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Hepatic biotransformation enzymes in a sentinel species, the painted turtle (*Chrysemys picta*), from Cape Cod, Massachusetts: seasonal-, sex- and location related differences

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We report changes in biomarker enzymes (cytochrome P4501A, glutathione-S-transferase) and protein expression (cytochrome P4501A) in adult painted turtles, *Chrysemys picta*, as part of a study of the potential impact of contaminants originating from the Massachusetts Military Reservation on Cape, Cod, MA. In animals from both Moody Pond, a site potentially impacted by contaminants, and Washburn Pond, a non-impacted site, rates of hepatic microsomal ethoxyresorufin-*O*-deethylase (EROD) activity were very low yet differences were detected. In Moody Pond, rates in both females and males peaked in June (4.3 ± 0.5 pmol min^{-1} mg^{-1} and 5.9 ± 1.5 pmol min^{-1} mg^{-1} (mean ± sem) respectively). At Washburn Pond, EROD rates in males peaked in May (3.8 ± 0.6 pmol min^{-1} mg^{-1}), and in females were highest in August (2.5 pmol min^{-1} mg^{-1}). There were differences in EROD rates between turtles from the two locations (ANOVA; $p < 0.05$), with higher activity at the impacted site. Western blot analysis for CYP1A protein showed a seasonal pattern of expression at the impacted site and a significant difference in expression between the two sites in June, with values being higher at the potentially impacted site ($p < 0.05$). Hepatic cytosolic GST activity, measured by 1,2-chlorodinitrobenzene conjugation, also showed a seasonal pattern in females, but not males. In Moody Pond, rates in females were highest in June ($8.05 \pm 1 \times 10^{-5}$ μmol min^{-1} mg^{-1}) and rates in males were highest in July ($7.74 \pm 2 \times 10$-5 μmol min^{-1} mg^{-1}). Rates were highest in females from Washburn Pond in May ($8.2 \pm 1.1 \times 10^{-5}$ μmol min^{-1} mg^{-1}) and, in males in June ($6.63 \pm 1 \times 10^{-5}$ μmol min^{-1} mg^{-1}). There was no significant difference in activity between males and females, but there were significant ($p<0.05$) differences between females from the two sites, with activity being higher at the potentially impacted site. The elevated activity of hepatic enzymes at the impacted site indicates that responses to unidentified pollutants or pollutant mixtures are superimposed upon a normal seasonal rhythm of enzyme activity at this location.

Keywords:

Abbreviations: AhR, arylhydrocarbon receptor; CYP1A, cytochrome P4501A; EROD, ethoxyresorufin-*O*-deethylase; GST, glutathione-S-transferase; MMR, Massachusetts Military Reservation; PCB, polychlorinated biphenyls; sem, standard error measurement.

Introduction

Negative effects of environmental pollutants on wildlife populations in some regions have been documented (see Colborn and Clement 1992 for review). The aquatic environment, in particular, is subject to a great deal of contamination, both from isolated leaks and spills and the continuous input from land run-off and

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agricultural and waste water sources (Goldfarb *et al*. 1998). There are, however, difficulties associated with assessing the effects of contaminants on a wild population, because of the continual changes in both the physical and chemical environment. Potential confounding factors such as temperature, feeding activity, light and reproductive activity often are not considered when assessing the effects of xenobiotics on a given population. In this study, we are assessing the effects of contamination and physical and physiological variables on the potential of the painted turtle, *Chrysemys picta,* to metabolize a mixture of contaminants.

We have a specific interest in the Massachusetts Military Reservation (MMR), a Superfund site on Cape Cod, MA, where disposal of organic and inorganic chemical waste has resulted in the formation of contaminated groundwater plumes. In some areas the plumes are interfacing with recreational surface water and wildlife habitats, and potentially may contaminate drinking water supplies. Because approximately 80% of Cape Cod residents depend on groundwater as the municipal drinking water supply (Rudel *et al*. 1998), there is a possible threat to not only the endemic biota, but also human health. The objectives of this study were: (a) to determine the seasonal variations in biotransformation enzymes in reptiles from a natural environment and (b) to determine, using biomarkers, potential exposure of the turtles to organic contaminants.

The species used here is the painted turtle, *C. picta*, a freshwater reptile common throughout much of the United States. Turtles are long-lived pelagic species that are likely to be exposed to contaminants through sediment or the water column. Turtles are also appropriately placed in the food chain to provide good indices of low-level contaminant exposure, which may be magnified by longevity. Further, turtles have been used by a number of investigators as biomonitors for heavy metal as well as PCB exposure (Albers *et al*. 1986).

