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Ecotoxicological assessment of diamondback terrapin (*Malaclemys terrapin*) pond habitat, prey and eggs in Bermuda

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ABSTRACT

Total petroleum hydrocarbons, PAH and various trace metal residues were extracted and analyzed from fresh whole diamondback terrapin (*Malaclemys terrapin*) eggs, whole brackish-water gastropods (terrapin prey) and benthic sediment from anchialine pond environments in Bermuda inhabited by terrapins. Gastropods and terrapin eggs showed higher concentrations of trace metals and organic contaminants than sediments. Conversely, PAHs were mostly found within the sediment and smaller amounts detected in gastropods and terrapin eggs. Results indicated that contaminants in prey were transferred to terrapin eggs, and that concentrations of several contaminants exceeded potentially toxic concentrations for aquatic vertebrates. Necropsy of unhatched eggs from nests that had yielded viable hatchlings showed significantly compromised embryonic development. Bermudian diamondback terrapins reside and feed in brackish wetland habitats characterized by widespread, multifactorial contamination. This study suggests that environmental contamination plays a role in the recorded low hatching success in terrapin eggs in Bermuda.

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1. Introduction

The archipelago of Bermuda is located at 32° 19'N and 64° 46'W — some 965 km ESE of Cape Hatteras, North Carolina, USA (Fig. 1). It is basically composed of porous biogenic limestone atop a volcanic seamount. Below sea level the interstices of limestone are filled with seawater; freshwater delivered by rainfall forms a lens above the saline layers; ponds wholly above sea level can be fresh. However, many landlocked ponds whose bottoms are below sea level are anchialine (water bodies with a subterranean connection with the ocean resulting in surface freshwater and deeper saline water).

Bermuda has a long history of environmental contamination by chemicals of various types (Linzey et al., 2003; Bacon et al., 2006, 2013; Fort et al., 2006a, 2006b, 2015). Insecticides have been used for decades to control mosquitoes (D. Kendall, personal communication), while the great popularity of golf courses on the islands (there are nine in total) has promoted herbicide use (Bacon et al., 2006, 2013). In addition, despite its small size (54 km^2), Bermuda has a high human population (approximately 65,000) and is home to around 44,000 licensed road vehicles of various types. Bermuda also has over 7600 powered recreational watercraft (motorboats and jet skis) that heavily use the surrounding waters (Adwick et al., 2005). Many of such craft have two-stroke engines that are known to emit far greater quantities of hydrocarbons to the atmosphere than road vehicles (Davenport and Switalski, 2006). Throughout much of the 20th century, garbage (including metallic objects, and batteries) was disposed of in shallow landfills near or on top of ponds and coastal waters (Sterrer and Wingate, 1981). High temperature incineration is now the main method of disposal of municipal solid waste from residential and commercial sources. Although not industrialized, Bermuda is consequently characterized by high concentrations of localized anthropogenic contaminants (Jones, 2011).

Recent investigations of the health status of the pond environment in Bermuda suggested a suite of contaminants of concern that are having detrimental effects on the resident fauna (Fort et al., 2006a, 2006b; Bacon, 2010; Bacon et al., 2013). These contaminants include total petroleum hydrocarbons (gasoline-range organics (TPH-GRO), diesel-range organics (TPH-DRO)), polycyclic aromatic hydrocarbons (PAH) and trace metals. Entry of contaminants into the wetlands comes through storm-water run-off from adjacent roadways, car parks and house drives, aerial deposition and leachate from nearby landfills and ground-water sources (Fort et al., 2006a, 2006b). Ponds located within and adjacent to golf courses are among the most contaminated wetlands in Bermuda (Bacon et al., 2013). Tissue residue analyses

