2007 flounder report for fish and shellfish monitoring

Massachusetts Water Resources Authority

Environmental Quality Department

Report 2008-05

Citation:

Nestler EC, Moore M, and Pembroke A. 2008. 2007 flounder report for fish and shellfish monitoring. Boston: Massachusetts Water Resources Authority. Report 2008-05. 18 p.

2007 flounder report for fish and shellfish monitoring

submitted to

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

prepared by

Eric Nestler[1]
Dr. Michael Moore[2]
Ann Pembroke[1]

[1]**Normandeau Associates, Inc.**
25 Nashua Road
Bedford, NH 03110

[2]**Woods Hole Oceanographic Institution**
Woods Hole, MA 02543

March 2008

This report is dedicated to Bill Crossen, Gloucester skipper and owner of the F/V Odessa. He first worked with me in 1988, and undertook the flounder survey every year until he died in the summer of 2007. Initially it was only Deer Island Flats, but then Bill and I developed the other stations in the light of the survey needs and Bill's knowledge of the resource. His daily cost was fixed. He often left port at 330am and returned home at 9 at night. He had a deep interest in the science, and truly made a substantial contribution to the MWRA project as a whole. He was an independent, free thinking soul, who genuinely cared about the work we did together. It is typical that before he died he ensured that there was a comparable vessel with an identical net willing to carry on the work. F/V Harvest Moon, skipper and owner Mark Carrol, also from Gloucester, will be doing the 2008 survey. Mark was the mate on three previous surveys on the Odessa. ~ Michael Moore

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

1.0 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 projections in the US Environmental Protection Agency's 1988 Supplemental Environmental Impact Statement 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 fish and shellfish (MWRA 1991). The goal of this program is to provide data that can be used to assess potential impacts to fish and shellfish from effluent discharged into Massachusetts Bay. Three indicator species have been used in the program: winter flounder (*Pseudopleuronectes americanus*), lobster (*Homarus americanus*), and blue mussel (*Mytilus edulis*). Resident flounder and lobster 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 and lobster include length, weight, biological condition, the presence of external or internal disease, and concentrations of inorganic and organic contaminants in body tissues. Mussels are collected from a reference location then deployed near the outfall and at monitoring sites in Boston Harbor and the Bays. After a 60-day deployment, mussel tissues are analyzed to assess bioaccumulation of contaminants. Data have been collected for these organisms since 1991 for mussels, and 1992 for flounder and lobster. 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. During 2007, only the flounder monitoring component was required.

This report presents results for the 2007 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 2007 data represent the seventh consecutive year of flounder monitoring since the start up of the Massachusetts Bay outfall in September of 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 2007, along with comparisons to historical flounder data, are presented in Section 3. Finally, conclusions drawn from the 2007 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 and shellfish.

2.0 METHODS

Winter flounder (*Pseudopleuronectes americanus*) were collected from four locations in Boston Harbor and the Bays to obtain specimens for age, weight, and length determination, gross examination of health, and histology of livers.

The methods and protocols used during the 2007 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 Fish and Shellfish Monitoring 2006-2007 (Pembroke et al. 2006).

2.1 STATIONS AND SAMPLING

The 2007 flounder survey was conducted between April 23 and May 7, 2007. Four sites were sampled to collect winter flounder for histological and chemical analyses:

- Deer Island Flats (DIF)
- Off Nantasket Beach (NB)
- Outfall Site (OS)
- East Cape Cod Bay (ECCB).

Table 2-1 provides the planned and actual sampling sites and locations for the 2007 flounder sampling. Adjustments in location and time were made to maximize collection efforts in an attempt to collect the required 50 flounder per site. Figure 2-1 shows the actual monitoring locations. Otter-trawl tows were conducted from the F/V *Odessa*, owned and operated by Captain William Crossen. The scientific crew consisted of principal investigator Dr. Michael Moore from WHOI and biologist Mindy Sweeny from Normandeau. 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 April 23rd, monitoring was completed at OS and NB, while only six fish were collected at DIF. On April 25th ECCB was sampled. Two additional trawls were conducted at DIF on May 7th to complete the station.

2.2 EXTERNAL EXAMINATION

Flounder were processed shipboard immediately after each trawl. Each flounder was assigned a unique identification number to indicate date, time, and site of collection. All specimens were weighed, and standard and total length determined. Flounder were examined externally for fin erosion, bent fin ray, ulcers, net damage, and lymphocystis. Each external condition was noted on a scale from 0 (absent) to 4 (severe). In the case of ulcers, a score of 1 indicated a single ulcer present; a score of 2 indicated 2 ulcers present; etc.

2.3 AGE DETERMINATION

Scales from each specimen were collected for age determination. Scales were removed after first removing any mucus, debris, and epidermis from the dorsum of the caudal peduncle by wiping in the direction of the tail with a blunt-edged table knife. Scales were then collected from the cleaned area by applying quick, firm, scraping motions in the direction of the head. The loosened scales were placed in the labeled age-sample envelope by inserting the knife between the liner of the sample envelope and scraping off the scales. The age of each flounder was determined by scientists at the National Marine Fisheries Services (NMFS) in Woods Hole, Massachusetts through analysis of growth rings (annuli).

2.4 HISTOLOGICAL PROCESSING

Following external examination and removal of scales, fish were processed at sea for histological analysis. Each flounder was killed by cervical section and then dissected shipboard. Livers were removed and examined for grossly visible abnormalities ("Gross Liver Lesion"), then serially sliced, with three equidistant slices preserved in 10% neutral buffered formalin for histological analysis. The gonads of each flounder were examined during the dissection to determine sexual maturity and gender.

