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IMPACTS OF MULTIPLE STRESSORS ON GROWTH AND METABOLIC RATE OF *MALACLEMYS TERRAPIN*

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Abstract—Coastal species encounter numerous physiological stressors ranging from daily fluctuations in salinity and temperature to anthropogenic contaminants, yet the effects of such stressor combinations on aquatic organisms remain largely unknown. Exposure to environmental contaminants, such as polychlorinated biphenyls (PCBs), can disrupt physiological processes, and while physiological responses to salinity change are well understood, the combined effects of salinity change and contaminants on these processes are unknown. Marine and brackish water turtles are often simultaneously exposed to both stressors. We exposed male, eight-month-old diamondback terrapins to one of four salinity treatments (0, 10, 20, and 30 parts per thousand) in the presence and absence of the anthropogenic stressor 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126, 20 μg/g via intraperitoneal injection) and monitored growth (carapace length and mass) and metabolic rate for six months. Exposure to PCB 126 significantly reduced growth ($p < 0.0001$), lowered standard metabolic rates (SMRs; $p < 0.0001$), and altered respiratory pattern ($p < 0.0001$). Salinity stress reduced growth ($p < 0.0001$) and altered the respiratory pattern ($p < 0.0001$) but had no overall effect on metabolic rate ($p = 0.33$). No interactive effects of PCBs and salinity were seen on either growth or metabolic rate. Our data indicate terrapins may be able to cope with some effects of salinity change through physiological adjustments but are less able to cope with PCBs. We show that PCB 126 disrupts the ecophysiological mechanisms that affect life history traits and thus ultimately could alter population structure and dynamics. The present study enriches our understanding of the environmental toxicology of reptiles and aids in the interpretation of health conditions documented in field-collected turtles contaminated with PCBs.

Keywords—Turtles Pentachlorobiphenyl 126 Salinity Metabolic rate and growth Hematocrit

INTRODUCTION

Organisms encounter stressors from natural environmental fluctuations and, in the presence of human populations, from anthropogenic contaminants. Some responses to stress include physiological and evolutionary modifications to avoid or tolerate stress [1,2]. These responses can be stressor specific [3] or can differ with experience [4], and organisms may respond differently to novel stressors than to stressors that have an evolutionary context. In addition, natural and anthropogenic stressors can interact in ways that alter energetic requirements and ultimately affect survival [5]. The ubiquity of stressors in modern environments and their potential interactive effects dictate the need for studies of multiple-stressor effects on growth, metabolic rate, and other physiological processes that can affect life histories.

Because stressors can cause long-term changes in physiology and ultimately can affect life histories, it is important to analyze ecophysiological variables that affect organism energetics [6]. For example, if up-regulation of physiological processes necessary to cope with stressors is energetically expensive, then stressed organisms should exhibit higher metabolic rates [7,8]. However, if stressed organisms are unable to increase their energy intake to meet increased physiological demands, then individuals with higher maintenance costs may exhibit decreased growth due to reallocation of energy from growth to maintenance [6].

Many coastal areas inhabited by terrapins are replete with natural stressors and anthropogenic stressors. Terrapins typically inhabit tidal areas where fluctuating salinities can affect physiological processes. Terrapins, like many other marine vertebrates, have an orbital salt gland, which functions to remove excess salt from the blood via a countercurrent exchange system [9]. However, in laboratory studies, continued exposure to high salinity (34 parts per thousand, or ppt) caused mature male terrapins to decrease their food intake [10]. In addition, exposure to high salinities causes changes in growth [11]. Thus, despite osmoregulatory organs for the removal of salt, salinity stress still affects basic physiological processes.

Anthropogenic toxicants contaminate many of these same coastal areas. Polychlorinated biphenyls (PCBs) comprise a mixture of 209 persistent, lipophilic congeners that bioaccumulate and biomagnify in organisms [12] and are common in coastal areas, including Chesapeake Bay, Maryland (USA) [13]. Many of these organochlorines have been reported in wild-caught turtles, including the dioxin-like congener 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) [14], which is one of the most biologically active congeners found in PCB mixtures and is responsible for much of the mixture's toxicity [15]. In green sea turtles from Hawaii (USA), PCB 126 accounted for 89% of the toxic equivalents in the tissues [16]. The biological effects of coplanar PCBs, including PCB 126, in aquatic vertebrates include altered thyroid hormone levels [17] and elevated CYP1A activity [18]. Organochlorine concentrations correlate with reduced packed blood cell volume

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(hematocrit) [19], which indicates that PCB exposure could result in a reduced carrying capacity for metabolic gases. Together, all of these responses suggest a significant impact of PCBs on energetics.

Salinity stress [11] and chemical body burdens influence growth [20] and development [14] in reptiles. However, studies examining the effects of combined stressors on the physiology of coastal reptiles are lacking. In the present study, we exposed juvenile diamondback terrapins to two potential stressors, PCB 126 and salinity, both alone and in combination, and measured responses in growth and respiration over a six-month period. Because terrapins have evolved passive, metabolically efficient responses to cope with salinity changes, we expected the toxic effects of PCB 126 to incur a metabolic cost greater than exposure to higher salinities. In addition, we hypothesized that the multiple-stressor combination of PCB 126 plus salinity would have more pronounced effects on terrapin growth and metabolic rate than salinity stress alone.

