Harmful Algae 101 (2021) 101973

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Original Article

Exposure to domoic acid is an ecological driver of cardiac disease in southern sea otters☆

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ARTICLE INFO

Keywords:
Domoic acid
Sea otter
Cardiac disease
Prey preference
Risk model
Climate change

ABSTRACT

Harmful algal blooms produce toxins that bioaccumulate in the food web and adversely affect humans, animals, and entire marine ecosystems. Blooms of the diatom *Pseudo-nitzschia* can produce domoic acid (DA), a toxin that most commonly causes neurological disease in endothermic animals, with cardiovascular effects that were first recognized in southern sea otters. Over the last 20 years, DA toxicosis has caused significant morbidity and mortality in marine mammals and seabirds along the west coast of the USA. Identifying DA exposure has been limited to toxin detection in biological fluids using biochemical assays, yet measurement of systemic toxin levels is an unreliable indicator of exposure dose or timing. Furthermore, there is little information regarding repeated DA exposure in marine wildlife. Here, the association between long-term environmental DA exposure and fatal cardiac disease was investigated in a longitudinal study of 186 free-ranging sea otters in California from 2001 – 2017, highlighting the chronic health effects of a marine toxin. A novel Bayesian spatiotemporal approach was used to characterize environmental DA exposure by combining several DA surveillance datasets and integrating this with life history data from radio-tagged otters in a time-dependent survival model. In this study, a sea otter with high DA exposure had a 1.7-fold increased hazard of fatal cardiomyopathy compared to an otter with low exposure. Otters that consumed a high proportion of crab and clam had a 2.5- and 1.2-times greater hazard of death due to cardiomyopathy than otters that consumed low proportions. Increasing age is a well-established predictor of cardiac disease, but this study is the first to identify that DA exposure affects the risk of cardiomyopathy more substantially in prime-age adults than aged adults. A 4-year-old otter with high DA exposure had 2.3 times greater risk of fatal cardiomyopathy than an otter with low exposure, while a 10-year old otter with high DA exposure had just 1.2 times greater risk. High *Toxoplasma gondii* titers also increased the hazard of death due to heart disease 2.4-fold. Domoic acid exposure was most detrimental for prime-age adults, whose survival and reproduction are vital for population growth, suggesting that persistent DA exposure will likely impact long-term viability of this threatened species. These results offer insight into the pervasiveness of DA in the food web and raise awareness of under-recognized chronic health effects of DA for wildlife at a time when toxic blooms are on the rise.

☆**Author Contributions:** M.E.M., M.T.T., M.A.M., and C.K.J. designed research; M.E.M., M.T.T., M.A.M., J.A.T., M.M.A., J.A.F., F.I.B., E.M.D., R.M.K., V.Z.W., and C.K.J. performed research and contributed data; M.E.M. and M.T.T. contributed new reagents/analytic tools; M.E.M. and M.T.T. analyzed data; C.K.J. advised on all analyses, M.E.M. wrote the manuscript and all authors contributed substantially to revisions and have approved the final article.

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https://doi.org/10.1016/j.hal.2020.101973
Received 31 August 2020; Received in revised form 18 December 2020; Accepted 19 December 2020
Available online 12 January 2021

1. Introduction

Ecosystem disturbances, due to climate change and loss of natural habitat, lead to increased disease risk and novel threats to public health and wildlife populations (Acevedo-Whitehouse and Duffus, 2009; Altizer et al., 2013; Borremans et al., 2019; Johnson et al., 2020). Harmful algal blooms are predicted to become more widespread, frequent, and persistent in relation to future climate projections and human influences (Van Dolah, 2000; Moore et al., 2008; Wells et al., 2015). Anthropogenic disruption of the nearshore system, such as terrestrial runoff and eutrophication (Anderson et al., 2002; Parsons and Dortch, 2002; Kudela et al., 2008), and rising temperature (McCabe et al., 2016; McKibben et al., 2017) have resulted in environmental conditions that favor bloom production. Present day examples of extreme weather and anomalously warm ocean events offer a glimpse into future climate conditions and their impact on harmful algal blooms (Trainer et al., 2020). For example, a massive marine heat wave in the northeast Pacific Ocean initiated an extensive and prolonged bloom of the toxigenic diatom *Pseudo-nitzschia* in spring 2015. This resulted in the largest recorded outbreak of the neurotoxin domoic acid (DA) along the North American west coast, impacting marine life and fisheries from Alaska to Baja California (McCabe et al., 2016).

