2023 Flounder Monitoring Results

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EXECUTIVE SUMMARY

The Massachusetts Water Resources Authority (MWRA) has implemented a long-term monitoring program for Massachusetts and Cape Cod Bays. The objectives of this program are to test whether the environmental impacts of the MWRA discharge from the Deer Island Wastewater Treatment Plant meet the limits of its discharge permit issued by the U.S. Environmental Protection Agency.

Before MWRA moved the treatment plant discharge to Massachusetts Bay in 2000, treated wastewater, or effluent, from Greater Boston communities was sent to outfalls in Boston Harbor. In the late 1980s, the high prevalence of liver disease in the harbor’s winter flounder contributed to concerns about the harbor’s ecological health. Up to 77% of flounder collected in Boston Harbor showed evidence of disease in liver tissue and up to 12% contained liver tumors, both known to be associated with contaminant exposure (Moore et al. 1996). In 2023, more than two decades after the effluent discharge was moved to Massachusetts Bay, 0% of flounder collected in Boston Harbor showed the mildest evidence of similar liver disease, and none contained tumors.

Moving MWRA’s treatment plant outfall to Massachusetts Bay caused concerns that winter flounder in the bay might also start to show health problems from exposure to contaminants. A monitoring program was established to provide data that can be used to assess potential impacts to winter flounder near the bay outfall, and to track their improving health in Boston Harbor (MWRA 1997, 2004, 2010, 2021). In 2021, after consistent evidence that the outfall did not result in early liver disease or tumors in winter flounder collected near the outfall, the monitoring plan was revised to discontinue monitoring at the two stations distant from the outfall: Nantasket Beach and eastern Cape Cod Bay (Figure 2-1).

In 2023, flounder were sampled from Boston Harbor and in Massachusetts Bay near the outfall and examined externally and internally for abnormalities. The data are controlled for age. The 2023 data represent the twenty-third consecutive year of flounder monitoring since the start-up of the Massachusetts Bay outfall in September 2000, following ten years of baseline monitoring (Moore et al. 2018, Moore et al. 2021).

Boston Harbor flounder - In 2023, for the first time in this program early liver disease, centrotubular hydropic vacuolation (CHV) was absent. Despite the small recent sample sizes, these data suggest that there has generally been a steady reduction in this contaminant-associated pathology in winter flounder collected at Deer Island Flats (DIF) during the past two decades. The high prevalence of neoplasia (tumors) characteristic of fish from Deer Island Flats in the mid- to late-1980s (Moore et al. 1996) has disappeared. Tumors have not been observed in any fish from Boston Harbor since 2004 and have never been observed in fish collected at the outfall site.

Massachusetts Bay flounder – In 2023 CHV prevalence remained low in bay winter flounder. In fact, during most years since the bay discharge began, CHV prevalence has been lower than it was before the bay outfall came online. Early liver disease in flounder from the outfall site increased consistently between 2005 and 2010, but since 2014 levels have been low and relatively stable (Figure 3-12).

1. INTRODUCTION

The detection of high prevalence of contaminant-associated liver disease (a condition known as centrotubular hydropic vacuolation, or CHV) in winter flounder (*Pseudopleuronectes americanus*) from Boston Harbor in the late 1980s was one of the findings that contributed to the concern about the ecological health of the Harbor. Up to 77% of flounder collected in Boston Harbor showed evidence of CHV and up to 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 was established (MWRA 1991). 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. Resident flounder have been collected from near the outfall and from sites in Boston Harbor and Massachusetts Bay (hereafter: Boston Harbor and the Bay) since 1991. Measured parameters for flounder include length, weight, age, biological condition, the presence of external or internal disease; and every third year since 2003, concentrations of inorganic and organic contaminants in body tissues (Nestler et al. 2016, Madray and Nestler 2023). 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 2023 winter 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 2023 data represent the twenty-third consecutive year of winter flounder monitoring since the start-up of the Massachusetts Bay outfall in September 2000, and the thirty-third year since the program began. A summary of the survey and laboratory methods used for winter flounder monitoring is provided in Section 2. The results of monitoring data from the survey conducted during 2023, along with comparisons to historical flounder data, are presented in Section 3. Finally, conclusions drawn from the 2023 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 were collected from one location in Boston Harbor and one in Massachusetts Bay (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 2023 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 2023 flounder survey was conducted between April 24 and May 30, 2023. Two sites were sampled to collect winter flounder for histological analyses:

- Deer Island Flats (DIF), historically impacted by contaminants
- Outfall Site (OS), to detect potential impacts from MWRA's treated wastewater

Figure 2-1 shows the 2023 monitoring locations as well as discontinued monitoring locations. Table 2-1 provides the planned and actual sampling sites and locations for the 2023 winter flounder sampling.

Otter-trawl tows were conducted from the F/V *Mystique Lady* operated by Captain Joe Jurek. 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). A secondary collection took place aboard the R/V *Gloria Michelle* from a Massachusetts Division of Marine Fisheries (MA DMF) survey station, 34-Z, by Chief Scientist Steven Wilcox on May 11th. Station 34-Z is approximately 0.6 nautical miles (nm) away from the planned Outfall Site location. Otter-trawl tows conducted for the survey are offset by about 1,000m (0.54 nm), therefore Site 34-Z is considered collected from the Outfall Site.

