WINTER FLOUNDER ULCER FINAL REPORT

FOR

FISH AND SHELLFISH MONITORING

July 25, 2003

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Submitted to
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1. Introduction

Between April 28th and 30th 2003, the Flounder Survey Cruise was conducted at stations located in the Massachusetts and Cape Cod Bays: close to the offshore outfall site, at Deer Island Flats, Nantasket Beach, Broad Sound, and Eastern Cape Cod Bay. This survey represents the continuation of flounder monitoring as part of MWRA's Harbor and Outfall Monitoring Program (HOM). The F/V *Odessa*, owned and operated by Mr. William B. Crossen served as the sampling platform during the survey. The survey report and preliminary Outfall Site histology data reports have already been submitted. During the 2003 survey a marked prevalence of ulcers was observed on the blind surface of a number of flounder (flatfish have both eyes on the upper 'eyed' surface, with the blind surface resting on the ocean bottom). This was regarded as a novel finding. This report provides an interpretation of the ulcer prevalence observed during the survey in the context of other available information.

2. Methods

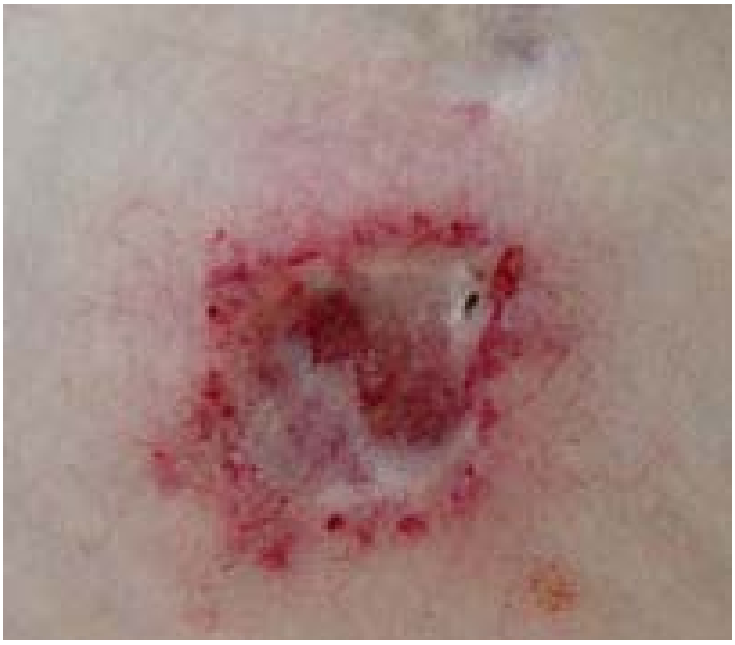
Field data for presence or absence of blind side skin ulcers were analyzed by station for 2003[1]. The "external lesion" data from past MWRA surveys, and field notes and comments kept by Joanne Lahey of Battelle were also examined. Other field biologists from NMFS, Mass DMF and USEPA were later asked for their observations on ulcers in winter flounder in the region. Stations for which data are available are shown in Figure 2.

Representative samples of fish with these ulcers from Nantasket Beach and the Outfall Site were placed in an ice filled cooler, immediately after initial examination, and delivered to the Marine Biological Laboratory and examined by Dr. Roxanna Smolowitz 16-19 hours after collection. They were sampled the same day for microbiological culture and histology. Bacterial cultures were held at room temperature for 4-5 days before sub-culturing and submission to Micro Technologies of Wiscasset, Maine for further identification.

[1] This data set is incomplete. Prior to the survey a discussion occurred between WHOI, Battelle and MWRA as to the consistency of the recording of external lesions in flounder throughout the Harbor and Outfall Monitoring Program. It was apparent that there had been significant between-year inconsistencies, thus it was agreed to drop this data field. Thus the field forms for 2003 did not have a data entry field for scoring external lesions. Station locations are shown in Figure 2. By the end of day 1 of the 2003 survey, by which time Deer Island and the Outfall had been sampled, it became apparent that there was a significant prevalence of ulcers present on the blind side of the fish. Thus on day 2 presence or absence of ulcers was recorded for Broad Sound and Nantasket Beach. The Outfall Site was revisited and 70 flounder visually screened for ulcers. There was no time to re-visit Deer Island that day. However of the 15 fish sampled at Battelle for chemistry from Deer Island, approximate ulcer prevalence was recalled post facto by Joanne Lahey at Battelle. After Day 2, the need to return to Deer Island to survey for ulcer prevalence was discussed with Battelle and the decision was made to not incur further vessel costs until requested to do so by MWRA.

