Appendix C

ATTACHMENT C-3

FISH HEALTH ASSESSMENT

Introduction

The CCH's Ocean Monitoring Program requires regular assessments of fish health to determine if there is an adverse effect from the Barber's Point wastewater discharge on representative fish species. Assessments of physical abnormalities and diseases in rig-caught fish species is performed by CCH monitoring personnel and specialists under contract to address specific monitoring requirements and permit conditions. Visual examination of fish is specified because a number of physical abnormalities and diseases have gross external manifestations, such as epidermal papillomas and fin lesions in fish (Murchelano 1982).

Fish health studies usually target demersal species (such as flatfish) because they are particularly susceptible to physical abnormalities and diseases, which appear to be associated with contaminated sediments (Johnson et al. 1992, 1993; Moore et al. 1997; Myers et al. 1993, 1994; Stehr et al. 1997,1998). This is because they live in direct contact or in close association with sediments, many of them feeding either directly or indirectly on benthic infauna, which also may be contaminated with pollutants from wastewater particles and/or sediments. This has been particularly true to mainland estuarine and nearshore waters where conditions allow for accumulation of organics.

In Hawaii, particularly in the waters of Mamala Bay, sedimentation and accumulation of organic matter of a wastewater origin has not been observed to have any significant measurable impact on sediment quality. However, the CCH does perform histopathological examination of the livers of fish caught near the outfall (caught at depths of 160-270 feet (48.8 and 82.3 meters) and at control sites in Maunalua Bay (to the south of Diamond) (depths of 50 feet and 220 feet (15.2 and 36.6 meters) to determine if there are any signs of chronic effects on fish from wastewater disposal practices.

Purpose and Objectives

These fish health studies are conducted to answer the following questions:

1) Is the outfall an epicenter of disease (external and liver parasitism, epidermal tumors, fin and skin lesions, and liver lesions)?

2) Are chemical contaminants in sediments (or prey organisms) positively associated with the prevalence of certain liver lesions?

The CCH determines compliance with this monitoring requirement by assessing the external condition of each rig-caught fish and the internal health of selected fish taken live to the laboratory and necropsied. Assessments of external conditions include visual examinations to document abnormal growths, atypical color patterns, parasites, fin lesions, and other types of lesions.

In the laboratory, liver tissues from each specimen are evaluated for parasites and pathological conditions and a report prepared on an annual basis.

Pollution-Induced Fish Diseases

Fishes residing in proximity to sewage outfalls could be at an increased risk for pollution related diseases or stress induced influences from changes in water quality (ie low dissolved oxygen, toxic chemicals, etc.)(U.S. EPA, 1987).

The literature on the relationship of fish pathology to pollution in marine and estuarine environments has identified -at least four grossly visible conditions as acceptable for immediate use in monitoring programs (Sindermann et al., 1980 and EPA, 1987) and which has been documented in associated with wastewater discharges or marine and estuarine pollution (EPA, 1987 and OCSD, 2002):

Fin erosion
Skin ulcers
Skeletal anomalies
Neoplasms (i.e., tumors).

Fin erosion is found in a variety of fishes from contaminated environments. It probably is the most frequently observed gross abnormality in polluted areas (Sindermann 1983). In demersal fishes, the dorsal and anal fins are the ones most frequently affected whereas in pelagic fishes, the caudal fin is the one primarily affected (U.S. EPA, 1987). The causes of fin erosion are unknown and likely complex. They may include chemical contaminants, low dissolved oxygen, and pathogens. Fin erosion has been induced in fishes after laboratory exposure to petroleum and PCBs (EPA, 1987).

Skin ulcers have been found in a variety of fishes from polluted habitat, and next to fin erosion, they are the most frequently reported gross abnormalities in polluted areas (Sindermann 1983). Prevalence of ulcers generally varies with season, and is often associated with organic enrichment. The primary cause of skin ulcers may be pathogenic organisms (e.g.*Vibrio* pp.) associated with pollution.

Skeletal anomalies frequently are more prevalent in fishes from polluted areas than in fishes from uncontaminated areas. Most observed skeletal anomalies involve the spinal column and include fusions, flexures, and vertebral compressions. Skeletal anomalies also include abnormalities of the head, fins, and gills. Skeletal anomalies have been induced in fishes after laboratory exposure to kepone and heavy metals (Sindermann et al. 1980).

Neoplasms or tumors have been found in elevated prevalence in a variety of polluted areas throughout the world (U.S. EPA, 1987). The most frequently reported grossly visible tumors include liver tumors, skin tumors (i.e., epidermal papillomas and/or carcinomas), and neurilemmomas. Liver tumors have been induced in fishes after laboratory exposure to a variety of chemicals. Two kinds of growths have been described as epidermal "papillomas" and pseudobranchial "tumors" in the literature (Sindermann et al. 1980). The predominant cell type in these growths is the presently unidentified X-cell which has been suggested is probably is a protozoan parasite, possibly an amoeba of the family Harmanellidae (Dawe 1981; Myers 1981). No relationship between the prevalence of these skin anomalies and pollution has been demonstrated conclusively.

