C-220

THE MICRONUCLEUS TEST: A SIMPLE GENOTOXICITY SCREENING FOR MARINE FISHES

by

Jeffrey N. Cross
Southern California Coastal Water Research Project
646 W. Pacific Coast Highway
Long Beach, CA 90806

and

Jo Ellen Hose
VANTUNA Research Group
Occidental College
1600 Campus Road
Los Angeles, CA 90041

Final Report

to

National Oceanic and Atmospheric Administration
7600 Sand Point Way, N.E.
Seattle, WA 98115

Contract No. 50ABNC700091

March, 1988

I. INTRODUCTION

This research is part of a larger program to asses biological monitoring methods indicative of exposure to mixtures of toxciants. In this report we describe the micronucleus test as applied to teleosts.

Micronuclei are smaller, secondary nuclei formed following chromosome breaks (Schmid 1976). Although they may arise spontaneously, the induction of micronuclei can detect genotoxic damage as a result of exposure to mutagens (Heddle et al 1983). The micronucleus test was originally described in rodent polychromatic erythrocytes (MacGregor et al. 1980). It has also been applied to teleost erythrocytes in laborartory exposures (Hooftman and de Raat 1982, Hose et al. 1984). In the field, micronucleus frequency was higher in two species of fish from contaminated coastal areas near Los Angeles (Hose et al. 1987).

The objective of this study was to quantify micronuclei in peripheral circulating erythrocytes of starry flounder, Platichthys stellatus, from four stations in San Francisco Bay and two stations on the outer coast and, to correlate micronucleus frequency with body burdens of organic contaminants.

II. METHODS

Starry flounder (Pleuronectidae: Platichthys stellatus) were collected by otter trawl from four sites within San Francisco Bay and two sites on the outer coast during the winter of 1986-87 (Figure 1). Details of trawl collecting methods and dates are presented in Spies et al. (1988).

Blood was collected from each fish immediately after capture in a heparinized syringe and blood smears were prepared. The smears were air dried and fixed in absolute methanol for 15 minutes aboard ship.

In the laboratory, the smears were stained with May-Grunwald-Giemsa and examined with a high power (1000x) microscope according to the method of Hose et al. (1987). The number of micronucleated erythrocytes (MN) was determined on coded slides and scored in a blind review. Two types of micronuclei were included in the counts: detached and attached. The number of micronucleated erythrocytes was the average of two counts of 1000 erythrocytes each and is expressed as MN^{o}/oo.

The degree of nuclear pleomorphism (loss of the usual elliptical shape of the nucleus) was determined for each slide and coded: 1 (<5% of erythrocytes), 2 (5-50%), or 3 (>50%). Severely pleomorphic nuclei had indentations and/or projections. If projection was greater than about

one-fourth the nuclear diameter and terminated in a chromatin mass, it was counted as an attached micronucleus.

Concentrations of 34 organic contaminants were determined in liver tissue of 80 starry flounders collected in the present study; the analytical methods and the compounds quantified are found in Spies et al. (1988). Because the resulting matrix of 80 fish by 34 contaminants was quite large (2720 entries), principal component analysis (PCA) was used to describe the similarity among the samples.

PCA is one of several ordination procedures for creating two- or three-dimensional projections from a multidimensional aggregation of data points in a way that preserves most of the systematic variation among the objects (fish) and variables (contaminants). In the simplest of terms, a fish (f) by contaminant (c) matrix containing n data points can be thought of as a cloud of n data points in f-dimensional space where the coordinates of each point (representing a contaminant) are the amounts measured in each of the f fish. The cloud of data points is treated as a single entity and rotated rigidly around its origin so that the pattern is as simple as possible. The new axes are the principal components and the new coordinates of the data points are the principal component scores (Pielou 1984).

Principal component analysis was performed in SYSTAT (Wilkinson 1986). A Pearson correlation matrix was calculated for log-transformed [$\log_{10}(x+1)$] lipid-corrected contaminant concentrations. All principal components with eigenvalues greater than 1.0 were examined in the first pass. Four principal components with eigenvalues greater than 1.0 were calculated and rotated, but the variance of the total data set explained by the third and fourth components was small (<14%). On the subsequent pass, only the first two principal components (those with the greatest eigenvalues) were calculated and rotated. Principal component scores were computed for each data point and normalized to zero mean and unit variance. Results of the PCA were used to examine the relationship between contaminant body burdens and micronucleus frequency.

Before the contaminant data were analyzed, the matrix was reduced in size by deleting compounds that were near or below detection limits in fish from all of the sites. The number of PCB congeners included in the analysis was further reduced by selecting those congeners with high certainty of resolution based on data in Eganhouse et al. (1987). The resulting data set contained 14 compounds: p,p'-DDE, p,p'-DDD, chlordane, dieldrin, heptachlorepoxide, lindane, and PCB congeners 44, 101, 118, 128, 138, 153, 180, and 206 (IUPAC congener numbers).

III. RESULTS

One hundred and fifty-eight starry flounder were collected and analyzed for erythrocytic micronuclei during the two sampling periods in the winter of 1986-87. Of the total, 116 fish were caught at four stations in San Francisco Bay and 42 fish were caught at two stations on the outer coast (Figure 1).

A. Size and Sex

The size and sex of starry flounder varied among the stations (Table 1). Females were obtained from all six stations. Males were not obtained from Santa Cruz. Females outnumbered males in collections from every site except San Pablo Bay. The size range of females (20.5 to 53.0 mm SL) was larger than the size range of males (19.7 to 40.5 mm SL). The largest fish of both sexes were caught on the outer coast.

Female size was not normally distributed (Kolmogorov-Smirnov test, $p<0.001$) and the variances were not homogeneous (Bartlett test, $X = 20.27$, $p=0.001$). The size of females captured was significantly different among the six stations (Kruskal-Wallis test, $H=27.59$, $p<0.001$) but not among the four stations within the bay ($H=3.56$, $p=0.31$).

Male size was not normally distributed (K-S test, $p<0.001$) but the variances were homogeneous (Bartlett test, $X = 1.43$, $p=0.84$). Male size was significantly different among the five stations where they were captured (Kruskal-Wallis test, $H=22.25$, $p<0.001$) but not among the four stations within the bay ($H=1.81$, $p=0.61$).

B. Micronucleus Frequencies

Starry flounder erythrocytes are elliptical with central elliptical nuclei (Figure 2). Starry flounder erythrocytes contained two types of micronuclei similar to those described by Hooftman and de Raat (1982): classical detached, circular pieces of chromatin (Figures 3 and 4) and chromatin projections attached to the nucleus (Figure 5). Detached micronuclei ranged from about 1/20 to 1/10 the diameter of the erythrocyte nucleus.

MN frequencies were not normally distributed (Kolmogorov-Smirnov one sample test, $p<0.001$) (Figure 6) and variances were not homogenous among the sites (Bartlett test, $X = 50.67$, $p<0.001$) (Table 2). The variance was substantially larger than the mean for Berkeley and Oakland fish suggesting a negative binomial distribution. The variance at the remaining stations was approximately equal to the mean at the remaining stations suggesting a Poisson distribution (Elliot 1979).

MN frequencies transformed to their logarithms [$\log_{10}$(MN+1)], appropriate for the negative binomial distribution, were not normally distributed (Kolmogorov-Smirnov test, $p<0.001$) although the variances were homogeneous (Bartlett test, $X=9.95$, $p=0.077$). MN frequencies transformed to their square roots [$(MN+0.5)^{-.5}$], appropriate for the Poisson distribution, were not normally distributed (Kolmogorov-Smirnov test, $p<0.001$) and the variances were not homogeneous (Bartlett test, $X=19.40$, $p=0.002$). Consequently, the MN data were analyzed by nonparametric (distribution-free) methods.

The frequency of micronuclei was significantly different among starry flounder collected in San Francisco Bay and on the outer coast (Kruskal-Wallis test, $H=25.67$, $p<0.001$). MN frequencies were higher among fish from San Francisco Bay (Table 2). There were no significant differences in MN frequencies among fish from the four stations in San Francisco Bay (Kruskal-Wallis test, $H=2.35$, $p=0.503$) or between fish from the two stations on the outer coast (Mann-Whitney U test, $U=229$, $p=0.213$).

The proportion of fish with no micronucleated erythrocytes ranged from .09 to .35 at the four stations in the Bay and from .46 to .66 at the sites on the outer coast (Table 2). The frequency of zeros was significantly different among the six stations ($X=46.39$, $p<0.001$) but not among the four stations in the Bay ($X=7.82$, $0.05<p<0.10$) or between the two stations on the outer coast ($X=0.71$, $0.25<p<0.50$) (Figure 7).

The frequency of MN were examined for each sex separately. There was no significant difference in MN frequency among male starry flounder at the five sites where they were collected (Kruskal-Wallis test, $H=7.05$, $p=0.11$; Table 3).

There was a significant difference in MN frequency among female starry flounder at the six sites (Kruskal-Wallis test, $H=24.37$, $p<0.001$; Table 3). MN frequency was not significantly different among females collected in San Francisco Bay ($H=2.91$, $p=0.41$) but it was significantly different between females collected in the bay and on the outer coast (Mann-Whitney U test, $U=359$, $p<0.001$). MN frequency was also significantly different between females collected at Santa Cruz and those collected at the Russian River (Mann-Whitney U test, $U=144$, $p=0.007$).

MN frequencies were compared between males and females at five stations where both sexes were collected. The differences were significant only among fish from the Russian River (Mann-Whitney U test, $U=29$, $p=0.004$) where males had higher MN frequencies than females (Table 3).