MATERIAL AND METHODS

In June 2002, we collected 12 clutches of recently laid (less than 24 h) diamondback terrapin eggs from the western shores of the Patuxent River, Chesapeake Bay. The middle Patuxent River supports some commercial fishing and agriculture, but unlike some of the other, more polluted tributaries, it is not listed as a region of concern by the Chesapeake Bay Program [21; www.chesapeakebay.net/pubs/792.pdf] and concentrations of most sediment contaminants, including PCBs, are well below the effects range low as defined by the National Oceanic and Atmospheric Administration's National Status and Trends Program [22; ccma.nos.noaa.gov/publications/NCCOSTM47.pdf]. Terrapin eggs previously collected from this area showed low concentrations of polycyclic aromatic hydrocarbons following an oil pipeline rupture in 2000 but were not screened for any other contaminants at that time [23].

Eggs were transported to Ohio University and incubated at male-producing temperatures (28°C). We confirmed sex by gross morphological examination in the subset of individuals used for hematocrit determination. We chose to examine stress responses only in males to keep sample sizes robust within treatment groups. After hatching, we individually reared animals for eight months before experimental testing, which could allow for breakdown and elimination of any PCBs obtained directly from the field. Only those individuals exhibiting normal growth were included in the present study.

Exposures

We randomly assigned 104 male, juvenile turtles to one of eight treatments. Salinities of 30, 20, 10, and 0 ppt of salt span the range of aquatic salinities normally encountered by terrapins in the field. Terrapins were exposed to salinity treatments in the presence and absence of 20 μg/g of PCB 126. This PCB dose was based on our previous experiment using male terrapins of similar age and held under nearly equivalent laboratory conditions [24] and the intraperitoneal dosages chosen by Yawetz, Woodin, and Stegeman [18]. Sample sizes (n = 13 for each of the eight combinations) were determined based on a power analysis from our previous study [24]. Turtles in the PCB treatments received one intraperitoneal injection (27-gauge tuberculin syringe) of 20 μg/g of PCB 126 (Ultra Scientific, Kingstown, RI, USA) dissolved in dimethylsulfoxide (Fisher Scientific, Pittsburgh, PA, USA) and corn oil (7% final dimethylsulfoxide concentration). Because our previous studies with terrapins showed no significant effect of the dimethylsulfoxide–corn oil vehicle on hatchling growth or survival [24] and studies with other turtles similarly showed no effect of corn oil on biochemical reactions [18], terrapins in the uncontaminated (0 μg/g) treatment received a sham (empty syringe) injection.

Although dietary exposure of PCBs is more ecologically relevant, we chose to deliver the PCB 126 using intraperitoneal injections for a number of reasons. First, no published data examine dietary absorption efficiencies of PCBs in turtles or reptiles in general. Huang and Karasov [25] report species-specific and chemical-dependent differences in absorption efficiency and observed much individual variation of uptake within a species. Second, terrapins eat while submerged in water and have messy feeding habits. Thus, dietary exposure in the present study could cause considerable variation in PCB exposure. Third, at the onset of the experiment, turtles were small (less than 20 g), making oral gavage difficult. Finally, many studies have administered coplanar PCBs via intraperitoneal injections in a number of taxa with good results [18]. Although PCB 126 concentrations administered in the present study are higher than those reported from wild terrapins [26], Stone, Kiviat, and Butkas [27] reported far higher concentrations of total PCBs in the livers of snapping turtles (*Chelydra serpentina*) from New York (USA). Furthermore, similar congeners have been administered via intraperitoneal injections to examine biochemical processes in turtles [18], producing results still relevant to wild populations.

Turtles were housed individually at 28°C (±2°C) in 12.5 cm × 17.5 cm × 6 cm plastic containers in approximately 300 ml of water under a full-spectrum Reptisun® (Zoo Med, San Luis Obispo, CA, USA) 12:12-h light:dark cycle in a walk-in environmental chamber. All treatments were randomized within the environmental chamber. We used laboratory grade reverse osmosis water mixed with Coralife® (Oceanic Systems, Walnut Creek, CA, USA) to attain the treatment salinities and stored the water in large carboys in the environmental chamber to ensure constant and equivalent temperature. Before water changes, the carboy water was aerated, mixed to resuspend any salt that may have settled during storage, and retested for appropriate salinity using a refractometer. Based on previous research suggesting terrapins cannot survive long periods in full strength seawater [11], we cycled turtles through the treatment salinities. Cycling from the salinity of the source population (10 ppt) to the treatment test salinities not only ensured survival of the animals but increased ecological realism because tidal coastal areas are subject to fluctuations in salinity. All turtles spent the first 10 d of a 30-d cycle at an acclimation salinity (10 ppt) and then were switched to ecologically relevant test salinities (0, 10, 20, or 30 ppt) for the remaining 20 d. We repeated this salinity cycle every 30 d for six months. Due to equipment constraints, turtles were randomly assigned to groups and each group was staggered in the onset of the six-month experimental protocol. Thus, PCB exposure, all measurements, and all feedings occurred on specific days of the 30-d cycle rather than specific calendar dates. Twice a week we fed turtles two tablespoons of Kordon® frozen brine shrimp (Novalek, Hayward, CA, USA) and 24 h later completely changed their water. All turtles ate throughout the study, and no mortality was observed. To determine whether size differences among treatments were caused by appetite, we measured the amount of food consumed calculated as food offered