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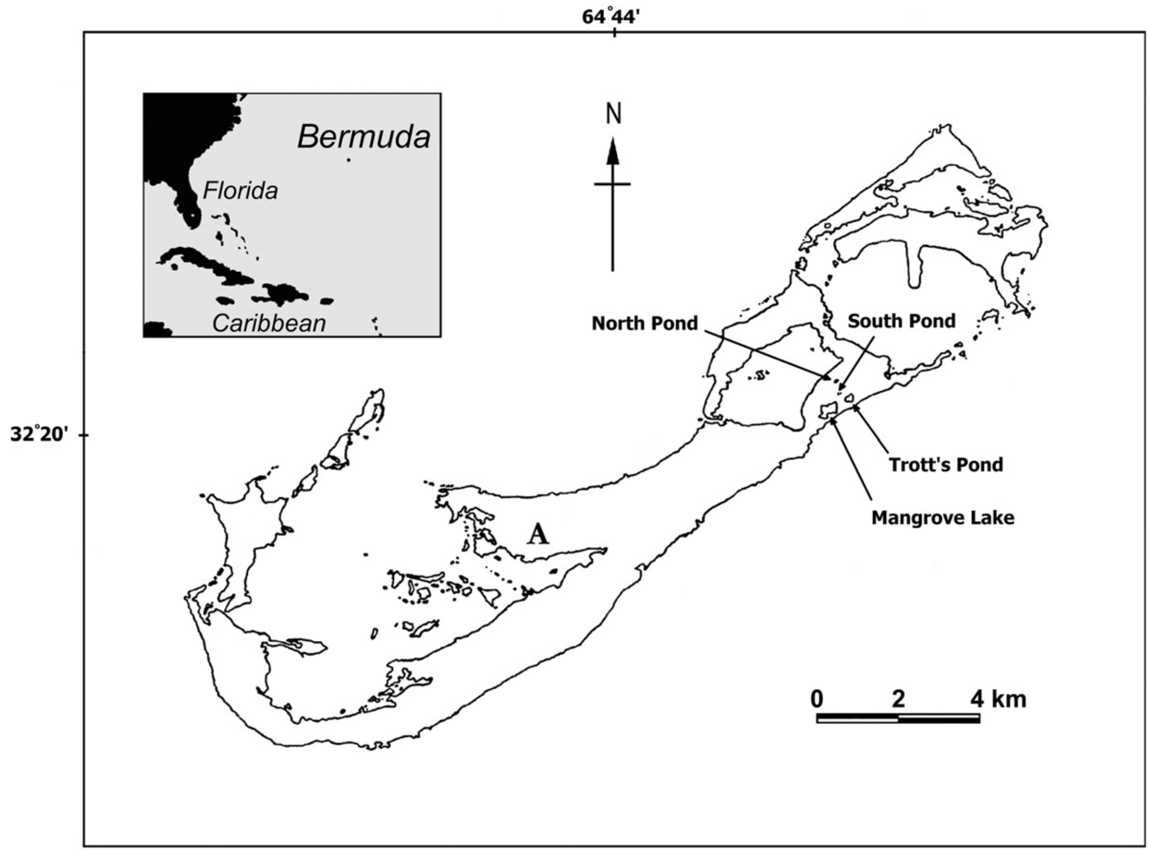

Fig. 1. Map of Bermuda showing the location of the ponds inhabited by diamondback terrapins; Mangrove Lake, South Pond, North Pond and Trott's Pond. (A = city of Hamilton).

from a range of taxa, including cane toads (*Rhinella marinus*), mosquitofish (*Gambusia holbrooki*), killifish (*Fundulus* spp.), and red-eared sliders (*Trachemys scripta elegans*) collected from a variety of contaminated wetlands across Bermuda have shown that petroleum hydrocarbons, PAH and trace metals were accumulated and are associated with developmental malformations, endocrine disruption, liver and gonad abnormalities, plus immunological stress (Bacon, 2010; Bacon et al., 2013).

Diamondback terrapins (*Malaclemys terrapin*) are native to Bermuda (Parham et al., 2008) and are residents of the land-locked, brackish-water pond environment (Davenport et al., 2005) (Fig. 1). They are vulnerable to local extirpation given a) the small population size (approximately 100 adult/subadult individuals), b) a highly-localized distribution (four brackish-water ponds situated on 1 km^2 of land), c) low annual hatching success (ca. 19%; Outerbridge, 2014) despite an absence of nest predators.

Diamondback terrapins in North America are known molluscivores (Tucker et al., 1995), and investigations into the feeding ecology of Bermuda's diamondback terrapins have shown that they ingest substantial quantities of small gastropods, which are known bio-accumulators of both inorganic and organic compounds (e.g. Walsh et al., 1995), together with appreciable quantities of surface benthic sediments (Outerbridge and Davenport, 2013). Terrapins accumulate trace metals in liver and muscle tissue (Burger, 2002), PAHs in eggs (Holliday

Table 1
Summary of the metal and total petroleum hydrocarbon residues (mg kg^{-1} dry weight values) found in composite samples of pond sediment, aquatic gastropods and diamondback terrapin eggs from Bermuda.