Fish Histopathology Studies

Studying the diseases of fish around ocean outfalls utilizing histopathology was initiated in 1969 in studies of the Orange County Sanitation District outfall (old 20 meter outfall) in Southern California. The fish around the outfall were shown to have a high incidence of fin erosion and epidermal papillomas and other symptoms of severe stress. Other outfalls were also studied by the scientists of the Southern California Coastal Water Research Project (Mearns and Sherwood, 1974 and 1977). The National Oceanic and Atmospheric Administration had scientists who also initiated studies in Washington state which eventually were performed throughout the country incorporating fish histopathology into monitoring programs (NOAA Status and Trends Program).

Fish Diseases Associated with Contaminated Sites

Histopathological examination of fish, such as has been done by the CCH for fish caught near the Barber's Point outfall is done to evaluate fish health and any signs of chronic disease. In national studies done by the National Oceanic and Atmospheric Administration as part of its Status and Trends program, liver neoplasms (cancerous tumors) were found in 10 fish species collected from 1984 to 1988 from sites near urban centers along the west and northeast coasts where sediments were highly contaminated with pesticides or hydrocarbons (Turgeon et al. 1992). Scientists concluded that the contaminants most likely to be factors in the development of these tumors were the PAHs, PCBs, and DDTs (Myers et al. 1993).

Tumors In Fish

To date, incidences of cancerous tumors are generally low in fish from U.S. coastal waters, other liver disease conditions, some of which may progress to neoplasms, occur more frequently in areas where contaminants are high. Neoplasms and pre-neoplasms (pre-cancerous tumors) were found in up to 15% of the winter flounder from sites in Boston Harbor (Murchelano and Wolke

1991). Along the west coast, neoplasm incidences are well below 10% in most fish species (Myers et al. 1993). Relatively high incidences of nontumorous disease conditions occur in fish from contaminated sites. For example, in English sole (*Parophrys vetulus*) from Elliott Bay, Washington, incidences of 42% for specific degeneration and necrosis (SDN) of liver cells and 1% proliferative disorders (cells duplicating out of control) have been recorded; and in white croaker from San Pedro Outer Harbor, California, 22% SDN and 7% for proliferative disorders have been found (Varanasi et al. 1989; Myers et al. 1993). At Morris Cove, a highly contaminated site in New Haven, Connecticut, up to 90% of the cells in winter flounder livers have been found to be vacuolated cells (large areas of apparently empty, nonfunctioning cells; Gronlund et al. 1991).

Fin Erosion

Although fin erosion (fish with reduced fins or in extreme stages of disease with no fins) has been found in all species at all sites, this condition is still unusual, except in a few highly contaminated areas. Eroded fins occurred in 27% of the black croaker (*Cheilotrema saturnum*) and 22% of barred sand bass (*Paralabrax nebulifer*) from the West Harbor site in San Diego Bay, California (McCain et al. 1989). Up to 90% of Atlantic croaker, 100% of sand seatrout (*Cynoscion arenarius*), and 17% of spot sampled from the Houston Ship Channel at Green Bayou, Texas, experienced fin loss due to disease

Reproductive impairment occurred in fish from Eagle Harbor and Duwamish Waterway in Puget Sound, San Francisco and San Pedro bays, and in Morris Cove. Significantly lower levels of estradiol (a reproductive hormone) and vitellogenin (yolk protein critical to the development of fertile eggs for reproduction) have been found in English sole from contaminated sites in Puget Sound than those at relatively clean sites (Johnson et al. 1989). Also, a significant proportion of fish from contaminated sites failed to produce yolked eggs and undergo normal ovarian development. Moreover, fewer English sole spawned from the Duwamish Waterway (54%) in comparison with those from Port Susan during the 1987 and 1988 reproductive seasons (Casillas et al. 1991).

White croaker from a site near Los Angeles and kelp bass (*Paralabrax clathratus*) from San Pedro Bay had lower reproductive success than those from less contaminated sites at Dana Point and Santa Catalina Island (Cross and Hose 1989). In this study, the percentage of spawning fish was 24%-68% lower, batch fecundity (number of eggs produced) was 36%-44% lower, and the proportion of eggs fertilized was 14%-45% lower at the contaminated site. Gonadally mature female starry flounders from an urbanized central San Francisco Bay site off Berkeley had a reduced proportion of floating eggs and poorer fertilization success than those captured at a site in northern San Pablo Bay (Spies and Rice 1988). In Long Island Sound, embryo abnormalities were most frequent and hatching success was lowest in female winter flounders from more contaminated sites near Milford and New Haven; larvae were smallest off Deer Island, a highly contaminated site in Boston Harbor (Nelson et al. 1991).

Thus, there is evidence from collections of benthic fish at estuarine sites with contaminated sediments that lower reproductive success may occur and this could have long-term effects on spawning populations of some sensitive species, particularly flatfish species.

Chemically Induced Liver Lesions in Fish

As discussed above, organic chemicals, particularly hydrocarbons and persistent fat-soluble organics like organochlorine pesticides, PCBs, and (polyaromatic hydrocarbons or PAH s) have been found to be associated with diseases in fish. Most often these situations have occurred in estuarine areas or enclosed water bodies with highly contaminated sediments. However, the potential for detecting the effects of chemicals on fish can be determined using histopathology to examine liver (hepatic) tissues from fish. This monitoring tool has been applied in some instances to the 301(h) permittees. The CCH initiated the use of this monitoring tool and used the EPA protocols (U.S. EPA, 1987) to undertake studies to determine fish health.

