Flounder Monitoring Report:
2010 Results

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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).

One aspect of the MWRA HOM program is a long-term monitoring program for winter flounder (MWRA 1991). The goal of this program is to provide data that can be used to assess potential impacts to winter flounder. 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, 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, and sampling is now done every third year (last implemented in 2009), except for flounder morphology and histopathology, which remain on an annual schedule.

This report presents results for the 2010 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 2010 data represent the tenth 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 2010, along with comparisons to historical flounder data, are presented in Section 3. Finally, conclusions drawn from the 2010 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 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 2010 flounder survey were similar to and consistent with previously used methods. Detailed descriptions of the methods are contained in the quality assurance plan (QAPP) for Flounder Monitoring 2008–2009 (Maciolek et al. 2008).

2.1 Stations and Sampling

The 2010 flounder survey was conducted between April 24 and May 1, 2010. 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 2010 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 (April 24 and 30, May 1) and biologists Paula Winchell, AECOM (April 24 and 30) and Rebecca Gast, WHOI (April 24 and May 1).

Fifty sexually mature (4–5 years old, total length ≥30 cm) winter flounder were collected at three stations, and 38 mature flounder at the outfall site (OS). Mobilization for the survey was conducted on April 24, when 50 fish were collected from the Deer Island and Nantasket Beach sites. Ten fish were collected from the Outfall site that same day. On April 30, only a further 28 fish were sampled from OS, despite a total of 3 hours and 31 minutes of bottom time at that site. It was agreed with MWRA that sufficient effort had been expended at OS. ECCB was sampled on May 1 and 50 fish were collected from that station.

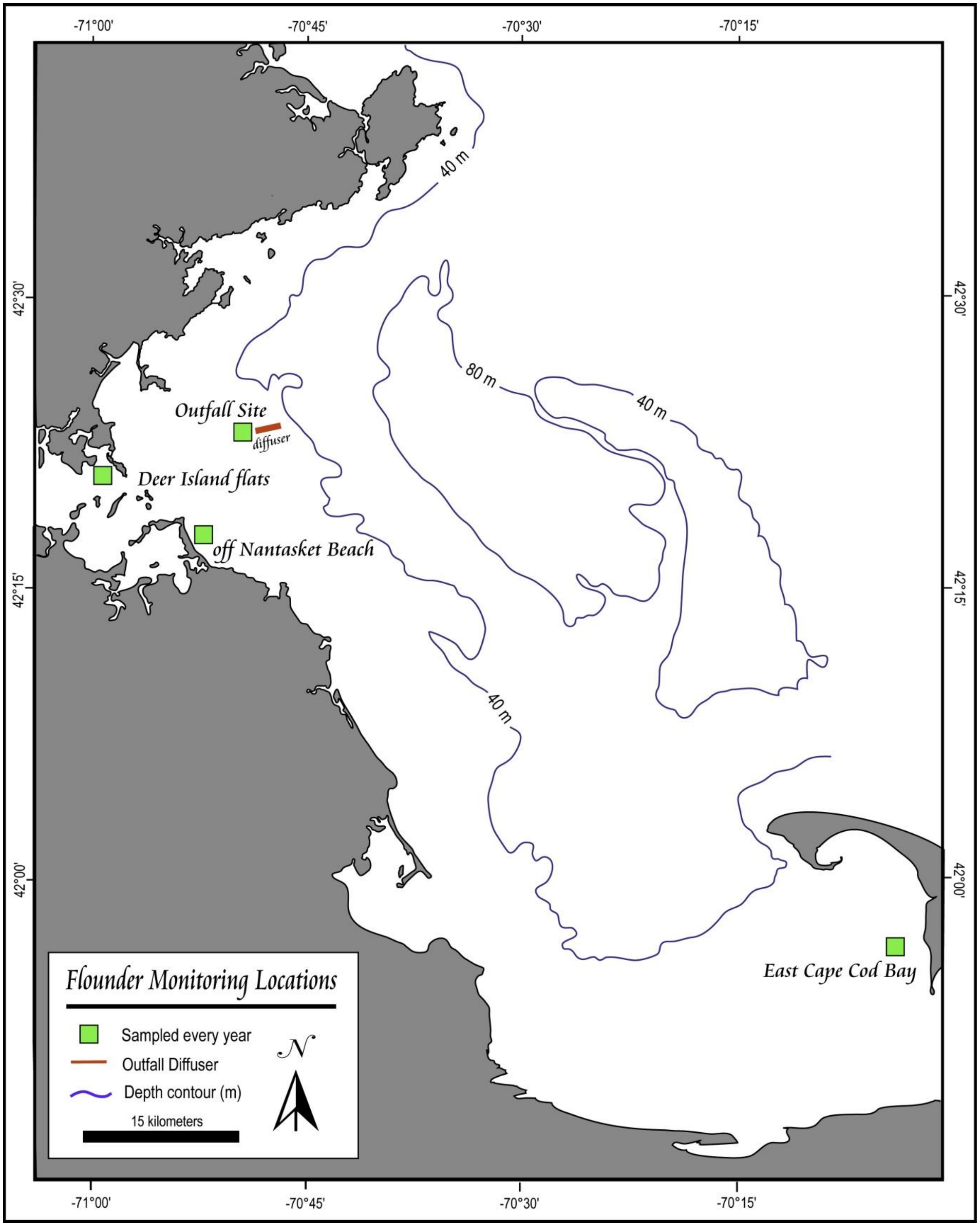

Figure 2-1. Flounder monitoring locations for 2010.

Table 2-1. Flounder Sampling Locations in 2010.

