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Edwin M. Perkins, and Richard W. Gossett.

HISTOLOGICAL CONDITION OF FISH LIVERS

Examination of liver tissue can give an early indication of changes in the health of fish. This study determined the histological condition of fish livers from a wide range of geographical areas including Santa Monica Bay, Palos Verdes, San Diego, San Clemente Island, and Cortes Bank. Results obtained using a subjective rating system showed that most fish had extensive hepatocyte (liver cell) hypertrophy (increased size) and vacuolation. These conditions appeared to be related to liver DDT concentrations. Closer examination using an objective histological measurement procedure showed that hepatocyte hypertrophy correlated more closely with oxygenated metabolites of chlorinated hydrocarbons than with parent compounds. These results indicate that the state of health of fish may be affected by chlorinated hydrocarbons in widespread areas of the southern California Bight.

MATERIALS AND METHODS

Sampling

Fish were collected from nine sites in coastal southern California. The collection sites included the three examined in detail for contaminants by Brown et al. (this volume). These represented a gradation of contamination from highly contaminated Palos Verdes (PV) Station 7-3, through moderately contaminated Santa Monica Bay (SMB) Station 6-4, to less contaminated SMB Station 2-3. Selected fish species were also examined from the following locations: San Clemente Island, approximately 100 km offshore; Cortes Bank, approximately 170 km offshore; the San Diego SD-4 Point Loma outfall station; the SD-1 reference site near Mission Bay; and San Mateo Point and Torrey Pines, both north of SD-1 (see Figure 1 in Schafer, this volume).

Longspine combfish *(Zaniolepis latipinnis)*, Pacific sanddab *(Citharichthys sordidus)*, yellowchine sculpin *(Icelinus quadriseriatus)*, California scorpionfish *(Scorpaena guttata)*, and California tonguefish *(Symphurus atricauda)* were collected by otter trawl from the San Clemente Island, Palos Verdes, Santa Monica Bay, San Diego, San Mateo Point, and Torrey Pines stations. Scorpionfish from Cortes Bank and kelp bass *(Paralabrax clathratus)* from San Mateo Point and Palos Verdes were collected by hook and line. Tissue dissections were made either on board ship or back in the laboratory. All dissections at the laboratory were performed on the same day as collection, except for scorpionfish from Cortes Bank which were dissected the following day. Samples for histology were collected during the following time periods: PV 7-3, June 1983; SMB 6-4, July and August 1983; SMB 2-3, September 1983; San Clemente Island and Cortes Bank, January 1984; San Mateo Point, May 1984; SD-1 and -4, June 1984; and Torrey Pines, August 1984. Samples for chemistry were collected at the same time as histology samples, except for PV 7-3, SMB 6-4, and SMB 2-3 chemistry samples which were collected in December 1982 (Brown et al., this volume).

Liver samples for histology were placed in 10% buffered formalin for approximately 24 hours. Samples were rinsed in running water for 6 hours and stored in 70% ethyl alcohol until needed. Later, samples were dehydrated with ethyl alcohol, cleared in xylene, vacuum infiltrated, embedded in paraffin, sectioned with a rotary microtome at 6 µm, and mounted on glass slides. Slides were stained with Harris's hematoxylin and eosin (Humason 1977), cover-slipped, and examined using either a subjective rating system or an objective measurement method.

Samples for chemical analysis were placed in kilned (600°C) glass jars sealed with teflon-lined lids. Samples were frozen at -20°C until analyzed. Analytical procedures were the same as those described in Brown et al. (this volume) and Gossett et al. (this volume).

Subjective Rating Methods

A blind rating system was used in which each slide was assigned a random code number, masking the identity of the collection site. Then slides were examined for hepatocellular alterations and alterations in hepatic foci, nuclei, and cholangiols. A subjective rating of 0, 1, 2, or 3, indicating degree of severity, was assigned for each condition in each tissue sample. For example, in liver tissue a rating of 0 indicated the absence of hepatocyte vacuolation; 1 indicated vacuolar occlusion of about one-third of hepatocyte cytoplasm; 2 indicated vacuolar occlusion of about two-thirds of cytoplasm, or severe hepatocellular vacuolation (Malins 1977); while 3 indicated complete vacuolar occlusion of cytoplasm (Haensly et al. 1982).

Objective Measurement Methods

Objective measurement methods for determining degree of hepatocyte hypertrophy were developed and compared to the subjective rating method using a sample of 25 scorpionfish collected from Cortes Bank. This sample was considered to be optimal for this purpose since it was a large sample of a single species with a wide range of liver cell sizes and contaminant concentrations, but without seasonal effects.

To determine the number of microscope fields which needed to be examined, five cells were measured from 20 randomly selected fields per slide for each scorpionfish at a magnification of 1000x using a Leitz SM-LUX light microscope equipped with a calibrated ocular micrometer. The cumulative variance of each of the measurements was plotted against the cumulative number of fields measured. For example, for hepatocyte diameter, the variance was determined for one field of five cells, for two fields of 10 cells, for three fields of 15 cells, and so on for 20 fields. The number of fields to be measured was chosen where the plot of variance against number of fields stabilized. This number of microscopic fields was then used to determine liver cell size, melanin macrophage center size, and numbers of melanin macrophage centers in scorpionfish and kelp bass. For all other species, numbers and size of melanin macrophage centers were determined using an arbitrarily chosen number of five fields per slide.

Statistical Analysis

The Kruskal-Wallis analysis of variance was applied to test differences between samples analyzed using subjective ratings. The Mann-Whitney U test was applied to test differences between samples analyzed using measurements (Zar 1974). The correlation between the subjective rating method and the measurement method was determined with Spearman's rank correlation coefficient. The correlations between liver cell size and mass and concentration of parent chlorinated hydrocarbons and oxygenated metabolites were determined using parametric correlation coefficients.