Mobilization for the survey was conducted on April 24th when 13 fish were collected from the Outfall site. On May 11th an 11 additional fish were collected from the Outfall site. Then on May 30th a total of 15 fish were collected at Deer Island Flats, and five more fish from the Outfall site. 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 in buffered formalin for histological analysis.

Figure 2-1. Flounder monitoring locations since 1991. Flounder were collected at the Deer Island Flats (DIF) and Outfall Site (OS) locations in 2023. The Nantasket Beach (NB) station was discontinued in 2021, and the East Cape Cod Bay (ECCB) station was discontinued in 2022.

Table 2-1. Flounder Sampling Locations in 2023.

Site (Station ID)/Date/Time			Actual Location		Planned Location	
			Latitude	Longitude	Latitude	Longitude
Deer Island Flats (DIF)	**30-May-23**	**8:00**	42.3467	-70.9667	42.3400	-70.9733
		8:40	42.3541	-70.9608	42.3400	-70.9733
		9:45	42.3467	-70.9701	42.3400	-70.9733
		10:22	42.3533	-70.9750	42.3400	-70.9733
Outfall Site (OS)	**24-Apr-23**	**7:04**	42.3915	-70.8216	42.3850	-70.8217
		8:15	42.3918	-70.8600	42.3850	-70.8217
		8:43	42.3882	-70.8317	42.3850	-70.8217
	11-May-23	**12:41**	42.3898	-70.8099	42.3850	-70.8217
	30-May-23	**11:50**	42.3877	-70.8335	42.3850	-70.8217
		13:00	42.3917	-70.8245	42.3850	-70.8217

2.2 Histological Analysis

Livers of 29 flounder from the Outfall Site and 15 from Deer Island Flats were prepared for histological (tissue) 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 44 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
- Apoptotic lesions (*i.e.,* balloons)

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.

The presence of liver flukes also recorded and scored as follows: 0 = absent, 1 = rare, 2 = common, and 3 = abundant.

2.3 Data Reduction and General Data Treatment

All fish data (1991 to 2023) 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 2020–2023 (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

Mechanical failures occurred in 2023 after three tows aboard the *Mystique Lady* when the sampling net was damaged beyond ship-side repair on April 24th. This resulted in additional sampling days (May 11th and May 30th) having only retrieved 13 fish from the Outfall site and none from Deer Island Flats on April 24th.

Due to the very low number of fish, a contingency plan was developed which included additional fish being supplied by MA DMF during their Spring Trawl Survey from their Station 34-Z located within the target radius of the Outfall site. This resulted in the retrieval of 11 additional fish.

The *Mystique Lady*, after net repairs, became available again on May 30th during which four tows were made at Deer Island resulting in a total of 15 fish. Tows were hindered by ghost traps and kelp, resulting in unusually short tows. An additional trip was taken later that day to the Outfall site in an effort to meet the 50 fish quota, which included two additional tows of a total bottom time of just a little over three hours that resulted in only 5 additional fish being collected. In total, 44 fish were collected during the 2023 winter flounder survey with 15 at Deer Island and 29 at the Outfall site. Consequently, the following analysis should be considered in light of those small sample sizes, especially for DIF.

3. RESULTS AND DISCUSSION

3.1 Fish Collected

Winter flounder, each a minimum of 30 centimeters (cm) in length, were collected between April 24 and May 30, 2023, at two 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 1.04x wider and for ECCB the F/V *Explorer 2* (84' sweep rope) was used with a net that was 1.2x wider. Between 2009 and 2022 the F/V *Harvest Moon* was used for all stations. In 2023 the *R/V Mystique Lady* was used with a 72' sweep length. 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, and CPUE's for the *R/V Mystique Lady* were multiplied by 70/72 to get CPUE units in *Odessa* equivalents). CPUE in 2023 was lower at OS and DIF compared to 2022 (Figure 3-1). In accordance with monitoring plan changes made in 2021, no fish were collected at sites NB or ECCB in 2022 and 2023.