FIGURE 1. ULCERS ON BLIND SIDE OF WINTER FLOUNDER COLLECTED AT THE OUTFALL SITE, 04/29/03

a) Typical ulcers on blind side of flounder.



b) Dermal ulcer penetrating to the skeletal muscle

3. Results

The typical appearance of ulcers on the blind surface of flounder in the 2003 survey is shown in Figure 1.

3.1 MWRA Sources: The field observations over the duration of the MWRA's monitoring program were made as follows: 1990 – 1992 WHOI (Moore), 1993-1994 Battelle (Lahey et al), 1995-2000 WHOI (Moore), 2001 WHOI (Woodin), 2002-2003 WHOI (Moore). The samples of 15 fish from stations analyzed for contaminants were dissected at Battelle and examined by Battelle staff. This author (Moore) does not recall seeing these ulcers prior to 2003. Lahey felt that the ulcers were present at a low level in previous years. Woodin, who only collected fish in 2001, when asked recently to describe the nature of the lesions observed depicted 'a circular "sore" of varying penetration through the skin, and mainly (if not solely) on the blind side'. When shown pictures of the ulcers photographed in 2003 he immediately recognized them as the lesions he had scored in 2001. However, the data are insufficient to conclude that all the external lesions recorded that year were ulcers, as the data are not specific for that lesion type alone.[2]

3.1a 2003 Data: The prevalence of ulcers observed on flounder in the 2003 survey is shown in Table 1 and included in Figure 2.

Table 1 – Prevalence of external ulcers on the blind surface of winter flounder sampled in April 2003.

Station	Ulcer Prevalence Percent (sample size)
Outfall Site	24% (70)
Broad Sound	16% (50)
Nantasket Beach	6% (50)
Eastern Cape Cod Bay	0% (50)
Deer Island	20-27% (15)*

*3 or 4 Flounder sampled for chemistry were recalled in hindsight to bear ulcers – Joanne Lahey pers. comm.

[2] There is no definition of what constitutes an 'External Lesion' in the Fish and Shellfish QAPP Lefkovitz, L., S. Abramson, and M. Moore. 2002. Combined Work/Quality Assurance Project Plan (CW/QAPP) for Fish and Shellfish Monitoring: 2002/2005. Boston: Massachusetts Water Resources Authority. Report ENQUAD ms-078. 71 p. External lesion score parameters in this database were not defined beyond a scale of severity, thus the data parameter is of limited usefulness, as it can include a diversity of lesion types such as lymphocystis (a virally induced swelling of epithelial cells), ulcers, and other conditions. Trauma induced by otter trawling, although this is routinely recognized, is not recorded. Thus any visible pathology could trigger a non-zero score for a particular fish. The prevalence of 'external lesions' from 1998 to 2002 in the MWRA database is shown in Table 2. Note that these lesions are poorly defined as to type.

3.1b. MWRA Database: The prevalence of external lesions as reported in the MWRA database is shown in Table 2.

Table 2 – 'External Lesions' percent prevalence (sample size) from MWRA database

Year	Deer Island	Nantasket Beach	Broad Sound	Outfall Site	Eastern Cape Cod Bay
1998	7 (15)	7 (15)	0 (15)	7 (15)	0 (15)
1999	0 (15)	7 (15)	27 (15)	0 (15)	0 (15)
2000	0 (15)	0 (15)	0 (15)	0 (15)	0 (15)
2001	22 (50)	0 (50)	10 (50)	6 (50)	0 (50)
2002	0 (50)	2 (50)	2 (50)	0 (50)	0 (50)

3.1c Battelle Logbook. Review of the comments written in the Battelle field logbook by Joanne Lahey for 1993 and 1994 reveals no evidence of blind surface ulcers. There are numerous references to small red and white spots, primarily on the eyed surface, but nothing that reflects the ulcers clearly observed in the 2003 survey. Much of what is described in these notes is probably the acute net trauma that is frequently seen in trawl caught flounder. These lesions are small ecyhmotic hemorrhages induced by the fish being crammed into the net twine by the motion of the net and the pressure of other fish. The "Gross score", a presumed index of external lesions, recorded for these two years in the MWRA database was upwards of 80% for all stations, so it is unlikely that these observations are in any way comparable to those made in later years.

3.2 Other Sources

NOAA Milford Lab - John Ziskowski, (pers.comm.)

Ziskowski observed ulcers in the Spring and Fall of 2002 (Table 3 and Figure 2).

