

# **Flounder Monitoring Report: 2013 Results**

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Massachusetts Water Resources Authority

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Report 2013-17



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**Submitted to**

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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 SEIS projections 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, 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 (for example, in 2012). Flounder morphology and histopathology remain on an annual schedule.

This report presents morphology and histopathology results for the 2013 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 2013 data represent the thirteenth 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 2013, along with comparisons to historical flounder data, are presented in Section 3. Finally, conclusions drawn from the 2013 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 2013 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 2011–2013, Revision 1 (Nestler et al. 2013).

### 2.1 Stations and Sampling

The 2013 flounder survey was conducted between April 26 and 29, 2013. Four sites were sampled to collect winter flounder for histological analyses:

- Deer Island Flats (DIF)
- Off Nantasket Beach (NB)
- Outfall Site (OS)
- East Cape Cod Bay (ECCB)

Figure 2-1 shows the monitoring locations. Table 2-1 provides the planned and actual sampling sites and locations for the 2013 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, WHOI, and biologists Eric Rydbeck and Joe Battaglia, both from Normandeau.

Mobilization for the survey was conducted on April 26<sup>th</sup>, when 50 fish were collected from each of Deer Island and Nantasket Beach. Fifty fish were collected from the Outfall on April 28<sup>th</sup>. On April 29<sup>th</sup>, 50 fish were collected from Eastern Cape Cod Bay. 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 fixed.

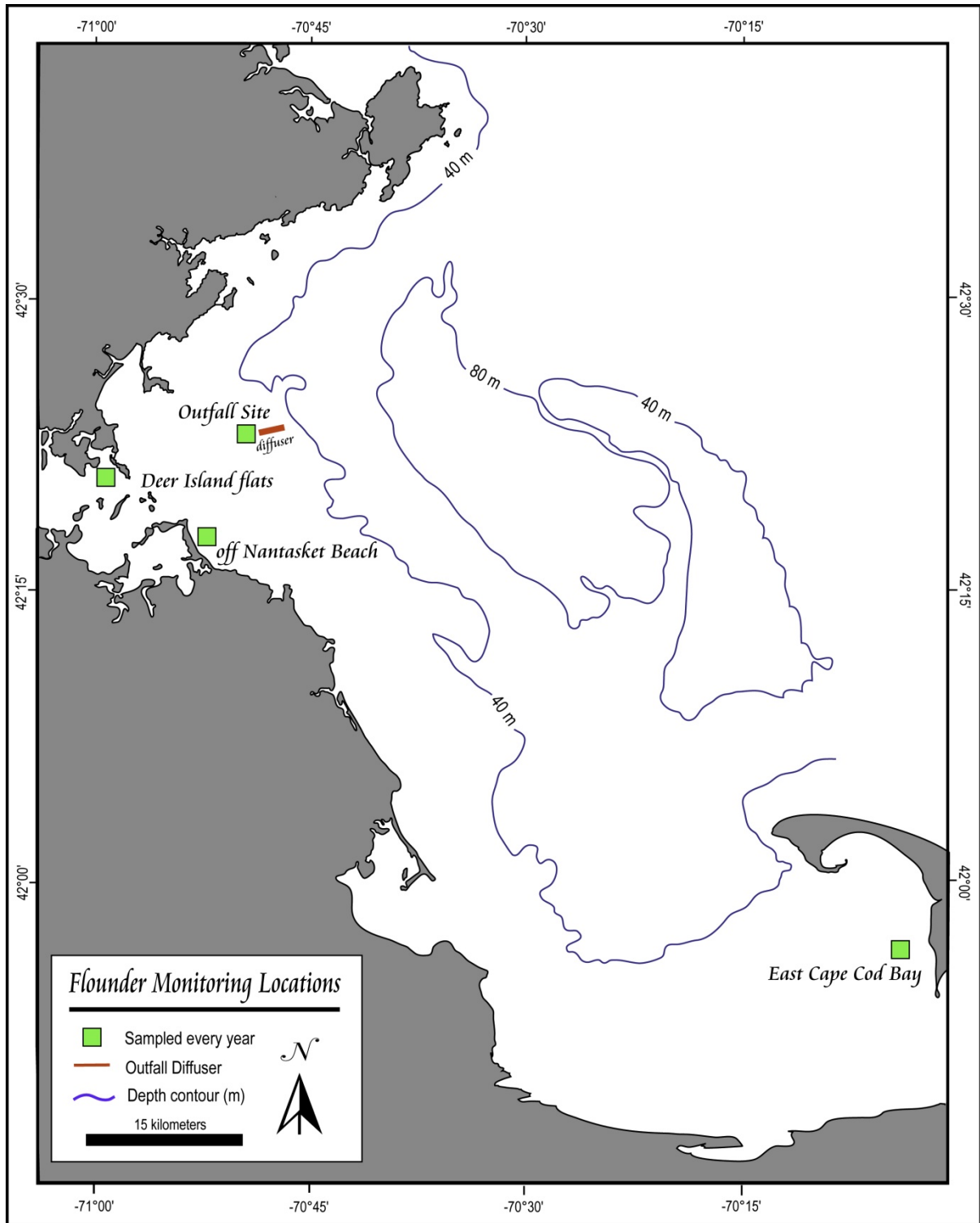


Figure 2-1. Flounder monitoring locations for 2013.

Table 2-1. Flounder Sampling Locations in 2013.

