[CANCER RESEARCH 53, 1761–1769, April 15, 1993]

Chromatophoromas and Chromatophore Hyperplasia in Pacific Rockfish (*Sebastes* spp.)[1]

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ABSTRACT

Pacific rockfish from Cordell Bank, off central California (United States), were collected and histologically examined from 1985 to 1990. Hyperplastic and neoplastic cutaneous lesions, involving dermal chromatophores, were observed in five species; yellowtail rockfish (*Sebastes flavidus*), bocaccio (*S. paucispinis*), olive rockfish (*S. serranoides*), widow rockfish (*S. entomelas*), and chilipepper rockfish (*S. goodei*). Yearly prevalences were highest in *S. paucispinis* (29–38%). Prevalence was initially low in *S. flavidus*, but increased more than 3-fold from 1985 (7.5%) to 1990 (25%). The majority of lesions were black, but white, yellow, orange, red, and mixed-color variants were also seen. Lesions were found in skin, fins, lips, gingiva, tongue, urogenital papilla, conjunctiva, and cornea of the eye. Flat lesions were consistent with melanophore (black), xanthophore (yellow or orange), and erythrophore (red) hyperplasia. Neoplastic lesions included melanophoromas, amelanotic melanophoromas, xanthophoromas, erythrophoromas, and mixed chromatophoromas. Although etiology has not been determined, interest is currently focused on potential exposure to chemical and radioactive carcinogens from the Farallon Island Radioactive Waste Dump, 30 km to the south.

INTRODUCTION

Pacific rockfish (*Sebastes spp.*) are long-lived (some with life spans in excess of 100 years), ovoviviparous marine fish belonging to the family *Scorpaenidae* (1). *Sebastes* is the most common genus found off the North American Pacific coast, and it inhabits a variety of ecosystems ranging from shallow coastal kelp beds to offshore reefs in 750 m of water (2, 3). A popular food fish, they are extensively harvested by both commercial and sport fishermen.

One of the most prolific fishing grounds off central California (United States) is Cordell Bank. Cordell Bank is the northernmost sea mount in the California continental shelf and is located 80 km northwest of San Francisco (Fig. 1). The bank has been the primary site of ongoing rockfish recruitment studies by the Tiburon Laboratory (Tiburon, CA) of the National Marine Fisheries Service. Over the course of numerous sampling trips, pigmented lesions consistent with chromatophore hyperplasia and neoplasia (chromatophoromas) were observed in five species of rockfish: yellowtail rockfish (*S. flavidus*); bocaccio (*S. paucispinis*); olive rockfish (*S. serranoides*); widow rockfish (*S. entomelas*); and chilipepper rockfish (*S. goodei*).

Chromatophoromas are cutaneous pigment cell tumors that arise from dermal chromatophores normally present in the skin of fish, amphibians, and reptiles (4–6). The four chromatophore types commonly found in fish skin are melanophores with black or brown pigment (melanin), iridophores with colorless pigments (purines), erythrophores with red pigments, and xanthophores with yellow pigments. Xanthophores and erythrophores are closely related and both contain carotenoids, pteridines, and flavins. Orange chromatophores are arbitrarily classified as either xanthophores or erythrophores, depending on which cell type they most closely resemble.

Chromatophoromas are common tumors of fish, and large epizootics have been reported in both marine and freshwater environments. Marine species involved with chromatophoroma epizootics include croakers (*Nibea mitsukurii*) off the Pacific coast of Japan (7), deepwater redfish (*Sebastes mentella*) in the North Atlantic (8), and butterflyfish (*Chaetodon multicinctus* and *C. miliaris*) from the Hawaiian Islands (9). Freshwater epizootics have involved drum (*Aplodinotus grunniens*) from the Great Lakes[3] and domestic goldfish (*Carassius auratus*) (10, 11).

The purpose of this study was to characterize the gross, histological, and electron microscopic features associated with hyperplastic and neoplastic pigmented lesions found in Pacific rockfish from Cordell Bank and to assess prevalence with respect to year, species, sex, age, and lesion type.

MATERIALS AND METHODS

Sampling. Rockfish from Cordell Bank were collected on a monthly basis, with some interruptions, from 1985 to 1990. Fish were located in the water column using an electronic fish finder and were caught using a hook-and-line technique. Sampling was biased toward mature female *S. flavidus* (collected for another study), but sex, age, number, and species varied with depth of fishing and time of year.

Scoring of Lesions. All *S. flavidus*, *S. paucispinis*, *S. entomelas*, and *S. goodei* with and without pigmented skin lesions were scored by a standardized method. The scoring sheet consisted of detailed drawings of the right and left sides of the fish. Drawings were divided into four quadrants based on a horizontal line drawn from the nose through the middle of the tail and a second vertical line extending from a point midway between the fifth and sixth dorsal spines down to the ventral body wall. Lesions were copied onto individual drawings, and each quadrant was subjectively scored on a scale of 1–6 based on the degree of black pigmentation and neoplastic development (1 = normal skin; 2–5 = mild to severe melanosis; 6 = raised black tumor).

Yellow or orange (xanthophore) and red (erythrophore) lesions were scored separately on a simplified basis of color, location, and number.

Statistical Analyses. Nonparametric tests were applied to prevalence and scoring data using the Number Cruncher Statistical System, version 5.03. Analyses were performed for *S. flavidus* and *S. paucispinis* where sample size was sufficiently large. Prevalence was tested using χ^2 analyses for differences between years, species, sexes, ages, and lesion types. Lesion scores were evaluated using the Kruskal-Wallis test for differences between years, species, sexes, and ages. Differences in lesion scores between dorsal and ventral, anterior and posterior were also compared using the Wilcoxon matched pairs test.