Algal toxins bioaccumulate in food webs and impact higher trophic organisms like marine predators and humans (Lefebvre et al., 1999, 2002; Scholin et al., 2000). Domoic acid causes significant wildlife mortality (Fritz et al., 1992; Work et al., 1993; Scholin et al., 2000) and amnesic shellfish poisoning, a severe neurological illness in people (Bates et al., 1989; Perl et al., 1990). Marine wildlife are sentinels of environmental change and have been useful in early detection of harmful algal blooms and their biological effects (Goldstein et al., 2008; Bargu et al., 2010; Bossart, 2011). Southern sea otters (*Enhydra lutris nereis*), a keystone nearshore species (Estes and Palmisano, 1974), face several threats from land-sea pathogen flow and terrestrial runoff causing nutrient loading that promotes algal toxin production, like DA (Miller et al., 2002a, 2010, 2020a; Kreuder et al., 2003, 2005; Jessup et al., 2004). Their unique ecology, physiology, and behavior make them bioindicators of DA trophic transfer: otters consume large amounts of invertebrates that bioconcentrate DA (Kenyon, 1969; Kizer et al., 1993; Wekell et al., 1994; Ferdin et al., 2002; Kvitek et al., 2008) and live in a region with recurrent toxic blooms (Trainer et al., 2000; Bargu et al., 2010).

Lasting health effects of repeated DA exposure in humans and wildlife have been increasingly recognized (Goldstein et al., 2008; Lefebvre et al., 2012; Grattan et al., 2016). Acute, subacute, and chronic DA exposure can affect the central nervous system, gastrointestinal tract, and cardiovascular system (Perl et al., 1990; Teitelbaum et al., 1990; Pulido, 2008;) and long-term impacts of the toxin were initially discovered in wildlife (Kreuder et al., 2005; Goldstein et al., 2008; Zabka et al., 2009). The chronic effects of DA exposure were first highlighted in California sea lions (*Zalophus californianus*) with a novel neurological syndrome characterized by epilepsy (Goldstein et al., 2008). The relationship between DA and cardiac disease was first uncovered in sea otters when DA was identified as a risk factor for cardiomyopathy (Kreuder et al., 2005). Degenerative cardiomyopathy is also a common finding in sea lions with DA toxicity (Zabka et al., 2009). Wildlife monitoring has demonstrated benefits for public health through recognition of new syndromes related to environmental toxins.

Cardiomyopathy is a disease of heart muscle that is progressive , sometimes leading to cardiac dilation and congestive heart failure. Previous epidemiological studies based on sea otter postmortem examinations have described cardiomyopathy as a multifactorial disease because of its associations with DA and protozoal infection with *Toxoplasma gondii* and *Sarcocystis neurona* (Kreuder et al., 2005; Miller et al., 2020a). Recent advances in sea otter pathology have improved the ability to distinguish between cardiac lesions associated with DA toxicosis from those caused by protozoal infection (Miller et al., 2020b). Cardiomyopathy and DA toxicosis are prevalent causes of sea otter mortality (Miller et al., 2020a), yet much remains unknown about the timeline of disease development, effect of environmental DA exposure, and impact of individual behaviors. Earlier research has been limited to cross-sectional studies that represent DA exposure at a single timepoint (Kreuder et al., 2005; Miller et al., 2020a. Domoic acid is excreted within hours of ingestion and measurement of systemic toxin levels are not reliable indicators of exposure dose or timing (Lefebvre et al., 2012; Jing et al., 2018). Environmental monitoring programs routinely collect *Pseudo-nitzschia* and DA data; unfortunately, sampling frequency is variable and geographically clustered, such that no single dataset reflects acute and chronic exposure through DA retention in food webs. The spatial and temporal nearshore ecology of DA is complex, but essential for understanding the pathophysiological effects on sea otters.

Southern sea otters are a federally listed threatened species (USFWS 2003) and an extensively studied wild carnivore. Long-term telemetry-based field studies and comprehensive postmortem examinations have generated longitudinal datasets (Tinker et al., 2019; Miller et al., 2020a). In the present study, a novel approach was developed to assess environmental DA exposure by combining several DA surveillance datasets and integrating this with detailed life history data from free-ranging sea otters to identify ecological drivers of cardiac disease. This research is the first to evaluate long-term environmental DA exposure in a wildlife species in order to characterize the chronic health effects of a pervasive marine toxin.

