Marine Pollution Bulletin 137 (2018) 129–136

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Examining the role of estrogenic activity and ocean temperature on declines of a coastal demersal flatfish population near the municipal wastewater outfall of Orange County, California, USA

Nicolette Andrzejczyk[a,*], Ken Sakamoto[b], Jeff Armstrong[b], Daniel Schlenk[a,*]

[a] Department of Environmental Sciences, University of California, Riverside, CA 92521, USA
[b] Orange County Sanitation District, Fountain Valley, CA 92708, USA

ARTICLE INFO

Keywords:
Wastewater
Ecotoxicology
Endocrine disruptors
Demersal flatfish
Climate change
Multiple stressors

ABSTRACT

Wastewater treatment plant effluent introduces a mixture of pollutants into marine environments; however, the impacts of chronic sublethal exposures on populations are often unclear. Presence of estrogenic agents in sediments and uptake of these compounds by demersal flatfishes has been reported at the Orange County Sanitation District (OCSD) wastewater outfall. Furthermore, estrogenic activity has been identified in male flatfish in the area, potentially contributing to observed population declines in the OCSD region. Rising ocean temperatures may further contribute to flatfish declines as relationships between temperature and abundance have been reported in the Southern California Bight. To investigate declines, sex ratios, condition factor, organ health indices, hormones, and vitellogenin were quantified in flatfish collected at OCSD outfall and reference sites. Additionally, historical temperature data was examined for trends with population abundances. Rather than being linked to estrogenic activity, results indicated that population declines were more correlated to increases in ocean temperature.

1. Introduction

Exposure to compounds with estrogenic modes of action has been linked to endocrine disrupting effects among wild fish populations in freshwater and marine environments (Lye et al., 1997; Jobling et al., 1998; Tyler et al., 1998; Allen et al., 1999; Palace et al., 2002; Kidd et al., 2007). The presence of estrogenic agents in aquatic environments is a result of anthropogenic activities, with wastewater treatment plant effluent being one of the major contributors (Jobling et al., 1995; Jobling et al., 1998; Thomas et al., 2001). As a result of large-scale urbanization, wastewater treatment plant effluent may pose risks to aquatic ecosystems receiving effluent from highly developed areas, such as Southern California. The Southern California Bight alone receives a total of over 1 billion gallons of treated wastewater per day, often containing several contaminants of emerging concern not removed by the treatment process (Lyon et al., 2006; Bay et al., 2012; Vidal-Dorsch et al., 2012).

At the Orange County Sanitation District (OCSD) wastewater treatment plant outfall, a variety of estrogenic agents have been identified in sediments, including 17β-estradiol (E_2), nonylphenol, alkylphenol ethoxylates, triclosan, and various pharmaceutical and personal care products, among others (Schlenk et al., 2005; Maruya et al., 2012). Furthermore, bioaccumulation of compounds deposited in sediments at the OCSD has been observed in the demersal flatfish hornyhead turbot (*Pleuronichthys verticalis*) (Bay et al., 2011; OCSD, 2012; Maruya et al., 2012). Compounds identified in sediments and organisms at the OCSD outfall may pose risks to marine health as E_2, nonylphenol, and alkylphenol ethoxylates have been shown to alter steroidogenesis in several fish species (Jobling et al., 1996; Nimrod and Benson, 1996; Arukwe et al., 1997; Desbrow et al., 1998; Nakamura et al., 2002).

Previous studies have investigated the occurrence of endocrine disrupting effects in hornyhead turbot residing at the OCSD outfall. These studies reported elevated E_2 and vitellogenin levels in hornyhead turbot males as well as skewed population sex ratios (Roy et al., 2003; Rempel et al., 2006; Deng et al., 2007; Bay et al., 2012; Forsgren et al., 2012). Although estrogenic activity has been identified, population abundances of hornyhead turbot have remained unchanged indicating little or no population-level effects (Forsgren et al., 2012). While hornyhead turbot populations appear to be stable, there may be other more sensitive species that respond differently to contaminants deposited in sediments at the OCSD outfall.

In addition to anthropogenic chemical inputs, trends in

* Corresponding authors.
E-mail addresses: nandr009@ucr.edu (N. Andrzejczyk), dschlenk@ucr.edu (D. Schlenk).

https://doi.org/10.1016/j.marpolbul.2018.10.005
Received 30 May 2018; Received in revised form 17 September 2018; Accepted 2 October 2018

environmental variables may further impact population abundances. Of these environmental variables, temperature and shifts in climate cycles are likely to mediate demersal flatfish populations as we are currently in the 3rd consecutive decade of record high ocean temperatures (Levitus et al., 2000; Hansen et al., 2010). In the Southern California Bight specifically, demersal flatfish abundances have shown to correlate with shoreline and offshore ocean temperatures, the Pacific Decadal Oscillation (PDO) index, and the El Niño Southern Oscillation (ENSO) cycle (Allen et al., 2004); these results indicate that temperature plays an important role in population fluctuations.

Unlike previously studied demersal flatfish species, Pacific sanddab (*Citharichthys sordidus*) have experienced population declines throughout the OCSD monitoring region (OCSD, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017). Pacific sanddab and other demersal flatfishes are ideal study species as they are exposed to compounds deposited in sediments through direct dermal absorption and through their diet of infaunal and epibenthic organisms (Allen, 1982; Love and Goldberg, 2009). Having identified population-level disturbances in Pacific sanddab, this study aims to identify potential mechanisms responsible for population declines. We hypothesized that declines may be linked to estrogenic activity given the previous detection of estrogenic agents in OCSD sediments and the identification of estrogenic activity in other flatfish species. Additionally, it was hypothesized that population declines may be correlated to shifts in ocean temperature as significant relationships between abundance and temperature as well as broader climate cycles have previously been identified among demersal flatfish populations in the Southern California Bight.

