A REPORT OF THE NECROPSY AND LIVER HISTOPATHOLOGY FINDINGS FOR FISH SAMPLED FROM THE SAND ISLAND OUTFALL SEPTEMBER 1994

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INTRODUCTION

The discharge of treated domestic sewage into the ocean gives rise to a concern that aquatic species which live in the area around the outfall are at an increased risk for pollution related diseases. One approach to assess if such an impact has occurred is to periodically monitor fish that have been captured from the immediate vicinity of the ocean outfall for liver neoplasms and pre-neoplastic changes. The following is a summary of gross necropsy and liver histopathology findings for 44 fish collected by staff of the Division of Wastewater Management on September 11 & 15, 1994 from the area of the Sand Island municipal sewage outfall.

PROCEDURES

For evaluation of liver histopathology, five species of fish were collected and include the akule, *Selar crumenophthalmus* (20 specimens, mean individual fish weight = 229.5 g); ta'ape, *Lutjanus kasmira* (16 specimens, mean individual fish weight = 189.4 g); menpachi, *Myripristis berndti* (3 specimens, mean individual fish weight = 152.7 g); and two species of moray eels, *Gymnothorax flavimarginatus* (3 specimens, mean individual fish weight = 1393.3 g) and *Gymnothorax undulatus* (two specimens, mean individual fish weight = 1450.0 g). The fish were captured either by hook and line or fish trap (the five eels) along the diffuser at roughly a depth of 220-240 feet. All the fish were received live and killed immediately before necropsy. Specimens were measured, weighed and examined for external and internal gross abnormalities. Liver, gonad or other tissue were preserved in Davidson Fixative. Otoliths were collected for aging the fish. Sections of liver, gonad and epidermal tissues were processed by standard histopathology methods. Tissue sections were stained with hematoxylin and eosin.

NECROPSY FINDINGS

Necropsy and histopathology findings are presented in Tables I-X at the end of this report. A spherical mass was found protruding from the dorsal fin (Figure 1) on one of the *Gymnothorax undulatus*, specimen 94-248E. The mass contained a clear fluid and deflated when opened to allow fixative to penetrate internally. Histologically, this mass was an epidermal cyst and not a tumor.

LIVER HISTOPATHOLOGY FINDINGS

Brief descriptions of the microscopic observations for liver tissues from the three fish species are summarized below.

Selar crumenophthalmus: (Table VI) In general, hepatocytes were non-vacuolated in these fish. Melanized macrophage centers were not observed in the liver sections from the 20 fish. Mononuclear infiltrates were occasionally noted around small veins. Venous and sinusoid congestion, subcapsular hemorrhage and acute hepatocellular degeneration were noted in many of the liver specimens. Hematin deposits were infrequent. Nodules of endocrine pancreas were present bordering the liver sections of three specimens. Neoplastic changes were not found in the liver tissues of the 20 specimens.

Lutjanus kasmira: (Table VII) Liver hepatocyte vacuolation was usually rather uniform for individual fish in the sample but variations between specimens was found that ranged from hepatocytes with granular cytoplasm, hepatocytes that had a few vacuoles to hepatocytes with vesicular cytoplasm. Mononuclear infiltrates were common in the sections of liver from the ta'ape. Such infiltrates were usually adjacent to bile ducts or portal veins. Larval metazoa and granulomata were occasionally noted in the liver or mesenteries of the fish. Venous and sinusoid congestion, hemorrhage and acute hepatocellular degeneration were common microscopic changes observed in the livers of these fish. Neoplastic changes were not found in the liver tissues of the 16 specimens.

Myripristis berndti: (Table VIII) Acute hepatocellular degeneration, congestion, hemorrhage, melanized macrophage centers and mononuclear infiltrates were observed in the sections of liver from the three specimens. Neoplastic changes were not found.

Gymnothorax flavimarginatus: (Table IX) The livers of these fish were lobulated and circumscribed with a prominent layer of connective tissue. Bile pigment and small melanized macrophage centers were characteristic findings. Acute hepatocellular degeneration in a zone adjacent to the fibrous surface capsule was a common finding in the livers of the three specimens. Specimen 94-248C had a foci of proliferative tissue in the liver that may be a pre-neoplastic change.

