

**REDUCED GONADAL SOMATIC INDEX AND EXTERNAL  
COLORATION FOLLOWING EXPOSURE  
TO P,P'-DDE IN ADULT MALE *FUNDULUS HETEROCLITUS***

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**EXTENDED ABSTRACT ONLY – DO NOT CITE**

The persistent organochlorine, p,p'-DDE (a lipophilic metabolite of DDT) is a potent antiandrogen that binds the androgen receptor (AR) in rats (Kelce et al. 1995). p,p'-DDE also binds ARs in fish *in vitro* (Thomas 2000) although *in vivo* studies investigating the antiandrogenic activity of p,p'-DDE in fish have produced conflicting results. Carlson et al. (2000) reported a lack of effects by either p,p'DDE or o,p'DDT on sexual development in rainbow trout embryos, whereas Baatrup and Junge (2001) recently demonstrated that p,p'DDE can act as an antiandrogen in male guppies. We evaluated the effects of p,p'DDE exposure on gonadal growth, coloration (male *Fundulus* develop yellow coloration when mature), and blood plasma concentrations of testosterone (T) and 11-ketotestosterone (KT).

Two similar studies were conducted in 1998 and 1999. *Fundulus* collected from Stony Brook, NY in the fall of 1997 were held approximately six months prior to the 1998 study (10 ppt salinity, 10°C) and for 1.5 years prior to the 1999 study. In March 1998, 56 *Fundulus* were allocated to tanks (12 fish per tank, with two tanks per dose), acclimated for five days and injected with either 0, 10 or 100 ppm p,p'DDE dissolved in olive oil. Temperature was then increased on the day of injection over a period of 4 days to 20°C to induce maturation. Fish were sampled 0, 2 and 4 weeks after injection, visually inspected for color development, and condition factor (CF), gonadal somatic index (GSI) and hepatic somatic index (HSI) were recorded. Na<sup>+</sup>,K<sup>+</sup>-ATPase activity was

measured in gill tissue as a general indicator osmoregulatory status. In 1999 we again rated coloration on a scale from 1 (little or no yellow) to 3 (bright yellow), since ratings were qualitative they were confirmed 'blind' by others in the lab. Blood plasma concentrations of T and KT were also measured using previously established radioimmunoassays. Statistical results were calculated using ANOVA, with HSD for unequal cell numbers. A nonparametric analysis employing a Kruskal-Wallis ANOVA by ranks post-hoc test in STATISTICA was used for hormone analysis since a large number of samples were below detection limits.

Results from both 1998 and 1999 were fairly consistent. In general p,p'-DDE did not affect general indicators of health including weight, HSI, or  $\text{Na}^+, \text{K}^+$ -ATPase activity (range from 6.2 to 7.0  $\mu\text{mole/mg protein/min}$ ). There was, however, a decrease in CF in 1998 in fish dosed with 100 ppm p,p'-DDE (from 1.12 to 1.05), although we did not observe a reduced CF in 1999. Exposure to 100 ppm p,p'-DDE caused a 28% decline in GSI in the maturing male fish in 1999 and a 22% decline (which was not significant) in

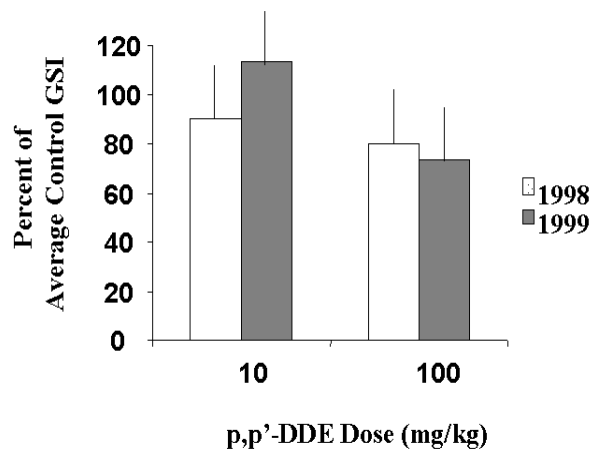


Fig. 1. Gonadal (testicular) somatic index (GSI) following four weeks of exposure to p,p'-DDE in *Fundulus heteroclitus* shown as a percent of mean control GSI (+/- STDEV). Data for studies conducted in 1998 and 1999.

