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Comparison of reproductive parameters in male yellow-blotched map turtles (*Graptemys flavimaculata*) from a historically contaminated site and a reference site

J.A. Shelby, M.T. Mendonça*

Department of Biological Sciences, Auburn University, 331 Funchess Hall, Auburn, AL 36849, USA

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Abstract

From May to September of 1998, we collected monthly plasma samples from male yellow-blotched map turtles captured at two sites in the Pascagoula River drainage, Mississippi. One site (Vancleave) has a documented history of pollution from industrial sources (principally 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD). Fish consumption advisories at the Vancleave site were lifted in 1996 and current impacts appear minimal. However, the yellow-blotched map turtle, a federally protected species, continues to decline in numbers. To determine if endocrine disruption could be a factor in the low reproductive rates observed in Vancleave turtles, we examined levels of plasma testosterone (T) and estradiol-17β (E_2) from males at this site and a second site (Leaksville), which has no known source of industrial pollution. Plasma was also tested for vitellogenin (VTG), which, in males, can be a biomarker of exposure to estrogenic contaminants. No males had detectable plasma VTG nor did mean monthly E_2 levels differ between sites. However, 10% of males from the historically polluted site were found to have high levels of E_2 (equivalent to levels found in females) and T was significantly lower for males captured at this site for 3 of 5 months. Our data suggest that the current impact of contaminants on reproduction in this population is limited. However, a portion of the population may have been affected developmentally, as represented by differences in reproductive parameters detected between sites.

Keywords: Reptile; *Graptemys flavimaculata*; Threatened species; Reproduction; Endocrine disruption; Pulp mill; TCDD; PCBs

1. Introduction

The detrimental effects of environmental contaminants on reproduction have been documented in many riverine vertebrates. The majority of studies examining wildlife species as bioindicators of environmental health focus on fish (Andersson et al., 1988; Munkittrick et al., 1991, 1992; McMaster et al., 1992, 1995; Van der Kraak et al., 1992; Van den Heuvel et al., 1994; Goodbred et al., 1997; Orlando et al., 1999). However, many fish species are relatively short-lived and do not provide much needed informa-

* Corresponding author. Tel.: +1-334-844-9246; fax: +1-334-844-9234.
E-mail address: mendomt@auburn.edu (M.T. Mendonça).

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tion on the persistent effects of pollution. Longer-lived species, such as turtles, can serve as indicators of environmental status on a larger time scale. The long-term sub-lethal impact of pollutants on reproduction may ultimately limit population growth, possibly leading to severe population declines and even extirpation. New techniques in pulp bleaching (e.g. elemental chlorine-free processing) have led to recovery of some rivers in North America, yet there may be persistent effects from historical industrial activities.

The yellow-blotched map turtle, *Graptemys flavimaculata*, is a species endemic to the Pascagoula River and its tributaries, the Leaf and Chickasawhay Rivers in Mississippi. Historical evidence suggests that this riverine species has been heavily affected by pollution. In 1986, 2 years after the operations of a wood pulp processing plant began, populations of yellow-blotched map turtles disappeared immediately downstream from the plant on the Leaf River (Ernst et al., 1994). Because of declining population densities, this species was listed as threatened by the US Fish and Wildlife Service in 1991 (Jones, 1991).

To examine the possibility that population declines in this species might be due to persistent effects of pollution on reproduction, we sampled plasma from male turtles from two sites for analysis. The two sites we chose for sampling differed in pollution history. The impacted site we chose was located on the Pascagoula River near Vancleave, Mississippi (Fig. 1). This river and its main tributary to the west, the Leaf River, were placed under fish consumption advisories from 1989 until 1995 due to elevated levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in fish (Mississippi Department of Environmental Quality, 1995; US EPA, 1997). These advisories encompassed areas downstream from a pulp mill on the Leaf River. This is one of the 10 largest bleached kraft pulp mills in operation in the United States, currently operating at a rate to produce 1550 tons of baled pulp per day. Furthermore, release of wastes from secondary treatment ponds by this mill in 1992 lead to mortality in fish and turtles along the length of the Leaf and Pascagoula Rivers. The site we designated as impacted is located 90 km downstream from the mill and has the last remaining substantial population of yellow-blotched map turtles located in the Leaf/Pascagoula River system.