(grams, dry wt) less orts (grams, dry wt) at six months after exposure.

Growth

All 104 turtles were measured every 30 d for six months. We measured straight line carapace length with digital calipers (Mitutoyo, Mason, OH, USA) to the nearest 0.1 mm and determined mass to the nearest 0.1 g with an electronic balance (A&D®, San Jose, CA, USA).

Standard metabolic rate

We measured terrapin CO_2 production using a flow-through respirometer (Sable Systems, Las Vegas, NV, USA). Metabolic studies of aquatic reptiles are typically conducted in air [7]. Although this procedure may be slightly desiccating, all turtles in this experiment lost less than 5% of their body mass during metabolic determinations regardless of PCB or salinity treatment.

Metabolic rate of all turtles was measured at the end of the 30-d salinity cycle (i.e., after 20 d of exposure to the test salinity). Juvenile terrapins were held at 28°C (±2°C) and fasted for 7 d before SMR determination to ensure metabolic rates did not include metabolic increases associated with digestion. Turtles were towel dried, weighed, and placed individually into 100-ml glass respirometry chambers in a 26°C incubator. Turtles were acclimated to the chamber and allowed to recover from any handling stress for 4 h before SMR measurement. The respirometry chamber received dry, CO_2-free ambient air flowing at 110 ml/min as determined by a Sierra Sidetrak® flow meter (Sierra Instruments, Monterey, CA, USA), which was continuously monitored by Datacan Software (Sable Systems) and periodically confirmed using a Gilmont® calibrated flow meter (Thermo Scientific, Waltham, MA, USA). Carbon dioxide production was measured during the quiescent period of the dark cycle. The Datacan Software program sampled every chamber individually and determined a baseline before and after each measurement period. Datacan measured the amount of CO_2 produced over a period of 15 min and plotted production as a function of time. To determine the total amount produced, we integrated under the curve, adjusted for flow rate, and took the mean of at least two 15-min sampling periods.

In addition to total CO_2 production, we measured the number of breaths taken by each individual during the SMR determinations. We analyzed each Datacan file for the number of distinct CO_2 peaks for each individual during each measurement period. A peak was defined as a distinct increase in CO_2 (released from the animal into the chamber) bounded on each side by a distinct decrease in CO_2 that measured at least half the height of the peak. Each peak was considered to represent one breath. Only those files containing a series of distinct peaks (breaths) over the duration of the 15-min measurement period were included. Thus, total sample sizes were 86 terrapins at 0 d, 97 terrapins at 30 d, 89 terrapins at 90 d, 91 terrapins at 150 d, and 92 terrapins at 180 d. Many turtles are capable of gas exchange through the skin, buccopharynx, and cloaca [28], and although this has not been shown specifically for *Malaclemys*, it is possible that a CO_2 peak may also represent one of these extrapulmonary bouts.

Hematocrit

At the end of six months, a subset of turtles ($n = 40$) was euthanized by decapitation. Immediately, we collected a blood sample in a heparinized capillary tube for hematocrit determination. Capillary tubes were spun in a microhematocrit Readacrit™ (BD, Franklin Lakes, NJ, USA) centrifuge at 8,000 rpm for 5 min. Hematocrit was determined from a direct reading scale.

Statistical analyses

Statistical analyses were performed using SAS release 8.02 (SAS Institute, Cary, NC, USA) [29]. All data were normally distributed and met the assumptions required for parametric statistics. To examine differences in growth, two repeated measures analysis of variance were performed using treatments as the factors and carapace length and mass as the dependent variables. A Bonferroni adjustment was used to decrease the experiment-wise error rate. To examine differences in SMR, we used a repeated measures analysis of covariance with treatments as factors, mean CO_2 production as the dependent variable, and body mass as a covariate. Breath data were analyzed using a repeated measures analysis of covariance with treatments as the factors, number of CO_2 peaks as the dependent variable, and body mass as a covariate to determine differences among the treatment groups. Instead of using a full analysis of variance model that considers all possible interactions, we analyzed growth and metabolic data using a reduced model to increase the degrees of freedom and overall statistical power. The reduced model included only the specific terms and interactions of interest. We used Tukey-Kramer post hoc tests following the analyses of variance to evaluate multiple comparisons. Analysis of variance was used to determine differences in hematocrit among the treatment groups. Values in figures are means ± 1 standard error with the exception of the total mean CO_2 production values, which are least-squares means that reduce the effect of body size on the variable of interest.