MN frequency was significantly positvely correlated with size for females (Spearman r_s=0.295, 0.02<p<0.05) and but not for males (Spearman r_s=0.107, p>0.50) (Figure 8) caught within San Francisco Bay.

C. Detached and Attached Micronuclei

Counts of detached and attached micronuclei suffered from the same distributional problems encountered with total micronuclei: non-normality and heterogeneous variances (Table 4). Among individual fish, detached micronuclei were not systematically more or less abundant than attached micronuclei (Wilcoxon matched pairs test, p=0.509).

The frequency of detached micronuclei was significantly different among starry flounders from the six stations (Kruskal Wallis test, H=22.30, p<0.001). Detached MN frequencies were higher among fish from San Francisco Bay Table 4). There were no significant differences among fish from the four stations in San Francisco Bay (Kruskal-Wallis test, H=1.81, p=0.613). There was a significant difference between fish from the two stations on the outer coast (Mann-Whitney U test, U=252, p=0.043); fish from Santa Cruz had higher detached MN frequencies than fish from the Russian River. The frequency of attached micronuclei was nearly significantly different among the sites (Kruskal Wallis test, H=10.54, p=0.061) (Table 4).

The frequency of detached micronuclei was significantly correlated to the frequency attached micronuclei among starry flounders from both San Francisco Bay (Spearman r_s=0.198, 0.02<p<0.05) and the outer coast (Spearman r_s=0.526, p<0.001) (Figure 9).

D. Nuclear Pleomorphism

Nuclear pleomorphism was observed in starry flounder at every station; fish with the severest cases (more than 50% of erythrocytes pleomorphic) were caught at the Berkeley, Oakland, and San Pablo Bay sites (Table 5; Figures 2,3, and 4).

There was a significant difference in the incidence of nuclear pleomorphism among fish from the six stations (X =11.07, p<0.001) (because of the high frequency of zeros for class 3 pleomorphism, classes 2 and 3 were combined). Fish from the Russian River had the lowest incidence of pleomorphic nuclei (Table 5). With those fish removed, the incidence of pleomorphic nuclei was not significantly different at the remaining stations (X =2.42, df=4, p>0.50).

E. Contaminant Body Burdens

Contaminant data expressed on a lipid weight basis (g/g lipid) were not normally distributed (Kolmogorov-Smirnov test, $p<0.05$) and variances were not homogeneous (Bartlett test, $p<0.05$) among the sites. The data were transformed to logarithms [$\log_{10}(x+1)$] to reduce the effect of the few fish with relatively high contaminant body burdens.

In general, starry flounder from central San Francisco Bay (Berkeley and Oakland) had the highest concentrations of organic contaminants, fish from the outer coast (Russian River and Santa Cruz) had the lowest, and fish from the northern part of the bay (San Pablo Bay and Vallejo) were intermediate (Table 6). Total organic contaminant concentration (sum of the 14 contaminants on a lipid weight basis) was not correlated with fish size for either females ($r_s=0.225$, $p>0.10$) or males ($r_s=0.396$, $p>0.10$) from San Francisco Bay.

Univariate analysis revealed strong correlations among the PCB congeners, DDE, and DDD; there were weaker but significant correlations among DDD, chlordane, dieldrin, and lindane (Table 7). The univariate pattern of normalized mean concentrations was similar among fish collected from the six sites (Figure 10).

The contaminant correlation matrix was examined by principal components analysis (PCA) for patterns among the sites. The first two principal components accounted for nearly 60% of the variance in the total data set (Table 8). The eight PCB congeners and p,p'-DDE were more heavily weighted on factor 1 and accounted for 46.5% of the variance (Figure 11). Dieldrin, chlordane, lindane and heptachlor-epoxide were more heavily weighted on factor 2 and accounted for 13.1% of the variance. The DDT isomer, p,p'-DDD was weighted similarly on both factors.

The principal component scores for individual fish revealed differences in the degree of contamination, but not patterns of contamination, among the sites (Figure 12). [Russian River female MSB 5227 was an outlier (factor 1 score 6.94 and factor 2 score -2.55) and was deleted from all factor plots.] Starry flounders from Berkeley and Oakland had the highest scores on both principal components. Fifty percent of the fish from the central bay (n=34) had positive scores on factors 1 and 2. Only 11% of the fish from the northern bay (n=28) and none of the fish from the outer coast (n=18) had positive scores on both factors. Fish from northern San Francisco Bay and the outer coast had the lowest scores. Fifty percent of the fish from the northern bay and 56% of the fish from the outer coast had negative scores on both factors.

There was no significant difference between prinicpal component scores of males or females from stations within San Francisco Bay (t-test, $p>0.20$). There were no significant correlations between scores and size for either males or females from stations within San Francisco Bay (Spearman r_s, $p>0.10$) or from the outer coast (Spearman r_s, $p>0.10$) (Figure 13).

F. Micronuclei and Contaminant Body Burdens

Micronuclei frequencies were not correlated with liver concentrations of organic contaminants (Table 9) or with prinicpal component scores of factor 1 (Spearman r_s, $p>0.50$) or factor 2 (Spearman r_s, $p>0.20$) (Figure 14). But MN frequencies of fish with positive scores on both factors (mean=2.5, SD=2.29, n=19) were significantly greater than fish with negative scores on both factors (mean=1.2, SD=2.10, n=34) (Mann-Whitney test, U=405, $p=0.013$).

IV. DISCUSSION

Micronucleus frequencies were significantly higher among starry flounders collected in San Francisco Bay than among fish collected on the outer coast. A significantly higher proportion of fish from the outer coast had no visible micronuclei comparted to fish from the bay. Within San Francisco Bay, there were no significant differences in MN frequencies. Fish with high MN frequencies also had a higher incidence of misshapen nuclei (nuclear pleomorphism).

Micronucleus frequencies were not significantly different among male starry flounders from the bay and the outer coast. MN frequencies were significantly higher among females from the bay than among females from the outer coast. MN frequencies were positively correlated with fish size among females, but not among males, from San Francisco Bay stations.

Two types of micronuclei were quantified in this study: detached and attached. Although attached micronuclei do not conform to the classical definition of a micronucleus in mammalian cells (Schmid 1976), they are quantifiable manifestations of genotoxicity in lower vertebrates (Hoofman and de Raat 1982, Hose et al. 1986) and were included in the MN counts. The frequencies of detached and attached micronuclei were positively correlated. Detached MN frequencies were significantly higher among bay fish than among fish from the outer coast; attached MN frequencies were nearly significantly different.

The attached micronuclei in starry flounder were morphologically different from micronuclei described for white croaker (Hose et al. 1986) and Eastern mudminnow

(Hoofman and de Raat 1982). In the croaker and mudminnow, attached MN appeared as a chromatin ball on a thin stalk. MN on thin stalks were not observed in starry flounder. Instead, thicker projections, many with a terminal clump of chromatin, were common.

The composition of organic contaminants in the livers of starry flounders was similar among the six sites. The main difference was the level of contamination: fish from central San Francisco Bay had higher mean levels than fish from northern San Francisco Bay which had higher mean levels than fish from the outer coast. Contaminant concentrations were not correlated with fish size or sex.

Some individuals at each site had relatively low contaminant concentrations (approximately 30% of the central bay fish and 50% of the fish at the remaining sites). Males and females were present in this group and, their size distributions were not significantly different from fish with the higest levels of contamination.

The lack of compositional differences among fish and the presence of fish with low organic contaminant concentrations at all of the sites suggests that: 1) chlorinated hydrocarbons have spread throughout the system and to the outer coast and, 2) some starry flounders are moving throughout the bay and from the bay to the outer coast. Phillips and Spies (ms) reviewed the data on chlorinated hydrocarbons in sediments and biota and concluded that some compounds, particularly PCBs, are widespread in the San Francisco estuarine system.

Micronucleus frequencies were not directly related to organic contaminant concentrations. However, fish with the highest scores from the prinicpal component analysis had significantly higher MN frequencies compared to fish with the lowest scores. The results are consistent with the non-clastogenic (chromosome breaking) properties of DDTs and PCBs (Heddle et al. 1983). The mutagenic potential of their metabolites is not adequately known. It may be that other potent clastogens (e.g., chlorinated benzenes and polycyclic aromatic hydrocarbons) not measured in this study but present in San Francisco Bay (Phillips and Spies, ms) would be more strongly correlated with MN frequency.

V. LITERATURE CITED

Elliot, J.M. 1979. Some methods for the statistical analysis of samples of benthic invertebrates. Freshwater Biological Assoc., Scientific Publ. No. 25, 156 p.

Eganhouse, R., B. Gould, P. Sherblom, and C. Phinney. 1987. Analytical procedures for the congener-specific determination of chlorobiphenyls in biological tissues. Report for Contract No. 85-122 to U.S. E.P.A. University of Massachusetts, Boston, 79 pp.

Heddle, J.A., M. Hite, B. Kirkhart, K. Mavourin, J.T. MacGregor, G.W. Newell, and M.F. Salamone. 1983. The induction of micronuclei as a measure of genotoxicity. Mutation Res. 123:61-118.

Hoofman, R.N. and W.K. de Raat. 1982. Induction of nuclear anomalies (micronuclei) in the peripheral blood erythrocytes of the eastern mudminnow (Umbra pygmaea) by ethyl methanesulphonate. Mutation Res. 104:147-152.

Hose, J.E., J.N. Cross, S.G. Smith, and D. Diehl. 1987. Elevated circulating erythrocyte micronuclei in fishes from contaminated sites off southern California. Mar. Environ. Res. 22:167-176.

