagocytosis is a defense employed when the foreign agent is smaller than the haemocyte. For larger particles, a multicellular defense is required to encapsulate the agent. Encapsulation reactions by haemocyte preparations obtained from *Panulirus interruptus* were studied by Hose *et al*. (1990) who showed that the reaction involved the semi-granular haemocytes and fibrocytes. Haemocytes (granulocytes and semigranulocytes) cluster around the foreign body, forming encapsulations many cell layers thick. The outer cells retain a more normal shape while inner cells become flattened. Diffuse melanisation occurs in the compact core and in the intracellular matrix forming a thick brown leathery capsule. Such capsules are not resorbed. Haemocytes also cluster around clots which are presumably resolved in time.

Degranulation is also a neglected area of study in spiny lobsters. Observation of histological material shows that mature granulocytes appear to aggregate near foreign agents and degranulate in the same way as molluscan haemocytes. This process was studied in quahog *(Mercenaria mercenaria*) by Mohandas *et al*. (1985) who showed that bacteria stimulate haemocytes to extrude intact lysosomes into the haemolymph, a process referred to as degranulation. The resulting release of lysosomal hydrolases is assumed responsible for associated host and non-host tissue damage (Feng 1988, Hose & Martin 1989, Watanabe 1999) and may be one mechanism by which bactericidal activity is seen to rise in lobster haemolymph after inoculation of formalin-killed bacteria (see Sinderman (1971) for review). Recent work on degranulation of human eosinophils has suggested that the eosinophils do not discharge granules to the cell surface (exocytosis) but by lysis (Watanabe 1999), and the process in lobster haemocytes may be the same.

Stress-related opportunistic infections result in an observed reduction in haemocyte numbers presumably by attrition (Stewart *et al.* 1967; Stewart & Rabin 1970; Newman & Feng 1982; Field & Apple 1995; Jussila *et al*. 1998). Low haemocyte counts result in long clotting times (Sinderman 1971). It should, however, be noted that the same effect can occur through an

increase in blood volume, and such changes are seldom measured. Changes in haemocyte counts with moulting may also be volume related (Tsing *et al*. 1989).

Is haemolymph sterile? There is ongoing debate. While many hold that the presence of bacteria in the haemolymph is indicative of septicaemia (Lightner 1977) and is a common result of stress (Lightner 1988), bacteria can be isolated from haemolymph of apparently healthy crustaceans. These include *Procambarus clarkii* (Scott & Thune 1986), *Homarus americanus* (Cornick & Stewart, 1966, 1968), *Callinectes sapidus* (Colwell *et al*. 1975), and *Penaeus vannamei* (Gomez-Gil *et al*. 1998). However, bacterial infection following stress can occur rapidly during capture and transport (Johnson 1976; Messick & Kennedy 1990) making it extremely difficult to ensure that unstressed 'healthy' crustaceans have been sampled. In addition, some bacteria, such as *Aerococcus viridans* var. *homari* appear to be difficult for the host to kill and eliminate (Stewart & Rabin 1970). Lesions associated with foreign body rejection can be used as an indicator of health status (L. Evans, Curtin University, pers. comm).

Cellular defense mechanisms- toxic insults

Toxins come from three main sources - environmental contaminants; toxins associated with foreign invaders (Bowser *et al*. 1981); and toxins resulting from tissue damage and haemocyte degranulation. Reactions to toxins have been studied using injected irritant substances such as turpentine (Fontaine *et al*. 1975). The heart is the organ most affected by circulating turpentine in the haemolymph (Fontaine *et al*. 1975). An acute inflammatory reaction produces melanized hemocytic nodules in the heart followed by influx of haemocytes and fibrocytes. Scar tissue is also formed as numerous collagin like fibres replace myocardial fibres in which numerous melanized nodules are interspersed. The myocarditis reported by Wada *et al*. (1994) in *Panulirus japonicus* may represent the end result of such a toxic insult.

Conclusions

Rapid advances are being made in the understanding of the humoral mechanisms of host defence in decapod crustaceans. However, the histopathology of cellular defense mechanisms, though first studied over 100 years ago, is still poorly studied. The influence of environmental stress, nutritional and moult status of the host on defence responses, all areas of critical importance to animal husbandry and production in aquaculture, have only recently drawn attention (Jussila *et al.,* 1998; Hall & van Dam, 1998) and are difficult to interpret in the absence of a basic understanding of the cellular defense mechanisms. Much remains to be discovered.

REFERENCES

Aono, H., & Mori, K. (1996) Interaction between hemocytes and plasma is necessary for hemolymph coagulation in the spiny lobster, *Panulirus japonicus*. *Comp. Biochem. Physiol.*, **113A**, 301-305.

Bauchau, A.G. (1981) Crustaceans. In *Invertebrate Blood Cells*, Vol. 2 (Ed. by N.A. Ratcliffe & A.F. Rowley), pp. 385-420. Academic Press, London.

Barracco, M., & Amirante, G.A. (1992) Morphological and cytochemical studies of the haemocytes of *Squilla mantis* (Stomatopoda). *J. Crust. Biol.*, **12,** 372-382.

Bell, T.A., & Lightner, D.V. (1988) *A handbook of normal penaeid shrimp histology*. World Aquaculture Society, Louisiana.

Bodammer, J.E. (1978) Cytological observations on the blood and haematopoetic tissue in the crab, *Callinectes sapidus*. *Cell & Tissue Res.,* **187,** 79-96.

Bowser, P.R., Rosemark, R., & Reiner, C.R. (1981) A preliminary report of vibriosis in cultured American lobsters, *Homarus americanus. J. Invertebr. Pathol.,* **37**, 80-85.

Colwell, R.R., Wicks, T.C., & Tubiash, H.S. (1975) A comparative study of the bacterial flora of the haemolymph of *Callinectes sapidus*. *Mar. Fish. Rev.,* **37**, 29-33.

Cornick, J.W., & Stewart, J.E. (1966) Microorganisms isolated from the haemolymph of the lobster *Homarus americanus*. *J. Fish. Res. Bd. Can.*, **23**, 1451-1454.

Cornick, J.W., & Stewart, J.E. (1968) Interaction of the pathogen *Gaffkya homari* with natural defense mechanisms of *Homarus americanus*. *J. Fish. Res. Bd. Can.*, **25**, 697-709.

Cornick, J.W., & Stewart, J.E. (1978) Lobster (*Homarus americanus*) haemocytes: classification, differential counts, and associated agglutinin activity. *J. invertebr. Pathol*., **31**, 194-203.

Durliat, M., & Vranckx, R. (1981) Action of various anticoagulants on hemolymphs of lobsters and spiny lobsters, *Biol. Bull. Mar. Biol. Lab., Woods Hole,* **160**, 55-68.

Feng, S.Y. (1988) Cellular defense mechanisms of oysters and mussels. American Fisheries Society Special Publication **18**, 153-168.

Field, R.H., & Appleton, P.L. (1995) A *Hematodinium*-like dinoflagellate infection of the Norway lobster *Nephrops norvegicus* observations on pathology and progress of infection. *Dis. Aquat. Org.*, **22**, 115-28.

Fisher, W.S. (1988) Shell disease of lobsters. In *Disease Diagnosis and Control in North American Marine Aquaculture*, 2nd edn (Ed. by C.J. Sindermann & D.V. Lightner), pp. 236-9. Elsevier, Amsterdam.

Fisher, W.S., Rosemark, T.R., & Nilson, E.H. (1976) The susceptability of cultured American lobsters to a chitinolytic bacterium. *Proc. World Maricult. Soc*., **7**: 511-520.

Fontaine, C.T. (1975) Observations on the wound repair processes in the freshwater crayfish *Procambarus* sp. *J. invert. Pathol*., **25**, 391-393.

Fontaine, C.T., & Lightner, D.V. (1974) Observations on the phagocytosis and elimination of carmine particles injected into the musculature of the white shrimp *Penaeus setiferus*. *J. invert. Pathol*., **24**, 141-148.

Fontaine, C.T., & Lightner, D.V. (1975) Cellular response to injury in penaeid shrimp. *Mar. Fish. Rev.* **37**, 4-10.

Fontaine, C.T., & Bruss, R.G., Sanderson, I.A., Lightner, D. V. (1975) Histopathological response to turpentine in the white shrimp *Penaeus setiferus*. *J. invert. Pathol*., **25**, 321-330.

Fuller, G.M., & Doolittle R.F. (1971a) Studies of invertebrate fibrinogen, I. Purification and characterization of fibrinogen from the spiny lobster. *Biochemistry,* **10**, 1305-11.

Fuller, G.M. & Doolittle R.F. (1971b) Studies of invertebrate fibrinogen, II. Transformation of lobster fibrinogen into fibrin. *Biochemistry,* **10**, 1311-18.

Ghidalia, W., Vendrely, R., Montmory, C., Coirault, Y., & Brouard, M.O. (1981) Coagulation in decapod crustacea. *J. Comp. Physiol.,* **142**, 473-8.

Goldberg, P.Z., Greenberg, A.H., & Gerrard, J.M. (1986) Activation of lobster haemocytes: cytoarchitectural aspects. *J. invert. Pathol*., **47**, 143-154.

Goldberg, P.Z., Huebner, E., & Greenberg, A.H (1984) Activation of lobster haemocytes for phagocytosis. *J. invert. Pathol*., **43**, 77-88.

Gomez-Gill, B., Tron-Mayén, L., Roque, A., Turnbull, J.F., Inglis, V., & Guerra-Flores, A.L. (1998) Species of *Vibrio* isolated from hepatopancreas, haemolymph and digestive tract of a population of healthy juvenile *Penaeus vannamei*. *Aquaculture*, **163**, 1-9.

Hall, M.R., & van Dam, E.H. (1998) The effects of different types of stress on blood glucose in the giant tiger prawn *Penaeus monodon*. *J. World Aquacult. Soc.*, **29**, 290-300.

Hearing, V., & Vernick, S.H. (1967) Fine structure of the blood cells of the lobster, *Homarus americanus*. *Chesapeake Science*, **8**, 170-186.

Hose, J.E., & Martin, G.G. (1989) Defense functions of granulocytes in the ridgeback prawn *Sicyonia ingentis*. *J. invert. Pathol*., **53**, 335-346.

Hose, J.E., Martin, G.G., & Gerard, A.S. (1990) A decapod hemocyte classification scheme integrating morphology, cytochemistry, and function. *Biol. Bull. Mar. Biol. Lab., Woods. Hole,* **178**, 33-45.

Johnson, P.T. (1976) Bacterial infection in the blue crab, *Callinectes sapidus*: Course of infection and histopathology. *J. invert. Pathol.,* **28**, 25-36.

Johnson, M.A., Elder, H.Y., & Davies, P.S. (1976) Cytology of *Carcinus* haemocytes and their function in carbohydrate metabolism. *Comp. Biochem. Physiol.,* **46A**: 569-581.

Jussila, J., Jago, J., Tsvetnenko, E., Dunstan, R., & Evans, L.H. (1998) Total and differential haemocyte counts in western rock lobster (*Panulirus cygnus* George) under post-harvest stress. *Mar. Freshwat. Res.*, **48**, 863-7.

Lanz Mendoza, H., & Faye, I. (1996) Immunoglobulin superfamily proteins in invertebrates. In *New Directions in Invertebrate Immunology*. (Ed. by K. Söderhäll, S. Iwanaga & G.R. Vasta) pp. 285-302. SOS Publications, Fair Haven.

Lightner, D.V. (1977) Shrimp diseases. p 10-77. In. Sindermann, C.J. (editor) *Disease diagnosis and control in North American Aquaculture*. Elsevier, Amsterdam.

Lightner, D.V. (1988) Diseases of cultured penaeid shrimp and prawns. In *Disease Diagnosis and Control in North American Marine Aquaculture,* 2nd edn (Ed. by C.J. Sindermann & D.V. Lightner), pp. 8-127. Elsevier, Amsterdam.

Malloy, S.C. (1978) Bacterial induced shell disease of lobsters (*Homarus americanus*). *J. wildl. Dis.* **14**, 2-10.

Martin, G.G., Quintero, M., Quigley, M. & Khosrovian, H. (2000) Elimination of sequestered material from the gills of decapod crustaceans. *J. Crust. Biol.* **20**, 209-217.

Messick, G.A. & Kennedy, V.S., (1990) Putative bacterial and viral infections in blue crabs, *Callinectes sapidus* Rathbun, 1896 held in flow-through or a recirculating system. *J. Shellfish Res.,* **9,** 33-40.

Mix, M.C. & Sparks, A.K. (1980) Hemocyte classification and differential counts in the Dungness crab *Cancer magister*. *J. invert. Pathol.*, **35**, 134-143.

Mohandas, A., Cheng, T.C., & Cheng, J.B. 1985: Mechanism of lysosomal enzyme release from *Mercenaria mercenaria* granulocytes: a scanning electron microscope study. *J. invert. Pathol.,* **46**, 189-197.

Newman, M.C., & Feng, S.Y. (1982) Susceptability and resistance of the rock crab *Cancer irroratus* to natural and experimental bacterial infection. *J. invert. Pathol.,* **40**, 75-88.

Nyhlen, L., & Unestam, T. (1980) Wound reactions and *Aphanomyces astaci* growth in crayfish cuticle. *J. invert. Pathol.,* **36**, 187-97.

Roch, P. (1999) Defense mechanisms and disease prevention in farmed marine invertebrates. *Aquaculture*, **172**, 125-146.

Scott, J.B., & Thune, R.L. (1986)Bacterial flora of haemolymph of red swamp crawfish *Procambarus clarkii* (Girard) from commercial ponds. *Aquaculture,* **58**, 161-165.

Sindermann, C.J. (1971) Internal defenses of Crustacea: A review. *Fish. Bull.,* **69**: 455-489.

Smith, V.J., & Ratcliffe, N.A. (1980a) Cellular defense reactions of the shore crab, *Carcinus maenas*: in vivo hemocytic and histopathological response to injected bacteria. *J. invert. Pathol*., **35**, 65-74.

Smith, V.J., & Ratcliffe, N.A. (1980b) Host defense reactions of the shore crab, *Carcinus maenas* (L.): clearance and distribution of infected test particles. *J. mar. biol. Ass. U.K.*, **60**, 89-102.

Söderhäll, K., & Ajaxon, R. (1982) Effects of quinones and melanin on mycelial growth of *Aphanomyces* spp. and extracellular protease of *Aphanomyces astaci,* a parasite on crayfish. *J. invert. Pathol.,* **39**, 105-9.

Söderhäll, K., & Smith, V.J. (1986) The prophenoloxidase activating system: the biochemistry of its activation and role in arthropod cellular immunity with special reference to crustaceans. In *Immunity in Invertebrates* (Ed. by M. Brehelin), pp. 208-23. Springer-Verlag, Berlin.

Söderhäll, K., Cerenius, L., & Johansson, M.W. (1996) The prophenoloxidase activating system in Invertebrate Immunology. In *Invertebrates In New Directions* (Ed. by K. Söderhäll, S. Iwanaga, and G.R. Vasta) pp. 229-253. SOS Publications, Fair Haven.

Stewart, J.E. (1993) Infectious diseases of marine crustaceans. In Couch, J.A. & Fournie, J.W. (editors). *Pathobiology of marine and estuarine organisms*. Pp. 319-342. CRC, Ann Arbour.

Stewart, J.E., & Rabin, H. (1970) Gaffkaemia, a bacterial disease of lobsters (genus *Homarus*). In. Snieszko, S.F. (editor). *A symposium on diseases of fishes and shellfishes. Am. Fish. Soc. Spec. Publ.,* **5**, 431-439.

Stewart, J.E., Cornick, J.W., & Dingle, J.R. (1967) An electronic method for counting lobster (*Homarus americanus* Milne Edwards) hemocytes and the influence of diet on hemocyte numbers and hemolymph proteins. *Can. J. Zool.,* **45**, 291-304.

Tsing, A., Arcier, J.-M., & Berhélin, M. (1989) Hemocytes of penaeid and palaemonid shrimps: Morphology, cytochemistry, and hemograms. *J. invert. Pathol*., **53**, 64-77.

Unestam, T. (1973) Fungal diseases of Crustacea. *Rev. Med. Vet. Mycology,* **8**, 1-20.

Unestam, T., & Nylund, J.E. (1972) Blood reactions in vitro in crayfish against a fungal parasite *Aphanomyces astaci. J. invert. Pathol.,* **19**, 94-106.

Wada, S., Takayama, A., Hatai, K., Shima, Y., & Fushimi, H. (1994) A pathological study on cardiac disease found in spiny lobsters. *Fish. Sci.*, **60**, 129-31.

Watanabe, K. (1999) The degranulation of eosinophils by cell lysis. *Asia/Pacific Microscopy and Analysis*, **10**, 9-10.

Williams, A.J., & Lutz, P.L. (1975) Blood cell types in *Carcinus maenas* and their physiological role. *J. mar. biol. ass. U.K.,* **55**, 671-674.

Diseases of spiny lobsters in New Zealand

B. K. Diggles[1]

[1]National Institute of Water and Atmospheric Research, PO Box 14-901 Kilbirnie, Wellington, New Zealand
Corresponding author. Tel.: +64 4 386 0300. Fax: +64 4 386 0574.
E-mail: b.diggles@niwa.cri.nz

ABSTRACT

A significant amount of research has been conducted in New Zealand investigating methods of culturing two species of spiny lobsters *Jasus edwardsii* and *Jasus verreauxi*. Various disease agents have contributed to morbidity and mortality in each of these species, especially during the early stages of development and refinement of rearing techniques. Mortalities of puerulus and juvenile *Jasus edwardsii* in experimental holding facilities were due to the invasive fungus *Haliphthoros* sp. and secondary vibriosis. Fouling of gills of *J. edwardsii* juveniles with a filamentous *Leucothrix*-like bacterium, thin septate fungi, free living nematodes and ectocommensal ciliates were probably due to poor water quality and system design in lobster rearing systems utilising recirculating seawater. Low level mortalities of adult *J. edwardsii* in experimental holding tanks were associated with symptoms of swelling, a condition termed Turgid Lobster Syndrome (TLS). Bacteria isolated from lobsters displaying TLS included *Vibrio harveyi* and *V. splendidus* I, however the aetiology of TLS remains undetermined at present. A small number of moribund adult *J. edwardsii* in a dietary experiment presented symptoms of bacterial necrosis and mummification of hepatopancreas tubules. The bacterium *Vibrio harveyi* was reminiscent of necrotising hepatopancreatitis. The bacterium *Vibrio harveyi* was isolated from moribund phyllosomas of *J. verreauxi* exhibiting luminous vibriosis. The gross signs of each disease and some suggestions for their prevention and control are described.

Keywords: mariculture, diseases, Paniluridae, mycosis, fungi, bacteria

I. INTRODUCTION

Two commercial species of spiny lobster, *Jasus edwardsii* (Hutton, 1875) and *Jasus verreauxi* (Milne Edwards, 1851) occur in New Zealand waters. These species support a commercial fishery with an annual export value of around $100 million NZD, based mainly on wild caught lobsters marketed live in Asian markets. The increasing commercial value of these crustaceans, together with quota restrictions on wild fisheries, has resulted in interest in their mariculture. A significant amount of research has been conducted in New Zealand determining conditions suitable for ongrowing of pueruli (Booth and Kittaka 1994). Within the last few years these experiments have been followed by the establishment of small scale commercial mariculture ventures using closed systems and recirculating water. Preliminary studies of methods for rearing larvae of *J. verreauxi* from egg through to puerulus have also been undertaken at the National Institute of Water and Atmospheric Research (NIWA) aquaculture research facility at Mahanga Bay, Wellington.

Few diseases have been recorded from wild lobsters in New Zealand (Booth and Kittaka, 1994), however novel disease agents have become apparent during the early stages of rearing of both species, causing differing degrees of morbidity and mortality. This paper provides an overview of some of the diseases encountered to date during the initial stages of confinement and ongrowing of the two species of spiny lobster in New Zealand, and provides a brief guide to the gross signs of disease, the disease agents, and in some cases, potential methods for their treatment and prophylaxis.

II. MATERIALS AND METHODS

Diseased lobsters were obtained between November 1997 and October 1999 from various sources including experiments at NIWA facilities at Greta Point and Mahanga Bay, Wellington, and from commercial suppliers. Typical dissection protocols included euthanasing animals by medial section with a pair of scissors, then excising and examining organs of interest with wet squashes and smears. Samples for histopathology were immediately fixed in 10% (v/v) formalin in seawater filtered to 0.22 µm for at least 24 hours. All histopathology samples were embedded in paraffin wax, processed using standard histological procedures and 6 µm sections were stained with hematoxylin and eosin. Bacteriology was performed on hemolymph drawn from the base of the 5th walking leg with a sterile 25 gauge hypodermic needle after surface sterilisation using 70% ethanol. Hemolymph samples were inoculated onto either marine agar (Difco), thiosulphate citrate bile salt sucrose agar (TCBS), (Oxoid), or Tryptic Soy Agar with 2% NaCl (TSA+2) (Oxoid), then incubated at either 15 or 20°C. Bacteria isolated in moderate to heavy growths were subcultured to ensure purity before storage in long term preservation medium (Beuchat 1974) for up to 2 weeks prior to identification. Biochemical characterisation was undertaken on isolates recovered from long term preservation medium and subjected to 52 phenotypic tests using methods described by Baumann *et al.* (1971), Furniss *et al.* (1978), and West and Colwell (1984). Small sections of gill were also inoculated into marine agar, Saubarose Dextrose Agar (SDA) (Oxoid) made up with seawater, Potato dextrose agar (PDA) (Gibco BRL), PDA +2 % NaCl (PDA +2), or a modified Vishnac medium (Fuller *et al.* 1964) to examine for marine fungi. All media used for fungus cultures included 0.5 mg/ml penicillin/streptomycin to minimise bacterial contamination.

III. RESULTS & DISCUSSION

Diseases of *Jasus edwardsii*

Invasive fungal infection

On several occasions significant mortalities were observed in puerulus and juvenile *J. edwardsii* held for ongrowing in experimental tanks at 10 to 15°C. Gross signs of disease included brown/black lesions at the base of the gills near insertion of the walking legs. In some instances between 30 and 50% of animals were affected. Wet mounts of gill lesions showed the presence of invasive fungal mycelia (Figure 1) in 100% of moribund lobsters. Apparently healthy and control lobsters were uninfected and exhibited no lesions. Histology

of gill lesions showed the presence of fungal mycelia inside the gill cuticle, while in many lobsters numerous epibionts (*Leucothrix*-like filamentous bacteria, sessile ciliates (*Carchesium* sp., *Epistylis* sp.)) were attached to the outside of the gill cuticle. Massive hemocyte infiltration and melanisation was apparent at the base of the walking leg adjacent to infected gill filaments (Figure 2). Additional lesions were evident in the hepatopancreas in some animals, including abnormal vacuolation of hepatopancreas tubules (Figure 3) when compared to healthy control animals (Figure 4), sloughing of necrotic hepatopancreocytes into the tubule lumen, and in some cases moderate numbers of bacteria in the tubule lumen (Figure 5).

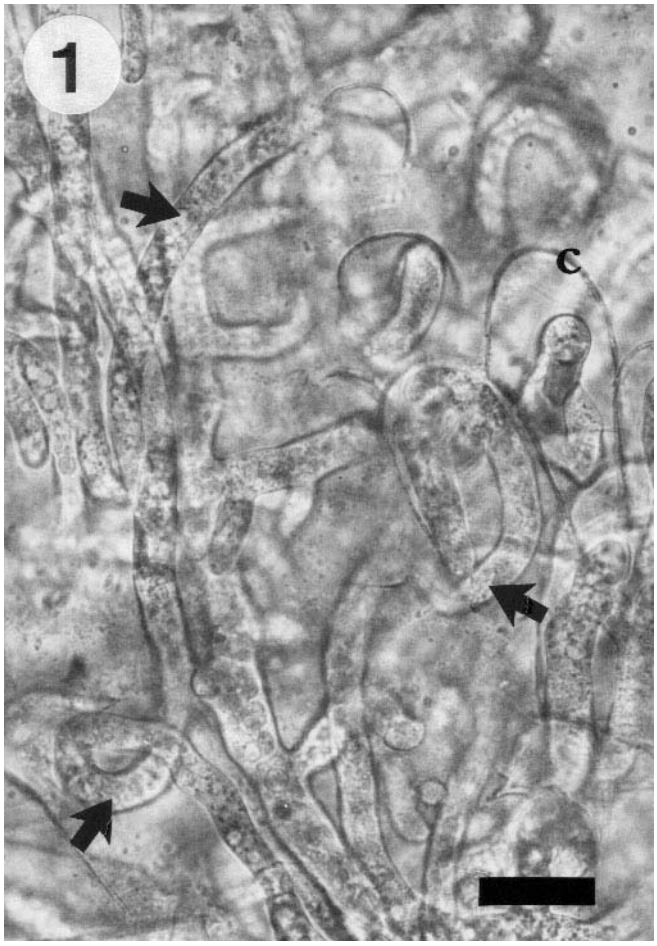


Figure 1 Wet squash of gill of *J. edwardsii* juvenile with an invasive fungal infection by *Haliphthoros* sp. Note mass of fungal mycelia (arrows) inside gill cuticle (c). Scale bar = 35 µm.

Figure 2 H&E section of base of gill of *J. edwardsii* infected with *Haliphthoros* sp. showing massive hemocyte infiltration (I) and melanisation (M) at the insertion of the walking leg. Note fungal mycelia inside gill cuticle (arrows). Scale bar = 175 µm.

Figure 3 Severe vacuolation in the hepatopancreas of *J. edwardsii* infected with *Haliphthoros* sp. Note eosinophillic amorphous substance (arrowed) in some vacuoles. Scale bar = 35 µm.

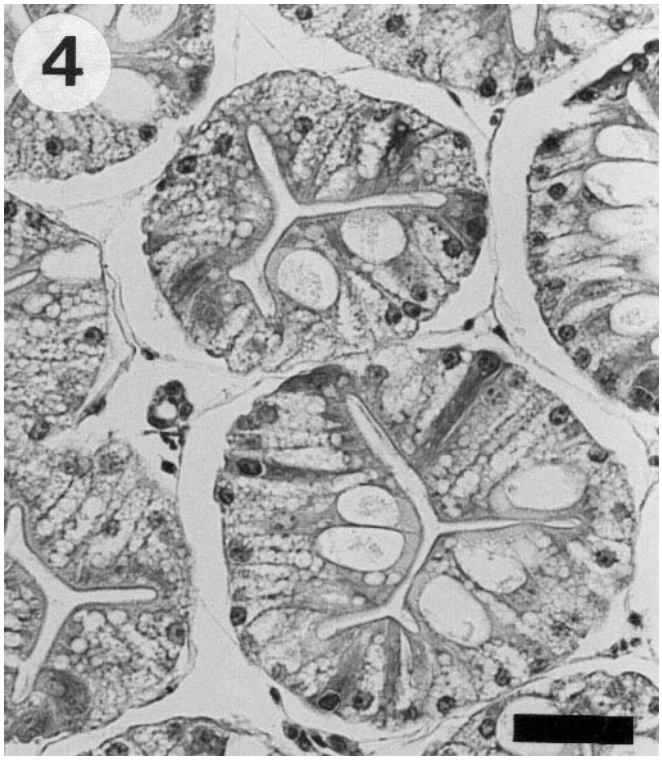


Figure 4 Control hepatopancreas from a healthy, well fed *J. edwardsii* showing normal vacuolation in R cells. Scale bar = 45 μm

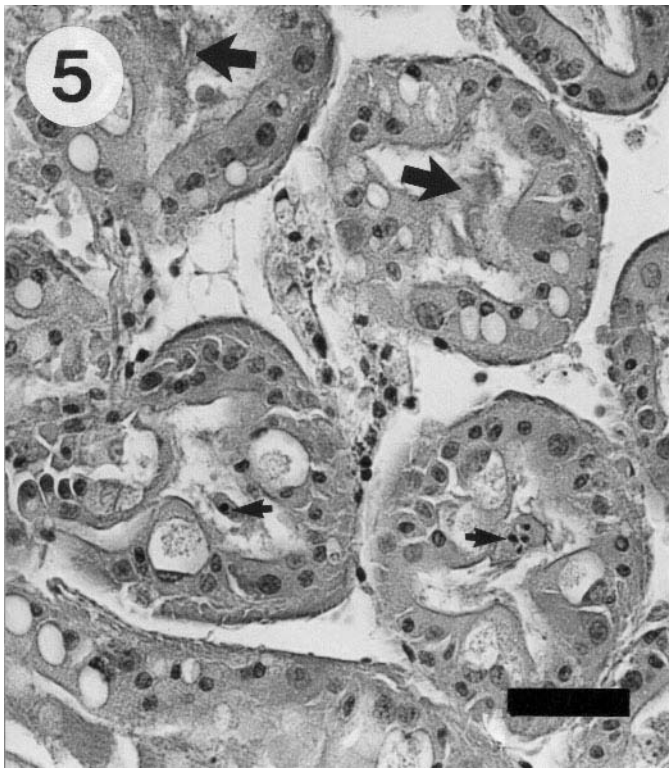


Figure 5 Hepatopancreas of a *J. edwardsii* infected with *Haliphthoros* sp. Note bacterial plaques (large arrows) and necrotic hepatopancreocytes (small arrows) sloughed into the tubule lumen. Scale bar = 45 μm

Cultures of the invasive fungus were successfully obtained when infected gill filaments were inoculated into marine agar. Growth was best in marine agar compared to PDA, PDA+2, SDA and modified Vishnac medium. The fungus was identified as a phycomycete with sparingly septate, highly branched vegetative mycelia 12 to 15 µm diameter. Biflagellate motile spores between 6 and 10 µm diameter were produced between 48 and 96 hours after introducing cultures into seawater at 15°C. The morphology of the fungal mycelia and spore discharge tubes observed in lobster gills (Figure 6) suggests a phycomycetous fungus of the genus *Haliphthoros* was the causative agent. An isolate of the fungus has been placed in the American Type Culture Collection under accession number MYA-847 (Diggles 2001). The specific identity of the fungus remains to be determined. Time to sporulation of the New Zealand isolate (48-96 hours) more closely resembles that reported for *H. milfordensis* (see Vishnac 1958, Fuller *et al.* 1964, Tharp and Bland 1977), rather than the 2 - 3 hours reported by Hatai *et al.* (1980) for *H. philippinensis*. *Haliphthoros milfordensis* is a phycomycetous fungus which can infect juvenile homarid lobsters, causing red-brown necrotic lesions in the gills at the base of walking legs (Fisher *et al.* 1975, 1978). *Haliphthoros milfordensis* is reportedly only problematic in American lobsters less than 25 mm carapace length (Fisher and Nilson, 1977), which generally agrees with observations of the size of susceptible *J. edwardsii* in New Zealand. Death of affected *J. edwardsii* usually occurred during the moult, perhaps from restriction of ecdysis due to extensive melanisation (Fisher and Nilson, 1977). However, the pathology in the hepatopancreas of some of the infected *J. edwardsii* (e.g. Figures 4 and 5) indicated that in some cases systemic effects could be implicated, perhaps by-products of fungal metabolism and/or secondary bacterial infection.

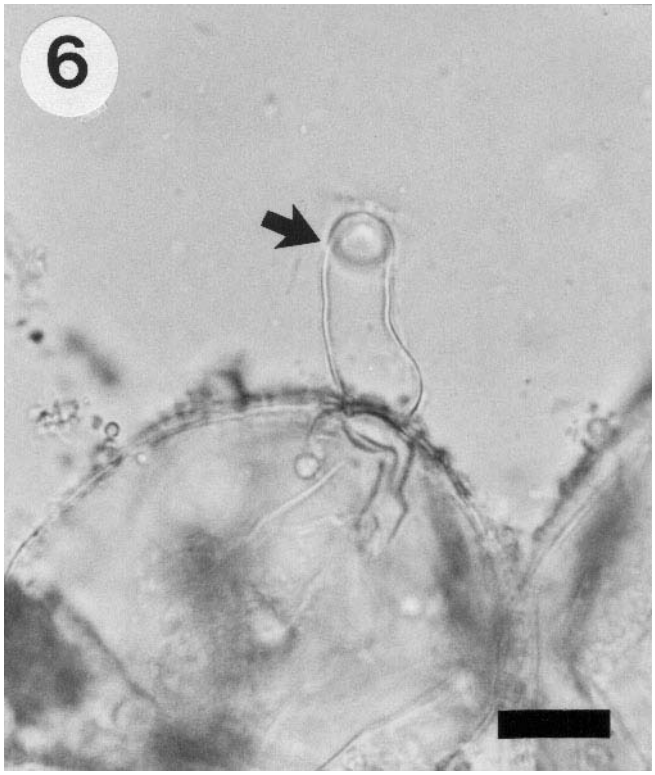


Figure 6 Discharge tube (arrow) of *Haliphthoros* sp. penetrating gill of *J. edwardsii*. Scale bar = 18 µm.

Prevention

Fungal infections of lobsters are usually husbandry related diseases attributable to handling damage, poor water quality and/or poor cleanliness of rearing systems (Fisher and Nilson, 1977, Fisher *et al.* 1978), hence attention to these factors appears warranted. Modification of the design of lobster rearing systems using recirculated water to improve biological and particle filtration, optimise water circulation (prevent "dead spots") and simplify cleaning should be investigated.

Treatment

Effective treatments for *Haliphthoros* are available (Abrahams and Brown, 1977, Lio-Po *et al.* 1985), and include baths of malachite green and Trifuralin, which kill zoospores and prevent infections from spreading (Lightner 1983). Once fungi are inside the cuticle, however, the survival chances of infected lobsters are poor, hence prevention is a far more desirable goal.

Epibiont fouling

Persistent low level mortalities of juvenile lobsters *Jasus edwardsii* in rearing systems utilising recirculated seawater were associated with moderate to heavy growths of epibionts. Affected animals showed sluggish behaviour and foci of light brown colouration in the gills. Most deaths of affected lobsters occurred at night during the moult. Moderate to heavy mixed growths of filamentous *Leucothrix*-like bacteria, a thin septate fungi, free living nematodes and sessile stalked ciliates (*Carchesium* sp., *Epistylis* sp., *Zoothamnium* sp.) were evident when affected areas of gills were examined using wet squashes (Figure 7). Deposits of organic detritus (dirt, faeces and other debris) were also commonly found. Examination of animals which had been reared in affected systems for varying periods of time showed that the epibionts were gradually accumulated. Scrapes of tank surfaces showed a buildup of organic detritus and moderate to heavy fouling with all of the types of epibionts found on lobster gills.

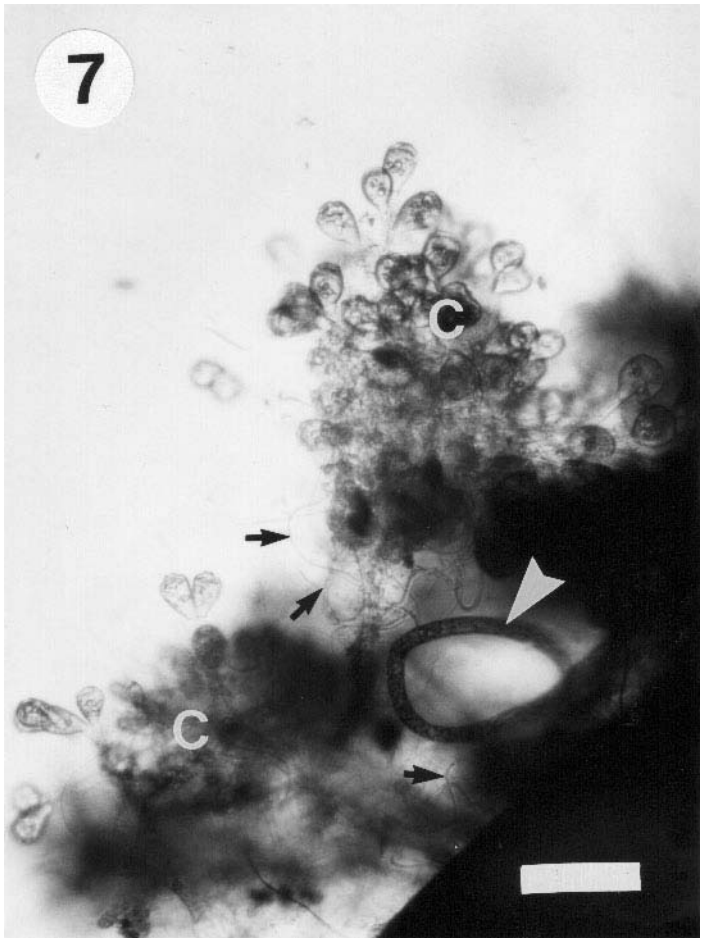


Figure 7 Epibiont growths on gills of *J. edwardsii*. Amongst two colonies of stalked ciliates (*Carchesium* sp.) (C) are *Leucothrix*-like filamentous bacteria (small arrows) and a free living nematode (large arrowhead). Scale bar = 130 μm.

