Environment: Peculiar Pigment Cell Neoplasm in Fish

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Chromatophoroma in the croaker *(Nibea mitsukurii)* showed a unique geographic distribution. The contribution of environmental chemicals to the cause of chromatophoroma in the feral croaker is considered likely on the basis of the following results in our studies. 1) Chromatophoroma was induced in tank-reared *N. mitsukurii* by administration of certain kinds of known carcinogens such as 7,12-dimethyl-benz(a)anthracene, N-methyl-N′-nitro-N-nitrosoguanidine, and nifurpirinol. 2) Local accumulation of pigment-cell hyperplasia in the catfish *(Protosus anguillaris)* showed similar tendencies to those of chromatophoroma in *N. mitsukurii.* 3) Removal of contaminated sediment from the harbor and the river appeared to reduce the incidence from 47% in 1973–1983 to about 20% in 1985–1987. 4) Waste water from a factory located at the station where the incidence of the neoplasm was the highest contained mutagenic substances such as chloroacetones and glyoxals [5]. Exposure of catfish to the waste water induced pigment-cell hyperplasia on the skin.
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Epizootiological studies of spontaneous tumors in fish may have unique significance for cancer research in the following ways: a) Early detection of and monitoring systems for environmental carcinogens; b) Animal models for use in correlative studies of epidemiology with experimental oncology; and c) Introduction to the discoveries of new problems and of new experimental systems.

The possible role of environmental chemicals to the existence of tumors in fish was first suggested by Lucke and Schumberger in 1941 [1] in relation to oral papilloma/carcinoma in brown bullhead caught in polluted rivers. The role of chemicals was clearly demonstrated by the studies on the hepatoms in hatchery-grown rainbow trout, which were detected in the United States around 1960 [2]. Since then, various types of tumors in feral populations of fish have been studied from the viewpoint of environmental carcinogenesis [3–5]. Pigment-cell neoplasms are some of the common types of fish neoplasms and are known to occur in 50 of the approximately 300 fish species in which neoplasms have been reported [5]. Tumors in the following fish species have been studied from the aspect of environmental carcinogenesis: Croakers (*Nibea mitsukurii* and *Nibea albiflora*), sea catfish *(Plotosus anguillaris)* inhabiting the coastal waters of Japan [6], gulf croaker *(Micropogon megalops)* [7], freshwater drum *(Aplodinodus grunniens)* [8], and brown bullhead *(Ictalurus nebulosis)* [8] distributed in the United States.

In the present paper, we will briefly review our studies on the pigment-cell neoplasm in the croaker *(Nibea mitsukurii)* with emphasis on the possible role of environmental chemicals, which were detected in water in areas where the tumor incidence was extremely high. The data presented here are as follows: 1) geographic distribution of chromatophoroma in *Nibea mitsukurii,* 2) localized increases in the incidence of pigment-cell hyperplasia in a sea catfish *(Plotosus anguillaris),* 3) chemical induction of chromatophoroma in tank reared *N. mitsukurii,* 4) annual change in the incidence of chromatophoroma in *N. mitsukurii,* 5) mutagenic activity of water samples and the types of mutagenic substances detected in waste water from a factory, and 6) induction of pigment cell hyperplasia in *Plotosus anguillaris* by exposure to waste water derived from the factory.

The morphology of the chromatophoroma in the croaker and the pigment-cell hyperplasia in the sea catfish have been reported earlier [6] with detailed data concerning items 1), 2), and 3) mentioned above.

Geographic Distribution of Chromatophoroma in the Croaker *(Nibea Mitsukurii)* *Nibea mitsukurii* (Sciaenidae, Percina, Percida, Teleostei) is a common fish inhabiting shallow waters along the Pacific coast from the middle to southwestern portions of Japan. The chromatophoroma in *N. mitsukurii* was first observed in 1973. A representative from a group of sportfishermen brought several fish with tumors to our laboratory, and we began our surveys on tumors in feral fish in cooperation with the group of sportfishermen (All Japan Surf Casting Federation).

During the period from 1973 to 1981, we fished at 70 stations in Honshu, Shikoku, and Kyushu. *N. mitsukurii* were caught at 25 stations, most of them located close to estuaries of rivers along the Pacific coast. In the Seto-inland sea, a croaker *(Nibea albiflora)* was encountered, and chromatophoroma was also found (Fig 1). Because no tumors were observed in fish less than 209 mm in total body length, fish larger than 210 mm were tentatively designated as the target population, and the incidence of chromatophoroma in such fish was compared. Tumor incidence was less than 5% at the water areas from *A* to *D* and *E* to *G,* which were located along the shore of Suruga Bay and facing the open sea, respectively. The fish caught at stations *P, Q, R,* and *S* along the coast of Tosa Bay in Shikoku showed relatively high tumor incidences (from 6.4% to 8.5%). The incidence was 0% in *N. mitsukurii* caught at stations *U* to *Y,* which were located along the eastern coast of Kyushu. The geographic distribution of the chromatophoromas was unique to Kii Peninsula; about 47% (1415 to 2991) of effective fish at station I developed chromatophoromas on their skin. The incidence at station *H* was also high, whereas it was significantly lower in the fish at

