EFFECT OF p,p′-DDT ON DEVELOPMENTAL STABILITY OF PECTORAL FIN RAYS IN THE GRUNION, *LEURESTHES TENUIS*

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ABSTRACT

Experiments are described that test the validity and utility of using bilateral asymmetry for the early detection of environmental deterioration. The first experiment, using a continuously flowing water system, was designed to test the effects of a ubiquitous pollutant, p,p′-DDT, on the pectoral fin ray asymmetry of grunion, *Leuresthes tenuis*, fry. Toxicant concentrations ranged from a low of 0.001 to a high of 500 parts per billion. The two highest p,p′-DDT concentrations, 100 and 500 parts per billion, proved lethal to grunion fry. For the other concentrations, increases in pectoral fin ray asymmetry with increasing toxicant concentration were observed. Increases in asymmetry were statistically significant.

The second experiment, which utilized a recirculating static water system, was designed to determine inherent differences in the level of pectoral ray fin asymmetry in fish from different localities. Results for grunion fry paralleled previously published results for adult grunion. Grunion from the relatively polluted southern California region had higher asymmetry levels than did those from Baja California. The significance of these results as they relate to the general question of asymmetry as a statistical indicator of environmental stress is discussed.

Asymmetry of bilateral characters has been proposed as a morphological measure of environmental stress in fish populations (Valentine, Soulé, and Samollow, 1973). The theory underlying such an analysis is that various environmental stresses may reduce the efficiency of developmental homeostatic mechanisms, thus leading to an average increase in the within-individual variation of morphological structures (Adams and Niswander, 1967). An asymmetry index, then, might provide a relative estimate of the physiological stress to which a population is being subjected.

Data from three wild-caught fish species supporting this hypothesis have previously been presented (Valentine, Soulé, and Samollow, 1973). We now present experimental results supporting the theory.

METHODS AND MATERIALS

Grunion, *Leuresthes tenuis*, were chosen as our experimental organism. Grunion are small, rapidly maturing, marine atherinids endemic to the Californias. They possess many features which make them attractive as a laboratory organism. Grunion spawn biweekly on sandy beaches during the high tides from late February or early March through August or September, and wild-spawned eggs may be easily collected by digging in wet sand after such a spawning run. The eggs mature into fry in about 10 days (Walker, 1952). Fry are large and easily maintained in the laboratory.

The approximate straight-line distances in miles to the localities mentioned in this paper from Point Fermin, the prominent point located adjacent to the entrance of the Los Angeles Harbor, are Belmont Shore, 9; Del Mar, 87; La Jolla Shores, 91; San Diego, 101; and Bahia San Quintin, 270. All of these localities are south of Point Fermin.

The toxicant chosen was p,p′-DDT(1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane), the metabolic products of which are known to be ubiquitous in the world ecosystem (Risebrough, Hugget, Griffin, and Goldberg, 1968). Such compounds are found in fairly high concentrations in marine organisms from California waters

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(Risebrough et al., 1967; Anas and Wilson, 1970; Wolman and Wilson, 1970; Burnett, 1971; Shaw, 1971, 1972; Castle and Woods, 1972; Munson, 1972; Risebrough, Menzel, Martin, and Olcott[3]).

Two types of apparatus were used: a flowing water system and a static water system. The flowing water apparatus was used for experiments in which fish were subjected to different toxicant concentrations. Flowing water mitigates differential toxicant uptake in experimental aquaria where unequal numbers of organisms are present. The static water apparatus was used to examine differences in congenital (untreated) asymmetry in populations; it employed filters to remove metabolic by-products.

