Flounder Monitoring Report: 2012 Results

Massachusetts Water Resources Authority

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Massachusetts Water Resources Authority
Environmental Quality Department
100 First Avenue
Charleston Navy Yard
Boston, MA 02129
(617) 242-6000

prepared by

Michael Moore[1]
Eric C. Nestler[2]
Ann E. Pembroke[2]

[1]Woods Hole Oceanographic Institution
Woods Hole, MA 02543

[2]Normandeau Associates, Inc.
25 Nashua Road
Bedford, NH 30110

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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. Since then sampling is now done every third year (implemented in 2012), except for flounder morphology and histopathology, which remain on an annual schedule.

This report presents morphology and histopathology results for the 2012 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 2012 data represent the twelfth 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 2012, along with comparisons to historical flounder data, are presented in Section 3. Finally, conclusions drawn from the 2012 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 2012 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 2012 flounder survey was conducted between April 24 and 26, 2012. 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 2012 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 biologist Eric Rydbeck, Normandeau.

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 24, when 50 fish were collected from the Nantasket Beach site and the Deer Island Site. Seven fish were collected from the Outfall site on April 24 and 43 on April 25. On April 26, 50 fish were collected from the Eastern Cape Cod Bay site. Fish were weighed and measured individually in the field. The first 15 fish from each station were placed in coolers on ice and delivered by Normandeau to Envirosystems for scale and otolith sampling and dissection for meat and liver chemical analysis in addition to liver histology. In the field, scales and otoliths were removed from each of the other 35 fish from each station for aging and livers were removed, sliced, examined and fixed.

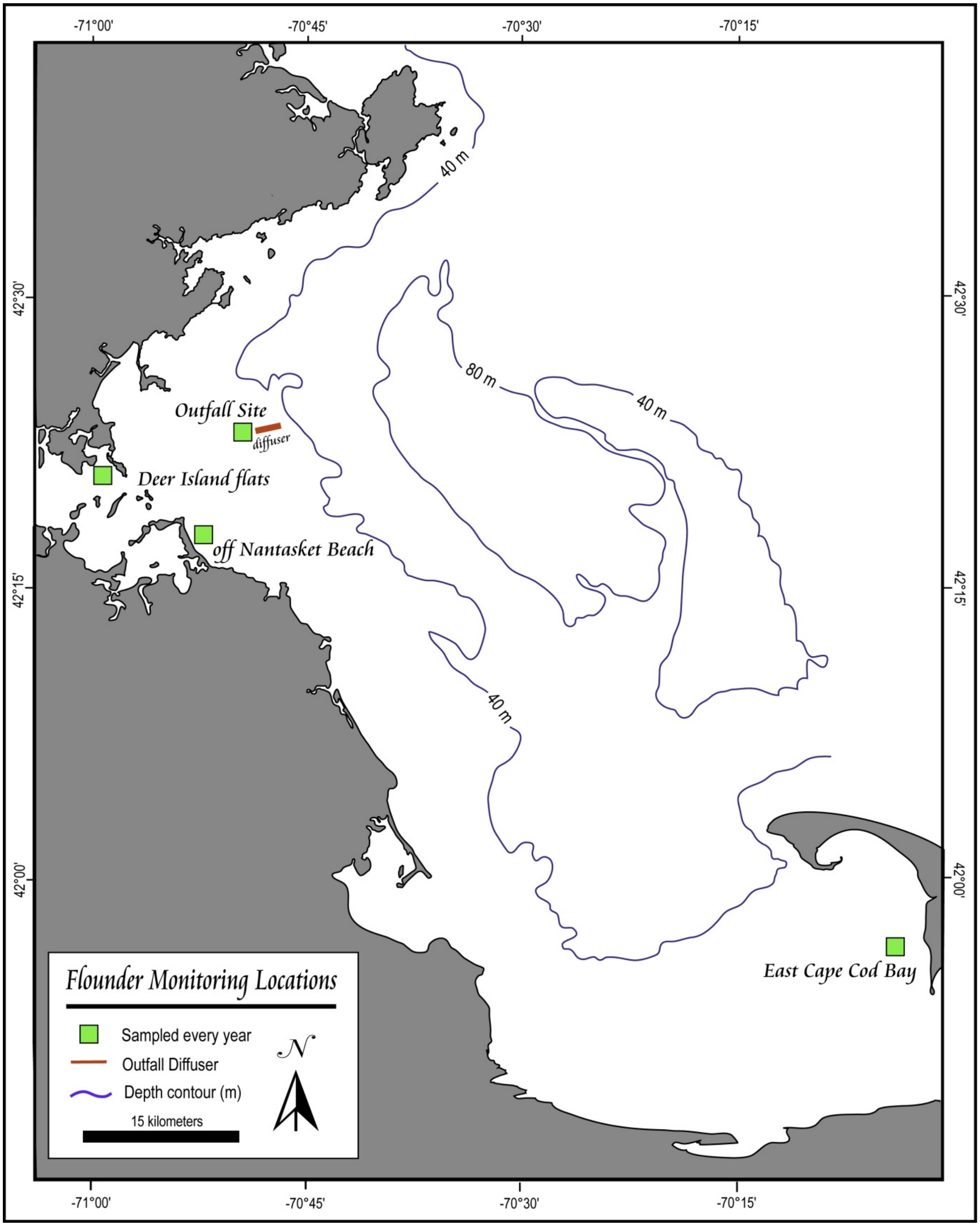

Figure 2-1. Flounder monitoring locations for 2012.

Table 2-1. Flounder Sampling Locations in 2012.

