# Flounder ulcer studies

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**Final Report**

**for**

# Flounder Ulcer Studies

**Task 29.16**

**MWRA Harbor and Outfall Monitoring Project**

**Submitted to**
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This report includes draft unpublished data from:

1) Massachusetts Division of Marine Fisheries Resource Assessment Program Spring Benthic Trawl Survey. Courtesy of Jeremy King, Jack Schwartz and David Pierce, MA DMF
2) National Marine Fisheries Service Bottom Trawl Resource Survey – courtesy of John Ziskowski, NOAA Fisheries

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# APPENDIX

# 1.0 INTRODUCTION

A novel prevalence of blind side ulcerative dermatitis in winter flounder was observed in the 2003 MWRA flounder survey cruise (Figures 1 and 2). Affected fish were in western Massachusetts Bay (Moore 2003). That report included 2002 and 2003 data from the Massachusetts Division of Marine Fisheries Spring Trawl Survey and the National Marine Fisheries Service Bottom Trawl Resource Surveys, whose data both concurred with the MWRA ulcer distribution findings, suggesting that the prevalence of the condition was novel since 2001 and focused on western Massachusetts Bay.


**Figure 1. Typical blind side ulcer of a winter flounder**

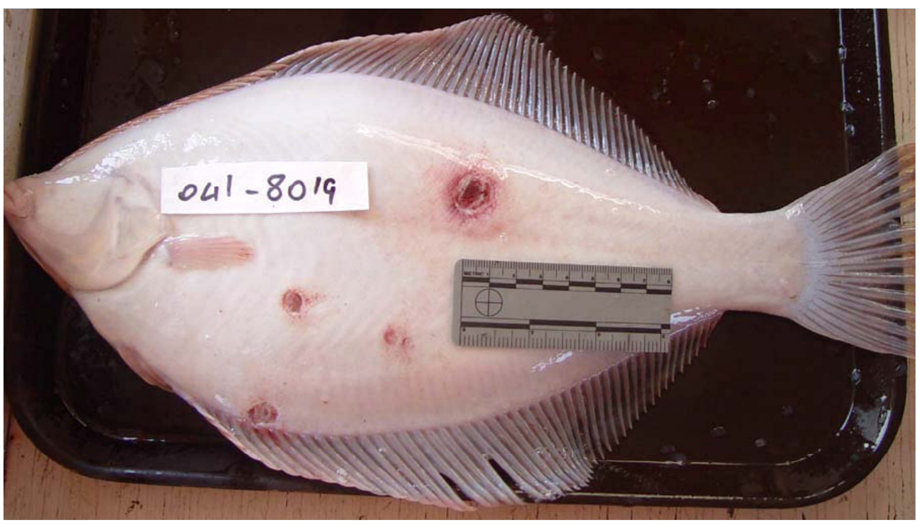



**Figure 2. Multiple blind side ulcers of a winter flounder**

At the request of the Outfall Monitoring Science Advisory Panel (OMSAP) a meeting was held in November 2003 at which fisheries researchers were requested to develop a plan of action to increase understanding of the lesions. The outcome of the meeting was that the MWRA was mandated to expand its normal April flounder survey to: a) develop and circulate a field guide that addresses how to discern the lesion from other skin conditions, and a protocol for recording the severity and incidence of the lesion; b) develop a plan that provides greater spatial coverage to better determine the geographic distribution of the lesion; c) to develop a more pro-active plan for conducting intensive microbiological study of the ulcer lesions. Details of these expansions are found elsewhere (Moore 2004).

Preliminary data from the expanded April flounder survey, as well as data from National Marine Fisheries Service and the Massachusetts Division of Marine Resources indicated the continued presence of the lesion on flounder collected from western Massachusetts Bay, but at a greater prevalence than in 2003. As a result the MWRA determined that it needed better temporal resolution of the incidence of the lesion to better understand its impact on the flounder population. Several additional surveys were planned at approximate three-month intervals at three stations. The first of the additional surveys was completed on June 23. A summary of the results from both the April and June surveys are included in this report.

# 2.0 METHODS

## 2.1 April 12th to May 2nd 2004 MWRA Flounder Survey FF 041

At each of the nine designated sampling sites (Table 1), Outfall Site (OS), Deer Island (DI), Nantasket Beach (NB) and Broad Sound (BS), Eastern Cape Cod Bay (ECCB), FF09, FF14, FF01A, and FF11, otter-trawl tows were conducted to collect a target of 50 sexually mature (4-5 years old) winter flounder. All specimens were weighed, and standard and fork length determined. Scales were taken from each specimen. Each flounder was examined externally and their fin erosion, bent fin ray, ulcer, net damage and lymphocystis condition 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. In addition for fish from the 5 histology stations, the liver was removed and examined for grossly visible abnormalities. Histology data will be reported in a separate data report. Additional details of methods can be found in Lefkovitz *et. al.,* 2002.

