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TUMORS IN FISH COLLECTED ON THE PALOS VERDES SHELF

The status of epidermal tumors in fish populations in the coastal waters off Los Angeles has not been reviewed since the mid-1970's. The objectives of this study were to determine the incidence and long-term trends of epidermal tumors among fish populations on the Palos Verdes shelf and to review current hypotheses on their etiology. Between 1971 and 1983, tumors were found on 0.3% of all individuals and 12% of all species collected. Over 90% of the tumorous individuals were Dover sole *(Microstomus pacificus)* less than 2 years old. The presence of tumors in this species appeared to be enhanced by proximity to the outfalls. Recent evidence suggests that tumors in Dover sole are caused by a parasitic protozoan, thus the lesion produced is not a true neoplasm but an amoebic pseudotumor.

METHODS

The data analyzed in this study were collected by Los Angeles County Sanitation District during regular monitoring cruises. The station numbers used herein are their designations. The data consisted of catch records of fishes and the frequency of tumors by species along seven transects on the Palos Verdes shelf (Figure 1). The trawls were made during daylight hours at three depths (23, 61, and 137 m) with an otter trawl towed along a depth isobath at 1.1 m/sec for 10 min. A 7.3-m (headrope length) otter trawl was used from 1971 to 1974 when it was replaced with a 7.6-m trawl; a 1.25- cm mesh cod-end liner was used in both nets. From 1971 through 1978, two samples were collected annually at each depth--one between April and June, the other between October and December. Additional trawls made at irregular intervals were included in the analyses. Quarterly trawling began in 1979 and has continued to the present. Trawling was discontinued at transects T2, T3, and T6 in 1977.

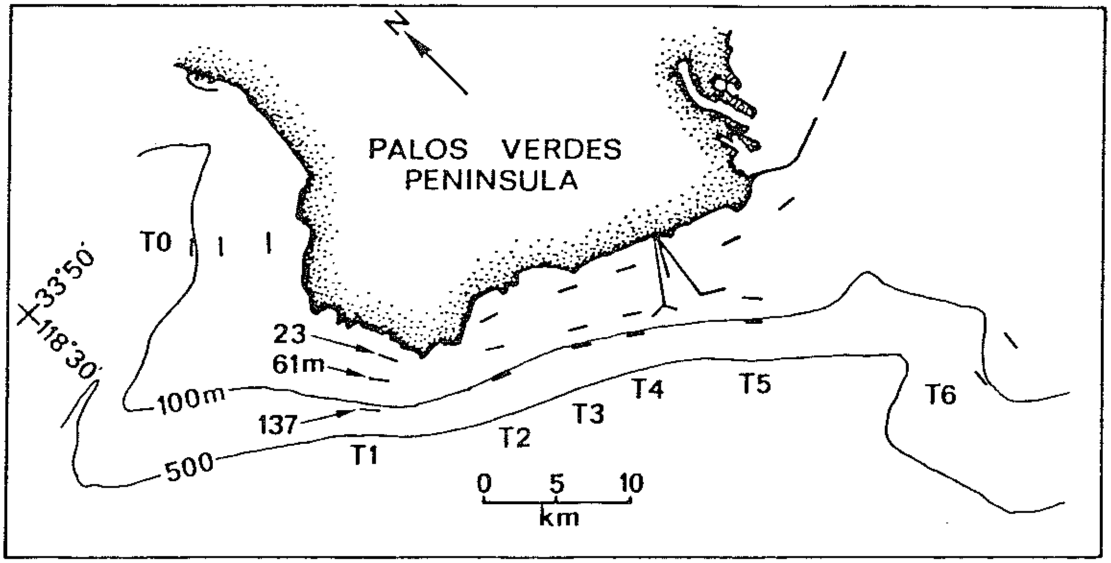

Figure 1. Map of the study stations on the Palos Verdes shelf.