These symptoms of death during the moult appear similar to that reported by Bowser and Rosemark (1981) for a nutritionally related moult death syndrome (MDS) in *Homarus americanus* fed purified diets. MDS has been suspected in *J. edwardsii* held in captivity (Booth and Kittaka, 1994), however most lobsters in New Zealand are fed mussels (James and Tong 1997), not purified diets, and the abnormal calcium deposits embedded in exuvial exoskeleton which characterise MDS have not been observed to date. Oxygen demand in *J. edwardsii* increases at night (Crear and Forteath 1998), when moulting usually occurs. In the cases examined by the author, it is considered more likely that heavy epibiont growth contributed to overnight deaths during moulting by reducing respiratory effectiveness, as reported for *Leucothrix mucor* infections of penaeid shrimp (Lightner, 1983), though nutritional inadequacy has yet to be ruled out as a predisposing factor. Cannibalism of moulting lobsters also appeared particularly prevalent in systems where heavy epibiont growth was evident. It is considered that the physical presence of fouling organisms on the gills of affected lobsters may have contributed to difficulty with moult shedding, and thus may contribute to mortalities from cannibalism by prolonging the time taken to moult.

Prevention

Improvement of system hygiene by improving water quality (e.g. more frequent water changes), regular cleaning of tank surfaces and improved particle filtration to remove circulating organic detritus are the recommended methods of minimising epibiont fouling. Modification of system design to improve biological filtration, optimise water circulation (prevent "dead spots") and simplify cleaning should also be investigated.

Treatment

Management of *Leucothrix* infestations using antibiotics and copper compounds has been reported (Lightner 1983). Formalin baths (25 ppm indefinite) are reportedly effective against ciliate infestations (Lightner 1983).

Hepatopancreas disease

A hepatopancreas disease was observed in a small percentage of sub-adult *J. edwardsii* used in a dietary experiment. Affected lobsters were reportedly lethargic, stopped feeding and eventually died. Upon dissection affected lobsters exhibited large blackened, necrotic areas in the hepatopancreas. Histopathology showed complete necrosis of affected hepatopancreas tubules which were surrounded by a melanised layer with a granulomatous response in interstitial areas composed mainly of granulocytes and fibrocyte-like cells (Figure 8). Some tubules contained a mass of gram negative bacteria, and also small protozoa, possibly flagellates, often peripheral to the bacterial masses, in closer approximation to the tubule wall. The pathology appeared reminiscent in some respects of necrotizing hepatopancreatitis of penaeid shrimp (Frelier *et al.* 1992), or bacterial necrosis and mummification of hepatopancreas tubules of *Cherax tenuimanus* caused by failure to digest an inadequate diet (Langdon *et al.* 1992). Whether the presumptive protozoa or bacteria play a primary or secondary role is at this stage unclear.

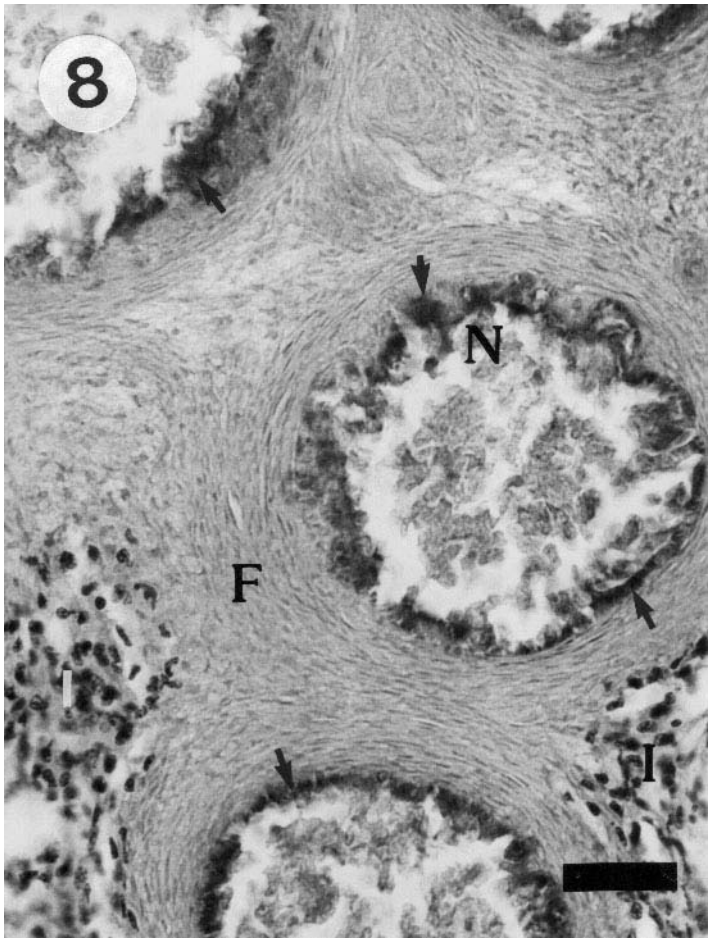


Figure 8 Hepatopancreas tubules of *J. edwardsii* with hepatopancreas disease. Note complete necrosis of tubule epithelium (N), melanisation (arrows), and hemocyte infiltration (I) and fibrosis (F) completely surrounding tubule remnants. Scale bar = 40 μm.

Prevention

If the disease is related to diet, provision of an adequate diet may reduce the incidence of hepatopancreas disease.

Treatment

None known

Turgid Lobster Syndrome

Turgid Lobster Syndrome (TLS) is a term used to describe a syndrome which occurs in adult *J. edwardsii* at low prevalence in laboratory holding tanks. The turgid condition is characterized by lobsters exhibiting fluid-filled, swollen arthrodial membranes caused by an apparent increase in hemolymph volume. In early stages the affected lobsters stop feeding, show limited swelling and become lethargic, while in later stages they cannot flex their abdomens. These symptoms may subside, but around 30 to 50% of affected lobsters develop

terminal stages of the condition, become moribund and die. The syndrome does not appear to be readily transferred from lobster to lobster.

An increase in the number of circulating granulocytes and pre-granulocytes has been noted in lobsters displaying symptoms of TLS. Bacteria such as *Vibrio harveyi* and *V. splendidus* I are occasionally, but not always, isolated from the hemolymph. Both *V. harveyi* and *V. splendidus* do not cause swelling when re-injected into healthy lobsters at up to 10^8 bacteria/animal. Transfer of hemolymph from affected lobsters to healthy individuals does not cause swelling. Symptoms of TLS are not associated with salinity fluctuations, nor with handling or other known stressors. The cause of the syndrome is unknown at present. Possible causes may include starvation, as this causes increases in hemolymph volume (Dall, 1974), or the syndrome may represent a physiological abnormality associated with the moult cycle, as animals in early stages of premoult are more likely to be affected.

Prevention and treatment

None known

Diseases of *Jasus verreauxi*

Luminous vibriosis of phyllosoma larvae

An outbreak of luminous vibriosis caused up to 80% mortality in a batch of *J. verreauxi* phyllosoma (instar 8 -10) reared in an experimental culture facility (Diggles *et al*., 2000). Affected larvae became opaque, exhibited small red spots throughout the body and pereiopods, and were faintly luminous when viewed in the dark. Histopathology showed massive bacterial plaques in the gut and hepatopancreas tubules (Figure 9). Pure cultures of a bacterium identified as *Vibrio harveyi* were isolated from moribund larvae. The disease syndrome was reproduced and *V. harveyi* was successfully reisolated from diseased larvae when healthy larvae were exposed by immersion to baths of more than 10^4 *V. harveyi* / ml at 23°C. A potential source of the *V. harveyi* was thought to be the *Artemia* given as live food. Phyllosomas of *Jasus edwardsii* had been reared in the same facility previously without evidence of infection with *V. harveyi*, however these were reared at lower temperatures (18 to 20°C). The outbreak of luminous vibriosis in *J. verreauxi* phyllosomas followed an increase in water temperature from 19 to 23°C, consistent with *V. harveyi* acting as a pathogen at warmer water temperatures.

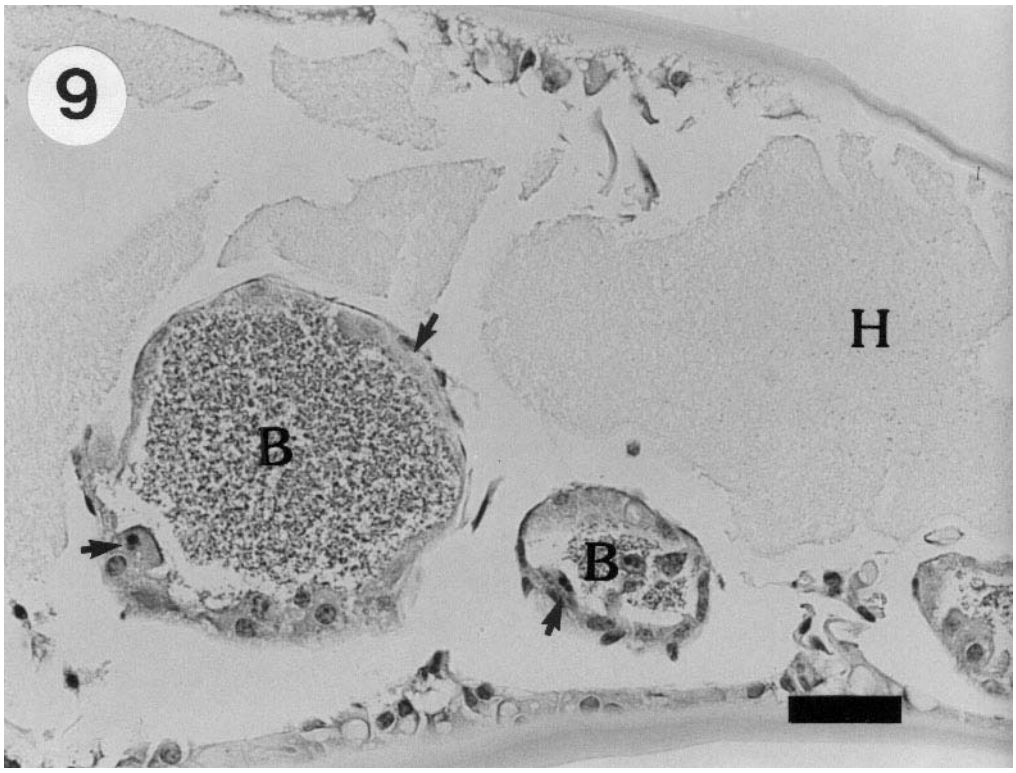


Figure 9 Hepatopancreas tubules of *J. verreauxi* phyllosoma with luminous vibriosis. Note completely atrophy and necrosis of tubules (arrows) which are filled with bacteria (B), and scattered bacteria in hemolymph (H). Scale bar = 40µm.

Prevention

Control of bacterial flora of culture water and live food is suggested.

Treatment

Antibiotics are often relied upon for treatment of luminous vibriosis in penaeid shrimp, however the emergence of antibiotic resistant strains of *V. harveyi* is becoming an increasing problem (Karunasagar *et al.* 1994). Alternative methods such as control of bacterial flora of culture water and live food through administration of probiotic bacteria (Rengpipat *et al.* 1998) is worth investigation.

Shell disease

Shell disease is ubiquitous in crustaceans and New Zealand is no exception. It has been recorded in wild and captive *Jasus edwardsii* (juveniles and adults), and captive *J. verreauxi* (adults), usually occurring on the ventral part of the tail fan and other areas of the carapace in contact with bottom surfaces. The cause is assumed to be chitinoclastic bacteria, however some melanised fungal hyphae have been observed in shell disease lesions in adult *J. verreauxi*. Shell disease does not appear to be pathogenic to either species of lobster, but is nonetheless important as it reduces their marketability.

Prevention and treatment

Maintenance of optimal water quality and regular drying, cleaning and disinfection of holding tanks are suggested.

Few, if any, diseases have been recorded from wild spiny lobsters in New Zealand (Booth and Kittaka 1994). The vast majority of diseases and disease syndromes recorded in New Zealand lobsters are from animals confined for rearing experiments and ongrowing in shorebased facilities. To date there have been few diseases of adult lobsters, none of which are economically important. Most of the diseases of juveniles and larvae recorded to date can, to varying extents, be attributed to husbandry related problems such as inadequate water quality or holding system design (Table I). Many have been recorded in culture of homarid lobsters and other crustaceans such as penaeid shrimp. Invasive fungi are well known pathogens of both larval (Nilson *et al.* 1976) and juvenile lobsters (Fisher *et al.* 1975, Lightner and Fontaine, 1975, Fisher and Nilson, 1977). Luminous vibriosis is one of the more significant bacterial pathogens of penaeid shrimp (Lavilla-Pitogo *et al.* 1990, 1992), while *V. harveyi* and *V. harveyi*-like bacteria have also been reported to cause mortalities in captive spiny lobster *Panulirus* sp. (see Chong and Chao 1986, Abraham *et al.* 1996). Epibiont fouling is also a common problem in crustacean culture (Fisher *et al.* 1978, Lightner 1983, Brock and Lightner 1990). While a significant amount of research will be required to determine best practice methods for culturing both *Jasus edwardsii* and *J. verreauxi*, none of the disease problems experienced to date appear to pose a serious threat to commercial industry provided due attention is paid to basic husbandry practices.

Table I Summary of diseases and disease syndromes encountered in captive spiny lobsters in New Zealand

Species	Disease	Size range affected	Causative agent	Gross signs	Possible methods of prophylaxis
Jasus edwardsii	Invasive fungal infection	Puerulus, juveniles	*Haliphthoros* sp.	Blackened, necrotic lesions in gills near insertion of walking legs, lethargy, death	Improve water quality, system design and husbandry practices
	Epibiont infestation of gills and other surfaces	Puerulus, juveniles	*Leucothrix* - like bacteria, thin septate fungi, free living nematodes, sessile ciliates	Brown areas on gills, lethargy, associated with death during moult	Improve water quality, system design and husbandry practices
	Hepatopancreas disease	Sub adults	Unknown, possibly related to diet	Blackened, foci in hepatopancreas with necrosis and mummification of tubules, lethargy, death	Provide with adequate diet
	Turgid lobster syndrome	Adults	Unknown	Swelling characterised by protrusion of arthrodial membranes, lethargy, sometimes death	Unknown
	Shell disease	Juveniles, Sub adults and adult	Chitinoclastic bacteria, fungi	Foci of blackened areas on the carapace, especially areas in contact with the bottom	Improve water quality, system design and husbandry practices
Jasus verreauxi	Luminous vibriosis	Phyllosoma larvae	*Vibrio harveyi*	Moribund larvae are luminescent with reddened tips of perieopods	Control bacterial flora of culture water and live food
	Shell disease	Adults	Chitinoclastic bacteria, fungi	Foci of eroded or blackened areas on the carapace, especially areas in contact with the bottom	Improve water quality, system design and husbandry practices

ACKNOWLEDGEMENTS

The author thanks C. Barker, J. Booth, J. Foreman and G. Moss for supplying lobsters on which much of this work was based. Thanks also to J. Carson for assistance with bacteriology, J. Shields for advice on crustacean diseases, and P. M. Hine, J. Handlinger, B. Jones, and D. Lightner for helpful discussions on various disease syndromes. J. Booth provided helpful suggestions on the manuscript. This research was conducted as part of an augmenting PGSF-funded programme CO1606.

REFERENCES

Abraham, T.J., Rahman, M.K. & Joseph, M.T.L. (1996) Bacterial disease in cultured spiny lobster, *Panulirus homarus* (L.). *J. Aquacult. Trop.,* **11**, 187-192.

Abrahams, D. & Brown, W.D. (1977) Evaluation of fungicides for *Haliphthoros milfordensis* and their toxicity to juvenile European lobsters. *Aquacult.,* **12**, 31-40.

Baumann, P., Baumann, L. & Mandel, M. (1971) Taxonomy of marine bacteria: the genus *Beneckea*. *J. Bacteriol*., 268-294.

Beuchat, L.R. (1974) Combined effects of water activity, solute, and temperature on the growth of *Vibrio parahaemolyticus*. *Appl. Microbiol*., **27**, 1075-1080.

Booth, J.D. & Kittaka, J. (1994) Growout of juvenile spiny lobster. In *Spiny Lobster Management* (Ed. By B.F. Phillips, J.S. Cobb & J. Kittaka), pp 424-445. Blackwell Scientific Publications, Oxford.

Bowser, P. & Rosemark, R. (1981) Mortalities of cultured lobsters, *Homarus*, associated with a moult death syndrome. *Aquacult.,* **23**, 11-18.

Brock, J.A. & Lightner, D.V. (1990) Diseases of Crustacea - Diseases caused by Microorganisms. In *Diseases of Marine Animals Volume III* (Ed. O. Kinne), pp 245-349. Biologische Anstalt Helgoland, Hamburg.

Chong, Y.C. & Chao, T.M. (1986) Septicemias of marine crabs and shrimp. In *The First Asian Fisheries Forum* (Ed. J.L. Maclean, L.B. Dizon, & L.V. Hosillos). pp 331-332. Asian Fisheries Society, Manila.

Dall, W. (1974) Indices of nutritional state in the western rock lobster, *Panulirus longipes* (Milne Edwards). I. Blood and tissue constituents and water content. *J. Exp. Mar. Biol. Ecol*., **16**, 167-180.

Diggles, B.K. (2001) A mycosis of juvenile spiny rock lobster, *Jasus edwardsii* (Hutton 1875) caused by *Haliphthoros* sp., and possible methods of chemical control. *J. Fish. Dis.* **24**: (in press).

Diggles, B.K., Moss, G.A., Carson, J. & Anderson, C.D. (2000) Luminous vibriosis in rock lobster *Jasus verreauxi* (Decapoda:Palinuridae) phyllosoma larvae caused by infection with *Vibrio harveyi*. *Dis. Aquat. Org*.

Fisher, W.S. & Nilson, E.H. (1977) *Haliphthoros* disease of lobsters. In *Disease Diagnosis and Control in North American Marine Aquaculture* (Ed. C.J. Sindermann), pp 173-177. Elsevier, New York.

Fisher, W.S., Nilson, E.H. & Shleser, R.A. (1975) Effect of the fungus *Haliphthoros milfordensis* on the juvenile stages of the American lobster *Homarus americanus*. *J. Invert. Pathol*., **26**, 41-45.

Fisher, W.S., Nilson, E.H., Steenbergen, J.F. & Lightner, D.V. (1978) Microbial diseases of cultured lobsters: a review. *Aquacult.,* **14**, 115-140.

Frelier, P.F., Sis, R.F., Bell, T.A. & Lewis, D.H. (1992) Microscopic and ultrastructural studies of necrotizing hepatopancreatitis in pacific white shrimp (*Penaeus vannamei*) cultured in Texas. Vet. Pathol., **29**, 269-277.

Fuller, M.S., Fowles, B.E. & McLaughlin, D.J. (1964) Isolation and pure culture study of marine phycomycetes. *Mycologia*, **56**, 745-756.

Furniss, A.L., Lee, J.V. & Donovan, T.J. (1978) *The Vibrios*. Monograph Series 11, Public Health Laboratory Service. Published by Her Majesty's Stationery Office, London.

Hatai, K., Bian, B.Z., Baticados, C.L. & Egusa, S. (1980) Studies on the fungal diseases in crustaceans. II. *Haliphthoros philippinensis* sp. nov. isolated from cultivated larvae of the jumbo tiger prawn (*Penaeus monodon*). *Trans. Mycol. Soc. Japan*, **21**, 47-55.

James, P. & Tong, L. (1997) Differences in growth and moult frequency amongst post-pueruli of *Jasus edwardsii* fed fresh, aged, or frozen mussels. *Mar. Freshw. Res.,* **48**, 931-934.

Karunasagar, I., Pai, R., Malathi, G.R. & Karunasagar, I. (1994) Mass mortality of *Penaeus monodon* larvae due to antibiotic resistant *Vibrio harveyi* infection. *Aquacult*., **128**, 203-209.

Langdon, J.S., Buller, N., Ostle, C. & Thorne, T. (1992) Bacterial necrosis and mummification of the digestive gland associated with feeding peas, *Pisum sativum*, to freshwater crayfish, *Cherax tenuimanus*. In *Diseases in Asian Aquaculture I*. (Ed. by M. Shariff, R.P. Subasinghe & J.R. Arthur), pp 199-205. Fish Health Section, Asian Fisheries Society, Manila.

Lavilla-Pitogo, C.R., Albright, L.J., Paner, M.G. & Sunaz, N.A. (1992) Studies on the source of luminescent *Vibrio harveyi* in *Penaeus monodon* hatcheries. In *Diseases in Asian Aquaculture I*. (Ed. by M. Shariff, R.P. Subasinghe & J.R. Arthur), pp 157-164. Fish Health Section, Asian Fisheries Society, Manila.

Lavilla-Pitogo, C.R., Leano, E.M. & Paner, M.G. (1998) Mortalities of pond-cultured juvenile shrimp, *Penaeus monodon*, associated with dominance of luminescent vibrios in the rearing environment. *Aquacult*., **164**, 337-349

Lightner, D.V. (1983) Diseases of cultured Penaeid shrimp. In: *CRC Handbook of Mariculture, Volume 1, Crustacean Aquaculture* (Ed. By J.P. McVey & J.R. Moore), pp 289-320. CRC Press Inc., Boca Raton.

Lightner, D.V. & Fontaine, C.T. (1975) A mycosis of the American Lobster, *Homarus americanus*, caused by *Fusarium* sp. *J. Invert. Pathol*., **25**, 239-245.

Lio-Po, G.D., Baticados, M.C.L., Lavilla, C.R. & Sanvictores, M.E.G. (1985) *In-vitro* effects of fungicides on *Haliphthoros philippinensis*. *J. Fish Dis*., **8**, 359-365.

Nilson, E.H., Fisher, W.S. & Shleser, R.A. (1976) A new mycosis of larval lobster (*Homarus americanus*). *J. Invert. Pathol*., **27**, 177-183.

Rengpipat, S., Phianphak, W., Piyatiratitivorakul, S. & Menasveta, P. (1998) Effects of probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. *Aquacult*., **167**, 301-313.

Tharp, T.P. & Bland, C.E. (1977) Biology and host range of *Haliphthoros milfordensis*. *Can. J. Bot*., **55**, 2936-2944.

Vishnac, H.S. (1958) A new marine phycomycete. *Mycologia,* **50**, 66-79.

West, P.A. & Colwell, R.R. (1984) Identification and classification of Vibrionaceae - an overview. In *Vibrios in the Environment* (Ed. R.R. Colwell). p 285-363, John Wiley & Sons, New York.

Identifying stress when western rock lobsters are stored out of water: the average and individual blood lactate concentrations

[1]Brian D. Paterson, [1,2]Glen W. Davidson and [1,2]Patrick T. Spanoghe

[1] Centre for Food Technology, 19 Hercules St Hamilton QLD 4007 Australia (patersonb@dpi.qld.gov.au)
[2] School of Biomedical Sciences, Curtin University of Technology, GPO Box U 1987, Perth WA 6845, Australia

ABSTRACT

Currently western rock lobsters, *Panulirus cygnus,* are graded a number of times during post-harvest handling, to remove injured or damaged lobsters, as well as to remove individuals, on the basis of posture and responsiveness, that experience shows may die during factory storage. Trials have been undertaken, using blood tests, to try to identify lobsters that survive a handling treatment but which die subsequently. We have subjected large numbers of lobsters to a controlled period of stress, then sampled their blood immediately to measure a number of physiological parameters. The lobsters were tagged and stored in the factory and their fate recorded. In this paper we examine the lactate results in detail to see how well results for individual lobsters compare with average findings. The group of lobsters that later survived had significantly lower blood lactate concentration leaving the treatment than those lobsters that did not. Clearly, reducing the stress that leads to lactate accumulation will improve the outcome. However, examining the frequency distributions of this parameter shows that the average lactate concentration in lobsters that did not survive following treatment does not convey well the diverse responses of individual lobsters and shows how difficult it is to use one parameter to predict death. Lobsters may die for more than one reason and using several parameters in combination may explain more of the mortality. Counting mortality is undeniably the simplest way to measure lethal stress, but stress indicators help explain why the lobsters died, and knowing that may suggest ways to alter practices to reduce mortality.

Keywords: Mortality, stress, lactic acid, live handling

I. INTRODUCTION

Western rock lobsters (*Panulirus cygnus* George) are graded a number of times during post-harvest handling to remove injured or damaged lobsters as well as to remove individuals that are considered to be "weak" on the basis of their posture and responsiveness. These stressed lobsters are singled out because it is thought that they are unlikely to survive for long in the factory. While the number of lobsters involved might only be a small fraction of the average annual catch of 10,500 tonnes, reducing this stress and ensuring that the bulk of the catch arrives fit for live export remains a priority. To reduce this stress we need to be able to measure it first.

Lobsters are stressed when a factor, sometimes called a stressor, causes their internal physiology to deviate from normal. Many of the parameters that can be measured in

crustacean blood are stress indicators of some kind (Paterson and Spanoghe, 1997). Of course, simply knowing that these lobsters are stressed after harvest may not be of much practical use. We want to identify lobsters that survive a handling treatment, but that die subsequently. Having early indicators for this kind of stress or delayed mortality would allow us to objectively compare handling methods using key tests, ones associated with the processes that kill lobsters. The results of these tests will also help to tell us why lobsters die or weaken following handling.

To find these indicators we have undertaken two major trials where we subjected large numbers of lobsters to a controlled period of stress, then sampled their blood immediately to measure a number of physiological parameters. The lobsters were then tagged and stored in the factory and their fate recorded. A full exposition of the data collected in these trials is beyond the scope of this article. Here we report data for one parameter, blood lactate concentration, in greater detail than would perhaps normally be the case. As this symposium is aimed at a broad audience, and the paper examines the implications of statistics when applied to individual lobsters, some readers may find more than usual attention given to explaining basic statistics.

Lactate, or lactic acid, was not chosen at random. When living cells are short of oxygen, they produce lactic acid. This is the same chemical produced when human muscles are deprived of oxygen and contributes to the fatigue experienced during strenuous exercise. Lactate is present in very low amounts in tissues and blood of resting unstressed lobsters and it accumulates when lobsters are stored and transported out of water (Spanoghe and Bourne, this volume). Indeed, a number of studies would have it that this progressive rise in lactate concentration, and the associated acidification of the blood and tissues, is a major cause of mortality when lobsters are transported (Vermeer, 1987; Whiteley and Taylor, 1990).

But does lactate concentration allow us to predict mortality, thus establishing the supposed cause and effect relationship proposed? This paper focuses upon the extent to which the test results of individual lobsters, and not so much the average for a group of lobsters, may or may not allow us to predict whether they will live or die following an imposed stress.

II. MATERIALS & METHODS

Two replicated studies of lobsters in different transport/storage environments were completed in Geraldton, Western Australia in November 1998 and March 1999. ‘A’ sized lobsters (approx. 450g) with pink shell colour, that had been in factory tanks for at least 24 h, and which originated from boats delivering directly to the factory were used in the experiments. On the evening before use, the lobsters were fed with chopped fish at approximately 3% of lobster weight, to simulate feeding on bait.

Each time, the basic design required that about 200 lobsters were distributed equally across five different treatments (submerged in flow-through seawater, submerged in recirculated seawater, humid air, sprayed with flow through seawater, sprayed with recirculating seawater) and stored under these conditions for six hours at ambient temperature. Following treatment, all lobsters from each of the 5 treatments were graded (acceptable or rejected), and half of the accepted and the rejected lobsters (about 100 lobsters in total) were tagged and their blood

sampled before placing them in a factory storage tank. The remaining lobsters were returned to the factory tanks without being sampled to act as controls for sampling mortality. The trials were repeated on three consecutive days, using three different tanks of lobsters so that a total of about 300 lobsters were treated, tagged, sampled and survival noted for each of the November and March replicates of the study.

After the treatments, lobster mortality (tank rejects) was monitored for up to a week following treatment. At the end of this observation period, the remaining lobsters were packed as if for export and stored for 36 hours in an air-conditioned room, a period similar to that required for live export. The lobsters were unpacked and placed back in the factory tanks and monitored for a further 24 h. Mortality while in the box or immediately upon re-tanking was monitored (box mortality). Lobsters that remained alive at the end of the process were designated as 'survivors.'

The blood samples were analysed for a number of the physiological and hematological parameters, some of which are mentioned elsewhere at this symposium; for example protein, lactate, glucose, calcium, magnesium, potassium, total hemocyte count, percentage granulocytes, and anti-bacterial factor (Evans *et al*., this volume). The results were then considered in terms of individual tests, to compare the average responses of lobsters that survived and those that did not. Groups of blood tests were also considered together using a statistical technique known as discriminant analysis to try to develop a short list of parameters that explain most, if not all, of the mortality. Rather than attempting to give the complete account of these studies (Paterson *et al*., in preparation), this paper discusses the results of lactate assays conducted during the November trial, to explore the shortcomings of using one parameter alone to predict mortality. Only the data for lobsters stored out of water (in moist air or under sprays) is presented, as mortality was low for lobsters following submerged storage.

Lactate was measured using a standard enzymatic test kit method (Boehringer Mannheim catalogue number 139 084). Differences between the averages of the different groups were tested using Analysis of Variance (Statistix For Windows, Analytical Software, Tallahassie, Florida, USA). The results were transformed by taking the square root of the blood lactate concentration prior to Analysis of Variance. This transformation of the data was necessary prior to Analysis of Variance because the distribution of results was not symmetrical about the average.

III. RESULTS

On average, lobsters that died in the days following the treatment emerged from the treatment with a blood lactate concentration higher than that of lobsters that later survived (compare tank rejects and survivors in Fig. 1). This difference was statistically significant. This means that if two groups of lobsters were picked at random from a larger population of lobsters we'd expect both groups to have a similar average lactate concentration. When they were sampled leaving the treatments, chance alone is unlikely to give the two groups such widely divergent average lactate concentrations, and we conclude that significantly elevated lactate concentration immediately after treatment is associated with lobsters that later die. At the risk of labouring the point, this bare, statistical conclusion refers only to the differences between

the averages. It makes no claims at all about the biological significance of the averages themselves.

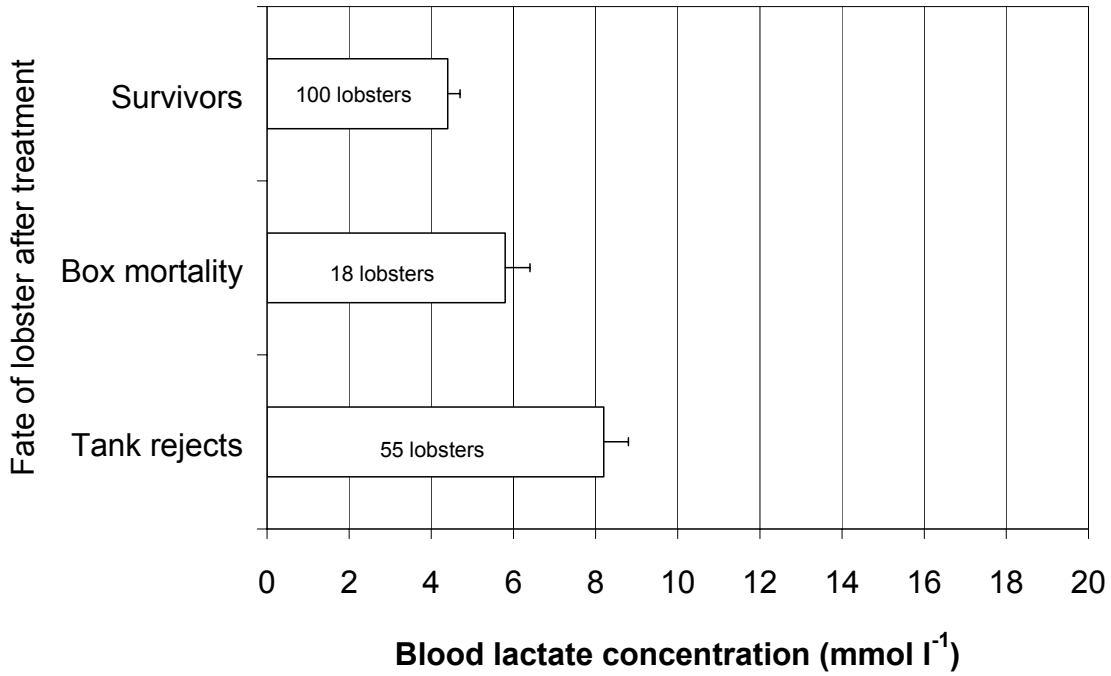


Figure 1 Average blood lactate concentration of lobsters sampled immediately after the stress treatment, and grouped according to their fate during later storage in factory tanks.

Nevertheless, the case for a lactate level of around 8 mmol l^{-1} predicting future mortality (the average for 'tank rejects' taken from Fig. 1) seems pretty watertight at this stage. But consider now the frequency distribution of the same data set (Fig. 2). This figure shows the number of lobsters sampled (the height of the bars) whose blood lactate concentration lies within the 1 mmol l^{-1} concentration interval chosen. This distribution is plotted for the three categories used to group the lobsters.

Notice that the lobsters that eventually died ('tank rejects') have a distribution that is much flatter, and its upper 'tail' extends to higher concentrations (up to 20 mmol l^{-1}) than the other distributions. This is a common response when animals deviate from normal resting levels for a parameter, they tend to show no typical degree of deviation but instead get spread across a considerable range of concentrations. In this case, lobsters with the highest lactate concentration were the most stressed. In contrast, the distribution of 'survivors' is weighted toward lower, more 'normal' concentrations. A large number of lobsters that were survivors had, immediately after treatment, a blood lactate concentration that was at or below 4 mmol l^{-1}. In some 'survivors' however, the blood lactate level had risen to levels of up to 11 mmol l^{-1} by the end of the 6 hour treatment, similar in fact to many of the lobsters that later died.

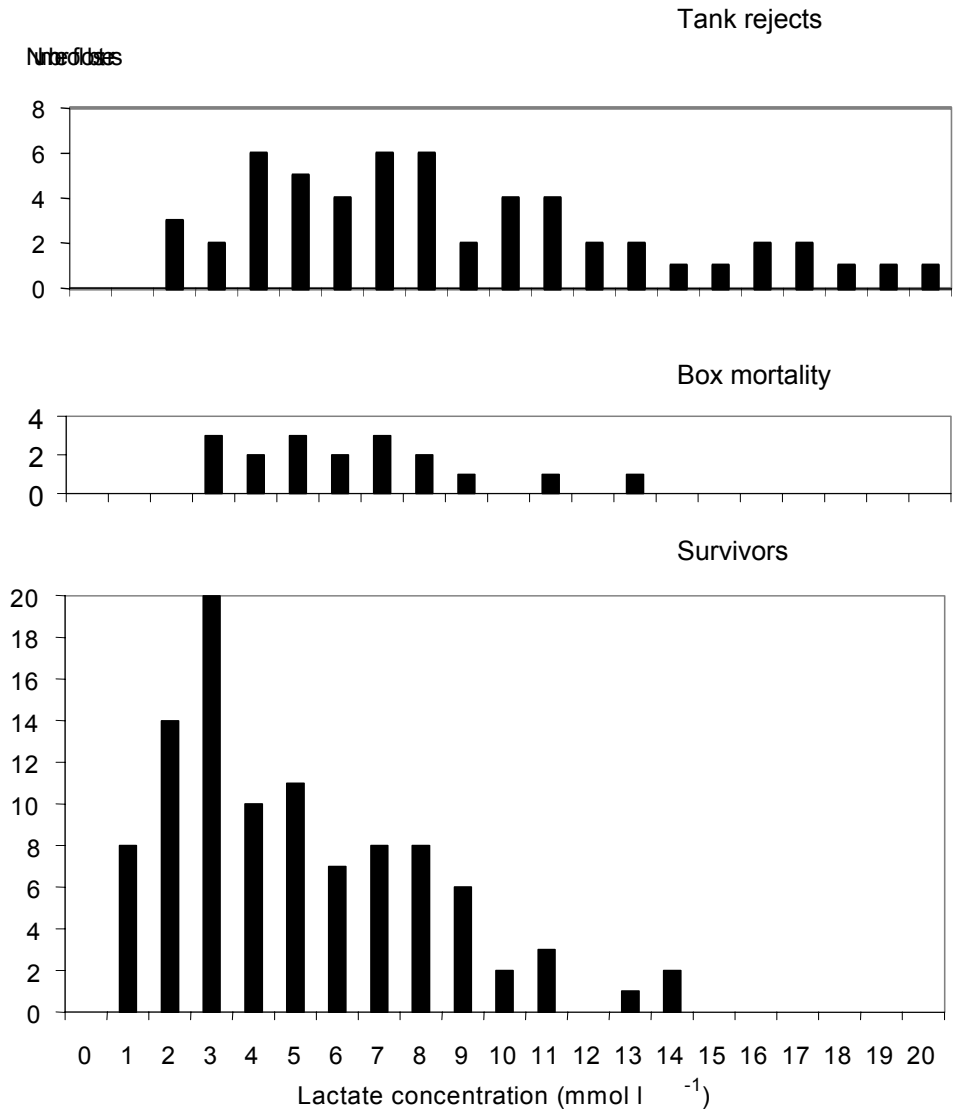


Figure 2 Frequency distributions of blood lactate concentrations of lobsters sampled immediately after the stress treatment, but grouped according to their fate during later storage in factory tanks

No lobster survived in this trial if its blood lactate concentration was greater than 14 mmol l^{-1} after the stress treatment. Comparing the group averages from Fig. 1 to the distributions in Fig. 2 shows the extent of overlap between the groups. The average lactate level in the lobsters that later died (Fig. 1), which could be concluded to be 'undesirable' is apparently physiologically acceptable, judging by the number of survivors which also had around 8 mmol l^{-1} lactate when sampled immediately after the treatment (Fig. 2). Indeed, a lobster with a blood lactate concentration of 10 mmol l^{-1} following treatment might live or might die, and some lobsters with low lactate concentrations in their blood, nevertheless died afterwards.