The potential biomarkers evaluated here are two of several enzymes involved in the two-phase process of xenobiotic biotransformation. The induction of cytochrome P4501A (CYP1A), as measured by ethoxyresorufin-*O*-deethylase (EROD) activity and immunoblot assay (Yawetz *et al*. 1998) and glutathione-S-transferase (GST) activity measured as 1,2-chlorodinitrobenze conjugation. The induction of the CYP1A enzyme, as measured by EROD activity, has long been used as a biomarker for organic contamination in aquatic systems (Stegeman *et al*. 1990, Goksøyr and Forlin 1992). Often the degree of hepatic CYP1A induction can be closely related to the level of organic contamination, specifically PAHs and PCBs, in the environment (Stegeman and Hahn 1994). The induction of GSTs has also been used extensively as a biomarker for contamination in several aquatic species (Collier and Varanasi 1991, van Veld *et al*. 1991). Hepatic biotransformation enzymes were therefore examined in field-trapped turtles from an impacted and a reference site on Cape Cod, MA. Seasonal and sex-related differences in CYP1A and GST enzyme activity were identified in these animals. Differences were also identified between the two sites, suggesting an effect of known groundwater pollution on enzyme induction in this species.

Materials and methods

Description of study site

The study site is the MMR, an active military base located on a 20000 acre site on Cape Cod, MA (figure 1). Assessment of the chemical environment thus far has not revealed excessive levels of organic

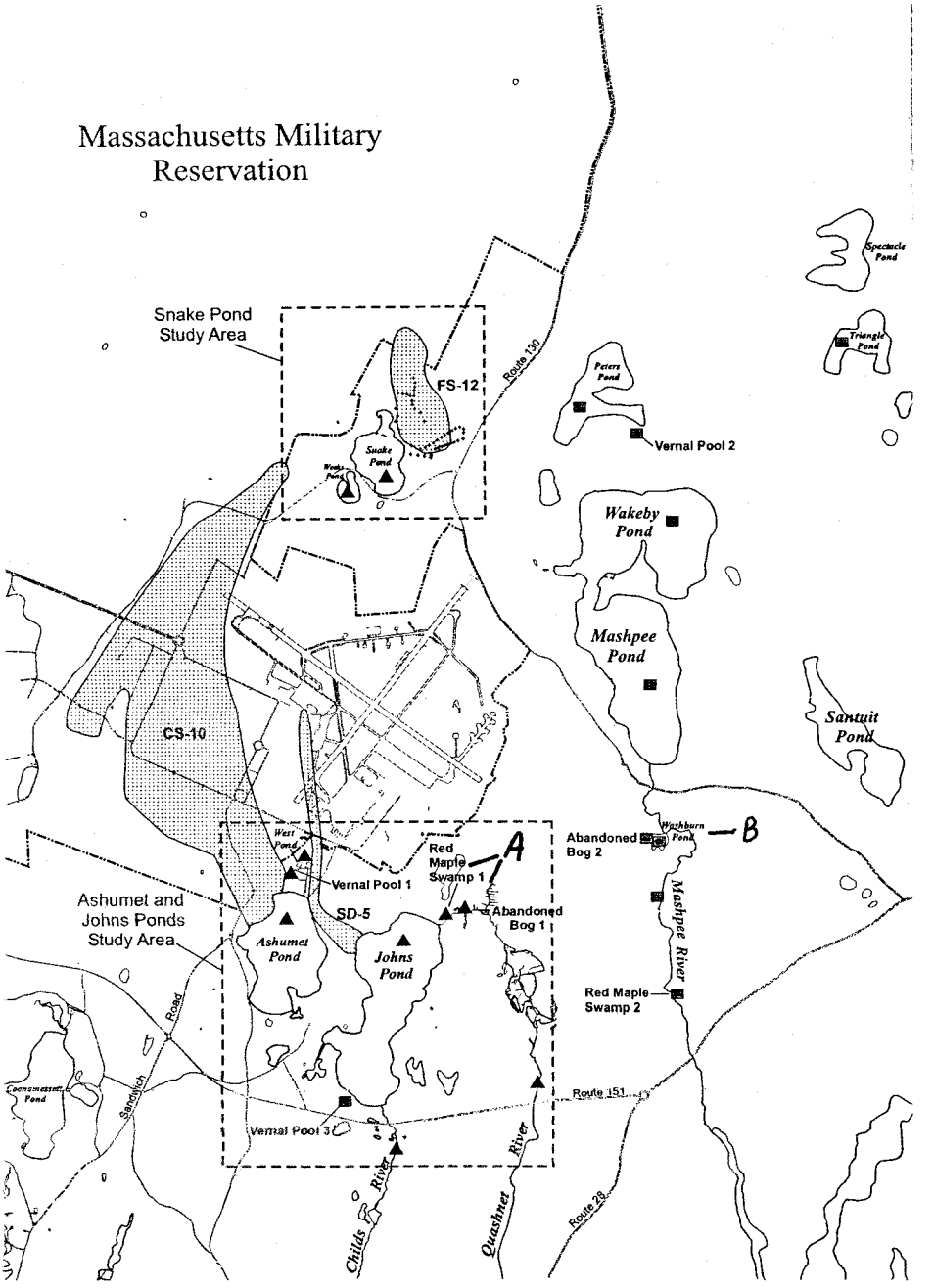

Figure 1. Map of the Massachusetts Military Reservation (MMR) on Cape Cod, Massachusetts. Animals were trapped over a 4-year period from both putative impacted (Moody Pond/Pond X) and non-impacted (Washburn Pond) areas. The putative impacted area (---) is marked A and the non-impacted area is marked B. (Map reproduced from Ecological Summary Report (1997) prepared by Jacob's Engineering).