2. Material and methods

2.1. Study population

A longitudinal dataset from telemetry-based field studies that followed 186 free-ranging sea otters from 2001 – 2017 was used to evaluate individual histories, behaviors, and environmental threats that increase the risk of fatal cardiomyopathy (Fig. 1). Sea otters were captured during field studies located in Elkhorn Slough (2013–2016), Monterey Bay/Peninsula (2000–2017), Monterey Bay and Big Sur (2008–2011), Cambria/San Simeon (2000–2004), San Luis Obispo (2012 – 2014), and the Santa Barbara Channel (2012–2014) (Tinker et al., 2007, 2008, 2012, 2017, 2019; Hughes et al., 2013). Age estimates were based on previously established parameters like dentition, tail length, and pelage characteristics (Tinker et al., 2017, 2019). Otters were anesthetized and implanted with intra-abdominal very high frequency (VHF) radio-transmitters (ATS Inc., Isanti, MN) and archival time depth recorders (Mk-9 model; Wildlife Computers, Redmond, WA) (Tinker et al., 2008). Sea otters were fitted with color coded flipper tags and released immediately after recovery at the capture location.

2.2. Sea otter observational data

Sea otters were directly observed in the wild 4–7 times per week for up to 14 years, enabling the characterization of age- and sex-specific survival, diet preferences, reproduction, movements, protozoal (*T. gondii* and *S. neurona*) exposure by serology, and causes of death. To assess previous DA exposure and obtain representative behavioral data, only individuals observed for at least one year after initial capture, with at least 300 foraging dives, were included. Detailed diet assessments were performed using direct observation (Tinker et al., 2008) and archival data from time depth recorders (Tinker et al., 2007), when available. Individuals were located by radio signal with VHF receivers and observers recorded their locations using a compass, laser rangefinder, and global positioning satellite technology (Tinker et al., 2006, 2019). Observers used high-powered spotting scopes to record the identity, size, and frequency of prey items consumed while otters handled prey at the surface after each foraging dive (Tinker et al., 2008).

The relative frequency of 13 main prey types (urchin, abalone, mussel, clam, snail, crab [including kelp crab, cancer crab, and others],

sand crab, sea star, worm, chiton, sand dollar, octopus, and squid) in an individual's diet was calculated. To obtain quantitative measures of the prevalence of prey species in the diet, prey frequency and size were converted to estimates of consumed biomass (Oftedal et al., 2007). These data were analyzed using a Monte-Carlo procedure to estimate each individual's diet composition (in terms of the proportion of consumed biomass contributed by each prey taxa), which explicitly incorporates sampling uncertainty and adjusts for recognized biases associated with missed data points in direct observations of sea otter foraging (Tinker et al., 2012).

Blood was collected at the time of capture and serum was evaluated for IgG antibodies to *T. gondii* and *S. neurona* using an indirect fluorescent antibody test optimized for sea otters, with a titer of ≥1:320 used to identify *T. gondii* infection (Miller et al., 2002b). In this study, a titer of ≥1:5120 was used to identify sea otters with a high level of antibodies as indicative of severe or active infection with *T. gondii* or *S. neurona,* which have been shown to cause cardiac pathology, such as myocarditis; additionally, elevated *T. gondii* titers could indicate recrudescence in debilitated animals (Miller et al., 2018). If an otter had a high titer at least once, it was considered positive for the duration of the study because of the focus on potential protozoa-caused cardiac lesions that could develop and worsen over time following infection; previous studies have demonstrated that protozoal infection can cause morbidity by inciting chronic cardiac inflammation (Kreuder et al., 2003; Miller et al., 2010, 2018, 2020a). Survival studies only include events as they unfold in time and for this reason, titers collected at postmortem examination were excluded because this information was not predictive of the outcome since it was not available prior to the animal's death.