The quarterly trawl data (1979-83) taken at 61 and 137 m were examined for seasonal trends in the incidence of tumors and total catch of Dover sole. Transects T0, T1, T4, and T5 were combined for each quarter because of their low frequency of tumors in the Dover sole population. Seasonal trends were estimated from:

$$P_t = f\,(T_t, S_t, R_t)$$

where P_t = the proportion of Dover sole with tumors at time t, T_t = the trend component at time t, S_t = the seasonal component at time t, R_t = the remaining components (cyclical and aperiodic) at time t, and f is a function relating the observed value of the time series to the trend, seasonal, and remaining components (Harnett and Murphy 1980). Regressions were fitted to the incidence data to estimate T (the regression coefficient) for each quarter. The trend is eliminated by dividing the quarterly P values by T. S is then estimated for each quarter, assuming R is small. Multiplicative and additive models were fitted to the data; multiplicative models gave a better fit (i.e., a lower residual sum of squares). Trend analyses were performed on the data for Dover sole, the most frequently affected species. Only data on individuals less than 120 mm BSL (79.8% of all tumored Dover sole) were used in the analyses. Trends in the proportion of Dover sole with tumors from 1971 through 1983 were determined by regressing the annual proportion with tumors against year. Because Dover sole were rarely collected at 23 m, this depth was dropped. All trawls made within a year at 61 m and 137 m at transects T0, T1, T4, and T5 were combined because of the low frequency of tumors in the Dover sole population. Proportions (p) were transformed to the arcsin $\sqrt{p}$, and years were numbered consecutively from 1971 through 1983 (e.g., 1, 2, 3, ..., 13).

RESULTS

General

From 1971 through 1983, 539 tumorous individuals from 15 species of fish were collected in 672 otter trawls on the Palos Verdes shelf (Table 1). Tumorous individuals comprised 0.3% of all individuals and 12% of all species collected during those years. The number of species affected by tumors declined from six in 1971 to one in 1976 and remained at one or two species through 1983. The correlation between the number of species with tumors and year is significant ($r = -0.802$, $n = 13$, $p = 0.001$).

Table 1. Taxonomic list of fish affected by tumors collected in 672 otter trawls on the Palos Verdes shelf from 1971 through 1983.

Common Name	Scientific Name	Number Collected	Number with Tumors	Percent with Tumors	Percent Occurrence in Trawl Collections
Specklefin midshipman	*Porichthys myriaster*	83	1	1.2	7.3
Blackbelly eelpout	*Lycodopsis pacifica*	3,273	1	<0.1	21.6
Calico rockfish	*Sebastes dallii*	9,235	1	<0.1	23.7
Cowcod	*Sebastes levis*	221	2	0.9	14.3
Flag rockfish	*Sebastes rubrivinctus*	59	1	1.7	4.8
Pink rockfish	*Sebastes eos*	37	1	2.7	1.0
Shortbelly rockfish	*Sebastes jordani*	3,260	2	0.1	23.8
Unidentified juvenile rockfish	*Sebastes sp.*	1,348	14	1.0	0.3
Shortspine combfish	*Zaniolepis frenata*	823	1	0.1	20.4
White croakers	*Genyonemus lineatus*	11,627	10	0.1	22.6
Shiner surfperch	*Cymatogaster aggregata*	9,553	1	<0.1	26.0
Speckled sanddab	*Citharichthys stigmaeus*	15,914	1	<0.1	39.0
Dover sole	*Microstomus pacificus*	43,266	501	1.2	60.9
English sole	*Parophrys vetulus*	1,278	1	0.1	43.2
Hornyhead turbot	*Pleuronichthys verticalis*	5,666	1	0.2	27.4

Tumors in Dover Sole

Tumors in Dover sole were confined primarily to smaller individuals (Figure 2). Tumors were first observed in fish 50-59 mm BSL and most frequently encountered in fish 100-109 mm BSL; of all tumored Dover sole, only 5.5% were larger than 150 mm BSL. The proportion of Dover sole with tumors increased rapidly with increasing fish size (Figure 3), peaked at greater than 5% in fish 70-109 mm BSL, and declined rapidly thereafter.

