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Novel associations between contaminant body burdens and biomarkers of reproductive condition in male Common Carp along multiple gradients of contaminant exposure in Lake Mead National Recreation Area, USA

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ABSTRACT

Adult male Common Carp were sampled in 2007/08 over a full reproductive cycle at Lake Mead National Recreation Area. Sites sampled included a stream dominated by treated wastewater effluent, a lake basin receiving the streamflow, an upstream lake basin (reference), and a site below Hoover Dam. Individual body burdens for 252 contaminants were measured, and biological variables assessed included physiological [plasma vitellogenin (VTG), estradiol-17β (E2), 11-ketotestosterone (11KT)] and organ [gonadosomatic index (GSI)] endpoints. Patterns in contaminant composition and biological condition were determined by Principal Component Analysis, and their associations modeled by Principal Component Regression. Three spatially distinct but temporally stable gradients of contaminant distribution were recognized: a contaminant mixture typical of wastewaters (PBDEs, methyl triclosan, galaxolide), PCBs, and DDTs. Two spatiotemporally variable patterns of biological condition were recognized: a primary pattern consisting of reproductive condition variables (11KT, E2, GSI), and a secondary pattern including general condition traits (condition factor, hematocrit, fork length). VTG was low in all fish, indicating low estrogenic activity of water at all sites. Wastewater contaminants associated negatively with GSI, 11KT and E2; PCBs associated negatively with GSI and 11KT; and DDTs associated positively with GSI and 11KT. Regression of GSI on sex steroids revealed a novel, nonlinear association between these variables. Inclusion of sex steroids in the GSI regression on contaminants rendered wastewater contaminants nonsignificant in the model and reduced the influence of PCBs and DDTs. Thus, the influence of contaminants on GSI may have been partially driven by organismal modes-of-action that include changes in sex steroid production. The positive association of DDTs with 11KT and GSI suggests that lifetime, sub-lethal exposures to DDTs have effects on male carp opposite of those reported by studies where exposure concentrations were relatively high. Lastly, this study highlighted advantages of multivariate/multiple regression approaches for exploring associations between complex contaminant mixtures and gradients and reproductive condition in wild fishes.

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1. Introduction

Laboratory studies of endocrine-disruptive chemicals (EDCs) have been largely based on experimental exposures to single compounds or to simple mixtures. These studies have yielded a wealth

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of information about mechanisms and modes of action (MOAs) by which EDCs may lead to adverse effects on health and reproduction, especially for EDCs with estrogenic activity (Segner et al., 2013). There is growing recognition, however, of the need to characterize the cumulative effect of environmentally relevant, complex EDC mixtures (Brander, 2013; Hotchkiss et al., 2008). Some investigators have also cautioned against the strong emphasis on MOAs that typifies many of the recent EDC studies related to toxicity test development at the expense of – or with relatively little direct attention given to – adverse physiological or ecological outcomes (Marty et al., 2011).

Field studies provide realistic settings for probing associations between actual EDC mixtures present in the environment and wildlife health. Results of field studies cannot provide conclusive evidence of cause-effect associations because of the complexity of biotic and abiotic variables that affect organismal functions. They can, however, reveal emerging contaminant problems with potential adverse outcomes and inform the selection of environmentally relevant EDCs (single compounds or mixtures) and their concentrations for experimental studies. Furthermore, field studies provide an ecological context to further extend findings from laboratory studies. For example, field EDC studies conducted in the 1990s revealed that many surface waters receiving effluent from wastewater treatment plants contained compounds with estrogenic activity that potentially could impair reproduction in teleost fishes (Bevans et al., 1996; Folmar et al., 1996; Jobling et al., 1998; Purdom et al., 1994). These early studies contributed to the emergence of widespread and intense interest in environmental EDCs although this interest was, and still is, largely biased towards EDCs with estrogenic activity (Propper, 2005; Segner et al., 2013; Sumpter and Jobling, 2013).