Site (Station ID)/Date/Time (Eastern Standard Time)			Actual Location		Planned Location	
			Latitude	Longitude	Latitude	Longitude
Deer Island Flats (DIF)	24-Apr-12	7:40	42.3483	-70.9683	42.34	-70.9733
		7:59	42.3508	-70.9728	42.34	-70.9733
		8:56	42.3514	-70.9737	42.34	-70.9733
East Cape Cod Bay (ECCB)	26-Apr-12	8:50	41.9468	-70.122	41.9367	-70.11
Off Nantasket Beach (NB)	24-Apr-12	10:52	42.2899	-70.8661	42.2933	-70.87
		11:44	42.2896	-70.8643	42.2933	-70.87
		12:51	42.2832	-70.8626	42.2933	-70.87
		14:03	42.2905	-70.8828	42.2933	-70.87
Outfall Site (OS)	24-Apr-12	16:04	42.3804	-70.8239	42.385	-70.8217
		16:57	42.3902	-70.8253	42.385	-70.8217
	25-Apr-12	9:16	42.3901	-70.8302	42.385	-70.8217
		10:23	42.3922	-70.8204	42.385	-70.8217
		11:08	42.3829	-70.824	42.385	-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 (2012) 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 (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.

2.4 Deviations from the QAPP

Flounder age data were estimated using both scales and otoliths during 2012. Age data reported herein are from scales, with the exception of 3 fish for which scale data were missing. Otolith data were used for two of those and the third has missing age data since both scale and otolith data were missing for that fish.

3. RESULTS AND DISCUSSION

3.1 Fish Collected

Winter flounder, each a minimum 30 cm in length, were collected between April 24 and 26, 2012, 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 at ECCB, while slightly lower than the past three years remains the highest of all stations. At NB and OS CPUE remains low, while at DIF CPUE rose to the highest level since 2005.

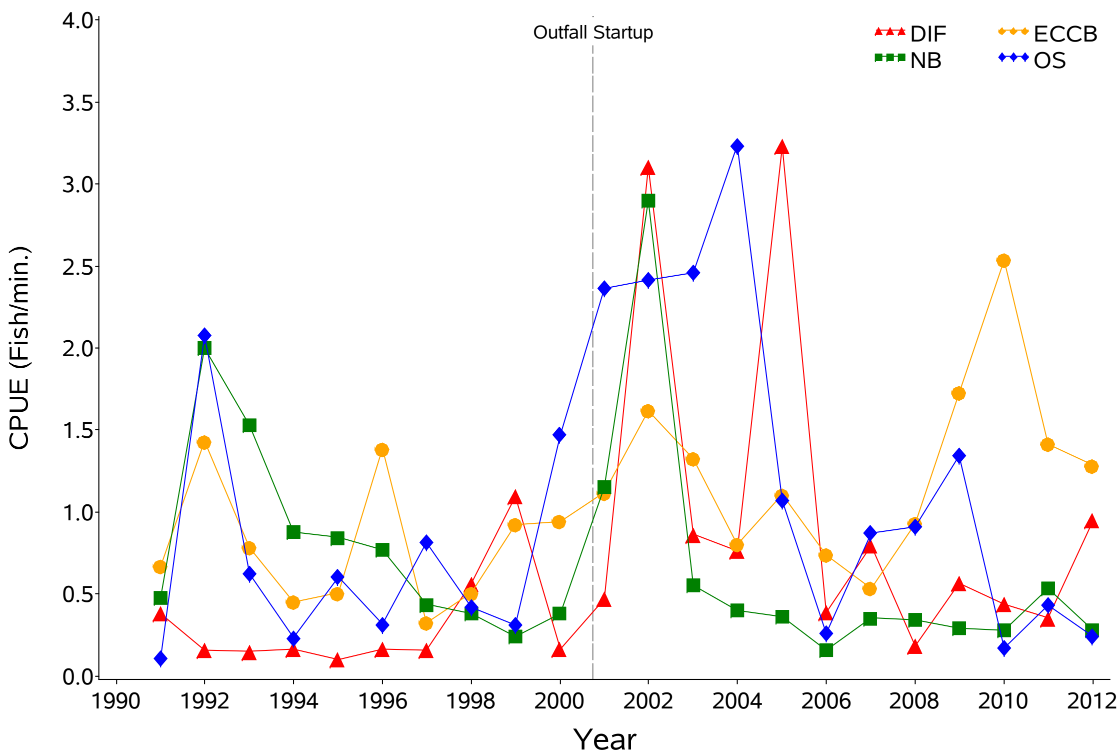

Figure 3-1. Catch Per Unit Effort (CPUE) for winter flounder trawled 1991–2012. Data for 2008 to 2012 have been normalized (see Section 3.1).

3.2 Physical Characteristics

Mean values for physical characteristics of the winter flounder collected in 2012 are reported in Table 3-1. These values reflect the project requirement to collect sexually mature specimens (>30cm total length). Age ranged from 4.3 to 5.0 years across the stations. Standard length ranged from a mean of 272 to 309 mm and total length from 336 to 377 mm; weight ranged from a mean of 447 to 607 g.

Between-year comparison of mean age (Figure 3-2) showed a reversal of an upward trend since 2008 except for DIF which increased slightly. Compared to 2011 standard length (Figure 3-3) showed a decrease for OS, increase for DIF and little change for NB and ECCB. Weights (Figure 3-4) decreased at OS, increased at DIF and were largely unchanged at ECCB and NB. Percent females (Figure 3-5) increased at all stations, especially DIF, after having fallen markedly in 2011.

Table 3-1. Summary of Physical Characteristics of Winter Flounder Collected in 2012.