Hose, J.E., J.B. Hannah, H.W. Puffer, and M.L. Landolt. 1984. Histologic and skeletal abnormalities in benzo(a)pyrene-treated rainbow trout alevins. Arch. Environ. Contam. Toxicol. 13:675-684.

MacGregor, J.T. C.M. Wehr, and D.H. Gould. 1980. Clastogen-induced micronuclei in peripheral blood erythrocytes: The basis of an imporved micronucleus test. Environ. Mutagen. 2:509-514.

Phillips, D.J.H. and R.B. Spies. ms. Chlorinated hydrocarbons in the San Francisco estuary.

Pielou, E.C. 1984. The interpretation of ecological data. John Wiley & Sons, New York, 263 pp.

Schmid, W. 1976. The micronucleus test for cytogenetic analysis. pp. 31-53, In: Chemical mutagens: Principles and methods for their detection. Vol. 6. A. Hollander (ed.). Plenum Press, New York.

Spies, R.B., D.W. Rice, M. Prieto, and P. Thomas. 1988. The response of the reproductive system in Platichthys stellatus to organic contamination in San Francisco Bay. Draft Final Report for Contract No. 40-ABNL-5759 to NOAA. Lawrence Livermore Laboratories, Livermore California, 77 pp.

Wilkinson, L. 1986. SYSTAT: The system for statistics. SYSTAT, Inc. Evanston, Illinois (pages unnumbered).

Table 1. Summary of starry flounder size data. SL=standard length; SD=one standard deviation; VAR=variance; N=sample size.

		FEMALES		
STATION	SL	SD	VAR	N
Berkeley	33.4	6.7	44.6	22
Oakland	32.6	7.2	51.2	18
San Pablo Bay	27.9	3.8	14.8	7
Vallejo	34.3	11.6	134.3	9
Santa Cruz	44.1	3.2	10.7	14
Russian River	36.6	4.9	23.7	15
		MALES		
STATION	SL	SD	VAR	N
Berkeley	26.1	3.7	13.7	17
Oakland	25.3	2.0	4.1	3
San Pablo Bay	25.5	3.3	10.6	17
Vallejo	24.9	3.4	11.5	7
Santa Cruz		no males collected		
Russian River	34.8	2.8	7.9	9

Table 2. Starry flounder micronucleus frequencies per 1000 erythrocytes. MN^{o}/oo=mean; SD=one standard deviation; VAR=variance; N=sample size; PROP=proportion of zeros.

STATION	MN^{o}/oo	SD	VAR	N	PROP
Berkeley	1.9	2.33	5.41	42	.119
Oakland	1.5	2.06	4.24	23	.348
San Pablo Bay	1.3	1.19	1.42	29	.172
Vallejo	2.2	1.49	2.23	22	.091
Santa Cruz	0.6	0.77	0.60	13	.462
Russian River	0.4	0.74	0.54	29	.655

Table 3. Starry flounder male and female micronucleus frequencies per 1000 erythrocytes. MN^{o}/oo=mean; SD=one standard deviation; VAR=variance; N=sample size.

		FEMALES		
STATION	MN^{o}/oo	SD	VAR	N
Berkeley	2.3	2.8	8.1	22
Oakland	1.6	2.2	5.0	18
San Pablo Bay	1.1	1.6	2.5	7
Vallejo	1.8	2.1	4.6	9
Santa Cruz	0.7	0.8	0.6	13
Russian River	0.1	0.3	0.1	15

		MALES		
STATION	MN^{o}/oo	SD	VAR	N
Berkeley	1.6	1.6	2.5	17
Oakland	1.2	1.6	2.5	3
San Pablo Bay	1.5	1.0	1.1	17
Vallejo	1.1	0.8	0.6	7
Santa Cruz		no males collected		
Russian River	0.6	0.6	0.4	9

Table 4. Starry flounder detached and attached micronucleus frequencies per 1000 erythrocytes. MN^{o}/oo=mean; SD=one standard deviation; VAR=variance; sample sizes in Table 2.

	DETACHED		
STATION	MN^{o}/oo	SD	VAR
Berkeley	0.7	0.74	0.54
Oakland	0.5	0.77	0.59
San Pablo Bay	0.7	0.74	0.54
Vallejo	0.9	1.24	1.53
Santa Cruz	0.3	0.33	0.11
Russian River	0.1	0.37	0.14

	ATTACHED		
STATION	MN^{o}/oo	SD	VAR
Berkeley	1.2	2.17	4.71
Oakland	1.0	1.72	2.97
San Pablo Bay	0.6	0.77	0.59
Vallejo	0.5	0.50	0.25
Santa Cruz	0.4	0.58	0.34
Russian River	0.3	0.43	0.19

Table 5. Percent of starry flounder with pleomorphic nuclei(NP). Each fish was rated: 1 if <5% of erythocytes were pleomorphic; 2 if 5-50% of erythocytes were pleomorphic; or 3 if >50% of erythocytes were pleomorphic. N=sample size.

	Percent with NP rating			
STATION	1	2	3	N
Berkeley	55	29	17	42
Oakland	65	22	13	23
San Pablo Bay	66	31	3	29
Vallejo	64	36	0	22
Santa Cruz	77	23	0	13
Russian River	97	3	0	29

Table 6. Summary of starry flounder liver contaminant concentrations (g/kg wet weight) by site. Data are means and one standard deviation (in parentheses); N=sample size. DDT is the sum of p,p'-DDE and p,p'-DDD. Pesticides is the sum of chlordane, dieldrin, heptachlorepoxide, and lindane. PCB is the sum of PCB congeners 44, 101, 118, 128, 138, 153, 180, and 206.

	N	DDT	Pesticides	PCB
Berkeley	18	202 (145)	50 (32)	422 (306)
Oakland	16	189 (120)	47 (34)	438 (312)
San Pablo Bay	14	161 (90)	48 (32)	110 (53)
Vallejo	14	160 (112)	30 (17)	110 (73)
Russian River	14	152 (245)	34 (44)	152 (253)
Santa Cruz	4	73 (73)	119 (142)	89 (63)

Table 7. Pearson correlation matrix among 14 organic contaminants (wet weight concentrations) measured in starry flounder livers (n=80). Correlation coefficients (r) greater than or equal to 0.220 are significant at p=0.05; r<0.220 have been replaced by < in the table.

	ppDDE	ppDDD	Hepte	Linda	Chlor
ppDDD	0.341				
Heptachlorepoxide	<	<			
Lindane	<	0.394	<		
Chlordane	<	0.521	<	0.418	
Dieldrin	<	0.288	<	<	<
PCB44	0.492	<	<	<	<
PCB101	0.525	0.632	<	0.257	<
PCB118	0.491	0.645	<	0.349	<
PCB128	0.270	<	<	<	<
PCB138	0.528	0.645	<	0.306	<
PCB153	0.616	0.580	<	0.283	<
PCB180	0.392	0.577	<	0.276	<
PCB206	0.540	<	<	<	<

	Dield	PCB44	PCB101	PCB118	PCB128
PCB44	<				
PCB101	<	<			
PCB118	<	0.244	0.842		
PCB128	<	0.231	0.271	0.308	
PCB138	<	0.232	0.896	0.844	0.247
PCB153	<	0.271	0.884	0.826	0.265
PCB180	<	<	0.821	0.712	0.224
PCB206	<	<	0.387	0.423	<

	PCB138	PCB153	PCB180
PCB153	0.971		
PCB180	0.916	0.917	
PCB206	0.445	0.474	0.378

Table 8. Variable loadings (coefficients) for the first two principle components and percent of the total variance in the data set that they explain.

	Factors	
	1	2
PCB153	0.923	0.227
PCB101	0.906	0.172
PCB138	0.885	0.305
PCB118	0.879	0.265
PCB180	0.839	0.280
ppDDE	0.816	-0.163
PCB206	0.815	-0.157
PCB44	0.750	-0.340
PCB128	0.630	-0.183
ppDDD	0.530	0.598
Dieldrin	0.002	0.667
Chlordane	0.049	0.648
Lindane	-0.003	0.254
Heptachlorepoxide	-0.009	0.161
Percent of total variance explained	46.5%	13.1%

Table 9. Spearman rank correlation coefficients between micronucleus frequency and contaminant concentrations. N=sample size. DDT is the sum of p,p'-DDE and p,p'-DDD. Pesticides is the sum of chlordane, dieldrin, heptachlorepoxide, and lindane. PCB is the sum of PCB congeners 44, 101, 118, 128, 138, 153, 180, and 206. r(.05) is the correlation coefficient significant at p=0.05 for the given sample size.