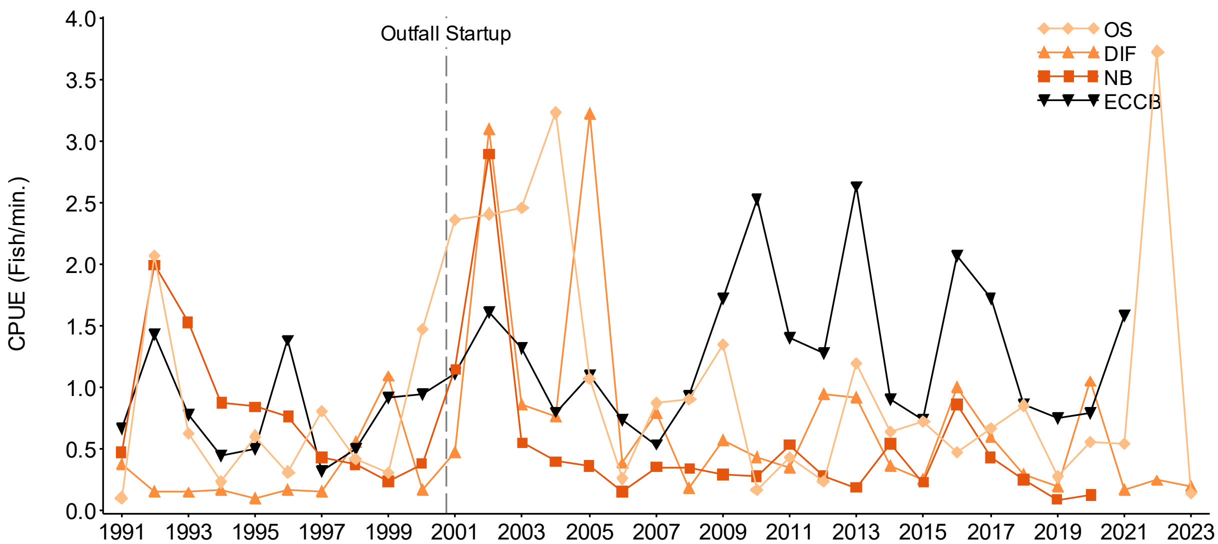

Figure 3-1. Catch Per Unit Effort (CPUE) for winter flounder trawled 1991–2023. Data for 2008 to 2023 have been normalized (see Section 3.1).

3.2 Physical Characteristics

Mean values for physical characteristics of the winter flounder collected in 2023 are reported in Table 3-1. These values reflect the project requirement to collect sexually mature specimens (>30 cm total length). Mean age was approximately 5.7 years across both stations. Mean standard length ranged from 281 to 299 millimeters (mm) and mean total length from 343 to 363 mm; weight ranged from a mean of 483 to 639 grams (g). See footnote a of Table 3-1 for an explanation of the difference between standard length and total length.

Mean age in 2023 compared to 2022 (Figure 3-2) increased slightly for OS and substantially for DIF. 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 2022, standard length (Figure 3-3) in 2023 increased for both OS and DIF. Weights (Figure 3-4) increased slightly for OS and substantially for DIF compared to 2022. Percent females (Figure 3-5) decreased slightly for DIF.

Table 3-1. Summary of Physical Characteristics of Winter Flounder Collected in 2023.

	DIF			OS		
Parameter	Mean	STDDEV[b]	N[b]	Mean	STDDEV[b]	N[b]
Age (years)	5.71	1.54	14	5.69	1.37	29
Standard Length (mm)[a]	299.13	33.87	15	281.21	25.52	29
Total Length (mm)[a]	363.40	41.28	15	343.34	30.00	29
Weight (g)	639.64	223.18	14	483.28	159.04	29

[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).
[b] STDDEV: standard deviation, N: number of fish.

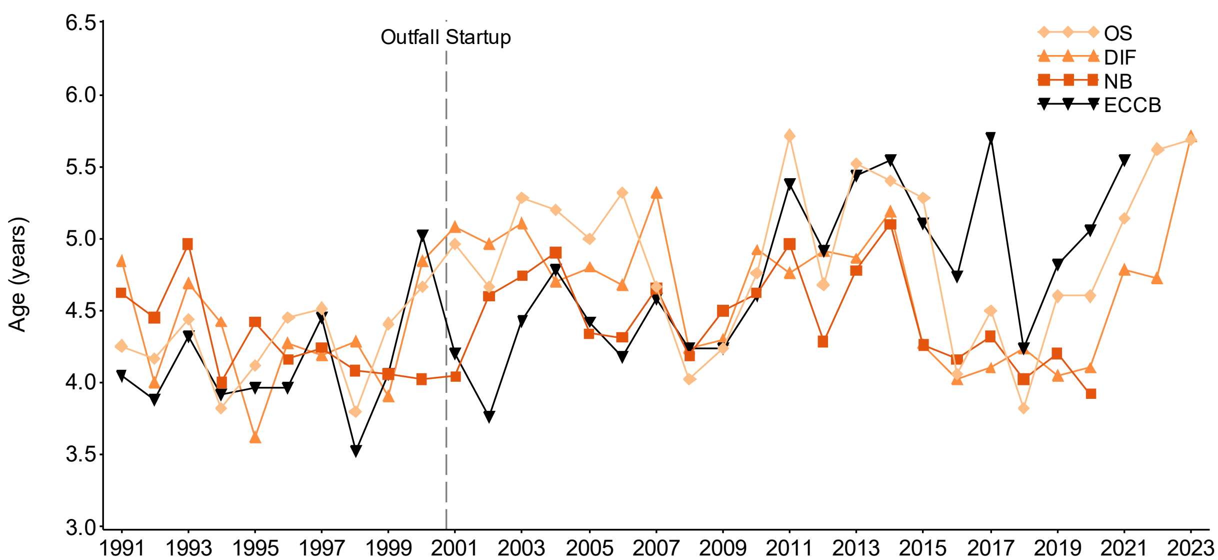

Figure 3-2. Average flounder age (years) compared by station and year.

