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UNPUBLISHED REPORT
RAPPORT NON PUBLIÉ

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for

THE MICROBIOLOGY LABORATORIES
APPLIED RESEARCH DIVISION

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PHASE II - STAGE 3

by

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CANADA CENTRE FOR INLAND WATERS
ENVIRONMENT CANADA
BURLINGTON, ONTARIO

March - 1976

Supported by Contract No. 02SU. KL347-5-0315, 1975-76.

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SUMMARY

The potential utility of monitoring neoplasia in fishes and their associated oncogenic viruses as sentinel animals for the early detection of waterborne factors which have the ability to induce oncogenic virus expression was explored. The occurrence, epizootiology, pathology, and etiology of the following tumours in Great Lakes fishes was investigated, with particular reference to polluted and non-polluted waters.

Field epizootiological studies were conducted on white suckers in Lake Ontario. Specimens were collected at varying distances from Burlington Harbour in which increased skin tumours (papillomas) were found in last year's investigations. These studies revealed elevation of the frequency of tumours (i.e., 60% of nine-year-old suckers) in the Burlington-Oakville region. The tumour frequency was found to decline in specimen collections made at varying distances in both directions from this region. These findings suggest that extrinsic environmental factors in these waters and/or bottom sediments are contributing to the elevated tumour frequencies. The presence of C-type virus was reconfirmed in electron microcopic studies and the viral enzyme (reverse transcriptase) was discovered to be associated with the tumours. Skin tumours (papillomas) in Lake Ontario brown bullheads were also found to exhibit "clustering" in Burlington Harbour.

Investigations of the etiology and pathogenesis of thyroid hyperplasia (goiter) in Lake Ontario coho salmon revealed that the condition develops rapidly during the anadromus migration of sexually mature fish. The disease is characterized by severe hypothyroidism as evidenced by low levels of thyroid hormone, thyrotroph proliferation, and hyperplastic thyroid follicles. Comparative studies with Lake Ontario coho salmon exhibiting the disease last

year and with those in Lake Michigan strongly suggest that extrinsic environmental factors are involved in the etiology of the disease. Inasmuch as hypothyroidism has been associated with neoplasia and reproductive failure, the elucidation of the etiology of the disease is one of the urgent concern.

A skin tumour which had Herpes virus associated with it was discovered in carp. The disease is infectious; normal carp held in the same tank as tumour-bearing fish develop the tumour. Inasmuch as Herpes viruses are prime candidates as etiological agents in human malignancy, the etiologic significance of the virus should be confirmed.

Field epizootiological studies of the Sertoli cell gonadal tumour in carp, goldfish, and goldfish x carp hybrids reconfirmed the presence of epizootics of the tumour in a variety of regions on the Great Lakes. The tumour was found in high frequencies in a relatively non-polluted area on Lake Huron. The widespread geographical distribution of the tumour within the Great Lakes, coupled with the absence of the disease in museum specimens collected before 1952, suggest that an ubiquitous environmental pollutant, i.e., polychlorinated biphenyls (PCB's) may be involved in the etiology of the disease. Carp fed a diet containing PCB's did not develop the tumour. Tumour-bearing fish fed a diet containing a pituitary gonadatroph block did not affect the maintenance of the tumour, although there was regression of the hyperplastic gonadatrophs. Fish injected with LH hormone also did not develop the tumour. These studies suggest that tumour development is independent of the pituitary gonadal axis, although the disease is characterized by massive gonadatroph proliferation.

INTRODUCTION

It has been estimated that 60 to 90% of the cancers in man are environmentally induced (directly or indirectly) by extrinsic environmental factors in the air, food, and water he consumes (Epstein, 1974). As the result of our mushrooming technological society, there has been an unbridled increase in the use of tens of thousands of new and often structurally complex synthetic compounds in industry and agriculture. Few of these have been screened for carcinogenic activity (i.e. 5,000 up to 1972) which represents a small fraction of the tens of thousands or perhaps hundreds of thousands of chemicals which have extensive environmental contact with man and ought to be screened for carcinogenic potential (Sarriotti, 1974).

Very likely, in lieu of the above, the incidence of neoplasia in man could increase because of new carcinogens, increased levels of carcinogens already in the environment, or the combined effects of the same which might reveal their effects decades after their introduction. Furthermore, in man, environmental carcinogens have a tendency to express themselves some 30 years post-exposure. It is apparent that there is an urgent need to detect and identify those factors in the environment which could be contributors to the etiology of cancer in man.

At present, the screening of compounds for carcinogenic potential is progressing at a rapidly accelerating rate due to advances in a number of areas in biology. Carcinogen screening is, however, largely confined to short-term testing of individual compounds in a battery of *in vitro* and *in vivo* assays. It is increasingly apparent, however, that evaluation of the carcinogenic potential of chemicals found in the environment is very complex. Even if the specific carcinogenic potential of a defined industrial or agricultural chemical is

established, the possible effects of breakdown products in the environment might be even more difficult to assess. Natural biodegradation or specific treatment processes of industrial and domestic effluents may lead to the development of new compounds having carcinogenic properties quite different from the present compound (i.e., chlorination). Another factor confounding laboratory evaluation of environmental agents is the combined and possible synergistic effects resulting from a mixture of carcinogenic and non-carcinogenic compounds.

Viruses have been shown to be the etiological agent or cause of a number of cancers in animals and have been implicated in the etiology of cancer in man. Cancer may be conceived as being a multi-factorial syndrome, caused by the breakdown of a complex system of feed-back mechanisms leading to the expression of cancer causing (oncogenic) viruses. Several carcinogens are known to act in concert with oncogenic viruses to produce neoplasms (Haran-Ghera, 1966; Igel et al., 1969; and Toth, 1963). Similarly, there are numerous examples of the combined effects of chemical co-carcinogens and viruses producing neoplasms, of which neither alone could induce tumour formation. It would seem a logical hypothesis, therefore, to suspect that alteration of an animal's environment by a battery of extrinsic factors might result in induction of oncogenic viruses. Although knowledge of such mechanisms is obscure, it is very likely that they play an important role in the etiology of cancer.

With regards to water, it has been documented that our aquatic environments are fouled with chemicals and classes of chemicals which have carcinogenic potential (Brown et al., 1973; Maugh, 1973; Morrison, 1971; Saffiotti, 1974; and Wogan, 1969). Although there is little evidence that carcinogens in water have produced widespread cancer problems in man, it is not difficult to envision such a possibility. Presently, due to ground water depletion,

many cities are processing for drinking purposes, water which may be in excess of 30% recycled water (Cillie et al., 1966). Many inorganic and organic compounds (some known carcinogens) are not removed in current water treatment facilities (Bornoff and Fisher, 1962; Ongerth et al., 1973). In fact, treatment may be producing carcinogens (i.e., chlorination may produce chloroform and carbon tetrachloride, both known carcinogens (Jolley, 1973; Bellar et al., 1974; Rook, 1974).

Of particular concern with regards to the above, is the report of Harris (1974) on the possible implications of cancer causing substances in Mississippi River water. The study presents presumptive epidemiological evidence which suggests a significant relationship between cancer mortality in white males drinking water obtained from the Mississippi River in the New Orleans area. The report strongly suggests that drinking water from the Mississippi River is causally related to cancer mortality in more than one million persons in Louisiana who depend on that source for their drinking water supply. Although the results of the New Orleans study cannot as yet be considered as conclusive evidence that cancer is in fact being caused by consuming contaminated water, these very suggestive findings must be fully taken into consideration.