Sample (sample size)		As	Cd	Cr	Cu	Fe	Pb	Ni	Zn	Hg	Gasoline-range petroleum hydrocarbons	Diesel-range petroleum hydrocarbons
Pond	max.	56.27	4.04	125.00	112.28	12884.62	42.20	8.79	64.95	0.27	0.77	148.33
sediment	min.	19.04	<0.01	27.02	21.63	2543.86	14.55	3.64	25.22	<0.01	<0.05	<0.5
(n = 12 × 4.5 l)	mean	35.18	2.94	58.29	67.73	5990.40	27.96	5.61	46.05	0.21	0.77	104.58
	SD	13.31	0.86	32.97	27.75	3465.33	8.05	1.57	12.03	0.04	–	35.27
Pond	max.	69.03	80.96	428.71	545.10	353.49	673.13	23.43	1585.84	29.93	<0.1	467.51
gastropods	min.	33.46	47.33	125.26	129.49	138.71	269.53	6.82	597.96	4.36	–	152.04
(30 g)	mean	51.24	65.79	272.09	321.94	230.44	388.79	16.85	1043.19	13.48	–	278.49
	SD	18.95	13.86	125.39	183.07	89.85	190.57	7.60	488.66	11.52	–	134.85
Terrapin	max.	58.49	86.32	65.41	131.60	147.17	469.86	3.64	227.70	10.52	<0.1	417.88
eggs	min.	5.40	12.59	6.13	66.29	64.78	69.08	0.84	98.62	1.65	–	80.74
(n = 11)	mean	28.16	37.74	26.17	90.21	100.66	167.66	1.97	165.11	4.37	–	225.57
	SD	15.09	23.42	16.95	22.20	25.97	117.53	0.78	36.53	2.79	–	117.01

SD = standard deviation.

Table 2
Summary of the polycyclic aromatic hydrocarbon residues (μg kg^{-1} dry weight values) found in composite samples of pond sediment, aquatic gastropods and diamondback terrapin eggs from Bermuda.

Sample (sample size)		Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene	Anthracene	Fluoranthene	Pyrene
Pond	max	<15	1567	3789	<7.5	2684	4333	<48.8	2982
sediment	min	<15	<7.5	<7.5	<7.5	<10	1091	<48.8	789
(n = 12 × 4.5 l)	mean	–	1567	2462	–	1856	2628	–	1828
	SD	–	–	1088	–	621	1165	–	756
Pond	max	<15	<7.5	2755	262	2219	1986	136	2667
gastropods	min	<15	<7.5	226	<7.5	445	699	<70.4	620
(30 g)	mean	–	–	1417	158	1611	1503	136	1869
	SD	–	–	1074	91	793	558	–	902
Terrapin	max	<15	<7.5	370	193	361	390	355	667
eggs	min	<15	<7.5	29	<7.5	58	46	77	149
(n = 11)	mean	–	–	136	88	171	155	207	403
	SD	–	–	122	50	101	112	94	182

SD = standard deviation.

et al., 2008) and have been used as bio-indicators of environmental contaminants in salt marsh ecosystems (Blanvillain et al., 2007; Basile et al., 2011). However, long-term effects of such exposure are unknown.

We hypothesized that Bermudian terrapins, like other Bermudian aquatic fauna, might be negatively affected by TPHs, PAHs and trace metals which could put the population at risk. Knowing whether this is the case is critical to the design of appropriate management initiatives and wetland remediation activities. Given the small population size of Bermudian terrapins, destructive sampling of adults, juveniles or hatchlings was unethical on conservation grounds. To investigate exposure to, and absorption of, contaminants, samples of pond benthic sediment, aquatic gastropods as well as whole diamondback terrapin eggs were analyzed for TPH, PAH and trace metal residues. In addition, terrapin eggs were necropsied to evaluate the internal structure of the egg, fertilization, and early embryo development.

2. Methods

The Department of Conservation Services (Bermuda Government) authorized all aspects of the study. The work conducted did not require formal permit under vivisection legislation of either Bermuda or Ireland.

2.1. Study sites

Mangrove Lake (10 ha) and Trott's Pond (3 ha) are among the largest ponds on Bermuda. Situated <200 m from the coast, both are surrounded by narrow fringes of red mangrove (*Rhizophora mangle*) swamps (2.3 ha and 0.8 ha respectively; Thomas et al., 1991; Thomas, 1993). Both are saline (annual mean salinity 28.6 psu) and connected to the ocean via small subterranean fissures (Thomas et al., 1991). South Pond is one of the smallest (0.2 ha) ponds on Bermuda and is associated with 0.3 ha of saw-grass (*Cladium jamaicense*) marsh. It is brackish (mean annual surface salinity 10.8 psu). Not being connected to the ocean, its salinity and water level are greatly affected by rainfall. All three ponds are shallow (mean depth range 35–269 cm) and have been incorporated into the Mid Ocean golf course as water hazards since the 1930s. Mangrove Lake has deep deposits of gelatinous, highly organic benthic sediment (Hatcher et al., 1982). By inspection this is also true of the other ponds (Outerbridge, personal observation).

