General and Comparative Endocrinology 219 (2015) 125–135

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Are endocrine and reproductive biomarkers altered in contaminant-exposed wild male Largemouth Bass (*Micropterus salmoides*) of Lake Mead, Nevada/Arizona, USA?

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ARTICLE INFO

Article history:
Available online 27 February 2015

Keywords:
Teleost
Endocrine disruption
Sex steroids
Gonadosomatic index
Gonadal development
Sperm quality

ABSTRACT

Male Largemouth Bass were sampled from two locations in Lake Mead (USA), a site influenced by treated municipal wastewater effluent and urban runoff (Las Vegas Bay), and a reference site (Overton Arm). Samples were collected in summer (July '07) and spring (March '08) to assess general health, endocrine and reproductive biomarkers, and compare contaminant body burdens by analyzing 252 organic chemicals. Sperm count and motility were measured in spring. Contaminants were detected at much higher frequencies and concentrations in fish from Las Vegas Bay than Overton Arm. Those with the highest concentrations included PCBs, DDTs, PBDEs, galaxolide, and methyl triclosan. Fish from Las Vegas Bay also had higher Fulton condition factor, hepatosomatic index, and hematocrit, and lower plasma 11-ketotestosterone concentration (KT). Gonadosomatic index (GSI) and sperm motility did not differ between sites, but sperm count was lower by nearly 50% in fish from Las Vegas Bay. A positive association between KT and GSI was identified, but this association was nonlinear. On average, maximal GSI was reached at sub-maximal KT concentrations. In conclusion, the higher concentration of contaminant body burdens coupled with reduced levels of KT and sperm count in fish from Las Vegas Bay suggest that male reproductive condition was influenced by contaminant exposures. Also, the nonlinear KT-GSI association provided a framework to understand why GSI was similar between male bass from both sites despite their large difference in KT, and also suggested the existence of post-gonadal growth functions of KT at high concentrations.

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1. Introduction

Lake Mead was formed by the construction of Hoover Dam in 1935 and is the largest reservoir by volume in the United States. It is also a vital resource for the region, supplying drinking water for more than 25 million people in the states of California, Arizona and Nevada, generating over 2 gigawatts of electricity through hydropower, and providing irrigation water for over 2.5 million acres of agricultural land (Turner et al., 2012). The lake provides habitat for over 14 species of fish, including two federally-listed as endangered species, the Bonytail Chub (*Gila elegans*) and the Razorback Sucker (*Xyrauchen texanus*) (Mueller and Marsh, 2002). In addition, Lake Mead supports large populations of non-native sportfishes including Largemouth Bass (*Micropterus salmoides*), Striped Bass (*Morone saxatilis*), and Channel Catfish (*Ictalurus punctatus*) as well as other introduced species like Common Carp (*Cyprinus carpio*).

Given the importance of Lake Mead in the western United States, it has been intensely monitored and studied. A synthesis of knowledge in aquatic sciences for the lake was recently

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http://dx.doi.org/10.1016/j.ygcen.2015.02.015
0016-6480/Published by Elsevier Inc.

published (Rosen et al., 2012). In spite of generally good water quality in Lake Mead, a combination of flows that include tertiary-treated municipal effluent and urban and industrial runoff delivered to Las Vegas Bay via the Las Vegas Wash (a stream draining the Las Vegas Valley) has regionally compromised the lake's water quality (Rosen et al., 2010). More than 100 organic chemicals have been detected in water, sediment and fish tissues from Lake Mead, particularly in the Las Vegas Bay area, many of which are known to have endocrine-disrupting properties (Rosen et al., 2012). They include legacy pollutants such as polychlorinated biphenyls (PCBs) and dichloro-diphenyl-trichloroethane and its derivatives (DDTs), and emerging contaminants like polybrominated diphenyl ethers (PBDEs, aka flame retardants), triclosan (antimicrobial), and polycyclic musks (fragrances).

A number of field studies on endocrine disruption have been conducted in Common Carp of Lake Mead (Bevans et al., 1996; Goodbred et al., 2007; Hinck et al., 2007; Patiño et al., 2003, 2012, 2015). These studies reported reduced concentrations of sex steroid hormones, reduced gonadosomatic index, increased incidence of testicular pathologies, and altered sperm quality in male fish from sites impacted by urban and industrial pollutants (Bevans et al., 1996; Goodbred et al., 2007; Patiño et al., 2003, 2012, 2015). Thus, endocrine disruption has been documented in areas of Lake Mead that receive urban runoff and treated wastewater effluent for what is generally considered a species with high tolerance for poor water quality conditions (Edwards and Twomey, 1982; Weber et al., 2010).

