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CONTINUED EVALUATION OF THE ATLANTIC TOMCOD AS AN ASSAY FOR CHEMICAL CARCINOGENS IN THE HUDSON RIVER (04/86A/13)

FINAL REPORT

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SUMMARY

Atlantic tomcod, one year or older, were collected from the Hudson River, NY, Pawcatuck River, CT, Saco, Penobscot, Sheepscot, Royal, and Cousins Rivers, ME. Histological examination of their livers revealed cancers in 52% of the Hudson River, 4% of the Pawcatuck River and 2% of the Saco River samples. No hepatocellular carcinoma was detected in the four other rivers in Maine. This indicates that hepatocellular carcinoma of tomcod is not restricted to the Hudson race of tomcod or to warmer, southern rivers. All three rivers with detectable carcinomas have had known inputs of xenobiotics; whereas, the other four rivers have only had inputs from agricultural runoff.

Juvenile tomcod were collected from the Hudson River and from two rivers in Maine at 6 and 7 months of age, respectively. Both groups were reared in the laboratory in an aquaculture system using recirculating water. In December of the same year, approximately six months later, all fish were autopsied for hepatocellular carcinoma. No carcinoma was detected for either group although cohorts in the Hudson River had 10% and 39% incidences. This suggests that hepatocellular carcinoma that develops in Hudson River tomcod is not due to maternal loading or early exposures in the upper river, but that extended exposure is required probably through ingested Gammarus or sediments in the lower Hudson Estuary. Confirmation of these findings are required because of the lack of control for the influence of reproductive hormones and the small sample size of the study.

INTRODUCTION

Ever since the high incidence of liver cancer in the Hudson River population of tomcod became known, people have wanted to know what factors caused this alarming pathology. Some are interested because they may have contributed to the cause via the disposal of toxic materials and they fear reprisal. Some worry that they will share the fate of the tomcod because they drink water from the Hudson River or eat its fish. Still others are simply angry that humans in general have been systematically destroying the environment and view the tomcod as another example of our disregard for the planet.

All of these concerns assume that cancer of the tomcod is due to an etiology involving xenobiotics, compounds created by humans that do not naturally form in nature. Indeed, xenobiotics are highly suspect; however, several other etiologies are also possible. These include thermal stress, viral transformation, or a race of Hudson River tomcod that is genetically susceptible to hepatocellular carcinoma.

This study examined several of these potential causes using different avenues of investigation including sampling tomcod from New England rivers and rearing fish in the laboratory.

IDENTIFICATION OF HEPATOCELLULAR-FREE POPULATION

Although the original intent for sampling tomcod outside of the Hudson River was to find a hepatocellular free population to use as a control group in rearing experiments, the collections directly revealed important information including the following:

1. established population and physiological baselines for tomcod.
2. determined if a lower yearly temperature precluded hepatocellular carcinoma from a population.
3. discovered if tomcod outside the Hudson River can potentially develop hepatocellular carcinoma or if this pathology is restricted to Hudson River fish.

Rivers Sampled

The Sheepscot and Penobscot Rivers were selected since these are larger rivers and are considered among the cleanest in northeastern United States; however, these rivers receive pesticide inputs from spraying of forest. Although they are small, the Royal and Cousins Rivers were sampled because they are believed to be free of industrial and agricultural input. The Saco was selected because this river had historical inputs from tannery mills and its northern location permitted evaluation of the effects of colder temperatures on the occurrence of hepatocellular carcinoma. The Pawcatuck River was used because it also has historical inputs from textile mills and its southern location is more similar to the Hudson River although it is a much smaller river (Fig. 1 and 2).

