Flounder Monitoring Report: 2014 Results

Massachusetts Water Resources Authority

Environmental Quality Department
Report 2014-16

Citation:

Moore MJ, Nestler EC, Pembroke, AE. 2014. Flounder Monitoring Report: 2014 Results. Boston: Massachusetts Water Resources Authority. Report 2014-16. 18 pp.

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Submitted to

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October 2014
Report 2014-16

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1. INTRODUCTION

The Massachusetts Water Resources Authority (MWRA) has implemented a long-term Harbor and Outfall Monitoring (HOM) Program for Massachusetts and Cape Cod Bays. The objectives of the HOM Program are to test whether the environmental impacts of the MWRA discharge are consistent with projections made in EPA's Supplemental Environmental Impact Statement approving the outfall siting and do not exceed Contingency Plan thresholds (MWRA 2001). A detailed description of the monitoring and its rationale is provided in the Effluent Outfall Monitoring Plan developed for the baseline period and the post-discharge monitoring plan (MWRA 1997, 2004, 2010).

The detection of high prevalence of contaminant-associated liver disease (a condition known as centrotubular hydropic vacuolation) in winter flounder from Boston Harbor in the late 1980s was one of the findings that contributed to the concern about the ecological health of the Harbor. For example, in 1988, over 75% of flounder collected in Boston Harbor showed evidence of disease in liver tissue associated with contaminant exposure, and 12% of the fish contained liver tumors, also associated with exposure to contaminants (Moore et al. 1996).

Following the design of the MWRA Deer Island Treatment Plant and the siting of the Massachusetts Bay outfall, concerns were raised that flounder in Massachusetts Bay exposed to the relocated effluent discharge might over time show substantially increased prevalence of these contaminant-associated lesions. Therefore, a long-term monitoring program for winter flounder (MWRA 1991) was established. The goals of this program are to provide data that can be used to assess potential impacts to winter flounder in the vicinity of the outfall and to track the expected long-term improvements in flounder in Boston Harbor. Resident flounder are collected from near the outfall and from sites in Boston Harbor, Massachusetts Bay, and Cape Cod Bay (hereafter: Boston Harbor and the Bays). Measured parameters for flounder include length, weight, age, biological condition, gender, the presence of external or internal disease, and concentrations of inorganic and organic contaminants in body tissues. Data have been collected since 1992. The full program was conducted annually until 2003. Since then tissue contaminant monitoring is now done every third year (most recently in 2012). Flounder morphology and histopathology remain on an annual schedule.

This report presents morphology and histopathology results for the 2014 flounder survey. The scope of the report is focused on assessing changes to flounder condition that may have resulted from the relocation of the outfall discharge. The 2014 data represent the fourteenth consecutive year of flounder monitoring since the start-up of the Massachusetts Bay outfall in September 2000. A summary of the survey and laboratory methods used for flounder monitoring is provided in Section 2. The results of monitoring data from the survey conducted during 2014, along with comparisons to historical flounder data, are presented in Section 3. Finally, conclusions drawn from the 2014 results and historical trends are summarized in Section 4. By comparing values with established thresholds and evaluating trends over time, these flounder data are used to ensure that discharge of effluent into the Bay does not result in measured adverse impacts to fish.

2. METHODS

Winter flounder (*Pseudopleuronectes americanus*) were collected from four locations in Boston Harbor and the Bays (Figure 2-1) to obtain specimens for age, weight, and length determination, gross examination of health, and histology of livers. The methods and protocols used during the 2014 flounder survey were similar to and consistent with previously used methods. Detailed descriptions of the methods are contained in the Quality Assurance Project Plan (QAPP) for Fish and Shellfish Monitoring 2014–2016 (Geoghegan et al. 2014).

2.1 Stations and Sampling

The 2014 flounder survey was conducted between April 21 and May 12, 2014. Four sites were sampled to collect winter flounder for histological analyses:

- Deer Island Flats (DIF) – former discharge site.
- Off Nantasket Beach (NB) – reference site.
- Outfall Site (OS) – new discharge site.
- East Cape Cod Bay (ECCB) – reference site.

Figure 2-1 shows the monitoring locations. Table 2-1 provides the planned and actual sampling sites and locations for the 2014 flounder sampling.

Otter-trawl tows were conducted from the F/V *Harvest Moon* operated by Captain Mark Carroll. The scientific crew consisted of principal investigator Dr. Michael Moore from WHOI, and biologist Eric Rydbeck from Normandeau.

Mobilization for the survey was conducted on April 21st, when 12 fish were collected from Deer Island and 50 from each of Nantasket Beach and the Outfall Site. On April 22nd 50 fish were collected from Eastern Cape Cod Bay. On May 12th, an additional 38 fish were collected from Deer Island Flats to complete the targeted 50 fish. Fish were weighed and measured individually in the field. Scales and otoliths were removed from each fish for aging and livers were removed, sliced, examined and three slices fixed.