Table 3 - Prevalence of skin ulcers in winter flounder: 2002 NMFS survey (J. Ziskowksi, Pers. comm.)

Date	Station #	Lat/Long	% (N) Ulcer Prevalence
April 24 2002	400	42 20 N 070 36 W	15 (27)
Oct. 24 2002	53 (~ 400)	42 20 N 070 36 W	6 (67)
April 24 2002	327	42 07 N 070 31 W	1 (140)
April 24 2002	328	42 03 N 070 28 W	4 (24)

This was the first year Ziskowski had noticed these lesions. John conducted careful external examination of all flounders caught from Boston Harbor during the 'Fish Day' series in 1989, 1992 and 1995 and never saw any such ulcers. Ulcers were also seen in 3/130 American plaice (*Hippogloissoides platessoides*) in October 2002 at Station 53 shown in Table 3 above.

Massachusetts Division of Marine Fisheries - Thomas Currier (pers. comm.).

In one tow on May 7 2003 in 9-10 meters of water off Nantasket Beach, 5/44 winter flounder had ulcers. The fish ranged from 30 to 39 cm in length and the ulcers were apparently comparable to those photographed on the MWRA 2003 survey cruise. In addition, in 27-28 m of water off Brant Rock one lesion, in a 30 cm fish, in a sample of 45 winter flounder was observed. Currier observed other winter flounder with ulcers at other stations but they were not part of a designated pathology sample. They did record one ulcer on a yellowtail flounder that was part of a 12 fish sample and taken in 32-34 m of water off Manomet. In their survey time series database (1978-2002) they have only three other instances where ulcers of whatever kind were recorded, all within the last two years: a winter flounder in a sample of 25 off Barnstable Harbor in May 2001, a summer flounder in Nantucket Sound in a sample of 9 in May 2002, and a winter flounder in a sample of 25 east of Salem. Between 8 and 10 May 2003, the Division Resource Assessment Project conducted about 15 tows between Nahant and the New Hampshire boundary. Currier was aboard during that time and he does remember seeing ulcers like that described for the 2003 MWRA survey. He did not believe that the incidence was as high as was recorded for the shallow Nantasket Beach tow. Quantitative gross external pathological observations were conducted for several species including winter flounder, yellowtail flounder and American plaice. When the observations were made was determined prior to taking a length measurement for that species and is dependent on other sampling priorities. There were pathology observations made on a 62 fish sub sample of winter flounder at a depth of 53 meters east of Eastern Point
(42° 35.48’ 70° 34.88’) and on a 54 winter flounder sample near Burnham Rocks (42° 31.32’ 70° 40.60’) that indicated a high incidence of lymphocystis. Ulcers were not observed during either of these observations. There were three samples of three winter flounder each (27 individuals) taken in Massachusetts Bay, Cape Cod Bay and North of Cape Ann during their May 2003 survey. Those samples were of whole fish and were frozen for an ongoing contaminant analysis study and did not have any ulcers.

USEPA Narragansett – George Gardner (pers.comm.)

The USEPA approach was to collect 100 animals per site in the spring of the years 1986 to 1991. The sites included Long Island Sound, Black Rock Harbor CT (1986 only), Fox Island and Gaspee Point in Narragansett Bay, New Bedford Harbor, Quincy Bay, East Cape Cod Bay, Martha's Vineyard and Georges Bank. They also have a sizable 1990 – 1992 EMAP data base that includes collection sites between the Chesapeake Bay and New York City. Ulcers, if present, were recorded in these EMAP collections. Winter flounder ulcers were seldom seen in their pathology studies. Gardner recalled that Black Rock Harbor, CT was the only urban site where they found winter flounder with ulcers.

3.3 Laboratory examination of ulcers found in 2003 Flounder Survey – Dr Roxanna Smolowitz.

A report from Dr Smolowitz at the Marine Biological Laboratory is appended. The report confirms that histologically the fish were suffering from an ulcerative dermatitis that lacked any fungal or acid fast bacterial involvement. A number of bacteria were cultured, none of which were regarded to be pathogenic by the microbiology laboratory. Two isolates were common to

all the the fish examined: one was identified as *Pseudomonas/Flavobacterium* spp, the other as *Shewanella* sp. Neither of these is considered a pathogen. A number of different biotypes of bacteria identified as *Pseudomonas* sp were found; different biotypes were isolated from different fish. The genus *Pseudomonas* includes many ubiquitous aquatic bacteria and some species have been reported to cause disease in fish. Further testing would be required to identify the species of *Pseudomonas*. There was no evidence of *Pseudomonas aeruginosa,* which is the species of *Pseudomonas* typically found in wastewater.