2. Materials and methods

2.1. Sampling area

All samples were collected within the OCSD ocean monitoring area located on the southern portion of the San Pedro Shelf within the Southern California Bight (Fig. 1). The OCSD outfall, T1, is located 7 km offshore at a depth of 55 m (33° 34.641′ N, 118° 00.567′ W), while the reference site, T11, is located 7.7 km north of the outfall at a depth of 60 m (33° 36.055′ N, 118° 05.199′ W). The reference site has over 25 years of monitoring data by the OCSD Ocean Monitoring division and was confirmed by USEPA Region IX. Trawling paths were determined using differential Global Positioning System (GPS) navigation to accurately locate the sampling sites and to control the speed of the trawl (2.0–2.5 knots over the bottom). Currents in the lower water column layer, where effluent is discharged, are directed upcoast (OCSD, 2009). Full secondary treatment for all OCSD effluent has been implemented since 2011.

2.2. Fish collection

Sexually mature Pacific sanddab were collected in February 2017 and March 2017 from the OCSD outfall and reference sites using a 7.6 m wide semi-balloon otter trawl. Individuals were counted, weighed, and measured upon collection. For February 2017 sampling, n = 11 individuals were sampled at the outfall with average lengths of 17.59 ± 2.02 cm and average weights of 87.55 ± 39.56 g; n = 12 individuals were sampled at the reference with average lengths of 19.23 ± 2.10 cm and average weights of 119.83 ± 48.89 g. For March 2017 sampling, n = 17 individuals were sampled at the outfall with average lengths of 16.38 ± 1.59 cm and average weights of 66.25 ± 10.85 g; no Pacific sanddab were caught at the reference site. Additionally, sex ratios were tallied at the outfall site of each individual collected during March 2017 sampling (n males = 39, n females = 41). Sex of each individual was determined by gross morphology of the gonads. Fish were kept in a flow-through holding tank receiving fresh seawater prior to processing. About 1 mL of blood was collected from the dorsal aorta using a 22-gauge syringe and was immediately centrifuged for 10 min at 10,000 rpm. The plasma supernatant was transferred to a new centrifuge tube and kept on dry ice before long-term storage at −80 °C for further analysis. Individuals were sacrificed by severing of the spinal cord, followed by harvesting of liver and gonad tissues. Tissues were kept on dry ice until they were transferred for long-term storage at −80 °C.

2.3. Current data

For individuals collected during February 2017 sampling, hepatic mRNA transcript levels for vitellogenin were quantified through real-time quantitative PCR (RT-qPCR) and normalized to 18S rRNA expression using primers in Table 1. Total RNA extractions from liver tissue were performed using Qiagen RNeasy® Mini Kit (Valencia, CA) according to the manufacturer's protocol. RNA concentration and integrity was measured on a NanoDrop® ND-1000 UV–Vis Spectrophotometer (Wilmington, DE) prior to cDNA synthesis. First strand cDNA was synthesized using Promega™ Reverse Transcription System (Madison, WI) with random primers according to manufacturer's protocol. cDNA products were then utilized for the RT-qPCR reaction using Bio-Rad SsoAdvanced™ Universal SYBR® Green Supermix (Hercules, CA) on a Bio-Rad CFX96 real-time PCR Detection System (Hercules, CA). The thermal cycling profile consisted of an initial denaturation at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 55 °C for 30 s, and extension at 95 °C for 10 s. qPCR products were verified using gel electrophoresis. Each sample was run in duplicate and mean values were calculated. Relative gene expression of vitellogenin between fish from outfall and reference sites was derived using the $2^{-\Delta\Delta CT}$ method among males and females individually.

Plasma testosterone and E_2 were quantified in male Pacific sanddab plasma samples collected in February and March 2017 using ELISA assay kits from Cayman Chemical (Ann Arbor, MI). Standard curves were produced following the manufacturer's protocol to obtain plasma testosterone and E_2 levels.

GSI and LSI values were calculated using the equation $GSI/LSI = \frac{organ\ weight}{body\ weight} \times 100$ for all Pacific sanddab collected during February 2017 sampling. Additionally, population sex ratios from February and March 2017 sampling were tabulated using gross morphology of gonads.

2.4. Historical population data

Historical OCSD trawl data, containing length and weight information on individuals captured during trawls, was used to calculate Fulton's condition factor (K) for Pacific sanddab using the equation $K = \frac{body\ weight}{length^3} \times 100$. Average yearly condition factor was calculated for all individuals collected at outfall and reference sites from 2006 to 2014. Historical trawl data was used to obtain Pacific sanddab abundances at outfall and reference sites from August 2005 to January 2017. These abundance values represent the total number of Pacific sanddab individuals collected during each biannual OCSD trawl at a given site. Fish community Shannon Weiner diversity indices (H) were gathered from OCSD Ocean Monitoring Annual reports (www.ocsd.com) from 2009 to 2015 for both outfall and reference sites from summer trawls.

2.5. Oceanographic variable data

Long-term ocean temperature data at the outfall and reference sites were acquired from OCSD from August 2005 to January 2017. Temperatures for the 50 to 60 m water column range were averaged for each year at outfall and reference sites to reflect temperatures Pacific sanddab are exposed to. Additional temperature data from OCSD Ocean Monitoring Annual Reports were used to substitute missing values in the long-term temperature data. Ocean temperature data were also