RESULTS AND DISCUSSION

Subjective Ratings

Results of subjective ratings of liver revealed the presence of hypertrophy and vacuolation in hepatocytes of California scorpionfish, California tonguefish, yellowchin sculpin, and Pacific sanddab from all stations in the southern California Bight (Table 1; Figure 1). Statistical analysis using the Kruskal-Wallis test indicated that, except for

Table 1. Subjective histological ratings of liver cell vacuolation and hypertrophy (size) and objective counts and measurements of melanin macrophage centers in four species of fish from four contaminated stations and several reference stations. These show no significant differences between species or stations, except for vacuolation and hypertrophy in California tonguefish.

Hepatocyte Vacuolation (numbers of fish)

	Longspine Combfish							Pacific Sanddab							California Tonguefish							Yellowchin Sculpin						
	0	0.5	1.0	1.5	2.0	2.5	3.0	0	0.5	1.0	1.5	2.0	2.5	3.0	0	0.5	1.0	1.5	2.0	2.5	3.0	0	0.5	1.0	1.5	2.0	2.5	3.0
PV 7-3	0 *	0	1	0	0	2	0	2	1	1	3	0	2	0	2	1	0	0	0	0	1	0	0	2	0	0	2	3
SMB 6-4	0	0	0	2	4	0	0	0	1	1	0	2	2	0	0	0	1	1	0	2	1							
SMB 2-3	0	0	2	4	6	1	0								1	2	0	1	1	0	0	0	0	1	0	0	2	3
SD-4	0	1	1	2	6	0	0								0	0	3	1	1	1	0							
Reference																												
Torrey Pines	0	0	1	2	1	2	0								1	3	0	0	0	1	0	0	1	0	2	1	1	1
San Mateo																												
San Clemente								2	0	1	1	2	3	0														
SD-1															3	2	1	0	0	0	0							

Hepatocyte Hypertrophy (numbers of fish)

	0	0.5	1.0	1.5	2.0	2.5	3.0	0	0.5	1.0	1.5	2.0	2.5	3.0	0	0.5	1.0	1.5	2.0	2.5	3.0	0	0.5	1.0	1.5	2.0	2.5	3.0
PV 7-3	1	0	1	1	0	0	0	6	0	1	1	1	0	0	3	0	0	1	0	0	0	1	0	1	0	3	2	0
SMB 6-4	0	2	3	1	0	0	0	2	0	2	0	2	0	0	2	1	2	0	0	0	0							
SMB 2-3	7	2	2	2	0	0	0								1	2	1	1	0	0	0	1	0	0	0	2	2	1
SD-4	2	3	3	1	1	0	0								2	2	1	0	1	0	0							
Reference																												
Torrey Pines	1	1	1	3	0	0	0								4	1	0	0	0	0	0	2	1	1	1	1	0	0
San Mateo																												
San Clemente								3	1	4	1	0	0	0														
SD-1															6	0	0	0	0	0	0							

Melanin Macrophage Center Numbers

	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PV 7-3	0.1	0.1	1.5	1.2	2.8	3.1	0.02	0.1
SMB 6-4	1.4	1.7	2.1	1.5	0.6	0.6		
SMB 2-3	1.4	1.0					0	0
SD-4	0.02	0.1			1.5	1.2		
Reference								
Torrey Pines	0.1	0.2			0	0	0	0
San Mateo								
San Clemente			2.6	3.2				
SD-1					1.8	1.5		

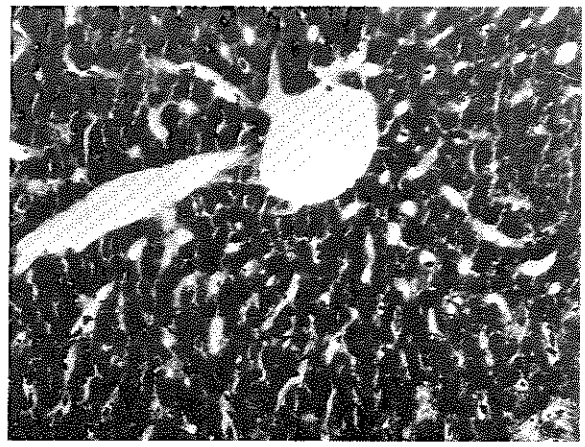
Melanin Macrophage Center Size (µm)

	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PV 7-3	22.5	22.5	24.1	6.2	20.1	14.0	3.2	8.5
SMB 6-4	28.1	17.3	25.5	11.6	28.9	12.2		
SMB 2-3	28.7	13.3			22.2	22.2	0	0
SD-4	3.2	10.0			22.1	14.4		
Reference								
Torrey Pines	5.6	13.8			0	0	0	0
San Mateo								
San Clemente			26.1	15.9				
SD-1					21.1	18.1		