Site (Station ID)/Date/Time			Actual Location		Planned Location	
			Latitude	Longitude	Latitude	Longitude
Deer Island Flats (DIF)	26-Apr-13	7:57	42.3466	-70.9635	42.3400	-70.9733
		8:42	42.3524	-70.9728	42.3400	-70.9733
		9:35	42.3525	-70.9733	42.3400	-70.9733
East Cape Cod Bay (ECCB)	29-Apr-13	8:25	41.9645	-70.1176	41.9367	-70.1100
Off Nantasket Beach (NB)	26-Apr-13	10:42	42.2911	-70.8682	42.2933	-70.8700
		11:28	42.2903	-70.8675	42.2933	-70.8700
		12:39	42.2839	-70.8605	42.2933	-70.8700
		13:55	42.2822	-70.8585	42.2933	-70.8700
Outfall Site (OS)	28-Apr-13	8:34	42.3926	-70.8360	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  $\mu\text{m}$ . 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 (2013) 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 2011–2013, Revision 1 (Nestler et al. 2013). 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

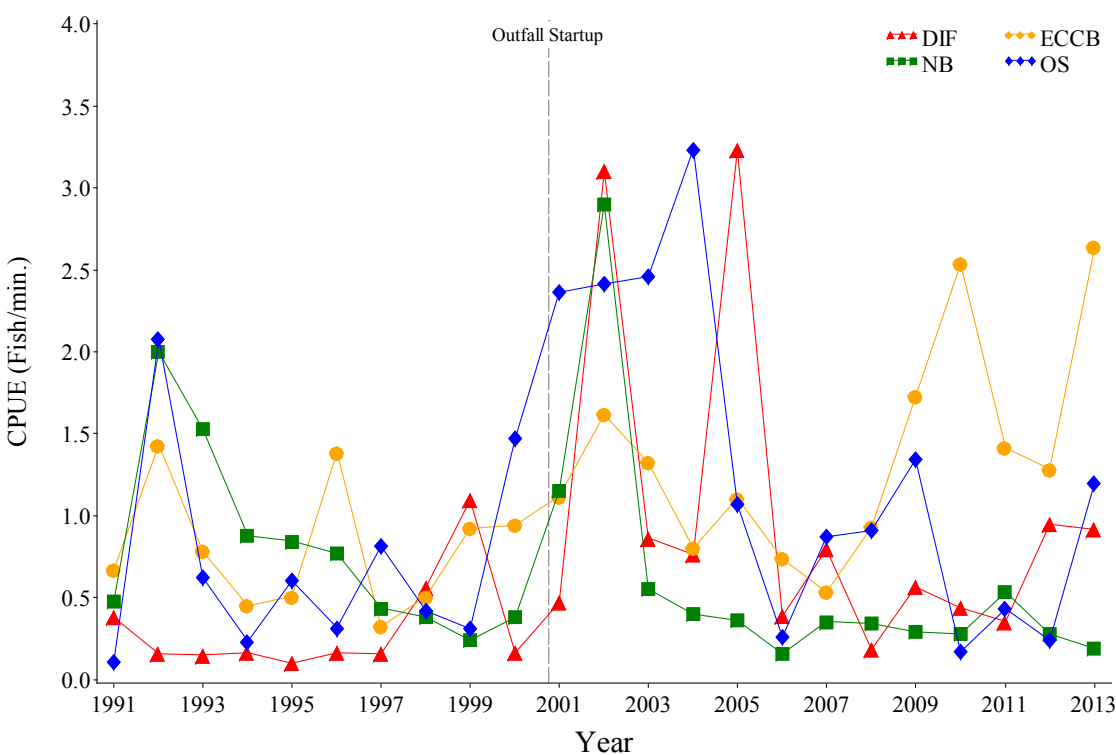
Age was measured with otoliths, but questions about comparability with the scale method, for older fish, caused MWRA to request that Grace Thornton of NOAA also measure 6 fish using the previous method of aging with scales. For these fish this changed the ages in the ANALYTICAL\_RESULTS table. The original values using the otolith method are stored in the EXPERIMENT\_RESULTS table.

Scatter plots of morphometric data show one obvious mis-recorded value. Fish number 13-1046 had a standard length recorded on the data sheet as 230mm. This has to be an erroneous record, given that animal falls in line for Total Length/ Weight and Age plots. This data point was therefore flagged as erroneous. A comment to that effect was appended to the data submission. Furthermore liver color and gross score were not recorded for fish number 13-5034.

### 3. RESULTS AND DISCUSSION

#### 3.1 Fish Collected

Winter flounder, each a minimum 30 cm in length, were collected between April 26 and 29, 2013, 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 was highest at ECCB, moderate at OS and DIF and remained low at NB.



**Figure 3-1. Catch Per Unit Effort (CPUE) for winter flounder trawled 1991–2013. Data for 2008 to 2013 have been normalized (see Section 3.1).**

#### 3.2 Physical Characteristics

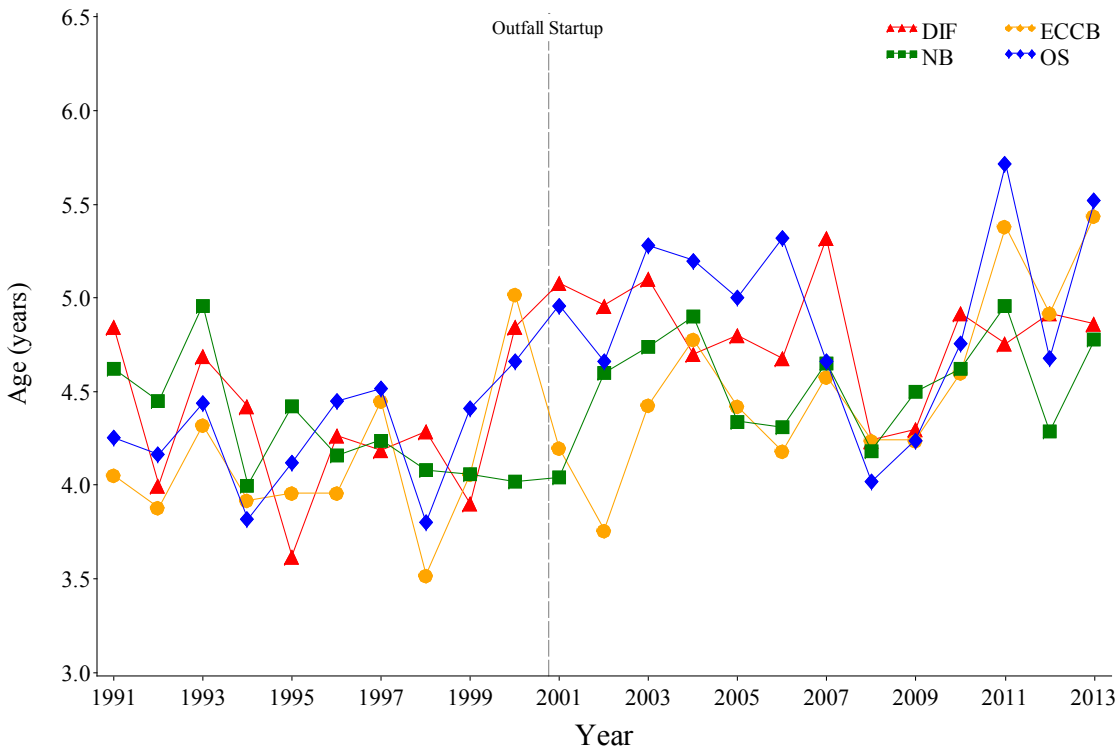
Mean values for physical characteristics of the winter flounder collected in 2013 are reported in Table 3-1. These values reflect the project requirement to collect sexually mature specimens (>30cm total

