Explaining patterns of deformity in freshwater turtles using MacCulloch’s hypothesis

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Abstract: A growing body of literature details the effects of teratogenic chemicals on embryonic development in freshwater turtles. However, other factors affecting developmental deformities have not been recently considered and evaluation of the significance of deformities in adults is lacking. We collected 193 wild Midland Painted Turtles (Chrysemys picta marginata Agassiz, 1857) and 39 Common Snapping Turtles (Chelydra serpentina (L., 1758)) from an uncontaminated site in Ontario and recorded incidence of deformity of the shell, limbs, face, and tail. We tested MacCulloch’s hypothesis (that incidence of deformity increases along a latitudinal gradient) by comparing our data with previously published deformity records from both uncontaminated and heavily polluted sites at varying latitudes. Incidence of nonembryonic deformity varied among wild populations and was not correlated with pollution levels. Thus adult deformity cannot be used as an indicator of site quality. Frequency of deformity increased with latitude in C. picta, supporting MacCulloch’s hypothesis, whereas deformities in C. serpentina did not. We refer to essential differences in the biology of the two species to explain this disparity and recommend that latitudinal variation be included as a covariate in the future when developmental trends are compared among distant sites.

Introduction

Developmental deformities with varying severity are frequently observed in wild populations of freshwater turtles (e.g., Zangerl and Johnson 1957; Ernst 1971; Ewert 1979; MacCulloch 1981; Mosimann 2002; Alarcos et al. 2005; Bell et al. 2006). Deformities range from being lethal, e.g., brain protruding from head (Bell et al. 2006), to very minor, e.g., extra scutes (Pavaliko 1986). Lethal deformities and deformities that reduce survival probability or reproductive fitness affect population dynamics, especially if the frequency of such deformities is very high. However, minor deformities, such as missing digits or abnormally shaped scutes, are unlikely to have a significant impact on fitness. No evidence suggests that minor deformities are indicators of reduced reproductive fitness.

Several factors have been suggested as causes of developmental deformities in turtles. Chemical teratogens are thought to cause severe deformity in turtle embryos, as was recently shown in wild populations of Painted Turtles (Chrysemys picta (Schneider, 1783)) and Common Snapping Turtles (Chelydra serpentina (L., 1758)) at a highly polluted site in Pennsylvania (Bell et al. 2006). Kyphosis, a dorsal–ventral contortion of the spine, is thought to be a congenital anomaly (Wyneken et al. 2008). Lynn and Ullrich (1950) found that suboptimal moisture levels during incubation increase deformity frequency in C. picta; a lack of moisture during incubation can also cause a shell abnormality known as pyramiding (Wiesner and Iben 2003). Yntema (1960) also found that eggs of C. serpentina incubated at low temperatures produce abnormal hatchlings. Yntema’s observations led MacCulloch (1981) to hypothesize that turtles in colder parts of their ranges might show higher levels of deformities, but this has not been tested.
Shell and spinal deformities in turtles range in severity. Severe deformities include missing plastron and extreme kyphosis (e.g., Bell et al. 2006; Wyneken et al. 2008). These are usually lethal and thus not observed in adults, and are probably rare under normal circumstances. However, mild deformities, such as abnormally shaped or extra scutes, are common in wild adult turtles (Ewert 1979; MacCulloch 1981; Ayres Fernández and Cordero Rivera 2004; Najbar and Szuszkiewicz 2006). Minor shell or spinal abnormalities are not known to affect fitness.

Bell et al. (2006) provide a beautifully illustrated example of the devastating effects that pollutants can have on turtle embryonic development. Their data show that, on average, 55% of C. picta embryos taken from a site with high pollution contamination developed lethal deformities, some involving the shell. But the frequency of minor deformities in adults (i.e., those deformities that probably do not decrease survivorship) was actually higher at their control site. The control site (the E.S. George Reserve, Michigan) was graphically distant, had “no indication of contamination by … pollutants in the water, sediments or turtles”, and no agricultural chemicals have been applied to the area since 1930 (E. Werner, personal communication).

