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NOTE

Toxics source reduction and sewage upgrades eliminated winter flounder liver neoplasia (1984–2017) from Boston Harbor, MA, USA

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ABSTRACT: Chemical carcinogen biomarkers can validate public investment in environmental remediation. A major factor driving the clean-up of Boston Harbor, MA, USA, induced by the federal Clean Water Act legislation of 1972, was the high prevalence of petroleum and halogenated aromatic hydrocarbon contaminant-associated liver neoplasia in winter flounder *Pseudopleuronectes americanus* in the harbor in the 1980s. In the present study, we examined the spatial and temporal relationships between the suspended solids and contaminants in the municipal sewage discharge, and liver neoplasia and histopathology in flounder, from 1987 to 2017. Toxics source reduction, sewage treatment, and sludge removal in the 1990s and outfall relocation offshore in 2000 enabled a decreasing prevalence of persistent toxic chemicals in flounder, effluent, and sediment, and consequent disappearance of liver neoplasia and reduction of neoplasm-associated, hydropically vacuolated biliary epithelial cells to background levels. This supports long-term investment in elimination and treatment of anthropogenic waste streams and the value of federal regulatory mandates to maintain and improve regional environmental quality.

KEY WORDS: Boston · Sewage · Solids · Flounder · Neoplasia · Health

INTRODUCTION

Prior to the initiation of a major court-mandated (Savage 1994), publicly funded sewage remediation and toxics source reduction effort, Boston Harbor (Massachusetts, USA) was described as 'America's dirtiest harbor' (Hecht 1992). Winter flounder *Pseudopleuronectes americanus* from Boston Harbor had an 8% (n = 200) prevalence of hepatic (hepatocellular and/or cholangiocellular) carcinomas that resembled experimental carcinogen-induced lesions in rodents (Murchelano & Wolke 1985). A study of 22 sites on the US northeast coast showed polycyclic aromatic hydrocarbons, DDTs, and chlordanes in sediments, stomach contents, liver, or bile of winter flounder to be significant risk factors for the development of hydropic vacuolation (HV) and other lesions in the flounder liver (Johnson et al. 1993). HV was shown to progress from single biliary preductular cells (centrotubular HV or CHV), which proliferated (Moore & Stegeman 1992) to replace entire hepatic tubules (tubular HV), and then to grossly visible foci of HV (Moore et al. 1997). Neoplasms were observed within some of these foci (Moore & Stegeman 1994). Therefore, the prevalence

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of CHV (a neoplasm-associated lesion only seen in certain flatfish species; Stehr et al. 1998) and hepatic neoplasms were then used respectively as biomarkers for early and late stages of the effects of the above suite of contaminants. Annual flounder trawl surveys in Massachusetts and Cape Cod Bays were initiated in 1991 to document the efficacy of efforts to remediate the environmental health of Boston Harbor and adjacent waters (Moore et al. 1996, 2005).

Waste management milestones in Boston Harbor's remediation included cessation of sewage sludge discharge in 1991, completion of a new primary sewage treatment facility in 1995, and upgrading to secondary treatment beginning in 1997 (Taylor 2010). As a result, daily input of total suspended solids and particulate nitrogen and carbon decreased by 80–90% between 1991 and 2000 (Taylor 2010). In September 2000, treated effluent was diverted from the outfall at Deer Island at the mouth of Boston Harbor to an offshore outfall 18 km to the northeast. Overall loadings of lipophilic toxic contaminants decreased as a result of targeted source reduction programs (Hunt et al. 2006, Delaney 2010, Werme et al. 2017), sludge removal, enhanced treatment, and outfall relocation offshore. Reductions in flounder liver pathology were also observed, from 1987 to 2003 for Boston Harbor and from 1991 to 2003 for other stations (Moore et al. 2005), but there was little analysis of these changes in the context of toxics loading through the period. In the present study, we added recent (2004–2017) data to compare the entire 33 yr winter flounder data series, with total suspended solids (Werme et al. 2017) as an indicator of overall toxic loading and specific contaminant groups in flounder tissues (Nestler et al. 2016), to examine the environmental improvement of this public works project.