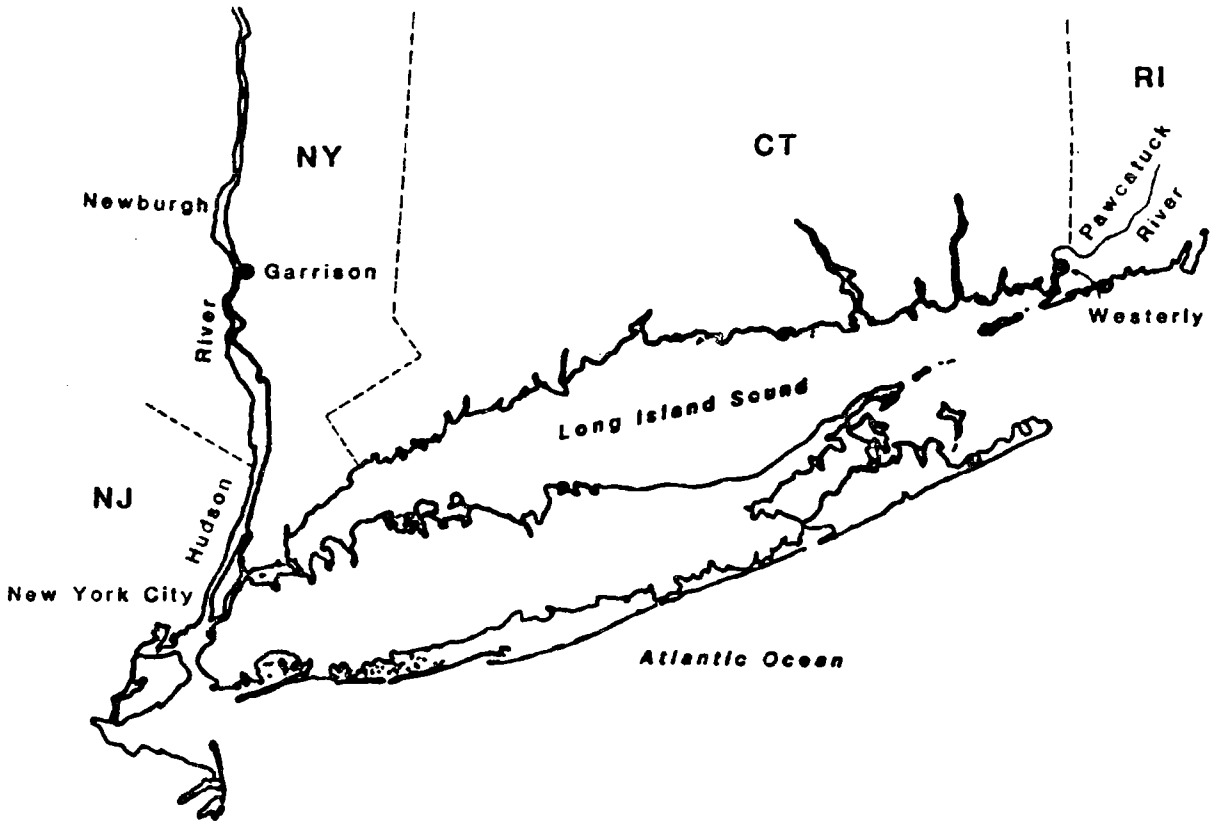

Figure 1. Map showing collection sites on the Hudson and Pawcatuck Rivers.

