



Harmful algal and cyanobacterial toxins in foraging green turtles (*Chelonia mydas*) in Florida's Big Bend

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A B S T R A C T

Numerous toxin-producing harmful algal (HAB) species occur in Florida's coastal waters. Exposure to these toxins has been shown to have sublethal effects in sea turtles. The objective of this study was to establish concentrations of 10 HAB toxins in plasma samples from green turtles (*Chelonia mydas*) foraging in Florida's Big Bend. Domoic acid, lyngbyatoxin-A, microcystins, nodularin, and okadaic acid were detected, demonstrating exposure to these HAB toxins, which are also a public health concern.

Harmful algal and/or cyanobacterial species have a global distribution, are found in all aquatic environments (e.g., marine, brackish, freshwater) (Abbott et al., 2009), and can pose significant threats to wildlife species (Landsberg, 2002). When conditions are favorable, these species proliferate and present at high concentrations (Abbott et al., 2009) in phenomena known as harmful algal blooms, or HABs, which appear to be increasing in frequency, duration, and range due to agriculture runoff, overfishing, and climate change (Glibert et al., 2005; Hallegraeff, 2010; Anderson et al., 2012). Toxins released from the single-celled organisms that cause these blooms can negatively impact wildlife health and survival (Landsberg, 2002; Fire and Van Dolah, 2012). Exposure to these toxins can result in strandings (dead or alive) of endangered species such as sea turtles, in addition to sublethal impairments including alterations in blood biochemistry, immunity, and neurologic function, thereby impacting their health (Arthur et al., 2006; Walsh et al., 2010, 2019; Perrault et al., 2014, 2016, 2017; Cocilova and Milton, 2016; Foley et al., 2019). This is of particular concern in Florida's coastal waters, where >70 freshwater, estuarine, and marine harmful algal species have been documented, and five species of threatened or endangered sea turtles (leatherbacks, *Dermochelys coriacea*; loggerheads, *Caretta caretta*; green turtles, *Chelonia mydas*; Kemp's ridleys, *Lepidochelys kempii*; hawksbills, *Eretmochelys imbricata*) inhabit coastal waters year-round (Foley et al., 2019). As many of Florida's turtle species inhabit both marine and estuarine environments (Ehrhart, 1983), there is the potential for exposure to numerous algal toxins.

Documented HAB species in waters of Florida's east and west coasts include *Karenia brevis* (associated toxin(s): brevetoxins), *Pseudo-nitzschia* spp. (domoic acid), *Lyngbya* spp. (lyngbyatoxins), *Microcystis* spp. (microcystins), *Prorocentrum* spp. (okadaic acid), and *Pyrodinium* spp. (saxitoxins) (Steidinger, 1993; Holloway-Adkins, 2001; Landsberg, 2002; Jacobson et al., 2006; Geselbracht, 2007; Paul et al., 2008; Philips et al., 2015; WHOI, 2016; Foley et al., 2019, Table 1). Sea turtles and other marine fauna become exposed to HAB toxins primarily through ingestion of contaminated prey (Flewelling et al., 2005; Manire et al., 2017). Immature green turtles are omnivorous and thus can secondarily ingest these toxins through the consumption of (1) primary consumers that have accumulated toxins, and/or (2) benthic algae and seagrasses and their associated epibiota (Bjorndal, 1997; Landsberg et al., 1999; Holloway-Adkins, 2001; Williams et al., 2014). Toxin exposure has been documented long after a bloom has dissipated, indicating persistence in both the environment and within organismal tissues (Flewelling et al., 2005; Capper et al., 2013; Fauquier et al., 2013; Perrault et al., 2014, 2016). With the exception of brevetoxins, studies investigating the prevalence and impacts of HAB toxins on sea turtles are few; without directed monitoring of toxin concentrations, our understanding of exposure dynamics will remain limited. To address this knowledge gap, the objective of this study was to establish concentrations of HAB toxins in plasma samples collected from green turtles captured in Florida's Big Bend during a non-bloom period.

The sampling site for this study was located in and adjacent to the

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shallow seagrass (e.g., *Halodule wrightii*, *Siringodium filidorme*, *Thalassia testudinum*, etc.) habitat of the St. Martins Marsh Aquatic Preserve. This relatively pristine area serves as an important foraging habitat for five sea turtle species in addition to containing the second largest expanse of seagrass habitat in the eastern Gulf of Mexico (Hale et al., 2004). In the Big Bend region, land coverage is primarily natural, much of the coastline and inland areas are under conservation protection, and human population densities are among the lowest in the state (Main and Allen, 2007; Seavey et al., 2011). Despite these factors, loss of oyster beds and seagrasses and a high estimated prevalence rate of fibropapillomatosis (FP; a tumor disease linked to chelonid herpesvirus 5 infection and degraded habitats; Herbst and Klein, 1995; Herbst et al., 1995; Lackovich et al., 1999; Van Houtan et al., 2010) in green turtles still occur in this area (Hale et al., 2004; Seavey et al., 2011). Even though this region has a perceived “pristine” ecosystem status, HABs are still considered a “very high-rated threat” to Big Bend coastal waters (Geselbracht, 2007).

Juvenile green turtles were captured using dip nets and sampled in Big Bend (28.8324°N, -82.7596°W) from 18 to 23 June 2015. Upon capture, blood (up to 10 mL, depending on the turtles’ size) was sampled from the external jugular vein using a lithium heparin Vacutainer® (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) blood collection system fitted with a 22-gauge, 1-inch needle. The

venipuncture site was swabbed with alternating applications of betadine and 70% isopropyl alcohol before and after blood collection. The blood samples were immediately chilled on ice until return to land, whereupon whole blood was centrifuged at 1318 g (3400 rpm) for 8 min for plasma separation and harvest. Plasma was stored frozen at -80 °C until analysis.

Turtles were weighed and minimum straight carapace length (SCL_{min}) was measured. Additionally, we visually assessed and recorded the presence, number, and size of FP tumors to assign a tumor severity score of 0–3 (Work and Balazs, 1999). After sampling, turtles were tagged with flipper (metal Inconel; National Band and Tag Co., Newport, Kentucky, USA) and passive integrated transponder (PIT; Biomark®, Inc., Boise, Idaho, USA) tags for identification. Turtles were released into the water at their capture site.