IV. DISCUSSION

We should approach with considerable caution any statement that lobsters die because *this* or *that* parameter changes. This is the key message to draw from Fig. 2. A similar story emerges if we look at other parameters measured in these studies, but these aren't explored here simply for the sake of brevity. So what does it mean if lobsters that die following stress earlier had a significantly higher average level of a certain parameter? Certainly we can conclude that high stress leads to mortality, and that deviation of parameters such as lactate can be linked to increased mortality in lobsters. The statistics are still valid when applied to groups of lobsters. We compare averages in order to draw objective conclusions about samples of lobsters. But we cannot use this approach to make judgements about individuals. It is arguable that it is not the 'average' individuals that we should be concerned about in this case, but rather the individuals with extremely above average levels of lactate following treatment.

Incidently, the average levels of lactate found here in all treatments were similar to those reported in emersed Florida lobster *Panulirus argus* (Latreille) and European lobster *Homarus gammarus* (L.) (Vermeer 1987; Whiteley and Taylor 1990). To put this in context, relatively high levels of lactate (an average of 16.5 mmol l^{-1}) have been reported in tropical rock lobsters, *P. ornatus* (Fabricius) kept briefly in air on small dinghy following capture, (Paterson, *et al*. 1997). Lactate accumulation, or the processes underlying it, may only be killing some of the lobsters. There could be some very simple reasons why no one parameter explains all of the lobster mortality. The average lactate concentration for tank rejects in Fig. 1 includes levels for all lobsters that died, many of which may have been killed by something unrelated to lactate. The added stress associated with handling and blood sampling the lobsters is clearly one possible source of mortality. The mortality rate of the sampled lobsters in the trial from which this data was drawn was about 10% higher than that of the unsampled ones. Sampling mortality might account for why some lobsters die without any pointers in the blood sample for their eventual fate. But is it that simple? A case could be put that the highly stressed lobsters, which we already expect to die, are even more likely to die because of the added manipulation involved in sampling? If sampling mortality alone was a major problem, we wouldn't have seen the low mortality that occurred following the submerged storage treatments (Paterson *et al*., unpublished data). Those lobsters were also sampled.

Other factors besides blood sampling may also be killing some of the lobsters. This would account for why lobsters which apparently weren't stressed, that is, had low lactate levels, still died following the trial. The lobsters might have been injured during the treatment, or perhaps became 'sick' following the trial. Using several indicators may help to account for more of these deaths.

Of course, the more parameters that have to be measured, the more complex the task becomes and even after that effort it is still possible that some mortality will remain unexplained. A point is very soon reached at which it is simpler to use mortality itself as the measure of lethal stress. However, stress indicators help us to understand why the lobsters died and knowing that may suggest ways to alter practices to reduce mortality.

ACKNOWLEDGEMENTS

Thanks to Glenn O'Brien, Wayne Hosking and other staff of Geraldton Fishermen's Cooperative, and Shannon McBride and Japo Jussila for assistance with the factory experiments. This research was supported by the Australian Fisheries Research and Development Corporation (FRDC 96/345) and forms part of the FRDC Rock Lobster Post-harvest Sub-program.

REFERENCES

Paterson, B. D., Grauf, S. G., & Smith, R.A. (1997). Hemolymph chemistry of tropical rock lobsters, *Panulirus ornatus* brought onto a mother ship from a catching dinghy in Torres Strait. *Mar. Freshw. Res.* **48**, 835-838.

Paterson, B. D. & Spanoghe, P. T. (1997). Stress indicators in marine decapod crustaceans, with particular reference to the grading of western rock lobsters (*Panulirus cygnus*) during commercial handling. *Mar. Freshw. Res.* **48**, 829-834.

Vermeer, G. K. (1987). Effects of air exposure on desiccation rate, hemolymph chemistry, and escape behaviour of the spiny lobster, *Panulirus argus*. *Fish. Bull.* **85**, 45-51.

Whiteley, N. M. & Taylor, E. W. (1990). The acid-base consequences of aerial exposure of the lobster, *Homarus gammarus*, at 10 and 20 °C. *J. Therm. Biol.* **15**, 47-56.

Physiological profiles and vigour indices of lobsters (*Panulirus cygnus*) delivered to processing facilities

Patrick T. Spanoghe[1] and P.K. Bourne[2]

[1]Centre for Food Technology, Queensland Department of Primary Industries, C/O School of Biomedical Sciences, Curtin University of Technology, GPO Box U1987, Perth, WA 6845, Australia
P.Spanoghe@curtin.edu.au
[2]School of Biomedical Sciences, Curtin University of Technology, GPO Box U1987, Perth, WA 6845, Australia

ABSTRACT

In this study, conducted in collaboration with the Western Australian rock lobster industry, the efficiency of a procedure for grading lobsters for suitability for live export trade, based on a selection of behavioural criteria, was examined. From the comparison of the physiological variables of lobsters graded for their vigour, it appeared that the phosphagen content of the muscle tissues was best correlated to vigour. On this basis, it is suggested that, i) grading lobsters for future suitability, using such vigour indices, could be misleading due to the labile nature of phosphagen, influenced as it is by muscular fatigue and, ii) that lobsters should be held in good quality seawater to allow recovery to occur prior to being graded**.**

Key words: Lobster, grading, vigour, phosphagen

I. INTRODUCTION

In Western Australia, lobsters delivered to processing premises are routinely subjected to a grading procedure which is aimed at selecting from the catch thoses animals best suited for live export. This assessment is based on a range of criteria referring to the morphological appearance of the animals such as, shell colour, number of missing appendages and behaviuoral responses to physical stimulation.

This study was conducted during the 1993-94 western rock lobster fishing season. Its aim was to study a sample of lobsters delivered to a live processing factory; to subject these lobsters to a grading procedure with reference to a vigour index based on a selection of behavioural observations; to use hemolymph and muscle samples to describe the physiological profiles of specific vigour classes, and to identify the physiological variables best correlated to the vigour index. The physiological variables studied were selected on the basis of the existing literature on emersed and/or disturbed crustaceans.

II. MATERIAL AND METHODS

Lobsters

A total of eighty nine specimens of western rock lobsters were collected upon delivery at the receival ramp of a lobster factory. The lobsters were selected at random from a population of red "A" size animals (76-79 mm carapace length). Lobsters exhibiting physical damage and/or signs of ecdysis (i.e., soft shell) were excluded. The vigour of the animals was immediately assessed, with reference to the method described by Spanoghe and Bourne (1997), modified by the introduction of a sixth class of somatic response, best suited to describe those animals exhibiting a "defensive/aggressive" behaviour (Table I). All animals were molt-staged by visual examination of the distal half of one abdominal pleopod according to the method of Lyle and McDonald (1983).

Table I Classes of lobster vigour indices
a, aggessive, vh, very healthy; h, healthy; w, weak; m, moribund; d, dead; na, not applicable

	Class					
Somatic response	0(d)	1(m)	2(w)	3(h)	4(vh)	5(a)
Defensive antenna response	no	no	no	no	no	yes
Vigourous tail flip	no	no	no	no	yes	yes
Appendage movements	no	no	yes	yes	yes	yes
Tail hanging flaccidly	yes	yes	yes	no	no	no
Eyestalk response	no	yes	na	na	na	na

Sampling and extraction

Prebranchial hemolymph samples were withdrawn from a venous sinus by the puncture of the arthrodial membrane at the base of the fifth walking leg. The samples (±1.5 ml) were immediately transferred into microcentrifuge tubes maintained on ice (0ºC). Immediately, 500 ml of hemolymph was withdrawn and mixed with 1000 ml pre-chilled 1mol L^{-1} perchloric acid, and frozen in liquid nitrogen prior to being stored at -80 ºC.

Muscle samples from the first thoracic leg were dissected rapidly on ice, with about 600 mg of muscle tissue excised and transferred into microcentrifuge tubes prior to being frozen in liquid nitrogen and stored at -80ºC for subsequent analyses. Metabolite and adenylate nucleotide extraction were adapted from a method described by Ryder (1985), with 200 mg of muscle tissue homogenised in 1.5 ml of 1M perchloric acid in a pre-chilled polypropylene tube for 40 seconds, using a Ultra Turrax T25 homogeniser, with a 10 mm diameter shaft (Janke & Kunkel GMBH and Co., Staton, Germany). After 25-35 minutes at 4ºC, the homogenate was centrifuged at 5000g for 10 minutes at 5 ºC, using a Beckman GS-6R centrifuge. Neutral perchlorate extracts (NPE) were prepared on ice with 3 mol L^{-1} $KHCO_3$ and frozen at -80ºC for subsequent analyses.

At a latter date, the frozen stored perchloric acid extracts prepared from hemolymph samples were thawed and centrifuged at 5000 g for 10 minutes at 5 ºC, the supernatants extracted and neutralised with 3 mol L^{-1} $KHCO_3$. Each deproteinised neutral perchlorate extract (NPE) was aliquoted and stored at -80 ºC until further analysis.

Physiological measurements

pH measurements on hemolymph samples were carried-out immediately after sampling at 4ºC using a micro-electrode (6 mm pH electrode, ref. N14-3854, Broadley-James Corp. Santa Ana, California 92705, USA). Standard pH calibrations were checked between samples and the standard buffer solutions renewed after every series of measurements.

Hemolymph and muscle lactate was measured enzymatically on NPE, employing a method described by Gutman and Wahlefeld (1974) and modified according to the recommendations of Engel and Jones (1978).

Hemolymph glucose was measured enzymatically on NPE, employing a method described by Bergmeyer *et al.* (1974).

Hemolymph ammonia titre was determined colourimetrically on NPE, using the Berthelot reactions, as described by Konitzer and Voigt (1963) and modified by Gips and Wibbens-Albert (1968).

Free total calcium was measured colourimetrically on NPE, by using the method described by Varley *et al*. (1980), using cresophtalein complexone (CPC) as a complexing agent. Interference of magnesium was eliminated by including 8-hydroxyquinoline in the CPC reagent.

All enzymatic and colourimetric assays were performed using an Multistat III Fluorescence/Light Scatter Microcentrifugal Analyser (Instrumentation Laboratory, Microchemical Division, North 3939 Freya, Spokam, WA 99207).

Muscle arginine phosphate concentrations were determined on neutralised extracts of muscle samples using an enzymatic method by monitoring spectrophotometrically the stoichiometric reduction of nicotinamide dinucleotide phosphate (NADP) at 340 nm (Lamprecht and Trauschold, 1974; Lamprecht *et al*., 1974). Tris buffer (pH= 7.6, 50 mmol L^{-1}) was substituted for the ethanolamine buffer, as recommended by Dehn *et al*., (1985).

Adenine nucleotide analysis was performed on neutralised extracts of muscle samples by an HPLC method described by Ryder (1985), using an isocratic mobile phase of 0.04 mol L^{-1} KH_2PO_4 and 0.06 mol L^{-1} K_2HPO_4, at pH 7.0 containing 5% (v/v) methanol, at a flow rate of 1 ml min^{-1}. The HPLC system consisted of two LKB Bromma 2150 HPLC pumps, an LKB Bromma LC controller, an LKB Bromma 2158 Uvicord SD detector (LKB-Produkter AB, S-161 25 Bromma 1, Sweden), a Rheodyne 7161 syringe-loading sample injector fitted with a 20 ml loop (Cotati California), and a 250mm x 4.6mm reversed-phase Econosil C18 stainless steel column (Altech associates, Inc. 2051 Waukegan Rd, Deerfield, Il 60015) equilibrated to 18 ºC. Data collection and processing were performed on a PC computer

using the Data Acquisition Plotting & Analysis (DAPA) software (DAPA Scientific Pty. Ltd., Kalamunda, Western Australia 6076). Standard stock solutions (10 mmol L^{-1}) of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP) and inosine monophosphate (IMP) were prepared weekly with aliquots stored at -80ºC. One aliquot per day was used, diluted, and discarded after use.

All enzymes, substrates and reagents were purchased from Sigma Chemicals Pty. Ltd. (St Louis, MO, USA) or Boehringer Mannheim Australia Pty. Ltd. (Castle Hill, NSW, 2154) and Precinorm standards were used for standardisation of techniques.

III. RESULTS

Physiological profiles of the 6 vigour classes of lobsters

For the 89 lobsters studied, 6.8% were classified as moribund, 55% weak, 21.3% healthy, 10.2% very healthy and 6.8% aggressive. Table II presents the results (mean ± s.e.m.) for the 8 physiological variables measured for each of the vigour classes.

Table II Relative proportion and physiological profiles of a sample of 89 lobsters graded for vigour, upon delivery at the receival ramp of a live lobster factory. Muscle tissue and hemolymph concentrations expressed as mmol g^{-1} and mmol L^{-1}, respectively. Data presented as mean ± s.e.m.

	Vigour Index				
	1(m)	2(w)	3(h)	4(vh)	5(a)
No. lobsters	6	49	19	9	6
Proportion	6.7%	55.1%	21.3%	10.1%	6.8%
pH_{hm}	7.56 ± 0.12	7.47 ± 0.03	7.45 ± 0.04	7.74 ± 0.05	7.77 ± 0.03
$Calcium_{hm}$	7.19 ± 0.77	8.04 ± 0.45	9.71 ± 0.51	8.96 ± 0.73	6.49 ± 0.62
$Glucose_{hm}$	0.99 ± 0.21	1.25 ± 1.12	1.01 ± 0.25	0.16 ± 0.05	0.62 ± 0.16
$Ammonia_{hm}$	0.76 ± 0.24	0.87 ± 0.06	0.55 ± 0.05	0.59 ± 0.03	0.32 ± 0.02
$Lactate_{msc}$	7.08 ± 0.61	7.61 ± 0.46	7.68 ± 0.54	6.30 ± 0.42	5.59 ± 0.94
$Lactate_{hm}$	8.67 ± 2.47	8.44 ± 0.61	6.54 ± 0.93	2.82 ± 0.22	3.14 ± 0.49
ATP_{msc}	2.92 ± 0.39	3.18 ± 0.15	4.18 ± 0.29	5.84 ± 0.68	6.07 ± 0.15
Ap_{msc}	2.06 ± 0.29	1.99 ± 0.11	4.08 ± 0.48	6.19 ± 0.89	7.73 ± 0.26

The data were submitted to regression analyses (Statview II package, Abacus Concept Inc., 1984, Bonita Ave., Berkeley CA 94704) and Table III presents the Pearson correlation coefficients and significance p levels for the physiological variables compared to vigour indices. Significant univariate relationships ($p \leq 0.05$) were identified between six of the physiological variables studied and VI class. These variables were pH_{hm}, $Ammonia_{hm}$, $Lactate_{hm}$,, $Glucose_{hm}$, AP_{msc} and ATP_{msc}. Negative relationships were identified between the dependent variable VI and the variables: $Ammonia_{hm}$ (r= -392), $Lactate_{hm}$ (r= -0.470), $Glucose_{hm}$ (r= -0.305). Fig. 1a suggest that signs of "weakness" in lobsters, resulting in low VI values (≤ 2), do not necessarily correspond to high lactate titre values, as demonstrated by

the very wide range of values recorded for these animals (3.0 to 17.2 mmol L^{-1}). Similarly, these "weak lobsters" recorded for the variable $Ammonia_{hm}$ values ranging between 0.45 and 2.42 mmol L^{-1} (Fig. 1b). In other respects, Fig.1c suggests that quite comparable glucose titres could be recorded in the group of lobsters graded as "1" or "5" with 0.99 ± 0.21 mmol L^{-1} and 0.62 ± 0.15 mmol L^{-1}, respectively.

Table III Pearson's correlation coefficients and significance levels (p) associated with visual estimates of vigour (VI) compared to physiological variables

Variables	**R** (89)	**p**
pH_{hm}	+0.355	0.0007
$Calcium_{hm}$	+0.034	0.7543
$Lactate_{hm}$	-0.468	0.0001
$Glucose_{hm}$	-0.305	0.005
$Ammonia_{hm}$	-0.392	0.0003
$Lactate_{msc}$	-0.159	0.1497
ATP_{msc}	0.645	0.0001
AP_{msc}	0.778	0.0001

Highly significant positive correlation relationships were identified between the variables pH_{hm}, ATP_{msc} and AP_{msc} with the variable VI (r= 0.355, r= 0.77 and r= 0.89, respectively). Fig. 1d suggests that the lobsters classified as VI "2" and "3" recorded a much wider range of pH values (6.90 to 7.99), when compared to the range of values recorded for the groups "4" and "5" (from 7.50 to 7.95). As far as ATP_{msc} and AP_{msc} variables are concerned, the positive relationships identified (Figs.1e and 1f) suggest that the behavioural response of lobsters, as used in the visual assessment of vigour, is closely dependent upon the ATP and AP concentration in the muscle tissues.

In order to define the best predictor of VI, the data for the 6 independent variables and the dependent variable VI were subjected to a multiple linear regression analysis using a forward stepwise regression (Statview II) with elimination of unnecessary variables. The analysis reveals that among the 6 independent potential variables, 2 were retained in the regression equation defined as: $VI = 0.29\ [AP_{msc}] - 0.56\ [NH3_{hm}] + 2.00$, with the variable AP_{msc} selected as the best predictor of vigour index, with a $r^2 = 0.63$. The inclusion of the second variable ($NH3_{hm}$) in the equation was reflected by an increased value for the correlation coefficient to reach $r^2 = 0.68$.

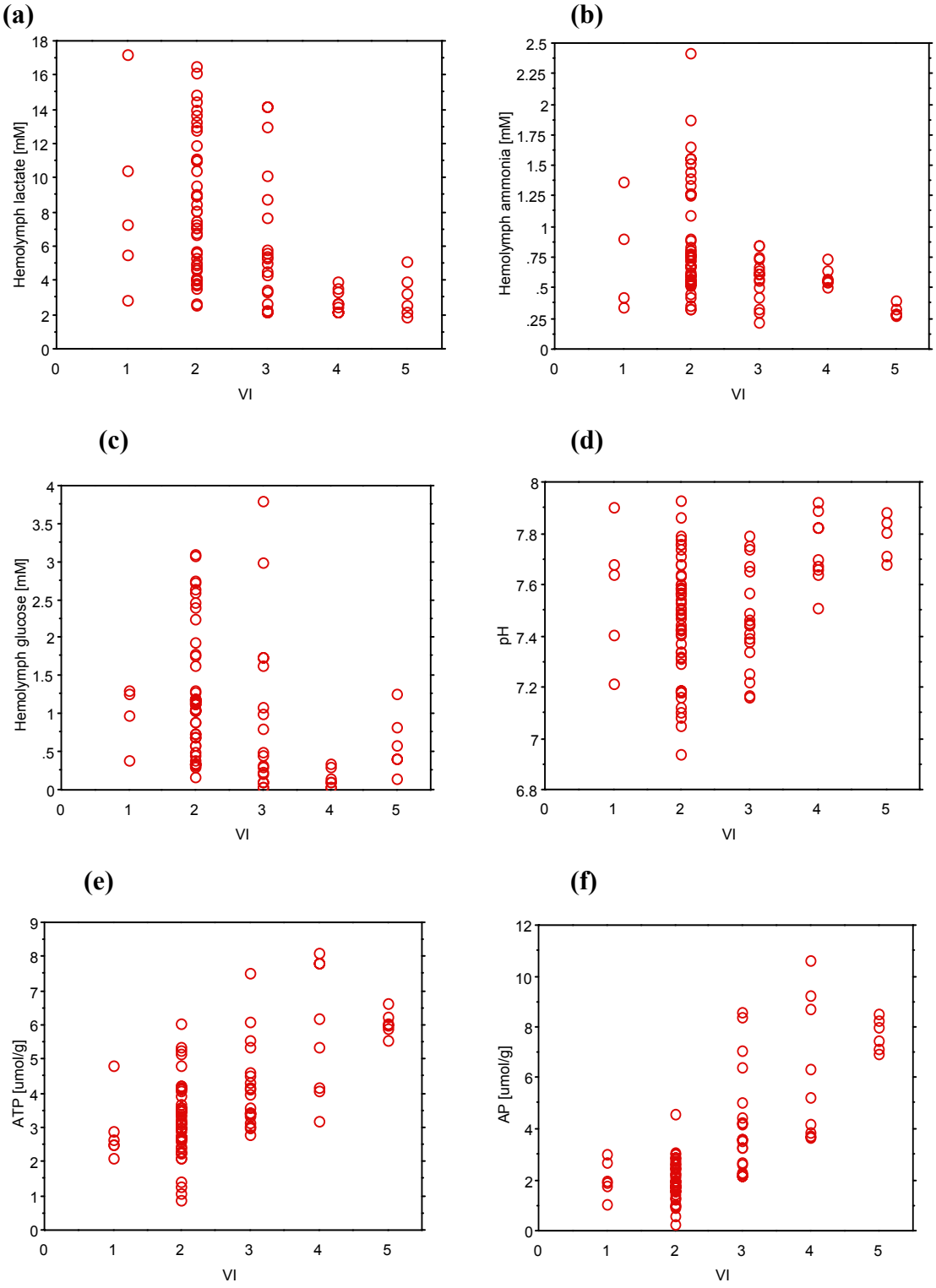


Figure 1 Physiological variables in hemolymph and muscle from individual lobsters in each vigour indices (VI) class. a) Hemolymph lactate, b) hemolymph ammonia, c) hemolymph glucose, d) hemolymph pH, e) muscle ATP, f) muscle AP.

IV. DISCUSSION

The assessment of lobster vigour, using the set of criteria described in Table 1, was found to be closely correlated with a range of physiological variables. As a general rule, lobsters demonstrating signs of low vigour were characterised as having a low blood pH, a high lactate concentration in their hemolymph and high hemolymph glucose and ammonia titres. ATP and AP concentrations in the muscle tissue of these animals were also lowered.

High concentrations of lactate in hemolymph and muscle tissues of crustaceans have been extensively described as being associated with conditions of aerial exposure, increased locomotor activity and disturbance. When comparing the values recorded in the present study with data reported by other authors, it appears that hemolymph lactate concentrations above 6 mmol L^{-1} are likely to occur in homarid and panilurid lobsters, when a period of more than two hours aerial exposure is experienced (Taylor and Whiteley, 1989; Vermeer, 1987). Higher values were recorded in the present study, suggesting that those animals recording high lactate concentration values were subjected to either long periods of aerial exposure or to extensive disturbance or both. Comparable findings have been reported by Whiteley and Taylor (1990) who identified that lobsters *(Homarus gammarus)* subjected to the combined effects of aerial exposure (7 hours) and a brief (5 min) period of disturbance recorded 12.9 ± 1.0 mmol L^{-1} for hemolymph lactate titre, compared to 10.6 ± 1.9 mmol L^{-1} in undisturbed animals. Similarly, a rise in ammonia concentration in the hemolymph of lobsters is likely to reflect extended periods of air exposure with the concomitant impaired ability to execrete this metabolite into the surrounding water, either via active ion exchange (Na^+/NH_4^+) or by passive diffusion, through the gill epithelium (Regnault, 1987). When comparing the values for the hemolymph ammonia titres recorded in this study (0.22 to 2.42 mmol L^{-1}) to that reported by Vermeer (1987) in *P. argus* for animals subjected to a period of 2 hours of aerial exposure (0.810 mmol L^{-1}), it would appear that those lobsters recording high values of ammonia in their hemolymph had probably been exposed to air for a longer period of time, which can occur when animals are transported in baskets, in trucks or in carrier-boats.

A rise in glucose titre is a classical response of crustaceans to disturbance (Dall, 1975), but has also been identified as a result of exposure to air by Johnson and Uglow (1985) and Telford (1974), with the concomitant shift to anaerobic glycolysis, which can itself result in an increased mobilisation of glucose if a co-ordinated metabolic rate depression by a factor 10 to 20 fold does not occur (Storey and Storey, 1990).

A depletion in the AP reserve could either correspond to burst of muscular activity, such as the “tail flicks” characterising the escape behaviour of decapods (England and Baldwin, 1985), or be explained by the role of this molecule in maintaining appropriate ATP levels (Paterson, 1993). In this instance, it is reasonable to suggest that the low values recorded for the lobsters graded as weak or moribund resulted from stress induced by either extended periods of aerial exposure, or by excessive periods of disturbance and rough handling, which can occur at different steps along the post-harvest chain. Access to oxygen probably became limited for these animals whilst being kept out of water and, as suggested by Onnen and Zebe (1983), the replenishment of the phosphagen reserves was impaired. Lowered ATP concentration is also indicative of a depletion of energy reserves (Dehn *et al*., 1985; Gade, 1984) and suggests that the energy generated through both aerobic and anaerobic pathways

was not sufficient to match the needs of the animals. Similar findings have been reported by Paterson (1993) in *Penaeus monodon* and *Penaeus japonicus*.

The results suggested that among the pool of lobsters demonstrating average to below average signs of vigour (VI $\leq$3), a wide variability existed with regard to most of the variables studied. However, the stepwise regression analyses revealed that 2 variables could account for 63% of the variation of the variable VI. These were primarily the phosphagen content of the muscle and to a lesser extent the ammonia concentration in the hemolymph. This suggests that an assessment of the condition of lobsters established on the basis of behavioural signs of vigour is most likely to reflect a state of muscular fatigue of the animal. In this respect, and considering that the phosphagen content of the muscle tissue can be depleted very rapidly, as a result of escape behaviour, but can also be replenished within 30-60 min, provided that oxygen supply to the tissues is maintained (or re-established) (Onnen and Zebe, 1983), it would appear that a subdued behaviour could reflect a transient state of fatigue rather than morbidity or poor health. This is particularly true in lobsters at the time of delivery to the live processing facilities, as these animals are likely to have endured adverse conditions of exposure to air, high temperature and rough handling, all conducive of energy depletion.

The fact that the ammonia titre was identified as the second variable closely correlated to the VI variable, emphasizes that, in addition to the debilitating effect of rough handling and disturbance, extended periods of air exposure are deleterious to lobsters.

From this, it appears that from an industry perspective, where an accurate selection of lobsters for the live export trade is of paramount importance, opportunity should be given to the animals to have access to good quality seawater, prior to grading. This would allow for oxygen uptake, execretion of metabolites and replenishment of the phosphagen reserve.

ACKNOWLEDGEMENTS

P.T.S. was funded by an Australian Postgraduate Research Award. We thank Mr Paul Tod and Mr Stephen Dawson for technical assistance and Dr N. Caputi of the Western Australian Marine Research Laboratories for advice on statistical analyses.

REFERENCES

Bergmeyer, H. U., Bernt, E., Schmidt, F., and Stork, H. (1974) D-glucose, determination with hexokinase and glucose-6-phosphate dehydrogenase. *Methods of Enzymatic Analysis*. New York, Academic Press: 1197-1229.

Dall, W. (1975). Blood carbohydrates in the western rock lobster *Panulirus longipes* (Milne Edwards). *J. Exp. Mar. Biol. Ecol.* **18**: 227-238.

Dehn, P. F., Haya, K., and Aiken, D. E. (1985). Adenylate energy charge, arginine phosphate and ATPase activity in juvenile *Homarus americanus* during the molt cycle. *Comp. Biochem. Physiol*. **81B**(3): 629-633.

Engel, P. C. and Jones, J.B. (1978). Causes and elimination of erratic blanks in enzymatic metabolite assays involving the use of NAD^+ in alkaline hydrazine buffers: improved conditions for the assay of L-glutamate, L-lactate and other metabolites. *Anal. Biochem.* **88**: 475- 484.

England, W. R., and Baldwin, J. (1985). Anaerobic energy metabolism in the tail musculature of the Australian yabby *Cherax destructor* (Crustacea, Decapoda, Parasticidae). Regulation of anaerobic glycolysis. *Comp. Biochem. Physiol.* **80**B: 327-335.

Gade, G. (1984). Effects of oxygen deprivation during anoxia and muscular work on the energy metabolism of the crayfish, *Orconectes Limosus*. *Comp. Biochem. Physiol.* **77**A: 495-502.

Gips and Wibbens-Albert (1968). *Clin. Chim. Acta,* **22**: 183.

Gutmann, I., and Wahlefeld, A.W. (1974). L-(+)-lactate determination with lactate dehydrogenase and NAD. *Methods of Enzymatic Analysis*. New York, Academic Press: 1465-1468.

Johnson, I., and Uglow, R.F. (1985). Some effects of aerial exposure on the respiratory physiology and blood chemistry of *Carcinus maenas* (L.) and *Liocarcinus puber* (L.). J. *Exp. Mar. Biol. Ecol.* **94**: 151-165.

Konitzer and Voigt (1963). *Clin. Chim. Acta*: **8**, 5.

Lamprecht, W., Stein, P., Heinz, F. and Weisser, H. (1974). Creatine phosphate. Determination with creatine kinase, hexokinase, and glucose-6-phosphate dehydrogenase. *Methods of Enzymatic Analysis*. New York, Academic Press: 1778-1781.

Lamprecht, W., and Trauschold, I. (1974). Adenosine-5-triphosphate. Determination with hexokinase and glucose-6-phosphate dehydrogenase. *Methods of Enzymatic Analysis*. New York, Academic Press: 2101-2110.

Lyle, W. G., and McDonald, C.D. (1983). Molt stage determination in the Hawaiian spiny lobster, *Panulirus marginatus*. *J. Crust. Biol.* **3**: 208-216.

Onnen, T., and Zebe, E. (1983). Energy metabolism on the tail muscles of the shrimp *Crangon crangon* during work and subsequent recovery. *Comp. Biochem. Physiol*. **74A**(4): 833-838.

Paterson, B.D. (1993) The rise in inosine monophosphate and L-lactate concentrations in muscle of live penaeid prawns (*Penaeus japonicus, Penaeus monodon*) stressed by storage out of the water. *Comp. Biochem. Physiol*. **106B**: 395-400.

Regnault, M. (1987). Nitrogen excretion in marine and fresh-water Crustacea. *Biol. Rev*. **62**: 1-24.

Ryder, J. M. (1985). Determination of adenosine triphosphate and its breakdown products in fish muscle by high-performance liquid chromatography. *J. Agric. Food Chem.* **33**: 676-680.

Spanoghe, P.T., and Bourne, P.K. (1997). Relative influence of environmental factors and processing techniques on *Panulirus cygnus* morbidity and mortality during simulated live shipments. *Mar. Freshw. Res.* **48**: 839-844.

Storey K.B., and Storey, J.M. (1990). Metabolic rate depression and biochemical adaptation in anaerobiosis, hibernation and estivation. *The Quart. Rev. Biol.* **65**(2): 145-174.

Taylor, E., W., and Whiteley, N. M. (1989). Oxygen transport and acid-base balance in the hemolymph of the lobster, *Homarus gammarus*, during aerial exposure and re-submersion. *J. Exp. Biol*. **144**: 417-436.

Telford, M. (1974). Blood glucose in crayfish -II. Variations induced by artificial stress. *Comp. Biochem. Physiol.* **48A**: 555-560.

Varley, H., Gowenlock, A.H., and Bell, M. (1980). The urease method using the Berthelot reaction. *Practical Clinical Biochemistry.* London, William Heinemann Medical Books LTD.:160-161.

Vermeer, G. K. (1987). Effects of air exposure on desiccation rate, hemolymph chemistry, and escape behavior of the spiny lobster, *Panulirus argus*. *Fish. Bull.* **85**: 45-51.

Whiteley, N. M., and Taylor, E.W. (1990). The acid-base consequences of aerial exposure in the lobster, *Homarus gammarus* (L.) at 10 and 20ºC. *J. Therm. Biol.* **15(1)**: 47-56.

Effects of handling or injury disturbance on total hemocyte counts in western rock lobster (*Panulirus cygnus* George)

Japo Jussila[1], Jeff Jago[2], Elena Tsvetnenko[3] & Louis H. Evans[3]

[1]Institute of Applied Biotechnology, University of Kuopio, Kuopio, Suomi-Finland
[2]School of Biomedical Sciences, Curtin University, Bentley, Western Australia
[3]Aquatic Science Research Unit, Muresk Institute of Agriculture, Curtin University, Perth, Western Australia

ABSTRACT

Preliminary experiments were carried out to investigate the effects of air exposure, handling and injury on the total (THCs) and differential hemocyte counts (DHCs), plasma protein content and vigor in western rock lobster (*Panulirus cygnus*). Lobsters were obtained from a commercial catch in Geraldton, Western Australia and held in Muresk Marine Laboratory in Fremantle in recirculating seawater tanks and fed using West Australian pilchards (mulies, *Sardinops neopilchardus*) *ad libidum* for several weeks prior to the experiments. Lobsters were acclimated to experimental systems' conditions for a week and feeding ceased 48 hours before the experiments. In the air exposure and handling experiment, lobsters were either held in the air in cages for 5 min or 120 min or held in the air in cages and shaken several times for 5 min or 120 min and sampled for THCs, plasma glucose and vigor. Controls were sampled both before and after the experimental lobsters were sampled. In the injury experiment, lobsters were kept individually in cages, tail membrane and tail muscle was penetrated using a scalpel causing a wound 10 mm in width and 25 mm in depth. Lobsters were sampled 10 min, 30 min, 1 h, 1 d and 5 d after injury for THCs, DHCs, vigor and hemolymph protein. Control samples were taken also in undisturbed control lobsters both before and after the experimental sampling. The results showed that air exposure alone did not cause changes in THCs while handling combined with air exposure increased THCs and that injured lobsters' THCs increased quicker than control groups THCs. DHCs and vigor index did not show significant response to treatments. The main conclusions were that THCs were responding to physical disturbance readily while air exposure alone did not evoke such a response.

Keywords: lobster, hemocyte, glucose, stress, air exposure, handling

I. INTRODUCTION

Post-harvest handling stress has a wide range of impacts on the lobster fishery in Western Australia. There have been several attempts to improve lobster handling, both in fishing boats and lobster holding facilities through a code of practice (Anon 1995), which is based on applied research. The improvements in lobster survival and wellbeing have led to a situation where both critical points in the post-harvest handling chain and further improvements in handling practices are harder to identify. Instead of obvious signs such as mortalities, one has to monitor stress or disturbance in lobsters using either traditional stress indicators, such as plasma glucose (Dall 1974; Santos & Keller 1993; Spanoghe 1996) or lactate (Albert &

Ellington 1985; Johnson & Uglow 1985), or develop new means, such as monitoring immune system responses to disturbance.

Immune system parameters, such as hemocyte counts (Stewart *et al*. 1967; Persson *et al*. 1987; Evans *et al*. 1992; Sequiera *et al*. 1996; Jussila *et al*. 1997; 1999), bactericidal activity (Söderhäll & Cerenius 1992, Ueda *et al*. 1994, 1999) and hemolymph clotting (our unpublished data) have been used as rough stress or disturbance indicators in crustaceans. Preliminary indications were that both hemocyte counts and hemolymph clotting could be used under certain conditions as indications of either stress or disturbance, but the detailed responses have not yet been studied.

The aim of the studies discussed in this paper is to establish a relationship between mild physiological disturbance and total or differential hemocyte counts to form as a basis for detailed future research. These studies are part of a wider range of investigations focusing on the hemocyte responses to various challenges of post-harvest handling of western rock lobsters (*Panulirus cygnus* George) carried out in Aquatic Science Research Unit, Curtin University on FRDC funding.

II. MATERIALS AND METHODS

Western rock lobsters (*P. cygnus*) used in the experiments were obtained either from commercial catch or from Fisheries Western Australia's research vessels. All lobsters used in the studies were mature and graded 'commercial size A' or slightly smaller. Lobsters were stored in Muresk Marine Laboratory premises for several weeks prior to experiments.

The experimental system consisted of eight 250 l seawater tanks in a recirculating system connected to a sump tank, biofilter, protein skimmer and heat exchanger. Water temperature was maintained at 21±1°C, photo period at 14 h light and 10 h dark. Water quality parameters (ammonia, nitrate, pH and temperature) were monitored daily during the experiments. Tanks were siphoned of excess food and debris twice a week. Lobsters were acclimated in the experimental system for one week before the experiment and were fed *ad libidum* using pilchards (mulies, *Sardinops neopilchardus*) once a day. Feeding ceased 48 h before the experiments started.

In the experiments, hemolymph samples (200 µl) were taken into 200 µl of precooled Na-cacodylate based anticoagulant (4.28 g of Na-cacodylate added to 75 ml of distilled water, 0.4 ml of stock 25% glutaraldehyde is then added, pH adjusted to 7.0 and volume adjusted to 100 mL) using a 23G needle and 1 ml syringe. Samples were taken either from pericardial sinus or ventral sinus on the base of the fifth walking leg. Total hemocyte counts (THCs) were then analyzed using either hemocytometer (Jussila *et al*. 1997) or Coulter Counter. Differential hemocyte counts (DHCs) were analyzed after smears were stained with Giemsa and May-Grünwald. A minimum of 150 cells were analyzed in each smear. Plasma glucose was analyzed using Sigma® kit procedure nr 501. Hemolymph protein was analyzed indirectly using refractometer (Shibuya® S-1) and converting refractive index to protein concentration using the formula established by Paterson *et al*. (1999). Vigor index was assessed following the criteria introduced by Spanoghe (1996).