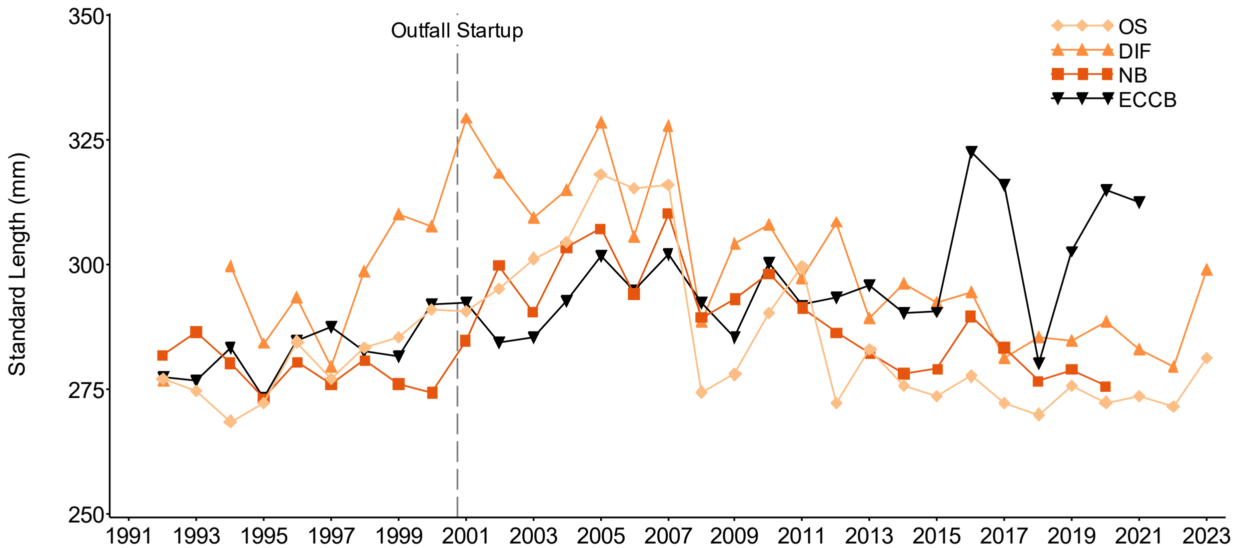

Figure 3-3. Average flounder standard length (mm) compared by station and year.

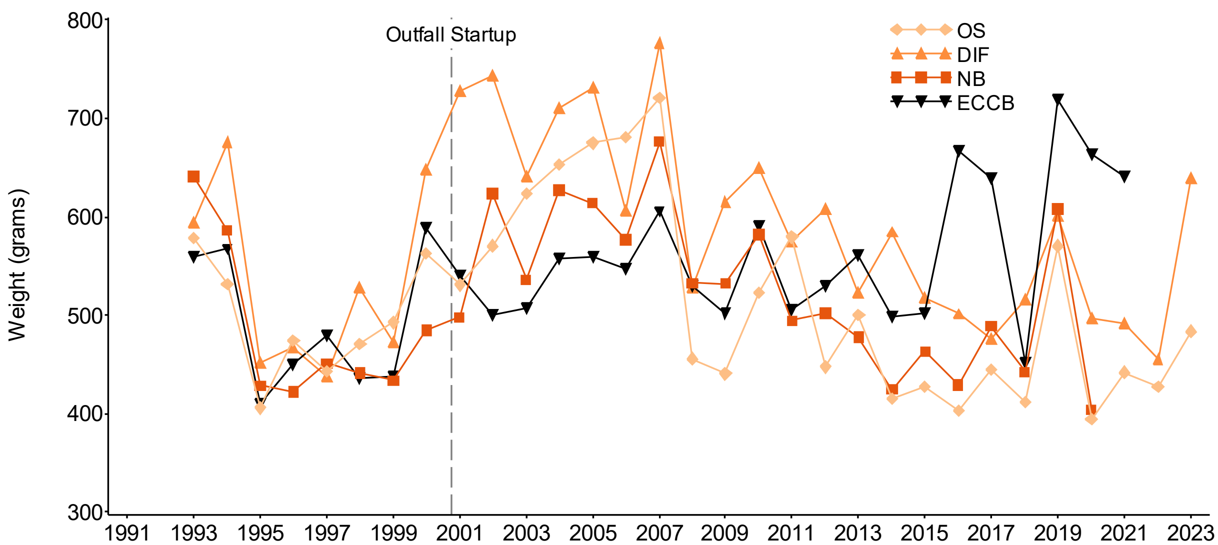

Figure 3-4. Average flounder weight (grams) compared by station and year.