It is apparent that a sentinel system for the early detection and identification of waterborne environmental carcinogens is urgently needed. In these studies, we have made a pilot study exploring the potential utility of monitoring fish and their associated neoplasms as indicators of waterborne environmental carcinogens. These studies have revealed alarmingly high tumour frequencies, some of which exhibit apparent clustering in waters receiving industrial and domestic wastes and which epizootiological findings support the role of extrinsic environmental factors. These studies stimulate the following

thought-providing questions: Do they reflect the presence of carcinogens in the environment? Oncogenic viruses and chemicals? Oncogenic viruses alone? The New Orleans "experience" gives urgency to these questions and gives credence as to the potential utility of fish as sentinel animals for the early detection of waterborne carcinogens.

White-Sucker Papilloma (Catostomus commersoni)

White suckers in the Great Lakes suffer epizootics of papillomatous growths. The tumours usually present themselves as cauliflower-like growths which may be found anywhere on the fish's body. The tumour is benign, and no evidence of invasiveness has been found. The tumour is widespread in white suckers in the Great Lakes; the tumour having been diagnosed in Lakes Superior, Michigan, Huron, Erie, Ontario and the St. Lawrence River. The disease has also been diagnosed in a number of Lakes in Ontario, Alberta, and Michigan (Sonstegard, 1975b).

During the course of field epizootiological studies conducted on Lake Simcoe (Ontario) white suckers, the majority of the tumours found were on the head region (lips, snout, and eyes), however, the tumours were commonly found on the fins and flank. Nevertheless, last year's field epizootiological studies in Burlington Harbour on Lake Ontario revealed that 100% of the specimens with the disease had tumours exclusively associated with the lips. Furthermore, an inordinately high proportion of the Burlington Harbour white suckers (29.6%) exhibited the tumour "clustering" compared to other areas surveyed on the Great Lakes (i.e., 2.6% in Toronto Harbour on Lake Ontario; and 0.7% in Southampton on Lake Huron) (Sonstegard, 1975b).

With regard to the anatomical predilection of the papillomas to the lips of the Burlington Harbour white suckers, it should be noted that the species

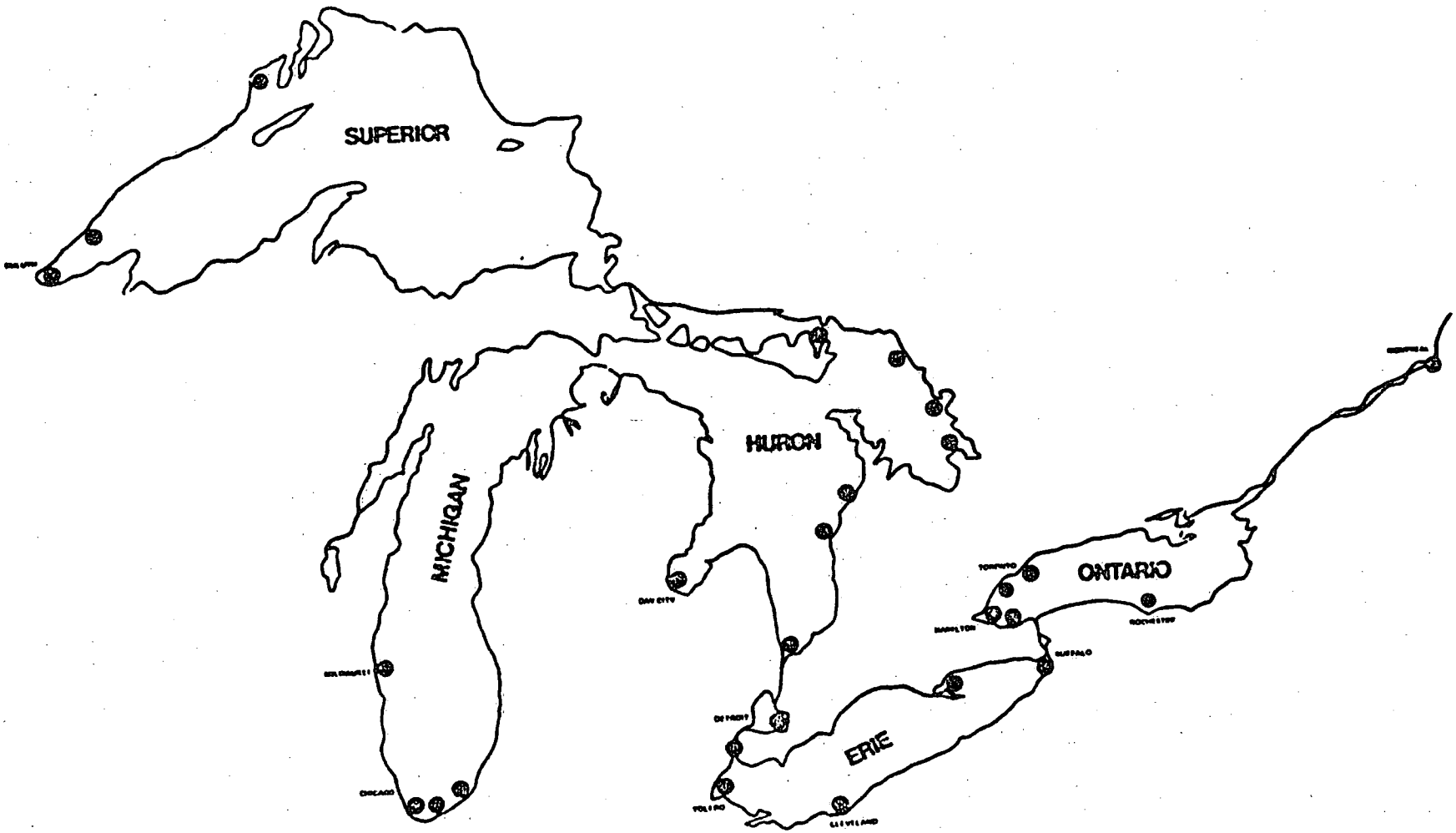
is a bottom-dwelling fish, spending its entire life feeding and resting on the bottom. These activities could either expose their mouth parts to tumour-inducing agents which may accumulate in bottom sediments, or they might cause mechanical abrasions to the mouth region, thus facilitating the transmission of virus. Such factors either singly or in combination might give rise to the neoplasm. The anatomical shift in the location of the neoplasm occurring in white suckers recovered from the Burlington Harbour, together with the clustering recorded in this watershed, strongly suggest that extrinsic environmental factors (carcinogens) in association with C-type virus play a role in the etiology of the syndrome (Sonstegard, 1973).

Field epizootiological studies were then made on selected sites on Lake Ontario to resolve whether "clustering" occurs in Burlington Harbour. The following collection sites were monitored: Dufferin Creek, Humber River, Oakville Creek, Burlington Harbour, Jordan Harbour, and Rochester, and are shown on the enclosed map. No tumours were detected in the collections made at Dufferin's Creek or Rochester, N.Y. The age of the suckers was determined by sectioning the pectoral fin and counting the anuli. The tumour frequencies found are represented in the enclosed graphs (Fig. 1 - 4).

These epizootiological studies indicate elevation of the frequency of tumours "clustering" in the Oakville-Burlington region on Lake Ontario. It is particularly relevant to note that the frequency of occurrence decreased dramatically in collections made at varying distances in both directions from this region. In addition to the apparent clustering recorded, the tumours were, in general, larger in size. Furthermore, a higher proportion of the catch exhibited multiple tumours.