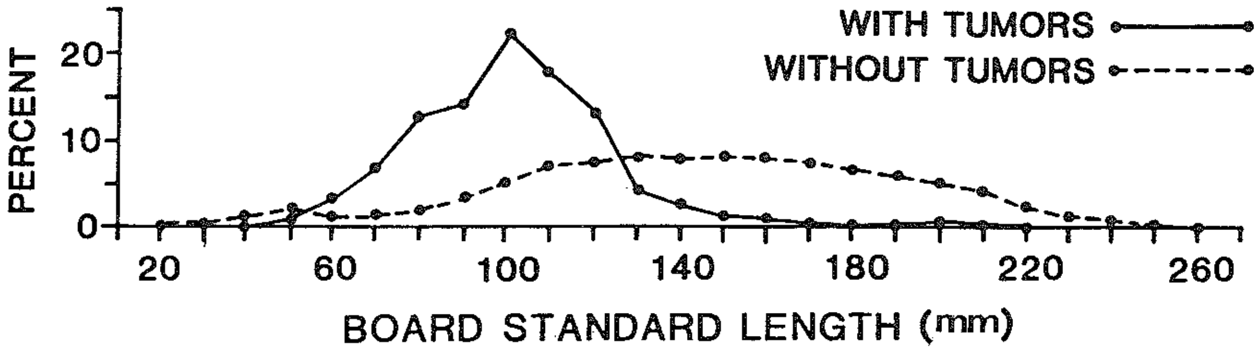

Figure 2. Size distribution of Dover sole *(Microstomus pacificus)* with and without tumors collected on the Palos Verdes shelf between 1971 and 1983.

Between 1971 and 1972, 103 Dover sole with tumors were collected by otter trawls from the Palos Verdes shelf, Santa Monica Bay, San Pedro Bay, and off Newport. The fish were measured (BSL); the number of tumors on each fish was counted and, for all but three individuals, measured. The fish ranged from 61-208 mm BSL with a mode between 100-119 mm BSL; 3.9% were larger than 150 mm BSL. There was no correlation between the number of tumors per fish and fish size ($r = -0.148$, $n = 103$, $0.10<p<0.20$). There was a significant correlation between tumor size and fish size ($r = 0.643$, $n = 100$, $p<<0.001$). Of individuals with only one tumor ($n = 46$), 54.3% had it on the eyed side, 37.9% had it on the blind side, and 8.7% had it on both sides. If individuals with one tumor on both sides were disregarded, there was no difference in the distribution of tumors between the eyed and blind side ($\chi^2 = 1.52$, $n = 42$, $0.20<p<0.30$). For individuals with more than one tumor ($n = 54$), 50.0% had tumors on both sides, 25.9% had tumors only on the eyed side, 13.0% had tumors only on the blind side, and 11.1% had at least one tumor on both sides. If individuals with one tumor on both sides were disregarded, there were significantly fewer fish with tumors on only one side ($\chi^2 = 12.88$, $n = 48$, $0.001<p<0.01$).

The time series analyses of the quarterly trawl data (1979-83) showed seasonal trends in both the proportion of Dover sole with tumors and the number of Dover sole caught per 10-min trawl (Figure 4). Interestingly, the trends were opposite: catch was highest in spring and summer, while the proportion with tumors was highest in fall and winter.

The trend analysis of data from transects T0, T1, T4, and T5 revealed no significant changes in the proportion of Dover sole with tumors from 1971 through 1983 (Table 2). Analysis of covariance revealed no significant differences among the slopes of the regressions ($F = 2.09$,

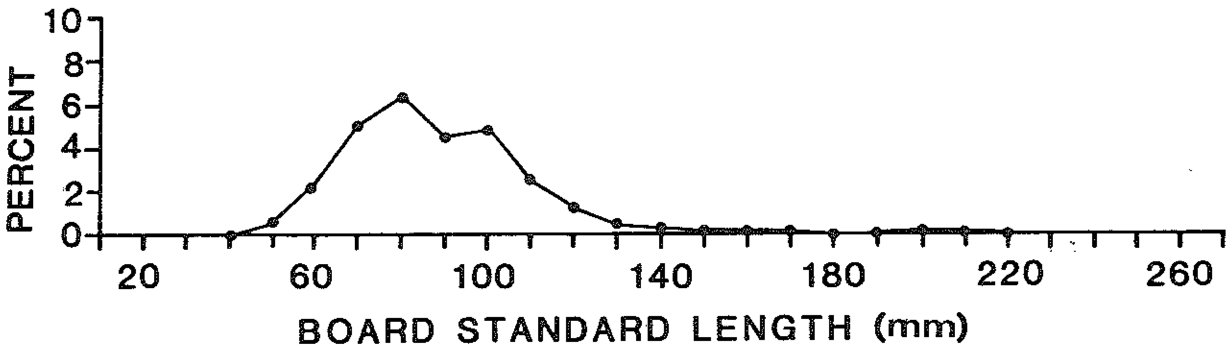

Figure 3. Proportion of Dover sole *(Microstomus pacificus)* collected on the Palos Verdes shelf between 1971 and 1983 with tumors by 10-mm size class.