2.3. Postmortem examination data

If an individual died, every effort was made to collect the carcass and send it to the veterinary pathologist for necropsy, which included comprehensive gross and microscopic examination of all major organs and tissues, diagnostic testing, and cause of death determination. Estimated age at death ranged from two to > 15 years old (mean: 10.6 years, SD: 2.8 years). Cases were excluded if the carcass was significantly decomposed, no histopathology was performed, heart pathology could not be accurately assessed, or there was postmortem scavenging. Cardiomyopathy was characterized grossly as diffuse orange-white-tan myocardial mottling, ventricular and atrial dilation, and congestive heart failure. Microscopic lesions included myocardial congestion and hemorrhage, cardiomyocyte swelling, vacuolation, necrosis or apoptosis, myophagia, depletion, interstitial fibrosis, vascular pathology, and variable nonsuppurative inflammation. This pattern of cardiac pathology has been previously described as characteristic of DA-associated cardiomyopathy, in contrast with primary protozoal heart disease, which causes a more inflammatory cardiac process, such as lymphoplasmacytic myocarditis (Miller et al., 2020b). All cases were subjectively graded by a veterinary pathologist to evaluate severity and chronicity of cardiac lesions, as well as the likelihood of DA involvement in cardiac disease, based on a case definition of DA toxicosis developed for sea otters based on gross necropsy, microscopic examination, and diagnostic testing of biological samples (Miller et al., 2020b). A description of the predominant inflammatory cell types (lymphoplasmacytic or pleocellular) and presence of protozoal tissue cysts, schizonts, or zoites in the heart, brain, or skeletal muscle were also included in case evaluations. If cardiomyopathy was severe enough to cause mortality, it was considered a case, regardless of its designation as a primary or contributing cause of death.

2.4. Environmental DA data

There are a variety of environmental monitoring programs that collect harmful algal bloom data in the nearshore ecosystem of California (Fig. 1). The California Department of Public Health leads the Marine Biotoxin Monitoring Program, which tests bivalve shellfish for DA (https://www.cdph.ca.gov/Programs/CEH/DRSEM/Pages/EMB/Shellfish/Marine-Biotoxin-Monitoring-Reports.aspx) using high performance liquid chromatography (Dhoot et al., 1993). Samples were

