Flounder Monitoring Report: 2011 Results

Massachusetts Water Resources Authority

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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).

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, 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, 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 2011 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 2011 data represent the eleventh 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 2011, along with comparisons to historical flounder data, are presented in Section 3. Finally, conclusions drawn from the 2011 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 2011 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 (Nestler et al. 2011).

2.1 Stations and Sampling

The 2011 flounder survey was conducted between April 25 and 27, 2011. 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 2011 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 25, 26, and 27) and biologists Eric Rydbeck, NAI (April 25 and 26) and Melinda Sweeny, NAI (April 27).

Fifty sexually mature (4–5 years old, total length ≥30 cm) winter flounder were collected at each station. Mobilization for the survey was conducted on April 25, when 50 fish were collected from the Nantasket Beach site and 34 fish from the Deer Island Site. Sixteen fish were collected from the Deer Island Site and 50 fish from the Outfall site on April 26. On April 27, 50 fish were collected from the Eastern Cape Cod Bay site. Fish were weighed and measured individually in the field. Scales were removed from each fish for aging. Livers were removed, sliced, examined and fixed in the field.

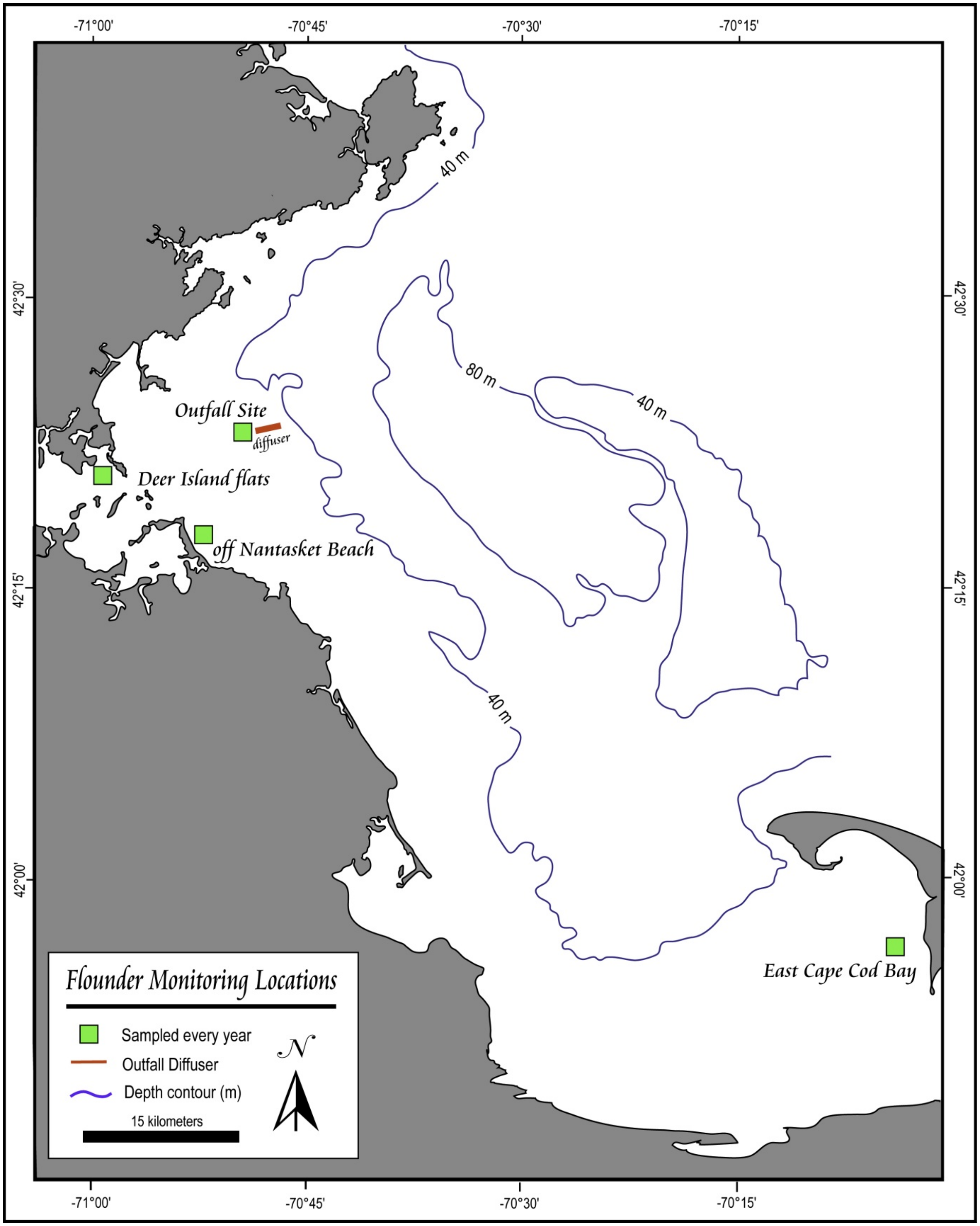

Figure 2-1. Flounder monitoring locations for 2011.

Table 2-1. Flounder Sampling Locations in 2011.

Trawl			Actual Location		Planned Location	
Site (Station ID)	**Date**	**Time**	**Latitude**	**Longitude**	**Latitude**	**Longitude**
Deer Island Flats (DIF)	**25-Apr-11**	**10:44**	42.3470	-70.9641	42.3400	-70.9733
		11:40	42.3516	-70.9731	42.3400	-70.9733
		13:12	42.3498	-70.9687	42.3400	-70.9733
	26-Apr-11	**7:18**	42.3481	-70.9661	42.3400	-70.9733
East Cape Cod Bay (ECCB)	**27-Apr-11**	**8:55**	41.9384	-70.1250	41.9367	-70.1100
Off Nantasket Beach (NB)	**25-Apr-11**	**7:38**	42.2890	-70.8658	42.2933	-70.8700
		8:51	42.2819	-70.8587	42.2933	-70.8700
Outfall Site (OS)	**26-Apr-11**	**10:12**	42.3895	-70.8267	42.3850	-70.8217
		11:10	42.3836	-70.8238	42.3850	-70.8217
		13:08	42.3910	-70.8294	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 (2011) 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 Quality Assurance Project Plan (QAPP) for Fish and Shellfish Monitoring 2011–2013 (Nestler et al. 2011). 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. During MWRA's review of the data it was noted that the weights and lengths for a given aged fish tended to be in the lower part of the historical range. Also, the average flounder age for the study was the highest we have observed for OS and ECCB. Upon inquiry from the lab responsible for the data we were informed that roughly two thirds of the scales were checked by the analyst who had done the work in previous years, suggesting that the analytical methodology was not responsible for the increase in age at those stations.