Furthermore, studies of the teratogenic effects of polychlorinated biphenyls (PCBs) and organochlorides on developing C. serpentina produce equivocal results. Bishop et al. (1998) indicate that teratogenic pollutants, especially polycyclic aromatic hydrocarbons, can significantly affect developing C. serpentina, although the impact may not be as severe as that documented for C. picta. However, exposure to other chemicals such as non-ortho PCBs and organochloride pesticides does not appear to increase rates of deformity. More recently, de Solla et al. (2008) found that although hatching deformity levels in Ontario were higher at some polluted sites than at their control sites, other polluted sites did not show the same trend. They conclude that chemicals such as PCBs and organochlorides may not have a biologically significant effect and that other factors may also be acting as teratogenic agents. In adult turtles, they found deformity levels ranging from 0% (at a polluted site) to 21.1%, with a rate of 7% deformed adults at their “clean” reference site. Clearly, factors other than chemical pollutants are involved. Emphasis on embryonic deformities in the literature makes it difficult to assess what the natural background rate of deformity in wild populations might be. Data on “normal” levels of deformity in wild turtles from a variety of sites are necessary to provide context for more specialized studies, such as those mentioned above.

This study compares published levels of deformities in juvenile and adult Midland Painted Turtles (Chrysemys picta marginata Agassiz, 1857) and C. serpentina with those observed at a natural site with good water quality. Our objectives were to describe deformities in wild adult turtles at a site not contaminated by teratogens and to determine (i) whether MacCulloch’s hypothesis (i.e., levels of deformity increase along a latitudinal (temperature) gradient) is supported by the current data for C. picta or C. serpentina and (ii) whether frequencies of minor deformity are similar among populations from sites with varying levels of pollution, in which case they might provide a general indicator of site quality.

Materials and methods

We collected C. p. marginata and C. serpentina from the Old Ausable Channel (OAC), Pinery Provincial Park, Ontario (43°16′N). Water quality in the OAC is excellent (Killins 2008; K. Jean, personal communication (2008)), as it is spring-fed and does not collect drainage from agricultural lands. The OAC is home to a large, healthy population of C. p. marginata and a high number of adult C. serpentina.

Turtles were caught by hand and with dip nets from 22 May to 7 August 2008. Each turtle was measured (curved carapace length) and marked by shell notching following a method modified from Cagle (1939) to identify recaptures. Each turtle was sexed if possible and any deformities of the face, tail, limbs, or shell were recorded and photographed. We considered any deviations from the normal body plan of the two species that did not appear to be caused by traumatic injury (e.g., extra scutes, fused or extra digits, missing digits with no evidence of scarring to explain their absence) to be deformities. The types of deformities we observed were consistent with abnormalities termed deformities in previous studies. All turtles were released at their capture site. All fieldwork was conducted using an animal use protocol approved by the Animal Care Committee of the Royal Ontario Museum.

Data from other C. picta populations were taken from Bell et al. (2006) and MacCulloch (1981). These included the John Heinz National Wildlife Refuge (JHNWR) in Pennsylvania (a heavily polluted site; 40°00′N), the E.S. George Reserve (ESGR), Michigan (a site with no evidence of significant pollution; 42°28′N), and the Qu’Appelle River (50°42′N; a site with some agricultural drainage).

Data from C. serpentina for comparison were taken from Bell et al. (2006) and de Solla et al. (2008). Sites used for comparison were the JHNWR, the ESGR, Tiny Marsh (a “clean” control site from de Solla et al. 2008; 44°35′N), and Dead Creek and Raisin River (contaminated sites from the same study; 44°00′N and 45°11′N, respectively). We chose to use the Dead Creek and Raisin River sites because they represented the contaminated sites with the highest and lowest recorded rates of deformity, and therefore provided a wide range for comparison.