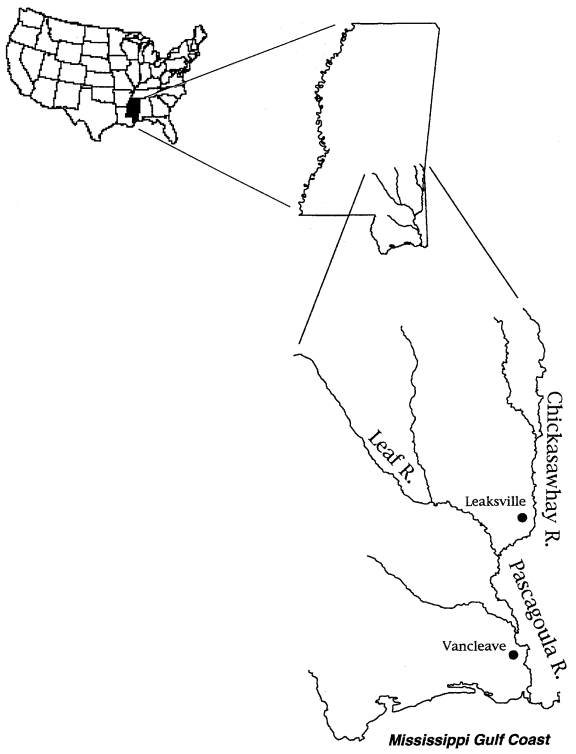

Fig. 1. Map of the Pascagoula River drainage in Mississippi with sampling locations: Leaksville (unimpacted); and Vancleave (historically impacted).

The unimpacted site located near Leaksville, Mississippi, on the Chickasawhay River is in a rural area, with few sources of pollution upstream (Fig. 1). The only documented source that has affected this site is an oil field in Clarke Co., MS, located approximately 100 river km upstream from the sample site, which released salinized water into the Chickasawhay during drilling operations 22 years ago for a 4-month period (McCoy and Vogt, 1980). There have been no fish consumption advisories issued for the Chickasawhay River. Turtles are not likely to have moved into this area from the impacted site due to limited seasonal movement (Bob Jones, unpublished data).

Turtles in the Leaf and Pascagoula rivers were exposed to elevated levels of industrial waste for approximately 11 years, from 1984 when the mill operations began until 1995 when EPA advisories were lifted. Levels of TCDD in fish tissues were reported to fall to background levels by 1996 (Justus et al., 1999). TCDD, even at low concentrations, disrupts endocrine functioning and development in many vertebrate species (Safe et al.,

1991; Peterson et al., 1993; Birnbaum, 1995). Since yellow-blotched map turtles may live 30 years or longer, a significant proportion of the reproducing population from the Vancleave site would have been exposed to elevated contaminant levels during their pre-adult development. Exposure to TCDD during this sensitive life stage in rats has been shown to have a life-long impact on reproduction (Mably et al., 1992a,b).

Yellow-blotched map turtle liver tissues collected at both sites currently have low levels of total polychlorinated biphenyls (PCB) and TCDD (Kannan et al., 2000). The pollutant levels in tissues today are similar to those observed at most sites without point source pollution (Beyer et al., 1996). There are no data on historical levels of these contaminants in turtle tissues. However, we propose that since elevated TCDD was documented in fish tissues, this turtle, which feeds mainly on filter feeding organisms such as freshwater sponges and mollusks (Seigel and Brauman, 1994), would also have been exposed to elevated TCDD levels, as well as other possible compounds found in pulp mill effluent that were not documented.

The purpose of the present study was to investigate the potential impact of this historical contaminant exposure (developmental and sublethal) on present-day endocrine and morphological parameters in adult yellow-blotched map turtles. Study of long-lived animals such as turtles may provide insight on the long-term effects of developmental exposure to pollutants on subsequent adult physiology. Therefore, we compared plasma sex steroid and vitellogenin level differences in adults to determine if these characteristics differed between sites.

2. Materials and methods

2.1. Sampling location and techniques

In 1998, adult male yellow-blotched map turtles were captured from April through October at a site located in Greene County near the town of Leaksville on a 3-km section of the Chickasawhay River (reference population), and from May through September at a site in Jackson County near the town of Vancleave on a 5-km section of the Pascagoula River (impacted population), Mississippi (Fig. 1). The capture period encompasses the time of year that these turtles undergo gonadal recrudescence, mating and reproduction.