2.5 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 25x, 100x, and 200x magnification to quantify the presence and extent of:

- Three types of vacuolation (centrotubular, tubular, and focal)
- Macrophage aggregation
- Biliary duct proliferation
- 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.

2.6 DATA REDUCTION AND GENERAL DATA TREATMENT

All fish and shellfish data (2007 and historical) were extracted directly from the HOM database and imported into SAS software, where data reduction, graphical presentations and statistical analyses were preformed. Data reduction was conducted as described in the Fish and Shellfish Monitoring QAPP (Pembroke et al. 2006). 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.

2.7 DEVIATIONS FROM THE QAPP

Sufficient numbers of flounder were not available at DIF on April 23rd, the first day of sampling. After 70 minutes of trawling, only six fish were collected. Additional trawling at DIF was postponed for two weeks to await inshore movement of flounder. Flounder abundance was adequate to complete the station on May 7th, when two additional trawls were conducted.

Figure 2-1. Flounder Monitoring Locations.

Table 2-1. Planned and Actual Sampling and Locations for Flounder Surveys.

Site (Station ID)	Number of Tows	Planned Location		Actual Location	
		Latitude	Longitude	Latitude	Longitude
Deer Island Flats (DIF)	3	42° 20.4'N	70° 58.4'W	42° 20.9'N	70° 58.5'W
East Cape Cod Bay (ECCB)	2	41° 56.2'N	70° 06.6'W	41° 57.3'N	70° 07.8'W
Off Nantasket Beach (NB)	2	42° 17.6'N	70° 52.2'W	42° 17.5'N	70° 51.5'W
Outfall Site (OS)	2	42° 23.1'N	70° 49.3'W	42° 23.3'N	70° 49.7'W

3.0 RESULTS AND DISCUSSION

3.1 FISH COLLECTED

Winter flounder, each a minimum 30 cm in length, were collected between April 23rd and May 7th, 2007 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 2007, CPUE values at all stations were comparable to those reported the previous year. Catch rates were highest at the Outfall Site. Nonetheless, CPUE at the OS during 2007 remained considerably lower than values reported during 2001 to 2004. Values at all stations were within the range of historical variation.

3.2 PHYSICAL CHARACTERISTICS

Mean values for physical characteristics of the winter flounder collected in 2007 are reported in Table 3-1. These values reflect the project requirement to collect sexually mature specimens (>30cm total length). The highest average values for length, weight, and age were from flounder collected at DIF, while the lowest values were from ECCB.

Mean age for each year was calculated for each station and plotted in Figure 3-2. The average age of flounder collected at OS, NB, and ECCB in 2007 was consistent with historical averages of between four and five years. Mean age at DIF was slightly higher than historical averages. Other exceptions over time include the higher average ages observed at the OS in some recent years, and collections of younger fish from DIF in 1995 and ECCB in 1998 and 2002. Standard length was slightly higher at most stations in 2007 as compared to 2006 (Figure 3-3). The 2007 results continue to suggest that standard lengths have increased since the late 1990’s. Mean weight also appears to have increased over this time period, at least compared to data from 1995 to 1999 (Figure 3-4). Weight data for 2007 were among the highest reported at all stations since monitoring began. At the OS site, a consistent upward trend in mean flounder weight has continued since 2002. These increases in overall size may relate to age, but may also reflect gender to some extent. 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).

3.3 EXTERNAL CONDITION

The external conditions of winter flounder collected in 2007 are presented as prevalence (% of individuals) per station in Table 3-2. The incidence of bent fin ray and fin erosion was highest in flounder collected at DIF, while lymphocystis was highest at OS. Only two flounder with blind side ulcers were collected in 2007, one each from DIF and OS.

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 2007 survey suggests a continuing decrease over recent years, declining at the outfall from 36% in 2004 to 2% in 2007. The highest prevalence observed was the first year it was recorded (2004).

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 in fish collected at DIF during 2007 was the highest in the history of the program. The incidence of fin rot in 2007 was higher than the previous year at all stations except for ECCB. Nevertheless, 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 1980’s when some winter flounder essentially lacked any fins.

3.4 LIVER LESION PREVALENCE

The prevalence (% of individuals) of liver lesions in winter flounder from each of the four stations sampled in 2007 is presented in Table 3-3. Neoplasms were not observed in any of the winter flounder collected during 2007. 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 2007, CHV prevalence at Deer Island dropped slightly from the 2006 level, continuing a general downward trend of contaminant-associated lesions at this site (Figure 3-8). The prevalence at Nantasket Beach rose slightly in 2007, but remained lower than the peak years of 1991 and 1999. CHV for 2007 at the Outfall Site was comparable to the low levels reported during the past two years, and is 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 2007 than the 1994 and 2001 peaks (Figure 3-9). CHV severity at Nantasket Beach in 2007 was slightly higher than the previous year. Severity remained low at the other stations sampled in 2007. 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. Nonetheless, such concern is minimized by the consistent use of one observer for the entire program, except for the years 1993 and 1994 when a second observer was used, and only a susbset of the samples were 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). At the Outfall Site, the pre-discharge and post-discharge years were analyzed separately. Sample size for the oldest age classes was low, so the analysis was restricted to fish of 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 two most common liver lesions at OS in 2007 were biliary proliferation and macrophage aggregation (Table 3-3). Biliary proliferation is another marker of general substandard health. It can reflect chronic hepatic toxicity, in hand with hydropic vacuolation, but it can also follow chronic liver parasitism, such as with flukes. Such flukes are often absent from the histological image so it is not possible to attribute environmental quality impacts to changes in biliary proliferation. Similarly macrophage aggregations accumulate with a variety of substandard liver health situations: parasitism, suboptimal nutrition, and contaminant exposure to mention a few. Thus neither biliary proliferation nor macrophage aggregation is as specific for chronic chemical exposures as hydropic vacuolation appears to be.