Gross Examination, Tissue Fixation, and Processing. Standard length, total wet weight, sex, and age (based on otolith examination) were determined, and complete necropsy was performed on representative fish. Tissues for light microscopy were fixed in 10% buffered formalin, and some were demineralized in formic acid. Light microscopy tissues were routinely paraffin processed, sectioned at 5–7 μm, and stained with hematoxylin and eosin. Tissues for transmission electron microscopy were either fixed in half-strength Karnovsky's solution or were subsampled from formalin-fixed tissues. EM[4] tissues were processed and embedded in epon, semithin-sectioned at 1 μm, and stained with toluidine blue. Following an initial screen of semithin sections, selected

Received 10/4/91; accepted 2/10/93.

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[1] Supported in part by USPHS Grant CA45131 from the National Cancer Institute.

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[3] M. S. Okihiro and P. C. Baumann, U. S. Fish and Wildlife Service, Columbus, OH, unpublished data.

[4] The abbreviations used are: EM, electron microscopy; FIRWD, Farallon Island Radioactive Waste Dump.

blocks were sectioned at 800–900 Å, stained with 2% uranyl acetate and Reynolds' lead citrate, and viewed with a Zeiss 10 or Phillips 410 electron microscope.

RESULTS

Prevalence

Prevalence data on *S. flavidus* reflect the full 6 years of the study (1985–1990). Prevalence data were determined for 4 years (1985–1988) for *S. paucispinis* and *S. entomelas* and were compiled for 2 years (1985–1986) for *S. goodei*. No prevalence data were obtained for *S. serranoides*. Yearly prevalences were consistently higher in *S. paucispinis* compared to all other species and were significantly ($P < 0.0001$) higher than lesion prevalence in *S. flavidus* (Table 1). Although lesion prevalence peaked in 1987 at 38.6%, there were no significant ($P = 0.1875$) differences between years in *S. paucispinis*. Prevalence in *S. flavidus* was initially low but has significantly ($P < 0.0001$) increased from 1985 (7.5%) to 1990 (25%).

Melanophore lesions (both hyperplastic and neoplastic) were the most common lesion type found in *S. flavidus*, and prevalences were significantly ($P < 0.0001$) higher than the combined total of xanthophore and erythrophore lesions in every year of the study (Table 2). No seasonal pattern was determined, but yearly prevalences followed the same general pattern of decreasing prevalence from melanophore to xanthophore to erythrophore lesions. The prevalences of melanophore lesions, melanophoromas, and combined xantho/erythrophore lesions were all significantly ($P < 0.0001$) higher in 1990 compared to earlier years. The prevalence of melanophoromas and xantho/erythrophore lesions was probably underestimated during the first year of the study, and no attempt was made to determine prevalence of xanthophore or erythrophore tumors.

The prevalence of melanophore lesions, melanophoromas, and xantho/erythrophore lesions increased significantly ($P < 0.0001$) with age in *S. flavidus* (Table 3). The increase was most prominent with melanophore lesions (1.3%, 1–10-year group; and 66.7%, >40-year group). Similar patterns of higher prevalences of melanophore lesions in older fish were also observed with *S. paucispinis*, *S. entomelas*, and *S. goodei*, but sample sizes of older fish were insufficient to test statistically.

Table 1 *Prevalence of pigmented skin lesions in S. flavidus, S. paucispinis, S. entomelas, and S. goodei collected from Cordell Bank from 1985 to 1990*

	% prevalence (no. fish with lesions/no. fish examined)			
Year	*S. flavidus*	*S. paucispinis*	*S. entomelas*	*S. goodei*
1985	7.5% (7/94)	35.1% (13/37)	6.3% (1.16)	8.2% (4/49)
1986	11.3% (15/133)	29.0% (11/38)	5.3% (1/19)	16.7% (3/18)
1987	9.8% (15/153)	38.6% (17/44)	0.0% (0/8)	ND
1988	11.0% (12/109)	37.8% (14/37)	0.0% (0/2)	ND
1989	16.8% (17/101)	ND[a]	ND	ND
1990	25.5% (56/220)	ND	ND	ND

[a] Not done.

Table 2 *Prevalence of pigmented skin lesions in S. flavidus collected from Cordell Bank from 1985 to 1990*

	% prevalence (no. fish with lesions/no. fish examined)		
Year	Melanophore lesions	Melanophormoas	Xantho/erythrophore lesions
1985	7.5% (7/94)	0.0% (0/94)	0.0% (0/94)
1986	9.8% (13/133)	1.5% (2/133)	2.3% (3/133)
1987	6.5% (10/153)	1.3% (2/153)	3.3% (5/153)
1988	9.2% (10/109)	0.9% (1/109)	1.8% (2/109)
1989	15.8% (16/101)	2.0% (2/101)	2.0% (2/101)
1990	22.7% (50/220)	8.6% (19/220)	11.4% (25/220)

Prevalence of melanophore lesions and melanophoromas was consistently higher in male *versus* female *S. flavidus* in almost all age groups. The difference was not significant, however ($P = 0.483$), and there was little difference in prevalence of xantho/erythrophore lesions between sexes in *S. flavidus*. There was also no significant difference in melanophore lesion prevalence between sexes in *S. paucispinis*. The prevalence of melanophore lesions was three times higher in male (23.1%) *versus* female (7.4%) *S. goodei*, and both *S. entomelas* with lesions were female. Sample sizes of *S. goodei* and *S. entomelas* were insufficient, however, to test statistically.

An extensive survey of the U.S. Pacific coast was not conducted, but periodic port sampling of trawl catches in Oregon and Washington by National Marine Fisheries Service personnel over the past 6 years has revealed only occasional rare *S. flavidus* with chromatophore lesions.[5]

Scoring

Scoring data from 106 *S. flavidus*, 45 *S. paucispinis*, 2 *S. entomelas*, and 7 *S. goodei* with melanophore lesions were evaluated based on age and sex. The average melanophore lesion score for all age groups was significantly ($P < 0.001$) higher in *S. flavidus* (19.8) compared to *S. paucispinus* (10.8). Average scores for *S. flavidus* increased significantly ($P < 0.0001$) with age (9.3, 1–10-year group; 25.0, >40-year group) (Table 4). Male *S. flavidus* scored consistently higher than females in all age groups, and the overall melanophore lesion score was significantly higher ($P < 0.001$) in males (24.3) than females (17.4). In contrast, there were minimal differences in lesion scores between male and female *S. paucispinis* or *S. goodei* and no appreciable change in lesion scores as these fish grew older.