length). Mean age ranged from 4.9 to 5.5 years across the stations. Mean standard length ranged from 282 to 296 mm and mean total length from 344 to 360 mm; weight ranged from a mean of 477 to 560 g.

Between-year comparison of mean age in 2012 (Figure 3-2) showed a reversal of an upward trend since 2008 except for DIF which increased slightly. In 2013, NB, OS and ECCB all returned to 2011 levels. DIF has remained similar since 2010. Compared to 2012, standard length (Figure 3-3) in 2013 showed a decrease for DIF and NB and an increase for OS and ECCB. Weights (Figure 3-4) decreased at DIF and NB and increased at OS and ECCB. Percent females (Figure 3-5) increased at OS, remained stable at ECCB, and fell at NB and DIF.

**Table 3-1. Summary of Physical Characteristics of Winter Flounder Collected in 2013.**

Parameter	DIF			ECCB			NB			OS		
	Mean	STDDEV	N	Mean	STDDEV	N	Mean	STDDEV	N	Mean	STDDEV	N
Age (years)	4.9	1.3	50	5.4	1.5	50	4.8	1.3	50	5.5	1.5	50
Standard Length (mm)	289	25.2	50	296	18.3	49	282	24.7	50	283	28.8	49
Total Length (mm)	353	29.4	50	360	22.8	50	344	29.7	50	347	33.9	50
Weight (g)	522	124.6	50	560	125.9	50	477	140.8	50	501	145.8	50



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

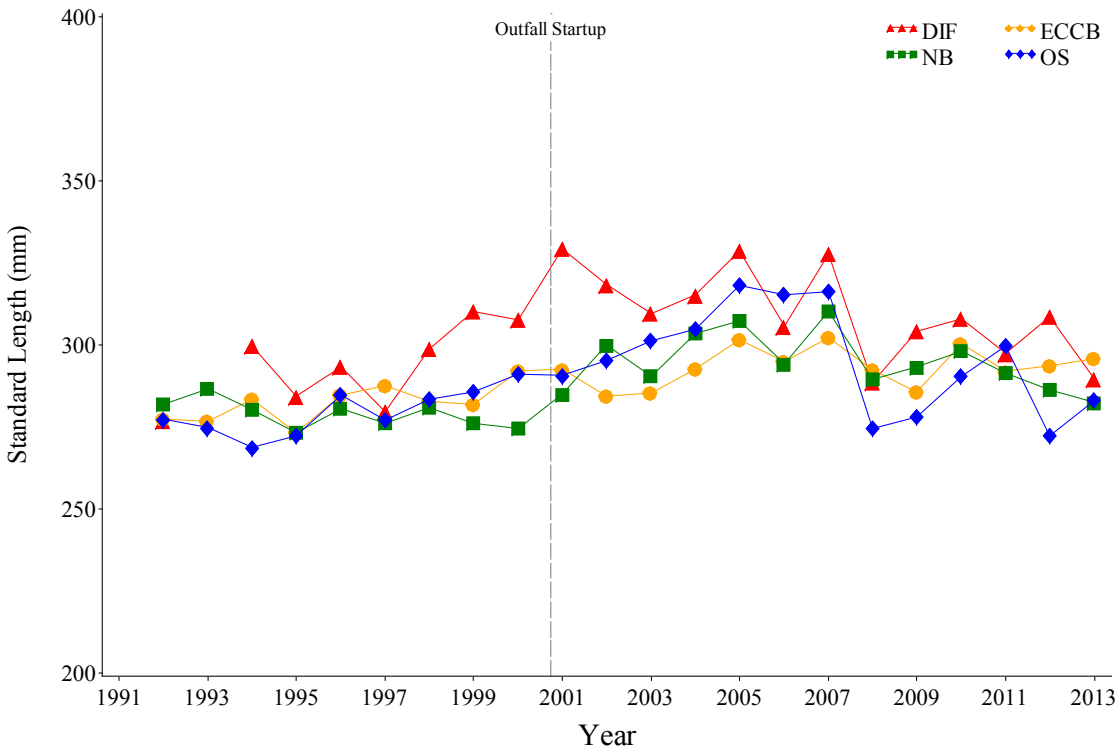


Figure 3-3. Average flounder standard length (mm) compared by station and year.