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 incidence 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 2007 was 12%, well below the threshold level.

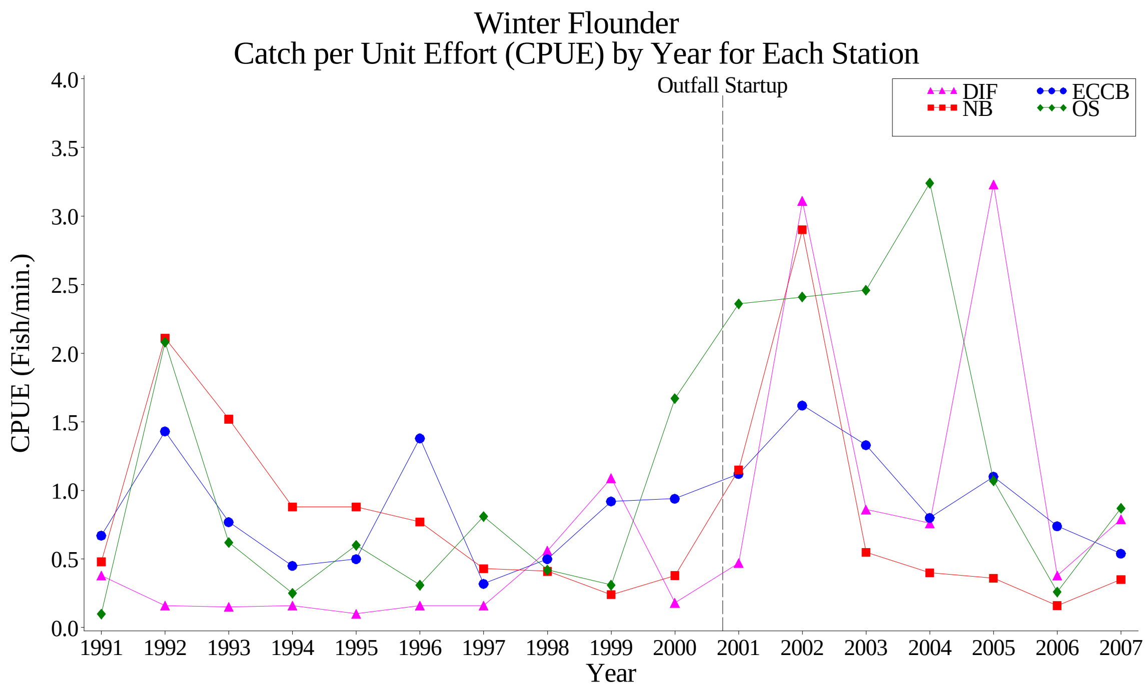

Figure 3-1. Catch per Unit Effort (CPUE) for Winter Flounder Trawled (1991- 2007).

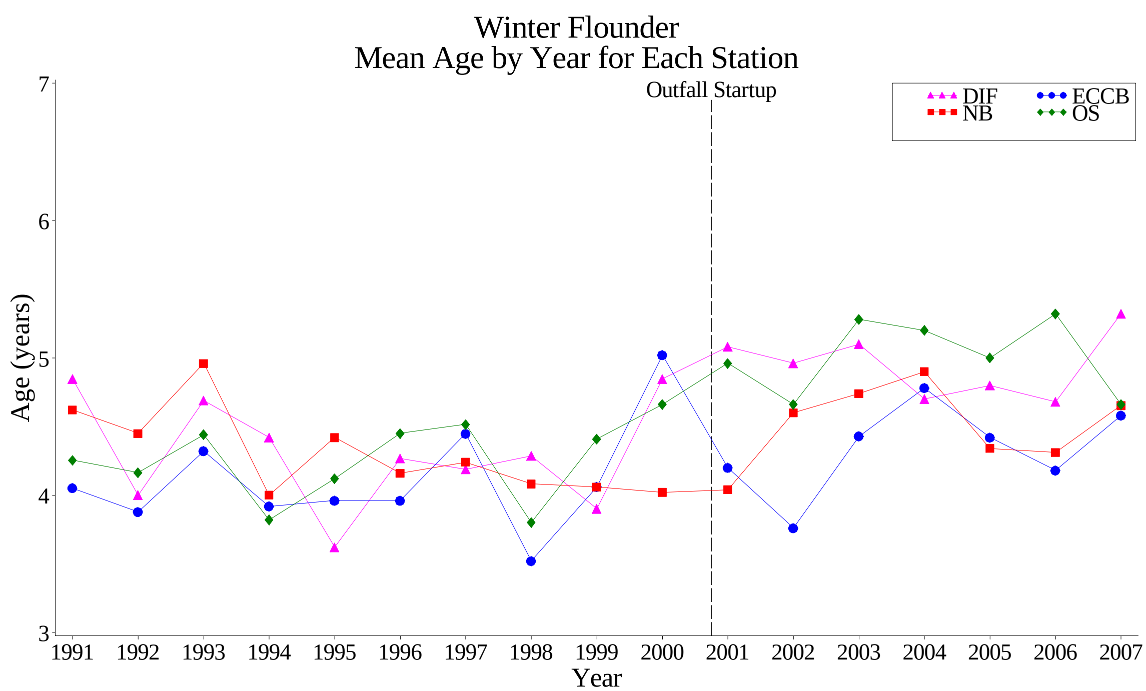

Figure 3-2. Average Flounder Age (years) Compared by Station and Year.