	DIF			ECCB			NB			OS		
Parameter	Mean	STDDEV	N	Mean	STDDEV	N	Mean	STDDEV	N	Mean	STDDEV	N
Age (years)	4.92	0.86	49	4.98	1.17	50	4.26	0.88	50	4.68	0.77	50
Standard Length (mm)	308.6	26.03	50	293.64	29.91	50	286.4	25.73	50	272.3	22.91	50
Total Length (mm)	377.08	30.81	50	360.04	36.79	50	351.88	31.38	50	336.48	27.62	50
Weight (g)	607.1	140.76	50	529	159.71	50	501.6	129.97	50	447.2	126.6	50

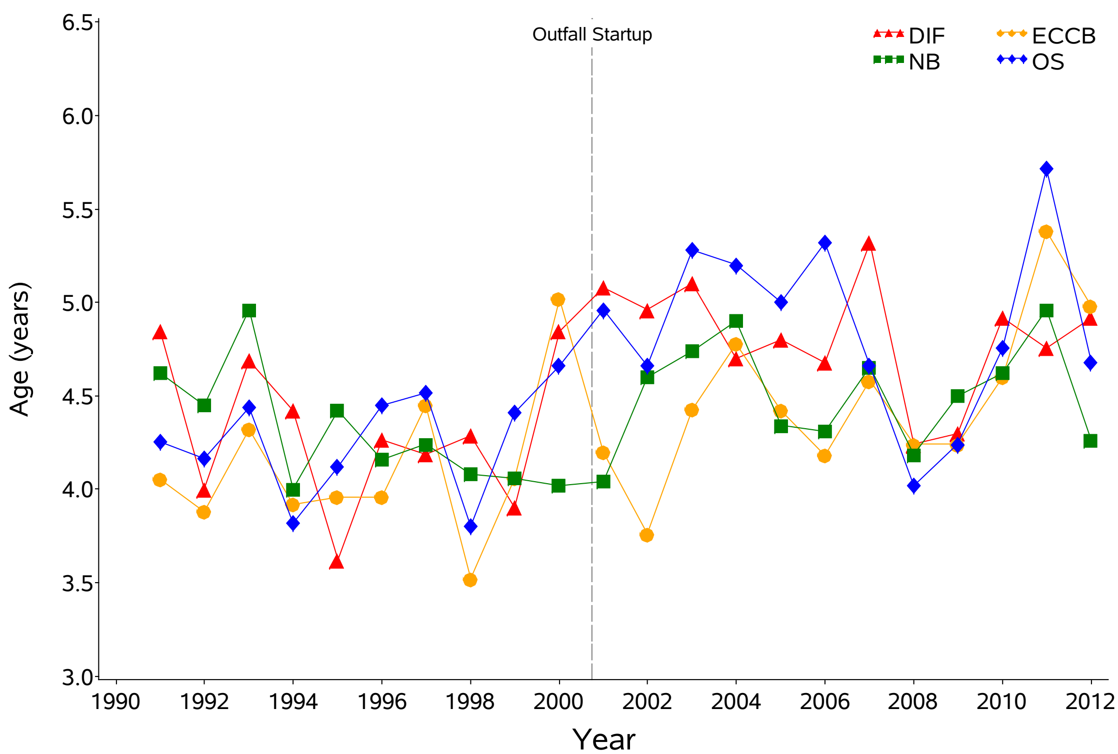

Figure 3-2. Average flounder age (years) compared by station and year.

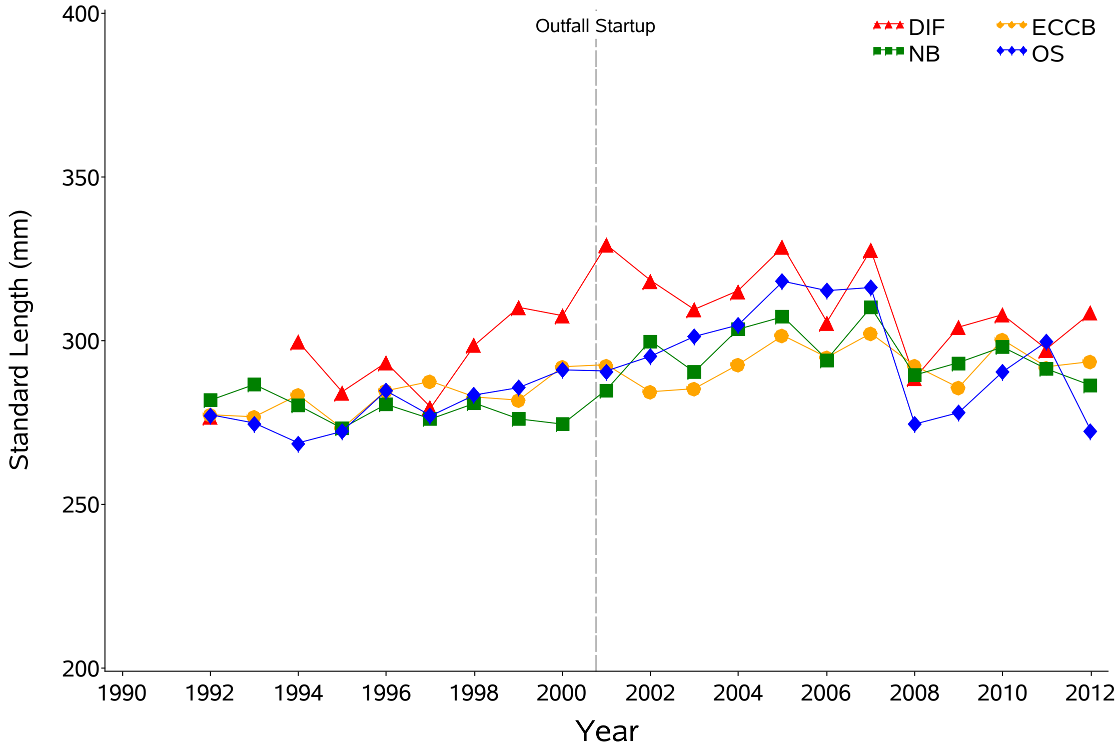

Figure 3-3. Average flounder standard length (mm) compared by station and year.