We tested the relationship between latitude and frequency of deformity by calculating a standard Pearson’s correlation coefficient ($r^2$). However, we were not able to use this approach to assess the role of pollutants. Previously published literature did not always provide measurements of pollution levels at all sites and the chemicals evaluated varied between studies. Thus, although comparable quantitative assessment of contamination would be ideal, this was not available for all sites. Therefore, we used Spearman’s rank correlation coefficient ($\rho$) to test for correlations between relative contamination levels and frequency of deformities at sites. Grouping of sites and rationale for this classification are presented in Table 1.

Results

Chrysemys picta marginata

We collected data from 193 C. p. marginata in the OAC.
# Table 1. Evidence for site contamination and contamination score used to rank sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Source</th>
<th>Contamination score*</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. Werner, personal communication, 2008</td>
<td></td>
<td>E. Werner, personal communication, 2008</td>
</tr>
<tr>
<td>ESGR, Michigan</td>
<td>Bell et al. 2006; E. Werner, personal communication, 2008</td>
<td>1</td>
<td>Bell et al. 2006; E. Werner, personal communication, 2008</td>
</tr>
<tr>
<td>Tiny Marsh, Ontario</td>
<td>de Solla et al. 2008</td>
<td>2</td>
<td>de Solla et al. 2008</td>
</tr>
<tr>
<td>Qu'appelle River, Saskatchewan</td>
<td>Low levels of PCBs, PBDEs, and chlordanes detected in eggs from this site (~200 ng/g wet mass)</td>
<td>1</td>
<td>de Solla et al. 2008</td>
</tr>
<tr>
<td>Dead Creek, Ontario</td>
<td>de Solla et al. 2008</td>
<td>2</td>
<td>de Solla et al. 2008</td>
</tr>
<tr>
<td>JHNWR, Pennsylvania</td>
<td>Bell et al. 2006</td>
<td>3</td>
<td>Bell et al. 2006</td>
</tr>
</tbody>
</table>

Note: OAC, Old Ausable Channel; ESGR, E.S. George Reserve; JHNWR, John Hume National Wildlife Refuge; NM, not measured (no quantitative assessment available); PCB, polychlorinated biphenyl; PBDE, polybrominated diphenyl ether; PAH, polycyclic aromatic hydrocarbons.

Fifty-three (27.5%) individuals had some type of developmental abnormality or asymmetry. Of the 53 abnormal individuals, 35.8% were male, 58.5% were female, and 5.7% were juveniles too small to sex accurately. In total, 97 abnormalities were recorded (some turtles had more than one visible abnormality). All abnormal individuals appeared healthy and behaved normally when handled and released.

The most frequently observed abnormalities (n = 28 individuals) involved the scutes. Some of these turtles had extra or merged scutes on the carapace and plastron. Ten of these 28 individuals had abnormally shaped scutes, indicating developmental stress that nevertheless did not cause extra scutes to form. In three cases, turtles with abnormally shaped scutes also had extra scutes, and in one of these three, spinal malformation was also present.

We also found several individuals with a convoluted central seam on the plastron (n = 22; Fig. 1); these are included in the “other” category of Table 2 to maintain consistency among compared studies. If these 22 turtles showing asymmetrical development of the plastral seam were included in the total count for shell abnormalities, then 42 individuals (21.7%) would be included. One turtle had a spinal defect (scoliosis), while four had deformed tail tips, such that the tails ended in spiky, modified scales, similar to those described in Bell et al. (2006). One individual had an unfused mandibular symphysis and one had a deformed limb, which appeared to have an extra process growing from the carpal joints (Fig. 1). We did not observe any incidence of ankylosis or kyphosis.

Frequency of shell deformities in *C. p. marginata* among four sites increased with latitude (Fig. 2). There was a significant correlation between latitude and incidence of minor shell deformity (r² = 0.9741, P < 0.05). The frequency of extra scutes is probably underestimated in our OAC data, as the number of scutes in the plastral bridge was not always recorded; this was the area that most frequently showed extra scutes in the Saskatchewan population (MacCulloch 1981).

**Chelydra serpentina**

Of 39 *C. serpentina* captured in the OAC, 9 (23.1%) had visible deformities (Table 2). Two of these were males and 7 were females. The heavy algal loads on the turtles’ carapaces made it difficult to assess carapacial scute patterns, so only extra scutes on the plastron were recorded. The most noteworthy deformities we observed were a cleft palate (unfused maxillae) in an adult female and an unarticulated plastral bridge in another adult female.

