2021 Flounder Monitoring Results

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EXECUTIVE SUMMARY

The detection of high prevalence of contaminant-associated liver disease such as liver tumors and centrotubular hydropic vacuolation (CHV) in winter flounder from Boston Harbor in the 1980s was one of the findings that contributed to the concern about the ecological health of the Harbor. In 1988, over 75% of flounder collected in Boston Harbor showed evidence of disease in liver tissue known to be associated with contaminant exposure, and 12% of the fish contained neoplasias (liver tumors), which also have a contaminant related etiology (Moore et al. 1996). In 2021, only 9% of flounder collected in Boston Harbor showed evidence of similar liver disease, and none contained neoplasias.

The siting of MWRA's Massachusetts Bay outfall caused concerns 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 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 health in Boston Harbor. Flounder have been collected from near the outfall and from sites in Boston Harbor, off Nantasket Beach, and in Cape Cod Bay. After consistent evidence that the outfall did not result in neoplasias or CHB in nearby flounder, the 2010 Ambient Monitoring Plan was revised in January 2021 to discontinue monitoring the two reference stations, Nantasket Beach and eastern Cape Cod Bay. However, the eastern Cape Cod Bay station was sampled in 2021 to help evaluate the addition of per- and polyfluoroalkyl substances (PFAS) contaminant analysis to the monitoring program. Flounder from each site are sampled annually for length, weight, age, biological condition, and the presence of external or internal disease. Concentrations of inorganic and organic contaminants in body tissues are determined every third year (Nestler et al. 2016; undertaken in 2018 and 2021, report in progress).

The 2021 data represent the twenty-first consecutive year of flounder monitoring since the start-up of the Massachusetts Bay outfall in September 2000. Results of the histological analyses in 2021 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 (DIF) during the past two decades, with 2021 being the lowest recorded yet.
- 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.
- The prevalence of CHV in flounder from the vicinity of MWRA's Massachusetts Bay outfall has not shown increases over levels observed during baseline monitoring. During most years since offshore discharge was initiated, prevalence has been less than that observed during the baseline monitoring before 2001. CHV prevalence increased consistently between 2005 and 2010, but since 2014 levels have been low and relatively stable (Figure 3-12). The 2021 value was the lowest yet recorded.
- There were no fish recorded with blind side ulcers at any of the stations in 2021.

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 the USEPA Environmental Impact Statement (SEIS) projections and do not exceed Contingency Plan thresholds (USEPA 1988, MWRA 2001). A detailed description of the monitoring and its rationale are provided in the Effluent Outfall Monitoring Plan developed for the baseline period and the post-discharge monitoring plan (MWRA 1997, 2004, 2010, 2021).

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. 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 1991. The full program was conducted annually until 2003. Since then, tissue contaminant monitoring is now done every third year (Nestler et al. 2016; undertaken in 2018 and 2021, report in progress). Flounder morphology and histopathology remain on an annual schedule. A summary of this and earlier studies was published by Moore et al. (2018).

This report presents morphology and histopathology results for the 2021 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 2021 data represent the twenty-first consecutive year of flounder monitoring since the start-up of the Massachusetts Bay outfall in September 2000, and the thirty-first year since the program began. 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 2021, along with comparisons to historical flounder data, are presented in Section 3. Finally, conclusions drawn from the 2021 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 winter flounder, and, by proxy, other similar species.

2. METHODS

Winter flounder (*Pseudopleuronectes americanus*) were collected from three 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 2021 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 2020–2022 (Rutecki et al. 2020).

2.1 Stations and Sampling

The 2021 flounder survey was conducted between April 27th and 28th, 2021. Three sites were sampled to collect winter flounder for histological and chemical (reported elsewhere) analyses:

- Deer Island Flats (DIF), historically impacted by contaminants
- Outfall Site (OS), to detect potential impacts from MWRA's treated wastewater
- East Cape Cod Bay (ECCB), a clean coastal reference station

Figure 2-1 shows the monitoring locations. Table 2-1 provides the planned and actual sampling sites and locations for the 2021 flounder sampling.

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

Mobilization for the survey was conducted on April 27th when 50 fish were collected from the Outfall site. On April 27th a total of 33 fish were collected from Deer Island Flats, and on April 28th there were 50 fish collected from eastern Cape Cod Bay. Fish were weighed and measured individually in the field. Scales were removed from each fish for aging and livers were removed, sliced, examined and three slices fixed.