Sta. Name	Trawl Date	Start		Finish		Bottom Time (hh:min.)	# Flounder >300 mm	Total Flounder/ Station	Total time (min/stn)	CPUE* (fish/min)
		Lat (N)	Long (W)	Lat (N)	Long (W)					
DIF	24 April	42°20.748′	70°57.782′	42°20.770′	70°57.807′	30	16			
DIF	24 April	42°20.847′	70°57.924′	42°20.772′	70°57.747′	31	4			
DIF	24 April	42°20.870′	70°57.989′	42°20.759′	70°57.819′	51	29			
DIF	24 April	42°20.844′	70°58.011′	42°21.244′	70°58.498′	14	9	58	126	0.46
NB	24 April	42°17.353′	70°51.924′	42°17.343′	70°51.867′	77	24			
NB	24 April	42°17.523′	70°51.943′	42°17.504′	70°52.058′	34	6			
NB	24 April	42°17.472′	70°52.097′	42°17.504′	70°50.088′	76	25	55	187	0.29
OS	24 April	42°22.908′	70°48.371′	42°22.675′	70°49.240′	50	10			
OS	30 April	42°22.566′	70°49.422′	42°22.981′	70°48.566′	26	11			
OS	30 April	42°22.863′	70°49.352′	42°22.717′	70°43.320′	50	13			
OS	30 April	42°22.817′	70°49.347′	42°22.948′	70°48.338′	85	4	38	211	0.18
ECCB	1 May	41°56.666′	70°07.374′	41°53.914′	70°07.795′	66	177	177	66	2.68

* For 2009, the FV *Harvest Moon* (74' sweep rope) was used for all stations, with a net that was thus 1.04 x wider when compared to the 70' sweep rope for the FV *Odessa* used in all years of the survey prior to 2008.

2.2 Histological Analysis

Livers of flounder from each site (50 from DIF, ECCB, and NB and 38 from OS) 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 188 slides (50 fish x 3 sites and 38 fish x 1 site). 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 (2010) were extracted directly from the HOM database and imported into Excel, where data reduction, graphical presentations and statistical analyses were performed. Data reduction was conducted as described in the Flounder Monitoring QAPP (Maciolek et al. 2008). 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. Three morphological results were marked “use with caution” (age and weight for one fish from OS and standard length for a second fish from OS); however, these results have been included in the means of those parameters for OS.

2.4 Deviations from the QAPP

Only 38 flounder were collected the Outfall Station in 2010. Therefore the power of this year’s survey is somewhat reduced at this station. In addition, the scales for FF1014032 were unreadable for age.

3. RESULTS AND DISCUSSION

3.1 Fish Collected

Winter flounder, each a minimum 30 cm in length, were collected between April 20 and May 1, 2010, 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. CPUE showed a continuation of a steady increase at ECCB, a return to pre-outfall startup historic lows at OS, and continued recent low levels for DIF and NB.

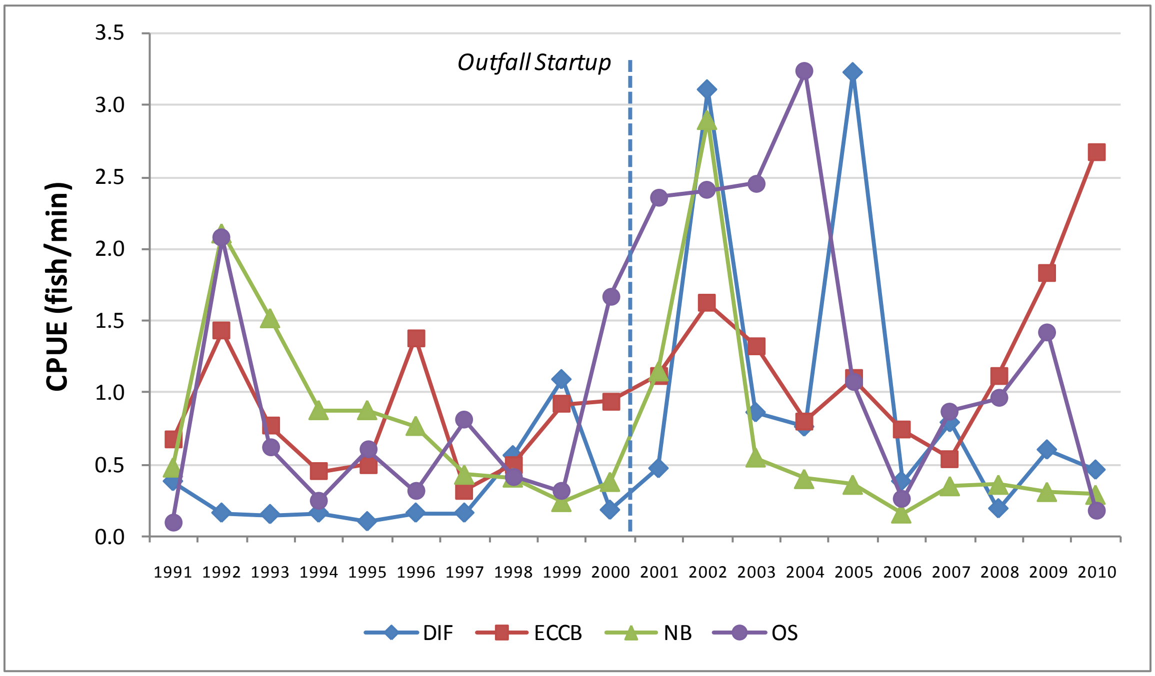

Figure 3-1. Catch Per Unit Effort (CPUE) for winter flounder trawled 1991–2010. Effort was constant up to and including 2007 with the FV *Odessa* (70' sweep rope). For 2008, the FV *Harvest Moon* (74' sweep rope) was used for DI, NB, and OS, with a net that was 1.04 x wider and for ECCB the FV *Explorer 2* (84' sweep rope) was used with a net that was 1.2 x wider. For 2009 and 2010, the FV *Harvest Moon* was used for all stations.

