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Nutritional status of *Carcinus maenas* (Crustacea: Decapoda) influences susceptibility to contaminant exposure

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ABSTRACT

Using the shore crab *Carcinus maenas* as a model, this study tested the hypothesis that nutritional status influences susceptibility of adult crabs (>60 mm carapace width (CW)) to environmental contamination. In the laboratory, crabs were either starved, given a restricted diet (fed on alternate days) or fully fed (fed each day). In addition, crabs under each feeding regime were exposed to a sublethal concentration (200 $\mu g\,l^{-1}$) of pyrene (PYR) as a model organic (PAH (polyaromatic hydrocarbon)) contaminant. Various physiological end points were measured after 7 and 14 days. Results indicated that adult shore crab physiology was relatively robust to short-term (7 days) nutritional changes as multivariate analysis (ANOSIM) showed no significant difference in shore crab physiological condition between control and pyrene-exposed crabs, irrespective of dietary feeding regime [Global $R = 0.018$, $P(\%) = 19.2$]. After 14 days, however, starved crabs showed significant impacts to physiological condition (as revealed by multivariate analysis) [Global $R = 0.134$, $P(\%) = 0.1$], [$R = 0.209$, $P(\%) = 0.1$]; starved individuals had significantly lower antioxidant status ($F_{2,48} = 5.35$, $P < 0.01$) compared to crabs under both types of feeding regime. Exposure to pyrene resulted in significantly elevated pyrene metabolite concentrations in the urine at 7 and 14 days compared with control individuals ($P < 0.001$), validating contaminant bioavailability, and this was found for all dietary treatments. Also, exposed crabs had significantly increased protein levels (proteinuria) than controls ($P < 0.001$) in their urine after 7 and 14 days, irrespective of dietary regime. After 7 days, pyrene-exposed crabs showed significantly increased antioxidant status ($P < 0.001$) and cellular functioning (increased cellular viability and decreased phagocytosis) ($P < 0.001$) compared to control crabs; however, after 14 days, antioxidant status ($P < 0.01$) and cellular viability ($P < 0.001$) were significantly decreased in pyrene-exposed compared to unexposed crabs. Results indicate that differences in nutritional status of adult crabs result in shore crabs being robust to short-term sublethal (7 days) pyrene exposure. Susceptibility to contaminant exposure, however, was measured after prolonged exposure (14 days) as indicated by reduced ability to combat oxidative stress. These results indicate that ecotoxicological studies need to take into account the nutritional state of the test organism to achieve the full assessment of contaminant impact. In addition, the results highlight that subtle seasonal biotic features of an organism can influence biomarker responses, and these need to be considered when interpreting field data and during the routine application of biological-effects tools in environmental monitoring.

1. Introduction

The shore crab *Carcinus maenas* experiences periods of reduced feeding during moulting (Lipcius and Herrnkind, 1982; Sanchez-Paz et al., 2006) and at times of limited food availability (Crothers, 1967). Crustacean growth is limited by the exoskeleton and only through moulting can an increase in body size be achieved (Crothers, 1967, 1968). Active feeding occurs during the intermoult stage when energy reserves are built up prior to moulting (Crothers, 1967). Approaching ecdysis, feeding declines and ceases during the process of moulting. The feeding cycle resumes postmoult when the exoskeleton hardens (Sanchez-Paz et al., 2006). The implications of fluctuating nutritional status for the ability of crabs to maintain optimal physiological function, especially at times of stress, remain to be studied. However, it is likely that the nutritional status of an organism is paramount to maintaining its biochemical and cellular homeostasis. Indeed, Sanchez-Paz et al. (2006) proposed that starvation in crustaceans provided a tractable model to understand the molecular and enzymatic changes which determine the energetic and metabolic function of the organism.

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In all eukaryotic cells, metabolism converts the energy in nutrients (or from storage) into a form which supports biosynthesis of molecules and maintenance of the internal environment; driven by generation of energy (in the form of ATP) via the Krebs cycle (or TCA cycle) (Bronk, 1999). The energy generated is used to oxidise pyruvate formed during the glycolytic breakdown of glucose and also oxidises acetyl CoA arising from fatty acid degradation and proteolysis to form amino acids; the latter are then used to synthesise new proteins required for survival or catabolised into Krebs cycle intermediates (e.g. citrate) to supply cells with energy (Hames and Hooper, 2000). The intermediates in the cycle provide precursor molecules for various biosynthetic pathways; synthesis of fatty acids from citrate, amino acid synthesis, synthesis of purine and pyrimidine nucleotides and glucose conversion by gluconeogenesis. With regard to *C. maenas*, glycogen is stored in granules of the alpha cells, where glucose is subsequently released upon breakdown of this storage polysaccharide (Johnstone et al., 1973). Fatty acid (lipid) breakdown occurs within the mitochondria, whereas protein degradation occurs within the lysosomes of cells (Sanchez-Paz et al., 2006). Nutrient deprivation or starvation may cause alteration to cellular metabolism, which may affect cellular functioning (de Duve and Wattiaux, 1966; Moore, 2004). The effect of starvation in *C. maenas* has been studied previously and effects such as suppression of metabolism (Marsden et al., 1973) and susceptibility to metal exposure [e.g. copper (0.5 mg l^{-1}) (Scottfordsmand and Depledge, 1993; Taylor and Anstiss, 1999) and cadmium (6 μM) (Styrishave et al., 2000)] have been reported.