2.2. Sediment and tissue collection/analysis

Benthic sediment was collected from five widely-separated locations within Mangrove Lake, three within Trott's Pond and four within South Pond in 2009 as part of an island-wide assessment of Bermuda's wetland health that has been in progress since the late 1990s (J. Bacon, personal communication). The sediment was collected from a boat using a long-handled dip net with a mesh size of 1 mm. Each sample comprised sediment skimmed from the surface of the pond bottom. The sediment sampler was rinsed (in-situ, using pond water) three times after each sample was collected. Only one sample was collected at each location. Samples were decanted into sterile 4.5 l glass bottles and refrigerated at 4 °C prior to shipment. One operative performed the actual sediment sampling, another labeled bottles and documented collection information.

Whole body samples of aquatic gastropods were collected in July 2011 from Mangrove Lake and South Pond during a series of benthic biotic transect surveys (Outerbridge, 2014). Hydrobiid snails (*Heleobops bermudensis*) were collected from 20 locations haphazardly chosen along two straight-line transects in Mangrove Lake (10 locations per transect, each approx. 400 m in length and perpendicular to each other (i.e. one north–south, the other east–west)) and also from 10 locations haphazardly chosen along one transect line in South Pond (approx. 260 m in length). Red-rimmed melania snails (*Melanoides tuberculata*) were also collected from the latter location. Collection consisted of sweeping a dip net with a 1 mm mesh and a square opening of 25 cm × 25 cm for a distance of 1 m and a depth of approx. 2.5 cm along the surface of the pond sediment. Collected sediment was passed through a 1 mm mesh sieve at the surface of the pond and remaining material transferred to a sterile 1 l plastic bottle for later inspection. Coffee bean snails (*Melampus coffeus*) were collected from 16 locations (no transects, but from within a 25 cm × 25 cm quadrat placed on the leaf litter) haphazardly chosen within the mangrove swamp community surrounding Mangrove Lake. Approximately 6 g of live hydrobiid snails were collected from these combined ponds, 6 g of live red-rimmed melania snails from South Pond and 18 g of live coffee bean marsh snails from Mangrove Lake. All snails were washed with distilled water. They were pooled together according to species, stored in sterile glass bottles (20 ml) and frozen at −18 °C until analyzed.

Eleven newly-laid whole diamondback terrapin eggs were collected from 11 different nests during nesting surveys in June and July 2011, within sand bunkers on the Mid Ocean golf course. This represented about 4.5% of annual egg-laying by the population (Outerbridge, 2014). All gastropods and eggs were frozen following collection. Collected samples were shipped to Fort Environmental Laboratories Inc. (Stillwater, Oklahoma, USA) for analytical analyses and necropsy. Total Petroleum Hydrocarbons (TPH), both diesel range (DRO) and gasoline range (GRO), were extracted and analyzed in duplicate accordance with SW-846 under EPA method 3510 (DRO extraction) and OK8000/81 and OK 8020/80, respectively using GC-MS and 1 g of each sample (USEPA, 2008). PAH analyses were also performed in duplicate in accordance with SW-846 (USEPA, 2008). One g of sample was extracted in duplicate in 100 mL of hexane using supercritical fluid extraction (SFE) in accordance with EPA method 3560 and analyzed by GC-MS in accordance with EPA method 8270 corrected for small volumes. Metal analyses were performed in duplicate in accordance with SW-846

Benzo(a)anthracene	Chrysene	Benzo(b)fluoranthene	Benzo(k)fluoranthene	Benzo(a)pyrene	Indeno(1,2,3-Cd)pyrene	Dibenz(a,h)anthracene	Benzo(g,h,i)perylene
2754	<48.8	<10	<10	<10	<10	2105	2456
<71	<48.8	<10	<10	<10	<10	<10	<10
1761	–	–	–	–	–	1397	2301
735	–	–	–	–	–	572	220
474	173	<10	<10	690	<10	1070	244
<70.4	<78.4	<10	<10	108	<10	348	<10
247	161	–	–	314	–	619	158
197	17	–	–	260	–	344	75
350	387	<10	<10	122	<10	219	<10
74	89	<10	<10	<10	<10	<10	<10
193	220	–	–	84	–	118	–
92	102	–	–	22	–	55	–

under EPA method 200.7 and corrected for small volumes (USEPA, 2008). One g of sample was digested in duplicate in HNO_3/HCl and analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES) (USEPA, 2008). Surrogates and internal standards used for TPH, PAH, and metals analyses were nonane and pentacosane (TPH); 4-bromofluorobenzene, 2,4,6-dibromophenol, 2-fluorobiphenyl, toluene d8, and terphenyl d14(PAHs); and Cu and Hg (metals). Standard recoveries for all analyses were between 80 and 120%. Quality Control (QC) results met laboratory specifications for each analysis reported.