A subset of the ulcerated and selected unulcerated flounder were also cultured on board aerobically (Marine Brain/Heart infusion Agar: MBHIA) and an-aerobically (Bacto: Difco Anaerobic Agar, BD, Franklin Lakes NJ). The area to be cultured was first irrigated in sterile sea water. Then a disposable sterile microbiological loop was used to pick up a sample of mucous and debris from the lesion edge. Control plates exposed only to the sterile sea water were also prepared. Anaerobic plates were sealed within 15 minutes of sampling in a GasPak Pouch. Ulcer bearing fish were photographed. Where a fish had more than one ulcer, the most anterior ulcer was sampled. The cultured fish were then individually bagged in ziplock bags, labeled and delivered the following morning on ice to Dr Roxanna Smolowitz, Marine Biological Laboratory, Woods Hole MA, for further bacteriological study. The detailed results of the laboratory study are reported in the appendix to this report.

## 2.2 June 23rd 2004 Additional Trawl Survey Cruise

Flounder were collected using the above methods for external examination only, from the OS, DI, NB and BS (Table 1).

**Table 1. Summary of Winter Flounder Ulcer Prevalence in 2003 and 2004**

| Agency | Station ID | Lat (N) | Long (W) | Ulcer % April 28 - 30 2003 | N | Ulcer % March 2 - May 2 2004 | N | Ulcer % June 23 2004 | N |
|---|---|---|---|---|---|---|---|---|---|
| MWRA | OS (4) | 42.39 | -70.82 | 24 | 50 | 36 | 50 | 14 | 50 |
| MWRA | BS (3) | 42.41 | -70.95 | 16 | 50 | 12 | 50 | 0 | 48 |
| MWRA | NB (2) | 42.29 | -70.86 | 6 | 50 | 30 | 50 | 6 | 16 |
| MWRA | ECCB (9) | 41.96 | -70.12 | 0 | 50 | 0 | 50 | NS | |
| MWRA | DI (1) | 42.35 | -70.97 | 20-27 | 15 | 22 | 50 | 0 | 50 |
| MWRA | FF11 (6) | 42.63 | -70.47 | NS | | 2 | 50 | NS | |
| MWRA | FF01A (7) | 42.52 | -70.61 | NS | | 4 | 50 | NS | |
| MWRA | FF09 (8) | 42.35 | -70.65 | NS | | 42 | 50 | NF | |
| MWRA | FF14 (9) | 42.42 | -70.66 | NS | | 32 | 50 | NF | |
| NMFS | 285 | 43.31 | -66.57 | NS | | 1 | 72 | NS | |
| NMFS | 286 | 43.41 | -66.24 | NS | | 0 | 17 | NS | |
| NMFS | 287 | 43.75 | -66.52 | NS | | 0 | 28 | NS | |
| NMFS | 293 | 44.02 | -66.72 | NS | | 0 | 29 | NS | |
| NMFS | 295 | 44.24 | -66.94 | NS | | 0 | 11 | NS | |
| NMFS | 296 | 44.36 | -66.97 | NS | | 0 | 3 | NS | |
| NMFS | 300 | 43.95 | -68.35 | NS | | 0 | 1 | NS | |
| NMFS | 301 | 43.92 | -68.62 | NS | | 0 | 1 | NS | |
| NMFS | 307 | 43.49 | -69.95 | NS | | 0 | 1 | NS | |
| NMFS | 308 | 43.51 | -70.03 | NS | | 13 | 8 | NS | |
| NMFS | 310 | 43.26 | -70.14 | NS | | 0 | 1 | NS | |
| NMFS | 311 | 43.19 | -70.31 | NS | | 0 | 1 | NS | |
| NMFS | 312 | 42.86 | -70.71 | NS | | 0 | 24 | NS | |
| NMFS | 318 | 42.55 | -70.36 | NS | | 0 | 1 | NS | |
| NMFS | 319 | 42.56 | -70.45 | NS | | 10 | 20 | NS | |
| NMFS | 320 | 42.44 | -70.83 | NS | | 1 | 95 | NS | |
| NMFS | 321 | 42.30 | -70.84 | NS | | 18 | 33 | NS | |
| NMFS | 322 | 42.34 | -70.66 | NS | | 17 | 64 | NS | |
| NMFS | 323 | 42.38 | -70.52 | NS | | 17 | 12 | NS | |
| NMFS | 324 | 42.20 | -70.47 | NS | | 22 | 9 | NS | |
| NMFS | 325 | 42.04 | -70.37 | NS | | 0 | 3 | NS | |
| NMFS | 326 | 42.07 | -70.41 | NS | | 0 | 4 | NS | |
| NMFS | 327 | 42.01 | -70.47 | NS | | 0 | 36 | NS | |
| NMFS | 328 | 41.97 | -70.54 | NS | | 0 | 21 | NS | |
| NMFS | 329 | 41.85 | -70.49 | NS | | 0 | 22 | NS | |
| NMFS | 330 | 41.96 | -70.23 | NS | | 0 | 15 | NS | |
| NMFS | 331 | 41.40 | -71.38 | NS | | 0 | 10 | NS | |
| NMFS | 332 | 41.33 | -71.41 | NS | | 0 | 13 | NS | |
| DMF | 1 | 41.83 | -70.37 | NS | | 0 | 1 | NS | |
| DMF | 2 | 41.88 | -70.36 | NS | | 0 | 5 | NS | |
| DMF | 3 | 41.85 | -70.42 | NS | | 0 | 1 | NS | |
| DMF | 4 | 41.86 | -70.50 | NS | | 0 | 19 | NS | |
| DMF | 5 | 41.82 | -70.52 | NS | | 0 | 15 | NS | |