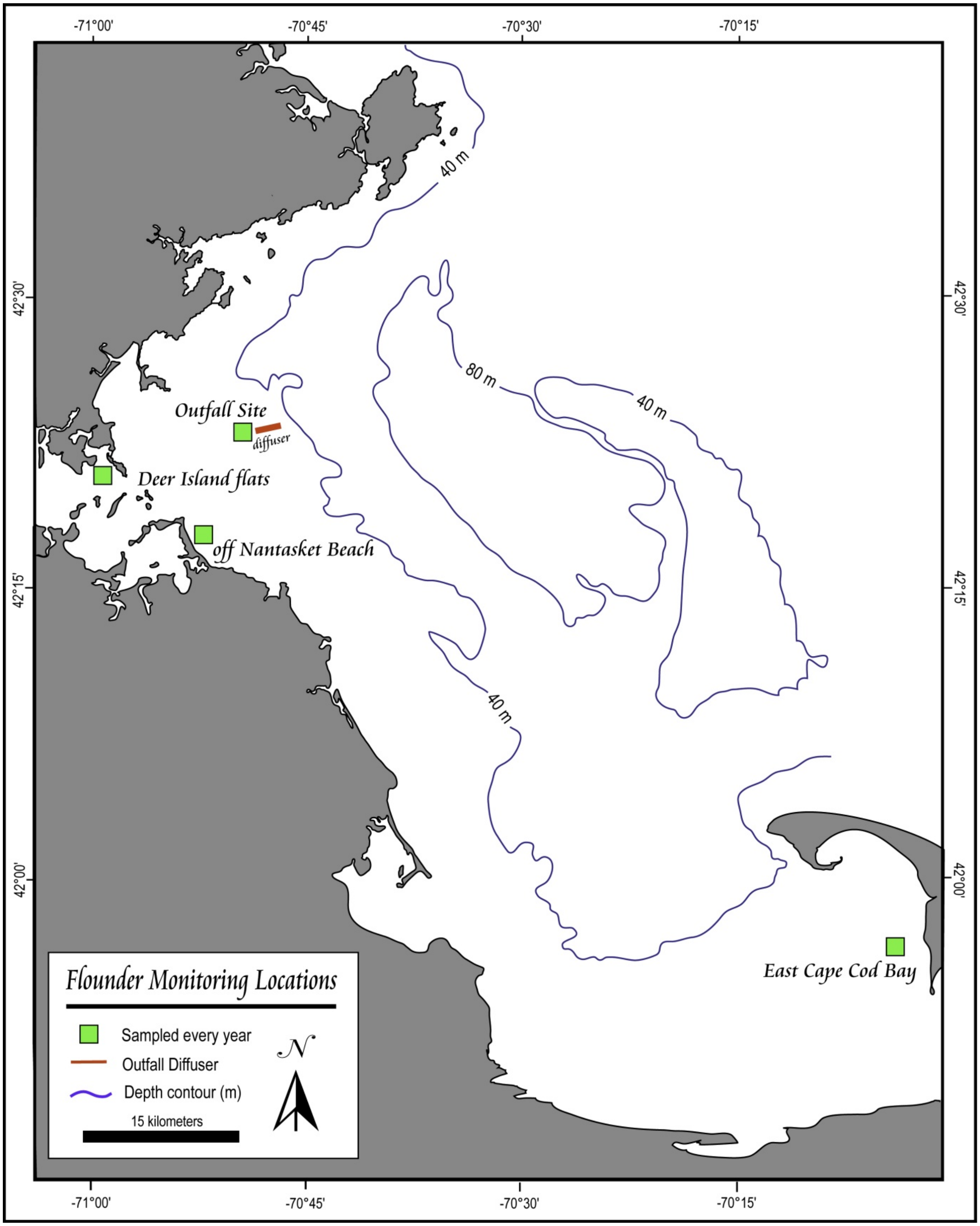

Figure 2-1. Flounder monitoring locations for 2014.

Table 2-1. Flounder Sampling Locations in 2014.

Site (Station ID)/Date/Time			Actual Location		Planned Location	
			Latitude	Longitude	Latitude	Longitude
Deer Island Flats (DIF)	21-Apr-14	7:02	42.3467	-70.9650	42.3400	-70.9733
		8:05	42.3483	-70.9650	42.3400	-70.9733
		8:58	42.3517	-70.9717	42.3400	-70.9733
		10:00	42.3483	-70.9667	42.3400	-70.9733
	12-May-14	8:34	42.3483	-70.9633	42.3400	-70.9733
East Cape Cod Bay (ECCB)	22-Apr-14	9:03	41.9483	-70.1283	41.9367	-70.1100
Off Nantasket Beach (NB)	21-Apr-14	11:49	42.2900	-70.8667	42.2933	-70.8700
Outfall Site (OS)	21-Apr-14	14:25	42.3817	-70.8250	42.3850	-70.8217

2.2 Histological Analysis

Livers of 50 flounder from each site were prepared for histological analysis by Experimental Pathology Laboratories in Herndon, VA. Transverse sections of flounder livers fixed as part of tissue sample processing were removed from the buffered formalin after at least 24 hours, rinsed in running tap water, dehydrated through a series of ethanols, cleared in xylene, and embedded in paraffin. Paraffin-embedded material was sectioned on a rotary microtome at a thickness of 5 µm. Each block contained three liver slices, resulting in one slide with three slices per slide per fish, for a total of 200 slides (50 fish x 4 sites). The sections were stained in hematoxylin and eosin. Each slide was examined by Dr. Moore under bright-field illumination at 25 x, 100 x, and 200 x magnification to quantify the presence and extent of

- Three types of vacuolation (centrotubular, tubular, and focal)
- Macrophage aggregation
- Biliary duct proliferation and trematode parasitism
- Neoplasia

The severity of each lesion was rated on a scale of 0 to 4, where: 0 = absent, 1 = minor, 2 = moderate, 3 = severe, and 4 = extreme.

2.3 Data Reduction and General Data Treatment

All fish data (1991 to 2014) were extracted directly from the HOM database and imported into SAS (version 9.2), where data reduction, graphical presentations and statistical analyses were performed. Data reduction was conducted as described in the Quality Assurance Project Plan (QAPP) for Fish and Shellfish Monitoring 2014–2016 (Geoghegan et al. 2014). For each liver lesion and each fish, a histopathological index was calculated as a mean of scores from three slices on one slide.

Histopathological indices and prevalence of lesions were compared among groups of flounder by differences in station and age. Flounder monitoring parameters were presented graphically and compared among stations and over time.

2.4 Deviations from the QAPP

All protocols outlined in the QAPP were followed. Flounder 141-1-28 had unreadable otolith and scale age. It was thus not given an age value.

3. RESULTS AND DISCUSSION

3.1 Fish Collected

Winter flounder, each a minimum 30 cm in length, were collected between April 21 and May 12, 2014, at four stations in the study area (Figure 2-1). The catch per unit effort (CPUE), defined as the number of fish at least 30 cm long obtained per minute of bottom trawling time, is reported per station in Figure 3-1. Effort was constant up to and including 2007 with the FV Odessa (70' sweep rope). For 2008, the FV Harvest Moon (74' sweep rope) was used for DIF, NB, and OS, with a net that was 1.04 x wider and for ECCB the FV Explorer 2 (84' sweep rope) was used with a net that was 1.2 x wider. Since 2009, the FV Harvest Moon has been used for all stations. Thus, data presented in Figure 3-1 have been normalized to the Odessa sweep length by using the ratio of sweep lengths as a multiplier (i.e., CPUE's for the FV Explorer net were multiplied by 70/84, and CPUE's for the Harvest Moon net by 70/74, to get CPUE units in Odessa equivalents). CPUE in 2014 was lower compared to 2013 at all stations except for NB (Figure 3-1). CPUE was highest at ECCB, moderate at OS and NB, and lowest at DIF.