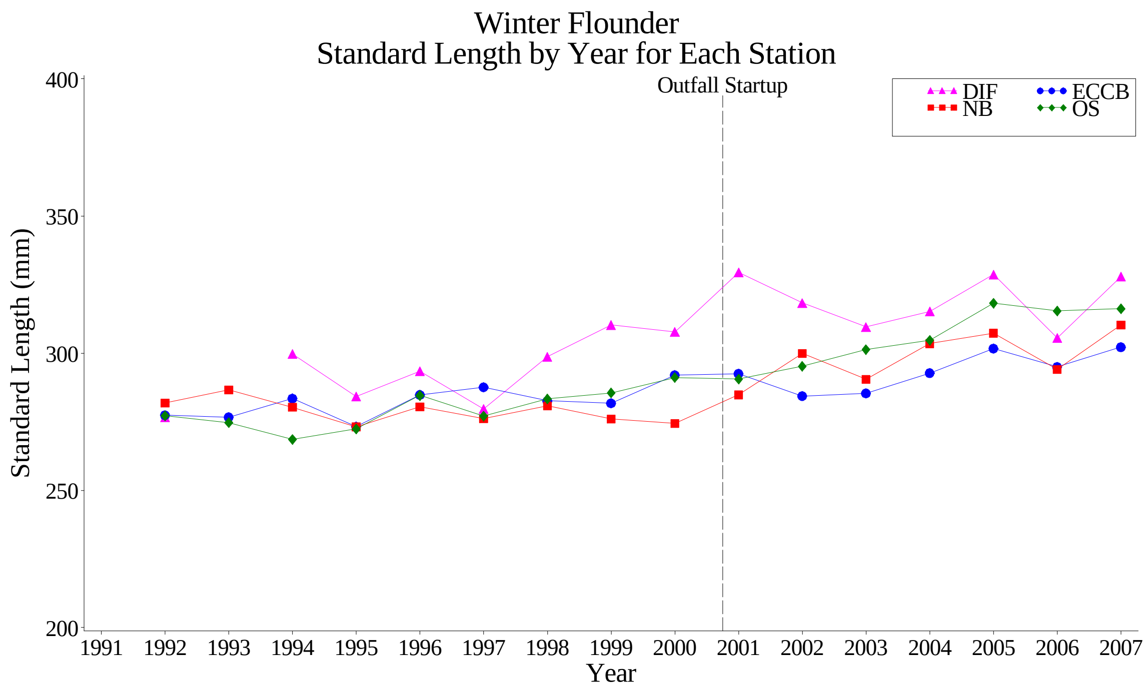

Figure 3-3. Average Flounder Standard Length (mm) Compared by Station and Year.

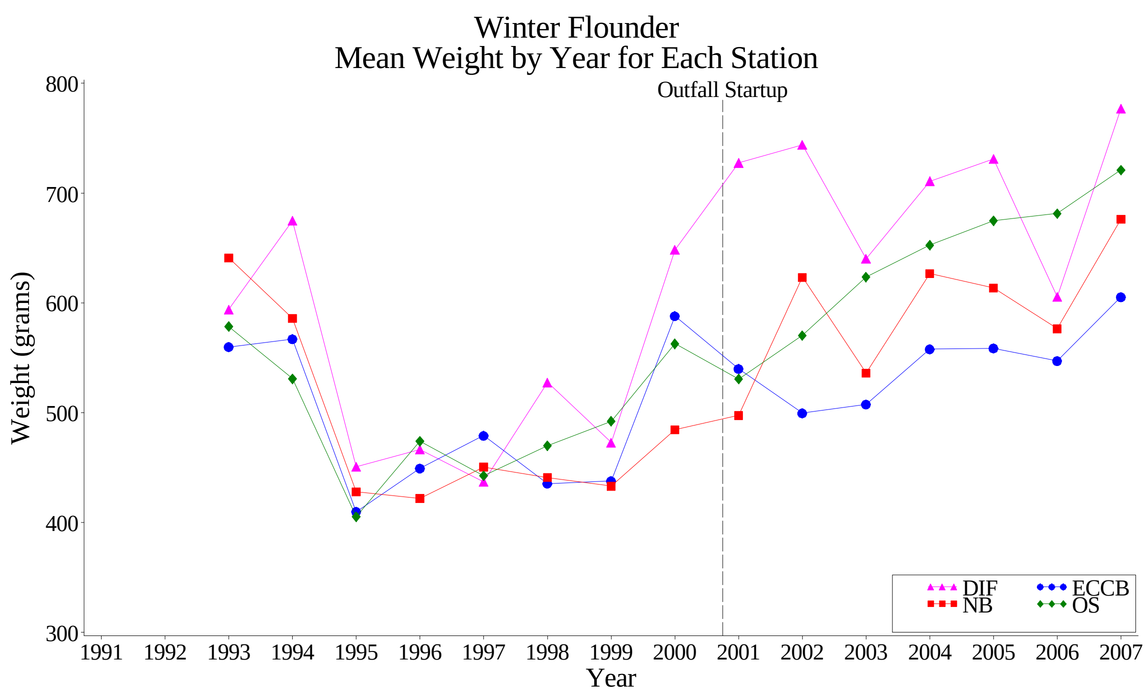

Figure 3-4. Average Flounder Weight (grams) Compared by Station and Year.