2.4 Deviations from the QAPP

Two scale samples were unreadable and the data cells marked 'e'.

3. RESULTS AND DISCUSSION

3.1 Fish Collected

Winter flounder, each a minimum 30 cm in length, were collected between April 25 and 27, 2011, 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. For 2009 through 2011, the FV Harvest Moon was used for all stations. Thus, data presented in Figure 3-1 for 2008 to 2011 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 showed a reversal of a prior 3 year increase at ECCB, a slight increase from 2010 lows for NB and OS, and a continuation of the recent decline for DIF.

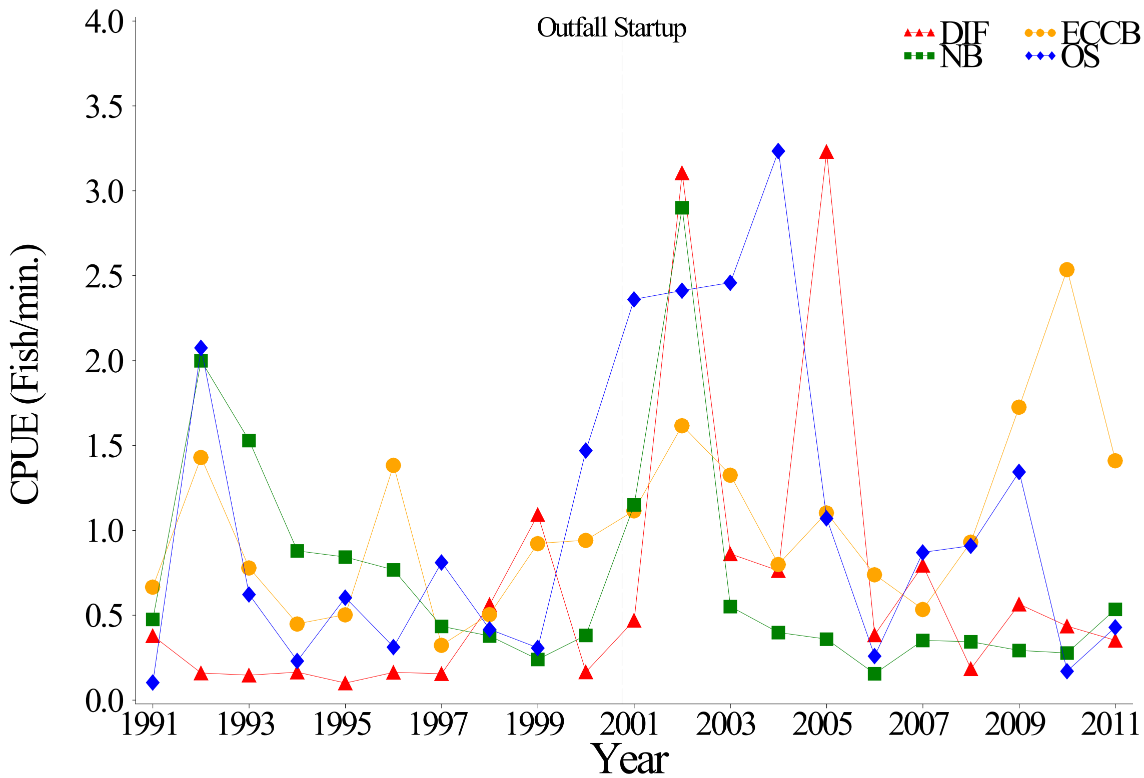

Figure 3-1. Catch Per Unit Effort (CPUE) for winter flounder trawled 1991–2011. Data for 2008 to 2011 have been normalized (see Section 3.1).

3.2 Physical Characteristics

Mean values for physical characteristics of the winter flounder collected in 2011 are reported in Table 3-1. These values reflect the project requirement to collect sexually mature specimens (>30cm total length). Age ranged from 4.8 to 5.7 years across the stations. Standard length ranged from a mean of 291 to 300 cm and total length from 354 to 366 cm; weight ranged from a mean of 494 to 579 g.

Between-year comparison of mean age (Figure 3-2) showed a continuation of an upward trend since 2008 except for DIF which showed a mild reversal. Standard length (Figure 3-3) showed an increase for OS, but a mild decrease for the other sites. Weights (Figure 3-4) increased at OS but decreased at all other stations relative to recent years. Percent females (Figure 3-5) were low compared to historical levels except at ECCB.

Table 3-1. Summary of Physical Characteristics of Winter Flounder Collected in 2011.

Parameter	DIF			ECCB			NB			OS		
	Mean	STDDEV	N	Mean	STDDEV	N	Mean	STDDEV	N	Mean	STDDEV	N
Age (years)	4.8	1.0	49	5.4	1.0	50	5.0	1.1	50	5.7	1.5	49
Standard Length (mm)	297.3	36.5	50	291.9	24.6	50	291.3	25.0	50	299.6	28.2	50
Total Length (mm)	361.7	42.4	50	354.9	28.4	50	355.8	30.7	50	365.9	32.1	50
Weight (g)	574.0	241.6	50	505.2	124.0	50	494.0	111.1	50	579.4	163.4	50