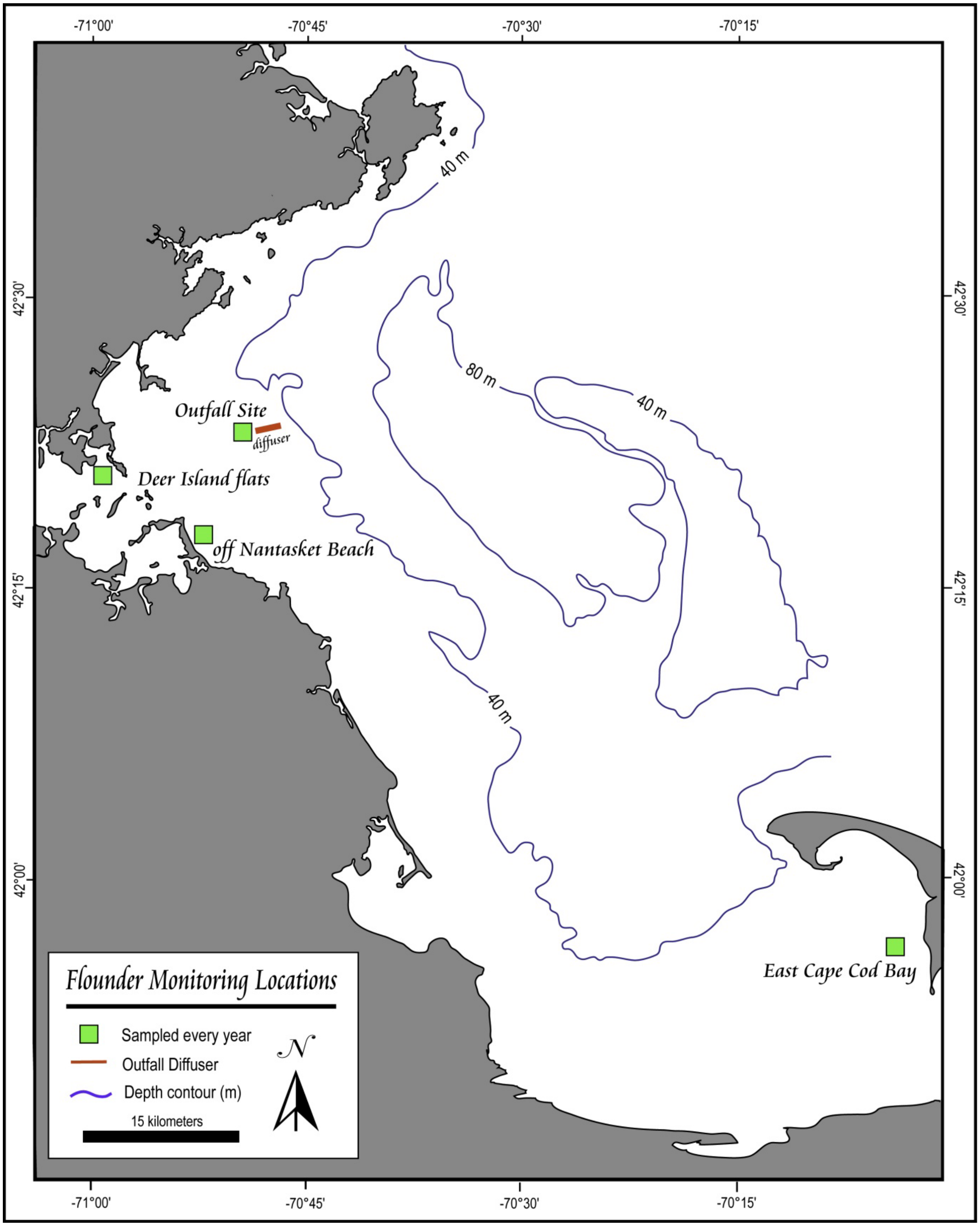

Figure 2-1. Flounder monitoring locations since 1991. The Nantasket Beach station was discontinued in 2021.

Table 2-1. Flounder Sampling Locations in 2021.

Site (Station ID)/Date/Time			Actual Location		Planned Location	
			Latitude	Longitude	Latitude	Longitude
Deer Island Flats (DIF)	27-Apr-21	11:43	42.3467	-70.9640	42.3400	-70.9733
		12:21	42.3533	-70.9750	42.3400	-70.9733
		12:52	42.3474	-70.9660	42.3400	-70.9733
		14:11	42.3469	-70.9660	42.3400	-70.9733
		15:21	42.3473	-70.9670	42.3400	-70.9733
		16:05	42.3477	-70.9660	42.3400	-70.9733
East Cape Cod Bay (ECCB)	28-Apr-21	9:03	41.9291	-70.1310	41.9367	-70.1100
Outfall Site (OS)	27-Apr-21	8:14	42.3920	-70.8350	42.3850	-70.8217
		9:30	42.3936	-70.8320	42.3850	-70.8217

2.2 Histological Analysis

Livers of 50 flounder from each site (33 at Deer Island Flats) 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 133 slides. 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 2021) were extracted directly from the HOM database and imported into SAS (version 9.3), 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 2020–2022 (Rutecki et al. 2020). 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

The cruise plan contingency for a consultation with Normandeau/MWRA after three hours of bottom time yielding less than fifty fish was invoked for the following station in 2021:

Deer Island Flats (DIF): Flounder availability on Deer Island was very low. In 3:00 (hr:min) of bottom time, 33 fish were collected at DIF.

3. RESULTS AND DISCUSSION

3.1 Fish Collected

Winter flounder, each a minimum 30 centimeters (cm) in length, were collected between April 27th and 28th, 2021, at three 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 F/V *Odessa* (70' sweep rope). For 2008, the F/V *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 F/V *Explorer 2* (84' sweep rope) was used with a net that was 1.2 x wider. Since 2009, the F/V *Harvest Moon* has been used for all stations. Thus, data presented in Figure 3-1 have been normalized to the F/V *Odessa* sweep length by using the ratio of sweep lengths as a multiplier (i.e., CPUE's for the F/V *Explorer 2* net were multiplied by 70/84, and CPUE's for the F/V *Harvest Moon* net by 70/74, to get CPUE units in Odessa equivalents). CPUE in 2021 increased for ECCB and was similar at OS compared to 2020, but decreased substantially for DIF (Figure 3-1). In accordance with changes made in 2021, no fish were collected at site NB in 2021.