Gymnothorax undulatus: (Table X) The livers of the two specimens were lobulated and circumscribed with a prominent layer of connective tissue. Bile pigment and small melanized macrophage centers were characteristic findings. Acute hepatocellular degeneration in a zone adjacent to the fibrous surface capsule was a common finding. A focal area of vacuolated hepatocytes and an area where the hepatocytes were pale staining was observed in the liver of eel 94-248E. Multiple, eosinophilic fusiform shaped

inclusions were noted in the cytoplasm of liver cells in specimen 94-248E. Neoplastic changes were not found in the liver sections of the two specimens.

The skin mass of eel 94-248E was found by microscopy to be an epidermal cyst.

DISCUSSION

The water depth is over 200 ft. (60m) in the area of the Sand Island Outfall. The fish in this study were subjected to acute decompression trauma during capture. In spite of this, City and County personnel were able to hold the specimens alive for delivery to Anuenue Fisheries Research Center (several hours from time of capture).

Several histologic alterations were recognized in the liver tissue from fish examined in the survey. The microscopic changes found include venous and sinusoid congestion; subcapsular and parenchymal hemorrhages; clear circular spaces resembling sites of gas emboli; scattered hematin deposits; in some specimens variation in the pattern of cytoplasmic vacuolation of hepatocytes; encysted metazoan parasites; melanized macrophage centers (MMCs); mononuclear cell infiltrates and granulomata.

The congestion, hemorrhage and hepatocellular degeneration found are acute changes and most likely reflect decompression trauma these fish received during capture and over the holding period prior to necropsy. Given the location of the outfall in deep water which necessitates rapid decompression of fish during retrieval from the bottom, these tissue changes are an expected finding. The hematin deposits are the result of use of an acid fixative, thus, represent fixation artifact.

SUMMARY

Forty four fish were collected from the area of the Sand Island Outfall and examined grossly and liver tissues histologically. An epidermal mass was found on one of the specimens. Microscopically, this was found to be a epidermal cyst and not a neoplastic lesion. A single, small site of putative proliferative cells (pre-neoplastic?) was observed in the liver of one specimen (94-248C). Histological changes diagnostic for neoplasms were not observed in the liver tissues of these specimens.

TABLE I
OUTFALL: SAND ISLAND, OAHU
SPECIES: *Selar crumenophthalmus*
DATE OF COLLECTION: 9/11/94
CASE NUMBER: 94-242A

SPECIMEN #	TOTAL LENGTH (cm)	TOTAL WEIGHT (gm)	LIVER WEIGHT (gm)	SEX
94-242A1	28.1	236	1.934	F
94-242A2	27.8	240	1.564	M
94-242A3	28.9	260	1.943	F
94-242A4	22.7	107	0.818	UD
94-242A5	22.2	93	0.554	F
94-242A6	29.6	284	2.050	M
94-242A7	29.3	246	1.723	M
94-242A8	29.5	274	2.023	F
94-242A9	29.6	280	2.034	M
94-242A10	28.9	254	2.123	F
94-242A11	29.3	254	1.910	M
94-242A12	18.2	42	0.200	UD
94-242A13	29.9	280	2.032	M
94-242A14	30.8	310	1.671	M
94-242A15	29.7	256	0.916	F
94-242A16	27.7	224	1.646	F
94-242A17	28.1	234	1.360	M
94-242A18	26.8	186	1.319	F
94-242A19	29.8	244	1.378	M
94-242A20	30.1	286	2.370	F
MEAN	27.850	229.5	1.578	
±S.D.	3.189	70.311	0.575	

F = female; M = male; UD = undetermined, gonad was not found

TABLE II
OUTFALL: SAND ISLAND, OAHU
SPECIES: *Lutjanus kasmira*
DATE OF COLLECTION: 9/11/94
CASE NUMBER: 94-242B

SPECIMEN #	TOTAL LENGTH (cm)	TOTAL WEIGHT (gm)	LIVER WEIGHT (gm)	SEX
94-242B1	22.1	152	1.233	F
94-242B2	22.5	160	1.450	F
94-242B3	25.9	212	1.536	M
94-242B4	22.0	132	1.326	F
94-242B5	19.6	108	0.467	M
94-242B6	23.5	170	1.127	M
94-242B7	22.7	158	1.186	F
94-242B8	30.5	404	2.737	M
94-242B9	23.2	160	1.207	M
94-242B10	23.2	164	1.546	M
94-242B11	20.5	104	0.584	F
94-242B12	28.7	306	2.049	M
94-242B13	22.7	152	0.994	F
94-242B14	27.5	256	1.986	M
94-242B15	24.1	180	1.526	M
94-242B16	24.8	212	1.606	M
MEAN	23.97	189.4	1.410	
±S.D.	2.912	77.003	0.550	