Plasma samples were shipped overnight on dry ice to the Center for Environmental Sciences and Engineering at the University of Connecticut, a lab certified by the Connecticut Department of Public Health for the analysis of organic compounds in biological tissues. Plasma samples (approximately 1 g) were homogenized and split into 2 aliquots of an equal mass to separately prepare for lipophilic and hydrophilic toxin analyses. The lipophilic fraction was suspended in 1 mL methanol (0.1% formic acid), sonicated in the dark for 30 min, and vortexed for an additional 30 min. The resulting extract was centrifuged at 5443 g for

Table 1

Summary of harmful algal toxins, their causative organisms with a Florida distribution, habitats, mechanisms of action, hydrophilic or lipophilic characteristics, and associated poisoning names. Data below are summarized from Landsberg (2002) and Abbott et al. (2009).

Toxin or toxin class	Causative organism(s)	Habitat(s)	Primary mechanism(s) of action (associated illness)	Hydrophilic or lipophilic	Associated sea turtle studies
Anatoxins	<i>Anabaena circinalis</i> <i>Anabaena flos-aquae</i> <i>Anabaena planctonica</i> <i>Aphanizomenon flos-aquae</i> <i>Cylindrospermum</i> sp. <i>Microcystis</i> spp. <i>Oscillatoria</i> spp. <i>Phormidium</i> spp. <i>Planktothrix</i> spp.	Freshwater estuarine	Neurotoxic	Hydrophilic	25
Brevetoxins	<i>Karenia brevis</i>	Estuarine, marine	Neurotoxic (NSP: neurotoxic shellfish poisoning); respiratory irritant	Lipophilic	4, 9, 11, 12, 15, 18, 19, 21, 23–25
Cylindrospermopsin	<i>Anabaena bergii</i> <i>Aphanizomenon flos-aquae</i> <i>Aphanizomenon ovalisporum</i> <i>Cylindrospermopsis raciborskii</i>	Freshwater, estuarine	Hepatotoxic	Hydrophilic	25
Domoic acid	<i>Pseudo-nitzschia</i> spp.	Estuarine, marine	Neurotoxic (ASP: amnesic shellfish poisoning)	Hydrophilic	4, 10, 13, 16, 17, 25
Lyngbyatoxins	<i>Lyngbya</i> spp.	Freshwater, estuarine, marine	Dermatotoxic	Lipophilic	1, 3, 5, 6, 11, 25
Microcystins	<i>Anabaena</i> spp. <i>Microcystis</i> spp. <i>Nostoc</i> spp. <i>Oscillatoria</i> spp. <i>Planktothrix</i> spp.	Freshwater, estuarine, marine	Hepatotoxic; tumor promoter	Lipophilic	11, 25
Nodularins	<i>Nodularia spumigena</i>	Estuarine	Hepatotoxic	Lipophilic	25
Okadaic acid	<i>Dinophysis</i> spp. <i>Prorocentrum</i> spp.	Freshwater, estuarine	Gastrotoxic (DSP: diarrhetic shellfish poisoning)	Lipophilic	2, 4, 8, 11, 25
Saxitoxins/ Neosaxitoxins	<i>Alexandrium minutum</i> <i>An. circinalis</i> <i>Ap. flos-aquae</i> <i>Cylindrospermopsis raciborskii</i> <i>Gymnodinium</i> spp. <i>Lyngbya</i> spp. <i>Planktothrix</i> spp. <i>Pyrodinium bahamense</i>	Freshwater, estuarine	Neurotoxic (PSP: paralytic shellfish poisoning)	Hydrophilic	4, 7, 11, 14, 16–18, 20, 22, 25

References: 1, Yasumoto, 1998; 2, Anderson et al. (2001); 3, Arthur et al. (2006); 4, Jacobson et al., (2006); 5, Arthur et al. (2008a); 6, Arthur et al. (2008b); 7, Licea-Duran et al. (2006); 8, Takahashi et al. (2008); 9, Walsh et al. (2010); 10, Harris et al. (2011); 11, Capper et al. (2013); 12, Fauquier et al. (2013); 13, Harris et al. (2013); 14, Amaya et al. (2014); 15, Perrault et al. (2014); 16, Manire et al. (2015); 17, Manire et al. (2016); 18, Reckendorf et al. (2016); 19, Perrault et al. (2016); 20, Trejo et al., 2016; 21, Perrault et al. (2017); 22, Amaya et al. (2018); 23, Walker et al. (2018); 24, Foley et al. (2019); 25, this study.

10 min and 190 μ L supernatant transferred to a chromatography vial and internal standard was added. The hydrophilic fraction was extracted in 1 mL of methanol:water (50:50), vortexed for 30 min, followed by centrifugation ($5443\text{ g} \times 10\text{ min}$), and 190 μ L of the supernatant transferred to a liquid chromatography vial. The hydrophilic and the lipophilic extracts were characterized for HAB toxins separately using a Waters, Inc. (Milford, Massachusetts, USA) Acquity ultra-performance liquid chromatograph/tandem mass spectrometer (UPLC-MS/MS) (Yang et al., 2017; Rodríguez et al., 2018). The UPLC was equipped with an HSS T3 column (2.1 mm \times 100 mm, particle size 1.8 μ m; Waters, Inc.), with water (0.1% formic acid) and acetonitrile (0.1% formic acid) used as mobile phases for separation of the HAB toxins (Provatas et al., 2014).

Standard quality assurance procedures were employed including the analysis of surrogate, laboratory controls, method blanks, duplicates, matrix spikes, and matrix spike duplicate samples. All quality control results were within the acceptance criteria. Duplicate and matrix spike duplicate recoveries were within 20% relative percent difference from each other. Laboratory control and matrix spike recoveries were within $\pm 25\%$ of the anticipated value. Instrument response was evaluated initially and after every 10 samples using calibration verification standards and a blank. All calibration verifications were within the acceptance criterion of 85–115% recovery, and all method blank values were below the reporting limit.