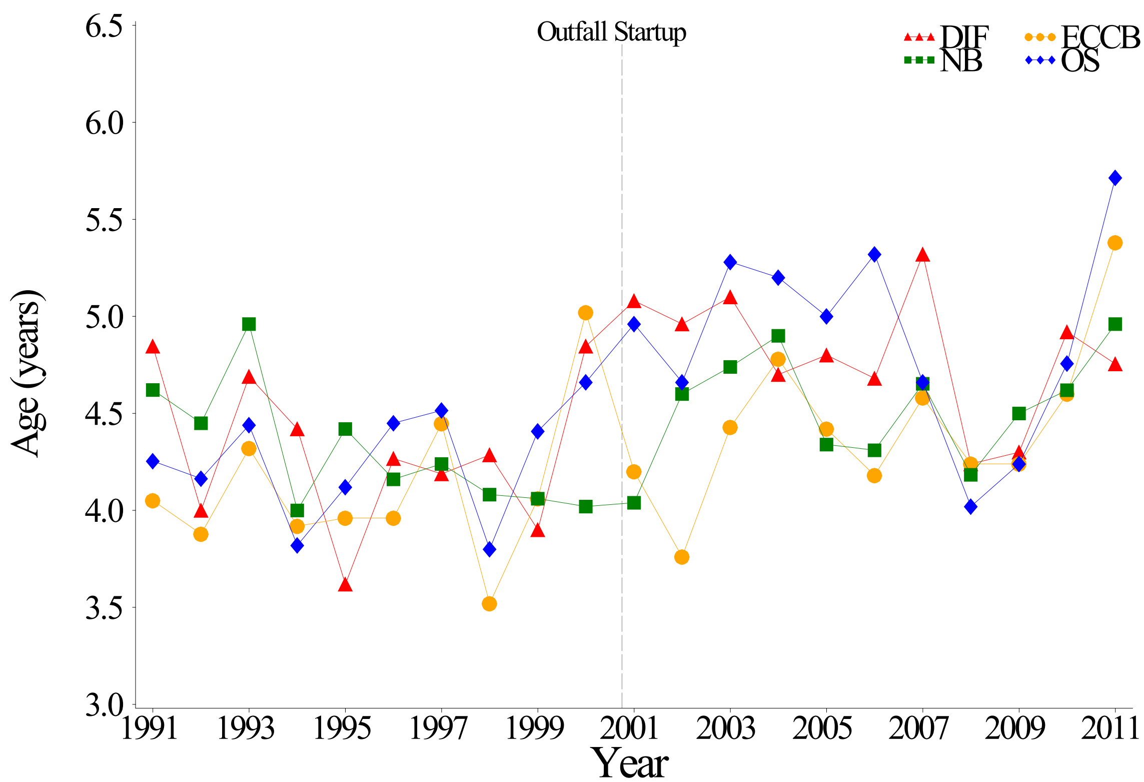

Figure 3-2. Average flounder age (years) compared by station and year.

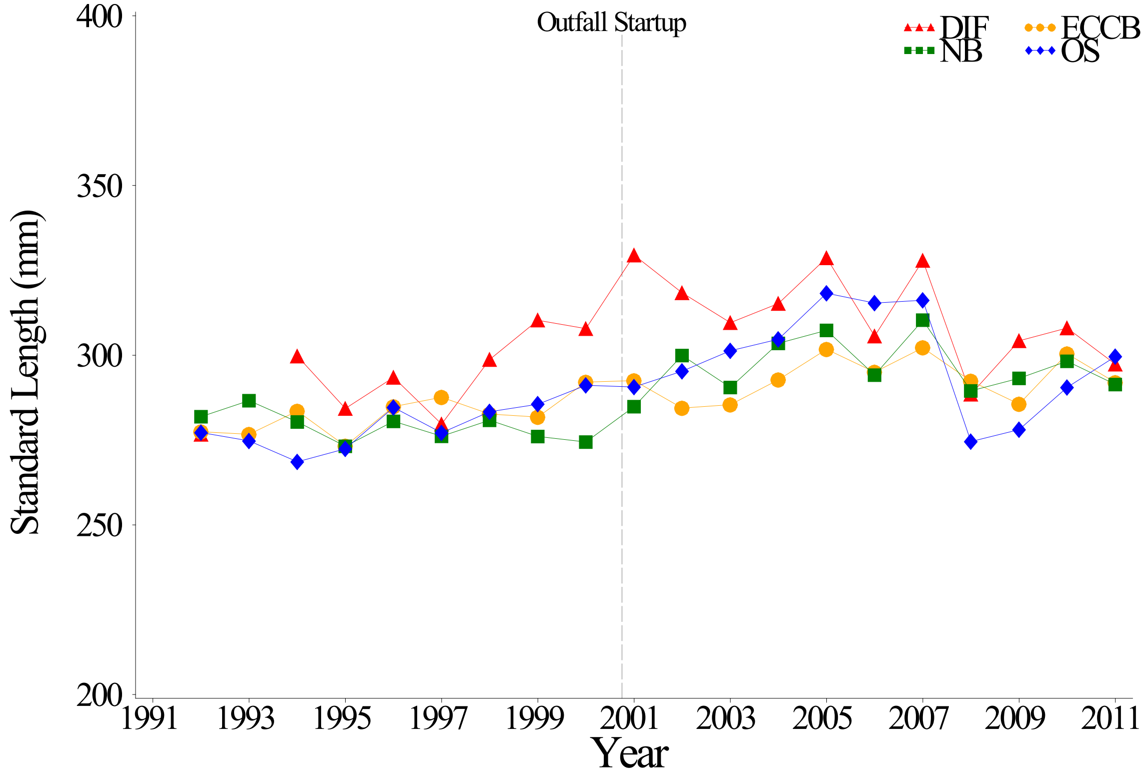

Figure 3-3. Average flounder standard length (mm) compared by station and year.

