# 2009 flounder monitoring report

Massachusetts Water Resources Authority

Environmental Quality Department

Report 2010-01

**Citation:**

Moore MJ, Maciolek NJ, and PS Winchell. 2010. 2009 flounder monitoring report. Boston: Massachusetts Water Resources Authority. Report 2010-01. 18 pp.

# Flounder Monitoring Report: 2009 Results

**Submitted to**

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**February 2010**
**Report No. 2010-01**

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# 1. INTRODUCTION

The Massachusetts Water Resources Authority (MWRA) has implemented a long-term Harbor and Outfall Monitoring (HOM) Program for Massachusetts and Cape Cod Bays. The objectives of the HOM Program are to test whether the environmental impacts of the MWRA discharge are consistent with SEIS projections and do not exceed Contingency Plan thresholds (MWRA 2001). A detailed description of the monitoring and its rationale is provided in the Effluent Outfall Monitoring Plan developed for the baseline period and the post-discharge monitoring plan (MWRA 1997, 2004).

One aspect of the MWRA HOM program is a long-term monitoring program for winter flounder (MWRA 1991). The goal of this program is to provide data that can be used to assess potential impacts to winter flounder. Resident flounder are collected from near the outfall and from sites in Boston Harbor, Massachusetts Bay, and Cape Cod Bay (hereafter: Boston Harbor and the Bays). Measured parameters for flounder include length, weight, biological condition, the presence of external or internal disease, and concentrations of inorganic and organic contaminants in body tissues. Data have been collected since 1992. The full program was conducted annually until 2003, and sampling is now done every third year (last implemented in 2006), except for flounder morphology and histopathology, which remain on an annual schedule.

This report presents results for the 2009 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 2009 data represent the eighth 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 2009, along with comparisons to historical flounder data, are presented in Section 3. Finally, conclusions drawn from the 2009 results and historical trends are summarized in Section 4. By comparing values with established thresholds and evaluating trends over time, these flounder data are used to ensure that discharge of effluent into the Bay does not result in adverse impacts to fish.

## 2. METHODS

Winter flounder (*Pseudopleuronectes americanus*) were collected from four locations in Boston Harbor and the Bays (Figure 2-1) to obtain specimens for age, weight, and length determination, gross examination of health, and histology of livers. The methods and protocols used during the 2009 flounder survey were similar to and consistent with previously used methods. More detailed descriptions of the methods are contained in the quality assurance plan (QAPP) for Flounder Monitoring 2008–2009 (Maciolek *et al.* 2008).

### 2.1 Stations and Sampling

The 2009 flounder survey was conducted between April 20 and May 5, 2009. 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 details of the actual trawling locations for the 2009 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 from WHOI and biologist Paula Winchell from AECOM. Fifty sexually mature (4–5 years old, total length ≥30 cm) winter flounder were collected at each station. During the first day of sampling on 20 April, monitoring was initiated at DI and NB and completed at OS. Low flounder abundance precluded completion of DI and NB at that time, and it was agreed to delay until fish could move inshore in sufficient numbers. DI and NB were completed on 4 May and ECCB was sampled on 5 May.