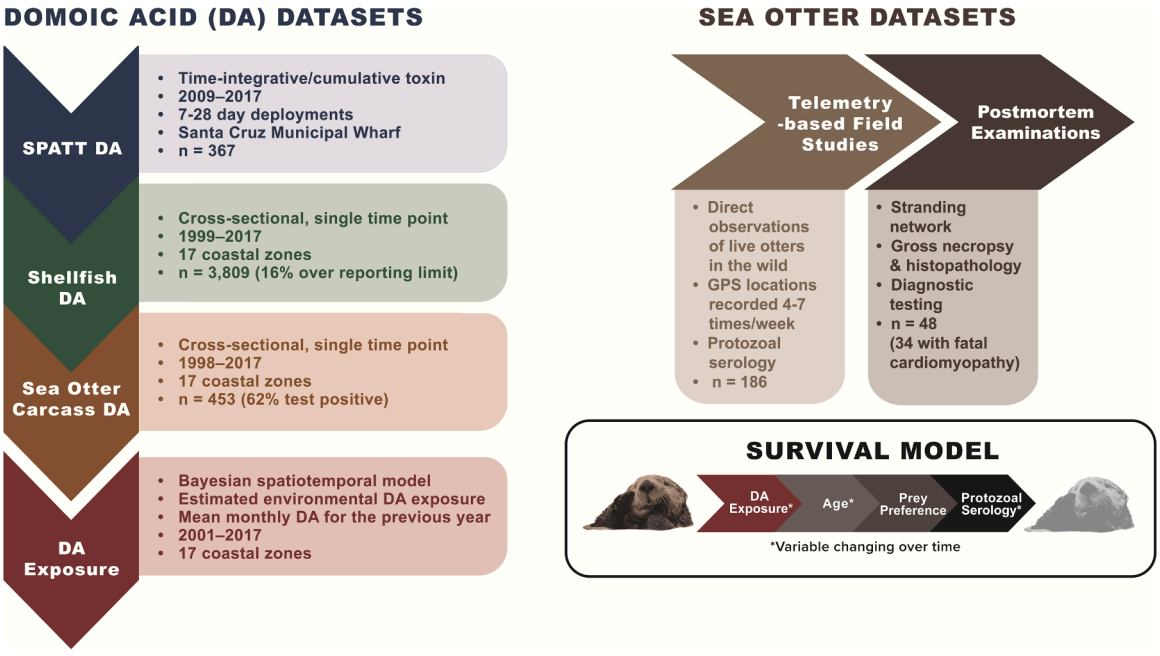

Fig. 1. Environmental domoic acid (DA) and southern sea otter (*Enhydra lutris nereis*) datasets were incorporated into a survival model that followed free-ranging sea otters through time to assess risk factors for fatal cardiac disease. Three DA monitoring datasets of varying spatial and temporal scale were combined in a Bayesian spatiotemporal model that estimated composite DA exposure values. This and other potential risk factors were included in a survival model utilizing sea otter datasets from 2001 – 2017 that included longitudinal telemetry-based field studies of 186 live otters and comprehensive postmortem examinations on 48 that died. Data sources include: Solid Phase Adsorption Toxin Tracking (SPATT) DA (Lane et al., 2010), shellfish DA (https://www.cdph.ca.gov/Programs/CEH/DRSEM/Pages/EMB/Shellfish/Marine-Biotoxin-Monitoring-Reports.aspx), and sea otter carcass DA (Tor et al., 2003; Miller et al., 2020a).

collected by volunteers with variable sampling frequency, such that shellfish growing areas were sampled once a week, while non-commercial sites were sampled sporadically based on the timing of known toxic blooms. Within the sea otter geographic range, shellfish sampling spanned from Half Moon Bay to Santa Barbara. Between 1999 – 2017, 3809 shellfish samples were tested for DA, with 16% ($n = 605$) over the reporting limit (2.5 ppm).

The California Department of Fish and Wildlife leads southern sea otter mortality investigations by conducting postmortem examinations and providing diagnostic pathology services at the Marine Wildlife Veterinary Care and Research Center (https://wildlife.ca.gov/OSPR/Science/MWVCRC). During sea otter postmortem examinations from 1998 – 2017, urine, feces, or gastrointestinal content were tested for DA using liquid chromatography-tandem mass spectrometery according to established methods (Tor et al., 2003). A concerted effort to perform systematic DA testing during sea otter necropsy was initiated as part of a large-scale mortality study from 1998 – 2012 (Miller et al., 2020a), but continued beyond that time frame to include sea otters necropsied from 2013 – 2017. Sea otter carcasses were recovered from San Francisco to Carlsbad, although most were within the established range of sea otters from Pigeon Point to Gaviota (Hatfield et al., 2019). Between 1998 – 2017, 453 otters were tested for DA during necropsy, with 62% ($n = 279$) testing positive at the method detection limit (5 ppb).

Solid Phase Adsorption Toxin Tracking (SPATT) is a sampling technique that employs a synthetic resin contained in a mesh bag, which was suspended from ropes under the Santa Cruz Municipal Wharf to absorb dissolved DA from the water (Lane et al., 2010). It allows for spatially discrete and time-integrative detection of dissolved toxin. Between 2009 – 2017, 367 SPATT samples were collected over deployments of 7 – 28 days. In order to assess sampling effort for each type of environmental DA data, all data were aggregated into 17 coastal zones, each 30 km in length, an appropriate spatial scale to capture variation in demographic effects, given sea otter range use patterns (Tinker et al., 2008; Tarjan and Tinker, 2016) (Fig. 2).

2.5. Bayesian spatiotemporal model for estimating DA exposure

Domoic acid is a spatiotemporally explicit environmental hazard, yet sampling variability makes it challenging to estimate toxin exposure. Here, a novel, composite metric for DA exposure was developed, which combined three environmental monitoring datasets using a Bayesian hierarchical model. The purpose of this analysis was to estimate long-term environmental DA exposure by month and coastal zone for use in a survival model that investigates the relationship between DA and fatal heart disease in sea otters. This Bayesian approach allowed for simultaneous fitting of the latent variable ("true" DA concentration, by month and coastal zone) to three observed variables: shellfish DA, sea otter DA, and SPATT DA. Spatial conditional autoregressive (CAR) methods were used to describe spatiotemporal variation in DA concentration, following the BYM2 model (Riebler et al., 2016), with temporal autocorrelation incorporated using the AR(1) approach (Liu et al., 2017). The CAR method is useful for describing complex spatiotemporal patterns and allows information from neighboring areas to inform reliable zone-specific estimates for areas or times that are data-poor.