| Agency | Station ID | Lat (N) | Long (W) | Ulcer % April 28 - 30 2003 | N | Ulcer % March 2 - May 2 2004 | N | Ulcer % June 23 2004 | N |
|---|---|---|---|---|---|---|---|---|---|
| DMF | 6 | 41.77 | -70.36 | NS | | 0 | 4 | NS | |
| DMF | 7 | 41.78 | -70.25 | NS | | 0 | 21 | NS | |
| DMF | 8 | 41.80 | -70.17 | NS | | 0 | 21 | NS | |
| DMF | 9 | 41.81 | -70.07 | NS | | 0 | 38 | NS | |
| DMF | 10 | 41.88 | -70.15 | NS | | 0 | 21 | NS | |
| DMF | 11 | 41.94 | -70.14 | NS | | 0 | 5 | NS | |
| DMF | 12 | 42.01 | -70.14 | NS | | NS | 0 | NS | |
| DMF | 13 | 42.07 | -70.30 | NS | | 0 | 14 | NS | |
| DMF | 14 | 42.07 | -70.39 | NS | | 0 | 2 | NS | |
| DMF | 15 | 41.97 | -70.45 | NS | | 0 | 1 | NS | |
| DMF | 16 | 41.98 | -70.52 | NS | | 0 | 7 | NS | |
| DMF | 17 | 41.98 | -70.58 | NS | | 0 | 14 | NS | |
| DMF | 18 | 42.14 | -70.68 | NS | | 0 | 11 | NS | |
| DMF | 19 | 42.28 | -70.86 | NS | | 0 | 16 | NS | |
| DMF | 20 | 42.32 | -70.79 | NS | | 14 | 22 | NS | |
| DMF | 21 | 42.31 | -70.71 | NS | | 11 | 18 | NS | |
| DMF | 22 | 42.29 | -70.80 | NS | | 11 | 9 | NS | |
| DMF | 23 | 42.39 | -70.82 | NS | | 0 | 14 | NS | |
| DMF | 24 | 42.45 | -70.91 | NS | | 16 | 31 | NS | |
| DMF | 25 | 42.46 | -70.81 | NS | | 0 | 46 | NS | |
| DMF | 26 | 42.46 | -70.72 | NS | | 0 | 2 | NS | |
| DMF | 27 | 42.51 | -70.71 | NS | | 0 | 6 | NS | |
| DMF | 28 | 42.49 | -70.65 | NS | | 0 | 2 | NS | |
| DMF | 29 | 42.53 | -70.67 | NS | | 0 | 8 | NS | |
| DMF | 30 | 42.70 | -70.65 | NS | | 0 | 2 | NS | |
| DMF | 31 | 42.69 | -70.66 | NS | | 0 | 1 | NS | |
| DMF | 32 | 42.73 | -70.67 | NS | | 0 | 4 | NS | |
| DMF | 33 | 42.77 | -70.75 | NS | | 100 | 1 | NS | |
| DMF | 34 | 42.84 | -70.81 | NS | | 0 | 6 | NS | |
| DMF | 35 | 42.78 | -70.79 | NS | | 0 | 5 | NS | |
| DMF | 36 | 42.71 | -70.75 | NS | | 0 | 8 | NS | |
| DMF | 37 | 42.61 | -70.54 | NS | | 0 | 9 | NS | |
| DMF | 38 | 42.65 | -70.54 | NS | | 0 | 13 | NS | |
| DMF | 39 | 41.95 | -70.48 | NS | | 0 | 2 | NS | |
| DMF | 40 | 41.94 | -70.39 | NS | | 100 | 1 | NS | |
| DMF | 41 | 41.96 | -70.35 | NS | | NS | 0 | NS | |
| DMF | 42 | 41.89 | -70.27 | NS | | NS | 0 | NS | |
| DMF | 43 | 41.95 | -70.22 | NS | | 0 | 2 | NS | |
| DMF | 44 | 42.04 | -70.32 | NS | | 0 | 2 | NS | |
| DMF | 45 | 41.99 | -70.22 | NS | | 0 | 2 | NS | |

NF = No flounder detected NS = Not sampled

### 2.3 Massachusetts Division of Marine Fisheries Resource Assessment Program Benthic Trawl Survey

Stations for this survey are shown in Table 1. The survey was made from May 8-10, 2004.

### 2.4 Ulcer prevalence in winter flounder from National Marine Fisheries Service March 2nd – April 22nd 2004 Bottom Trawl Resource Survey Stations

Stations for this survey are shown in Table 1. Full details are available at: http://www.nefsc.noaa.gov/esb/Resource_Survey_Reports.htm. The survey was conducted between the March 2 and April 22, 2004. Massachusetts Bay stations were sampled on April 20 and 21.