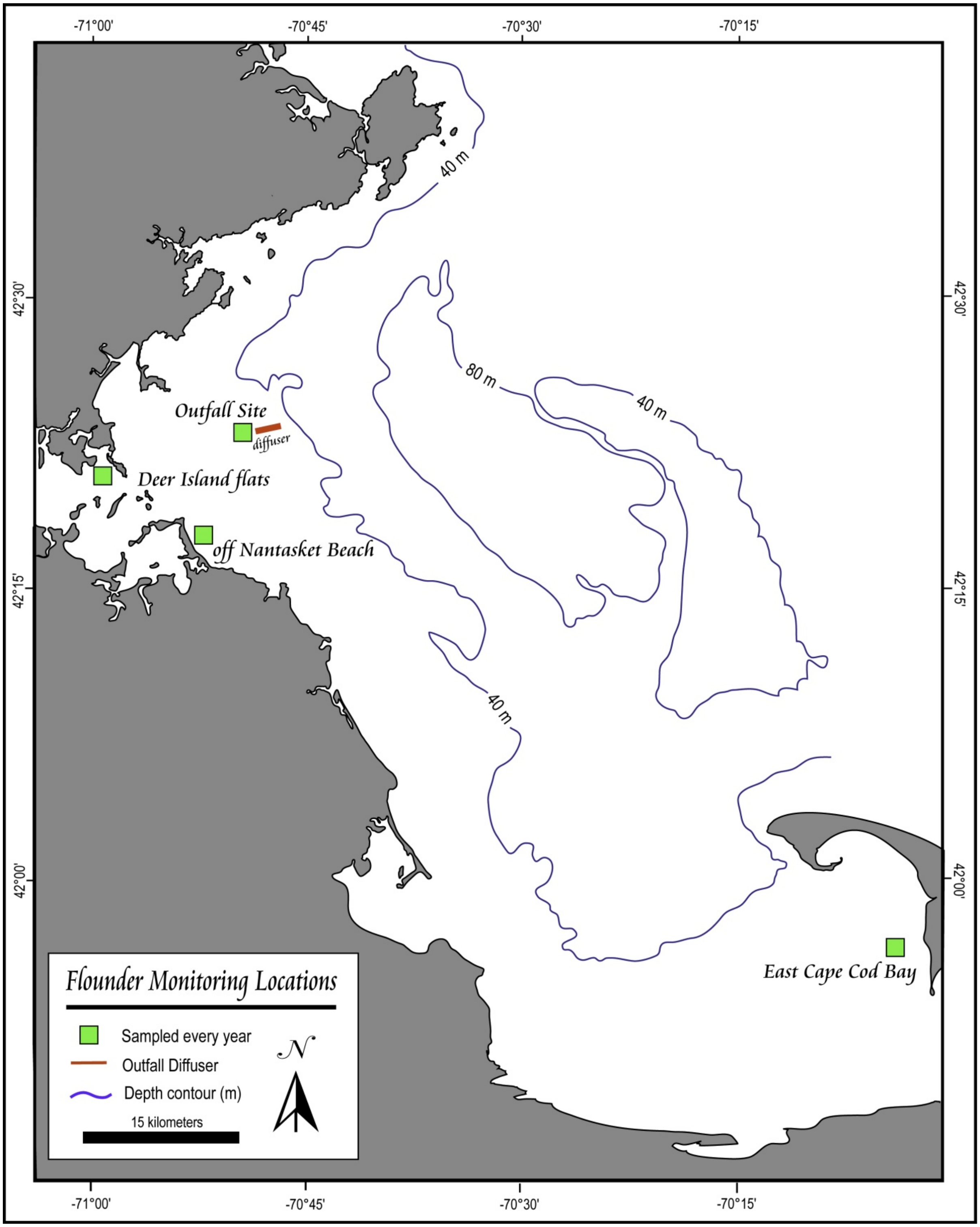


**Figure 2-1. Flounder monitoring locations for 2009.**

**Table 2-1. Sampling Dates, Locations, and CPUE in 2009.**

| Site | Trawl Date 2009 | Start | | Finish | | Bottom Time (hh:min.) | # Flounder >300 mm | Total Flounder | Total time (min/stn) | CPUE (fish/min) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Lat (N) | Long (W) | Lat (N) | Long (W) | | | | | |
| DIF | 20 Apr | 42° 20.672' | 70° 57.763' | 42° 20.728' | 70° 57.839' | 0:36 | 9 | 64 | 107 | 0.60 |
| DIF | 20 Apr | 42° 20.870' | 70° 57.972' | 42° 21.172' | 70° 58.599' | 0:53 | 11 | | | |
| DIF | 04 May | 42° 20.831' | 70° 57.908' | 42° 21.070' | 70° 58.593' | 0:18 | 44 | | | |
| NB | 20 Apr | 42° 17.329' | 70° 51.916' | 42° 17.458' | 70° 51.975' | 0:53 | 3 | 57 | 184 | 0.31 |
| NB | 20 Apr | 42° 17.384' | 70° 51.924' | 42° 17.496' | 70° 51.973' | 0:53 | 12 | | | |
| NB | 20 Apr | 42° 17.543' | 70° 51.475' | 42° 17.544' | 70° 51.950' | 0:36 | 7 | | | |
| NB | 04 May | 42° 17.299' | 70° 51.981' | 42° 16.925' | 70° 51.669' | 0:42 | 35 | | | |
| OS | 20 Apr | 42° 22.978' | 70° 48.346' | 42° 23.348' | 70° 49.041' | 0:16 | 24 | 64 | 45 | 1.42 |
| OS | 20 Apr | 42° 23.598' | 70° 49.282' | 42° 22.720' | 70° 48.330' | 0:29 | 40 | | | |
| ECCB | 05 May | 41° 56.978' | 70° 07.489' | 41° 55.243' | 70° 07.773' | 0:40 | 73 | 73 | 40 | 1.83 |

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

## 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 (2009 and historical) were extracted directly from the HOM database and imported into Excel, where data reduction, graphical presentations and statistical analyses were performed. Data reduction was conducted as described in the Flounder Monitoring QAPP (Maciolek *et al.* 2008). For each liver lesion and each fish, a histopathological index was calculated as a mean of scores from three slices on one slide. Histopathological indices and prevalence of lesions were compared among groups of flounder by differences in station and age. Flounder monitoring parameters were presented graphically and compared among stations and over time.

## 2.4 Deviations from the QAPP

All stations were sampled using the vessel *Harvest Moon*. Thus, the net used differed from years prior to 2008 on the vessel *Odessa* in the following regard. Sweep and head rope lengths were as follows: *Odessa*: 70' and 50', *Harvest Moon*: 74' and 52'. It would be reasonable to regard the *Odessa* sweep length as the standard and normalize CPUE data from other net to this using a simple ratio of sweep lengths as a multiplier—i.e., multiply CPUEs for the *Harvest Moon* net by 70/74, to get CPUE units in *Odessa* equivalents. Data presented below were not normalized with these factors.

# 3. RESULTS AND DISCUSSION

## 3.1 Fish Collected

Winter flounder, each a minimum 30 cm in length, were collected between April 20 and May 5, 2009, 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. In 2009, CPUE values at all stations increased compared to the previous year except for Nantasket Beach. Catch rates remained highest at the Outfall Site and Eastern Cape Cod Bay. CPUE at the ECCB during 2009 was higher than at any time in the study. Values at the other stations were within the range of historical variation.

## 3.2 Physical Characteristics

Mean values for physical characteristics of the winter flounder collected in 2009 are reported in Table 3-1. These values reflect the project requirement to collect sexually mature specimens (>30 cm total length). The values for length, weight, and age for flounder collected at the different stations showed no consistent patterns between parameters between stations.

Mean age for each year was calculated for each station and plotted in Figure 3-2. The average age of flounder collected at all stations in 2009 was consistent with historical averages of between four and five years, with all stations except ECCB showing a slight increase from 2008. Likewise, with the exception of ECCB, standard length was higher at all stations than in 2008 (Figure 3-3). Mean weights varied in trend at the different stations compared to 2008 (Figure 3-4). At the OS site, a consistent upward trend in mean flounder weight up to 2007 was reversed in 2008 and continued a mild gentle decline in 2009. Winter flounder display dimorphic growth patterns with females reaching a larger adult size than males (Pereira et al. 1999). The percentage of females has increased at all stations since monitoring began, with collections in recent years having been composed almost entirely of females (Figure 3-5). Although in 2008 there was a moderate reversal at DIF for this parameter, in 2009 DIF was again almost entirely females, while NB and OS showed a few more males.