Green turtles sampled for this study were all determined to be juveniles (Bjorndal et al., 2000) based on their minimum straight carapace length (mean \pm SE: 36.0 \pm 1.0 cm; range: 27.2–43.4 cm) and mass (mean \pm SE: 6.6 \pm 0.5 kg; range: 2.8–11.3 kg). All turtles appeared to be in good body condition based on visual assessment/subjective body condition estimation using plastron shape/concavity and observation of neck and limb base thickness. Additionally, 18/21 (85.7%) turtles had barnacles on the skin, carapace, and/or plastron; 17/21 (81.0%) had external evidence of FP; 4/21 (19.1%) had flipper damage; 4/21 (19.1%) had leeches on the skin, mouth, and/or cloaca; 3/21 (14.3%) had leech cocoons on the skin and/or plastron; and 2/21 (9.5%) had carapace damage.

To the authors' knowledge, this is the first report of toxin concentrations in sea turtles residing in Florida's Big Bend, and represents the

most comprehensive toxin analysis of sea turtles to date with regards to the number of toxins/toxin classes analyzed (Table 1). Overall, 13/21 (61.9%) green turtles tested positive for at least one toxin, with 4/21 (19.1%) testing positive for two toxins (Table 2). 8/21 (38.1%) turtles had plasma with all toxins below the detection limits. The most frequently detected toxin was domoic acid (6/21 turtles, 28.6%), followed by lyngbyatoxin-A, microcystin-LR, microcystin-RR, nodularin, and okadaic acid (2/21 turtles, 9.5% each), and microcystin-LA detected in 1/21 turtles (4.8%). Anatoxin-A, brevetoxin-B, brevetoxin-3, cylindrospermopsin, microcystin-YR, neosaxitoxin, and saxitoxin were not detected in any samples (Table 2). Due to low sample size, in-depth statistical tests could not be conducted; however, using SPSS (v. 25, SPSS, Inc. Chicago, Illinois, USA), a Fisher's exact test revealed that turtles with external evidence of FP were not more likely ($P = 0.617$) to test positive for at least one plasma toxin (11/17 turtles, 64.7%) than turtles that did not have external FP (2/4 turtles, 50%).

There are >5000 described marine phytoplankton species, with ~ 80 known to produce toxins (Hallegraeff, 2014); at least 50 marine and 20 freshwater algal/cyanobacterial species are found in Florida (Abbott et al., 2009). Ingestion of seagrasses, macroalgae, and benthic dinoflagellates and cyanobacteria that accumulate and/or produce these toxins is the most plausible route of exposure in the green turtles from this study (Landsberg et al., 1999; Holloway-Adkins, 2001; Flewelling et al., 2005; Capper et al., 2013). Cyanobacterial and red tide blooms have occurred in Florida's Big Bend in previous years (Geselbracht, 2007; Hu et al., 2015; Perrault et al., 2017; FFWCC, 2019); however, no active HABs were reported from this region during June 2015 when sampling occurred, aside from *Pseudo-nitzschia* detected in a few samples at low concentrations (FFWCC-FWRI HAB Monitoring Database). It is worth noting that the majority of phytoplankton abundance data is collected in response to HABs and sampling efforts vary with funding availability. Additionally, there is a relatively small amount of available HAB data for Florida's Big Bend in comparison to other areas of the state where HABs are more common (FFWCC-FWRI HAB Monitoring Database). Despite this apparent lack of preceding HAB(s), more than half of the green turtles from this study tested positive for at least one algal or cyanobacterial toxin. Brevetoxins can remain in the plasma of loggerhead sea turtles for ≤ 80 days post-exposure (Fauquier et al., 2013);

Table 2

Concentrations (in ng/g wet weight) of harmful algal/cyanobacterial toxins detected in plasma of juvenile green turtles from Florida's Big Bend. Samples with a positive result are bolded. Anatoxin-A (<1.7), brevetoxin-B (<75), brevetoxin-3 (<75), cylindrospermopsin (<3), microcystin-YR (<15), neosaxitoxin (<18.8), and saxitoxin (<30) were below the limits of detection (included parenthetically next to the toxin name) for all samples.

Turtle ID	Domoic acid	Lyngbyatoxin-A	Microcystin-LA	Microcystin-LR	Microcystin-RR	Nodularin	Okadaic acid
LLA459	<5	<0.2	<7.5	<15	<1.5	<10	<1.5
LLA471	<5	<0.2	<7.5	<15	<1.5	<10	9.1
LLA488	12.5	<0.2	<7.5	<15	<1.5	<10	<1.5
LLA480	<5	<0.2	<7.5	<15	<1.5	<10	<1.5
LLA490	22.4	<0.2	<7.5	<15	<1.5	19.9	<1.5
LLA495	<5	<0.2	<7.5	<15	<1.5	<10	26.5
LLA496	<5	0.40	<7.5	<15	<1.5	<10	<1.5
LLA499	16.9	<0.2	<7.5	<15	<1.5	31.7	<1.5
LLH804	<5	<0.2	<7.5	<15	<1.5	<10	<1.5
LLH806	<5	<0.2	<7.5	63.2	22.5	<10	<1.5
LLH810	<5	<0.2	<7.5	<15	<1.5	<10	<1.5
LLH817	<5	<0.2	<7.5	<15	<1.5	<10	<1.5
LLH821	<5	<0.2	<7.5	<15	<1.5	<10	<1.5
LLH823	24.0	<0.2	<7.5	<15	<1.5	<10	<1.5
LLH825	<5	<0.2	<7.5	<15	20.2	<10	<1.5
LLH826	<5	<0.2	<7.5	51.6	<1.5	<10	<1.5
LLH828	41.7	0.64	<7.5	<15	<1.5	<10	<1.5
LLH830	<5	<0.2	<7.5	<15	<1.5	<10	<1.5
LLH837	<5	<0.2	<7.5	<15	<1.5	<10	<1.5
LLH841	50.6	<0.2	<7.5	<15	<1.5	<10	<1.5
LLH843	<5	<0.2	22.8	<15	<1.5	<10	<1.5
Median	<5	<0.2	<7.5	<15	<1.5	<10	<1.5
Range	<5–50.6	<0.2–0.64	<7.5–22.8	<15–63.2	<1.5–22.5	<10–21.7	<1.5–26.5
# positives	6/21 (28.6%)	2/21 (9.5%)	1/21 (4.8%)	2/21 (9.5%)	2/21 (9.5%)	2/21 (9.5%)	2/21 (9.5%)
# positives with FP	6 (100%)	2 (100%)	1 (100%)	2 (100%)	1 (50%)	2 (100%)	1 (50%)

