Aquatic Toxicology 156 (2014) 116–124

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Behavioural and physical effects of arsenic exposure in fish are aggravated by aquatic algae

Kit Magellan*, Laura Barral-Fraga, Marona Rovira, Pao Srean, Gemma Urrea, Emili García-Berthou, Helena Guasch

Institute of Aquatic Ecology, University of Girona, E-17071 Girona, Spain

ARTICLE INFO

Article history:
Received 28 March 2014
Received in revised form 4 August 2014
Accepted 9 August 2014
Available online 17 August 2014

Keywords:
Aggression
Arsenic
Bioaccumulation
Gambusia holbrooki
Algae
Nutrient cycling

ABSTRACT

Arsenic contamination has global impacts and freshwaters are major arsenic repositories. Arsenic toxicity depends on numerous interacting factors which makes effects difficult to estimate. The use of aquatic algae is often advocated for bioremediation of arsenic contaminated waters as they absorb arsenate and transform it into arsenite and methylated chemical species. Fish are another key constituent of aquatic ecosystems. Contamination in natural systems is often too low to cause mortality but sufficient to interfere with normal functioning. Alteration of complex, naturally occurring fish behaviours such as foraging and aggression are ecologically relevant indicators of toxicity and ideal for assessing sublethal impacts. We examined the effects of arsenic exposure in the invasive mosquitofish, *Gambusia holbrooki*, in a laboratory experiment incorporating some of the complexity of natural systems by including the interacting effects of aquatic algae. Our aims were to quantify the effects of arsenic on some complex behaviours and physical parameters in mosquitofish, and to assess whether the detoxifying mechanisms of algae would ameliorate any effects of arsenic exposure. Aggression increased significantly with arsenic whereas operculum movement decreased non-significantly and neither food capture efficiency nor consumption were notably affected. Bioaccumulation increased with arsenic and unexpectedly so did fish biomass. Possibly increased aggression facilitated food resource defence allowing fish to gain weight. The presence of algae aggravated the effects of arsenic exposure. For increase in fish biomass, algae acted antagonistically with arsenic, resulting in a disadvantageous reduction in weight gained. For bioaccumulation the effects were even more severe, as algae operated additively with arsenic to increase arsenic uptake and/or assimilation. Aggression was also highest in the presence of both algae and arsenic. Bioremediation of arsenic contaminated waters using aquatic algae should therefore be carried out with consideration of entire ecosystem effects. We highlight that multidisciplinary, cross-taxon research, particularly integrating behavioural and other effects, is crucial for understanding the impacts of arsenic toxicity and thus restoration of aquatic ecosystems.

1. Introduction

Arsenic (As) from both anthropogenic and natural sources has global impacts (Mandal and Suzuki, 2002; Nordstrom, 2002; Rahman and Hasegawa, 2012; Rahman et al., 2012; Smedley and Kinniburgh, 2002) and aquatic systems, including freshwaters, are major repositories for arsenic (Nordstrom, 2002; Smedley and Kinniburgh, 2002). Although some national and international standards are in effect, for example the World Health Organization safe limit for drinking water is 10 $\mu g\,L^{-1}$ (Smith et al., 2002), the toxicity of As is dependent on numerous interacting factors such as its source, concentration and bioavailability; environmental parameters; and organisms' resistance ability and detoxifying mechanisms (Mandal and Suzuki, 2002; Rahman and Hasegawa, 2012; Smedley and Kinniburgh, 2002). A key factor is its chemical speciation. Inorganic As (iAs) is generally more toxic than organic As, while of the iAs species, arsenite (As^{III}) is more toxic than arsenate (As^{V}). However, the organic methylated species (dimethylarsinous acid, $DMAA^{III}$, and monomethylarsonous acid, $MMAA^{III}$) are more toxic than their iA parent compounds (Rahman et al., 2012; Smedley and Kinniburgh, 2002). Quantifying total arsenic in environmental and biological samples is therefore not synonymous with assessment of associated risks. The main chemical species in freshwaters are inorganic arsenics but

* Corresponding author. Tel.: +34 972 418 369; mobile: +34 666 785 381.
E-mail addresses: kit8x@hotmail.com, kit.magellan@udg.edu (K. Magellan).