F = female; M = male

TABLE III
OUTFALL: SAND ISLAND, OAHU
SPECIES: *Myripristis berndti*
DATE OF COLLECTION: 9/11/94
CASE NUMBER: 94-242C

SPECIMEN #	TOTAL LENGTH (cm)	TOTAL WEIGHT (gm)	LIVER WEIGHT (gm)	SEX
94-242C1	21.9	158	1.027	F
94-242C2	18.6	112	0.733	F
94-242C3	22.3	188	1.111	F
MEAN	20.93	152.67	0.957	
±S.D.	2.031	38.280	0.198	

F = female

TABLE IV
OUTFALL: SAND ISLAND, OAHU
SPECIES: *Gymnothorax flavimarginatus*
DATE OF COLLECTION: 9/15/94
CASE NUMBER: 94-248A-C

SPECIMEN #	TOTAL LENGTH (cm)	TOTAL WEIGHT (gm)	LIVER WEIGHT (gm)	SEX
94-248A	100	1,400	13.234	M
94-248B	94.5	1,240	8.898	F
94-248C	94.5	1,540	16.032	M
MEAN	96.33	1,393.33	12.721	
±S.D.	3.175	150.111	3.595	

F = female; M = male

TABLE V
OUTFALL: SAND ISLAND, OAHU
SPECIES: *Gymnothorax undulatus*
DATE OF COLLECTION: 9/15/94
CASE NUMBER: 92-248D-E

SPECIMEN #	TOTAL LENGTH (cm)	TOTAL WEIGHT (gm)	LIVER WEIGHT (gm)	SEX
94-248D	92.1	1,400	13.808	M
94-248E	92.0	1,500	8.579	M
MEAN	92.05	1,450.0	11.194	
±S.D.	0.071	70.711	3.697	

M = male

TABLE VI
OUTFALL: SAND ISLAND, OAHU
SPECIES: *Selar crumenophthalmus*
DATE OF COLLECTION: 9/11/94
CASE NUMBER: 94-242A

SPECIMEN #	MICROSCOPIC FINDINGS FOR THE LIVER
A1	Hepatocytes are non-vacuolated; hepatic cords uniform; venous congestion; sinusoids dilated in some areas; hemorrhage and subcapsular acute necrosis of hepatocytes. Metazoan parasite encysted in the wall of a bile duct.
A2	Hepatocytes are non-vacuolated; uniform hepatic cords; spherical spaces suggestive of air emboli; congestion.
A3	Hepatocytes are non-vacuolated; uniform hepatic cords; extensive congestion and hemorrhage; hemosiderin deposits; mononuclear infiltrate along the border of one side of a vein.
A4	Hepatocytes are non-vacuolated; uniform hepatic cords; mononuclear infiltrate around small veins in several sites; nodule of endocrine pancreas adjacent to a major bile duct near the surface of the liver.
A5	Hepatocytes are non-vacuolated; uniform hepatic cords; variable venous congestion and several spherical spaces suggestive of gas emboli.
A6	Hepatocytes are non-vacuolated; uniform hepatic cords; mononuclear infiltrate around several small veins.
A7	Hepatocytes are non-vacuolated; uniform hepatic cords; venous congestion; hemorrhages and apparent gas emboli; subcapsular acute necrosis of hepatocytes.
A8	Hepatocytes are non-vacuolated; uniform hepatic cords; hemorrhages and dilated sinusoids; subcapsular acute necrosis of hepatocytes.
A9	Hepatocytes are non-vacuolated; uniform hepatic cords; hemorrhages and dilated sinusoids; subcapsular acute necrosis of hepatocytes.
A10	Hepatocytes are non-vacuolated; uniform hepatic cords; hemorrhage; dilated sinusoids and apparent gas emboli.
A11	Hepatocytes are non-vacuolated; uniform hepatic cords; hemorrhage; subcapsular and deep acute necrosis of hepatocytes; single cyst (parasite?).
A12	Hepatocytes are non-vacuolated; uniform hepatic cords; hemorrhage; subcapsular acute necrosis of hepatocytes; nodule of endocrine pancreas.

