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THE INFLUENCE OF CHLORINATED HYDROCARBONS ON SCORPIONFISH LIVERS

Hypertrophy, meaning increased cell size in an organ, is widespread in livers of fish in the southern California Bight (Perkins and Rosenthal, this volume). It is not known whether this is a normal state related to the reproductive cycle or caused by exposure to manmade contaminants. Contaminant-related hypertrophy usually results from increased lipid accumulation (Sherwood 1977). This condition represents a reaction to injury which can lead to fibrosis, cirrhosis, and necrosis (Dianzani 1979; Zimmerman 1978). The livers of fish in the southern California Bight are larger in fish near municipal wastewater outfalls than in those taken from less contaminated areas, presumably because of the influence of synthetic organic chemicals. To understand the causes of this size increase, liver changes due to natural causes--i.e. reproductive changes--must be separated from those caused by the presence of outfall-related contaminants.

The objectives of this research were to discover the effects of contaminants on livers of the California spotted scorpionfish *(Scorpaena guttata)* and to develop a model that can be used to predict liver weight based on a combination of many factors. Scorpionfish have many advantages as an experimental animal, and we routinely use them in the laboratory. Our finding, that the most important liver size increase correlates with the oxygenated metabolites of DDT, contradicted past hypotheses that DDT parent compounds were the prime cause. After additional research has ascertained seasonal changes, there is a good possibility that natural changes in fish livers can be differentiated from those changes caused by synthetic organic contaminants, allowing us to interpret future histological and biochemical results.

BASIS FOR MODEL DEVELOPMENT

The functions of the liver include the metabolism of proteins, carbohydrates, lipids, hormones, and chemical contaminants. The production of a fatty liver is mainly dependent upon a disproportion between the synthesis of triglycerides and their secretion (Dianzani 1979). Equilibrium between synthesis and secretion can be affected by many factors including diet, stress, the presence of contaminants, and the reproductive cycle. Allen et al. (1976) stated that the initial response of rodents to exposure to hydrocarbons is a proliferation of the endoplasmic reticulum and an increased synthesis of lipids within these cytoplasmic membranes. The metabolism of hydrocarbons to their oxygenated metabolites may cause changes in the system that controls the transport of lipids out of the liver, resulting in higher accumulation of lipids than normal (Dianzani 1979).

Significant changes in livers collected from the southern California Bight have been observed by SCCWRP researchers in the past. Sherwood (1977) indicated a good correlation between the lipid content in Dover sole livers and their concentrations of DDTs and PCBs. The liver somatic index (LSI: liver weight divided by whole-body weight), which probably reflects increased lipid content, was consistently higher in Dover sole from the more contaminated Palos Verdes region than from the less contaminated Dana Point region. This difference suggests that the high level of contaminants at Palos Verdes (presumably DDT and its primary metabolites DDD and DDE) caused an increase in the lipid content in fish livers.

However, recent data (Figure 1) indicate that the reproductive cycle also has a profound effect on the liver size of scorpionfish. This effect may be due to the mobilization into the liver of lipids which are used in the production of reproductive tissues. For example, although the whole-body weight and concentrations of DDTs and PCBs were not significantly different between fish caught at Dana Point during two sampling periods, the mean liver weight of postspawning scorpionfish was 8.2 g while the mean liver weight of prespawning fish was 22.8 g. Moreover, the mass of parent compounds in the liver was increased by 2.3 times in the prespawning fish. This higher mass of contaminants may indicate that the DDTs and PCBs were mobilized from other tissues along with lipids and taken up by the liver.

Recently we sampled 25 scorpionfish during the postspawning season from what was considered to be a relatively clean area at Cortes Bank, which is approximately 145 km off the southern California coast. Liver samples were collected for contaminant analysis (including the oxygenated metabolites of DDTs and PCBs) and histological slides. This procedure provided us with a large sample size for developing a model to predict the liver size in scorpionfish. The data collected provided us with a choice of many predictor variables, such as liver cell volume, parent compounds, oxy-metabolites, and natural factors such as body weight and standard length (Table 1).