http://dx.doi.org/10.1016/j.aquatox.2014.08.006
0166-445X/

methylated and other organic As species are also found (Rahman and Hasegawa, 2012; Rahman et al., 2012). Freshwater ecosystems are extensive and highly dynamic (Moss, 1998) which together with the variable nature of As toxicity makes effects difficult to estimate (Rahman et al., 2012; Smedley and Kinniburgh, 2002; Smith et al., 2002). However, assessment and prediction are essential. In addition to providing water and nutrients for human consumption (Mandal and Suzuki, 2002; Smith et al., 2002; Villéger et al., 2012), freshwater ecosystems may themselves suffer severe impacts from arsenic toxicity (e.g. Rahman and Hasegawa, 2012; Rahman et al., 2012; Scott and Sloman, 2004; Smedley and Kinniburgh, 2002).

Biological activity plays a vital role in As speciation, distribution and cycling in freshwaters (Rahman and Hasegawa, 2012; Rahman et al., 2012). Organismal uptake of arsenic may be direct, through ingestion, inhalation and absorption, or indirect through the food chain (Mandal and Suzuki, 2002; Moss, 1998; Smedley and Kinniburgh, 2002; Smith et al., 2002). Aquatic plants (and bacteria) have important functions in these processes through biotransformation of As species (Hellweger and Lall, 2004; Rahman and Hasegawa, 2012; Rahman et al., 2012). As^{V}, the stable and predominant species of arsenic in aquatic environments, has chemical and structural similarities to phosphate (PO_4^{3-}). Algae mistake As^{V} for PO_4^{3-} and actively absorb it via the same pathways. Once inside the algal cells, As^{V} becomes toxic as this resemblance breaks down, and algae reduce As^{V} to As^{III}, methylate it to $MMAA^{III}$ and $DMAA^{III}$, and excrete it mostly as As^{III} and/or $DMAA^{III}$, which is thought to be a detoxifying mechanism (Hellweger and Lall, 2004; Rahman and Hasegawa, 2012; Rahman et al., 2012). Several factors influence this process. Different algal species have different methylation abilities (Rahman and Hasegawa, 2012) and tolerances to As^{V} (e.g. Favas et al., 2012; Levy et al., 2005; Wang et al., 2013), and not all algae excrete As^{III}. For example, both *Chlorella* sp. and *Monoraphidium arcuatum* take up As^{V} and reduce it to As^{III} but only *M. arcuatum* excretes it (Levy et al., 2005). Moreover, recent studies indicate that methylation may not be the primary mode of detoxification in freshwater algae. Instead, arsenic is taken up by cells using the phosphate transport system, reduced to As^{III} in the cell and then excreted into the growth medium, probably by an active transport system (Levy et al., 2005; Wang et al., 2013). For example, after exposing *Chlamydomonas reinhardtii* and *Scenedesmus obliquus* to different arsenate concentrations, no methylated species could be detected (Wang et al., 2013). Similarly, arsenate and arsenite were the dominant species in the freshwater algae *Synechocystis* and *C. reinhardtii* (Yin et al., 2011, 2012). This transformation reaction is suggested to be correlated with algal growth rate and P nutrient status, leading to almost complete methylation under P-limiting conditions and slower methylation and excretion of As^{III} into the media if P is in excess (Hellweger and Lall, 2004). Nonetheless, these studies confirm that P has a key role in arsenate toxicity and that biotransformation of As by algae is a central component of aquatic As cycling. Indeed, the use of algae is often advocated for bioremediation of As contaminated water (e.g. Levy et al., 2005; Favas et al., 2012; Rahman and Hasegawa, 2012; Rahman et al., 2012; Wang et al., 2013).