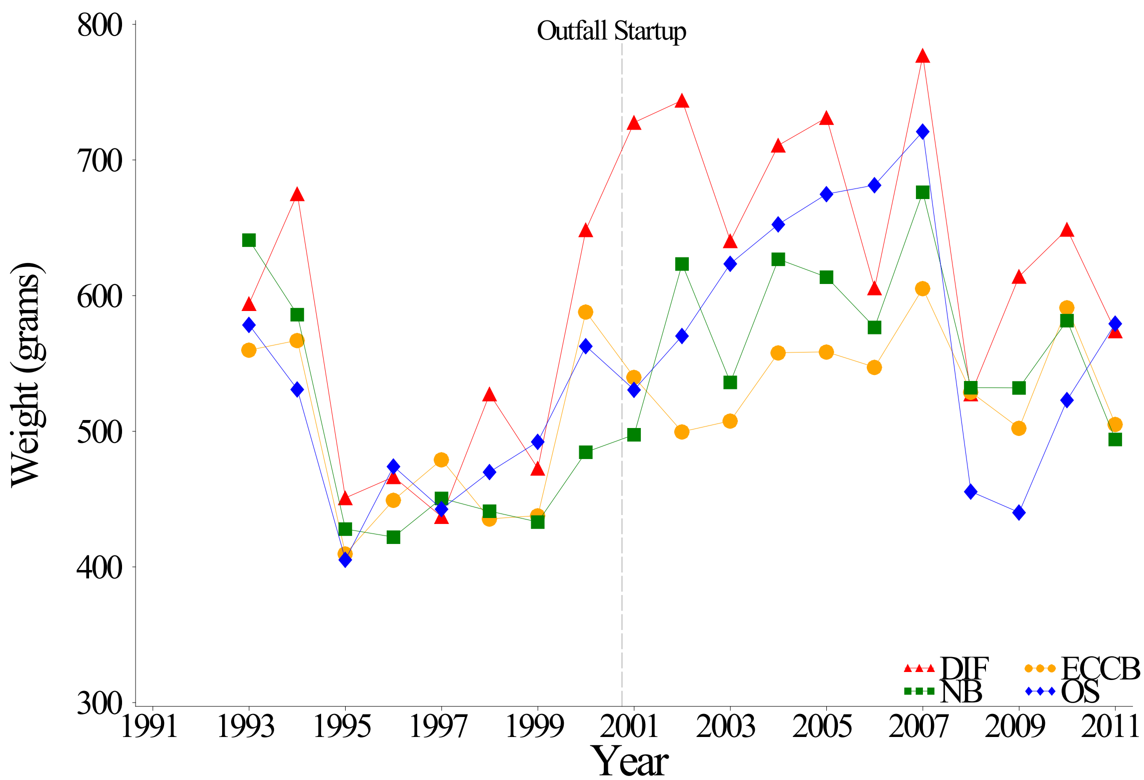

Figure 3-4. Average flounder weight (grams) compared by station and year.

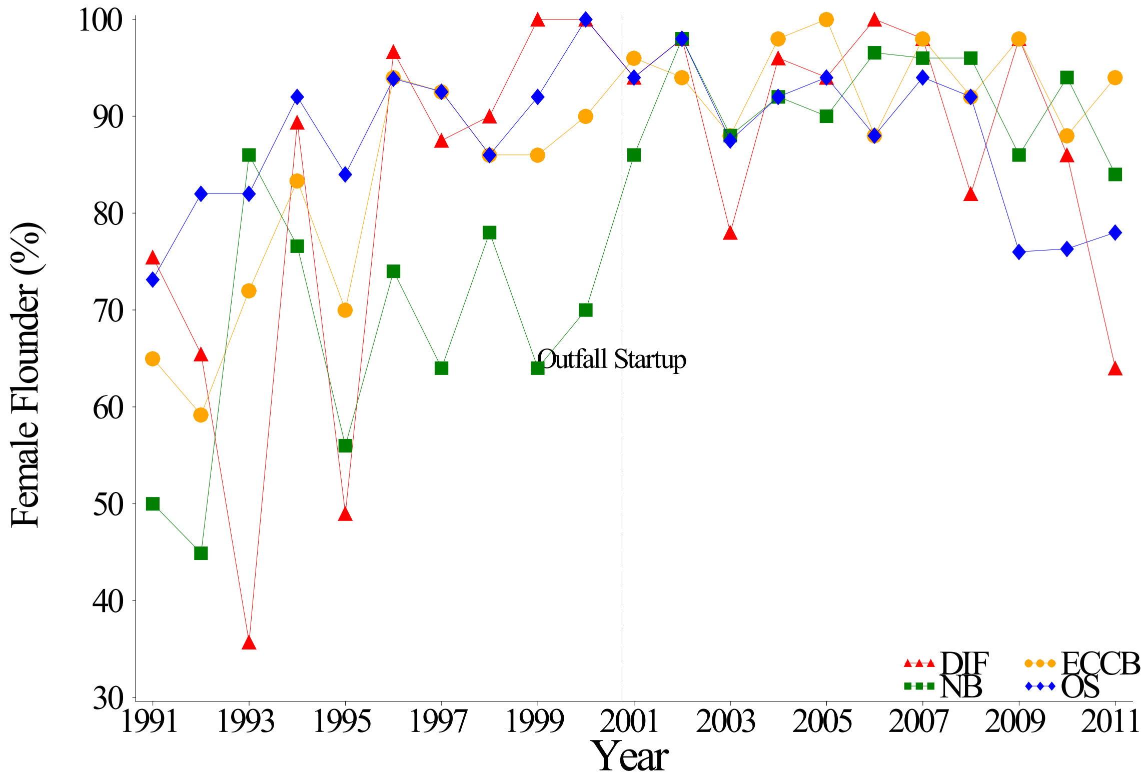

Figure 3-5. Proportion of female flounder compared by station and year.

3.3 External Condition

The external conditions of winter flounder collected in 2011 are presented as prevalence (% of individuals) per station in Table 3-2. Bent fin ray ranged from 16 to 24%, being highest at DIF and NB. The prevalence of blind side ulcers was higher in 2011 than in recent years at all stations except NB where they were absent: 2% at ECCB, 12% at DIF and 20% at OS. Fin erosion ranged from 20 to 28%, being highest at ECCB. Lymphocystis ranged from 22 to 40%, being highest at OS.

Table 3-2. Prevalence (%) of External Conditions Assessed for Winter Flounder Collected in 2011.