Evaluation of PAH and metals data for potential hazard followed marine sediment guidelines adopted by the New Jersey Department of Environmental Protection (NJ DEP). The effects range low (ERL) represents a concentration at which adverse effects on benthos were found in 10% of cases reviewed, whereas the effects range median (ERM) represents a concentration at which adverse effects on benthos were found in more than 50% of cases examined. As the state of New Jersey does not have guideline values for TPH-GRO and TPH-DRO, the low effects level guidelines were values established by the state of Oklahoma (USA) for soil remediation at petroleum-contaminated sites and the severe effects level guidelines were values established by the state of California (USA) for soil screening. There were no comparable guidelines for safe amounts of TPHs, PAHs and metals in biological samples.

2.3. Embryo necropsy

Nests were inspected after hatchling emergence was completed and 25 unhatched eggs from 15 randomly-chosen nests were subsequently opened to determine whether they showed signs of development and potential abnormalities. Opened eggs were examined for yolk consistency, embryonic streaks, and embryonic development using a dissection microscope under 10× magnification. Eggs with congealed and frothy yolks were considered to be heat-stressed. Eggs with more significant embryonic development were carefully removed from the yolk and examined externally for stage of development and developmental abnormalities.

3. Results

3.1. Contaminant concentrations

Tables 1 and 2 summarise the concentrations of TPH, trace metal and PAH residues found in composite samples of pond sediment from Mangrove Lake, South Pond and Trott's Pond, aquatic gastropods collected from Mangrove Lake and South Pond, and freshly-laid terrapin eggs collected from the sand bunkers on the Mid Ocean golf course. Table 3 summarizes the benchmark values for trace metals and TPH established by the states of New Jersey, Oklahoma and California (USA). Table 4 summarizes the benchmark values for PAHs established by the state of New Jersey.

In general terms, the TPHs and trace metals were found in greater concentrations in the aquatic gastropods and terrapin eggs than in the pond sediment, whereas the greatest amounts of PAHs were mostly found within the sediment and lesser amounts were detected in the gastropods and eggs.

3.1.1. Sediment

Sediment from Mangrove Lake, South Pond and Trott's Pond was highly contaminated with a variety of compounds. Elevated concentrations of TPH-DRO and trace metals were detected in all three ponds (see Table 1). The mean composited value for TPH-DRO was close to, and the maximum value exceeded, the severe effects concentration; the mean composited values for As, Cd, Cu and Hg exceeded the low effects concentration range for marine sediment screening guidelines established by the NJ DEP. However, the maximum concentrations detected did not exceed the median effects range guidelines (Table 3). PAHs were also detected in all three ponds at concentrations that exceed the low effects and median effects ranges. Eight (50%) of the PAHs examined had mean values that exceeded the low effects range and six PAHs exceeded the median effects range for sediment quality guidelines (SQG) (compare Tables 2 and 4). Examination of the maximum PAH values shows that eight compounds, acenaphthylene, acenaphthene, phenanthrene, anthracene, pyrene, benzo(a)anthracene, benzo(g,h,i)perylene and dibenz(a,h)anthracene, were found to greatly exceed (≤8-fold) the median effects range (see Table 4).

3.1.2. Gastropods

The data show that the aquatic gastropods accumulated significant amounts of TPH-DRO and all metals except Fe. In some cases the mean values of metal residues found in the composite gastropod samples were 10- to 20-fold greater (e.g Pb, Cd and Zn), and Hg residues were 64-fold greater, than the mean values found in the composite sediment samples (Tables 1 and 2). Of the three different gastropod species examined, the coffee bean marsh snail (*Melampus coffeus*) was found to have the greatest concentrations of TPH and metals. A number of PAHs that were found at elevated concentrations in the benthic sediment were not detected in the gastropods, but mean fluorene, pyrene, chrysene and benzo(a)anthracene concentrations were detected at higher concentrations in the composite aquatic gastropod samples.

3.1.3. Diamondback terrapin eggs

The data show that mean concentrations of Cd, Cu, Pb, Zn and Hg plus TPH-DRO were significantly higher in terrapin eggs than in the benthic sediment, but lower than those detected in aquatic gastropods

Table 3
Benchmark values for metals (mg kg^{-1} dry weight values) established for marine and estuarine environments by the New Jersey Department of Environmental Protection and total petroleum hydrocarbons (mg kg^{-1} dw values) established for soil screening and remediation by the states of Oklahoma and California. Effects range low (ERL) and effects range median (ERM) are specific chemical concentrations that are derived from compiled biological toxicity assays and synoptic sampling of marine sediment.