Turtles were captured with basking traps. These open baskets were placed below water level, on logs where turtles were frequently observed to bask. Turtles were removed immediately after they were startled into the trap by an approaching boat. Blood (1 ml) was collected from the caudal artery within 10 min of capture (heparinized 1-ml syringe; 26 gauge needle). Blood samples were stored on ice for less than 6 h, then centrifuged for 10 min at $1000 \times g$. Plasma was pipetted into cryovials and frozen in liquid nitrogen for transport. All plasma samples were stored at $-20^{\circ}C$ until processed. Body size was measured as straight-line maximum carapace length (CL). Turtles were determined to be adults if CL exceeded 7.5 cm. Juveniles could be identified by the presence of distinct annuli on their carapace and were excluded from the analysis. Adult individuals have indistinct annuli, making them impossible to age. Animals were released at the site of capture.

2.2. Hormone analysis

Plasma testosterone (T) and 17-β estradiol (E_2) were measured via radioimmunoassay with the methods previously described and validated for use in yellow-blotched map turtles (Shelby et al., 2000). In brief, for T and E_2, volumes of 75 and 125 μl, respectively, of plasma were extracted with 3 ml of anhydrous diethyl ether. E_2 and T antibodies were obtained from Endocrine Sciences, Tarzana, CA. Extraction efficiencies averaged 96.4 and 87.8%, and interassay variations with respect to spiked controls were 8.0 and 8.6%, respectively. The intra-assay variation averaged 7.1%. Sensitivity of the assays averaged 9 and 12 pg/ml, respectively.

2.3. VTG analysis

VTG from E_2-treated female yellow-blotched map turtle plasma was purified by DEAE-Sephacel (diethylaminoethyl sephacel) column separation followed by Mg^{2+}/EDTA precipitation (as described in Wiley et al., 1979). Vitellogenin concentration was analyzed in plasma samples with an antibody-capture enzyme linked immunosorbant assay (ELISA). Rabbit polyclonal anti-turtle VTG (*Trachemys scripta*) IgG was obtained from

the lab of Brent Palmer, University of Kentucky. Plasma was serially diluted fivefold from 1:75 to 1:234 375 and 100 μl of each was loaded in duplicate into Immulon II brand ELISA plates. Purified vitellogenin was serially diluted from 582.5 to 2.27 μg/ml and dilutions were loaded in duplicate, as were VTG positive (E_2 treated female) and negative (captive male) controls. Interassay variation averaged 10.66% and intra-assay variation averaged 13.62%. The average sensitivity of the assays was 0.10 μg/ml. This assay was validated by demonstrating parallel binding slopes of dilution curves of the purified VTG as compared to E_2 treated female yellow-blotched map turtle plasma, and by demonstrating that control male plasma was not reactive with the antiserum. Furthermore, VTG measured using this technique was positively correlated to plasma E_2 levels in wild caught female yellow-blotched map turtles collected at the reference site (J. Shelby, unpublished data). VTG positive plasma, as determined by ELISA, showed visible bands at ~210 kDa using Western blot techniques whereas VTG negative plasma showed no band at this weight (Fig. 2). VTG has been found to have a similar MW in other turtle species (Selcer and Palmer, 1995; Heck et al., 1997). Procedure for Western blot included incubating the blot with primary polyclonal antibody specific for turtle VTG (Rabbit polyclonal anti- turtle VTG IgG, Brent Palmer, University of Kentucky), diluted 1:15 000 (v/v) at room temperature (RT) for 1.5 h and a secondary antibody (HRP conjugated goat anti-rabbit IgG, Bio-Rad, Winston, NY) diluted 1:3000 incubated 1.5 h at RT. Vitellogenin was visualized using ECL Western blotting reagents and Hyperfilm-ECL (Amersham Life Science Inc., Arlington Heights, IL).

2.4. Statistical analysis

Plasma T, E_2, and CL were grouped by site and monthly sample period. Bartlett's test of homogeneity of variances indicated that all of these groups had homogenous variances (Sokal and Rohlf, 1981). Simple linear regression was used to determine if CL accounted for a significant proportion of the variance in levels of T and E_2. However, there was no effect of CL on hormone levels ($P \geq 0.50$). Thus, size was not used as a covariate in analyses of steroid variance. For each site, monthly T and E_2 means were analyzed with one-way analysis of variance (ANOVA) followed by a post hoc Fisher's least significant difference test. Differences in mean levels of E_2 and T by sample period between sites were determined with two-way ANOVAs. No statistical analyses were used for VTG data, since values were determined to be below detectable levels. Significance was determined at $P < 0.05$. Statistical analysis was performed with Statview statistical software program (SAS Institute, Cary, NC).