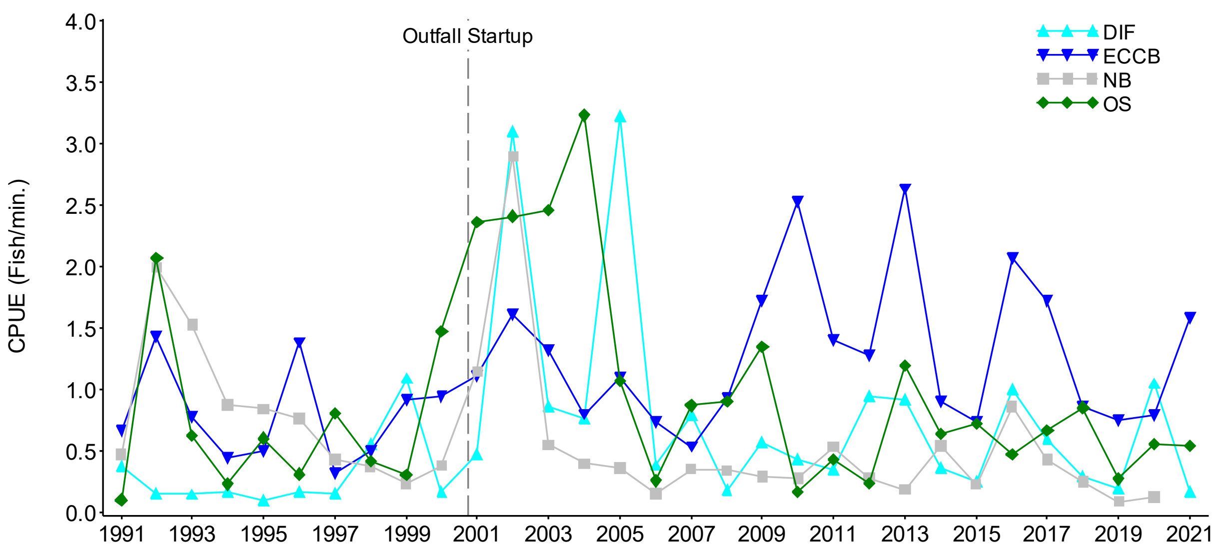

Figure 3-1. Catch Per Unit Effort (CPUE) for winter flounder trawled 1991–2021. Data for 2008 to 2021 have been normalized (see Section 3.1).

3.2 Physical Characteristics

Mean values for physical characteristics of the winter flounder collected in 2021 are reported in Table 3-1. These values reflect the project requirement to collect sexually mature specimens (>30cm total length). Mean age ranged from 4.8 to 5.5 years across the stations. Mean standard length ranged from 274 to 312 millimeters (mm) and mean total length from 333 to 377 mm; weight ranged from a mean of 394 to 663 grams (g).

Mean age in 2021 compared to 2020 (Figure 3-2) increased for DIF, OS and ECCB. Scale analysis was used for age determination since 2016 consistent with the methods followed historically for this program (Fields 1988, Rutecki et al. 2017). Otoliths were used for age determination in 2014 and 2015. Comparisons between the two methods indicate that for older fish the otolith method may provide an older age than the scale method. Compared to 2020, standard length (Figure 3-3) in 2021 was quite stable for all three stations. Weights (Figure 3-4) were comparable at all stations compared to 2020. Percent females (Figure 3-5) increased at all stations.

Table 3-1. Summary of Physical Characteristics of Winter Flounder Collected in 2021.

	DIF			ECCB			OS		
Parameter	**Mean**	**STDDEV**	**N**	**Mean**	**STDDEV**	**N**	**Mean**	**STDDEV**	**N**
Age (years)	4.78	0.94	32	5.54	1.3	50	5.14	1.06	49
Standard Length (mm)[a]	283.09	23.63	33	312.48	27.01	50	273.68	21.53	50
Total Length (mm) [a]	343.45	28.21	33	376.74	31.8	50	332.54	24.68	50
Weight (g)	490.61	141.29	33	640.5	151.02	50	441.56	103.39	50

[a] Lengths: from the most forward point of the head, with the mouth closed: to the base of the caudal fin (Standard), and to the farthest tip of the tail (Total).

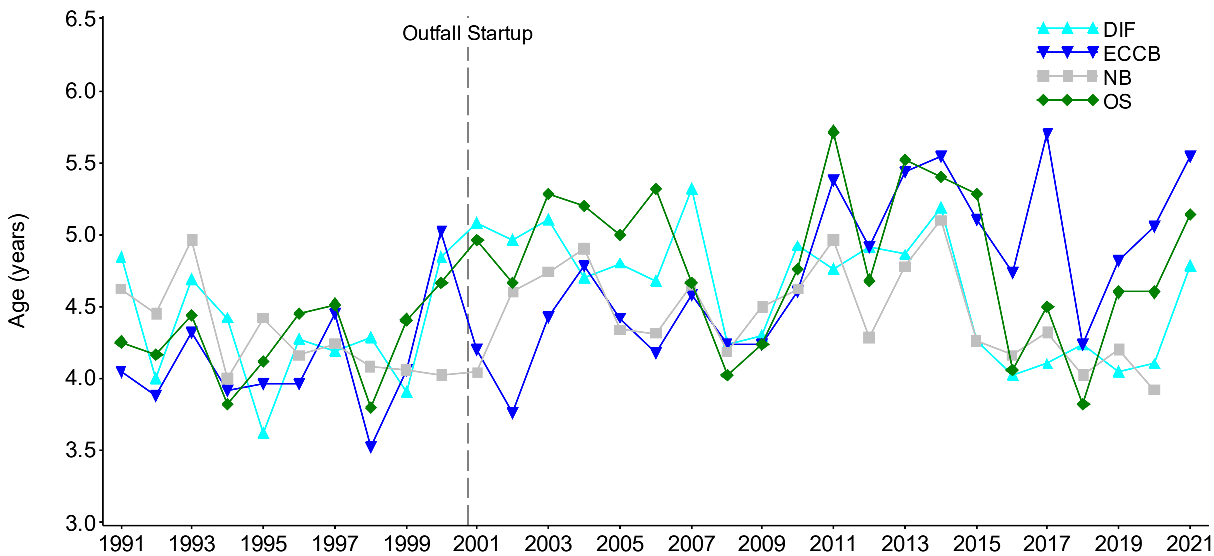

Figure 3-2. Average flounder age (years) compared by station and year.

