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Haemolymph chemistry of tropical rock lobsters (*Panulirus ornatus*) brought onto a mother ship from a catching dinghy in Torres Strait

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Abstract. For export of live *Panulirus ornatus* from northern Queensland, divers catch the lobsters by hand and keep them in small tanks on dinghies before draining the tanks and returning at speed to a mother ship that has a larger storage tank. The lobsters are sometimes too weak for export. The physiological state of lobsters stored in a tank on the mother ship was studied by measuring the concentrations of L-lactate, D-glucose and ammonia in the haemolymph. Oxygen levels in the dinghy tanks were normally acceptable but fell rapidly below 50% saturation when flow was stopped and the tank was draining. The concentration of lactate in the haemolymph of lobsters arriving from the dinghy was 16.4 ± 5.7 mmol L^{-1} (mean ± s.d.,n = 9); this fell during storage on the mother ship. On the mother ship, serum concentrations of calcium, potassium and magnesium ions all increased, haemolymph glucose concentration increased slightly and then decreased, and ammonia concentration did not change. Future work may identify which aspects of prior handling are responsible for the elevated lactate concentrations in captive lobsters, but improvements could be made meanwhile to water flow through the dinghy tanks.

Introduction

The lobster fishery in Torres Strait, based on the tropical rock lobster (*Panulirus ornatus*), had an annual catch in 1993 of 190 t (of tails), with a commercial value of $A4.5 million (Pitcher and McLoughlin 1994). Recently, the premium paid for live tropical lobsters has stimulated increased export of live lobsters. Divers can catch the lobsters by hand (rather than spearing them) and keep them alive in small tanks on the dinghies before draining the tanks and returning at speed to a mother ship that has a larger storage tank.

The advantage of using dinghies is that the mother ship can anchor in the lee of a reef and the dinghies and divers can then fan out over a large area of ocean. The main problem, however, is that the dinghy tanks are drained for short periods when the dinghies are travelling at speed, either from dive site to dive site or when returning to the mother ship. Although keeping the lobsters in air for even a few minutes may stress them, the most immediate problem may be that lobsters at the bottom of the pile are likely to be trapped in nearly anoxic water if the tank does not drain completely. But it is worth noting that for all their apparent or potential faults, the dinghy tanks usually deliver apparently healthy lobsters to the mother ships.

Occasional episodes in which captured lobsters proved to be too weak for export have prompted this study of the post-harvest handling and physiology of *P. ornatus*, particularly to see whether the small tanks on the dinghies are properly oxygenated.

The design of the tanks varies considerably from dinghy to dinghy. The dinghy studied here uses a bilge pump on the stern to pump sea water into an above-deck tank that has other pipe s to drain the water away. The lobsters stay in these tanks for up to an hour before being transferred to the mother ship. The below-deck storage tank on the mother ship is quite large and easily able to accommodate catches of a couple of hundred kilograms gathered over a cruise of four to five days.

There is little physiological information on *P. ornatus* other than a study of the physiological deterioration in this species during the breeding migration (Trendall and Prescott 1989). However, there are some comparative physiological data available from other lobsters and crabs during live transport and handling that indicate that the stressed crustaceans are likely to show disturbances in lactate (an indicator of tissue hypoxia), glucose (a general stress indicator in crustaceans), ammonia (an end product of protein degradation), and serum electrolytes (indicators of ionic balance) (Vermeer 1987; DeFur *et al.* 1988; Spicer *et al.* 1990; Whiteley and Taylor 1992; Hunter and Uglow 1993).

In order to learn about the physiological condition of the lobsters, we measured oxygen concentrations in the dinghy tank and took haemolymph samples from lobsters arriving on the mother ship and following different periods of recovery and analysed these for parameters known to respond to capture and handling in other crustacean species.

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Materials and methods

This work was conducted on a live-lobster boat (mother ship) and a lobster dinghy fishing at Beka and Numa Reefs (9˚45′S,142˚22′E and 9˚40′S,142˚20′E respectively) in central Torres Strait in June 1996.

The concentration of dissolved oxygen in the water leaving the dinghy tank was monitored with a DO 300 submersible oxygen sensor modified with a rapid-response membrane (Greenspan, Warwick, Qld). The sensor was placed inside the tank so that the membrane was adjacent to the outlet. It remained in place, recording oxygen concentration and temperature every 2 min, during a day of fishing.