however, in laboratory and domestic animals experimentally dosed with algal toxins, plasma clearance rates appear to be much faster (i.e., less than 2 h to one week) for brevetoxin-3, domoic acid, microcystin-LR, and saxitoxin (Robinson et al., 1991; Andrinolo et al., 1999; Benson et al., 1999; Fuquay et al., 2012). Additionally, brevetoxin-3 was absent in plasma of red-eared sliders (*Trachemys scripta*) and diamondback terrapins (*Malaclemys terrapin*) one week after seven doses administered orally over 14 days (Cocilova et al., 2017). Because no known blooms occurred before or during the sampling period aside from the presence of some *Pseudo-nitzschii* spp. and because of known rapid clearance rates in laboratory-exposed organisms, we can deduce that toxin detection described here indicates that numerous harmful algal and cyanobacterial species (e.g., *Dinophysis* spp., *Lyngbya* spp., *Microcystis* spp., *Nodularia* sp., *Prorocentrum* spp., *Pseudo-nitzschia* spp.) are typically present in the Gulf waters of Florida's Big Bend (Abbott et al., 2009) and that exposure to these toxins can likely occur in the absence of a large-scale bloom (Perrault et al., 2014, 2016). It is also possible that some local blooms go undetected (Rines et al., 2002; McManus et al., 2008). Brevetoxins from *K. brevis* were previously detected in green and Kemp's ridley turtles from Florida's Big Bend (Perrault et al., 2017), but those turtles were sampled immediately after a red tide bloom and occurrence of these blooms in Big Bend is relatively rare (Hu et al., 2015; FWC, 2019; Foley et al., 2019). The relatively high detection limits for brevetoxins (75 ng/g) and saxitoxin (30 ng/g) in this study likely impeded detection of biologically relevant toxin concentrations. This is particularly true for detection during non-bloom periods, since previously reported brevetoxin and saxitoxin concentrations in plasma and other tissues of naturally exposed sea turtles in Florida are typically below these levels (Walsh et al., 2010; Capper et al., 2013; Fauquier et al., 2013).

Harmful algal toxins have been previously documented in the diet and tissues of sea turtles (see Table 1 for a complete list of those studies and analyzed toxins) from Madagascar (Yasumoto, 1998), Australia (Arthur et al., 2006, 2008a; 2008b; Takahashi et al., 2008), El Salvador, Guatemala, Mexico (Amaya et al., 2014, 2018; Licea-Duran et al., 2006; Trejo et al., 2016), Florida USA (Anderson et al., 2001; Jacobson et al., 2006; Walsh et al., 2010; Capper et al., 2013; Fauquier et al., 2013; Perrault et al., 2014, 2016; 2017; Manire et al., 2015), Hawaii USA (Anderson et al., 2001), California USA (Harris et al., 2011, 2013), Massachusetts USA (Reckendorf et al., 2016), and Texas USA (Walker et al., 2018). Concentrations of saxitoxins and brevetoxins have been reported in sea turtle plasma (Manire et al., 2016, 2017; Perrault et al., 2014, 2016, 2017; Trejo et al., 2016; Walsh et al., 2010); however, the concentrations of these toxins reported here fell below detection limits for all samples analyzed, preventing comparisons.

The positive results seen here are concerning, especially in the absence of a temporally related, detected HAB, as several toxins (e.g., anatoxins, lyngbyatoxins, microcystins, nodularins, okadaic acid) have been implicated as tumor promoters in laboratory cell lines and mammalian models and/or are of public health concern (Landsberg, 2002; Humpage, 2008). Toxin exposure has been hypothesized to be a co-factor in FP tumor development in green turtles, since harmful algal and cyanobacterial species tend to occur in regions where FP prevalence rates are high (Landsberg et al., 1999; Anderson et al., 2001; Arthur et al., 2008a; Perrault et al., 2017). Here, we did not find evidence that turtles with FP tumors were more likely to test positive for toxin exposure. Identifying causal relationships between toxin exposure and FP development is problematic due to various unknown factors such as virus exposure timing and duration, and the possibility that biological factors or synergistic exposure to multiple toxins may potentiate or mask any observable effects (Herbst and Klein, 1995).

Numerous harmful algal species regularly occur in Florida's coastal waters; therefore, co-occurrence of these HAB organisms and subsequent exposure to their associated toxins is possible, and in some areas is likely, for sea turtles that reside there (Kubanek et al., 2005; Paerl et al., 2008; Fire et al., 2011; Twiner et al., 2011; Capper et al., 2013). Out of

the published studies to date that have documented toxin exposure in sea turtles (Table 1), only five (20.8%) examined more than one toxin/class of toxins (e.g., brevetoxins) with regards to cause of death or stranding (Jacobson et al., 2006; Capper et al., 2013; Manire et al., 2015, 2016; Reckendorf et al., 2016). We can expect HAB toxin exposure and subsequent sea turtle strandings in Florida to increase in future years as HAB-related impacts to public health, tourism, fisheries, organismal health, and ecosystem functioning have increased in the last several decades and are expected to continue rising (Anderson et al., 2012). Therefore, it is imperative to gain a better understanding of toxin concentrations in sea turtles from Florida's Big Bend, a region vulnerable to the effects of anthropogenic disturbances including nutrient enrichment, increased agricultural use, and surface drainage (Frazer et al., 2001; Seavey et al., 2011; USFWS, 2013). Ongoing and future research by the authors of this study will explore relationships between toxin exposure and specific health biomarkers in sea turtles, which may point to toxin-related disruption of key physiological systems and elucidate any effects that exist between toxin exposure and disease risk or severity (Capper et al., 2013). Effective conservation measures must take species health into consideration, because a population cannot be sustainable if it consists primarily of unhealthy individuals (Karesh and Cook, 1995). Because sea turtles are sentinels of ecosystem health (Aguirre and Lutz, 2004), more research is needed to determine the prevalence of HAB species, HAB-associated toxins, and their impacts on sea turtle health.