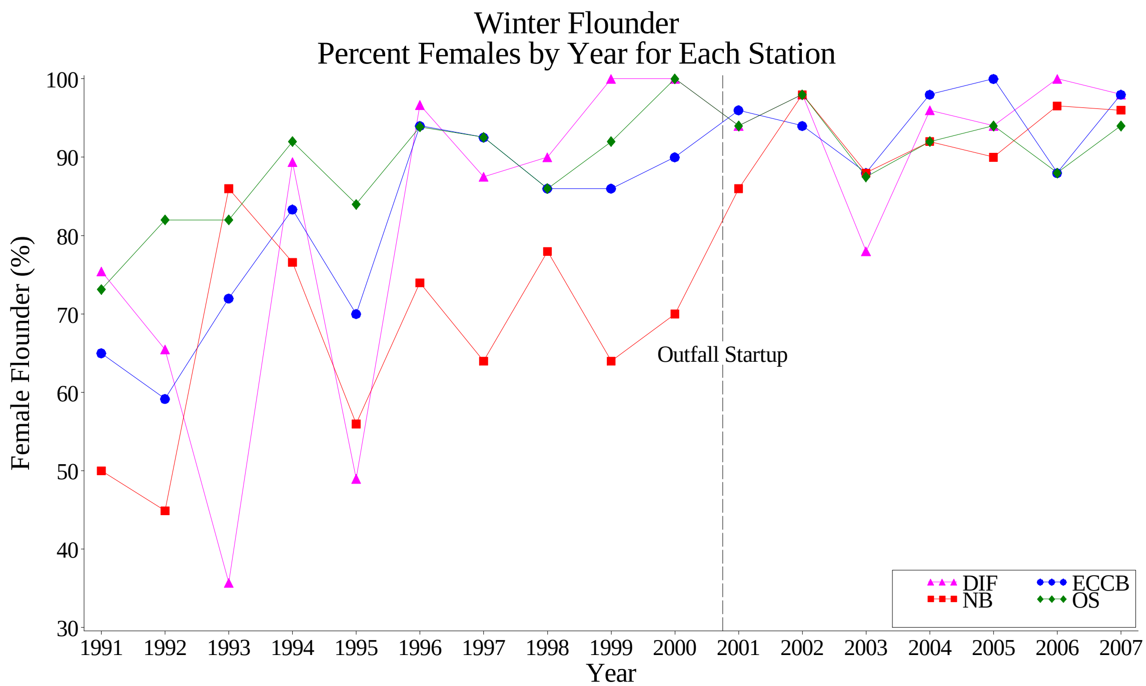

Figure 3-5. Proportion (%) of Female Flounder Compared by Station and Year.

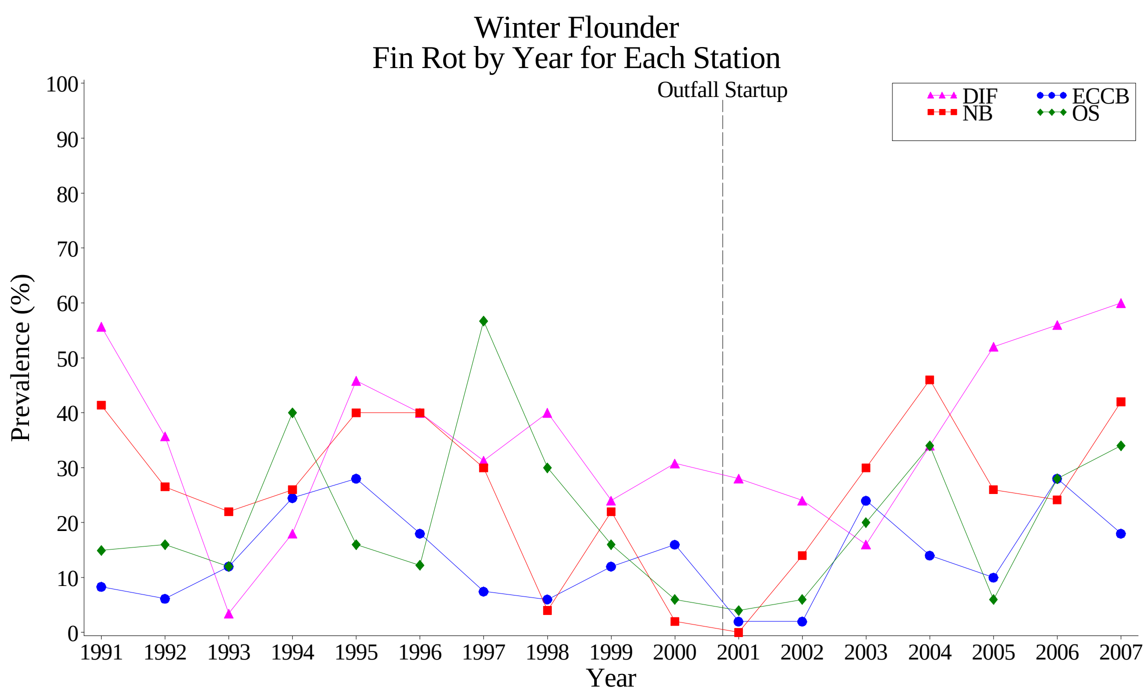

Figure 3-6. Temporal Comparison of Fin Rot Prevalence (%) in Winter Flounder by Station over Time.