3.2 Physical Characteristics

Mean values for physical characteristics of the winter flounder collected in 2010 are reported in Table 3-1. These values reflect the project requirement to collect sexually mature specimens (>30cm total length). Age was comparable at a mean of 4.6 to 4.9 years across the stations. Standard length ranged from a mean of 291 to 308 cm and total length from 357 to 375 cm; weight ranged from a mean of 523 to 649 g.

Between-year comparison of mean age (Figure 3-2) and standard length (Figure 3-3) showed a continuation of an upward trend since 2008. Age was more comparable between stations than in many prior years, and at close to the historical mean. Weight (Figure 3-4) showed the same upward trend since 2008 for all stations, and again was within the middle of the historical range. Percent female (Figure 3-5) showed a reduction from historic highs for DIF and ECCB. NB increased to close to historic highs, while OS remained comparable to 2009 at a level lower than the other stations.

Table 3-1. Summary of Physical Characteristics of Winter Flounder Collected in 2010.

	Station (Sample size)							
	DIF (50)		ECCB (50)		NB (50)		OS (38)	
Parameter	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Age (years)	4.9	1.4	4.6	0.8	4.6	1.2	4.8	1.1
Standard Length (mm)	308.0	33.3	300.4	26.8	298.2	34.7	290.5	26.9
Total Length (mm)	375.0	38.9	366.8	31.2	364.7	41.1	357.1	31.2
Weight (g)	648.9	197.6	591.2	172.4	581.6	194.1	523.0	143.6

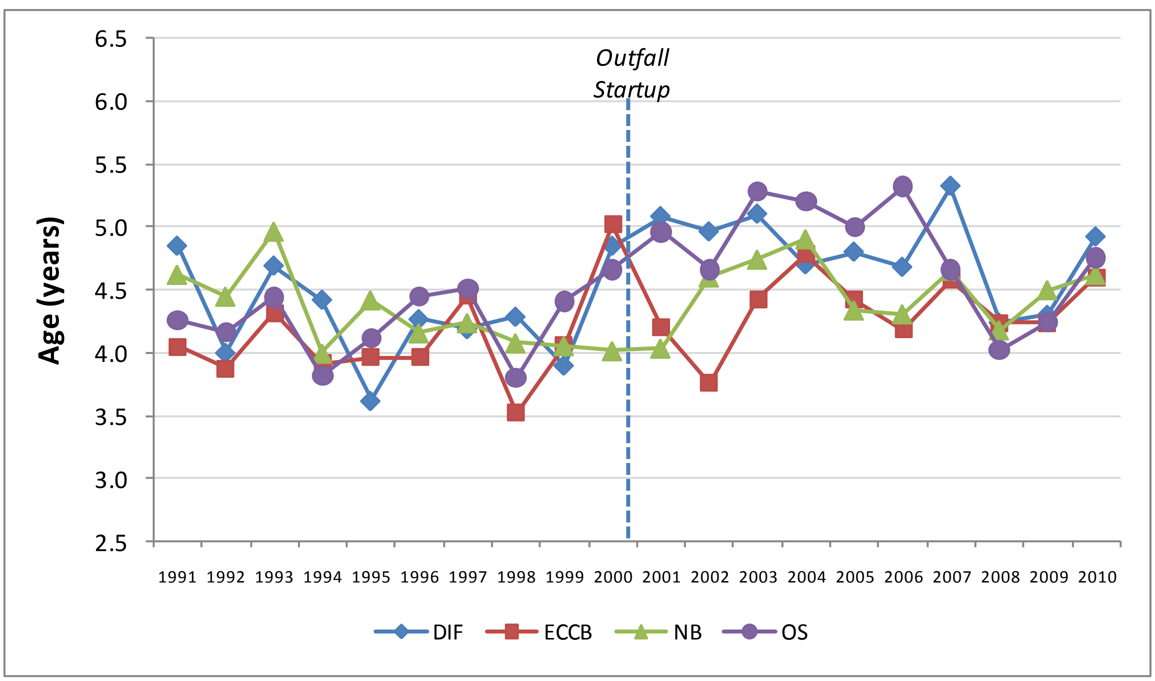

Figure 3-2. Average flounder age (years) compared by station and year.