	N	DDT	Pest	PCB	r(.05)
All fish	76	0.152	-0.018	0.155	0.229
Bay fish	59	0.110	-0.015	0.115	0.261
Bay females	37	0.065	-0.207	0.066	0.335
Bay males	20	0.203	0.463	0.136	0.472

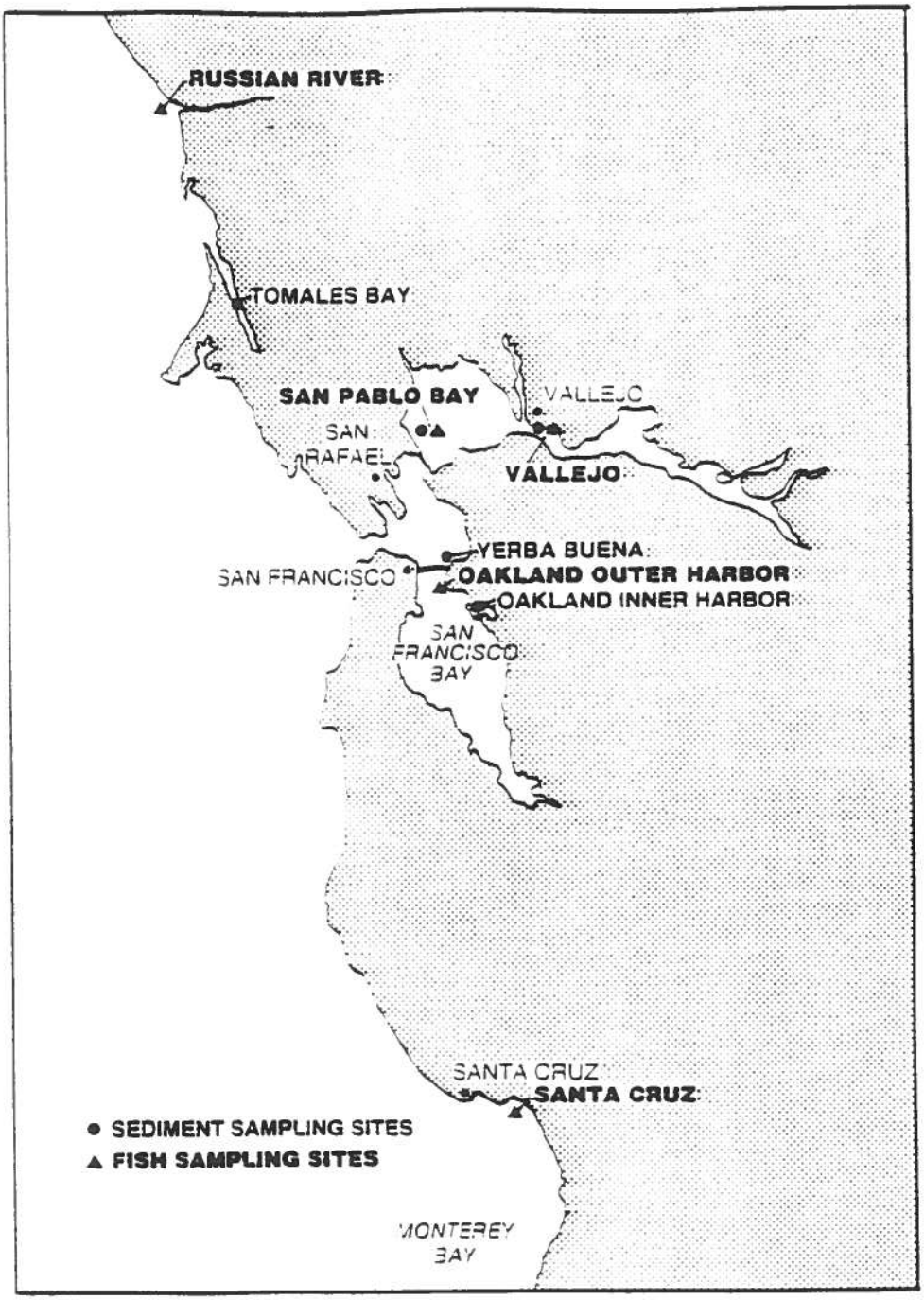

Figure 1. Map of the study sites

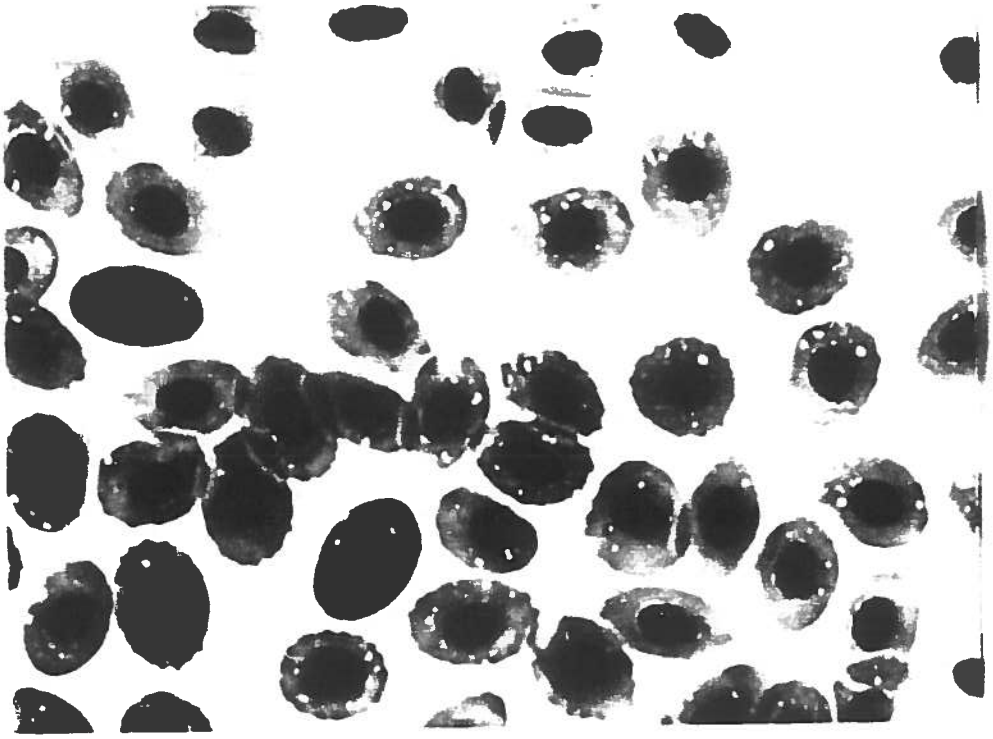

Figure 2. Erythrocytes of starry flounder from the Russian River. Note the uniform elliptical appearance of the nuclei. White circles in the cytoplasm are artifacts. 5600 X. May Grunwald-Giemsa.

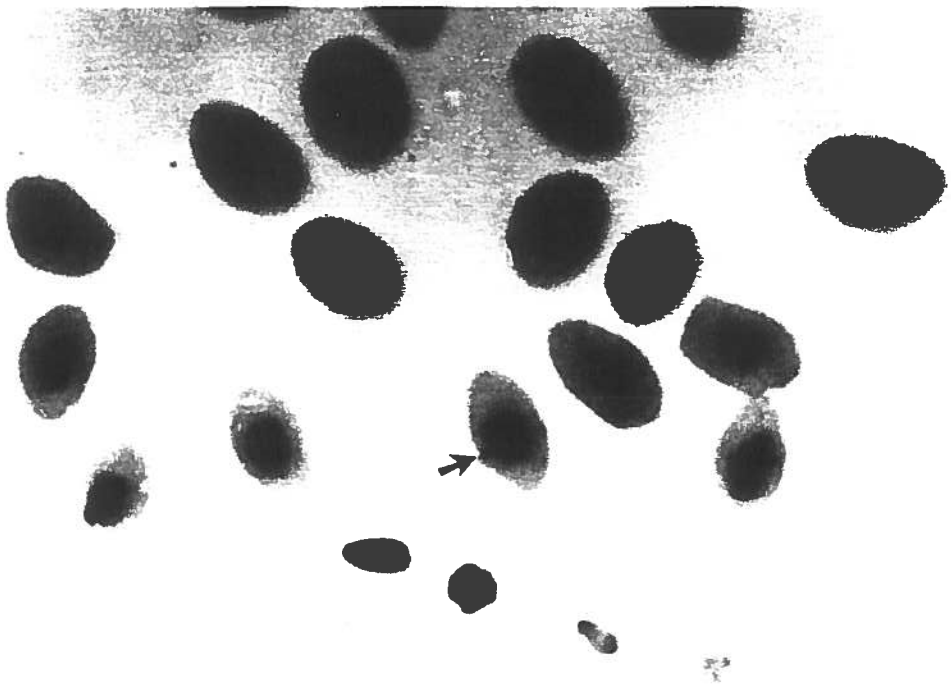

Figure 3. Erythrocytes of starry flounder from Oakland. Note the high incidence of nuclear pleomorphism. One erythrocyte contains a detached micronucleus (arrow). Small, dark spots in cytoplasm are bacteria. 5600 X. May Grunwald-Giemsa.

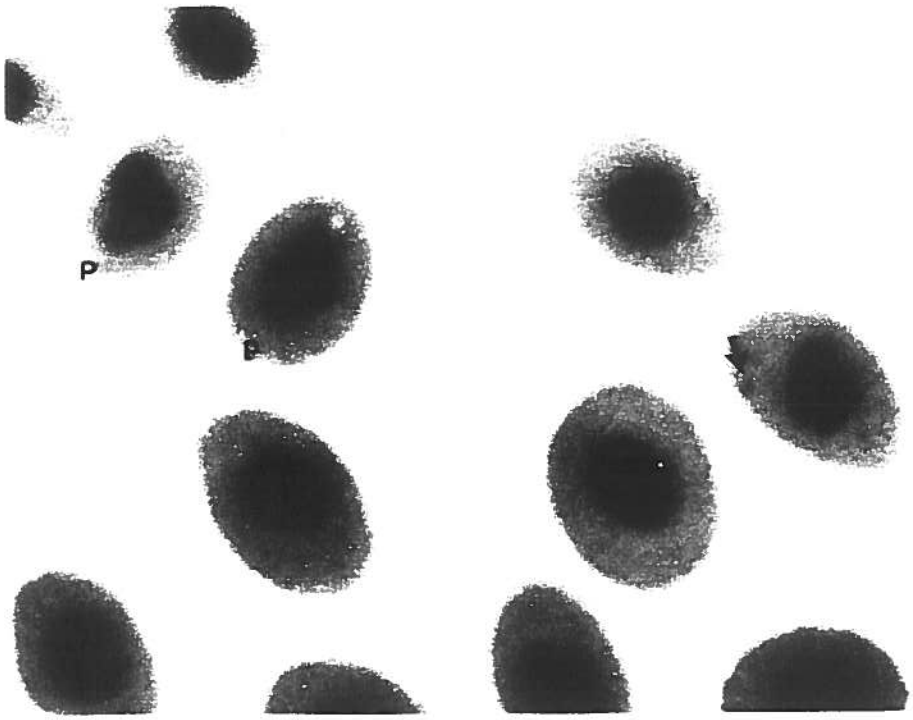

Figure 4. Erythrocyte from Figure 3 containing detached micronucleus (arrow). Nuclei of three erythrocytes are pleomorphic (P). 9450 X. May Grunwald-Giemsa.