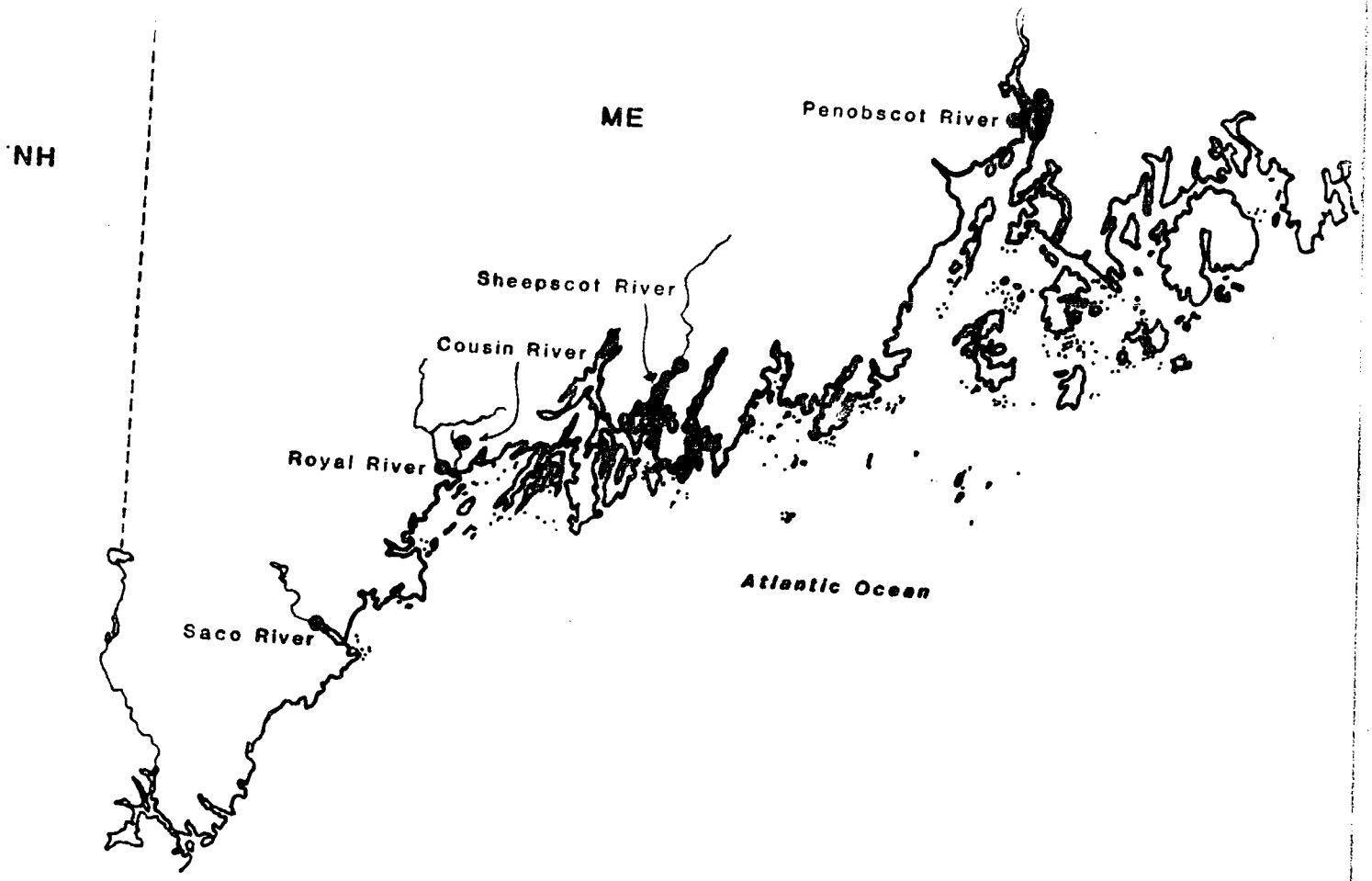

Figure 2. Map showing collection sites on the Saco, Royal, Cousin, Sheepscot and Penobscot Rivers in Maine.

Collections

During the winter months (Nov.-Jan.) tomcod spawn in many of the coastal rivers of northeastern United States and Canada, and many remain in the rivers throughout the year. Tomcod were collected during the spawning seasons (1985-89) using both large and small box traps set along the shore of selected sites of the Hudson River, NY (n = 64), Pawcatuck River, CT (n = 35), and the Saco (n = 68) and Royal (n = 4) Rivers, ME. Tomcod spawning occurs earlier in Maine, then further south and finally in the Hudson River allowing a single research team to follow the spawning runs and collect at all sites. Theoretically, earlier collections could also be made further north in Maine and Canada. Summer collections (1987) for juveniles using an otter trawl in the Sheepscot and Penobscot Rivers, ME also confirmed the presence of adults (n = 2 and 19, respectively) in the rivers during the summer.

Collected fish were measured and sexed. The livers were excised and examined for gross pathology. Liver samples of fish collected during the winter months were fixed in Bouin's solution or buffered formaldehyde. Tissue samples were dehydrated in ethanol and then embedded in paraffin. Sections were cut and stained with hematoxylin and eosin (H & E). H & E stained sections were evaluated for the presence of areas of cellular alteration and hepatocellular carcinoma. Encysted protozoan parasites were also enumerated.

Size and Age differences

Age. Although hepatocellular carcinoma is the most obvious pathology affecting the Hudson River tomcod, other factors may also be affected. Of the more than 560 tomcod collected from the Hudson, only one was found to be 3 years old (Dey et al., 1986). We found one three year old in the Saco out of 68 collected. Two of the 19 fish collected from the Penobscot were 2+ in the summer and would be considered age 3 in December. Three-year-old and even older tomcod have been more commonly reported from rivers north of the Hudson and in Canada (Howe, 1971; Legendre and Lagueux, 1948; Roy et al., 1975). Since the incidence of hepatocellular carcinoma is almost 100% in two year old Hudson tomcod (Dey et al., 1986) this may now be the upper age limit for this population.