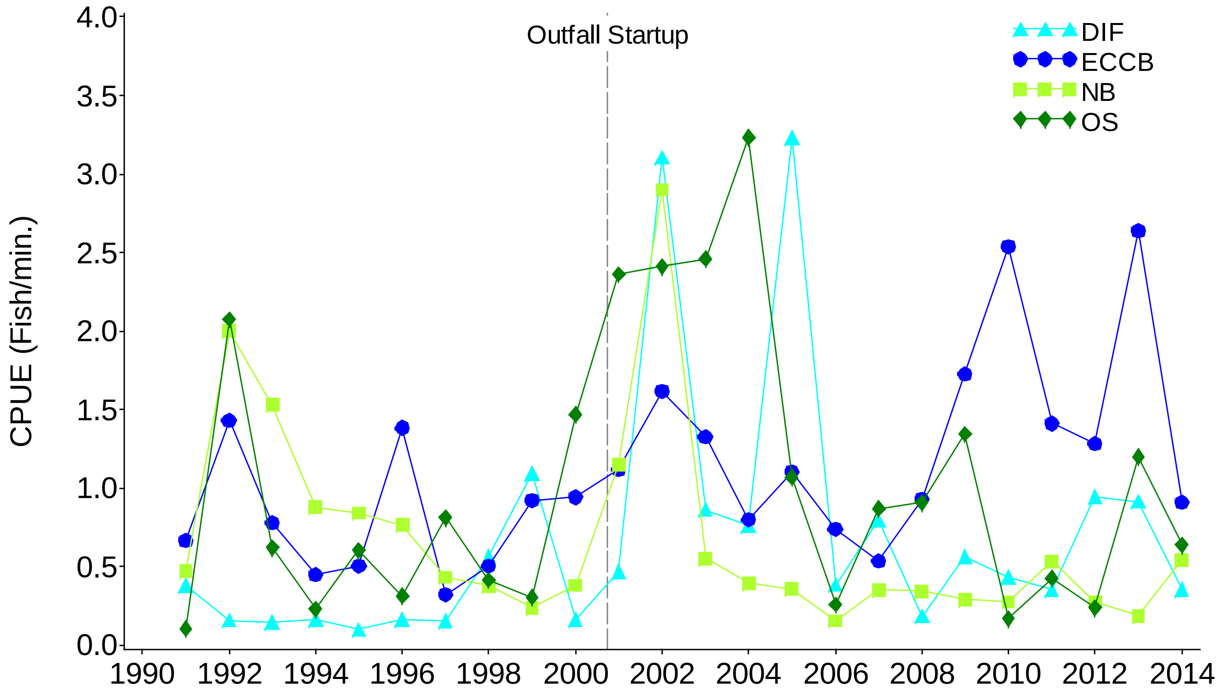

Figure 3-1. Catch Per Unit Effort (CPUE) for winter flounder trawled 1991–2014. Data for 2008 to 2014 have been normalized (see Section 3.1).

3.2 Physical Characteristics

Mean values for physical characteristics of the winter flounder collected in 2014 are reported in Table 3-1. These values reflect the project requirement to collect sexually mature specimens (>30cm total length). Mean age ranged from 5.1 to 5.5 years across the stations. Mean standard length ranged from 276 to 296 mm and mean total length from 337 to 362 mm; weight ranged from a mean of 415 to 584 g.

Mean age in 2014 compared to 2013 (Figure 3-2) was comparable for OS and ECCB, and higher for DIF and NB. 2014 was the first year when all age data were determined from otoliths, which is considered a more robust method for age determination than the previously used method of scale analysis. Comparisons between the two methods indicate that for older fish the otolith method may provide an older age than the scale method. Compared to 2013, standard length (Figure 3-3) in 2014 showed a slight increase for DIF and a slight decrease for NB, OS and ECCB. Weights (Figure 3-4) decreased at OS, ECCB and NB and increased at DIF. Percent females (Figure 3-5) increased at DIF and NB, and fell at ECCB and OS.

Table 3-1. Summary of Physical Characteristics of Winter Flounder Collected in 2014.

	DIF			ECCB			NB			OS		
Parameter	**Mean**	**STDDEV**	**N**	**Mean**	**STDDEV**	**N**	**Mean**	**STDDEV**	**N**	**Mean**	**STDDEV**	**N**
Age (years)	5.2	1.6	49	5.5	1.5	50	5.1	1.5	50	5.4	1.5	50
Standard Length (mm)	296	22.7	50	290	25.1	50	278	25.1	50	276	24.6	50
Total Length (mm)	362	28.1	50	354	30.2	50	340	28.5	50	337	29.5	50
Weight (g)	584	126.3	50	498	150.1	50	424	106.5	50	415	124.8	50

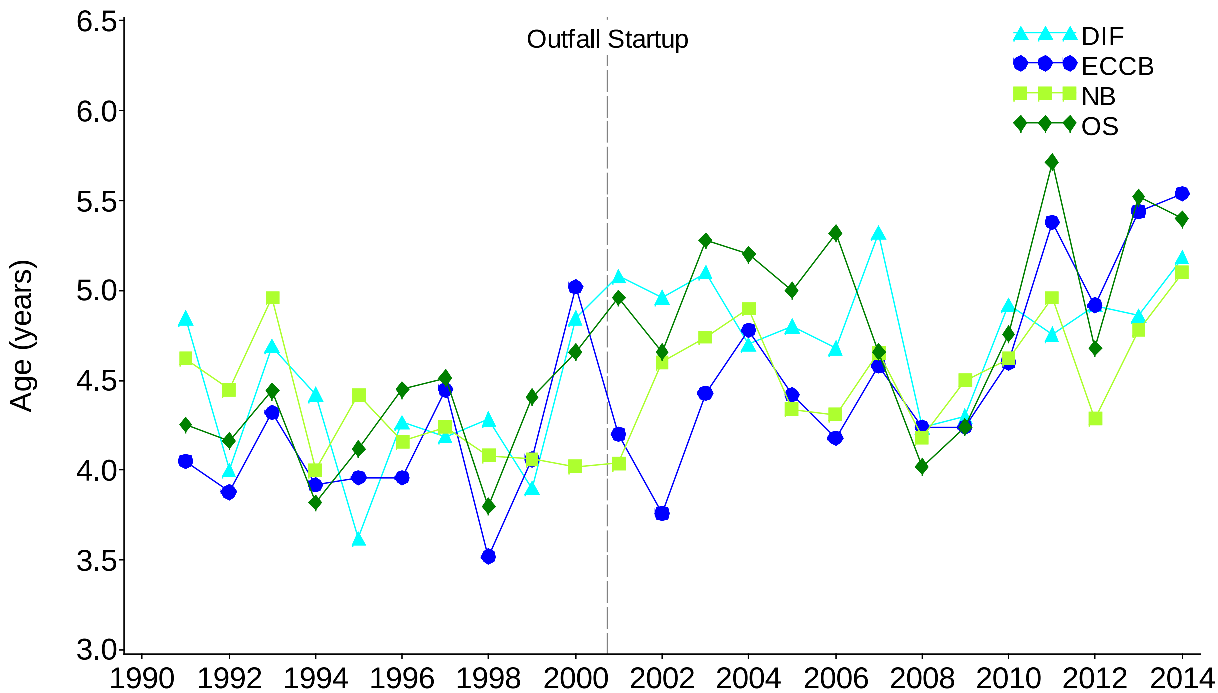

Figure 3-2. Average flounder age (years) compared by station and year.

