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Histopathology and contaminant concentrations in fish from Kuwait's marine environment

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ABSTRACT

Kuwait has witnessed major socioeconomic and industrial development in recent decades. Consequently, a variety of contaminants related to these activities have been discharged directly into the marine environment. This paper describes the application of a histopathology baseline survey in two potential sentinel species, the Giant sea catfish (*Arius thalassinus*) and the Fourlined terapon (*Pelates quadrilineatus*) to assess the health of biota inhabiting Kuwait's marine environment. Histological analysis revealed several lesion types in both species, although the prevalence was generally considered low with no discernible differences between sampling locations. The analysis of contaminant burdens (metals, PCBs, PBDEs, HBCDD) in *A. thalassinus*, along with the analysis of bile for PAH metabolites in both species, indicated that levels of contaminant exposure was low. Overall the data show that both species appear to be susceptible to pathologies associated with environmental contaminants and therefore suitable for further investigation as sentinel organisms for biological effects monitoring.

1. Introduction

Kuwait has witnessed a huge increase in coastal development that has been linked to its rapid industrial growth over recent decades (Al-Abdulghani et al., 2013). This has resulted in increases in anthropogenic pressures including eutrophication, inputs of domestic sewage and discharges of industrial waste (Ahmed et al., 1998; Al-Ghadban et al., 2002; Al-Abdulghani et al., 2013; Al-Sarawi et al., this issue). Water pollution is one of the most critical environmental threats facing Kuwait and the wider Gulf region (Halpern et al., 2008; Sheppard et al., 2010). One source of pollution in Kuwait's marine environment is derived from sewage effluent discharge, which along with microbial contamination can contain a range of chemical contaminants such as, organic substances, inorganic substances and heavy metals (Ghannoum et al., 1991; Bou-Olayan and Al-Sarawi, 1993; El-Desouki and Abdulraheem, 1998; Gevao et al., 2006; Lyons et al., this issue). The threats these pose to biota inhabiting Kuwait's marine environment has yet to be fully assessed, although this subject is becoming an increasing concern to policy makers, the general public and fishing communities following a number of well documented mass fish mortalities in the region (Heil et al., 2001; Glibert et al., 2002; Al-Mutairi et al., 2014).

The Gulf's fisheries industry represents the second most important natural resource after oil and is of great socioeconomic significance (Ahmed et al., 1998; Al-Zaidan et al., 2013). The regions geography and climate coupled with the rapid development of its coastal margins leave it prone to various types of environmental degradation (Sale et al. 2011). In recent years Kuwait's total fisheries landings have steadily declined with the depletion of valuable fish species (Dadzie et al. 2005; Sheppard et al., 2010; Al-Zaidan et al., 2013). Such a decline is thought to be a result of various factors or stressors, including overfishing, destruction of nursery grounds, reduction of flow of the Shatt-Al-Arab river and the discharge of high quantities of partially-treated or untreated sewage water into the environment (Sheppard et al. 2010; Al-Abdulghani et al., 2013). In addition, the level of chemical contaminants in marine biota from Kuwait has recently been reviewed and a number of metals (e.g. As, cd and Hg) were reported as failing international food safety limits (Al-Sarawi et al., this issue). Major point sources of pollution include the numerous sewage outfalls around Kuwait city and inputs associated with industry and desalination/power plants to the north and south of the city

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(Heil et al., 2001; Al-Abdulghani et al., 2013). In combination, these various activities have resulted in the high influx of water containing different types of pollutants, including heavy metals, polycyclic aromatic hydrocarbons, (PAHs) and polychlorinated biphenyls (PCBs) (Al-Ghadban et al., 2002; Al-Abdulghani et al., 2013; Al-Sarawi et al., this issue). The hydrological features of Kuwait Bay and the surrounding coastal environment (being semi-enclosed with shallow depths and weak water circulation) make it ideal for the accumulation of pollutants resulting from anthropogenic activities. There is currently limited published material relating to pollution incidents and the impact they may have on fish health in Kuwait's marine systems (Stentiford et al., 2014). However, several studies have linked the environmental degradation, due to the increase nutrient input from effluent water discharge, to the deterioration in the fish health and disease outbreaks (Heil et al., 2001; Glibert et al., 2002).

Monitoring the health of species inhabiting aquatic systems can act as early warning indicators of environmental quality and potential human health effects resulting from the consumption of contaminated seafood. Biomarkers being either biochemical, physiological or histological are important to interpret the toxicity of the aquatic systems and have been widely used to assess environmental health (Myers et al., 1998; Fracácio et al., 2003; Costa et al., 2009; Stentiford et al., 2010; Brooks et al., 2009; Hutchinson et al., 2013; Stentiford et al., 2014). Significantly a number of field, laboratory and mesocosm studies have also demonstrated causal links between exposure to chemical contamination and the development of toxicopathic hepatic lesions (Hinton and Lauren, 1990; Hinton et al., 1992; Myers et al., 1998; Stehr et al., 2004). Following studies of this type, it is generally accepted that certain liver lesions in marine fish can be induced by environmental contaminants and that these represent an ecologically relevant biological endpoint of exposure to pollution. Thus histological and biochemical biomarkers provide a powerful tool for detection and characterisation of the biological end points of toxicant and carcinogen exposure (Hinton and Lauren, 1990; Hinton et al., 1992; Moore and Simpson, 1992). Furthermore, the utility of histopathology as a sensitive indicator of health in wild fish populations has been demonstrated in Europe and North America (Myers et al., 1998; Costa et al., 2009; Stentiford et al., 2009; Vethaak et al., 2009; Stentiford et al., 2010).