We were not able to isolate and compare shell-only deformities for *C. serpentina*, since deformity types for adults were not defined for all comparison sites. We therefore compared total frequency of observed deformity at the six sites. Latitude and frequency of deformity were not significantly correlated (Fig. 3), although P was approaching significance (r² = 0.5058, P = 0.056). As in *C. p. marginata*, there was no apparent correlation between site contamination and minor deformities. There was no correlation between contamination score (Table 1) and incidence of deformities in either *C. picta* (ρ = −0.105, P > 0.05) or *C. serpentina* (ρ = −0.639, P > 0.05); the lowest observed frequency of deformity in adults occurred at the most heavily polluted site.
Discussion

We found that site contamination does not explain the variation in frequency of deformity in either *C. p. marginata* or *C. serpentina*. Therefore, the incidence of deformity at a site cannot be used as an indicator of habitat quality or contamination. However, the strong correlation between latitude and incidence of minor deformities in wild *C. p. marginata* supports MacCulloch’s hypothesis, and is most likely caused by 2004 climatic factors, including temperature. These factors may be involved during incubation. Correlations between experimental suboptimal moisture or temperature conditions and abnormal development of turtle embryos (Lynn and Ullrich 1950; Yntema 1960) are well documented. Temperature may also play an important role in the development of eggs within the female, since egg content (including water content) is correlated with climatic factors such as temperature and rainfall (Finkler et al. 1994), and can affect embryo development. Interestingly, MacCulloch’s hypothesis is only weakly supported by the data for *C. serpentina*. The correlation between latitude and deformity in this species was only approaching statistical significance, but given the number of other factors that may influence deformity frequency, the correlation may be biologically significant.

The incidence of deformities in adult *C. serpentina* in the OAC is higher than that reported for this species elsewhere. We may be underestimating deformity levels in this species at our site for the reasons previously stated. Adult *C. serpentina* in the OAC move in and out of the channel (C.M. Davy, unpublished data), thus our sampled adults may have been incubated elsewhere. Nevertheless, teratogenic compounds cannot be convincingly implicated in the deformities we report here. Genetic factors may be responsible for some deformities, although this possibility remains to be tested. Regardless, our data will contribute to future comparative studies.

Where data are available, *C. serpentina* populations consistently show lower incidence of deformity compared with the sympatric *C. p. marginata*. Bell et al. (2006) suggest that the higher rate of deformities in *C. picta* might reflect a greater susceptibility to pollution in this species; their embryonic data support this hypothesis. However, their adult data do not support the pollution hypothesis, and yet
Fig. 2. Frequency of shell (scute and spinal) deformities in juvenile and adult Midland Painted Turtles (*Chrysemys picta marginata*) from four sites, in order of increasing latitude. Latitude and deformity frequency are significantly correlated ($r^2 = 0.9741, P < 0.05$). JHNWR, John Heinz National Wildlife Refuge; ESGR, E.S. George Reserve.

Fig. 3. Frequency of observed external deformities in Common Snapping Turtles (*Chelydra serpentina*) from six sites, in order of increasing latitude. Latitude and frequency of deformity are positively correlated ($r^2 = 0.5058, P = 0.056$). JHNWR, John Heinz National Wildlife Refuge; ESGR, E.S. George Reserve.
Table 2. Frequency of types of externally visible deformities in wild Painted Turtles (Chrysemys pica and Chrysemys pica marginata) and Common Snapping Turtles (Chelydra serpentina) from sites at varying latitudes.