	As	Cd	Cr	Cu	Pb	Ni	Zn	Hg	Gasoline-range petroleum hydrocarbons	Diesel-range petroleum hydrocarbons
Effects range low (ERL)	8.2	1.2	81	34	47	21	150	0.15	–	–
Effects range median (ERM)	70	9.6	370	270	218	52	410	0.71	–	–
Oklahoma (low effects)	–	–	–	–	–	–	–	–	50	50
California (severe effects)	–	–	–	–	–	–	–	–	110	110

(see Table 1). Four PAHs (fluorene, fluoranthene, chrysene and benzo(a)anthracene) were also detected in greater amounts in the composite egg samples than in the sediment (see Table 2), including fluoranthene and chrysene that demonstrated accumulation from sediment to gastropods to terrapin eggs.

3.2. Egg and embryo necropsies

Of the 25 eggs necropsied, ten showed no sign of development, may have been unfertilized and appeared to have been heat damaged. A further ten showed very limited (arrested) embryonic development (presence of primitive streak only), some appearing heat stressed based on the congealed consistency of the yolk. The remaining five eggs contained recognisable embryos. One was a pre-scute embryo with apparently normal development of eyes and forelimbs (hence also representing arrested development as its nest mates had developed far more and emerged). Another exhibited facial, eye, forelimb and shell malformations; it also had a protruding vertebral column. A third showed no signs of normal development, being grossly malformed and undersized. A fourth exhibited scute damage, while the final embryo was necrotic and had head and eye malformations.

4. Discussion

Although the study was limited by ethical and conservation constraints that prohibited the sacrifice of hatchling, juvenile or adult diamondback terrapins, the results obtained confirmed that the sediments of the pond environments inhabited by the terrapins of Bermuda were heavily contaminated by trace metals and hydrocarbons. These findings are consistent with previous assessments of Bermudian wetlands (Fort et al., 2006a, 2006b; Bacon, 2010; Bacon et al., 2012). The small benthic gastropods that inhabited the golf course ponds which serve as the main food source for the terrapins were also contaminated, showing accumulation of all trace metals (except Fe) and TPH-DRO. Similar accumulation has been repeatedly reported for freshwater and marine gastropods (e.g. Walsh et al., 1995). A wide range of PAHs were present in the gastropods' tissues. Although most of the specific PAHs were detected at lower concentrations than found in the sediments, marked accumulation of the potent carcinogen benzo(a)pyrene (Lee and Shim, 2007) was observed.

Terrapin eggs contained lower concentrations of all metals than did the gastropods. However, concentrations of Hg, Zn, Pb and Cd were all above those detected in the sediments. In contrast, concentrations of TPH-DRO in gastropods and terrapin eggs were similar, while PAH concentrations tended to be lower in eggs than either gastropods or sediments. Taken with earlier studies, these data suggested that concentrations of contaminants in the Bermudian eggs were remarkably high. Burger (2002) measured metal concentrations (wet weight specific) in terrapin eggs, liver and muscle in material collected from Tuckerton, New Jersey, a rural and coastal area distant from contaminant sources. Ricklefs (1977) reported that water comprised 68.9% of terrapin egg mass; Roosenburg and Dennis (2005) found water composition values ranging from 66.5% to 73.5% (mean 70.9%). The comparison of Bermudian and New Jersey studies (Table 5) was based on the assumption that 70% of wet egg mass is made up of water. The eggs of Bermudian diamondbacks were heavily contaminated by trace metals, with concentrations ×20 (Cr) to ×42,000 (Cd) greater than at the relatively pristine site of Tuckerton. Burger (2002) reported that egg metal concentrations were generally equal to or lower than maternal tissue concentrations, so the egg data provide strong indications that adult diamondbacks on Bermuda also have high concentrations of trace metals. Female slider turtles (*Trachemys scripta*) are reported to sequester some trace metals in their eggs as an excretion method to rid their bodies of chemicals (Burger and Gibbons, 1998) and the same may be true of diamondback terrapins, thus exposing their embryos to contaminants. It is also worth noting that eggs of green sea turtles (*Chelonia mydas*), collected for public health analysis, contained far lower concentrations of As (ca. 0.32 mg kg^{-1} dry weight (dw)), Cd (0.03 mg kg^{-1} dw) and Pb (0.10 mg kg^{-1} dw) (van de Merwe et al., 2009) than Bermudian diamondback eggs.