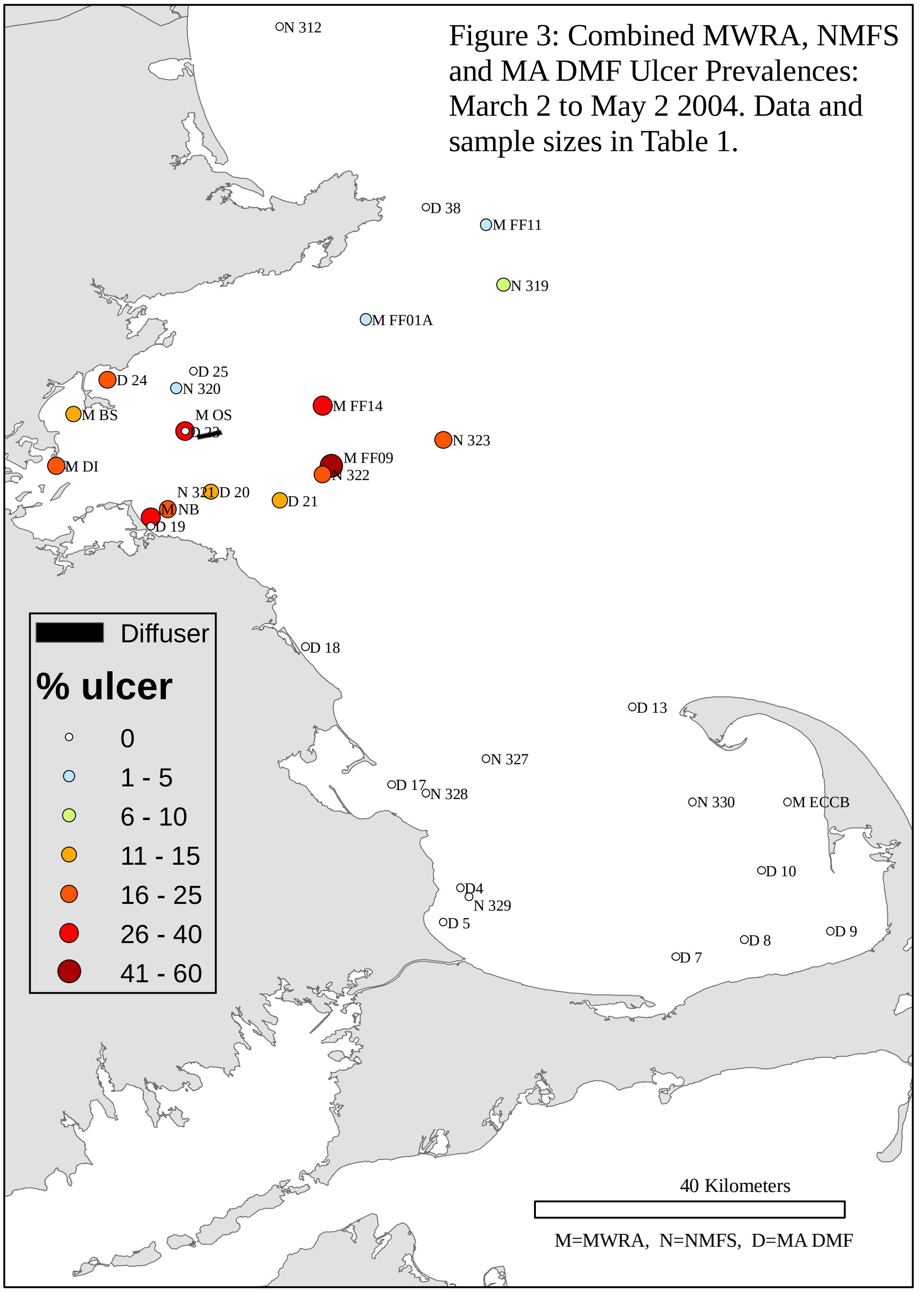
## 3.0 RESULTS

### 3.1 Ulcer Prevalences

The prevalence of ulcers on the blind-side surface of flounder in the 2004 MWRA surveys is shown in Table 1 and Figure 3. Ulcers were present in the April 12 to May 2 survey at the following sites, in decreasing order of prevalence: FF09, FF14, OS, NB, DI, BS, FF01A, and FF11. They were absent from ECCB. Ulcers were also present at a lower prevalence at OS and NB on June 23. Most interesting at the latter date was the changed appearance of the ulcers, where healing of the ulcers was commonly evident (Figure 4). Only one such case was found in the earlier cruise.