In the current study we have undertaken a histopathological survey to assess the health of two fish species, the bottom feeding Giant sea catfish (*Arius thalassinus*) and the pelagic Fourlined terapon (*Pelates quadrilineatus*), commonly found in Kuwait waters. In addition, we report the concentrations of metal and organohalogen compounds in *A. thalassinus*, along with the concentration of PAH bile metabolites in both *A. thalassinus* and *P. quadrilineatus*. Such studies will help facilitate the use of histopathology as part of a suite of endpoints for assessing the health status of marine biota and will act as a baseline for future monitoring programmes.

2. Materials and methods

2.1. Sampling and site information

Fish were collected from 5 stations located within Kuwait Bay and along the cities eastern coastline during April 2014 (see Fig. 1). Sites were selected to provide representative locations of environments within and external to Kuwait Bay and at which fishing had been conducted successfully in the past (e.g. free of bottom obstructions). In addition, due to the restriction of fishing within Kuwait Bay appropriate permissions were obtain from Public Authority for Agriculture Affairs and Fish Resources (PAAFR). Fishing at each location was undertaken using the Kuwait Institute of Scientific Research (KISR) vessel *Bahith-II* equipped with a stern otter trawl. Fishing tows were conducted for 20 min after which fish were transferred to aerated seawater tanks prior to sampling. The sex, size (total length) and presence of external signs of disease were recorded for each fish (see Supplementary data file 1).

2.2. Histopathology

Fish were euthanised by a blow to the head, followed immediately by severing of the spinal chord, after which visceral organs were removed and a 4 mm liver cross section (in addition to gonad, kidney and spleen tissues) was placed in tissue cassettes and transferred to 10% Neutral Buffered Formalin (NBF) for a minimum of 24 h. Specimens were then transferred to 70% industrial methylated spirit (IMS) prior to histological processing.

Fixed samples were processed to wax in a vacuum infiltration processor using standard protocols. Sections were cut at 3–5 μm on a rotary microtome and resulting tissue sections were mounted onto glass slides before staining with haematoxylin and eosin (H&E). Stained sections were analysed by light microscopy (Nikon Eclipse E800) and digital images were taken using the Nikon Elements BR system (Nikon).

Liver pathology criteria applied to oriental sole and large-toothed flounder was derived from those specified under the BEQUALM programme and following guidelines set out by Feist et al. (2004). All other pathologies were recorded according to criteria discussed in Stentiford et al. (2003).

2.3. Bile metabolite analysis

Following euthanasia the gall bladder from both *A. thalassinus* and *P. quabrilineatus* was removed and immediately placed in a −20 °C freezer. Bile samples were analysed for fluorescent bile metabolites using synchronous fluorescence spectrometry (SFS), as described by Ariese et al. (2005). Briefly, bile samples were thawed in an ultrasonic bath and diluted with ethanol/water (50:50 v/v). SFS spectra were measured in a 1 cm quartz cuvette using a Perkin Elmer LS50 spectrofluorimeter. For quantification, the net peak area from excitation wavelength 323–423 nm was measured and expressed as μg kg^{-1} wet weight 1-hydroxypyrene (1-OH pyrene) equivalents.

2.4. Chemical analysis

Whole samples of *A. thalassinus* were stored frozen at −20 °C until required for analysis. In preparation for analysis, samples were defrosted and dissected in a strictly controlled, contaminant-free environment. For each site, 4–5 individual fish had muscle and liver tissue removed and these were bulked and homogenised to produce a single homogenate of each tissue per site. Sub-samples of fish liver were processed for PCBs, PBDEs, HBCDD (~5 g) and metals (~3 g) analysis; whereas sub-samples of catfish muscle (~3 g) were processed for metals analysis only. Tissue samples were stored frozen at −20 °C until required for analysis.

2.4.1. Biota metals analysis

The biota samples underwent an acid digestion using an enclosed vessel microwave (Multiwave 3000, Anton Paar, Hertford, UK). Typically, approximately 3 g of homogenised sample was weighed out and pre-digested overnight in 6 mL of nitric acid (Aristar grade 69%, VWR, Leicestershire, UK). The digestion was performed using a temperature-controlled microwave programme specific for the sample matrix. The digest was then further diluted prior to analysis by inductively-coupled plasma-mass spectrometry

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