As discussed earlier, the white sucker is a bottom-dwelling species, spending its entire life feeding and resting on the bottom. These activities

SURVEY SITES FOR TUMOUR-BEARING FISH IN THE GREAT LAKES



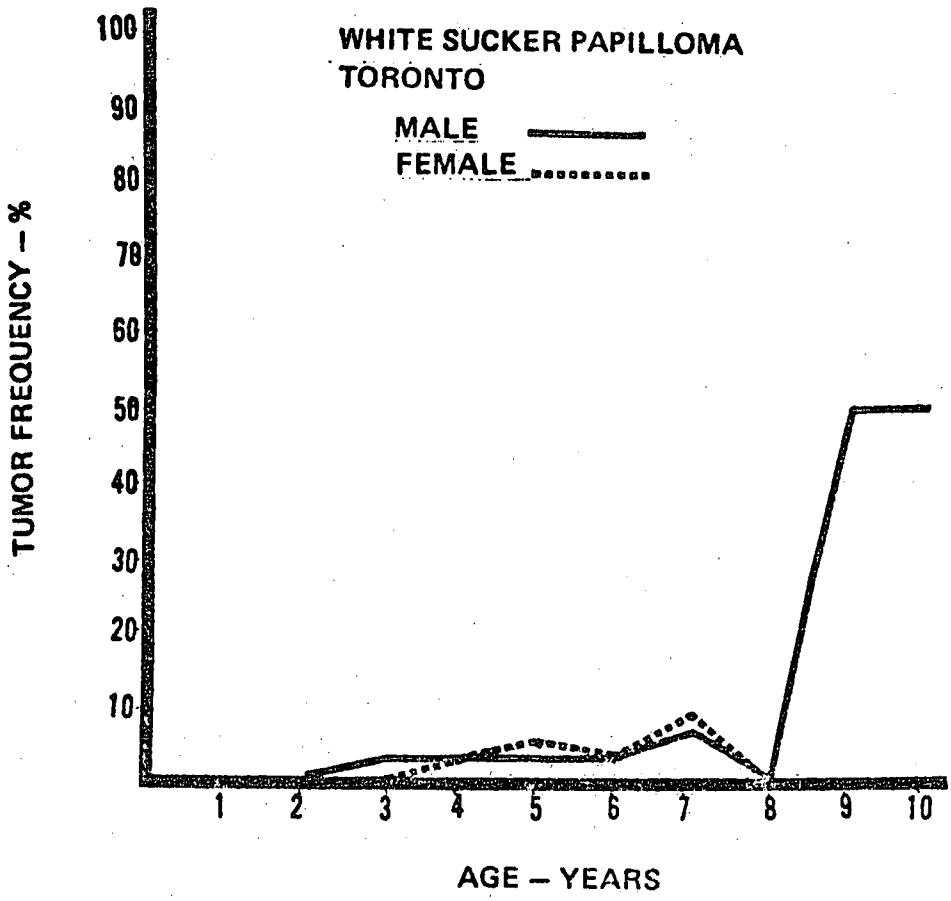


FIGURE 1

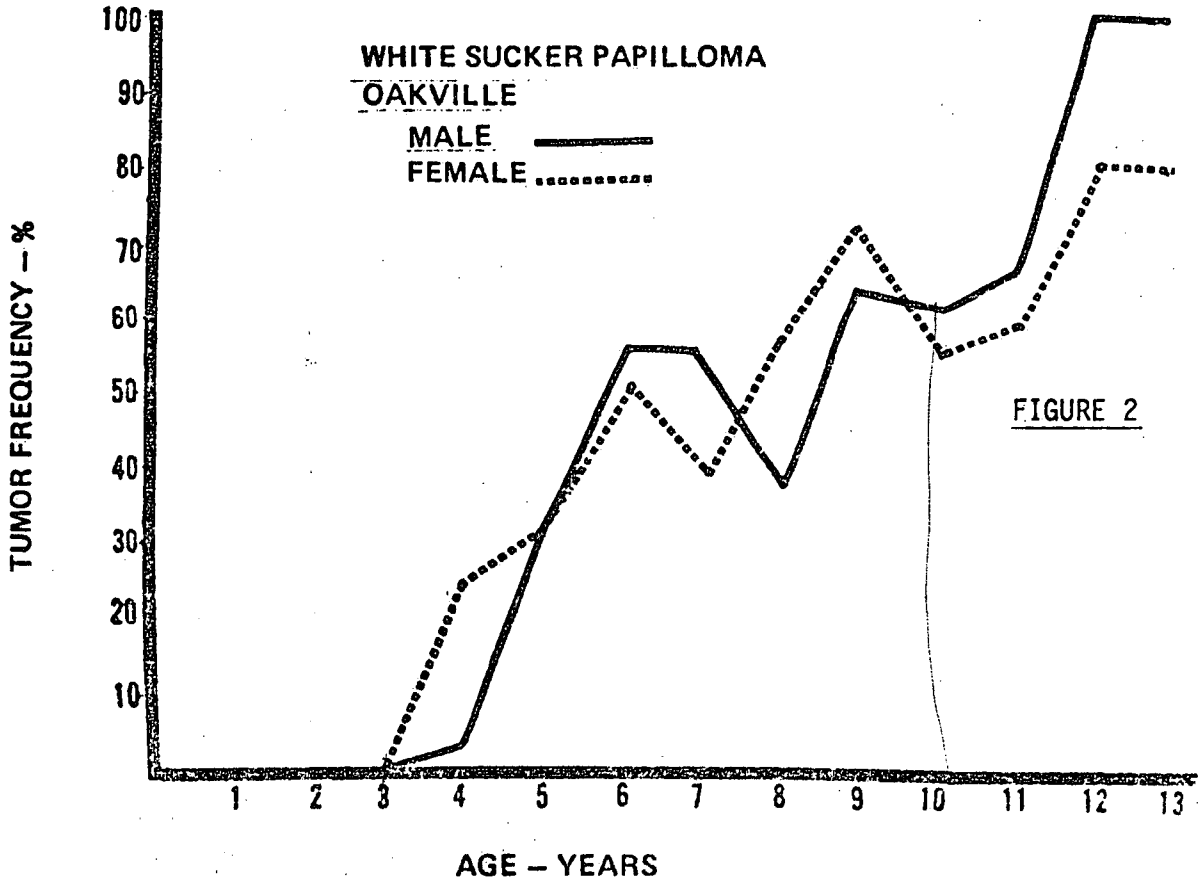


FIGURE 2

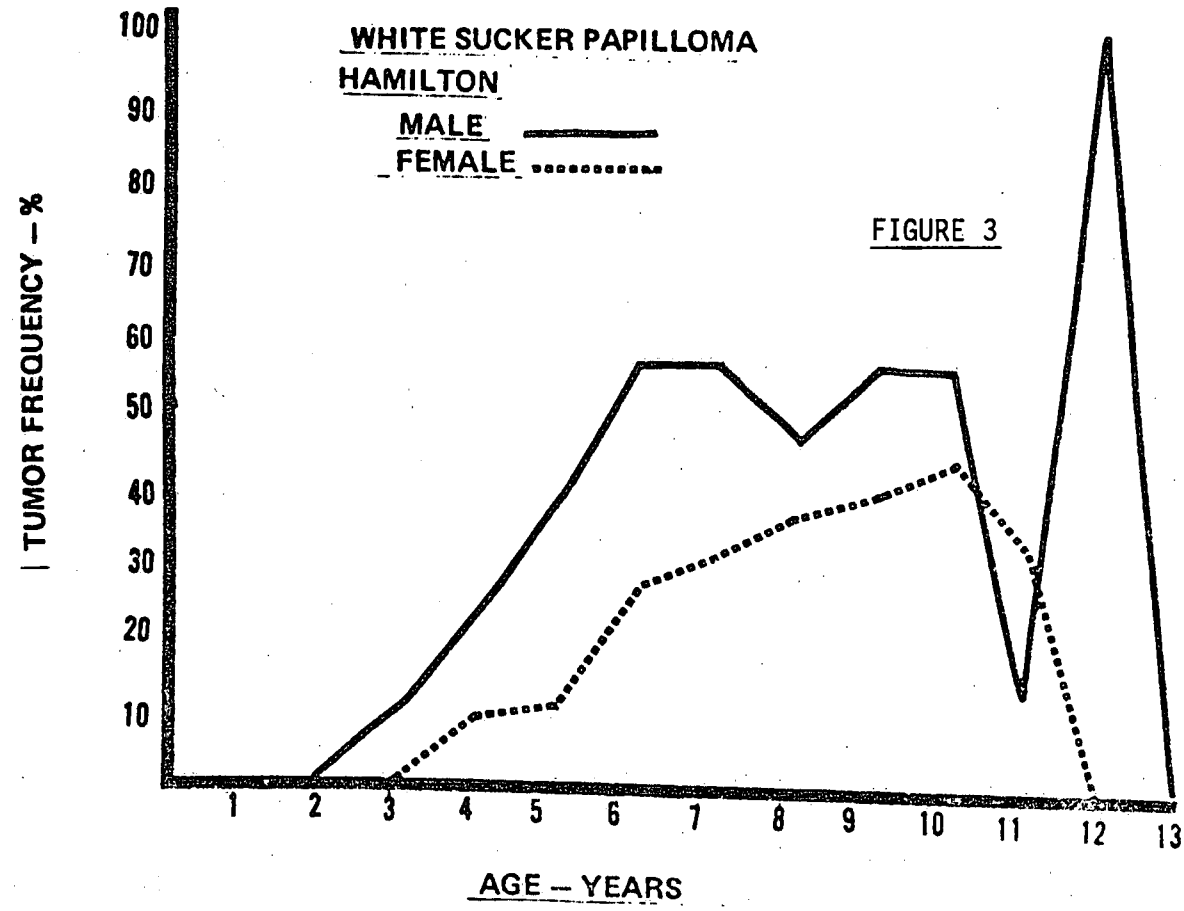


FIGURE 3

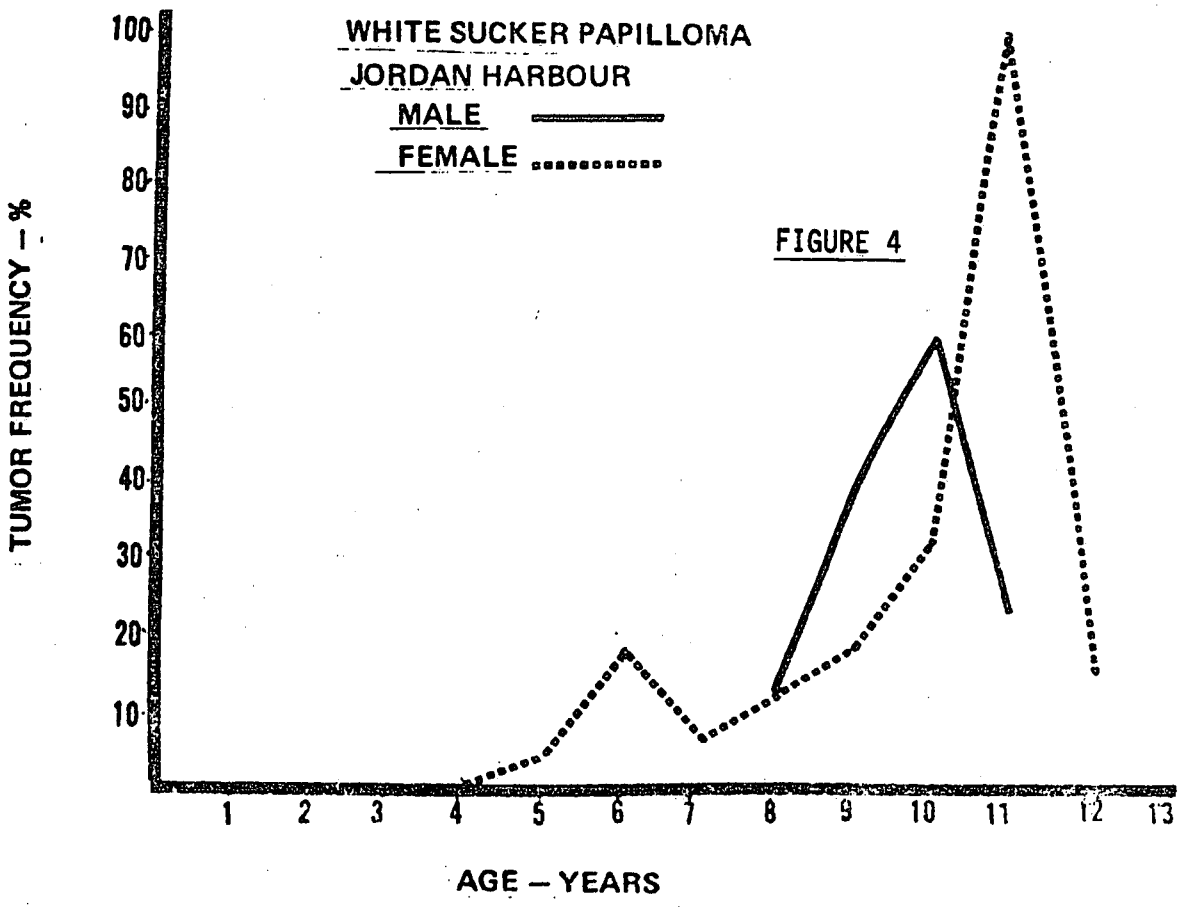


FIGURE 4

could expose their mouth parts (lips) to carcinogens (natural and industrial) which may accumulate in the bottom sediment. Alternatively such substances might cause mechanical abrasions to this anatomical region thus facilitating transmission of virus; both of which alone or in concert might give rise to the neoplasm. With regard to the above, polynuclear aromatic hydrocarbons (PAH's) which are potent carcinogens have been reported in Great Lakes sediments at levels of 54 µg/gm dry weight (Strosher and Hodgson, 1975). Similarly, polychlorinated hydrocarbons and a variety of heavy metals also accumulate at high levels in bottom sediments, which may play a role in the etiology of the tumour.

No behavioural pattern (i.e., courtship or spawning) have been observed which would facilitate mechanical transmission of an infectious agent (i.e., virus) or transplantation of tumour cells and thus explain this anatomical predeliction for the lips. Another confounding consideration with regard to mechanical horizontal transmission by contact by an infectious process is the distribution of the tumours on the lips. For example, of the white suckers exhibiting tumours in the Oakville collection, only 17.4% of the tumour-bearing suckers had tumours in opposition (i.e., tumour on the opposite lip at a site corresponding to the tumour). Considering that the opposite lip would be in almost constant contact with the tumour "kissing effect", it seems unlikely that the high frequency of occurrence of the tumour is due solely to an extremely efficient infectious process. Another interesting epizootiological observation was that the upper lip was more frequently involved than the lower. Of the Oakville tumour-bearing fish 61.4% had tumours on the upper lip whereas only 38.6% had tumours on the lower lip. Laboratory observations of resting and actively feeding white suckers suggest that the upper lip has more direct contact with bottom sediments and might be more prone to abrasions during the act of feeding.