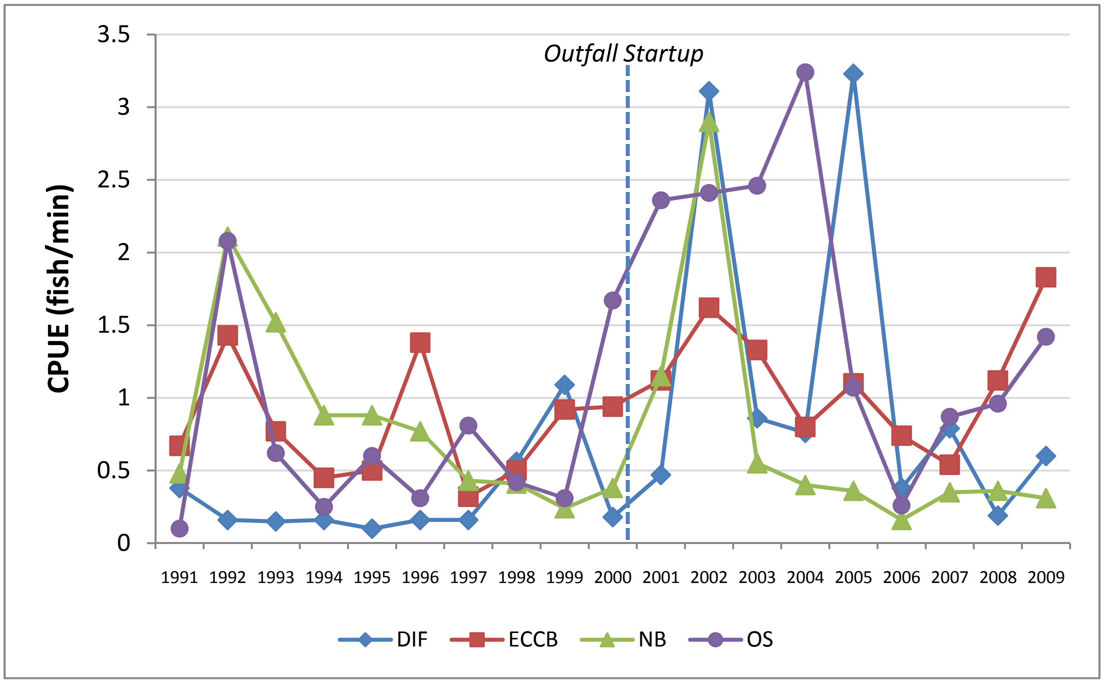


**Figure 3-1. Catch Per Unit Effort (CPUE) for winter flounder trawled 1991–2009. Effort was constant up to and including 2007 with the FV *Odessa* (70' sweep rope). For 2008, the FV *Harvest Moon* (74' sweep rope) was used for DI, NB, and OS, with a net that was 1.04 x wider and for ECCB the FV *Explorer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

**Table 3-1. Summary of Physical Characteristics of Winter Flounder Collected in 2009.**

| | Station (Sample size) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | DIF (50) | | ECCB (50) | | NB (50) | | OS (50) | |
| **Parameter** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** |
| Age (years) | 4.3 | 1.2 | 4.2 | 1.0 | 4.5 | 1.1 | 4.2 | 0.9 |
| Standard Length (mm) | 304.3 | 34.2 | 285.6 | 22.8 | 293.2 | 28.7 | 278.0 | 19.4 |
| Total Length (mm) | 370.8[1] | 42.7 | 348.1 | 27.2 | 356.2 | 34.2 | 339.7 | 22.2 |
| Weight (g) | 614.1 | 182.7 | 502.4 | 109.6 | 532.0 | 155.1 | 440.2 | 95.3 |

[1]Based on 49 of 50 fish; one was missing the tail and was not included.