or inorganic contaminants, however, some hydrological samples reveal certain metals (Ni, Zn, Cr) and organics (phthalates, benzo[*a*]pyrene) above levels deemed safe by the United States Environmental Protection Agency (US EPA). In our studies, animals were collected from both potentially impacted (figure 1, A) and non-impacted (figure1, B) sites in the vicinity of the MMR. The potentially impacted

site, Moody Pond and its surrounding area, is a semi-developed relatively large site with proximity to the Eastern Briarwood plume, a fuel spill plume that has been characterized by the Air Force Center for Environmental Excellence (AFCEE, April 1998). The primary contaminants in this plume are TCE (trichloroethylene) and EDB (ethylene dibromide), both of which have been measured at levels above the Massachusetts state drinking water standards (MMR IRP update, December 1998). The non-impacted site, Washburn Pond, is east of Moody Pond and is not in the path of a groundwater plume. The sites chosen have similar yearly temperature cycles, pH (M. Rie, unpublished data), and surrounding vegetation (M. Rie, personal observation).

Animals

Adult turtles (*C. picta*) were collected using sardine-baited circular mesh traps (Memphis Net and Twine, Memphis, TN) deployed for 48 h. Animals were collected monthly from April to November, with bimonthly catches during May of each year. In each pond, traps were set at specific sites, which were used for each trapping episode. Animals were sexed, weighed and sacrificed by decapitation within 24 h of collection (body weight range: females [156–530 g], males [113–346 g]). Liver samples of approximately 1 g (weight range for liver samples: 0.8–1.1 g) were immediately removed and placed into sterile tubes on ice.

Microsome preparation

All microsomal and cytosolic fractions were prepared from fresh liver tissue at 4 °C. Microsomes were prepared by homogenizing liver tissue in four volumes of buffer (50 mM Tris–HCl, 0.15 M KCl, pH 7.4) followed by differential centrifugation (Yawetz *et al.* 1997). Aliquots of the cytosolic supernatant were collected and stored at –80 °C until use. The microsomal pellet was resuspended in 1.5 ml of buffer (50 mM Tris–HCl, 1 mM EDTA, 1 mM DTT and 20% glycerol, pH 7.4) with care being taken not to disturb the underlying glycogen pellet. The resuspension was homogenized using a Teflon pestle, with aliquots taken and stored at –80 °C until use. Cytosolic and microsomal protein content was determined using the method of Lowry *et al.* (1951) with bovine serum albumin as the standard.

Measurement of EROD activity

EROD activity was measured using a Cytofluor™ multiwell fluorescence plate reader as previously described (Hahn *et al.* 1993). The reaction, carried out in a 96-well plate, consisted of 150 µl of ER (7-ethoxyresorufin, final concentration 2 µM), 10 µl of microsomes, and was initiated with 40 µl of NADPH (final concentration 1.67 mM). The formation of resorufin was measured at room temperature for 6 min. Data are expressed as pmol of resorufin formed min^{-1} mg^{-1} protein.

Western blot analysis for CYP1A

An aliquot of the microsomal fraction (30–50 µg of total protein) was taken, mixed with sample treatment buffer (4×, 0.25 M Tris, 40% glycerol, 4% SDS, 0.008% bromophenol blue, 5% β-mercaptoethanol) and boiled for 4 min. Samples were loaded onto 8–16% Tris–glycine gradient gels (Novex, SanDiego, CA) and run at 125 mV for 2 h at 4 °C. Transfer to 0.05 µm nitrocellulose was done at 250 mAmp for 3 h at 4 °C. Membranes were stained in Ponceau S to confirm the presence of bands and photographed. After rinsing in deionized water, blots were placed in 5% non-fat dry milk dissolved in TBS and incubated overnight. Blots were rinsed and incubated in a primary antibody (monoclonal 1-12-3 against P450E (CYP1A) from scup, *Stenotomus chrysops* (Park *et al.* 1986)), which had been dissolved in TBS/5% non-fat dry milk by shaking for 1 h at room temperature. Mab 1-12-3 specifically recognizes putative CYP1A in the turtle, *C. picta* (Yawetz *et al.* 1997). After washing three times in TBS/0.1% Tween 20, blots were incubated in a sheep anti-mouse HRP conjugated secondary antibody (Amersham, CA) for 1 h at room temperature. Following six washes in TBS/0.1% Tween 20, blots were developed using the enhanced chemiluminesence (ECL) system according to the manufacturer's instructions (Amersham, CA). Densitometric analysis of stained bands was done using digital photography (Kodak DCS camera) and NIH image software. Intensity of the bands was determined based on comparison with a range (0.05–0.4 pmol) of known CYP1A standards. Data are expressed as relative amount of CYP1A protein mg^{-1} total loaded protein.