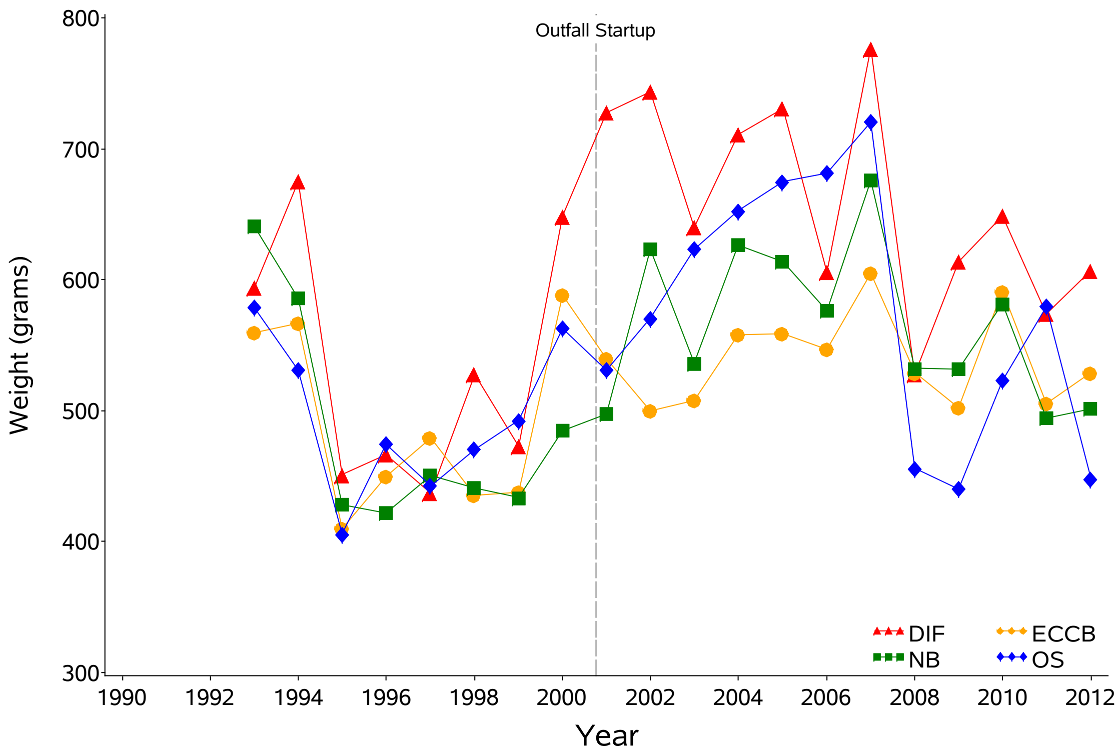

Figure 3-4. Average flounder weight (grams) compared by station and year.

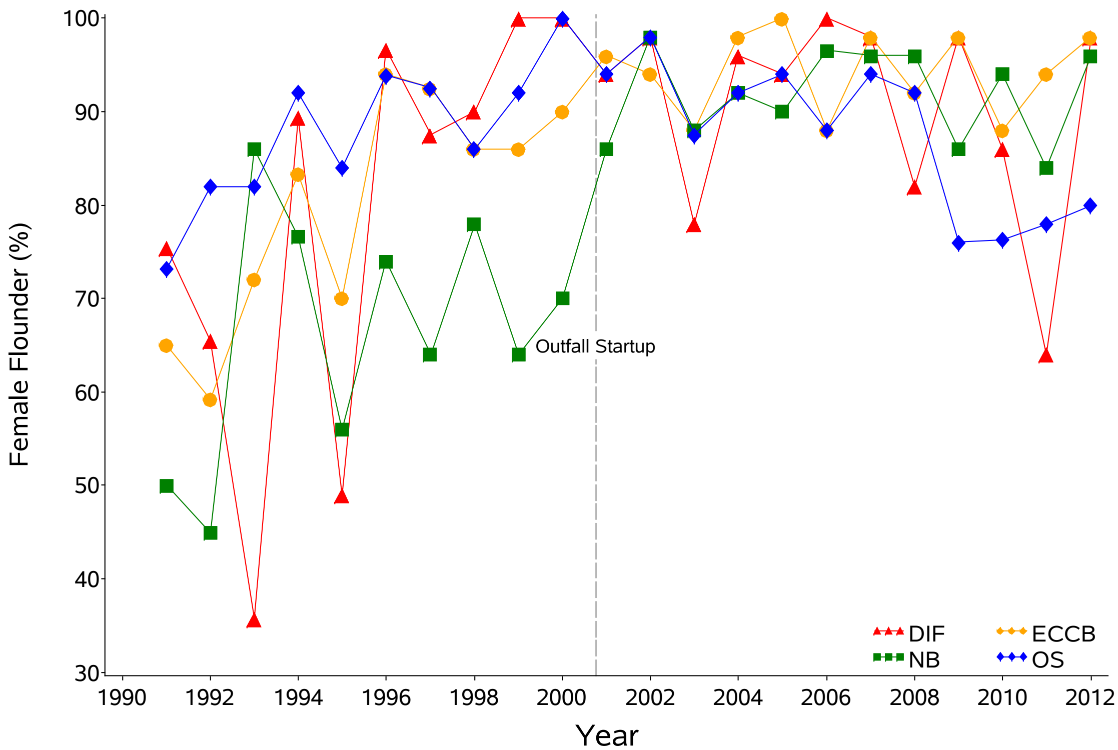

Figure 3-5. Proportion of female flounder compared by station and year.