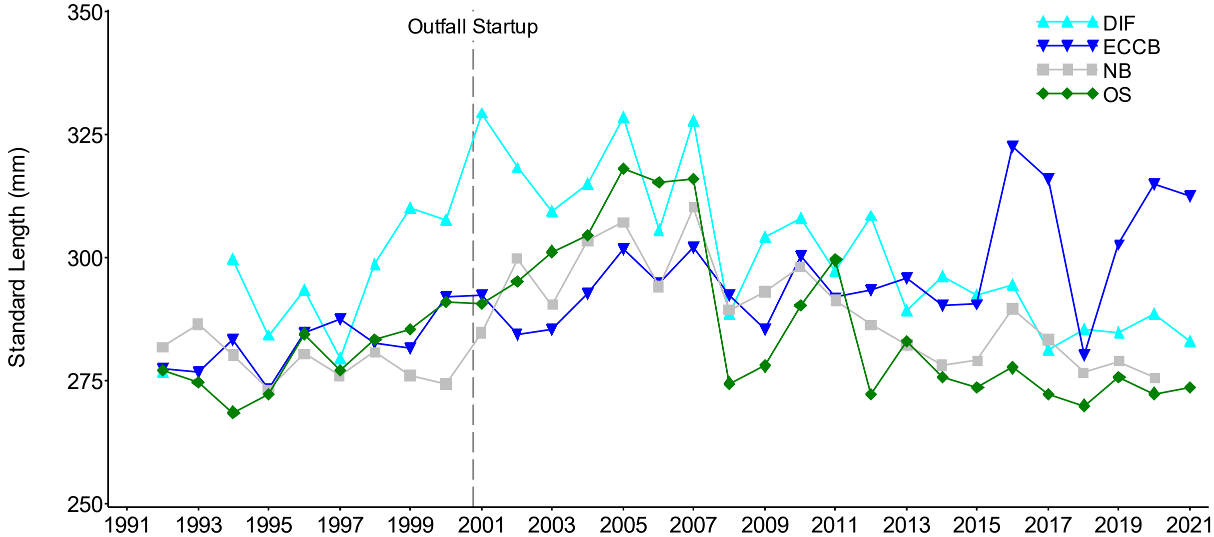

Figure 3-3. Average flounder standard length (mm) compared by station and year.

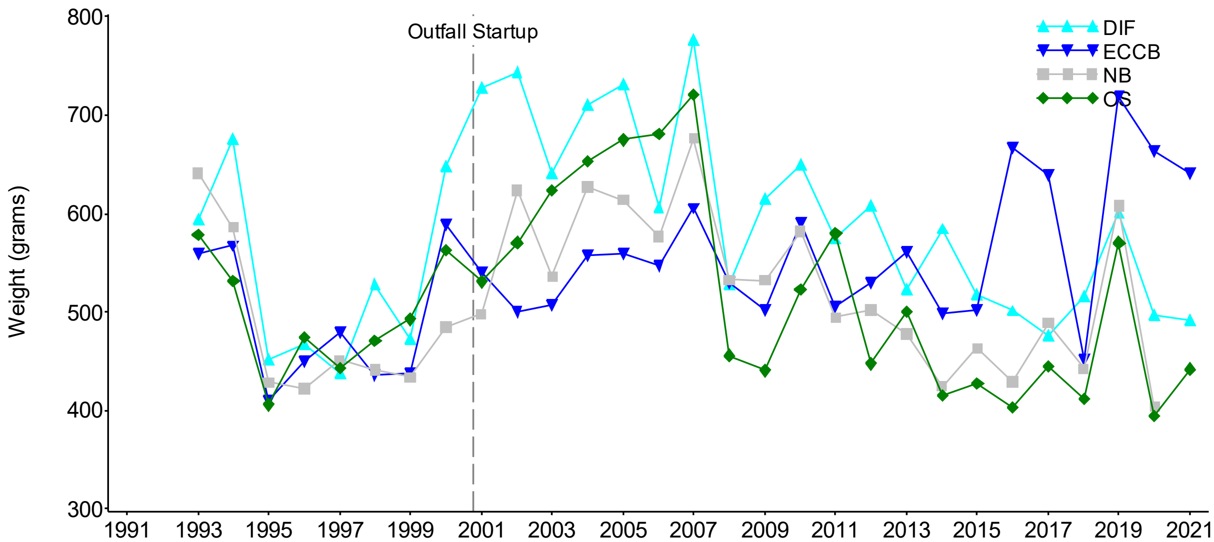

Figure 3-4. Average flounder weight (grams) compared by station and year.