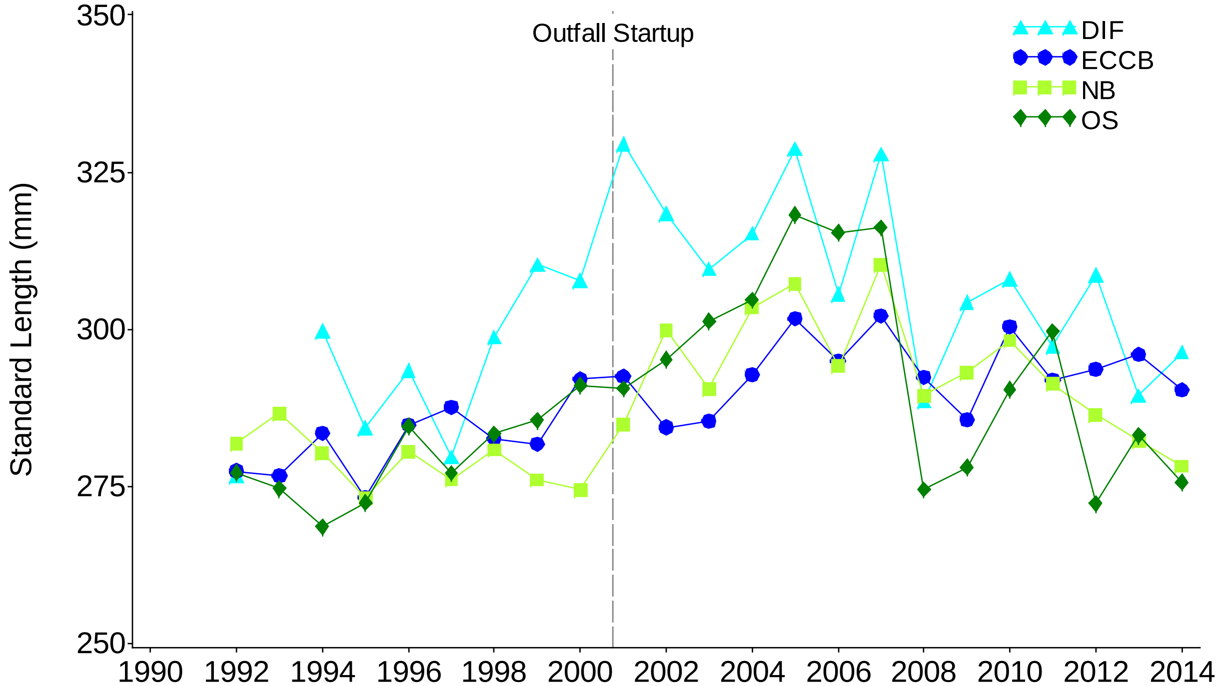

Figure 3-3. Average flounder standard length (mm) compared by station and year.

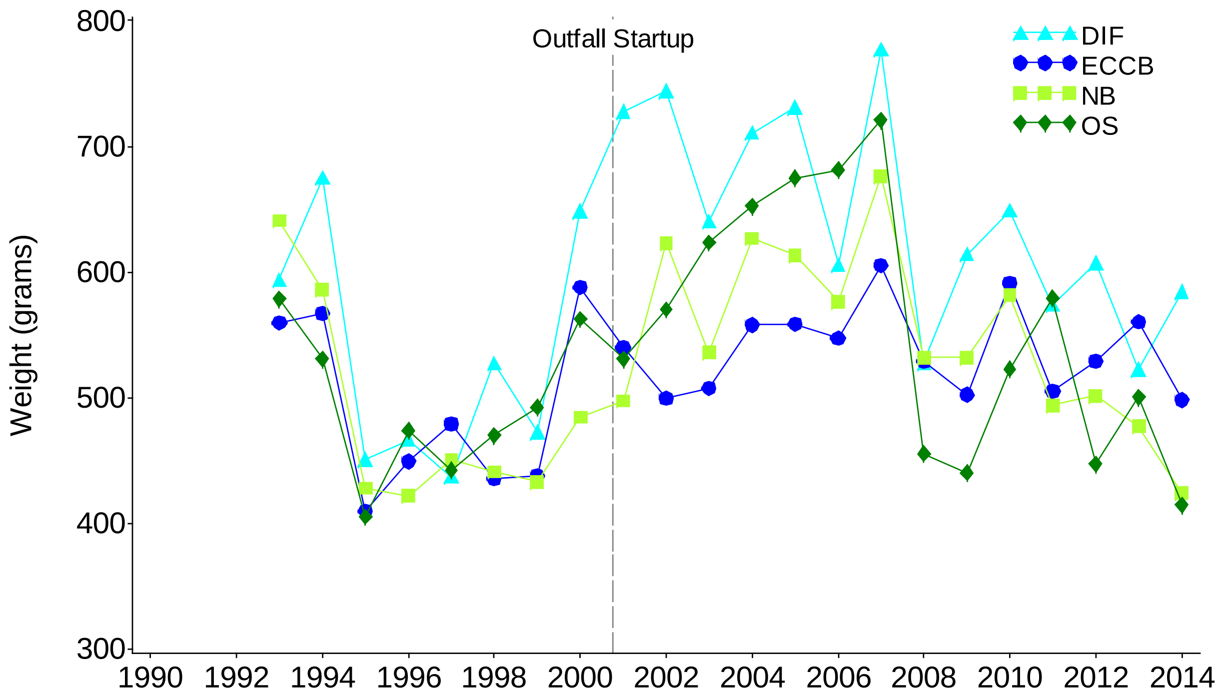

Figure 3-4. Average flounder weight (grams) compared by station and year.

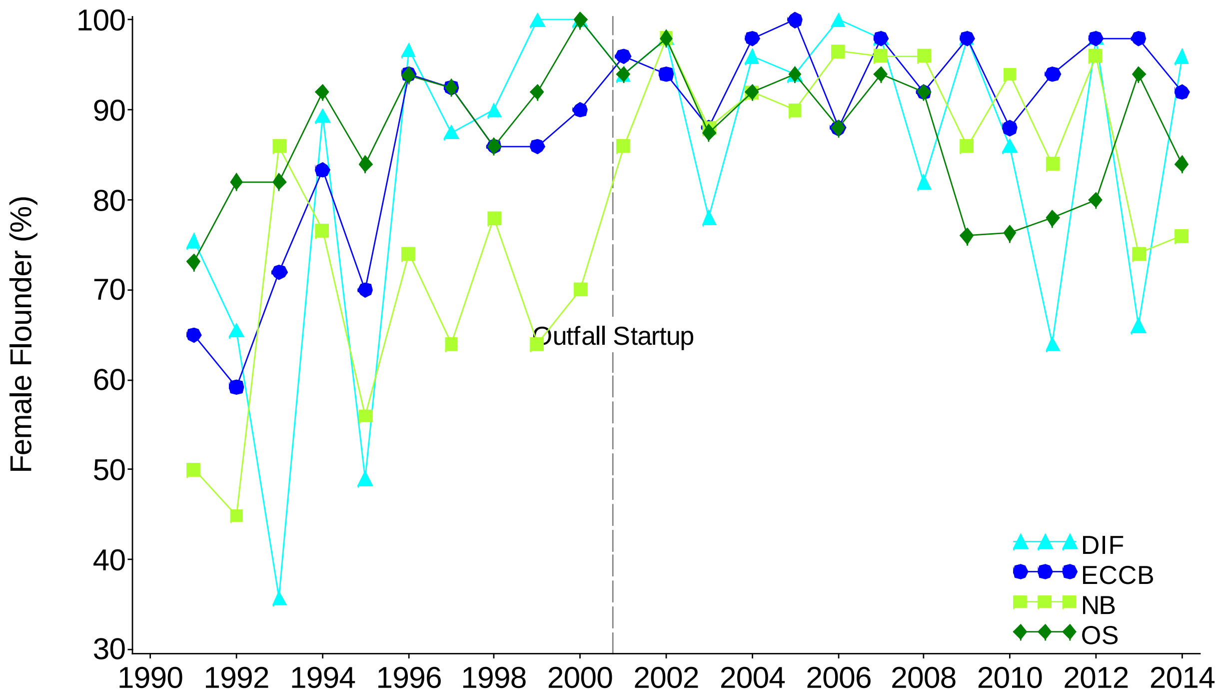

Figure 3-5. Proportion of female flounder compared by station and year.