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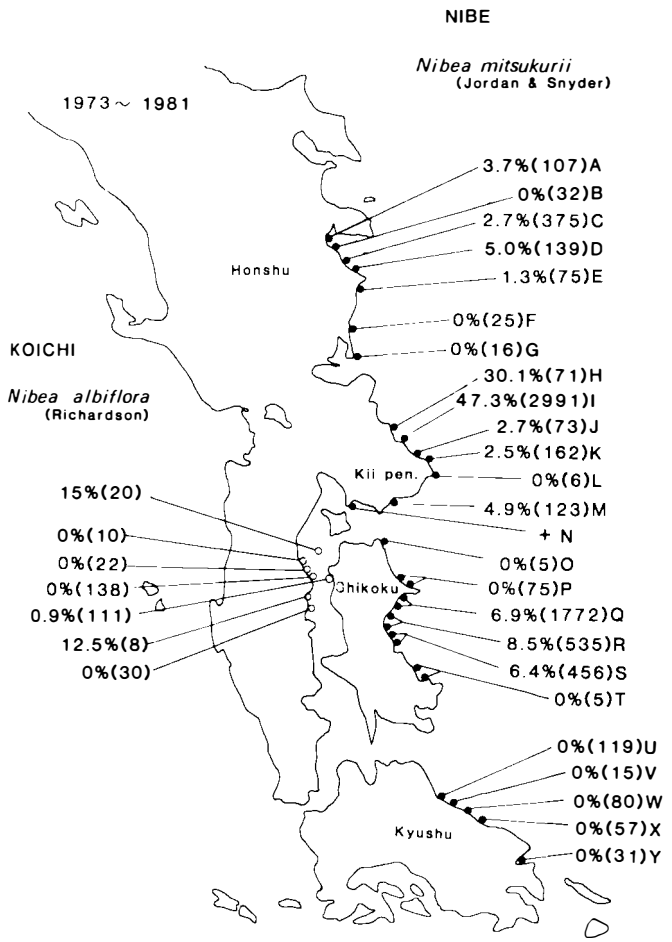

Figure 1. Geographic Distribution of Chromatophoroma Incidence in the Croakers, Nibe *Nibea mitsukurii)* and *(N. albiflora),* which were caught from 1973 to 1981. Numbers in parentheses represent the numbers of fish larger than 210 mm in total length examined.

stations *J* (2.7%) and *K* (2.5%), which were located 18 and 30 km away from station *I,* respectively.

The incidence of chromatophoromas was correlated to the size of the fish. The relationship of incidences in *N. mitsukurii* at different survey stations to body length are shown in Fig 2. The smallest tumor-bearing fish was 211 mm in total body length and about 1.5 years old. The largest *N. mitsukurii* caught during our surveys was 396 mm long and over 5 years old. The incidences of tumors tended to increase with the size of the fish at most sites, although the incidence at station *I* reached a plateau in fish longer than 270 mm. *N. mitsukurii* inhabiting station *I* clearly had a uniquely high incidence of chromatophoroma.

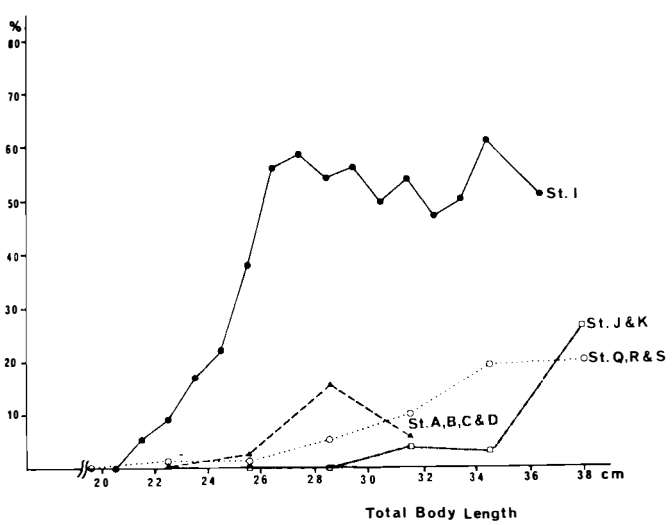

Figure 2. Correlation between body size and chromatophoroma incidence in *N. mitsukurii* caught at several areas from 1973 to 1981. Average tumor incidences are shown graphically.