$0.05<p<0.10$); however, there were significant differences among the elevations ($F = 6.27$, $0.001<p<0.0025$). A Newman-Keuls multiple-range test produced the following:

T0 T1 T4 T5

indicating that the proportion of tumorous individuals was higher at stations closer to the outfall (T1, T4, and T5) than at the station that was farthest away (T0).

Table 2. Results of trend analyses for Dover sole *(Microstomus pacificus)* less than 120 mm BSL collected at four transects on the Palos Verdes shelf from 1971 through 1983. Y = arcsin $\sqrt{a}$ where a = proportion with tumors (determined by combining data from the 61- and 137-m stations for each year); X = time in years numbered consecutively from 1971 through 1983 (e.g., 1, 2, 3,..., 13); CI = confidence interval ; p = probability that β = 0.

Transect	Regression	95% CI for slope	p
T0	Y = 1.455 + 0.387X	-0.467 to 1.241	$p>0.50$
T1	Y = 17.220 - 0.958X	-2.132 to 0.216	$p=0.10$
T4	Y = 19.048 - 0.706X	-1.680 to 0.268	$0.10<p<0.25$
T5	Y = 13.801 - 0.321X	-1.034 to 0.392	$p>0.50$

DISCUSSION

Approximately 12% of the fish species and 0.3% of the individuals collected between 1971 and 1983 had epidermal tumors. The decline in the number of species affected by tumors was most rapid between 1971 and 1976 following the wastewater treatment modifications made in the early 1970's. This pattern suggests that the decline was related to reduced surface sediment contamination. Unfortunately, it is not known whether all of the tumors in all of the species have the same etiology, or whether several types of tumors are involved.

Dover sole accounted for 93% of all tumorous fish, but only 1.2% of all Dover sole possessed tumors (Table 1). Table 3, which compares recent data on the incidence of epidermal tumors of presumedly similar etiology among three species of pleuronectid flatfishes common on the West Coast, suggests that tumors are restricted to very young fish and that the incidence varies among areas and years.

Table 3. Incidence (%) of epidermal tumors by age among English sole *(Parophrys vetulus)*, starry flounder *(Platichthys stellatus)*, and Dover sole *(Microstomus pacificus)* from the West Coast. The age of tumorous Dover sole in the present study was estimated from an age-length key developed from age data on 328 individuals collected on the Palos Verdes shelf in 1972 and 1973 (Cross 1984).

		Age (years)				
Species	Location (year)	0	1	2	3	Source
English sole	Northern Puget Sound (1966-67)	← 4.8 →		0	0	McArn and Wellings 1971
Starry flounder	Northern Puget Sound (1966-67)	26.9	← 1.5 →			
English sole	Northern San Francisco Bay (1965-66)	15.5	-	-	-	Cooper and Keller 1969
	Southern San Francisco Bay (1965-66)	8.9	-	-	-	
Starry flounder	Northern Puget Sound (1979-81)	37.4	12.7	1.7	-	Campana 1983
Dover sole	Palos Verdes shelf (1971-83)	3.1	2.0	0.2	0.1	Present study

Tumors in Dover Sole

Analysis of tumor incidence among Dover sole showed that 1) there was no correlation between fish size and number of tumors and 2) tumors were no more abundant on the eyed side than on the blind side, as has been reported for English sole *(Parophrys vetulus)* and flathead sole *(Hippoglossoides elassodon)* (Wellings et al. 1976). However, larger fish had larger tumors (Sherwood and Mearns 1976). (Campana (1983), in a study of tumorous starry flounder *(Platichthys stellatus)* from Puget Sound, showed that tumors continued to grow as the fish grew; he found no evidence for tumor regression.)