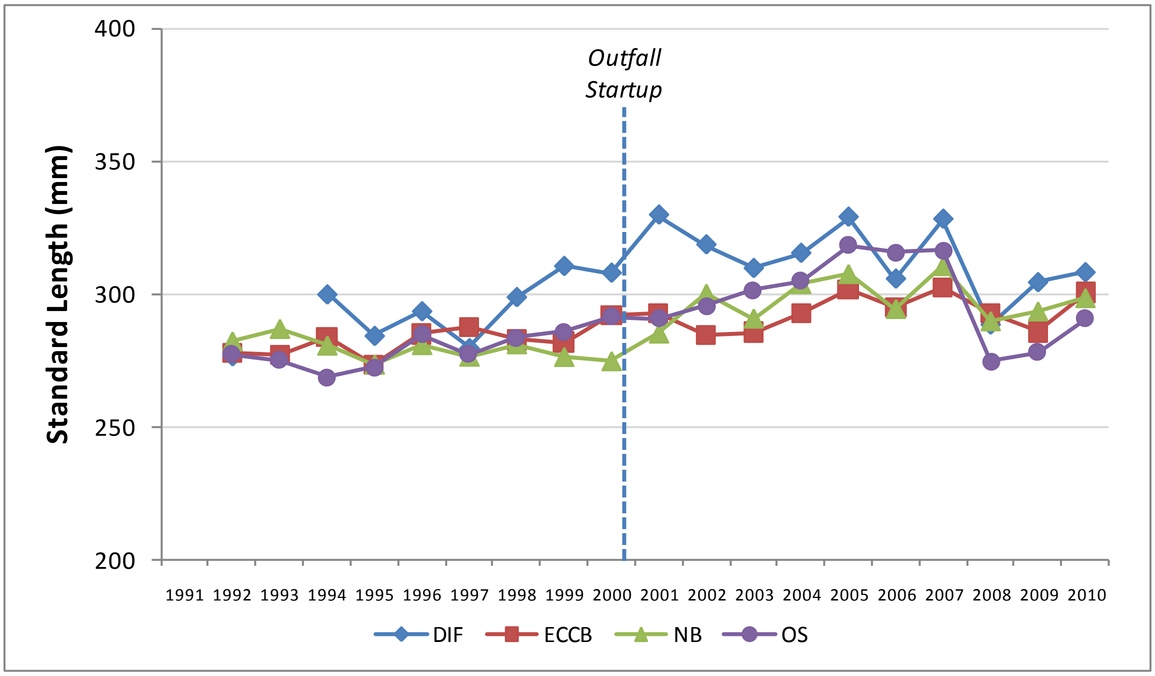

Figure 3-3. Average flounder standard length (mm) compared by station and year.

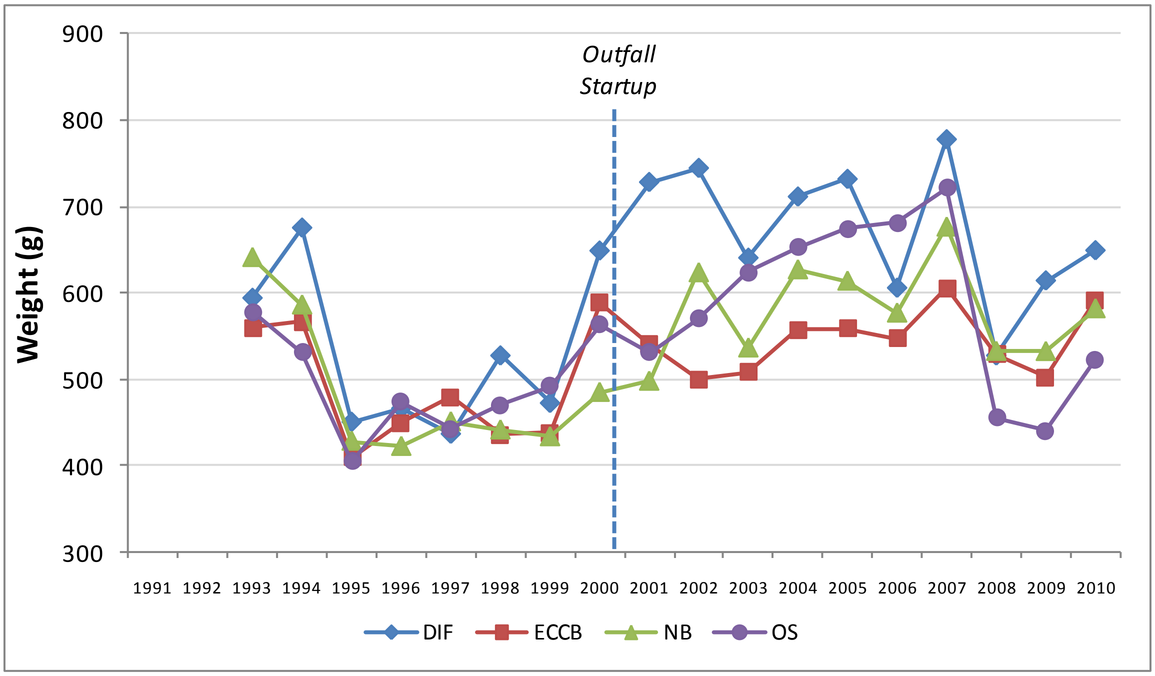

Figure 3-4. Average flounder weight (grams) compared by station and year.