SPECIMEN #	MICROSCOPIC FINDINGS FOR THE LIVER
A13	Hepatocytes are non-vacuolated; uniform hepatic cords; hemorrhages; dilated sinusoids; acute degeneration.
A14	Hepatocytes are non-vacuolated; uniform hepatic cords; hemorrhage; congestion and acute degeneration.
A15	Hepatocytes are non-vacuolated; uniform hepatic cords; congestion; hemorrhage; apparent gas emboli and a nodule of endocrine pancreas.
A16	Hepatocytes are non-vacuolated; uniform hepatic cords; hemorrhage; apparent gas emboli; congestion; dilated sinusoids and acute degeneration.
A17	Hepatocytes are non-vacuolated; uniform hepatic cords; hemorrhage; apparent gas emboli; dilated sinusoids; mononuclear infiltrate around several veins; acute degeneration.
A18	Hepatocytes are non-vacuolated; uniform hepatic cords; hemorrhage; apparent gas emboli; dilated sinusoids and acute degeneration.
A19	Hepatocytes are non-vacuolated; uniform hepatic cords; hemorrhage; apparent gas emboli; dilated sinusoids and acute degeneration.
A20	Hepatocytes are non-vacuolated; uniform hepatic cords; hemorrhage; apparent gas emboli; dilated sinusoids and acute degeneration.

TABLE VII
OUTFALL: SAND ISLAND, OAHU
SPECIES: *Lutjanus kasmira*
DATE OF COLLECTION: 9/11/94
CASE NUMBER: 94-242B

SPECIMEN #	MICROSCOPIC FINDINGS FOR THE LIVER
B1	Hepatocytes are uniform with some vacuolation; dilated sinusoids; mononuclear infiltrate bordering exocrine pancreatic islets or bile ducts.
B2	Hepatocytes are uniform with some vacuolation; some congestion.
B3	Hepatocytes are uniform with some vacuolation; encysted metazoan parasite in the mesenteries.
B4	Hepatocytes are uniform with some vacuolation; mononuclear infiltrate rimmed bile ducts.
B5	Hepatocytes are uniform with some vacuolation; congestion and acute hepatocellular degeneration; mononuclear infiltrate rimmed bile ducts; granuloma in mesenteries.
B6	Hepatocytes are uniform with some vacuolation; congestion and acute hepatocellular degeneration; mononuclear infiltrate rimmed bile ducts; granuloma in mesenteries.
B7	Hepatocytes are uniform with some vacuolation; hemorrhage and acute hepatocellular degeneration; dilated sinusoids and encysted metazoan parasite in the mesenteries.
B8	Hepatocytes are uniform with some vacuolation; hemorrhage and acute hepatocellular degeneration.
B9	Hepatocytes are uniform with some vacuolation; hemorrhage and acute hepatocellular degeneration.
B10	Hepatocytes are uniform with some vacuolation; dilated bile ducts.
B11	Hepatocytes are uniform with some vacuolation; hemorrhage and acute hepatocellular degeneration.
B12	Hepatocytes are uniform with some vacuolation; hemorrhage and acute hepatocellular degeneration.
B13	Hepatocytes are uniform with some vacuolation; hemorrhage and acute hepatocellular degeneration; granuloma in the mesenteries.
B14	Hepatocytes are uniform with some vacuolation; hemorrhage and acute hepatocellular degeneration.

SPECIMEN #	MICROSCOPIC FINDINGS FOR THE LIVER
B15	Hepatocytes are uniform with some vacuolation; hemorrhage; acute hepatocellular degeneration; mononuclear infiltrate rimmed bile ducts.
B16	Hepatocytes are uniform with some vacuolation; hemorrhage and acute hepatocellular degeneration; dilated sinusoids; mononuclear infiltrate rimmed bile ducts.