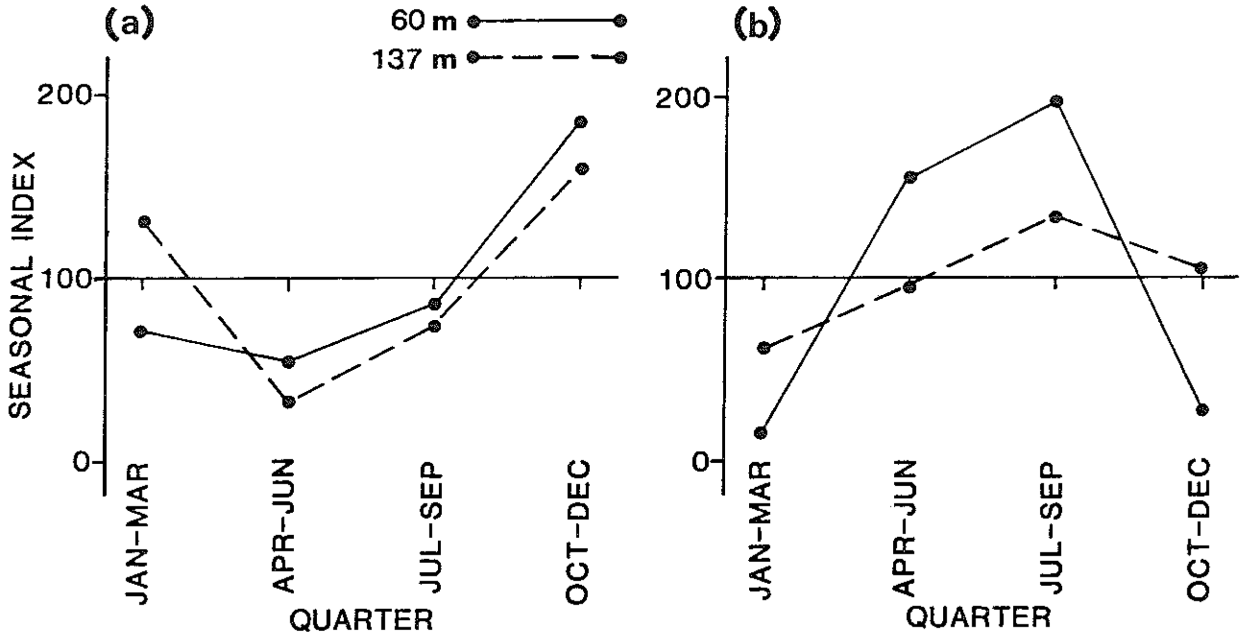

Figure 4. Seasonal trends at two depths (a) in the proportion of Dover sole *(Microstomus pacificus)* with epidermal tumors and (b) in the catch of Dover sole in one 10-min trawl (data from 1979 through 1983).

Tumor incidence among Dover sole was highest in those less than a year old (Table 3); only 6.5% of the tumorous individuals collected between 1971 and 1983 were age two or older. Tumors were not evident on the smallest recruits (40-49 mm BSL) in the first quarter (January-March), but appeared in larger fish (50-59 mm BSL) in the second quarter (April-June). Similarly, Campana's (1983) study found that starry flounder in Puget Sound settled out of the plankton as early as May but the first tumors did not appear until July.

The rapid decline in the proportion of tumorous Dover sole with increasing fish size suggests that either 1) tumorous fish have higher mortality rates than nontumorous fish or 2) tumorous fish have higher emigration rates. Seasonal analysis of the quarterly trawl data showed that the proportion of Dover sole with tumors was highest in the fall and winter when the catch of Dover sole was lowest (Figure 4). This seasonal pattern of catch occurred because Dover sole migrate offshore into deeper water in the fall and winter and onshore into shallower water in the spring and summer (Hagerman 1952). (Note that the magnitude of the seasonal swing is greater at 60 m than at 137 m.) The high proportion of tumorous fish on the Palos Verdes shelf in the fall and winter suggests that tumorous fish do not participate in the offshore movement to the same extent as do nontumorous fish. An alteration of this type of migratory behavior was noted for tumorous English sole (Stich et al. 1976).

The decline in the proportion of tumorous Dover sole with increasing fish size is probably the result of increased mortality. Campana (1983) showed that tumorous starry flounder were more susceptible to stress, had lower growth rates, and had much higher mortality rates than nontumorous individuals.

Trend analyses of the incidence of tumorous Dover sole indicated no significant change from 1971 thorugh 1983. Lack of significance probably resulted from the low proportion of Dover sole with tumors and the rather high variability among years. There was, however, a significant difference (Figure 1) in the proportion of tumorous individuals smaller than 120 mm BSL between T0, the station farthest from the outfalls (1.2%), and T1, T4, and T5 (4.1%). The slopes of the trend lines flattened out from T1 to T5 (Table 2) in a pattern remarkably similar to the pattern produced by the same type of analysis on Dover sole with fin erosion (Cross 1984), further suggesting that tumors are related to the outfalls.