Flowing Water Experiments

The experimental apparatus used consisted of 10 pairs of 6-liter, solid, molded, all-glass aquaria. One pair of aquaria was used as a control, the other nine pairs had the following p,p'-DDT concentrations: 0.001, 0.01, 0.1, 0.4, 0.7, 1, 10, 100, and 500 ppb (parts per billion). Water was gravity-fed from an 18-liter seamless all-glass reservoir tank to a pair of aquaria, thus ensuring that each experimental pair received the same quantity of toxicant. Water levels in the aquaria were maintained at 4 liters by individual all-glass, air-lift water pumps, each pump having the capacity to remove more water from the aquaria than was entering from the reservoir. Aquaria were covered with a ¼-inch plastic cover, and air was removed by vacuum (discharged to the outside of the building) to prevent contamination between tanks. Waste water from each tank was discharged into a central collecting tube and deposited into a sealed drain. The water flow to each tank was such that its water was completely replaced daily.

To obtain the desired toxicant concentrations, as mentioned above, standard solutions of p,p'-DDT in pesticide quality acetone were prepared. The quantity of p,p'-DDT in each standard solution was such that when 100 μl of acetone was dissolved in 18 liters of seawater (the size of our reservoir tanks), the concentration of p,p'-DDT in each reservoir tank was brought to one of the desired experimental concentrations. Filtered seawater was used for these experiments (obtained from the Scripps Institution of Oceanography Aquarium supply).

Grunion eggs for this experiment were collected from Belmont Shore. The eggs were obtained by digging in the sand the morning after a major grunion run. Clutches of eggs were placed in damp sand for an incubation period of 10 days. Eggs were hatched by placing handfuls of egg-bearing sand in seawater and agitating for a short period of time.

The flowing water experiments were begun with two control tanks and two replicate tanks for each treatment. Each tank initially contained approximately 400 fry. Thirty-eight days after hatching (48 days after being spawned), fish in one of the control tanks and one each of the replicate treatment tanks were killed. Fifty-one days after hatching (61 days after being spawned), the remaining fish were killed.

Static Water Experiments

The static water experiments consisted of raising grunion fry from different localities in continuously filtered recirculating seawater. Seawater in these tanks was changed weekly. Approximately 400 fry were used from each of three localities, Belmont Shore, Del Mar, and Bahia San Quintin, 200 fry being placed in each of two replicate tanks. Collection of eggs, hatching, and experiment termination periods were identical to those previously described.

All grunion were fed several times weekly on a diet consisting of newly hatched brine shrimp nauplii. Dead brine shrimp were removed from all tanks daily. Water temperature was ambient (ca. 20°C). No attempt was made to adhere to a light-dark cycle.

Analyses

Upon termination of each experiment, live

[3] Risebrough, R. W., D. B. Menzel, D. J. Martin, and H. S. Olcott. 1970. DDT residues in Pacific marine fish. Unpubl. manuscr., 32 p. Bodega Bay Mar. Lab., Bodega Bay, CA 94923.

specimens were placed in a 10% Formalin[4] solution. The fish died with their pectoral fins spread. After a minimum of 24 h in the fixative, specimens were rinsed in fresh water and stained for several hours in a 5% KOH-alizarin red solution. Pectoral fin rays were scored using a binocular dissecting microscope. The standard length of each specimen was determined by placing individuals on a flat plastic ruler.

We have chosen to use the variance, V_a, of the right (R) minus left (L) values to quantify the amount of asymmetry. This statistic is simply $\Sigma(d - \bar{d})^2/N - 1$, where d is an individual's asymmetry (right minus left value), $\bar{d}$ is the mean of the asymmetry distribution, and N is the sample size. In our previous paper we used a similar statistic, the squared coefficient of asymmetry variation,

$$CV_a^2 = (s_d \times 100/\bar{x}_{R+L})^2,$$

where the scale correction, $\bar{x}_{R+L}$, is the mean value of the character itself. In contrast to the earlier study, we found that the amount of asymmetry was uncorrelated with the mean character state, probably because the fins of the grunion in this study were still developing. Even though smaller grunion fry had fewer fin rays, they were not less asymmetrical in an absolute sense.