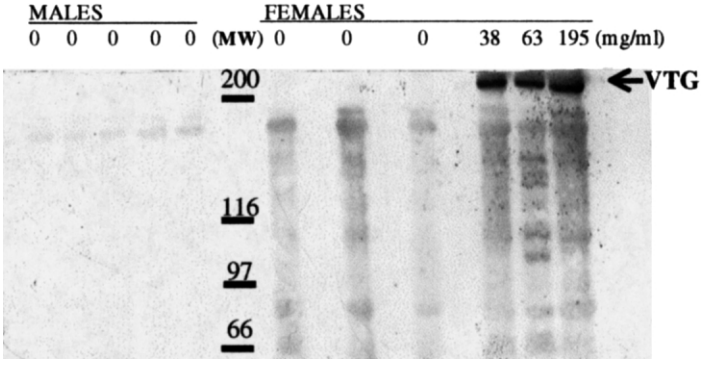

Fig. 2. Validation of enzyme linked immunosorbant assay (ELISA) with Western blot techniques. VTG represents vitellogenin (VTG) protein band; MW represents molecular weight markers. Lanes 1–5 were loaded with wild caught male *Graptemys flavimaculata* plasma diluted 1:1500 (labeled as MALES). Lanes 6–11 were loaded with wild caught female *Graptemys flavimaculata* plasma diluted 1:1500 (labeled as FEMALES). Values at the top of each lane represent amount of VTG in mg/ml as determined for each plasma sample by ELISA.

3. Results

3.1. Plasma testosterone

There was significant variation in mean monthly T for males captured at the Leaksville site ($F_{4,43} = 8.06$; $P < 0.0001$) (Fig. 3). Mean T levels in May were significantly higher than levels in July or August ($P = 0.008$; $P = 0.031$, respectively) and mean T for September was significantly higher than levels observed in June, July and August ($P \leq 0.0005$). There was also significant variation in mean monthly T for males captured at Vancleave ($F_{4,52} = 13.32$; $P < 0.0001$) (Fig. 3). Mean T levels were significantly higher in June than May, July and August ($P \leq 0.009$). Mean T in June was significantly lower than peak T occurring in September ($P = 0.005$).

When the two sites were compared by month, overall levels of T were found to be significantly higher in Leaksville males ($F_{1,91} = 9.8$, $P = 0.002$). During May, July and August, T was significantly higher in Leaksville males ($P \leq 0.01$). However, in June ($F_{1,19} = 0.24$, $P = 0.63$) and September ($F_{1,17} = 4.02$, $P = 0.06$) there was no difference in mean T levels (Fig. 3). Peak spring T levels, occurring in May for Leaksville and June for Vancleave, were not significantly different ($F_{1,19} = 3.77$, $P = 0.068$) even though Leaksville mean T was more than double the mean values seen for Vancleave (3.15 vs. 1.44 ng/ml, respectively). This discrepancy was probably due to high variance in T levels for males captured in Leaksville during May. The peak in mean T levels observed in the fall for Leaksville males occurred in October and was equivalent to peak T levels observed for Vancleave males in a previous study (7.12 ± 0.78 ng/ml for Leaksville, and 8.08 ± 1.29 for Vancleave) (Shelby et al., 2000).

Carapace length was significantly larger in males from the Vancleave site as compared to males captured at the Leaksville site ($F_{1,94} = 54.15$; $P < 0.0001$). However, T was not correlated to CL for males from the Leaksville site ($r = 0.005$, $F_{1,41} = 0.001$, $P = 0.97$), and was negatively correlated to CL for males from Vancleave ($r = -0.30$, $F_{1,52} = 5.00$, $P = 0.03$). However, since the Vancleave data was limited, analysis was redone including information from males collected in a previous study (Shelby et al., 2000). When the T and CL data collected from males captured in Vancleave in 1997 were included, the relationship between T and CL was not significant ($r = 0.06$, $F_{1,117} = 0.45$, $P = 0.50$).