External Conditions	Station (Sample size)			
	DIF (50)	ECCB (50)	NB (50)	OS (50)
Bent Fin Ray	24	16	24	22
Blind Side Ulcers	12	2	0	20
Fin Erosion (Fin Rot)	26	28	22	20
Lymphocystis	22	22	26	40

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. By 2007 the elevated levels observed from 2003-06 decreased and remained low through 2010 (Figure 3-6). In 2011, ulcers were again observed at OS and DIF and for the first time an ulcerated fish was observed at ECCB.

The results of additional lab and field studies were unable to determine a cause for the ulcers (Moore 2003 and Moore et al., 2004). OMSAP review of this material in 2004-05 and again in 2011 resulted in no requests for further study other than to continue careful tracking of this condition during future flounder surveys.

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 3-7. Fin erosion fell at DIF and ECCB; climbed slightly at OS and NB; and was comparable across all stations.

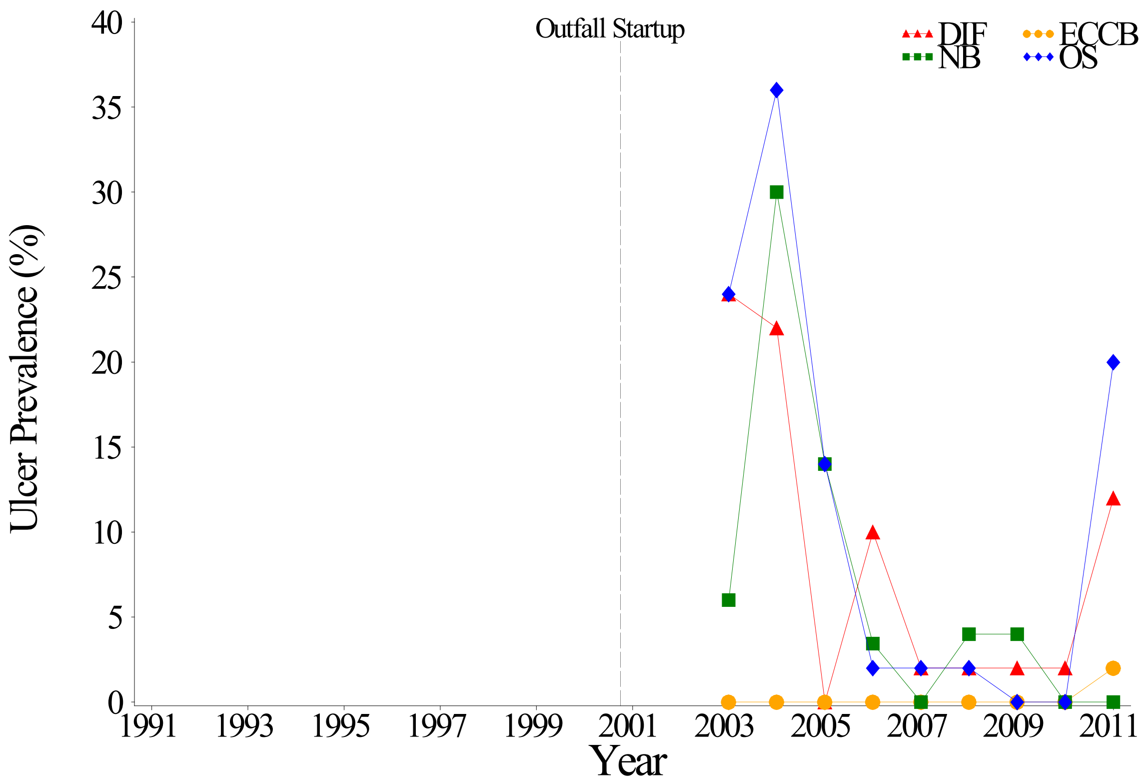

Figure 3-6. Temporal comparison of blind side ulcer prevalence (%) in winter flounder by station.

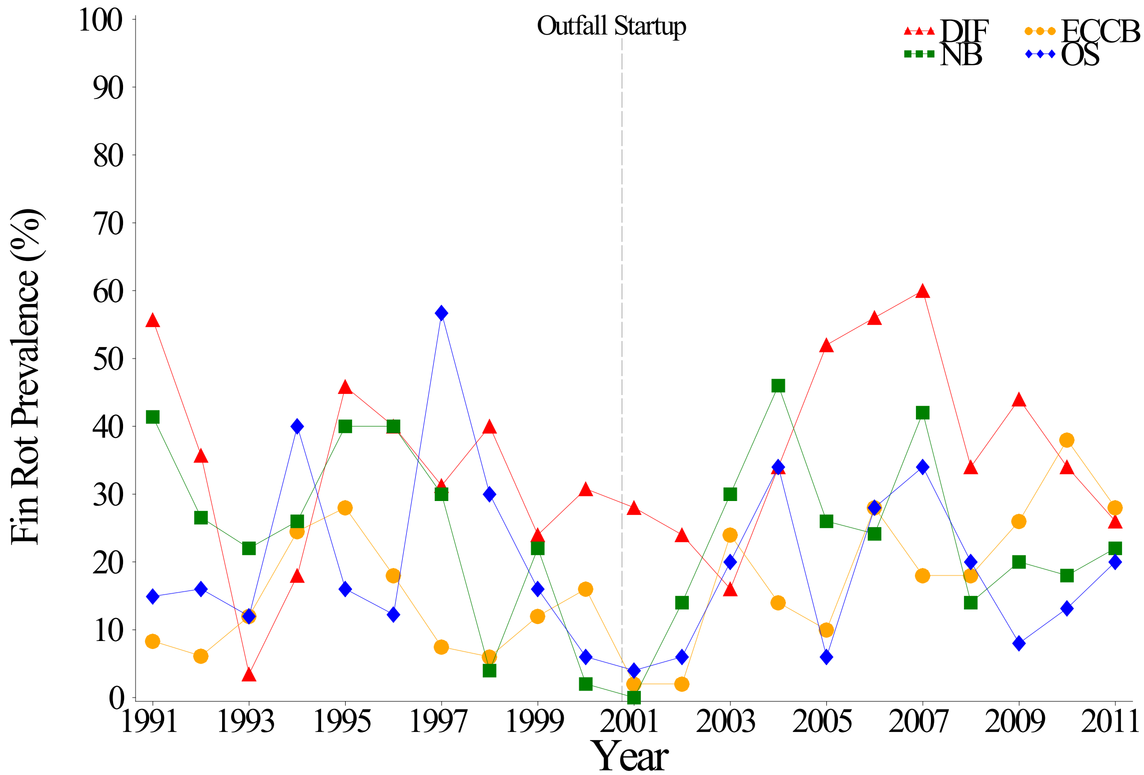

Figure 3-7. Temporal comparison of fin rot prevalence (%) in winter flounder by station.