The tumour frequencies recorded within specific age-sex groups at the various sites monitored provided some relevant epizootiological indications. White suckers reach sexual maturity at four years of age (Beamish, 1970) when the tumours begin to be detected. The tumour frequency (see Oakville graph) then progressively increase with age. The tumours in the younger fish were generally smaller and both the frequency of occurrence and size increased progressively with age. These results suggest that the tumour persists for the life of the fish. The development of the tumours post-sexual maturity suggests that the virus associated with the tumour may be horizontally transmitted during the act of spawning or that the expression of the virus may be linked to the endocrine system. The age at which the tumour begins to be detected must also be considered in the light of the migratory and/or behavioural patterns of the species in question. It is believed by this investigator that the suckers stay in streams for the first 1-3 years of life and then migrate into the open lake environment which might, in turn, be where the animal is exposed to carcinogens giving rise to tumour development. The catch data of the white suckers collected indicate that statistically significant catches were in the 5-9 year age class. Within these age classes, there is evidence of clustering in the Oakville and Burlington sucker compared to the other sites monitored.

To date, transmission trials have been performed on groups, each consisting of 200 fish. The trials involved holding tumour-bearing with normal fish, suckers which had been exposed by making mechanical abrasions on the lip and rubbing the abraded area with tumour, and fish which had been inoculated percutaneously with cell free preparations of the tumour and all have been refractory. No evidence of <u>in vitro</u> transformation was found in trials with the following fish cell lines: rainbow trout gonad, fathead

minnow, brown bullhead and bluegill fry.

Electron microscopic studies of tissues collected from the tumours have revealed large numbers of C-type virus particles associated with the white sucker papilloma. The virus (about 100 nanometer) was found budding from the cytoplasmic membrane and aggregates of virus were found in intracellular spaces. C-type viruses have been found associated with cancers in a variety of animals (i.e., mice, birds, cats, monkeys, hamsters, and humans) and are prime candidates as etiological agents in human cancers (Temin and Mizutani, 1974).

C-type viruses are unique in that their replication involves a DNA polymerase which is coded for by the RNA of the C-type virus (Temin and Mizutani, 1970; Baltimore, 1970). In an attempt to elucidate the etiological role of the C-type virus visualized in electron microscopic studies of the tumour, we sought for and found reverse transcriptase activity associated with the white sucker papilloma. These observations strongly suggest that C-type virus (a known cancer-causing virus) is involved in the etiology of the disease.

The oncogene theory of Hubner and Todaro (1969) states that in most vertebrate species, including man, cells carry C-type virus genomes as repressed genes. These are transmitted vertically and may be "triggered" depending on the hosts genotype, age, and exposure to environmental carcinogens. Once "triggered" viruses are produced and/or tumours are formed. According to this theory, genetic susceptibility is widespread and may be sine qua non in certain neoplasms. It would follow, that waterborne carcinogens could serve as "triggering" mechanisms of either horizontally or vertically transmitted C-type viral genomes. The result is the higher tumour frequencies in polluted as opposed to non-polluted waters as evidenced by the clustering reported here.

Brown Bullhead Papilloma (Ictalurus nebulosus)

Field epizootiological studies of the brown bullhead papilloma were made in Burlington and Jordan Harbours, on Lake Ontario, and Port Rowan on Lake Erie. The anatomical distribution of the papillomas in the brown bullhead are similar to the white sucker papilloma discussed earlier in that both diseases exhibit a propensity to develop as tumours on the lips. Until recently no satisfactory technique to determine the age of these fish by sectioning the pectoral fins has been developed in our laboratory. This confounded comparative tumour frequency studies in polluted vs non-polluted waters.

In the field investigations conducted, only large old bullheads were found with the tumour. The netting test on Lake Ontario bullheads is particularly interesting. A large catch (338) of very large brown bullheads was made at Jordan Harbour (a relatively non-polluted bay on Lake Ontario) and a low tumour frequency (1.6%) was found in these fish. In similar studies conducted in Burlington Harbour (heavily polluted bay on Lake Ontario, separated from Jordan Harbour by about 30 miles), the tumour frequency in the large bullheads (similar size to those collected at Jordan Harbour) was about 80%, suggesting possible clustering in Burlington Harbour. These observations closely parallel the clustering of the white sucker papilloma from the same collection sites.

Although classified as a papilloma (benign skin tumour) evidence of invasiveness was found in histopathological studies and the tumour may have to be reclassified as a carcinoma (malignant tumour of epithelial origin). The disease in brown bullheads exhibits high tumour frequencies in nature. However experiments involving 650 fish showed no evidence of transmission in laboratory contagion, transplantation, and cell free transmission trials. These studies suggest that the frequency of occurrence of the disease in brown

bullheads may be environmentally related. The brown bullhead papilloma as a sentinel animal for environmental carcinogens has distinct advantage. The fish is non-migratory in nature and spends its entire life in a geographically restricted area. Thus, it may help to recognize areas where cancer causing pollution may be present.

Hypothyroidism (Goiter) - Coho Salmon (Oncorynchus kisutch)

Lake Ontario coho salmon were found to suffer an epizootic of thyroid (goiter) during the 1974 spawning run (Sonstegard, 1975b). In that study 42.9% of the coho necropsied exhibited distinct nodes (goiter) on the gill arches and nearly all had gross evidence of thyroid hyperplasia as evidenced by diffuse swelling of the base of the gill arches. Earlier in 1974 (June), a large catch of coho of the same age group in which the epizootic of goiter was found in the fall spawning runs were examined, and no goiter was found. These field observations suggest that the condition develops during the period of rapid gonad development. In an attempt to resolve the etiology of the condition, the following summarizes collaborative investigations conducted with Dr. John Leatherland (fish endocrinologist, University of Guelph) in 1975.

On August 18, 1975, a gill net survey (5 inch stretch mesh) was made in Lake Ontario off the mouth of the Credit River. The following information and/or data was collected in this test netting and in subsequent collections reported here: plasma, Bouins fixed tissues from the base of the gill arches of normal and goiter-bearing coho for characterization of thyroid histology, Bouins Hollande fixed pituitaries of both normal and goiter-bearing coho, and sex. At the time of the August 18 test netting, the coho were actively feeding as evidenced by gut examinations and angler success.

In this netting, 52 coho were captured, of which three exhibited gross overt goiter (5%).

A second gill net collection was made at the mouth of the Credit River on September 5, 1975. At the time of this collection, the fish had gone off feed as evidenced by gut examination and a drastic drop in angling success. In this collection a total of 68 fish were captured, of which 12 exhibited gross overt goiter (17.6%).

A third collection was made by seining and snagging on October 24, 1975 on the Credit River, near the O.M.N.R. weir at Streetsville. In this collection a total of 51 fish were examined of which 12 exhibited gross overt goiter (24%). At the time of these collections the coho were "ripe" and could readily by manually spawned.

Histopathological examinations were made on the thyroid with particular reference to characterizing the gland at various stages of their anadromic run. In the first collection, the thyroid in the non-goitered fish was composed of relatively small numbers of follicles with low cuboidal cells. The colloid was homogeneous with little or no vacuolation. The above description is that of a normal telost thyroid gland. In the goitered condition the epithelial cells were markedly hyperplastic as evidenced by tall columnar cells. The epithelial cells were commonly arranged into what appeared to be tubules with the lumen follicle forming the empty cavity of the tubule. There was little or no colloid in the goitered coho.

The "normal" coho captured in the second netting exhibited a thickening of the epithelial cells, some of which began to take on the appearance of columnar cells. Colloid was present in the follicles, although less than in the August netting. The thyroids of the goitered specimens captured in the second netting were similar to those described above.

The "normal" coho captured in the third collections all exhibited tall columnar cells accompanied by a loss of colloid. This is the histological picture of a hypothyroid animal. The goitered fish exhibited severely hyperplastic columnar epithelium, loss of follicular arrangement, and were devoid of colloid.