To analyze the causes for this unique geographic distribution of tumorous fish, we conducted several studies investigating genetic, viral, and chemical factors. No evidence was obtained to support the viral etiology hypothesis or the genetic etiology hypothesis [6], but several results which suggested a chemical etiology were obtained.

Localized Increases in Pigment Cell Hyperplasia in the Catfish If environmental chemicals are one of the etiologic factors in the development of pigment-cell neoplasms in the croaker, the same substances might cause neoplasms or related disorders in other species of fish that inhabit the same area. Therefore, we examined tumors and related disorders in various fish species. Hepatomas, gastric adenomas, and a single case of neurilenmoma were observed in the tongue sole (*Areliscus joyneri,* Gunther), the black porgy (*Acanthopagrus schlegeli,* Blecker), Japanese sea perch (*Lateolobrax japonicus,* Cuvier), and the jack (*Trachurus japonicus,* Temminck and Schlegel). However, these tumors were seen only in large fish, and we could not catch sufficient numbers of large fish of these species to study these tumors epizootiologically. We could, however, obtain epizootiologic data concerning skin pigment cell hyperplasia in the catfish *(Protosus anguillaris).* The hyperplastic lesions of pigment cells were noticeable as black or gray nevus-like spots with slight swelling. Histologically, a few to more than ten layers of pigment cells with spindle shape and dendrites grew parallel to the skin surface in the pigmented lesions. On the other hand, the pigment cell layer was single in the normal skin.

The catfish were caught at 11 survey stations from 1979 to 1980, and the incidence of fish with unusual chromatophore hyperplasia is shown in Table I. Fish larger than 150 mm long were designated as the target population. The incidence at station *I* was significantly higher than at station *K* ($X2 = 6.57$).

Induction of Chromatophoroma in *N. Mitsukurii* by Administration of Chemical Carcinogens The techniques for hatching and rearing *N. mitsukurii* were established in 1978 by Taniguchi and a year later by Kumai. Chromatophoroma was induced in tank-reared *N. mitsukurii* by administration of known carcinogens, such as 7, 12 dimethylbenz-(a)anthracene (DMBA), N-methyl-N′-nitro-N-nitrosoguanidine(MNNG), and 6-hydroxymethyl-2-(2-5-nitro-2-furylvinyl) pyridine [also known as nifurpirinol (NP)].

Table II shows the results of the experiments in which NP was given to tank-reared *N. mitsukurii.* DMBA and MNNG are well known classic carcinogens. However, NP was commonly used in fish cultures in Japan until 1981 as an antibacterial and antiprotozooal agent for prevention or cure of a variety of fish diseases. The potent carcinogenicity of nifurpirinol was first observed during the course of the experiments using *Nibea mitsukurii.* Later we found

Table I. Incidence of Unusual Chromatophore Hyperplasia in the Catfish *(Plotosus anguillaris)* Larger than 150 mm in Length.

Station	Incidence, %	No./Total
I	13.5	15/111
K	4.8	10/210
R	2.5	1/40
D	0	0/19
H	0	0/39
J	0	0/10
L	0	0/43
M	0	0/44
Y	0	0/32
Toba	0	0/28
Kii-nagashima	0	0/123

Table II. Induction of Proliferative Pigmented Lesions in *N. mitsukurii* by Nifurpirinol

Initial No. of Fish	Dose of NP ppm[a]	No. of Fish with Pigmented Lesions/Average No. of Lesions/Fish					
		11 Months	%	13 Months	%	20 Months[b]	%
50	2	3/4 2.0	70	2/2 10.0	100		
50	1	23/37 1.8	62	20/23 7.0	87	16/16 11.5	100
52	0.5	22/52 0.5	42	36/49 1.9	73	27/38 4.0	71
100	0	0/39 0	0	2/38 0.2	5	5/32 0.8	16
50	Untreated group I	0/40 0	0	—		1/39 0.025	2.5
204	Untreated group II	—		6/204 0.034	2.9	—	

[a] NP was administered 14 times for 1 h each time.
[b] All fish were killed and examined histologically.

that it induced various types of tumors in mice [6], hepatomas, gastric adenomas and unusual skin pigmentation in rainbow trout [9], hepatomas in medaka [10], and pigment-cell hyperplasia in the red-color fancy carp *(Cyprinus carpio)* [11]. Nifurpirinol was also a potent mutagen in bacteria [12].

Five-month-old *N. mitsukurii,* reared in tanks, were divided into six groups (70 each) and exposed to sea water containing 0, 0.5, 1, and 2 ppm NP for 1 h, twice a day for 7 d. These groups were kept in 500-l tanks. Because the untreated group of fish had their water supply cut off for 1 h during the experiment, two other control groups were prepared as untreated groups (untreated group I) with approximately 204 fish (untreated group II). The untreated group II was kept in a 3,000-l tank, and the other groups were kept in separate 500-l tanks. The fish of untreated group II were used for other purposes 13 months later. Surface examinations were done at 11 and 13 months, and histologic examinations of the lesions were performed on the fish necropsied at 20 months.

