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Seasonal differences in the physiology of *Carcinus maenas* (Crustacea: Decapoda) from estuaries with varying levels of anthropogenic contamination

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ABSTRACT

This study reports the seasonal variability in aspects of the physiology of the shore crab *Carcinus maenas* from three estuaries in South-west England, each with varying anthropogenic inputs: Avon Estuary ('relatively low' impact), Yealm Estuary ('intermediate' impact) and Plym Estuary ('relatively high' impact). Crabs collected over 12 months from the Avon had a significantly 'lower' physiological condition in winter and spring compared to summer and autumn; in particular, haemocyte phagocytic capability (a general indicator of immune function) was significantly higher in winter and spring compared to summer and autumn, and total haemolymph antioxidant status (an indicator of oxidative stress) was significantly lower in winter compared to the remainder of the year. Potentially, shore crabs may be more susceptible to the effects of contaminant exposure, such as increased immunotoxicity (thus, reduction of immune function) and/or oxyradicals (or reactive oxygen species) exposure) especially in seasons of increased susceptibility i.e. summer/autumn (lower phagocytic capability) and winter (lowest antioxidant function). As the Avon was taken to represent the 'reference' site, this pattern is considered to reflect the 'normal' seasonal variability in shore crab physiology. Shore crab physiological condition from the 'relatively high' impact estuary (Plym) revealed increased cellular viability and antioxidant status in autumn and winter compared with that of the 'standard' pattern (Avon) However, crabs from the intermediate impact estuary (Yealm) only demonstrated significant physiological differences in summer as shown by a lower cellular viability. All crabs had been exposed to PAHs (confirmed by the presence of PAH metabolites in their urine) which may account for the observed differences in shore crab physiology. In conclusion, to aid understanding of the potential contaminant impacts on biota it is imperative that the 'normal' seasonal variability of physiological condition be established. Biological effects-based monitoring studies should therefore be employed seasonally to potentially highlight 'windows of sensitivity' to contaminant impact.

1. Introduction

Coastal marine waters and sediments, including estuaries, continually receive anthropogenic inputs and contain many chemicals that are potentially toxic to aquatic organisms (Ridgway and Shimmield, 2002; Salazar-Coria et al., 2010). Accelerated human population growth and development around these coastal and estuarine zones have placed pressure on these sensitive habitats, such as eutrophication, decreased biodiversity, habitat loss and alteration (Kennish, 2002), especially as these coastal areas are deemed 'ecological nurseries' where the most sensitive juvenile life-stages of organisms are found (Haywood et al., 1998; Epifanio et al., 2003; Moksnes et al., 2003). The recent emphasis of marine environmental monitoring has been to develop biological measurements of anthropogenic impact, including physiological assessments of the 'health' of chosen test species (Galloway et al., 2002, 2004; 2006). Unfortunately, implementation of this approach is impeded by the lack of knowledge of the basic biochemistry of the organisms, including "normal" physiological ranges, such as seasonal variations (Mehrle and Mayer, 1980) before the impact of polluting chemicals can be assessed. Seasonal patterns are summarised as temporal variations which are "usually responses to particular cues that tend to coincide with time of year, if such cues do not arise in any particular year, the response will not be elicited" (Crowe, 1999).

The shore crab *Carcinus maenas* (Decapoda: Brachyura) is a common inhabitant of various coastal habitats, including estuaries (Crothers, 1968; Hunter and Naylor, 1993), throughout Europe

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(Hayward and Ryland, 1995), and is used widely as a test organism in ecotoxicology (Bamber and Depledge, 1997b; Fossi et al., 2000). *C. maenas* responds quickly to environmental change, through changes in osmoregulation (Bjerregaard and Visle, 1985, 1986), respiration rates and capability (Arudpragasm and Naylor, 1964a,b; Dawirs, 1983; Depledge, 1985; Spicer and Weber, 1991), and metabolic and cardiac activity (Wallace, 1972; Cumberlidge and Uglow, 1977a,b; Depledge, 1984). The physiological plasticity of *C. maenas* is a major factor accounting for its widespread distribution (Rainbow, 1997). However, low genetic variability has been shown for shore crab populations around the U.K. coasts, indicating that patterns of phenotypic variability among shore crab populations are likely to reflect differences between local environments (Brian et al., 2006), making this species a good bioindicator of environmental contamination. Shore crab 'health' assessments using various physiological techniques (including cellular viability and immune function) have been employed previously in both laboratory and field scenarios demonstrating contaminant impact this species (Brown et al., 2004; Galloway et al., 2004; Hagger et al., 2009). The aims of this present study were, therefore, to firstly, elucidate the 'natural' seasonal pattern in shore crab physiology in crabs from a relatively clean estuary (Avon Estuary) and secondly, to evaluate whether such seasonal patterns were altered under conditions of anthropogenic input i.e. maritime activity i.e. 'intermediate' Yealm Estuary and 'relatively high' Plym Estuary.