Measurement of glutathione-S-transferase activity

Activity of cytosolic glutathione-S-transferase (GST) was determined spectrophotometrically using the method of Habig *et al.* (1974). The reaction mixture consisted of 1 mM 1,2 chlorodinitrobenzene (CDNB) dissolved in 100 mM Tris–HCl (pH 7.4), 1 mM glutathione and 10 µl of the hepatic cytosolic fraction in 1 ml total volume. The activity of GST was determined at 340 nm at 25 °C over 5 min. Data are expressed as µmol min^{-1} mg^{-1} of cytosolic protein.

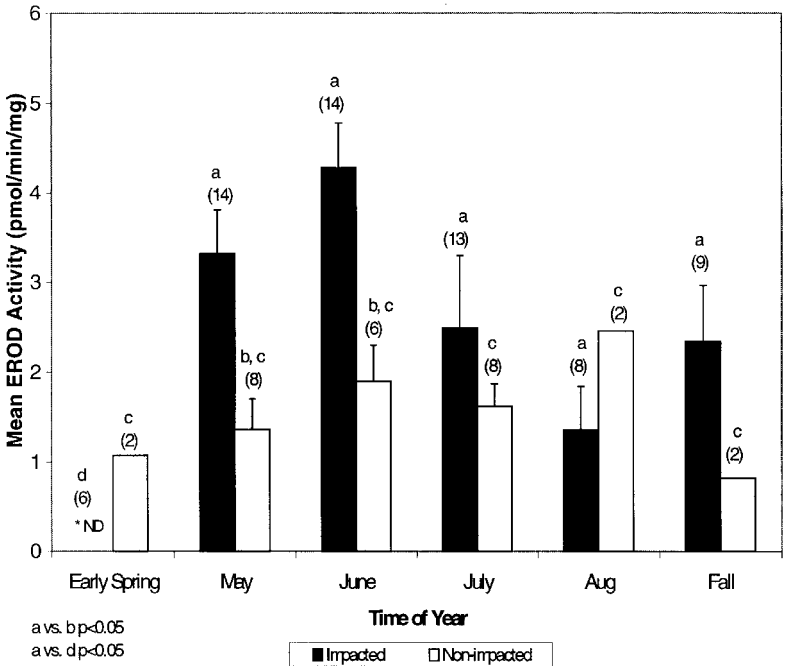

Figure 2. Mean ethoxyresorfuin-*O*-deethylase (EROD) activity in adult female turtles (*Chrysemys picta*) taken from putative impacted and non-impacted sites. Hepatic microsomes were analysed for EROD activity using a kinetic cytofluor assay. Data are expressed as mean EROD activity min^{-1} mg^{-1} microsomal protein. Values are given as mean activity ± sem (*n*).

Data analysis and statistics

One way ANOVA was done to analyse the seasonal differences in enzyme activity. Kruskal–Wallis ANOVA on ranks with Dunn's multiple comparison was done in cases where the data were not normally distributed. When comparing two groups, each from a different site, Student's *t*-test was used. Regression analysis was done to determine any linear relationship between EROD activity and CYP1A protein level for animals at each site. In all cases, significance is reported at $p < 0.05$.

Results

Seasonal changes in EROD activity in females (figure 2)

At the potentially impacted site (Moody Pond), the rate of hepatic microsomal EROD activity showed a seasonal pattern. In early spring, levels were low to non-detectable. In May there was a significant ($p < 0.05$) rise in EROD activity to 3.3 ± 0.5 pmol min^{-1} mg^{-1}. This increase continued in June, when the peak level of activity was seen (4.2 ± 0.5 pmol min^{-1} mg^{-1}). The level of EROD activity in June was, on average, higher than at all other times of the year. Through the summer and autumn (July–October), mean EROD activity decreased significantly ($p < 0.05$) from the levels seen in May and June, however no significant differences were seen between these two months. At the non-impacted site, hepatic EROD activity showed no significant seasonal changes ($p > 0.05$) and the highest mean value was found in August (2.5 pmol min^{-1} mg^{-1}).

There were significant differences between the potentially impacted and non-impacted sites. In both May and June significantly ($p < 0.05$) higher levels of hepatic microsomal EROD activity were seen in females from the impacted site.