Most of the fish in the 2-ppm NP group died within 40 d from subacute toxicity of NP. When examined at 11, 13, and 20 months, the 1- and 0.5-ppm NP group showed high incidences of pigmented lesions, whereas the incidences in the control groups were extremely low. The percentage of fish with pigmented lesions, as well as the number of lesions on individual fish, showed a dose response in the experiments. The average number of pigmented lesions per fish in the 1-ppm group was threefold or greater than that of the 0.5-ppm group. The incidences and numbers of lesions increased with the length of experimental period and reached 100% and 11.5 in the 1-ppm group and 71% and 4.0 in the 0.5-ppm group, respectively, at 20 months. The incidences of the 0-ppm and of the untreated *(I)* group seem to be different; however, the difference was not significant ($X2 = 2.3$). When the lesions were observed histologically at 20 months, most of them were similar to those of the smallest pigmented lesions in wild *N. mitsukurii.* The incidence of typical chromatophoromas which developed whorling or star-burst patterns was 38% (six fish) in the 1-ppm group, 29% (11 fish) in the 0.5-ppm group, 6% (two fish) in the 0-ppm group, and 0% in the untreated *(I)* group (Table III).

Annual Incidences of the Chromatophoroma in *N. Mitsukurii* The coast of the Kii Peninsula is generally clear, free of urban waste, and sparsely populated. Large factories are rare, but three large pulp and paper factories are located near station *I*. Therefore, if certain chemicals are involved in the development of the neoplasm, they might originate from the industrial waste of these factories. For this reason, the discharged sewage and sediment were chemically analyzed by the Rec assay [13] and/or Ames test [14].

When a dried sample of the waste water and the extracts from the sediment were examined for their mutagenicity in 1976, both extracts originating from one of the three factories showed a positive reaction both in the Rec assay and the Ames test. Similar factories also exist close to stations *A, C, D,* and *X*. Therefore, corresponding samples, waste water, and sediment samples from the factories and *N. mitsukurii* at stations *A* and *D* were examined by the same methods. All the samples at station *D* were negative in the Ames test and Rec assay. The waste water and sediment samples at station *A* showed weak DNA damage and mutagenic potency [6,15]. Until

Table III. Compounds Identified from HPLC FR.1 of Water Samples

Compounds	M.W.	Fr. 1[a] From		
		B	E-1	E-2
1,2-Dimethoxybenzene (Veratrole)	138			1
3-Acetylthiophene	126			2
2-Acetyl-5-methylthiophene	140			3
4-Hydroxy-3-methoxyacetophenone	166		1	
5,8-Dimethylquinoline	157		2	
Benzothiazole	135			4
2-Methyl-5-propanoylthiophene	154		3	
1,2,3-Trimethoxybenzene	168	1	4	5
3-Methyl-1-indanone	146		5	
1-Indanone	132		6	6
Isoeugenol	164		7	
p-Isopropylbenzoic acid	164		8	
Methyleugenol	178		9	7
Benzylacetone	148	2		
8,β,H-Cedran-8-ol	222	3		
3,5-Dimethoxy-4-hydroxyacetophenone	196	4	10	
p-Methoxyacetophenone	150		11	8
Unknown	(162)		12	9
δ-Cadinol	222	5		
Methylisoeugenol	178		13	10
1,6-Dimethyl-4-isopropylnaphthalene	198	6	14	11
β-Eudesmol	222	7		
Dimethyl phthalate	194	8		
Methyl hippurate	193		15	12
Unknown	(198)	9	16	13
Unknown	(146)		17	14
Diethyl phthalate	222	10		
2-(Methylthio)benzothiazole	181			15
2,4,5-Trimethoxypropenylbenzene (Asarone)	208	11	18	16
Benzophenone	182			17
3,5-Dimethoxyacetophenone (Acetoveratrone)	180	12	19	18
Unknown	(210)	13	20	19
Dibutyl phthalate	278	14	21	20
Unknown	(196)		22	21
Unknown	(—)			22
5-Hydroxycalamenene	218	15	23	23
Tosyldimethylamine	199	16		
Unknown	(226)		24	24
Tosylethylamine	199	17		
Di(2-ethylhexyl) phthalate	390	18	25	25

[a] The figures showed the peak number on gas-chromatogram.

1977, the factory at station *I* had discharged untreated waste water into river *D* and the adjacent harbor. The bottom of the harbor and some portions of the river estuary located at the area had been covered by sludge containing potent mutagens which were discharged from the pulp factory. During the period from 1977 to 1979, the factory set up a waste water purification system, removed sediment from the harbor, and changed the place of waste-water drains from the river and harbor to the coast. In 1982, the city near station *I* removed the sediment at the mouth of the river, and we could no longer obtain sediment samples.

