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Hepatic transcriptional responses to copper in the three-spined stickleback are affected by their pollution exposure history

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ABSTRACT

Some fish populations inhabiting contaminated environments show evidence of increased chemical tolerance, however the mechanisms contributing to this tolerance, and whether this is heritable, are poorly understood. We investigated the responses of two populations of wild three-spined stickleback (*Gasterosteus aculeatus*) with different histories of contaminant exposure to an oestrogen and copper, two widespread aquatic pollutants. Male stickleback originating from two sites, the River Aire, with a history of complex pollution discharges, and Siblyback Lake, with a history of metal contamination, were depurated and then exposed to copper (46 μg/L) and the synthetic oestrogen ethinyloestradiol (22 ng/L). The hepatic transcriptomic response was compared between the two populations and to a reference population with no known history of exposure (Houghton Springs, Dorset). Gene responses included those typical for both copper and oestrogen, with no discernable difference in response to oestrogen between populations. There was, however, some difference in the magnitude of response to copper between populations. Siblyback fish showed an elevated baseline transcription of genes encoding metallothioneins and a lower level of metallothionein induction following copper exposure, compared to those from the River Aire. Similarly, a further experiment with an F1 generation of Siblyback fish bred in the laboratory found evidence for elevated transcription of genes encoding metallothioneins in unexposed fish, together with an altered transcriptional response to 125 μg/L copper, compared with F1 fish originating from the clean reference population exposed to the same copper concentration. These data suggest that the stickleback from Siblyback Lake have a differential response to copper, which is inherited by the F1 generation in laboratory conditions, and for which the underlying mechanism may include an elevation of baseline transcription of genes encoding metallothioneins. The genetic and/or epigenetic mechanisms contributing to this inherited alteration of *metallothionein* transcription have yet to be established.

1. Introduction

Metals and oestrogenic chemicals are widespread contaminants of freshwater systems worldwide. Anthropogenic sources of metal pollution include mining and industrial activity, and oestrogenic pollution derives from industrial, agricultural and domestic sources. Toxic effects of chronic metal exposure in wild fish populations include impaired metabolic activity, growth, immunity and genetic diversity (Bourret et al., 2008; Couture and Kumar, 2003; Levesque et al., 2003; Pierron et al., 2009; Rajotte and Couture, 2002). Exposure to environmental oestrogens has been reported to induce intersex in some fish species (Jobling et al., 1998; van Aerle et al., 2001) whilst roach (*Rutilus rutilus*) inhabiting some of the more contaminated sections of UK rivers may have reduced reproductive success and genetic diversity (Hamilton et al., 2014; Harris et al., 2011; Jobling et al., 2002). Viable populations of fish, however, do exist in some heavily polluted environments, including those contaminated with metals and/or oestrogens (Hamilton et al., 2014; Uren Webster et al., 2013).

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Selective pressures favouring increased tolerance to pollution are likely to drive adaptive change in populations inhabiting contaminated environments. One of the best characterised examples of this are populations of North American Atlantic killifish (*Fundulus heteroclitus*), which have adapted to estuarine environments heavily contaminated with aromatic hydrocarbons. These killifish showed reduced sensitivity to aromatic hydrocarbon exposure, primarily due to a lack of induction of cytochrome P4501a (CYP1A) through suppression of the aryl hydrocarbon receptor (AHR) signalling pathway (Whitehead et al., 2010; Wirgin and Waldman, 2004), and this mechanism of tolerance was inherited to F1, and in some cases F2, generations (Whitehead et al., 2012). Evidence of a genetic basis of this tolerance to aromatic hydrocarbons includes functional differences in the *CYP1A* promoter (Williams and Oleksiak, 2011) and altered SNP frequency in *AHRs* (Reitzel et al., 2014), although this tolerance may not be entirely due to genetic selection, and is also likely to vary with the contaminant (Clark et al., 2013).

Altered response to oestrogen exposure has also been demonstrated in several populations of killifish in New Bedford Harbour and Newark Bay with a history of exposure to chemicals affecting oestrogen signalling, including PCBs. Depurated male killifish from polluted environments showed reduced transcription of oestrogen-dependent genes including *vitellogenins*, *chorion proteins* and *aromatase* following exposure to 17β-oestradiol (E2) compared to those from a clean, reference population (Bugel et al., 2014; Greytak et al., 2010). Their F1 larvae showed attenuation of the *ERα* transcriptional response, but not the other oestrogen-response biomarkers (Greytak et al., 2010). This suggests that chronic environmental exposure drives a reduced sensitivity to oestrogen, through modulation of oestrogen receptor signalling, but this response can vary with life stage (Bugel et al., 2014; Greytak et al., 2010). Studies on roach populations originating from river stretches in the UK heavily contaminated by oestrogenic chemicals have found the opposite, with a sensitisation of oestrogen responsiveness (Lange et al., 2009). Genetic and epigenetic mechanisms contributing to these responses are suspected but have not yet been established.