Size. The tomcod from Maine also appear to be larger (Table I). This size difference was reflected in both our field collections and our laboratory reared fish (field data: $t = 2.60$, $df = 67$, $p < 0.02$; laboratory data: $t = 2.26$, $df = 19$, $p < 0.05$). Since spawning occurs earlier in Maine, the difference may just be due to age differences but we also collected earlier in Maine. Further effort on these population parameters is certainly needed.

Table I.--Lengths of one-year-old adult Atlantic tomcod collected from rivers in Maine and New York during the annual spawning run and final lengths of laboratory reared juveniles.

Site	Date	n	Mean (mm)	SD	Range (mm)
Adults					
Saco, Royal	12/86	38	194.5	22.38	151-253
Saco	12/87	16	190.1	14.84	164-218
Hudson	1/87	4	153.5	35.62	117-193
Hudson	1/88	11	176.7	28.42	130-216
Hudson[a]	12/83-3/84	458	155.2	[b]	109-235
Laboratory					
Sheepscot	12/87	3	151.7	10.62	139-165
Hudson	12/87	18	135.3	16.28	107-170

[a](Dey et al., 1986)
[b]not provided

Eggs were also significantly smaller from Hudson River females than from the Saco River (Table II). Tomcod eggs from the Pawcatuck River also appeared larger, although these were not actually measured.

TABLE II. Tomcod egg diameters in millimeters.

	Hudson	Saco
n	27	18
mean	1.26[a]	1.47[a]
SD	0.35	0.49

[a] $p < 0.001$, $t = 8.579$, $df = 43$

Cancer Incidences

The liver of the Atlantic tomcod possesses two large lobes and one smaller central lobe that is suspended over the digestive tract. Normal appearing liver is brown to tan (Class 1) while hepatocellular carcinomas appear as translucent areas, light spots or lumpy masses (Class 2). Very large tumors can encompass the entire lobe of a liver and often are cystic with mixtures of blood and bile (Class 3).

Table III. Incidence of liver tumors in Atlantic tomcod based on gross observations and histological analyses.

River	Gross (Class 2, 3)	Histology (Class 1)	Total
	All Fish		
Field collection			
Hudson	27/58 (47%)	6/38[1] (16%)	33/64 (52%)
Saco	0/68	1/56 (2%)	1/56 (2%)
Royal	0/4	0/4	0/4
Penobscot	0/19	0/17	0/17
Pawcatuck	0/35	1/25 (4%)	1/25 (4%)
	Fish < 175 mm		
Field collection			
Hudson	0/21	2/21 (10%)	2/21 (10%)
Laboratory reared			
Hudson	0/18	0/18	0/18
Sheepscot	0/3	0/3	0/3

[1]Includes 8 fish used for breeding stock at SP, Inc., MA that were not grossly classified--2/8 were positive.

Gross evidence of hepatocellular carcinoma was only detected in tomcod from the Hudson River. No gross tumors or small lesions were seen in any of the other rivers. However, histological examination showed two small carcinomas in one fish from the Saco, a 2% incidence and 1 carcinoma in 1 fish form the Pawcatuck, a 4% incidence. None of the other rivers sampled had detectable carcinoma (Table III). Dey et al. (1986) also detected 2 fish with neoplastic nodules in the Pawcatuck, an incidence of 5%. The low, but never-the-less detectible, incidence of carcinoma in some of these other rivers indicates that the Hudson River population is not an isolated case or due solely to some genetic predisposition. Since the Hudson is on the southern extreme of the species range, the synergistic effect of temperature is possible, but thermal stress is not the likely cause of these tumors since fish in colder waters also developed carcinomas. Both the Pawcatuck and the Saco have had past industrial inputs, textile mills and a tannery, respectively, so xenobiotics are also suspect in these rivers.

Normal livers in paraffin section were typically composed of cuboidal epithelial cells having an oval or round nucleus with a vacuolated cytoplasm. Tubules of cells were separated by blood sinuses. Pancreatic

tissue was normally present within the liver, but was generally located near the margins of the liver and near ducts. Pancreatic tissue was more darkly stained but was easily distinguished from hepatocellular carcinoma cells by their smaller nuclei, uniform cell shape, and the presence of pink staining zymogen granules.