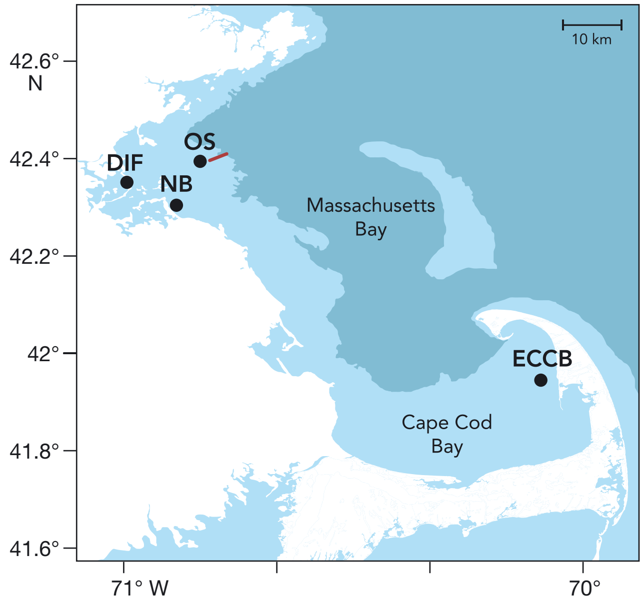

Fig. 1. Winter flounder *Pseudopleuronectes americanus* monitoring stations in Boston Harbor (see Table S1 in the Supplement for geographic coordinates). DIF: Deer Island Flats; NB: Nantasket Beach; OS: Outfall Site (red line: outfall diffusers: black dot: flounder sampling station); ECCB: Eastern Cape Cod Bay. Light/dark blue shading indicates waters shallower/deeper than 40 m

MATERIALS AND METHODS

The objective of the study was to examine the spatial and temporal relationships between suspended solids and contaminants in a major municipal sewage discharge, and associated flounder liver neoplasia and related histopathology, in the context of a major remediation project. Winter flounder were collected from Deer Island Flats (Stn DIF) between 1987 and 1990. Between 1991 and 2017, at least 50 adult winter flounder were examined annually (usually late April, early May) from each of 4 stations (Fig. 1; Tables S1 & S2 in the Supplement at www.int-res.com/articles/suppl/d131p239_supp.pdf): Stn DIF, Nantasket Beach (Stn NB, 10 km from Boston Harbor), Outfall Site (Stn OS, near the new outfall, 18 km from Boston Harbor, which was activated in 2000), and Eastern Cape Cod Bay (Stn ECCB, 85 km from Boston Harbor). As previously described (Moore et al. 1996, 2005), the winter flounder were collected using a commercial otter trawl, evaluated grossly, and then sampled. Samples (3 equally spaced liver slices per fish) were analyzed using routine histopathological methods, for examination of hematoxylin and eosin stained slides using bright field microscopy. Pesticides and PCB congeners were analyzed by gas chromatography-electron capture detector (until 2006) and then gas chromatography-mass spectrometry operating in the selected-ion-monitoring mode, using a 60 m Rtx-5 column (or equivalent) and an Agilent 5973 detector (or equivalent) (Taylor 2013).

For the statistical analysis, data were analyzed using a stepwise linear regression.

RESULTS

Hepatic neoplasm prevalence in winter flounder at Stn DIF in Boston Harbor, adjacent to the original Deer Island outfall, dropped from a peak of 11.5% in 1988 to 0% in 1992, then 3 cases were seen in 1996 and a single case in 2004 (Fig. 2). No cases have been observed at Stn DIF since 2004 up to 2017. A single case was observed at Stn NB in 1994. Fig. 3 shows that CHV prevalence at Stn DIF has also declined, from a peak of 77% in 1987 to the 20–30% range in recent years. Less dramatic declines are also apparent for Stns NB and OS. At Stn ECCB, CHV has maintained a low baseline prevalence with no trend throughout the study period. Tubular and focal HV prevalence also declined comparably (MWRA-ENQUAD 2018). Moderate to severe cases of HV were generally widespread throughout the liver, whereas mild cases, which predominated in recent years, were more localized.