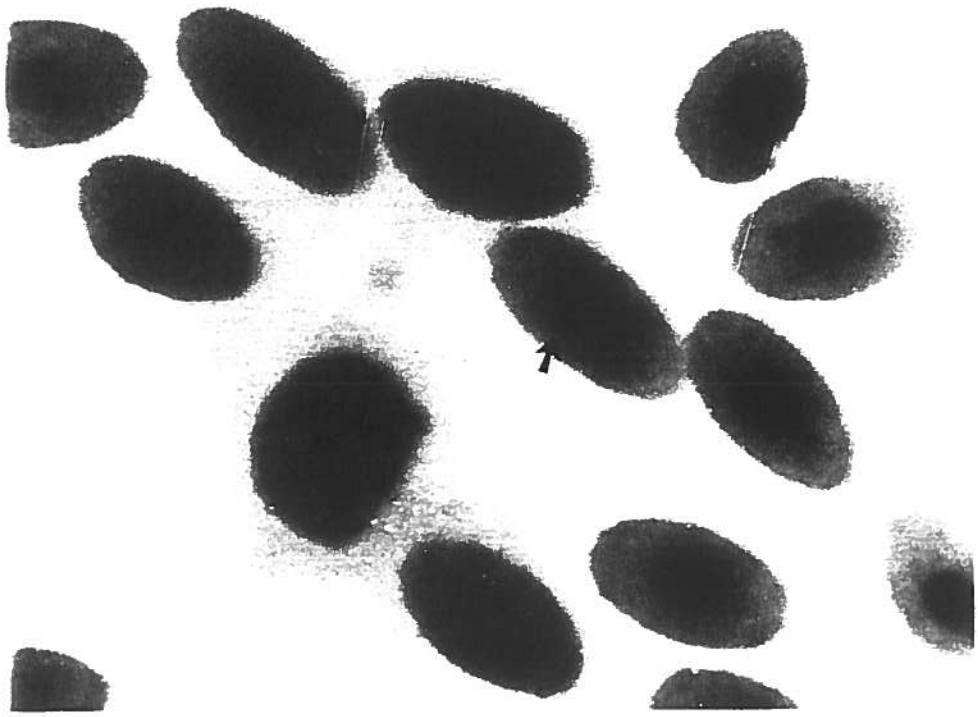

Figure 5. Erythrocyte of starry flounder from San Pablo Bay with an attached micronucleus (arrow). Many nuclei are pleomorphic. 9450 X. May Grunwald-Giemsa.

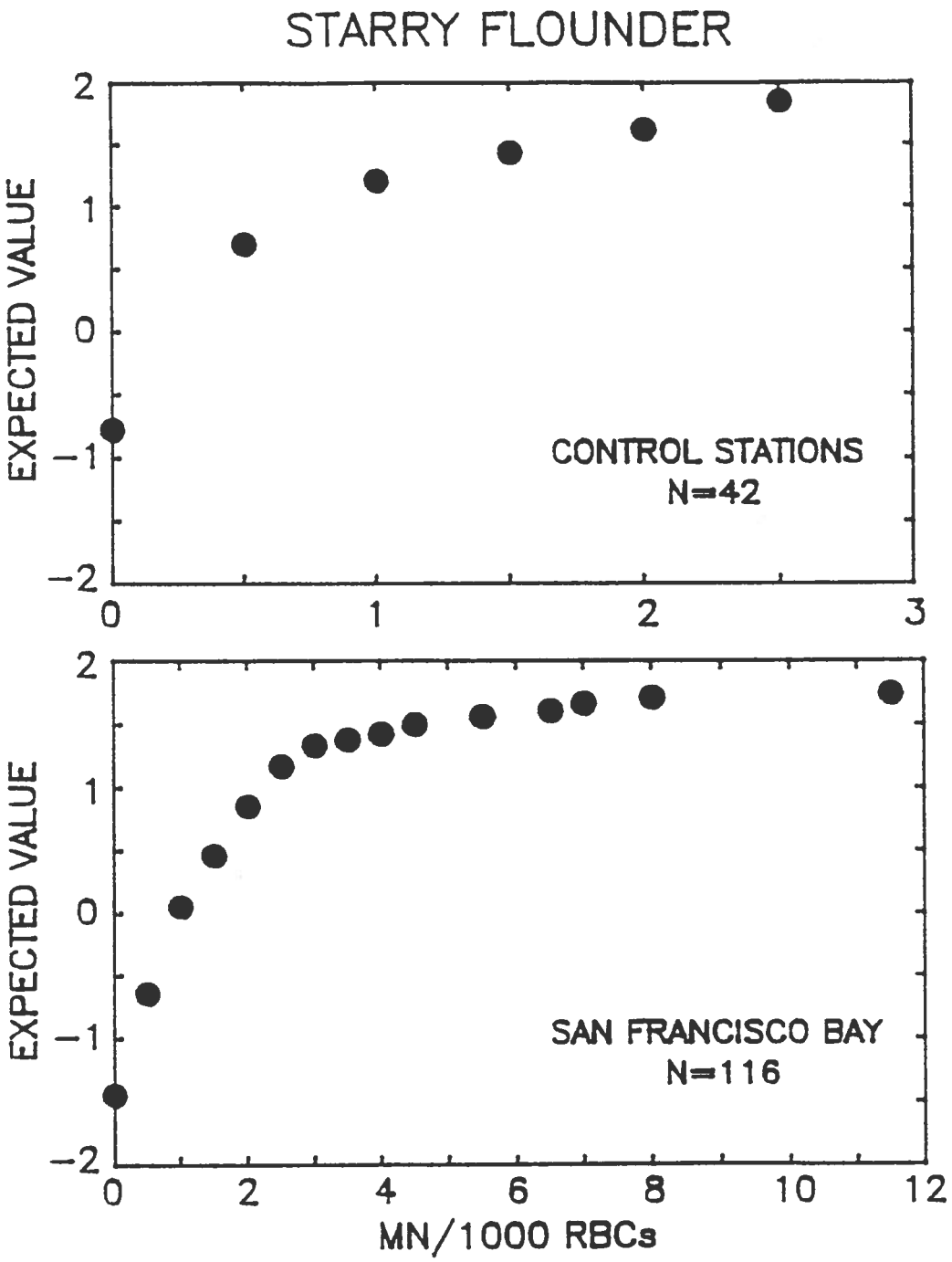

Figure 6. Normal probability plots of untransformed micronuclei counts of starry flounder collected in San Francisco Bay and at control stations on the outer coast.