Abnormalities in hepatic tissue included excessive vacuolation and basophilic foci that appeared as cellular areas with a thin cytoplasm that stained more basophilic than the surrounding tissue. Basophilic areas of cellular alteration and hepatocellular carcinoma were very similar in appearance and seemed to differ only in the amount of area affected and the degree of invasion. The cells of these two pathologies were larger and possessed larger nuclei. They were less vacuolated and were more basophilic than the surrounding cells. The cells often were not cuboidal but were elongated in the same plane of section. Large areas of these cells compressed and displaced the normal hepatic parenchyma. Necrosis, hemorrhage, and eosinophilic cysts were characteristic of large tumors.

REARING EXPERIMENTS

Juvenile tomcod were collected from the Hudson River and Sheepscot and Penobscot Rivers to evaluate the effect of living in the Hudson on tumor incidence. Hudson River fish reared in the laboratory could exhibit three possible incidences of hepatocellular carcinoma incidences in comparison to their cohorts in the Hudson River:

1. the same cancer incidence.
2. a lower cancer incidence.
3. no detectable cancer incidence.

Although these results would not identify a specific cause, they could eliminate some possible causes including maternal loading of eggs, genetic effects, viral etiology, thermal stress, exposure period required, and upper and lower Hudson River involvement (Table IV).

TABLE IV. Interpretation of tumor Incidences in Reared Tomcod

none	reduced	same
not genetic	not genetic	possible genetic
not viral	not viral	possible viral
not maternal	possible maternal	possible maternal
not upper-Hudson	possible upper-Hudson	possible upper-Hudson
may need longer exposure	may need longer exposure	six months exposure
or lower-Hudson exposure	or lower-Hudson exposure	lower-Hudson?

Collections of Juveniles

Sub-adult or juvenile tomcod (n = 362) having a mean length of 54.1 mm (based on early mortalities, n = 17, SD = 6.68) were collected from the Hudson River on June 2, 1987 at the cooling water intakes of the Indian Point Nuclear Power Plant, Indian Point, New York (Fig. 1). One of the intakes was equipped with a modified Ristoff screen that entrained live fish and then flushed the fish into a trough of water. Tomcod were removed from the 20°C water of the trough with dip nets, transferred to plastic bags, sealed with pure oxygen, packed with ice in styrofoam coolers and then driven to Louisville.

Slightly older fish (n = 99) having a mean length of 79.5 mm (based on early mortalities, n = 16, SD = 10.02) were collected between July 11 and 15, 1987 from the Sheepscot and Penobscot Rivers in Maine (Fig. 2) using a fifteen foot otter trawl with a liner in the cod end. Fish were held in aerated, plastic garbage cans prior to shipping as previously described above. A small sample was caught in the Penobscot River using the 15 ft otter trawl; however, extreme fog conditions hampered the effort. Collections were also attempted in the Royal River using a hoop net, but this primarily caught Alosa and no tomcod.

Another collection was made from the Sheepscot River in Aug, 1987 using larger equipment. A shrimp boat with motorized winch and a 60 ft seine caught about 50 juvenile tomcod, but the larger size of the net was inconvenient for the size of the trawling area. The net was also severely damaged during towing because of rocky outcrops which snagged the net. As a result, our effort was prematurely terminated before we could obtain sufficient juveniles for our experiments. Faster towing speed also seems to have abraded more scales from the juveniles collected. We also had a record heat wave which fatally stressed the few juveniles that we did catch so the entire effort proved futile.

Juvenile tomcod were collected from the Hudson River in June of 1987 were reared in the laboratory in de-chlorinated water obtained from the municipal water supply for Louisville, KY. Juvenile tomcod were also collected from the Sheepscot and Penobscot Rivers, ME to serve as a negative control. Both groups of fish were reared in circular tanks using recirculating water systems and fed an artificial diet. Details of the aquaculture methods have been previously reported (Cormier and Racine, 1988) and are reprinted in the Appendix for convenience. In December of 1987, surviving fish were sacrificed, autopsied and their livers were fixed in buffered formalin.