Injury and handling experiment: Treatment group lobsters were injured by penetrating the ventral tail membrane with a sterile scalpel to make a 10 mm wide and 25 mm deep cut into the tail muscle. The wound was made in the third joint laterally 2 cm from abdominal artery and ventral nerve cord. After the treatment, lobsters were placed in the tanks until sampled after 10, 30 and 60 min and 1 and 5 d for THCs, DHCs, hemolymph protein and vigor. Controls were handled similarly except for wounding. Undisturbed controls were sampled both before and after the experiment.

Air exposure and handling experiment: Lobsters were held in individual oyster mesh cages. In the air exposed group, the water level in the tanks was lowered below the bottom of the cage by siphoning without disturbing the lobsters and they were sampled after set time lag (5 or 120 min) for THCs, plasma glucose and vigor. Lobsters that were both exposed to air and handled were removed from the tanks, shaken (mild shaking of the cage so that the lobster could not hold to the sides of the cage) initially for one min and left out of water. The lobsters that were sampled after 5 min were then left alone until sampled, while the lobsters sampled after 120 min were shaken after every 30 min. Control groups were sampled before and after all treatment groups were sampled, as soon as they were taken out of the tanks.

The statistical analyses was carried using SPSS for Windows v.8.0.2. The tests used were Explore, X^2-test, Oneway Anova and Correlation. The results are expressed as mean±SE unless otherwise indicated.

III. RESULTS & DISCUSSION

Water quality parameters showed acceptable water quality during the experiments, with oxygen saturation being above 95%, pH between 7.6-8.1, ammonia, and nitrite levels low and water temperature 21±1ºC.

In the injury and handling study, total hemocyte counts (THCs) increased in the injured group rapidly being after 10 min at 5.4×10^6 cells/mL and peaked after 30 min (7.7×10^6 cells/ml), decreasing later to control levels of 5.5 – 5.6×10^6 cells/ml (Fig. 1). In handled controls the THCs increased similarly being after 10 min at 3.8×10^6 cells/mL, with the peak after 60 min (7.1×10^6 cells/ml) and a following decrease to control levels of 4.3 – 4.8×10^6 cells/ml. There was a significant difference (Oneway Anova, LSD, $p<0.05$) between treatment and control groups after 30 min. There were no significant differences (Oneway Anova, LSD, $p>0.05$) in differential hemocyte counts (DHCs) among the studied groups, even though there was considerable variation in all three hemocyte subpopulations from one sampling time to another (Table I). Hemolymph protein was slightly increased to 93 mg/ml in injured group after 30 min and then decreased to control levels in the groups sampled later in the experiment (Fig. 2), while the trends in the control groups were inverse compared to injured groups. There was a significant difference (Oneway Anova, LSD, $p<0.05$) between injured and handled control groups in hemolymph protein after 30 min and 5 d. There were no significant differences (X^2 -test, $p>0.05$) between studied groups in the vigor index, with both of the groups having a slight increase in vigor ratings initially up to 30 min and then the injured group gaining higher vigor ratings than controls towards the end of the project, after 1 and 5 d (Table II). Control lobsters did not gain highest vigor rating, i.e. 5, during days 1 and 5. THCs

correlated with hemolymph protein and granulocyte proportion, hemolymph protein correlated with granulocyte and semigranulocyte proportion and finally semigranulocyte proportions correlated with both hyalinocyte and granulocyte proportions (Table III).

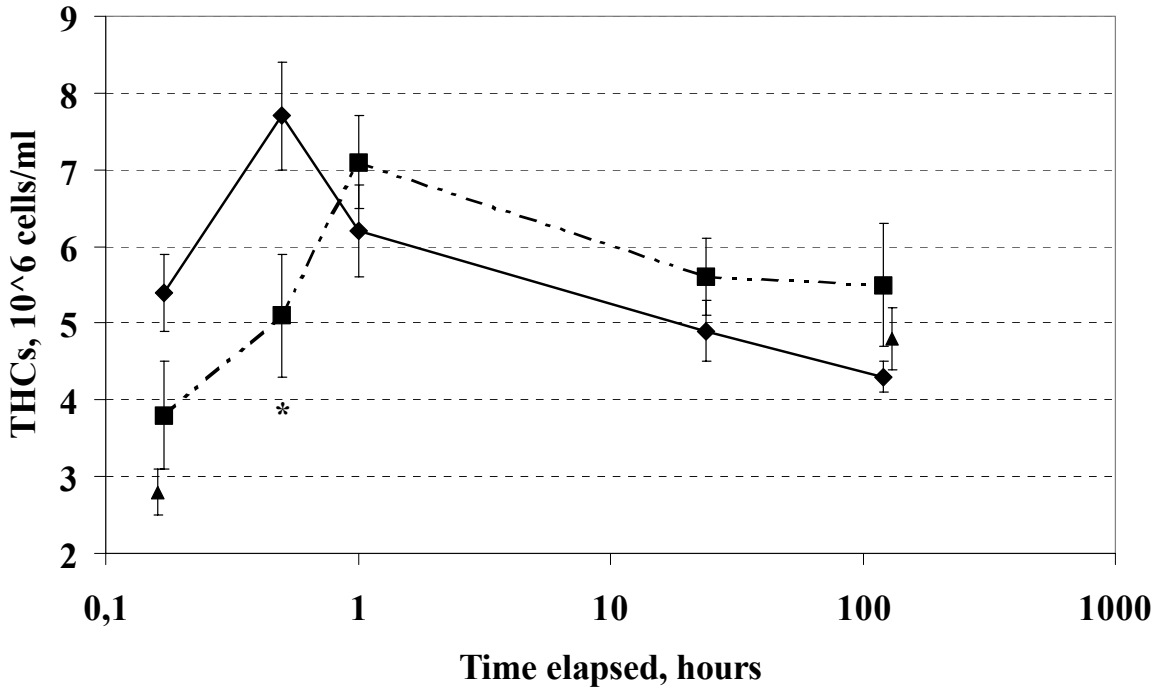


Figure 1 The effect of wounding and handling on total hemocyte counts (THCs, $x10^6$ cells/ml) in western rock lobster (*Panulirus cygnus*) under experimental conditions. Logarithmic scale in x axis. Symbols are: * = significant difference, ◆ = treatment group (solid line), ■ = handled control group (dashed line) and ▲ = undisturbed control group.

Table I Differential hemocyte counts (DHCs) in the injury study. HCs = hyalinocyte proportion; GCs = granulocyte proportion; SGCs = semigranulocyte proportion.

		Initial control	10 min	30 min	60 min	1 d	5 d
HCs, %	treatment	12.8±2.1	9.3±1.7	12.4±3.8	9.7±0.9	13.5±2.4	7.5±2.0
	control		9.3±2.8	13.4±1.3	11.3±1.8	11.7±1.1	10.3±1.6
GCs, %	treatment	8.6±1.3	13.1±1.0	9.4±1.0	12.7±2.1	11.0±1.9	6.6±1.5
	control		9.1±0.8	7.4±2.5	13.1±2.0	7.8±1.5	9.5±2.1
SGCs, %	treatment	78.6±2.2	77.5±2.3	78.2±4.3	77.6±2.1	75.5±2.6	85.7±1.9
	control		81.5±2.3	78.2±4.3	79.1±3.2	80.4±1.4	80.1±2.9

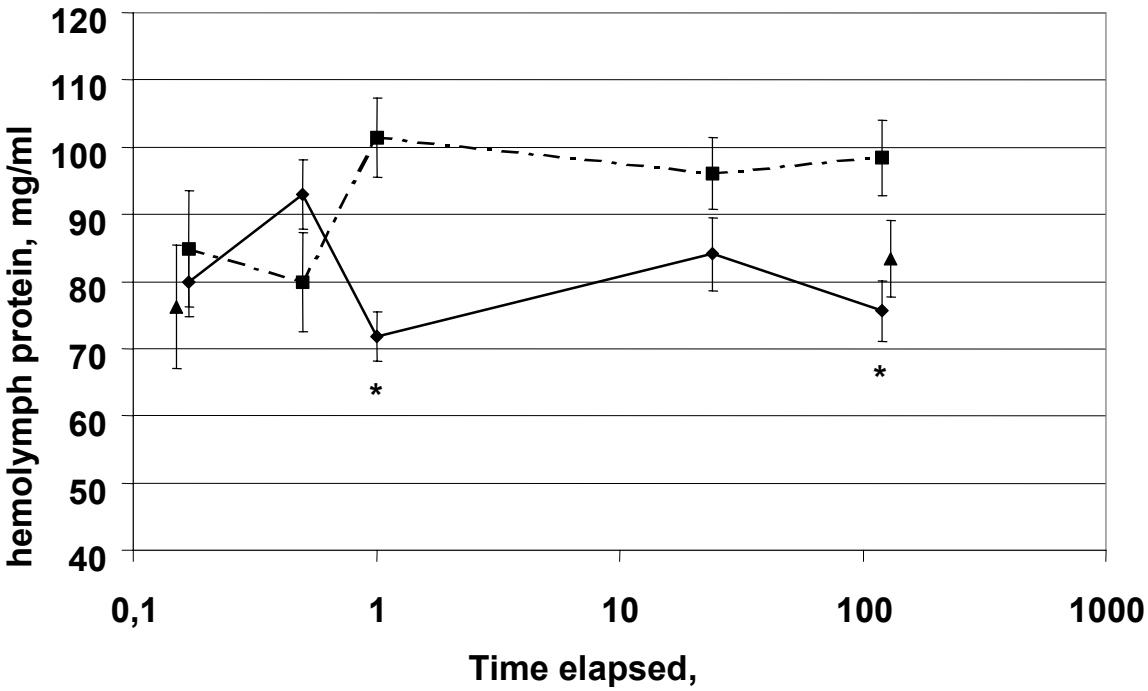


Figure 2 The effect of wounding and handling on hemolymph protein content (mg/ml) in western rock lobster (*Panulirus cygnus*) under experimental conditions. Logarithmic scale in x axis. Symbols are * = significant difference, ◆ = treatment group (solid line), ■ = handled control group (dashed line) and ▲ = undisturbed control group.

Table II Vigor index rating proportions in the injury study. Vigor index ratings are: 3 = healthy; 4 = very healthy; 5 = aggressive (Spanoghe 1996).

		Vigor index rating proportion, %		
		3	4	5
Initial	control	63	37	0
10 min	treatment	29	71	0
	control	29	71	0
30 min	treatment	44	28	28
	control	29	29	42
60 min	treatment	71	29	0
	control	14	43	43
1 d	treatment	29	57	14
	control	14	86	0
5 d	treatment	0	57	43
	control	14	86	0
Final	control	43	43	14

Table III Correlations among parameters studied in the injury study. The statistical significance is expressed as ** = $p<0.01$. Abbreviations are: THCs = total hemocyte counts; Protein = hemolymph protein concentration; HCs = hyalinocyte proportion; GCs = granulocyte proportion; SGCs = semigranulocyte proportion.

	Vigor	THCs 10^6 cells/ml	Protein mg/dl	HCs %	GCs %
THCs	NS				
Protein	NS	0.51**			
HCs	NS	NS	NS		
GCs	NS	0.31**	0.34**	NS	
SGCs	NS	NS	-0.34**	-0.73**	-0.67**

Table IV Vigor index rating proportions in air exposure study. Vigor index ratings are: 3 = healthy; 4 = very healthy; 5 = aggressive (Spanoghe 1996).

	Vigor index rating proportion, %		
	3	4	5
Initial control	13	50	37
Emersion, 5 min	13	50	37
Emersion, 120 min	13	25	62
Handling & emersion, 5 min	25	75	0
Handling & emersion, 120 min	50	38	12
Final control	13	62	25

In the air exposure and handling study, total hemocyte counts (THCs) were similar or slightly lower in the air exposed lobsters compared to the control groups' THCs, while THCs in the lobsters that were both exposed to air and handled were significantly higher after 5 min (Oneway Anova, LSD, $p<0.05$) (Fig. 3). The THCs in final control group were slightly increased compared to the initial control group's THCs. Plasma glucose was significantly elevated (Oneway Anova, LSD, $p<0.05$) in both treatments after 120 min (Fig. 4), while in 5 min groups plasma glucose was only slightly increased. In the 5 min treatment group, SE's were significantly larger than SE's in 120 min treatment groups (T-test distribution based test (Sokal & Brauman 1980), $p<0.05$). There were no significant correlations among THCs, plasma glucose and vigor index in the air exposure experiment, nor were there significant differences in vigor index among the studied groups (X^2 -test), while the handled and emersed groups tended to have lower mean vigor ratings than emersed or control groups (Table IV).

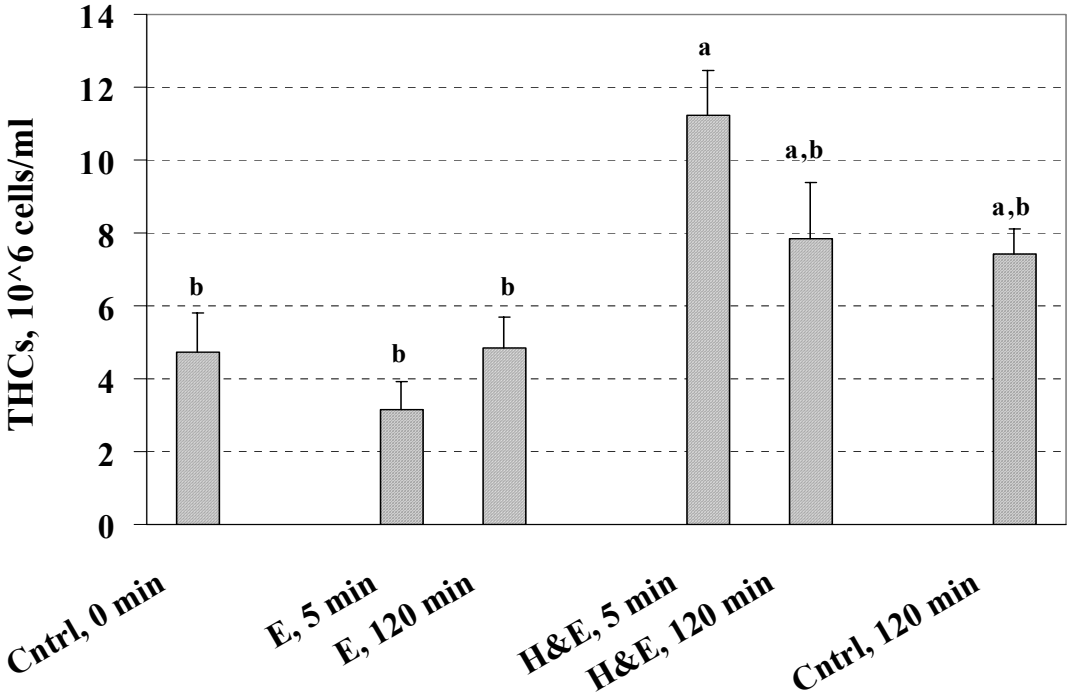


Figure 3 The effect of handling and air exposure on the total hemocyte counts (THCs) in western rock lobster (*Panulirus cygnus*) under experimental conditions. Group abbreviations are E = air exposed group, H&E = handled and air exposed group and Cntrl = control group. Different letters on top of the bars indicate statistically significant differences (Oneway Anova, LSD, $p<0.05$).

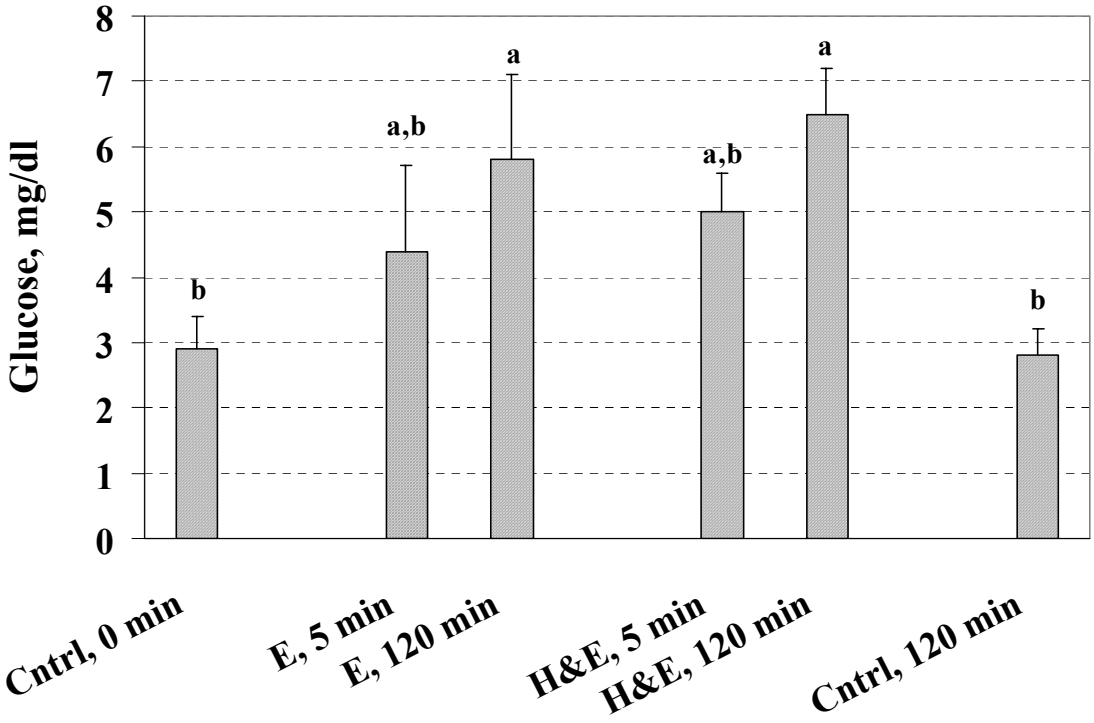


Figure 4 The effect of handling and air exposure on plasma glucose in western rock lobster (*Panulirus cygnus*) under experimental conditions. Group abbreviations are E = air exposed group, H&E = handled and air exposed group and Cntrl = control group. Different letters on top of the bars indicate statistically significant differences (Oneway Anova, LSD, $p<0.05$).

Table IV Vigor index rating proportions in air exposure study. Vigor index ratings are: 3 = healthy; 4 = very healthy; 5 = aggressive (Spanoghe 1996).

	Vigor index rating proportion, %		
	3	4	5
Initial control	13	50	37
Emersion, 5 min	13	50	37
Emersion, 120 min	13	25	62
Handling & emersion, 5 min	25	75	0
Handling & emersion, 120 min	50	38	12
Final control	13	62	25

The most significant findings of these studies were that air exposure alone did not evoke a response in total hemocyte counts in western rock lobsters (*P. cygnus*), while handling combined with other disturbances could cause changes in them. The practical relevance of the finding is that total hemocyte counts could be used to distinguish between different types of stressors. Furthermore, it might be that short term air exposure as such does not cause stress in the lobster immune system. Physical disturbance caused an immune system alarm reaction in western rock lobster, with changes occurring in hemocyte counts along with similar changes in plasma glucose. On the other hand, air exposure as sole stressor did not seem to cause a response in the immune system variables, despite a significant response in plasma glucose to air exposure in the present and previous studies (Spanoghe 1996; Jussila *et al*. 1999). Air exposure might decrease activity in lobsters and thus provoke a system shut-down (our unpublished data) instead of invoking an alarm or stress reaction.

Total hemocyte counts showed significantly faster response in injured lobsters compared to the controls. This could have indicated that mobilization of hemocytes is induced by injury and can act even faster than mobilization due to exercise alone. Hemocytes, in addition to being the main defense against invasion by disease causing agents, take part in wound healing (Söderhäll & Cerenius 1992). The delayed and less marked peak in handled lobsters total hemocyte counts further indicated that mobilization of the hemocytes is less important if lobsters are not injured when handling. Regardless of the treatment, lobsters were recovering at the same rate in the injury experiment.

The rapid increase in total hemocyte counts in both groups in the injury study could have been caused by release of the hemocytes into the circulation from storage sites, since the time frame, 30 min, is much too short to enable a massive production of hemocytes in the hematopoietic tissue. It has been argued, that hematopoietic tissue could store large numbers of mature hemocytes (Kenneth Söderhäll, oral communication) and thus act as the storage site. The differential hemocyte counts had complicated patterns after the injury and changes similar to those of treatment group could also be seen in control group. In the present study, the treatments could not explain the observed changes in differential hemocyte counts.

Part of the circulating protein takes part in hemolymph clotting, and changes in the protein content could also be due to changes in the total hemolymph volume in lobsters (Smith & Dall 1982). Based on their observation that hemolymph protein concentration reflects hemolymph volume, injured lobsters in the present study tended first to dehydrate and then there was an increase in hemolymph volume. In other studies, Chen *et al*. (1993) have observed changes in

clottable protein concentrations during and after injury, when proteins are presumably taking part in healing processes. Furthermore, Xue *et al*. (1993) have observed an increase in stress proteins right after injury, and they presumed that proteins are then used in wound healing, which could also have happened in the present study.

In the injury experiment, lobsters were disturbed after initial wounding and handling trauma with both experimental groups having a slight increase in vigor index, of which the control group seemed to recover. High vigor ratings, 4-5, are indications of lobsters being alerted by treatments, still in their full strength (Spanoghe 1996). The injured group showed high vigor ratings towards the end of the study period, which could indicate that they were still alert after 5 days of recovery. This was seen also in slightly higher total hemocyte counts in the injured group compared to controls, possibly due to the ongoing healing in the wound site.

In the air exposure experiment, vigor index ratings indicated that handling combined with air exposure was more exhausting than air exposure alone, similarly to what could be seen in total hemocyte counts. Total hemocyte counts did not show significant response to the air exposure, which, on the contrary, was clearly seen in glucose levels. We have observed similar responses to air exposure in our previous studies in freshwater crayfish (Jussila *et al*. 1999), with the physical disturbance required to induce hemocyte responses. Thus air exposure alone does not seem to cause immune system to respond. This selective reaction would allow the usage of hemocyte responses to distinguish between different types of stressors.

Disturbance during the sampling could have caused the observed differences in total hemocyte counts between initial and final controls. Under experimental laboratory conditions, both physical disturbance, such as noises, vibrations, visions of persons moving around, etc., and chemical communication through the water could have disturbed lobsters. We have observed also in other, later studies, that lobsters seem to get agitated easily while the sampling is proceeding (unpublished data). These responses could be indicating sensitive nature of hemocyte responses. Thus the design of the stress experiments could easily interfere with the treatments, and interpretation of the results. It can be clearly seen, that when using indicators, such as hemocyte counts, which have not yet been established as stress indicators, there should also be classic stress indicators, such as plasma glucose or lactate (Telford 1974; Johnson & Uglow 1985; Santos & Keller 1993), measured concurrently.

We observed total hemocyte counts below 4×10^6 cells/ml in both experiments discussed here, which have previously been reported as indications of poor condition or health in lobsters (Jussila *et al*. 1997). These low total hemocyte counts could also have been caused by other factors, and comparisons to other studies have to be made with caution, since total hemocyte count baseline seem to be partially dependent on the rearing conditions. Especially the type of nutrition seems to have an impact on total hemocyte counts even after short period of time (Stewart *et al*. 1967, Jussila 1997), i.e. few days, which further emphasizes proper usage of controls in the experiments.

In the present experiments we have observed changes in circulating hemocyte counts due to handling, while air exposure seemed to cause only minimal response. The changes in total hemocyte counts due to physical disturbance were quick and significant. Based on these studies, there are indications that total hemocyte counts could be used as stress or disturbance

indicators in lobsters and that short term air exposure is a minor challenge to immune system. It could also be clearly seen, that further studies are required for proper understanding of the hemocyte stress responses.

ACKNOWLEDGMENTS

We would like to express our gratitude to FRDC for funding the project. Professor Bruce Phillips made valuable comments before, during and after the experiments, thanks for that.

REFERENCES

Albert, J. L. & Ellington, W. R. (1985) Patters and energy metabolism in the stone crab, *Menippe mercenaria*, during severe hypoxia and subsequent recovery. *J. Exp. Zool*. 234: 175-183.

Anon (1995) Code of practice. Rock lobster industry. Western Australian Fishing Industry Council. 43 pp.

Chen, Y.-L., Huang, S.-H., Cheng, J.-H. & Tsai, I.-H. (1993) Relationship between hemolymph-coagulatin and disease in shrimps. 2. Purification and characterization of the hemolymph coagulogen of penaeids. *COA Fish. Ser. Taipei* **40,** 1-9.

Dall, W. (1974) Indices of nutritional state in the western rock lobster, *Panulirus longipes* (Milne Edwards). I. Blood and tissue constituents and water content. *J. Exp. Mar. Biol. Ecol*. **16**, 167-180.

Evans, L. H., Fan, A., Finn, S. P., Dawson, S. A., Siva, C. J. & Lee, I. R. (1992) Nutritional status assessment studies in the freshwater crayfish, *Cherax tenuimanus*. In *Proceedings of the Aquaculture Nutrition Workshop, Salamander Bay, 15-17 April 1991* (Ed. by G. L. Allan & W. Dall), pp. 87-91. NSW Fisheries, Brackish Water Fish Research Station, Salamander Bay, Australia.

Johnson, I. & Uglow, R. F. (1985) Some effects of aerial exposure on the respiratory physiology and blood chemistry of *Carcinus maenas* (L.) and *Liocarcinus puber* (L.). *J. Exp. Mar. Biol. Ecol.* **94**, 151-165.

Jussila, J. (1997) Physiological responses of Astacid and Parastacid crayfishes (Crustacea: Decapoda) to conditions of intensive culture. Doctoral dissertation. Kuopio University Publications C. Natural and Environmental Sciences 67. 137 p.

Jussila, J., Tsvetnenko, E., Jago, J., Dunstan, B., & Evans, L.H. (1997) Total and differential hemocyte counts in western rock lobsters (*Panulirus cygnus*) under post-harvest handling stress. *Mar. Freshwat. Res.* **48**, 863-867.

Jussila, J., Paganini, M., Mansfield, S. & Evans, L. H. (1999) On physiological responses, hemolymph glucose, total hemocyte count and dehydration, of marron (*Cherax*

tenuimanus) to handling and transportation under simulated conditions. *Freshwat. Crayf.* **12**, 154-167.

Paterson, B., Davidson, G. W. & Spanoghe, P. T. (1999) Determination of total protein in hemolymph of the western rock lobster (*Panulirus cygnus* George) by refractometry. In *International Symposium on Lobster Health Management. Abstract Proceedings*. pp 28. Adelaide 19-21 September 1999. Aquatic Science Research Unit. Curtin University of Technology.

Persson, M., Cerenius, L. & Söderhäll, K. (1987) The influence of haemocyte number on the resistance of the freshwater crayfish, *Pacifastacus leniusculus* Dana, to the parasite *Aphanomyces astaci*. *J. Fish Dis.* **10,** 471-477.

Santos, E. A. & Keller, R. (1993) Effect of exposure to atmospheric air on blood glucose and lactate concentrations in two crustacean species: a role for crustacean hyperglyceamic hormone (CHH). *Comp. Biochem. Physiol.* **106A,** 343-347.

Sequiera, T., Tavares, D. & Aralachaves, M. (1996) Evidence for circulation hemocyte proliferation in the shrimp *Penaeus japonicus*. *Develop. Comp. Immunol.* **20**, 97-104.

Smith, D. M. & Dall, W. (1982) Blood protein, blood volume and extracellular space relationships in two *Penaeus* spp. (Decapoda, Crustacea). J. Exp. Mar. Biol. Ecol. **63**, 1-15.

Sokal, R. R. & Brauman, C. A. (1980) Significance tests for coefficients of variation and variability profiles. *Systemat. Zool.* **29,** 50-66.

Spanoghe, P. T. (1996) An investigation of the physiological and biochemical responses elicited by Panulirus cygnus to harvesting, holding and live transport. Doctoral thesis, School of Biomedical Sciences, Curtin University, Perth, Australia. 378 p.

Stewart, J. E., Cornick, J. W. & Dingle, J. R. (1967) An electronic method for counting lobster (*Homarus americanus*) hemocytes and the influence of diet on hemocyte numbers and hemolymph proteins. *Can. J. Zool.* **45**, 291-304.

Söderhäll, K. & Cerenius, L. (1992) Crustacean immunity. *Ann. Rev. Fish Dis.* **2,** 3-23.

Telford, M. (1974) Blood glucose in crayfish – II. Variations induced by artificial stress. *Comp. Biochem. Physiol.* **48A,** 555-560.

Ueda, R., Sugita, H. & Deguchi, Y. (1994) Bactericidal activities of the hemolymph of the Japanese spiny lobster, *Panulirus japonicus* (Decapoda, palinuridae). *Crustaceana* **67(2),** 256-258.

Ueda, R., Sugita, H. & Deguchi, Y. (1999) Effect of transportation on the serum bactericidal activity of *Penaeus japonicus* and *Ovalipes punctatus*. *Aquaculture* **171(3-4),** 221-225.

Xue, Z.-Y., Grossfeld, R. M. (1993) Stress protein synthesis and accumulation after traumatic injury of crayfish CNS. *Neurochem. Res.* **18(2),** 209-218.

Can compromised condition explain early mortalities in spiny lobster culture?

Andrew Jeffs[1]

[1]National Institute of Water and Atmospheric Research, P.O. Box 109-695, Newmarket, Auckland, New Zealand

Corresponding author. Tel.: +64-9-375-2048. Fax: +64-9-375-2051. E-mail: a.jeffs@niwa.cri.nz

ABSTRACT

Energy reserves in the form of lipids are thought to be critical for fuelling the onshore movement, settlement, and subsequent development of the puerulus stage of spiny lobsters. From previous research there are indications that a proportion of pueruli may be bereft of energy stores upon settlement. This may greatly affect the post-settlement survival of lobsters by preventing development to the moult or exposing them to increased disease risk as a consequence of a compromised immune response. This possibility was investigated for a situation where high mortalities were experienced in catches of pueruli taken from the wild as the seed source for spiny lobster aquaculture. Biochemical techniques previously developed for assessing the condition of pueruli were used for lively and moribund lobsters sampled from different collection locations. The results of this study confirm the importance of lipids to the post-settlement development of spiny lobsters and suggest that in this instance the high mortalities that were observed were not related to depleted lipid reserves.

Keywords: condition, puerulus, lobster, aquaculture

I. INTRODUCTION

The lifecycle of spiny lobsters involves a long larval period that can last for over two years in some species (Lipcius and Cobb 1994). Planktonic lobster larvae metamorphose to nektonic pueruli in oceanic waters beyond the continental shelf (Booth and Stewart 1992; Chiswell and Booth 1999). The pueruli then make their way into shallow waters to settle and later moult to become benthic juveniles. The distance travelled by the pueruli of many species is estimated to be in the order of tens of kilometres, a journey thought to take up to several weeks (Booth 1989; Kittaka 1990). Interestingly, the puerulus in many species appears to be non-feeding, relying entirely on reserves accumulated during the larval phase (Kittaka 1990; Nishida *et al*. 1990; Lemmens 1994a; Lemmens 1994b; Takahashi *et al*. 1994). Recent research has indicated that stored lipid is critical for fuelling the onshore movement, settlement and subsequent moulting of pueruli (Takahashi *et al*. 1994; Pearce 1997; Jeffs *et al*. 1999). Furthermore, some of this research on *Jasus edwardsii* (Hutton 1875) in New Zealand suggests that a proportion of pueruli may be enervated at the end of the settlement process, especially in coastal areas with a wide continental shelf (Jeffs *et al*. 1999). It was suggested that the depletion of energy-rich lipid reserves may greatly affect the subsequent chances of survival for pueruli by preventing development to the moult or exposing them to increased

disease risks as a consequence of a lowered immune response. This phenomenon has been suggested to occur in a range of other marine species with a larval phase and could have profound ecological and fisheries significance (Holland 1978; Boidron-Métairon 1995; Stobutzki 1997). This possibility was investigated among the early post settlement stages of *J. edwardsii* in New Zealand that were harvested from the wild as the seed stock for aquaculture. This investigation was conducted after some catches of pueruli were found to be experiencing high mortalities shortly after capture during the winter of 1998. The investigation relied on the use of a biochemical assay for lipids developed during previous ecological research on this species (Jeffs *et al*. 1990).

II. MATERIALS AND METHODS

Animal Collection

Early stages of the spiny lobster, *J. edwardsii*, were collected over the summer of 1997 - 1998 from Castle Point on the south-eastern coast of the North Island of New Zealand (Fig. 1). At Castle Point, pueruli were caught by two methods; puerulus collectors and a small plankton net. Pueruli that had arrived in the last 12 hours and first instar juveniles were taken from standard puerulus collectors deployed in shallow water (<3m) on the lee side of a reef (Booth 1979; Hayakawa *et al*. 1990). The puerulus collectors consisted of artificial crevices in which the pueruli settle from the plankton (Booth and Tarring 1986). The puerulus collectors were emptied on 16 December 1997 and daily over 31 January – 2 February 1998. A small plankton net (1 m^2 opening, 10 mm mesh) was towed at a depth of 1 – 20 m behind a 3 m boat at a speed of around 4 m s^{-1} within 1 km of the shore on the nights of 31 January and 1 February 1998. A sample of 25 first instar juveniles were transferred to shallow tanks in a seawater system in Auckland that was held at ambient seawater temperature. The juvenile lobsters were offered freshly opened mussels (*Perna canaliculus*) (Gmelin 1791) on which to feed. Over the following fortnight records were kept of lobster mortalities.

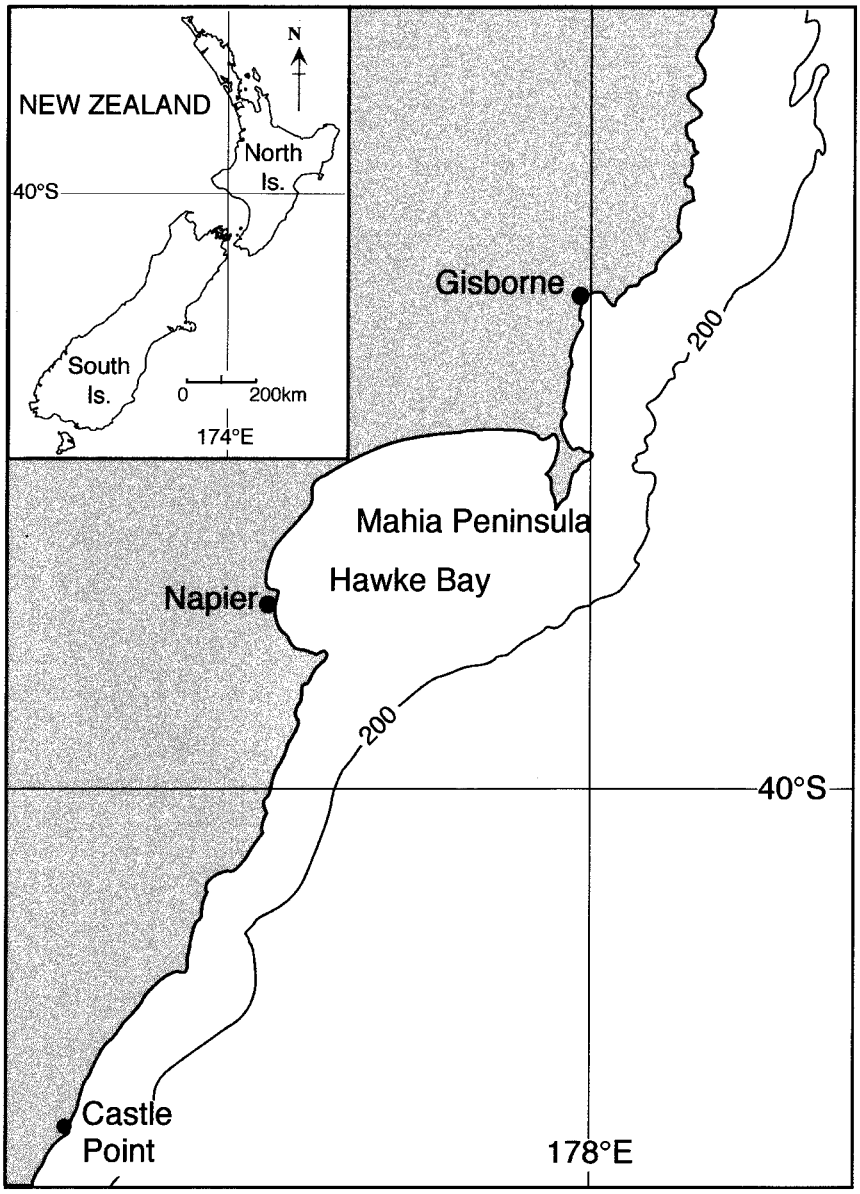


Figure 1 The sampling sites for the early stages of lobsters on the south-eastern coast of the North Island of New Zealand

During the winter of 1998 catches of pueruli were made in the ports of Napier and Gisborne on the south-eastern coast of the North Island of New Zealand (Fig. 1). Pueruli were taken from standard puerulus collectors deployed in shallow water (<10 m) by suspending them by ropes from wharf structures. The puerulus collectors were emptied between 25 May 1998 – 3 June 1998. The pueruli were transferred to shallow tanks in a seawater system in Napier that was held at around 16°C. First instar juvenile lobsters were offered freshly opened mussels (*P. canaliculus*) on which to feed. Over the following fortnight records were kept of lobster mortalities and general observations were made of behaviour. On the 16 June 1998 lively and moribund first instar juveniles were sampled from the tanks for biochemical analysis.

On the 15 June 1998 the collectors at the Port of Napier were cleared and only pueruli that had settled in the last 12 hours were taken.