There is a well-established Largemouth Bass population in Lake Mead. Largemouth Bass are in the family Centrarchidae, and occupy a niche different than Common Carp (Umek et al., 2010). The fish feed at different trophic levels and have different food habits (bass is a top predator; carp is mid-level omnivore), and are therefore potentially exposed to different types and concentrations of contaminants. In addition, compared to Common Carp (Edwards and Twomey, 1982), Largemouth Bass are generally less tolerant of poor water quality (Stuber et al., 1982). Therefore, a study of Largemouth Bass in Lake Mead would provide valuable information to compare differences in exposure and responses to endocrine-disrupting chemicals among species in their natural environment, and to evaluate the extent of endocrine disruption in the teleost fauna of the lake. This study focused on Largemouth Bass collected from Las Vegas Bay and a reference site, Overton Arm, with limited upstream impacts from urban populations. The targets of this study were male fish, as earlier studies found the most significant impacts in males (Bevans et al., 1996; Patiño et al., 2003). Fish were collected in spring, just before the onset of spawning, and in summer. The primary objectives were to determine (1) differences between sites in whole-body concentrations of selected legacy and emerging organic contaminants, (2) differences between sites in health and reproductive biomarkers including sex steroid hormones and sperm quality, and (3) general relationships between these two sets of variables.

2. Materials and methods

2.1. Study sites and sampling

Based on results of previous studies of Lake Mead, Overton Arm was designated as the reference site due to its low inputs of point and non-point source pollutants compared to Las Vegas Bay (Goodbred et al., 2007; Patiño et al., 2003, 2012, 2015; Rosen et al., 2010) (Fig. 1). Male Largemouth Bass were collected in July 2007 (summer, before gonadal recrudescence) and March 2008 (spring, just before spawning). The collection period generally lasted 4 days for each sample event. Fish were captured by electrofishing (pulsed DC current) in shallow water <4 m (Largemouth Bass typically spawn at depths less than 3 m; Hunt et al., 2002) adjacent to shorelines where cover was present, and placed in a live well until further processed. It is difficult to collect Largemouth Bass from Lake Mead compared to other, more abundant species (e.g., Common Carp); thus, sample sizes differed between collection dates because of differences in success of capture. The desired number of fish per site and sampling event was 15, but only 6 male bass could be collected from Las Vegas Bay and 10 from Overton Arm in summer 2007, and 13 males were collected from each site in spring 2008. On shore, fish were immersed in a lethal dose of anesthesia (1 g tricaine methane sulfonate per liter of lake water) until movement ceased, euthanized by a blunt blow to the head, weighed to the nearest 5 g, and measured to the nearest mm. Gonads and liver were dissected and weighed to the nearest 0.1 g to determine somatic indices. Blood was sampled with a heparinized, 5-ml syringe using a 20-gage needle from the caudal vein for measurement of hematocrit and plasma sex steroid hormones.

Hematocrit was determined on site at the time of blood collection using a HemataSTAT II Microhematocrit centrifuge (Separation Technology, Inc., Altamonte Spring, Florida, USA). Blood for sex steroid hormone analysis was transferred to heparinized Vaccu-tubes™ and centrifuged at approximately 1000*g* for 10–15 min to separate the plasma. Plasma was transferred to cryovials and placed on dry ice, and then stored at −80 °C until analysis. During the spring 2008 collection, one testis from each fish was rinsed with Hanks' balanced salt solution containing penicillin/streptomycin (HBSS-PS), then placed intact in 500-ml sterile bottles filled with HBSS-PS, and shipped overnight at 4 °C to the U.S. Geological Survey National Wetlands Research Center for sperm quality analysis. Fish carcasses were wrapped in aluminum foil, including the remaining gonad, placed in plastic bags, frozen to −80 °C, and shipped to the U.S. Geological Survey Columbia Environmental Research Center for contaminant analysis.

2.2. Contaminant analysis

Individual fish were ground, homogenized and sub-sampled for analyses as described by Patiño et al. (2015) for carp, whose analytical procedures were based on Echols et al. (2013) and Peterman et al. (2006). A total of 252 chemicals were selected for analysis (for full list see Patiño et al., 2015); however, only 216 individual organic chemicals could be clearly resolved in bass. Unresolved groups of chemicals included 11 groups representing 2–4 PCB congener mixtures each and one group containing two PBDEs, and were also included as "individual" chemicals in the initial analysis. From this dataset of 228 chemicals, those with ⩽40% of values below their respective LOD were selected for further statistical analyses if fish from the contaminated site (Las Vegas Bay) had an average value greater than 5 ng g^{-1} ww for PCBs, 9 ng g^{-1} ww for PBDEs, or 10 ng g^{-1} ww for DDTs. This second selection criterion served to focus attention on the most prominent chemicals. The final list used in the present statistical analysis included 21 individual chemicals and 5 class sums ($\sum$s) (Table 1).

2.3. Plasma sex steroid assays

The androgen 11-ketotestorosterone (KT) and estrogen 17β-estradiol (E2) were assayed in plasma using commercial ELISA kits. The procedures used were those recommended by the kit manufacturer (No. 582751 for KT and 582251 for E2; Cayman Chemical Company, Ann Arbor, Michigan, USA). Plasma (KT) or extract (E2) diluted with assay buffer yielded nearly perfect parallelism to the standard curves (R^2 = 0.99–1.00) using plasma containing naturally high or low steroid concentrations. For