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The effect of lead on the metabolic and energetic status of the Yabby, *Cherax destructor*, during environmental hypoxia

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Abstract

The concomitant effects of Pb and hypoxia on respiration and muscle energy status were examined in the freshwater crayfish *Cherax destructor* to determine if Pb intoxication exacerbated the effects of hypoxia. *C. destructor*, either intoxicated for 14 days with 0.5 mg L^{-1} Pb, or from Pb-free control conditions, were subjected to progressive hypoxia at -2.7 kPa h^{-1} to a O_2 partial pressure in the water (P_wO_2) of 1.33 kPa. This hypoxia was then sustained for 3 h. Pb-exposure reduced O_2-uptake ($\dot{M}O_2$) at all P_wO_2 above 1.33 kPa but without any saving in ventilation, implying that Pb either unlinked ventilation from actual O_2 requirements or rendered O_2 transfer across the gill less efficient. Hypoxia alone induced no change in the adenylate energy charge (AEC), total adenylate (TAN), ATP/ADP ratio or in the equilibrium constant for adenylate kinase (K'_{ADEN}), apparently due to protection of ATP levels by arginine phosphate. Under maximal hypoxia (P_wO_2 = 1.33 kPa) the Pb-exposed crayfish increased muscle ADP by 73% (tail) and 158% (chelae) but without any change in AMP, ATP or TAN. Thus, AEC declined (chelae AEC = 0.71; tail AEC = 0.85), as did the ATP/ADP ratio and K'_{ADEN}. L-Lactate increased in the muscle tissues of control but not Pb-exposed crayfish, consistent with a lowered O_2 requirement in the Pb-exposed animals. The Pb intoxication slowed respiration and probably glycolysis, possibly altering the [ATP]:[ADP] equilibrium concentrations for adenylate kinase (K'_{AK}). Lowered $\dot{M}O_2$ during severe hypoxia slows oxidative phosphorylation and ADP accumulation could occur as non-utilised substrate and may reflect a transient disequilibrium. During this time ATP levels were protected by arginine phosphate. AEC is sensitive to Pb in hypoxic crayfish but the changes have low importance for the energetic competence of the crayfish. During sustained hypoxia the crayfish recovered their energy status regardless of the Pb-exposure and this was, therefore, not a feature of Pb intoxication. Consequently, the ADP was recovered into the ATP pool of the hypoxic crayfish, and demand on arginine phosphate relieved. The Pb exposure did not otherwise exacerbate the effect of sustained hypoxia and *C. destructor* appeared to cope well with Pb intoxication, apparently by a specific Pb-induced hypometabolism separate from hypoxic response. Lowered metabolism as a survival response has limitations in the longer term and the implications for crustaceans generally warrant further study.

Keywords: Crayfish; Hypoxia; Lead; Adenylate; AEC; Lactate; Metabolism

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1. Introduction

The 'Yabby' *Cherax destructor* is an endemic crayfish in eastern temperate Australia. Heavy metal contamination, including lead, is prevalent along and within the industrialized tributaries and inflows of the larger rivers in urban centers of eastern Australia (e.g. Birch and Taylor, 1999; Taylor et al., 2004a). Lead remains biologically active in the environment since it is not degraded, thus levels continue to increase (WHO, 1995). Macro-invertebrates now form an important part of almost all river health monitoring schemes (e.g. Barbour et al., 1999; Dickens and Graham, 2002; Clarke et al., 2003; Parsons et al., 2004). The increased storm-water drainage in urban areas can lead to species loss (Walsh et al., 2004) partly due to euthrophication (Taylor et al., 2004b) and the habitat of *C. destructor* can routinely become hypoxic (Merrick, 1993).

C. destructor may become hypometabolic in response to declining O_2 in the water (Morris and Callaghan, 1998a), or if possible, it will intermittently leave hypoxic water to breathe air (Morris and Callaghan, 1998b). For crustaceans generally, the response to either environmental hypoxia or impaired O_2 diffusion at the gills is to increase ventilation frequency (e.g. Taylor, 1988; Reiber, 1995; Morris, 1991). *C. destructor* maintained haemolymph PO_2 during progressive hypoxia by a four-fold hyperventilation (Morris and Callaghan, 1998a). The apparent critical partial pressure (P_c) of O_2 in the water (P_wO_2) for ventilation and anaerobiosis in *C. destructor* ($P_wO_2 < 2.7$ kPa) was comparable to that of other oxyregulating crustaceans (Morris and Callaghan, 1998a) but lower than for other crayfish (P_c of 5.32 and 2.7 kPa; e.g. Wheatly and Taylor, 1981; Massabuau and Burtin, 1984). *C. destructor* maintained a mass specific O_2-uptake ($\dot{M}O_2$) during sustained hypoxia ($P_wO_2 \sim 1.35$ kPa) of approximately 10% of the normoxic rate (Morris and Callaghan, 1998a). The progressive hypoxia elicited little lactacidosis but instead the pH increased as a consequence of the hypocapnia from hyperventilation.

The muscle adenylate levels of *C. destructor* did not change during progressive hypoxia and Morris and Callaghan (1998a) calculated that maintaining metabolism without anaerobiosis or depletion of ATP would require almost 45 mmol kg^{-1} of arginine phosphate. Arginine phosphate may buffer ATP levels during extended hypoxia (below). However, converting the actual $\dot{M}O_2$ of those hypoxic crayfish to ATP production gave an ATP turnover rate only 14% of the normoxic rate.

Metals such as copper and zinc in the water can cause or exacerbate the effects of hypoxia in crustaceans, probably due to gill damage (e.g. Anderson, 1978; Nonnotte et al., 1998; Lawson et al., 1995; Spicer and Weber, 1992). Similar effects have been documented for lead exposed crayfish e.g. *Procambarus clarkii* (Torreblanca et al., 1987). Exposure of *C. destructor* to 0.5 mg L^{-1} Pb caused a reduction in $\dot{M}O_2$ of at least 50% within 24 h, and of 70% after 21 days but did not elicit any compensatory increase in ventilation, and thus appeared to induce a hypometabolic state (Ahern and Morris, 1999). These authors suggested that this effect of Pb may also promote an elevation of the threshold PO_2 for anaerobiosis. Lactate is the end product of anaerobic metabolism in hypoxic crustaceans including crayfish (e.g. Jackson et al., 2001) and would normally accumulate, together with increased glycolysis and glycogenolysis associated with a hyperglycaemia (for review, see Storey and Storey, 1990). The haemolymph glucose concentrations of Pb-impacted crayfish were indistinguishable from control values, neither was metabolism supplemented by anaerobiosis and thus overall energetic demand must have been lowered (Ahern and Morris, 1999). A hypometabolic response to Pb exposure may be a survival mechanism to endure severe degradation of water quality and consequent impairment of O_2 uptake. Avoiding anaerobiosis via progressive hypometabolism (lowered respiration) is energetically conservative (Hochachka, 1988) but limits physiological processes and behaviour. An increase in the P_c, for either ventilation or anaerobiosis, will increase the water PO_2 at which *C. destructor* experiences acid–base perturbations (Reiber, 1995), further reducing the ability to survive in a hypoxic environment. Thus, Ahern and Morris (1999) concluded that Pb contamination could therefore exclude *C. destructor* from waters prone to hypoxia.

Consequently, it was important to establish the effects of Pb in crayfish exposed simultaneously to hypoxia. In particular, it was crucial to determine if combined Pb and hypoxia effects perturb the metabolic capacity and thereby the possible distribution of this, and probably other, crustaceans. Previous assessments of *C. destructor* during hypoxia have considered phos-