Potential adaptation to chronic metal exposure has been reported in various wild fish populations. For example, brown trout (*Salmo trutta*) from the River Hayle in Cornwall, UK, have shown tolerance of high concentrations of a mixture of metals that are lethal to naïve trout. These trout displayed relatively little evidence of overt toxicity, despite accumulating high tissue concentrations of metals, and showed evidence of several mechanisms of metal tolerance including up-regulation of metal-handing pathways and ion homeostasis (Uren Webster et al., 2013). Genetic analysis revealed differences between populations, both within this river and compared to clean rivers nearby, which were predicted to coincide with increases in local mining activity, suggesting that local adaption to metal contamination had contributed to a reduction in gene flow between these populations (Paris et al., 2015). Studies on yellow perch (*Perca flavescens*) populations inhabiting lakes in North America contaminated through industrial and mining activity have shown tolerance of high concentrations of metals including copper and cadmium, and the potential mechanisms of tolerance were reported to include elevated metallothionein and oxidative stress responses (Defo et al., 2015; Giguère et al., 2005; Pierron et al., 2009). There is also some evidence of selection in these perch populations driven by metal contamination (Bélanger-Deschênes et al., 2013; Bourret et al., 2008).

Evidence of considerable toxicity was also found in yellow perch chronically exposed to metals, including impaired metabolism and poor condition (Couture and Kumar, 2003; Levesque et al., 2003; Pierron et al., 2009; Rajotte and Couture, 2002), compared to less obvious signs of toxicity in the Hayle brown trout (Uren Webster et al., 2013). This may reflect a greater adaptive change in the trout following longer historical contamination in the river Hayle (~1000 years) compared to the ~100 years of contamination experienced by fish in the North American lakes. A genetic contribution to metal tolerance in fish therefore seems likely, but the precise mechanisms of this adaptive change and the potential relative contributions of phenotypic plasticity or other mechanisms are yet to be established. In addition, little is known about the response of depurated individuals to metal exposure or whether metal tolerance is inherited in F1 or subsequent generations raised in clean environments.

Three-spined stickleback (*Gasterosteus aculeatus*) inhabit many water systems worldwide, are relatively tolerant of stressors and can undergo rapid speciation in response to environmental change (McKinnon and Rundle, 2002). We have previously used global transcript profiling to establish the transcriptional signatures of response to copper and 17alpha-ethinylestradiol (EE2) in stickleback originating from a reference site (Houghton Springs, Dorset, UK), receiving borehole water with no known history of metal or oestrogenic contamination (Katsiadaki et al., 2010; Santos et al., 2010). Here, we examined the hypothesis that historical exposure to these classes of pollutants modifies the tolerance of exposed populations, altering their response to further exposure. We investigated the transcriptional responses to EE2 and copper in two populations of stickleback that originate from water systems with a history of contamination by a wide range of chemicals including oestrogens (River Aire, Leeds, UK) and metals (Siblyback Lake, Cornwall, UK), followed by periods of improved water quality. We found responses typical for both copper and oestrogen exposure in both populations, as identified previously for stickleback at Houghton Springs (Katsiadaki et al., 2010; Santos et al., 2010), but with differences in the magnitude of response to copper between populations, and differences in the baseline transcription of copper-responsive genes, including *metallothionein*. A further experiment with an F1 generation of Siblyback fish bred and maintained in a clean environment, and subsequently exposed to copper, showed evidence of an elevated baseline transcription of *metallothionein*, together with an altered transcriptional and behavioural response, compared with F1 fish originating from the reference (Houghton Springs) population.

2. Materials and methods

All experiments were performed using reagents from Sigma-Aldrich (Dorest, UK), unless otherwise stated.

2.1. F0 exposure

2.1.1. Site selection

Stickleback fish were collected from two sites chosen based on their history of oestrogen and metal pollution. The River Aire flows through a heavily industrialised area in Yorkshire, UK, and has a long history of heavy pollution. Considerable stretches of this river downstream of wastewater treatment outflows were reported to contain high concentrations of oestrogenic chemicals, particularly alkylphenol polyethoxylates (Harries et al., 1997), although improvements in wastewater treatment have since considerably reduced the degree of oestrogenic activity in this river (Sheahan et al., 2002). High prevalence and severity of feminisation in wild fish populations have been reported in this river (Jobling et al., 2002; Jobling et al., 1998; van Aerle et al., 2001). Resident stickleback populations are also known to have undergone significant population bottlenecks, although this has been shown not to have impacted on male reproductive competitiveness (Santos et al., 2013).