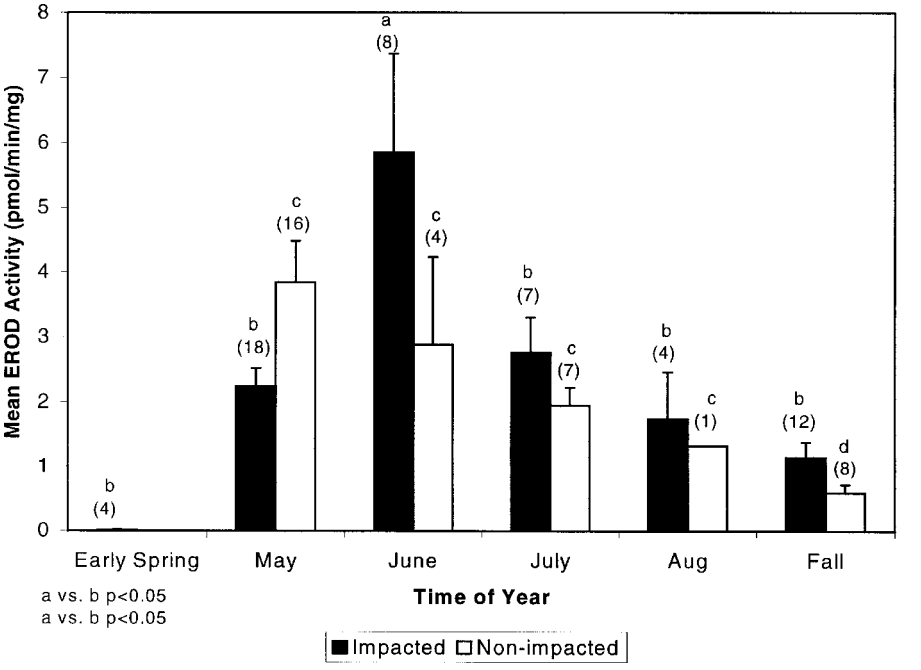

Figure 3. Mean ethoxyresorfuin-*O*-deethylase (EROD) activity in adult male turtles (*Chrysemys picta*) taken from putative impacted and non-impacted sites. Hepatic microsomes were analysed for EROD activity using a kinetic cytofluor assay. Data are expressed are mean EROD activity min^{-1} mg^{-1} microsomal protein. Values are given as mean ± sem (*n*).

Seasonal changes in EROD activity in males (figure 3)

As with females, a seasonal pattern in hepatic microsomal EROD activity was seen in males from the impacted site. In early spring, levels were low to non-detectable, but began to rise in May. The peak of EROD activity was seen in June (5.8 ± 1.5 pmol min^{-1} mg^{-1}), with levels declining in the summer and autumn. There was a significant ($p < 0.05$) difference in activity between the peak levels in June and those in the early spring and autumn.

At the non-impacted site, there was also a seasonal pattern of EROD activity. No data are shown for early spring, as males were not collected from this site until May. The levels of EROD activity in May were 3.8 ± 0.6 pmol min^{-1} mg^{-1}, the highest rates observed in males from this site ($p < 0.05$); levels declined over the season from this peak. There were no significant differences in activity in males between the two sites ($p > 0.05$), although, on average, the levels of activity are higher at the impacted site.

There were also significant ($p < 0.05$) differences in EROD activity between males and females from the two sites.

CYP1A protein determined by Western blot (table 1, figure 4)

As with the hepatic microsomal EROD data, the microsomal CYP1A protein content showed a seasonal pattern (see table 1). At the potentially impacted site, in the early spring, levels of CYP1A were non-detectable in females, but were detectable in males. During the trapping season there were significant ($p < 0.05$)

Table 1. Mean relative CYP1A levels, obtained by Western blot analysis using mouse anti scup monoclonal antibody, for adult male and female turtles (*Chrysemys picta*) taken from potentially impacted and non-impacted sites over a 4 year period. Values are expressed as relative CYP1A level mg^{-1} microsomal protein ± sem (*n*).

	Impacted		Non-impacted	
Time of Year	Males	Females	Males	Females
Early spring	3.2 ± 2.4 (*n* = 7)	n.d.	None collected	0.5 (*n* = 2)
May	1.5 ± 0.5 (*n* = 11)	3.2 ± 0.6 (*n* = 10)	3.8 ± 0.6 (*n* = 16)	1.2 ± 0.3 (*n* = 9)
June	9.6 ± 1.5* (*n* = 7)	6.5 ± 1.8* (*n* = 8)	2.5 ± 1 (*n* = 4)	0.9 ± 0.4 (*n* = 6)
July	5.8 ± 1.4 (*n* = 4)	5.4 ± 1.7 (*n* = 6)	4.1 ± 1.1 (*n* = 5)	1.9 ± 0.5 (*n* = 8)
August	2.7 (*n* = 3)	3.5 ± 1.3 (*n* = 5)	0.3 (*n* = 1)	5.4 (*n* = 2)
Autumn	6.9 ± 1.9 (*n* = 12)	6.3 ± 2.3 (*n* = 9)	0.7 ± 0.2 (*n* = 5)	1.5 (*n* = 3)

* $p < 0.05$ vs non-impacted animals.