3.3 External Condition

The external conditions of winter flounder collected in 2014 are presented as prevalence (% of individuals) per station in Table 3-2. Bent fin ray ranged from 0 to 20%, being highest at DIF. Blind side ulcers were absent on all fish at ECCB and NB in 2014, and found on one fish each at DIF and OS. Fin erosion ranged from 8 to 26%, being highest at DIF. Lymphocystis ranged from 14% at ECCB to 46% at OS.

Table 3-2. Prevalence (%) of External Conditions Assessed for Winter Flounder Collected in 2014.

External Conditions	Station (Sample size)			
	DIF (50)	ECCB (50)	NB (50)	OS (50)
Bent Fin Ray	20	6	14	0
Blind Side Ulcers	2	0	0	2
Fin Erosion (Fin Rot)	26	18	22	8
Lymphocystis	24	14	28	46

Ulcer prevalence has been recorded since 2003. It is unclear if ulcers were absent prior to 2003, given lack of a specific record, but if they were present, it was at a very low level. Elevated levels of ulcers were observed from 2003-06, then decreased from 2007-10, and were once again elevated in 2011 (Figure 3-6). In 2012 low levels of ulcers were present at OS and NB. Ulcers were absent from all stations in 2013. And in 2014, one ulcerated fish was found at DIF and another at OS.

Fin ray surface mucous and epithelia are impacted by increased levels of ammonia and other pollutants, making fin erosion a useful parameter for detecting deteriorating water quality conditions (Bosakowski and Wagner 1994). The prevalence of fin erosion for each year was calculated for each station and plotted in Figure 3-7. In 2014 fin erosion was comparable to that observed in 2013 at each station.

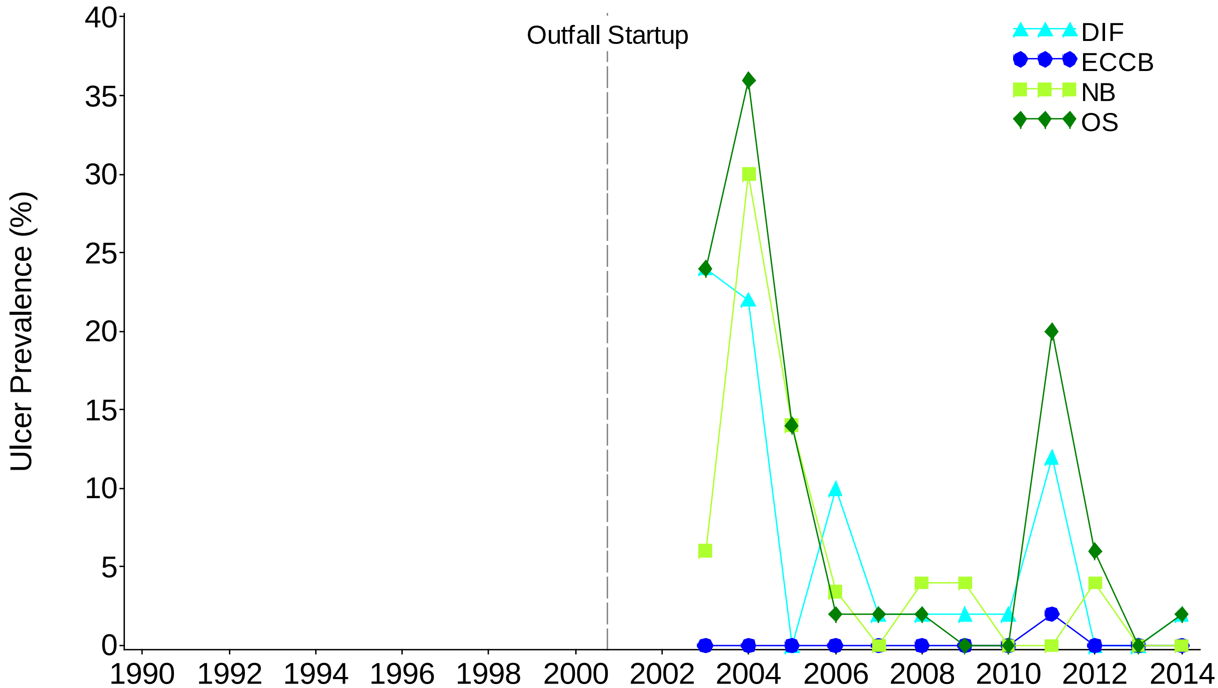

Figure 3-6. Temporal comparison of blind side ulcer prevalence (%) in winter flounder by station.

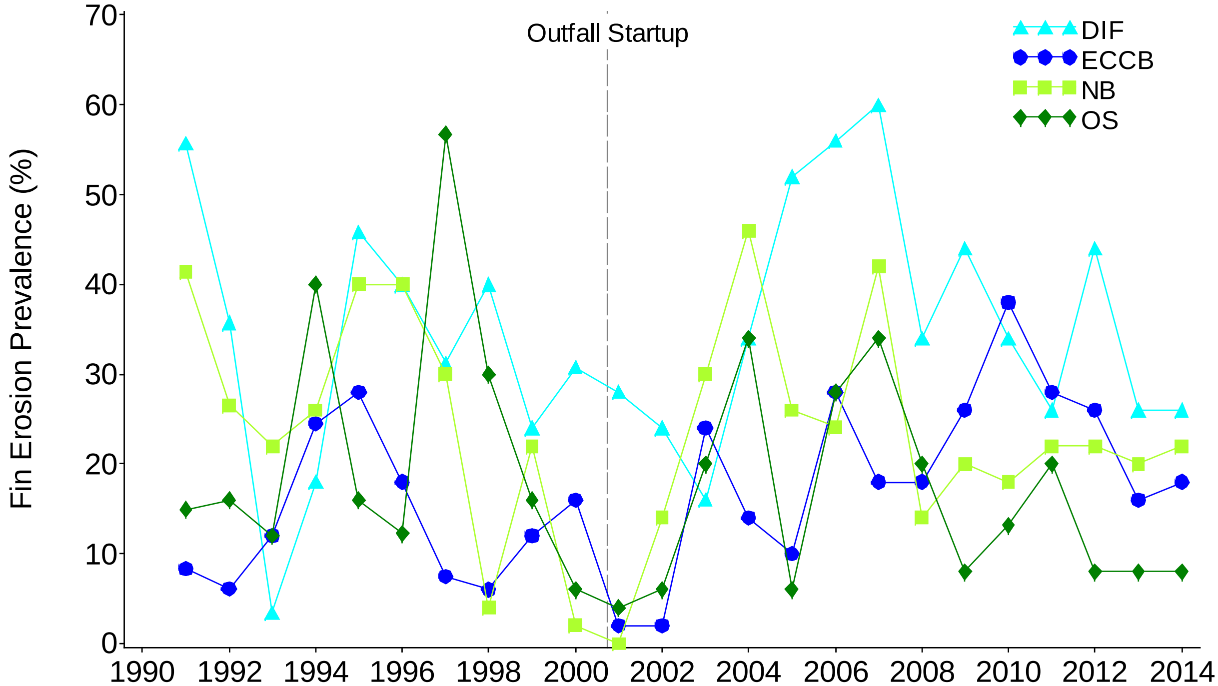

Figure 3-7. Temporal comparison of fin erosion prevalence (%) in winter flounder by station.

