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Charting a path towards non-destructive biomarkers in threatened wildlife: A systematic quantitative literature review☆

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ABSTRACT

Threatened species are susceptible to irreversible population decline caused by adverse sub-lethal effects of chemical contaminant exposure. It is therefore vital to develop the necessary tools to predict and detect these effects as early as possible. Biomarkers of contaminant exposure and effect are widely applied to this end, and a significant amount of research has focused on development and validation of sensitive and diagnostic biomarkers. However, progress in the use biomarkers that can be measured using non-destructive techniques has been relatively slow and there are still many difficulties to overcome in the development of sound methods. This paper systematically quantifies and reviews studies that have aimed to develop or validate non-destructive biomarkers in wildlife, and provides an analysis of the successes of these methods based on the invasiveness of the methods, the potential for universal application, cost, and the potential for new biomarker discovery. These data are then used to infer what methods and approaches appear the most effective for successful development of non-destructive biomarkers of contaminant exposure in wildlife. This review highlights that research on non-destructive biomarkers in wildlife is severely lacking, and suggests further exploration of *in vitro* methods in future studies.

1. Introduction

Elucidating current or potential negative effects of chemical pollutants on threatened wildlife is a vital aspect of conservation efforts. Populations of threatened species are already pressured by major anthropogenic impacts such as climate change, habitat destruction and overfishing, and the additional effect of exposure to chemical contaminants has been cited as a contributing factor to the decrease in global biodiversity (Bickham et al., 2000).

Aside from the obvious adverse effect of lethal toxicity (Oaks et al., 2004; Relyea and Diecks, 2008), contaminant exposure can have less immediately apparent negative effects in wildlife, such as immune suppression (Keller et al., 2006; Van Loveren et al., 2000), reproductive incapacity (Porte et al., 2006), endocrine disruption (Scott et al., 2014; Soffker and Tyler, 2012) and behavioural toxicity (Lanctot et al., 2016; Melvin and Wilson, 2013). Ultimately, these organism-level effects can lead to consequences at the population and ecosystem levels. For example, Kidd et al. (2007) demonstrated that reduction of reproductive output can lead to the complete collapse of a fish population after exposure to environmentally relevant concentrations of the synthetic hormone ethinylestradiol. The effects of chemical exposure on organisms can be varied, even between contaminants with the same mode of action. For example, endocrine disrupting chemicals can cause either feminisation of the males or masculinisation of the females, through either physical changes, such as abnormal gonad morphology (Kang et al., 2006; Leusch et al., 2006), or complex hormonal changes that reduce successful reproductive output (Denslow and Sepúlveda, 2007). A decrease in reproductive output can also be indirectly caused by immunotoxicant exposure through various adverse effects. Studies have connected immunotoxins to the deterioration of lymphoid tissue, such as the thymus, causing a weakened innate or adaptive immune response (Desforges et al., 2016; Galloway and Handy, 2003). This reduced immune function can lead to an increased parasite load or pathogen susceptibility, and the resulting accumulated energetic costs of this burden can detract from healthy reproductive function in the organism (Booth et al., 1993; Hillegass et al., 2010; Marcogliese and Pietrock, 2011). In more severe cases, the immune system may be so compromised that life span is dramatically decreased, as was the case with several mass mortality

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events where tens of thousands of seals became ill and died within a short period of time due to the contraction of a morbillivirus (Mahy et al., 1988; Osterhaus and Vedder, 1988). While the direct cause of death was the virus, it was later established through analysis of the carcasses and *in vivo* exposure experiments that exposure to mixtures of organochlorine contaminants such as polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzodioxins (PCDDs) were responsible for decreasing immune system functioning, rendering these individuals more susceptible to the virus (de Swart, 1994; de Swart et al., 1996; Ross et al., 1995; Van Loveren et al., 2000). It is therefore apparent that the effects of contaminant exposure can be subtle, yet cause serious adverse effects in wildlife populations.

Resulting population decline may be irreversible by the time these effects manifest, particularly for threatened species in which population numbers are already low or declining. In light of this, contaminant mitigation efforts need to focus on early detection of chemical exposure and negative effects. One approach to determine the effects of contaminant exposure in organisms is to perform controlled experiments on laboratory animals to correlate exposure and effect. This has been widely done with laboratory animals such as fish (Bandelj et al., 2006; Brockmeier et al., 2016; Melvin, 2016; van der Oost et al., 2003), and while this method is valuable, there are logistical and ethical considerations to this approach when dealing with threatened wildlife. An alternative may be to use closely related (but not endangered) model organisms and assume that effects to a related species would be similar (e.g. de la Casa-Resino et al., 2013). However, inter-species extrapolation is problematic due to the potential for significant differences in both toxicokinetic and toxicodynamic factors (Bokkers and Slob, 2007; Nyman et al., 2014).