All lobsters destined for biochemical analyses were frozen in liquid nitrogen shortly after removal from the tank or collectors and transferred to the laboratory for analyses.

Biochemical Analysis

The carapace length of each frozen puerulus was measured with callipers and then all of the appendages were removed to ensure that valid comparisons could be made between pueruli that had missing limbs and those that did not. The pueruli bodies were freeze dried to a constant mass and then ground to a fine powder in a pulverizer cooled with liquid nitrogen. Total lipid content was determined gravimetrically following a methanol-chloroform (2:1 v/v) extraction technique (Mann and Gallager 1985) that had been verified previously for use on pueruli (Jeffs *et al*. 1990). Duplicate samples were run for each puerulus and mean values taken.

Statistical Analysis

A comparison of the carapace sizes and lipid content of first instar juvenile lobsters that were lively and moribund, and caught from different locations was conducted using a one-factor analysis of variance (ANOVA). Statistical comparisons between mean values of the carapace sizes and lipid content of early pueruli were conducted with a Welch's approximate *t*-test after sample variances were found to be homogeneous with the F_{max} test (Sokal & Rohlf 1995). A statistical significance level of 0.05 was employed for all analyses.

III. RESULTS & DISCUSSION

Mortalities

Captive first instar juveniles taken from Castle Point in the late summer of 1997 - 1998 actively fed on the opened mussels and all successfully completed the second moult within four weeks. There were no mortalities among the 25 captive lobsters over this period.

Observations of pueruli and juveniles taken from Napier and Gisborne over the early winter of 1998 showed that most pueruli went through the first moult within four to ten days of arrival in the seawater system. Mortalities began to increase from around 14 days after capture, which was about the time of the second moult. These animals appeared to become weak, losing vigour and were usually unable to complete the second moult. Those juveniles that survived this second moult appeared to have much higher levels of survival through subsequent moults. In other catches of wild pueruli and juvenile lobsters over the winter from the same locations, held under the same conditions, mortalities often reached as high as 30%. This pattern of mortalities was common to catches of early lobsters from both Gisborne and Napier. Detailed mortality records were kept for a collection of 320 animals caught from Napier on 28 May 1998 (Fig. 2) and 1050 animals caught at Gisborne on 25 May 1998 (Fig. 3).

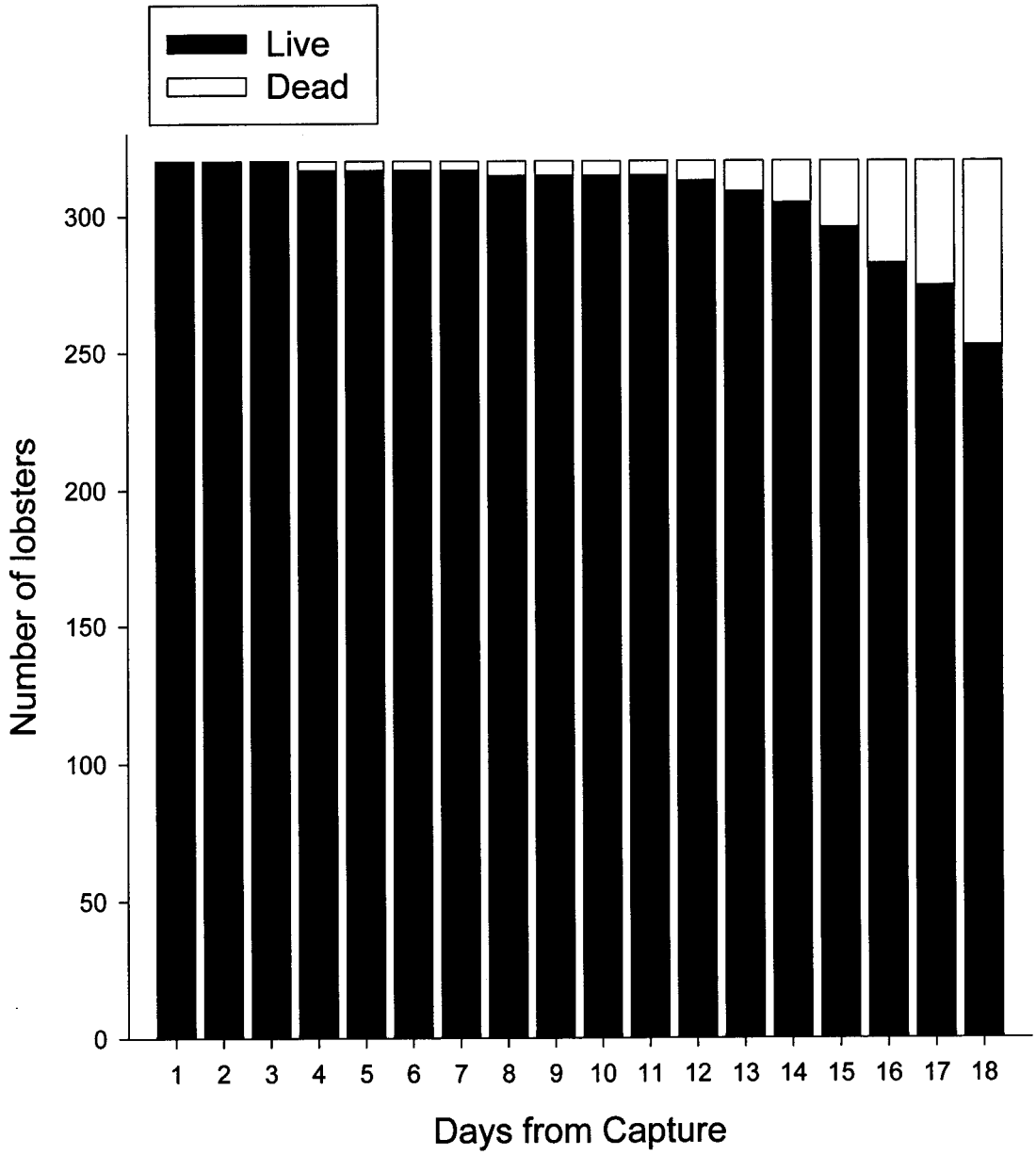


Figure 2 Mortality among 320 early post-settlement lobsters caught from Napier on 28 May 1998 and held in captivity in Napier

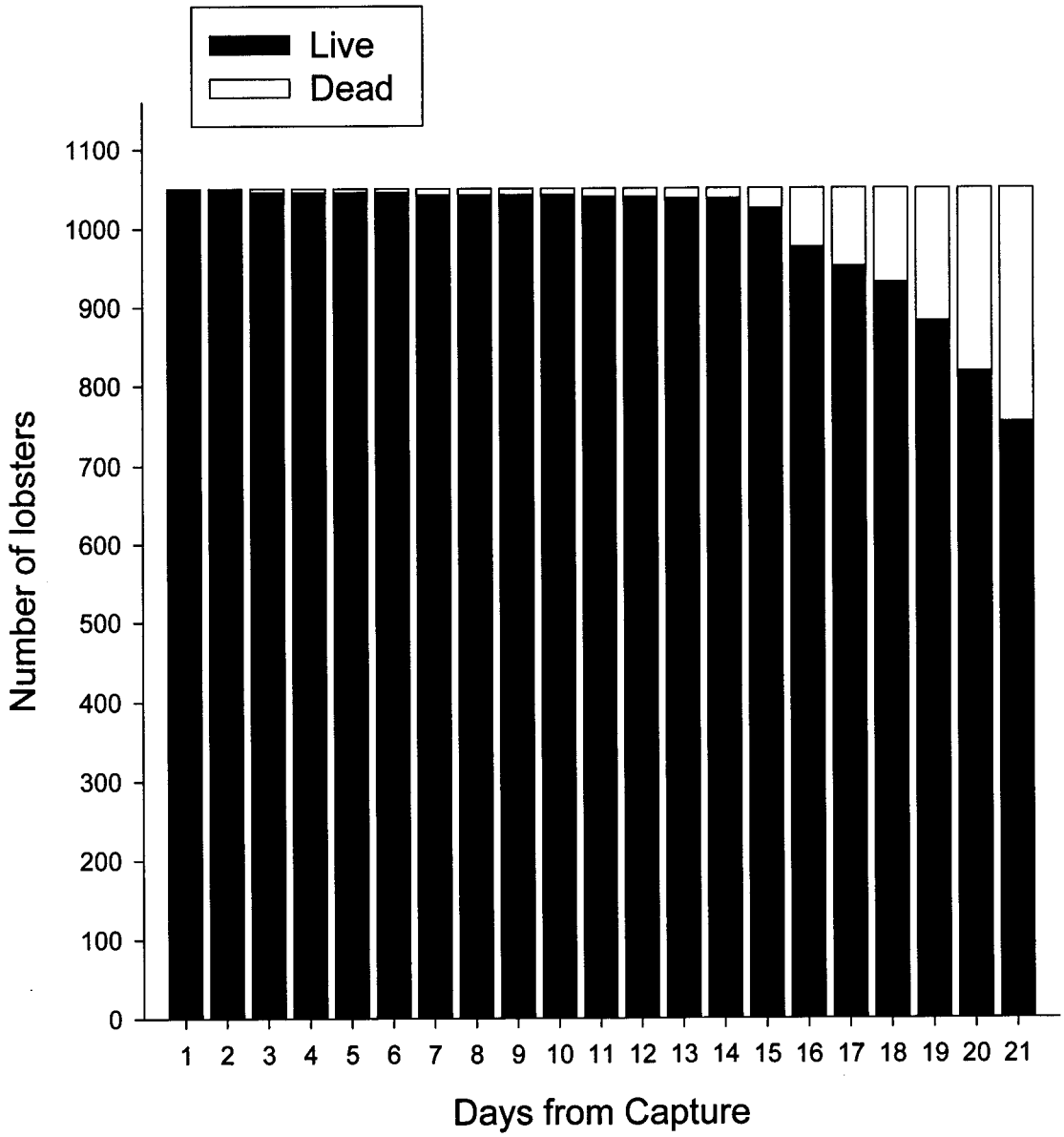


Figure 3 Mortality among 1050 early post-settlement lobsters caught from Gisborne on 25 May 1998 and held in captivity in Napier

Biochemical Analysis

A total of 70 pueruli and first instar juvenile lobsters were used for lipid analyses. The source, numbers, status, size and lipid contents of animals sampled for lipid analysis are presented in Table I.

Table I Number, locations, status and lipid content of early stages of spiny lobsters sampled for this study. Lipid content is expressed as a percentage of dry mass (mg(100mg dry mass)$^{-1}$). Carapace length and lipid content are the mean values derived from sampling individual pueruli ± SE. Measurements from the same developmental stage but from different collections that share the same symbol (♣ ♦ ♥ ♠) are not statistically different at $\alpha = 0.05$.

Lobster Stage	Number	Capture Location	Capture Date	Sampling Date	Status	Carapace length (mm)	% Lipid (mg %)
First instar juveniles	10	Gisborne & Napier	28 May – 3 June 1998	16 June 1998	Lively	11.2 ± 0.2 ♣	5.6 ± 0.5 ♣
First instar juveniles	9	Gisborne & Napier	25 May – 3 June 1998	16 June 1998	Moribund	11.6 ± 0.2 ♣	5.4 ± 0.5 ♣
First instar juveniles	10	Castle Point	31 January – 2 February 1998	Same day as capture	Lively	11.8 ± 0.3 ♣	3.5 ± 0.2 ♠
Pueruli (just settled)	5	Napier	15 June 1998	15 June 1998	Lively	10.6 ± 0.3 ♦	19.6 ± 1.4 ♦
Pueruli (just settled)	36	Castle Point	16 December 1997 – 2 February 1998	Same day as capture	Lively	10.3 ± 0.1 ♦	14.6 ± 0.5 ♥

The first instar juveniles were all of a similar size and there was no difference among the carapace lengths of the three groups of first instar juveniles that were lively or moribund, or caught at different times and places (ANOVA $F_{2,26} = 1.81$, $P > 0.05$). However, there was a difference in the lipid content between these three groups of first instar juveniles (ANOVA $F_{2,26} = 8.14$, $P < 0.002$). Tukey-Kramer Honestly Significant Difference ($\alpha = 0.05$) pairwise comparisons of means showed that there was no significant difference in the lipid content of lively and moribund first instar juveniles from Napier and Gisborne. However, lively first instar juveniles taken from Castle Point had significantly lower lipid content than both lively and moribund first instar juveniles from Napier and Gisborne (a mean difference of 1.9% of dry mass).

The early pueruli were all of a similar size as there was no difference between the carapace lengths of pueruli taken from Castle Point or Napier ($t_5 = 1.28$, $P > 0.05$). However, there was a significant difference in the lipid content of pueruli taken from Castle Point and Napier ($t_5 = 3.37$, $P < 0.02$). On average pueruli taken from Napier in the winter of 1998 contained significantly greater quantities of lipid (a mean difference of 5.0% of dry mass) than pueruli caught at Castle Point 4 - 6 months earlier.

There has been much research and speculation about the role of energy reserves in the successful settlement and subsequent recruitment of many marine fishes and some benthic marine invertebrates (Suthers 1992; Boidron-Métairon 1995; Hunt and Scheibling 1997). Despite the remarkably long and unusual larval development found among the spiny lobsters there has been relatively little research on the role of their energy reserves in post settlement survival (Jeffs *et al*. 1999). Previous work on several species of spiny lobster, including *J. edwardsii*, has indicated that a proportion of the non-feeding pueruli may be bereft of energy stores in the form of lipid upon settlement (Lemmens 1994a; Lemmens 1994b; Jeffs *et al*. 1999). This may greatly affect their subsequent chances of survival by preventing development to the moult or exposing them to increased disease risk as a consequence of a lowered immune response.

This study failed to find an association between lower levels of energy stores in the form of lipids and increased mortalities of post-settlement lobsters. Pueruli that had just settled in Napier during winter had significantly higher lipid content than pueruli taken at Castle Point in the proceeding summer period. Similarly, first instar juveniles caught at Castle Point in February 1998 had significantly lower lipid content than first instar juveniles from Napier and Gisborne taken during June 1998. Large numbers of first instar juveniles from Napier and Gisborne died in captivity, while there were no mortalities among a sample of first instar juveniles taken from Castle Point, despite them having less extensive lipid stores. Furthermore, there were no differences between the lipid stores of lively and moribund first instar juveniles that were captured from Gisborne and Napier during the winter of 1998 and held in captivity together.

The lipid levels recorded in all lobsters in this study in New Zealand were markedly higher than those recorded for identical stages of wild caught *J. edwardsii* from one site in Tasmania, Australia (Pearce, 1997). She found that early settling pueruli had a mean lipid content of around 8% while measured lipid levels in post-pueruli never exceeded 3.4%. Unfortunately, no information on post-settlement survival was recorded in relation to these generally lower lipid levels, however, it could be inferred that settling pueruli with a mean lipid content of

around 8% retained the energetic capacity to develop and moult to become first instar juveniles.

Overall, these results suggest that the differences in mortalities among captive lobsters observed in this study may be due to the holding conditions for the lobsters rather than their biochemical condition. This suggestion is supported by subsequent research showing that the mortalities of the early stages of lobsters in captivity, particularly around early moults, may be related to fungal infections associated with some holding conditions for lobsters (Diggles 1999a; Diggles 1999b). Visible symptoms of fungal infection were noted among moribund lobsters analysed for this study (Jeffs pers. obs.).

Pearce (1997) found marked differences in the lipid reserves between settling pueruli and first instar juveniles of *J. edwardsii* captured from one site in Tasmania. She attributed this difference to the very energetically demanding morphological changes lobsters undergo at this time of their development. Marked differences in lipids between these two developmental stages were also found in this New Zealand study at two sites, which reconfirms the critical importance of lipids in fuelling this stage of the lifecycle.

The significantly lower lipid content observed in lobsters sampled from Castle Point in summer compared to those from Gisborne and Napier in winter may be due to site and, or seasonal differences. Both Gisborne and Napier have greater shelf distances than Castle Point over which the pueruli need to travel in order to move inshore to settle (35 km Gisborne, 75 km Napier and 20 km Castle Point). Previous research has suggested that shelf distances may play a role in depletion of lipids, especially if pueruli are actively swimming inshore (Lemmens 1994a; Lemmens 1994b; Jeffs *et al*. 1999). If this were the case, it could be expected that lobsters sampled at Castle Point would have higher levels of remaining lipid, which was not the case.

Seasonal differences may also account for the differences in the lipid contents of the early stages of lobsters observed in this study. Pearce (1997) found no seasonal differences in the lipid levels of pueruli of *J. edwardsii* caught in Tasmania. However, Lemmens (1994a and 1994b) found seasonal differences in the energy content of the pueruli of *Panulirus cygnus* in Western Australia, with a trend for decreased energy levels to be found in summer. It was thought that this could be related to an increased energy demand from higher water temperatures raising the metabolic rate of pueruli. Clearly, the reason for, and the subsequent ecological implications of these marked differences in energy stores in the early post settlement stages of *J. edwardsii* requires further investigation.

ACKNOWLEDGEMENTS

Many thanks to Megan Willmott, Anthony Hickey and Rufus Wells who helped with the biochemical analyses and especially Vivien Ward for the illustrations. Thanks also to the many people who assisted with the collection of the animals for this project, especially Phil Davies, Niki Gladding, Jeff Forman and Dean Stotter. This research was supported by the Foundation for Research, Science and Technology.

REFERENCES

Boidron-Métairon, I.F. (1995) Larval nutrition. In *Ecology of Marine Invertebrate Larvae* (Ed. by L. McEdward), pp. 223-48. CRC Press, Boca Raton, U.S.A.

Booth, J.D. (1979) Settlement of the rock lobster, *Jasus edwardsii* (Decapoda: Palinuridae) at Castle Point, New Zealand. *N.Z. J. Mar. Freshwat. Res*., **13**, 395-406.

Booth, J.D. (1989) Occurrence of the puerulus stage of the rock lobster, *Jasus edwardsii* at the New Plymouth power station, New Zealand. *NZ J. Mar. Freshwat. Res*., **23**, 43-50.

Booth, J.D. & Stewart, R.A. (1992) Distribution of phyllosoma larvae of the red rock lobster *Jasus edwardsii* off the east coast of New Zealand in relation to the oceanography. In *Australian Society for Fish Biology Workshop Larval Biology* (Ed. by D.A. Hancock), pp. 138-48. Bureau of Rural Resources Proceedings No. 15, Australian Government Publishing Service, Canberra, Australia.

Booth, J.D. & Tarring, S.C. (1986) Settlement of the red rock lobster, *Jasus edwardsii*, near Gisborne, New Zealand. *N.Z. J. Mar. Freshwat. Res*., **20**, 291-97.

Chiswell, S.M. & Booth, J.D. (1999) Rock lobster *Jasus edwardsii* larval retention by the Wairarapa Eddy off New Zealand. *Mar. Ecol. Prog. Ser*., **183**, 227-40.

Diggles, B.K. (1999a) Mortalities of *Jasus edwardsii* maintained in captivity. *Lobster Newsletter*, **12(1)**, 11-13.

Diggles, B.K. (1999b) Diseases in spiny lobster holding in New Zealand. In *Proc. Int. Symp. on Lobster Health Management*. Fisheries Research and Development Corporation, Canberra, Australia (In press).

Hayakawa, Y., Booth, J.D., Nishida. S., Sekiguchi, H., Saisho, T. & Kittaka, J. (1990) Daily settlement of the puerulus stage of the red rock lobster *Jasus edwardsii* at Castlepoint, New Zealand. *Nippon Suisan Gakk*., **56**, 1703-16.

Holland, D.L. (1978) Lipid reserves and energy metabolism in the larvae of benthic marine invertebrates. In *Biochemical and Biophysical Perspectives in Marine Biology Vol 4* (Ed. by D.C. Malins & J.R. Sargent), pp. 85-123. Academic Press, London, U.K.

Hunt, H.L. & Scheibling, R.E. (1997) Role of early post-settlement mortality in recruitment of benthic marine invertebrates. *Mar. Ecol. Prog. Ser*., **155**, 269-301.

Jeffs, A.G., Willmott, M.E. & Wells, R.M.G. (1999) The use of energy stores in the puerulus of the spiny lobster *Jasus edwardsii* across the continental shelf of New Zealand. *Comp. Biochem. Physiol*., **123A**, 351-57.

Kittaka, J. (1990) Ecology and behaviour of puerulus of spiny lobsters. *La Mer*, **28**, 255-59.

Lemmens, J.W.T.J. (1994a) Biochemical evidence for absence of feeding in puerulus larvae of the western rock lobster *Panulirus cygnus* (Decapoda: Palinuridae). *Mar. Biol.*, **118**, 383-91.

Lemmens, J.W.T.J. (1994b) The western rock lobster *Panulirus cygnus* (George, 1962) (Decapoda: Palinuridae): the effect of temperature and developmental stage on energy requirements of pueruli. *J. Exp. Mar. Biol. Ecol.*, **180**, 221-34.

Lipcius, R.N. & Cobb, J.S. (1994) Introduction; Ecology and fishery biology of spiny lobsters. In *Spiny Lobster Management* (Ed. by B.F. Phillips, J.S. Cobb & J. Kittaka), pp 1-30. Fishing News Books, Oxford, U.K.

Mann, R. & Gallager, S.M. (1985) Physiological and biochemical energetics of larvae of *Teredo navalis* and *Bankia gouldi* (Bartsch) (Bivalvia: Teredinidae). *J. Exp. Mar. Biol. Ecol.*, **85**, 211-28.

Nishida, S., Quigley, B.D., Booth, J.D., Nemoto, T. & Kittaka, J. (1990) Comparative morphology of the final-stage phyllosoma, puerulus and postpuerulus of the rock lobster *Jasus edwardsii* (Decapoda: Palinuridae). *J. Crustac. Biol.*, **10**, 293-305.

Nishida, S., Takahashi, Y. & Kittaka, J. (1995) Structural changes in the hepatopancreas of the rock lobster, *Jasus edwardsii* (Crustacea: Palinuridae) during development from the puerulus to post-puerulus. *Mar. Biol.*, **123**, 837-44.

Pearce, A. (1997) The nutritional condition of newly settled *Jasus edwardsii* (Hutton 1875) (Palinuridae). BSc(Hons) thesis, University of Tasmania, Australia.

Sokal, R.R. & Rohlf, F.J. (1995) *Biometry; the Principles and Practice of Statistics in Biological Research*. W.H. Freeman & Co, New York, U.S.A.

Stobutzki, I.C. (1997) Energetic cost of sustained swimming in the late pelagic stages of reef fishes. *Mar. Ecol. Prog. Ser.*, **152**, 249-59.

Suthers, I.M. (1992) The use of condition indices in larval fish. In *Australian Society for Fish Biology Workshop Larval Biology* (Ed. by D.A. Hancock), pp. 49-58. Bureau of Rural Resources Proceedings No. 15, Australian Government Publishing Service, Canberra, Australia.

Takahashi, Y., Nishida, S. & Kittaka, J. (1994) Histological characteristics of fat bodies in the puerulus of the rock lobster *Jasus edwardsii* (Hutton, 1875) (Decapoda, Palinuridae). *Crustaceana*, **66**, 318-25.

Disease conditions of cultured phyllosoma larvae and juveniles of the southern rock lobster (*Jasus edwardsii,* Decapoda; Palinuridae)

J. Handlinger[1], J. Carson[1], A.J. Ritar[2], B.J. Crear[2], D.P. Taylor[1] and D. Johnston[3]*

[1]Fish Health Unit, Mt Pleasant
[2]Marine Research Laboratories, Taroona
[3]School of Aquaculture
Tasmanian Aquaculture & Fisheries Institute, University of Tasmania, Hobart, Tasmania.
[*]Corresponding author

ABSTRACT

Cultured phyllosoma larvae and juveniles of the southern rock lobster (*Jasus edwardsii)* were monitored for disease for almost two years. No major disease outbreaks and no specific pathogens were seen in either age group, though relatively few were examined. A variety of external fouling organisms and occasional deeper invasions were seen, which have contributed to either sporadic losses or to background low level mortality. Fouling consistently involved adhered *Leucothrix*-like bacteria that provided a habitat for a complex microbiota consisting of clumps of smaller bacteria, stalked peritrich ciliates, *Chilodonella*-like ciliates, amoebae, and occasional fungi. Bacteria isolated from animals held in culture, and possibly associated with focal degeneration and adhesion of the exoskeleton, particularly in appendages, included *Flavobacterium* species and mixed *Vibrio* species including *V. anguillarum, V. alginolyticus* and *V. tubiashii*, all recognised pathogens of several aquatic animals. Histological examination suggested these bacteria were largely in small granulomas in appendages (gills in juveniles), or in the hepatopancreas tubules. *V. harveyi* was isolated once from newly collected small juveniles with digestive tubule degeneration and occasional granulomas with visible bacteria. Isolation of bacteria from haemolymph of juveniles was rare, and there was histological evidence of bacteraemia in only the phyllosoma. Heavy fouling and gill tip necrosis reflected poor water quality (high ammonia, low dissolved oxygen) and was largely controlled by manipulation of environmental conditions.

I. INTRODUCTION

There is strong commercial interest in Australia in developing an aquaculture industry for rock lobster (Schaap 1997). In the long-term, this would be based on the closure of the life cycle i.e. farming of lobster from eggs to a marketable size. This interest has developed as a result of high demand and prices for the existing product, opportunities for new product (smaller size) and concerns regarding potential over fishing.

However, the larval culture of rock lobster from eggs has proven difficult. For the southern rock lobster (*Jasus edwardsii*), a small number have survived through to settlement of pueruli (Kittaka *et al.,* 1988, Tong *et al.,* 1997) indicating that there is scope for their successful propagation from eggs. This is despite the long, planktonic larval phase, lasting up to two years in the wild (Phillips & Sastry, 1980; Booth & Phillips, 1994) with 11 distinct morphological stages (Lesser, 1978) and 17 or more instars (Booth, 1994; Kittaka, 1994)

during development from newly-hatched larvae to the puerulus stage. A proposed rock lobster aquaculture industry in Tasmania initially intends to access pueruli and young juveniles from the wild but considers that hatchery production of seedstock will allow sustainability and future industry expansion (Schaap, 1997), so consistent production of pueruli from eggs will be important.

The principal diseases of lobsters comprise bacterial diseases (gaffkemia, shell disease, vibriosis), fungal infections (systemic and superficial mycoses) and parasitic infections (Sinderman, 1989; Evans & Brock, 1994; Takahashi, 1995; Abraham *et al*., 1996). The high stocking densities employed in aquaculture and the associated stress on cultured stock potentiates the outbreak and spread of infectious disease (Evans & Brock, 1994; Aguado & Bashirullah, 1996). Non-infectious disease states, particularly those caused by inadequate nutrition, also occur (Evans & Brock, 1994). However, there is little published information on diseases of Australian lobsters, although Edgerton (1996) has described a number of viruses and Edgerton & Prior (1999) described a rickettsial-like organism in the culture of the Australian crayfish, *Cherax quadricarinatus*. Hine & Jones (1994) report that there are no known parasitic diseases of *Jasus edwardsii* in New Zealand.

This study was undertaken to describe the diseases of cultured phyllosoma and juvenile southern rock lobsters (*Jasus edwardsii*) using histological and microbiological methods.

II. MATERIALS AND METHODS

Phyllosoma larvae and juveniles of southern rock lobsters cultured and held at the Tasmanian Aquaculture and Fisheries Institute, Marine Research Laboratory Hobart, from 1997 until 2000 constituted the study group. The lobsters were held in rearing facilities for investigations into the potential of this species for commercial aquaculture. Phyllosoma and juveniles were collected and submitted to the Fish Health Unit, Animal Health Laboratory, Department of Primary Industries, Water and Environment, Tasmania for examination during episodes of mortality and poor performance. These included moribund animals and apparently normal cohorts for comparison. Specimens were examined grossly for evidence of external infestations and then prepared for histopathology and microbiology. Following chill coma or anaesthetic overdose, phyllosoma were fixed whole, and juvenile lobsters were sagitally sectioned, and placed into 10% formalin in seawater, or Davidson's seawater fixative, or a combined fixative (1% glutaraldehyde and 4% formaldehyde in seawater). Phyllosoma beyond Stage V, and juvenile lobsters required decalcification in a standard decalcification fluid diluted 10 fold in tap water.

Histology

For initial preparation, phyllosoma larvae were embedded in bacteriological grade 2% agar. Briefly, whole phyllosoma were placed onto a slide and melted but cooled agar was droppered over the larvae until a mound developed. The larvae were then removed from the slide and a few drops of agar were used to seal the cutting surface. Selected larvae were also sectioned transversely between the thorax and the abdomen and the procedure followed as above. For histology, agar blocks were then processed routinely into paraffin wax, sections were cut at 5 μm and stained with haematoxylin and eosin (HE), or other stains such as Gram and giemsa.

Selected phyllosoma were also embedded into soft resin, sectioned at 1 μm and stained with HE.

Microbiology

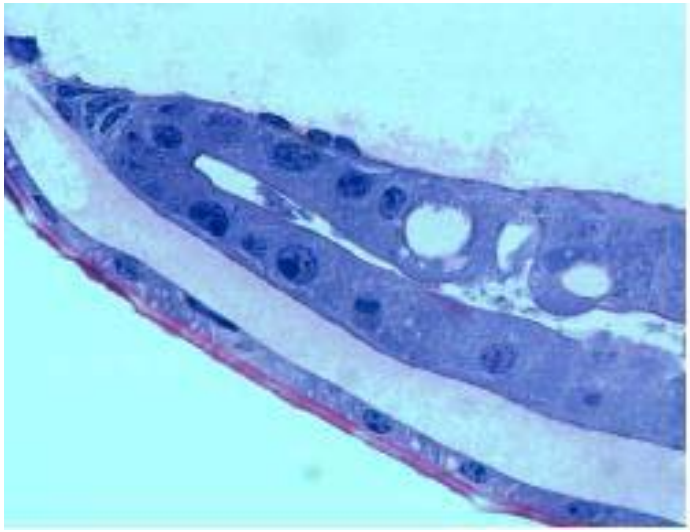
Phyllosoma were surface disinfected using 70% alcohol wipes and allowed to dry. The whole animal was then macerated and samples collected using sterile techniques were plated onto blood agar, tryptone soya agar (TSA), and thiosulphate citrate bile sucrose (TCBS) media. For juvenile lobsters, haemolymph was sampled directly from the heart. Haemolymph samples were plated onto media as above.

III. RESULTS

The diseases detected during this study were all bacterial in origin. In both phyllosoma and the juveniles, fouling of the shell with epibionts was the most prevalent disease. *Vibrio* sp. infections manifest as enteritis and/or septicaemia were also common. Lesions with dual or multiple pathogens present, such as concurrent fouling and infections with bacteria and fungi, were less common.

Normal digestive gland histology

The decapod digestive gland occupies much of the cephalothoracic cavity and is connected to the ventro-posterior region of the pyloric stomach by two small primary ducts. Each duct branches into numerous blind ending tubules which comprise the gland. The tubules have a central lumen surrounded by highly differentiated epithelial cells, all of which have a microvillous brush border. There are 4 epithelial cell types: E (embryonic), R (resorptive), B (blisterlike) and F (fibrillar). E-cells are located in the distal tips with F-, R- and B-cells dispersed throughout the proximal region of the tubules. F-cells have a basophilic cytoplasm and a large nucleus whereas B-cells are distinguished by a large digestive vacuole which occupies the majority of the cytoplasm. R-cells have several vesicles containing lipid although in routine histological processing the lipid is dissolved giving the vesicles an "empty appearance" (Icely & Nott, 1980) (Figures 1 and 2).



Figures 1 and 2 Phyllosoma. Resin sections. Normal microscopic anatomy of the hepatopancreas. Figure 1. Longitudinal section, x 400. Figure 2. Transverse section, x 400.

Disease conditions of phyllosoma larvae

External shell fouling

External shell fouling with epibionts occurred over both the body and the appendages. Newly hatched larvae were not found to be regularly fouled. Fouling contributed to sporadic losses in phyllosoma of nearly all stages. The intensity of infestation on individual phyllosoma was variable and not uniformly distributed. Dissection microscope and histological examination showed that fouling was usually mixed, and included colonies of bacteria including *Vibrio* species and *Flavobacterium* species and Leukothrix-like bacteria with a filamentous appearance. Chilodonella-like cililates, amoebae, and stalked peritrich ciliates were also present. Often, heavy fouling was associated with focal erosion of the exoskeleton by non-*Leucothrix*-like bacteria, resulting in sub-dermal granulomas (Figure 3). *Vibrio*-like bacteria, based on their morphology and gram reaction, were occasionally detected within granulomas. Fouling organisms were not routinely examined with microbiology but *Vibrio* bacteria including *V. anguillarum, V. alginolyticus* and *V. tubiashii* were isolated. Examination of *Artemia salina* nauplii fed to phyllosoma suggested these were a likely source of fouling organisms.

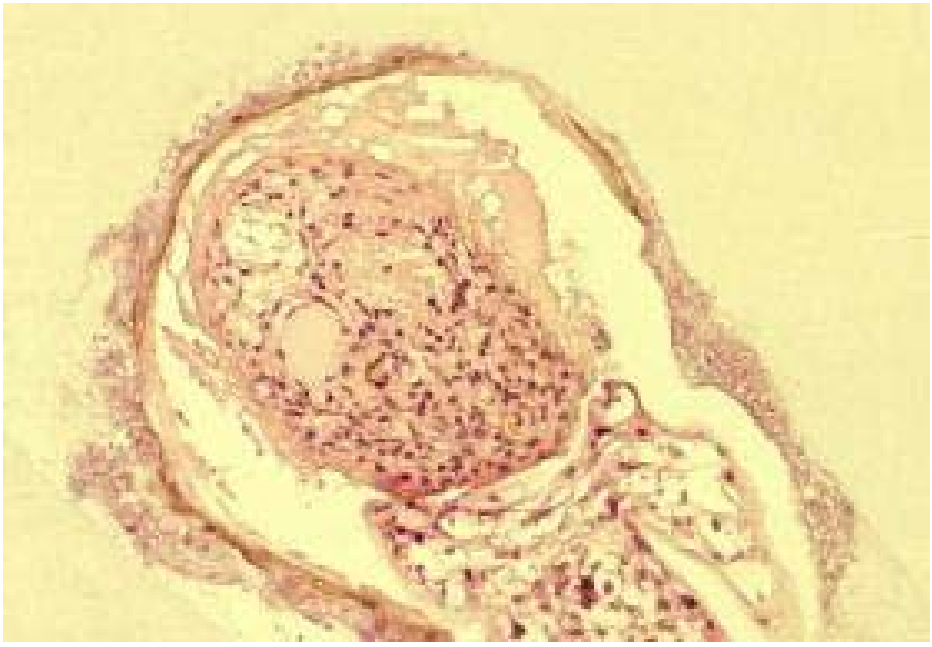


Figure 3 Phyllosoma. Paraffin section. External fouling of an appendage with granuloma formation beneath the exoskeleton, x 100.

Vibrio associated enteritis and/or septicaemia

Bacterial associated hepatopancreas epithelial erosion was seen as early as the second instar stage. A variety of *Vibrio* species including *V. anguillarum* were isolated. Affected phyllosoma were noticed to experience sporadic mortalities. Histologically, massive bacterial colonies were evident in hepatopancreas digestive tubules (Figure 4). In many cases, bacteria were in close apposition to the epithelial luminal surfaces. Hepatopancreas epithelium was often vacuolated and showed extensive degeneration and sloughing into the lumen to then expose the underlying basement membrane (Figures 5 and 6). Sections of affected gut prepared with resin showed ribbon-like eosinophilic structures in the cytoplasm of degenerating cells (Figures 7 and 8). Secondary septicaemia was observed following complete gut epithelial erosion and extension into the haemolymph of the coelomic cavity. In both cases, there was little appreciable haemocyte response.

Figure 4 Phyllosoma. Resin section. Degenerate gut tubule with *Vibrio*-like bacteria present within the lumen, epithelial cells and the haemolymph, x 400.

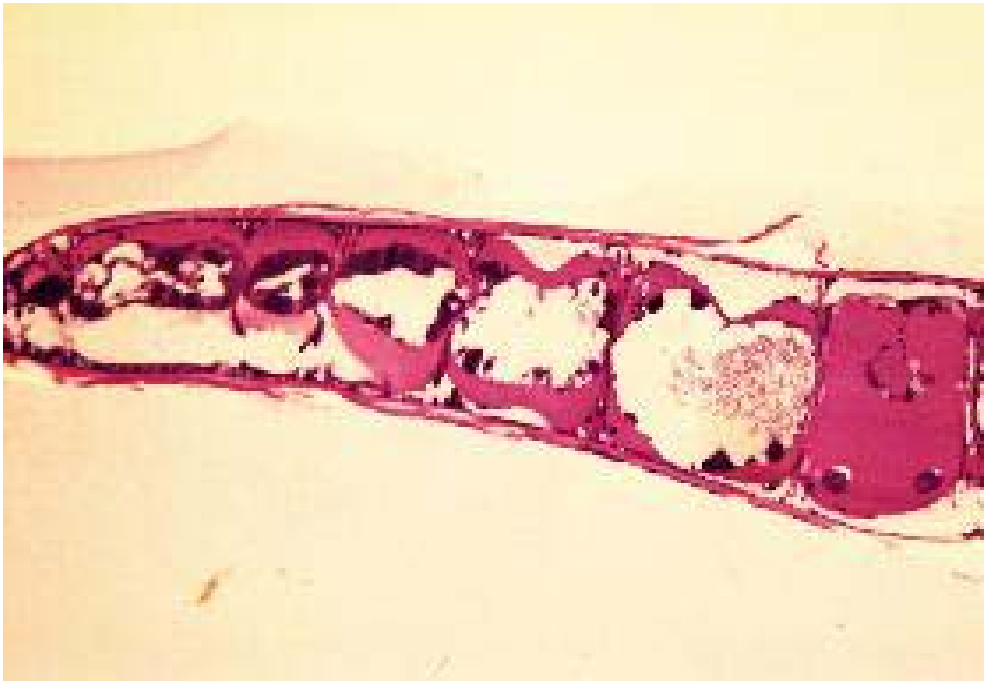


Figure 5 Phyllosoma. Paraffin section. Digestive tubules with varying epithelial degeneration. Note the relative sparing of the distal tubules, x 100.