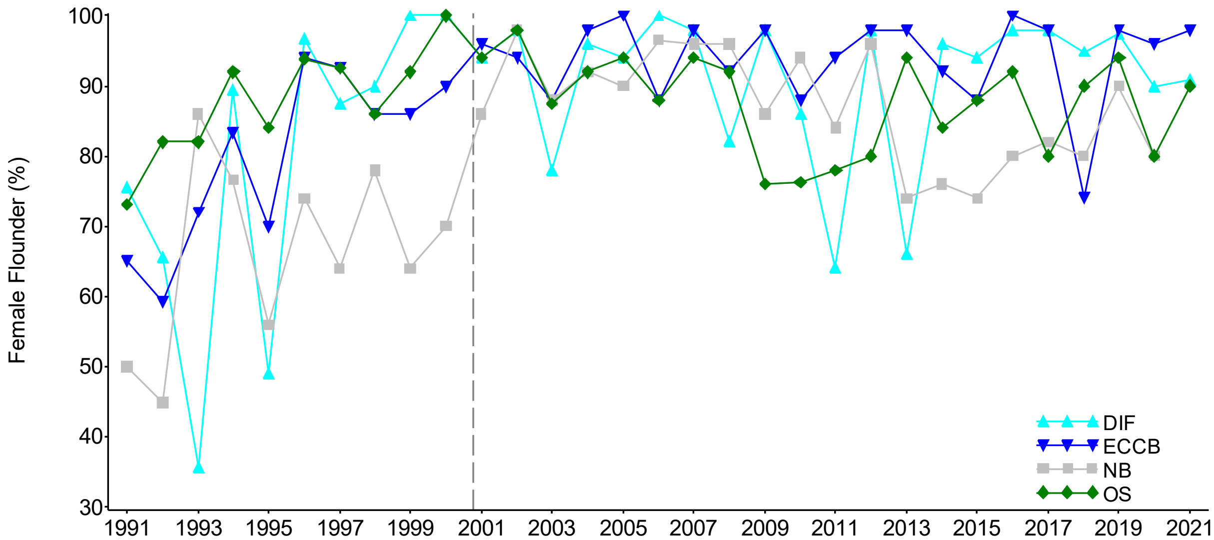

Figure 3-5. Proportion of female flounder compared by station and year.

3.3 External Condition

The external conditions of winter flounder collected in 2021 are presented as prevalence (% of individuals) per station in Table 3-2. Bent fin ray ranged from 6 to 8%, being highest at OS. Blind side ulcers were absent on all fish in 2021. Fin erosion ranged from 6 to 18%, being highest at OS. Lymphocystis ranged from 4% at ECCB to 38% at OS.

Table 3-2. Prevalence (%) of External Conditions Assessed for Winter Flounder Collected in 2021.

External Conditions	Station (Sample size)		
	DIF (33)	ECCB (50)	OS (50)
Bent Fin Ray	6	6	8
Blind Side Ulcers	0	0	0
Fin Erosion (Fin Rot)	6	14	18
Lymphocystis	12	4	38

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 2003-2006, then decreased from 2007-2010, and were once again elevated in 2011 (Figure 3-6). Since 2012, ulcers have remained at relatively low levels at all stations, although an increase was observed at NB in 2019. Ulcers were absent in 2021.

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. Fin erosion values for 2020 were high at DIF, NB and ECCB and moderate at OS. It increased at all stations compared to 2019, and may have resulted from collection of these data by an alternate analyst. The classification of these lesions is inevitably somewhat subjective. The 2020 fin erosion data have been flagged in the project database for further consideration in future years. In 2021, fin erosion levels were comparable to 2019 and earlier, except DIF in 2021 was lower than in 2019.

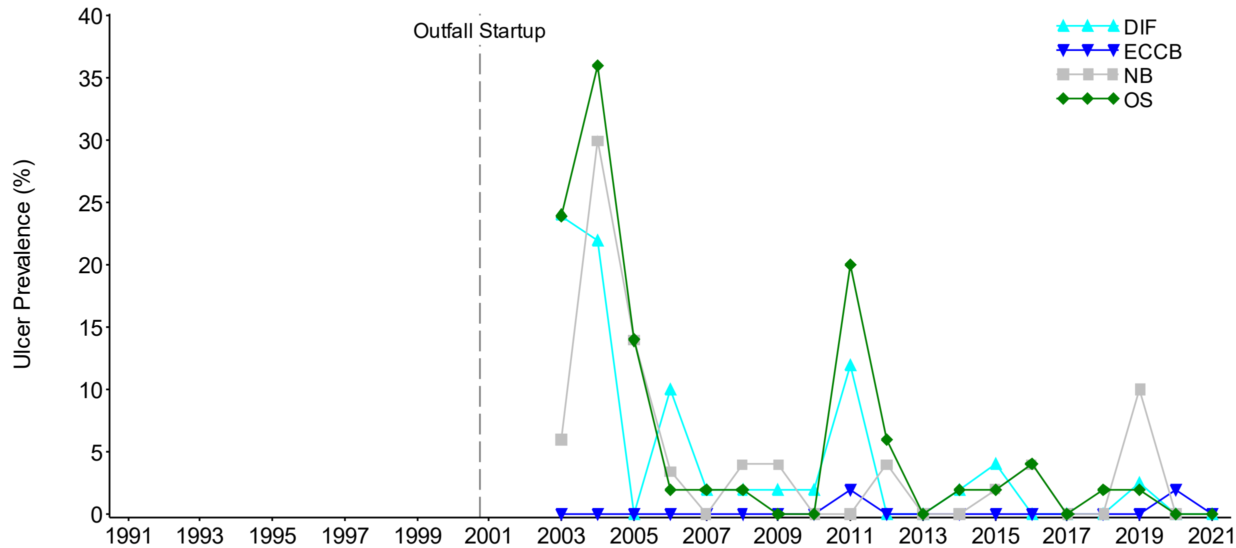

Figure 3-6. Temporal comparison of blind side ulcer prevalence (%) in winter flounder by station.