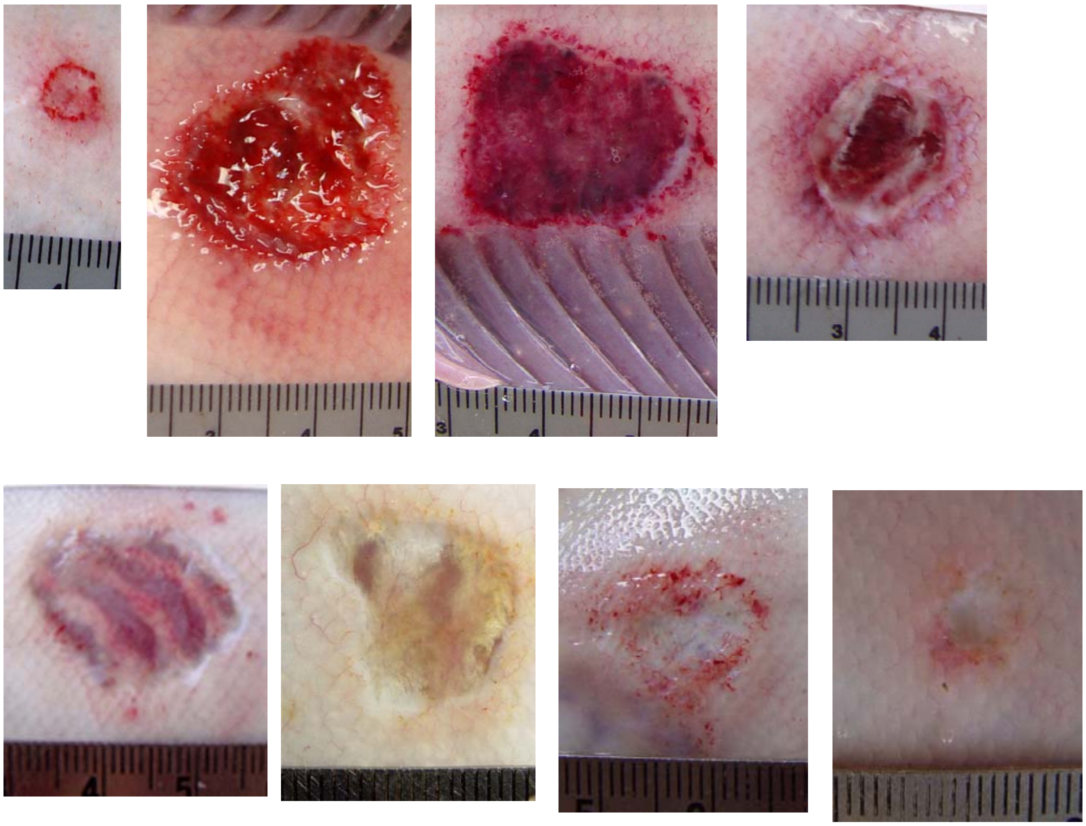
### 3.2 Histology and Microbiology (See Appendix for details)

While some lesions appeared to be trauma related, most were subacute to chronic ulcers characterized by severe deeply ulcerative, mildly to moderately hemorrhagic, macrophagic, lymphocytic and fibrosing superficial and deep dermatitis, with focal loss of epithelium, scales, superficial dermal connective




Data and samples sizes in Table 1; only stations where n ≥ 10 are plotted.

**Figure 3. Combined MWRA, NMFS and MA DMF Ulcer Prevalences: March 2 to May 2 2004**



These images have been arranged in an assumed series through the possible life stages of an ulcer, with the earliest presumed stage on the top left. The first four images were taken on April 26/28th, the last four on June 23rd. Small divisions on the scale are mm. The acute inflammatory pictures in the upper row are replaced by infilling and repair in the lower row.

**Figure 4. Assumed time series of winter flounder ulcer images from 2004 in Massachusetts Bay**

tissues and muscle and necrosis of remaining exposed deeper dermal muscle. Interestingly, in areas of healing that were undergoing re-epithelization, the underlying healing connective tissues showed the development of fat cells in upper dermis in place of scales and superficial dermal muscle. Normal repair would expect the tissue type to reflect that which was lost (i.e., scales and dermal muscle).

There was no significant difference in the histological appearance of the ulcers from different sampling sites. No obvious cause of the ulcers could be identified on any section from any animal in any location. Special stains from selected cases were examined for bacterial, fungal, or DNA- based viral infections in the ulcers. Special stains showed only diffusely spread, low numbers of gram negative bacteria present in the lesions. No viral, or fungal elements were identified in either H&E or specially stained tissue sections.

Other infections were noted in the fish including encysted trematodes (parasite) in the gills and skin, and lymphocystis (viral) and epitheliocystis (bacterial) infections in the gills. Abundance of melanomacrophage centers in the spleen (a potential indicator of subacute to chronic infections in fish) varied in abundance with Case Numbers 1575 (Station FF01A), 1586 (Station Broad Sound), 1591 (Station FF09) showing more macrophages per unit field than other stations.

Histology revealed that some animals showed hydropic vacuolation of bile tubules in the liver parenchyma as noted in winter flounder from Boston Harbor and elsewhere in previous studies. The gills, often reactive to poor water quality, did show parasite infection and in some fish chronic healed lesions, but did not show significant current histological abnormalities. This might indicate that the causes of the ulcers are not significantly affecting the gills.

Cultures of sterile sea water only showed no growth with one exception, suggesting that the sterile technique on board the vessel was generally adequate. Bacteria identified from colonies grown aerobically include Gram negative and positive isolates Table 2. Organisms were present on ulcerated and normal skin. However, some of the bacteria identified are potentially important opportunistic disease producing bacteria, as indicated in Table 2 in the right hand column. This might indicate that there is some other primary cause either for the occurrence of the ulcers (such as trauma) that might be associated with higher levels of opportunistic bacteria in the environment resulting in the occurrence of multi-focal irregularly occurring ulcers of various sizes in the skin.