Groups of nine lobsters had haemolymph samples taken from them at different times after their arrival on the mother ship. As the catching pattern of each dinghy trip differed, this source of variation was distributed between sampling times. Therefore, after each trip, nine healthy lobsters were taken at random; three were sampled immediately, and two groups of three lobsters were submerged and their haemolymph sampled after either 1, 3 or 9 h of recovery had elapsed. This continued until nine lobsters had been sampled in each time category.

Haemolymph was sampled (about 1.5 mL) from the pericardial sinus with the aid of 2-mL plastic syringes and 25-G hypodermic needles (38 mm long) kept ice-cold to retard clotting. Access to the pericardial sinus was achieved through the arthrodial membrane at the rear of the carapace (‘head’). Each lobster was sampled only once.

A subsample (0.7 mL) of each haemolymph sample was extracted in an equal volume of ice-cold 0.6 mol L^{-1} perchloric acid and centrifuged, and the supernatant was neutralized by addition of 3 mol L^{-1} KOH. The subsample extracts and remaining haemolymph were frozen in microcentrifuge vials in a freezer on the mother ship, flown to Brisbane, and stored at –70˚C until analysis.

The neutralized haemolymph extracts were analysed spectrophotometrically for L-lactate and D-glucose with the aid of Boehringer Mannheim Kits 139084 and 716251 respectively. The concentration of lactate in the extracts was often above or below the concentration range required for the assay, so these samples were brought onto the calibration curve by repeating the assays with a different volume of extract.

Ammonia concentration in whole haemolymph was determined by the Berthelot reaction (Sigma Diagnostics procedure No. 640). The ultrafrozen stored vials of whole haemolymph were thawed in ice water to discourage coagulation. Absorbances of assays of three replicate 40-µL subsamples were read against a reagent blank and were compared against a calibration curve prepared by assaying 10-µL samples of four ammonium chloride standards (0.5 to 5 mmol L^{-1}).

A Pye Unicam spectrophotometer and 1-cm cuvettes were used for all absorbance measurements during assays of metabolites.

Serum electrolyte concentration was determined in the thawed samples following ammonia determination. The remaining unused haemolymph samples were placed in the refrigerator and allowed to coagulate overnight. Then the clot in each was broken up with a clean stirring rod and the vials were centrifuged for 30 min at 13000 *g* to produce serum (Stewart *et al.* 1966) that was then diluted before analysis. Magnesium and calcium concentrations were determined colorimetrically on an Olympus Reply instrument by the Calgamite method and the Arsenazo III method respectively (Trace Scientific). Potassium was determined by flame atomic absorption spectroscopy on a Varian Techtron SpectrAA-40, using an air–acetylene flame and 1000 mg L^{-1} caesium chloride as a suppressant.

Results

Oxygen concentration in the dinghy tank

The concentrations of dissolved oxygen in the storage tank on the dinghy were generally good, except for brief periods when the oxygen concentration fell precipitously when sea water stopped entering the tank (Fig. 1). The period of apparent supersaturation in Fig. 1 is an artefact caused by removing the oxygen sensor temporarily from the water.

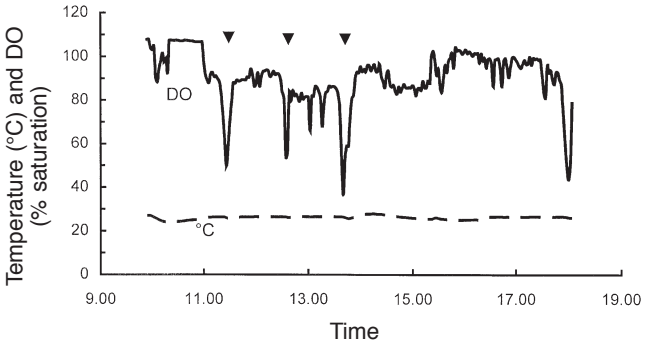

Fig. 1. Record of dissolved oxygen (DO) and temperature in a dinghy tank during a day of fishing. Marked falls in oxygen tension during interruptions to flow are marked with arrows.