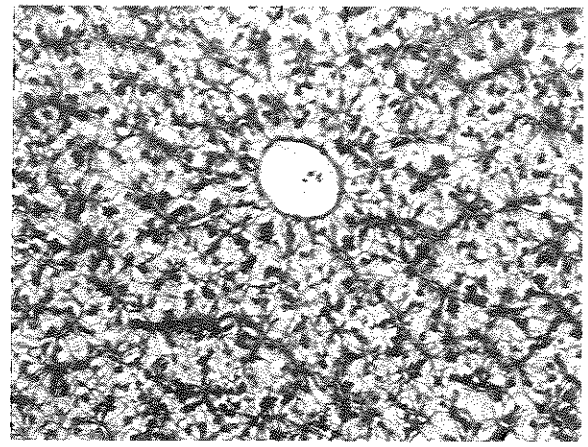
* 0 = none present

NORMAL

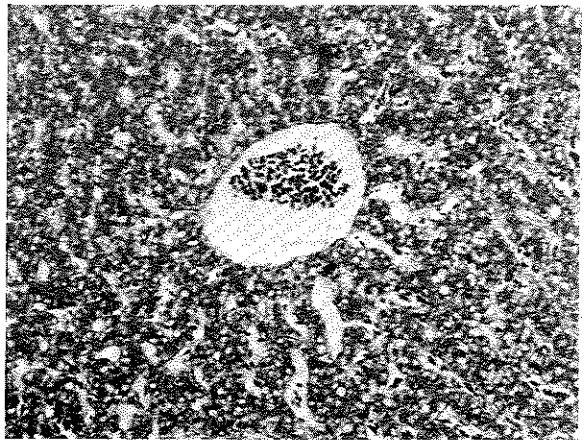
VACUOLATED



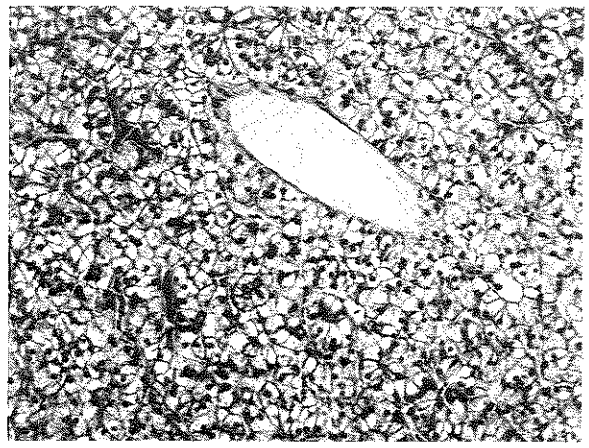
a. Kelp bass: San Mateo.



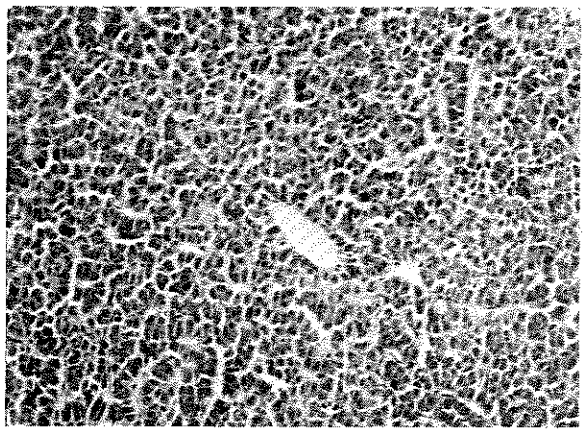
b. Kelp bass: PV 7-3.



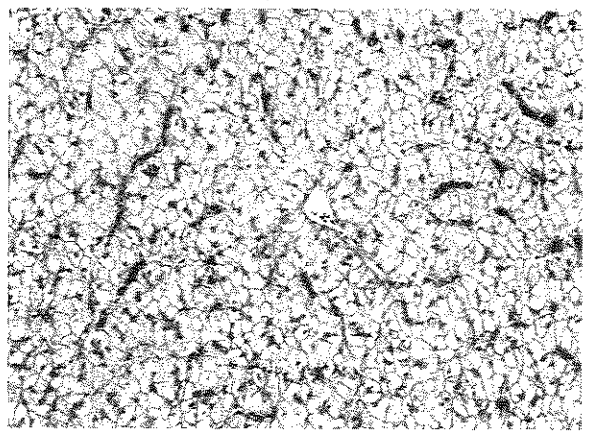
c. Longspine combfish: Torrey Pines.



d. Longspine combfish: PV 7-3.



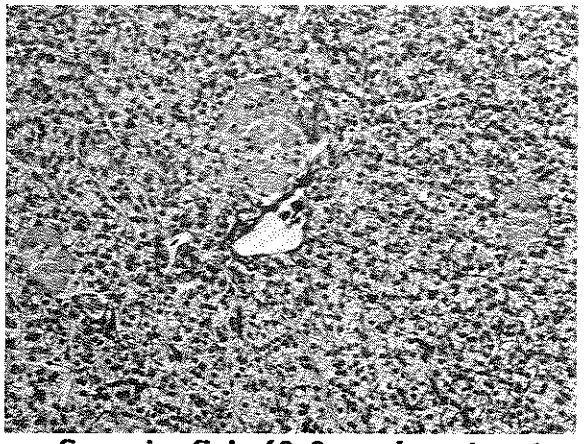
e. Pacific sanddab: San Clemente.



f. Pacific sanddab: PV 7-3.

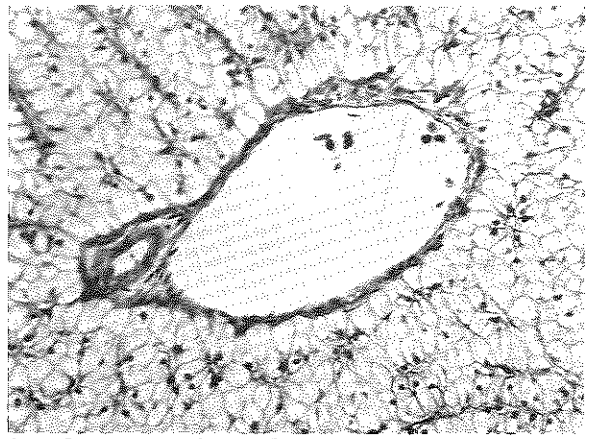
Figure 1. Photos a-l compare normal and vaculated liver parenchymas (x200) of fish species from sampling areas shown (continued).

