



Spatial patterns in markers of contaminant exposure, glucose and glycogen metabolism, and immunological response in juvenile winter flounder (*Pseudopleuronectes americanus*)

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ABSTRACT

Inshore winter flounder (*Pseudopleuronectes americanus*) populations in NY, USA have reached record low numbers in recent years, and recruitment into the fishery appears to be limited by survival of post-settlement juvenile fish. In order to identify cellular pathways associated with site-specific variation in condition and mortality, we examined differential mRNA expression in juvenile winter flounder collected from six different bays across a gradient in human population density and sewage inputs. Illumina sequencing of pooled samples of flounder from contrasting degraded sites and less impacted sites was used to guide our choice of targets for qPCR analysis. 253 transcripts of >100 bp were differentially expressed, with 60% showing strong homology to mostly teleost sequences within the NCBI database. Based on these data, transcripts representing nine genes of interest associated with contaminant exposure, immune response and glucose and glycogen metabolism were examined by qPCR in individual flounder from each site. Statistically significant site-specific differences were observed in expression of all but one gene, although patterns in expression were complex with only one (vitellogenin), demonstrating a west to east gradient consistent with known loadings of municipal sewage effluent. Principal components analysis (PCA) identified relationships among the genes evaluated. Our data indicate that juvenile winter flounder are responding to estrogenic chemicals in more urbanized coastal bays, and suggests potential mechanistic links between immune response, contaminant exposure and energy metabolism.

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1. Introduction

Winter flounder (*Pseudopleuronectes americanus*) once supported a dominant commercial and recreational fishery along the Atlantic coast of North America from the Gulf of St. Lawrence to Chesapeake Bay. Since peaking in the mid-1980s, catches have declined, particularly in NY waters (<http://www.st.nmfs.noaa.gov/web>; Sagarese et al., 2011), local stocks are at record lows in abundance (Socrates and Colvin, 2006; CTDEP, 2011; Nuttall et al., 2011), and recent data indicates an extremely small parental stock contributing to inshore populations (O'Leary et al., 2013). Likely driving these declines are decades of overfishing, environmental change, and declining habitat condition. Recruitment into the NY fishery appears to be limited by survival of post-settlement juveniles (Socrates and Colvin, 2006; Yencho et al., in review). Indeed estimates of young-of-the-year (YOY) mortality in two Long Island bays in 2007 and 2008 indicated higher levels than

observed in similar environments from adjacent states including NJ, CT, and RI (Yencho et al., in review). Earlier studies on winter flounder from urban bays of Long Island Sound have shown winter flounder to have reduced fitness in areas with high contaminant loadings (Nelson et al., 1991; Perry et al., 1991; Black et al., 1988), and more recent work has indicated YOY winter flounder and other resident species from western Long Island bays show evidence of feminization (Mena et al., 2006; McElroy et al., 2006; Duffy et al., 2009).

Winter flounder make a good sentinel species for examining the impact of environmental change as they tend to have a limited range, with inshore populations of adults undergoing a seasonal on/off shore migration to avoid excessively hot summer temperatures, although some bays may also support resident fish with even more limited ranges (Poole, 1966; Sagarese et al., 2011). Spawning of inshore stocks primarily occurs in winter and early spring with most migrating fish returning to their natal estuaries to spawn demersal eggs. Juvenile fish are thought to remain in shallow waters for their first couple of years of life (Klein-MacPhee, 2002). Because of their life history, winter flounder embryos and young fish are constantly exposed to sediment associated contaminants, as well as being subject to the highly variable environmental conditions of the near shore benthos where dissolved oxygen, pH, salinity, and temperature vary on daily to decadal time scales.

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In this study we used qPCR to evaluate relative gene expression in YOY winter flounder collected from bays representing an urban gradient along Long Island, NY. We hypothesized that environmental factors that might be evident along this gradient (such as contaminant exposure, temperature, salinity and dissolved oxygen), would cause site-specific shifts in metabolic and immunological status of winter flounder. This study was part of a larger investigation evaluating a suite of biological, environmental and anthropogenic factors associated with recruitment success in YOY winter flounder populations in south shore bays of Long Island (Frisk et al., 2013). Here we report results of Illumina sequencing on pooled liver samples from more and less degraded sites and on the site-specific expression of nine genes related to contaminant exposure, energy metabolism and immune response in the livers from individual flounder.

2. Materials and methods

2.1. Site selection

Juvenile winter flounder were collected from six bays along the south shore of Long Island, NY, USA from May through October during 2010 and 2011 (Fig. 1). These study locations represent a west to east gradient in urbanization and sewage inputs within a very short geographical range of only 200 km. Based on locally reported census data, population density differs by a factor of 100 from west to east over the breadth of the study sites, ranging from over 2000/km² in the west to 20/km² in the east (www.Census.com). An even larger gradient exists in sewage inputs. Jamaica Bay receives about 8×10^5 L of treated effluent per day, while Hempstead Bay receives only 2×10^2 L of effluent per day (IEC, 2009). Moriches Bay receives no municipally treated wastewater, although its primary tributary, the Forge River, is known to be significantly impacted by seepage leaching in from failing septic systems, and this area was historically impacted by excess nutrient loading from duck farms (Swanson et al., 2010). The three more eastern sites in this study, Shinnecock Bay, Cold Spring Pond, and Napeague Harbor have both low population densities and no reported evidence of impacts from sewage or seepage inputs. Very little monitoring data exist in most of these areas