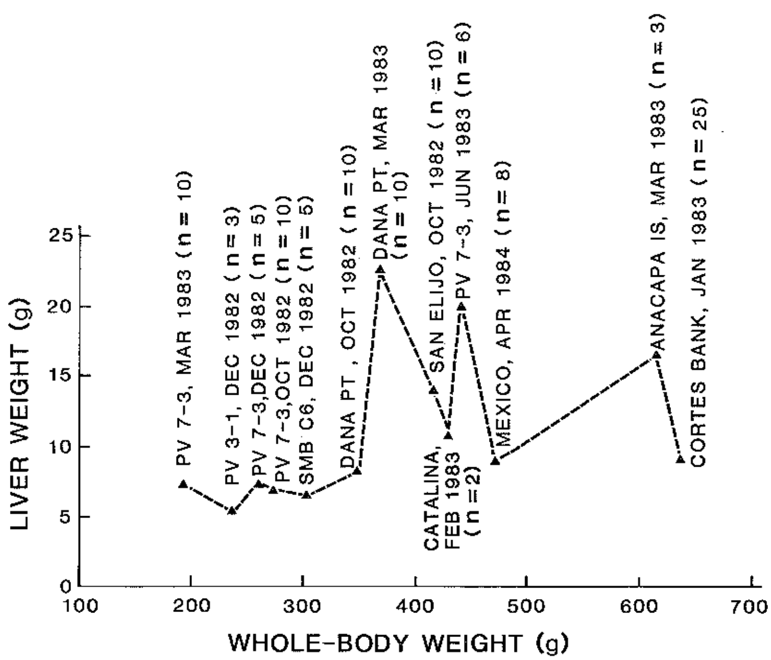

Figure 1. Plot of liver weight versus whole-body weight of scorpionfish collected from several areas in the southern California Bight showing the high seasonal variation in liver weights.

MODEL DEVELOPMENT

The first step was to develop a way to quantify our histopathological results so they could be used in building the model. This was accomplished by developing an objective method to replace the subjective hypertrophy rating previously used. Hypertrophy, a measure of the enlargement of a cell, has been indicated by a relative rating of 0-3 (Perkins et al. 1982). In this study we measured the diameters of cells, using a light microscope equipped with a micrometer, and calculated cell volumes (assuming cells are spherical). We determined that measuring five cells in each of 16 randomly chosen fields produced a stable variance estimate. Each of the 25 scorpionfish liver slides were measured using this technique. Cell volumes were normally distributed, but since the variance increased with the mean, the data were $\log_{10}$ transformed.

Next, we chose 15 different variables that might influence liver weight. These were standard length, whole-body weight, cell volume, total DDT concentration, total PCB concentration, total parent compound concentration (DDTs + PCBs), DDTol concentration (oxygenated metabolites of DDTs), PCBol concentration (oxygenated metabolites of PCBs), total metabolite concentration, mass of total DDTs in the liver,

Table 1. Summary of results from the livers of 25 postspawning scorpionfish collected from Cortes Bank in January 1984.

	Whole-Body Weight (g)	Standard Length (mm)	Liver Weight (g)	Cell Volume (μm^3)	Total DDT	Total PCB	DDTols[a]	PCBols[b]
					(ppm wet weight)			
Median	580	268	8.6	2325	4.1	0.31	8.8	3.4
Mean	636	263	9.1	2467	5.4	0.32	10.3	6.9
1 SD	260	36	5.2	1796	4.6	0.20	5.0	8.1
Minimum	262	204	3.3	467	0.5	0.03	4.5	0.4
Maximum	1126	320	28.3	7847	16.3	0.73	24.7	27.1

[a] p,p'-DDA + p,p'-DDOH.
[b] 2,2',5'-trichloro-4-biphenylol + 2',3',4',5'-tetrachloro-4-biphenylol + 2',3,3',4',5'-pentachloro-2-biphenylol + 2',3',4',5,5'-pentachloro-2-biphenylol.

mass of total PCBs in the liver, mass of parent compounds in the liver, mass of DDTols in the liver, mass of PCBols in the liver, and mass of total metabolites in the liver. This list was then reduced by removing the variance inflation factors (factors that were highly correlated with each other); for example, standard length and whole-body weight are highly correlated, and therefore only one need represent that parameter. After this analysis, the five parameters remaining that could be used in the model were whole-body weight, cell volume, mass of DDTols in the liver, mass of PCBols in the liver, and mass of parent compounds in the liver.

The five predictors were then analyzed in a stepwise regression against liver weight. This type of analysis regresses the predictor variables most highly correlated with liver weight (LW) and produces an equation that is tested for significance at the 0.05 level. The remaining variables are entered one at a time based on their correlation with liver weight and the significance of the relationship. For example, if whole-body weight was the predictor with the highest correlation with liver weight, it was chosen first and the regression equation was calculated. Then the predictor that had the highest partial correlation with the liver weight (after adjusting for predictors already in the regression) was entered, a new regression was calculated, and each predictor in the equation was tested for significance. This process continues until the latest predictor entered is not significant (Draper and Smith 1981). The results (Table 2) of this analysis indicated that three of the predictor variables, whole-body weight, liver cell volume, and mass of DDTols in the liver, fit the model and could predict 82.0 percent of the variance in liver weight of Cortes Bank scorpionfish. No other predictor variables fit within the significance limit. The final regression equation was:

$$\text{Log}_{10}\text{LW} = -0.361 + 0.0006X_1 + 0.244X_2 + 0.0012X_3 \pm s_{y \cdot x}$$

where X_1 = Whole-body weight (g)
X_2 = Log_{10} liver cell volume (μm^3)
X_3 = Mass of DDTols in the liver (μg)
$s_{y \cdot x}$ = Standard error of the estimate = 0.09424

This equation can be used to predict liver weight with very good accuracy (Figure 2). The amount of variance explained by each of these predictors indicates that the main determinant of liver weight was body size, as expected, but cell volume also had a significant effect. Since liver cell volume is considered to be an indicator of lipid content, the size of the liver can be considered to be affected by the amount of lipids present. Finally, since oxygenated metabolites, rather than parent compounds, made the only other significant contribution to liver size, the regression analysis indicated that liver weight is controlled more by the mass of oxygenated DDT metabolites than by parent compounds. This finding contradicts the past hypothesis that parent compounds are the main factor (Sherwood 1977). One possible

Table 2. Results of a stepwise regression of liver weight on five predictors, n = 25 .