4. Discussion

From the available data on skin ulceration in winter flounder in the study area, the following conclusions can be drawn: observations by MWRA and other researchers indicate that the lesions observed in 2003 have not been observed in marked numbers in winter flounder prior to 2001. The highest prevalence of lesions appears to be in the Boston Harbor – western Massachusetts Bay region (Figure 2)**.**

Skin ulceration in marine flatfish is not a rare occurrence; however the etiology of this condition is not understood. They have received particular attention over the past 30 years (Wiklund and Bylund 1993, Lang *et al.* 1999, Noga 2000). Wiklund and Bylund (1993) observed a 2-11% prevalence of ulceration in the European flounder (*Platichthys flesus*). The bacterium *Aeromonas salmonicida* was considered a significant organism in the condition. They discussed the possible etiologies that included fishing gear trauma, pollution and salinity gradients and poor body condition. They concluded that etiology was obscure in most cases, in spite of a greater prevalence in the more degraded habitats. It was difficult to relate ulcers to specific chemicals or pollutants. Sex, size and sampling season affected ulcer prevalence. Lang et al (1999) found a 4-11% prevalence in the same species in the Baltic. Salinity and temperature affected prevalence. They commented that the role of contaminants had been discussed and disputed for more than two decades. They could not discount the role of contaminants in the syndrome, but that prevalence observations needed interpretation in both host and site contexts. Noga (2000) reviewed the multitude of factors and agents associated with skin ulceration in fish. He concluded that skin ulceration is a well-recognized indicator of a polluted or otherwise stressed aquatic environment, that involves a complex array of biological and physical risk factors and host responses.

Only one study is known to the author to have provided historical data on skin lesions on winter flounder: skin ulceration prevalence in 1979-1983 was reported for commercially important fish in the NW Atlantic including winter flounder (Ziskowski et al. 1987). For winter flounder, 0.07% of 4240 winter flounder had skin ulceration in the greater Boston Harbor area, and 0.1% of 2027 had skin ulceration in the eastern portion of Massachusetts Bay. No cause for the reported ulcers was suggested.

Thus it would seem that the prevalence of ulcers in the western portion of Massachusetts Bay, has increased markedly, beginning in 2001. Given the general uncertainty about the specific etiology of skin lesions it is not possible to determine at this time the cause of the observed ulceration in winter flounder.