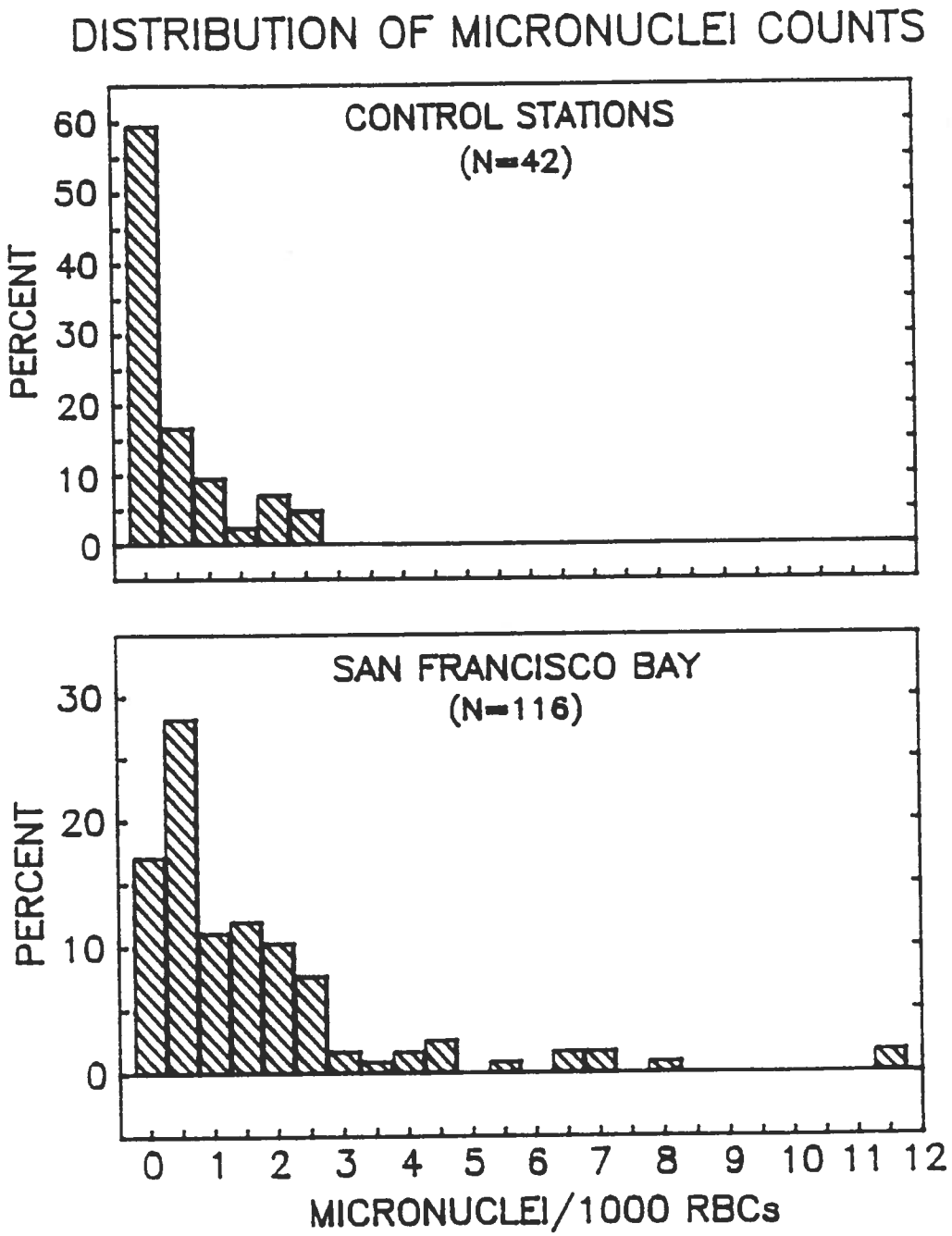

Figure 7. Percent frequency distribution of micronucleus counts of starry flounder collected in San Francisco Bay and at control stations on the outer coast.

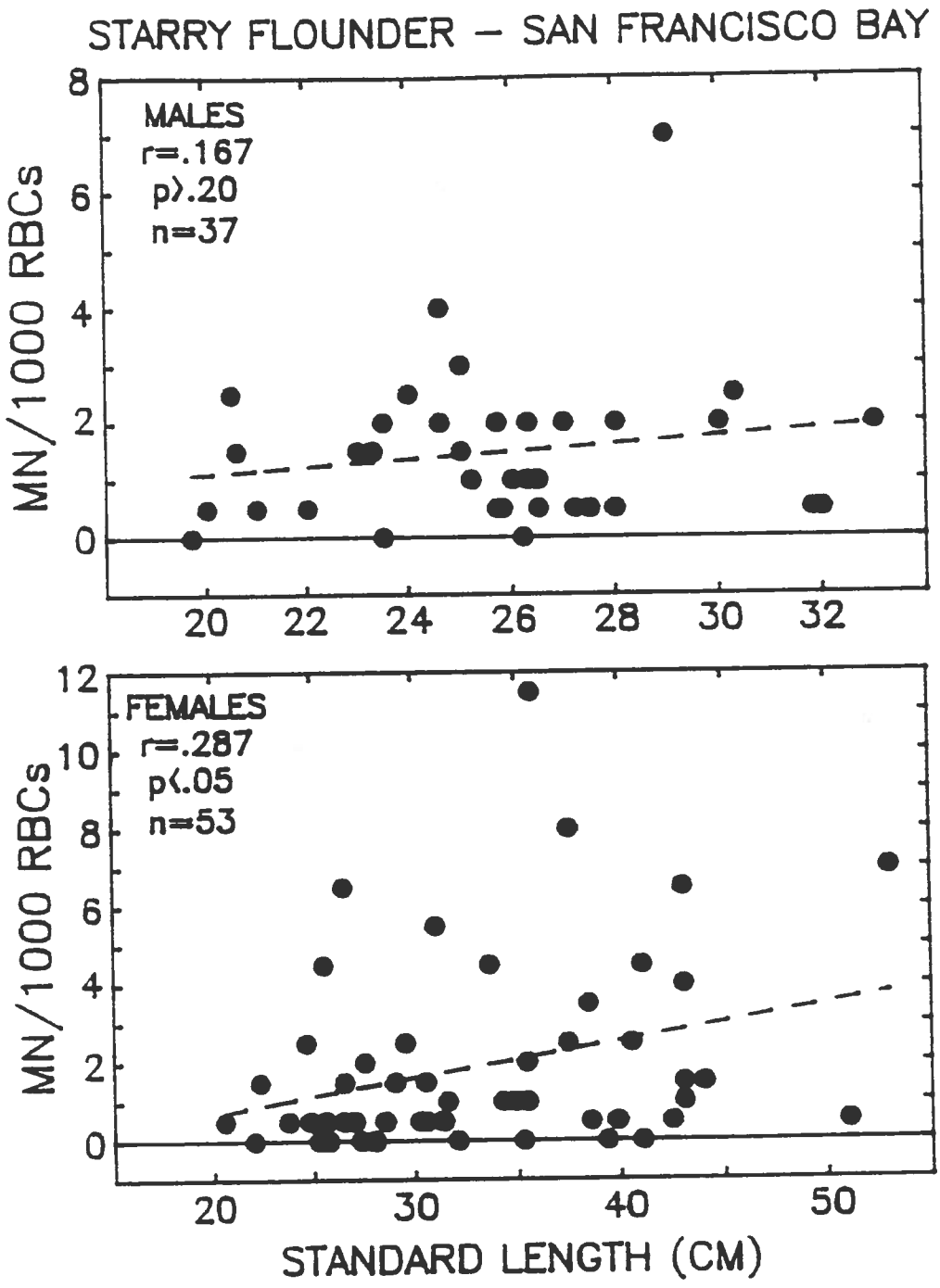

Figure 8. The relationship between micronucleus frequency and size for male and female starry flounders from stations within San Francisco Bay.

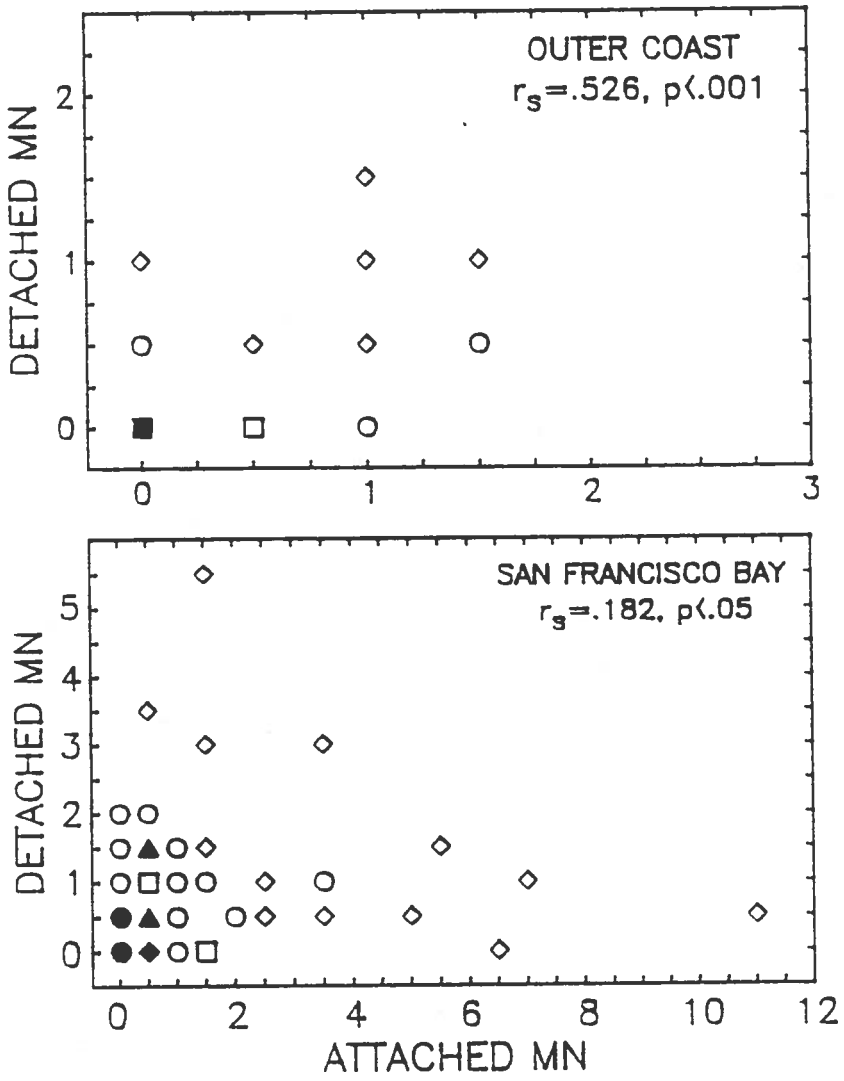

Figure 9. The relationship between detached and attached micronucleus frequency of starry flounder collected in San Francisco Bay and at control stations on the outer coast. Symbols reprsent number of fish. Filled square more than 20; filled circle 16-20; filled diamond 11-15; filled triangle 7-10; open square 4-6; open circle 2-3; open diamond 1.