Results of Rearing Experiment

Surprisingly, no hepatocellular carcinoma was found at the gross and histological levels for either group. Our samples from the Hudson for fish of a similar size, less than 170mm, had a 10% incidence of hepatocellular carcinoma (Table III) while Dey et al. (1986) had a 39% incidence of hepatocellular carcinoma for fish less than 175mm.

Almost all tomcod collected from the Hudson River and reared for six months were infested with cysts of a protozoan parasite. Cysts were very common in the liver and spleen, but could also be found in other internal organs and on the mouth and fins. Tomcod collected from the Sheepscot and Penobscot Rivers and reared had no parasitic cysts. Histological section from collections from the Penobscot and Hudson Rivers occasionally revealed similar cysts in the livers of adults. Although parasitic cysts were often extensive in the reared Hudson River sample, mortality during culturing occurred at the same rate as the group without parasitic cysts (Fig. 3).

DISCUSSION

Liver cancer in the Hudson River population of Atlantic tomcod was first identified and reported by Smith et al. (1979). Further confirmation of hepatocellular carcinoma and ultrastructural analyses were reported by Cormier (1986). All of these researchers postulated a chemical etiology for the very high incidence of cancer in tomcod since portions of the Hudson River are known to contain high levels of polyaromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), pesticides and heavy metals (Rohmann, 1985). In fact, high levels of PCB's in striped bass have kept that fishery closed to commercial exploitation in the Hudson for several years. A chemical survey of priority pollutants showed very high levels of PCB's, chlordane, DDT, and heavy metals in tomcod liver (Dey et al., 1986). PAH's were not detected; however, other workers have shown that PAH's have a rapid clearance rate in fish but the effects of metabolites can be very significant (Neff, 1985).

More than 560 tomcod have been evaluated for gross and histological evidence of hepatocellular carcinoma. Incidences from 24% to 100%, depending on age/size class, were reported for the Hudson River population as compared to 3% to 10% for another less impacted population from the Pawcatuck River, CT (Dey et al., 1986). However, a clear link with xenobiotics could not be made. One portion of the present study expanded histopathological monitoring to include evaluation of cancer incidence in tomcod from rivers in Maine.

Low levels of hepatocellular carcinoma were detected in the Pawcatuck and Saco Rivers indicating that the Hudson River tomcod is not a unique strain of tomcod prone to cancer, but that tomcod in other rivers can also develop carcinomas. Temperature is not the sole cause of cancer in Hudson River fish since hepatocellular carcinoma were found in the northern Saco and southern Pawcatuck Rivers. Thus, xenobiotics still seem to be the most likely cause not only for the Hudson River population, but for other tomcod populations as well.

Environmental Correlates

In the Saco and Pawcatuck Rivers where some cancer was found, there are potential sources of carcinogens. In the Saco River an upstream dam retains sediments from a tannery that contain potential carcinogens such as chromium. These sediments being upstream from the spawning area, however, are not directly accessible to the native tomcod population. The Pawcatuck