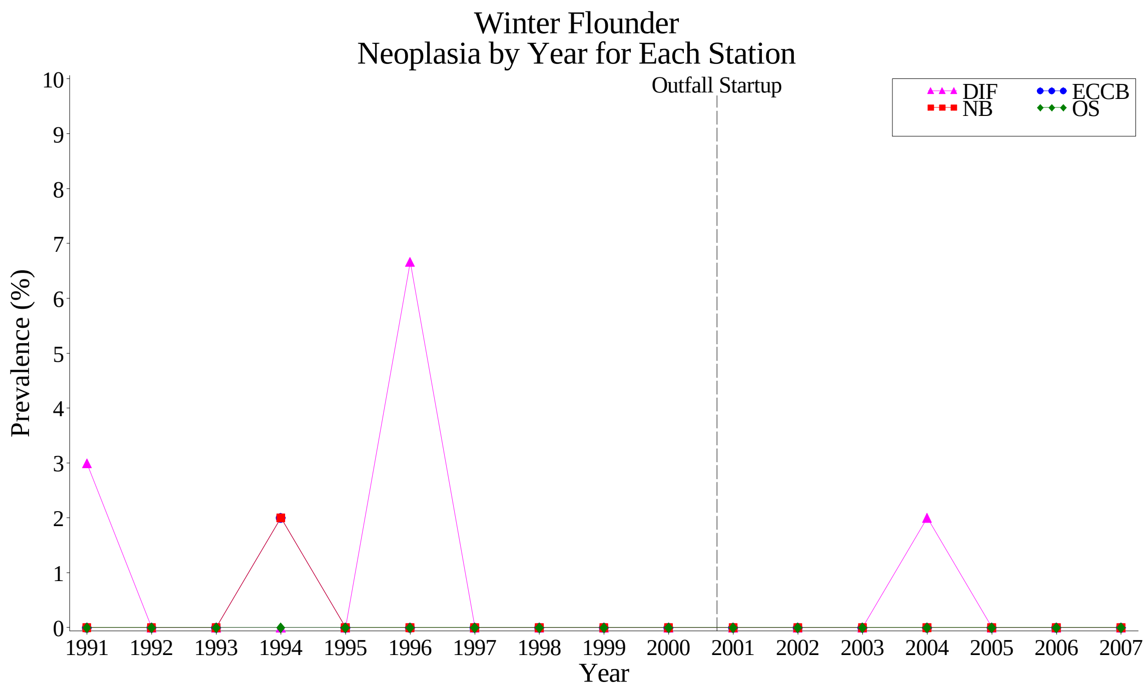

Figure 3-7. Temporal Comparison of Neoplasia Prevalence (%) in Winter Flounder by Station over Time.

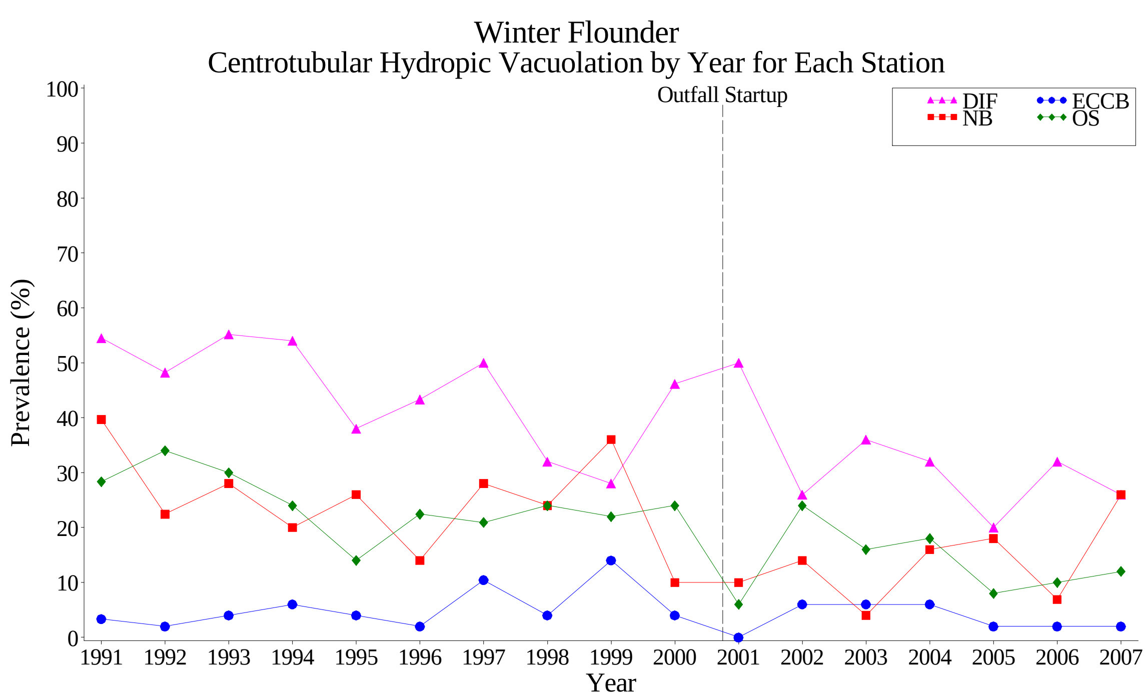

Figure 3-8 Temporal Comparison of Prevalence (%) of Centrotubular Hydropic Vacuolation in Winter Flounder by Station over Time.