The list of potential chemicals that have been found in laboratory studies to induce liver lesions is quite large. Most often however, these studies employ high doses and the levels to which the fish are exposed are many-fold greater than might be measured in the environment, particularly in well-mixed open ocean waters. However, the list does include some of the chemicals which have been found in fish muscle tissue in the fish in Mamala Bay (See Appendix H). This includes DDT and PCBs (although the particular PCB s found in the fish in Mamala Bay are not the 1260 and 1254 fractions most often found to be responsible for inducing lesions).

TABLE C-3-1
CHEMICALS THAT HAVE INDUCED HEPATIC LESIONS IN FISHES FOLLOWING LABORATORY EXPOSURE

<u>Organochlorine insecticides</u>
Chlordane
DDT
Dieldrin
Endosulfan
Endrin
Heptachlor
Hexachlorocyclohexane (beta isomer, lindane byproduct)
Kepone
Lindane
Methoxychlor
Toxaphene

<u>Organochlorine herbicides</u>
Dichlobenil
Dowicide G
2,4-D
Kuron (silvex)
Tordon 101 (picloram and 2,4-D as amine salts)
Tordon 22K (picloram, potassium salt)

<u>Industrial organochlorine compounds</u>
PCB-Aroclor 1248
PCB-Aroclor 1254
PCB-Miscellaneous
Carbon tetrachloride
Monochlorobenzene

<u>Organophosphate insecticides</u>
Abate (temphos)
Diazinon (Spectracide)
Dimethoate (Cygon)
Dursban (chlorpyrifos)
Dylox (trichlorfon)
Malathion
Methyl parathion

<u>Carbamate insecticides</u>
Aldicarb (Temik)
Carbaryl (Sevin)
Propoxur (Baygon)

<u>Miscellaneous herbicides</u>
Acrolein
Amitrole-T
Dinoseb
Diquat
Hydrothol 191
Paraquat-CL

<u>Fossil-fuel related compounds</u>
Benzo(a)pyrene (BaP)
Crude oil-whole
Crude oil-water soluble fraction
7-12 Dimethylbenz(a)anthracene (DMBA)
Oiled sediments

<u>Chemotherapeutic agents</u>
Copper sulfate
Diethylstilbestrol (DES)
Sulfamethazine
Thiabendazole

<u>Mycotoxins</u>

Aliatoxin B_1 (AFB)
Aflatoxin G_1 (AFG)
Aflatoxin M_1 (AFM)
Aflatoxin Q1 (AFQ)
Afl atoxicol (AFL)
Ochratoxin A + B
Sterigmatocystine
Versicolorin A

Plant derivatives
Cycad nut meal
Cycasin
Cyclopropenoid fatty acids (CPFA)
Gosypol
Methylazoxymethanol acetate (MAMA)
Pyrrolizidine alkaloids
Tannic acid

Nitroso- compounds
N,N'-dinitrosopiperazine (DNP)
N-nitrosodiethylamine (DEN)
N-nitrosodimethylamine (DMN
N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)
N-nitrosomorpholine (NM)

Miscellaneous nitrogenous compounds
2-Acetylaminofluorene (2-AAF)
o-Aminoazoto]uene (o-AAT)
Ammonia
Benzidine
Carbazone
p-Dimethylaminoazobenzene (DAAB
Thiourea
Urethane

Miscellaneous organic and organometallic compounds
Bis(tri-n-butyltin) oxide
Dimethylsulfoxide (DMSO)
Methylmercuric chloride
Nitro-3-(trifluoromethyl)phenol
Phenol

Inorganic compounds
Arsentates(sodium and disodium)
Cadmium chloride
Cupric sulfate
Lead nitrate
Mercuric chloride

Source: U.S. EPA, 1987.

Histopathological Evaluation of Fish

Histopathological analyses of fish livers are performed because:

> Pollutants tend to be most highly concentrated in livers;
> Livers are sites of metabolism for certain pollutants [i.e., chlorinated pesticides such as DDT and chlordane and polychlorinated biphenols (PCBs)]and the metabolic products of pollutants are known to have mutagenic and carcinogenic properties (Gmur and Varanasi 1982);
> Certain liver lesions are thought to be induced by contaminants and thus are useful indicators of contaminant exposure (as explained below); and
> Liver disease may occur in fish that have no external manifestations.

Some liver pathologies have been shown to be positively correlated with exposure to chemical contaminants (typically, related to fish age and/or size) and, therefore, are useful indicators of contaminant exposure effects (Bodammer and Murchelano 1990; Hinton and Lauren 1990; Hinton et al. 1992; Johnson et al. 1992; Moore et al. 1997: Myers et al. 1993, 1994; Stehr et al.1997,1998). Pathologies of particular concern are neoplasms (tumors) and cholangioma along with preneoplastic foci of cellular alteration (FCA), and severe hydropic vacuolation (SHV) of hepatopancreatic parenchyma. These changes are observed microscopically by trained specialists (fish pathologists) after the tissues are preserved, stained, imbedded in paraffin blocks, thinly cut and mounted on glass slides for microscopic examination.