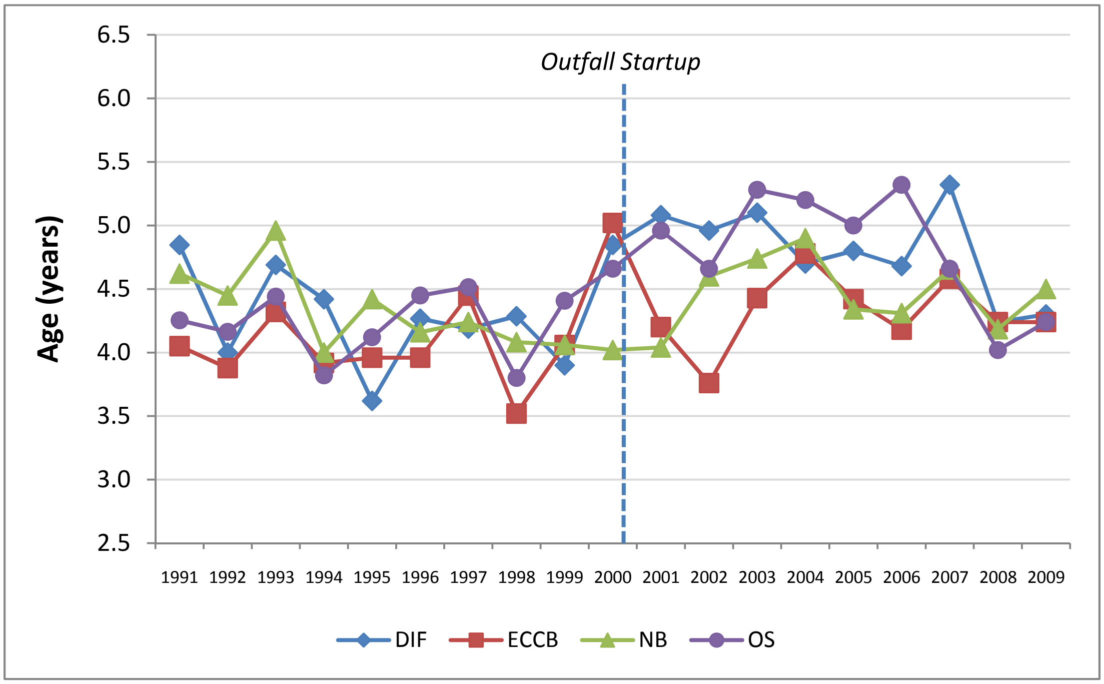


**Figure 3-2. Average flounder age (years) compared by station and year.**

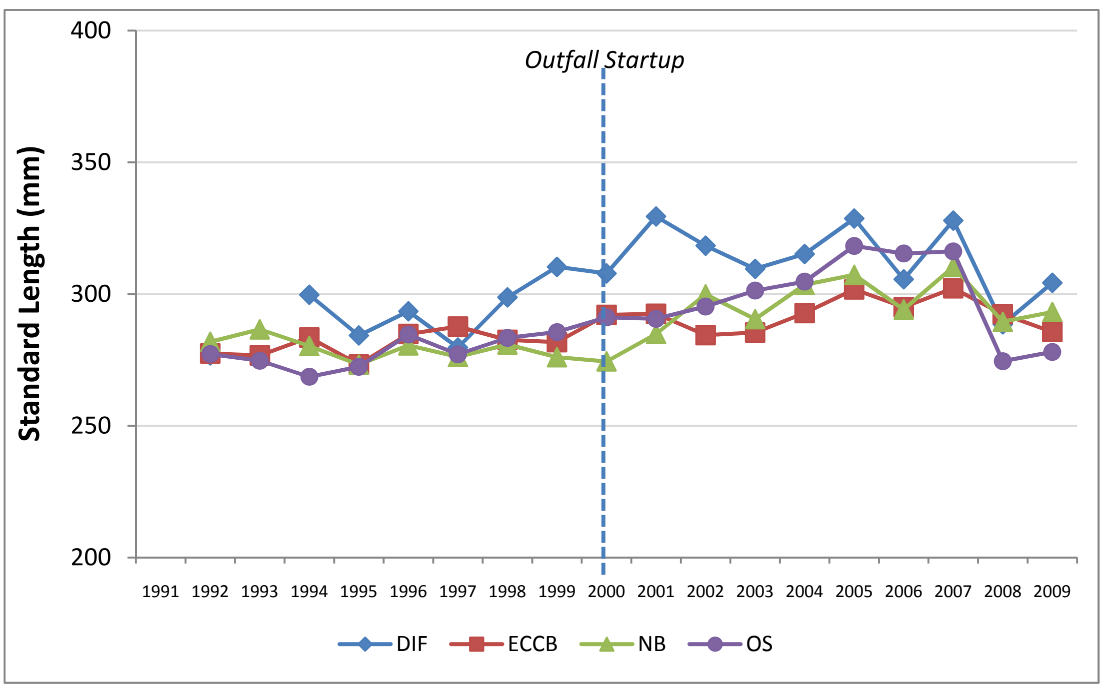


**Figure 3-3. Average flounder standard length (mm) compared by station and year.**

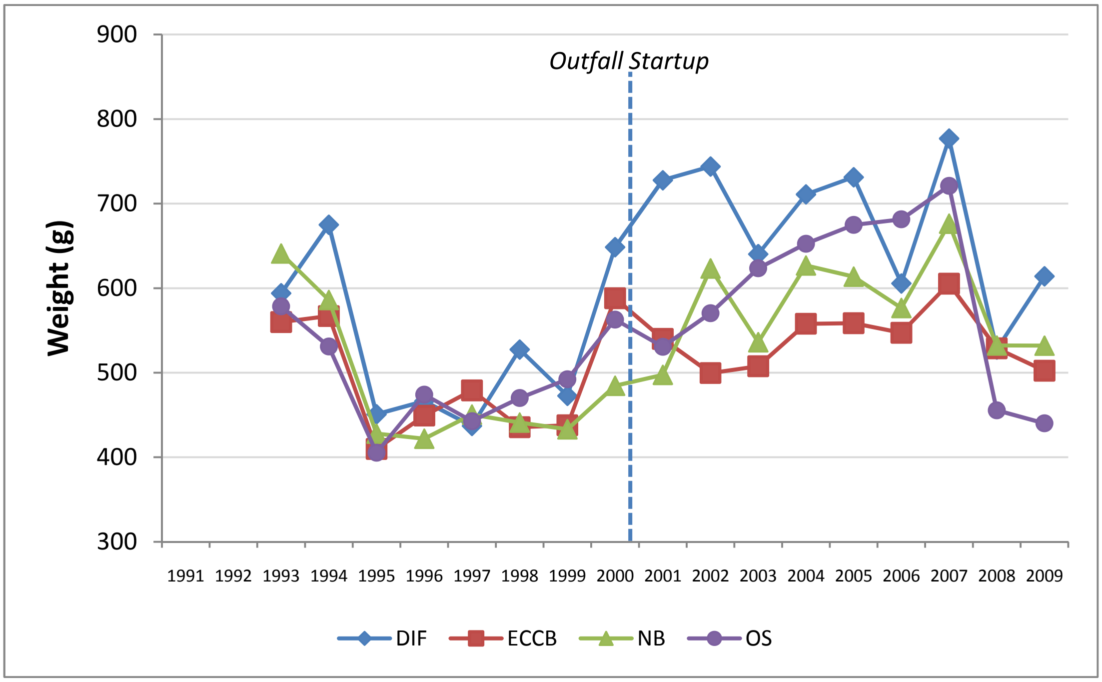


**Figure 3-4. Average flounder weight (grams) compared by station and year.**

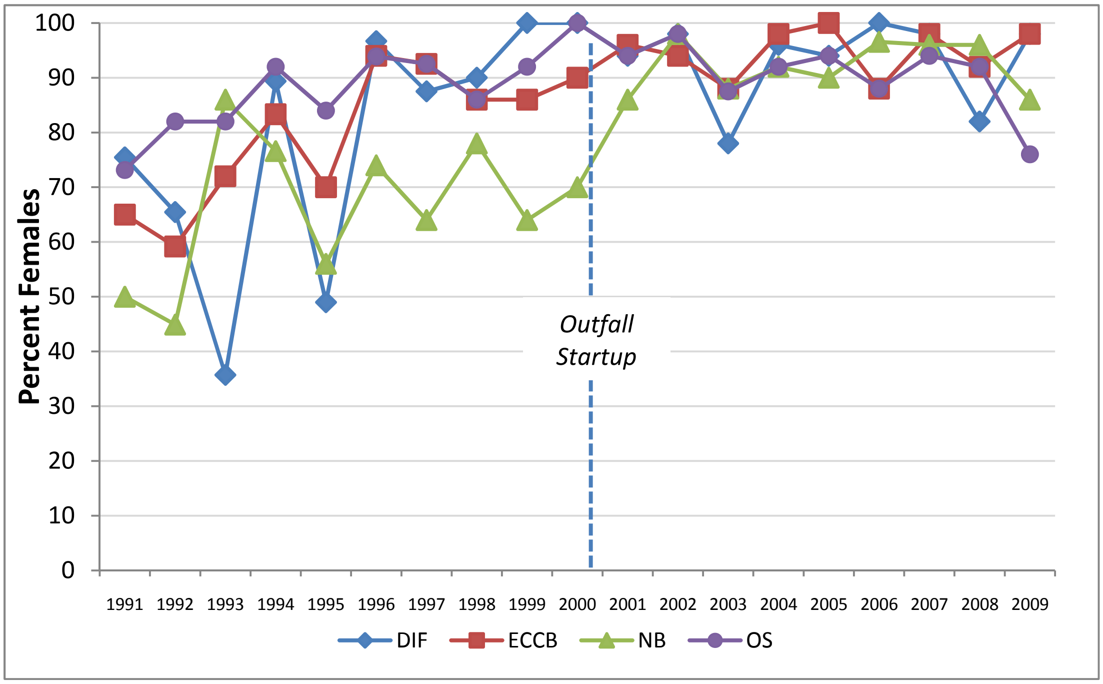


**Figure 3-5. Proportion of female flounder compared by station and year.**

### 3.3 External Condition

The external conditions of winter flounder collected in 2009 are presented as prevalence (% of individuals) per station in Table 3-2. The prevalence of fin erosion was highest in flounder collected at DIF, while lymphocystis was highest at OS. Bent fin ray was highest at DIF. Blind side ulcers were present at a low prevalence at DIF and NB.