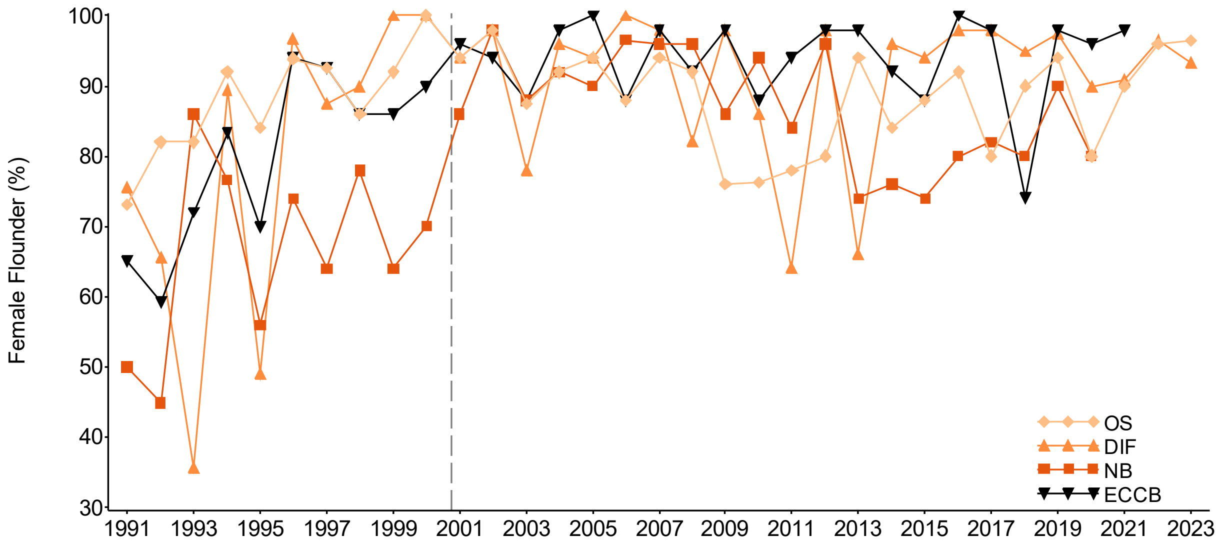

Figure 3-5. Proportion of female flounder compared by station and year.

3.3 External Condition

The external conditions of winter flounder collected in 2023 are presented as prevalence (% of individuals) per station in Table 3-2. Bent fin ray ranged from 27 to 0%, being highest at DIF. Blind side ulcers were absent on all fish in 2023. Fin erosion ranged from 27 to 14%, being highest at DIF. Lymphocystis ranged from 80% at DIF to 55% at OS.

Table 3-2. Prevalence (%) of External Conditions Assessed for Winter Flounder Collected in 2023.

External Conditions	Station (Sample size)	
	DIF (15)	OS (29)
Bent Fin Ray	26.7	0.0
Blind Side Ulcers	0.0	0.0
Fin Erosion (Fin Rot)	26.7	13.8
Lymphocystis	80.0	55.2

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 2023.

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 2023 were comparable and quite low at OS. In 2023 DIF continued to increase from 2022.

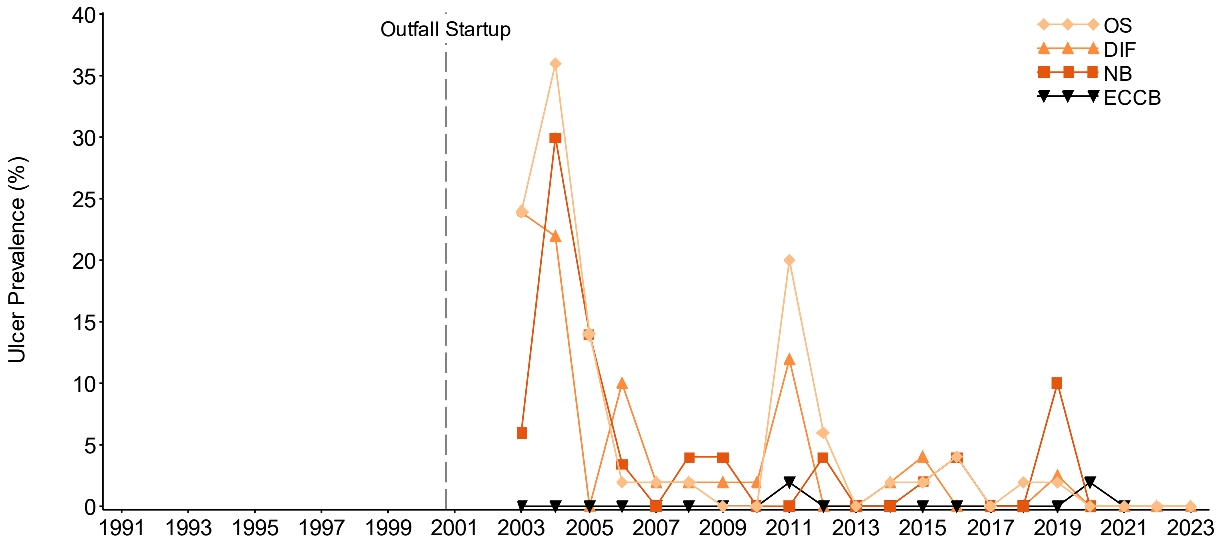

Figure 3-6. Temporal comparison of blind side ulcer prevalence (%) in winter flounder by station.