Histopathological examination of the pituitaries collected from the August to October collections exhibited a progressive proliferation of thyrotrophs. This supports the clinical picture of the rapid onset of hypothyroidism as evidenced by the increased frequency of occurrence of overt goiters and thyroid histopathology.

Serum thyroid hormones (thyroxin and triiodothyronine) were measured from coho collected in September and October. Thyroxin and triiodothyronine levels in the September collection were 1.0 and 400.3 respectively. The thyroxin and triiodothyronine levels in the October collection were 0.4 and 80.2 respectively. These measurements are indicative of severe hypothyroid problems.

It is well established that the thyroid gland in teleosts shows marked seasonal variations in appearance with periods of greatest apparent activity associated with the time of reproduction. Similarly, since in anadromous salmonids, the thyroid appears most active at the time when the fish are moving into fresh water, the thyroid has been implicated in osmotic and ionic regulation. This apparent increase in thyroid activity (derived from histological studies) may reflect the response to iodide deficiency rather than a true increase in thyroid hormone requirements. Thus the gland is hyperplastic and hypertrophic due to hypersecretion of thyroid stimulating hormone (TSH), the latter resulting from lowered thyroxine (and T_3) levels.

The iodine concentration in the Great Lakes is 20-60 times lower than in sea water (Winchester, 1970). Fresh water fishes indigenous to the Great Lakes basin apparently have adapted to this condition, but this adaptation may be less successful for anadromous fishes such as the coho. Land locked forms of anadromous fishes making a prolonged stay in a low iodine fresh water environment are likely under added osmotic stress which depends on thyroid hormone.

The seasonal requirement of thyroid hormones in concert with the low iodine fresh water environment and stress of osmoregulation may partially account for the previous appearance of goiters in coho studies in this investigation. The coho demonstrate several symptoms of hypothyroidism, low serum thyroxin and T_3 levels, hyperplastic and hypertrophic thyrotrophs and large nodular goiters. The histological studies suggest that the goiter became progressively more extensive between August and October. The serum levels of thyroid hormones fell significantly between September and October, while the incidence of overt (nodular) goiters increased from 5% in August to 24% in October, all of which suggest an increasing need for thyroid hormone during this period of anadromic migration preceeding spawning.

The low iodine background in the Great Lakes basin has historically been associated with endemic goiter in man before the introduction of iodized salt. The development of thyroid goiter in the adult coho is in one sense endemic due in part to compensatory hyperplasia in response to low iodine, together with stress of physiologic processes increasing thyroid activity as discussed earlier. However, the following observations suggest that extrinsic environmental factors are involved in the etiology of the goiters.

According to Hnath (1975) Lake Michigan field biologists reported that in 1967 and 1968 adult coho exhibited a higher frequency of occurrence of

"gill tumours" than in recent years, although no records are available to document these observations. Hnath, in 1971, examined 111 adult coho at the Platte River Spawning Station and found no gross goiters. Similarly, we examined 200 adult coho at the same station in 1973 and found no goiters. Goiter does, however, occur in Lake Michigan coho as documented by histopathological diagnosis of several specimens forwarded to this laboratory. It is interesting to note that goiter frequency in Lake Michigan coho has apparently declined over the years. Similarly, during the 1974 spawning run of the Lake Ontario adult coho, we found a frequency of occurrence of the goiter of 42.8%, whereas coho monitored at the same collection site this year had a frequency of occurrence of 24%. Furthermore, the histological appearance of the thyroid gland and thyrotroph proliferation in the 1974 collections were more severe than those recorded this year. This constitutes strong epizootiological evidence that the goiters are not due solely to low iodine levels in the environment, as seasonal fluctuations of iodine levels in a large lake environment is highly unlikely.

The seasonal variation in frequency of occurrence and severity of goiters may reflect exposure of different populations to dietary and/or waterborne goiterogens. The coho is a far ranging school fish in the open lake environment, and depending on a variety of environmental and chance phenomena, might from season to season or individual to individual, be exposed to varying levels of extrinsic environmental factors depending on the lake environment that it occupied.

While these studies suggest that extrinsic environmental factors are acting as goiterogens, potentiating the development of goiter in a low iodine environment, no environmental goiterogens have been identified to date. Coho in Lake Ontario have high levels of polychlorinated biphenyls which have been

reported to stimulate the thyroid gland (Mayer et al., 1972). A PCB stimulated thyroid gland may increase the rate of depletion of an hypothyroid fish, possibly triggering development of goiter. This mechanism or role may be supported by the seasonal development of the goiters, as there is rapid mobilization of lipid depots during migration. The mobilization of these depots (PCB's accumulate in lipid depots) may increase the levels of PCB's in target tissues, which could stress an already depleted thyroid gland, resulting in goiter formation. Similarly, several other environmental pollutants known to be in Lake Ontario coho have known effects on thyroid (deldrin and DDT).

Drongowski and co-workers (1975) reported low plasma thyroxin and T_3 values for adult coho salmon collected at the Platte River Spawning Station in Michigan after their migration out of Lake Michigan. However, the reported thyroxine values (1.8 µg/100 ml plasma) were 1.8 times as high as the Lake Ontario coho sampled during their anadromic migration before spawning, and 4.5 times as high as those monitored on the spawning grounds. Similarly, the histological appearance of the thyroid reported by Drongowski appeared less severely hypertrophic than that of the Lake Ontario coho.

The iodine concentration in Lake Ontario water (2.9 µg/l) (Winchester, 1970). Therefore, if the hypothyroid condition were solely due to low iodine levels in the environment (endemic goiter), one would expect Lake Michigan coho to exhibit a higher frequency of goiter and exhibit more severe hypothyroidism which is at variance to the data discussed here. Similarly, Lake Erie has higher iodine levels than Lake Michigan (1.7 µg/l) and Lake Erie coho were reported to have a frequency of occurrence of 44% of goiter (Black and Simpson, 1975).

The potential importance of waterborne or dietary goiterogens (natural or as pollutants) is the fact that the fish are consumed by humans and the lake water is increasingly being utilized for drinking water. The suspect goiterogen discussed here (PCB's) has been reported in human lipid depots at a concentration of 1 ppm in 45% of the U.S.A. population (Price and Welch, 1972). Inasmuch as thyroid hyperplasia may readily progress to neoplasia, particularly in the presence of carcinogen, the etiology of the goiters need to be resolved (Axelrad and Leblond, 1955; Furth, 1969).

These studies raise significant questions regarding the effects of constant low or chronic levels of environmental pollutants on animal health. There are in the literature, growing data to suggest many endocrine parallels between teleosts and mammals in the regulation of endocrine functions. Fish, by the process of biological magnification, are exposed to environmental insults often higher than those to which other animals are exposed. It is apparent that monitoring the endocrine disorders and neoplasia in these animals has obvious merit.

Epidermal Hyperplasia - Carp (Cyprinus carpio)

During the course of laboratory experimentation involving the holding of fingerling carp in static tanks into which the carcinogen diethylnitrosamine had been added, slightly elevated gray-white growths were noted developing on the skin. Electron microscopic studies of the hyperplastic epithelium revealed tremendous quantities of Herpes virus. Contagion trials in which tumour-bearing carp were held with normal fish were successful as the tumour developed in the normal carp. Cell free transmission trials are underway. Cell culture transformation trials using established fish cell lines from goldfish, fathead minnow, and rainbow trout gonad were refractory.

Some evidence of invasiveness has been found in histopathological studies, and the disease may have to be reclassified as an epidermoid carcinoma (malignant tumour of skin).