L-Lactate and D-glucose

Lobsters arriving on the mother ship had a lactate concentration of 16.4 ± 5.7 mmol L^{-1} (mean ± s.d., $n = 9$) in their haemolymph. Lactate concentration decreased while the lobsters were stored on the mother ship, although the wide variation between lobsters early during storage violates the assumption of homogeneity of variances (Table 1). The concentration of glucose in the haemolymph of *P. ornatus* arriving on the mother ship was 1.8 ± 0.9 mmol L^{-1}. The concentration of glucose in haemolymph of lobsters sampled after 1 h of storage was significantly higher ($P < 0.001$) than that of lobsters sampled on arrival or after longer periods of storage. There was considerable variation in both glucose and lactate, as indicated by the relatively wide standard deviations. Glucose concentration was not correlated with lactate concentration ($r^2 = 0.25$). One lobster sampled 9 h after being submerged on the mother ship had a glucose concentration of 3.1 mmol L^{-1}, outside the range obtained from the other eight lobsters (0.5 to 1.5 mmol L^{-1}). The lobsters sampled in this study showed no symptoms of morbidity, such as flaccid abdomen or legs.

Table 1. Haemolymph chemistry of tropical rock lobsters (*Panulirus ornatus*) stored on a mother ship for up to 9 h after arriving from a catching dinghy

Concentrations are given as mean ± s.d., in units of mmol L^{-1}. Means with different letters are significantly different at 5%

	Hours stored on mother ship			
	Arrival	1	3	9
No. of lobsters	9	9	9	9
L-Lactate	16.4 ± 5.70	15.6 ± 5.49	8.9 ± 5.98	1.0 ± 0.78
D-Glucose	1.8 ± 0.86[a]	2.9 ± 0.67[b]	2.3 ± 0.53[ab]	1.3 ± 0.76[a]
Ammonia	1.2 ± 0.27	1.3 ± 0.14	1.3 ± 0.16	1.2 ± 0.16

Ammonia

No significant change in ammonia concentration occurred for the range of storage periods examined (Table 1).

Serum electrolytes

The concentrations of all three ions analysed increased while the lobsters were stored on the mother ship (Table 2). Magnesium concentrations (log-transformed because of heterogeneity of variances) were significantly higher after 1 h of storage ($P < 0.05$), whereas lobsters sampled 9 h after arrival had significantly elevated potassium concentrations ($P < 0.05$). Calcium concentrations in lobsters arriving on the mother ship were relatively uniform and increased variably in the stored lobsters (Table 2).

Table 2. Electrolytes in serum of tropical rock lobsters (*Panulirus ornatus*) stored on a mother ship for up to 9 h after arriving from a catching dinghy

Concentrations are given as mean ± s.d., in units of mmol L^{-1}. Means with different letters are significantly different at 5%

	Hours stored on mother ship			
	Arrival	1	3	9
No. of lobsters	9	9	9	9
Calcium	14.2 ± 1.58	25.9 ± 6.39	27.4 ± 7.08	27.8 ± 3.96
Magnesium	8.7 ± 1.23[a]	14.5 ± 3.50[b]	14.3 ± 3.77[b]	13.1 ± 1.57[b]
Potassium	8.7 ± 1.65[a]	10.8 ± 3.40[b]	10.5 ± 4.12[b]	17.1 ± 3.60[b]

Discussion

Lobsters arriving on the mother ship have, on average, very high concentrations of lactate in their haemolymph, indicating that they are in a state of oxygen debt, which could be a result of a number of aspects of their prior handling, such as exercise (Field *et al.* 1991), emersion (Whiteley and Taylor 1990; Whiteley *et al.* 1990), or aquatic hypoxia (Lowery and Tate 1986).

The divers say that the lobsters flap their abdomens when they are captured and put in the net bag, and the lobsters also flap when they are placed in the dinghy tank. However, the concentrations of lactate found here seem excessive in comparison with concentrations in haemolymph of *P. argus* kept out of water for much longer (2 h) (Vermeer 1987) or concentrations in exercising crayfish (Phillips *et al.* 1977; Head and Baldwin 1986). A combination of several factors may be involved.