The study of the livers of fish was started in the late 1960's when it was observed that English sole in San Francisco Bay had abnormal livers and tumors (cancer) (Cooper, 1969). Since that time a lot of study has gone into studying various flatfish and looking at the pathogenesis of the liver diseases experiences in the English sole. The following major hepatic lesions that are thought to be related to or associated with the histogenesis of liver neoplasms in English sole, which has among the most complete studies on flatfish histogenesis done (Meyers, et al. 1987):

- Nonspecific necrotic lesions
    - Hepatocellular coagulation necrosis
    - Liquefactive necrosis
    - Hydropic degeneration
    - Pyknosis
    - Hyalinization
    - Cystic parenchymal degeneration

- Specific degenerative conditions
    - Nuclear pleomorphism
    - Megalocytic hepatosis

- Nonneoplastic proliferative conditions
  - Nonhyperplastic hepatocellular regeneration

- Foci of cellular alteration
  - Eosinophilic foci
  - Basophilic foci
  - Clear cell or vacuolated cell foci
  - Hyperplastic regenerative foci

- Neoplasms
  - Liver cell adenomas
  - Hepatocellular carcinomas
  - Cholangiomas
  - Cholangiocellular carcinomas
  - Mixed carcinomas.

This overview of fish diseases is provided for background and understanding of some of the terms and conditions used in evaluating fish health and what results have been found in other areas where such studies have been performed.

Results and Discussion of Other Pacific Coast Fish Health Assessments

External Parasites in Fish Near Outfalls

Changes in parasite prevalence are a species-specific population phenomenon and changes in the incidence may reveal altered levels of environmental stress and/or changes in resistance to these stresses (OCSD, 2001). However, as evidenced in extensive studies in southern California near a large 301(h) permitted wastewater discharge, the overall prevalence of external parasitism [(for fish collected off Orange County, California near the Orange County Sanitation District s outfall which is the largest 301(h)-permittted discharge (240 mgd) during July 2000 and January 2001] was 0.65% (161 out of 24,869 individuals), up from 0.11% from the previous year (OCSD 2001). Eye copepod parasites (*P. cincinnatus*) were the most prevalent external parasite accounting for most of the observed incidences (0.57%) and were only found on Pacific sanddabs (140). The prevalence of this eye parasite for Pacific sanddabs is well below the average of 1.4% reported for the Southern California Bight (SCB)(Perkins and Gartman 1997). Similarly, the 1994 Southern California Bight Pilot Project (SCBPP) found eye parasites only on Pacific sanddabs with a prevalence of 1.1% (47 out of 4123) (Allen et al. 1998).

Leeches and other types of external body parasites included copepods and other small unidentifiable parasites are also observed on occasion.There was no spatial pattern relative to the Orange County outfall for the prevalence of parasites. Over a 16 years period, external parasites were observed in 0.11 to 1.0% of the fish specimens (OCSD 1996, 1997 and 2001). Compared with other areas of the SCB (Mearns and Sherwood 1977; Robinson 1982;), the incidence of parasitism in the OCSD study area is low. Studies have also shown little evidence of a chemical contaminant relationship for parasitism (Johnson et al. 1993). Fin lesion is rare, and was found on a single California scorpionfish at an outfall monitoring station in 2001.

Fin Lesions in Fish Near Outfalls

Over the past 16 years, studies of fish have measured frequencies of fin lesions ranging from zero to 0.11% (OCSD 1997, 1998, 1996a, 2000). These indices are very low compared with the 9% incidence of fin lesions among more than 170,000 individual fish collected by otter trawl on the Palos Verdes shelf from 1971 1981 (Cross 1985), and results from other studies in the Souther California Bight (SCB) (OCSD 1996, Mearns and Sherwood 1974, 1977; McDermott-Ehrlich et al. 1977; Sherwood and Mearns 1977; Cross 1982; Allen et al. 1998). More recently, the 1994 SCB Pilot Project (similar in intent to the regional monitoring effort now being done in Mamala Bay) collected 18,912 fish and found one fin lesion on a spotted turbot and 7 body lesions on 5 different species giving an overall prevalence of 0.04%. Thus, there has been a dramatic reduction over the past three decades in these types of lesions throughout the SCB, including the OCSD study area. No such fin lesions have been found on fish caught near the Barber's Point outfall.

Tumors in Flatfish Near Outfalls

Epidermal tumors in Dover sole which were once prevalent have also declined dramatically and while the SCBPP project found only 1% prevalence throughout the SCB, this condition has not been observed in Dover sole from the Orange County study area in over three years. The present, low levels of epidermal tumors and fin lesions are consistent with the relatively low contaminant concentrations in bottom sediments and fish tissues . The low tumor incidences are also expected based on present low mass emissions from major outfalls, and improved source control and treatment practices that have occurred over the past two decades in southern California (OCSD 1997, 1998, 1996a, 2000). No fish with tumors have been caught to date near the Barber's Point outfall or in reference areas.

Liver Parasitism

A variety of hepatic (liver) parasites, including biliary myxidial spores, coccidia, unidentified helminthic parasites, nematodes, trematodes, and larval canthocephalans, otherwise known as "mesenteric acanthocysts", can typically be found in fish.

Prevalence of liver parasites appears to be species-specific and has ranged from a few percent in some species to 100% in chub mackerel (*Scomber japonicus*) (OCSD 1996, 1997, 1998a). The interest in liver parasites stems from the possibility that stressful environmental conditions might make individual fish more susceptible to parasitism. Thus, the spatial pattern of parasite prevalence can be evaluated to determine whether the wastewater discharge may be contributing to parasite prevalence.

Coccidia protozoans are intracellular parasites of epithelial cells of annelids, mollusks, arthropods, and vertebrates. In fishes, they are often found in the bile ducts, liver, kidneys, and sometimes in the testes.