NORMAL

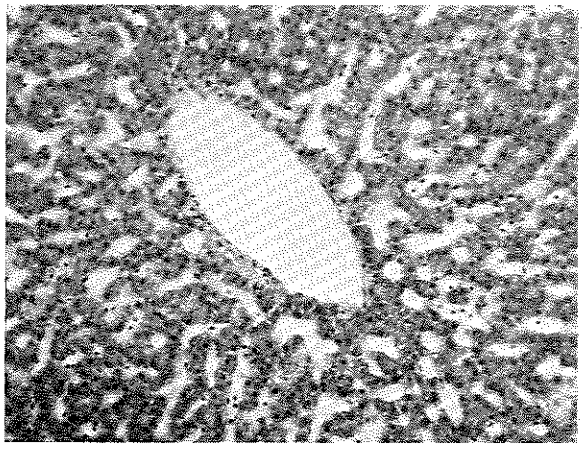


g. Scorpionfish (8.8-µm hepatocyte diameter): Cortes Bank.

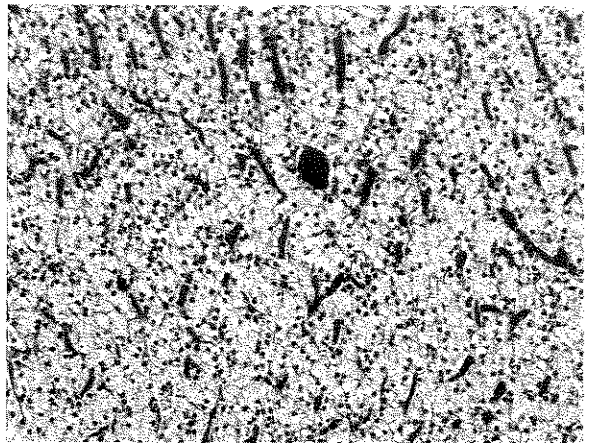
VACUOLATED



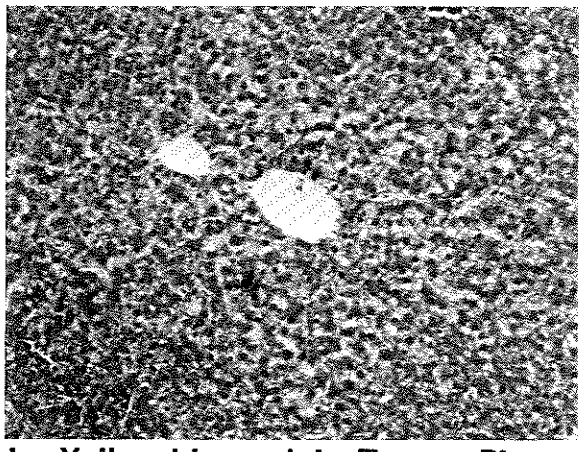
h. Scorpionfish (22.9-µm hepatocyte diameter): Cortes Bank.



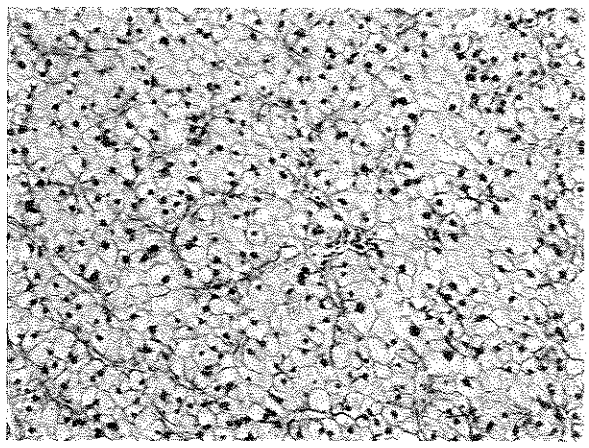
i. California tonguefish: SD-1.



j. California tonguefish: PV 7-3.

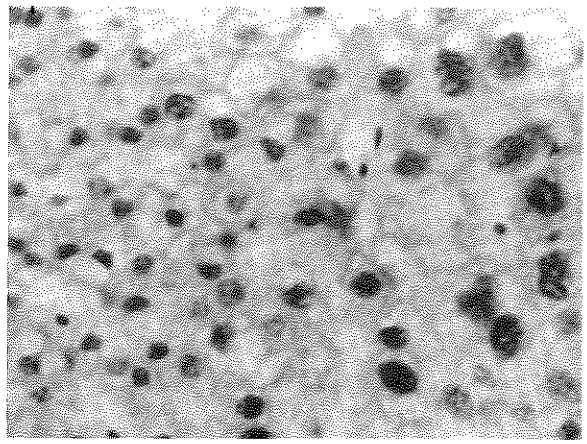


k. Yellowchin sculpin:Torrey Pines.



l. Yellowchin sculpin: PV 7-3.

Figure 1. (continued).



m.

Figure 1 (concluded). Photo m shows nuclear pleomorphism (x400) in the liver of a yellowchin sculpin from Torrey Pines.

California tonguefish, there were no differences between stations. The California tonguefish showed a significant difference in vacuolation ($p<0.026$) and hypertrophy ($p<0.051$) between stations. However, the presence of unequal sample sizes subjected to the nonparametric Kruskal-Wallis test prevented determination of which station or stations were different. Trends in data indicate that there was less vacuolation in California tonguefish from the reference stations.