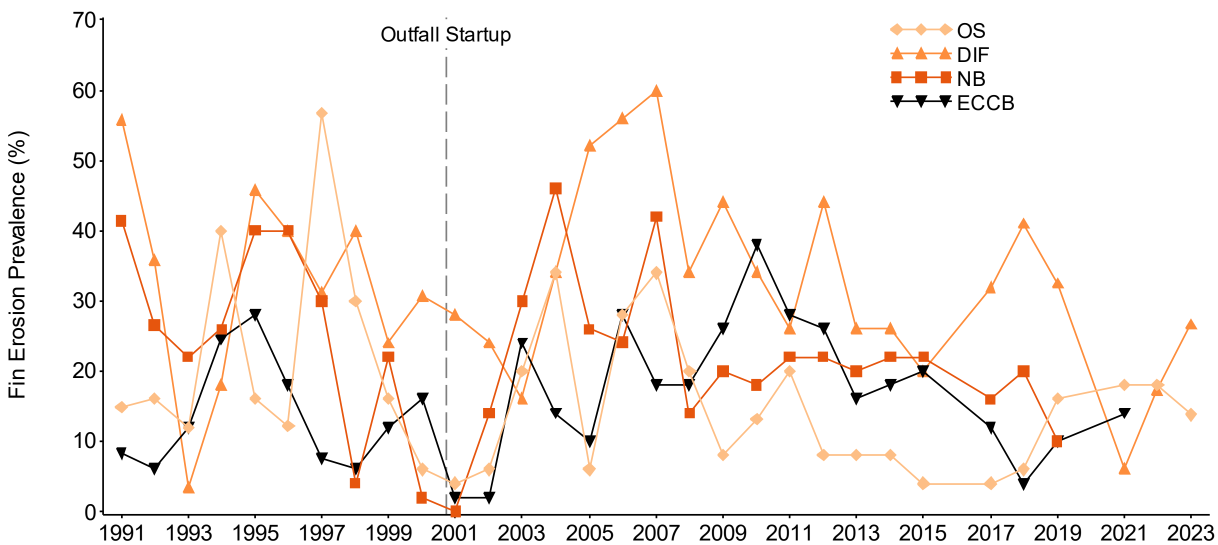

Figure 3-7. Temporal comparison of fin erosion prevalence (%) in winter flounder by station. 2016 and 2020 data for fin erosion were flagged and excluded from analyses due to inconsistency with this parameter from other years.

3.4 Liver Lesion Prevalence

The prevalence (% of individuals) of liver lesions in winter flounder from each of the two stations sampled in 2023 is presented in Table 3-3. Balloons ranged from 0 to 14%, bile duct protozoa were absent, biliary proliferation ranged from 0 to 10%, CHV ranged from 0 to 7%, focal hydropic vacuolation and liver flukes were absent from all stations. Macrophage aggregation ranged from 33 to 59%, tubular hydropic vacuolation ranged from 0 to 7%, and neoplasia was absent at all sites.

Compared to previous years, neoplasms remained absent at all sites (Figure 3-8), 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 2023.

Liver Conditions	Station (Sample Size)	
	DIF (15)	OS (29)
Balloons	0	13.79
Bile Duct Protozoan	0	0
Biliary Proliferation	0	10.34
Centrotubular Hydropic Vacuolation	0	6.9
Focal Hydropic Vacuolation	0	0
Liver Flukes	0	0
Macrophage Aggregation	33.33	58.62
Neoplasia (tumors)	0	0
Tubular Hydropic Vacuolation	0	6.9

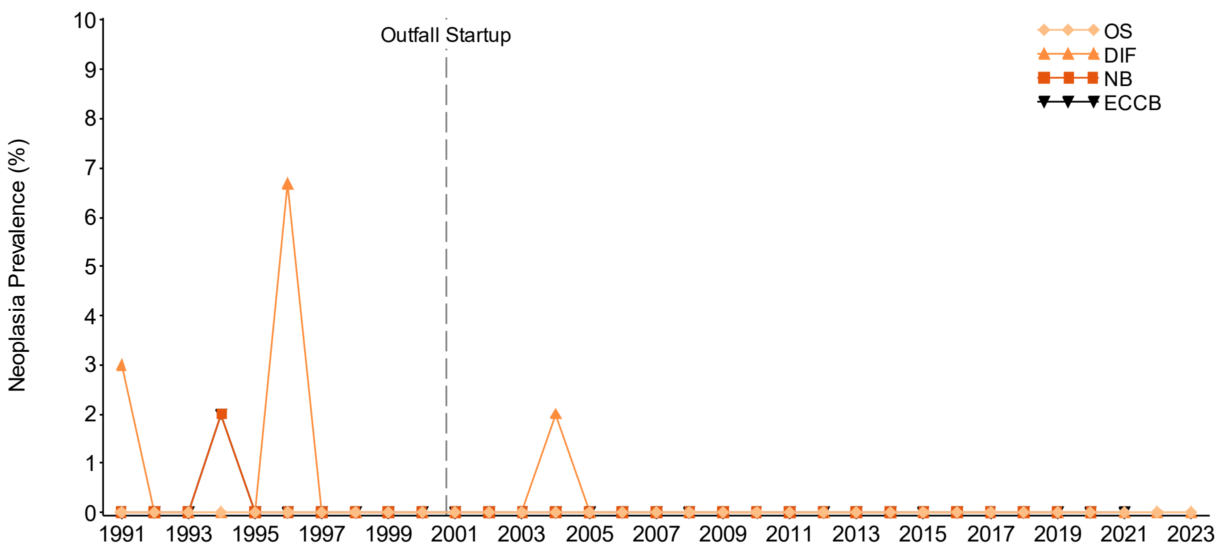

Figure 3-8. Temporal comparison of neoplasia prevalence (%) in winter flounder by station.

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. In 2023, CHV was absent from DIF for the first time in the study, while OS increased marginally. ECCB provided a good reference baseline.

The severity of centrotubular hydropic vacuolation (CHV; Figure 3-10) shows the same general trends downward for DIF and OS approaching the low levels of the ECCB baseline. There was a minor increase in CHV severity at OS in 2023.