Author contributions

Conceptualization: JRP, MJA, APK, Methodology: JRP, CRP, MJA, MJB, CRM, APK, Validation: CRP, Formal Analysis: JRP, CRP, Investigation: JRP, CRP, MJA, MJB, CRM, APK, Resources: JRP, CRP, MJA, MJB, CRM, APK, Data Curation: JRP, CRP, Writing – Original Draft Preparation: JRP, CRP, APK, Writing – Review & Editing: JRP, CRP, MJA, MJB, CRM, APK, Project Administration: MJA, APK, Funding Acquisition: JRP, MJA, MJB, CRM, APK.

Ethics statement

All sampling was conducted under Florida Fish and Wildlife Conservation Commission Marine Turtle Permit #125 and National Marine Fisheries Service Permit #16598.

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References

- Abbott, G.M., Landsberg, J.H., Reich, A.R., Steidinger, K.A., Ketchen, S., Blackmore, C., 2009. Resource Guide for Public Health Response to Harmful Algal Blooms in Florida. Fish and Wildlife. Research Institute Technical Report TR-14. viii +, p. 132.
- Aguirre, A.A., Lutz, P.L., 2004. Marine turtles as sentinels of ecosystem health: is fibropapillomatosis an indicator? *EcoHealth* 1, 275–283.
- Amaya, O., Quintanilla, R., Stacy, B.A., Bottein, M.-Y.D., Flewelling, L., Hardy, R., Dueñas, C., Ruiz, G., 2018. Large-scale sea turtle mortality events in El Salvador attributed to paralytic shellfish toxin-producing algae blooms. *Front. Mar. Sci.* 5, 411.
- Amaya, O., Ruiz, G., Espinoza, J., Rivera, W., 2014. Saxitoxin analyses with a receptor binding assay (RBA) suggest PSP intoxication of sea turtles in El Salvador. *Harmful Algae News* 48, 6–7.
- Anderson, D.M., Cembella, A.D., Hallegraef, G.M., 2012. Progress in understanding harmful algal blooms: paradigm shifts and new technologies for research, monitoring, and management. *Annu. Rev. Mar. Sci.* 4, 143–176.
- Anderson, Y., Landsberg, J., Balazs, G., Carthy, R., 2001. The relationship between the potential tumor-promoting dinoflagellates *Prorocentrum* spp. and green turtle fibropapillomatosis: preliminary results of a comparison between Hawaii and