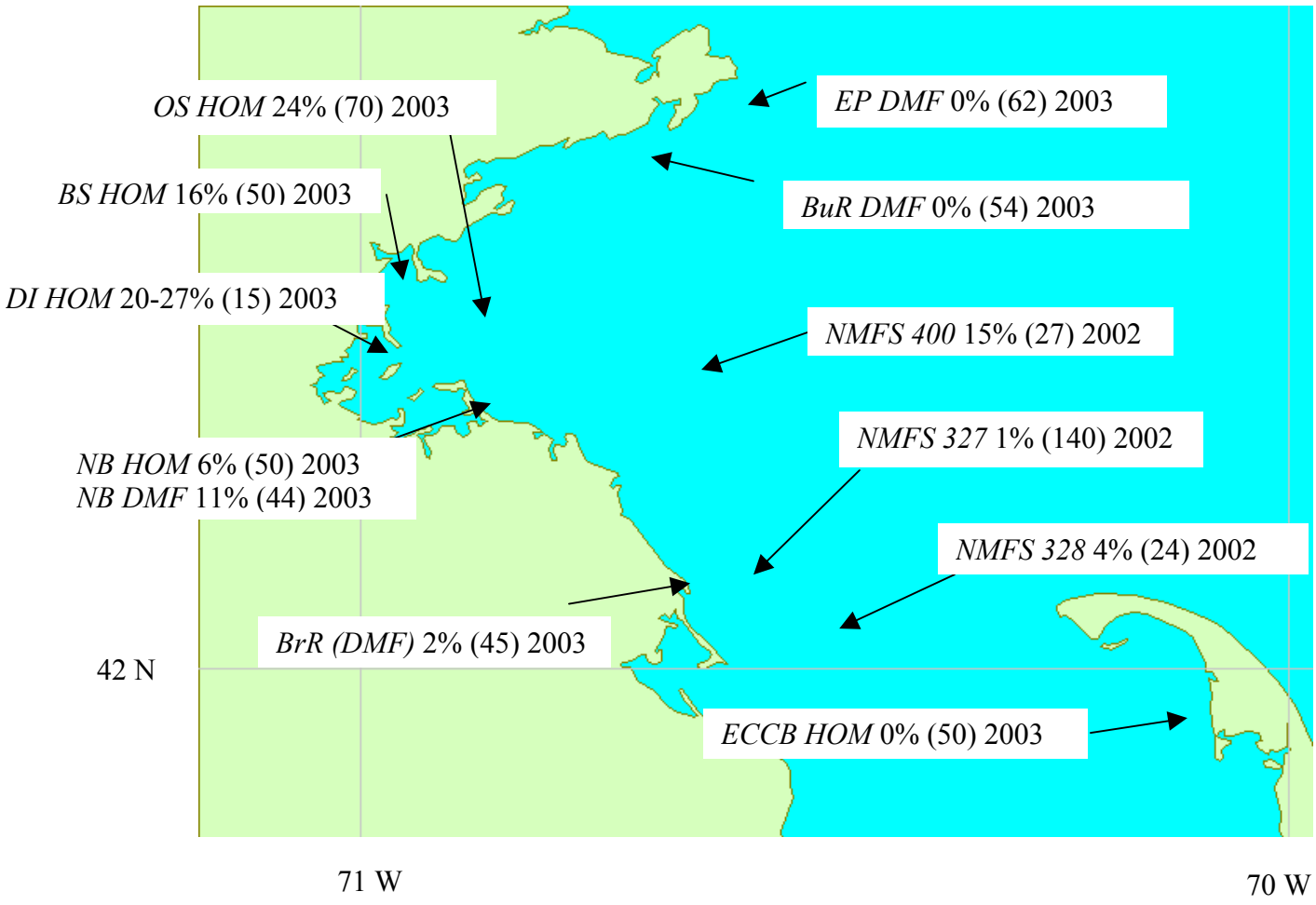


FIGURE 2. PREVALENCE OF FLOUNDER ULCERS IN SPRINC 2002/2003. DATA FROM HOM 2003 FLOUNDER SURVEY, NMFS BOTTOM FISH SURVEY 2002 (J. ZISKOWSKI PERS. COMM,) AND MASS *DMF* GROUNDFISH SURVEY (T CURRIER PERS. COMM.).

Data shown as: *Site Agency* Ulcer prevalence (Sample Size) and year collected.

EP = Eastern Point, BuR = Burnham Rocks, OS = Outfall Site, BS = Broad Sound, DI = Deer Island Flats, NB = Nantasket Beach, BrR = Brandt Rock, ECCB = Eastern Cape Cod Bay.

5. Problems experienced, actions taken, and recommendations

A method for categorizing and recording external lesions should be developed. There should be an illustrated definition for use in the field of a) net trauma, b) viral lymphocystis, c) degrees of fin erosion and d) skin ulceration, e) other lesion types with an opportunity to describe them sufficiently to allow data to be appropriately compared in hindsight.

It is recommended that: a) the eyed and blind side of all fish with lesions be photographed at the time of sampling; b) type photographs be assimilated to allow a set of visual lesion definitions and grades to be established, and c) future surveys record specific external lesion types rather than a generic score of external abnormality.

Acknowledgements

This report has been made possible by significant input from Tom Currier, Massachusetts Division of Marine Fisheries, John Ziskowski (National Marine Fisheries Service), George Gardner (US Environmental Protection Agency), and Roxanna Smolowitz (Marine Biological Laboratory).

Case Report for Winter Flounder (MBL Case No. 1264), submitted by Dr. Michael Moore on 4/30/03.

These fish were collected from Broad Sound (Bagged) and Boston Outfall (Unbagged) 04/29/30 places on ice in a cooler until necropsy on 04/30/03.

Gross Pathology:

Dermal ulcers were noted on fish numbers 1 (03-3029), 2 (03-3048), 3 (not labeled and unbagged), 4 (not labeled and bagged), 5 (not labeled and unbagged), 6 (not labeled and unbagged), 7 (not labeled and unbagged) on the down side (originally the right lateral side of the fish) of winter flounder submitted for examination. Fish number 8 (not labeled and unbagged) did not present with dermal ulcers. Animals 1 to 7 had at least two ulcers in the blind side dermis. Ulcers ranged from 0.5 to 1.5 cm in diameter. Most ulcers showed central depression and ulceration with hemorrhagic margins and petechial hemorrhages in the surrounding more normal dermis. In the center of some lesions, underlying exposed muscle was noted. Abdomen of some animals had been opened for prior sampling.