Although we cannot point out the exact cause of the lactate build-up, it is clear that these disturbed lobsters will have a respiration rate that is higher than normal (Nimura and Inoue 1969). Disturbed lobsters crowded into a small volume of static sea water at 25–30˚C will exhaust the available oxygen more rapidly than will resting, undisturbed lobsters. Haemolymph lactate concentration fell when lobsters were allowed to recover submerged on the mother ship. An oxygen debt is often repaid by a heightened rate of oxygen consumption during lactate oxidation or gluconeogenesis (Bridges and Brand 1980).

The concentration of glucose in the haemolymph of *P. ornatus* arriving on the mother ship was only slightly higher than that reported for fed, undisturbed western rock lobsters (*P. cygnus*) in the laboratory (1.2 mmol L^{-1}) (Dall 1974*a*). This finding is encouraging, if unexpected. Glucose concentration is recognized as a general indicator of stress in crustaceans, and on the basis of this previous work on *P. cygnus*, considerably higher concentrations of glucose were expected in the haemolymph of the present animals. However, baseline concentrations of glucose in wild lobsters are possibly lower than those in captive lobsters, as is suggested by observations of *Nephrops norvegicus* and *Homarus americanus* (Telford 1968; Spicer *et al.* 1990), so even the present concentrations may indicate a degree of stress.

The apparent brief rise in glucose concentrations in lobsters on the mother ship is at least consistent with the idea that the lobsters are disturbed by handling, but some caution is required when interpreting this peak primarily as a ‘stress’ response. Perhaps lobsters are simply converting some lactate to glucose. The high glucose concentration measured at 1 h could conceivably be the trailing shoulder of an even higher peak in glucose concentration that was missed by the sampling protocol.

Hypoxia and handling are reported to reduce the haemolymph ammonia concentration in decapods (Hagerman *et al.* 1990; Hunter and Uglow 1993). However, the ammonia concentration in lobsters arriving on the mother ship was higher than the range reported from various crustaceans (Hagerman *et al.* 1990) and higher than that reported from *P. argus* after 2 h in air (Vermeer 1987). Furthermore, the concentration in the haemolymph did not change during the period of sampling, though it is clear that information about ammonia excretion is required to interpret this.

There was considerable variation between individual lobsters in lactate and glucose concentrations in the haemolymph. This may be because the lobsters coming onto the mother ship have either been in captivity for different periods or been stressed to different degrees, or even because they show broad intrinsic variation. For example, a bag of lobsters brought onto the dinghy just before return to the mother ship may not actually be submerged until the dinghy reaches the mother ship.

Despite the high concentrations of lactate in the lobsters arriving on the mother ship, the concentrations of calcium, magnesium and potassium ions in the haemolymph of the newly caught tropical rock lobsters were close to what would be considered normal in other rock lobsters Travis 1955; Dall 1974*b*; Mercaldo Allen 1991). But the concentrations of these ions increased to abnormal levels while the lobsters were apparently recovering on the mother

ship. This may be a delayed response to the previous stress on the dinghy, particularly since a rise in calcium concentration is sometimes seen during buffering of haemolymph acidosis (Burnett 1992). Similarly, loss of ability to regulate haemolymph magnesium concentration accompanies commercial shipment and hypoxia in lobsters and crabs (Albert and Ellington 1985; Whiteley and Taylor 1992). The late rise in potassium concentration is cause for concern because resting lobsters normally tightly regulate the concentration of this ion (Dall 1974*b*). To clarify the significance of the potassium data, we need observations of lobsters that are in captivity for longer periods to find out whether these elevated concentrations are sustained.

It has been shown that lobsters arriving on the mother ship can have extraordinarily high concentrations of lactate in their haemolymph, but it remains to identify what aspects of their previous handling are responsible. This is not as easy to do as it sounds because of the practical difficulties of working on small dinghies. However, what is clear is that the disturbed lobsters are likely to have a high demand for oxygen, and this requires that the industry consider, as a priority, whether there is adequate water flow into the dinghy tanks. Given that the lobsters are usually in good enough condition for export, this suggests that only under some circumstances are the lobsters unable to tolerate this degree of physiological stress. Factors such as the stocking density and the physiological condition of lobsters being carried on the dinghies could be considered in future studies.

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