Figure 3 shows the annual tumor incidences of the croakers *(N. mitsukurii)* caught at station *I*. No significant differences were observed in the tumor incidences from 1973 to 1979. The average incidence for these years was 47%. In 1980, the 75% incidence was significantly higher than that noted from 1973 to 1979. The incidence fell to 18% – 21% in 1984 – 1987.

The tumors in croakers were observed in fish older than 1.5 years of age. The life-span of the feral fish is approximately 5 years. Changes in annual tumor incidences suggest that the dredging influenced the increased tumor incidence in 1980, and that the removal of the sludge resulted in a decrease in tumor incidence after 1984.

Detection of Mutagens from Water Sample The sea water near station *I* still contains potent mutagens [16]. Water samples were collected from the coastal seashore (point *B*), two rivers (points *C* and *D*), and a pulp and paper mill drainpipe (point *E*) from 1985 to 1987 (Fig 4). At point *E*, two kinds of effluent water (E-1 and E-2) were collected as samples before and after the final treatment, respectively. Organic compounds contained in the water samples were adsorbed on a XAD-2 resin column. The adsorbate was eluted with ether and then methanol using Soxhlet extracting apparatus. Each extract was evaporated, and a portion of the residue was subjected to the Ames test. An aliquot of the ether extract that showed mutagenic activity was fractionated into 5 – 6 fractions by gel filtration on a Sephadex LH-20 column. The mutagenic fractions were also subjected to the Ames test and gas chromatography – mass spectrometric computer (GC-MS) analysis to identify the mutagenic compounds. For the isolation of glyoxal, methylglyoxal, and diacetyl, which are direct mutagens to S. typhimurium TA100, o-phenylenediamine was added to each water sample (0.5 g/L) and stirred for 2 h at room temperature. Then the corresponding quinoxaline derivatives were extracted with chloroform (100 ml × 3). The solvent was evaporated, and the residue was subjected to GC-MS analysis.

The ether extract from sea water at point *B* showed weak mutagenic activity toward *S. typhimurium* TA100 without S9 mix. The ether extracts from rivers *C* and *D* did not show any mutagenicity toward either bacterial strain. Though the ether extract of E-1 showed high mutagenic activity toward S. typhimurium TA100, that of E-2 exhibited only weak mutagenicity, which was variable depending on the sampling time (Fig 4). The results suggest that the mutagenic substances in the sea water were derived from the waste water of the factory.

The third fraction obtained by gel filtration of E-1 ether extract was, furthermore, applied to HPLC. HPLC-1 eluted with n-hexane-ethanol (99:1) mixture showed the highest mutagenicity among all fractions (Fig 5). By GC-MS analysis of HPLC-1 of each sample, 16, 19, and 18 organic compounds were identified from *B*, E-1, and E-2, respectively (Table III).

Among them, trimethoxybenzene, 3-methylthiophene, and 2-(methylthio)benzothiazole induced His + revertant colonies more often than the control. Moreover, 1,3-dichloroacetone, 1,1,3-tri-chloroacetone, 1,2,3,3-tetrachloroacetone, and petachloroacetone were identified from HPLC-1 of effluent water from E-1 and E-2. They induced 250 rev/0.5 μg, 250 rev/360 μg, 400 rev/50 μg, and 450 rev/250 μg in *S. typhimurium* TA100, respectively.

When each water sample was treated with o-phenylenediamine, three decarbonyl compounds (glyoxal, methylglyoxal, and diacetyl) were isolated as corresponding quinozaline derivatives. These compounds (three type) were direct mutagens toward *S. typhimurium*

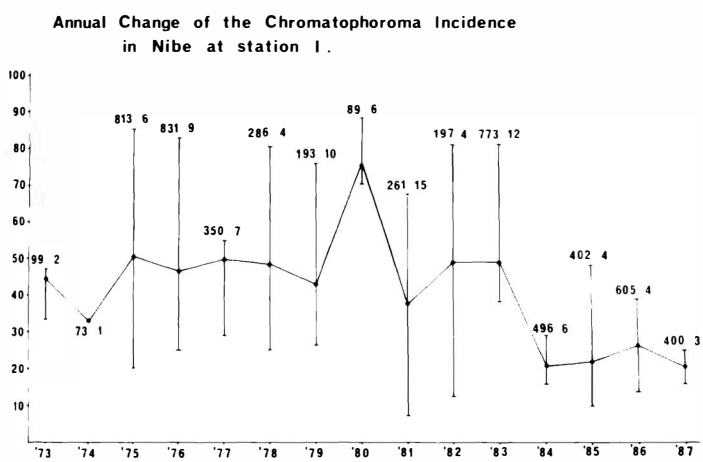

Figure 3. Annual change of the chromatophoroma incidence in *N. mitsukurii* at station *I*. *Numerators* of the fraction in the graph show the number of effective fish larger than 210 mm in body length, and the *denominators* show the time of the effective survey in which more than 10 effective fish were caught. *Vertical lines* indicate the highest and lowest incidences.