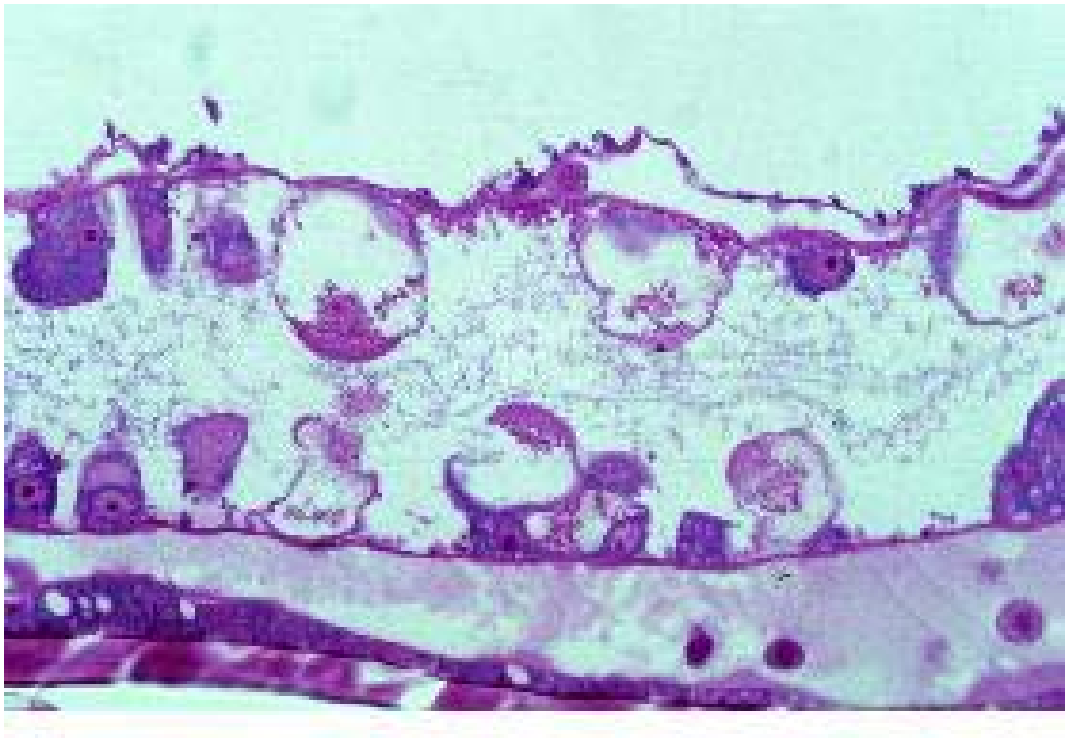
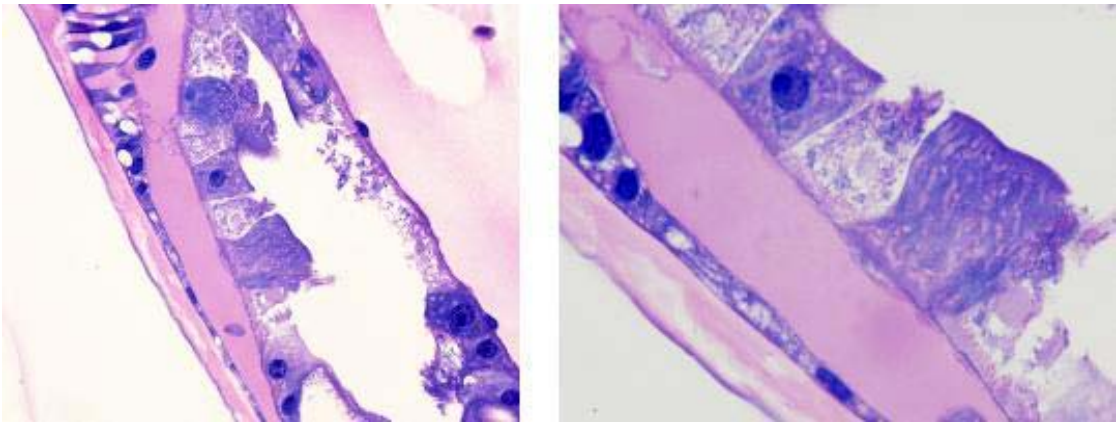


Figure 6 Phyllosoma. Resin section. Tubule epithelial degeneration and loss, x 400.



Figures 7 and 8 Phyllosoma. Resin sections.
Figure 7. Selective loss of tubule epithelia, x 400
Figure 8. Higher magnification of same area. Detail of unidentified eosinophilic bodies in the cytoplasm, x 1000.

Disease conditions of juvenile lobsters

External fouling

Fouling with epibionts including filamentous bacteria (*Leucothrix*-like) and sessile ciliated protozoa and organic debris were common observations. Fouling was present over the gill,

body and appendages. Histological examination showed similar changes to those seen in the phyllosoma. Additionally, heavy fouling was associated with gill necrosis (Figure 9). Heavy general fouling was also associated with ulceration of the exoskeleton. Ulceration was associated with juvenile lobsters kept at high densities and reflected a degree of trauma to the shell. Incidences of ulceration were only sporadic, and were mostly located dorsally, posterior to the carapace. Pathology was confined to the vicinity of the lesion and consisted of intense inflammatory infiltrates, granulomatous reaction, and degeneration of individual muscle fibres. The cuticular and subcuticular exoskeleton was thickened adjacent to the lesion and there was melanisation. There was no evidence of systemic involvement. Mixed bacteria including *Flavobacterium* spp. were isolated from the lesions. Moderate to heavy fouling with epibionts was associated with persistent low level mortality in juveniles kept in recirculated seawater rearing systems where overall water flow was adequate and outlet water quality measurements acceptable. Further investigations found water flow through certain areas of the culture vessel to be suboptimal.

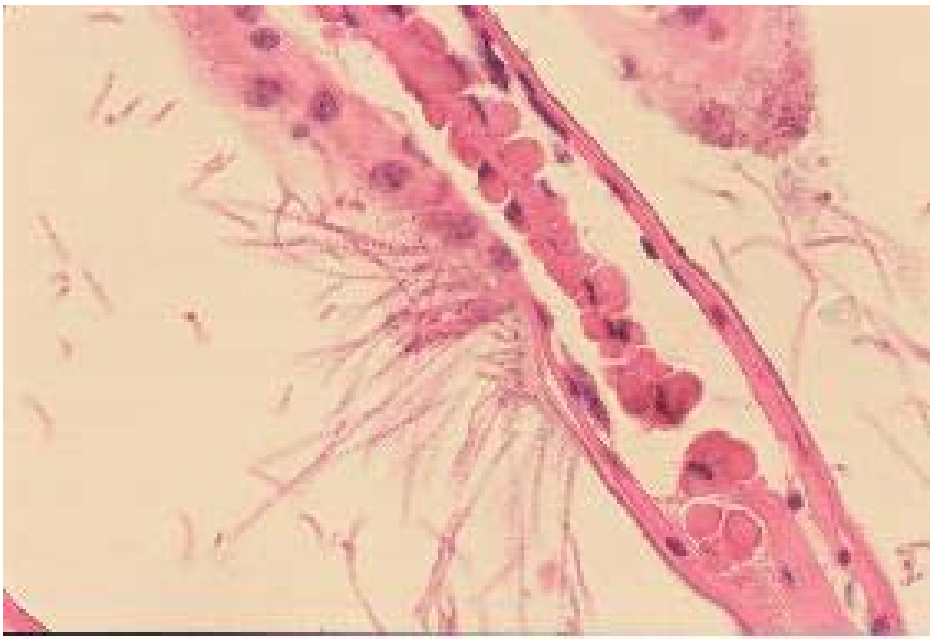


Figure 9 Juvenile. Paraffin section. Fouling of the gill with *Leucothrix*-like filamentous bacteria, x 400.

Shell disease caused by chitinoclastic bacteria and unidentified fungi

Shell disease was a relatively uncommon finding, apparently associated with trauma. Juveniles affected with shell disease developed erosion and/or, blackening of affected areas of the cuticle. Microscopic examination of tissue sections from the affected areas identified both bacterial and fungal infections. Shell disease lesions were most obvious on the ventral part of the tail fan and other areas of the carapace in contact with bottom surfaces subject to injury. The primary cause was assumed to be infection by chitinoclastic bacteria. However, some melanised fungal hyphae have been observed in shell disease lesions following injury.

Bacterial enteritis

Bacterial enteritis was diagnosed in a group of newly caught juveniles. Microscopic lesions were characterised by focal erosion of the digestive tubule epithelia and bacterial colonies within the tubule lumen, but usually without evidence of septicaemia. There was variable haemocytosis around the affected areas. Complete erosion of the epithelium was seen to elicit a granulomatous reaction containing variable numbers of bacteria in the interstitial tissue (Figure 10). Mixed bacteria including low levels of *V. harveyi* were isolated from some animals in this group.

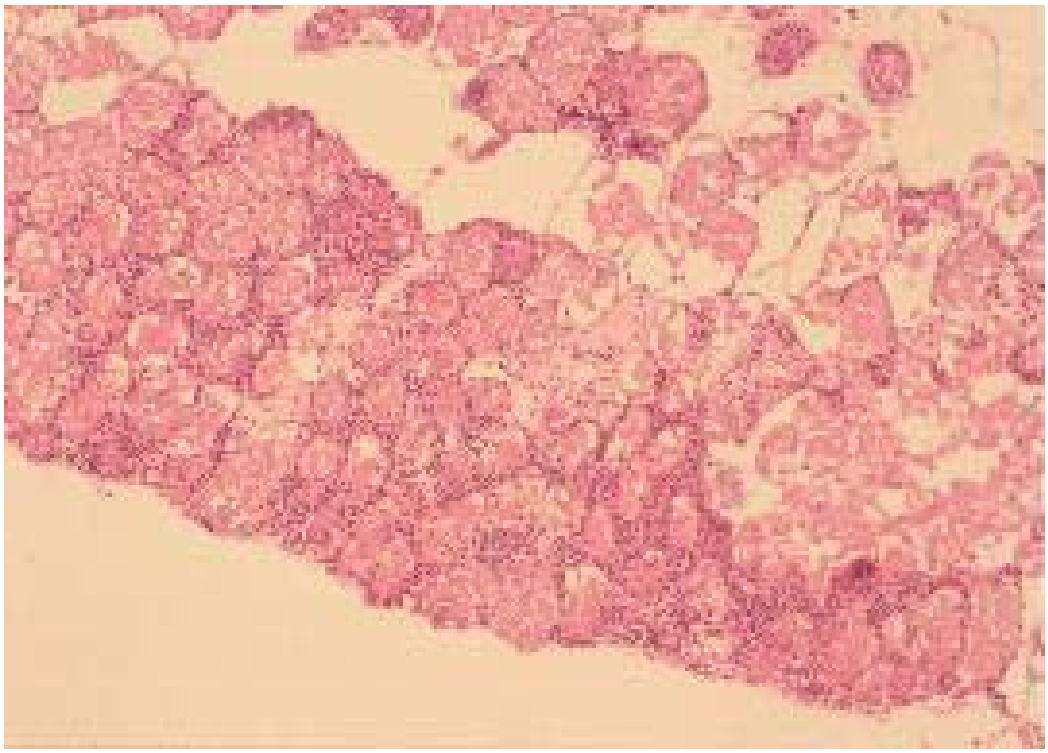


Figure 10 Juvenile. Paraffin section. Extensive degeneration of the digestive tubules accompanying bacterial enteritis. Granulomatous inflammation of the tubules and the interstitial tissues, x 40.

Miscellaneous conditions, dietary associated inappetance

Inappetance of juvenile lobsters was found to be associated with a batch of mussels (Mytilus edulis) used as food. The affected juveniles experienced 3-5% mortality over the period of a month, and would not consume the mussels for eight weeks. There was no evidence of bacterial infection. Histologically, changes were restricted to the digestive gland. The mucosal epithelial cells had variable fine vacuolation. Relatively few B-cells were present and a fine granular pigment was dispersed within the cytoplasm of tall epithelial cells.

IV. DISCUSSION

In this study, there were no significant disease outbreaks and no specific pathogens were seen in either age group. Fouling of the external body was common in both phyllosoma and juvenile lobsters and resulted in sporadic low level mortalities. External infestations with fouling organisms were common and this finding has been reported by other researchers (Sinderman, 1989; Evans & Brock, 1994; Takahashi, 1995; Abraham *et al*., 1996). All diseases reported during this study were of bacterial origin. Disease problems were often associated with elevation of seawater temperatures in juvenile tanks, and uneven water flow leading to locally poor water quality, with lower dissolved oxygen concentration and ammonia buildup. The high stocking densities employed in aquaculture and the associated stress on cultured stock potentiates the outbreak and spread of infectious disease (Evans & Brock, 1994; Aguado & Bashirullah, 1996). Under aquaculture conditions, various stresses caused by high stocking density and resultant environmental pollution with organic matter are known to damage the host defense system with resultant increased susceptibility to infection (Takahashi *et al*., 1995). *Jasus edwardsii* juveniles could tolerate seawater temperatures of 22°C when given sufficient water flow and quality (Thomas *et al*., 2000).

A shell disease syndrome has been reported from many freshwater and marine crustaceans of economic importance (Sindermann, 1989; Smolowitz *et al*., 1992). Signs of the disease syndrome included erosion and pitting of the exoskeleton, resulting from activities of chitin-destroying microorganisms-bacteria and fungi of several genera (Sindermann, 1989). Infection was usually limited to the exoskeleton, although underlying living tissues were occasionally invaded by other opportunistic microbial pathogens (Sindermann, 1989). Exoskeletal erosions are common in lobsters from areas where contaminated sediments occur (Sindermann, 1989). Shell erosion in Crustacea is a particular problem in impoundments, in aquaculture facilities, and in degraded habitats (Sindermann, 1989; Smolowitz *et al*., 1992). It is contagious but its etiology is complex (Sindermann, 1989). The lobsters in this study exhibited the typical defensive mechanisms reported in lobsters elsewhere (Smolowitz *et al*., 1992) of epicuticle deposition, melanisation, inflammatory cell infiltration and pseudomembrane formation.

Fouling with *Leucothrix*-like bacteria was a consistent feature within static culture vessels and was associated with low oxygen levels and high ammonia resulting from poor water flow patterns within holding tanks. Persistent low level mortalities of juvenile lobsters in rearing systems utilising recirculated seawater were associated with moderate to heavy growths of epibionts. Affected juveniles showed sluggish behaviour and foci of light brown colouration in the gills. Most deaths of affected lobsters occurred just prior to or during the moult. It appears likely that heavy epibiont growth reduces respiratory effectiveness, as observed in penaeid shrimp (Lightner, 1983). This may explain why animals die during the moult as oxygen demand in *J. edwardsii* increases at night when moulting usually occurs (Crear & Forteath, 1998). Examination of animals which had been reared in affected systems for varying periods of time showed that the epibionts were gradually accumulated. Scrapes of tank surfaces showed a buildup of organic detritus and moderate to heavy fouling with all of the types of epibionts found on lobster gills, indicating poor system hygiene as the cause of the condition. Poor oxygenation was not always obvious. Fouling associated losses were seen in a system where regular surface cleaning prevented heavy tank fouling and where oxygen concentration at the tank exit was adequate. However, the need to have hides in holding tanks

provided an opportunity for localised areas of poor water flow and hence decreased water quality, especially when the water temperature was high. Losses ceased within 48 h of increasing water flow and aeration. Despite fouling with epibionts being potentially damaging, embryos of the American lobster, *Homarus americanus* are remarkably resistant to infection by the fungus *Lagenidium callinectes*, a pathogen of many crustaceans, because the surface of the embryos are covered almost exclusively by a single gram negative bacterial epibiont (Gil-Turnes & Fenical, 1992).

Vibrio species *V. anguillarum, V. alginolyticus* and *V. tubiashii* were isolated from the exoskeleton and *V. harveyi* from the digestive tubules from lobsters in this study. *Vibrio alginolyticus* and *V. harveyi*-like bacteria were previously isolated from haemolymph and exoskeleton lesions of the spiny lobster, *Panulirus homarus* by Abraham *et al.,* (1996). We were not able to detect bacteremia with routine microbiological methods but were able to demonstrate this with histology. This feature would appear to be explained by the low overall incidence of systemic spread and the fact that not all phyllosoma larvae and juvenile lobsters were submitted for microbiological examination.

The mussels used to feed the juvenile lobsters were sourced from an area that has experienced dinoflagellate blooms in the recent past. The presence of neurotoxins such as those associated with paralytic shellfish poisoning or toxins produced from other species of phytoplankton might have accumulated in these shellfish. The mussels would be expected to have a high tolerance to these toxins and would appear grossly normal. What percentage of the mortalities would have been a direct effect of the toxins is not clear, however, the lobsters would be expected to avoid this diet and this might have culminated in mortalities from starvation. Increased susceptibility to other non-infectious disease states, particularly those caused by inadequate nutrition, would be expected to occur (Evans & Brock, 1994).

REFERENCES

Abraham, T.J., Rahman, M.K. & Joseph, M.T.L. (1996) Bacterial disease in cultured spiny lobster, *Panulirus homarus* (Linnaeus). *Journal of Aquaculture in the Tropics*. **11**; 187-192.

Aguado, N. & Bashirullah, A.K.M. (1996) Shell diseases in wild penaeid shrimps in eastern region of Venezuela. *Journal of Aquariculture & Aquatic Sciences*. **8**; 1-6.

Booth, J. D. (1994) *Jasus edwardsii* larval recruitment off the east coast of New Zealand. *Crustaceana.* **66**; 295-317.

Booth, J. D. & Phillips, B. F. (1994) Early life history of spiny lobster. *Crustaceana,* **66**; 271-294.

Crear, B.J. & Forteath, G.N.R. (1998) A physiological investigation into methods of improving post capture survival of both the southern rock lobster *Jasus edwardsii*, and the western rock lobster *Panulirus cygnus*. FRDC Project 94/134.03, University of Tasmania, Launceston, Tasmania.

Edgerton, B.F. (1996) Viruses of freshwater crayfish. PhD thesis, Department of Biomedical and Tropical Veterinary Science, James Cook University, Townsville.

Edgerton, B. F. & Prior, H. C. (1999) Description of a hepatopancreatic rickettsia-like organism in the redclaw crayfish *Cherax quadricarinatus*. *Diseases of Aquatic Organisms*. **36**; 77-80.

Evans, L.H. & Brock, J.A. (1994) Diseases of spiny lobsters. (Ed. B.F Phillips, J.S Cobb, J. Kittaka), In: Spiny Lobster Management. pp. 461-472, Blackwell Scientific Publications, London.

Gil-Turnes, M.S. & Fenical, W. (1992) Embryos of *Homarus americanus* are protected by epibiotic bacteria. *Biological Bulletin, Marine Biological Laboratory, Woods Hole*. **182**; 105-108.

Hine, P.M. & Jones, J.B. (1994) Bonamia and other aquatic parasites of importance to New Zealand. *New Zealand Journal of Zoology*. **21**; 49-56.

Icely, J.D. & Nott, J.A. (1980) Digestion and absorption: digestive system and associated organs. In, F.W. Harrison, treatise Ed. *Microscopical anatomy of invertebrates*. Vol. 10. F.W. Harrison and G.A. Humes, Eds. Decapod Crustacea. pp 147-201. Wiley-Liss, New York.

Kittaka, J. (1994) Larval rearing. In *Spiny Lobster Management* (Ed. B. F. Phillips, J. S. Cobb and J. Kittaka), pp. 402-423. Blackwell Scientific Publications Ltd, Oxford.

Kittaka, J., Iwai, M. & Yoshimura, M. (1988) Culture of a hybrid of spiny lobster genus *Jasus* from egg to puerulus stage. *Nippon Suisan Gakkaishi.* **54**; 413-417.

Lesser, J.H.R. (1978) Phyllosoma larvae of *Jasus edwardsii* (Hutton) (Crustacea: Decapoda: Palinuridae) and their distribution off the east coast of the North Island, New Zealand. *New Zealand Journal of Marine and Freshwater Research*. **12**; 357-370.

Lightner, D.V. (1983) Diseases of cultured penaeid shrimp. In *CRC Handbook of Mariculture Volume 1 Crustacean Aquaculture.* (Ed. by J. P. McVey and J. R. Moore), pp 289-320. CRC Press, Boca Raton, Florida.

Phillips, B. F. & Sastry, A. N. (1980) Larval Ecology. In *The Biology and Mangement of Lobsters* Vol. 2. (Ed. J. S. Cobb and B. F. Phillips), pp. 11-57. Academic Press, New York.

Schaap, A. (1997) Tasmania looks to rock lobster culture. *Austasia Aquaculture*. **11**; 40-45.

Sindermann, C.J. (1989) Shell disease syndrome in marine crustaceans. U.S. National Oceanic & Atmospheric Administration. Technical Memorandum. 51 pp.

Smolowitz, R.M., Bullis, R.A. & Abt, D.A. (1992) Pathologic cuticular changes of winter impoundment shell disease preceding and during intermolt in the American lobster, *Homarus americanus*. *Biological Bulletin, Marine Biological Laboratory, Woods Hole*. **183**; 99-112.

Takahashi, Y., Itami, T. & Kondo, M. (1995) Immunodefense system of Crustacea. *Fish Pathology/Gyobyo Kenkyu*. **30**; 141-150.

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

Tong, L. J., Moss, G. A., Paewai, M. M. & Pickering, T. D. (1997) Effect of brine-shrimp numbers on growth and survival of early-stage phyllosoma larvae of rock lobster *Jasus edwardsii*. *Marine and Freshwater Research.* **48**; 935-940.

Tail disease in southern rock lobsters (*Jasus edwardsii*)

Ruth E. Reuter[1], Michael C. Geddes[2], Louis H. Evans[3], and Simon R. Bryars[2]

[1]IDEXX Laboratories, 33 Flemington St., Glenside, South Australia, 5065;
[2]University of Adelaide, Adelaide, South Australia 5000;
[3]Aquatic Science Research Unit, Curtin University of Technology, Kent Street, Bentley, Western Australia 6102

ABSTRACT

Wild caught lobsters are commonly kept captive in holding facilities to maximise harvest returns. Tail disease due to chitin-destroying bacteria is considered a major problem overseas, in lobsters held in groups over winter. In South Australia the condition has been seen during the summer period. Tissue samples were collected in 1999 from five groups of lobsters being fed different diets, in sea-based and land-based holding systems at Port Lincoln, South Australia. Lesions were identified in the chitin on the tail and/or claw in 11 animals. Histopathologic examination of lesions showed inflammation often associated with cracks and fissures in the overlying chitin. Of the four samples cultured, *Vibrio alginolyticus* was isolated from all samples, with *Plesiomonas shigelloides* also obtained from one of the samples. *V. alginolyticus* and *Aeromonas hydrophila* had been cultured from similar lesions in lobsters from the same sea-based holding cages one year earlier. These organisms are commonly present in marine and estuarine environments. However it was considered that the handling and holding of the lobsters, in association with elevated water temperatures, could have predisposed them to invasion of damaged tissue by these organisms. *V. alginolyticus* has also been associated with skin damage, ulcers, anaemia, tail and fin disease in finfish, and mortality in eels after handling.

I. INTRODUCTION

Incidents of shell and tail disease in lobsters in holding facilities have been reported since the early 1930s (Hess, 1937). More recently, a survey of lobsters in lobster traps close to sewage disposal sites and relatively unpolluted areas identified a 7.9% incidence of shell disease. However, there was no significant statistical difference in prevalence between lobsters from the two sites (Ziskowski *et al*, 1994). A questionnaire circulated to producers by the Department of Marine Resources, University of Maine, USA revealed that shell disease was prevalent in lobsters in holding facilities, appearing between 3 weeks and 4 months of holding in up to 50% of the facilities surveyed (White, 1999). Chitinolytic bacterial disease of American lobsters (*Homarus americanus*) and shrimps (*Cancer* spp.) has been described as ubiquitous on the east coast of North America and common in lobsters stored in pounds (Bower *et al*, 1994).

The rock lobster fisheries in Southern Australia are a valuable commercial industry. Due to the variation in financial returns depending on season, market forces and other factors, there has been increasing interest in holding live lobsters for extended periods. However there have been problems experienced with mortalities, shell and tail disease, and feeding in these

situations. These factors have resulted in reduced condition and quality of the product. In December of 1997 a small pilot study was instituted to hold lobsters in cages over the Australian summer period, to identify some of the factors of importance in the production of shell and tail disease under these circumstances. Based on this study, an expanded trial was conducted in the 1998 summer period.

II. MATERIAL & METHODS

In November 1998 a feeding trial was initiated at Port Lincoln, South Australia, as part of the FRDC project 98/305 on live-holding of adult *Jasus edwardsii*. One hundred experimental lobsters were separated into 5 treatment groups of 20 animals each. Four of the groups were held in separate compartments in a sea-based cage system. One of these groups was not fed, while the other 3 groups were each fed on one of 3 diets: a hard, dry artificial pellet (SRLD1-98); a soft, moist artificial pellet (SRLD1-98); and live mussels (*Mytilus edulis*). The fifth treatment group was held in a land-based raceway system and fed on the hard, dry artificial pellet diet. The details of the feeding trial will be reported separately.

At the completion of the trial, 10 animals were selected for detailed examination from each of the experimental groups and from a lobster processing facility. The 10 lobsters from the processing facility were used as a control group as they were wild-caught and had not been exposed to long-term holding. The external surface of each lobster was examined for evidence of blisters, pitting, erosions or other damage to the carapace, appendages and tail. Sections from 4 representative lesions from the tails were cultured on 7% horse blood agar, MacConkey's agar, TCBS cholera medium and Saboraud's agar*. The plates were incubated at 24°C. Slices of tissue were placed in 10% seawater formalin, fixed at least 24 hours, processed routinely and stained with haematoxylin and eosin for microscopic examination.

* Oxoid Australia Pty. Ltd., Melbourne, Australia.

III. RESULTS

Gross examination

Examination of the lobsters revealed changes in the tail from blistering of the ventral surface to ragged edges and complete loss of some sections. The exoskeleton exhibited variably sized areas of pitting and erosion on the shell of the claw. Damage of this type was found in 11 of the 60 animals sampled. Affected animals were found in all of the groups.

Bacteriology

Culture of the tail samples yielded a heavy growth of *V. alginolyticus* from all 4 samples and a moderate growth of *Plesiomonas shigelloides* from 1 sample. Both organisms were sensitive to all antibiotics tested. Fungal agents were not identified in the specimens.

Histopathology

Sections taken from 7 samples of tail or shell revealed microscopic cracks and fissures of varying sizes in the superficial chitin, extending into the underlying soft tissue. There was haemorrhage, oedema, collections of haemocytes and, in two cases, small pockets of Gram negative bacteria in the areas of erosion on the surface and inflammation underneath the remaining fragments of cuticle. Thrombosis of blood vessels was evident in two cases.

IV. DISCUSSION

In the Port Lincoln area the water temperatures are highest in late January and February, when they may reach 21-24ºC around the lobster facilities. The water temperatures in the locations from which the lobsters originate usually range from 16-18ºC. In one overseas study, an association between increased water temperature and development of lesions was described (Taylor, 1948). In Tasmania, a seasonal count of *Vibrio* sp. identified in sediment from sites along the east coast peaked in the summer (Cameron *et al*., 1988). *V. alginolyticus* is commonly found in marine and estuarine environments and has been isolated from the water in marine fish tanks (Gilmour, 1977). *Plesiomonas shigelloides*, another member of the Vibrionaceae, has been associated with disease in rainbow trout overseas (Cruz *et al.*, 1986) Numerous species of Gram negative bacteria possess the enzyme chitinase, which destroys the chitin of the shell, resulting in pitting, erosions and severe shell damage. Many of the *Vibrio* sp. are considered secondary opportunists, causing disease only when fish or shellfish are under stress, or when there is already damage to the shell, allowing the bacteria to invade the underlying tissue. High mortality, ulcers or "red spot" in farmed sea bream (Colorni *et al.*, 1981) and eels (Austin & Austin, 1993) have been associated with extensive handling, suggesting the *Vibrio* organisms isolated were opportunists. In the study reported here, many of the lobsters in the holding facilities developed a range of exoskeleton and tail lesions. The splitting and cracking of the chitin seen microscopically could have allowed entrance of bacteria such as *V. alginolyticus*, commonly present in the water. Predisposing factors could have included handling of the animals, the stress of holding resulting in decreased immunocompetence, injury from fighting or abrasions from the cage wire, and elevated water temperatures during the period of holding.

ACKNOWLEDGEMENTS

We would like to acknowledge the assistance of Dr. R. Musgrove, Mrs. A Barnes and Ms. S. Fotedar in sample collection and preparation; and Mrs. K. McLennan for the microbiological examinations.

REFERENCES

Austin, B & Austin, D.A. (1993) Vibrionaceae representatives. In *Bacterial Fish Pathogens; disease in farmed and wild fish*. 2nd ed. pp 265-307.

Bower, S., McGladdery, S.E. & Price, I.M. (1994) Chitinolytic bacterial disease. In Synopsis of infectious diseases and parasites of commercially exploited shellfish. *Annual Review Fish Disease* **4**,129-130.

Cameron, D.E., Garland, C.D., Lewis, T.E., & Machin, P.J. (1988) A survey of Vibrionaceae in Tasmanian coastal waters, with special reference to bacterial species pathogenic to fish or shellfish. *Australian Journal Marine Freshwater Research* **39**, 145-152.

Colorni, A., Paperna, I. & Gordin, H. (1981) Bacterial infections in gilthead sea bream *Sparus aurata* cultured in Elat. *Aquaculture* **23**, 257-267.

Cruz, J.M., Saraiva, A., Eiras, J.C., Branco, R., & Sousa, J.C. (1986) An outbreak of *Plesiomonas shigelloides* in farmed rainbow trout, *Salmo gairdneri* Richardson, in Portugal. *Bulletin of European Association of Fish Pathology* **6**, 20-22.

Gilmour, A. (1977) Characteristics of marine vibrios isolated from fish farm tanks. *Aquaculture* **11**, 51-62.

Hess, E. (1937) A shell disease in lobsters (*Homarus americanus*) caused by chitinovorous bacteria. *J. Biol. Board. Can.* **3**, 358-362.

Taylor, C.C. (1948) Shell disease as a mortality factor in the lobster (*Homarus americanus*). *Maine Department Sea Shore Fisheries, Fisheries Circular* 4,1-8.

White, S. (1999) Shell disease survey produces preliminary results. *Lobster Bulletin*, Lobster Institute, University of Maine, Orono, USA **13**, 4.

Ziskowski, J., Spallone, R., Kapareiko, D., Robohm, R., Calabrese, A., & Pereira, J. (1994). Shell disease in American lobster (*Homarus americanus*) in the offshore, Northwest-Atlantic region around the 106-mile sewage-sludge disposal site. Abstr. Marine Environmental *Engineering*. The Gordon and Breach Publishing Group. OPA (Overseas Publishers Association) Amsterdam B.V.

Health aspects in Norwegian lobster stock enhancement

Knut E Jørstad, Øivind Bergh and Kari Andersen*
Institute of Marine Research, P O Box 1870, N-5817 Bergen, Norway

*Corresponding author. Tel.: 47 55236347. Fax: 47 55236379
E-mail: knut.joerstad@imr.no

ABSTRACT

Large-scale releases of artificially produced organisms into the marine environment might represent an increased risk to spread diseases. In the national Norwegian sea-ranching program, run from 1990 to 1998, a requirement was to examine broodstock and juveniles for possible diseases before permission to release was given. In the enhancement project with European lobster, *Homarus gammarus*, in southwestern Norway the only potential disease associated with lobster was gaffkaemia, caused by the pathogen *Aerococcus viridans*. This disease is commonly known in American lobster, *Homarus americanus*, and has also spread to Europe possibly through commercial import of live specimens. It was first reported in imported American lobster in Norway in 1976, and extensive investigations in wild populations from 1981 to 1984 concluded that the pathogen was not endemic in Norwegian waters. In general, fish disease problems associated with the Norwegian aquaculture industry has required a more comprehensive legislation, and a new law was established in 1998. According to current regulations, all diseases in farmed and wild organisms, including the marine environment and lobsters, must be reported to the veterinary authorities. Proposals to establish lobster hatcheries for stock-enhancement and farming activities must be carefully evaluated before permission is given.

INTRODUCTION

Lobster fisheries in southern Norway have long traditions and have for several centuries represented a significant activity in coastal areas. Large fluctuations in the harvest have been observed. The annual catch was around 1,000 metric tonnes in the 1930's (Dannevig, 1936), and at that time this was the largest lobster fishery in Europe contributing to 30 to 40% of total harvest in this region. Some years after the World War II the landings fluctuated between 600 and 700 tonnes followed by a collapse in the lobster fishery between 1960 and 1970 (Fig. 1). After the depletion of the stocks, the total lobster harvest has in Norway officially only been about 30 tonnes annually.

The decrease in the Norwegian lobster stocks led to an increased interest in importing live lobsters from other lobster fisheries in the world. Already in the late 1950's, commercial interests in western Norway established contact to import lobsters of Scottish origin to Norway. Even American lobsters (*Homarus americanus*) were imported from the western Atlantic (Staveland & Kjos-Hansen, 1978; Egidius, 1978) and this practice continues. Both kinds of activities represent increased risks to introduce diseases, which potentially could spread to the wild populations in Norway.

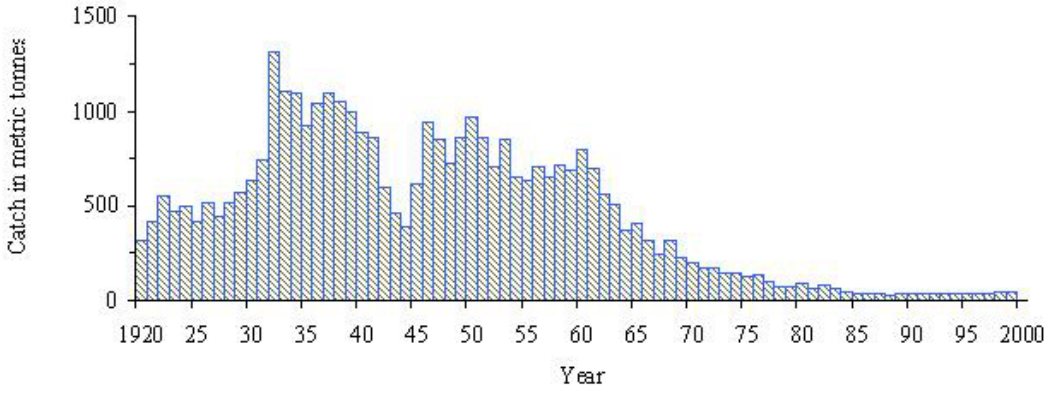


Figure 1 Lobster landings in Norway
Data obtained from Directorate of Fisheries. A minor increase in the landings has been observed in latest years.

Focus has also been given to culture activities to supplement lobster stocks, and already in the mid 1880's. G.M. Dannevig hatched larvae of the European lobster *(Homarus gammarus)* and obtained settlement (Appeløf, 1909). A large holding facility was established at Kvitsøy in southwestern Norway in one of the major fishing areas, as part of local stock enhancement based on releasing larvae. Evaluation of the effect of the early activities was quite controversial; in the last decades more attention has been placed on producing larger lobster juveniles for release. These activities were based on the large-scale lobster hatchery at Kyrksæterøra built by the commercial company Tiedeman in the early 1980's.

The purpose of this paper is to summarize the Norwegian experiences of the health situation in European lobsters and discuss health aspects in ongoing lobster-enhancement programs. In addition, the new law regulations are evaluated in relation to the development of future large-scale lobster-hatchery operations.

Introduction of Gaffkemia in Norwegian Lobster

Gaffkemia is a lobster disease caused by the bacterium *Aerococcus viridans* (for review see Stewart, 1980), and at higher summer temperatures this infection often causes high mortalities in lobster-holding ponds. The disease is well known and described in the American lobster, especially in holding ponds, and infected individuals are also found, though at variable frequencies, in wild populations (Stewart *et al.*, 1966).

According to Egidius (1972), high mortalities were observed in lobsters imported from Norway to Netherlands in 1957. Screening of healthy lobsters from holding ponds in Norway, however, revealed no evidence of the pathogen. Injection of the bacterium in controlled experiments caused 100% mortality. The first reported outbreak of gaffkemia in Norway occurred in 1976, when about 500 American lobsters were imported and held in a lobster pond in Stavanger, southwestern Norway (Håstein *et al.*; 1977; Egidius, 1978). During the first week after arrival, about 23% died, first believed to be due to polluted water. High mortalities continued even after transferring the lobster to other areas, and gaffkemia was later diagnosed as the cause. In the same area and ponds, several new outbreaks of this

disease were reported in 1977 and 1980. The disease is therefore believed to have been introduced through the import of the American lobsters as described.