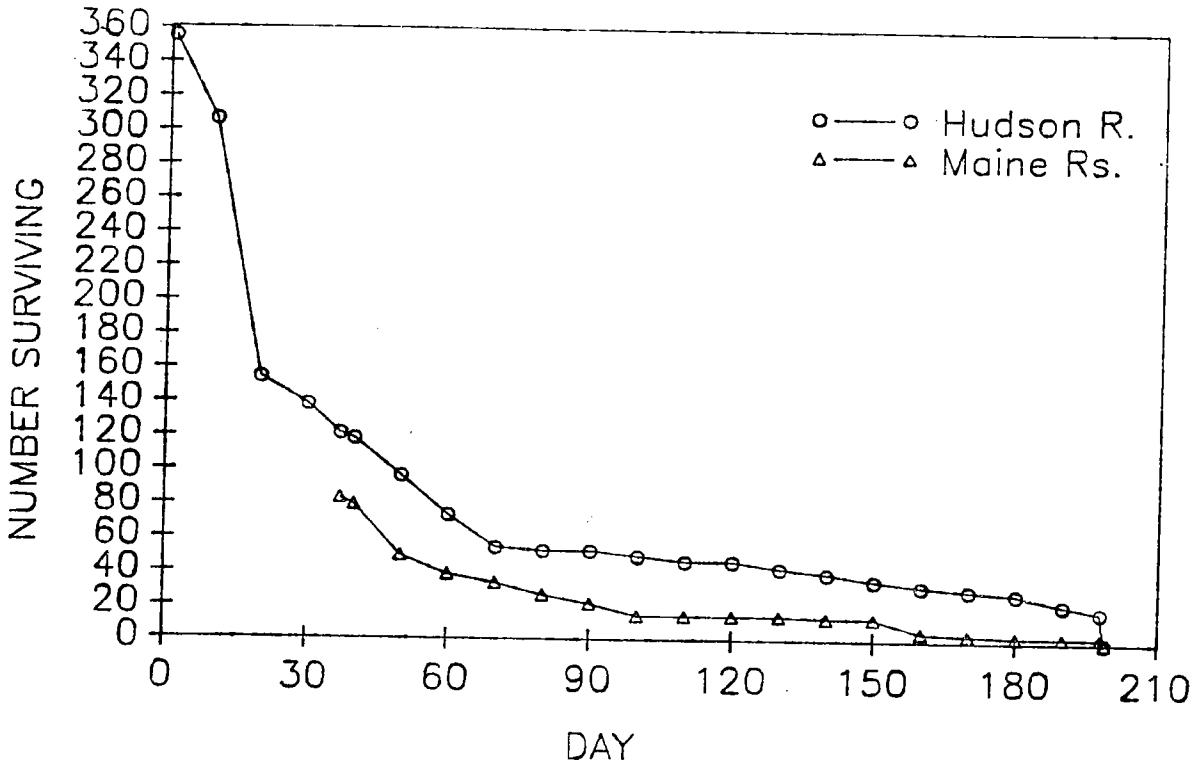

Figure 3. Survival curves of sub-adult tomcod collected from Maine (Sheepscot and Penobscot Rivers) and Hudson River. Initial group sizes for Maine (n = 83) and Hudson (n = 355). Change in slope at day 23 is believed to be due to improved feeding techniques. Change in slope after day 70 is coincidental with new filtration system. On day 198, n = 3 for Maine and n = 18 for Hudson. Day 1 = Jun. 4, 1987. (From Cormier and Racine, 1988).

has textile mills that have historically discharged wastes into the river, and may be still continuing the practice on a limited scale. Those rivers without detectable carcinoma do not have appreciable industrial input, but may be impacted by agricultural runoff; however, the Maine Dept. of Natural Resources considers these to be amongst the cleanest tidal rivers in the state (Flagg, L., personal communication, 1986). Pristine, chemical-free rivers probably no longer exist in the United States, however, this study would suggests that some levels of chemical mixtures can be tolerated, but not those as high as have been found in the Hudson River. The levels of contaminants are believed to be much lower in the other rivers studied than in the Hudson River and the minimal and lack of hepatocellular carcinoma would support this contention.

Although, the histopathologies and chemical analyses suggest a correlation between pollutants and tumor incidence, this comparison is currently limited since chemical analyses for sediment are only available from the Hudson River (Bopp et al., 1981; 1982) and chemical analyses of livers have only been performed on the tomcod from the Pawcatuck and Hudson Rivers (Dey et al., 1986). The presence of compounds in the liver indicate bioavailability and bioaccumulation, but do not prove these accumulated compounds caused the carcinomas. Also, the mitigating role of higher temperatures in the Hudson, which is at the southern extreme of the tomcod's range, can not be ignored as a possible synergistic factor.

Juveniles removed from the Hudson after six months and reared in clean water showed no incidence of hepatocellular carcinoma six months later when cohorts of the same size class collected from the River showed a 10 to 39% cancer incidence. Although caution must be exercised in interpreting these findings because of our low sample size, the lack of tumor formation in the laboratory reared Hudson River population suggests that maternal loading of yolk may not be sufficient to induce hepatocyte transformation, and that exposure during the first six months of life is not sufficient to induce high levels of tumor expression. This is interesting since laboratory experiments have shown that yolk sac larvae are more sensitive to carcinogens than older fish (Hendricks, 1982). The insufficient exposure period may simply be too short or it may be that tomcod are exposed to the causative agents closer to the mouth of the Hudson and the juveniles that we had collected had not yet migrated down to contaminated areas.