The prevalence of blind-side surface ulcers in the western portion of Massachusetts Bay increased markedly beginning in 2003 (Moore 2003). Extensive pathology and microbiology studies have been unable to determine a cause of the ulcers (Moore *et al*. 2004). Additional surveys conducted throughout 2004 and 2005 established that ulcer prevalence peaked in late winter to early spring, with evidence of healed ulcers and lower ulcer prevalence into early summer (Moore 2006). This apparent recovery sequence suggests that these lesions may be a non-lethal seasonal condition. Ulcer prevalence in the 2009 survey suggests a continuing decrease over recent years, declining at the outfall from 36% in 2004 to 0% in 2009. Nantasket Beach had a 4% ulcer prevalence in 2009. The highest prevalence observed was the first year it was recorded (2004).

**Table 3-2. Prevalence (%) of External Conditions Assessed for Winter Flounder Collected in 2009.**

| External Conditions | Station (Sample size) | | | |
|---|---|---|---|---|
| | DIF (50) | ECCB (50) | NB (50) | OS (50) |
| Bent Fin Ray | 34 | 14 | 10 | 2 |
| Blind Side Ulcers | 2 | 0 | 4 | 0 |
| Fin Erosion (Fin rot) | 44 | 26 | 20 | 8 |
| Lymphocystis | 16 | 10 | 24 | 54 |

Fin ray surface mucous and epithelia are impacted by increased levels of ammonia and other pollutants, making fin rot a useful parameter for detecting deteriorating water quality conditions (Bosakowski and Wagner 1994). The prevalence of fin erosion for each year was calculated for each station and plotted in Figure 3-6. This plot indicates that fin rot prevalence remained within historic ranges in 2009. These values have varied considerably over time and no clear trends in this condition are apparent for most locations. Furthermore, the severity of fin rot observed during the program period has never come close to that observed by Dr. Moore in the 1980s when some winter flounder essentially lacked any fins.

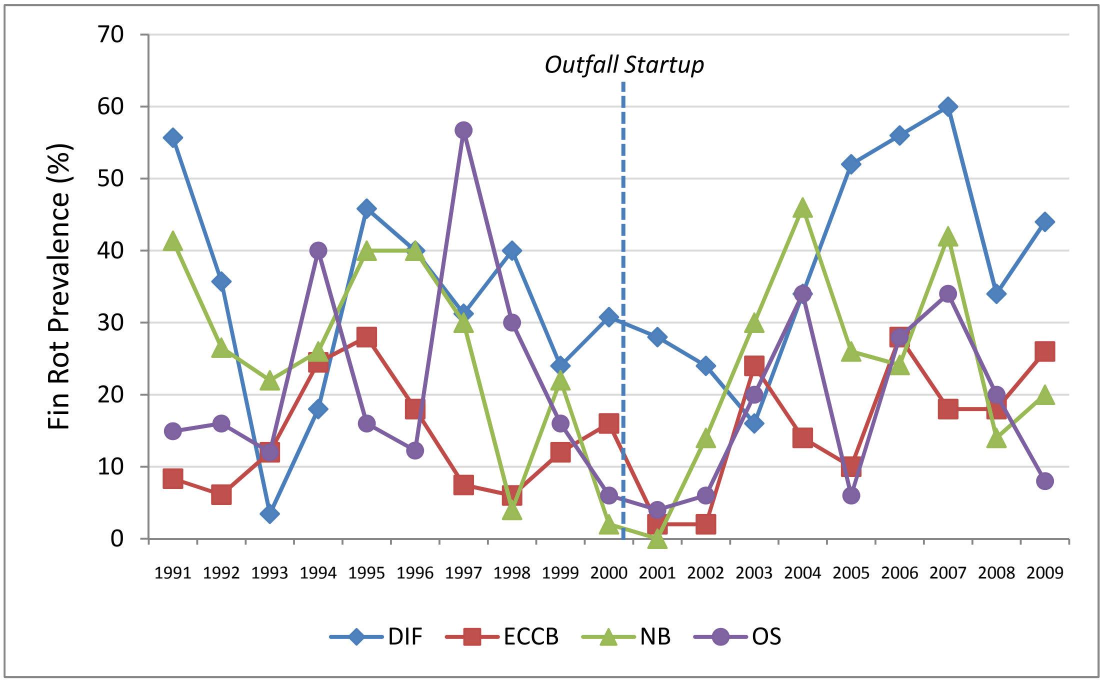


**Figure 3-6. Temporal comparison of fin rot prevalence (%) in winter flounder by station over time.**

### 3.4 Liver Lesion Prevalence

The prevalence (% of individuals) of liver lesions in winter flounder from each of the four stations sampled in 2009 is presented in Table 3-3. Neoplasms were not observed in any of the winter flounder collected during 2009. These lesions have always been rare or absent from all sites other than Deer Island, and none have ever been detected at the Outfall Site (Figure 3-7).

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. Centrotubular hydropic vacuolation (CHV) is the least severe and most common form observed in the collections (Table 3-3). In 2009, CHV prevalence at Deer Island increased slightly from the 2008 level, reversing a general downward trend of contaminant-associated lesions at this site (Figure 3-8), although in the longer term the 2009 uptick was relatively insignificant. The prevalence at Nantasket Beach remains low. CHV for 2009 at the Outfall Site was comparable to the low levels reported during the past three years, and remains below the typical prevalence in the pre-discharge period. At the reference site in Eastern Cape Cod Bay, CHV prevalence remained consistent with the low levels seen throughout the study (Figure 3-8).

The severity of CHV at Deer Island remained substantially lower in 2009 than the 1994 and 2001 peaks (Figure 3-9). CHV severity at Nantasket Beach in 2009 remained low.

**Table 3-3. Prevalence (%) of Liver Lesions in Winter Flounder Collected in 2009.**

| Lesion Type | Station (sample size) | | | |
|---|---|---|---|---|
| | DIF (49) | ECCB (50) | NB (48) | OS (48) |
| Balloons | 4 | 6 | 4 | 8 |
| Bile Duct Protozoan | 0 | 6 | 0 | 0 |
| Biliary Proliferation | 10 | 30 | 17 | 15 |
| Centrotubular Hydropic Vacuolation | 29 | 2 | 10 | 15 |
| Focal Hydropic Vacuolation | 0 | 0 | 0 | 0 |
| Liver Flukes | 0 | 0 | 0 | 2 |
| Macrophage Aggregation | 43 | 42 | 52 | 60 |
| Neoplasia | 0 | 0 | 0 | 0 |
| Tubular Hydropic Vacuolation | 27 | 2 | 10 | 15 |