3.3 External Condition

The external conditions of winter flounder collected in 2012 are presented as prevalence (% of individuals) per station in Table 3-2. Bent fin ray ranged from 2 to 22%, being highest at DIF. The prevalence of blind side ulcers in 2012 fell back to recent levels after a spike in 2011. Fin erosion ranged from 8 to 44%, being highest at DIF. Lymphocystis ranged from 20 to 54%, being highest at OS.

Table 3-2. Prevalence (%) of External Conditions Assessed for Winter Flounder Collected in 2012.

External Conditions	Station (Sample size)			
	DIF (50)	ECCB (50)	NB (50)	OS (50)
Bent Fin Ray	22	20	8	2
Blind Side Ulcers	0	0	4	6
Fin Erosion (Fin Rot)	44	26	22	8
Lymphocystis	34	26	20	54

Ulcer prevalence has been recorded since 2004. It is unclear if ulcers were absent prior to 2004, 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 2004-06, 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.

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. Compared to 2011 fin erosion rose at DIF, fell at OS, and remained practically unchanged at NB and ECCB.

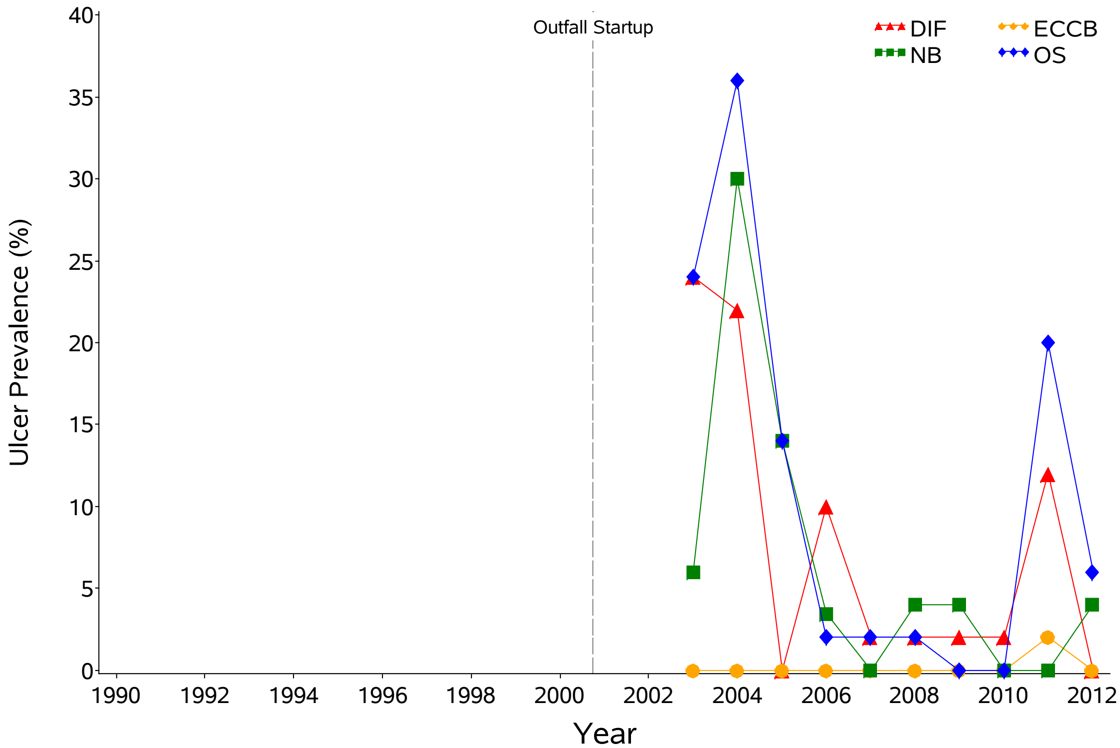

Figure 3-6. Temporal comparison of blind side ulcer prevalence (%) in winter flounder by station.