Fish are a key constituent of aquatic ecosystems and are involved in As mobilization. They are an important component of the aquatic food chain (Agah et al., 2009; Kumar and Banerjee, 2012; Zhang et al., 2013) and even small fish are a source of protein for human consumption (e.g. Moeller et al., 2003). Some fish are also used as bioindicators of various aquatic pollutants (Bhattacharya et al., 2007; Moeller et al., 2003; Moss, 1998; Scott and Sloman, 2004). Bioaccumulation of arsenic in fish occurs directly through absorption across the gills or skin and indirectly via consumption of prey (Rahman et al., 2012); and inorganic, methylated and other organic arsenicals are all found in various fish species (Rahman et al., 2012; Rahman and Hasegawa, 2012). The effects of arsenic toxicity have been examined in numerous species worldwide. For example, bioaccumulation of arsenic has been recorded in fish from California (Moeller et al., 2003), sub-Saharan Africa (Ouédraogo and Amyot, 2013), India (Kumar and Banerjee, 2012), France (Noël et al., 2013), China (Zhang et al., 2013) and the Persian Gulf (Agah et al., 2009). However, most research has focused on parameters such as bioaccumulation, and physiological parameters such as growth (e.g. Kumar and Banerjee, 2012) and metabolic and histopathological effects (e.g. Ahmed et al., 2013; Bhattacharya et al., 2007). One factor that has received much less attention is fish behaviour (e.g. Scott and Sloman, 2004; Weis and Candelmo, 2012; Weis et al., 2001). Contamination in natural systems is often at concentrations well below those that cause mortality, but even low levels of toxicity may be sufficient to interfere with normal functioning. Fish behaviour is ideal for assessing these sublethal impacts (Moss, 1998; Scott and Sloman, 2004; Weis and Candelmo, 2012). Much of the current research focusses on direct behavioural responses to contaminants, for example, avoidance of contaminated sites, respiratory changes and behaviour like body tremors associated with illness. However, alteration of complex, naturally occurring behaviours such as foraging and predation, agonistic interactions, shoaling and reproductive behaviours are more ecologically relevant indicators of toxicity (Scott and Sloman, 2004; Sopinka et al., 2010; Weis et al., 2001). Various environmental toxicants have been shown to affect complex behaviours (reviewed in Atchison et al., 1987; Scott and Sloman, 2004). Arsenic in particular reduces long-term memory in the zebrafish, *Danio rerio* (de Castro et al., 2009) and is part of a cocktail of chemicals that affects aggressive interactions in the round goby, *Neogobius melanostomus* (Sopinka et al., 2010). However, the effects of arsenic on fish behaviour have received little attention to date: arsenic is not listed in Scott and Sloman's (2004) comprehensive review of contaminant effects on fish behaviour. Given the global impacts of arsenic toxicity (e.g. Mandal and Suzuki, 2002; Smedley and Kinniburgh, 2002; Rahman et al., 2012) more work is needed in this field.

In this study, we examined the effects of arsenic on complex behaviours in the invasive mosquitofish, *Gambusia holbrooki*. This small fish has been introduced worldwide, primarily for mosquito control (Lever, 1996; Pyke, 2008). Although highly tolerant of a variety of stressors (e.g. Evans et al., 2011; Staub et al., 2004; Uliano et al., 2010), *G. holbrooki* and the closely related *Gambusia affinis* have been used in toxicity studies (e.g. Tatara et al., 1999, 2001) and are known to be affected by arsenic (e.g. Moeller et al., 2003; Newman et al., 1989). As behaviour links physiological functions with ecological processes, an understudied field of research (e.g. Scott and Sloman, 2004; Weis et al., 2001), we also included physiological parameters to assess interrelated effects of arsenic toxicity. Moreover, given the intricacies of the feedback and cycling interactions contributing to arsenic toxicity (e.g. Scott and Sloman, 2004; Weis et al., 2011), field studies may be more general and realistic about environmental effects (Moss, 1998), while laboratory studies allow more controlled quantification of effects, and both provide valuable insight (Weis and Candelmo, 2012). Therefore, we also examined the interacting effects of naturally occurring algae, thus incorporating some of the complexity of natural systems in a laboratory experiment and disentangling some specific processes from whole ecosystem effects.

We addressed two main aims: first to quantify the effects of arsenic on *G. holbrooki*, and second to assess the interacting affects of algae on arsenic toxicity in this fish species. We examined one direct behavioural response to stress, opercular ventilation rate (Brown et al., 2005; Hawkins et al., 2004), predicting that operculum movement would increase in response to the stress of arsenic exposure; and two complex behaviours, aggression and foraging. As both stress (Folkedal et al., 2012) and physiological effects of contaminants (Weis et al., 2001) can reduce feeding ability