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Stress indicators in marine decapod crustaceans, with particular reference to the grading of western rock lobsters (*Panulirus cygnus*) during commercial handling

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Abstract.   Good transport survival of western rock lobsters (*Panulirus cygnus*) is ensured by rigorous selection of healthy lobsters prior to packaging for transport. The rejects are attributed to stress during harvesting and handling. A major stressor, of variable severity throughout the fishery, is the storage and transport of the lobsters out of water with accompanying effects of temperature, disturbance and tail-flipping exercise on metabolic rate. Pointers to apparent fatigue or injury in weak lobsters may be found in lobster haemolymph. Published literature suggests a number of parameters that might prove to be predictors of mortality in *P. cygnus*, but these will have to be examined in detailed physiological studies. Information is also required from tissue metabolism and pathology to complete the picture. If the symptoms are the result of previous stress, then one obvious approach is to sample rock lobsters at key points along the harvesting and handling process, in conjunction with sampling of normal or 'baseline' lobsters and laboratory stress trials. Practical stress indicators, once identified, can be used both to test existing screening methods and in studies aimed at changing handling practices to reduce stress.

Extra keywords: live transport, live storage, emersion, hypoxia, ionic regulation, osmotic regulation, acid–base regulation, haemolymph volume regulation, metabolism

Introduction

Live transport is increasingly used to maximize returns from the Western Australian fishery for western rock lobster (*Panulirus cygnus*). Palinurid lobsters, like the homarids, are relatively easy to send by air-freight long distances to market, with only minor losses. Although even minor losses add up to a lot of lobsters over time and provide their own impetus for transport monitoring, little physiological research has been conducted in the post-harvest area.

Rigorous selection of healthy lobsters occurs before they are packaged for transport. For example, on average only about 80% of the western rock lobsters arriving at a factory might be thought fit for live export. Processors would have more options for processing lobsters if all lobsters were fit for live export, the more so because loss of physiological condition in very weak lobsters may be accompanied by a deterioration in flavour or texture (Boyd and Sumner 1973).

Stress associated with harvesting and handling has been blamed for these losses. A stress response occurs when a regulated physiological system is pushed beyond normal bounds by one or more external factors or 'stressors' acting upon it (Barton and Iwama 1991). To focus more clearly upon stress indicators, we should first consider what stressors western rock lobsters may experience during post-harvest handling.

Rock lobster harvesting and handling

Practices in the fishery for western rock lobster are similar to those applied to other species of rock lobster. The lobsters are caught in pots and begin to tail-flip vigorously as they leave the water and during sizing and handling on the boat. It is recommended that the rock lobsters stay in air on the boats for as short a period as possible to reduce stress (Brown and Caputi 1983), though very little is known about the physiological effects of keeping *P. cygnus* in air on boats. Lobsters generally cannot breathe properly in air, and this problem may certainly be compounded by high temperatures, disturbance and handling (Taylor and Whiteley 1989; Whiteley and Taylor 1990).

The catch of western rock lobster is stored on the boat in sea-water holding tanks. These tanks must be well supplied with well oxygenated sea water because disturbed lobsters have a respiration rate that is higher than normal (Crear and Forteath 1997). If a suitable storage environment is provided for harvested lobsters, most physiological parameters disturbed by emersion and/or tail-flipping will return to nearly normal levels within about an hour, depending upon the severity of the stress, though some aspects of recovery may take longer (Whiteley and Taylor 1992).

Following storage on the boats, some lobsters are unloaded directly at the factory, but others arrive at the factory after a journey by truck (stored cool in air or under a

sea-water spray). Still others are stored in floating pens and taken by fast carrier boats, again under chilled sea-water sprays, to the factory. The importance of controlling temperature when transporting lobsters out of water has been demonstrated in a recent study (Tod and Spanoghe 1997).

At the factory, the lobsters are weighed and sorted for size, injury and vigour, a process that involves further disturbance and handling in air. The lobsters are then placed in storage tanks, where they are kept for several days before they are packed for live export. The lobsters need an environment that allows them to recover from the physiological stress accompanying harvesting and transportation. This recovery may occur relatively rapidly (Taylor and Whiteley 1989; Whiteley and Taylor 1992; Spanoghe 1997) unless the lobsters have suffered permanent injury and later weaken and die in captivity.

Post-harvest handling of western rock lobsters therefore involves periods of storage or transport in air alternating with periods of submerged recovery. This apparent recovery needs to be studied carefully, particularly since some lobsters are already graded as weak on arrival at the factory and others may weaken and die subsequently. In order to find out why this is happening, we need to be able to quantify stress in rock lobsters.