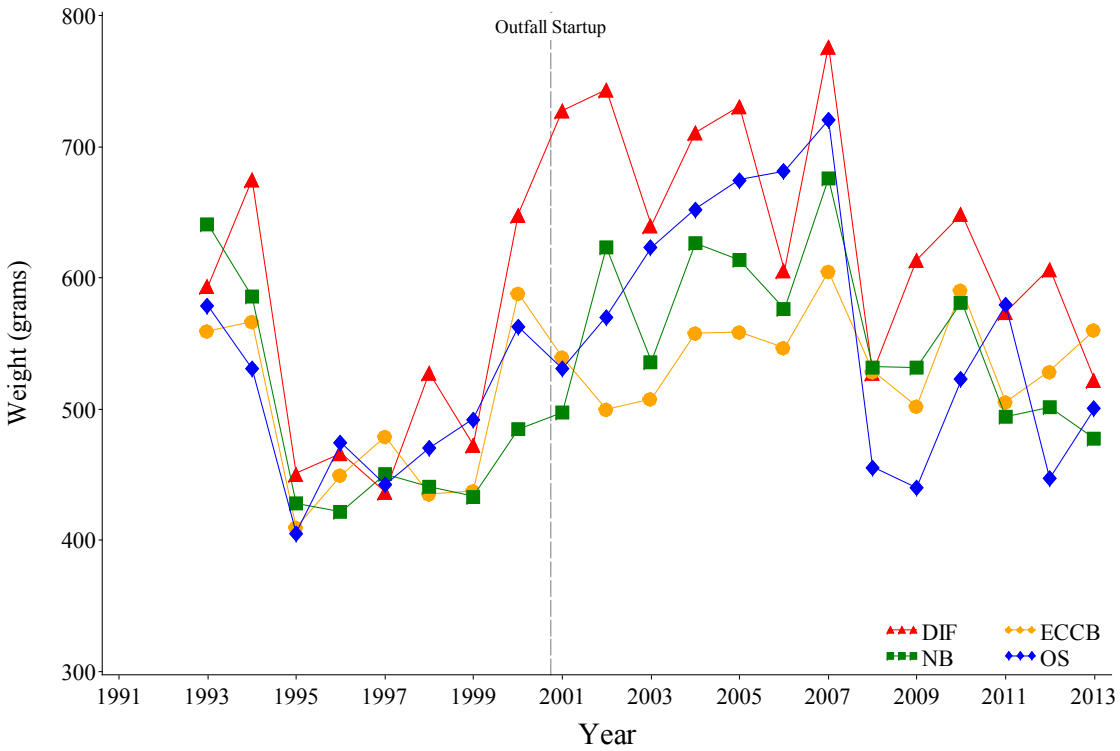


Figure 3-4. Average flounder weight (grams) compared by station and year.

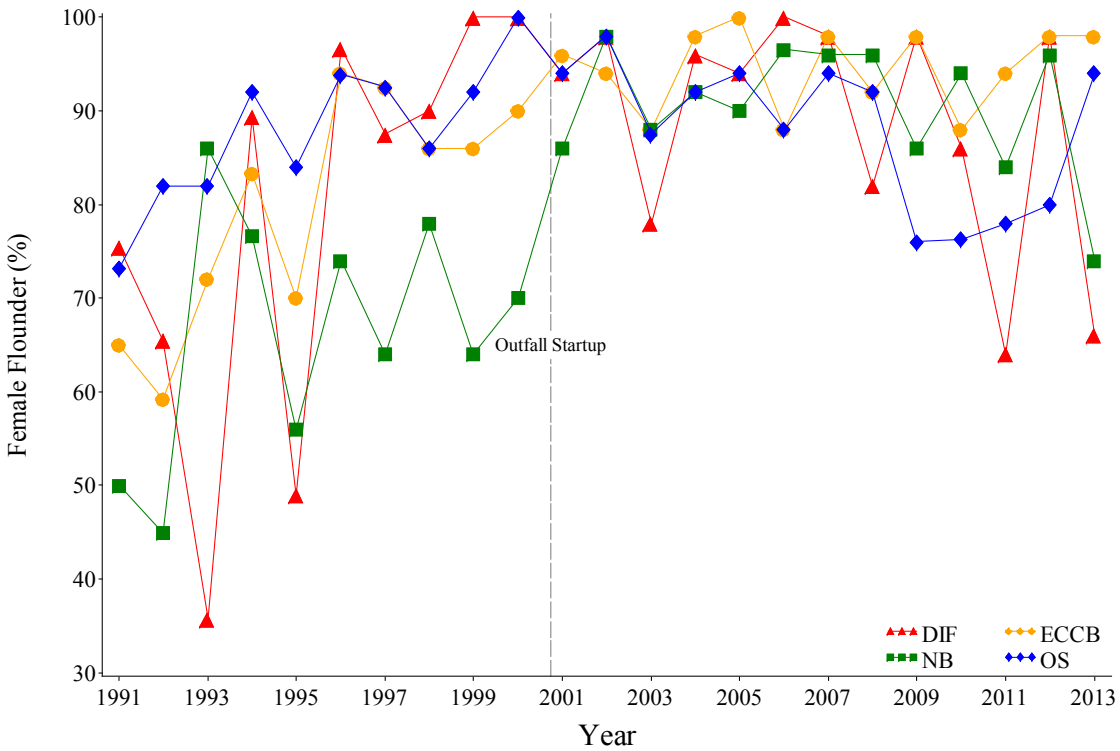


Figure 3-5. Proportion of female flounder compared by station and year.