3.2. Plasma E_2 and VTG

There was no significant variation in mean monthly E_2 for males captured at Leaksville ($F_{4,43} = 1.93$; $P = 0.12$) or Vancleave ($F_{4,52} = 2.13$; $P = 0.09$) (Fig. 4). There was no difference in E_2 levels between locations by sample period ($F_{1,91} = 1.45$; $P = 0.23$). However, there were several males from the Vancleave site with high levels of E_2. Four Vancleave males exhibited high E_2 levels (as compared to the maximum for any Leaksville site male of 70 pg/ml) at levels from 90 to 390 pg/ml (during June, July and August sample periods). Males captured at the Vancleave site in previous years were also observed to have elevated E_2 levels. A 10% proportion of Vancleave males exhibited elevated E_2 when data were pooled for 1996, 1997 and 1998 (20 males of 198) (J.A. Shelby, M.T. Mendonça, unpublished data). No VTG was detected in plasma from males captured at either site ($n = 40$, Vancleave; $n = 34$, Leaksville), as determined by ELISA. There is no significant relationship between CL and E_2 levels for males at Leaksville ($r = 0.002$, $F_{1,41} = 0.07$, $P = 0.80$) or Vancleave ($r = 0.005$, $F_{1,52} = 0.001$, $P = 0.97$).

4. Discussion

All studies focusing on the effects of pollution on wildlife species have been done while pollutants were elevated in the environment. The

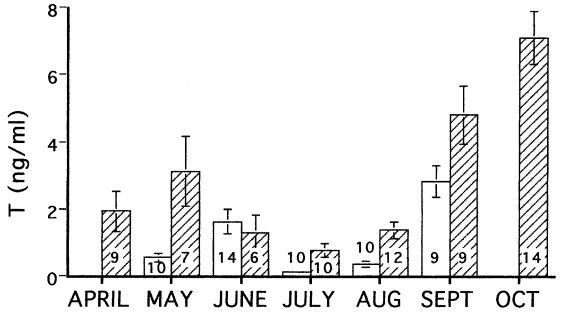

Fig. 3. Seasonal plasma testosterone (T) levels for wild caught male *Graptemys flavimaculata* captured at field sites in Vancleave (open bars) and Leaksville (shaded bars). Values are monthly means +1 S.E. and numbers at the base of the bars represent sample size.

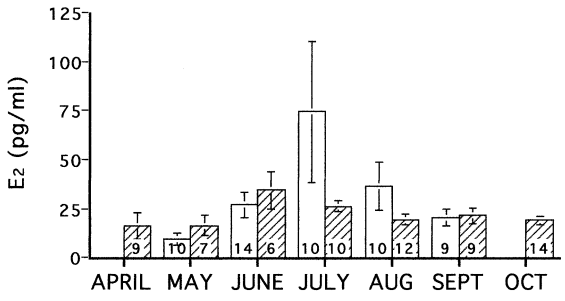

Fig. 4. Seasonal plasma estradiol-17β (E_2) levels for wild caught male *Graptemys flavimaculata* captured at field sites in Vancleave (open bars) and Leaksville (shaded bars). Values are monthly means +1 S.E. and numbers at the base of the bars represent sample size.

present study focuses on potential long-term effects, and addresses the question 'are the effects of a pollution event persistent after the pollutants are no longer at elevated levels?' Turtles are a good model for examination of this aspect of pollution impact because they are long-lived and relatively sedentary. Yellow-blotched map turtles seem to be particularly sensitive to these impacts on a population level as indicated by population decline when operations of a pulp mill located on the Leaf River began and by the lack of population recovery. This sensitivity may be due to the fact that turtles have a relatively slow rate of reproduction.

Male yellow-blotched map turtles require approximately 4 years to reach maturity. Many turtles present in the Vancleave population, especially those 4–14 years of age in 1998, were most likely exposed to elevated pollutant levels during their development. Those age 14 hatched when mill operations began and populations upstream began to disappear, and those age 4 hatched in 1994, the last year that fish consumption advisories were issued for the Pascagoula River drainage. Based on population structure and mortality estimates, this cohort represents a substantial portion of breeding males in the population, most likely more than 50% (R.A. Seigel, personal communication).

Recent analysis of TCDD and PCBs in female liver tissue and eggs leads us to believe that current impacts due to these contaminants are minimal (J.A. Shelby, M.T. Mendonça, unpublished data). However, elevated TCDD levels documented in the past in the Pascagoula River may have affected turtles during development, having lifelong reproductive consequences. Pulp mill effluent contains many toxic compounds such as phenols and resin acids, which are minimized during secondary treatment (Verta et al., 1996). Other pollutant sources at this site could include a wood treatment plant (inactive today) and sewage treatment plant (Justus et al., 1999). Therefore, other unknown contaminants may have also been elevated in the past or they may still be present at the contaminated site. We cannot discount the fact that the differences observed between sites may be due to some unmeasured pollutant or combination of pollutants present in the past or now. We do know, however, that TCDD levels were elevated in the past and propose that site differences could be due to this fact.