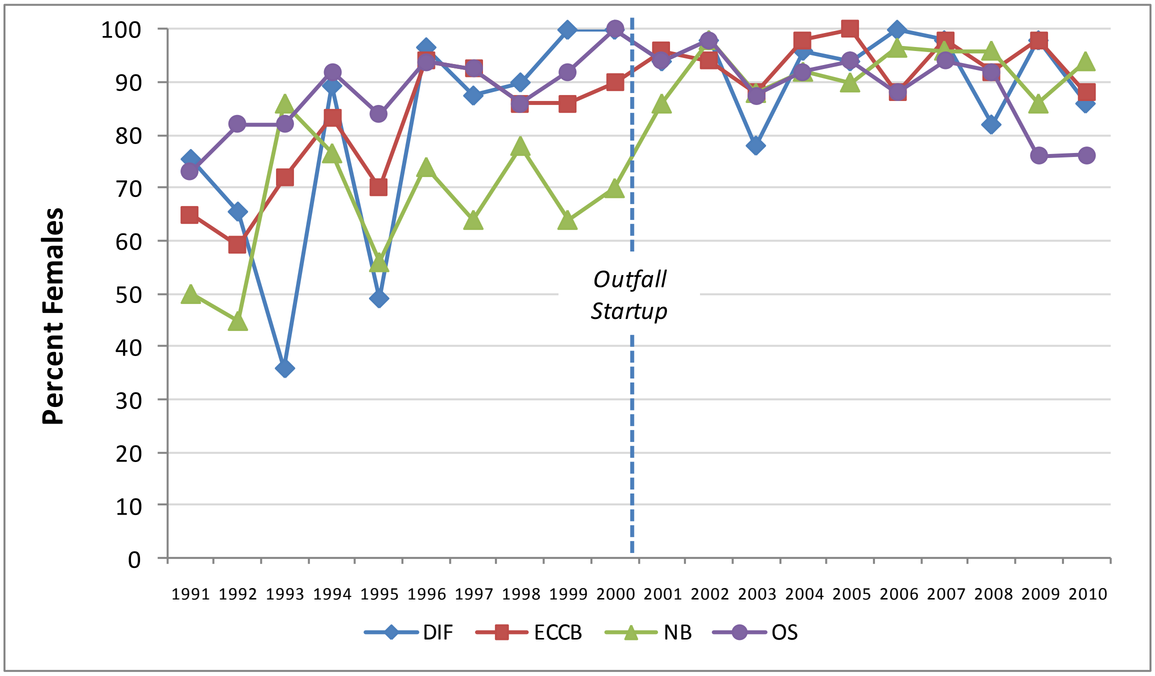

Figure 3-5. Proportion of female flounder compared by station and year.

3.3 External Condition

The external conditions of winter flounder collected in 2010 are presented as prevalence (% of individuals) per station in Table 3-2. Bent fin ray ranged from 4 to 24%, being highest at DIF. Blind side ulcers were absent except for one fish at DIF. Fin erosion ranged from 13 to 38%, being highest at ECCB. Lymphocystis ranged from 14 to 42, being highest at OS.

Table 3-2. Prevalence (%) of External Conditions Assessed for Winter Flounder Collected in 2010.

External Conditions	Station (Sample size)			
	DIF (50)	**ECCB (50)**	**NB (50)**	**OS (38)**
Bent Fin Ray	24	20	4	10.5
Blind Side Ulcers	2	0	0	0
Fin Erosion (Fin rot)	34	38	18	13.2
Lymphocystis	24	14	22	42.1

Fin ray surface mucous and epithelia are impacted by increased levels of ammonia and other pollutants, making fin rot 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 3-6. Fin erosion fell to close to historical mean level at DIF; remained low at NB; climbed slightly at OS, which still remained the lowest of the four stations; and continued to climb to an historic high at ECCB.

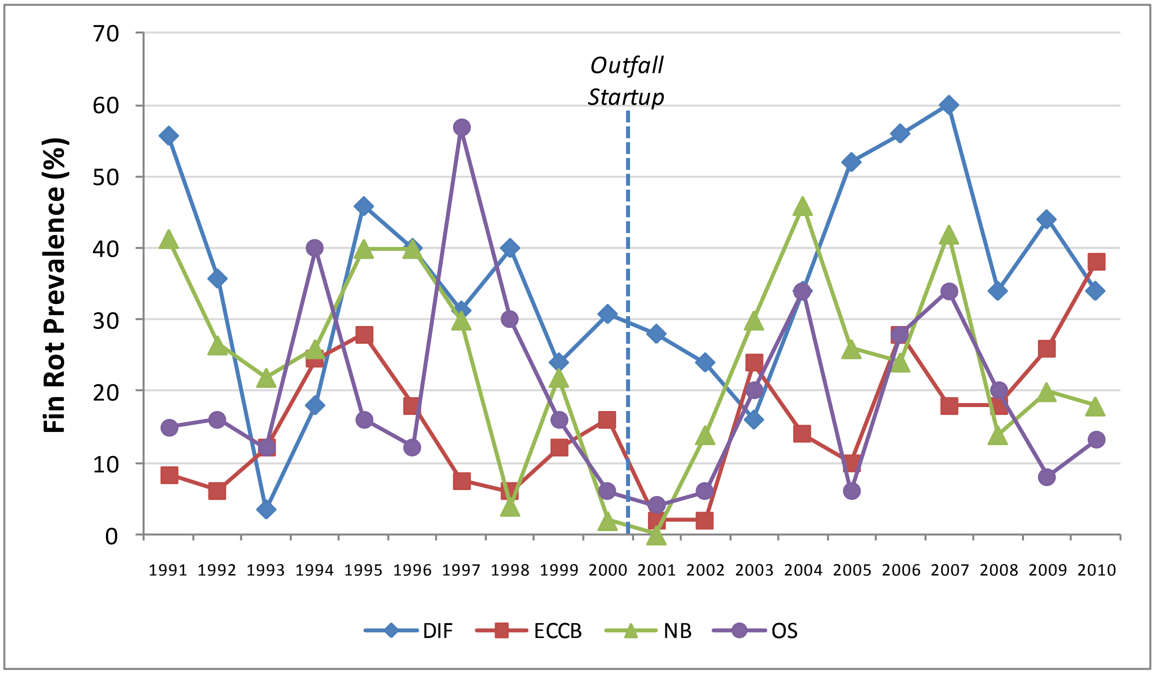

Figure 3-6. Temporal comparison of fin rot prevalence (%) in winter flounder by station.