### 3.3 External Condition

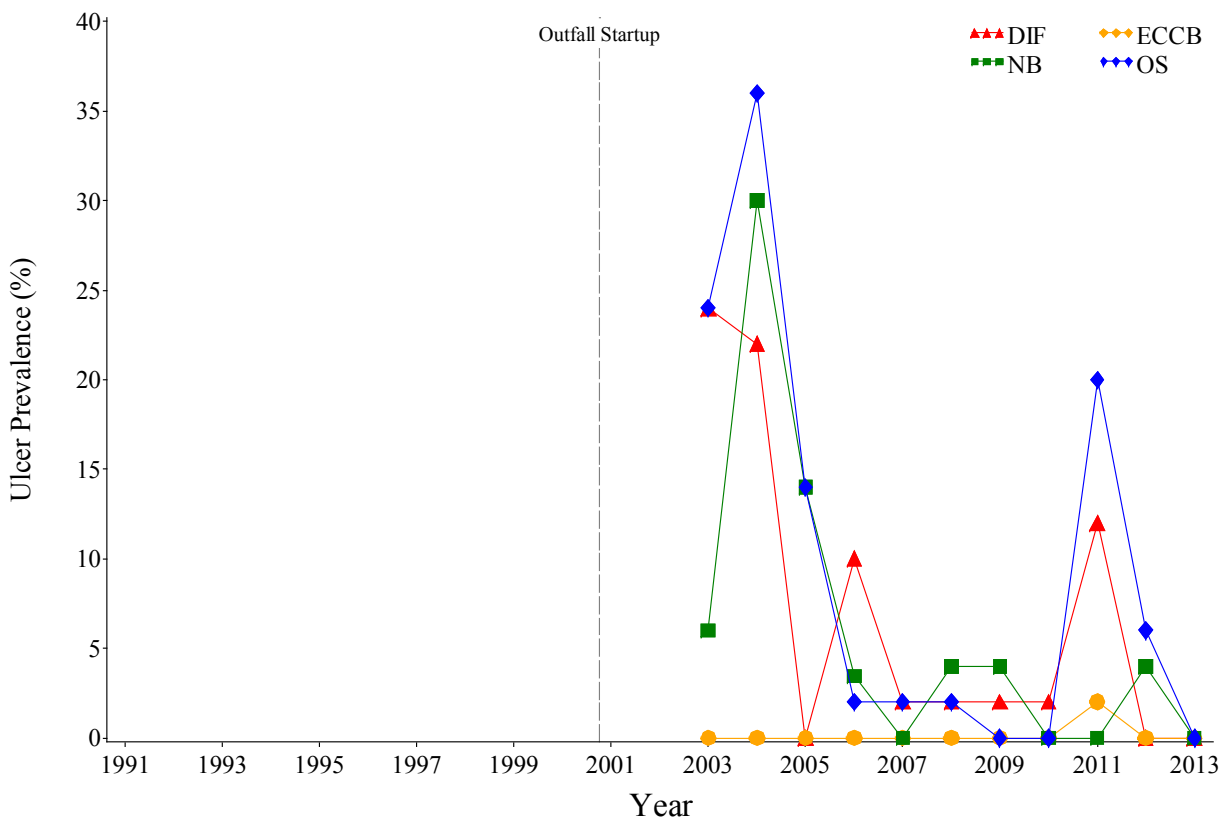
The external conditions of winter flounder collected in 2013 are presented as prevalence (% of individuals) per station in Table 3-2. Bent fin ray ranged from 2 to 16%, being highest at NB. The prevalence of blind side ulcers was zero at all stations in 2013. Fin erosion ranged from 8 to 26%, being highest at DIF. Lymphocystis ranged from 16 to 54%, being highest at OS.

Table 3-2. Prevalence (%) of External Conditions Assessed for Winter Flounder Collected in 2013.

External Conditions	Station (Sample size)			
	DIF (50)	ECCB (50)	NB (50)	OS (50)
Bent Fin Ray	14	8	16	2
Blind Side Ulcers	0	0	0	0
Fin Erosion (Fin Rot)	26	16	20	8
Lymphocystis	16	28	32	54

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. In 2013 ulcers were absent from all stations.

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. Compared to 2012, in 2013 fin erosion fell at DIF and ECCB, and remained practically unchanged at NB and OS.