3.4 Liver Lesion Prevalence

The prevalence (% of individuals) of liver lesions in winter flounder from each of the four stations sampled in 2011 is presented in Table 3-3. Balloons ranged from 4 to 26%, bile duct protozoa were absent at all stations, biliary proliferation ranged from 8 to 20%, centrotubular vacuolation ranged from 2 to 22% being lowest at ECCB and highest at DIF, focal hydropic vacuolation was absent at all sites, and liver flukes ranged from 0 to 2 %. Macrophage aggregation ranged from 52 to 80%, neoplasia was absent at all sites, and tubular hydropic vacuolation ranged from 2 to 16%.

Table 3-3. Prevalence (%) of Liver Lesions in Winter Flounder Collected in 2011.

Liver Conditions	Station (Sample size)			
	DIF (50)	ECCB (50)	NB (50)	OS (50)
Balloons	4	4	6	26
Bile Duct Protozoan	0	0	0	0
Biliary Proliferation	20	8	12	10
Centrotubular Hydropic Vacuolation	22	2	16	20
Focal Hydropic Vacuolation	0	0	0	0
Liver Flukes	2	2	0	0
Macrophage Aggregation	52	52	58	80
Neoplasia	0	0	0	0
Tubular Hydropic Vacuolation	16	0	2	12

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 MRWA project began remains totally absent.

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 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 continued gentle low-level increase at NB, and a continued background level at ECCB.

The severity of CHV (Figure 3-10) 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.

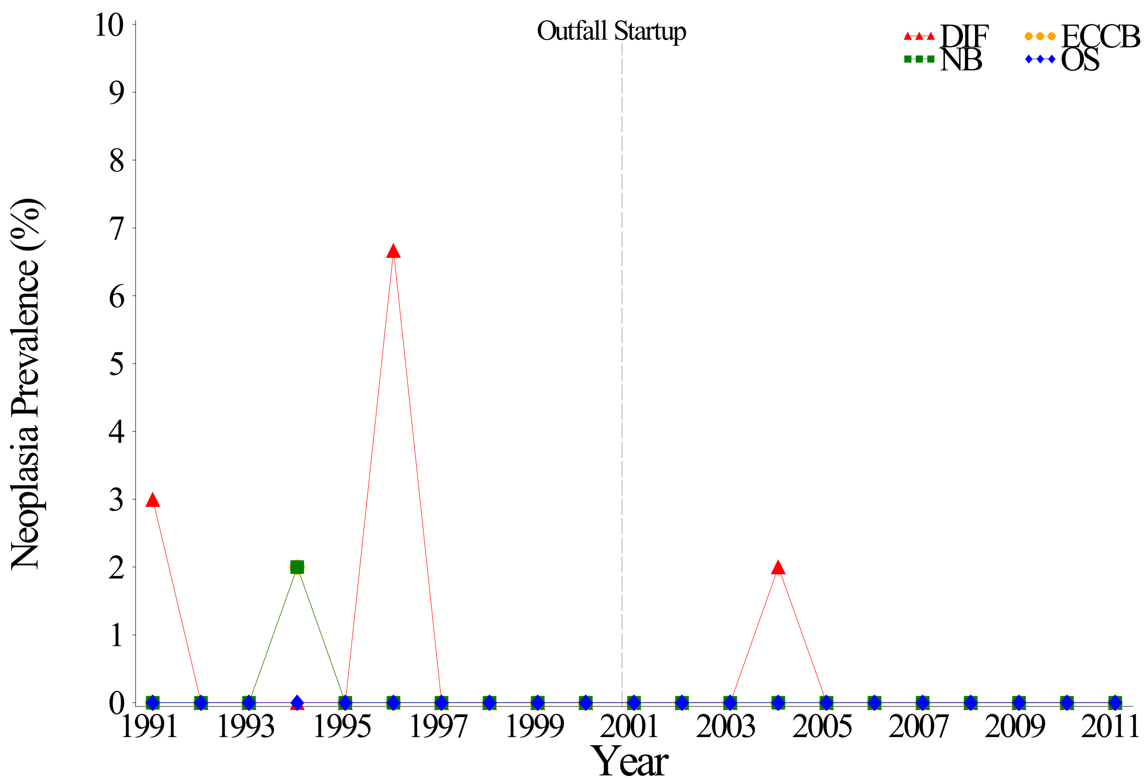

Figure 3-8. Temporal comparison of neoplasia prevalence (%) in winter flounder by station.