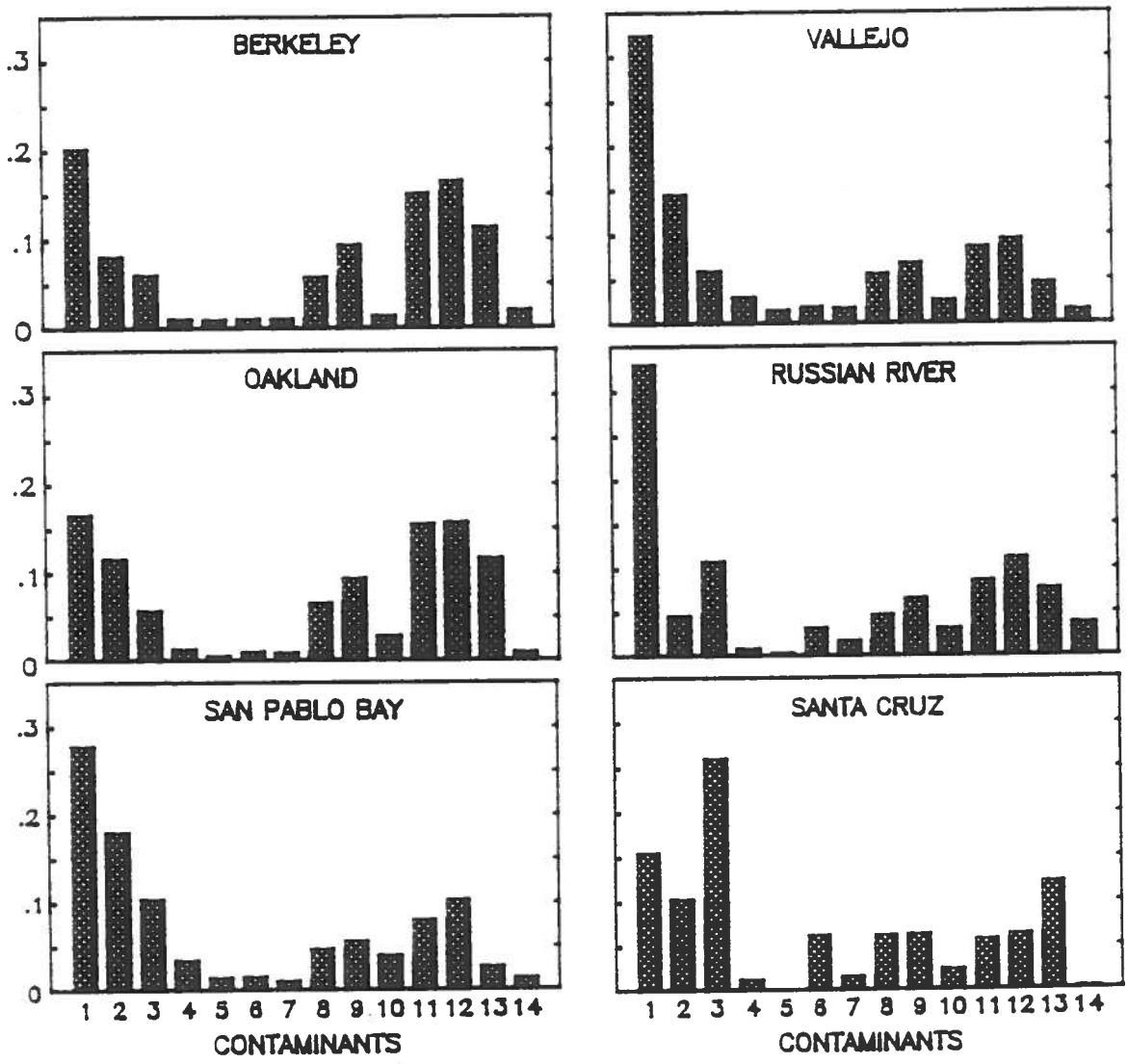

Figure 10. Normalized mean chlorinated hydrocarbon concentrations. Contaminant numbers on the abscissa are: 1) p,p'-DDE; 2) p,p'-DDD; 3) dieldrin; 4) chlordane; 5) lindane; 6) heptachlorepoxide; 7) PCB44; 8) PCB101; 9) PCB118; 10) PCB128; 11) PCB138; 12) PCB153; 13) PCB180 and 14) PCB206.

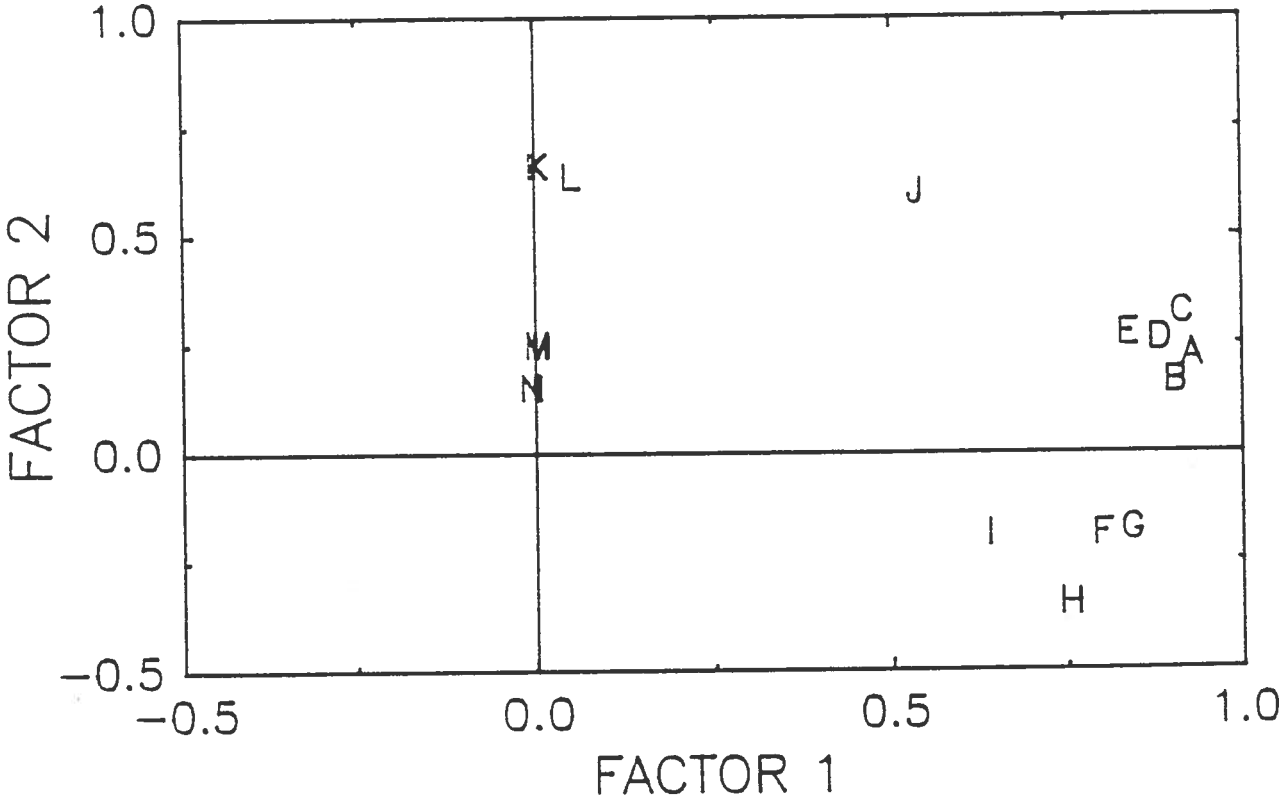

Figure 11. Contaminant loadings on the first two principal components (factors). Contaminants are: A) PCB153; B) PCB101; C) PCB138; D) PCB118; E) PCB180; F) p,p'-DDE; G) PCB206; H) PCB44; I) PCB128; J) p,p'-DDD; K) dieldrin; L) chlordane; M) lindane; N) Heptachlorepoxide.