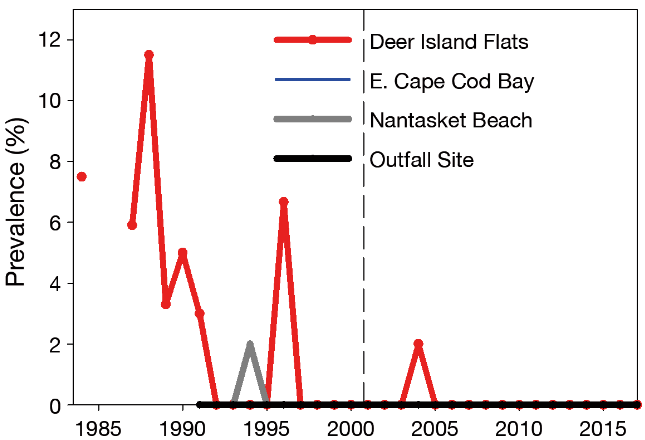

Fig. 2. Temporal comparison of neoplasia prevalence (%) in winter flounder *Pseudopleuronectes americanus* by station (see Fig. 1). Outfall discharge activated in 2000 (vertical dashed line). The 1984 data are from Murchelano & Wolke (1985)

The most inclusive, annually available, and relevant parameter that approximates the overall toxics burden in the metropolitan Boston sewage discharge is the mass of total suspended solids discharged by the Massachusetts Water Resources Authority outfall through time (Werme et al. 2017) (see our Fig. 1 for outfall locations: near Stn DIF until September 2000 and then at the offshore Stn OS). The solid phase is the primary reservoir for persistent organic and inorganic contaminants, as their very persistence depends on their relative hydrophobicity (Gschwend & Wu 1985). We therefore compared reduction in CHV prevalence in flounder from Stn DIF with the daily solids discharges in Boston wastewater discharge between 1991 and 2017 (Fig. 3). There was a significant correlation between Stn DIF's flounder CHV and total solids discharges between 1991 and 2017 (CHV = 25.17772 + 0.210081 × Total Solids, $r^2 = 0.5426$, $p < 0.0001$).

Flounder liver and muscle contaminant analyses were made annually (1992–2003), then every 3 yr (2006–2015) on a random sample of 15 of the 50 flounder examined histologically in those years. Those and many other related data are available in an extensive series of technical reports summarizing the ongoing Harbor and Outfall Monitoring Program undertaken by the Massachusetts Water Resources Authority and their contractors (MWRA-ENQUAD 2018). Table S3 in our Supplement shows a summary of Stn DIF flounder contaminant data in comparison to CHV prevalence. The relationship was analyzed between annual mean CHV prevalence (for all 50 of the fish examined histologically each year) and contaminant

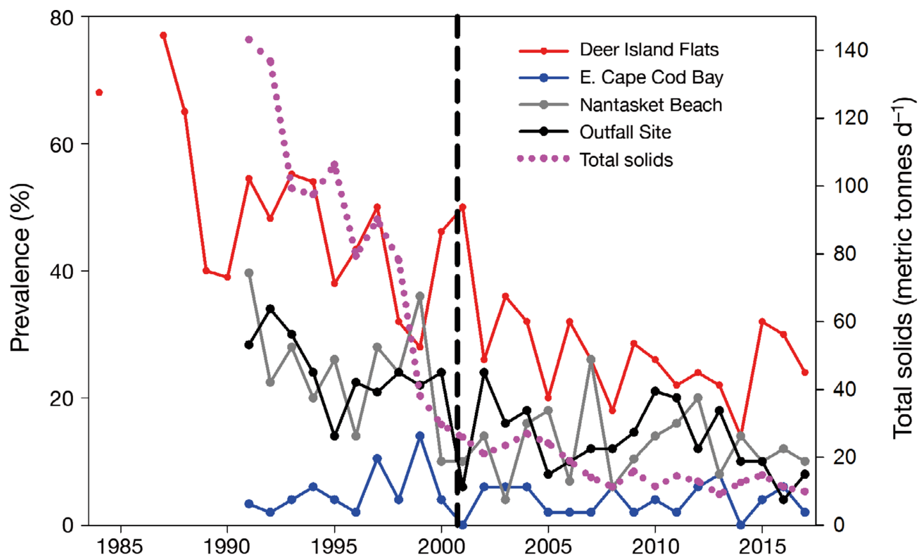

Fig. 3. Prevalence of centrotubular hydropic vacuolation (CHV) in winter flounder *Pseudopleuronectes americanus* by station (see Fig. 1) and total solids discharged by the Boston Outfall which was at Deer Island till September 2000 (vertical dashed line) and then at the offshore Outfall Site. The 1984 data are from Murchelano & Wolke (1985)

concentrations (for a 15-fish subset). HV prevalence for comparison to the 4 most recent contaminant surveys (2006–2015) was calculated as a 3 yr mean including the year sampled for chemistry and the subsequent 2 yr. The analyte most predictive of HV was total chlordanes in flounder fillet (Fig. 4), and to a lesser extent, other organic analytes, including 4,4′-DDE and total PCBs. Inorganic contaminants showed little relationship with HV.