Coccidia typically have most heavily parasitized white croaker, an important target species, having an average prevalence between 1998-2000 of 31.9%. Myxosporida represent another type of parasitic protozoan, which almost exclusively are found in fishes. Myxidial and their spores inhabit the hollow organs or live in the connective tissues of liver and kidneys and are mostly harmless to the host, but can cause damaging tumor-like masses. This type of parasite was most prevalent in English sole, parasitizing 15.6% of the specimens examined.

Trematodes are parasitic flatworms that attach to the host by means of suckers. The digenetic trematodes are responsible for many types of detrimental conditions in their hosts, including liver, lung, and blood flukes and tapeworms. Trematodes tend to be rare

Liver Pathology in Fish Near Outfalls

The prevalence of certain types of liver lesions have been shown to be positively correlated with exposure to chemical contaminants and, therefore, are useful bioindicators of exposure and environmental stress (Bodammer and Murchelano 1990; Hinton and Lauren 1990; Hinton et al. 1992; Johnson et al. 1992; Moore et al. 1997: Myers et al. 1993, 1994; Steer et al. 1997, 1998). Of the 392 fish livers evaluated from July 2000, 12 specimens (3.1%) had severe liver pathologies. Severe liver pathology is herein defined as those lesions related to disease and/or contaminant exposure, including neoplasms (cholangiocellular carcinoma, cholangioma, and liver cell adenoma), foci of cellular alteration (basophilic foci, clear cell foci, and esonophilic foci), and hydropic vacuolation, which pertains only to white croaker.

Other types of liver lesions are common, but these lesions may have multiple causes, including environmental contaminants, normal aging, infections, and parasite invasion. Classes of these type of lesions includes non-neoplastic proliferative lesions (biliary hyperplasia, cholangiofibrosis, hepatocellular regeneration, and parenchymal fibrosis), unique or specific degenerative/necrotic condition [hepatocelluar nuclear pleomorphism, and megalocytic hepatosis], and nonspecific necrotic lesions unassociated with visible infectious agents (hepatocellular coagulative necrosis, hepatocellular hyalization, hepatocellular hydropic degeneration, hepatocellular liquefactive necrosis, and non specific vacuolation). All liver lesions are considered detrimental to the health of the individual, but not all lesions are environmentally induced.

Of the 430 white croaker collected for the SPS in August 1999 and March 2000, 11 (2.6%) specimens were found with cholangiocellular carcinoma or cholangioma. None of the 26 specimens collected in July 2000 showed these lesions. This is in contrast to 1995 and 1996 when 5.3% and 4.3%, of the white croaker were found with these types of lesions, respectively.

Subsequent years have been very low with 0% in 1997, 0.7% (1 out of 143), and only one specimen caught in 1999 and this was without tumors. The overall prevalence of significant lesions has been decreasing since 1993 (12.6%) and suggests an improving trend. However, this apparent change in lesion prevalence can vary based on differences in the size/age of the individual collected. Annual changes in prevalence of SHV are influenced by several factors, such as the species sampled, specimen size, sex, and collection location, in addition to possible contaminant exposure. SHV in white croaker decreased from 32% in 1994, to 8.7% in 1997 and to 2.6% in 1998; but increased in the August 1999 and March 2000 samples to 9.3%. This condition was not observed in any white croaker collected in July 2000.

These recent values are comparable to the approximately 8% prevalence for this lesion reported from Dana Point, a NOAA Status and Trends reference area (Stehr et al. 1998).

An analysis of histopathology data from 1986 1999 near the OCSD outfall revealed that the three target species collected near the outfall (within the zone of initial dilution by rig fishing and the nearest otter trawl station [T1]) had the highest prevalence of severe liver pathology within the study area. These findings suggested that a spatial pattern may exist for fish pathology relative to the outfall area. While prevalence and type of liver lesions tended to be species specific, in all cases larger and presumably older fish had a greater prevalence of severe lesion types (e.g., OCSD 1996, 1999; Myers et al. 1993,1994; Stehr et al. 1998). Furthermore, since larger fish tend to be found at the outfall, it would be expected that the outfall area would have a higher prevalence of liver pathology. However, because growth rates are highly variable, age is a better indicator of potential exposure than fish length. Consequently, age/lesion prevalence regressions were established for white croaker and tested to determine whether there were differences in the age of lesion onset (i.e., the regression intercept) or the rate of lesion acquisition (i.e., the slope of the regression) at the outfall compared to the farfield area. The results of this analysis found that lesion prevalence increases significantly with age and there were no sex or location differences for SHV and neoplasms. Thus, the outfall has the same prevalence for these types of lesions as the farfield area indicating that the outfall area is not a disease epicenter for these lesion types. Significant sex/location effects were found for FCA lesion types and all Severe Lesions combined, but these results indicate that older females in the farfield have a greater prevalence for FCA than males or females at the outfall and farfield males. Additionally, middle-aged males at the outfall develop Severe Lesions at a later age than elsewhere and there were no sexual differences for Severe Lesion types. Thus, the outfall is not a disease epicenter for liver pathology in white croakers. A similar type of analysis was conducted for bigmouth sole and hornyhead turbot based on size-pathology relationships and a similar conclusion was reached that the outfall was not a disease epicenter..

The specific studies conducted to examine the fish health of fish caught near the Barber's Point outfall diffuser and at control sites removed from any potential sources of contaminants is discussed below.