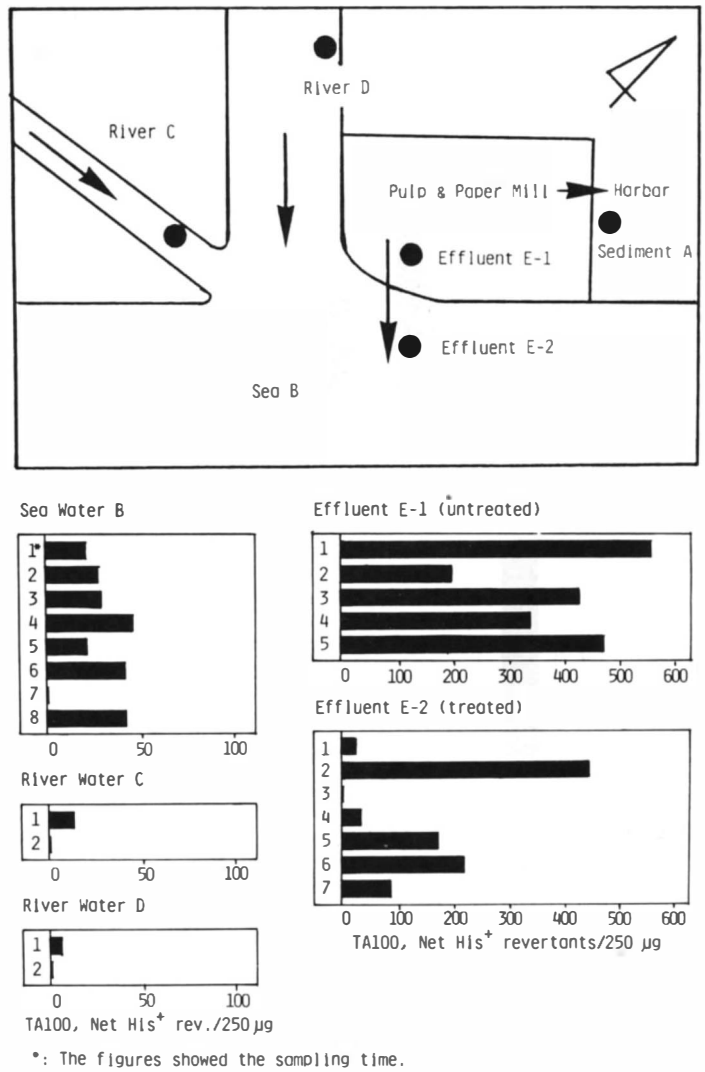

Figure 4. Sampling points and mutagenic activity of water samples at station *I* from 1985 to 1987.

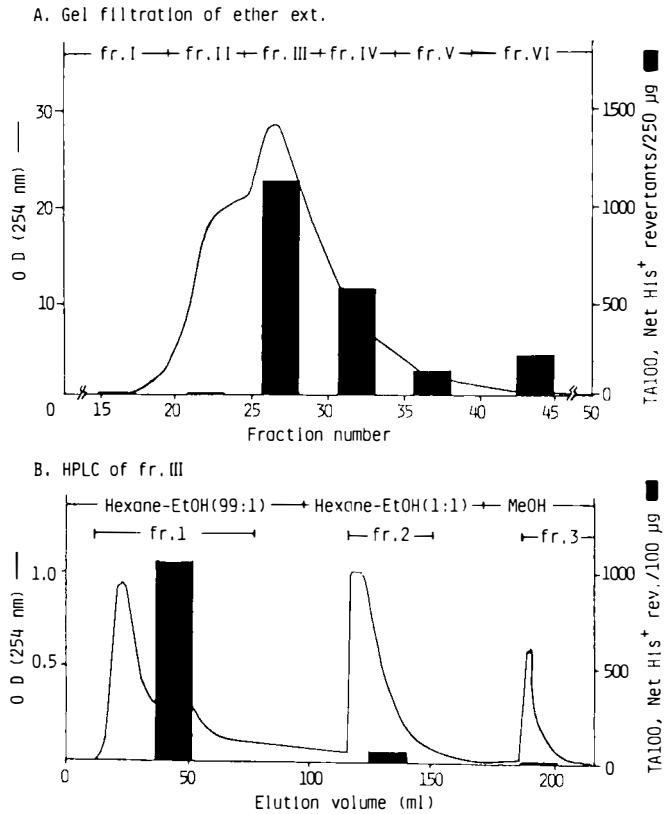

Figure 5. Fractionation and mutagenic activities of the ether extract from E-1.