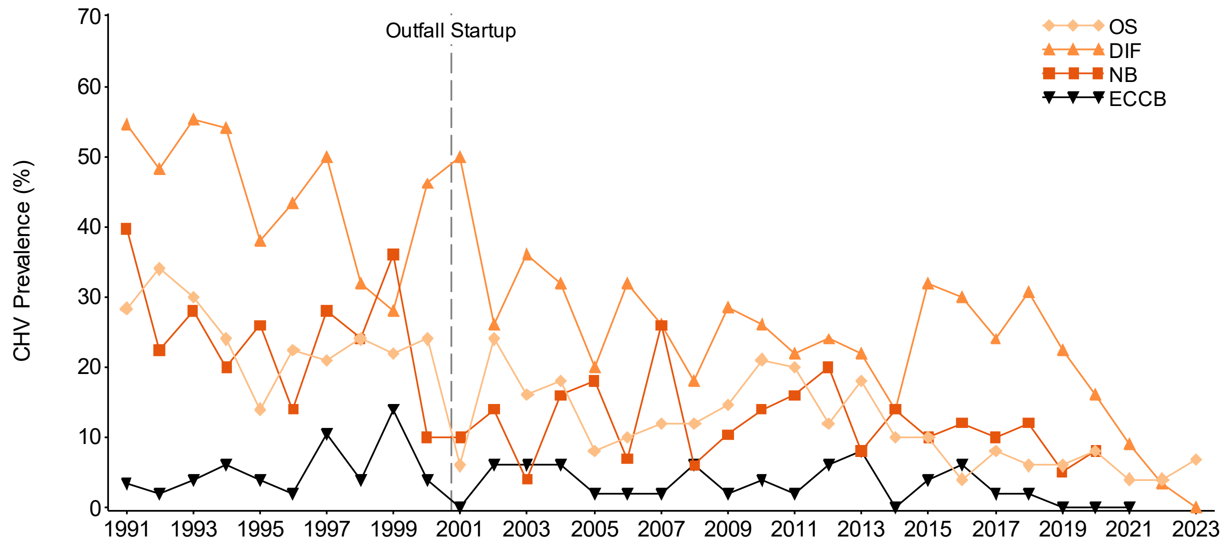

Figure 3-9. Temporal comparison of prevalence (%) of centrotubular hydropic vacuolation in winter flounder by station.

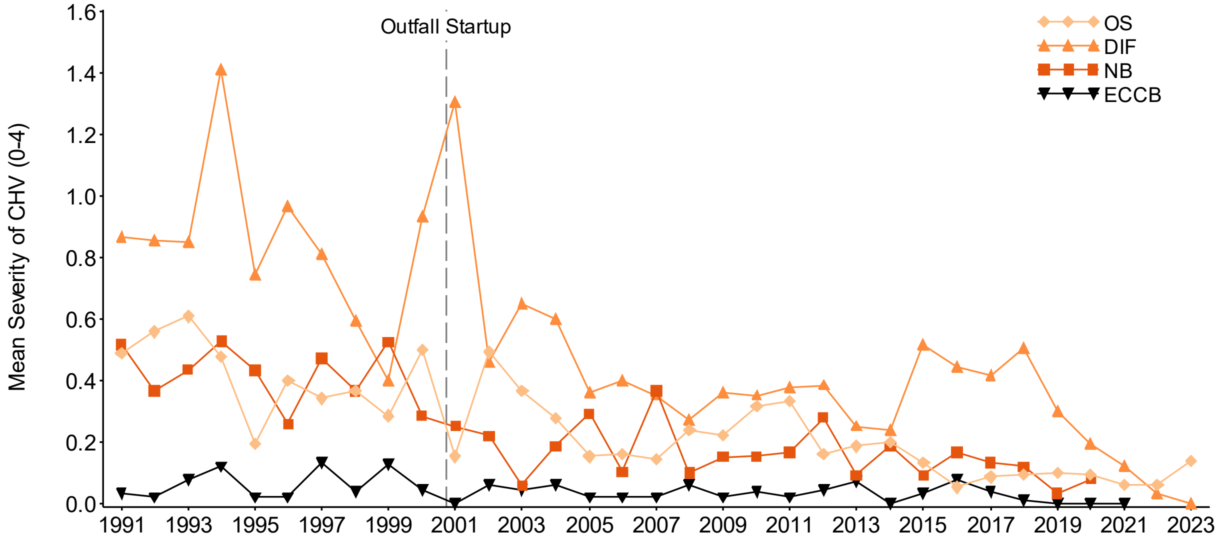

Figure 3-10. Centrotubular hydropic vacuolation severity (rank) in winter flounder compared between sites and years.

Relationships between age and lesion prevalence were also analyzed. The proportion of fish that had CHV 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. OS does 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 CHV absent at DIF in 2023. OS remained within the variability of recent years.