The high Cd concentrations recorded in sediments, gastropods and terrapin eggs are particularly disturbing, as this non-essential metal has been subject to world-wide emission control for decades and global environmental concentrations have declined for many years. Cd, which is an endocrine disrupter in fish (Vettilard and Bailhache, 2005), is carcinogenic and possibly mutagenic (Burger, 2008). The observed value of 38 mg kg^{-1} dw for terrapin eggs in Bermuda is about ×38 the maximum permitted concentration in human foodstuffs (Eisler, 1985).

Cr (in trivalent and especially hexavalent form) is known to be mutagenic, teratogenic, carcinogenic and an embryotoxin. Tissue concentrations in excess of 4 mg kg^{-1} dw indicate significant Cr contamination in a wide range of vertebrates (Eisler, 1986). The observed mean value of 28 mg kg^{-1} dw for terrapin eggs in Bermuda indicates that this metal is also present at possibly deleterious concentrations.

Anthropogenic As mainly enters the environment in the form of pesticides. Background As concentrations in living organisms are usually <3 mg kg^{-1} dw (calculated from Eisler, 1988), so the observed mean concentration of 28 mg kg^{-1} dw in terrapin eggs in Bermuda is nearly 10× background. However, these concentrations are lower than those often found in seafood (Eisler, 1988) and the effects of such

Table 4
Benchmark values for PAH (μg kg^{-1} dry weight values) established for marine and estuarine environments by the New Jersey Department of Environmental Protection. Effects range low (ERL) and effects range median (ERM) are specific chemical concentrations that are derived from compiled biological toxicity assays and synoptic sampling of marine sediment.

	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Benzo(a)anthracene
Effects range low ERL	160	44	16	19	240	85	600	665	261
Effects range median ERM	2,100	640	500	540	1,500	1,100	5,100	2,600	1,600

Table 5
Comparison of mean dry weight specific metal concentrations (mg kg^{-1} dw) recorded in diamondback terrapin eggs from Bermuda (this study) and Tuckerton, New Jersey (Burger, 2002). Only 5 metals were analyzed in both studies; those of Burger (2002) have been converted assuming 70% of egg mass is made up of water.

Site	As	Cd	Cr	Pb	Hg
New Jersey	0.04	0.0009	1.30	0.13	0.12
Bermuda	28	38	26	168	4.4

concentrations are unknown. As, which occurs in many forms, is generally elevated in marine organisms and often forms non-toxic complexes (Eisler, 1988).

Pb concentrations in pond sediments were below ERL and ERM concentrations and therefore not dissimilar to those of coastal estuarine sediments, but Hg concentrations were intermediate between ERL and ERM; both were accumulated by the gastropods and diamondback eggs. Hg is teratogenic, mutagenic and carcinogenic, and is known to cause embryocidal, cytochemical and histopathological effects in wildlife (Eisler, 1987), but there have been few toxicological studies of Hg in reptiles. The mean Hg concentration in the diamondback terrapin eggs from Bermuda (4.37 mg kg^{-1} dw) was significantly higher than that reported by Burger and Gibbons (1998) for the slider turtle (*Trachemys scripta*) from the Savannah River Site, South Carolina (0.04 mg kg^{-1} dw) as well as that reported by Burger (2002) for diamondback terrapins from New Jersey (0.12 mg kg^{-1} dw). The biological transformation of Hg to the highly toxic methylmercury form and its subsequent accumulation in food chains is a threat to many species, especially those inhabiting aquatic environments (USEPA, 1997).

Cu compounds (e.g. $CuSO_4$) are widely used as biocides to control nuisance algae and macrophytes (Bartley, 1967; Havens, 1994) and can concentrate in soil, water, and sediments after prolonged periods of application. Among marine organisms, the highest accumulations of copper are generally found in molluscan tissues rather than vertebrate tissues (Eisler, 1979, 1981). Diet appears to be the most important route of Cu accumulation in aquatic animals; however, data are scarce on Cu concentrations in field populations of amphibians and reptiles. Crocodile eggs may contain 60 mg kg^{-1} dw and livers of some toads may contain as much as 2100 mg kg^{-1} dw without apparent adverse effects (see Eisler, 1998); therefore, the amount of Cu detected in Bermuda's diamondback terrapin eggs (90.2 mg kg^{-1} dw) may be within tolerable limits.

No previous measurements of TPH-DRO appear to have been conducted on diamondback terrapin eggs and interpretation of the data is difficult because of the high lipid content (26–30% of egg dw; Ricklefs, 1977; Roosenburg and Dennis, 2005; this study). Despite this limitation, it is clear that terrapins accumulate TPH-DRO and transfer them to their eggs. In addition, the sedimentary TPH concentrations far exceed those already known to cause high concentrations of malformations in amphibians (Fort and McLaughlin, 2003).