The aim of this study was to investigate if nutritional status influenced the susceptibility of adult *C. maenas* to a sublethal exposure (200 μg l^{-1}) of pyrene (PYR), a highly lipophilic polyaromatic hydrocarbon (PAH). To achieve this aim, the nutritional status of adult crabs was evaluated in unexposed individuals and compared with individuals exposed to pyrene. There is concern over the fate and effect of PAHs in the marine environment due to their persistence, bioaccumulation potential, and acute and chronic toxicity to marine organisms (Dissanayake and Galloway, 2004).

2. Materials and methods

2.1. Shore crab collection and maintenance

In June 2005, *C. maenas* was collected from the Avon Estuary, Bantham, South-west England (grid reference: SX 6623 4380) using mackerel-baited traps. In the laboratory, adult male (green) intermoult individuals [>60 mm carapace width (CW)] (Crothers, 1967) were selected for experimentation; each was weighed (wet weight g) and individually labelled (labels attached to carapace with cyanoacrylate glue). Crabs were maintained in static holding tanks containing filtered (10 μm carbon filtered), aerated seawater (salinity of 34, 15 ± 1 °C) under a 12 h light:12 h dark photoperiod for 2 days before being transferred to the experimental tanks.

2.2. Experimental design

Crabs (n = 18 in each case) were maintained under one of the following nutritional treatments for 14 days: 'starved' (no food); 'diet restricted' (fed 1 g of food on alternate days after an initial starvation period of 3 days); and 'fully fed' (fed 2 g of food each day). The feeding rationale was based on values reported for adult blue crabs *Callinectes sapidus* which indicated that crabs reached satiation when ingesting about 3% of body weight per day (McGaw and Reiber, 200). The food consisted of γ-irradiated cockle (*Cerastoderma edule*) (Gamma foods, Tropical Marine Centre, Bristol, U.K.) and the water was changed within 18 h of each feeding. Crabs under each feeding regime (n = 9 per treatment) were exposed to a sublethal (nominal) concentration (200 μg l^{-1}) of pyrene (Watson et al., 2004a). After 7 and 14 days, various physiological endpoints, selected to gain an integrated, holistic summary of shore crab physiology at different levels of biological organisation, were measured.

2.3. Urine and haemolymph sampling

Urine was sampled from the antennal gland of individual crabs using a 1 ml syringe with a micropipette tip. Haemolymph samples (500 μl) from individual crabs were extracted by puncture of the arthrodial membrane at the base of the 4th walking leg using a pre-chilled 1 ml syringe and 21 gauge needle (Sigma–Aldrich, U.K.). Urine and haemolymph samples were snap frozen using liquid nitrogen and stored at −80 °C for subsequent biochemical analysis. Cellular assays were conducted immediately after haemolymph sampling.

2.4. Validation of pyrene exposure

As a surrogate indicator of contaminant exposure (Dissanayake and Galloway, 2004; Watson et al., 2004b), pyrene metabolites in the urine were determined by fluorescence analysis ($\lambda_{345/382}$) for 1-hydroxylpyrene-type metabolites (1-OH). Urine samples were diluted with 50% ethanol (10 μl in 50% ethanol, ratio 1:20). Pyrene toxicity results from membrane damage, and/or enzyme inactivation or damage by reactive oxygen species produced by pyrene metabolism (Livingstone, 1991). Exposure, and subsequent metabolism of PAH compounds by organisms, results in metabolites of the parent compound being concentrated in body fluids, tissues and excreta (Dissanayake and Galloway, 2004). The presence of such metabolites in crab urine is used here as a surrogate measurement for detecting exposure to bioavailable contaminants (Fillmann et al., 2002). Irrespective of diet, urinary concentrations of 1-hydroxypyrene-type metabolites were significantly higher in pyrene-exposed compared to unexposed individuals following 7 (ANOVA, $F_{2,50}$ = 10.19, P < 0.001) and 14 days exposure (ANOVA, $F_{2,50}$ = 51.79, P < 0.001 (Fig. 1a and b, respectively). There were no significant differences in pyrene metabolite levels when comparing 7 and 14 day exposed crabs for starved (ANOVA, $F_{1,16}$ = 1.86, P = 0.19), diet-restricted (ANOVA, $F_{1,16}$ = 1.51, P = 0.24) or fully fed crabs (ANOVA, $F_{1,16}$ = 3.22, P = 0.092), indicating that nutritional status and exposure duration had no influence on pyrene metabolism by shore crabs, and that production of pyrene metabolites was constant throughout the study, regardless of physiological condition.

2.5. Biochemical endpoints

Urinary protein concentration was quantified using a protein reagent kit (Pierce, U.K.) with Bovine Serum Albumin (Sigma–Aldrich, U.K.) as a standard protein solution.

Antioxidant status [FRAP (Ferric reducing ability of plasma)] was determined by measuring the combined reducing power of the electron donating antioxidants present in the haemolymph (Benzie and Strain, 1996; Rickwood and Galloway, 2004). A stoichiometric excess of the oxidant ferric tripyridyltriazine (Fe^{III}-TPTZ) was added to each 10 μl sample (300 μl of 10 mM in 300 mM sodium acetate, pH 3.6) and its reduction to the ferrous form (Fe^{II}) monitored over 10 min at 593 nm.

2.6. Cellular endpoints

Stress-induced pathological change (cellular viability) was determined in the lysosomal compartments of haemocytes using a dye uptake method (Galloway et al., 2004).