<table>
<thead>
<tr>
<th>Location</th>
<th>Chrysemys pica and Chrysemys pica marginata</th>
<th>Chelydra serpentina</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of abnormal individuals</td>
<td>No. of recorded abnormalities</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td></td>
</tr>
<tr>
<td>JHNWR, Pennsylvania</td>
<td>135</td>
<td>105</td>
</tr>
<tr>
<td>ESBR, Michigan</td>
<td>4036</td>
<td>4036</td>
</tr>
<tr>
<td>Qu’Appelle River, Saskatchewanan</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>OAC, Michigan</td>
<td>173</td>
<td>173</td>
</tr>
<tr>
<td>JHNWR, Pennsylvania</td>
<td>865</td>
<td>865</td>
</tr>
<tr>
<td>ESBR, Michigan</td>
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</tbody>
</table>

Note: Values from Table 1 of MacCulloch (1981) have been adjusted to include two individuals with kyphosis and two individuals with a convoluted central seam on the plastron, following the text.}

still show higher total deformity in C. plica, as do our data (27% for C. p. marginata vs. 23% for C. serpentina). We hypothesize that the variance reflects different nesting strategies between the two species. Chelydra serpentina generally lays only one clutch per season (Congdon et al. 2008), so the hatchlings usually emerge before temperatures drop significantly. In contrast, C. pica may lay one or two clutches per season (Congdon et al. 2003; Samson 2003), with the second clutch often overwintering in the nest (Breitenbach et al. 1984; Ernst et al. 1994). These later clutches can experience significant drops in temperature during their development, especially at higher latitudes. Thus, the higher frequency of deformity observed in wild C. pica may represent clutches laid late in the season; this hypothesis should be relatively simple to test.

We recorded a slightly higher proportion of females with deformities in both species, as did MacCulloch (1981), and this trend conforms to our expectations. Both our study species exhibit temperature-dependent sex determination. Both produce females when incubated at high (30–32°C) or very low (20–22°C) temperatures and males when incubated at intermediate temperatures (26–27°C; Yntema 1979; Gutzke and Paukstis 1984). Therefore, we would predict that if suboptimal temperatures are causing higher levels of deformity, then significantly more females (having developed at the lowest viable incubation temperatures) should develop deformities; the data support this prediction.

The effects of latitude and environmental pollutants on embryonic development of turtles likely interact in complex ways. Perhaps some individuals are genetically predisposed to develop asymmetrically. Such individuals might develop asymmetries (asymmetrical sculation, slight scoliosis) if incubated at suboptimal temperatures but would not develop major deformities under reasonably good conditions. This would explain the higher levels of minor deformity at less polluted sites. However, these individuals might be more likely to develop severe deformities if exposed to teratogens, and therefore not survive. Thus, we would expect a slightly lower incidence of minor deformities at severely contaminated sites because individuals predisposed to asymmetrical development will develop more extreme, lethal deformities instead of surviving to adulthood. Indeed, Bell et al. (2006) found that although the overall percentage of deformed adult C. pica did not differ greatly between their polluted and nonpolluted sites (17.9% and 14.2%, respectively), the severity of deformities in adults at the polluted JHNWR site was much higher. This suggests that genetic or climatic predispositions to asymmetrical development were aggravated by the presence of teratogenic chemicals.

We reiterate the findings of de Solla et al. (2008), keeping MacCulloch’s hypothesis in mind. Their data show that the frequency of deformity in hatched individuals (i.e., those who have a chance to survive to become deformed adults) is not significantly correlated with chemical contamination. However, they did find a significant difference between their two reference sites (both of which are in the northern part of their sample range and one of which is their most northern site) and three contaminated sites (all of which are southerly and two of which are their most southern sites). Latitudinal variation is a potential explanation for this trend; we recommend that future evaluations of deformities among...
variably contaminated sites should consider latitudinal variation among sites as a covariable.

Many factors clearly influence embryonic development; teasing these apart in wild populations may not always be possible. Clearly environmental pollutants can pose a threat to wild turtles and other wildlife; the recent studies showing this are valuable resources for population management and conservation. The maintenance or restoration of clean, high-quality habitat is crucial to the protection of many species. However, without detracting from the importance of conserving a pristine environment, we nevertheless hope that the analysis presented here provides reason to think twice before attributing the bulk of observed deformities in wild freshwater turtles primarily to the effects of chemical pollutants.

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References