2. Material and methods

2.1. Study sites

In 2006, *Carcinus maenas* was collected from three estuaries in South-west England U.K.), each with a varying anthropogenic input. Based on maritime activity, the Avon Estuary (50°16′43 N, 3°52′18 W) was designated as having 'relatively low' impact as it receives <20 vessels yr^{1} (QHM, 2006a), the Yealm Estuary (50°18′49 N, 4°03′08 W) was designated as 'intermediate', receiving <250 vessels yr^{1} (QHM, 2006c), and the Plym Estuary (50°21′54 N, 4°08′02.7 W) was designated as 'relatively high' due to >1500 vessels yr^{1} (QHM, 2006b). In addition to maritime activity, the concentrations of polycyclic aromatic hydrocarbons (PAHs) were chosen as an indicator of organic contamination as PAHs are ubiquitous pollutants of the marine environment (King et al., 2004). PAHs arrive in coastal waters from many sources including petrochemical pollution, incomplete combustion processes (Livingstone et al., 1992; Clarke et al., 2001), metal smelting (Naes et al., 1995; Beyer et al., 1996) and electrolytic production of aluminium using anode technology (Beyer et al., 1998; Aas et al., 2001; Tsibulsky, 2001). There are two broad and distinct PAH groups categorised dependent upon source; petrogenic PAHs are derived from petroleum products and are characterised by (relative) low molecular masses and by a 2- or 3-ringed structure, (King et al., 2004). Pyrolytic PAHs, however, are combustion derived and are formed as a result of high temperature combustion of organic matter and industrial processes (OSPAR, 2001); examples include, benzo[a]pyrene and pyrene, which in particular, is a 4-ringed PAH that is included in the USEPA priority pollutant list (Tsibulsky, 2001). PAH metabolites in crab urine are a good surrogate indicator of PAH exposure (Dissanayake and Galloway, 2004; Watson et al., 2004; Koenig et al., 2008) and King et al. (2004) reported increased PAH metabolites from areas of high maritime activity. PAH metabolites, measured from crabs from the three estuaries (see below for details about crab collection), revealed that all crabs had been exposed to PAHs but those from the Avon and Yealm Estuaries had significantly lower exposure than those from the Plym (*ANOVA*; Log-transformed, $F_{2,\ 14} = 4.70$, $P < 0.05$) (Fig. 1), corroborating previous evidence (Dissanayake et al., 2010). Based on maritime activity and PAH exposure, therefore, the contamination level of the three estuaries was ranked here as 'relatively low' (Avon), 'intermediate' (Yealm) and 'relatively high' (Plym).

2.2. Shore crab collection and maintenance

Shore crabs were collected, using mackerel-baited cages deployed (at random), at three time points within four seasons over 12 months (Winter (January–March), Spring (April–June), Summer (July–September) and Autumn (October–December)). Water temperatures (mean ± SE), taken with a hand-held Cellox 325-3 oxygen probe (Multi 340i/SET, WTW, Germany), for each season were respectively 7.5 °C ± 0.24 (winter), 13.7 °C ± 0.37 (spring), 16.9°C ± 0.33 (summer) and 14.7°C ± 1.66 (autumn). At each sampling, cages were deployed 3 h prior to high tide and collected at the predicted time of high tide (HWS), as crabs express a tidal migration entering the intertidal zone at high tide and leaving again before low tide (Hunter and Naylor, 1993). A maximum mean number of three adult male (green) intermoult individuals (>60 mm carapace width) per cage were sampled for the analysis of physiological condition. Only adult crabs were sampled (>60 mm CW) as variability in the physiological techniques, e.g. cellular viability, are known to vary between ontogenetic stage i.e. juveniles vs. adults (Dissanayake et al., 2008b), and patterns of phenotypic variability among shore crab populations are suggested to reflect differences between local environments (Brian et al., 2006). Crabs were transported to the laboratory in cooler boxes with damp absorbent paper. Previous studies have shown that crabs transported (<1 h) in cooler boxes and damp absorbent paper do not contribute significant bias (Dissanayake and Bamber, 2010; Dissanayake et al., 2010). Crabs heart rate levels, for example, have been observed to decrease from 'active' (i.e. handling stress) to 'resting' levels within 30 min (Dissanayake et al., 2008b). Urine and blood samples were withdrawn within 24 h and up to 48 h of arrival in the laboratory, respectively. PAH metabolites observed in urine and lysosomal stability reflect contaminant exposure up to six days previously (Watson et al., 2004; Dissanayake and Bamber, 2010). Urine and haemolymph sampling techniques are non-destructive; the periods between sampling the same individual for different physiological and behavioural parameters have been shown to allow sufficient recovery in previous studies (Bamber and

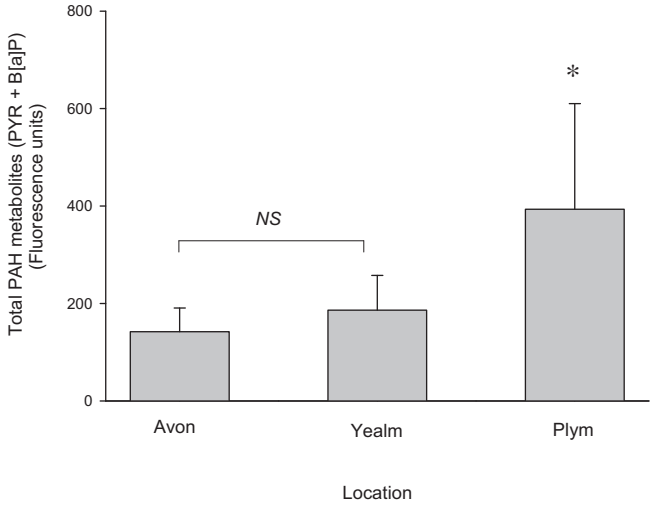

Fig. 1. Total PAH metabolites (PYR + B[a]P) (mean ± 1 S.E.) in the urine of adult *Carcinus maenas* ($n = 12$ time points throughout the year), asterisk signifies $P < 0.01$, *NS signifies* Not significant.