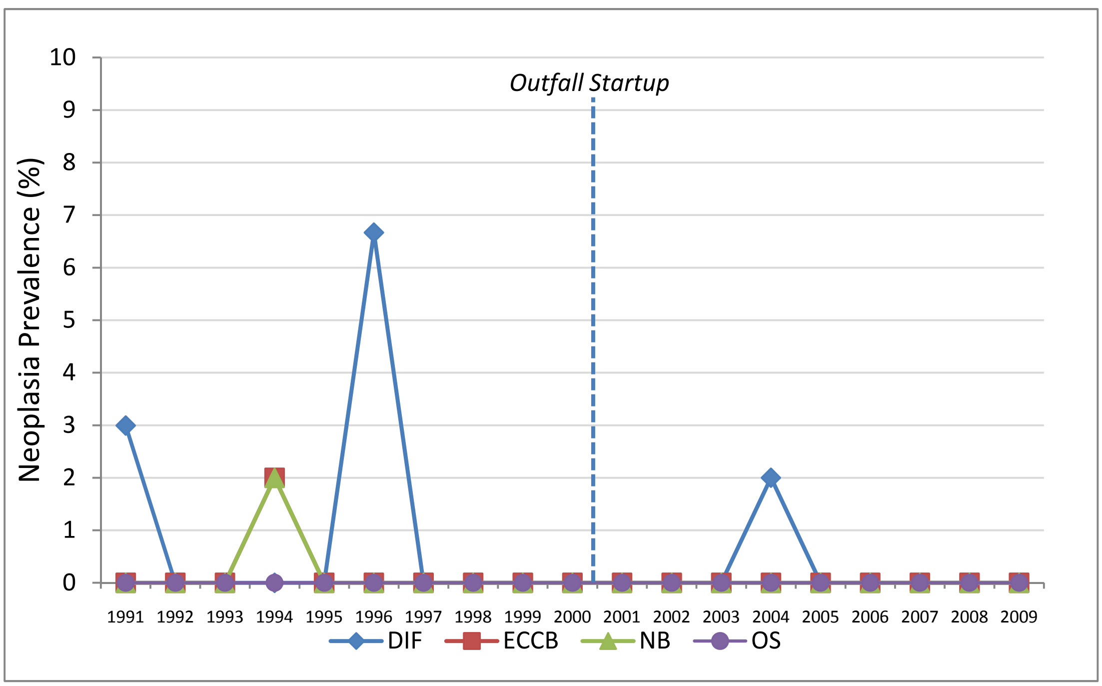


**Figure 3-7. Temporal comparison of neoplasia prevalence (%) in winter flounder by station over time.**

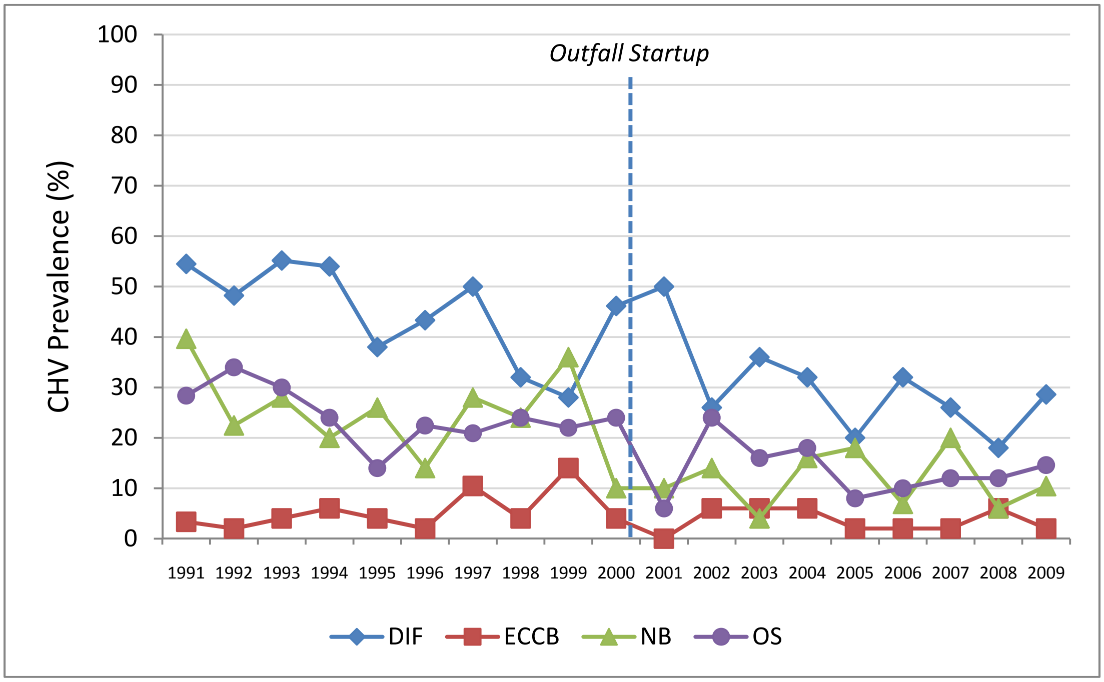


**Figure 3-8. Temporal comparison of prevalence (%) of centrotubular hydropic vacuolation in winter flounder by station over time.**