Evaluation based on lesion distribution revealed that melanophore lesions in *S. flavidus* were significantly ($P = 0.0001$) more severe dorsally (10.3 dorsal and 9.4 ventral). Anterior scores (10.2) were

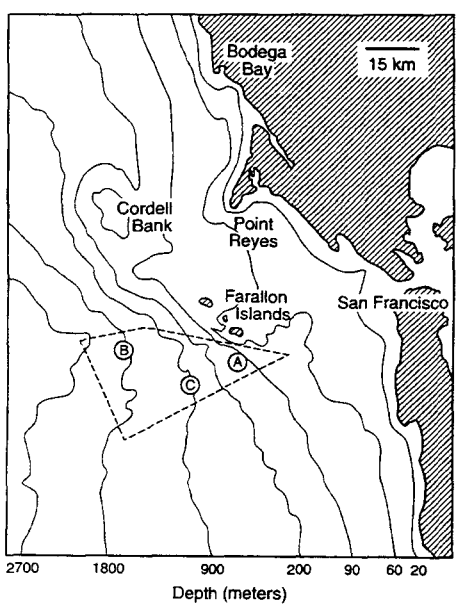

Fig. 1. The study site was located at Cordell bank, 80 km northwest of San Francisco. Farallon Islands Radioactive Waste Dump is delineated by *dashed lines*; *A*, *B*, and *C*, specific disposal sites in 90, 1800, and 900 m of water.

[5] M. Eldridge, National Marine Fisheries Service, Southwest Fisheries Science Center, Tiburon, CA, personal communication.

Table 3 *Age distribution of S. flavidus with respect to percentage prevalence (no. fish with lesions/no. fish examined) of melanophore lesions, melanophoromas, and xantho/erythrophore lesions*

	Age in years				
Lesion type	1–10	11–20	21–30	31–40	>40
Melanophore lesions	1.3% (3/225)	7.3% (25/344)	31.0% 65/210)	44.0% (11/25)	66.7% (2/3)
Melanophoromas	0% (0/225)	0.6% (2/344)	10.0% (21/210)	7.0% (2/25)	33.3% (1/3)
Xantho/erythrophore lesions	1.8% (4/225)	3.2% (11/344)	9.0% (19/210)	8.0% (2/25)	33.3% (1/3)

Table 4 *Age and sex distribution of S. flavidus with respect to average melanophore lesion score*

	Age in years				
S. flavidus	1–10	11–20	21–30	31–40	>40
All fish	9.3	14.2	21.8	22.6	25.0
Males	NA[a]	16.2	25.5	25.8	41.0
Females	9.3	13.6	19.9	20.0	9.0

[a] Not applicable.

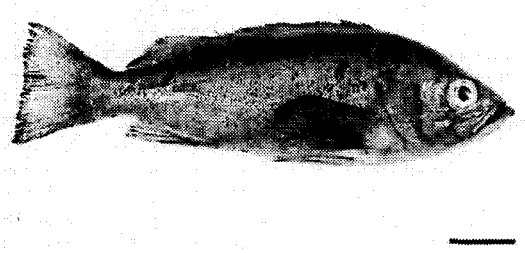

Fig. 2. *S. flavidus* with mild melanophore hyperplasia. *Bar* = 5 cm.

higher than posterior (9.6), but the difference was not as significant ($P = 0.1005$). The most severely affected quadrant was the dorsal-anterior (5.3), and the least affected was the ventral-posterior (4.5). Melanophoromas in *S. flavidus* also tended to occur dorsally (62 dorsal, 45 ventral) and cranially (60 anterior, 47 posterior). Again the most severely affected quadrant was the dorsal-anterior (34 tumors), and the least affected was the ventral-posterior (19 tumors).

Gross Pathology

S. flavidus (810 specimens), *S. paucispinis* (156 specimens), *S. entomelas* (45 specimens), *S. goodei* (67 specimens), and *S. serranoides* (5 specimens) with and without pigmented skin lesions were examined grossly.

Normal Pigmentation. *S. flavidus* without lesions were dark gray to greenish brown dorsally, with lighter colored flanks and a pale ventrum. There were often light grey to white patches at the base of the dorsal fin and small, indistinct, red-brown spots over the flanks. Fins (especially caudal) were yellow.

Melanophore Lesions. Black pigmented lesions in *S. flavidus* were found in the skin, fins, lips, gingiva, tongue, urogenital papilla, conjunctiva, and cornea of the eye. The smallest lesions were 0.1–2.0 cm in diameter, round to irregular flat foci (Fig. 2). In severely affected fish, foci coalesced to form large areas of melanosis covering up to 80% of skin and fins (Fig. 3). The surface of the smallest lesions was usually smooth, but larger lesions were often rough, with irregular displacement of scales. On section, foci and areas of melanosis were restricted to the skin.

Raised black masses varied from 0.2 to 2.0 cm in diameter and were usually sessile, broad based, and nonencapsulated (Fig. 4). Although some masses arose in areas of normal pigmentation, the majority were centered within larger areas of melanosis. Some black tumors had central regions that were opaque white (Fig. 5) or were mixed with yellow and/or orange tissue. Large masses occasionally extended through the dermis into the underlying subcutaneous tissue.

Pigmented lesions in *S. serranoides* and *S. entomelas* were similar to those in *S. flavidus*, with affected fish having a range of lesions from small foci of melanosis to large black tumors. Lesions in *S. paucispinis* (Fig. 6) and *S. goodei* were limited to irregular, flat foci and areas of melanosis.