It has been shown that exposure to TCDD during development can affect reproductive capabilities in organisms throughout their lifetime. For example, in rats a single maternal dose of 160 pg/g or more during pregnancy lowers plasma *T* and dihydrotestosterone, and reduces weights in seminal vesicles and ventral prostates in a dose-dependent manner in adult male offspring when compared to unexposed rats (Mably et al., 1992a,b). Overall, *T* levels were lower for males at the historically polluted site (Vancleave). Specifically, *T* levels were significantly lower for 3 of the 5 months that sampling coincided. One of the 2 months that did not show a significant difference in *T* levels between sites closely approached significance (September, $P = 0.06$). Seasonal fluctuations in *T* were essentially the same between sites. The seasonal cycle of *T* has been characterized for Vancleave males (Shelby et al., 2000). Generally, mean monthly *T* levels are slightly elevated in the spring, but never exceed 2.5 ng/ml. Testosterone then drops to basal levels until the fall when it rises to maximum levels (8.5 to 11.4 ng/ml, 1997 and 1996, respectively), most likely coincident with fall gonadal growth.

This study showed that a significant early *T* peak occurred in May for males captured in Leaksville, the historically unimpacted site. Levels of *T* for males caught at the Vancleave site were significantly lower when the two sites were compared during the May sample period. However, the levels of *T* were elevated in June 1998 for Vancleave males, a later spring peak than previously reported (April in 1996 and May in 1997) (Shelby et al., 2000). When the May peak in *T* for Leaksville males was compared to the June

peak in Vancleave males, it was higher, but not significantly ($P = 0.07$). During July and August, when levels were basal at both sites, the mean T levels for males captured in Leaksville were significantly higher than for males caught in Vancleave.

Comparison between the fall peak levels between the two sites was not possible since sampling for October was limited to the Leaksville site. However, when fall peak T levels for Vancleave males captured in October 1997 were compared to peak levels of T for males captured in Leaksville in October of 1998 there was no significant difference (Shelby et al., 2000).

Despite the slight differences in timing, the spring peak and basal T levels all show the same pattern: lower T levels at the Vancleave site. This overall difference in T levels may be the result of site differences or the result of historical pollution impacts at the Vancleave site. A potentially important difference between the two sites is water temperature. It is known that timing of steroid fluctuations in turtles can be influenced by temperature (Mendonça and Licht, 1986; Mahmoud and Licht, 1987). However, the difference in seasonal water temperature between the two sites is minimal (e.g. water temperature at the Vancleave site was cooler than the Leaksville site by 0.5–1.0°C when measured in 1997) (Plunkett et al., 1998). It seems unlikely that such small differences would affect the overall levels of plasma T. Furthermore, E_2 levels were not affected. Other differences between the sites are the width of the rivers and salinity of the water. At the Vancleave site the river is wider (100–250 m) and when last measured had a higher salinity (4 ppt chloride) than at the Leaksville site (30–150 m wide, 0.07 ppt chloride) (US Army Corps of Engineers, 1968). Given the relatively minor site differences, we believe that the pattern of lower T in adult males from the impacted site is most likely a consequence of developmental exposure to pulp mill effluent. This pattern of lowered T in response to exposure to various pollutants, including pulp mill effluent, has been seen in other species (Brookstaff et al., 1990; McMaster et al., 1991; Munkittrick et al., 1991, 1992; Peterson et al., 1993; Orlando et al., 1999). Exposure to a mixture of pollutants (pesticides, dicofol, DDT and sulfuric acid) has also been shown to reduce plasma T in juvenile alligators (Guillette et al., 1995).

Another reproductive steroid that has been shown to be affected by pollutant exposure is E_2. When the levels of E_2 for yellow-blotched map turtles were compared between sites, four males captured at the Vancleave site had high levels of plasma E_2 (from 90 to 390 pg/ml), which were equivalent to peak levels for females from the same site (Shelby et al., 2000). In previous years, other males captured at the Vancleave site were found to have high levels of E_2 giving a total of 20 males out of 198 (10%) sampled over a 3-year period (J.A. Shelby, M.T. Mendonça, unpublished data). Males captured at the Leaksville site were never documented with E_2 levels greater than 70 pg/ml. It could be that males with high levels of plasma E_2 were embryos or juveniles present at the Vancleave site during the time when pollutant levels were elevated.