3.4 Liver Lesion Prevalence

The prevalence (% of individuals) of liver lesions in winter flounder from each of the four stations sampled in 2014 is presented in Table 3-3. Balloons ranged from 2 to 16%, bile duct protozoa were absent at all stations, biliary proliferation ranged from 6 to 12%, centrotubular vacuolation ranged from 0 to 14% being lowest at ECCB and highest at DIF and NB, focal hydropic vacuolation was absent at all sites, and

Table 3-3. Prevalence (%) of Liver Lesions in Winter Flounder Collected in 2014.

Liver Conditions	Station (Sample size)			
	DIF (50)	ECCB (50)	NB (50)	OS (50)
Balloons	2	16	12	12
Bile Duct Protozoan	0	0	0	0
Biliary Proliferation	12	10	6	12
Centrotubular Hydropic Vacuolation	14	0	14	10
Focal Hydropic Vacuolation	0	0	0	0
Liver Flukes	0	0	0	4
Macrophage Aggregation	46	62	46	68
Neoplasia	0	0	0	0
Tubular Hydropic Vacuolation	8	0	4	8

liver flukes ranged from 0 to 4%. Macrophage aggregation ranged from 46 to 68%, neoplasia was absent at all sites, and tubular hydropic vacuolation ranged from 0 to 8%.

Neoplasms (Figure 3-8) remained absent at all sites, a situation that has persisted since 2005. Thus it continues to be true that the most significant histopathology associated with Deer Island Flats before the MWRA project began remains totally absent.

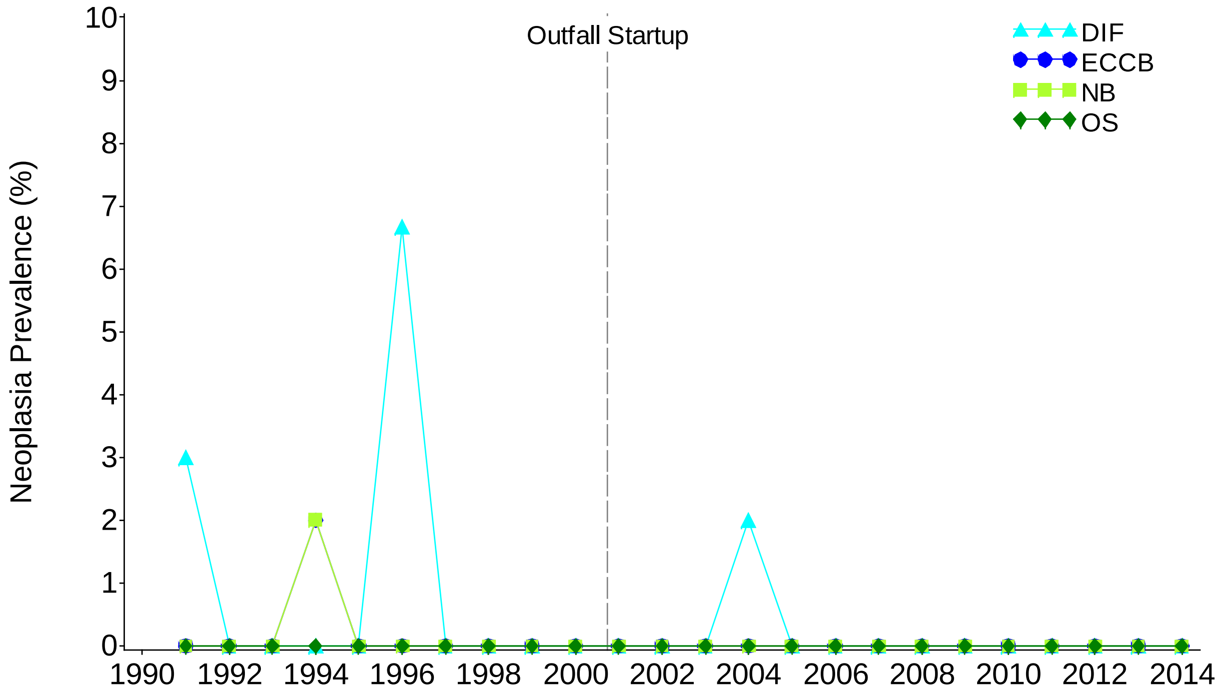

Figure 3-8. Temporal comparison of neoplasia prevalence (%) in winter flounder by station.

Along with neoplasms, hydropic vacuolation, because of its relationship to environmental contaminants, has been one of the principal lesions monitored in winter flounder throughout the program. Figure 3-9 shows a continuation in 2014 of the general decline in centrotubular hydropic vacuolation at DIF since 1991. An ongoing gentle increase at OS since 2005 reversed in 2012, increased again in 2013, but then decreased in 2014; 2014 levels at OS are now below all of the pre-discharge years. A continued gentle low-level increase at NB through 2012 reversed in 2013 and then increased again in 2014, and a background level continued at ECCB, although it crept up a bit in 2013, but it fell to zero again in 2014.

The severity of CHV (Figure 3-10) has also declined since 1991 at DIF. Severity at DIF has typically been higher than the intermediate levels found at NB and OS. Severity levels have remained relatively stable at all stations since 2005. During 2014, severity was comparable at DIF, NB, and OS, with continued background low levels at ECCB.