Xanthophore and Erythrophore Lesions. Hyperplastic (flat) and neoplastic (raised) yellow, orange, and red lesions were seen only in *S. flavidus* and, although much less common, were similar in distribution and gross morphology (except for color) to melanophore le-

Fig. 3. *S. flavidus* with severe melanophore hyperplasia. *Bar* = 5 cm.

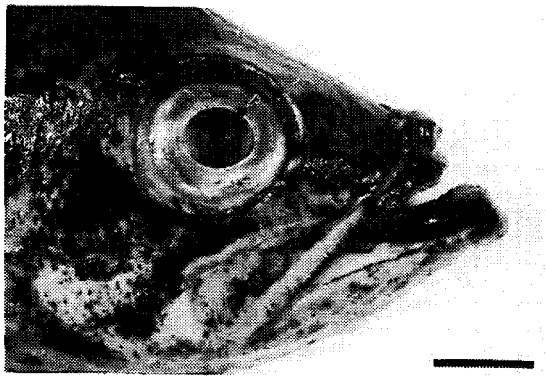

Fig. 4. *S. flavidus* with severe melanophore hyperplasia involving the entire head, focal melanophore hyperplasia in the cornea (*arrowhead*), and melanophoroma over the nose. *Bar* = 2 cm.

Fig. 5. *S. flavidus* with melanophore hyperplasia involving the pectoral fin (*F*) and melanophoroma with central depigmentation over the flank. *Scale* = 10 mm.

Fig. 6. *S. paucispinis* with multiple areas of melanophore hyperplasia. *Bar* = 10 cm.

sions. Orange lesions, often seen with yellow lesions, were closer in color to yellow than red. Red lesions were distinct with respect to color and rare.

Internal Organs. *S. flavidus* often had multiple, 1–6-mm-diameter, grey, firm, nodular masses in the kidneys, spleen, mesenteries, and occasionally the liver. A few *S. flavidus* with melanophoromas had multiple, 0.25–1.5-cm-diameter, translucent white to black, nodular masses in the liver. One *S. serranoides* with multiple melanophoromas had two nodular gill masses.

Histopathology

Cutaneous lesions were histologically examined from 32 *S. flavidus* and included 42 melanophore lesions, 7 xanthophore (2 yellow and 5 orange) lesions, 4 erythrophore (red) lesions, and 8 mixed melanoxanthophore (black mixed with yellow and/or orange) lesions. Melanophore lesions were also collected from 2 *S. paucispinis* and one *S. serranoides*. For comparison, skin (dorsal and flank) and fins (pectoral and caudal) from 3 *S. flavidus* without lesions were also examined.

Normal Pigmentation. Pigment cells in normal *S. flavidus* skin were present in four distinct dermal layers: (*a*) in the stratum spongiosum at the dermal-epidermal junction; (*b*) between the stratum spongiosum and scales; (*c*) between the scales and stratum compactum; and (*d*) between the stratum compactum and subcutaneous fat. Melanophores were present in all four layers and were arranged into single, sometimes discontinuous rows of spindle cells packed with granular black-brown pigment. There were proportionally more melanophores in superficial *versus* deep layers and in dorsal *versus* flank skin. Scattered throughout all layers were small numbers of finely tapered spindle cells, devoid of melanin (presumptive xanthophores). Some presumptive xanthophores contained small, clear to pale yellow, intracytoplasmic vacuoles.

In the fins, dermal chromatophores were reduced to a single, irregular, discontinuous layer adjacent to the dermal-epidermal junction. In areas where scales were present (primarily in the caudal fin), scattered chromatophores were also found in the stratum spongiosum adjacent to scales. In contrast to the skin, there were relatively few melanophores in the fins and proportionally greater numbers of presumptive xanthophores. Of the two fins examined, there were far fewer melanophores in the caudal fin.

Melanophore Lesions. The smallest foci of melanosis in the skin and fins of *S. flavidus* were characterized by hyperplasia of dermal melanophores in the most superficial layer of chromatophores, adjacent to the epidermis. Hyperplastic melanophores were heavily pigmented and densely packed into multiple layers. Melanophore hyperplasia varied from mild (Fig. 7) to severe (Fig. 8) and was restricted to the dermis. The epidermal stratified squamous epithelium was often hyperplastic and sometimes contained increased numbers of mucous and/or mononuclear inflammatory cells (primarily lymphocytes). Foci and areas of melanosis in *S. paucispinis* and *S. serranoides* were histologically similar to those in *S. flavidus*.

Areas of severe melanophore hyperplasia in *S. flavidus* often graded into small foci of dysplasia and early neoplasia. Neoplasms were classified as melanophoromas and were distinguished from hyperplastic foci by their accentuated disorganization, variability in pigmentation, and pleomorphism (Fig. 9). It was difficult to reliably predict histological appearance based on gross morphology, and many flat foci of apparent benign melanosis were later shown to be neoplastic.

All melanophoromas were composed of pleomorphic populations of stellate to spindle cells arranged into tightly packed, interweaving fascicles and whorling bundles (Fig. 10). The majority of tumors were well vascularized, and, in some, there was whorling of spindle cells around individual capillaries. Spindle cells had indistinct borders and varied from elongate and finely tapered to plump with abundant cytoplasm. Degree of pigmentation was highly variable, with some cells having only a fine dusting of granular black-brown pigment while others were packed with melanin. In some areas, tumor cells were completely devoid of pigment (Fig. 11), and even the most heavily melanized tumors were usually a mixture of pigmented and nonpigmented cells. Nuclei were oval to elongate, and although mitotic figures were usually rare, a few tumors had mitotic indices as high as 3–5 per 40× objective field. Grossly white tumors, classified

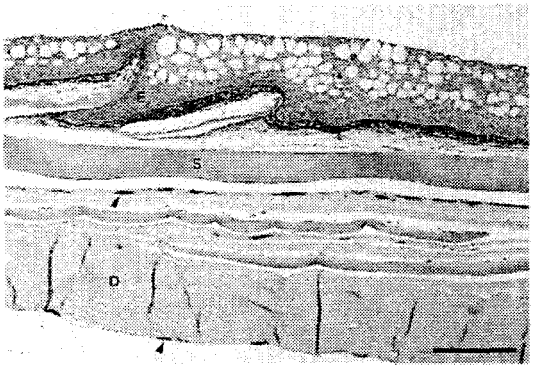

Fig. 7. Mild melanophore hyperplasia in the superficial dermis, adjacent to the epidermis (*E*), of *S. flavidus*. Normal melanophore layers (*arrowheads*) are located below the scale (*S*) and the stratum compactum of the dermis (*D*). H&E. *Bar* = 200 μm.