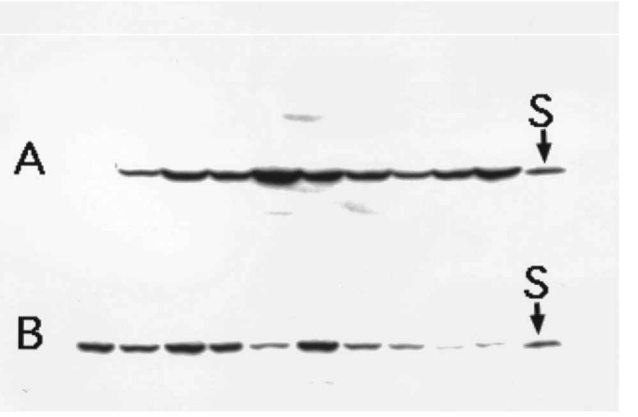

Figure 4. Immunoblot for hepatic microsomal CYP1A in adult male and female turtles taken from putative impacted (A) and non-impacted (B) sites. The last lane on both blots (S) contains 0.1 pmol of a scup CYP1A standard (electrophorectic mol. wt of 54.3). Samples were normalized for mg total microsomal protein.

changes in relative CYP1A protein levels in both males and females from the impacted site. CYP1A protein expression appeared to increase in May, but the highest level occurred, on average, in June and did not show a marked decrease until August. In females there was a trend toward increased CYP1A levels in the autumn, although the differences were not significant. In males, there appeared to be a steady decline in the level of CYP1A protein expression after July, which is consistent with the temporal changes in EROD activity.

At the non-impacted site, the levels of CYP1A protein did not change significantly during the season. In females, the expression of CYP1A did not vary significantly and was at relatively low levels throughout the year (the elevated levels seen in females during August may be related to the low number of animals collected at this time of the year). In males, the amount of CYP1A was highest in

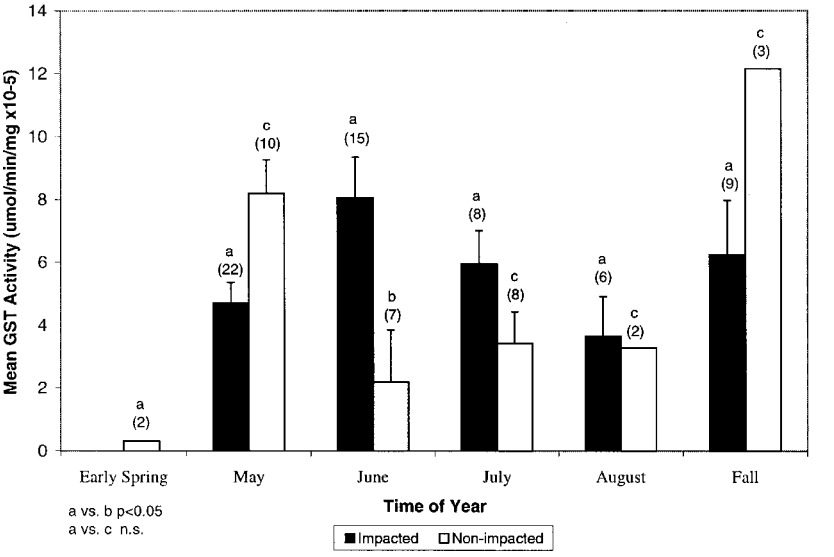

Figure 5. Seasonal changes in glutathione-S-transferase (GST) activity in adult female turtles (*Chrysemys picta*) taken from putative impacted and non-impacted sites. Hepatic cytosolic fractions were analysed using a spectrophotometric assay with CDNB as the substrate. Data are expressed as mean GST activity min^{-1} mg^{-1} cytosolic protein. Values are given as mean ± sem (*n*).

May, June and July, and lowest in August and the autumn; these changes were not, however, statistically significant. This is consistent with the rates of EROD activity, which show a similar pattern in these animals.

There were significant ($p < 0.05$) differences in relative levels of CYP1A between animals from the impacted and non-impacted sites. The levels of CYP1A seen in June are significantly higher in both males and females from the impacted site when compared with animals from the reference site.

The immunoblot data (figure 4) are an example of the differences in CYP1A content seen in animals taken from the impacted and non-impacted sites. The intensity of the bands is greater in animals taken from the impacted site (figure 4, A), when compared with those from the non-impacted sites (figure 4, B). There was no statistically significant ($p > 0.05$) correlation between EROD activity and relative CYP1A protein level for either the impacted or non-impacted animals.

Seasonal changes in GST activity in females (figure 5)

In females from both the potentially impacted and non-impacted site, the level of GST activity showed a seasonal pattern. At the impacted site, levels of GST activity began to rise in May, reaching peak levels in June (8.0 ± 1.3 μmol min^{-1} $mg^{-1} \times 10^{-5}$). There was an apparent decrease in the level of activity through the summer and autumn (July–October), but the differences were not significant. At the non-impacted site, levels of GST activity were lowest in the early spring and rose to a peak in May (8.2 ± 1.1 μmol min^{-1} $mg^{-1} \times 10^{-5}$). Through the summer, activity levels were significantly decreased from the May values and remained constant. There appeared to be a second peak in the autumn, however, this may be an artifact as the data are highly variable.