3.4 Liver Lesion Prevalence

The prevalence (% of individuals) of liver lesions in winter flounder from each of the four stations sampled in 2010 is presented in Table 3-3. Balloons ranged from 4 to 11%, bile duct protozoa were absent at all stations, biliary proliferation ranged from 8 to 24%, centrotubular vacuolation ranged from 4 to 26% being lowest at ECCB and highest at DIF, focal hydropic vacuolation was absent at all sites, and liver flukes ranged from 0 to 8 %. Macrophage aggregation ranged from 50 to 79%, neoplasia was absent at all sites, and tubular hydropic vacuolation ranged from 4 to 24%.

Neoplasms (Figure 3-7) 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 MRWA project began remains totally absent.

Table 3-3. Prevalence (%) of Liver Lesions in Winter Flounder Collected in 2010.

Lesion Type	Station (sample size)			
	DIF (50)	ECCB (50)	NB (50)	OS (38)
Balloons	4	8	8	11
Bile Duct Protozoan	0	0	0	0
Biliary Proliferation	24	8	8	18
Centrotubular Hydropic Vacuolation	26	4	14	21
Focal Hydropic Vacuolation	0	0	0	0
Liver Flukes	2	0	2	8
Macrophage Aggregation	56	50	74	79
Neoplasia	0	0	0	0
Tubular Hydropic Vacuolation	24	4	12	21

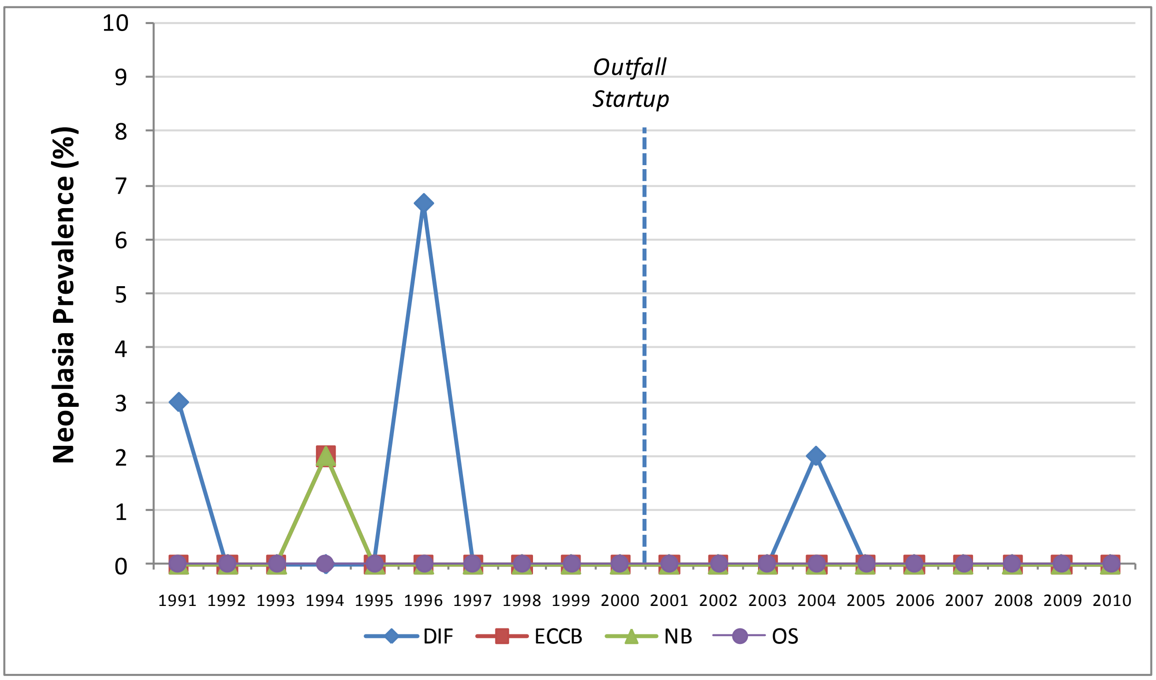

Figure 3-7. Temporal comparison of neoplasia prevalence (%) in winter flounder by station.

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 3-8 shows a continuation of the general decline in centrotubular hydropic vacuolation at DIF since 1991, a recent ongoing gentle increase at OS, but still at a level below most of the pre-discharge years, a gentle increase of low recent levels at NB, and a continued background level at ECCB.

The severity of CHV (Figure 3-9) shows a stable level at DIF since 2005, a gentle increase since 2006 at OS, a low level at NB, and a background low level at ECCB.