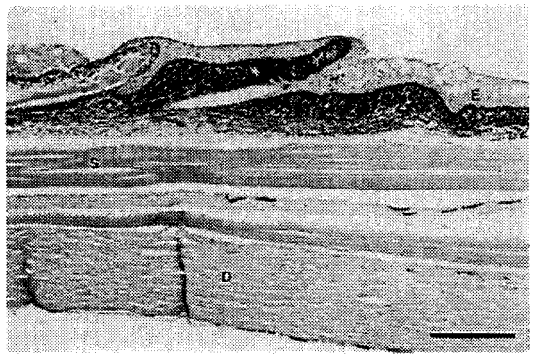

Fig. 8. Severe melanophore hyperplasia in *S. flavidus*. Hyperplasia is limited to the superficial dermis, adjacent to the epidermis (*E*). Normal melanophore layers below the scale (*S*) and stratum compactum of the dermis (*D*) are not involved. H&E. *Bar* = 200 μm.

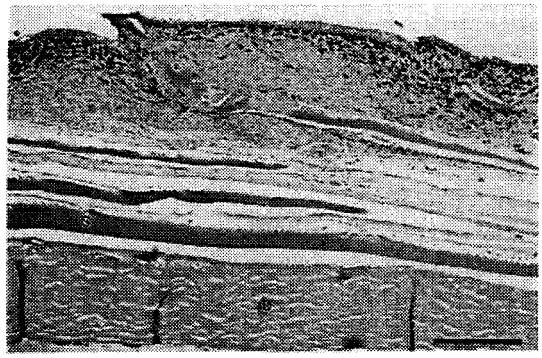

Fig. 9. Early melanophoroma in *S. flavidus*. Neoplastic melanophores are pleomorphic, variably pigmented, and arranged into irregular tightly fascicles but are still resticted to the superficial dermis above the scale (*S*) and stratum compactum of the dermis (*D*). H&E. *Bar* = 200 μm.

as amelanotic melanophoromas, were primarily composed of nonpigmented spindle cells, but almost all had small foci of melanin-bearing cells.

Small melanophoromas were restricted to the superficial dermis, but with increasing size there was a progressive extension through the stratum compactum and occasional invasion of the subcutis. Fin tumors often eroded into bony fin rays, and extensive invasion frequently resulted in severe bone destruction and variable new periosteal bone formation. Reactive bone formation was also occasionally seen in scales isolated by tumor cells.

The majority of melanophoromas were infiltrated with moderate to large numbers of lymphocytes, and many contained large macrophage aggregates packed with melanin (Fig. 10). The overlying epidermis was often eroded or ulcerated and infiltrated with lymphocytes and macrophages. All black-and-white lesions were negative for reflecting platelets (characteristic of iridophores) when examined with polarized light. Melanophoromas and amelanotic melanophoromas were also found in *S. serranoides* and were identical to those in *S. flavidus*.

Xanthophore and Erythrophore Lesions. Nonraised yellow, orange, and red lesions in *S. flavidus* were difficult to detect and interpret with routine histological processing. Alcohol dehydration resulted in the extraction of fatty carotenoid pigments, and presumptive xanthophores and erythrophores were almost indistinguishable from other spindle cells normally present in the dermis. Some xanthophores were identified by the presence of clear to pale yellow intracytoplasmic vacuoles, but in general, hyperplastic foci involving these cells could not be reliably diagnosed with hematoxylin and eosin-stained paraffin sections.

Mass lesions, involving xanthophores (xanthophoromas) and erythrophores (erythrophoromas), were not histologically distinguishable either from each other or from amelanotic melanophoromas in routine hematoxylin and eosin sections. All three tumor types were composed of finely tapered spindle cells devoid of melanin. Because of this, the classification of tumors was primarily based on coloration prior to fixation. Yellow and orange tumors were classified as xanthophoromas, and red tumors were identified as erythrophoromas. Tumors with more than one color (usually black and yellow) were classified as mixed chromatophoromas.

The only way to reliably evaluate yellow, orange, and red lesions histologically was via EM semithin sections. Toluidine blue-stained semithin sections revealed the presence of small to large amounts of distinct green lipid droplets within presumptive xanthophores and

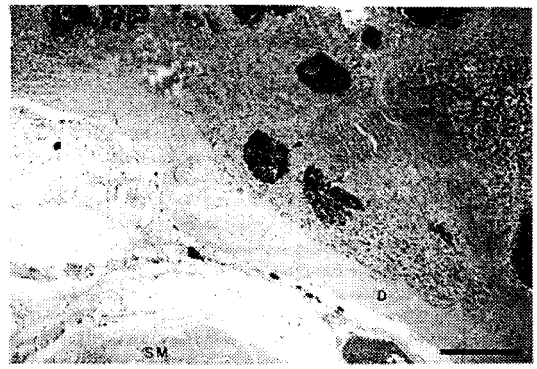

Fig. 10. Advanced melanophoroma in *S. flavidus*. The tumor, composed of heavily pigmented spindle cells arranged into densely packed fascicles, has invaded the stratum compactum of the dermis (*D*) but not the underlying skeletal muscle (*SM*). Numerous large aggregates of melanin laden macrophages (*arrowheads*) are present. H&E. *Bar* = 500 μm.