Heptocyte hypertrophy and vacuolation can result from a variety of causes including accumulation of lipids, glycogen, and water, or a proliferation of cellular organelles. When hepatocyte hypertrophy and vacuolation result from exposure to contaminants, these conditions usually indicate excess lipid accumulation and proliferation of endoplasmic reticulum (Brown et al. 1982; Perkins et al. 1982). Lipid accumulation has been shown to result from a variety of causes including diet, stress, seasonal changes related to reproduction, and exposure to chlorinated and petroleum hydrocarbons (Malins 1977; Dianzani 1979; Gossett et al., this volume). The relative contributions of seasonality and chlorinated hydrocarbons to liver size have been discused by Gossett et al. (this volume). The relationship of chlorinated hydrocarbons to liver cell size will be discussed in a later section of this report.

Other parameters which were investigated but failed to show any trends between stations were as follows: hepatic cord structure; perisinusoidal nuclei; sinusoidal compression and congestion; nuclear, parenchymal or cholangiolar alterations; and parasite infestation (Table 2). However, 13 out of the 19 yellowchin sculpin hepatocyte nuclei exhibited nuclear pleomorphism (Figure 1); frequency of occurrence was the same at outfall and reference stations (Table 2). This condition is indicative of

Table 2. Subjective histological ratings and chlorinated hydrocarbon concentrations (mg/wet kg) in fish livers from contaminated and reference sites. These revealed significant differences in contaminant concentrations between stations using the Kruskal-Wallis test.

		Subjective Rating						
							Chlorinated Hydrocarbons	
	n	Parasites	Adenomatous Foci	Nuclear Pleomorphism	Sinusoidal Congestion	n	Total DDT	Total PCB
Yellowchin sculpin								
Contaminated								
PV 7-3	7	0	0	4	0	4	74 ± 13	2.3 ± 0.4
SMB 6-4	6	0	0	5	0	5	20 ± 9	2.3 ± 0.8
SMB 2-3	NA[a]	NA	NA	NA	NA	4	13 ± 4	1.2 ± 0.5
Reference								
Torrey Pines	6	0	0	4	0	6	1.4 ± 0.9	0.034 ± 0.083
California tonguefish								
Contaminated								
PV 7-3	4	0	1	1	1	NA	NA	NA
SMB 6-4	5	0	1	1	2	6	35 ± 25	7.3 ± 5.2
SMB 2-3	5	0	0	1	1	NA	NA	NA
SD-4	6	0	0	0	1	6	3.6 ± 4.0	0.67 ± 0.6
Reference								
Torrey Pines	5	1	0	0	0	6	2.5 ± 1.4	0.37 ± 0.51
SD-1	6	0	0	0	0	6	7.9 ± 5.6	1.4 ± 1.6
Pacific sanddab								
Contaminated								
PV 7-3	9	2	0	0	1	4	608 ± 207	14 ± 5
SMB 6-4	NA	NA	NA	NA	NA	6	71 ± 36	16 ± 6
SMB 2-3	6	1	0	0	1	5	42 ± 19	4.8 ± 2.1
Reference								
San Clemente Island	9	0	0	0	0	10	1.9 ± 0.7	0.19 ± 0.36
Longspine combfish								
Contaminated								
PV 7-3	3	0	0	0	2	7	86 ± 18	5.9 ± 1.3
SMB 6-4	13	1	0	1	2	6	22 ± 4	4.0 ± 0.9
SMB 2-3	6	0	0	0	3	4	12 ± 4	1.3 ± 0.3
SD-4	10	0	0	0	2	6	2.6 ± 0.9	0.60 ± 0.4
Reference								
Torrey Pines	6	0	0	0	0	6	5.0 ± 4.6	0.10 ± 0.09

[a]Not analyzed.

a regressive anaplastic change, or reversal of cells towards more primitive types; it is one of the criteria for malignancy of tumors (Cowdry 1940). Adenomatous foci (benign epithelial glandular tumors) were noted in several California tonguefish from PV 7-3 and SMB 6-4 (Table 2).

Objective Measurements

The cumulative mean and variance stabilized at approximately 16 fields (Figure 2). This number of fields was used for all measurements and counts reported for scorpionfish and kelp bass.

Results from these measurements (Table 3) showed the mean hepatocyte diameter of scorpionfish collected from the Palos Verdes shelf was significantly larger than the mean hepatocyte diameter of scorpionfish from Cortes Bank. Despite the fact that fish from Palos Verdes had larger hepatocytes than those from Cortes Bank, almost all fish including those from Cortes Bank had hepatocyte hypertrophy and vacuolation (Figure 1). There was no difference in the size of liver cells of kelp bass from Palos Verdes and the San Mateo reference site (Table 3). Some kelp bass from San Mateo did not have hepatocyte hypertrophy or vacuolation (Figure 1).

The objective measurement method was used to determine sizes and numbers of melanin macrophage centers in livers of all fish collected. No statistically significant differences between stations were noted except for scorpionfish (Tables 1 and 3). The diameters of melanin macrophage centers in livers of scorpionfish collected from the Palos Verdes shelf were significantly smaller than the diameters of melanin macrophage centers from Cortes Bank. The numbers of melanin macrophage centers (per microscopic field) in scorpionfish collected from Palos Verdes shelf were significantly lower than the numbers of melanin macrophage centers from Cortes Bank.

Previous studies have shown that stress can cause a decline in white blood cell-thrombocyte (WBC-T) counts in fish blood (McLeay 1975). Since macrophage measurements are a component of WBC-T counts, the lower numbers of melanin macrophage centers in Palos Verdes scorpionfish livers compared to those from Cortes Bank could suggest relatively stressful conditions at Palos Verdes. This stress could be related to environmental factors, including contaminants (Wedermeyer and McLeay 1981). Low WBC-T counts, which have been related by previous studies to the susceptability of fish to disease (McLeay 1975; Wedermeyer and McLeay 1981), could include the parasitic infestations, tumors, and fin erosion which are prevalent in fish from the Palos Verdes shelf (Cross et al. 1984).