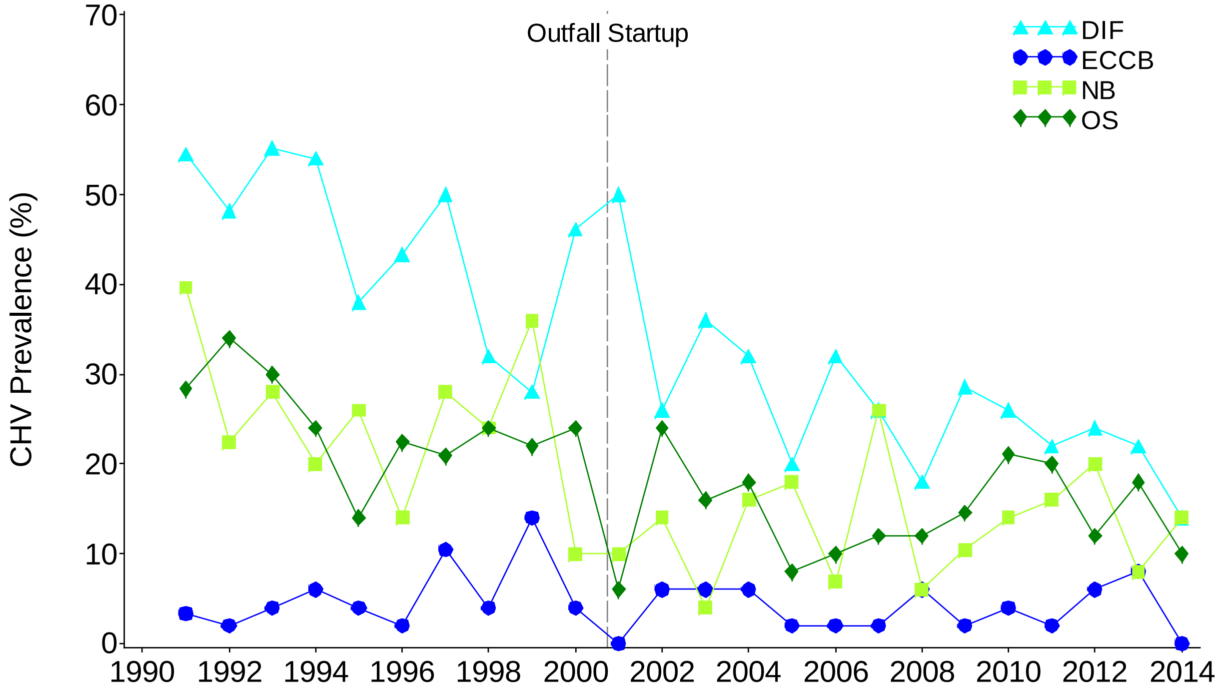

Figure 3-9. Temporal comparison of prevalence (%) of centrotubular hydropic vacuolation in winter flounder by station.

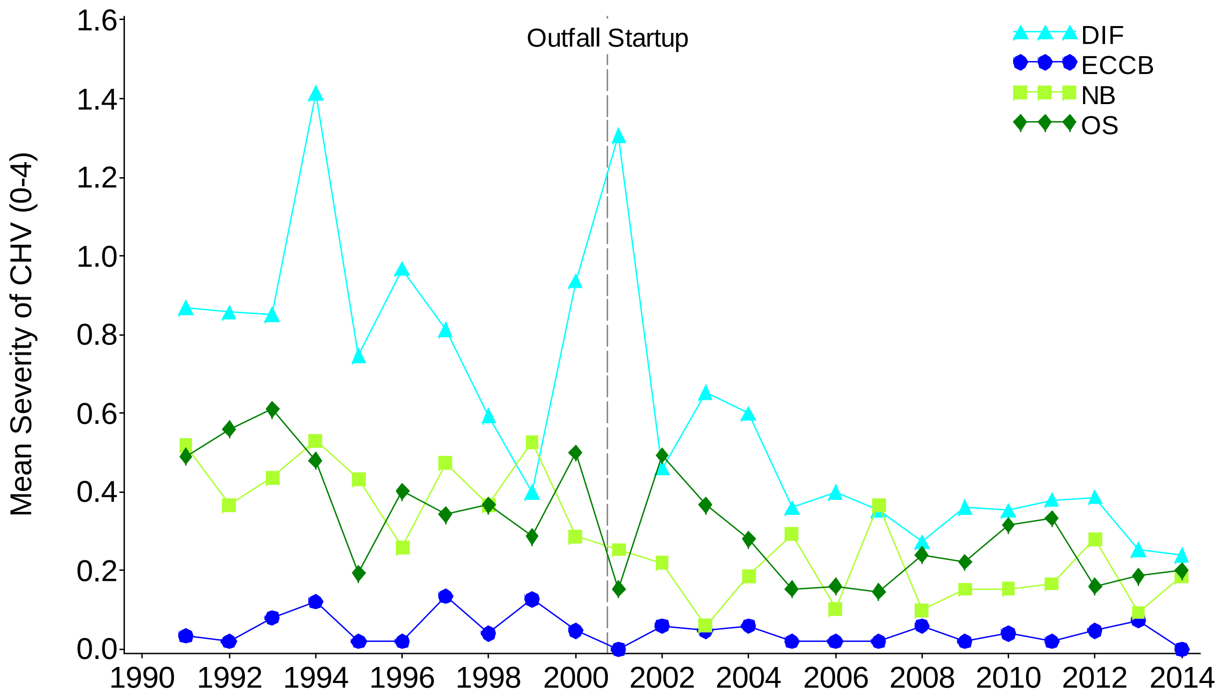

Figure 3-10.Centrotubular hydropic vacuolation severity (rank) in winter flounder compared between sites and years.

Relationships between age and lesion prevalence were also analyzed. The proportion of fish that had CHV (using data collected since 1997) was calculated for each age class at all stations (Figure 3-11). A modest increase in CHV, as might be expected with increased age, was found at ECCB. DIF shows a greater increase with age pre-discharge, compared to post-discharge, suggesting a reducing cumulative impact of remaining toxicants thought to induce this lesion. A slight increase with age is perhaps also seen at OS pre-discharge.

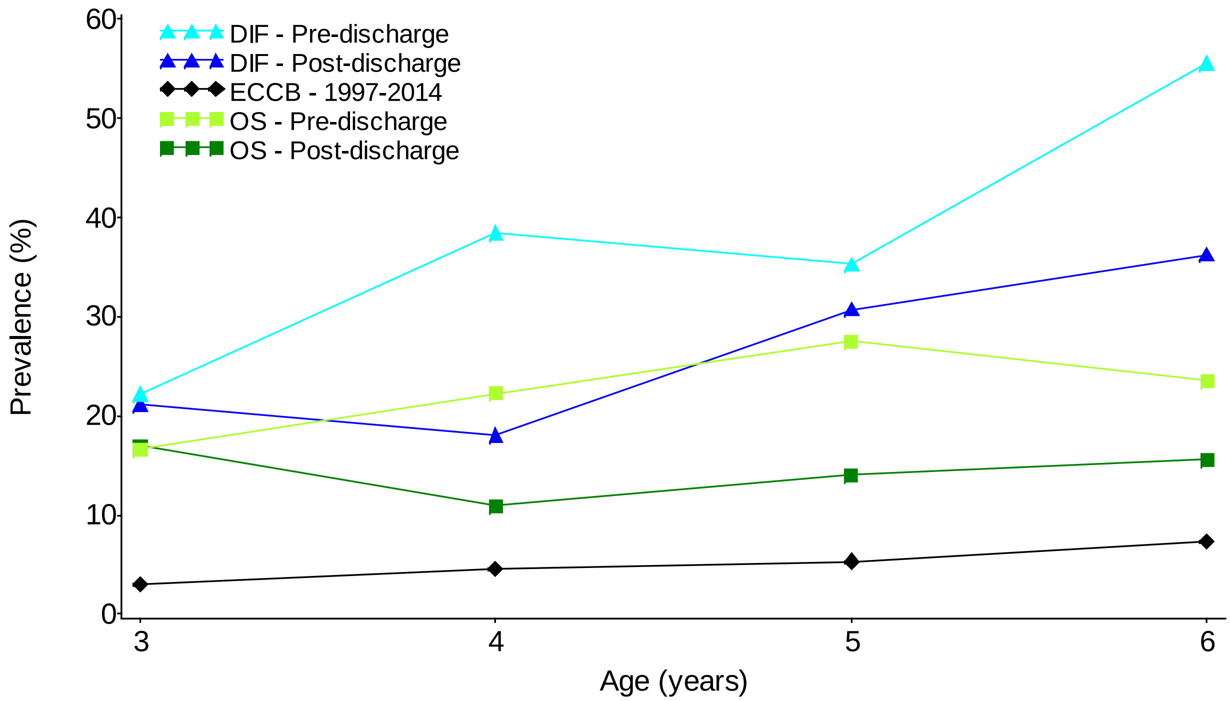

Figure 3-11. Proportion (%) of winter flounder showing hydropic vacuolation for each age.