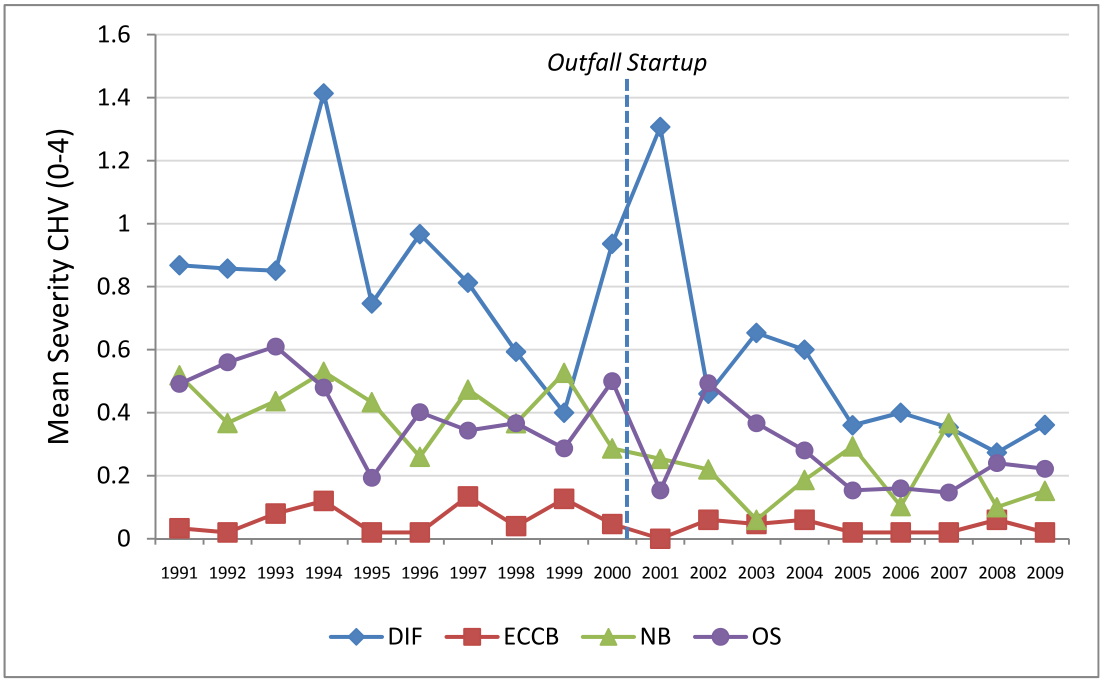


**Figure 3-9.Centrotubular hydropic vacuolation severity (rank) in winter flounder compared between sites and years.**

Assessment of severity is subjective as contrasted with the objective observation of presence or absence of the lesion. The subjectivity of the assessment should be kept in mind when considering the significance of slight changes in the severity index from year to year. Such concern is minimized by the consistent use of one observer for the entire program, with the exception of the years 1993 and 1994, when a second observer was used; however, a subset of those samples were also examined for quality control by the primary observer.

Relationships between age and lesion prevalence were also analyzed. The proportion of fish that had CHV (using data collected since 1997) was calculated for each age class at all stations (Figure 3-10). Sample size for the oldest age classes was low, so the analysis was restricted to fish eight years old or less. At all stations, the prevalence of CHV disease generally increased with age over the first several reproductive years. CHV prevalence in older fish was inconsistent among stations, continuing to increase with age at Deer Island, while leveling-off or declining in older age flounder at most other stations. To further assess the impact of changes in age on hydropic vacuolation prevalence, the percentage of fish at each station in each year that showed some degree of hydropic vacuolation was divided by the average age of fish for that year at that station. This generated an age-corrected index for the presence of hydropic vacuolation (Figure 3-11). The general trend compares well with that of the overall prevalence plot, unweighted for age.

The most common liver lesion at OS in 2009 was macrophage aggregation (Table 3-3). Biliary proliferation is another marker of general substandard health that remained elevated in 2009, being highest at ECCB. This was not accompanied by elevated levels of hydropic vacuolation, a situation one might expect if the biliary proliferation was following chemical insult to biliary epithelial cells, as has been seen at more contaminated sites during this study. In recent years, a data field was added for bile duct trematode prevalence. None were seen this year at any station. However, in three fish from ECCB (5009, 5014 and 5047), unicellular parasites were observed in the bile duct cells of the bile duct lumen. A representative image is shown in Figure 3-12. Dr Mark Myers (NOAA NWFSC) examined this image and made the following comment: “These are probably spores of a myxosporean parasite, and most likely are some species of *Myxidium*.” Thus the biliary proliferation seen at ECCB may be associated with these unicellular parasites.