- Florida. In: Coyne, M.S., Clark, R.D. (Eds.), Proceedings of the Twenty-First Annual Symposium on Sea Turtle Biology and Conservation, vol. 528. NOAA Technical Memorandum NMFS-SEFSC.
- Andrinolo, D., Michea, L.F., Lagos, N., 1999. Toxic effects, pharmacokinetics and clearance of saxitoxin, a component of paralytic shellfish poison (PSP), in cats. *Toxicol. X* 37, 447–464.
- Arthur, K., Limpus, C., Balazs, G., Capper, A., Udy, J., Shaw, G., Keuper-Bennett, U., Bennet, P., 2008. The exposure of green turtles (*Chelonia mydas*) to tumour promoting compounds produced by the cyanobacterium *Lyngbya majuscula* and their potential role in the aetiology of fibropapillomatosis. *Harmful Algae* 7, 114–124.
- Arthur, K.E., Limpus, C.J., Whittier, J.M., 2008. Baseline blood biochemistry of Australian green turtle (*Chelonia mydas*) and effects of exposure to the toxic cyanobacterium *Lyngbya majuscula*. *Aust. J. Zool.* 56, 23–32.
- Arthur, K.E., Limpus, C.J., Roelfsema, C.M., Udy, J.W., Shaw, G.R., 2006. A bloom of *Lyngbya majuscula* in Shoalwater Bay, Queensland, Australia: an important feeding ground for the green turtle (*Chelonia mydas*). *Harmful Algae* 5, 251–265.
- Benson, J.M., Tischler, D.L., Baden, D.G., 1999. Uptake, tissue distribution, and excretion of brevetoxin. 3 administered to rates by intratracheal instillation. *J. Toxicol. Environ. Health* 56, 345–355.
- Bjorndal, K., 1997. Foraging ecology and nutrition of sea turtles. In: Lutz, P.L., Musick, J. A. (Eds.), *The Biology of Sea Turtles*. CRC Press, Boca Raton, pp. 199–232.
- Bjorndal, K.A., Bolten, A.B., Chaloupka, M.Y., 2000. Green turtle somatic growth model: evidence for density dependence. *Ecol. Appl.* 10, 269–282.
- Capper, A., Flewelling, L.J., Arthur, K., 2013. Dietary exposure to harmful algal bloom (HAB) toxins in the endangered manatee (*Trichechus manatus latirostris*) and green sea turtle (*Chelonia mydas*) in Florida, USA. *Harmful Algae* 28, 1–9.
- Cocilova, C.C., Flewelling, L.J., Bossart, G.D., Granholm, A.A., Milton, S.L., 2017. Tissue uptake, distribution and excretion of brevetoxin-3 after oraland intratracheal exposure in the freshwater turtle *Trachemys scripta* and the diamondback terrapin *Malaclemys terrapin*. *Aquat. Toxicol.* 187, 29–37.
- Cocilova, C.C., Milton, S.L., 2016. Characterization of brevetoxin (PbTx-3) exposure in neurons of the anoxia-tolerant freshwater turtle (*Trachemys scripta*). *Aquat. Toxicol.* 180, 115–122.
- Ehrhart, L.M., 1983. Marine turtles of the Indian River lagoon system. *Fla. Sci.* 46, 337–346.
- Fauquier, D.A., Flewelling, L.J., Maucher, J., Manire, C.A., Socha, V., Kinsel, M.J., Stacy, B.A., Henry, M., Gannon, J., Ramsdell, J.S., Landsberg, J.H., 2013. Brevetoxin in blood, biological fluids, and tissues of sea turtles naturally exposed to *Karenia brevis* blooms in central west Florida. *J. Zoo Wildl. Med.* 44, 364–375.
- Fire, S.E., Wang, Z., Byrd, M., Whitehead, H.R., Paternoster, J., Morton, S.L., 2011. Co-occurrence of multiple classes of harmful algal toxins in bottlenose dolphins (*Tursiops truncatus*) stranding during an unusual mortality event in Texas, USA. *Harmful Algae* 10, 330–336.
- Fire, S.E., Van Dolah, F.M., 2012. Marine biotoxins: emergence of harmful algal blooms as health threats to marine wildlife. In: Aguirre, A.A., Ostfield, R.S., Daszak, P. (Eds.), *New Directions in Conservation Medicine: Applied Cases in Ecological Health*. Oxford University Press, New York, pp. 374–389.
- Flewelling, L.J., Naar, J.P., Abbott, J.P., Baden, D.G., Barros, N.B., Bossart, G.D., Bottein, M.Y., Hammond, D.G., Haubold, E.M., Heil, C.A., Henry, M.S., Jacobs, H. M., Leighfield, T.A., Pierce, R.H., Pitchford, T.D., Rommel, S.A., Scott, P.S., Steidinger, K.A., Truby, E.W., Van Dolah, F.M., Landsberg, J.H., 2005. Brevetoxicosis: red tides and marine mammal mortalities. *Nature* 435, 755–756.
- Florida Fish and Wildlife Conservation Commission (FFWCC), 2019. Red Tide-Statewide Status. Available via: <https://myfwc.com/research/red-tide/statewide/>.
- Foley, A.M., Stacy, B.A., Schueller, P., Flewelling, L.J., Schroeder, B., Minch, K., Fauquier, D.A., Foote, J.J., Manire, C.A., Atwood, K.E., Granholm, A.A., Landsberg, J.H., 2019. Assessing *Karenia brevis* red tide as a mortality factor of sea turtles in Florida, USA. *Dis. Aquat. Org.* 132, 109–124.
- Frazier, T.K., Hoyer, M.V., Notestein, S.K., Hale, J.A., Canfield Jr., D.E., 2001. Physical, Chemical and Vegetative Characteristics of Five Gulf Coast Rivers. Final Report. Southwest Florida Water Management District, Brooksville, Florida. Available via: https://www.researchgate.net/publication/286449050_Physical_Chemical_and_Vegetative_e_Characteristics_of_Five_Gulf_Coast_Rivers.
- Fuquay, J.M., Muha, N., Wang, Z., Ramsdell, J.S., 2012. Toxicokinetics of domoic acid in the fetal rat. *Toxicology* 294, 36–41.
- Geselbracht, L., 2007. Conservation Action Plan for Marine and Estuarine Resources of the Big Bend Area of Florida. The Nature Conservancy, Florida Chapter. Available via: https://public.myfwc.com/crossdoi/fundedprojects/SWG_Final_Geselbracht_05047.pdf.
- Glibert, P.M., Anderson, D.M., Gentien, P., Granéli, E., Sellner, K.G., 2005. The global, complex phenomena of harmful algal blooms. *Oceanography* 18, 136–147.
- Hale, J.A., Frazier, T.K., Tomasko, D.A., Hall, M.O., 2004. Changes in the distribution of seagrass species along Florida's central Gulf coast: iverson and bittaker revisited. *Estuaries* 27, 36–43.
- Hallegraeff, G.M., 2010. Ocean climate change, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge. *J. Phycol.* 46, 220–235.
- Hallegraeff, G.M., 2014. Harmful algae and their toxins: progress, paradoxes and paradigm shifts. In: Rossini, G.P. (Ed.), *Toxins and Biologically Active Compounds from Microalgae, Volume I: Origin, Chemistry and Detection*. CRC Press, Boca Raton, pp. 3–20.
- Harris, H.S., Benson, S.R., Gilardi, K.V., Poppenga, R.H., Work, T.M., Dutton, P.H., Mazet, J.A.K., 2011. Comparative health assessment of Western Pacific leatherback turtles (*Dermochelys coriacea*) foraging off the coast of California, 2005–2007. *J. Wildl. Dis.* 47, 321–337.