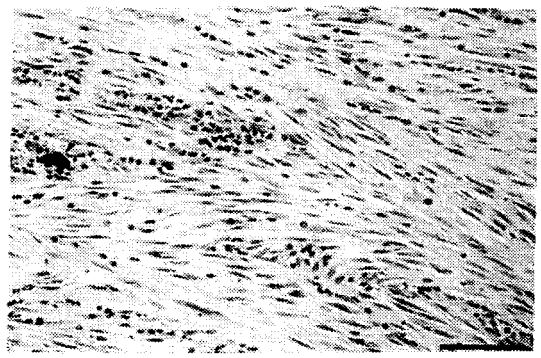

Fig. 11. Amelanotic portion of an advanced melanophoroma in *S. flavidus*. Except for a single melanophore (*arrowhead*), interweaving fascicles of neoplastic spindle cells are devoid of pigment. H&E. *Bar* = 50 μm.

erythrophores. All yellow, orange, and red lesions were negative for reflecting platelets when examined with polarized light.

Internal Organs. Nodular grey masses found in the internal organs of many *S. flavidus* were identified as foci of cartilaginous metaplasia in response to chronic *Ichthyophonus sp.* infection. Liver masses from one *S. flavidus* were consistent with metastasis from one of several cutaneous melanophoromas. The masses, composed of plump nonpigmented spindle cells, were found throughout the hepatic parenchyma and within blood vessels. The two gill masses from one *S. serranoides* were also believed to represent metastatic lesions from a cutaneous melanophoroma. The gill masses were somewhat pleocellular, but both were in part composed of nonpigmented spindle cells arranged into fascicles or whorling bundles.

Electron Microscopy

EM tissues were collected from 12 *S. flavidus* with lesions and included 2 foci of melanophore hyperplasia, 9 melanophoromas, one amelanotic melanophoroma, 2 xanthophoromas, one focus of erythrophore hyperplasia, one erythrophoroma, and 2 mixed (melano-xanthophore) chromatophoromas. For comparison, EM sections of skin (dorsal and flank) and fins (pectoral and caudal) from three *S. flavidus* without lesions were also examined.

Normal Pigmentation. Normal melanophores in the skin and fins were characterized by smooth cell membranes with few pinocytotic vesicles, small numbers of mitochondria, small to moderate amounts of ER, and large numbers of membrane-bound, electron-dense, round to oval melanosomes. Melanosomes were evenly distributed throughout the cytoplasm and varied from 0.2 to 0.6 μm in diameter. Xanthophores in normal skin and fins were similar ultrastructurally except for the absence of melanosomes and the presence of small to moderate amounts of cytoplasmic lipid. The lipid was randomly distributed, and pterinosomes were not observed.

Melanophore Lesions. Both melanophore hyperplasia and neoplasia were characterized by pleomorphic stellate to spindle cells with variable numbers of electron-dense melanosomes ranging from 0.15 to 0.60 μm (Fig. 12). Mixed in were some cells with lipid droplets and others with membrane-bound organelles intermediate in electron density to fat and melanin. Mitochondria were often distorted, and ring forms were common. Irregular membrane fragments and myelin figures were often seen both within and between cells. Nuclei were pleomorphic, and pinocytotic vesicles were common. One melanophoroma had widely scattered iridophores characterized by an empty latticework of membranes resulting from the dissolution of reflecting platelets during processing. The amelanotic melanophoroma was predominantly composed of spindle cells completely devoid of pigment.

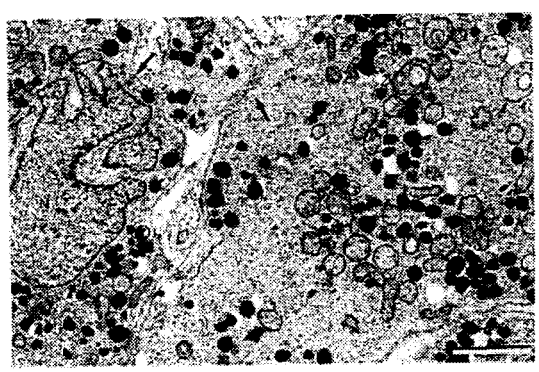

Fig. 12. Malignant melanophores with pleomorphic nuclei (*N*), scattered electron-dense melanosomes, dysplastic ring-shaped mitochondria (*arrowheads*), and numerous pinocytotic vesicles along cell margins (*arrows*). Uranyl acetate and lead citrate. *Bar* = 2 μm.

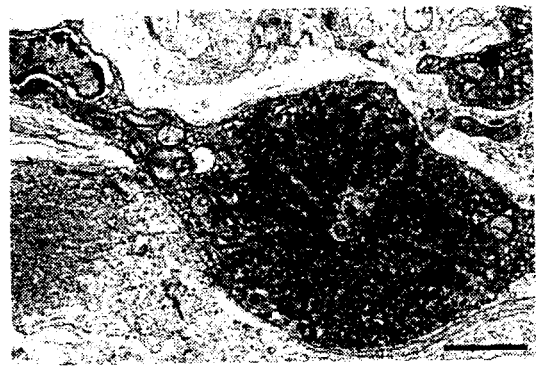

Fig. 13. Malignant xanthophore packed with lipid droplets arranged in radial fashion around a central focus of filaments. Adjacent cell contains several Golgi complexes (*arrowhead*), myelin figures (*arrow*), and lipid that is less electron dense. Uranyl acetate and lead citrate. *Bar* = 2 μm.

Xanthophore and Erythrophore Lesions. The two xanthophoromas examined differed markedly ultrastructurally. The first was characterized by pleomorphic spindle cells packed with lipid droplets (0.03–0.30 μm) which were often radially arranged around a central focus of filaments (Fig. 13). The second xanthophoroma had almost no lipid but was instead composed of cells filled with pterinosomes and large numbers of pinocytotic vesicles (Fig. 14). Pterinosomes ranged from 0.3 to 0.7 μm and were filled with amorphous flocculent material and disorganized fibrils. The second xanthophoroma also contained scattered iridophores.