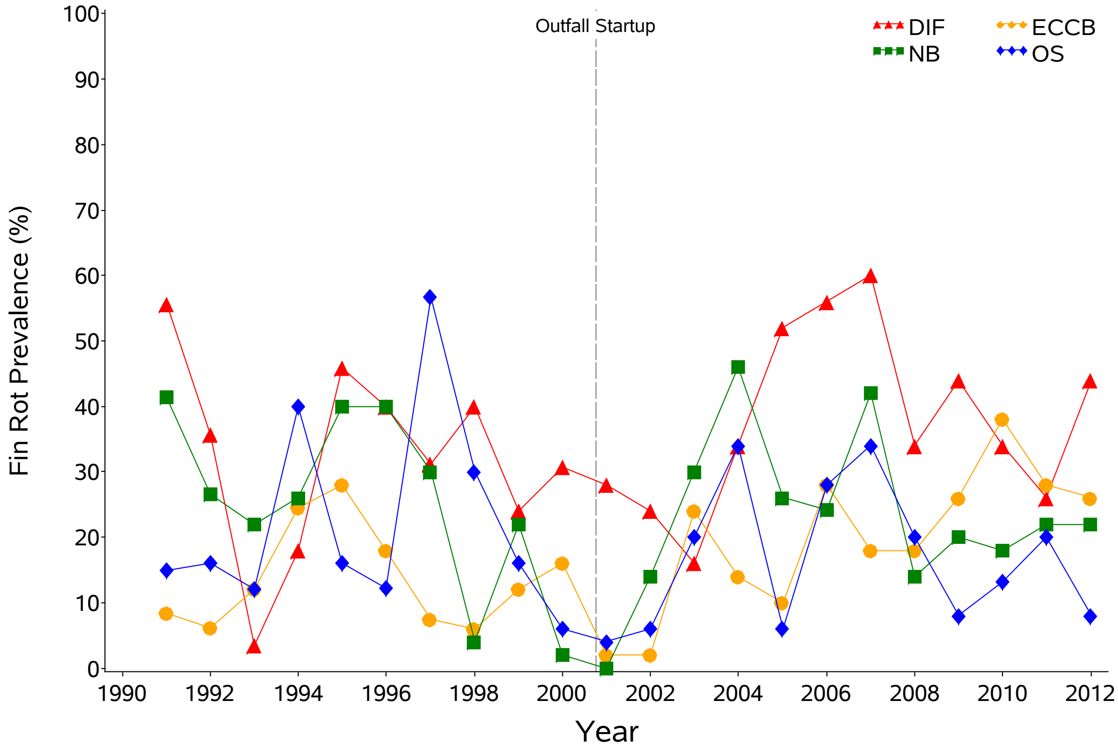

Figure 3-7. Temporal comparison of fin rot prevalence (%) in winter flounder by station.

3.4 Liver Lesion Prevalence

The prevalence (% of individuals) of liver lesions in winter flounder from each of the four stations sampled in 2012 is presented in Table 3-3. Balloons ranged from 2 to 8%, bile duct protozoa were absent at all stations, biliary proliferation ranged from 4 to 26%, centrotubular vacuolation ranged from 6 to 24% 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 50 to 70%, neoplasia was absent at all sites, and tubular hydropic vacuolation ranged from 0 to 14%.

Table 3-3. Prevalence (%) of Liver Lesions in Winter Flounder Collected in 2012.

Liver Conditions	Station (Sample size)			
	DIF (50)	ECCB (50)	NB (50)	OS (50)
Balloons	2	2	8	8
Bile Duct Protozoan	0	0	0	0
Biliary Proliferation	14	4	8	26
Centrotubular Hydropic Vacuolation	24	6	20	12
Focal Hydropic Vacuolation	0	0	0	0
Liver Flukes	0	0	2	2
Macrophage Aggregation	62	50	60	70
Neoplasia	0	0	0	0
Tubular Hydropic Vacuolation	14	0	8	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 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 mild reversal of the general decline in centrotubular hydropic vacuolation at DIF since 1991, a recent ongoing gentle increase at OS reversed in 2012 and remains 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 NB, a drop at OS, and a background low level at ECCB.

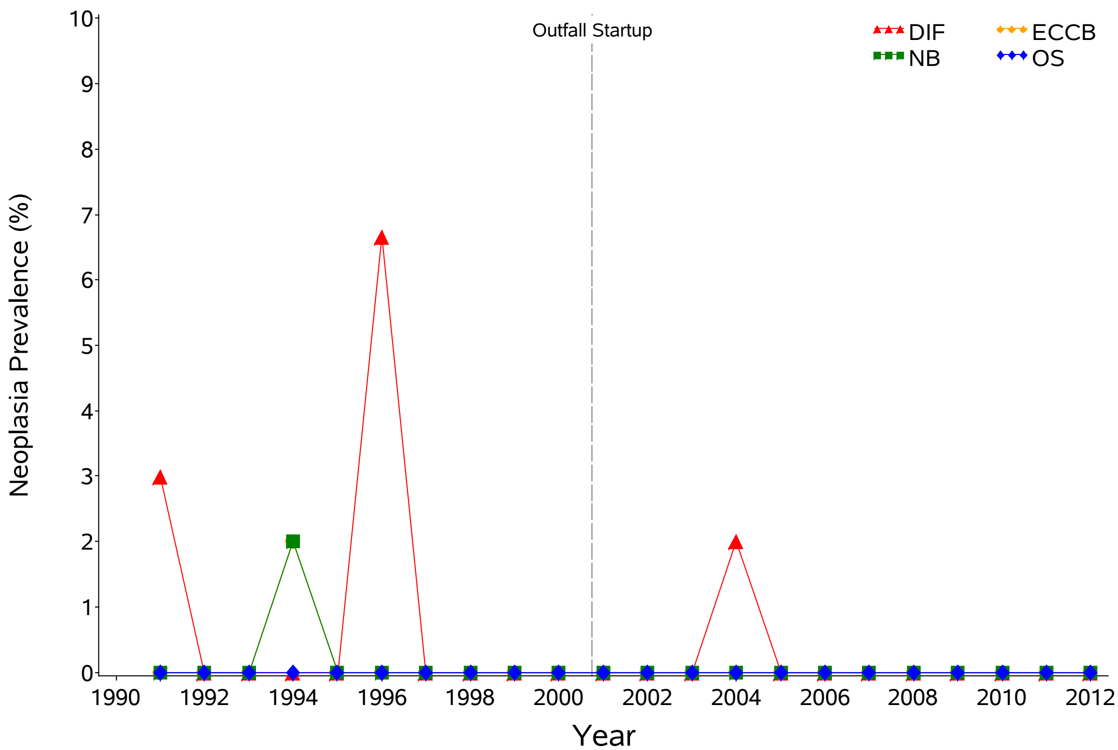

Figure 3-8. Temporal comparison of neoplasia prevalence (%) in winter flounder by station.