TA100 and induced 520 rev/80 µg, 1200 rev/20 µg, and 45 rev/50 µg, respectively.

The mutagenic compounds isolated from water samples E-1 and E-2 are summarized in Table IV.

Induction of Pigment Cell Hyperplasia in the Catfish *(Plotosus Anguillaris)* Because the waste water from the factory showed a mutagenic activity, we started carcinogenicity testing of it using tank-reared *N. mitsukurii*. However, all the experimental fish died a few months later due to an outbreak of severe red tide which occurred in the sea near our experimental station. Therefore, we resumed experiments using the catfish *(Plotosus anguillaris)*.

Three kinds of waste water received from the factory were tentatively designated K-5, K-8, and K-9. K-5 is untreated waste water of bleached kraft pulp mill; K-8 is untreated mixed waste water of bleached kraft pulp mill and from the paper production process in which several kinds of dye were used to produce color paper; and K-9 is discharged effluent water which has undergone the final treatment.

P. anguillaris, several months old and about 5 cm in length, were caught at the coast near our experimental station and divided into five groups (50 each). Waste water was diluted twentyfold with sea water, and catfish were exposed to the solution for 6 h, bi-weekly, for a total of seven times. Two kinds of control groups were prepared: One that was exposed to the sea water containing 5% of fresh water instead of the waste water, and a positive control which was exposed to 1% of NP for 1 h, seven times. These fish were kept in 500-l tanks, given krill and fish meat, and necropsied 15 months after the initiation of the experiments, because pigment cell hyperplasia was seen in the fish larger than 150 mm and older than approximately 2 years in feral circumstances.

Histologic observations of the inner organs are not yet completed. However, nevus-like pigmented lesions, 1–7 mm in diameter, were observed on the skin of the fish. Histologically, these lesions contained hyperplasia of pigmented cells, similar to those in the feral fish. Because we did not use any fishery drugs, the effective numbers of groups were relatively few. However, the incidences of the fish with pigmented lesions in groups given K-8, K-9, and K-5 were not higher than in the positive control group, while the differences in incidence were significant between groups given NP, K-8, K-5, or K-9 and the control group given fresh water instead of waste water. Probability values by Fisher's exact test were 0.042, 0.000, 0.016, and 0.003, respectively.

In addition, multiple pigmented lesions tended to occur in the experimental groups. The number of pigmented lesions on the skin did not differ significantly among NP, K-8, K-9, and K-5 groups, but was significantly different between the fresh-water control and the other groups. K-8 group, which was exposed to the waste water before the final treatment, showed the highest incidence of pigmented lesions (Table V).

DISCUSSION

A previous paper [6] has reported on the geographic distribution of chromatophoroma in *N. mitsukurii,* a localized increase in pigment-cell hyperplasia in *P. anguillaris,* and chemical inductions of chromatophoroma in tank-reared *N. mitsukurii,* in addition to the studies of ecology and population genetics of *N. mitsukurii* and the virology of chromatophoroma. The induction of pigment-cell neoplasms by the administration of chemical carcinogens is also known in medaka *(Oryzias latipes)* [17,18]. The induction of hyperplasia has been reported in rainbow trout *(Salmo gairdneri)* [19] and common carp *(Cyprinus carpio)* [20]. Reports concerning chemical induction of unusual growths of fish pigment cells are rather rare. However, in our unpublished data, nine of nine species, including one inter-species F1 hybrid, exposed to NP or MNNG, developed pigment-cell neoplasms or hyperplasia (Table VI). Therefore, pigment cells in many species of fish seem to be highly susceptibile to the development of unusual growths by the administration of certain kinds of chemical carcinogens.

New data reported in the present paper are: 1) annual change in the incidence of the chromatophoroma in *N. mitsukurii,* 2) detection of mutagens from water samples, and 3) induction of pigment cell hyperplasia in the catfish *(P. anguillaris)*.

The croaker *(N. mitsukurii)* is a bottom feeder. The annual changes in the incidence of the croaker chromatophoroma suggest that the removal of contaminated sediments from the harbor and the river estuary have reduced tumor incidences in the fish. Nevertheless, the incidence is still the highest in all the survey stations

Table IV. Mutagenic Compounds Detected from E-1 and E-2

Compounds	Structures	Mutagenic Activity (TA100, -S9)	
		Dose (µg)	Net His^+ Rev
Glyoxal	CHOCHO	80	520
Methylglyoxal	CH_3COCHO	20	1200
Diacetyl	$CH_3COCOCH_3$	50	45
1,3-Dichloroacetone	$ClCH_2COCH_2Cl$	0.5	250
1,1,3-Trichloroacetone	$Cl_2CHCOCH_2Cl$	360	250
1,1,3,3-Tetrachloroacetone	$Cl_2CHCOCHCl_2$	50	400
Pentachloroacetone	$Cl_3CCOCHCl_2$	200	450