RESULTS

Exposures

We randomly selected four uncontaminated individuals and three individuals that received the 20 μg/g PCB 126 exposure. To determine the PCB body burdens of the turtles used in the present study, liver concentrations of PCB 126 were measured by gas chromatography with electron capture detection by S.D. Holladay (Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA, USA). Liver concentrations of PCB 126 were less than 0.03 μg/g and averaged 13.2 μg/g (wet wt; detection limit = 0.02 μg/g) in uncontaminated and PCB-exposed individuals, respectively.

Growth

Food consumed was not significantly different between PCB-exposed and control turtles ($F_{1,58} = 3.66$, $p = 0.06$), and there was no interaction between PCB and salinity ($F_{3,58} = 0.48$, $p = 0.69$). However, salinity treatment alone did reduce food consumption ($F_{3,58} = 10.84$, $p < 0.01$). Animals held at higher salinities (20 and 30 ppt) consumed less food than those at lower salinities (0 and 10 ppt; Tukey-Kramer $q = 3.7$, $p < 0.05$).

Both PCB 126 and salinity affected growth (Fig. 1). At the onset of the experiment, turtles in all treatment groups were similar in carapace length ($F_{7,103} = 0.36$, $p = 0.92$; Fig. 1A) and mass ($F_{7,88} = 0.22$, $p = 0.98$; Fig. 1B). After six months, turtles exposed to PCB 126 in combination with various salinities exhibited less variance in body size among the different

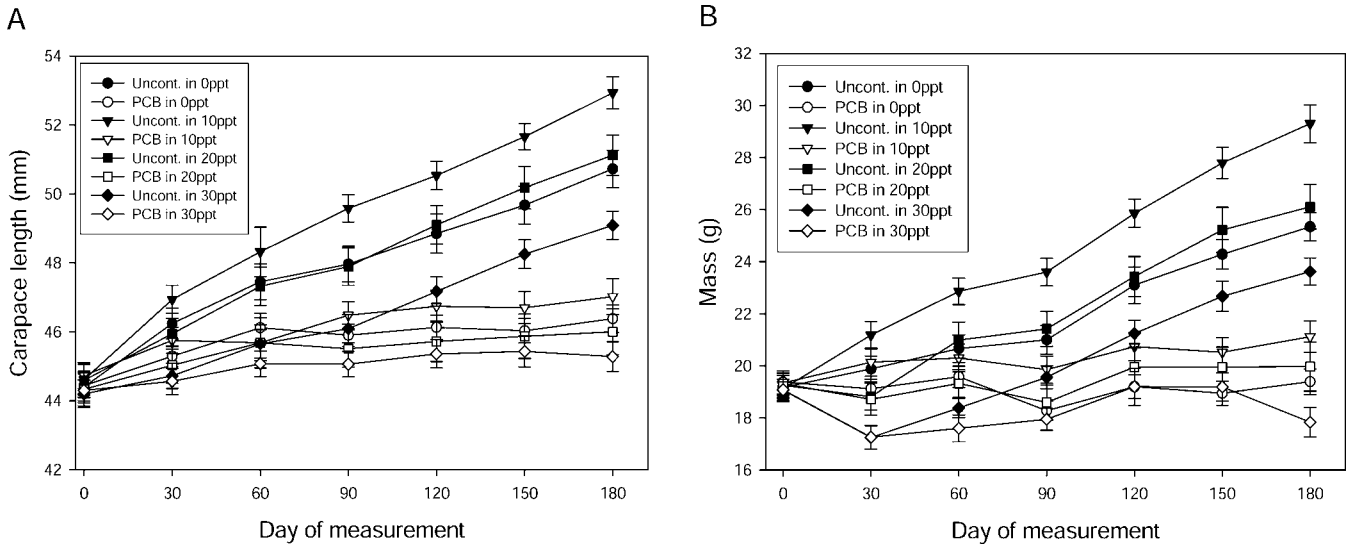

Fig. 1. Growth rates of juvenile diamondback terrapins measured by carapace length (**A**) and body mass (**B**) after exposure to one of four salinities and in the absence and presence of intraperitoneal injections of pentachlorobiphenyl (PCB 126). Error bars = 1 standard error; uncont. = uncontaminated.

salinity levels than did uncontaminated turtles. Furthermore, PCB-exposed turtles at all test salinities other than the acclimation salinity (PCB + 10 ppt) were significantly smaller in mass and length than all uncontaminated turtles except those experiencing the highest (uncontaminated in 30 ppt) salinity stress (Tukey-Kramer $q = 4.38$, $p < 0.05$). During the experiment, there was a PCB 126 by salinity by time interaction for mass ($p = 0.005$) but not for length ($p = 0.09$). However, no significant PCB 126 by salinity interaction was seen for mass once time was removed ($p = 0.33$; Table 1).