The focus of erythrophore hyperplasia was characterized by large polygonal cells packed with lipid. The majority of lipid was in the form of small (0.01–0.05 μm) round droplets, but in some cells there was coalescence into large irregular "lakes" of fat up to 4 μm in diameter. The erythrophoroma contained few cells with lipid and was largely composed of nonpigmented cells with variable amounts of intermediate filaments, polyribosomes, and rough endoplasmic reticulum (Fig. 15).

Mixed chromatophoromas were characterized by the presence of both melanophores with melanosomes and presumptive xanthophores with variable amounts of lipid. No viral particles were seen in any of the tissue examined.

DISCUSSION

The gross, histological, and electron microscopic features of pigmented cutaneous lesions in Pacific rockfish are all consistent with the diagnosis of chromatophore hyperplasia and neoplasia. Although it has been proposed that these tumors be called chromatoblastomas (12), because of the evidence that all chromatophores arise from a neural crest-derived, pluripotent stem cell (*i.e.*, chromatoblast), we prefer to identify these tumors as chromatophoromas because the majority do show varying degrees of differentiation with respect to specific pigment cell types.

Subclassification of chromatophoromas is primarily based on the identification of specific pigment organelles (*e.g.*, melanosomes with melanophoromas or reflecting platelets with iridophoromas), but difficulty can arise when trying to differentiate xanthophore from erythrophore lesions because these cell types share many of the same pigments and pigment organelles. Our EM findings reflected this

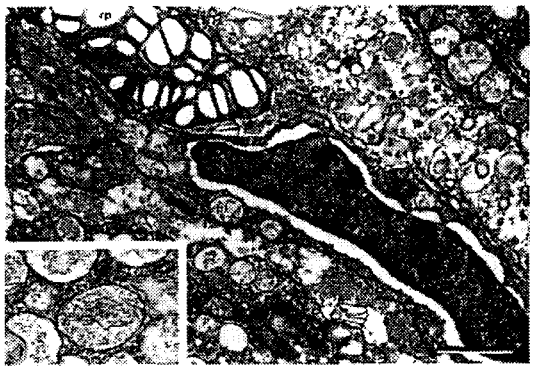

Fig. 14. Malignant xanthophores and one iridophore. Reflecting platelets (*rp*) in the iridophore were dissolved out during processing, leaving behind an empty latticework of empty membranes. Xanthophores contain numerous vesicles and pterinosomes (*pt*). Uranyl acetate and lead citrate. *Bar* = 1 μm. *Inset*, pterinosome with disorganized meshwork of fibrils.

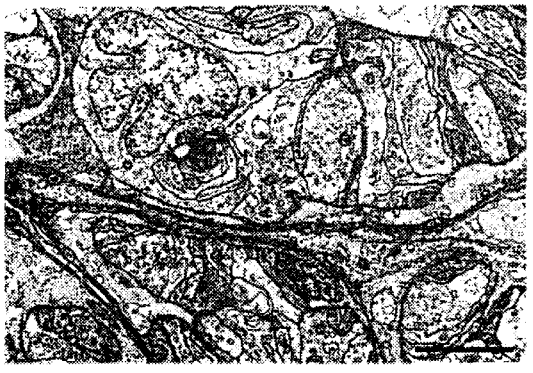

Fig. 15. Malignant erythrophores, with scattered filaments and small vesicles, are completely devoid of pigment. Uranyl acetate and lead citrate. *Bar* = 2 μm.

because although we did demonstrate organelles (pterinosomes and presumptive carotenoid lipid droplets) typical of both xanthophores and erythrophores, there was no consistency with respect to ultrastructure, and subclassification was therefore made on the basis of gross coloration.

Hyperplastic lesions are likely precursors to chromatophoromas in at least 3 of 5 species (*S. flavidus*, *S. serranoides*, and *S. entomelas*). Progression appears to occur stepwise from mild to severe hyperplasia, dysplasia, and eventual neoplasia. While the majority of chromatophoromas were benign and restricted to the dermis, some of the largest were malignant as evidenced by increasing anaplasia, invasion, and occasional metastasis to liver (in *S. flavidus*) or gill (in *S. serranoides*). Anaplastic chromatophoromas were quite variable histologically and, with severe pigment loss, must be differentiated from other cutaneous spindle cell tumors including fibromas, fibrosarcomas, neurofibromas, schwannomas, and hemangiopericytomas (13–16).

The prevalence and distribution of lesions in *S. flavidus* appear to reflect the relative number and distribution of chromatophores in normal skin. *S. flavidus* has a normal predominance of dermal melanophores (concentrated dorsally), followed in number by xanthophores (primarily in fins) and then erythrophores (located in small red-brown spots over the flanks). Accordingly, melanophore lesions were most common, followed by xanthophore lesions, and then erythrophore lesions (the least common). The dorsal and anterior distribution of melanophore hyperplasia and melanophoromas in *S. flavidus* is also probably a function of normal melanophore location.

Etiology is currently unknown. Potential etiologies include oncogenic viruses, genetic predisposition, normal aging phenomena, UV and ionizing radiation, and exposure to xenobiotic compounds. Although oncogenic retroviruses have been clearly implicated in mammalian lymphoma, leukemia, and sarcomas (17), there has been little evidence to support a viral etiology with respect to melanomas in mammals. The association between oncogenic viruses and neoplasia in fish is less common but has been established with lymphoma in northern pike (*Esox lucius*) (18–21), epithelial tumors in masou salmon (*Onchorhynchus masou*) (22–24), and dermal sarcomas in walleye (*Stizostedion vitreum*) (25–27). No viral particles were seen in any rockfish lesions, but the amount of tissue examined was limited, and a viral etiology cannot be definitively ruled out.