The use of CV_a^2 would, in fact, give misleading results. For example, examination of Table 1 will show that fry in untreated tanks have pectoral fin ray means varying from a low of 13.491 (Control) to a high of 23.220 (Del Mar). The V_a values are 0.554 and 0.596, respectively. The corresponding CV_a^2 values are 30.5 and 11.0.

RESULTS

One-half of the replicates were killed at 38 days after hatching because we were uncertain how long the fry would live. The remaining replicates were terminated 51 days after hatching. This latter time period was chosen because examination of grunion fry from Bahia San Quintin at that age (this experiment was started 2 wk before the others) indicated that the number of pectoral fin rays was near the same mean as was found in wild adults. Mortality, due to an unknown cause, was also reducing the numbers of fry in some tanks. The 38-day experiments were terminated too early to yield useful data as many fish had not yet begun to develop ossified pectoral fin rays.

Grunion fry receiving the largest dose of p,p'-DDT, 500 ppb, began showing signs of neuromuscular dysfunction the day after the experi-

[4] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

TABLE 1.—Grunion statistical information.

Treatment	N	Mean length ± standard error	Pectoral fin rays: Mean count (right + left) ± standard error	Pectoral fin rays: [1]V_a
		Static water experiments		
Bahia San Quintin	75	13.811 ± 0.084	22.800 ± 0.332	0.132
Del Mar	82	14.734 ± 0.096	23.220 ± 0.267	0.596
Belmont Shore	79	12.457 ± 0.099	16.684 ± 0.296	0.590
		Flowing water experiments		
Control[2]	53	11.728 ± 0.122	13.491 ± 0.557	0.554
0.001 ppb DDT	66	12.625 ± 0.105	20.303 ± 0.381	0.519
0.01 ppb DDT	7	13.486 ± 0.531	22.714 ± 1.266	0.576
0.1 ppb DDT	64	12.358 ± 0.114	19.031 ± 0.442	1.499
0.4 ppb DDT	65	12.602 ± 0.127	20.154 ± 0.473	0.898
0.7 ppb DDT	78	12.096 ± 0.088	17.679 ± 0.384	0.918
1.0 ppb DDT	51	11.402 ± 0.137	11.409 ± 0.737	2.401
10.0 ppb DDT	65	12.409 ± 0.139	18.985 ± 0.606	2.902

[1] Asymmetry variance.
[2] Control for the flowing water experiments.

ment began. Fry began swimming erratically, moving jerkily and, unlike untreated fry, swam on or near the bottom of the tank, sometimes upside down. Within a week all fry died. After 1 wk, fry in the 100-ppb treatment tanks began showing similar signs. Mortality reached 100% after 2 wk.

Asymmetry data are presented in Table 1. Grunion from Bahia San Quintin have the lowest variance of all groups examined. Fish from Del Mar and Belmont Shore, as well as the Control 0.001 and 0.01 fish, have intermediate variances (the 0.01-ppb sample should perhaps be eliminated because of its small size). Slightly greater variances are found in the 0.1-, 0.4-, and 0.7-ppb treatments. Finally, the 1- and 10-ppb treatment fish have the highest variances. It should also be pointed out that while the progression of asymmetry variances for p,p′-DDT-treated fish in the flowing water experiments is not perfect, the trend is statistically significant. Applying the Tau coefficient (Sokal and Rohlf, 1969) to these data (Control through 10-ppb treatment) yields $0.05 > P > 0.02$.

Comparing the V_a values for fry from Belmont Shore and Bahia San Quintin with the V_a values for adult fish (from data presented by Valentine, Soulé and Samollow, 1973) indicates that the ratio of asymmetry for adults is nearly the same as it is for fry (Table 2).

mont Shore grunion contain almost twice as much DDT as do La Jolla Shores grunion: Belmont Shore females 2.45 ppm ± 0.97, $N = 5$; La Jolla Shores females 1.40 ± 0.77, $N = 2$ (values on a whole body, wet weight basis, and include all isomers and metabolic products). DDT values reported here for Belmont Shore fish were obtained from specimens caught during the first grunion run of the season, whereas the experimental eggs were collected almost 6 wk later, from the third run.