	Step 1	**Step 2**	**Step 3**
Constant	0.5172	-0.5383	-0.3606
Whole-body weight,	0.00061	0.00070	0.00059
T-ratio	5.76	8.19	6.74
Log_{10} cell volume,		0.310	0.244
T-ratio		4.04	3.33
DDTol mass,			0.00120
T-ratio			2.55
$S_{y \cdot x}$	0.136	0.105	0.0942
R-squared	59.02	76.47	82.09

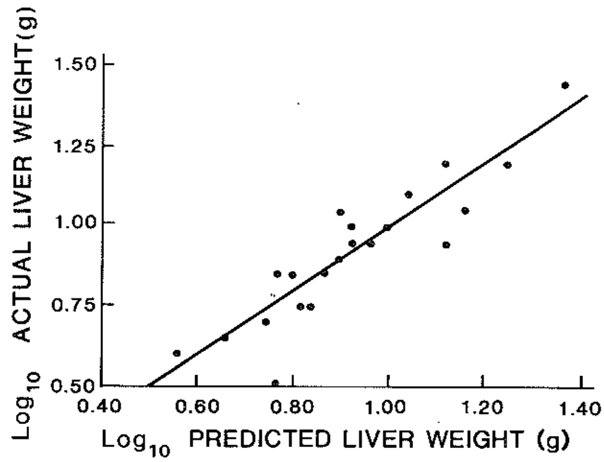

Figure 2. Plot of the $\log_{10}$ measured liver weight versus the $\log_{10}$ of the predicted liver weight results used to develop the model for predicting liver weight in scorpionfish.

explanation for why PCB metabolites were not significant as well is that the number of possible PCB metabolites is significantly higher and we are not measuring enough for a representative fraction.

The final step in model development is verification. Scorpionfish collected from Dana Point and the Channel Islands were measured for whole-body weight, cell volume, and oxygenated metabolites. Using these data we predicted liver weights with our equation and compared the predicted values to the actual values. The result was a good correlation ($r = 0.827$; $p<0.001$) between predicted and actual values (Figure 3). However, most of the predicted values were below the measured values. After further analysis it was determined that the slopes of the regression lines of $\log_{10}$ liver weights versus $\log_{10}$ whole-body weights were the same, but the y-intercepts of the regression lines were significantly different. This difference indicates that livers were a smaller part of the whole-body weights of the Dana Point plus Channel Island fish, than of the Cortes Bank fish, even though the rate of increase in liver weights per whole-body weights was the same. These results substantiate the need for a seasonal and spatial study to improve the model.

DISCUSSION

This scorpionfish model is a good start at attempting to understand the effects of manmade contaminants and natural processes on liver weight and to better interpret the widespread hypertrophy observed in fish livers from the southern California coast. We suggest the following improvements: 1) enlarge the model to include data from a seasonal

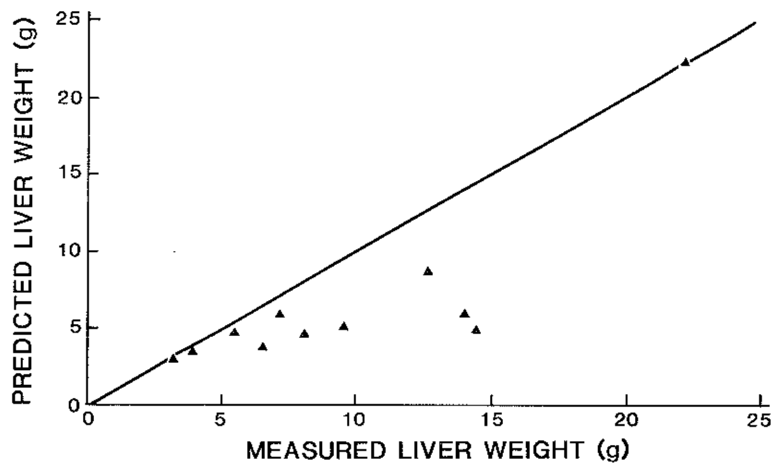

Figure 3. Results from applying the model to scorpionfish collected from Dana Point (n = 10) and the Channel Islands (n = 2) comparing predicted liver weight value to measured value (correlation coefficient r = 0.827, p<0.001).

study throughout the scorpionfish reproductive cycle to account for the changes in liver weight due to reproduction; 2) test and develop models for other species as well. Once these models are developed, it may be possible to determine whether or not manmade contaminants are a major factor in the generation of liver pathology.

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