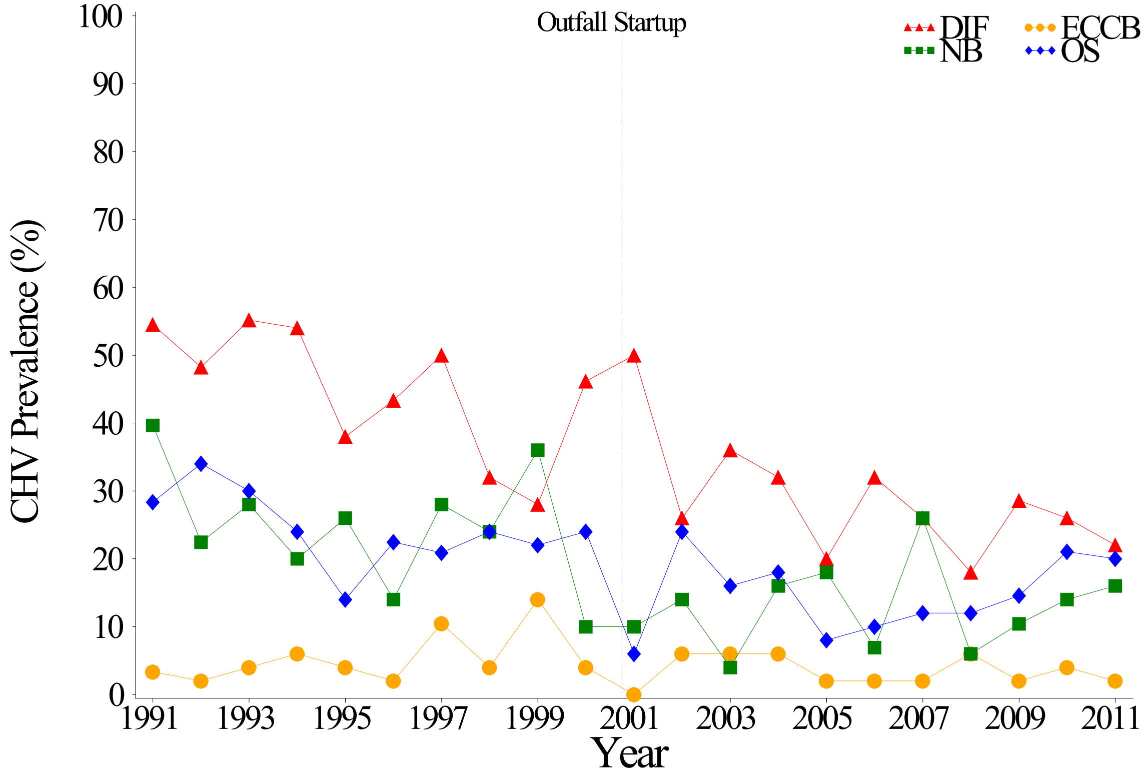

Figure 3-9. Temporal comparison of prevalence (%) of centrotubular hydropic vacuolation in winter flounder by station.

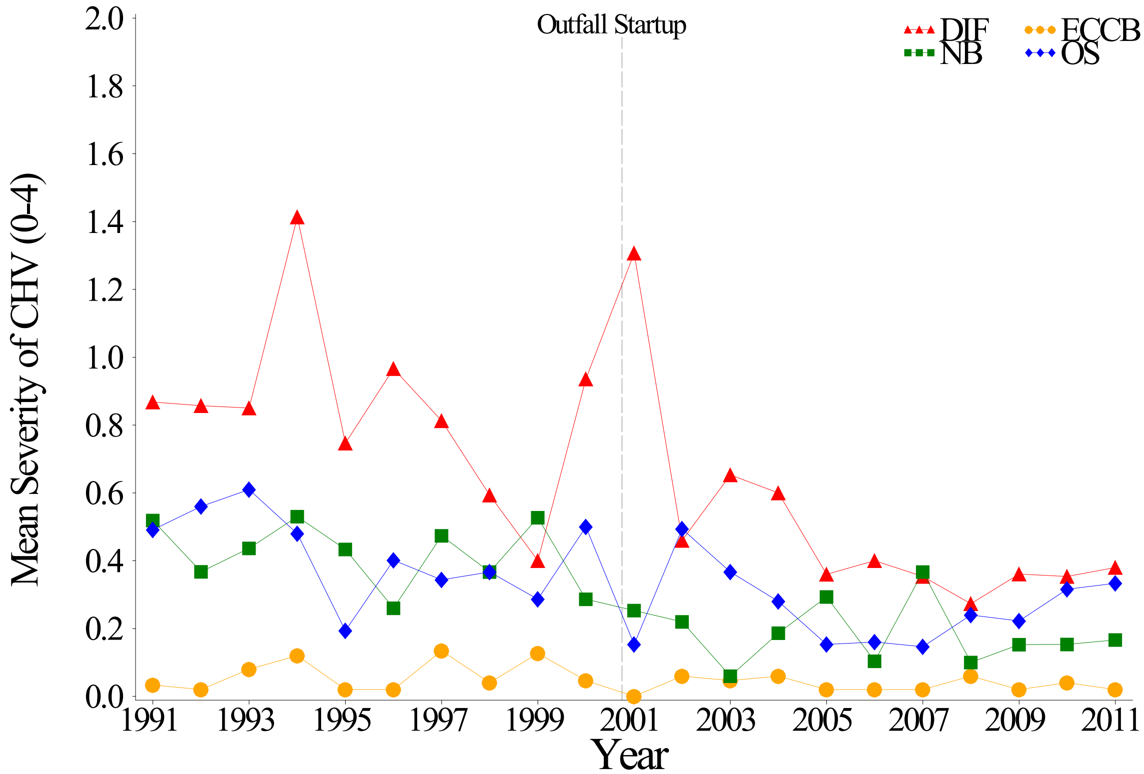

Figure 3-10.Centrotubular hydropic vacuolation severity (rank) in winter flounder compared between sites and years.

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. The remaining stations do not show this trend.