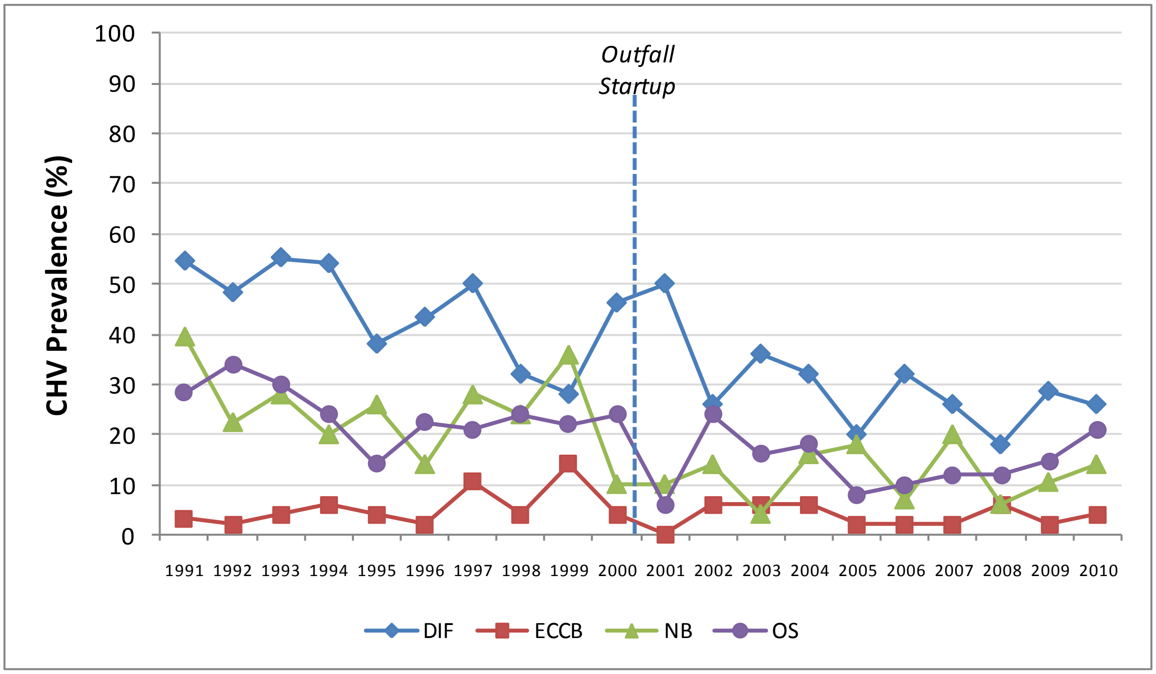

Figure 3-8. Temporal comparison of prevalence (%) of centrotubular hydropic vacuolation in winter flounder by station.

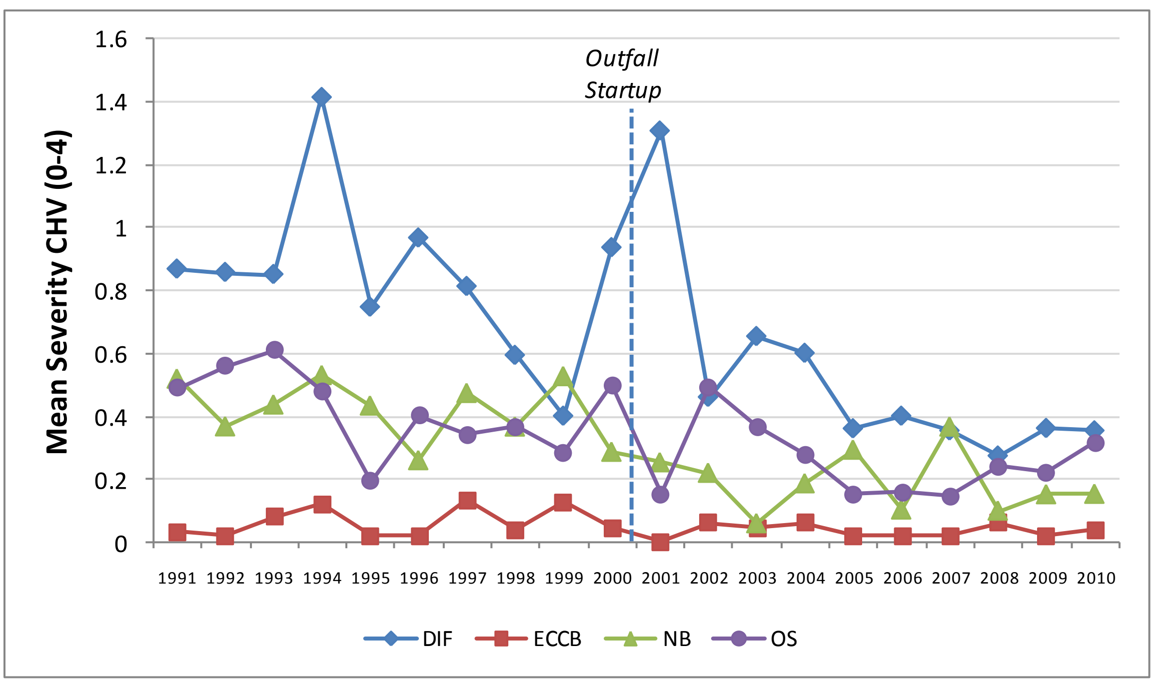

Figure 3-9.Centrotubular hydropic vacuolation severity (rank) in winter flounder compared between sites and years.

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 3-10). DIF continues to show a slow increase with age, suggesting a cumulative impact of remaining toxicants thought to induce this lesion. The remaining stations do not show this trend.