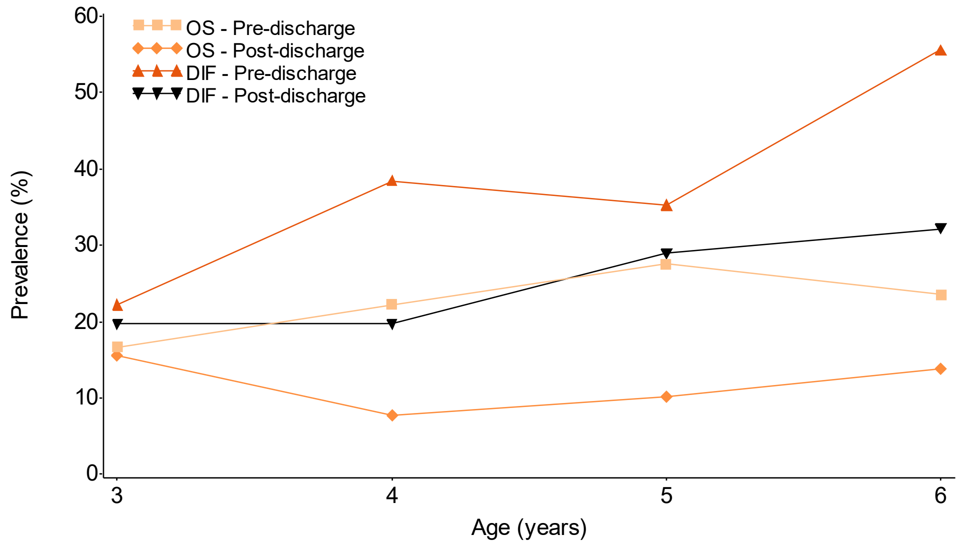

Figure 3-11. Proportion (%) of winter flounder showing hydropic vacuolation for each age.

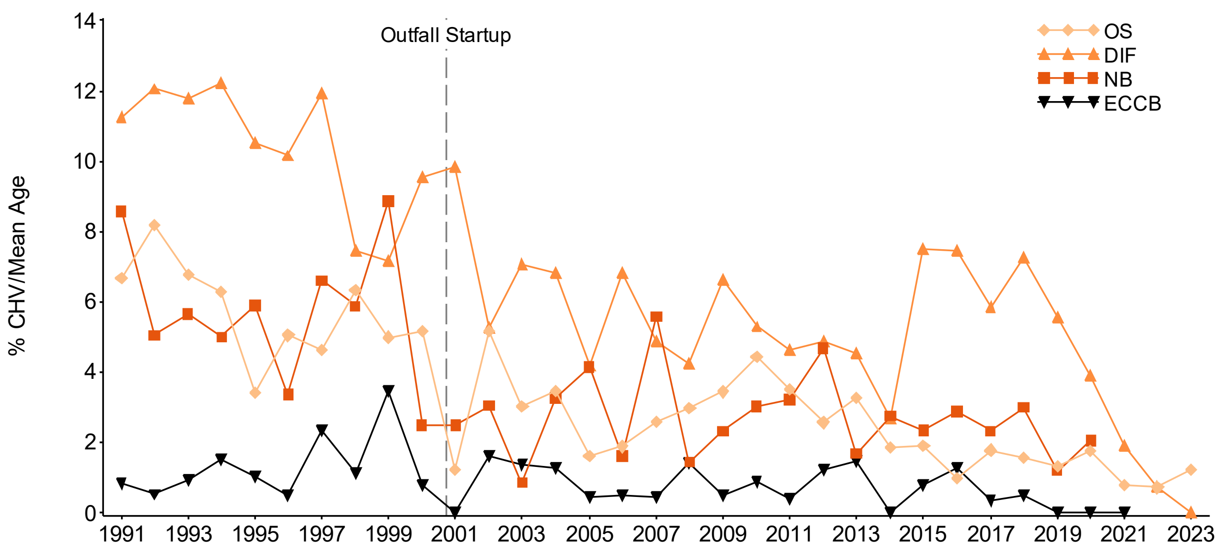

Figure 3-12. Hydropic vacuolation index (CHV%/age) for each station by year.

3.5 Threshold Comparison

The MWRA Contingency Plan includes threshold levels against which key potential indicators of wastewater impacts are evaluated (MWRA 2001). Due to the concerns that effluent discharge might increase the prevalence of lesions in Massachusetts Bay populations of winter flounder, liver disease prevalence was selected as a key indicator, with a Caution Level threshold set at 44.94% for the prevalence of CHV in winter flounder collected at the Outfall Site. The threshold was based on the average CHV prevalence in flounder at DIF during the baseline monitoring period of 1991-2000. CHV prevalence at the Outfall Site during 2023 was 7% (Table 3-3), well below the threshold level.

4. CONCLUSIONS

The 2023 Flounder Survey provided samples from two locations (DIF and OS) in a manner consistent with previous surveys. Catch per unit effort was very low at OS and DIF. The overall length of the flounder collected increased until 2008, when size returned to levels seen at the beginning of the study, a trend that continued through 2023. As has been the case throughout the duration of the monitoring program, the 2023 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. Given the very strong correlation between sex and size in coastal populations of adult winter flounder, factors associated with temporal changes in the size of flounder found at these sampling sites are likely responsible for the patterns observed (Moore et al. 2016). The already high proportion of females increased at all sites during the baseline period, and since the Outfall came online, 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. 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. No ulcers were observed in 2023.

Results of the histological analyses in 2023 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, since 2019 the downward trend has resumed and is now within the range of the baseline established at the previously sampled East Cape Cod Bay station.
- 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 0% for the first time in 2023, albeit with a small sample size.
- 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).
- 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, with an all-time low in 2022, albeit with a mild reversal in 2023 (Figure 3-12).

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