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

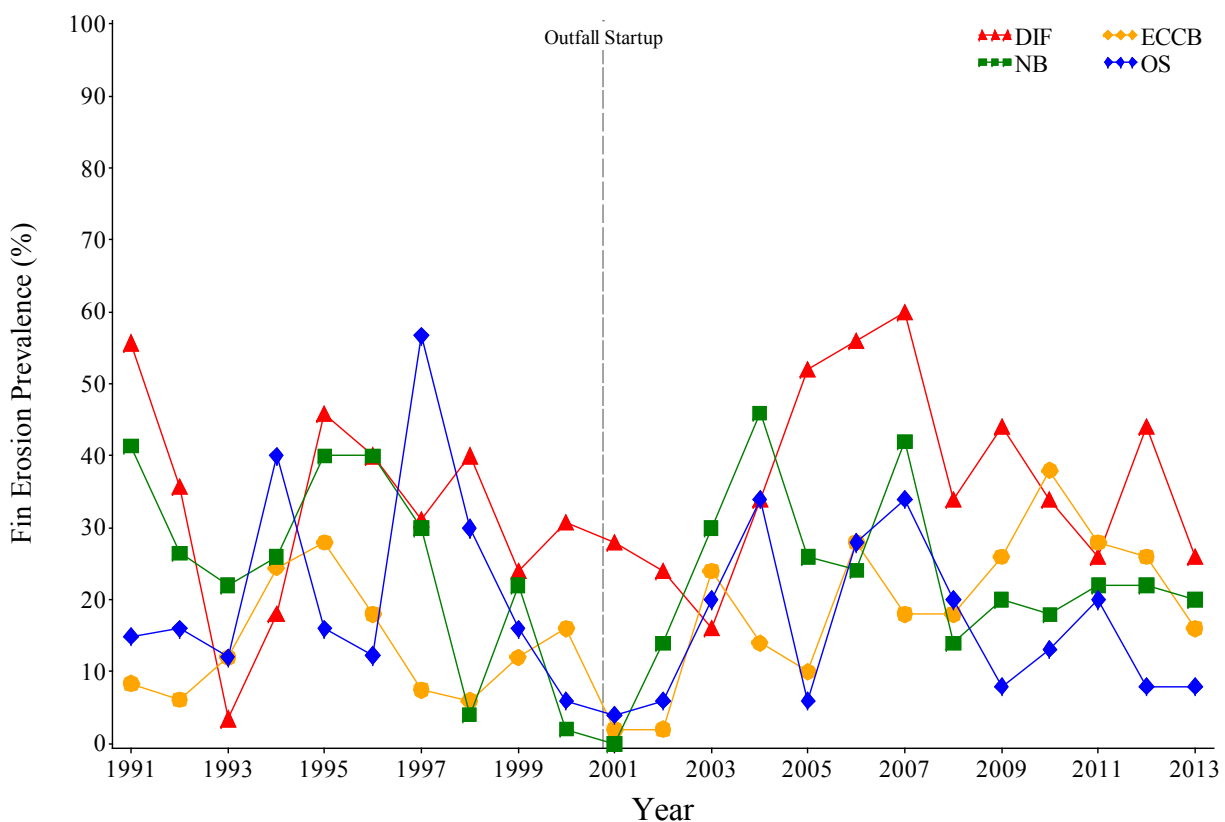


Figure 3-7. Temporal comparison of fin erosion prevalence (%) in winter flounder by station.

### 3.4 Liver Lesion Prevalence

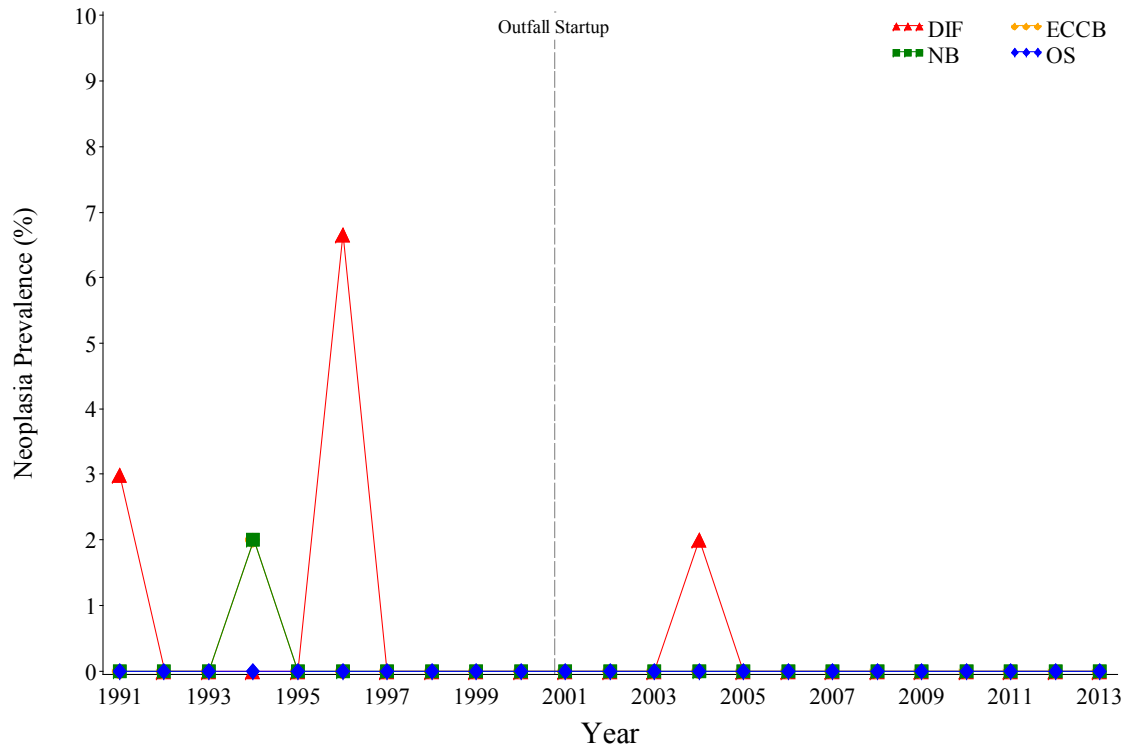
The prevalence (% of individuals) of liver lesions in winter flounder from each of the four stations sampled in 2013 is presented in Table 3-3. Balloons ranged from 6 to 16%, bile duct protozoa were absent

Table 3-3. Prevalence (%) of Liver Lesions in Winter Flounder Collected in 2013.

Liver Conditions	Station (Sample size)			
	DIF (50)	ECCB (50)	NB (50)	OS (50)
Balloons	6	16	10	10
Bile Duct Protozoan	2	0	0	0
Biliary Proliferation	8	8	8	12
Centrotubular Hydropic Vacuolation	22	8	8	18
Focal Hydropic Vacuolation	0	0	0	0
Liver Flukes	2	0	0	2
Macrophage Aggregation	56	64	42	82
Neoplasia	0	0	0	0
Tubular Hydropic Vacuolation	4	0	0	6

at all stations except for 2% (one animal) at DIF, biliary proliferation ranged from 8 to 12%, centrotubular vacuolation ranged from 8 to 22% being lowest at ECCB and NB and highest at DIF, focal hydropic vacuolation was absent at all sites, and liver flukes ranged from 0 to 2%. Macrophage aggregation ranged from 42 to 64%, neoplasia was absent at all sites, and tubular hydropic vacuolation ranged from 0 to 6%.

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 2013 of the general decline in centrotubular hydropic vacuolation at DIF since 1991. An ongoing gentle increase at OS since 2005 reversed in 2012 and then increased again in 2013; levels at OS remain below most of the pre-discharge years. A continued gentle low-level increase at NB through 2012 reversed in 2013, and a background level continued at ECCB, although it is creeping up a bit, but remains below an historic high in 1999.

The severity of CHV (Figure 3-10) shows a stable level at DIF 2005 -2012, which then dropped in 2013, a gentle increase since 2006 through 2012 at NB that reversed in 2013, little change in 2013 compared to 2012 at OS, and a continued background low level at ECCB.