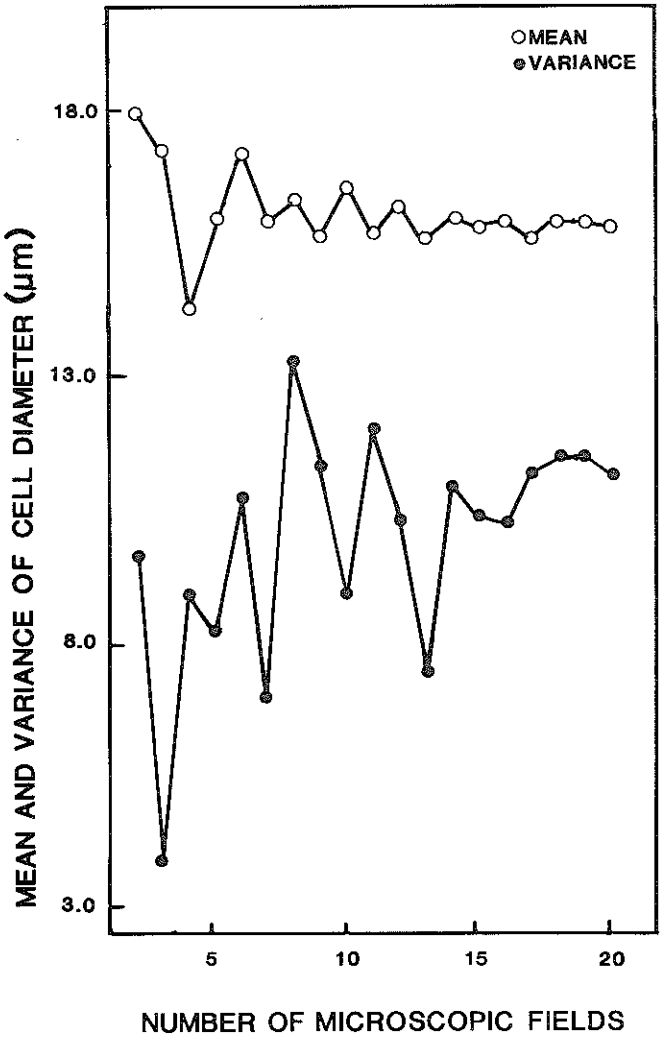


Figure 2. One example of the cumulative mean and variance of scorpionfish hepatocyte diameter plotted against the number of fields examined. The variance stabilized at approximately 16 fields.

Comparison of Subjective Ratings to Objective Measurements

The correlation between measured cell diameter and hypertrophy/ vacuolation ratings was positive but not significant (r_s = 0.337, $0.05<p<0.10$; Figure 3). Close examination of individual data points showed that there was a large range of measured cell diameters for each subjective rating (Figure 3). For example, subjective ratings of 3 corresponded to a range of measured cell diameters from 11 µm to 22 µm. This variation indicates that the subjective rating method is influenced by more than cell diameter; i.e., by staining intensity, different tissue section thickness, and/or individual interpretation.

As will be discussed in more detail in Part 5 (Perkins and Rosenthal, this volume), subjective histopathological rating systems suffer from

Table 3. Objective histological measurements and chlorinated hydrocarbon concentrations (mg/wet kg) in fish livers from contaminated and reference sites. These revealed significant differences in both contaminant concentrations using the Kruskal-Wallis test, and in histological measurements using the Mann-Whitney U test.

	Objective Measurements								Chlorinated Hydrocarbons			
	Cell Diameter				Melanin Macrophage Diameter		Melanin Macrophage Numbers		Total DDT		Total PCB	
	n	Kelp Bass	n	Scorpionfish	Kelp Bass	Scorpionfish	Kelp Bass	Scorpionfish	Kelp Bass	Scorpionfish	Kelp Bass	Scorpionfish
Contaminated												
PV 7-3	10	12.3 ± 2.0	6	21.6 ± 3.4	1.6 ± 2.2	3.2 ± 3.3	0.3 ± 0.5	0.1 ± 0.1	7.1 ± 3.2	58 ± 32	0.4 ± 0.4	2.7 ± 0.8
SMB 6-4	NA[a]	NA	1	13.0 ± 3.4	NA	4.5 ± 18.0	NA	0.1 ± 0.3	NA	23 ± 14	NA	2.0 ± 1.3
SMB 2-3	NA	NA	2	18.2 ± 1.8	NA	2.0 ± 2.8	NA	0.1 ± 0.3	NA	14 ± 11	NA	2.1 ± 1.4
Reference												
Cortes Bank	NA	NA	25	15.2 ± 3.4	NA	27.8 ± 17.2	NA	2.0 ± 1.9	NA	5.4 ± 4.6	NA	0.3 ± 0.2
San Mateo	10	11.1 ± 2.0	NA	NA	5.2 ± 7.0	NA	0.7 ± 0.6	NA	1.8 ± 2.2	NA	0.2 ± 0.5	NA
p		NS[b]		<0.001	NS	<0.001	NS	<0.001	<0.05	<0.05	NS	<0.05

[a] Not analyzed.
[b] Not significant.