Clearly, the outbreaks of the disease in holding ponds could lead to further spread of the pathogen into the wild populations in the Rogaland region. A large-scale screening program of wild lobsters from the area was initiated in 1981, and in four years, more than 3,000 specimens were analyzed (Wiik *et al*., 1987). Only one specimen, collected in 1981, was confirmed infected by the pathogen. No infected lobsters were detected in any other years. The pathogen was also isolated from sediments taken from one of the lobster ponds about 4 months after infected lobsters had been removed.

From studies with DNA-DNA hybridization, Wiik *et al*. (1986) concluded that the lobster pathogenic strains isolated from *H. gammarus* from Norway and the UK had high similarity to strains isolated from *H. americanus* (80 to 100% homology), despite intra-group variations in fermentative capabilities. This observation supports the view that the disease is introduced. The virulent *A. viridans* strains could be separated from other Gram positive marine cocci by means of DNA-DNA homology and the characteristic ability to form tetrads. A modified selection-enrichment isolation method was developed which was effective in detecting the pathogen in various samples from lobsters. Only two virulent strains were isolated from a dead lobster, and one from the sediments of a lobster pond (Wiik *et al*. 1987). The last observations demanded total disinfecting of the lobster-holding ponds after the outbreaks of gaffkemia.

Table I Pathogen screening *(Aerococcus viridans)* in wild caught Norwegian lobster in western Norway in the years after gaffkemia outbreaks in 1976. (Wiik *et al*., 1987).

Sampling time	Location	No. analyzed	Gaffkemia
1981, Dec	Kvitsøy	200	0
1981, Dec	Stavanger	579	1
1982, Dec	Kvitsøy	697	0
1983, June	Kvitsøy	297	0
1983, Dec	Kvitsøy	395	0
1984, Oct	Kvitsøy	876	0

The Large-Scale Lobster Hatchery

With regards to cultivation of lobsters, a new direction took place in the 1970's (see review, Aiken & Waddy, 1995). In Norway, S. Grimsen and J.G. Balchen at the Norwegian Institute of Technology in Trondheim, established co-operation with the commercial company Tiedemans and successfully raised about 1 year old lobster juveniles of *Homarus gammarus* for release purposes. As a result, a large-scale lobster hatchery was built at Kyrksæterøra (Grimsen *et al*., 1987) in connection to a thermal effluent from a ferro-silican smelting plant (Hølla Smelting Work). During the period from 1979 to 1987, a large number of lobster juveniles were produced and released along the Norwegian coast.

The hatchery was based on supply of wild-caught berried females purchased from fisherman and transferred to Kyrksæterøra for hatching. When the larvae reached the development stage IV, the last pelagic stage before settling, they were transferred to lobster trays with separate compartments. Each tray contained 120 individuals and was placed into a large circular pool with a diameter of 50m. The pool was divided into 11 concentric rings, and the trays circulated within each ring.

The on-growing system in the hatchery was designed to a maximum production of 120,000 juveniles per year. A detailed description of the nursery, and the experiences with hatchery operations, is given by Uglem & Grimsen (1994). From the period the commercial company operated the hatchery, little information is available about stock health and practical approaches to prevent spread of potential pathogens within the hatchery and the on-growing system, where broodstock were held and the juveniles were later raised. The water supply was shared between these two facilities. The system was therefore sensitive with respect to spread of introduced pathogens.

Figure 2 Kyrksæterøra Lobster Hatchery. (a) Section of concentric rings with floating lobster trays and (b) Lobster tray with individual compartments for juvenile lobster rearing.

The Institute of Marine Research in Bergen took over the large-scale Kyrksæterøra Lobster Hatchery in 1989, and European lobster was included as one of four species within the national Norwegian Ranching Program (PUSH), initiated in 1990. During the period from 1990 to 1994, the hatchery produced lobster juveniles for release purposes. The last release took place in 1994, and thereafter the hatchery was closed down.

The transport of wild-caught berried females to the hatchery at Kyrksæterøra involved some health risk. In order to reduce vertical transfer of diseases, experiments were conducted with various surface disinfection procedures for lobster eggs. The results indicated that the treatment with $150mg1^{-1}$ iodine could reduce bacterial growth on lobster eggs, but it was not possible to obtain complete surface disinfections. As this treatment could lead to decreased viability of larvae, it could only be recommended when massive egg mortality due to epibiotic bacteria was otherwise unavoidable (Uglem *et al*., 1996).

The Releases at Kvitsøy

The juvenile lobsters produced at the Kyrksæterøra hatchery were tagged with a micro-magnetic tag (Uglem & Grimsen, 1995), and transported to the release area at the Archipelago of Kvitsøy where they were released, mainly by local fishermen. During the period from 1990 to 1994, a total of 128,000 juveniles were released, of which 125,600 were tagged. The commercial landings in the release area were later closely monitored every year. The results showed that the fraction of cultured lobsters in the catches had increased to about 60% by 1998 (Agnalt *et al*., 1999).

Table II Screening of gaffkemia in samples of broodstock and lobster juveniles in relation to production of lobster juveniles for release purposes within the Norwegian Ranching Program 1990 – 1994

Year	Date	Location	Stage	Number Investigated	Gaffkemia Positive
1992	17.01	Kyrksæterøra	Juveniles	32	0
1992	01.06	Kvitsøy	Broodstock	145	0
1993	16.02	Kyrksæterøra	Juveniles	35	0
1993	30.03	Kyrksæterøra	Juveniles	39	0
1993	06.07	Lindå	Broodstock	12	0
1993	24.11	Kyrksæterøra	Juveniles	40	0
1993	24.11	Kvitsøy	Broodstock	30	0
1994	22.02	Kyrksæterøra	Juveniles	45	0
1994	18.04	Kyrksæterøra	Juveniles	30	0
1994	07.06	Kvitsøy	Broodstock	20	0

All enhancement experiments within the Norwegian Ranching Program had to act in accordance with national veterinarian requirements. In the lobster project, test samples were taken from the bloodstock and analyzed for gaffkemia infection. The results for some of these samples are summarized in Table 2. No positive specimens were found. Disease screening was a necessary requirement for transporting the wild, berried females from the Kvitsøy Islands to the Kyrksæterøra hatchery, and to obtain permission to release the cultivated juveniles into the wild environment. No positive samples were detected in the sub samples of the lobster juveniles released at Kvitsøy.

New Veterinary Law (1998)

During the last decades, the rapid developing salmon industry in Norway has experienced a number of health and disease problems. In order to control and reduce these problems, the veterinary authorities have developed strict regulations, and a new law covering all aquaculture activities, including marine species, was established in 1998. The purpose of the new legislation is to reduce disease problems in aquaculture industry, as a basis for developing an economically sustainable enterprise for the future. The new law has strict regulations and requirements from the veterinary authorities at different levels including:

1) Local veterinarian (and the farmer) performing health survey at the aquaculture farm;

2) The District and County veterinary authorities which could take legal actions according to the current law when there is an outbreak of a disease; and

3) The national veterinary authorities and the Directorate of Fisheries currently monitoring the health situation in the industry.

At present, gaffkemia is listed in the "B" class of fish/shellfish diseases. In case of a positive diagnosis of an infection in a farm in this disease category, the veterinary authorities give information to the Directorate of Fisheries. The law prohibits transfer of animals from the farm unless the County veterinary authorities have given written permission. Under normal conditions, plans must be developed and implemented with regards to controlled slaughter of the animals and disinfection approaches on the lobster farm/facility. Based on an evaluation of the risks for further spread of the pathogen, however, the veterinarian authorities have the option of imposing a "stamping out" strategy to prevent the disease from spreading.

There are, at present, no commercially operated lobster hatcheries in Norway, but there is interest in establishing such. The local community at the Kvitsøy Islands is now attempting to build a local lobster hatchery to continue to release lobster juveniles, focusing on enhancement of the local stock. Some small-scale lobster farming trials are being carried out as well as several experiments with research institutions. All the experiments carried out so far have used the local broodstock. This strategy is aimed at reducing the risk for introducing pathogens from other regions. Clearly, future developments towards a commercial operated lobster hatchery will require an all over evaluation of technical approaches from a health / disease point of view by the veterinary authorities.

Similarly, the commercial company "Norwegian Lobster AS" is now planning to establish an industrial scale lobster hatchery based on warm cooling water on Tjeldbergodden in mid-Norway. This new hatchery is a direct continuation of the Kyrksæterøra Lobster Hatchery described above. The automated industrial facility is planned to produce up to 2.4 million lobster juveniles (age 9 – 21 months) annually. In this scale of production, wild berried females have to be collected from a large geographic area and transported to the hatchery. The present plans represent interesting technology, but also involve large challenges with respect to lobster health and disease questions.

ACKNOWLEDGEMENTS

This work was supported by Norwegian Research Council travel grant to KEJ during sabbatical at CSIRO Marine Laboratories in Hobart, Tasmania. We are also indebted to Ann-Lisbeth Agnalt for valuable critiques and linguistic corrections.

REFERENCES

Agnalt, A.-L., van der Meeren G.I., Jørstad, K.E., Næss, H., Farestveit, E., Nøstvoll, E., Svåsand, T., Korsøen, E. & Ydstebø, L. (1999) Stock enhancement of European lobster (*Homarus gammarus*): A large scale experiment off south-western Norway (Kvitsøy). In: *Stock Enhancement and Sea Ranching* (Ed. by B. Howell, E. Moksness, & T. Svåsand) pp.401 – 419. Fishing News Books, Blackwell Science Oxford, UK.

Aiken, D.E. & Waddy, S.L. (1995) Aquaculture. In *Biology of the lobster*. (Ed. by J.R. Factor) pp 153 – 175. Academic Press, New York.

Appeløf, A. (1909) Undersøkelser over hummeren (*Homarus vulgaris*) med særskilt hensyn til dens optræden ved Norges kyster. (Investigations of lobster (*Homarus vulgaris*) with special emphasis on its behaviour along the Norwegian coast. Efter foranstaltning av Stavanger filial av selskapet for de Norske Fiskeries fremme. A.S. John Griegs Boktrykkeri, Bergen (in Norwegian).

Dannevig, A. (1936) Hummber og hummerkultur (Lobster and lobster culture). *FiskeriDirektoratets Skriftlige Serie HavUndersøkelser*, IV. No. 12 (in Norwegian).

Egidius, E. (1972) On the internal bacterial flora of the European lobster (*Homarus vulgaris* L.) and it susceptibility to Gaffkemia. *Aquaculture*, **1**, 183 – 197.

Egidius, E. (1978) Lobster import: two outbreaks of Gaffkemia in Norway, *International Council Exploration Sea*, Council Meeting 1978/ **K: 17**, 1–5.

Grimsen, S., Jaques, R.N., Erenst, V. & Balchen, J.G. (1987) Aspects of automation in a lobster farming plant. *Modeling, Identification and Control, Norway*, **8**, 61–68.

Håstein, T., Roald, S.O., Kjos-Hansen, B. & Staveland, K. (1997) Occurrence of Gaffkemia in lobsters in Norway. *Acta Veterinary Scandinavia*, **18**, 138–139.

Staveland, K. & Kjos-Hansen, B. (1978) Gaffkemia på norsk hummer i hummerparker. *Norsk veterinær-tidskrift*, **90**, 101–106 (in Norwegian).

Stewart, J.E. (1980) Diseases. In: *The biology and management of lobsters*, (Ed. by J.S. Cobb and B.F. Phillips). Vol 1, pp. 301–342. Academic Press, New York,

Stewart, J.E., Cornick, J.W., Spears, D.I. & McLeese, D.W. (1966). Incidence of Gaffkemia homari in natural lobster (*Homarus gammarus*) populations in the Atlantic Region of Canada. *Journal Fisheries Research Board Canada*, **23**, 1325–1330.

Uglem, I. & Grimsen, S. (1994) Large scale production of juvenile lobster; a commercial approach. *Progressing, Workshop and Training Course on Biology, Management, Cultivation and Stock Enhancement of Lobsters*. Galway, Ireland, April, 1994.

Uglem, I. & Grimsen, S. (1995) Tag retention and survival for lobster juveniles (*Homarus gammarus* (L.)) marked with coded wire tags. *Aquaculture Research*, **26**, 837– 841.

Uglem, I., Uksnøy, L.E. & Bergh, Ø. (1996) Chemical treatment of lobster eggs against epibiotic bacteria. *Aquaculture International*, **4**, 1–8.

Wiik, R., Egidius, E. & Goksøyr, J. (1987) Screening of Norwegian lobsters *Homarus gammarus* for the lobster pathogen *Aerococcus virdans*. *Diseases of Aquatic Organisms,* **3**, 97–100.

Wiik. R., Torsvik, V. & Egidius, E. (1986) Phenotypic and genotypic comparisons among strains of the lobster pathogen *Aerococcus viridans* and other marine *Aerococcus viridans*-like cocci. *International Journal of Systematic Bacteriology*, **36**, 431–434.

Measures of condition in dietary studies on western rock lobster post-pueruli

E. Tsvetnenko[1], J. Brown[2], B.D. Glencross[1] and L.H. Evans[1]*

[1]Aquatic Science Research Unit, Muresk Institute of Agriculture, Curtin University of Technology, Suite 3, Enterprise Unit 1, 11 Brodie Hall Drive, Technology Park, Bentley WA 6102
E-mail: tsvetnee@muresk.curtin.edu.au
[2]Fisheries Western Australia, Fremantle Maritime Centre, Fleet Street, Fremantle, WA 6160
*Corresponding author

ABSTRACT

A nutritional study was conducted for nine weeks on western rock lobster post-pueruli fed either fresh mussel diet (D1) or one of four artificial diets, two in moist (D2 and D3) and two in dry (D4 and D5) pelleted form. Growth rates, expressed as average daily gain and specific growth rate, and condition indices were determined for all treatment groups. At the commencement of the experiment the moisture content of the digestive gland and tail muscle and the hepatosomatic and muscle-somatic wet and dry indices were determined. At the end of the experiment animals from each treatment were examined for the same parameters. In addition, at the end of the experiment the lipid content of the digestive gland and tail muscle was determined in animals from all treatment groups. Clotting time, total number of hemocytes and percent of granulocytes were also determined in hemolymph samples from animals fed diets D1 and D2.

Lobsters fed the natural mussel diet grew significantly faster than those fed the artificial diets. Changes in the digestive gland and muscle indices indicated deterioration of the animals' condition during the course of the trial in all treatments. This deterioration was more pronounced in lobsters fed artificial diets than in lobsters fed mussel diet. While there were no significant differences between growth rates in animals fed artificial diets, significant differences were observed in several of the condition indices. The hemolymph parameters, total hemocyte count and proportion of granulocytes, were consistent with tissue indices. The use of condition indices for assessing lobster nutritional and health status is discussed.

Key words: condition, lobster, nutrition, aquaculture

I. INTRODUCTION

The western rock lobster is recognised as an extremely valuable commercial species, the value of its fishery being estimated at three hundred million dollars a year (Fisheries Department of Western Australia 1996). To protect this valuable commodity and increase the production, the idea of culturing the western rock lobster has become increasingly attractive. While aquaculture of other crustacean species has become relatively well established, aquaculture of western rock lobster is still in its infancy. To achieve progress in this area, the development of an adequate artificial diet and adequate culturing environment is required, together with the instruments that would allow assessment of condition of cultured animals. While the

assessment of morphological features collectively referred to as "condition" is readily achieved for vertebrate animals, condition status in crustaceans is difficult to determine from the external appearance or total weight (Dall 1974; Aiken & Waddy 1992). Despite this, certain measures of condition have been developed, such as organosomatic indices (Schrif *et al*. 1987; Evans *et al*. 1992; Jussila and Mannonen 1997), or blood protein concentrations (Dall 1974; Huner *et al*. 1990).

The aim of this study was to evaluate the use of organosomatic and hemolymph parameters as condition indices in nutritional studies on western rock lobster post-pueruli.

II. MATERIALS AND METHODS

Experimental Animals

One hundred and ninety three post-puerulus juvenile rock lobsters were collected during January from various locations from Lancelin to Shark Bay north of Perth. They were held in a tank at the Fisheries Marine Research Laboratories for a period of two months to acclimatise to laboratory conditions. The tank formed part of a seawater flow-through system using ocean waters of the Marmion Marine Reserve. During the acclimatisation period the lobsters were fed a diet of mussels (*Mytilis edulis planulatis*) and prawn pellets (Lucky Star).

Experimental Design

Ninety juvenile lobsters with body weight ranging between 0.8 and 2 grams were selected for a 5-treatment randomised block experiment with three replicates. Six animals were randomly assigned to each replicate.

Six 390-litre (655 x 375 x 1575 mm) rectangle tanks were arranged in three blocks of two. Each tank contained two equally distanced dividers separating the tank into three equal 130-litre compartments. No water mixing occurred between each of the compartments. Each compartment was provided with gravity-fed and temperature controlled (23ºC) flow-through filtered seawater (60 mL/minute/tank). Each compartment was individually aerated and contained two clay bricks with holes for sheltering lobsters.

The five treatment groups were represented by lobsters fed control diet (D1), consisting of fresh mussels (*Mytilus edulis planulatis*); artificial moist pelleted diet D2; artificial moist pelleted diet D3; artificial dry pelleted diet D4 with a formulation similar to D2 and artificial dry pelleted diet D5 with a formulation similar to D3. Diets were supplied by the CSIRO Division of Marine Research, Cleveland, Queensland. The proximate composition of the diets is shown in Table I. The diets were fed *ad libitum* (about 1-2% of biomass) twice daily during the 9-week experiment. Animals were individually weighed at 3-weekly intervals.

Table I Proximate composition of the experimental diets

Parameter	D2*	D3*	D4**	D5**
Dry matter (%)	60.0	60.0	90.0	90.0
Dig. energy (MJ/kg)	9.0	10.0	13.6	15.1
Crude protein (%)	30.1	33.8	45.3	50.6
Crude fat (%)	4.8	7.4	7.2	11.1

*Semi-moist pellets
** Dry pellets

Growth Performance Parameters

Growth rates were calculated by the following equations:

Average Daily Gain (ADG; g/d) = (final weight – initial weight)/growing period

Specific Growth Rate (SGR; %/d) = 100 x (ln final weight – ln initial weight)/growing period

Condition Parameters

At the commencement of the experiment, an additional 10 animals with body weights falling within the same range as experimental animals, were dissected to determine moisture content of digestive gland and tail muscle, hepatosomatic index (HSI), wet and dry, muscle-somatic index (MSI), wet and dry and lipid content of digestive gland and tail muscle

Moisture content of both tissues was determined by drying for 18 hours at 105ºC and reweighing, the difference between wet and dry weights being taken as moisture content and expressed as a percentage of total organ wet weight. Hepatosomatic index (HSI), wet, and muscle-somatic index (MSI), wet, were expressed as the weight of the wet digestive gland and the weight of wet tail muscle, respectively, as a proportion of the weight of the whole animal. Hepatosomatic index (HSI), dry, and muscle-somatic index (MSI), dry, were expressed as the weight of dry digestive gland and weight of dry tail muscle, respectively, as a proportion of the weight of the whole animal.

At the end of the experiment a similar number of animals from each treatment were examined for the same parameters.

In addition, at the end of the experiment the lipid content of the digestive gland and tail muscle was determined in animals from all treatment groups. Total lipid content (% of dry matter) was determined gravimetrically following a methanol-chloroform (1:2 v/v) extraction technique used previously on adult western rock lobster digestive gland (Tsvetnenko *et al*. 1996). Clotting time, total number of hemocytes and percent of granulocytes were determined in hemolymph samples from animals fed D1 and D2. Clotting time was determined by a

method based on a combination of those used by Battele and Kravitz (1978), Kopacek *et al*. (1993) and Evans *et al*. (2000). Hemolymph was withdrawn from a pericardial sinus and a sample immediately collected into a haematocrit tube. The tube was continually inverted until the clotting process had finished (ie. the haemolymph became motionless) and the time was recorded. Total number of circulating hemocytes (THC) were estimated in the hemolymph collected into sodium cacodylate-glutaraldehyde anticoagulant and diluted with physiological saline solution. This mixture was loaded onto a hemocytometer with improved Neubauer ruling, and hemocytes were counted at 200X magnification. THC was expressed as number of cells in 1 mL of hemolymph. Granulocytes were counted in the hemolymph smears following air-drying, fixation in the methanol and staining by the May-Grunwald/Giemsa technique.

Statistical Analysis

All data were analysed using one-way analysis of variance (ANOVA) and multiple comparisons among treatment means were made with the Tukey-HSD test using a statistical analysis software program SPSS (SPSS, 1995). Results were considered statistically significant at $P < 0.05$.

III. RESULTS

Results of the feeding trial are summarised in the Table II. ADG and SGR of lobsters fed the natural mussel diet D1 were significantly higher than those of lobsters fed the artificial diets. There were no significant differences between lobsters fed four artificial diets.

Table II Growth rates and condition indices in lobsters fed five different diets (Mean ± SE)

Parameter	Initial	D1	D2	D3	D4	D5
ADG (g/day)	-	0.097 ± 0.004^{a}	0.026 ± 0.001^{b}	0.026 ± 0.002^{b}	0.027 ± 0.002^{b}	0.027 ± 0.002^{b}
SGR (%/day)	-	2.64 ± 0.01^{a}	1.22 ± 0.05^{b}	1.25 ± 0.10^{b}	1.26 ± 0.13^{b}	1.29 ± 0.12^{b}
HSI_{wet}	6.11 ± 0.22^{a}	5.01 ± 0.42^{ab}	4.25 ± 0.21^{b}	4.73 ± 0.21^{b}	4.61 ± 0.27^{b}	5.01 ± 0.32^{ab}
HSI_{dry}	2.36 ± 0.24^{a}	1.48 ± 0.15^{b}	1.23 ± 0.10^{b}	1.18 ± 0.07^{b}	1.38 ± 0.14^{b}	1.46 ± 0.13^{b}
MSI_{wet}	23.82 ± 0.65^{a}	18.47 ± 0.97^{b}	15.13 ± 0.48^{c}	15.92 ± 0.55^{bc}	15.19 ± 0.44^{c}	17.05 ± 0.92^{bc}
MSI_{dry}	6.21 ± 0.27^{a}	4.57 ± 0.30^{abc}	3.73 ± 0.33^{bc}	3.36 ± 0.27^{b}	3.33 ± 0.18^{b}	4.92 ± 0.66^{ac}
Digestive gland moisture (%)	65.85 ± 6.78^{a}	70.86 ± 1.63^{ab}	71.31 ± 1.38^{ab}	75.12 ± 1.00^{b}	70.53 ± 1.36^{ab}	71.32 ± 1.34^{ab}
Muscle moisture (%)	73.97 ± 0.80^{a}	75.36 ± 0.51^{ab}	75.28 ± 2.11^{ab}	79.07 ± 1.27^{b}	78.04 ± 1.05^{ab}	71.97 ± 2.35^{a}
Digest.gland lipid (%)	N/D	14.49 ± 2.09^{a}	14.52 ± 4.17^{ac}	27.51 ± 7.01^{bc}	31.02 ± 6.32^{b}	15.10 ± 0.56^{ab}
Muscle lipid (%)	N/D	4.60 ± 0.40^{ab}	5.06 ± 1.37^{ab}	9.28 ± 4.57^{a}	3.14 ± 0.40^{bc}	2.06 ± 0.18^{c}
Clotting time (sec)	N/D	49.5 ± 3.3^{a}	51.3 ± 5.0^{a}	N/D	N/D	N/D
THC x 10^{6} cells/mL	N/D	8.05 ± 1.30^{a}	5.20 ± 0.80^{a}	N/D	N/D	N/D
% granular cells	N/D	2.96 ± 0.64^{a}	5.05 ± 0.55^{b}	N/D	N/D	N/D

ADG and SGR data are means of three tanks ± SE. Hepatosomatic and musclesomatic indices, % moisture, clotting time, THC (x 10^{6}) and % granular cells data are means of ten animals ± SE. % lipid data are means of five pooled samples ± SE. Values in the same row with different superscripts are significantly different ($P < 0.05$). N/D = not determined.

There were no significant differences in wet hepatosomatic indices of lobsters fed all diets. However, only in lobsters fed mussels (D1) and diet D5 this index did not change significantly from the initial level, while in other treatment groups the index decreased by the end of the experiment. The dry hepatosomatic indices were not significantly different among all treatment groups, but they were significantly lower than in initial lobsters.

The wet muscle-somatic index decreased by the end of the experiment in lobsters fed all diets. Lobsters fed diets D2 and D4 had significantly lower wet muscle-somatic index than lobsters fed control diet (D1). The dry muscle-somatic index decreased significantly in lobsters fed diets D2, D3 and D4, while in mussel and diet D5 fed lobsters it did not change from initial level.

Moisture content in the digestive gland of lobsters fed diet D3 was significantly higher than in initial lobsters. Similarly, lobsters fed diet D3 had the higher level of moisture in the muscle tissue compared with initial lobsters.

The highest lipid content (% of dry matter) was observed in the digestive gland of lobsters fed diets D3 and D4. However, lobsters fed diet D4 differed significantly in this parameter from lobsters fed diets D1 and D2, while lobsters fed diet D3 differed significantly only from lobsters fed D1 (mussels). In the muscle tissue the highest lipid content was observed in lobsters fed diet D3, although it differed significantly only from that in lobsters fed diets D4 and D5.

The hemolymph parameters of clotting time, THC and percentage of granulocytes were determined at the completion of the trial only in lobsters fed two diets, mussel diet D1 and artificial diet D2. The clotting time did not differ in lobsters fed the two diets. The total number of hemocytes was lower in lobsters fed the artificial diet than in lobsters fed mussels, but the difference was not statistically significant. The percent of granular cells was significantly higher in lobsters fed the artificial diet than in lobsters fed mussels.

IV. DISCUSSION

The growth rate results obtained in this experiment for lobsters fed mussel diet were similar to those reported by other authors for *Panulirus cygnus* reared in aquaria (Phillips *et al*. 1977, 1983, 1992). Lobsters fed the natural mussel diet (D1) grew significantly faster than those fed the artificial diets. These results agree with previous research in that natural diets produce significantly better growth rates in lobsters than artificial feeds (Jones *et al*. 1997; Kreider and Watts 1998). Western rock lobsters naturally eat molluscs in the wild and supplement this feed with coralline algae (Edgar 1990; Jernakoff *et al*. 1993).

Growth rates, although very important indicators of diet suitability in the lobster culturing process, do not provide a complete assessment of diet performance. Condition indices such as the ratios of tail muscle or digestive gland to body weight, tissue moisture content or lipid reserves can provide a further indication of the nutritional status and energy reserves of the animal (Mannonen and Henttonen 1995; McClain 1995a,b; Jussila and Mannonen 1997; Musgrove 1997). In addition, moisture and lipid content of the fish muscle are important

determinants of the quality of seafood for the consumer (Pigott and Tucker 1990; Ackman 1995; Paterson *et al*. 1997).

Low dry indices at the end of this feeding trial might indicate the depletion of energy reserves both in the digestive gland and in the tail muscle during the course of experiment. This depletion was mostly expressed in D3 fed lobsters, which had significantly higher moisture content in their digestive gland and muscle tissue than the initial lobsters. Comparison of lobsters at the trial conclusion to the initial lobsters sampled before the trial began shows that the condition of the lobsters had deteriorated by the end of the trial. This was especially true for lobsters fed artificial diets, in particular diet D3. Lobsters fed diet D5 were in marginally better condition than lobsters fed the other artificial diets. However, a reduction in dry matter content was compensated by increased lipid deposition (Table 2). Lobsters fed diet D3 had a higher percentage of lipid in their muscle tissue than lobsters fed other artificial diets. The digestive gland lipid concentration of lobsters fed diet D3 was even higher than that of lobsters fed mussel diet. Excessive accumulation of lipid in liver of vertebrates is regarded as a pathological feature (Robbins *et al*. 1981). It is possible that the excessive accumulation of fat found in D3 lobsters was an analogous condition. However, in prawns high lipid content of the digestive gland was not associated with any pathological conditions (Glencross *et al*. 1999) and the same may be the case for lobsters.

While there were no significant differences between growth rates in animals fed artificial diets, significant differences were observed in several of condition indices, and this observation proves the usefulness of tissues parameters as condition indicators.

While digestive gland and muscle indices are accepted as possible condition indicators, their determination has the major drawback of killing the animal and being time-consuming. Sampling of hemolymph and estimation of its constituents or properties which may be affected by nutritional or health status could be a preferrable approach (Dall 1974). Clotting of hemolymph is a mechanism involved in the self-defence of crustaceans (Holmblad and Soderhall 1999). Preliminary data obtained in this study do not suggest that this parameter would prove useful as an assessment of condition index. The reduced number of hemocytes in hemolymph of animals fed artificial diet concurs with previous findings on both freshwater crayfish marron and western rock lobster. The THCs in marron reared on nutritionally insufficient diets were lower than in semi-intensively reared marron (Jussila 1997). The THCs were suggested for use as stress or condition indicators in western rock lobster with the levels between 4 and 8 x 10^6 cells/mL considered as normal level, and those below 4 x 10^6 cells/mL indicating deteriorating condition (Jussila *et al*. 1999). Lobsters fed the artificial diet in our trial had THC on the lower side of the normal range and they differed from lobsters fed mussels at the level of significance of 0.060, which may represent a trend, while not showing a significant difference.

The increase in the proportion of granular cells in lobsters fed the artificial diet compared to that fed natural diet in this study is in agreement with a similar increase, which we also observed in a starvation trial with freshwater crayfish in which the animals were deprived of food. In that experiment the significant difference in proportion of granular cells was observed within 14 days of trial commencement (unpublished data).

Use of proportion of granulocytes in total hemocyte population of lobster hemolymph as a condition index appears to be quite promising though requires some more work to be done on lobsters under different rearing conditions.

ACKNOWLEDGEMENTS

The experiment was conducted as part of FRDC project "Feed development for rock lobster aquaculture" within Rock Lobster Enhancement and Aquaculture Subprogram with Dr Kevin Williams from CSIRO Division of Marine Research, Queensland as Principal Investigator.

REFERENCES

Ackman, R.G. (1995) Composition and nutritive value of fish and shellfish lipids. In *Fish and Fishery Products. Composition, Nutritive Properties and Stability* (Ed. by A.Ruiter), pp. 117-156. CAB International, UK.

Aiken, D.E. & Waddy, S.L. (1992) The growth process in crayfish. *Rev. Aquatic Sci*., **6**, 335-81.

Battele, B.A. & Kravitz, E.A. (1978) Targets of octopamine action in the lobster: cyclic nucleotide changes and physiological effects in hemolymph, heart and exoskeletal muscle. *J. Pharmacol. Exp. Therapeutics*, **205**, 438-48.

Dall, W. (1974) Indices of nutritional state in the western rock lobster, *Panulirus longipes* (Milne Edwards). J. Exp. Mar. Biol. Ecol. **16**, 97-125.

Edgar 1990.

Evans, L.H., Fan, A., Finn, S.P., Dawson, S.A., Siva, C.J. & Lee, I.R. (1992) Nutritional status assessment studies in the freshwater crayfish, *Cherax tenuimanus*. In *Proceedings of the Aquaculture Nutrition Workshop* (Ed. by G.L. Allan & W. Dall), pp.87-91. NSW Fisheries, Brackish Water Fish Culture Research Station, Salamander Bay, Australia.

Glencross, B.D., Smith, D.M., Tonks, M.L., Tabrett, S.J. & Williams, K.C. (1999) A reference diet for nutritional studies of the giant tiger prawn *Penaeus monodon. Aquaculture Nutrition*, **5**, 33-39.

Fisheries Department of Western Australia (1996) State of Fisheries Report 1995-1996 (Ed. By J. Penn), Perth, WA.

Holmblad, T. & Soderhall, K. (1999) Cell adhesion molecules and antioxidative enzymes in a crustacean, possible role in immunity. *Aquaculture*, **172**, 111-23.

Huner, J.V., Kononen, H. & Lindqvist, O.V. (1990) Variation in body composition and exoskeleton mineralisation as functions of the moult and reproductive cycles of the

noble crayfish, *Astacus astacus* L. (Decapoda, Astacidae), from a pond in Central Finland. *Comp. Biochem. Physiol*., **96A**, 235-40.

Jernakoff, P., Fitzpatrick, J.J. & Phillips, B.F. (1993) The diet of post-puerulus western rock lobster *Panulirus cygnus* George, at Seven Mile Beach, Western Australia. *Aust. J. Mar. Freshwater Res*. **44**, 649-55.

Jones, D.A., Kumlu, M., Le Vay, L. & Fletcher, D.J. (1997) The digestive physiology of herbivorous, omnivorous and carnivorous crustacean larvae: a review. *Aquaculture*, **155**, 289-91.

Jussila, J. (1997) Physiological responses of Astacid and Parastacid Crafishes (Crustacea, Decapoda) to conditions of intensive culture. Doctoral Dissertation, University of Kuopio.

Jussila, J. & Mannonen, A. (1997) Energy content of marron (*Cherax tenuimanus*) and noble crayfish (*Astacus astacus*) hepatopancreas and its relationship to hepatopancreas moisture content. *Aquaculture*, **149**, 157-61.

Jussila, J., Tsvetnenko, E., Jago, J. & Evans, L.H. (1999) Post-harvest handling stress in western rock lobster: Hemocytes' point of view. In *International Symposium on Lobster Health Management Abstract Proceedings*, p.19, Adelaide, Australia.

Kopacek, P., Hall, M. & Soderhall, K. (1993) Characterisation of a clotting protein, isolated from plasma of the freshwater crayfish *Pacifastacus leniusculus. Eur. J. Biochem.*, **213**, 591-97.

Kreider, J.L. & Watts, S.A. (1998) Behavioural responses of the crayfish, *Procambarus clarkii*, to natural dietary items and common components of formulated crustacean feeds. *J. Chem. Ecol*. **24**, 91-111.

Mannonen, A., & Henttonen, P. (1995) Some observations on the condition of crayfish (*Astacus astacus*) in a river affected by peat mining in Central Finland. *Freshwater crayfish*, **10**, 274-81.

McClain, W.R. (1995a) Effects of population density and feeding rate on growth and feeding consumption of red swamp crawfish *Procambarus clarkii . J.World Aquaculture Soc*., **26**, 14-22.

McClain, W.R. (1995b) Growth of crawfish *Procambarus clarkii* as a function of density and food resources. *J. World Aquaculture Soc*., **26**, 24-28.

Musgrove, R.J. (1997) The effect of food availability on condition in juvenile southern rock lobster, *Jasus edwardsii*. In *The Fifth International Conference and Workshop on Lobster Biology and Management Programme and Abstracts*, p.57, Queenstown, New Zealand.

Paterson, B., Goodrick, B. & Frost, S. (1997) Controlling the quality of aquacultured food products. *Trends in Food Science & Technology*, **8**, 253-257.

Pigott, G.M. & Tucker, B.W. (1990) Seafood: Effects of Technology on Nutrition. Marcel Dekker, Inc., New York and Basel.

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

Phillips, B.F., Joll, L.M., Sandland, R.L. & Wright, D. (1983) Longevity, reproductive condition and growth of the western rock lobster *Panulirus cygnus* George, reared in aquaria. *Aust. J. Mar. Freshwater Res*. **34**, 419-29.

Phillips, B.F., Palmer, M.J. & Cruz, R. (1992) Estimating growth of the spiny lobster *Panulirus cygnus, P. argus* and *P. ornatus. Aust. J. Mar. Freshwater Res*. **43** 1177-88.

Robbins, S.L., Angell, M. and Kumar, V. (1981) Basic Pathology, 3rd edition. W.B.Saunders Company.

Schrif, V.R., Turner, P., Selby, L., Hannapel, C. De La Cruz, P. & Dehn, P.F. (1987) Nutritional status and energy metabolism of crayfish *Procambarus clarkii*, Girard) muscle and hepatopancreas. *Comp. Biochem. Physiol*., **88A**, 383-86.

Tsvetnenko, E., Kailis, S., Evans, L. & Longmore, R. (1996) Fatty acid composition of lipids from the contents of rock lobster (Panulirus cygnus) cephalothorax. *JAOCS*, **73**, 259-261.

Measuring total protein concentration in blood of the western rock lobster (*Panulirus cygnus* George) by refractometry

Brian D. Paterson[1], Glen W. Davidson[2] and Patrick T. Spanoghe[2]

[1] Centre for Food Technology, 19 Hercules St Hamilton QLD 4007 Australia (patersonb@dpi.qld.gov.au)
[2] School of Biomedical Sciences, Curtin University of Technology, GPO Box 1987U, Perth WA 6845, Australia

ABSTRACT

Research on western rock lobsters *Panulirus cygnus* has shown that refractometry is a simple non-destructive field technique for assessing the blood protein concentration and hence the tissue mass or "condition" of rock lobsters. However, a conversion from refractive index (RI) to protein concentration has not been published for this species. In order to convert RI values to protein concentrations, the refractive index of a number of blood samples were measured at ambient temperature using a Shibuya S-1 salinometer calibrated at ambient temperature with distilled water. Total protein concentrations were determined colourimetrically by the biuret method. Comparing the data gave the following regression equation:

$$\text{Total protein (mg ml}^{-1}) = 5402.398 \times \text{RI} - 7214.877,\ r^2 = 0.947,\ n = 28$$

This analytical method represents a simple and useful way to obtain important information about the condition of rock lobsters entering commercial handling and transport. The measurement involves a straightforward physical phenomenon and it is probably not surprising that the conversion equation obtained in this study is similar to that derived from a study of the American lobster *Homarus americanus*. These conversions may be a satisfactory method of estimating blood protein concentration in other large marine crustaceans. Though if large numbers of measurements are to be made for other species using this method, it is relatively easy to establish a calibration to ensure the accuracy of the technique.