The tumour has been found in Burlington Harbour carp and similar to the observations made on the white sucker and brown bullhead papillomas, the tumour has exhibited a propensity for the lips and head region. This may suggest possible environmental induction or opportunity for horizontal transmission as discussed with the white sucker papilloma. Inasmuch as Herpe virus is a prime candidate for malignancy in man, the etiologic significance of the virus should be confirmed.

Gonadal Tumours (Goldfish, carp, goldfish x carp hybrids)

Epizootics of gonadal tumours (Sertoli cell origin) were found in Great Lakes goldfish (Cassarius auratus), carp (Cyprinus carpio), and goldfish x carp hybrids during the course of these investigations (Sonstegard, 1974; Sonstegard, 1975a, b). The enclosed graphs (Fig. 5, 6) represent gonadal tumour frequency in hybrids in several geographical areas. Hybrids up to 3-4 years of age (age of sexual maturity in carp) (McCrimmon, 1968) appeared to have normally developing gonads and in subsequent years develop the neoplasm. Tumour-bearing fish were invariably sterile. With regard to epizootiological studies to resolve whether extrinsic environmental factors were involved in the etiology of the disease a review of museum collections made prior to 1952 was made (Royal Ontario Museum, University of Michigan, Ohio State University, and the Smithsonian). No tumours were found in this review of museum collections (Sonstegard, 1975b).

The collections at Michigan and Ohio were particularly relevant because of the following. The University of Michigan had a moderately large

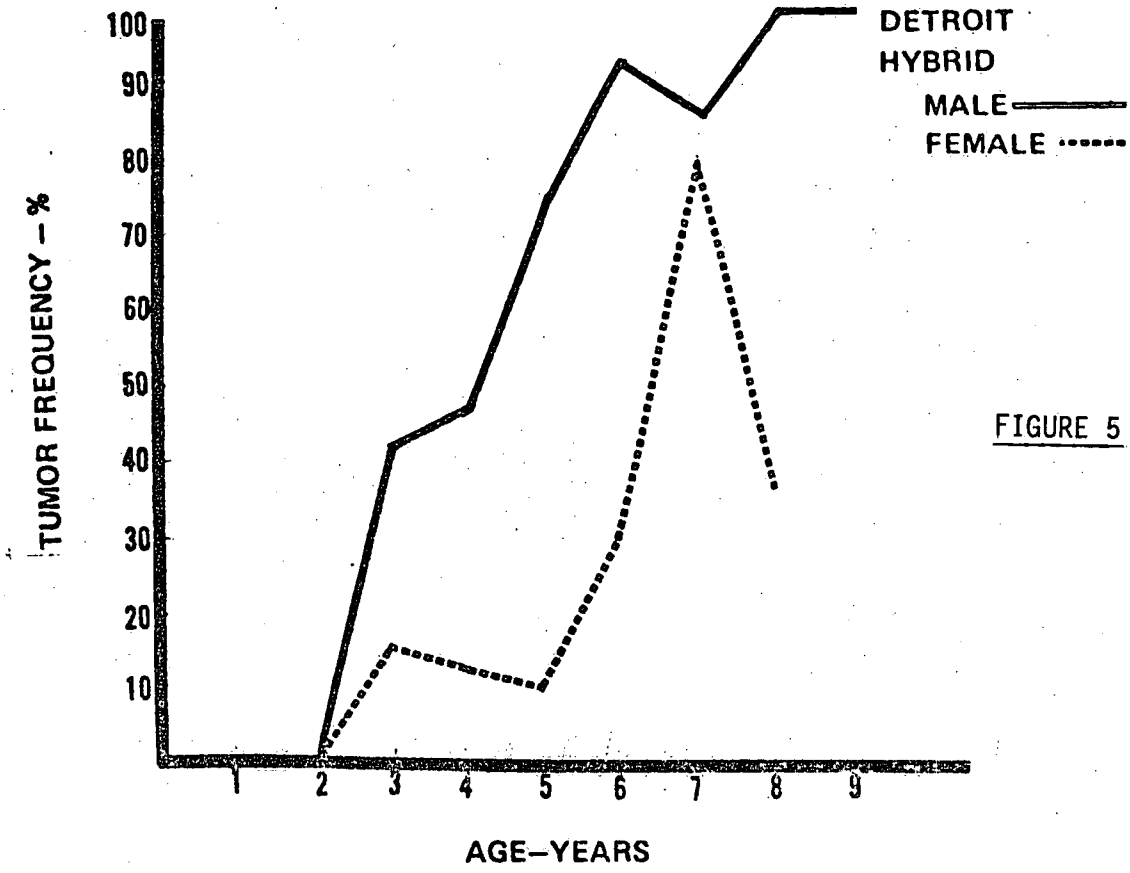


FIGURE 5

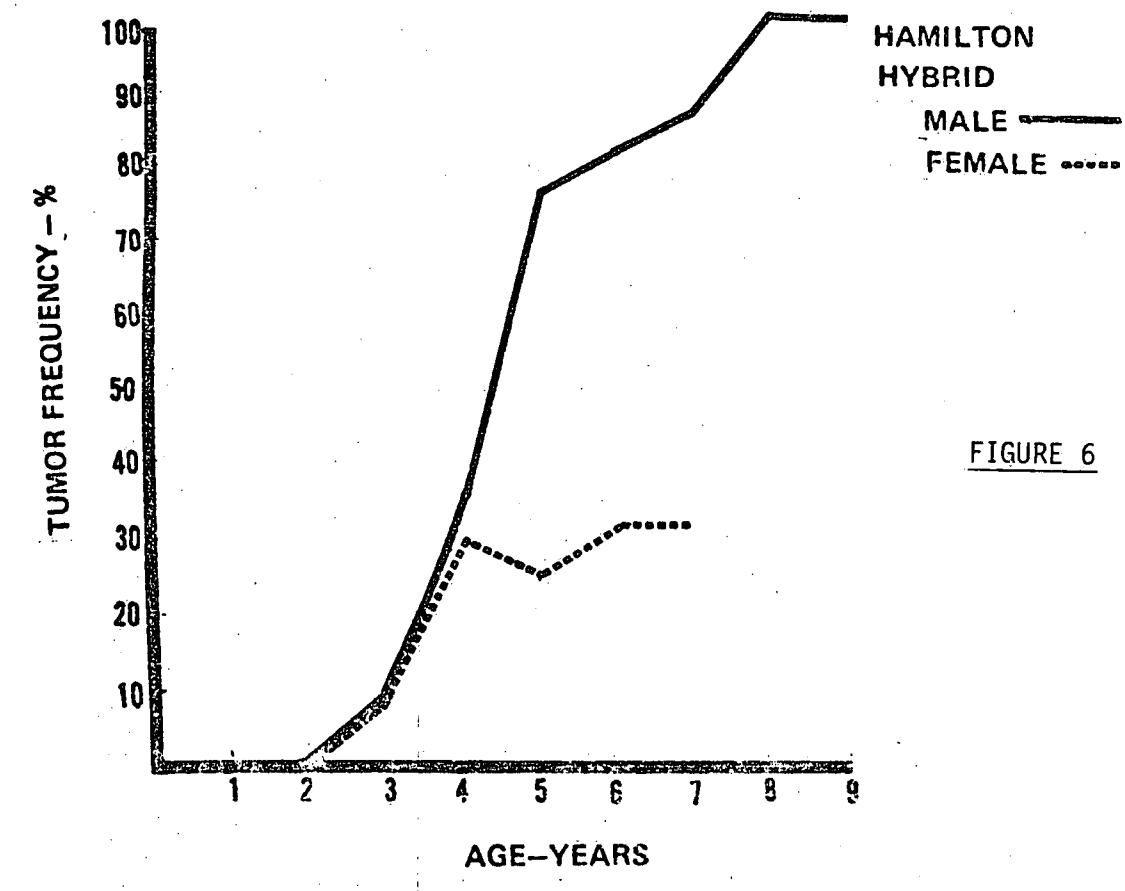


FIGURE 6

random collection of hybrids (38 fish) captured in 1952 off the mouth of the River Rouge in Detroit. Examinations of these fish revealed no tumours. In our investigations, collections from the same geographical site in 1974 and 1975 gave tumour incidence as high as 100% in the older males.