Alternatively, data linking size of fish with a higher tumor incidence (Dey et al., 1986) would suggest that carcinogen dosage through food is even more likely. In one year old tomcod the incidence of hepatocellular carcinoma increases with fish length (Dey et al., 1986) suggesting that the more food eaten, the greater the growth and the greater the chance of ingesting carcinogens. Another possible factor, that may be responsible for the lack of tumor expression in the laboratory reared fish, is that light cycle and temperature were not matched with those of the Hudson and none of the fish were in reproductive condition when sacrificed, as were most of the field collected fish. Hormonal stimulation of some cancers has been well documented and may be required for the promotion of tumor growth. Data from a small number of tomcod collected during the pre-spawning season showed a lower incidence of hepatocellular carcinoma compared to tomcod collected

post-spawning (Dey et al., 1986). Although titillating, these findings should be reproduced with a larger sample size and rearing conditions must be more closely matched with seasonal conditions normally found in the river.

ACKNOWLEDGMENTS

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APPENDIX

Culture of Sub-Adults

Upon arrival, sub-adults were placed in 1890 L circular fiberglass tanks (Red Ewald, Inc.) filled with 5 ppt artificial sea water (Dayno Manufacturing, Inc.) that was cooled with a chiller-aerator (Frigid Units, Inc). The water was gradually raised to room temperature. Initially, the water was conditioned by a large, modified undergravel-type system using clam and oyster shells, but this was replaced to facilitate cleaning of the tanks. A biological filter was made by layering shells and plastic coke rings (Norton, Inc.) into a cylinder constructed of clear fiberglass (30.48 cm diameter, 121.92 cm tall) with a 2.5 cm diameter exit hole at the base which was covered with fiberglass window screening. Coke rings were layered at the bottom to minimize obstruction of the hole and at the top to maximize aeration. The filter was inoculated with Fritz-Zyme #9 (Fritz Chemical Co.). A system of polyvinyl chloride (PVC) tubing was used to pump water at a flow rate of 3970 liters per hour from the tank through an Aquarium Life Support Filtration Unit (Aquanetics, Inc.) equipped with a 16um filter and two 25 watt ultraviolet lights. The filtered and sterilized water was then pumped into the biological filter. The pump intakes were covered with fiberglass screens that were removed daily and immediately replaced with clean screens to prevent tomcod from being sucked into the piping. The salinity of the water was raised over a period of one month to give a final salinity of 35 ppt for the Maine fish and 15 ppt for the Hudson River fish. The salinity was then maintained by adding dechlorinated water as needed. The tanks were never drained. Sub-adults were fed twice daily a diet of chopped shrimp and organ meats, but the diet was later shifted to an artificial liver-based feed.

Sub-Adult Nutrition

Sub-adult tomcod, collected from the wild, were fed twice daily with chopped shrimp, liver, kidney, gizzard and heart. Liver was most readily accepted; however, the collagen in the organs were not properly digested and several fish died with a large bolus of collagen obstructing their digestive tract. Others died with a prolapsed intestine probably due to excessive attempts to eliminate the collagen. Although shrimp did not cause these problems, it was not readily accepted by the fish. Some cannibalism was observed, especially in the early months, but no attempt was made to alter this behavior.

An artificial diet was developed using liver, shrimp and liver, or liver and flake fish food. Liver or shrimp was pureed in a Waring blender and the liver was filtered through a fine cloth to remove the collagen. The liver extract was used alone or mixed with pureed shrimp or flake food and solidified with gelatin or agar in the following proportions: 50 ml liver extract and 10 ml flake food in 6.5% agar or gelatin. The gelled liver was chilled until needed and then chopped into small pieces approximately 2 to 4 mm^3. Any collagen detected at this time was also removed. Ultimately, agar was used solely since the proteases in the liver extract liquified the

gelatin. The proteases could not be heat-inactivated since tomcod refused cooked liver.

Tomcod fed readily on the artificial mixture; however, they fed best if the food was added slowly to the tank and could be engulfed while still in the water column. Some food was eaten from the tank floor, but was often rejected after mouthing. Tomcod also preferred dim lighting, slow movements of the caretaker and minimal noise. When frightened they would rush to the opposite side of the tank and attempt to hide behind the filtration column.

Uneaten food and other waste material was manually removed by suction since our facility could not accommodate floor drains.