Keywords: condition, hemolymph, tail meat, portable instrument

I. INTRODUCTION

Blood protein concentration in lobsters changes when the blood volume changes in response to moulting or starvation. Research on western rock lobsters *Panulirus cygnus* George has shown that refractometry is a simple non-destructive field technique for assessing the blood protein concentration and hence parameters such as blood volume, tissue mass or "condition" of rock lobsters (Dall, 1974, 1975).

The use of refractometry to determine serum or blood protein concentration is an established clinical technique and has a number of benefits over more complex methods. 1) The refractometers themselves are relatively cheap, most cost less than $500. 2) The method provides immediate results, and 3) the data is at least as reliable as those of commonly employed colourimetric methods that require samples to be stored and later analysed (Dall,

1975; Alexander and Ingram, 1980). 4) Clotting of blood is not an issue as protein determinations can be performed rapidly using whole blood within the time it takes for the blood to clot. Of course, when using the instrument in the field, it is important to note that unless a temperature compensated refractometer is used, the temperature of the device should be kept as constant as possible and the calibration of the instrument must be checked periodically with distilled water during measurements.

II. MATERIALS & METHODS

Blood samples were obtained from the pericardial sinus (beside the heart) of lobsters via the thin arthrodial membrane between the posterior margin of the carapace and the abdomen using ice-cold disposable hypodermic syringes. The refractive index (RI) of each sample was measured immediately at ambient temperature (~25°C) using a Shibuya S-1 salinometer calibrated at ambient temperature with distilled water.

After drawing the sample, 2 or 3 drops of blood were immediately placed on the prism of the refractometer and the RI was recorded before the blood clotted. If clotting did occur, the reading did not appear to alter, but the demarcation line on the prism became indistinct and difficult to read. Between readings the prism was rinsed with distilled water and wiped with a tissue. Calibration checks demonstrated that in terms of the RI recorded, it did not matter if the temperature of the few drops of sample (eg. 17°C) was initially different from the operating temperature of the refractometer at ambient temperature. Therefore, realistic differentials in ambient air and seawater temperature were not a significant source of measurement error.

In order to convert RI values to protein concentrations, the refractive index of a number of fresh blood samples was measured and total protein concentrations were determined immediately by the biuret method (Varley, 1967) using a Shimadzu UV 1201 spectrophotometer. Serial dilution of a solution of bovine serum albumin (150 mg ml^{-1}) provided standards. A calibration relationship for the two parameters was then calculated by regressing RI on total protein concentration using least squares linear regression techniques.

III. RESULTS & DISCUSSION

Several studies have shown that the protein scale on clinical serum refractometers, while correlated to protein concentration, sometimes overestimates the values returned by colorimetric assays of protein in fish serum, (Alexander and Ingram, 1980; Hunn and Greer, 1990; Ikeda and Ozaki, 1982; Wells and Pankhurst, 1999). Further calibration of the device is required in these cases.

The instrument used here was chosen because it has a slightly wider refractive index scale than typical serum protein refractometers, allowing RI values to be measured above 1.360. In the approximately 3000 lobsters used in our studies, values of RI varied between 1.3430 - 1.3695.

The calibration relationship for predicting blood total protein from RI was determined to be:

(1) Total protein (mg ml^{-1}) = (RI - 1.3364379)/ 0.0001757, r^2 = 0.947, n = 28

A similar relationship has been calculated for *Homarus americanus* Milne-Edwards by Leavitt and Bayer (1977) but these authors regressed total protein concentration on RI yielding the following equation:

(2) Total Protein (mg ml^{-1}) = 5449.417 x RI - 7295.321

If, for comparison, we express our data the same way we arrive at the following equation:

(3) Total protein (mg ml^{-1}) = 5402.398 x RI - 7214.877, r^2 = 0.947, n = 28

For comparison, the data and equation obtained from this study are plotted alongside the regression for *Homarus americanus* in Fig. 1. Note that the slopes are quite similar but that the intercepts on the RI axis (the projected RI of a sample lacking any protein) differ slightly, implying a difference between the two species in the contribution to refractive index of solids other than protein. Dall (1975) also calculated this relationship in the western rock lobster but did not report the whole equation. In addition, Dall (1975) used a different method for calibrating the refractometer, setting RI to 1.3400 with a 3% salt solution. The reason for doing so is unclear, given that the RI of a 3% salt solution is 1.3380 on a refractometer calibrated with distilled water. Regardless of which calibration is used the slope of the line remains unaffected.

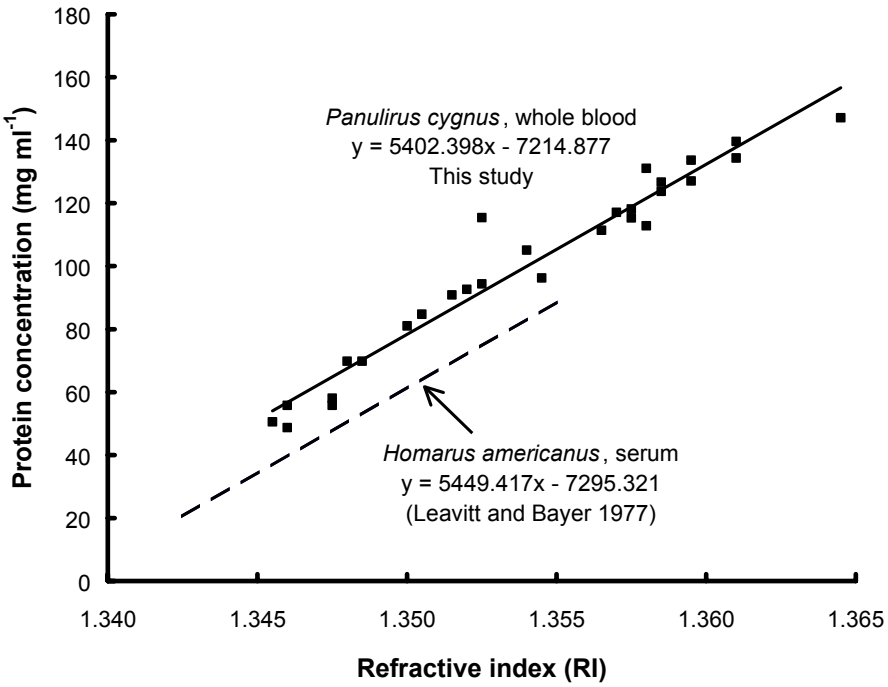


Figure 1 Comparing the relationship between protein concentration and blood refractive index (RI) for western rock lobster *Panulirus cygnus* and the published calibration for serum of American lobster *Homarus americanus.*

The biuret reagent reacts with peptide bonds to form a reddish-purple complex, the absorbance of which can be measured at 540 nm. The intensity of colour is proportional to the number of peptide bonds and hence the protein concentration (mg ml^{-1}). Whereas biuret reacts only with protein, RI is affected by all solutes in the sample. The use of refractometry for protein determinations relies upon the overwhelming contribution of protein to the total solute concentration and the relative constancy of the concentrations of other solutes. However, in human clinical studies there are a number of conditions that may interfere with the protein vs. RI relationship. For example RI is affected by, amongst other things, alterations in the albumin/globulin ratio and hyperglycaemia (Tietz, 1926). The first of these is probably of little concern in crustacea since by far the greatest proportion (75 - 95%) of blood protein is attributable to hemocyanin, the protein that transports oxygen in the blood. Hyperglycaemia (elevated glucose levels) in crustaceans is also likely to be less of a concern than in mammalian studies since hyperglycaemic values in mammals are much higher than in decapods. In addition, the concentration of salts in the blood of marine decapods is much greater than in mammals, therefore the relative effect on RI of an increase in glucose will be much less in decapods.

The method is already recommended for grading the condition of live *H. americanus* arriving at factories, allowing industry operators to make decisions about the marketing of individual lobsters based on information from a single drop of blood (Leavitt and Bayer, 1977). It has also been used for studies of live lobsters, crabs, penaeid shrimp and fish (Vezina, 1978; Smith and Dall, 1982; Alexander and Ingram, 1980, Moore *et al*., 2000). One other example of where protein refractometry has been used applied to commercial problems relates to monitoring the spoilage of fish (Vyncke, 1995).

This analytical method represents a simple and useful way to obtain important information about the condition of rock lobsters entering commercial handling and transport. The measurement involves a straightforward physical phenomenon and it is probably not surprising that the conversion equation obtained in this study (equation 2) is similar to that derived from a study of the American lobster (Leavitt and Bayer, 1977).

It is probably safe to assume therefore, that for some purposes, the equations reported here, or others published (e.g. equation 2), may be a satisfactory estimation of blood protein concentration in other large marine crustaceans, especially if knowing relative changes is sufficient. Though if large numbers of measurements are to be made for another species using this method, it is relatively easy to calculate a calibration relationship to ensure the accuracy of the technique.

Aside from being an index of condition (eg. blood volume and tissue mass), protein concentration in the blood of crustaceans also varies with the moult cycle, being lowest during post-moult and highest pre-moult. This needs to be taken into account when interpreting why a given lobster has low or high protein level (Dall, 1974). Preliminary data suggest that migratory sub-adult *P. cygnus*, or “whites”, have the characteristically low blood protein concentration expected of post-moult lobsters.

ACKNOWLEDGEMENTS

This research was supported by the Fisheries Research and Development Corporation (FRDC 96/345) and forms part of the FRDC Rock Lobster Post-harvest Sub-program.

REFERENCES

Alexander, J.B. & Ingram, G.A. (1980). A comparison of five of the methods commonly used to measure protein concentrations in fish sera. *J. Fish Biol.* **16**, 115-122.

Dall, W. (1974). Indices of nutritional state in the western rock lobster, *Panulirus longipes* (Milne Edwards). I. Blood and tissue constituents and water content. *J. Exp. Mar. Biol. Ecol.* **16**, 167-180.

Dall, W. (1975). Indices of nutritional state in the western rock lobster, *Panulirus longipes* (Milne Edwards). II. gastric fluid constituents. *J. Exp. Mar. Biol. Ecol.* **18**, 1-18.

Hunn, J.B. & Greer, I.E. (1990). Colorimetric and refractometer estimates of total plasma protein in striped bass, *Morone saxatilis* (Walbaum). *J. Fish Biol.* **36**, 617-618.

Ikeda, Y. & Ozaki, H. (1982). Application of a protein refractometer on fish blood. *Bull. Jap. Soc. Sci. Fish.* **48**, 1251-1256.

Leavitt, D.F. & Bayer, R.C. (1977). A refractometric method for determining serum protein concentration in the American lobster. *Aquaculture* **12**, 169-171.

Moore, L.E., Smith, D.M. & Loneragan, N.R. (2000). Blood refractive index and whole-body lipid content as indicators of nutritional condition for penaeid prawns (Decapoda: Penaeidae). *J. Exp. Mar. Biol. Ecol.* **244**, 131-143.

Smith, D.M. & Dall, W. (1982). Blood protein, blood volume, and extracellular space relationships in two *Penaeus* spp. (Decapoda: Crustacea). *J. Exp. Mar. Biol. Ecol.* **63**, 1-15.

Tietz, N.W. (ed.) (1926). *Fundamentals of Clinical Chemistry*. W.B. Saunders Co., Philadelphia.

Varley, H. (1967). *Practical Clinical Biochemistry*. 4th Ed., William Heinemann, Medical Books Ltd., London.

Vezina, B. (1978). Comparaison entre la methode au biuret et le refractometre pour les dosage des proteines seriques chez le homard et chez le crabe tourteau. *Naturaliste Canadien* **105**, 457-460.

Vyncke, W. (1995). The determination of total volatile bases in eye fluid as a non-destructive spoilage assessment test for fish. *Arch. Lebensmittel*. **46**, 96-97.

Wells, R.M.G. & Pankhurst, N.W. (1999). Evaluation of simple instruments for the measurement of blood glucose and lactate, and plasma protein as stress indicators in fish. *J. World Aquacult. Soc.* **30**, 276-284.

A preliminary evaluation of 3 haemolymph tests to assess health status in tropical rock lobsters *Panulirus ornatus*

John H. Norton, Naomi Levy and Kelly Field

Queensland Department of Primary Industries, Oonoonba Veterinary Laboratory
PO Box 1085 Townsville QLD 4810

ABSTRACT

Three hemolymph tests were evaluated on a small number of tropical rock lobsters *Panulirus ornatus* as possible indicators of health status. The tests included a phenoloxidase (PO) test, a red blood cell (RBC) agglutination test and an antibacterial (bactericidal/bacteriostatic) (AB) test. They were conducted on both clinically normal and sick adult lobsters. Highly significant differences ($p<0.01$) were obtained for the phenoloxidase and RBC agglutination tests. Although no significant difference was obtained for the antibacterial test, a high level of antibacterial activity was observed in one sick lobster. These pilot experiments strongly suggest that further experimentation with these three tests on both clinically normal and sick adult rock lobster hemolymph would be productive.

Key Words: P*anulirus ornatus,* phenoloxidase, agglutination test, antibacterial test

I. INTRODUCTION

With increasing interest in the aquaculture of rock lobsters, there is a need to develop ways of evaluating the health status of both wild-caught and cultured rock lobsters, and in particular of *Panulirus ornatus (*Fabricius*)*, Queensland's premier rock lobster. Our aim was to evaluate three hemolymph tests namely a phenoloxidase test, a red blood cell agglutination test and an antibacterial test for their suitability to separate healthy from sick lobsters. Reports of these tests in Crustacea are not new as documented in the review of non-cellular immunity in crustaceans by Smith and Chisholm (1992). However their use to evaluate the health status of stressed crustaceans, especially of lobsters, is not common (Smith and Chisholm 1992) though Ueda *et al*. (1990) attempted to use an antibacterial test to evaluate health status in *Panulirus japonicus* (Von Siebold). If successful, these tests would be a valuable aid to improved management of both wild-caught and cultured lobsters.

II. MATERIALS & METHODS

Hemolymph Collection

Initially, lobsters were divided into sick and healthy groups. The healthy lobsters exhibited normal reflexes when handled while the sick lobsters had either depressed reflexes when handled or, in one lobster, an extensive inflammation of the tail. Each lobster was bled from the pericardial sinus. The membrane at the rear of the carapace was disinfected with 70% alcohol or a similar antiseptic, prior to insertion of the needle. The hemolymph was collected

using a 21 gauge needle and a 5 ml disposable syringe. Hemolymph samples for the phenoloxidase test were chilled prior to analysis for phenoloxidase activity. Hemolymph samples for the RBC agglutination and antibacterial tests were frozen and maintained at -70ºC prior to testing.

Phenoloxidase Test

Thirty normal cultured adult rock lobsters and 6 sick adult, wild-caught lobsters were sampled. Lobster carapace size range was 35.8 to 73.5 mm (normal) and 78 to 104 mm (moribund) with an even distribution of both sexes and all moult stages including postmoult, intermoult and premoult. Hemolymph was assayed following the method of Perazzolo and Barracco (1997) with the following exceptions:- 1. The hemolymph was diluted 1:3 with phosphate buffer. 2. The L-3,4-Dihydroxyphenylalanine (L-DOPA), serum and elicitor were added to a 96 well microtitre plate, incubated for 60 mins at room temperature then the degree of absorbance due to formation of a black pigment was measured to determine phenoloxidase activity. Three methods of activating pro-phenoloxidase were used including laminarin, trypsin and spontaneous activation. Lobsters were moult staged according to the method of Turnbull (1989).

RBC Agglutination Test

Six normal adult lobsters, and 6 sick adult lobsters were sampled. Lobster carapace size range was 75 to 85 mm (normal) and 103 to 125 mm (moribund) with an even distribution of both sexes. The moult stages were not recorded. The method was based on the work of Marchalonis and Edelman (1968) and of Ueda *et al*. (1991) using 1% chicken red blood cells in serial dilutions of hemolymph with phosphate buffer. Final agglutination titres were determined at 18-24 hrs.

Antibacterial Test

The lobsters sampled were those used for the RBC agglutination test. The method was based on the work of Noga *et al*. (1994; 1996) with the following exceptions:- A local vibrio species was grown up in trypticase soy broth and turbidity adjusted to 0.75 on the McFarlane standard scale. 10 µl of each serially diluted hemolymph sample in phosphate buffer was spotted onto a trypticase soy agar plate that had been swabbed with the bacterial culture. Plates were incubated at 25°C for 18-24 hours. Clear zones represent bacterial growth inhibition.

Statistics

One way analysis of variance was used to compare phenoloxidase activity between the 30 normal and 6 sick adult lobsters in the phenoloxidase test. A one way analysis of variance was used to compare log transformed data between the 6 normal and the 6 sick/moribund lobsters in both the RBC agglutination and the antibacterial tests.

III. RESULTS

The differences between the normal and the sick lobsters were highly significant ($p<0.01$) for all 3 methods of activating pro-phenoloxidase to phenoloxidase (Table I). For the RBC agglutination test, the difference between the normal and the sick lobsters was also highly significant ($p<0.01$) (Table II). However, the difference between these 2 groups of lobsters for the antibacterial test was not significant (Table II). Nevertheless, one of the sick lobsters which had a chronic inflammation of the tail (telson and uropods) had a high antibacterial titre (16) compared to the other lobsters in the sick group (0-2).

Table I Haemolymph phenoloxidase activity for healthy and sick adult lobsters

Status (Number of Lobsters/Group)	Phenoloxidase activity (change in absorbance at 492 nm per ml per minute) mean (range)		
	Laminarin	Trypsin	Spontaneous
Healthy (30)	543[a*] (96-1191)	892[a] (352-1780)	473[a] (39-1046)
Sick (6)	165[b] (100-340)	447[b] (340-580)	143[b] (80-290)

* In each column, values followed by a different letter differ significantly ($p<0.01$)

Table II Haemolymph red blood cell agglutination test and antibacterial test results on healthy and sick adult lobsters.

Status (No. of Lobsters/Group)	RBC Agglutination Test	Antibacterial Test
	Means (log transformation of reciprocals of titres)	
Healthy (6)	51[a*]	0.4[a]
Sick (6)	102[b]	0.9[a]

* In each column, values followed by a different letter differ significantly ($p<0.01$).

IV. DISCUSSION

The prophenoloxidase (proPO) activating system acts as a pattern recognition and defence system in invertebrate blood (Johansson and Soderhall 1989). The great majority of phenoloxidase activity in crustaceans is found within the granulocytes and semigranulocytes. This activity can be determined from haemocyte lysate or serum using a variety of elicitors including laminarin, lipopolysaccharide and trypsin (Perazzolo and Barracco 1997). Spontaneous activation (without elicitors) has also been reported (Sung *et al*. 1998). Investigation into phenoloxidase levels relating to immune/health status has been limited. Some preliminary investigations suggest that phenoloxidase activity is reduced in crustaceans when stressed or infected with virus (Moullac *et al*. 1998; Walker pers. com. 1998). The highly significant reduction of phenoloxidase activity regardless of elicitor type in sick lobsters confers with previous work and suggests this test may be a useful indicator of crayfish health.

The highly significant difference obtained in the RBC agglutination test in a small group of lobsters suggests that this test will separate lobsters that have been stressed eg., by crowding, handling and transport, as well as the lobster with a chronically inflamed tail from clinically normal lobsters. Nevertheless, there is still scope for expanding the range of titres and increasing the sensitivity of this test by the use of other species of red blood cells, the addition of calcium, etc. (Marchalonis and Edelman 1968).

In the antibacterial test, the lack of a significant difference between the clinically normal and sick lobsters may have been partly due to the small numbers of lobsters tested but may also have been due to the lack of antibacterial activity in many of the lobsters especially the sick ones. As bacteria have frequently been cultured from the hemolymph of sick lobsters (Norton, unpublished data), it is probable that these bacteria would have neutralised much of the antibacterial activity in the hemolymph to give a false negative result. This latter finding was unexpected. Furthermore, a more sensitive bacterial strain may have produced higher titres (Ueda *et al*. 1994, Noga *et al*. 1996). The other unexpected finding was the increase in the antibacterial titre in the lobster with the inflamed tail. Noga *et al*. (1994), working with crabs, reported a decrease in antibacterial titres in crabs from more polluted areas compared to crabs from less polluted areas. However, the more polluted areas were also areas of lower salinity. Ueda *et al*. (1990) working with the spiny lobster *P.japonicus* also reported a reduction in the antibacterial titre in lobsters exposed to reduced salinity and to transport stress. Further evaluation of this test is required using larger numbers of lobsters. The fact that the sick lobsters were also larger and hence older than the normal lobsters may have had some influence on the results.

Each of the tests reported here also needs to evaluate possible effect of sex, age and moult stage on assay results. In conclusion, these tests give promise of practical ways of evaluating the health status of groups of lobsters. However extensive testing is needed before these tests could be used on an individual lobster basis.

ACKNOWLEDGMENTS

The authors thank Ms Larnie Linton of the Northern Fisheries Centre, Cairns for collection of samples and Mr R. Meyer of the Department of Primary Industries, Townsville for statistical analyses.

REFERENCES

Johansson, M.W. and Soderhall, K. (1989) Cellular immunity in crustaceans and the pro PO system. *Parasitology Today,* **5,** 171-176.

Marchalonis, J.J. and Edelman, G.M. (1968) Isolation and characterization of a hemagglutinin from *Limulus polyphemus. J. Mol. Biol.,* **32**, 453-465.

Moullac, G., Soyez, C., Sauliner, D., Ansqner, D., Avarre, J. & Levy P. (1998) The effect of hypoxic stress on the immune response and resistance to vibriosis of the shrimp *P.stylirostris. Fish Shellfish Immunol.,* **8***,* 621-629.

Noga, E.J., Engel, D.P., Arroll, T.W., McKenna, S. and Davidian, M. (1994) Low serum antibacterial activity coincides with increased prevalence of shell disease in blue crabs *Callinectes sapidus. Dis. Aquat. Org.,* **19**, 121-128.

Noga, E.J., Arroll, T.A. and Fan, Z. (1996) Specificity and some physicochemical characteristics of the antibacterial activity from blue crab *Callinectes sapidus*. *Fish and Shellfish Immunol.,* **6**, 403-412.

Perazzolo, L. & Barracco, M. (1997) The prophenoloxidase activating system of the shrimp *Penaeus paulensis* and associated factors. *Dev. Comp. Immunol.*, **21**, 385-395

Smith, V.J. and Chisholm, R.S. (1992) Non-cellular immunity in crustaceans. *Fish and Shellfish Immunol.,* **2**, 1-31.

Sung, H., Chang, H., Her, C., Chang, J. & Song, Y. (1998) Phenoloxidase Activity of Hemocytes Derived from *P. monodon. M. Rosenbergii J. Inv. Path.,* **71**, 26-33

Turnbull, C.T. (1989) Pleopod cuticular morphology as an index of moult stage in the ornate rock lobster, *Panulirus ornatus. Aust. J. Mar. Freshwat. Res.,* **40**, 285-293.

Ueda, R., Sugita, H. and Deguchi, Y. (1990) The serum bactericidal activity : a possible indicator as crustacean health conditions in *Proc. South Pacific Congr. Mar. Sci. Technol.,* Tokyo, pp 333-340.

Ueda, R., Sugita, H. and Deguchi, Y. (1991) Naturally occurring agglutinin in the hemolymph of Japanese coastal crustacea. *Nippon Suisan Gakkaishi,* **57**, 69-78.

Ueda, R., Sugita, H. and Deguchi, Y. (1994) Bactericidal activities of the hemolymph of the Japanese spiny lobster, *Panulirus japonicus* (Decapoda, Palinuridae) *Crustaceana*, **67**, 256-258.

Panel discussion summary 1

Health Management Issues in Lobster Aquaculture

1. INTRODUCTION

A panel discussion on 'Health Management Issues in Lobster Aquaculture' was held at the conclusion of the first day of the lobster health management symposium. The discussion was moderated by Dr Robert van Barneveld, Sub-program Leader, FRDC Rock Lobster Enhancement and Aquaculture Sub-program and was attended by approximately 70 symposium participants. Participants included industry personnel, rock lobster researchers, government and FRDC representatives and international visitors.

At the commencement of the panel discussion Dr van Barneveld reviewed the FRDC Rock Lobster Enhancement and Aquaculture Sub-program and suggested that discussion could be directed at four main topics:

- Research and industry priorities with respect to lobster aquaculture and enhancement
- Research directions
- Industry feedback on research program
- Other issues

Discussion was held on these and other topics raised by symposium participants. A summary of these discussions along with the names of principal contributors to the discussion is given below.

2. DISCUSSION TOPICS

2.1 Research Directions and Priorities

2.1.1 Health management practices in post-harvest storage and aquaculture

An audience member commented that health management is of prime importance to successful long term storage and aquaculture. In response, another participant stressed that while it was important to develop and use health management techniques, the major priority was in the education of fishers, handlers and airlines as well as in developing understanding of environmental conditions of holding/rearing systems which optimise health and survival.

A West Australian processing industry member described the approach taken in his company to optimise survival during live marketing. He indicated that extensive studies had been undertaken through working with individual fishers right through to markets in Japan. This work has yielded a good data base on lobsters from different sources and their fate with respect to survival during transport. He commented that the

most healthy lobsters were those delivered direct to the factory from local fishing boats – these had a low rejection rate at grading and an almost zero mortality on storage. Providing fishers follow best practice procedures mortalities can be reduced to a small proportion of residual health compromised animals. However, he stressed the need for education of fishers on best practice post-harvest handling procedures.

An audience member from New Zealand supported the above views and commented that the major factor affecting lobster health in post-harvest was how the animals were treated before they came into the factory.

(Mike Leach, Colin McDonald, Wayne Hoskins, Ben Diggles)

2.1.2 Disease investigations

The suggestion was made that spiny lobsters are very robust and that disease issues were not a high priority. Instead, it was suggested that identifying optimal conditions for aquaculture should be the main priority. This suggestion was hotly disputed with one audience member stating that this was a very shortsighted view. He went on to express the view that diseases were inevitable in aquaculture, and that generating data and information on occurrence of disease conditions through investigation and monitoring of mortalities was essential.

This opinion was supported by another participant who commented that there was a dearth of information on lobster diseases and the task of identifying pathogens and managing disease outbreaks would be simplified if more information was available. The issue of the need for identification of health indicators for use in health management and health monitoring was raised by another participant. The opportunity to improve health through the use of probiotics was also mentioned.

One audience member commented that there were three main areas where research was required:

- Knowledge of disease threats
- What effect diseases have on the host and the compounding effect of stress in disease outbreaks
- Treatment options

However, the same individual also commented that there was an urgent need to identify optimal conditions for rearing lobsters and went on to say that the emphasis between these two research areas would probably change if disease outbreaks occurred.

The need for investigations on aquaculture system design was supported by another audience member who suggested that a high priority should be given to documenting current rearing techniques for puerulus and juveniles and conduct research on improving these techniques.

(Andrew Jeffs, Judith Handlinger, Brian Jones, Kenneth Söderhäll, Ben Diggles, Louis Evans; two audience members)

2.1.3 Genetic and disease risks from lobster aquaculture and enhancement

The question was raised as to whether disease risks to wild stock lobsters exist from aquaculture or long term storage in coastal locations. This question stimulated a wide ranging discussion on issues relating to disease risks, genetic issues and risks to biodiversity through escape of cultured lobsters to the wild. A summary of comments and questions is given below:

Disease issues

- There is a real risk of disease from aquaculture or long term storage
- If the pilchard viral disease is used as an example, it can be confidently concluded that a similar scenario could occur in the rock lobster industry. Any unexplained mortalities should be investigated as quickly as possible.

Genetics issues

- Genetics is likely to be an important area for research - observations in the United States have suggested that the source of lobsters from the wild influences survival during long term storage
- Is there any genetic work being done on rock lobsters?
- Little work is currently being conducted on spiny lobster genetics
- Genetic analysis is being conducted in lobster stock enhancement trials in Europe but it is still too early for definitive results
- Will escape of genetic stocks into the wild affect wildstock?
- Whether or not escapees affect wildstock biodiversity isn't really known but ecologists tend to support the view that they do
- It is unlikely that cultured animals reared on artificial diets would outcompete wildstock animals
- There is likely to be little risk to the genetic integrity of wild stock lobsters from cultured escapees since lobster aquaculture is still in its infancy

The comment was made that effort should be placed in trying to enhance the lobster papulation in the wild rather than conducting on-shore aquaculture – issues such as maximising shelter, food supply, predator protection were of prime importance. Another audience member commented that research on artificial habitat for settlement and early juvenile predator protection was being conducted in New Zealand.
(Ross Gould, Gil Waller, Kenneth Söderhäll, Andrew Jeffs, Judith Handlinger, Mike Leach, Knut Jörstad, Wayne Hoskins)

2.2 Shell Disease

A discussion was held on the likely etiological factors in shell disease, tail rot and tail erosion. It was questioned whether shell disease is always preceded by a physical injury to the lobster. An outbreak of shell disease in which trauma due to poor trap

design in a southern rock lobster fishery was described while another participant commented that shell disease results from high stress, poor nutrition and high temperatures. The likelihood of the condition having varied and interacting causes was stressed.

(Judith Handlinger, Mike Geddes, Louis Evans, audience member)

2.3 The Use of Antibiotics in Lobster Aquaculture and Enhancement

Following on from one of the presentations during the paper sessions, a member of the audience commented that antibiotics should not be used in lobster aquaculture or enhancement. The question was raised as to responsibility for enforcement of antibiotic usage. It was indicated that enforcement was ensured through HACCP and through regulation by government agencies. (Bob Bayer, Steve Hood, audience member)

2.4 Technical Topics

Two questions were asked relating to the storage of lobsters in tanks.

2.4.1 Tank design

Q - How should a tank be set up so as not to get dead areas?

A – Refer to the Lobster Institute web page for information on holding systems for lobsters

2.4.2 Lobster holding systems

Q – What is the optimal holding system for lobsters?

A1 – The Clearwater system uses low temperatures for extended holding of Maine lobsters

A2 – Comment on experience in holding system at Port Lincoln – using natural and artificial feeds

2.4.3 Variation in lobster size

Q – Is there any information to explain why the size of lobsters from different catch zones differ?

A1 – Size differences are also seen in wildstock lobsters in New Zealand. It is probably due to differences in habitat. Have also looked at size differences in cultured phyllosoma and found that egg quality can influence lobster size.

A2 – There has been some work on *Panulirus argus* which showed that it was possible to identify which broodstock contributed to different groups of settled lobsters.

(Gil Waller, Mike Geddes, Colin McDonald, Glen Davidson, Andrew Jeffs, Bob Bayer, Jean Lavelle, audience member)

3. SUMMARY

Dr Robert van Barneveld summarised the discussions by concluding:

- Health management is an issue in rock lobster aquaculture and enhancement
- Holding conditions is also an issue, particularly as they influence health
- Resources should be directed towards monitoring mortalities

Meeting notes compiled by:

Associate Professor Louis Evans
Convenor
International Symposium on Lobster Health Management

Panel discussion summary 2

Lobster Live Export

1. INTRODUCTION

A panel discussion on 'Lobster Live Export' was held at the conclusion of the second day of the lobster health management symposium. The discussion was moderated Professor Bruce Phillips, Sub-program Leader, FRDC Rock Lobster Post-Harvest Sub-program, and was attended by approximately 50 symposium participants. Participants included industry personnel, rock lobster researchers, government and FRDC representatives and international visitors.

Professor Phillips introduced the topic by describing the current activities of the FRDC Rock Lobster Post-Harvest Sub-program. He indicated that the first major project in the program – Physiological Studies on Stress and Morbidity during Post-Harvest Handling and Storage of Western Rock Lobster *Panulirus cygnus* – was nearing completion. The project comprised two sub-projects, one managed by Dr Brian Patterson, QLD DPI, focussed on physiological parameters and the other by Assoc. Prof. Louis Evans, Aquatic Science Research Unit, Curtin University of Technology, focussed on immune parameters. In addition to this major project, two other projects within the Sub-program had been completed, one on the development of a Code of Practice for post-harvest handling of rock lobsters, conducted by Richard Stevens, WAFIC, and the other a study of the influence of environmental conditions in holding systems on lobster physiology, a PhD study conducted by Dr Brad Creer, University of Tasmania under the supervision of Professor Nigel Forteath.

An FRDC funded project aimed at publication of a rock lobster autopsy manual had also been recently approved. This project is being lead by Assoc. Prof. Louis Evans, Curtin University of Technology and is being jointly conducted with Dr Brian Jones, Fisheries WA and other fish health personnel from South Australia, Tasmania and Queensland.

Professor Phillips informed the group of a recent decision by the Sub-program Steering Committee to endorse three new projects for 2000 and beyond – investigations of leg loss in post-harvest lobsters, truck transport conditions and ammonia tolerance. The use of hides as rock lobster bait was also considered to be an important issue and had been investigated recently in another FRDC project conducted by Richard Stevens, WAFIC. He then opened the discussion by inviting participants to raise issues of relevance to live lobster transport.

Discussion was held on topics raised by symposium participants. A summary of these discussions along with the names of principal contributors to the discussion is given below.

2. DISCUSSION TOPICS

2.1 Use of Hides for Lobster Bait

The topic of the use of cow hides as bait for lobster traps was raised. Two issues were discussed in relation to this topic 1) a marketing concern, in particular the presence of hairs and hide fragments in the gut of lobsters when they are served at a restaurant; and 2) whether lobsters are adversely affected by eating hides. From comments by audience members it appeared that there had been no research on this topic.

Another issue which was raised related to possible adverse impacts of regurgitated hide material on water quality in holding systems. This query lead to a discussion on purging of lobsters. A comment was made that the presence of excess wastes in effluents would be a concern for regulatory authorities but this had not been in issue in the lobster processing industry to date. A lobster processor said that purging of lobsters took about three days.

(Glen O'Brien, Mick Olsen, Ross Gould, Bruce Phillips, Ross McGregor, Louis Evans)

2.2 Quality of Post-Harvest Lobsters

The question of how to improve the quality of post-harvest lobsters was raised by a lobster processor. One avenue was to pay a price differential for different quality of product. This suggestion stimulated considerable discussion. It was pointed out that small boats may not be able to install elaborate equipment required to maintain optimal condition of lobsters following capture. As a result it was not appropriate to pay a different price for different quality lobsters. Another audience member stated that it was clear in the South Australian industry that different boats delivered lobsters of different quality. A price differential would appear to be the only way to change this situation. Another processor disagree strongly with this view, saying that the best avenue for improving quality was through education of the fishers. He went on to describe the approach used in his company – assessing catch, providing published information, encouraging peer pressure – and stated that this approach had resulted in a significant improvement in product quality. Another participant agreed with this view and commented that in Western Australia there had been a marked improvement in post-harvest handling procedures. However, another audience member made the observation that while the information was available to fishers, installation of correct equipment and use of best practice handling procedures were not always the first priority.

(Stephen Hood, Glen O'Brien, Bruce Phillips, Wayne Hoskins, two audience members)

2.3 Live transport conditions

Some questions and comments related to issues of environmental conditions used to transport lobsters. A processor asked what avenues could be taken to enhance to survival of lobsters over long air flights - what is presently known with respect to optimal purging time, environmental temperature, treatment chemicals etc? Another participant enquired as to whether the optimum temperature and oxygen levels had been determined for live lobster transport. In reply, it was pointed out that these conditions were very species specific. The need for a better understanding of lobster biology was stressed. A comment was made that improvements in survival had been achieved through improvements in engineering but that what was now needed was a better understanding of lobster biology. Difficulties with attitudes of airline companies were also discussed – it was suggested that the airlines were more concerned with passengers than with freight.

(Bruce Phillips, Glen O'Brien, Mick Olsen, Louis Evans, Steven Hood, two audience members)

2.4 Education of Fishers

The need for improved approaches to education of fishers was a common thread through much of the discussions. The question was raised as to how fishers could be encouraged to use the Code of Practice to improve their handling procedures. It was commented that while they may read to information provided it did not necessarily follow that they would improve their practices. Another participant made the comment that coastal tours were not very effective and that research was required on the best approaches to ensuring fishers have access to and use information. It was commented that fishers listen most to other fishers and to staff from processing companies or Cooperatives.

Some suggestions for improving information transfer included:

- Training Fisheries WA staff in patrol boats and in depots in best practice procedures
- Conducting regular seminars for fishers as well as for researchers
- Developing information briefs similar to those used in the agriculture industry
- Setting up websites
- Encouraging more interaction between fishers and researchers
- Producing more video material
- Disseminating information in fisheries journals

(Ron Gould, Brian Jones, Bruce Phillips, Glen O'Brien, Mick Olsen, Louis Evans, Steven Hood, two audience members)

Meeting notes compiled by:
Associate Professor Louis Evans
Convenor
International Symposium on Lobster Health Management