Table V. Induction of Unusual Chromatophore Hyperplasia on the Skin of *Plotosus anguillaris* by Water Exposure of Waste Water from a Pulp Factory

Experimental Group	No. of Fish Examined	No. of Fish with Pigment Cell Hyperplasta	Incidence	Average No. of Pigmented Lesions Per Fish
K-5	19	11	57.9%	1.12
K-8	7	7	100.0%	3.00
K-9	20	14	70.0%	1.55
Control	10	1	10.0%	0.10
NP[a]	23	21	91.3%	3.09

[a] Positive control.

Table VI. Induction of Pigment Cell Neoplasm or Hyperplasia by Water Exposure of N-methyl-N′-nitro-N-Nitrosoguanidine (MNNG) or Nifurpirinol (NP)

Species: Academic Name; Common Name	Developmental Stage or Age	Chemicals and Dose	Skin Pigment Cell Neoplasm	Hyperplasia	Tumors in other organs
Salmo gairdnerii irideus; rainbow trout	Various stages from embryo to larva	5–20 ppm MNNG, 6 or 24 hr, single	0%	18–60%	Hepatoma, G.I. tract adenoma and adenocarcinoma, nephroblastoma, granulosa cell tumor, swim-bladder adenoma
		0.5–2 ppm NP, 1 hr, 6 times	0%	9–19%	Hepatoma, G.I. tract adenoma
	2 months old fry	5 ppm MNNG, 6 hr single	0%	0%	Hepatoma, G.I. tract adenoma, nephroblastoma
Oncorhynchus rhodyrus; amago in Japanese (a land-locked salmon)	Embryo	10 ppm MNNG, 6 hr, single	0%	12–19%	Hepatoma, G.I. tract adenoma
Cyprinus carpio: red-color fancy carp	5 months old	1–5 ppm MNNG, 1 hr, 12 times	0%	−100%	Retinoblastoma, subcutaneous fibroma, osteoma
		1–10 ppm NP, 1 hr, single	0%	−100%	Seminoma
Nibea mitsukurii; croaker, nibe in Japanese	5 months old	0.5–2 ppm, NP, 1 hr, 14 times	100%	100%	G.I. tract adenoma
Oryzias lapipes; medaka (killifish)	3 months old	0.5–20 ppm MNNG, 1 hr, 1–18 times	8–12%	not examined	Branchioblastoma, epithelioma of the skin, oral cavity and gill, rhabdomyoma
	3 months old	0.5–10 ppm NP, 1 hr, 1–18 times	4%	not examined	Neurofibroma, fibroma, hepatoma
Carasius auratus; goldfish	3 months old	10 ppm MNNG, 1 hr, 18 times	2%	0%	Skin papilloma, osteoma, gill tumors
Xiphophorus maculatus; inbred Sd platyfish	3 weeks old	5 ppm MNNG, 1 hr, 6 times	9%	34%	Skin papilloma, gill tumors
Xiphophorus helleri; inbred green swordtail	3 weeks old	5 ppm MNNG, 1 hr, 6 times	1%	16%	Skin papilloma, gill tumors
Inter-species F1 hybrid between *X. maculatus* and *X. helleri*	3 weeks old	MNNG	22–57%	100%	Skin papilloma, gill tumors

(approximately 20%). When water samples at station *I* were submitted to the Ames test for mutagenicity, the sea water and the waste water from a local factory proved to be mutagenic. The waste water before final treatment (K-8) showed the highest mutagenic activity. In experiments of carcinogenicity on *P. anguillaris,* the incidence of the pigment-cell hyperplasia on the skin was the highest in the group given K-8. These results suggest that the provision for cleaning the waste water from the factory also contributed to the reduced incidence of chromatophoroma. The following mutagenic substances were detected in the waste water from the factory: glyoxal, methylglyoxal, diacetyl, 1,3-dichloroacetone, 1,1,3-trichloroacetone, 1,1,3,3-tetrachloroacetone, and pentachloroacetone. Carcinogenicity testing of these substances is a further project.

The products of the factory are bleached kraft pulp and color paper. There are several factories producing similar products in Japan. However, it must be remembered that the factory is located at the shore near an estuary facing the Pacific ocean and had discharged waste water into this estuary and an adjacent harbor for more than 20 years.

N. mitsukurii mostly inhabit estuarine areas facing the open sea, probably because the areas are rich in food. Its spawning season is from late spring to early summer. Many of the juveniles are caught in the harbor and estuary and at the shore near the estuary in autumn. The special environment at station *I* and the ecological nature of *N. mitsukurii* seem to be one reason for the high incidence of the tumor in the fish at station *I*.

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