In June there was a significant ($p < 0.05$) difference in GST activity, in females,

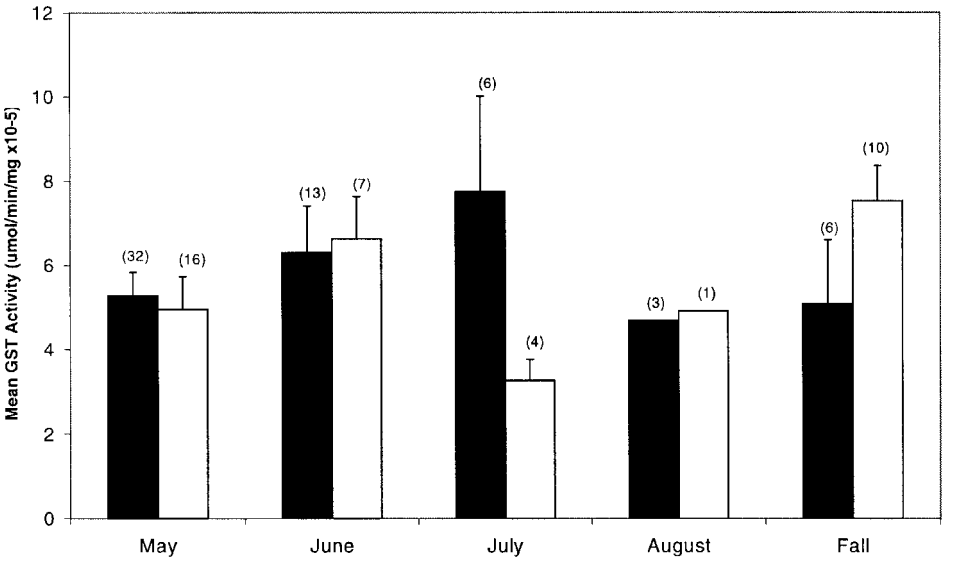

Figure 6. Seasonal changes in glutathione-S-transferase (GST) activity in adult male turtles (*Chrysemys picta*) from putative impacted and non-impacted sites. Hepatic cytosolic fractions were analysed using a spectrophotometric assay with CDNB as the substrate. Data are expressed as mean GST activity min^{-1} mg^{-1} cytosolic protein. Values are given as mean ± sem (*n*).

between the impacted and non-impacted site. There were no significant differences between the two sites at any other time of year.

Seasonal changes in GST activity in males (figure 6)

In males from both the impacted and non-impacted sites, there were no significant ($p > 0.05$) seasonal changes in the level of GST activity. At the impacted site, levels are highest in July (7.7 ± 2.3 μmol min^{-1} $mg^{-1} \times 10^{-5}$). Similarly, at the non-impacted site, the level of activity is highest in June (6.6 ± 1 μmol min^{-1} $mg^{-1} \times 10^{-5}$, $p > 0.05$). There were no significant sex differences in GST activity at either the impacted or non-impacted site.

Discussion

This study presents data for seasonal and sex-related differences in phase I (CYP1A) and phase II (GST) enzyme activities in two populations of turtles on Cape Cod, Massachusetts. In addition, we have demonstrated differences in the level of activity of these enzymes between the potentially impacted and non-impacted sites. The elevated rates of enzyme activity in the animals from the impacted site are of concern because of the known ground and surface water pollution at this location (AFCEE, April 1998, MMR, IRP update, December 1998). This represents the first report of the seasonal pattern of biotransformation enzyme activity and their use as bioindicators of exposure in the turtle, *C. picta*.

Physical and physiological factors, including temperature, sex and age, have been shown to influence the induction and metabolic activity of biotransformation enzymes in several non-mammalian species (see Kleinow *et al.* 1987, Ertl and Winston 1998 for review). Physiological and hormonal changes occur in reptiles

associated with variations in seasonal temperature (Harless and Morlock 1979, Licht, 1984). However, there are no studies characterizing potential seasonal and gender-related changes in biotransformation enzymes in reptiles. The seasonal pattern of phase I (CYP1A) activity in turtles taken from the impacted site shows that peak levels occurred in late spring in both males and females. The males from the non-impacted site also showed similar pattern of activity, with values peaking in May. In contrast, females taken from the non-impacted site did not show significant seasonal changes in EROD activity and, in general, were quite low (<2.5 pmol min^{-1} mg^{-1}) at all times sampled. Previous studies in the newt, *Pleurodeles walti*, have shown annual changes in biotransformation enzyme activity with highest levels seen in late spring (Marty *et al*. 1992), similar to the pattern seen in the animals used in this study. Although there were seasonal changes in water temperature at both the putative impacted and non-impacted areas, there were no significant differences in water temperature between the two sites (M. Rie, unpublished data). This suggests that the seasonal pattern of enzyme activity may be related to temperature changes within , rather than between, each site.

Animals from the impacted site also showed a seasonal pattern in the expression of CYP1A protein, which follows a pattern similar to the changes in EROD activity at this site. At the non-impacted site the females showed low to non-detectable levels of CYP1A throughout the season, which follows a trend similar to the changes in EROD activity seen in these animals. In contrast, although detectable levels of CYP1A protein were found in males, no seasonal pattern was seen despite changes in EROD activity. In *C. picta* treated with various inducers of CYP1A, the level of CYP1A expression and EROD activity show a linear relationship to each other (Yawetz *et al*. 1998). However, in the present study the relationship between EROD activity and CYP1A content was not linear ($p > 0.05$).