Barber's Point Outfall Fish Liver Histopathology Studies

To address this potential concern, histopathological studies of fish were initiated in 1997 to accompany visual observations being made for gross abnormalities of rig-caught fish collected for bioaccumulation studies. In 1997, thirty specimens of three species of fishes were collected from the area around the Barber's Point deep water diffuser. These fishes were examined for gross necropsy and liver histopathology by J.A. Brock (1997). The three species of fishes were the humuhumu mimi or bridled triggerfish (*Sufflamen fraenatus*), the blue-lined snapper or taape (*Lutjanus kasmira*) and the big-eyed scad or akule (*Selar crumenophthalmus*). Unlike other areas where these studies are done, Hawaii does not have a large number or abundance of flatfish species, and thus the target species for the studies are those species in most abundance near the outfall and are of commercial and recreational importance to the local fishery.

The food habits of *Sufflamen fraenatus* are unknown but other related balistids feed on small, heavy-bodied invertebrates such as crabs, mollusks and echinoids by use of their powerful jaws and heavy dentition (Hobson 1974).

Since the first study was completed in 1997 (Brock, J.A., 1997), several others have been completed to evaluate fish health (Brock, J. A., 1999 and Work T. M., 2001, 2002 and 2003). The results are submitted to the EPA and State DOH and reported in the Barber's Point WWTP Annual Assessment Reports.

1997 Fish Health Assessment Summary

Thirty fish from the Barbers Point Ocean Outfall were examined. Tumors or tumor-like lesions were not found during necropsy of the 30 fish. Gross evidence of neoplasia was not found in the integument or in the liver of the 30 fish evaluated. Microscopically, one specimen (*Lutjanus kasmira*, 97-176C8) had two alterations which may be pre-neoplastic changes. Dr. John Harshbarger of the Registry of Tumors in Lower Animals did a microscopic evaluation which confirmed the presence of bile duct hyperplasia in the liver of this fish.

The liver histopathology findings are presented in the full report (Brock, J. A. 1997). Brief descriptions of the microscopic observations (as direct quotes in italics) of liver tissues from the 10 individuals each of three fish species are summarized below.

Selar crumenophthalmus

In general, hepatocytes were mostly nonvacuolated in the fish, although small areas of vacuolated hepatocytes were present in most specimens. Small melanized macrophage centers *were often present in the sections of liver. Venous and sinusoid congestion, subcapsular hemorrhage, and perivascular cuffs of mononuclear cells were noted in many of the liver specimens. Small granulomata were noted in the sections of liver in two of the fish. A metazoan parasite was observed in the gall bladder of one specimen. Neoplastic changes were not found in the liver tissue of the 10 fish.*

Myripristis spp.

Hepatocellular vacuolation, congestion, hemorrhage, melanized macrophage centers, and mononuclear infiltrates were observed in the sections of liver from the 10 specimens. Neoplastic changes were not found.

Lutjanus kasmira

Hepatocellular necrosis, hepatocellular vacuolation, congestion, hemorrhage, melanized macrophage centers, mononuclear infiltrates, and a few parasites encysted on the surface of the liver were observed in the sections of liver from the 10 specimens. One specimen (97-176C8) had a few pleomorphic nuclei (Figure 1) in the parenchyma of the liver. This specimen also had what was tentatively identified as bile duct hyperplasia (Figure 2). Both of these changes may constitute pre-neoplastic alterations in the liver of this fish species.

The fish in this study were subjected to acute decompression trauma during capture. Despite this, City and County of Honolulu personnel were able to keep 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 of fish examined. The microscopic changes found include venous and sinusoid congestion, subcapsular and parenchymal hemorrhages, variation in the pattern of cytoplasmic vacuolation of hepatocytes, areas of acute hepatocellular degeneration and necrosis, melanized macrophage centers, mononuclear cell infiltrates, granulomata, and metazoan parasites.

The congestion, hemorrhage, and hepatocellular necrosis found are acute changes and most likely reflect the decompression trauma these fish received during capture and during the holding period prior to necropsy. Given the location of capture of the fish in deep water, which necessitates rapid decompression of fish during retrieval from the bottom, these tissue changes are an expected finding. The other tissue responses or changes included inflammatory reactions (granulomata, mononuclear infiltration, and melanized macrophage centers), putative altered metabolism (hepatocellular vacuolation), and infection by one or more metazoan parasites.

Two cellular alterations found in one specimen (Lutjanus kasmira, 97-176C8) have been identified tentatively as pre-neoplastic changes. The cellular changes were several liver parenchyma cells with pleomorphic nuclei and bile duct hyperplasia in several areas of the liver of this fish.

1999 Fish Health Assessment Summary

In 1999 gross necropsy and fish liver histopathology were conducted on 10 live specimens each of three different fish species: *Myripristis* spp., *Lutjanus kasmira*, and *Selar crumenophthalmus*. Hyperplasia and periductal fibrosis were seen in the liver of two *L. kasmira* and two *S. crumenophthalmus*. Putative Myxosporidea spores were observed in the biliary system of six fish. Gross evidence of neoplasia was not found in the integument or in the liver of the 30 fish evaluated. Tumors or tumor-like lesions were not found during necropsy of the 30 fish.

Liver Histopathology

Brief descriptions of the microscopic observations of liver tissues from the 10 individuals each of three fish species are provided below in italics as reported:.

Myripristis spp.