Despite their advantages, field EDC studies are relatively uncommon due in part to their high cost and labor requirements and often difficult logistics. In addition, most field studies that combine biological measurements with analytical chemistry have focused primarily on a single class of potential EDCs or on a limited number of compounds. A few studies have generated comprehensive analytical chemistry and biological datasets (e.g., Feist et al., 2005; Hinck et al., 2007, 2008; Sepúlveda et al., 2002), but their statistical approaches were limited to univariate and bivariate analyses, sometimes because contaminants were measured in composited samples, not individual fish. Multivariate statistical approaches have potential to uncover patterns in complex field datasets that may not be apparent from univariate analyses, and are commonly used in studies of water quality (Migliaccio et al., 2011). Also, to explore associations between contaminant and biological variables by multiple regression, the use of orthogonal (uncorrelated) variables derived from techniques such as Principal Component Analysis (PCA) would reduce problems of multicollinearity and model instability associated with the use of original independent variables (Jolliffe, 1982). Multivariate analyses have been applied in some field studies of contaminants (e.g., Long et al., 2014; Patiño et al., 2012; Torres et al., 2014) but, to our knowledge, only a handful of field EDC studies have used multivariate statistics on biological data (e.g., Patiño et al., 2012; Theodorakis et al., 2006; Torres et al., 2014). A field study of fish and amphibian populations exposed to perchlorate concluded that multivariate analyses are more powerful than univariate analyses for detecting spatiotemporal differences in biological condition (Theodorakis et al., 2006).

Lake Mead National Recreation Area (LMNRA), Arizona and Nevada (USA) includes Lake Mead, the largest reservoir by volume in the USA. The lake supplies water and hydroelectric power to the southwestern region of the country and is critical habitat for federally endangered fishes (Chandra et al., 2012; Turner et al., 2012). Plasma vitellogenin (VTG) is an estrogen-inducible hepatic lipoprotein typically detectable only in female fish. In 1995, VTG was recorded at concentrations as high as ~60 mg/mL in male Common Carp (*Cyprinus carpio*) from sub-basins of the Lake Mead receiving treated wastewater effluent and municipal runoff from the City of Las Vegas (Nevada), but was undetectable at a reference site (Bevans et al., 1996). A later study (1999–2000) determined the annual reproductive cycle of carp in Lake Mead and found that reproductive condition in impacted areas was affected to a larger degree in males than females (Patiño et al., 2003). Analyses of VTG in the 1999–2000 fish showed that while a few individuals still had high levels, males from impacted and reference areas had similarly low overall median values (<0.03 mg/mL; Goodbred et al., 2007). Based on this biomarker, therefore, the estrogenicity of water in impacted areas seems to have declined between 1995 and 1999–2000. In contrast, other studies of LMNRA conducted through the mid-2000s indicated the presence of a variety of legacy and emerging contaminants in water, sediment, and fish from the areas receiving treated effluent and runoff (Rosen et al., 2012). These observations suggest that fish condition may be impacted by a mix of EDCs in LMNRA but via organismal MOAs that do not involve VTG production in males.

The objective of this study was to determine associations between whole-body contaminant concentrations (indices of exposure) and biomarkers of reproductive condition in individual male carp from different locations within LMNRA and through a full reproductive cycle. Principal Component Analysis was used to identify spatiotemporal patterns in data distributions, and orthogonal variables derived from the contaminant PCA were used as independent variables to model their association with biological variables by Principal Component Regression. Results of this study were expected to provide information that will further our understanding of the impacts on wild fishes of municipal wastewater-influenced habitats containing emerging and legacy contaminants with negligible estrogenic activity, and a case-study for the application of multivariate statistics to complex contaminant and biological field datasets.

2. Methodology

2.1. Study sites and rationale for selection

Common Carp were collected at three sites within Lake Mead and its watershed along an expected concentration gradient of contaminants associated with treated municipal effluent and urban runoff, and at an additional site downstream of Hoover Dam. The four sites were: Las Vegas Wash, Las Vegas Bay, Overton Arm, and Willow Beach (Fig. 1). Las Vegas Wash collects non-point surface and ground discharges, non-point runoff from the City of Las Vegas metropolitan area, and municipal effluent from three wastewater treatment facilities (Turner et al., 2012). Las Vegas Wash enters Lake Mead at Las Vegas Bay, which is located on the western portion of Boulder Basin where flows from Las Vegas Wash mix with upstream Colorado River flows. Overton Arm receives flow from the Virgin and Muddy Rivers and merges with the Colorado River at Virgin Basin, upstream of Boulder Basin (Fig. 1). The watersheds of the Virgin and Muddy Rivers are covered mostly by rangeland or forest (Turner et al., 2012). Except for Las Vegas Bay, contaminant loading into Lake Mead is relatively low (Tietjen et al., 2012).

Willow Beach is a site on the Colorado River approximately 11 km below Hoover Dam (Fig. 1). Water from Las Vegas Bay continues to mix with upstream Colorado River flow in Boulder Basin as it moves towards the dam. Thus, discharges from Hoover Dam and consequently stream flows at Willow Beach are expected to contain lower concentrations of contaminants derived from Las