Circumstantial evidence supports the hypothesis that eggs spawned early in the season have relatively high levels of DDT. Walker (1952) states that older fish spawn first. If larger fish have higher body burdens of DDT than smaller fish, then eggs spawned by large fish might have more DDT. Alternatively, if the amount of DDT deposited in eggs is a constant proportion of the amount of DDT in the female body, then the first spawn would contain more DDT in absolute terms than the later spawns. We lay some stress on these possibilities because of our enigmatic observation that eggs from the first Belmont Shore run all failed to hatch, dying at an advanced stage of development.

If DDT increases asymmetry, then the low V_a values for grunion fry from Bahia San Quintin might be attributed to low adult and egg DDT burdens. Although we have no data

TABLE 2.—Comparison of pectoral fin ray V_a values for experimental and wild-caught grunion.

Locality	Wild adults			Experimental fry (untreated)		
	Date	N	V_a	Date	N	V_a
Bahia San Quintin	1970	57	0.070	1972	75	0.132
Belmont Shore	1969-70	27	0.286	1972	79	0.590

DISCUSSION

Egg and Adult DDT Burdens

We have measured the chlorinated hydrocarbon body burdens of several wild-caught grunion. These data will be published in a later paper and are here offered only for comparative purposes. Our small sample suggests that Belmont Shore grunion

on pesticides concentrations in adult grunion from Bahia San Quintin, we have gathered such data in barred sand bass, *Paralabrax nebulifer* (pertinent life history data for this species are given in Valentine, Soulé, and Samollow, 1973). DDT concentrations in barred sand bass from Bahia San Quintin are 0.199 ± 0.038 ppm ($N = 16$), from San Diego, 2.150 ± 0.184 ppm ($N = 28$), a difference of roughly an order of magnitude.

DDT and Asymmetry

In our earlier report we showed that adult grunion from Belmont Shore were 4.08 times as asymmetrical as San Quintin fish. In the present study, the ratio of asymmetry variances in untreated fry is almost the same, 4.46. This consistency may be more than coincidence. This result and our experimental data support our hypothesis based on data from wild-caught fish that environmental stress may produce statistically detectable changes in asymmetry (Valentine, Soulé, and Samollow, 1973). Perhaps the most important result is that extremely low concentrations of DDT (< 1 ppb) cause a very significant deterioration in the symmetry of fin rays. When it is noted that in all probability the control fry from Belmont Shore were already carrying a stressful load of DDT, the significance of the results is enhanced.

Our rationale for utilizing p,p'-DDT as a stressant was that it (and its metabolic products) are known to occur in high concentrations in southern California marine organisms (Burnett, 1971; Castle and Woods, 1972; Duke and Wilson, 1971; Munson, 1972; Risebrough et al., 1967). The White's Point outfall, situated about a mile north of Point Fermin, was shown to be releasing effluent containing DDT approaching 100 ppb in late 1970, and also similar quantities of polychlorinated biphenyls (Schmidt, Risebrough, and Gress, 1971). These polychlorinated biphenyls may themselves produce increases in asymmetry since they have properties similar to DDT (Risebrough, Reiche, Peakall, Herman, and Kirven, 1968; Gustafson, 1970; Peakall and Lincer, 1970). We would, in any case, expect to find a diversity of agents in southern California's marine waters capable of producing increases in asymmetry, among them DDT.

Grunion fry exposed to increasing levels of p,p'-DDT in water yielded results in accord with our environmental stress hypothesis (Valentine, Soulé, and Samollow, 1973). At p,p'-DDT levels greater than 0.01 ppb, asymmetry values increased. Increases in the asymmetry of bilateral structures are easily quantified and monitored. We hope other workers will test this system with other pollutants that may affect calcium metabolism. The funding situation precludes our doing so.

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