1998 (Figure 1). Additionally we observed a decrease in yellow coloration of the males in 1999 (Table 1). Differences in coloration, though not quantified, were noted in 1998 as well. Development of coloration is an androgen dependent process in most male fish often involving KT (reviewed in Borg 1994). The results of this work are consistent with effects of p,p-DDE in the guppy (Baatrup and Junge 2001) where high doses of p,p'-DDE caused reduced coloration, sperm count, gonad size and courtship behavior (1 ppm of p,p'-DDE in food was estimated to result in a daily 'dose' of 15 µg/g fish [Baatrup and Junge 2001] or a maximum of 450 ppm over 30 days.) Our exposure levels are closer to the lower dose used by Baatrup and Junge (2001) although our route of exposure was quite different (a single injection at the initiation of the study verses a daily exposure throughout the course of the study). Additionally we measured plasma concentrations of T and KT. Unfortunately too many values for T were below the detection limit of our assay to conduct statistical analysis. KT concentrations did not appear to be altered five weeks after p,p'-DDE exposure (Table 1). Since a large number of samples were below detection limits for T and KT it is difficult to draw conclusions regarding the effects of p,p'-DDE exposure on these androgens. Although by taking the conservative approach of assigning the detection limit to those samples below that limit, our data suggests that exposure to p,p'-DDE did not alter concentrations of KT. Nor did concentrations of KT appear to differ among groups when calculated as ng/ml steroid/g gonad (data not shown). The results of this study suggest that p,p'-DDE exposure in adult fish interferes with gonad growth and development of nuptial coloration in *Fundulus*, possibly by acting as an antiandrogen.

Table 1. Effects of p,p'-DDE on morphometric and steroidal endpoints in *Fundulus heteroclitus* following two or four weeks of exposure data are expressed as mean(stdev).

tmt mg/ kg	N	Wt <sup>a</sup> g	L cm	HSI %	GSI %	CF	KT <sup>b</sup> ng/ml	T <sup>c</sup> ng/ ml	Color Score
Results 1998 2 weeks after initial exposure									
0	8	5.2 (2.2)A	7.7 (1.0)A	1.0 (0.6)B	4.1 (1.1)B	1.10 (0.04)A	n	n	n
10	8	5.9 (1.3)A	8.1 (0.5)A	1.3 (0.3)B	3.7 (0.8)B	1.08 (0.04)A	n	n	n
100	8	4.6 (0.9)A	8.1 (0.8)A	1.5 (0.4)B	4.5 (0.9)B	1.19 (0.04)A	n	n	n
Results 1999 4 weeks after initial exposure									
0	5	4.6 (1.3)A	7.4 (0.8)A	0.9 (0.2)B	3.7 (0.2)B	1.12 (0.03)A	n	n	n
10	7	5.1 (2.0)A	7.5 (1.0)A	0.9 (0.3)B	3.2 (0.7)B	1.15 (0.03)A	n	n	n
100	8	4.6 (0.9)A	7.6 (0.5)A	0.8 (0.2)B	2.9 (0.7)B	1.05 (0.02)B	n	n	n
Results 1999 4 weeks after initial exposure									
0	12	10.9 (2.0)A	9.5 (0.5)A	1.7 (0.3)B	2.5 (0.5)A	1.25 (0.04)A	0.46 (0.08)B	0.14 (0.02) A	1.9 (0.7) B
10	11	11.0 (3.1)A	9.5 (0.8)A	1.7 (0.3)B	2.8 (0.5)A	1.26 (0.04)A	0.67 (0.30)B	0.21 (0.11) A	1.9 (0.7) B
100	12	12.9 (3.2)A	9.9 (0.7)A	1.8 (0.4)B	1.8 (0.5)B	1.31 (0.31)A	0.48 (0.17)B	0.16 (0.05) A	1.2 (0.5) A

<sup>a</sup>Different letters are significantly different ( $p < 0.05$ ) using ANOVA and HSD for an unequal N post-hoc test. <sup>b</sup> KT measurements are for individual fish. Sample detection limit was 0.40 ng/ml to be conservative we used 0.40 ng/ml for samples that were below detection limit. There were several samples below detection limit (0, 5, 4 and 8 in the Time 0, 0 ppm, 10 ppm and 100 ppm groups respectively). Because of the large number of samples below detection we used a nonparametric posthoc test for KT analysis (Kruskal-Wallis ANOVA by ranks) <sup>c</sup>Samples from 3-4 individuals were pooled for analysis of T, so N=4 per

treatment except Time 0 where N=3. Sample detection limit was 0.13 ng/ml, to be conservative we used 0.13 ng/ml for samples that were below detection limit. There were 3, 1 and 3 values below detection in the 0, 10 and 100 ppm groups respectively, so no statistical analysis was conducted. <sup>d</sup> n=not measured in this study.

## References

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