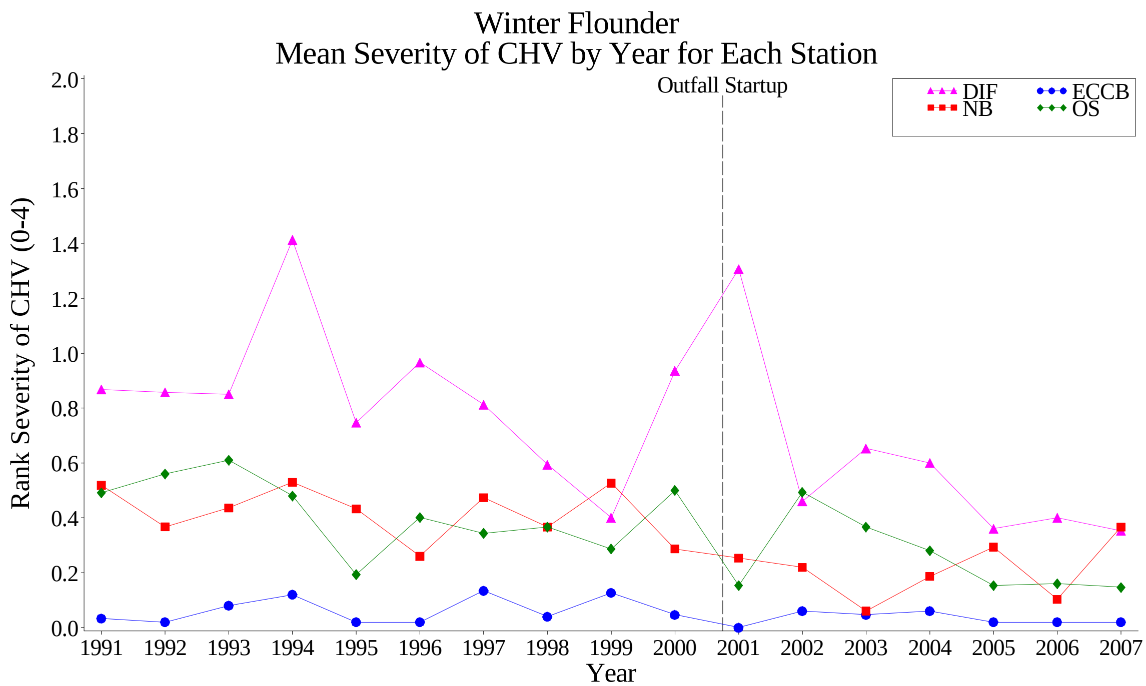

Figure 3-9. Centrotubular Hydropic Vacuolation Severity (rank) in Winter Flounder Compared Between Sites and Years.

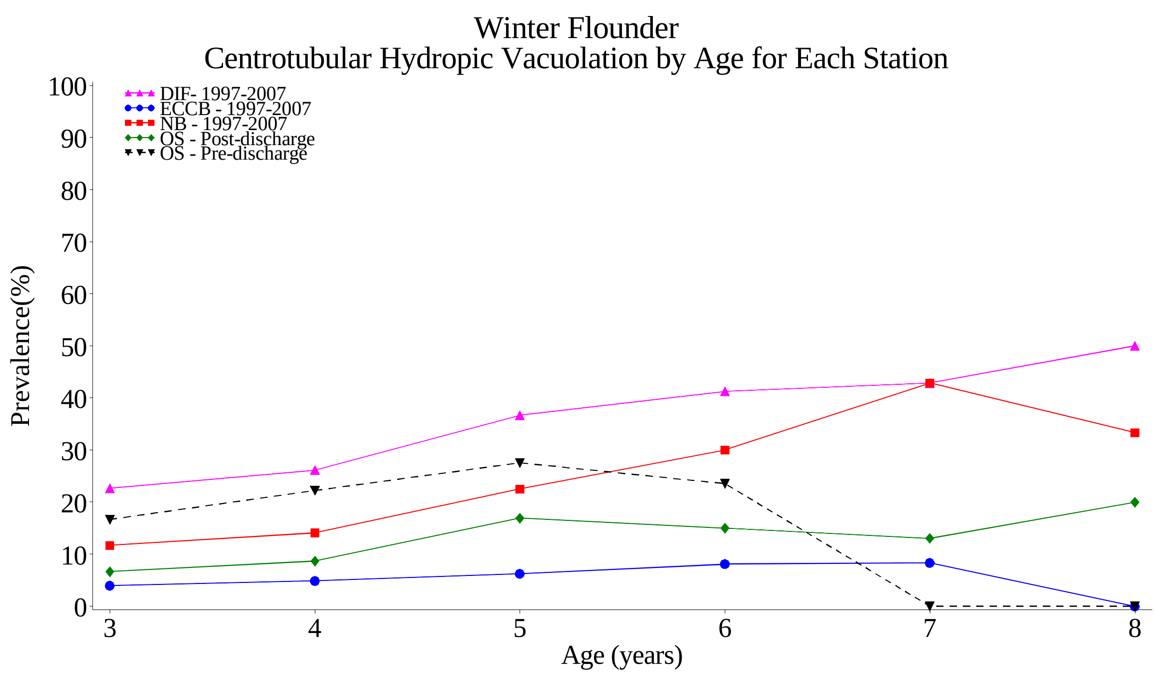

Figure 3-10. Proportion (%) of Winter Flounder Showing Hydropic Vacuolation for Each Age.