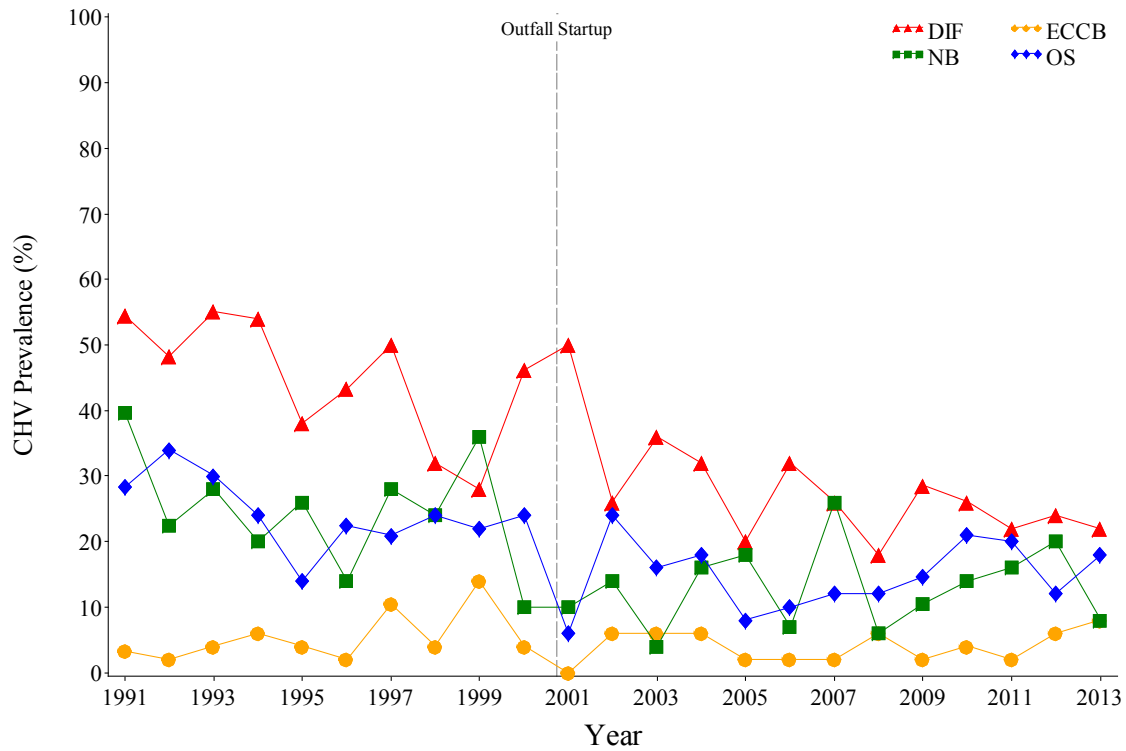


Figure 3-9. Temporal comparison of prevalence (%) of centrotubular hydropic vacuolation in winter flounder by station.

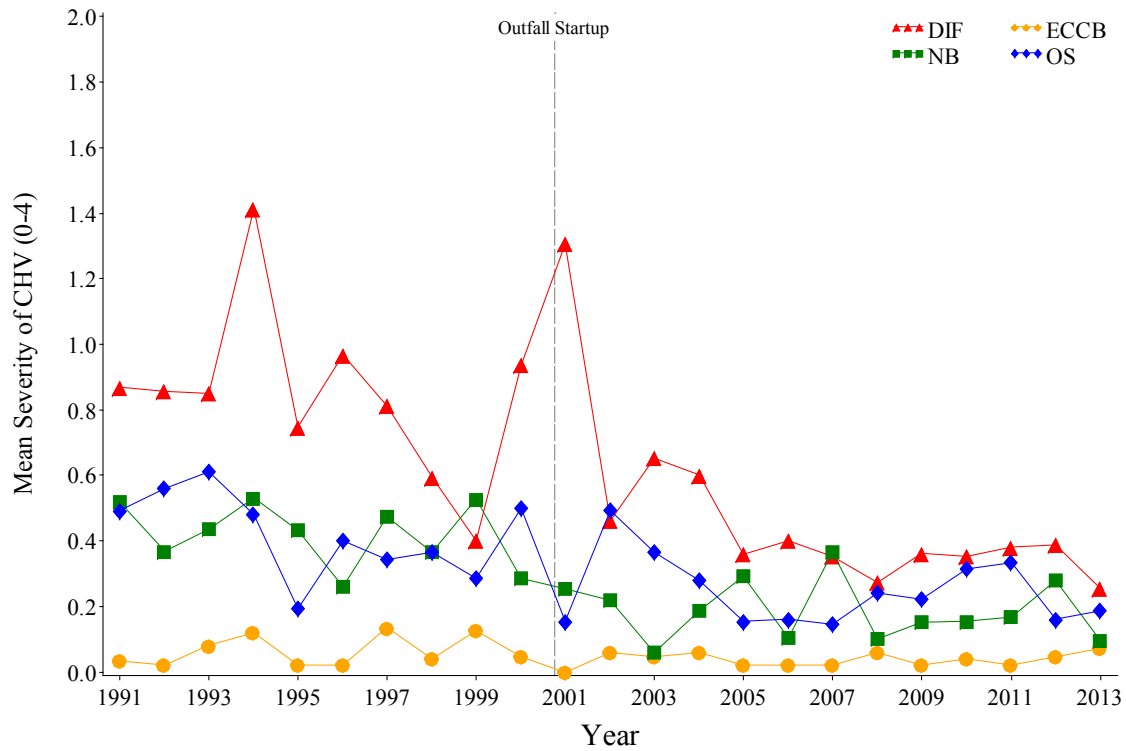
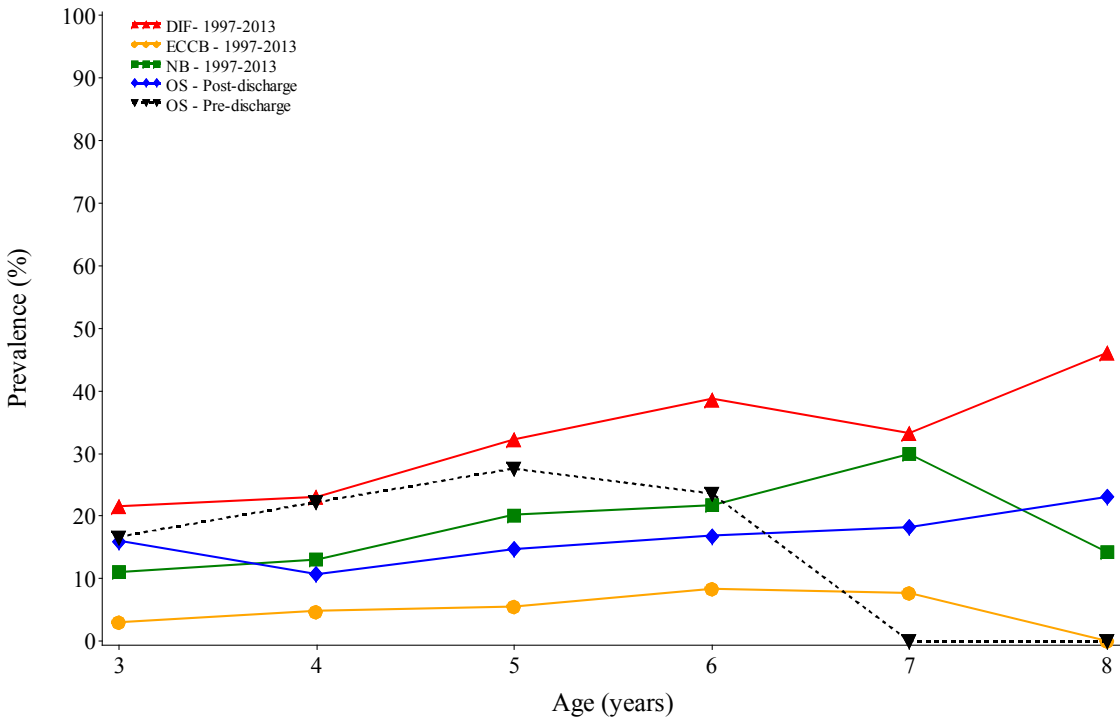