There are directly comparable data in the case of PAHs. Holliday et al. (2008) measured the PAH content of terrapin eggs collected from various shores around Swanson's Creek, Maryland one year after a serious spill of crude and fuel oil. Data for the cleanest and most contaminated sites are shown in comparison with Bermudian data in Table 6. Eggs from Bermudian diamondback terrapins feature similar PAH concentrations to those collected from the most contaminated Maryland sites and are around ×12 the concentrations of (relatively) uncontaminated eggs.

Table 6
Comparison of PAH concentrations (μg kg^{-1} dry weight) recorded in diamondback terrapin eggs from Bermuda (this study) and eggs collected from a Maryland creek subject to an oil spill one year earlier (Holliday et al., 2008).

PAH	Bermuda	Maryland	
		A	B
Naphthalene	BDL	BDL	107
Acenaphthylene	BDL	BDL	47
Acenaphthene	136	BDL	52
Fluorene	88	BDL	BDL
Phenanthrene	171	BDL	BDL
Anthracene	155	BDL	68
Fluoranthene	207	BDL	433
Pyrene	403	BDL	88
Benzo(a)anthracene	193	BDL	61
Chrysene	220	BDL	BDL
Benzo(b)fluoranthene	BDL	BDL	BDL
Benzo(k)fluoranthene	BDL	BDL	82
Benzo(a)pyrene	84	117	96
Perlyene	NM	26	113
Indeno(1,2,3-Cd)pyrene	BDL	BDL	402
Dibenz(a,h)anthracene	118	BDL	BDL
Benzo(g,h,i)perylene	BDL	BDL	140
TOTAL	1775	143	1689

A = 'Clean site' (Golden Beach), B = 'Contaminated site' (Sheridan Point), BDL = below detection limit, NM = not measured.

Van Meter et al. (2006) reported that exposure to crude oil and PAHs (particularly benzo[a]pyrene and 7,12-dimethylbenz-[a]anthracene) had a detrimental effect on the survival and development of common snapping turtle (*Chelydra serpentina*) embryos. Low hatching success and high deformity rates were reported from eggs collected from the John Heinz National Wildlife Refuge in Pennsylvania (a contaminated wetland). Bermuda's diamondback terrapin population has been characterized as having a very low annual hatching success rate (19%) despite an absence of nest depredation. Furthermore, this population is composed of individuals affected by a moderate level of minor deformities (e.g. misshapen carapace or plastron, extra scutes, misshapen scutes and deformed digits) (Outerbridge, 2014). Such deformities have been attributed to embryological exposure to high incubation temperatures (Wood and Herlands, 1997; Herlands et al., 2004) as well as petroleum crude oil and PAHs (Van Meter et al., 2006). One potential route of egg PAH exposure is via incubation in contaminated beach sands; another route of exposure is maternal transfer of lipophilic hydrocarbons (Nagle et al., 2001). The sources of the PAHs found within Bermuda's wetlands are currently being investigated.

The egg necropsy data, although limited because of small sample size (n = 25), suggest that the high observed contaminant concentrations of eggs may significantly compromise embryonic development in this threatened terrapin population. Fertilized eggs generally contained arrested primitive streaks, while embryos that developed further were grossly malformed. Identified heat stress effects (on structure and composition of yolk) probably followed embryo death. The source of contaminants at this early stage of development was presumably far more from maternal transfer than from the environment.

Chrysene	Benzo(k)fluoranthene	Benzo(a)pyrene	Indeno(1,2,3-Cd)pyrene	Dibenz(a,h)anthracene	Benzo(g,h,i)perylene
384	240	430	200	63	170
2,800	1,340	1,600	320	260	320

Overall, it is evident that the Bermudian diamondback terrapins live and feed in wetland habitats characterized by multifactorial contamination that includes their main food sources. While their own tissue contaminant concentrations are unknown, it is evident that contaminants are transferred to eggs, and that the concentrations of several of these exceed those known to cause damage. Some of the contaminants are known to have mutagenic and teratogenic effects at the observed concentrations, so may have reduced the incidence of successful embryonic development.

5. Concluding comments

This study suggests that environmental contamination may cause the low hatching success shown by diamondback terrapin eggs on Bermuda. Ideally, attempts should be made to store any hatchling, juvenile and adult material that results from mortalities, with a view to determining tissue contaminant concentrations. Similarly, it would be desirable to extend studies to measurements of persistent organic pollutants (POPS) in both benthic gastropods and terrapin eggs. However, from a conservation and precautionary perspective the existing data already indicate that a program of wetland remediation is urgently needed. Much of the observed contamination may be historical, but all efforts to reduce present and future contamination of the study ponds should be made.

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