A major issue arising from this study concerns the low rates of CYP1A (EROD) activity. The levels of enzyme activity determined here were, at most, < 30 pmol min^{-1} mg^{-1}. These rates are only 10% of rates found in many non-induced fish (Stegeman and Hahn 1994), birds (Walker and Ronis 1989) and mammals (Wroblewski and Olson 1985). The low level of CYP1A activity reported here for *C. picta* is similar to that reported by others for this species (Yawetz *et al*. 1997). Lizards (Gutman and Kidron 1971) and crocodilians (Ertl *et al*. 1999) also exhibit low CYP1A activity, suggesting a reptile-specific difference from other vertebrate groups. Even in animals treated with maximally-inducing doses of aryl hydrocarbon receptor (AhR) agonists, the rates of EROD in these reptiles are 10–100 times lower than those seen in the rat. There are several possible explanations for the poor correlation between CYP1A protein level and catalytic activity. First, there may be an inefficient interaction of the substrate (7-ethoxyresorufin) with the active site of the CYP1A enzyme in reptiles. Second, substrates such as highly chlorinated PCBs may be bound to the enzyme, thereby reducing its activity, but not affecting immunodetection of the protein. Finally, there may be another P450, other than or in addition to CYP1A that metabolizes EROD in this species. Nonetheless, even with the relatively low level of activity found here, there are clear seasonal-, sex- and site-related differences in CYP1A activity, using 7-ethoxyresorufin as the substrate. Thus, while other substrates or measurement of the CYP1A protein may yield stronger signals, EROD is still informative in assessing CYP1A induction in the turtle.

The phase II (GST) activity showed a seasonal trend in females, with peak

values seen at the impacted site in June, and at the non-impacted site in May. The temporal peak in GST activity in females from the impacted site corresponds to the peak in EROD activity. In males, however, there were no significant changes in GST activity, unlike EROD activity, throughout the season. As with EROD, the rates of GST activity in both males and females were low (μmol min^{-1} $mg^{-1} \times 10^{-5}$), when compared with reported values in fish (μmol min^{-1} mg^{-1}, Collier and Varanasi 1991) and other species (van Veld *et al.* 1991, George 1994, Egaas *et al.* 1999), and were, in addition, highly variable. Others have reported only modest induction and high individual variability in fish (Egaas *et al.* 1999). The specificity and sensitivity of GST as a biomarker has not been established to the same extent as CYP1A induction and activity (George 1994), and the data presented here suggest it may be less useful than CYP1A as a biomarker also in the turtle.

In addition to temperature, gender and reproductive status are also known to effect the activity of some biotransformation enzymes in the reptile (see Ertl and Winston 1998 for review). In male *C. picta*, androgen levels are highest in early spring and late autumn and spermatogenesis occurs in the autumn (Dubois *et al.* 1988). Thus, reported plasma androgen levels are inversely related to the seasonal pattern of EROD activity and unrelated to GST activity. In contrast, in female *C. picta,* reported changes in oestradiol and progesterone, which increase in the preovulatory period (May) and oestradiol which also increases in the autumn (Callard *et al.* 1978), may be correlated to EROD and GST activity. Others have shown that there are significant differences in microsomal P450 content and activity between males and females during the annual cycle (Forlin and Haux 1990), and it appears that some endogenous oestrogens can influence the activity of cytochrome P450s in fish (Pajor *et al.* 1990). Thus, the seasonal changes in EROD and possibly GST levels observed in the current study may be correlated with the hormonal and physiological changes related to reproduction in the turtle, *C. picta.*

The plumes of contamination present at the MMR Superfund site represent a low-level complex mixture of both organic and inorganic substances. The existence of fuel and chemical spill plumes originating within the MMR Superfund site has received much attention because of the population density and fragility of the water supply on Cape Cod. A report of the Air Force Center for Environmental Excellence (April 1998) indicates the presence of bis (3-ethyl) hexyl phthalate (BEP), in the surface water and sediment of most ponds in this area, at levels that exceed both Risk and Hazard based concentrations defined by the US EPA. Other organic contaminants, benzo[*a*]pyrene, *pp*-DDE, *pp*-DDD and dieldrin were also widely detected in Cape Cod surface waters. Therefore, the putative induction of CYP1A/GST in animals from this site may be a correlate of the elevated organic contamination detected in the plumes. However, due to the variety of plume components, the specific compounds potentially mediating this effect cannot be definitively identified.

There are many potential influences on the potential ability of an organism to metabolize xenobiotic compounds. The data presented here illustrate that although temperature, feeding and reproductive activity are indicated to be correlated with elevated hepatic phase I enzymatic activity, the differences between animals from the impacted versus non-impacted site may be possibly ascribed to increased xenobiotic load. Understanding the seasonal- and gender-related changes in biotransformation enzyme induction may allow for a more complete assessment of the effects of contamination on a natural population.

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