Stress indicators in marine decapod crustaceans

Factory staff use damage, posture and responsiveness of the rock lobsters to grade them for live export. Studies frequently show that clawed and spiny lobsters may lose the ability to tail-flip and may show other behavioural aberrations after on-board handling and transport (Brown and Caputi 1983; Vermeer 1987; van der Meeren 1991). However, some of these effects may be reversible, and the question is whether a rejected lobster is stressed or simply temporarily fatigued (Spanoghe 1997). Another issue is whether a fraction of the lobsters currently graded as fit are actually stressed and likely to die during export.

Muscle exhaustion and recovery can be demonstrated by measuring energy reserves following a tissue biopsy. However, a simple non-destructive method of measuring stress in lobsters would be of more practical benefit to the lobster industry. This limits observations to the vitality of the animal and to parameters in the haemolymph, particularly those that might be associated with irreversible physiological damage.

Crustacean haemolymph is a solution carrying inorganic ions and gases, products of digestion, and the substrates and wastes of metabolism, physiologically important organic compounds such as amines and steroid hormones, proteins and peptides, along with a population of cells, the haemocytes. Three kinds of haemocyte have been described on the basis of morphological and functional characteristics in the haemolymph of crustaceans: hyaline, semi-granular

and granular cells. These haemocytes participate in coagulation and mediate the phenoloxidase (melanization) response to foreign materials; however, the details are still poorly understood (Soderhall and Cerenius 1992). The numbers of haemocytes circulating in the haemolymph generally fall in response to bacterial and other infections (Johnson 1976). Incidentally, bacteria are often detected in the haemolymph of captive and apparently 'healthy' crustaceans; however, this may be due to the stress and physical injury associated with handling and capture (Messick and Kennedy 1990).

Specific aspects of the haemolymph physiology of crustaceans are covered separately in recent reviews (Regnault 1987; Depledge and Bjerregaard 1989; Burnett 1992; Mangum 1992; Wheatly and Henry 1992; Santos and Keller 1993a). The following review of stress responses in marine decapods is simply intended to consider which haemolymph components may prove to be useful indicators of stress or physiological dysfunction in rock lobsters.

Inorganic ions

Rock lobsters cannot regulate their haemolymph osmotic pressure independently of the environment (Dall 1974b), so we cannot use the loss of ability to osmo-regulate as an indicator of stress (Charmantier et al. 1994). However, western rock lobsters resemble other crustaceans in regulating the concentrations of certain physiologically important ions in the haemolymph independently of concentrations in the environment, a process typically disturbed by ecdysis or stress. Potassium concentration is normally tightly regulated in crustacean haemolymph (Dall 1974b). Calcium ion concentration in haemolymph changes during buffering of acidosis and during moulting as part of the process of calcification (Mercaldo Allen 1991). Paradoxically, changing the salinity in either direction causes a rise in magnesium concentration in the haemolymph of P. cygnus, suggesting that the role of the antennal gland in ridding the body of excess fluid at low salinity outstrips the capacity of the gland to regulate magnesium concentration (Dall 1974b). Rises in haemolymph magnesium concentration accompany ecdysis, commercial shipment or hypoxia in lobsters and crabs (Albert and Ellington 1985; Mercaldo Allen 1991; Whiteley and Taylor 1992).

Oxygen and oxygen uptake

Disturbed lobsters have a high demand for oxygen, and despite the high gill ventilation rates typical of 'stressed' crustaceans (Jouve-Duhamel and Truchot 1985), even a modest fall in oxygen concentration in the water curtails the rate of oxygen uptake (Nimura and Inoue 1969).

Haemolymph oxygen concentration falls when the concentration of oxygen in the environment falls, when

crustaceans are taken from the water for extended periods, and when gill function is compromised in other circumstances (such as during moulting), and this in turn influences the function of the respiratory pigment, haemocyanin (Taylor and Whiteley 1989; Burnett 1992; Mangum 1992). The haemolymph may become colourless during internal hypoxia, losing the characteristic blue-grey of oxygenated haemocyanin. Apart from this overt change in appearance, changes in the rate of oxygen delivery to the tissues during stress will also influence the entry of other potential stress indicators, such as metabolic wastes, into the haemolymph.

Metabolites and waste products

Glucose concentration in crustacean haemolymph generally rises in response to a number of stressors such as handling, emersion and disease (Dall 1974*a*; Spindler-Barth 1976; Santos and Keller 1993*b*). The changes in haemolymph glucose concentration during moulting are variable and no generalizations can be made from the species already studied (Telford 1968; Lacerda and Sawaya 1986; Chan *et al.* 1988; Mercaldo Allen 1991).