In addition, laboratory experiments often focus on single compounds or a small number of compounds in mixtures (e.g. Gilman et al., 2003; Solomon et al., 2008; Wang et al., 2016). However, chemicals are generally present as complex mixtures in the environment and subsequently in wildlife (Jin et al., 2015), which means that adverse effects may manifest at different concentrations and in a different manner in wild populations than in animals exposed in a laboratory setting (de Swart et al., 1996; Kelly et al., 2007). Examining species-specific *in situ* effects of contaminants in wildlife therefore represents a more meaningful reflection of the effect of real-world exposure, compared to effects observed in laboratory-based controlled environment (Spurgeon et al., 2010). However, moving towards examining *in situ* exposure presents a new set of challenges, largely due to the fact that many variables cannot be controlled for, and therefore the ability to assign causation of exposure to adverse effects is limited.

Biomarkers of exposure and/or effect are tools often used for early detection of health deterioration, although the term is very general and has been assigned a number of definitions depending on the context. In general, the term biomarker refers to the response of a biological system to a potential threat ranging from an ecosystem, to a population or individual level. Biomarkers of chemical exposure and effect have largely been studied in individuals, predominantly at the molecular level. Separating the terms 'exposure' and 'effect' can be challenging as is thoroughly discussed by van der Oost et al. (2003). In toxicology, a biomarker of exposure is often considered to be the detection of a contaminant itself or its metabolites, whereas a marker of effect is the alteration of a biochemical or physiological parameter associated with contaminant exposure (Dos Santos et al., 2016; Espín et al., 2015). However, if this altered response is not proven to be detrimental to the individual, it may not be an indicator of effect and hence will simply be a marker of exposure (e.g. da Silva Corrêa et al., 2016). Some studies do not define the detection of contaminant in an organism as a biomarker but rather more accurately as a 'bioaccumulation marker' (van der Oost et al., 2003). However, the reference to biomarkers in this paper will be to both endogenous molecules and chemicals detected in the tissue or blood of organisms with endogenous molecular responses that indicate a proven adverse effect referred to as "biomarkers of effect".

Biomarkers play an important role in early detection of disease in humans (Etzioni et al., 2003), and the methods for clinical biomarker discovery are well established (Spurgeon et al., 2010; Vaidya and Bonventre, 2010). The application of biomarkers in ecotoxicology provides an alternative to laboratory exposure experiments, and a potentially more effective way to detect negative effects of contaminants on the health of threatened wild populations (Kendall, 2016). Biomarkers of effect have been well established for some contaminants and validated in a number of species, especially aquatic organisms such as fish. These markers cover a number of molecules including biotransformation enzymes (e.g. cytochrome P450), stress proteins (e.g. HSP70), as well as haematological, immunological and reproductive factors (e.g. vitellogenin, a well-established marker of endocrine disruption in fish) (Sumpter and Jobling, 1995; Leusch et al., 2005). However, the validation of these biomarkers for use in wildlife has been limited, especially for those species with high conservation importance (e.g. sea turtles; Finlayson et al., 2016).

One significant hindrance in wildlife biomarker research is that traditional methods for the establishment of biomarkers require manipulation and often sacrifice of live animals (e.g. Jasinska et al., 2015), which is particularly not desirable for threatened species. However, studies attempting to establish and validate biomarkers of contaminant exposure using non-destructive samples from wildlife are emerging, and technological developments are inviting new and innovative ways to approach this problem (Martyniuk and Simmons, 2016). Despite this, there has not been a comprehensive review of the methods used to validate non-destructive measurement of biomarkers in wild vertebrate fauna since Fossi and Leonzio's (1993) review of this topic over 20 years ago. While there are countless studies that investigate the accumulation and adverse effects of contaminants in wildlife, studies that aim to validate non-destructive measurement of these markers for the detection of adverse effects are not as numerous, or successful. In light of the continuing decrease in global biodiversity, it is crucial that attempts to develop non-destructive tools that could be used for monitoring and early detection of negative impacts, and therefore help prevent further population decline, be as effective as possible. This paper aims to address this issue by systematically reviewing studies that have explicitly attempted to establish and/or validate biomarkers of contaminant exposure and effect in wildlife using non-destructive techniques. Within this, gaps in the knowledge are identified, and guidance on future research in this field are presented.

2. Methods

2.1. Search tools and parameters

A systematic literature search (Pickering and Byrne, 2014) was performed to find studies that have attempted to develop non-destructive biomarkers of contaminant exposure and effect in vertebrate wildlife. Google Scholar, Web of Science and Science Direct databases were used to search for studies that met a set of predetermined criteria. Each study must have included all of the following terms: 'biomarker' and 'contaminant' in combination with either of the following terms; 'non-destructive' or 'non-invasive'. All papers published prior to October 2016 that met all of these criteria, and that were relevant to vertebrate wildlife, were