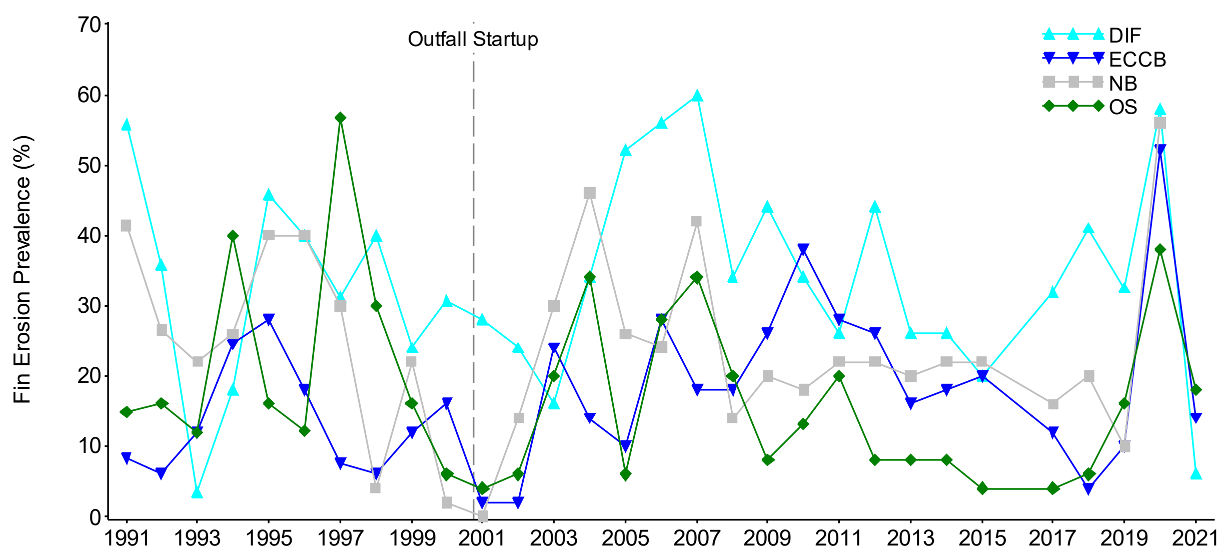

Figure 3-7. Temporal comparison of fin erosion prevalence (%) in winter flounder by station. 2016 data for fin erosion were flagged and excluded from analyses due to inconsistency with this parameter from other years.

3.4 Liver Lesion Prevalence

The prevalence (% of individuals) of liver lesions in winter flounder from each of the four stations sampled in 2021 is presented in Table 3-3. Balloons ranged from 0 to 8%, bile duct protozoa were in 6% of DIF fish, biliary proliferation ranged from 0 to 2%, centrotubular vacuolation ranged from 0 to 9% being lowest at ECCB and highest at DIF, focal hydropic vacuolation was absent at all stations, and liver flukes were absent from all stations. Macrophage aggregation ranged from 30 to 54%, tubular hydropic vacuolation ranged from 2 to 6%, and neoplasia was absent at all sites.

Compared to previous years, 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.

Table 3-3. Prevalence (%) of Liver Lesions in Winter Flounder Collected in 2021.

Liver Conditions	Station (Sample size)		
	DIF (33)	ECCB (50)	OS (50)
Balloons	3.03	0	8
Bile Duct Protozoan	6.06	0	0
Biliary Proliferation	9.09	0	2
Centrotubular Hydropic Vacuolation	9.09	0	4
Focal Hydropic Vacuolation	0	0	0
Liver Flukes	0	0	0
Macrophage Aggregation	30.3	44	54
Neoplasia	0	0	0
Tubular Hydropic Vacuolation	6.06	0	2

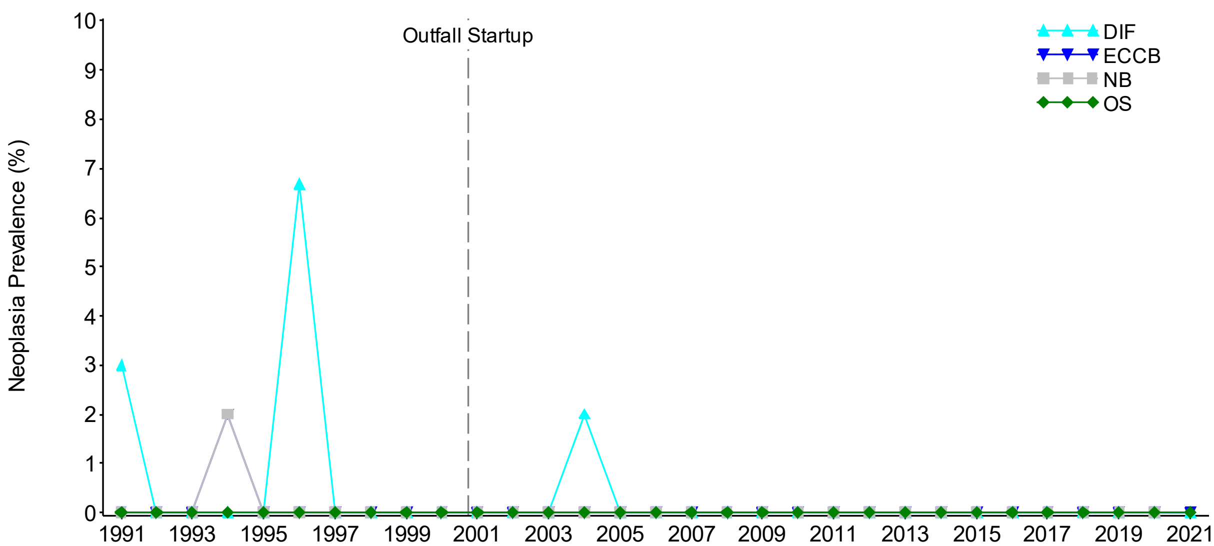

Figure 3-8. Temporal comparison of neoplasia prevalence (%) in winter flounder by station.

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 an overall reduction in hydropic vacuolation at DIF and OS during the study. ECCB remains a good reference station.

The severity of centrotubular hydropic vacuolation (CHV; Figure 3-10) shows the same general trends down for DIF and OS and low levels for ECCB.