All turtles exposed to PCB 126 were significantly smaller in length ($p < 0.0001$) and mass ($p < 0.0001$) than were uncontaminated individuals. Uncontaminated turtles that remained at the acclimation (and source population) salinity of 10 ppt were the largest in mass and length, followed by 20 ppt and 0 ppt, and individuals at 30 ppt were the smallest in both mass and length (Fig. 1 and Table 1). Interestingly, a 10-ppt change in salinity, either increasing (to 20 ppt) or decreasing (to 0 ppt) resulted in smaller, similarly sized turtles relative to the 10-ppt controls (Tukey-Kramer $q = 3.69$, $p < 0.05$). Furthermore, a salinity increase from 10 ppt to 20 ppt resulted in a turtle that was 2.6 g smaller, and an additional increase of 10 ppt (up to 30 ppt) resulted in a turtle that was 5.1 g smaller, suggesting salinity has an incremental effect on mass.

Standard metabolic rate

Although Figure 2 depicts CO_2 production increasing over the six-month study, this appears to be a residual effect of body size, and plotting the least-squares means only partially removed the effect of differences in body size. Salinity had no effect on mass-adjusted SMR as measured by total CO_2 production ($p = 0.33$; Fig. 2A and Table 1). However, exposure to PCB 126, regardless of the salinity treatment, caused a significant reduction in the amount of total CO_2 produced (Table 1), with differences becoming significant at 90 d after exposure (Fig. 2B). The decrease in metabolic rate was associated with a change in breathing behavior. At day 0, all individuals took equal numbers of breaths during the 15-min measurement period, as shown by the equal number of CO_2 peaks (Fig. 3A). Turtles held at 0 ppt took the fewest number of breaths compared with those at 20 and 30 ppt (Tukey-Kramer $q = 3.69$, $p < 0.05$; Fig. 3A and Table 1). Polychlorinated biphenyl–exposed turtles also took more breaths than uncontaminated turtles ($p < 0.0001$; Fig. 3B and Table 1), but no interaction was seen between PCB 126 and salinity ($p = 0.64$; Table 1).

Hematocrit

Hematocrit was lower ($F_{1,39} = 33.71$, $p < 0.001$) in PCB-exposed animals than in uncontaminated turtles (Fig. 4) but was not affected by salinity ($F_{3,39} = 0.57$, $p = 0.64$).

DISCUSSION

In the present study, we exposed estuarine diamondback terrapins from the Chesapeake Bay to PCB 126 and ecologically relevant salinities. We hypothesized that the multiple-

Table 1. Statistical analysis of the effects of treatment (pentachlorobiphenyl [PCB 126], salinity, and their interaction) on mass, carapace length (CL), carbon dioxide (CO_2) production, and number of CO_2 exhalations (peaks) in juvenile diamondback terrapins[a]

	Mass			CL			CO_2			Peaks		
Variable	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
PCB	1, 96	62.27	<0.0001	1, 96	70.8	<0.0001	1, 147	26.56	<0.0001	1, 179	22.74	<0.0001
Salinity	3, 96	11.53	<0.0001	3, 96	9.02	<0.0001	3, 147	1.15	0.33	3, 179	11.64	<0.0001
PCB × salinity	3, 96	1.17	0.33	3, 96	1.37	0.26	3, 147	2.61	0.31	3, 179	0.56	0.64
PCB × salinity × time	21, 95	2.19	0.005	21, 95	1.53	0.09	12, 138	1.46	0.15	12, 146	1.04	0.41

[a] A repeated measures analysis of variance was used for mass and CL, and a repeated measures analysis of covariance with body mass as a covariate was used for CO_2 and peaks. Presented are degrees of freedom *(df), F* statistics, and associated *p* values.