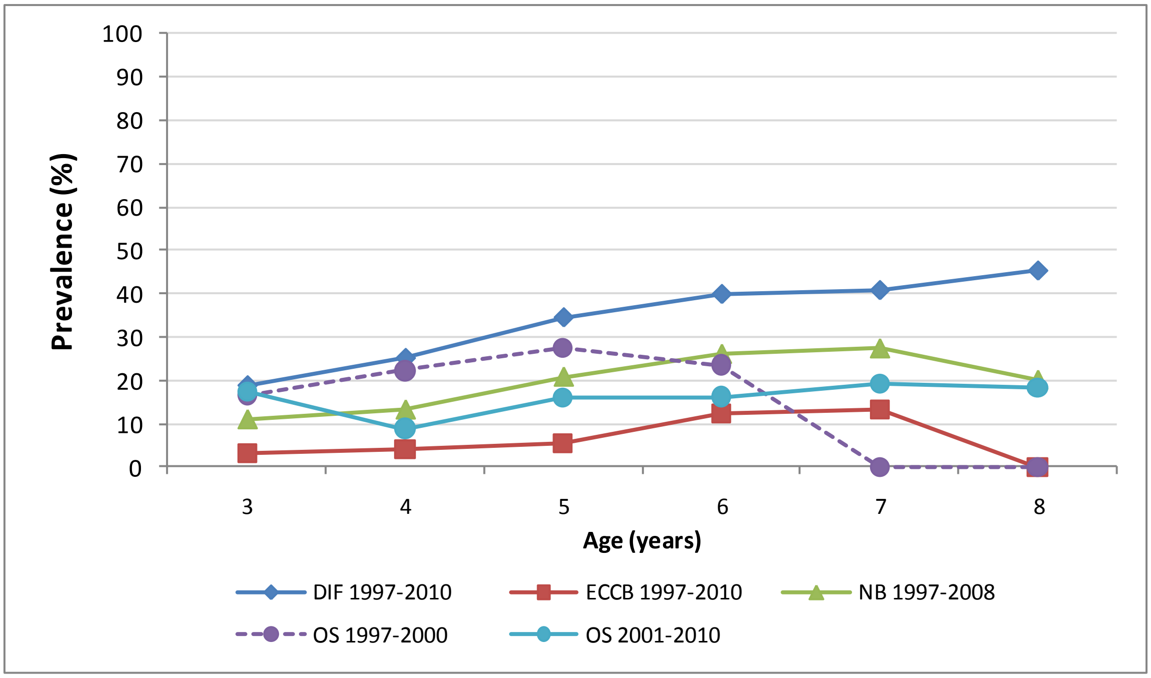

Figure 3-10. Proportion (%) of winter flounder showing hydropic vacuolation for each age.

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 3-11). The overall trend for DIF remains downward, OS shows a steady upward trend since 2005, but it is still below most pre-discharge levels, NB remains low, but with a recent increase since 2008, and ECCB remains at historic background levels.

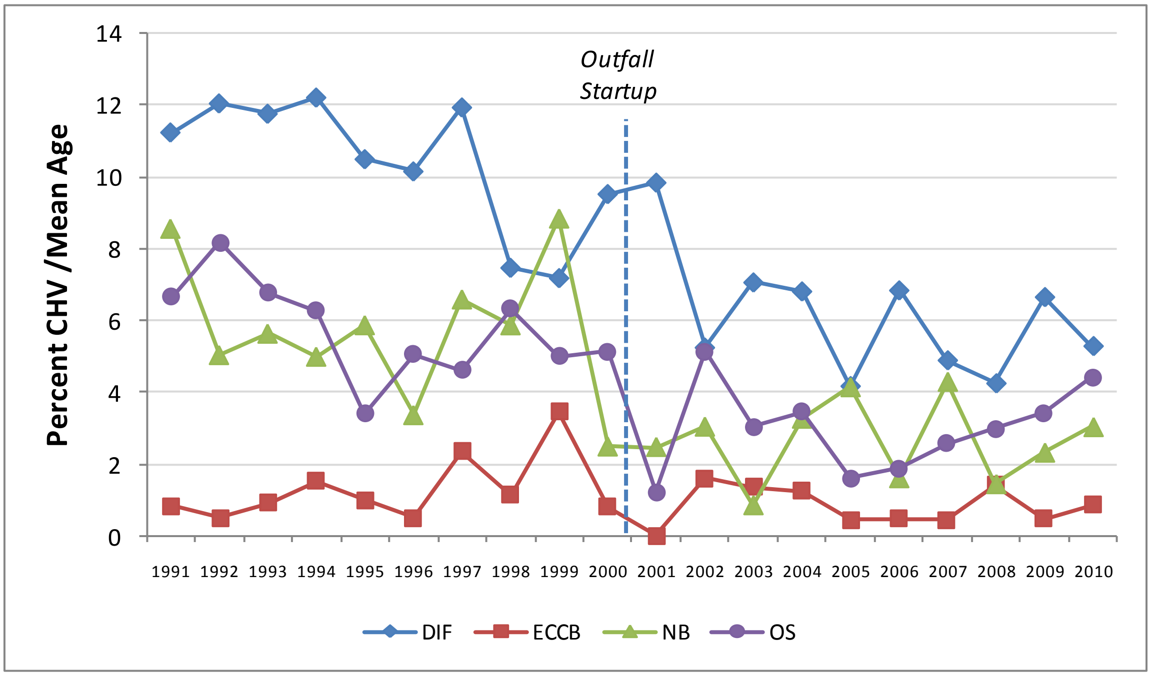

Figure 3-11. Hydropic vacuolation index (CHV%/age) for each station by year.

3.5 Threshold Comparison

The MWRA Monitoring Program has established threshold levels against which to measure key indicators of wastewater impacts (MWRA 2001). 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 2010 was 21%, well below the threshold level.

4. CONCLUSIONS

The 2010 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 fell from 2009 but was not the lowest on record (1991), following higher catch rates reported between 2000 and 2004. 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. In 2009, that trend gently reversed for all stations other than ECCB. In 2010 that trend continued with ECCB showing an uptick as well. The proportions of females in the populations sampled remain high at all stations.

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). Results of the 2010 survey suggest a very low ulcer prevalence at OS, from 36% in 2004 to 0% in 2010. One ulcerated fish was seen, at DIF, in 2010.

The age-corrected hydropic vacuolation prevalence suggested that there has been a steady reduction in the contaminant-associated pathology in winter flounder at DIF during the past decade. There was none of the high neoplasm prevalence characteristic of fish from DIF in the mid- to late-1980s. The most notable trend is that of the slow rise of age-corrected CHV at the OS site. This however should be taken in perspective of the pre-discharge values, all except one of which exceeded the 2010 value (Figure 3-11).

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