- Harris, H.S., Benson, S.R., Miller, M.A., Fire, S.E., Stacy, B.A., Kudela, R.M., Fahy, C.C., Seminoff, J.A., 2013. Domoic acid in the leatherback turtle food web on critical foraging grounds in central California. In: 44th Annual Conference of the International Association for Aquatic Animal Medicine. Available via: <https://www.vin.com/apputil/content/defaultadv1.aspx?id=5768604&pid=11375&print=1>.
- Herbst, L.H., Jacobson, E.R., Moretti, R., Brown, T., Sundberg, J.P., Klein, P.A., 1995. Experimental transmission of green turtle fibropapillomatosis using cell-free tumor extracts. *Dis. Aquat. Org.* 22, 1–12.
- Herbst, L.H., Klein, P.A., 1995. Green turtle fibropapillomatosis: challenges to assessing the role of environmental cofactors. *Environ. Health Perspect.* 103, 27–30.
- Holloway-Adkins, K.G., 2001. A Comparative Study of the Breeding Ecology of *Chelonia mydas*. (Green Turtle) and the Incidental Ingestion of *Proocentrum* Spp. Master's Thesis. University of Central Florida, Orlando, p. 168.
- Hu, C., Barnes, B.B., Qi, L., Corcoran, A.A., 2015. A harmful algal bloom of *Karenia brevis* in the northeastern Gulf of Mexico as revealed by MODIS and VIIRS: a comparison. *Sensors* 15, 2873–2887.
- Humpage, A.R., 2008. Toxin types, toxicokinetics and toxicodynamics (Chapter 16). In: Hudnell, K.H. (Ed.), *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs*. Adv. Exp. Med. Biol. 619. Springer Press, New York, pp. 383–415.
- Jacobson, E.R., Homer, B.L., Stacy, B.A., Greiner, E.C., Szabo, N.J., Chrisman, C.L., Origg, F., Coberley, S., Foley, A.M., Landsberg, J.H., Flewelling, L., Ewing, R.Y., Moretti, R., Schaf, S., Rose, C., Mader, D.R., Harman, G.R., Manire, C.A., Mettee, N. S., Mizisin, A.P., Shelton, G.D., 2006. Neurological disease in wild loggerhead sea turtles *Caretta caretta*. *Dis. Aquat. Org.* 70, 139–154.
- Karesh, W.B., Cook, R.A., 1995. Applications of veterinary medicine to in situ conservation efforts. *J. Zoo Wildl. Med.* 26, 2–9.
- Kubaneck, J., Hicks, M.K., Naar, J., Villareal, T.A., 2005. Does the red tide dinoflagellate *Karenia brevis* use allelopathy to outcompete other phytoplankton? *Limnol. Oceanogr.* 50, 883–895.
- Lackovich, J.K., Brown, D.R., Homer, B.L., Garber, R.L., Mader, D.R., Moretti, R.H., Patterson, A.D., Herbst, L.H., Oros, J., Jacobson, E.R., Curry, S.S., Klein, P.A., 1999. Association of herpesvirus with fibropapillomatosis of the green turtle *Chelonia mydas* and the *Caretta caretta* in Florida. *Dis. Aquat. Org.* 37, 89–97.
- Landsberg, J.H., 2002. The effects of harmful algal blooms on aquatic organisms. *Rev. Fish. Sci.* 10, 113–390.
- Landsberg, J.H., Balazs, G.H., Steidinger, K.A., Baden, D.G., Work, T.M., Russell, D.J., 1999. The potential role of natural tumor promoters in marine turtle fibropapillomatosis. *J. Aquat. Anim. Health* 11, 199–210.
- Licea-Duran, S., Navarrete, A., Rodríguez, R., Bustillos, J., Martínez, B., Ramírez, C., 2006. Monitoring a bloom of *Pyrodinium bahamense* var. *compressum* in El Salvador, Guatemala and Mexico (November 2005–March 2006). In: Proceedings of 12th International Conference on Harmful Algae, Copenhagen, Denmark. I.O.C. UNESCO, pp. 86–89.
- Main, M.B., Allen, G.M., 2007. Florida's Environment: North Central Region. Wildlife Ecology and Conservation Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida, USA. Available via: <http://ufdcimages.uflib.ufl.edu/IR/00/00/34/72/00001/UW27100.pdf>.
- Manire, C.A., Flewelling, L.J., Fauquier, D.A., 2017. Harmful algae and biotoxins. In: Manire, C.A., Norton, T.M., Stacy, B.A., Innis, C.J., Harms, C.A. (Eds.), *Sea Turtle Health and Rehabilitation*. Plantation, J. Ross Publishing, pp. 791–804.
- Manire, C.A., Stacy, B., Flewelling, L., Rodriguez, M., Foley, A., Young, M., Keene, J., 2016. Follow-up report on unusual strandings of small juvenile green sea turtles (*Chelonia mydas*) in southeastern Florida. In: 47th Annual Conference of the International Association for Aquatic Animal Medicine. Available via: <https://www.vin.com/apputil/content/defaultadv1.aspx?id=7312314&pid=14818>.
- Manire, C.A., Stacy, B., Flewelling, L., Rodriguez, M., Perry, S., 2015. Unusual strandings of small juvenile green sea turtles, *Chelonia mydas*, in southeast Florida. In: 46th Annual Conference of the International Association for Aquatic Animal Medicine. Available via: <https://www.vin.com/apputil/content/defaultadv1.aspx?pid=12676&meta=Generic&catId=52978&id=6651439&ind=166&objTypeID=17>.
- McManus, M.A., Kudela, R.M., Silver, M.W., Steward, G.F., Donaghay, P.L., Sullivan, J. M., 2008. Cryptic blooms: are thin layers the missing connection? *Estuar. Coasts* 31, 396–401.
- Paerl, H.W., Joyner, J.J., Joyner, A.R., Arthur, K., Paul, V., O'Neil, J.M., Heil, C.A., 2008. Co-occurrence of dinoflagellate and cyanobacterial harmful algal blooms in southwest Florida coastal waters: dual nutrient (N and P) input controls. *Mar. Ecol. Prog. Ser.* 371, 143–153.
- Paul, V., Arthur, K., Capper, A., Ross, C., Banks, K., Bartels, E., Clementz, M., Semon, K., 2008. Impact of Toxic Cyanobacterial Blooms (*Lyngbya* spp.) on Coral Reef Habitats off Southeastern Florida and the Florida Keys. Protect Our Reefs Grant Report #POR-2006-18. Available via: <https://dspace.mote.org/bitstream/2075/2943/1/Paul-%202006-18.pdf>.
- Perrault, J.R., Bauman, K.D., Greenan, T.M., Blum, P.C., Henry, M.S., Walsh, C.J., 2016. Maternal transfer and sublethal immune system effects of brevetoxin exposure in nesting loggerhead sea turtles (*Caretta caretta*) from western Florida. *Aquat. Toxicol.* 180, 131–140.
- Perrault, J.R., Schmid, J.R., Walsh, C.J., Yordy, J.E., Tucker, A.D., 2014. Brevetoxin exposure, superoxide dismutase activity and plasma protein electrophoretic profiles in wild-caught Kemp's ridley sea turtles (*Lepidochelys kempii*) in southwest Florida. *Harmful Algae* 37, 194–202.
- Perrault, J.R., Stacy, N.I., Lehner, A.F., Mott, C.R., Hirsch, S., Gorham, J.C., Buchweitz, J. P., Bresette, M.J., Walsh, C.J., 2017. Potential effects of brevetoxins and toxic elements on various health variables in Kemp's ridley (*Lepidochelys kempii*) and green (*Chelonia mydas*) sea turtles after a red tide bloom event. *Sci. Total Environ.* 605–606, 967–979.