Mild congestion, hemorrhage, hepatocellular necrosis, melanized macrophage centers, and mononuclear infiltrates were observed in the sections of liver from some of the 10 specimens. Neoplastic changes were not found.

Lutjanus kasmira

Hepatocellular vacuolation, congestion, hemorrhage, melanized macrophage centers, and mononuclear infiltrates were observed in the sections of liver from some of the 10 specimens. Two fish had hyperplasia and periductal fibrosis of the bile ducts (99-42B5 and 99-42B7). Putative Myxosporidea spores were observed in the biliary system of three fish (99-42B1, 99-42B4, and 99-42B9). One specimen had several small granulomata in the liver (99-42B9). Neoplastic changes were not found.

Selar crumenophthalmus

In general, hepatocytes were mostly nonvacuolated in the fish, although small areas of vacuolated hepatocytes were present in most specimens. Small melanized macrophage centers were often present in the sections of liver. Venous and sinusoid congestion, and perivascular cuffs of mononuclear cells were noted in many of the liver sections. Mild bile duct hyperplasia and periductal fibrosis were found in the liver of two fish (99-42C7 and 99-42C9). Putative Myxosporidea spores were observed in the gall bladder of three specimens (99-42C2, 99-42C7, and 99-42C8). A metazoan parasite was found in the biliary system of two fish (99-42C3 and 99-42C6). Neoplastic changes were not found in the liver tissue of the 10 fish.

2000 Fish Health Assessment Summary

In 2000 gross necropsy and fish liver histopathology were conducted on 10 live specimens each of three different fish species: *Myripristis* spp., *Lutjanus kasmira*, and *Selar crumenophthalmus*. Bile duct hyperplasia and periductal fibrosis were seen in the liver of four *L. kasmira* and one *S. crumenophthalmus*. Putative Myxosporidea spores were observed in the biliary system of two fish. Metazoan parasites were observed encysted in the liver or within the biliary system of six fish. Gross or microscopic evidence of neoplasia was not found in the integument or in the liver of the 30 fish evaluated.

2001 Fish Health Assessment Summary

In 2001 gross necropsy and fish liver histopathology were conducted on 10 live specimens each of three different fish species: *Lutjanus kasmira*, *Myripristis* sp., and *Selar crumenophthalmus*. Putative Myxosporidea spores were observed in the biliary system of ten fish. Metazoan parasites were observed encysted in the liver or within the biliary system of one fish. Gross or microscopic evidence of neoplasia was not found in the 30 fish evaluated.
Tumors or tumor-like lesions were not seen in any internal or external organs of the 30 fish.

Brief descriptions of the microscopic observations of liver tissues from the 10 individuals each of three fish species are provided below.
Lutjanus kasmira

> *Mild to marked congestion, hemorrhage, hepatocellular necrosis, melanized macrophage centers, and mononuclear infiltrates were the lesions most often observed. Suspect myxosporidians associated with no inflammation were seen in the bile duct epithelium, and one fish had an encysted metazoan of unknown identity. Neoplastic changes were not found.*

Myripristis sp.

> *Mild hepatocellular vacuolation, focal acute and chronic necrosis and inflammation, melanized macrophage centers, mononuclear infiltrates, and rare suspect myxosporidians in the bile duct epithelium were the most common lesions. Neoplastic changes were not found.*

Selar crumenophthalmus

> *Nonvacuolated hepatocytes, small melanized macrophage centers, occasional mononuclear infiltrates, and acute hemorrhage and necrosis were most commonly seen. Neoplastic changes were not found.*

The congestion, hemorrhage, and hepatocellular necrosis seen are acute changes that most likely reflect the decompression trauma the fish received during capture and during the holding period prior to necropsy. Given the location of capture of the fish in deep water, which necessitates their rapid decompression during retrieval from the bottom, these tissue changes are an expected finding.

Other lesions seen are considered incidental. The inflammatory changes (both acute and chronic) were mild and nonspecific and could have been caused by a variety of etiologies, including an infectious, toxic, or other type of agent that can injure the tissue. The hepatocellular vacuolation may be an indication of physiologic storage of lipid or glycogen, or, in extreme cases, metabolic or toxic anomalies that can lead to damage to cytoplasmic organelles.

Myxosporidians and metazoan parasites were also seen. Myxosporidians have complex lifecycles that involve specific fish and invertebrate hosts. Subclinical infection by one or more myxosporidians is very common for fish in the wild. Additionally, wild fish harbor many other parasites (e.g., trematodes, nematodes, and cestodes), some of which migrate through the liver and most of which are species-specific in their life cycles and thus are unlikely related to the effluent.

2002 Fish Health Assessment Summary

Brief descriptions of the microscopic observations of liver tissues from the 10 individuals each of three fish species are provided below (in italics as reported in Brock, J.A. 2002).

Lutjanus kasmira

Melanized macrophage centers, lymphoid infiltrates, emphysema, necrosis, hemorrhage, and atrophy were the most commonly encountered findings. Vacuolated cells, protozoa, and unidentified metazoans were less commonly seen. Neoplastic changes were not found.

Myripristis spp.

Mild to marked hepatocellular vacuolation, lymphoid infiltrates, and melanized melanized macrophage centers were the most common lesions. Congestion, unidentified protozoa, and emphysema were less commonly seen. Neoplastic changes were not found.