### *3.3 Massachusetts Division of Marine Fisheries Resource Assessment Program Benthic Trawl Survey*

Results of this survey are summarized in Table 1 and Figure 3. Ulcer prevalence in winter flounder greater than 300 mm in total length was elevated in Nahant Bay, and to the south and south east of the OS. No ulcers were observed at the station nearest the outfall.

### *3.4 Ulcer prevalence in winter flounder from National Marine Fisheries Service March 2 – April 22 2004 Bottom Trawl Resource Survey*

Results from this survey are shown in Table 1 and Figure 3. The primary location for significant ulcer prevalence was in western Massachusetts Bay at sites to the south of the outfall. A station east of Cape Ann did show a prevalence of 10% (2 of 20 fish) and ulcers were also present off Portland ME (1 of 8 fish) and west of Cape Sable NS (1 of 72 fish).

### *3.5 Ulcer Prevalence in winter flounder in Newfoundland waters*

Examination of the photographs in this report lead to the statement by Dr Jerry Payne, Department of Fisheries and Oceans, St John's Newfoundland, Canada that he had not seen or heard of such lesions in winter flounder from the waters off Newfoundland.

## 4.0 DISCUSSION

There are a number of background topics that need consideration before the significance of the above observations can be discussed.

### *4.1 Winter flounder migration off Massachusetts North of Cape Cod.*

Interpretation of the observations in this report requires an understanding of the seasonal migration of winter flounder in the western Massachusetts Bay region. The most detailed study of this issue was by Howe and Coates (1975). Their tagging study found that in Boston Harbor 'sexually mature fish concentrated in shoal water during winter and spring. Following spawning, spent fish dispersed and wandered, relocating to deeper water near the Harbor mouth by midsummer.' Overall their returns from release sites north of Cape Cod showed limited movement from inshore grounds. Their working hypothesis was that fish move short distances to deeper water to avoid summer water temperatures greater than 15 deg. C. McCracken (1963) suggested that north of Cape Cod immature and adult fish move to deeper, relatively warmer water in mid-winter. Howe and Coates (1975) suggest that these

**Table 2. Summary of Organisms Cultured from Ulcerated and Normal Winter Flounder Skin**

| Organism | Case (Fish #) - All from ulcers unless marked by * which designates normal skin | Gram | Shape | Virulence |
|---|---|---|---|---|
| Psychrobacter spp. | 1575B (7003)*, 1576A (6010), 1576B (6001)*, 1585A (1016), 1585B (1016), 1586A (3016), 1586B (3016) , 1587A (2018), 1587B (2017), 1592Ab (9003) | - | Coccobacillus | Opportunistic pathogen |
| Pseudoalteromonas spp. | 1587 (2019), 1588 (4016), 1590 (1028), 1591 (8003), 1592 (9003), 1596 (5017)* | - | Bacillus | |
| Pseudomonas spp. | 1575 (7001), 1585 (1016), 1586 (3016), 1588 (4016), 1592 (9003), | - | Bacillus | Some pathogens, some probionts |
| Carnobacterium mobile | 1575Ab (7001), 1586E (3018) , 1596Aa (5017)* | + | Bacillus | Used as a probiotic† |
| Brevibacterium mcbrellneri | 1587G (2108), 1590A (1028), 1596Fa(5017)* | + | Bacillus | Can be antifungal |
| Microbacteriacae spp. | 1575C (7001), 1585E (1016) , 1588B (4018) | + | Coccus | Normal Gastrointestinal flora |
| Sphingomonas sanguinis | 1587 (2020), 1591 (8001), 1592 (9003) | - | Bacillus | |
| Bacillus sp. | 1575Db (7003)* , 1591C (8003) | + | Bacilli | Used as a probiotic |
| Deinococcus spp? | 1576D (6010) , 1586C (3701) | + | Coccus | |
| Actinomyces spp. | 1576E (6004)* , 1588C (4019) | + | Bacillus | |
| Arthrobacter spp. | 1585C (1017) , 1592E (9003) | +/- | Bacillus | Normal Gastrointestinal flora |
| Staphylococcus spp. | 1585D (1017), 1587C (2018) | + | Coccus | Normal Gastrointestinal flora |
| Deinobacter spp. | 1588A (4016) , 1588H (4019) | + | Coccus | |
| Kytococcus sedentarius | 1591A (8002), 1591B (8003) | + | Bacillus | Pitted keratolysis in humans |
| Rhodococcus spp. | 1576C (6001)* | + | Bacillus | *R. coprophilus* is a human sewage marker |
| Pasteurella spp. | 1571 (7001) | - | Bacillus | |
| Chryseomonas indologenes | 1575 (7003)* | - | Bacillus | |
| Shewanella putrefaciens | 1575 (7001) | - | Bacillus | Normal Gastrointestinal flora |
| Vibrio spp. | 1590 (1026) | - | Bacillus | |
| Sphingomonas paucimobilis | 1590 (1026) | - | Bacillus | |
| Aeromonasspp. | 1596 (5016) | - | Bacillus | |

*Normal skin a opposed to ulcer. † A probiotic is a microbe that interferes with the growth of a pathogen.

animals may indicate offshore spawning activity. The early spring presence of both pre-spawning and post-spawning winter flounder at sites such as the Outfall site would tend to support this hypothesis of an offshore spawning population. Anecdotal evidence from draggermen that offshore winter flounder come into shallower water as the inshore water warms up in late spring (pers. comm. Bill Crossen) seems consistent with this view. As noted above, this can only be true for part of the stock as pre-spawning and post-spawning winter flounder can be caught on Deer Island Flats in the February to April period. Thus the fine details of winter flounder movements at the Boston latitude are still something of an enigma. The ongoing series of additional trawl surveys in the coming year may shed some light in this regard. The June 23rd survey showed flounder to be absent from FF09 and FF14 (pers. comm. Bill Crossen), present at OS and BS, rare at NB and abundant at DI.