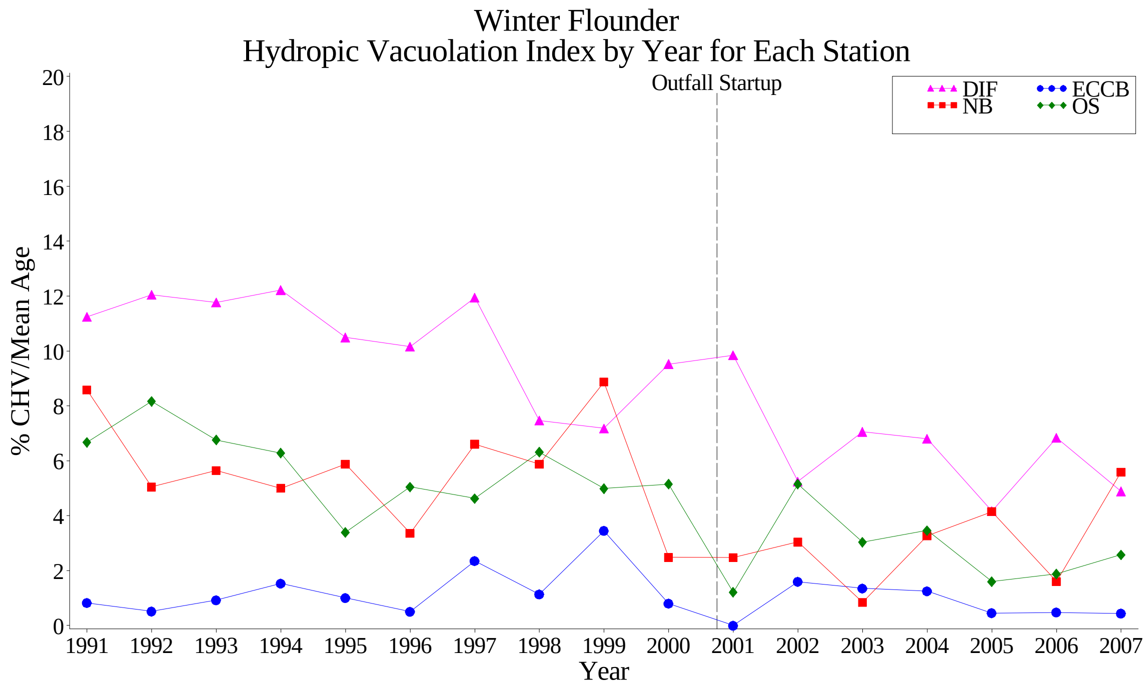

Figure 3-11. Hydropic Vacuolation Index (CHV%/Age) for Each Station by Year.

Table 3-1. Summary of Physical Characteristics of Winter Flounder Collected in 2007.

Parameter	DIF Mean	STDDEV	N	ECCB Mean	STDDEV	N	NB Mean	STDDEV	N	OS Mean	STDDEV	N
Age (years)	5.3	1.0	50	4.6	1.1	50	4.7	1.1	49	4.7	1.1	50
Standard Length (mm)	327.9	29.5	50	302.2	29.0	50	310.3	34.8	50	316.2	31.5	50
Total Length (mm)	398.9	35.3	50	368.5	34.9	50	377.7	40.9	50	387.0	37.7	50
Weight (g)	777.0	207.1	50	605.3	179.5	50	676.3	270.1	50	721.0	248.0	50

Table 3-2. Prevalence (%) of External Conditions Assessed for Winter Flounder Collected in 2007.

External Conditions	Station (sample size)			
	DIF (50)	ECCB (50)	NB (50)	OS (50)
Bent Fin Ray	18	8	10	0
Blind Side Ulcers	2	0	0	2
Fin Erosion	60	18	42	34
Lymphocystis	44	20	16	58

Table 3-3. Prevalence (%) of Liver Lesions in Winter Flounder Collected in 2007.

Lesion Type	Station (sample size)			
	DIF (50)	ECCB (50)	NB (50)	OS (50)
Neoplasm	0	0	0	0
Focal Hydropic Vacuolation	0	0	0	0
Tubular Hydropic Vacuolation	12	0	8	4
Centrotubular Hydropic Vacuolation	26	2	26	12
Biliary Proliferation	18	32	34	26
Macrophage Aggregation	66	52	56	88

4.0 CONCLUSIONS

The 2007 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, and this may be related to changes in the gender ratio. The proportion of females in the populations sampled is at or near an all-time high.

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 2007 survey suggest a continuing decrease in ulcer prevalence at OS, from 36% in 2004 to 2% in 2007.

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.

5.0 REFERENCES

Bosakowski T. and E.J. Wagner. 1994. A survey of trout fin erosion, water quality, and rearing conditions at state fish hatcheries in Utah. Journal of the World Aquaculture Society. 25(2):308-316.

Moore M.J. 2003. Winter flounder ulcer final report for fish and shellfish monitoring. Boston: Massachusetts Water Resources Authority. Report ms-088. 16 p.

Moore M.J., 2006. 2005 Flounder Report for Fish and Shellfish Monitoring. Boston: Massachusetts Water Resources Authority. Report ENQUAD 2006-14. 18 p.

Moore M.J., R.M. Smolowitz, and K. Uhlinger. 2004. Flounder Ulcer Studies. Boston: Massachusetts Water Resources Authority. Report ENQUAD 2004-10. 19 p.

MWRA. 1991. Massachusetts Water Resources Authority effluent outfall monitoring plan. Phase I: baseline studies. MWRA Environmental Quality Department, November 1991. Boston: Massachusetts Water Resources Authority. 95 p.

MWRA. 1997. Massachusetts Water Resources Authority effluent outfall monitoring plan: Phase II postdischarge monitoring. MWRA Environmental Quality Department Miscellaneous Report No. ms-44 Boston: Massachusetts Water Resources Authority. 61 p.

MWRA. 2001. Massachusetts Water Resources Authority Contingency Plan Revision 1. Boston: Massachusetts Water Resources Authority. Report ENQUAD ms-071. 47 p.

MWRA. 2004. Massachusetts Water Resources Authority effluent outfall monitoring plan Revision 1, March 2004. Boston: Massachusetts Water Resources Authority. Report 0-ms-092. 65 p.

Pembroke, A, R Hasevlat, EC Nestler, SD Chapnick, NC Rothman, G Salata, S Kane Driscoll, and MJ Moore. 2006. Combined Work/Quality Assurance Project Plan (QAPP) for Fish and Shellfish Monitoring: 2006-2007. Boston: Massachusetts Water Resources Authority. 97 p.

Pereira, J.J., R. Goldberg, J.J. Ziskowski, P.L. Berrien, W.W. Morse, and D.L. Johnson. 1999. Essential Fish Habitat Source Document: Winter flounder, *Pseudopleuronectes americanus*, life history and habitat characteristics. NOAA Technical Memorandum NMFS-NE-138. National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center.