Other external lesions noted were few to moderate numbers of white nodules of approximately 1 mm in diameter in the dermis of the fins of fish 3, 4, 6, 7 and 8 (Lymphocystis). Internally, approx. 0.5 mm diameter, round, white nodules (microsporidial granulomas) were noted in the anterior intestine and mesentery of fish 2 and 3 and were associated with mild small adhesions of the mesenteries to other peritoneal organs.

Examination of gills showed no unusual findings. Scraps of the ulcers showed mucus, cellular debris and bacteria.

Bacterial Evaluation:

One ulcer in each fish was sampled bacterially by Dr Smolowitz. At each sampling, the lesion was flushed with sterile sea water then sampled with a sterile loop. The samples were plated on a marine brain heart infusion agar and held at room temperature for 4-5 days. Individual colonies types (based on color and colony formation) were identified and counted in each sample then isolated and sent to Micro Technologies Inc. for identification. Only microbial samples 1264 C and E were present in all fish sampled (colony numbers on the original plates ranged from 2 to 15 for #C and from 2 to 20 for #E). Isolate B was identified in fish 3 to 7 only and ranged from colony counts of 1 to 3 per plate). B was identifed as *Pseudomonas* sp. C was identified as *Pseudomonas/Flavobacterium* spp and E was identified as *Shewanella. Shewanella* sp. is often present in dead fish (putrefaction bacteria). *Pseudomonas* spp. are ubiquitous aquatic bacteria (Horsley 1977) with some species common in wastewater (de Vicente *et al.* 1991). Some species have been reported to have caused disease in fish (Larsen and Jensen 1977, Wawrzyniak and Grawinski 1991). Further identification of these bacteria (esp. *Pseudomonas*) using DNA sequencing may be helpful. There was no evidence of *Pseudomonas aeruginosa.*

Histological examination:

Focally extensive dermatitis with central ulceration and numerous bacteria noted in the lesions. Peripherally alternating hyperplasia or thinning of stratified squamous epithelium with inter and intra cellular edema, necrosis and sloughing of surface layers of the stratified squamous epithelium (resulting pitted profile of the surface layers of the epithelium), and edema of underlying squamous cells with breakage of tonofilaments and small edematous clefts between cells. Scales and scale pockets are missing in areas adjacent to the ulcers in many foci. In other foci, hemorrhage and inflammation are noted in the scale pocket tissues and adjacent to the scale itself. The underlying dermis in affected areas shows moderate to severe hemorrhage, prominent vessels with congestion and mild to moderate edema with focally severe necrosis of dermal and adventitial connective tissues and in some areas the associated muscles bundles. Chronic diffuse moderate lymphocytic, macrophagic and lesser heterophilic, fibrosing dermatitis is noted in the affected dermis of most lesions. Rarely granulation tissue is noted at the base of the ulcers. Hemorrhage and inflammation extended into the perimysium and adventitial connective tissues surrounding and lying between muscle bundles in areas underlying lesions.

Liver (3 animals examined)- no significant lesions
Kidney (3animals examined) - no significant lesions
Spleen (2 animals examined)- Possible depletion of lymphocytes (2 animals) and mild increase in melanomacrophage centers (1 animal).
Gill (3 animals examined)- no significant lesions
Tail fin (1 animals) - lymphocytsis
Intestine (1 animal examined) - multifocal large submucosal caseous granulomas containing possible organisms and cell debris