## 4.2 Previous study of ulceration in winter flounder

Robohm and Brown (978) found an ulcerative condition of the eyed side in recently caught summer flounder that apparently also developed in other flatfish species including winter flounder in adjacent tanks. The authors concluded that the agent was likely a new *Vibrio* species, which required some prior damage (i.e. net trauma to summer flounder) to the scale barrier to take hold. It could be acutely lethal with fish dying of septicemia. In terms of the current ulcer outbreak this study is important as it indicates that there are pathogenic ulcerogenic bacteria that seem to need concomitant trauma to the surface for the disease to take hold. In Robohm and Brown's case, for the summer flounder at least, it would seem that net trauma was the precipitating event.

## 4.3 Previous Studies of flatfish ulcers in Europe

- (HELCOM 1996) http://www.baltic.vtt.fi/balticinfo/text_files/chapter6.3.html Section 6.3.2.1

  "Various bacteria considered to be involved in the etiology of the ulcer disease of Baltic flounder (*Platichthys flesus*), have been isolated (Dalsgaard 1994, Kroon 1994, Wiklund 1994). However, particularly 'atypical' *Aeromonas salmonicida* was consistently isolated from diseased Baltic Sea flounder (Wiklund 1994), and it is therefore likely, that this bacterium is the main cause of the skin-ulcer disease. Acute as well as healed skin ulcers are more prevalent in male than in female flounder (Wiklund and Bylund 1993, Anonymous 1994). Seasonal changes in the prevalence were reported (Wiklund and Bylund 1993, Kadakas 1994). No temporal trends in the prevalence of acute/healing skin ulcers in flounder from the southwestern parts of the Baltic Sea, i.e., southwest and southeast of Bornholm, were found in the period 1986-93 (Lang and Dethlefsen 1994)".

- (Wiklund and Bylund 1993)

  "Males were more affected (10.7%) than females (3.1%) and larger fish showed higher disease prevalences than smaller ones. The length of the ulcerated flounders varied between 19 and 42 cm. The ulcers were not randomly distributed on the fish trunk. They occurred mainly on the blind side of the males and on the eye side of the females. Ulcers on males tended to occur close to the lateral line; on females, the ulcers were scattered over the whole trunk. Bacterial examination of diseased specimens resulted in the isolation of a cytochrome oxidase-negative, atypical Aeromonas salmonicida, primarily from the ulcers."

## 4.4 Etiology of the ulcers described in winter flounder in Western Massachusetts Bay.

### 4.4.1 Microbiology and histology findings.

The microbiological and histological testing did not provide a clear etiology of the lesions, but suggest that there is no "epizootic" of a primary pathogen causing the lesions. No fungal elements were identified in culture, stained histological sections or scrapings of the ulcers. These results strongly suggest that fungus is not involved in these lesions. Viral particles were not observed histologically and lesions did not show viral associated histological lesions (such as ballooning degeneration of the epithelial cells, margination of chromatin, inclusions, etc). So, while viral causes cannot be totally eliminated (no Transmission Electron Microscopy was done), they are very unlikely to be the cause of these lesions. Histologically, there was no evidence of parasitic infections such as *Pfisteria* in the lesions, although a few of the fish were affected in other areas of the body by viral infections and carried normal loads of parasites.

Histological sections showed that the lesions were infected by bacteria. Evaluations involved a thorough look at potential microbiological causes including aerobic, anaerobic, and acid-fast bacteria. More than 20 different bacteria species, including gram negative and gram positive bacilli and gram positive cocci, isolated from the lesions were identified. Although there was one *Aeromonas sp* and one *Vibrio sp.* isolated from one individual fish, these were not confirmed as typical known marine fish pathogens such as *Aeromonas salmonicida,* (Wiklund 1994, Wiklund and Byland 1993) or *Vibrio anguillarum*.

The testing done should have been able to detect potentially pathogenic bacteria commonly found in wastewater, including *Clostridium perfingens* and *Pseudomonas aeruginosa* if they were present, but these species were not found in the lesions. All the bacterial isolates found are organisms that would normally be found on or in the fish as part of their normal flora, or expected to be found in the normal marine environment.

Since no one bacteria was consistently present in the lesions (as would be expected if these lesions were primarily caused by bacteria), it is likely that some other prior insult to the dermis (puncture wound, etc), allowed bacteria present in the environment to enter into the tissues and cause infection. Opportunistic agents could be present on the fish themselves, in the Massachusetts Bay sediments or present in the water column or treated wastewater, but there would seem to be the need for some other primary insult, which allowed the agent to penetrate the scale barrier.