Respiring crustacean tissues oxidize metabolic substrates completely to carbon dioxide when oxygen is plentiful and incompletely to lactic acid when it is not (Albert and Ellington 1985). Both carbon dioxide and lactate can accumulate in the haemolymph during emersion (Johnson and Uglow 1985; DeFur *et al.* 1988; Whiteley *et al.* 1990) and the resulting acidosis must be corrected primarily by buffering in the haemolymph and then, when the crustaceans are resubmerged, by carbon dioxide excretion and ionic exchanges at the gills (Wheatly and Henry 1992). The lactate itself is not usually excreted (Gade *et al.* 1986). Instead, when sufficient oxygen is available, this waste is converted back into other metabolic intermediates and either oxidized further to carbon dioxide or used to resynthesize storage sugars such as glycogen (Gade *et al.* 1986).

Aquatic crustaceans excrete the nitrogen derived from protein and amino acid catabolism primarily through the gills as ammonia (ammonotely). The concentration of this waste in the haemolymph changes in response to stress and ecdysis (Hunter and Uglow 1993*b*). Ammonia concentration falls when the excretion rate through the gills rises in response to stressors such as handling, noise or low salinity (Regnault and Lagardere 1983; Regnault 1987). Conversely, both high environmental ammonia concentrations and emersion impair gill ammonia excretion and lead to accumulation of this waste (Vermeer 1987; Chen *et al.* 1994; Schmitt and Uglow 1997*a*, 1997*b*). In addition to changes in ammonia concentration, partial departures from ammonotely have been reported in other crustaceans in response to salinity change, high environmental ammonia concentrations, hypoxia and handling (Spaargaren *et al.* 1982; Hagerman *et al.* 1990; Hunter and Uglow 1993*a*;

Chen *et al.* 1994), causing other nitrogenous wastes such as urate to accumulate in the haemolymph (Dykens 1991). When western rock lobsters are challenged by a drop in salinity, they mobilize nitrogenous osmolytes rapidly into their haemolymph and then lose most of this by excretion (Dall 1975*b*). Perhaps disturbances in the osmotic balance of decapods underlie other apparent departures from ammonotely occurring in response to various stressors.

Hormones

Various peptide, amine and steroid hormones circulate at times in the haemolymph and modulate the physiological state of crustaceans (Fingerman 1995). Hormones already implicated in stress or handling reactions include crustacean hyperglycaemic hormone (CHH) and neurohormones that modulate rates of heartbeat and gill ventilation. CHH, an eyestalk-derived factor, raises the glucose concentration in the haemolymph in response to handling and emersion, though the mechanism of this response is still being elucidated (Santos and Keller 1993*b*). Other neurohormones apparently contribute to the prolonged periods of elevated rates of heartbeat and gill ventilation following handling or experimental surgery (Wilkens *et al.* 1985). By influencing the dynamics of tissue perfusion and exchange of materials at the gill, these hormones may influence rates of waste excretion and possibly even the concentrations of other haemolymph components in circulation (Regnault 1987).

Proteins

Protein concentration in the haemolymph is diluted by water uptake and the rise in haemolymph volume during moulting (Mercaldo Allen 1991), and it also falls in starved lobsters, in which the wasting of muscle tissue increases the haemolymph volume (Stewart *et al.* 1967; Dall 1975*a*). There is some disagreement about other cases in which protein concentration changes. Dall (1975*a*) reported a rise in haemolymph volume and consequential fall in protein concentration in recently captured western rock lobsters. Protein catabolism has been offered as an explanation for the fall in total protein concentration in haemolymph of shrimp exposed to high concentrations of ammonia and nitrite (Chen *et al.* 1994; Chen and Cheng 1995). However, an increase in haemolymph volume is possible if the haemolymph osmotic pressure falls in these cases.

Haemolymph proteins collectively, along with the carbonate–bicarbonate system, have a broad physiological role in acid–base regulation (Wheatly and Henry 1992); however, the bulk of the protein fraction is concerned either with oxygen transport or, in the case of the haemocytes, in host-defence reactions. Enzymes also occur in the haemolymph (Najafabadi *et al.* 1992), and the activity of one of these—acid phosphatase—has already been linked to stress-related cell lysis in shrimp (Dillon and Fisher 1983).

The oxygen-binding macromolecule, haemocyanin, is usually the largest fraction of protein (Depledge and Bjerregaard 1989). Stressors affect the ability of haemocyanin to bind and release oxygen through haemolymph factors that change in response to stress, such as pH and concentrations of inorganic ions (particularly calcium) and organic ions (e.g. lactate and urate) (Lallier and Truchot 1989; Burnett 1992). Haemocyanin is disabled by some stressors, such as extreme oxygen deprivation; however, compensatory synthesis of additional haemocyanin in response to hypoxia has also been reported (Hagerman and Pihl Baden 1988; Hagerman *et al.* 1990).