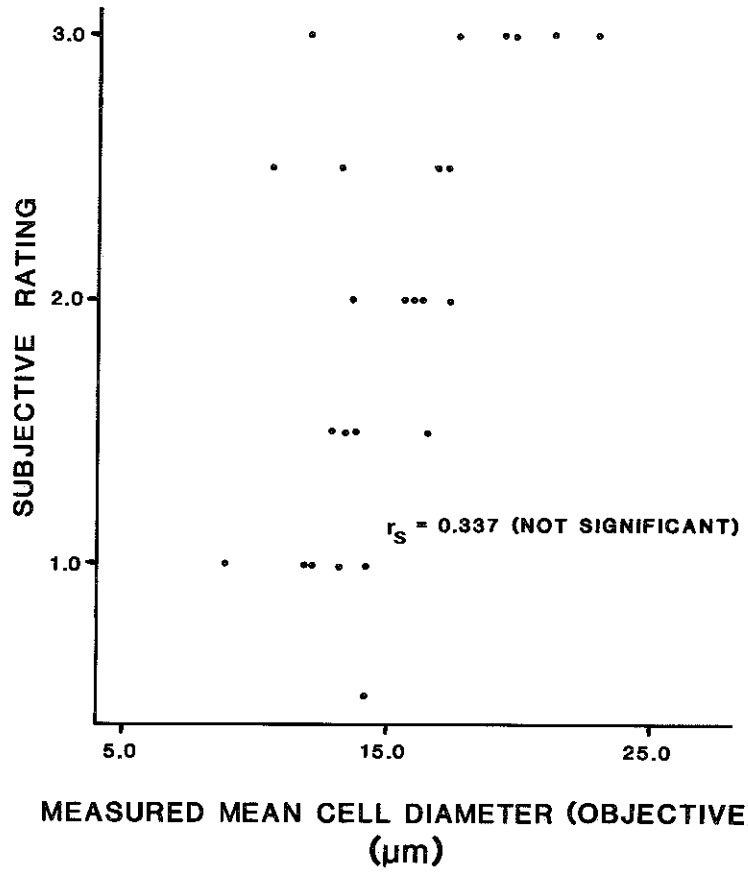


Figure 3. Comparison of the methods for determining cell size. Results show no significant correlation between the two methods.

certain inconsistencies due to lack of methodological standardization and differences of opinions regarding pathological nomenclature. By contrast, the objective measurement method utilized in this study provides an accurate estimate of cell size that is statistically verifiable and thereby offers considerable promise in improving the accuracy of histopathological evaluations.

One disadvantage of the objective measurement method described in this paper is time involved in evaluating each slide. It takes approximately one hour and 30 min to measure and count all parameters on a given slide, whereas the subjective rating system takes only 15 min. Therefore, in future studies the information gained from utilizing the objective measurement method should be weighed against the time involved. It should be noted that the study by Gossett et al. (this volume) would not have been possible without the objective measurement method.

COMPARISON OF SUBJECTIVE RATINGS AND OBJECTIVE MEASUREMENTS TO TISSUE CHEMISTRY

Subjective Ratings

Comparison of subjective ratings with tissue chemistry values was impared by the fact that chemical analysis was not always performed on the same fish as was histological examination. As described previously, samples from PV 7-3, SMB 6-4, and SMB 2-3 for chemistry were collected in December 1982, while samples for histology were collected from June until August 1983. Samples for chemistry and histology from other areas were collected at the same time.

Statistical analysis of data indicated that chlorinated hydrocarbons at PV 7-3, SMB 6-4, and SMB 2-3 were often elevated above reference site values (Table 2). However, subjective histological ratings did not show such differences, except perhaps in the case of California tonguefish as described previously (Table 1). The presence of chemical differences between stations in the absence of histological differences could indicate that either of the following is true: 1) chlorinated hydrocarbons are not the causative agent for the observed pathology, or 2) fish from all areas have concentrations of chlorinated hydrocarbons that exceed the threshold for such pathology. The latter possibility is supported by Zeh's (1982) analysis which showed a threshold of 0.900 mg pesticide/dry kg (or approximately 0.2 mg/wt kg) of liver as the threshold for occurrence of pathology. However, the presence of other possible agents such as petroleum hydrocarbons has not been investigated in southern California coastal fish. Nor have the effects of diet, stress, and lipid cycles related to reproduction been thoroughly examined.

Objective Measurements

Livers of kelp bass from San Mateo Point and Palos Verdes showed wide variations of liver cell size (Table 3) which appeared to be related to liver total DDT concentration. For example, the smallest kelp bass liver from San Mateo Point had a cell diameter of 8.5 µm and a DDT concentration of 0.087 ppm, while the largest liver from this station had a cell diameter of 14.4 µm and a DDT concentration of 5.90 ppm.

However, a closer analysis of our Cortes Bank scorpionfish data revealed that liver cell size was correlated with the mass of oxygenated metabolites of chlorinated hydrocarbons, rather than with either the mass or concentrations of parent compounds or concentrations of oxygenated metabolites (Figure 4). We believe that liver cell size was