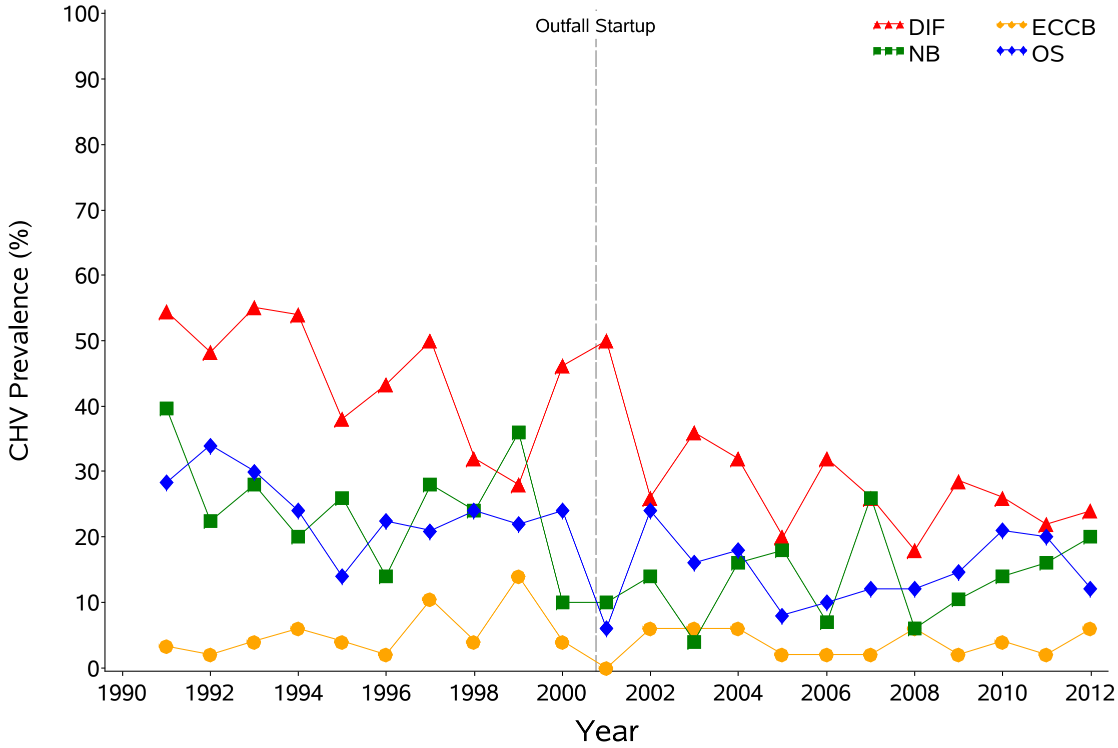

Figure 3-9. Temporal comparison of prevalence (%) of centrotubular hydropic vacuolation in winter flounder by station.

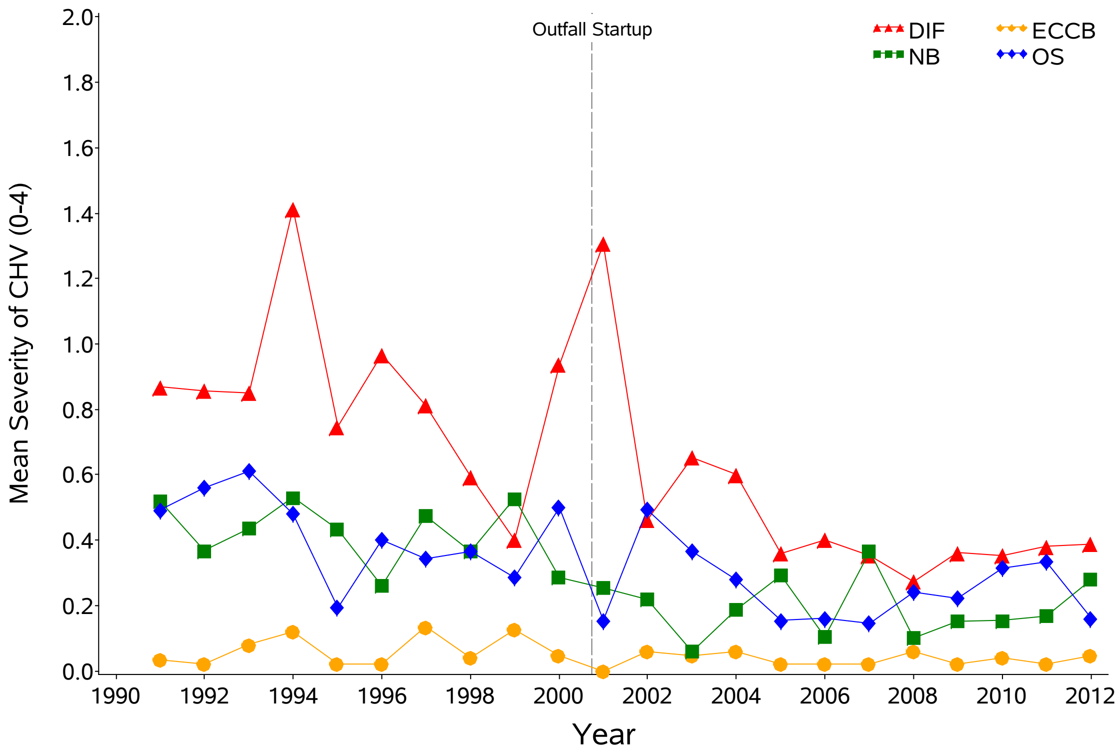

Figure 3-10.Centrotubular hydropic vacuolation severity (rank) in winter flounder compared between sites and years.

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. A lesser increase is perhaps seen at OS. NB and ECCB do not show this trend.