Host-defence proteins in crustacean haemolymph include a protein associated with coagulation (at sites of both wound repair and encapsulation of foreign materials) and putative antibacterial agents such as bactericidins and agglutinins (Soderhall and Cerenius 1992). Stress may leave crustaceans more vulnerable to bacterial infection (Sugita and Deguchi 1994), and once disease strikes, disturbances have been reported in total protein concentration, the concentrations of individual protein components in the haemolymph, and the ability of haemolymph to coagulate (Johnson 1976; Spindler-Barth 1976; Henke 1985).

Future work

Haemolymph components may provide important information about the physiological condition of lobsters, though experiments are clearly required to test the efficacy of particular indicators in rock lobsters. If lobsters arrive weak at the factory because of previous stress, then an obvious way to examine this is to sample lobsters at key points along the handling chain to identify parameters that deviate from normal values and see whether these deviations occur in lobsters rejected by the existing grading practices. Normal or 'baseline' values of parameters must be established by sampling the haemolymph of lobsters *in situ*, at various times of the year and in different parts of the fishery, as well as by bringing lobsters into the laboratory to allow them to recover before they are sampled. Understanding the seasonal and biological changes in the fishery may also clarify how this variability affects the stress susceptibility of lobsters.

Commercial handling can involve numerous uncontrolled stressors. Changes seen in the lobsters on boats and in factories can therefore be better interpreted through controlled stress tests in the laboratory. Major stressors to test in the laboratory are clearly emersion and accompanying factors such as disturbance and temperature. Knowing the effects of these stressors on other marine decapods determines to some extent the list of parameters that should be measured. Studies of crabs and lobsters during commercial handling in air have concentrated largely on the effects of lactic acid accumulation and acidosis (DeFur *et al.* 1988; Spicer *et al.* 1990; Varley and Greenaway 1992;

Whiteley and Taylor 1992), though recently attention has also been paid to the concentrations and excretion of nitrogenous wastes (Hunter and Uglow 1993*a*; Schmitt and Uglow 1997*a*). Although ammonia concentration in the haemolymph would be an obvious parameter to study, studies of other decapods suggest that other nitrogenous wastes such as urate or urea would have more potential as stress indicators (Dykens 1991; Chen *et al.* 1994).

Inorganic ions must also be considered. Calcium concentration must be measured because it is associated with the buffering of acidosis, and potassium concentration must also be measured because it would be unusual for such a tightly regulated parameter to deviate. Beyond concentrations of particular ions, reports of haemolymph volume changes in captive rock lobsters (Dall 1975*a*) mean that we cannot rule out disturbances in the osmotic or fluid balance of these osmotic conformers in response to stress. Measuring osmotic pressure and total protein and magnesium concentrations in the haemolymph would provide useful checks of body volume regulation and antennal gland function.

A wide selection of haemolymph parameters must be tested because the range for some parameters will be relatively wide in unstressed lobsters and because some stress indicators may not be simple and inexpensive enough for factory research. A certain degree of acidosis and lactate and calcium accumulation in the haemolymph is a normal consequence of tail-flipping exercise or moulting, so a change in these parameters is hardly an incontrovertible indicator of stress. Sophisticated analytical techniques can be used in the laboratory, but parameters amenable to physical or colorimetric tests also need to be considered because of the simplicity and economy of these tests under factory conditions.

Concentrations of materials in the haemolymph change as a consequence of other processes, so information about the production of these compounds in the tissues and the excretion of some materials may be required to provide a complete picture. Because histological changes may occur in parallel with physiological changes, a collaborative study combining the diverse approaches of haemolymph chemistry, tissue biochemistry and histopathology may make it easier to interpret changes in the haemolymph.

Conclusions

Almost anything that can be measured in crustacean haemolymph may be a stress indicator of some kind. Stress in rock lobsters and other commercially harvested decapods has been considered in the past from the relatively narrow perspective of a particular discipline or a small set of parameters. More progress could be made by a collaborative study combining the diverse approaches of haemolymph chemistry, tissue biochemistry and histology.

Identifying stress indicators for western rock lobsters in

this way can have two benefits. Firstly, by showing how stress develops in the lobsters and indicating what aspects of handling cause most problems after harvesting, it may be possible to change the handling regime to ensure that lobsters are stressed as little as possible. Secondly, the physiological stress indicators for this species will be a useful tool for examining existing grading methods in order to ensure that lobsters selected as fit for live marketing are just that.

These benefits would obviously be most useful in the short-term storage of rock lobsters for live export. However, a thorough understanding of the stress physiology of rock lobsters would also benefit the long-term storage, moulting and growth of western rock lobsters in captivity.

Acknowledgments

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