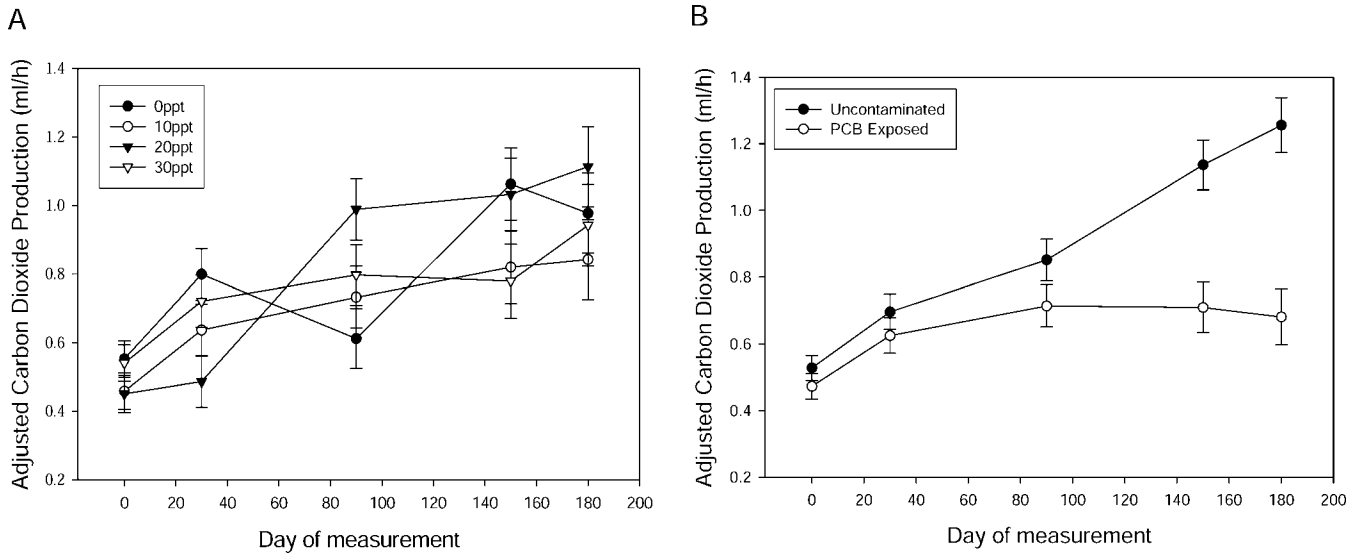

Fig. 2. Carbon dioxide production measured in juvenile diamondback terrapins. Each line represents uncontaminated and pentachlorobiphenyl (PCB 126)–exposed individuals combined to show there was no effect of the various salinity treatments (**A**). Turtles from all salinity treatments were combined to show the difference in carbon dioxide production in the presence and absence of intraperitoneal injections of PCB 126 (**B**). Error bars = 1 standard error.

stressor combination, PCB 126 and salinity, would have more pronounced effects on growth and metabolic rate than salinity stress alone due to an increased energy demand in multiple-stressed terrapins relative to those experiencing only a single stressor. Acute exposure to PCB 126 significantly decreased growth and metabolic rate and significantly increased ventilation frequency over time. Six months after PCB exposure, terrapins remained unable to physiologically recover from the PCB 126 and maintained growth and metabolic rates significantly lower than those of uncontaminated individuals. Chronic exposure to high and low salinity treatments also acted as stressors but only caused a modest decrease in growth and growth rate recovered during the experiment. The initial differences in growth in salinity-stressed animals may reflect an acclimation or adjustment period during which mechanisms necessary to cope with salinity stress are up-regulated. Although interactive stressor effects have been shown in other taxa, no interaction was seen between PCB 126 and salinity once the effect of time was removed. Our results indicate that physiological responses were stressor specific and PCB 126 exposure elicited a greater physiological response than that of exposure to high salinity or of combined exposure to salinity and PCB.

Growth

To our knowledge, our experimental data are the first under controlled laboratory conditions to provide direct evidence that PCB 126 exposure significantly reduces growth in reptiles. Growth effects were not evident until at least 90 d after PCB exposure, indicating a lag between exposure and the onset of whole-organism growth effects. Moreover, it is likely that if the growth trajectories for the PCB-treated and sham turtles

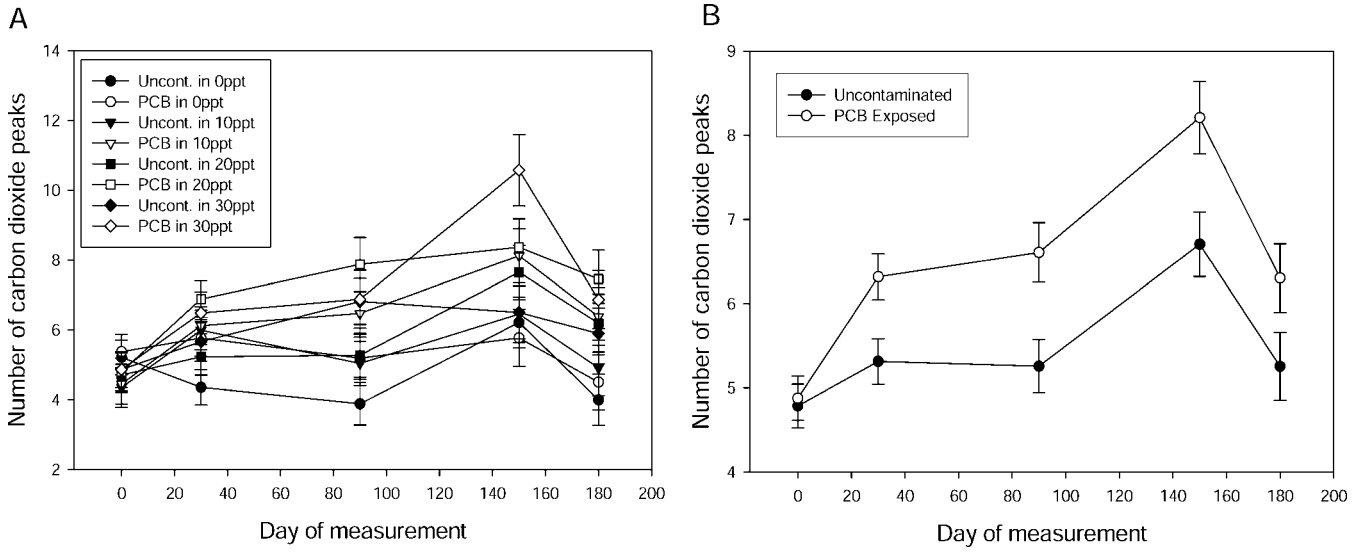

Fig. 3. The respiratory pattern as measured by the number of carbon dioxide peaks (considered to represent breaths) in juvenile diamondback terrapins exposed to various salinity treatments and in the presence and absence of intraperitoneal injections of pentachlorobiphenyl (PCB 126). Error bars = 1 standard error.