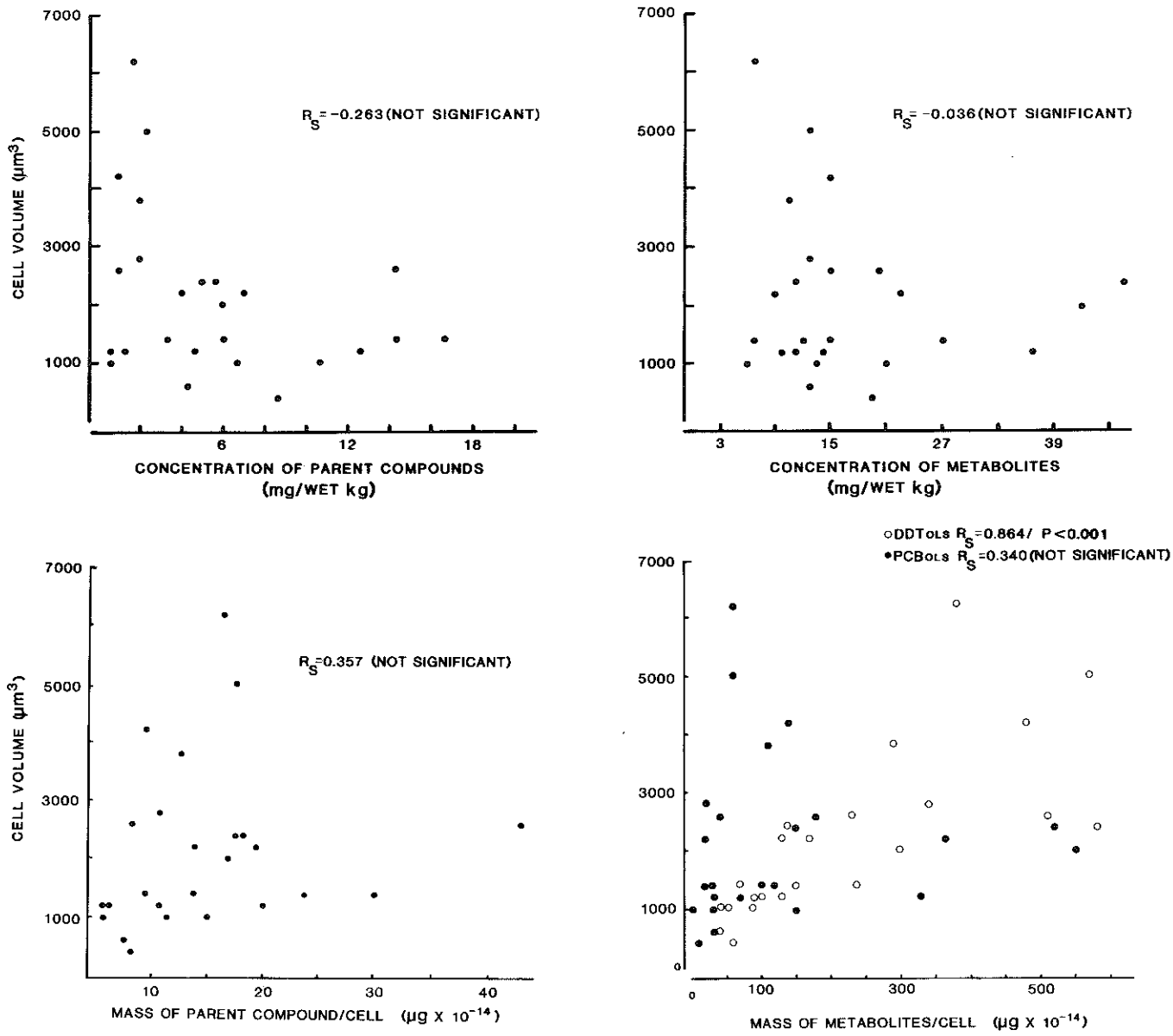
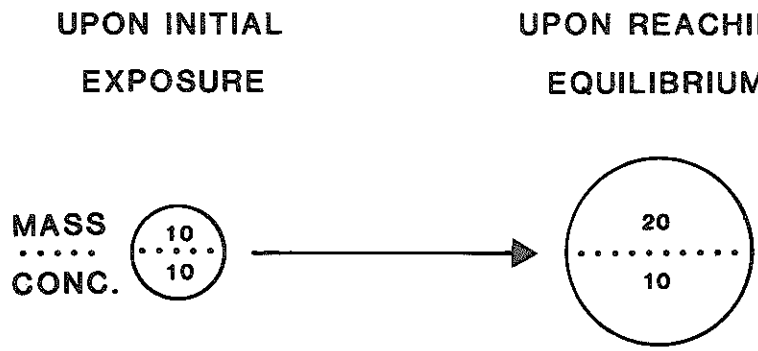


Figure 4. Cell volumes related to contaminants. Volume does not correlate with the concentration of parent compounds or metabolites of chlorinated hydrocarbons, or with the mass of parent compounds per cell; but volume does correlate with the mass of metabolites per cell. The mass of parent compounds or metabolites per cell was calculated by dividing the mass of each in whole liver by the number of cells in whole liver. The number of cells in whole liver was calculated by dividing the volume of the liver (where 1 g ≈ 1 ml) by the mean volume of one liver cell. Correlations were done using Spearman's rank correlation test.

not related to concentration of parent or metabolite forms of chlorinated hydrocarbons because, as the mass of metabolites increases in cells, it causes an increase in cell size that results in no apparent change in concentration. In other words, as shown in this diagram, the size of



the cell increases to accommodate the mass of chlorinated hydrocarbons in the cell, resulting in a larger cell with no change in concentration. These results are in accordance with previous studies (reviewed in Brown et al. 1982) which indicate that oxygenated metabolites, not parent compounds, are responsible for chronic toxicity.

Comparisons with other Geographical Areas

Results from this study showed that extensive vacuolation (50% vacuolar occlusion of cytoplasm (Pierce et al. 1980)), or a rating of 1.5 or higher) occurred in an average of 53% of reference fish and in an average of 70% of fish from more contaminated areas of the southern California Bight (Table 1). In contrast, Malins et al. (1984) found storage disorders (steatosis, or fatty liver, and hemosiderosis) in proportions ranging from 0% in fish from relatively uncontaminated reference areas of Puget Sound, Washington, to 23.5% in some parts of highly contaminated embayments. This difference in percent of occurrence may result from different rating schemes, or from greater contamination in the southern California Bight than in Puget Sound (Brown et al., this volume).

CONCLUSIONS

These findings indicate that the state of health of fish may be affected by chlorinated hydrocarbons in widespread areas of the southern California Bight. Further studies are needed to determine the geographical extent of these effects and the relative contribution of seasonality and other contaminants to the observed conditions.

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