TABLE VIII
OUTFALL: SAND ISLAND, OAHU
SPECIES: *Myripristis berndti*
DATE OF COLLECTION: 9/11/94
CASE NUMBER: 94-242C

SPECIMEN #	MICROSCOPIC FINDINGS FOR THE LIVER
C1	Hepatocytes are uniform with some vacuolation; congestion and melanized macrophage centers.
C2	Hepatocytes are uniform with some vacuolation; congestion and melanized macrophage centers.
C3	Hepatocytes are uniform with some vacuolation; congestion,; melanized macrophage centers; dilated sinusoids; area of acute degeneration and a small foci of infiltrating cells.

TABLE IX
OUTFALL: SAND ISLAND, OAHU
SPECIES: *Gymnothorax flavimarginatus*
DATE OF COLLECTION: 9/15/94
CASE NUMBER: 94-248A-C

SPECIMEN #	MICROSCOPIC FINDINGS FOR THE LIVER
A	Hepatocytes are uniform; bile pigment within the cytoplasm; melanized macrophage centers; thick fibrous capsule surrounds liver lobules; acute hepatocellular degeneration adjacent to the capsule; small foci of cellular infiltrate.
B	Hepatocytes are uniform; bile pigment within the cytoplasm; melanized macrophage centers; thick fibrous capsule surrounds liver lobules; acute hepatocellular degeneration adjacent to the capsule; dilated sinusoids.
C	Hepatocytes are uniform; bile pigment within the cytoplasm; melanized macrophage centers; thick fibrous capsule surrounds liver lobules; acute hepatocellular degeneration adjacent to the capsule; focal area of cellular proliferation in the liver parenchyma.

TABLE X
OUTFALL: SAND ISLAND, OAHU
SPECIES: *Gymnothorax undulatus*
DATE OF COLLECTION: 9/15/94
CASE NUMBER: 92-248D-E

SPECIMEN #	MICROSCOPIC FINDINGS FOR THE LIVER AND DORSAL FIN (94-248E)
D	Hepatocytes are uniform; bile pigment within the cytoplasm; melanized macrophage centers; thick fibrous capsule surrounds liver lobules; acute hepatocellular degeneration adjacent to the capsule.
E	Hepatocytes are uniform; bile pigment within the cytoplasm; focal area of vacuolated hepatocytes; large area of hepatocytes with reduced cytoplasmic staining; multiple, eosinophilic, fusiform-shaped inclusions with some hepatocytes. Cyst structure of the dorsal fin is lined by thin epidermis with prominent goblet cells.

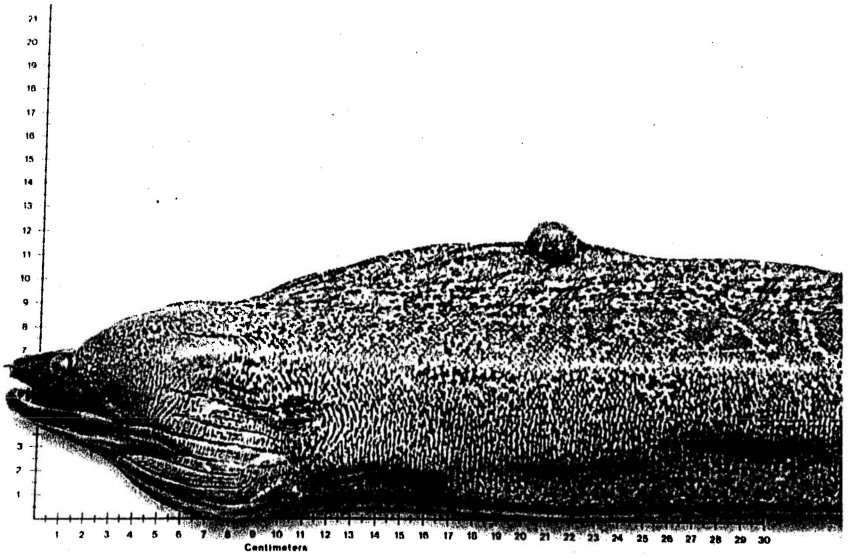

FIGURE 1: Photo of epidermal cyst on the dorsal fin of *Gymnothorax undulatus* (specimen 94-248E)