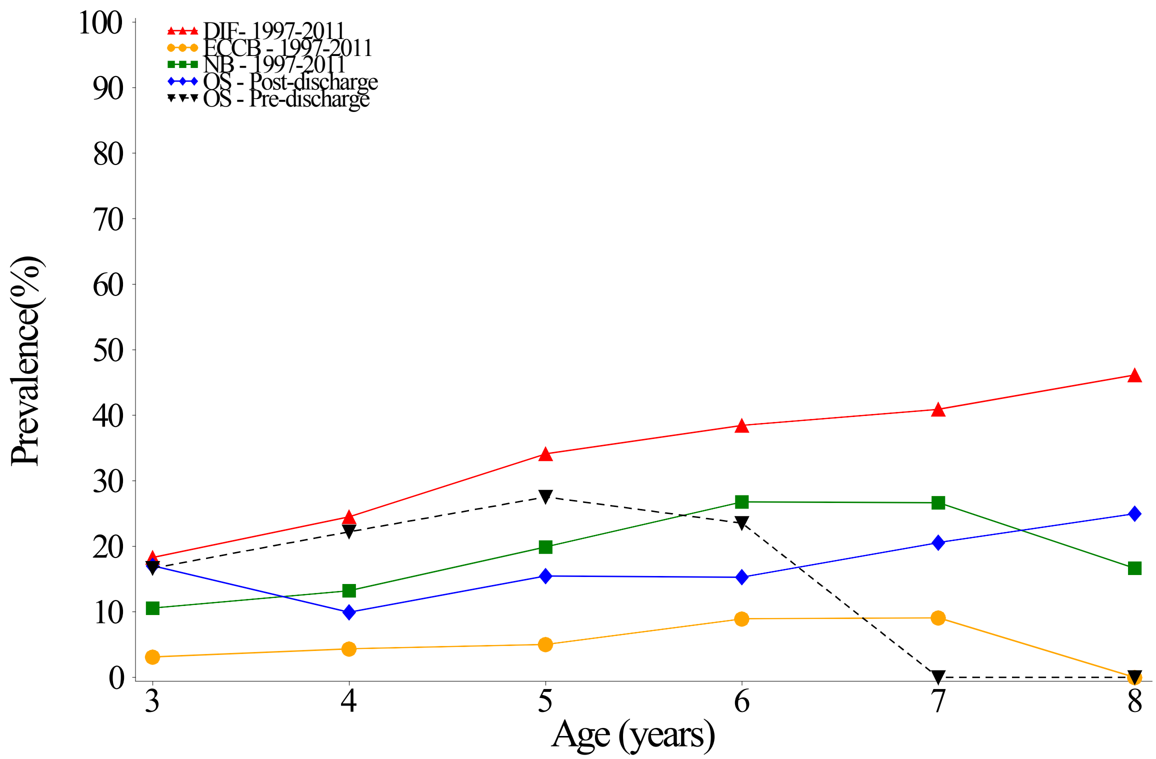

Figure 3-11. Proportion (%) of winter flounder showing hydropic vacuolation for each age.

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 trend for DIF remains downward, OS showed a reversal of a steady upward trend since 2005, NB remains low, but with recent increases since 2008 leveling off, and ECCB remains at historic background levels.

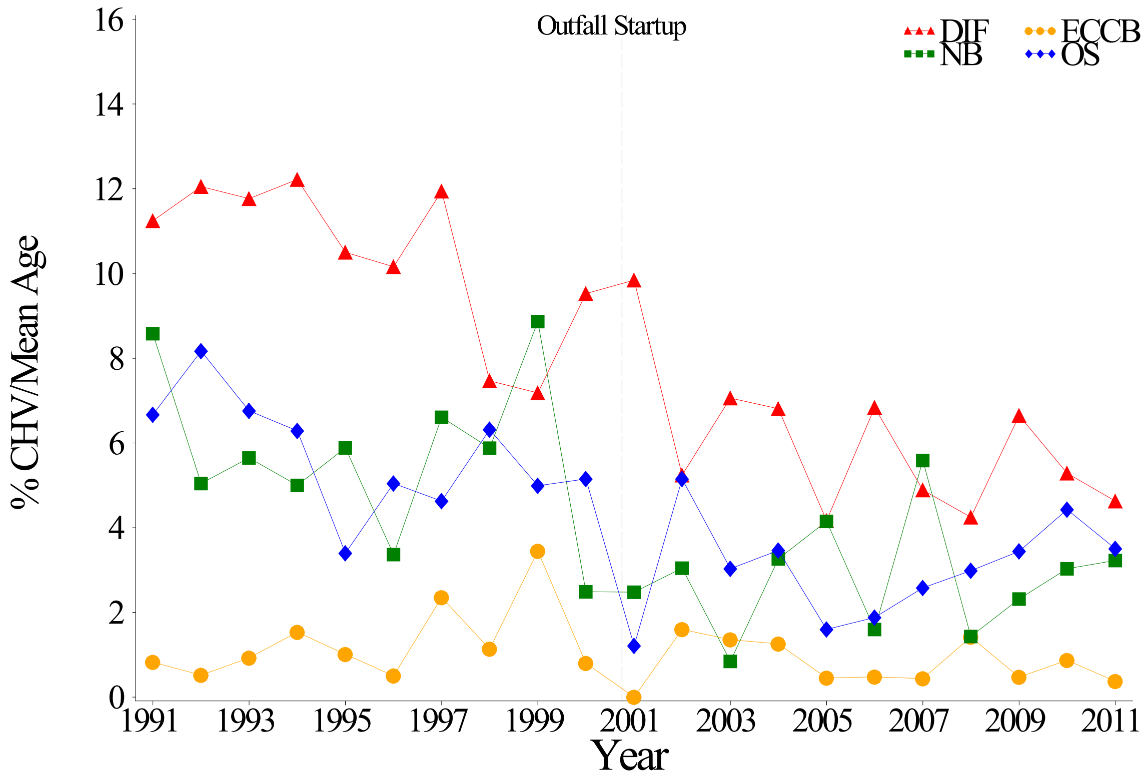

Figure 3-12. Hydropic vacuolation index (CHV%/age) for each station by year.

3.5 Threshold Comparison

The MWRA Monitoring Program has established threshold levels against which to measure key indicators of wastewater impacts (MWRA 2001). 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 2011 was 20%, well below the threshold level.

4. CONCLUSIONS

The 2011 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 climbed from the low of 2010. 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. In 2011 all stations were comparable for flounder size. The proportions of females in the populations sampled remain high at all stations except DIF which returned to a level not seen the mid 1990's.

Following increased ulcer prevalence beginning in 2003, extensive pathology and microbiology studies were unable to determine a cause of the ulcers (Moore 2003, Moore et al. 2004). Results of the 2010 survey showed a very low ulcer prevalence at OS, from 36% in 2004 to 0% in 2010. One ulcerated fish was seen, at DIF, in 2010. However, in 2011 this trend was again reversed with significant prevalence at DIF and OS and with an ulcerated fish at ECCB for the first time. The MWRA should continue careful tracking of this condition.

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 (Moore et al. 1996). The most notable trend has been that of the slow rise of age-corrected CHV at the OS site, which was gently reversed in 2011 (Figure 3-12).

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