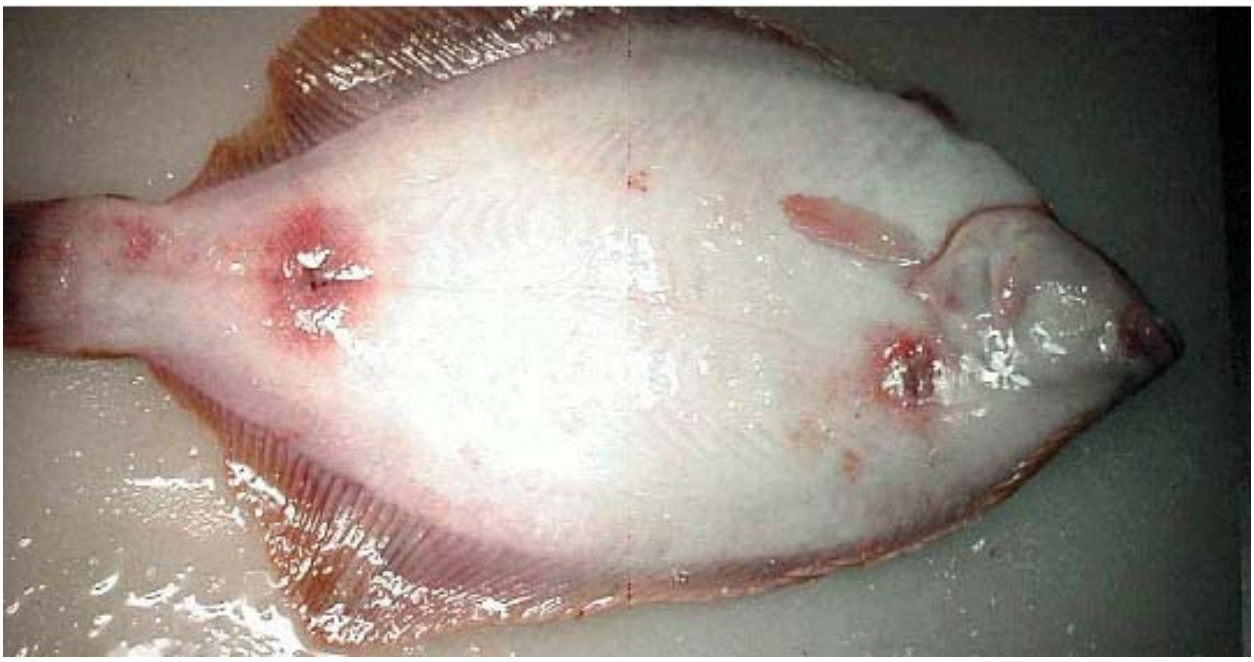
Special Stains (on paraffin sections):

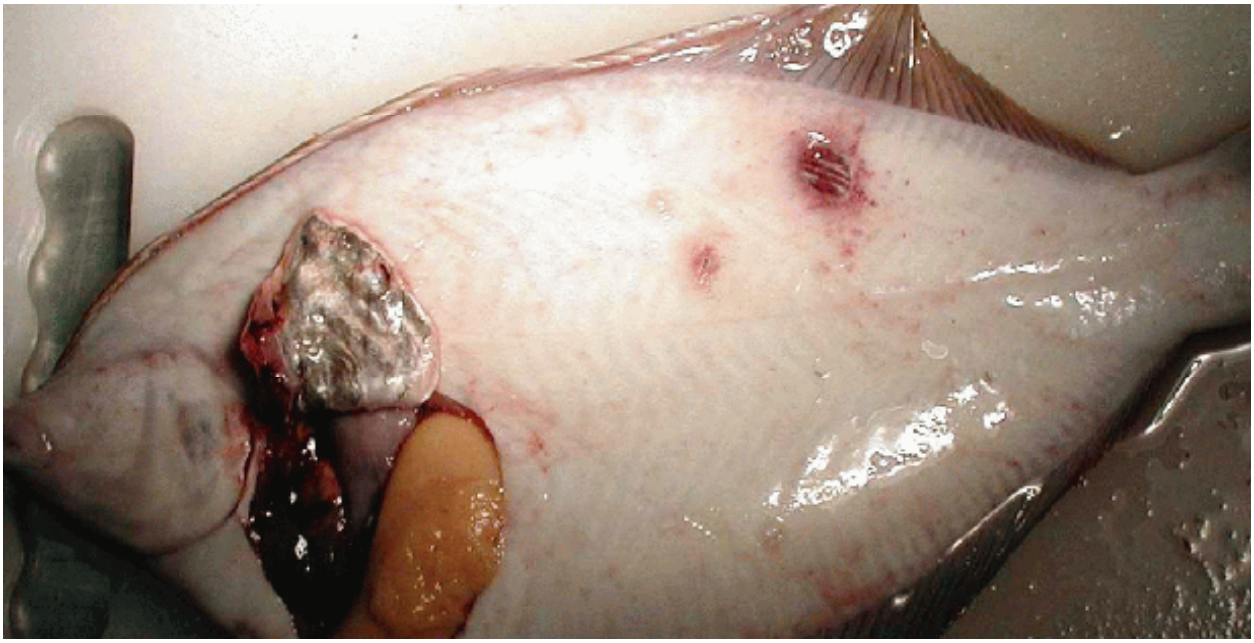
Tissue Gram stain on dermal lesions- Rod and coccoid rod bacteria noted in small numbers (1-10 per location) in the epithelial cells, free in necrotic tissue and in macrophages in affected dermis.
PAS (fungal stain) on dermal lesions - negative
AFB (acid fast) on dermal lesions (Nocardia or mycobacteria) - negative
Feulgens (possible dna virus) on dermal lesions - negative

PAS on intestinal granulomas - neg
AFB on intestinal granulomas - positive staining of microsporidial spores (sparsely present in some granulomas and numerous in others).