Etiology in Tumors in Dover Sole

Papillomatous skin tumors have been reported in flatfishes (Bothidae, Pleuronectidae, Hippoglossidae) and gobies (Gobiidae) (Cooper and Keller 1969; McArn and Wellings 1971; Miller and Wellings 1971; Mearns and Sherwood 1974; Ito et al. 1976; Oishi et al. 1976; Wellings et al. 1976; McCain et al. 1978; Campana 1983) and tumors of the parabranchial gland have been reported in cod (Gadidae) and rockfish (Scorpaenidae) (McCain et al. 1979; Dawe et al. 1979; Myers 1981; Watermann and Dethlefsen 1982) from the North Pacific and North Atlantic oceans. All of these tumors are characterized by a unique cell type originally termed the X-cell (Brooks et al. 1969), the nature and etiology of which have been controversial. X-cell tumors were thought to be autonomous neoplasms similar to papillomas in mammals, virus or virus-like particles, neoplastic epidermal or other cells altered in appearance, or unicellular parasites (McCain et al. 1978; Myers 1981). Recent evidence supports the hypothesis that the X-cell is a unicellular protozoan parasite resembling a parasitic amoeba (Dawe et al. 1979; Myers 1981; Watermann and Dethlefsen 1982). The lesion produced by the X-cell is not a true neoplasm but an amoebic pseudotumor (Dawe et al. 1979; Watermann and Dethlefsen 1982) or a parasitic xenoma (Myers 1981), and the epidermal proliferation is reactive hyperplasia (Wellings et al. 1976). The X-cells can be seen in a histological section through a tumor from a Dover sole collected on the Palos Verdes shelf (Figure 5).

The hypothesized life history of the amoeba is as follows. From a pool of encysted, infectious organisms (free-floating or in marine sediments), the parasite enters a host through the epidermis. The mature cysts divide and proliferate in subepithelial sites, thereby producing an

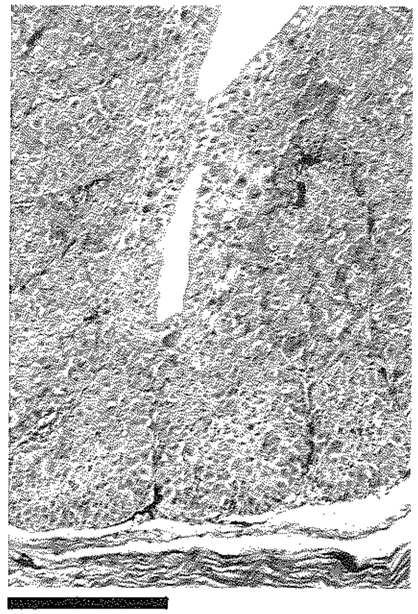

Figure 5. Histological section through a tumor from a Dover sole *(Microstomus pacificus)* collected on the Palos Verdes shelf showing the characteristic nests of parasitic amoebae. (Bar = 0.5 mm)

inflammatory response in the host. Establishment of the parasite in a susceptible host stimulates vascularization and formation of a fibrous network (stroma) that subdivides the xenoma until it eventually becomes papillomatous. The parasite may prevent phagocytosis by stimulating extensive envelope cell formation around itself. The xenoma continues to grow or may regress. X-cells may be shed and then reencyst, encyst within the host prior to exit, or be shed when the host dies (Myers 1981; Watermann and Dethlefsen 1982).

As suggested earlier, it appears that the outfalls on the Palos Verdes shelf enhance the incidence of the parasitic xenomas in Dover sole. Amoebae morphologically similar to the X-cell (Myers 1981) are common in marine sediments (Sawyer 1971) and sewage sludge (Singh and Das 1972) and have been isolated from sewage sludge dump sites in the ocean (Sawyer et al. 1977).

ACKNOWLEDGEMENTS

I would like to thank Los Angeles County Sanitation District for access to the monitoring data and Dr. Alan J. Mearns and Margie Sherwood for access to the tumor data on Dover sole collected in 1971 and 1972. Dr. John C. Harshbarger of the Registry of Tumors in Lower Animals, Smithsonian Museum, confirmed that the X-cells are parasitic amoebae.

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