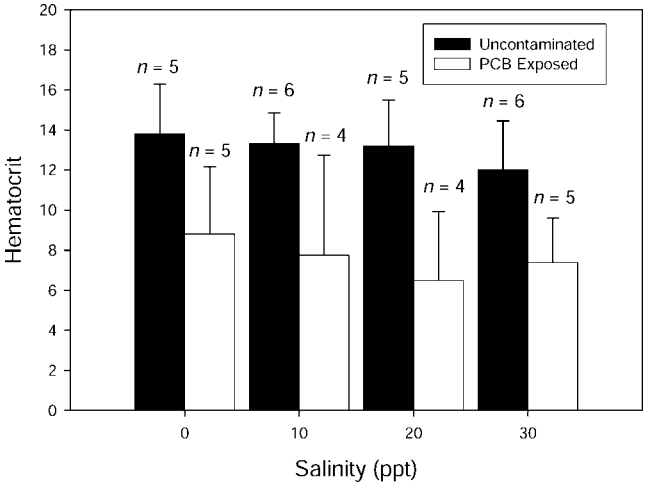

Fig. 4. Hematocrit measured in juvenile diamondback terrapins exposed to various salinities and intraperitoneal injections of pentachlorobiphenyl (PCB 126). Numbers above each bar indicate sample size; error bars = 1 standard error.

were extended beyond the 180 d of the present study they would continue to diverge, resulting in increased differences in size between PCB-exposed and uncontaminated turtles over time. Exposures in the present study resulted in a PCB 126 liver concentration of 13.2 μg/g wet weight. These values are higher than concentrations of PCB 126 (0.2–5.6 ng/g wet wt) recently measured in snapping turtle fat tissue [30] but within the range of the repeated high-dose intraperitoneal exposures (5–50 μg/g) employed by Yawetz, Woodin, and Stegeman [18]. Although the dose was high, our data support correlations found in ecotoxicology field studies that suggest PCBs inhibit growth in snapping turtles [31]. Whether this growth effect is the result of PCB 126–mediated alterations on the ability of the gut to extract nutrients from food, a disruption of growth hormones, or some other mechanism remains to be demonstrated. The exact mechanism likely depends on the specific dose and toxicant and may be affected by the energetic state of the individual [32].

In contrast to the effects of PCB exposure, terrapins appeared to physiologically adjust to salinity changes, thereby preventing permanent effects on growth rate. At each experimental measurement period, turtles that remained at 10 ppt (the acclimation salinity and the approximate salinity the Patuxent population normally experiences in the field) attained the largest size. With exposure to a different salinity (0, 20, or 30 ppt) growth initially slowed, but over time growth rates recovered and slopes were similar among salinity treatments by 120 d (Fig. 1). Interestingly, the initial decrease in growth caused by salinity appeared to have a long-term effect in that turtles at all stressor salinities (0, 20, and 30 ppt) remained smaller than those held at 10 ppt (Fig. 1A) despite the recovery of the growth rate by day 120. If this salinity effect persists until maturity, then it may partially explain why terrapins from coastal populations with higher salinity are smaller than those from inland, lower-salinity populations (W.M. Roosenburg, personal observation, Ohio University, Athens, OH, USA). These findings are similar to those of Dunson [11], who exposed hatchling terrapins to five salinity levels (0–35 ppt) for 33 d and showed that turtles held at approximately 9 ppt attained the greatest mass gain whereas higher salinities caused a reduction in mass gain. In other laboratory studies, continued exposure to high salinity (34 ppt) caused mature male terrapins to decrease their food intake [10]. In the present study, smaller size was not solely due to differences in food intake. Although individuals at the higher-salinity treatments (20 and 30 ppt) ate less, growth rates were still similar, perhaps accomplished through an adjustment in digestive processing. Interestingly, terrapins at 0 ppt consumed as much as those at 10 ppt but were smaller and more similar in size to those at 20 ppt, which were eating less, suggesting these differences in size are being driven by more than food intake alone. We cannot predict how these differences in growth might affect age or size at first reproduction, but should the salinity-stressed turtles show such differences when they reach reproductive maturity, then salinity stress and its physiological compensation would have had long-term effects and altered the life history of these populations. Understanding the fitness trade-off associated with these differences remains an interesting question.