on levels of common chemical contaminants in sediments. The U.S. Environmental Protection Agency's National Coastal Assessment (<http://www.epa.gov/emap/nca/html/data/index.html>) provides the most comprehensive dataset available for common organic and inorganic non-nutrient contaminants. A summary of these data evaluating contaminant levels in fine (>2% total organic carbon and >10,000 µg/g Fe) sediments from the study sites or nearby areas measured between 2000 and 2005 (the most recent data available) verifies the general west to east gradient in contaminant loadings (Supplementary File 1). These data not only identify Jamaica Bay as being the most contaminated, but also indicate that fish from all sites are likely to experience some level of chemical contaminant exposure. It is important to point out that by only looking at fine sediments, these data represent the high end of general chemical contamination at these sites. Levels of contaminants in sandy sediments would be much lower, and sandy sediments are common, particularly in the more eastern sites. Even considering the worst case scenario of exposure to fine sediments, average contaminant levels generally only exceed Effects Range Low (ERL) values designated by the National Oceanic and Atmospheric Administration (NOAA) at some sites, and the Effects Range Median (ERM) levels are not exceeded for any contaminants (Long et al., 1995), indicating that most of these sites are representative of the more wide-spread contamination found in all but remote coastal areas both in the U.S. and world-wide.

2.2. Fish collection and processing

Flounder ranging in size from 0.2 to 36 g and 24 to 135 mm total length were collected using either a 1 m beam trawl or with 3–60 m beach seines from May through October in 2010 and 2011. Attempts were made to collect fish every other week from each site during the sampling season. All sampling was done during daylight, generally between 9 am and 2 pm. We attempted to collect 10 fish per location per sampling day, but low flounder abundance at individual sites often prevented this. Flounder were collected at three of the sites (Jamaica, Moriches, and Shinnecock Bays) in both 2010 and 2011, while Cold Spring Pond and Napeague Harbor were only sampled in 2010, and Hempstead Bay was only sampled in 2011. A total of 221 flounder were used in these analyses with at least 19 individuals from each location evaluated. Detailed information on the dates of collection and number of fish analyzed from each location is shown in Supplementary File 2. Due to low abundance and high mortality at many of the sites, very few flounder could be caught after mid-August, making analysis of expression patterns as a function of fish age or size problematic. Analysis of year-to-year differences in mRNA expression at the three sites where data were available for both years (Jamaica, Moriches and Shinnecock Bays) indicated no consistent statistical differences between years nor between early and late season fish (data not shown), so all data from both years were pooled for site-specific analysis at these sites. All winter flounder collected were flash frozen immediately upon retrieval from the water between two blocks of dry ice, and maintained in a –80 °C freezer until processed. Temperature, salinity, and dissolved oxygen were recorded at the time of collection using a YSI Model 85 probe (Yellow Springs, AK). During the 2011 sampling season, continuously recording Hach Hydromet data sondes were moored 0.5 m off the bottom during June, July and August, providing a continuous record of dissolved oxygen, temperature and salinity in Jamaica, Moriches and Shinnecock Bays. Fish collection and processing was conducted in accordance with permits issued to M. Frisk by the New York State Department of Environmental Conservation (#1030 and 1644), and by Stony Brook University's Institutional Animal Care and Use Committee to A. McElroy (IRBNet #260837).

2.3. RNA isolation, RNA-sequencing and differential analysis

Livers were removed from fish while still frozen and RNA extracted from <130 mg of tissue after homogenization in TRIzol® reagent

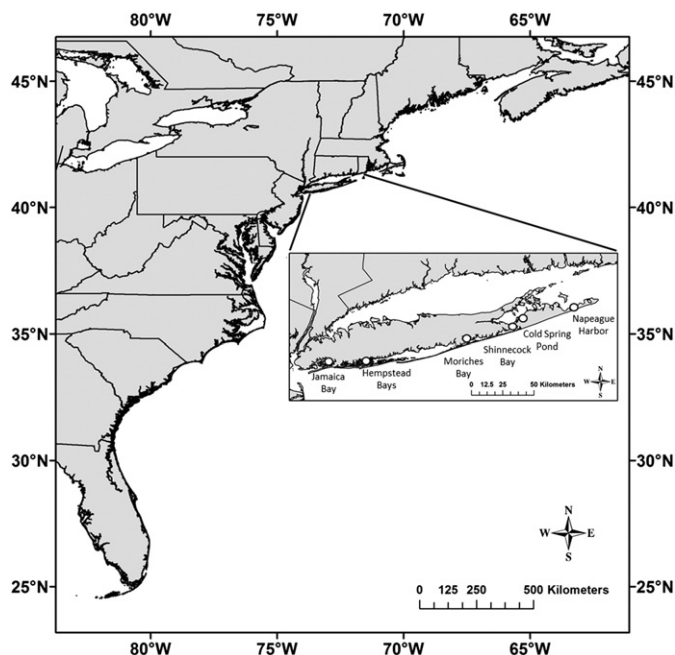


Fig. 1. Map of northeast Atlantic coast of North America showing study sites on Long Island, NY.

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