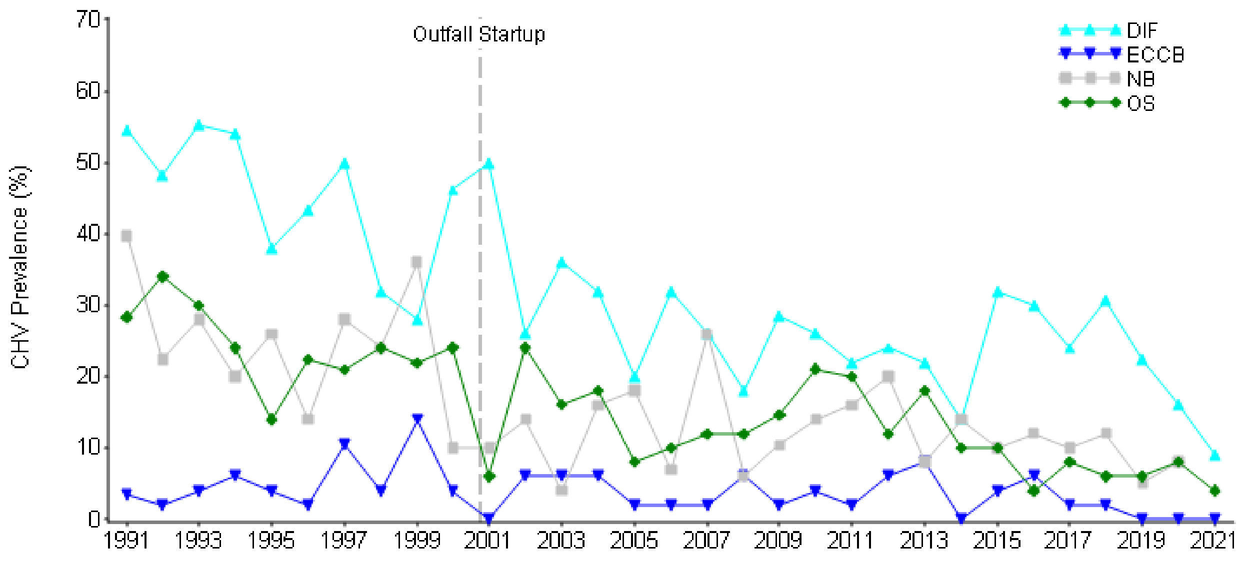

Figure 3-9. Temporal comparison of prevalence (%) of centrotubular hydropic vacuolation in winter flounder by station.

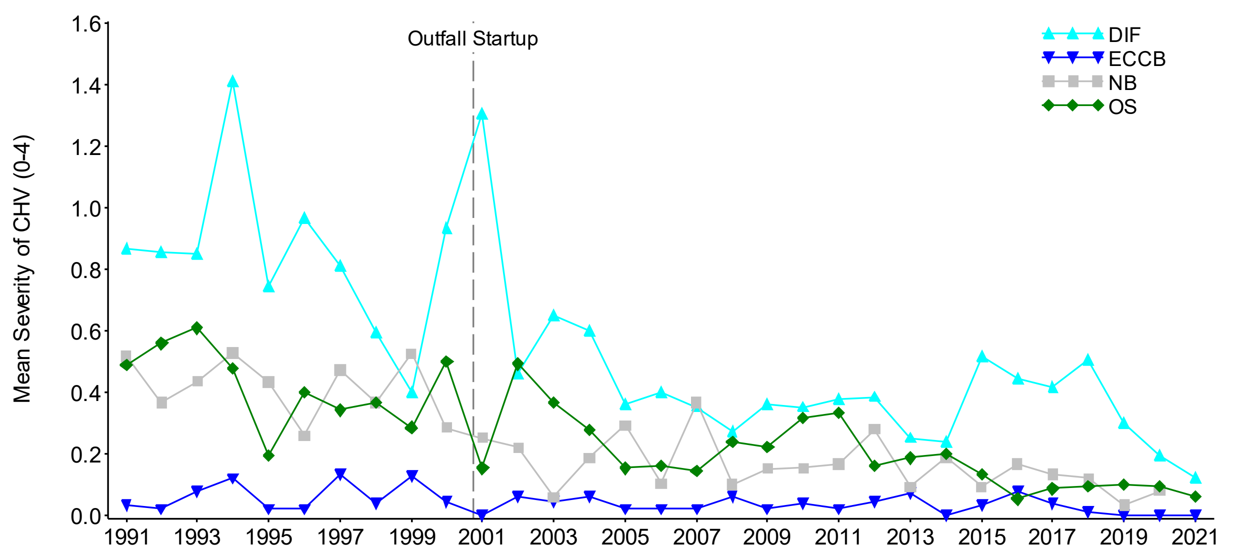

Figure 3-10. Centrotubular hydropic vacuolation severity (rank) in winter flounder compared between sites and years.

Relationships between age and lesion prevalence were also analyzed. The proportion of fish that had CHV (using data collected since 1997 for ECCB) was calculated for each age class at all stations (Figure 3-11). DIF shows a greater increase with age pre-discharge, compared to post-discharge, suggesting the cumulative impact of remaining toxicants thought to induce this lesion has decreased over time. The other stations do not show obvious increases in severity with 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 stable downward trend for DIF, with some inter-annual variability, was maintained, with DIF in 2021 showing the lowest value yet recorded.