Metabolic rate

Physiological mechanisms to cope with stressors can be energetically expensive and lead to increased metabolic rate. Hopkins, Rowe, and Congdon [7] showed that contaminant-exposed snakes exhibited higher oxygen consumption than those from reference populations. Similarly, we hypothesized salinity stress, PCB 126 exposure, or both would require the up-regulation of physiologically expensive pathways, thus increasing terrapin metabolic rate.

We found that salinity stress, either alone or in combination with PCB 126, did not significantly affect metabolic rate (Table 1), perhaps because removal of excess salt can be accomplished passively via the orbital salt gland [9]. Yet, despite this lack of effect on metabolic rate, the salinity-stressed turtles were significantly smaller in size than those held at the acclimation salinity (10 ppt).

If there was no energetic trade-off from an increased metabolic rate causing reduced growth and the differences are not completely explained by a decreased appetite, then why were salt-stressed turtles smaller? It is possible that if salinity affected energy assimilation or digestive parameters not measured in the present study, the overall amount of energy available for assimilation would be less, which may result in decreased growth. Another possible explanation is that any metabolic cost of removing salt may occur in association with feeding and thus be a component of specific dynamic action. Because we fasted animals 7 d before measuring metabolic rate, the physiological cost of osmoregulation may have gone undetected.

In contrast to the effects of salinity stress, and contrary to our predictions, PCB-exposed individuals had decreased CO_2 production (Fig. 2B), a difference not significant until 90 d after exposure. Because we measured CO_2 production during the dark cycle when metabolic rates can be 50% lower [33], a lowered metabolic rate could allow PCB-stressed turtles to conserve energy for future activities, whereas uncontaminated individuals with lower energy demands overall would be able to maintain higher metabolic rates. A depressed metabolic rate could also be a symptom of overall illness, thus not an adaptive response for conserving energy, although all turtles appeared healthy at the end of the present 180-d study. Turtle metabolic rate may be dose dependent, as has been seen in pigeons treated with the chlorinated pesticide *p,p′*-DDT [34], where low *p,p′*-DDT doses increased metabolic rate and high doses significantly reduced metabolic rate. Thus, doses lower than those

used in the present study might produce the expected increased metabolic rate and associated physiological trade-offs. These dose-dependent metabolic effects may be the result of contaminants altering thyroid hormones. Jefferies and French [35] suggest low doses of chlorinated contaminants cause hyperthyroidism, leading to an increase in metabolic rate, whereas higher doses result in hypothyroidism and thus a reduced metabolic rate. Recent research has documented wide-ranging effects of organic pollutants on thyroid function, including the alteration of plasma T_3 and T_4 in alligators collected from contaminated lakes in Florida (USA) [36] and numerous laboratory studies directly linking PCB exposure to altered plasma T_4 in mammals [37]. Future ecophysiological studies of PCB stress in reptiles should include measures of thyroid function because thyroid hormones play an integral role in physiology and our current understanding of thyroid toxicology in reptiles is generally minimal.

Previous research has shown that turtles exposed to high salinities exhibit higher hematocrit except during the summer months [38]. In the present study, turtles were held under near-summer conditions and their hematocrit was unaffected by salinity stress, a pattern consistent with Gilles-Baillien [38]. In contrast to salinity stress, PCB exposure reduced hematocrit, consistent with previous studies linking exposure to organochlorines with anemia [19]. Lower hematocrit means a reduced ability to transport oxygen to and metabolic waste products from tissues to support cellular metabolism. A reduced ability to transport blood gases could lead to a lower metabolic state and partly explain the lower total CO_2 produced by PCB-stressed turtles.

The differential effects of PCB 126 and salinity on ventilation frequency indicate turtles exposed to PCB 126 exhibit a response consistent with stress while those exposed to salinity changes alone did not. Reduced metabolic rate in PCB 126–treated turtles was accompanied by increased ventilation frequency, indicating that a smaller amount of CO_2 must have been released with each ventilation. Faster, shallower breathing is typical of stressed organisms, including reptiles, which are attempting to maximize respiratory intake while minimizing energy expenditure [39]. In contrast, although salinity did not affect metabolic rate, it did affect ventilation frequency. Turtles held at 0 ppt ventilated at a lower frequency than those at higher salinities; thus, fewer CO_2 releases produced the same overall amount of CO_2 eliminated over a given period.

CONCLUSIONS

We have shown that exposure to salinity stress and PCBs, either alone or in combination, had significant effects on the growth and physiology of juvenile diamondback terrapins. Our findings further indicate that exposure to PCBs in coastal habitats may be having significant effects on terrapin populations. Additional research is needed regarding toxicant effects on terrapins inhabiting coastal areas if we are to predict how chemically contaminated habitats may be affecting these slow-growing species. Although we found no additive or synergistic effects of combining salinity and PCB stressors in the present study, future studies combining stressors typical of coastal habitats, such as salinity, temperature, organic pollutants, and metals, are needed to more fully assess the potential effects of the real-world, multiple-stressor conditions experienced by these marine reptiles. Such studies are vital if we are to develop predictive models that facilitate the development of more effective conservation practices in anthropogenically altered habitats.

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