At Ohio State University, Dr. Milton Trautman, an elderly eminent ecthylogist, was consulted regarding the hybrids. Dr. Trautman had made extensive field and laboratory investigations of the hybrids prior to 1950 in the Toledo, Ohio region of Lake Erie. He recorded hybrids exhibiting evidence of backcrosses. In our investigations, conducted some 30 years later, all hybrids found are sterile and are of a F_1 generation.

The field epizootiological studies conducted in 1974 and 1975 in conjunction with the review of museum collections made prior to 1952 suggest that since that time either chemical or environmental factors which have oncogenic potential have been discharged into the Great Lakes. In an attempt to resolve this question, exhaustive efforts were made to capture hybrids in the upper and relatively "unpolluted" waters of the Great Lakes. A total catch of 26 hybrids from the Kincardine region on Lake Huron represented the uppermost region (distance from industrial basins) where hybrids could be captured. An overall tumour frequency of 61% was found. These results suggest that all hybrids within the lake system suffer high frequencies of gonadal tumours. The widespread geographical distribution of the disease today suggests that the agent or agents are virtually ubiquitous. In lieu of the same, and the onset of the syndrome post 1952, it is hypothesized that polychlorinated biphenyls (PCB's) and/or DDT may be involved in the etiology of the tumour. Tables 1 and 2 give the heavy metals, pesticide and PCB analysis of carp and carp x goldfish hybrids collected in field epizootiological studies from various sites in Canada and U.S.A.

TABLE 1

HEAVY METAL ANALYSIS OF CARP KIDNEY HOMOGENATES - CONTENT IN HOMOGENATES (ppm)

Sample Site	Hg	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Montreal	0.440	3.54	0.303	1.49	2.10	168.	1.06	1.16	0.800	116.
Detroit	0.996	7.64	0.311	0.801	2.12	186.	0.54	0.86	0.510	120.
Parry Sound	0.429	10.1	0.228	1.49	2.71	129.	0.78	0.97	0.465	174.
Cleveland	0.179	3.49	0.378	1.33	3.76	169.	1.18	1.53	1.18	133.
Toledo	0.213	6.14	0.272	1.09	1.08	146.	0.52	0.95	1.03	276.
Sarnia	1.487	3.65	0.386	0.971	1.92	195.	0.67	1.26	1.06	124.
Port Rowan	0.164	3.32	0.303	0.861	1.48	151.	0.83	1.12	0.527	165.
Burlington Harbour*	0.514	3.74	0.205	0.983	1.82	115.	1.22	1.37	0.839	194.
Burlington Harbour**	0.174	1.37	0.303	1.51	1.88	101.	0.64	0.80	0.417	73.2
Burlington Harbour***	0.170	0.965	0.394	3.91	2.92	220.	1.97	2.64	0.362	315.
Toronto	0.367	2.65	0.583	1.24	2.34	153.	1.39	1.14	0.725	251.
Burlington Harbour****	0.091	0.039	0.713	0.861	1.05	59.6	0.44	0.60	1.03	89.1

* Collected off tailings of steel mills

** Tumor bearing hybrids, collected in Cootes Paradise

*** Tumor bearing hybrids, collected off tailings of steel mills

**** Tissue analysis made on gonads of tumor bearing hybrids

TABLE 2

PESTICIDE RESIDUE ANALYSIS OF CARP KIDNEY HOMOGENATES - CONTENT IN HOMOGENATES (ppm)

	Extracted Fat (ppm)				Tissue (ppm)				
	para-DDE	p-pDDD	Dieldrin	PCB	% Fat	p-DDE	p-pDDD	Dieldrin	PCB
Montreal	0.74	0.92	0.24	27.0	3.88	0.029	0.035	.009	1.0
Detroit	2.10	0.91	0.26	56.0	7.56	0.158	0.069	.019	4.2
Parry Sound	2.22	0.46	0.08	13.0	6.09	0.135	0.028	.005	0.79
Cleveland	0.80	0.91	0.26	47.0	6.82	0.054	0.062	.017	3.2
Toledo	1.03	1.50	0.16	30.0	7.20	0.074	0.108	.011	2.2
Sarnia	0.80	1.00	0.33	27.0	4.98	0.039	0.049	.016	1.3
Port Rowan	0.74	0.46	0.16	3.0	4.68	0.035	0.021	.007	0.14
Burlington Harbour*	5.60	2.72	0.26	47.0	9.06	0.507	0.246	.023	4.3
Burlington Harbour**	3.33	3.07	0.08	37.0	12.40	0.413	0.381	.010	4.6
Burlington Harbour***	2.18	1.81	0.26	73.0	7.40	0.161	0.133	.019	5.4
Toronto	3.70	1.53	0.16	33.0	2.58	0.095	0.039	.004	0.85
Burlington Harbour****	4.26	1.81	0.52	110.0	2.67	0.116	0.048	.014	2.9

* Collected off tailings of steel mills

** Tumor bearing hybrids, collected in Cootes Paradise

*** Tumor bearing hybrids, collected off tailings of steel mills

**** Tissue analysis made on gonads of tumor bearing hybrids

In an attempt to resolve the pathogenesis of the disease, the following summarizes investigations made to examine the effects of the tumours on the pituitary-gonad axis. The neoplasms in both males and females appeared to be entirely of Sertoli cell origin, however, some specimens exhibited characteristics of interstitial cells. The tumour-bearing animals were sterile and spermatogenesis and oogenesis were markedly reduced or absent. The pituitaries in fish having tumours were approximately 3 times the size of those in animals without tumours. This massive enlargement was the result of hyperplasia of the basophilic cell component (gonadotrophic cells) of the proximal pars distilis; the cells composed 80-90% of the region in tumour-bearing fish compared with 50% tumour-free animals. There were no apparent differences in the structure of fine structure of the gonadotrophs in tumour bearing and normal fish of comparable age and season. Twenty daily injections of luteinnizing hormone (LH) (1/2 µg/g body wt) given to 1 year old, tumour-free hybrids maintained in water at 25^{o}C under a 16 hr. light photoperiod failed to induce tumour formation. Moreover, treatment of tumoured hybrids with methallibure (gonadotrophic blocker) did not effect a reduction in the size or incidence of the tumours, although it did markedly reduce the size of the pituitary and the activity of the gonadotrophic cells. It thus appears that the Sertoli cell tumours are not directly regulated by the gonadotrophs, rather, that the gonadotrophic cell hyperplasia results from the presence of the tumour, possibly in response to a lowered circulating level of gonadal steroid (Sonstegard et al., 1975).

Cell free and transplantation trials of the tumours, each involving 400 fish, have been refractory (two years duration). Contagion studies in which tumour-bearing and normal specimens were held in the same tanks and were also refractory. Cell culture transformation trials were refractory as

well as reverse transcriptase assays. No evidence of virus has been found in electron microscopic studies. Attempts to induce tumour development by placing carp on a diet containing 10 ppm PCB's were refractory.

These investigations all point to the role of extrinsic environmental factors in the etiology of the tumour. The existence of environmental pollutants causing tumours and total reproductive failure is a matter of urgent concern. The etiology of the disease should be promptly resolved.

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