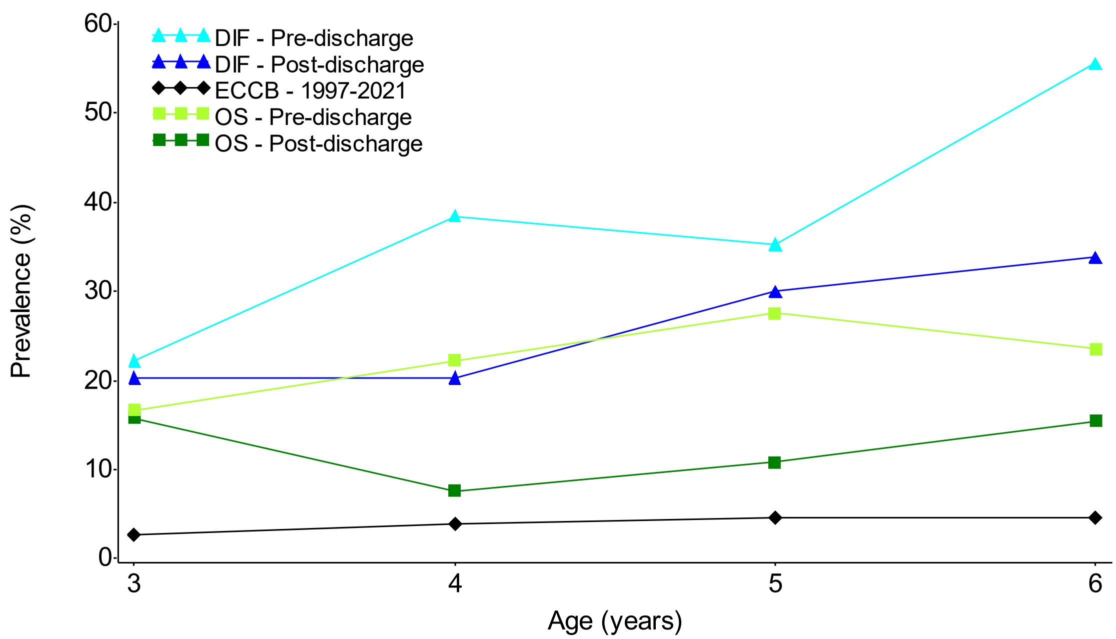

Figure 3-11. Proportion (%) of winter flounder showing hydropic vacuolation for each age.

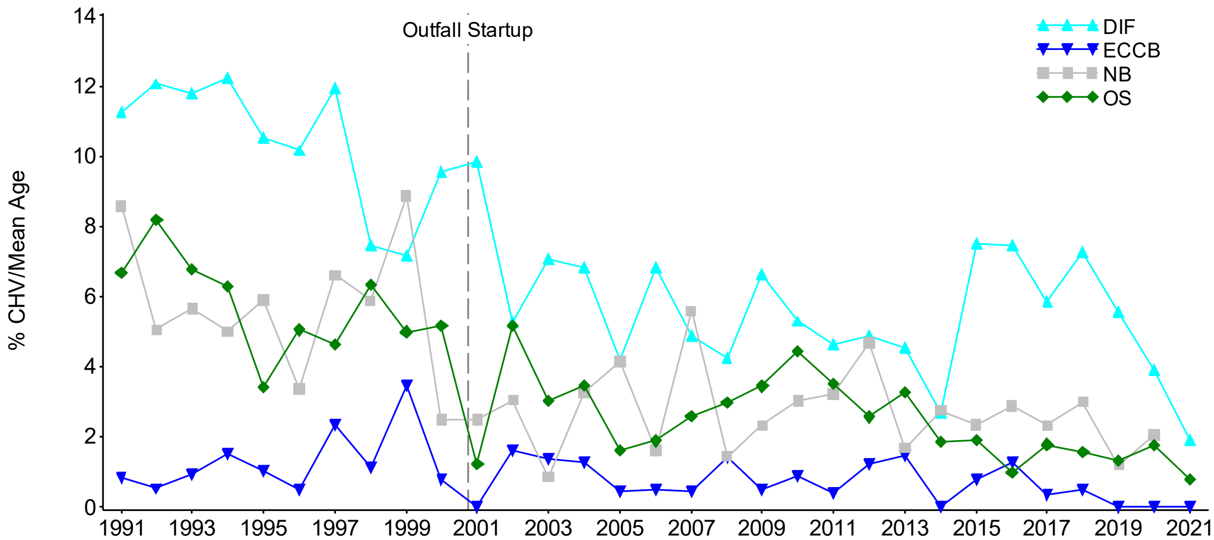

Figure 3-12. Hydropic vacuolation index (CHV%/age) for each station by year.

3.5 Threshold Comparison

The MWRA Contingency Plan includes threshold levels against which key potential indicators of wastewater impacts are tested (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% (the average prevalence in flounder at DIF during baseline monitoring (1991-2000)) for the prevalence of CHV in winter flounder collected at the Outfall Site. CHV prevalence at the Outfall Site during 2021 was 4% (Table 3-3), well below the threshold level, and the lowest yet recorded.

4. CONCLUSIONS

The 2021 Flounder Survey provided samples from three locations (DIF, OS, and ECCB) and was conducted in a manner consistent with previous surveys. Catch per unit effort increased at ECCB, was similar at OS, but decreased at DIF compared to 2020. The overall length 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 2020 except for ECCB which showed a marked increase in 2019 through 2021. As has been the case throughout the duration of the monitoring program, the 2021 catches were dominated by females. Factors influencing sex ratios are complex and poorly understood; however, the 2015 survey report concluded that there is no link between sewage releases into Boston Harbor and Massachusetts Bay and female biased sex ratios (Moore et al. 2016). The already high proportion of females increased at all sites during the baseline period, and since the Outfall came on line but there has been no sustained inter-station difference in proportion of females that could be related to distance from the outfall.

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. Ulcers have remained at relatively low levels at all stations since 2012, although NB showed a marked ulcer prevalence increase in 2019. No ulcers were observed in 2021.

Results of the histological analyses in 2021 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, although a general mild increase was present beginning in 2015, but since 2019, the downward trend has resumed again.
- 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 most recent years. This is a remarkable change. The mild reversal to closer to 30% between 2015 and 2018 returned to 16% in 2020, and to a record low of 9% in 2021.
- 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 (Moore et al., 2018).
- 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.
- The prevalence of CHV in flounder from the vicinity of MWRA's Massachusetts Bay outfall has not shown increases over levels observed during baseline monitoring. During most years since offshore discharge was initiated, prevalence has been less than that observed during the baseline monitoring before 2001. A slow rise in the prevalence of age-corrected CHV in flounder collected in the vicinity of the outfall was observed between 2005 and 2010. It has declined again in recent years with some year-to-year variability (Figure 3-12).

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