- Phlips, E.J., Badylak, S., Lasi, M.A., Chamberlain, R., Green, W.C., Hall, L.M., Hart, J.A., Lockwood, J.C., Miller, J.D., Morris, L.J., Steward, J.S., 2015. From red tides to green and brown tides: bloom dynamics in a restricted subtropical lagoon under shifting climatic conditions. *Estuar. Coasts* 38, 886–904.
- Provatas, A.A., Yeudakimau, A.V., Gatley, E.L., Guerrero, E.R., Stuart, J.D., Perkins, C.R., 2014. Rapid screening and quantitative determination of selected algal toxins in surface water samples by direct evaporation and UPLC-MS/MS analysis. *Trends Chromatogr.* 9, 29–37.
- Reckendorf, A., Tuxbury, K., Cavin, J., Silver, G., Brisson, J., McManus, C., Stacy, B., Merigo, C., Innis, C., 2016. Laryngeal paralysis in a loggerhead sea turtle (*Caretta caretta*). *J. Herpetol. Med. Surg.* 26, 20–25.
- Rines, J.E.B., Donaghay, P.L., Dekshenieks, M.M., Sullivan, J.M., Twardowski, M.S., 2002. Thin layers and camouflage: hidden *Pseudo-nitzschia* spp. (Bacillariophyceae) populations in a fjord in the San Juan Islands, Washington, USA. *Mar. Ecol. Prog. Ser.* 225, 123–137.
- Robinson, N.A., Pace, J.G., Matson, C.F., Miura, G.A., Lawrence, W.B., 1991. Tissue distribution, excretion and hepatic biotransformation of microcystin-LR in mice. *J. Pharmacol. Exp. Ther.* 256, 176–182.
- Rodríguez, I., Alfonso, A., González-Jartín, J.M., Vieytes, M.R., Botana, L.M., 2018. A single run UPLC-MS/MS method for detection of all EU-regulated marine toxins. *Talanta* 189, 622–628.
- Seavey, J.R., Pine III, W.E., Frederick, P., Sturmer, L., Berrigan, M., 2011. Decadal changes in oyster reefs in the Big Bend of Florida's Gulf coast. *Ecosphere* 2, 114.
- Steidinger, K.A., 1993. Some taxonomic and biological aspects of toxic dinoflagellates. In: Falconer, I.R. (Ed.), *Algal Toxins in Seafood and Drinking Water*. Academic Press, London, pp. 1–28.
- Takahashi, E.M., Arthur, K.E., Shaw, G.R., 2008. Occurrence of okadaic acid in the feeding grounds of dugongs (*Dugong dugon*) and green turtles (*Chelonia mydas*) in Moreton Bay, Australia. *Harmful Algae* 7, 430–437.
- Trejo, C.D., Hernández, A.M.G., Ochoa, C.B., 2016. Phytotoxins associated with mortality in subadult black turtles (*Chelonia mydas agassizii*) in Michoacan, Mexico. In: Mangel, J.C., Rees, A., Pajuelo, M., Córdova, F., Acuña, N. (Eds.), *Proceedings of the Thirty-Sixth Annual Symposium on Sea Turtle Biology and Conservation*. NOAA Technical Memorandum NMFS-SEFSC-734.
- Twiner, M.J., Fire, S., Schwacke, L., Davidson, L., Wang, Z., Morton, S., Roth, S., Balmer, B., Rowles, T.K., Wells, R.S., 2011. Concurrent exposure of bottlenose dolphins (*Tursiops truncatus*) to multiple algal toxins in Sarasota Bay, Florida, USA. *PLoS One* 6, e17394.
- United States Fish and Wildlife Service (USFWS), 2013. *Vision for a Healthy Gulf of Mexico Watershed*. Available via: <https://www.fws.gov/southeast/pdf/gulf-vision-document.pdf>.
- Van Houtan, K.S., Hargrove, S.K., Balazs, G.H., 2010. Land use, macroalgae, and a tumor-forming disease in marine turtles. *PLoS One* 5, e12900.
- Walker, J.S., Shaver, D.J., Stacy, B.A., Flewelling, L.J., Broadwater, M.H., Wang, Z., 2018. Brevetoxin exposure in sea turtles in south Texas (USA) during *Karenia brevis* red tide. *Dis. Aquat. Org.* 127, 145–150.
- Walsh, C.J., Cocilova, C., Restivo, J., Flewelling, L., Milton, S., 2019. Immune function in *Trachemys scripta* following exposure to a predominant brevetoxin congener, PbTx-3, as a model for potential health impacts for sea turtles naturally exposed to brevetoxins. *Ecotoxicology* 28, 1085–1104.
- Walsh, C.J., Leggett, S.R., Carter, B.J., Colle, C., 2010. Effects of brevetoxin exposure on the immune system of loggerhead sea turtles. *Aquat. Toxicol.* 97, 293–303.
- Williams, N.C., Bjørndal, K.A., Lamont, M.M., Carthy, R.R., 2014. Winter diets of immature green turtles (*Chelonia mydas*) on a northern feeding ground: integrating stomach contents and stable isotope analysis. *Estuar. Coasts* 37, 986–994.
- Woods Hole Oceanographic Institute (WHOI), 2016. *Harmful Algae. Recent Trends: National Changes*. Available via: <https://www.whoi.edu/redtide/regions/us/recent-trends>.
- Work, T.M., Balazs, G.H., 1999. Relating tumor score to hematology in green turtles with fibropapillomatosis in Hawaii. *J. Wildl. Dis.* 35, 804–807.
- Yang, X., Zhou, L., Tan, Y., Shi, X., Zhao, Z., Nie, D., Zhou, C., Liu, H., 2017. Development and validation of a liquid chromatography-tandem mass spectrometry method coupled with dispersive solid-phase extraction for simultaneous quantification of eight paralytic shellfish poisoning toxins in shellfish. *Toxins* 9, 206.
- Yasumoto, T., 1998. Fish poisoning due to toxins of microalgal origins in the Pacific. *Toxicon* 36, 1515–1518.