The role of E_2 in males is poorly understood. Estradiol-17β is formed in males by aromatization of T at specific target tissues such as the testes and brain (Kime, 1986). This aromatization step has been suggested as a target site for endocrine disruption in the follicles of female white suckers (McMaster et al., 1995). Additionally, male and female white suckers at sites contaminated with pulp mill effluent have lowered E_2 levels when compared to those at reference sites (McMaster et al., 1991; Munkittrick et al., 1991). For alligators, in vitro levels of E_2 produced by testes from individuals exposed to a mixture of pollutants (noted above) were significantly higher, when compared to testes of alligators from an uncontaminated site. However, plasma levels of E_2 did not reflect this difference (Guillette et al., 1994).

PCBs, a type of contaminant detected in low levels at both of our sites (Kannan et al., 2000; J. Shelby, unpublished data), can influence sex determination in turtles (Bergeron et al., 1994). This potentially could affect plasma E_2 levels in adults. Other pollutants have adversely affected gonadal development in reptiles. Abnormal gonadal development has been shown in hatchling alligators from a lake contaminated with the above-mentioned mixture of pollutants. These males appear to be normal in external morphology but have abnormal steroid production (abnormally low plasma T) and poorly organized seminiferous tubules (Guillette et al., 1995).

VTG was not detected in plasma collected from males from any site. VTG is an E_2-induced pro-

tein formed in the liver and transported in the blood to be deposited in the developing ovaries of females. Detectable levels of vitellogenin in plasma of males has been documented to indicate the presence of estrogen-like pollutants in fish habitats (Goodbred et al., 1997; Sumpter and Jobling, 1995). Many chemicals have been determined to be estrogenic, including PCBs and polychlorinated dibenzodioxins (Sumpter and Jobling, 1995). Detectable limits of our ELISA were well within the range of 10–800 μg/ml detected in the plasma of adult male carp from polluted streams (Goodbred et al., 1997). Furthermore, male painted turtles, *Chrysemys picta*, have been shown to respond to E_2 injection by an increase in plasma VTG (Ho et al., 1981; Palmer and Palmer, 1995). These data and the fact that no male yellow-blotched map turtles had plasma VTG suggest that current environmental levels of estrogen-like pollutants are too low to affect plasma VTG levels in male yellow-blotched map turtles.

Males captured at the Vancleave site were significantly larger than males captured at the reference site in Leaksville. The difference in size did not appear to be attributable to a missing cohort upon examination of the size distribution. More likely, the size difference is due to possible differences in food availability between sites, or competition with a closely related species (*Graptemys gibbonsi*, the Pascagoula map turtle) abundant at the Leaksville site.

This study indicates that a substantial proportion of turtles from the impacted site have lower plasma *T* and 10% have higher plasma E_2 than their counterparts at the unimpacted site. These differences were apparent in animals 90 km downstream from the suspected source of pollutants in the last remaining population years after measured pollutant levels dropped. We do not know to what extent these differences translate into reproductive impairment. However, population declines of this species at the impacted site are severe and have occurred since industrial activities on the river began. The 11-year time frame that pollutants are thought to have been elevated in the Pascagoula and Leaf Rivers may have affected as much as 50% of the present adult population during their developmental years. This population may not begin recovery from the described pollution event until affected individuals are no longer such a substantial proportion of the reproducing population. Males that matured in the 1999 and 2000 reproductive seasons were not exposed to elevated pollutants during development, given that the advisories were lifted in 1995 and males reach maturity at approximately 4 years.

Long-term monitoring of these populations is needed to determine the health of this species at both of these sites, now that steroid and VTG baselines have been established. Periodic monitoring of reproduction in this species could provide information on long term effects of historical pollution events. As younger individuals reach maturity, there could be an increase in reproductive success at the population level, indicating a decrease in the number of individuals exposed to elevated pollutant levels during development. Other changes in reproductive success could also indicate changes in ecosystem health. Information on reproduction in the yellow-blotched map turtle collected in the future will also aid in species management and hopefully lead to protection of habitat critical to the survival of this imperiled species.

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