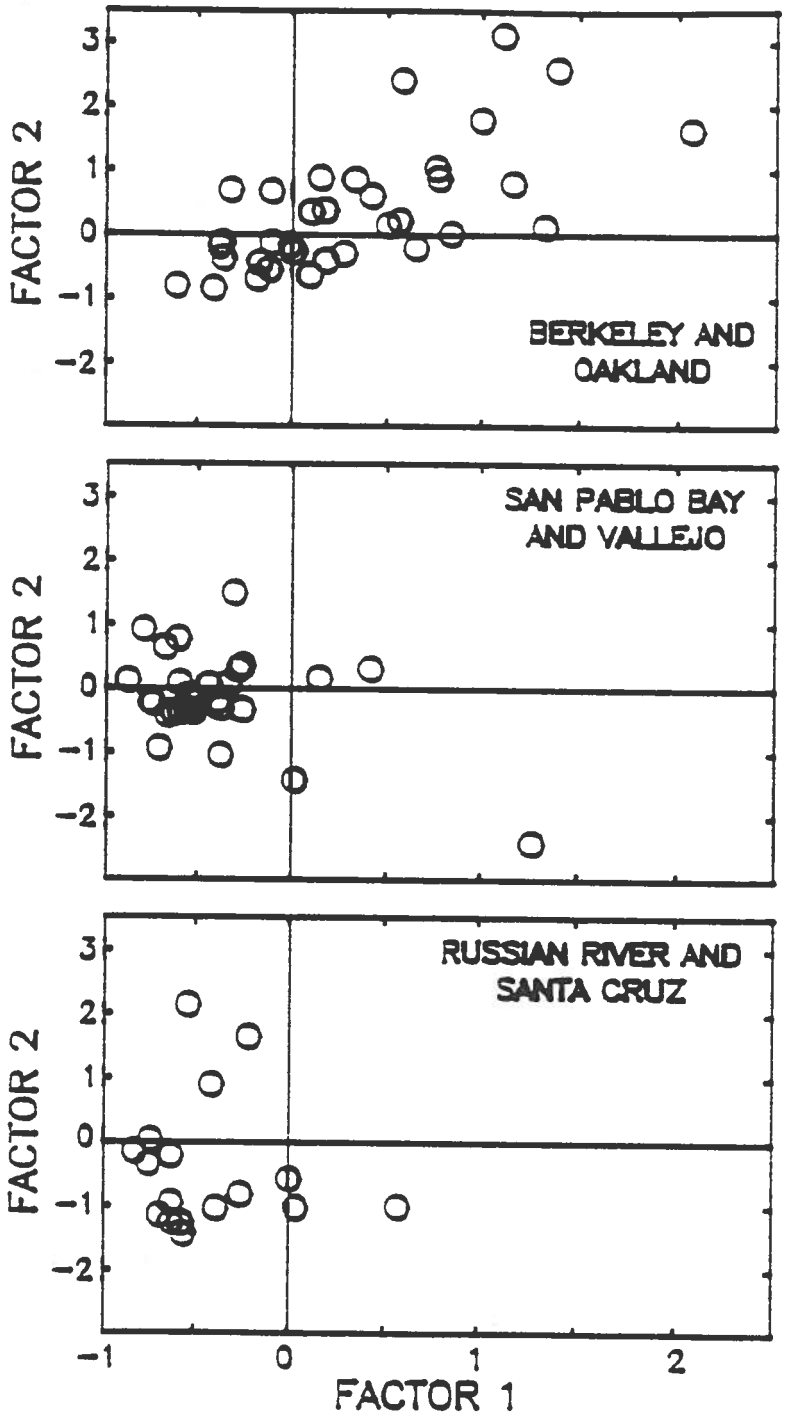

Figure 12. Principal component scores of individual starry flounder combined by location.

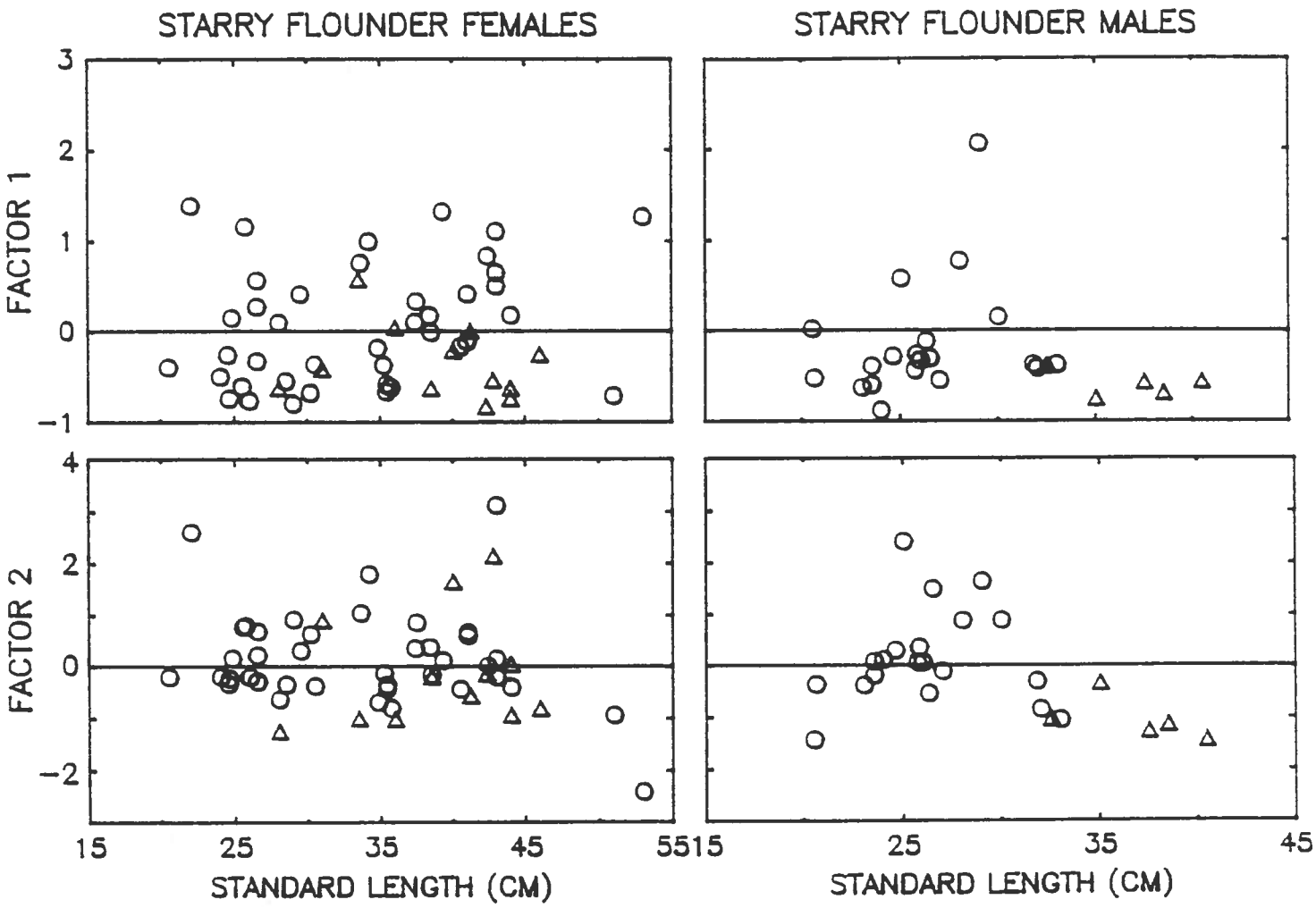

Figure 13. Principal component scores versus size of male and female starry flounder from San Francisco Bay (circles) and the outer coast (traingles).

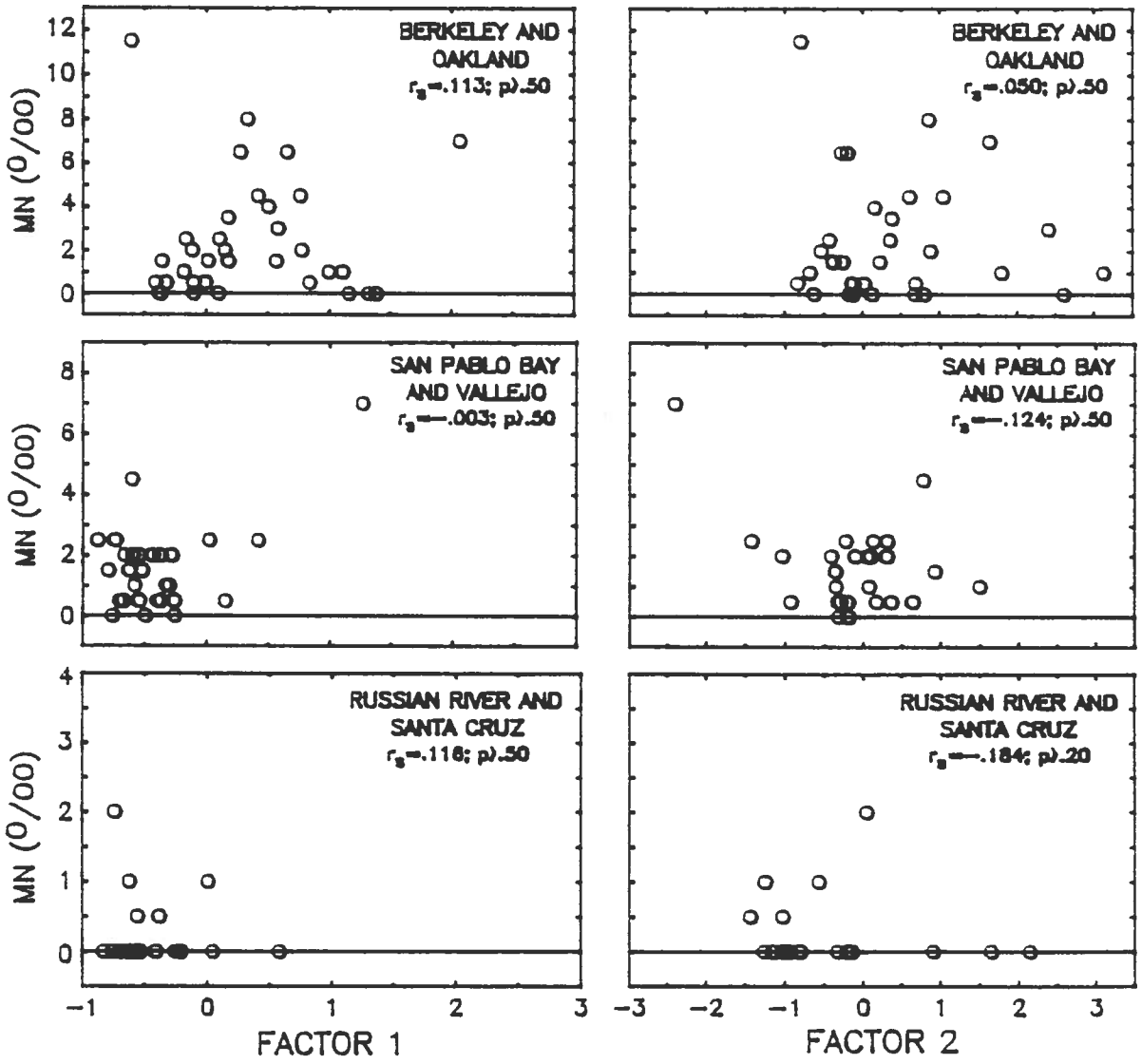

Figure 14. Principal component scores versus frequency of micronuclei of starry flounder combined by location.