Diagnoses: Multifocal bacterial dermatitis with deep ulceration on ventral (down side) of winter flounder. Whether bacteria are primary or secondary is not known, but they are present deep in the lesions. Multifocal Lymphocystis of dermis (primarily tail fin). Multifocal microsporidial granulomas of the intestine and mesentery.

Roxanna Smolowitz, DVM
6/25/03





Laboratory report for bacterial isolates. Note Isolate 1267 is not part of the flounder case series.

JUN-19-2003 11:12AM FROM-MAR BIO LAB MAR RSRC 508 289 7900 T-848 P.001/002 F-427

To: Michael Moore, WHOI,

Michael your case is 1264 A to L

micro technologies, inc.

Dr. Roxanna Smolowitz, DVM
Marine Biological Laboratory
7 MBL St.
Woods Hole, Massachusetts 02543

2 June 2003

Re: Micro Technologies Accession #M03052003

Dear Roxanna,

Following are results of bacterial identification performed on 12 slants received by Micro Technologies, Inc. on 20 May 2003:

Isolate 1264A: Gram positive cocci, 0-129 (150 mg) S, oxidase negative, catalase positive, motile, NaCl not required for growth.
Biolog ID: Not reactive.
ID: *Micrococcus spp.*

Isolate 1264B: Gram negative rod, 0-129 (150 mg) R, oxidase positive, TSI=K/K, motile, NaCl not required for growth.
API 20NE Biotype #1000045.
ID: *Pseudomonas spp.*

Isolate 1264C: Gram negative rod, 0-129 (150 mg) S, oxidase positive, TSI=K/K.
API 20NE Biotype #1050004.
ID: *Pseudomonas/Flavobacterium spp.*

Isolate 1264D: Gram positive cocci, 0-129 (150 mg) S, oxidase negative, catalase positive, motile.
ID: *Micrococcus spp.*

Isolate 1264E: Gram negative rod, 0-129 (150 mg) S, oxidase positive, TSI=K/K, motile, NaCl required for growth.
API 20NE Biotype #1454344.
ID: *Shewanella spp.*

Isolate 1264F: Gram negative rod, 0-129 (150 mg) S, oxidase positive, TSI=K/K, motile, NaCl required for growth.
API 20NE Biotype #1400344.
ID: *Shewanella spp.*

Isolate 1264G: Gram negative rod, 0-129 (150 mg) R, oxidase positive, TSI=K/K, motile, NaCl not required for growth.
API 20NE Biotype #1000055.
ID: *Pseudomonas spp.*

Isolate 1264H: Gram negative rod, 0-129 (150 mg) R, oxidase positive, TSI=K/K, motile, NaCl not required for growth.
API 20NE Biotype #1057575.
ID: *Pseudomonas spp.*

Isolate 1264J: Gram negative rod, 0-129 (150 mg) R, oxidase positive, TSI=K/K, motile, NaCl not required for growth.
API 20NE Biotype #1000004.
ID: *Pseudomonas spp.*

Isolate 1264K: Gram negative rod, 0-129 (150 mg) S, oxidase negative, TSI=K/K, non-motile, NaCl not required for growth.
API 20NE Biotype #1467300.
ID: *Sphingomonas spp.* – most similar to *S. paucimobilis*

Isolate 1264L: Gram negative rod, 0-129 (150 mg) S, oxidase negative, TSI=K/K, non-motile, NaCl not required for growth.
API 20NE Biotype #1046400.
ID: *Sphingomonas/Pseudomonas spp.*

Isolate 1267: Gram negative rod, 0-129 (150 mg) R, oxidase negative, TSI=A/A, motile, NaCl required for growth.
API 20E Biotype #4044000.
ID: *Edwardsiella tarda*

Testing was performed using only standard biochemical techniques. It was therefore not possible to further identify the less reactive isolates. The last isolate, #1267, was the only pathogenic organism identified. Isolate 1264A, although very distinctive in color, was very unreactive in our routine testing procedures and therefore difficult to identify with certainty. The information we were able to obtain does however place it most similar to a *Micrococcus* species; we will work on this isolate some more. You wanted to pursue some of the identifications with DNA sequencing; although there are several isolates identified as *Pseudomonas spp.*, they appear to differ from each other to varying extents, probably unfortunately making it difficult for you to pick a candidate for sequencing. Please let me know if you have any questions regarding these results or would like to have additional testing performed.

Sincerely,

Cem Giray, Ph.D.
Laboratory Director

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