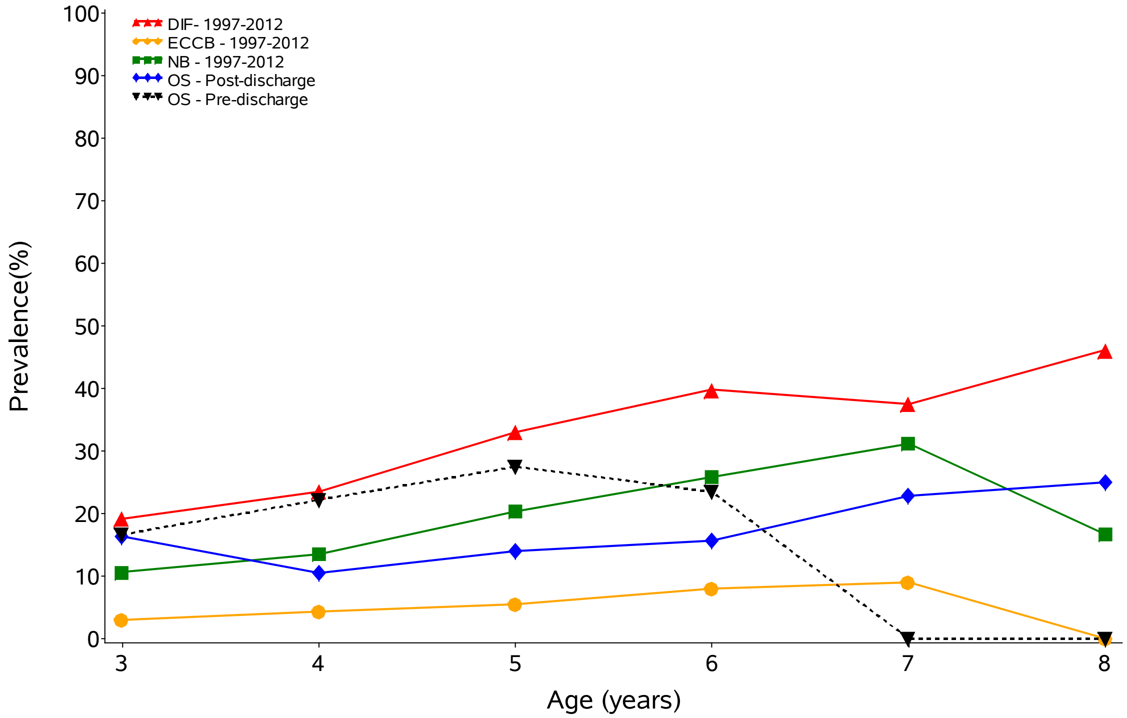

Figure 3-11. Proportion (%) of winter flounder showing hydropic vacuolation for each age.

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, OS continued a 2011 reversal of a steady upward trend since 2005, NB has shown recent increases since 2008, and ECCB remains at historic background levels.

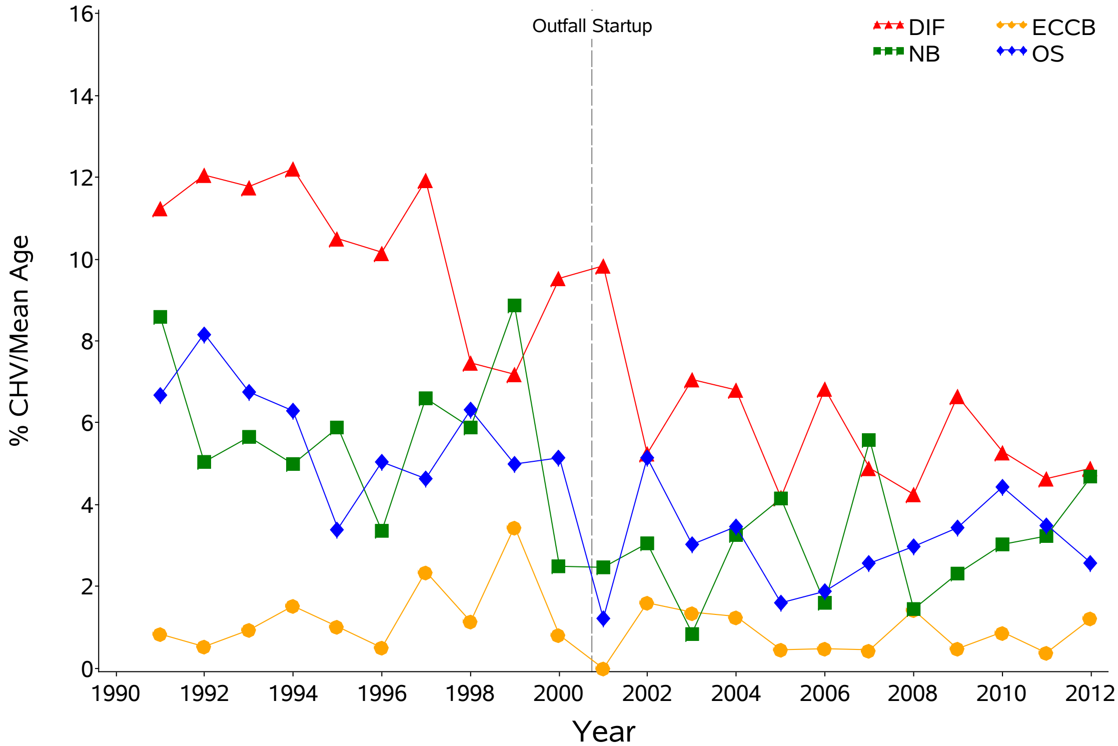

Figure 3-12. Hydropic vacuolation index (CHV%/age) for each station by year.

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 2012 was 12%, well below the threshold level.

4. CONCLUSIONS

The 2012 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 returned close to 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. In 2012 OS fish returned to historic lows. The proportions of females in the populations sampled remain high at all stations including DIF which returned to a high level after a 2011 drop.

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.

The age-corrected hydropic vacuolation prevalence suggested that there has generally 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 and 2012 (Figure 3-12).

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Massachusetts Water Resources Authority
Charlestown Navy Yard
100 First Avenue
Boston, MA 02129
(617) 242-6000
http://www.mwra.state.ma.us