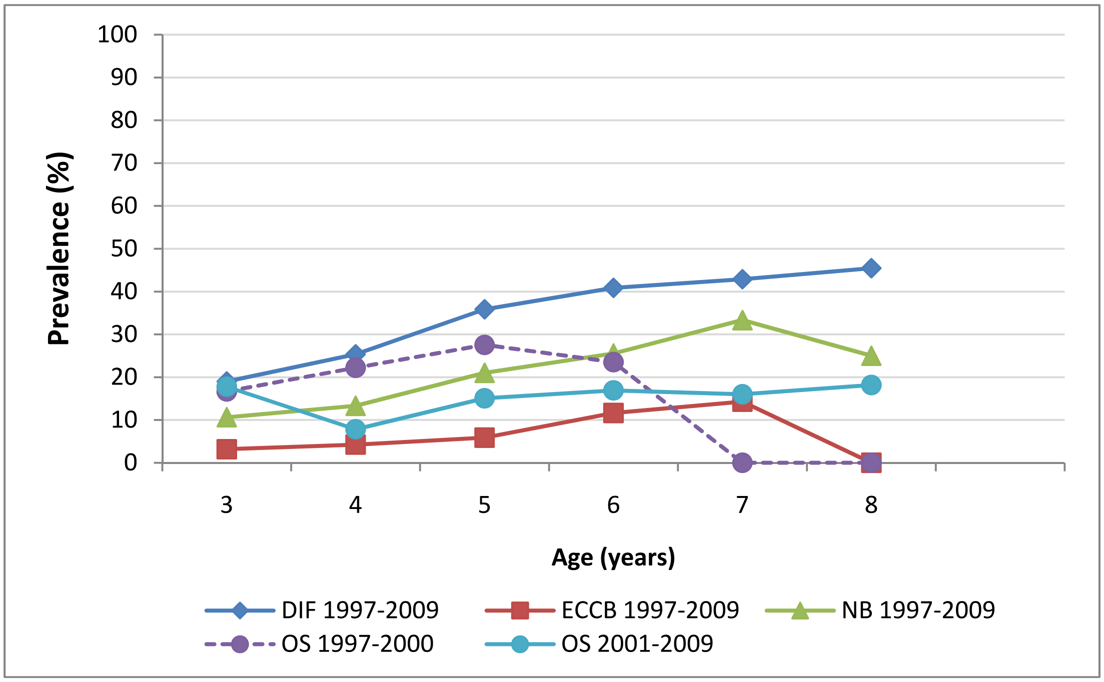


**Figure 3-10. Proportion (%) of winter flounder showing hydropic vacuolation for each age.**

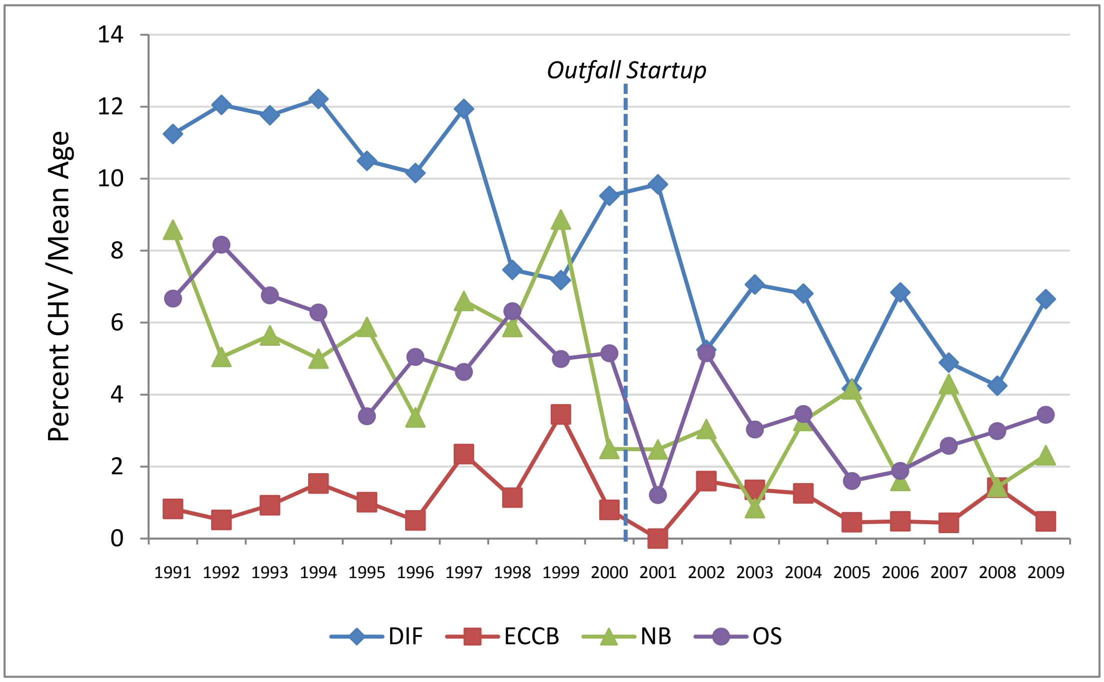


**Figure 3-11. Hydropic vacuolation index (CHV%/Age) for each station by year.**

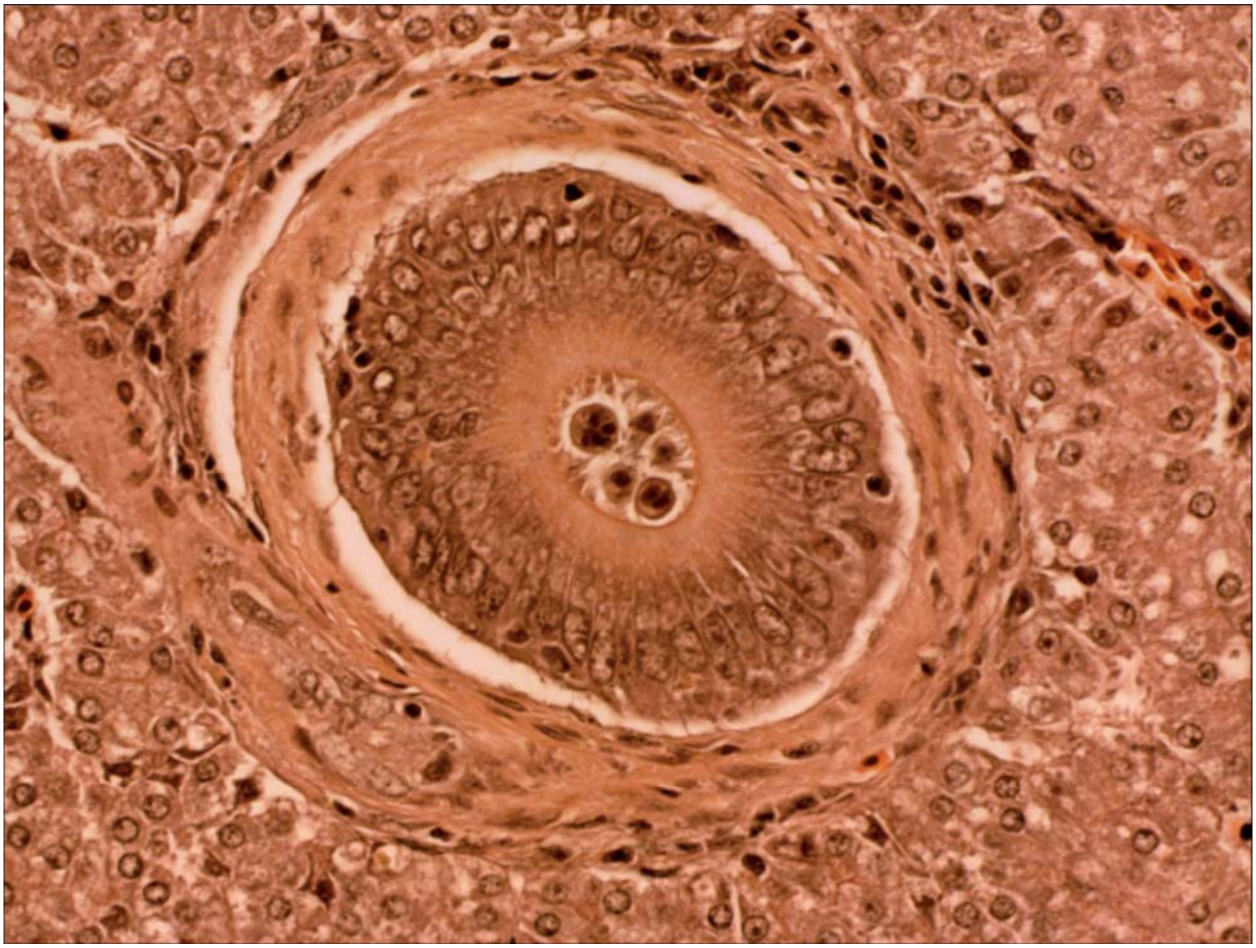

**Figure 3-12. Hematoxylin-and-eosin-stained section of liver from winter flounder FF091-5009. The multinucleate cells in the lumen of the bile duct are a protozoan parasite. Magnification 400 x.**

### 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 2009 was 15%, well below the threshold level.

## 4. CONCLUSIONS

The 2009 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 was consistent with typical predischarge levels, following higher catch rates reported between 2000 and 2004. The overall size of the flounder collected has 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. The proportions of females in the populations sampled remain high at all stations.

Following increased ulcer prevalence beginning in 2003, extensive pathology and microbiology studies were unable to determine a cause of the ulcers (Moore *et al.* 2004). Results of the 2009 survey suggest a continuing decrease in ulcer prevalence at OS, from 36% in 2004 to 0% in 2009. The highest prevalence of blind side ulcers in 2009 was 4% at NB.

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.

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