Detailed data on the flounder sampled in this long-term project, including age-to-length and weight-to-length ratios and other parameters, are available in previous reports (MWRA-ENQUAD 2018) and papers (Moore 1991, Moore et al. 1996, 2005), and are summarized in our Table S2. Age, length, and weight parameters are variable (Moore & Nestler 2017), but the general trend through time is an increase in age since 2000, which should if anything have biased the CHV prevalence higher through time, thus the decreasing trend is indeed marked. There has, however, been a trend towards a skewed sex ratio in favor of females in 1996 and thereafter, but this trend is regional to New England and not limited to those areas affected by the Boston outfall (Moore et al. 2016).

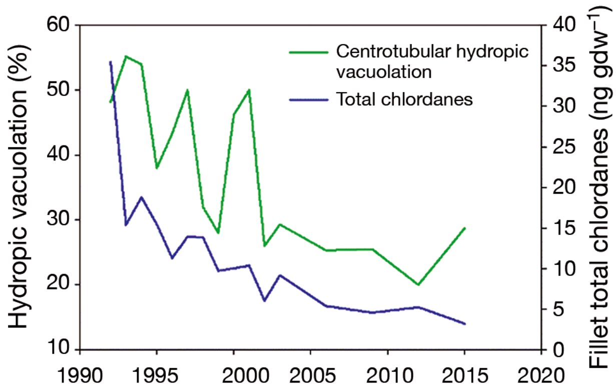

Fig. 4. Mean centrotubular hydropic vacuolation (CHV) prevalence in winter flounder *Pseudopleuronectes americanus* at Stn Deer Island Flats (DIF) compared to flounder fillet total chlordanes concentrations (using the 2 yr after the chemistry year plus the chemistry year to create the mean values for 2003 through 2015). Stepwise linear regression (r^2 = 0.3736, p = 0.0155; see Table S3 in the Supplement); gdw: grams dry weight

CONCLUSIONS

The current study shows that CHV has been a valuable biomarker for the long-term impacts of sewage toxics source reduction, sludge removal, enhanced treatment, and outfall relocation offshore for a major metropolitan area. Of the chemical contaminants analyzed in the flounder, total chlordanes were the most predictive of this change; however, the chronic carcinogenic risk in the waste stream presumably represents a combined exposure to many different compounds. It also suggests that the substantive source reduction, treatment, and removal actions that were undertaken were sufficient to address the problem of toxic chemical effects on flounder, even without the relocation of the outfall, given that the offshore Stn OS shows the same decreasing trend as other stations in the study. Other justifications for that outfall extension remain, given the need for nutrient dilution in deeper open waters, as the available data (Taylor 2013) indicate that Boston Harbor was unable to assimilate the nutrient input associated with treated wastewater, while that same nutrient load constitutes a small percentage of the nutrient budget in Massachusetts Bay (HydroQual 2000). Irrespective of the specific value of the outfall relocation, the present study validates public investment, proximately in removal of toxic contaminants from the marine environment, and ultimately in improved environmental health. Flounder tumors were a major part of the public concern that lead to the court-mandated restoration of Boston Harbor. Therefore, the public health benefit of minimizing toxic chemical exposure to coastal waters, and thereby to commercially and recreationally harvested seafood, would seem to strongly validate this major public works effort. This project has therefore met the expectations in 1983 of Norfolk County (Massachusetts, USA) Superior Court Judge Paul G. Garrity, the so-called 'sludge judge' who oversaw the court-mandated effort and wrote: 'The current and potential impact of pollution upon the health, welfare and safety of persons who live and work nearby Boston Harbor and who use it for commercial, recreational, and other purposes is staggering. The damage to that environment and to the creatures who live in it may very well become irreversible unless measures are taken to control, and at some point preclude, the pollution and consequent destruction of that very valuable resource' (Lehman 1983).

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enquad/trlist.html. They are also databased at Environmental Quality, Water and Wastewater Dept., Massachusetts Water Resources Authority, 100 First Avenue, Charlestown Navy Yard, Boston, MA 02129, USA. Flounder were collected and sampled with approval from the Institutional Animal Care and Use Committee of the Woods Hole Oceanographic Institution, and collected under Scientific Permit #057225 from the Massachusetts Division of Marine Fisheries. This article represents the opinions and conclusions of the authors and not necessarily those of the Massachusetts Water Resources Authority.

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