Figure 3-10. Centrotubular hydropic vacuolation severity (rank) in winter flounder compared between sites and years.

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). DIF continues to show a slow increase with age, suggesting a cumulative impact of remaining toxicants thought to induce this lesion. A lesser increase is perhaps seen at OS. NB and ECCB do not show this trend.



**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. In 2013 OS reversed a 2011/2012 downward trend. NB has shown recent increases from 2008-2012 that reversed in 2013, and ECCB remains at historic background levels.

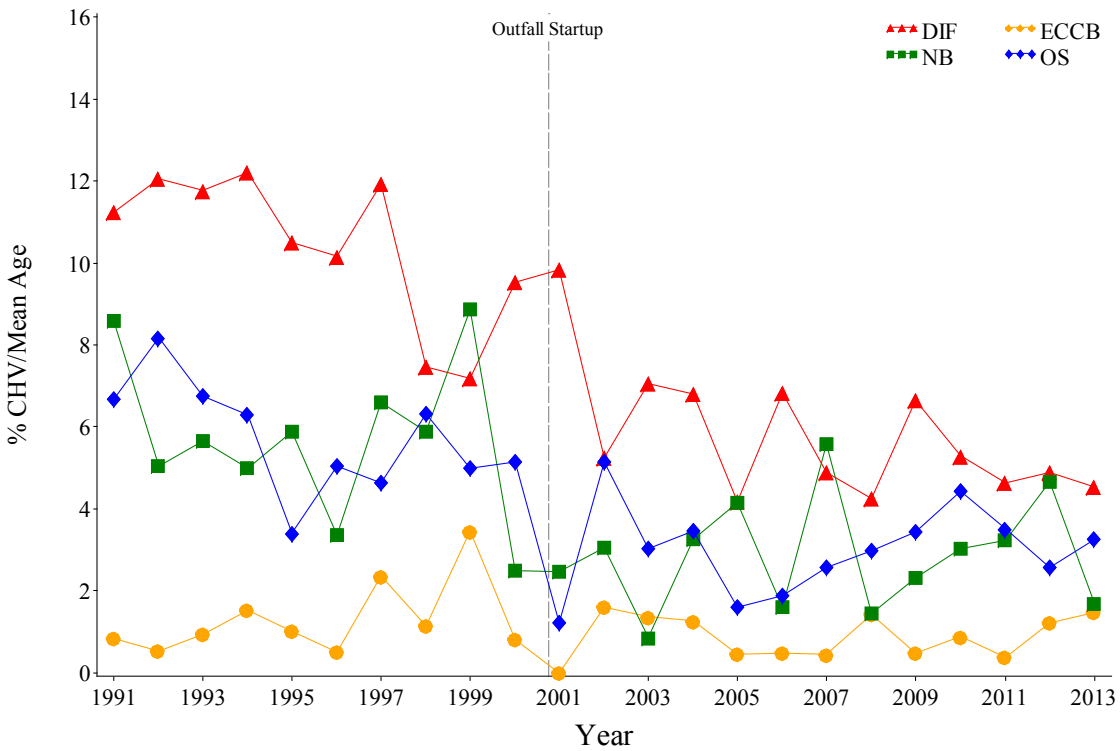


Figure 3-12. Hydropic vacuolation index (CHV%/age) for each station by year.

### 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 2013 was 18%, well below the threshold level.

#### 4. CONCLUSIONS

The 2013 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 2013. The proportions of females in the populations sampled remain high at all stations except DIF and NB.

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.

Results of the histological analyses in 2013 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 30% in recent years.
- Flounder collected off Nantasket Beach and in the vicinity of the outfall since discharge began in September 2000 also consistently show hydropic vacuolation prevalence lower than 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 again in 2012 but rose somewhat in 2013 (Figure 3-12). These values are still below baseline levels, prior to outfall startup.

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