### 4.4.2 Spatial distribution and sediment contact.

The highest prevalence was observed in northwestern Massachusetts Bay, but the fish having lesions were broadly distributed in a band running approximately 50 km ESE from Boston Harbor. Individual fish with lesions were found by researchers at stations in Cape Cod Bay, off Cape Ann, off Portland, and near Cape Sable, Nova Scotia. The presence of the lesions on the blind side of the flounder, which is normally in contact with the sea-floor, suggests contact with the sediments may play a factor in the initiation or development of the ulcers.

MWRA monitoring indicates that there has been no general increase in nearfield (or farfield) sediment contamination over historical levels (Maciolek *et al*. 2003) that could be related to epithelial damage allowing for the induction of the observed ulcerative dermatitis. Studies by the U.S. Geological Survey did document a detectable increase in *Clostridium perfringens* spores and silver, both of which are excellent tracers of wastewater solids, in nearfield sediment trap samples collected in the months after outfall startup (Bothner *et al*. 2002). However, these increases were not observed in seafloor sediments themselves, and tracer concentrations in the post-startup sediment trap samples were similar to those measured in the early 1990s (Bothner *et al.* 2002).

### 4.4.3 Temporal factor(s).

The falling prevalence of ulcers between April and June 2004, the increased prevalence of healed ulcers during the same timeframe, and the absence of ulcers in the Fall NMFS surveys in 2002 and 2003 suggest that there is a seasonal aspect to this syndrome. A large number of seasonal and interannual factors, many of which covary, could hypothetically be associated with the induction of the ulcers. A partial listing includes:

- Changes in the fish during spawning. The late winter/ early spring spawning of winter flounder and other seasonal movements of the fish, are summarized in Section 4.1 but are not completely understood.
- Seasonal precipitation. Increased precipitation in winter-spring leads to increased land runoff, and also to increases in river flows, treatment plant flows, and short-term increases in the proportion of MWRA flows receiving only primary treatment. Any of these could be a vector for seasonal variability in the delivery of solids, contaminants, or pathogens to the seafloor.
- Sediment resuspension and transport. The northeast storms responsible for much winter-spring precipitation and runoff cause substantial wave-induced sediment resuspension and transport in western Massachusetts Bay (Butman et al. 2002), moving both fine sediments and contaminants (Bothner et al. 2002) and possibly sediment-assocaated pathogens that could play a role in the induction or development of these lesions.
- Interannual climate variations. In addition to seasonal variability, factors associated with interannual variability in weather may or may not play a role in the presence or severity of this sydrome. For example, ulcers were observed but not quantified in the flounder survey in April 2001, and were observed in appreciable prevalence in the surveys in both 2003 and 2004. No ulcers were observed in April 2002. The winter of 2001-2002 occurred during an extreme drought, with almost no winter storms or precipitation, and therefore extremely low river flows and wastewater flows. Additionally, the winters of 2002-3 and 2003-4 were characterized by extremely cold weather and resulted in some of the coldest winter bottomwater temperatures in Massachusetts Bay in the last decade.

The long-term mooring and sediment studies carried out in western Mass. Bay by the US Geological Survey provide an excellent dataset on seasonal and interannual variability in western Massachusetts Bay (Bothner et al. 2002; Butman et al. 2002), that could be evaluated to help identify seasonal signals in the data that may or may not be associated with the syndrome. In addition to the contaminant results already obtained, the study has generated an archive of sediment and sediment trap samples that could be analyzed for new or emerging contaminants of concern, if any are identified while evaluating this condition.

# 5.0 SUMMARY

The blind-side winter flounder ulcers first noted during MWRA's survey in 2003 were found again in 2004. Using standardized protocols, MWRA, DMF and NMFS more completely documented the prevalence and geographic extent of the condition. Extensive pathology and microbiology studies have been unable to determine a cause of the ulcers.

With respect to MWRA's discharge, MWRA's monitoring has not found significant changes in either the waters or sediment quality of Massachusetts Bay that point to a likely cause of the ulcers. Flounder with ulcers have been found over a large geographic area, from Boston Harbor to Stellwagen Basin, making it difficult to understand how this localized, well-treated, and well-diluted discharge could be a cause. Conversely, MWRA's discharge is within the area where affected fish are found, and the ulcers were not observed until after the new outfall began operating. It is therefore impossible to either rule in or to rule out MWRA's outfall discharge as a contributing factor.

Evidence of healed ulcers and a lessened ulcer prevalence in early summer 2004 indicate that the lesions may be a non-lethal seasonal condition from which affected fish can recover. This finding is encouraging, and is under further investigation.

# 6.0 RECOMMENDATIONS

1. Continue the additional trawl surveys through until next spring to assess changing ulcer prevalence with time (the next survey is scheduled for the week of September 28, 2004).

2. Continue to quantify prevalence and severity of the lesion during April 2005 flounder monitoring studies.

3. Continue to cooperate with state and federal fisheries agencies and scientists to evaluate the condition.

4. Further evaluate the long-term USGS mooring, sediment trap, and surficial sediment data for seasonal or interannual changes in sediment quality, either natural or anthropogenic, that might be related to the syndrome.

# 7.0 REFERENCES

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