Selar crumenophthalmus

Vacuolization, unidentified protozoa, melanized macrophage centers, and hemorrhage were the most commonly seen lesions. Less commonly seen were lymphoid infiltrates and congestion. Neoplastic changes were not found.

DISCUSSION

As noted in previous years, the fish in this study were subjected to acute decompression trauma during capture. Despite this, City and County of Honolulu personnel were able to keep the specimens alive for delivery to Halawa necropsy facility (several hours from time of capture). The fish remained alive until they were euthanized for necropsy.

Neoplastic changes (tumors) were not seen. When present, neoplastic changes are characterized by uncontrolled cell growth, aberrant cytoplasmic and nuclear morphology, rapid cell division (mitotic figures), displacement of adjacent normal tissue, presence or absence of encapsulation, hemorrhage, necrosis, and inflammation.

The congestion, hemorrhage, hepatocellular necrosis, and emphysema seen are acute changes and most likely reflect the decompression trauma the fish received during capture and during the holding period prior to necropsy. Given the location of capture of the fish in deep water, which necessitates their rapid decompression during retrieval from the bottom, these tissue changes are an expected finding.

Other lesions seen are considered incidental. Inflammatory changes (both acute and chronic) were mild and nonspecific and could have been caused by a variety of etiologies, including an infectious, toxic, or other type of agent that can injure the tissue. The hepatocellular vacuolation could be an indication of physiologic storage of lipid or glycogen, or, in extreme cases, metabolic or toxic anomalies that can lead to damage to cytoplasmic organelles.

Protozoans and metazoan parasites were also seen. Coccidia and other protozoa have complex lifecycles that involve specific fish and invertebrate hosts. Subclinical infection by one or more protozoa is very common in wild fish. Additionally, wild fish harbor many other parasites (e.g., trematodes, nematodes, and cestodes), some of which migrate through the liver and most of which are species-specific in their life cycles and thus are unlikely related to exposure to effluent discharged from the outfall.

2003 Fish Health Assessment Summary

In 2003, gross necropsy and fish liver histopathology were done on ten specimens each of *Lutjanus kasmira, Myripristis berndti,* and *Selar crumenophthalmus* collected live near the terminus of the Barbers Point Ocean Outfall. An additional cohort often specimens each of *L. kasmira, M. berndti,* and *S. crumenophthalmus* were collected live at reference station FR2 in Maunalua Bay. Melanized macrophages centers or hepatocellular vacuolation were the most common change seen in the liver of all fish collected. Hemorrhage, acute necrosis, and lymphoid infiltrates were other changes seen. Emphysema or infection with Microsporidia was noted only in *L. kasmira* from the outfall area, whereas metazoan infection was seen only in one *L. kasmira* and one *M. berndti* from the reference station. Neoplastic changes were not seen. Wild fish have many parasites (Microsporidia, nematodes, cestodes), some of which migrate through the liver and most of which are species-specific in their life cycles. The emphysema, hemorrhage, and acute necrosis were rapid changes most likely due to decompression trauma the fish received during capture. Lymphoid infiltrates were mild and considered nonspecific. Gross or microscopic evidence of neoplasia was not found in the 30 fish collected near the Barbers Point outfall terminus or in the 30 fish collected at the reference station in Maunalua Bay

Discussion of Barber's Point Fish Histopathology Results

The fish caught in the deep water near the outfall are subjected to acute decompression trauma during capture. Despite this, City and County of Honolulu personnel are able to keep the specimens alive for delivery to the Halawa necropsy facility several hours later making for ideal conditions for euthanization and subsequent necropsy.

In the examination of the fish, neoplastic changes (tumors) were not seen. When present, however, neoplastic changes are characterized by uncontrolled cell growth, aberrant cytoplasmic and nuclear morphology, rapid cell division (mitotic figures), displacement of adjacent normal tissue, presence or absence of encapsulation, hemorrhage, necrosis, and inflammation.

The hemorrhage, acute hepatocellular necrosis (liver damage), and emphysema were acute changes and most likely reflected the decompression trauma the fish received during capture and during the holding period prior to necropsy. Given the location of capture of the fish in deep water, which necessitates their rapid decompression during retrieval from the bottom, these tissue changes are an expected finding.

Other lesions were considered incidental. Inflammatory changes (both acute ones manifested by granulocytes and chronic ones manifested by mononuclears) were mild and nonspecific and could have been caused by a variety of etiologies, including infectious, toxic, or other type of agents that can injure the tissue. Hepatocellular vacuolation could be an indication of physiologic storage of lipid or glycogen, or, in extreme cases, metabolic or toxic anomalies that can lead to damage to cytoplasmic organelles.

Myxosporidians and metazoans were rarely seen. Myxosporidians have complex life cycles that involve specific fish and invertebrate hosts. Subclinical infection by one or more myxosporidians is very common in wild fish. Additionally, wild fish harbor many other parasites (trematodes, nematodes, cestodes, various protozoa), some of which migrate through the liver and most of which are species-specific in their life cycles and thus are unlikely related to the effluent.

Conclusions

All the histopathological studies done on fish (three species) to date have shown that the two local species of commercial and recreational importance to local fishermen are healthy. There has been no evidence of acute or chronic disease symptoms which would indicate that the Barber's Point outfall is a disease epicenter. There is no evidence of fin erosion, tumors, increased parasitism, ambicoloration, vertebral abnormalities, or other signs of disease. All fish appear to be healthy.

References

Note that all references cited are listed in Section IV of the application.