To further assess the impact of changes in age on hydropic vacuolation prevalence, the percentage of fish at each station in each year that showed some degree of hydropic vacuolation was divided by the average age of fish for that year at that station. This generated an age-corrected index for the presence of hydropic vacuolation (Figure 3-12). The overall downward trend for DIF showed a mild reversal in 2012 that then reversed down again in 2013 and 2014. In 2013 OS reversed a 2011/2012 downward trend: this went down again in 2014. NB has shown recent increases from 2008-2012 that reversed in 2013 and then increased again in 2014, and ECCB remains at historic background levels.

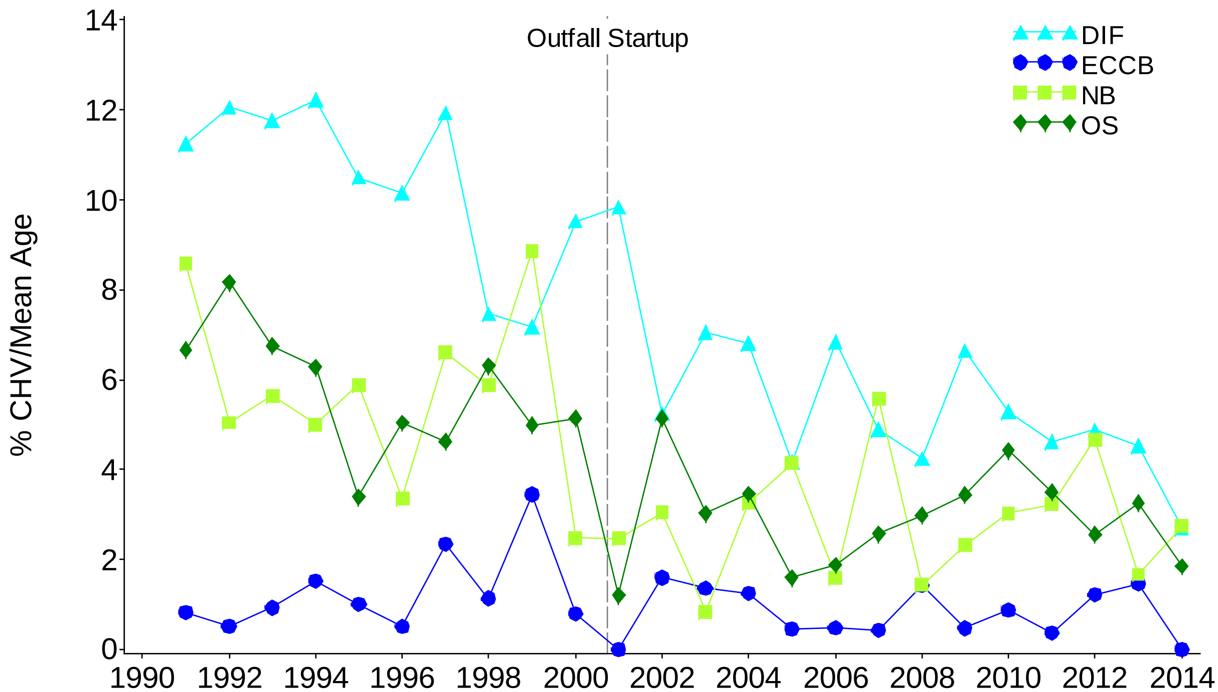

Figure 3-12. Hydropic vacuolation index (CHV%/age) for each station by year.

3.5 Threshold Comparison

The MWRA Monitoring Program has established threshold levels against which to measure key indicators of wastewater impacts (MWRA 2001). Because of the concerns that effluent discharge might increase the prevalence of lesions in Massachusetts Bay populations of winter flounder towards levels seen in Boston Harbor in the 1980s, liver disease prevalence was selected as a key indicator, with a Caution Level threshold set at 44.94% for the prevalence of centrotubular hydropic vacuolation (CHV) in winter flounder collected at the Outfall Site. CHV prevalence at the Outfall Site during 2014 was 10%, well below the threshold level.

4. CONCLUSIONS

The 2014 Flounder Survey provided samples from four locations (DIF, NB, OS, and ECCB) and was conducted in a manner consistent with previous surveys. Catch per unit effort at OS was close to median for that station. The overall size of the flounder collected increased during the past decade until 2008, when size returned to levels seen at the beginning of the study, a trend that continued through 2014. As has been the case throughout the duration of the monitoring program, the 2014 catches were dominated by females. Factors influencing sex ratios are complex and poorly understood. The already high proportion of females increased at all sites during the baseline period, and since the Outfall came on line the fish collected from eastern Cape Cod Bay, the site farthest from the Outfall, have had the highest proportion of female fish.

Following increased ulcer prevalence beginning in 2003, extensive pathology and microbiology studies were unable to determine a cause of the ulcers (Moore et al. 2004). Elevated levels of ulcers were observed from 2003 to 2006 at stations except for ECCB. Ulcer prevalence then decreased and remained low from 2007 to 2010, followed by an increase reported in 2011. In 2012 low levels of ulcers were present at OS and NB. No ulcers were observed in 2013, and two were observed in 2014.

Results of the histological analyses in 2014 support previous observations made from this long-term dataset.

- Age-corrected hydropic vacuolation prevalence data suggest that there has generally been a steady reduction in the contaminant-associated pathology in winter flounder collected at Deer Island Flats during the past two decades.

- The oldest Harbor data were not age-corrected. Uncorrected CHV prevalences in harbor flounder have decreased from over 75% in 1988 to approximately 20% or less in recent years. This is a remarkable change.

- Flounder collected off Nantasket Beach and in the vicinity of the outfall since discharge began in September 2000 also consistently show hydropic vacuolation prevalence at or lower than levels observed during baseline monitoring (1991-2000).

- The high neoplasm prevalence characteristic of fish from DIF in the mid- to late-1980s (Moore et al. 1996) has disappeared. Neoplasia has not been observed in a fish from Boston Harbor since 2004, and has never been observed in fish collected at the outfall site.

- Disease prevalence at the eastern Cape Cod Bay reference site has been relatively stable since monitoring began there in 1991 and is consistently the lowest of all areas sampled.

- One possible trend that will continue to be tracked is a slow rise in the prevalence of age-corrected CHV in flounder collected in the vicinity of the outfall that was observed between 2005 and 2010. CHV declined at the OS site in 2011 and 2012, rose somewhat in 2013, but then fell again in 2014 (Figure 3-12). These values are still below baseline levels, prior to outfall startup.

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