Genetic predisposition to melanoma is well documented in domestic animals (13) and humans (28, 29), and melanophoromas are readily induced in selective platyfish-swordtail hybrids (*Xiphophorus maculatus* crossed with *X. helleri*) (30–32). It is unlikely, however, that fish from Cordell Bank are a highly inbred, isolated group of fish. Rockfish have a pelagic larval stage and are probably randomly distributed by ocean currents following the fertilization of eggs. In addition, recent analysis of ribosomal genes from mitochondrial DNA revealed no significant differences between populations of *S. flavidus* from Cordell Bank, West Port in central Washington, and Vancouver Island, British Columbia.[6] The involvement of at least five different species would also appear to decrease the likelihood that genetics plays a critical etiological role.

Although both lesion prevalence and severity in *S. flavidus* increased with age, it is also unlikely that age is the only or predominant factor. Age-associated increases in pigment cell tumor prevalence have been documented in both goldfish (10) and *Xiphophorus* hybrids (33), and almost all species of mammals have higher prevalences of benign and malignant tumors with increasing age. The increased tumor prevalence in both fish and mammals, however, is usually more ---- -niform throughout a given population as it ages. If lesions were primarily age related, we would have expected to see rockfish with lesions throughout the Pacific coast. Instead, affected fish were concentrated at Cordell Bank. The higher prevalence in older *S. flavidus* may simply be a function of the slow progression of the disease following exposure and initiation at an early age.

One potential source of neoplastic initiation is exposure to UV radiation from sunlight. Although exposure to UV radiation alone will not induce melanoma in mice (34), and human melanoma is not correlated with exposed skin surfaces, there is an extensive body of research which indicates fairly conclusively that UV radiation is involved in melanoma formation (35). UV radiation has also been shown to induce melanophoromas in platyfish-swordtail hybrids, and these fish have been proposed as an animal model for human malignant melanoma (36). Wild fish species (including *X. maculatus* and *X. helleri*), however, have not been shown to be susceptible to UV radiation-induced neoplasia. Additionally, there is minimal penetration of UV radiation through water, and it would seem unlikely that deep-water species of rockfish are receiving any significant exposure.

Ionizing radiation is another potential etiological agent that must be considered because of the relatively close proximity (30 km) of Cordell Bank to the Farallon Islands Radioactive Waste Dump (Fig. 1). Approximately 14,500 Ci of radioactive wastes were disposed of from

[6] K. McGauley, Humboldt State University, CA, personal communication.

1946 to 1970 at three sites in 90 (site A), 900 (site C), and 1800 (site B) m of water (37–40). Several studies have already been conducted at the FIRWD (41–43), and most have concluded that sediments and biota are significantly contaminated with $^{239+240}$Pu and ^{137}Cs. Estimates of sediment levels range as high as 2,208 times background for $^{239+240}$Pu (42) and 134 times background for ^{137}Cs (43). Estimates of $^{239+240}$Pu levels in fish (various species) were as high as 8500 times background for muscle and 5071 times background for liver (42).

Although there is little evidence that ionizing radiation induces melanoma in either humans or laboratory mammals (44), radiation is linked with a wide range of mammalian neoplasms, and long-term effects in fish are unknown. It is interesting to note that scales in rockfish are often ossified and that exposure to ^{239}Pu, which is specifically recognized for its propensity to localize in bone and induce osteosarcomas in mammals (45), could result in localization in scales. Sequestration of ^{239}Pu immediately adjacent to dermal chromatophores would certainly enhance the possibility for neoplastic transformation or promotion.

In addition to radioactive compounds, the FIRWD was also extensively used as a chemical waste disposal site, with approximately 47,800 drums of unspecified waste being dumped from 1946 to 1970 (46). Limited sampling has already revealed high levels of chlorinated hydrocarbons (dichlorodiphenyltrichloroethanes and polychlorinated biphenyls) in sablefish (*Anoplopoma fimbria*) and dover sole (*Microstomus pacificus*) (47). While dichlorodiphenyltrichloroethanes and polychlorinated biphenyls have not been directly linked with melanoma in mammals, both are considered hepatic carcinogens in laboratory animals (48, 49), and there is at least one report of a slightly increased incidence of melanoma in a group of men occupationally exposed to polychlorinated biphenyls (50, 51).

Polyaromatic hydrocarbons have been definitively linked with melanoma in mammals, and 7,12-dimethylbenz[*a*]anthracene is capable of inducing melanoma via direct skin painting in hamsters, guinea pigs, and mice (35). Interestingly, Japanese researchers studying epizootics of chromatophoromas in *N. mitsukurii* were able to induce both chromatophore hyperplasia and neoplasia in laboratory-reared *N. mitsukuri* using several known carcinogens including 7,12-dimethylbenz[*a*]anthracene (7). Japanese researchers were also able to induce melanophore hyperplasia in 70–100% of marine catfish (*Plotosus anguillaris*) and chromatophoroma in one *N. mitsukurii* using effluent from a kraft pulp mill epidemiologically linked with identical lesions in wild fish (52). The Japanese study is one of the few examples where the hypothesis that chromatophoromas in wild fish are caused by exposure to carcinogenic compounds has been tested, at least partially, under controlled laboratory conditions.

There are a number of ways that fish from Cordell Bank may be exposed to radioactive or chemical compounds from the FIRWD. While the dominant surface current off California (the California current) runs north to south, surface waters near the Farallons are controlled from October through March by the northward-flowing Davidson counter-current (38). Deep bottom currents at the FIRWD are more complex, but Crabbs (53) did find a predominantly northward-flowing current from site B. There may also be some northward migration of rockfish in response to El Niño (warm water current), and adult Cordell rockfish may have been exposed as larvae or juveniles at the Farallons prior to being carried north.

Efforts are under way to expand the study area to include the FIRWD, but much additional work needs to be done in order to establish a more definitive link between the cutaneous lesions in rockfish and possible exposure to carcinogenic compounds in the environment.

ACKNOWLEDGMENTS

The authors thank the entire staff of the Tiburon Laboratory of the National Marine Fisheries Service for technical assistance and for help in the collection and aging of specimens.

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