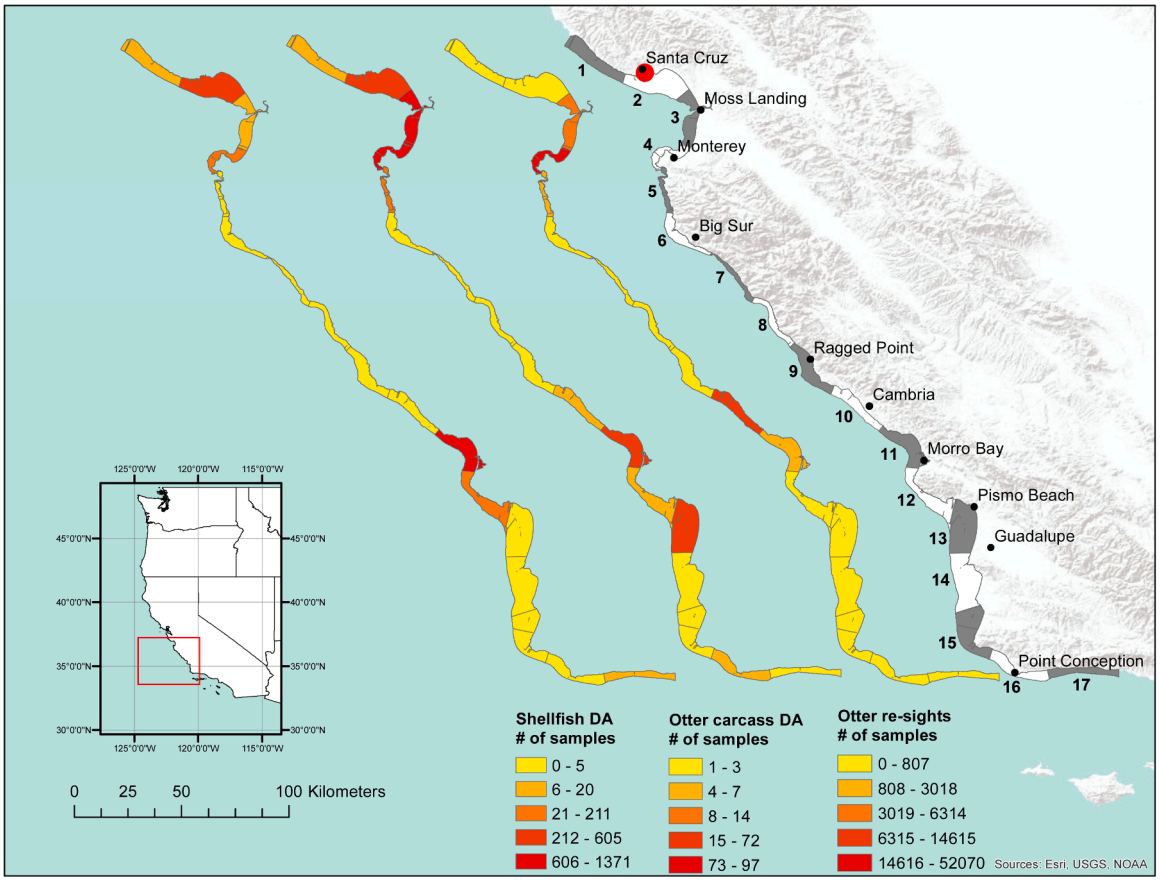

Fig. 2. Sampling effort for direct observations of free-ranging sea otters during telemetry-based field studies and environmental domoic acid (DA) surveillance in California as represented by 17 coastal zones from Año Nuevo to Point Conception (gray and white). To assess overlap among sea otter range use and environmental variables, data were aggregated within each zone. From left to right, datasets representing environmental DA exposure and sea otter locations during the study included: (1) shellfish DA test results, (2) postmortem sea otter DA test results, (3) live sea otter re-sight observations, (4) Solid Phase Adsorption Toxin Tracking (SPATT) DA testing (only at Santa Cruz Municipal Wharf, denoted by red circle). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

This model incorporated a time-lag between each dataset such that DA was first detected by SPATT, then two months later by shellfish toxicity, and two months later by acute DA toxicosis in sea otters. These lag periods were based on previous research demonstrating that SPATT provided a consistent signal-response to DA-producing blooms seven weeks prior to the detection of shellfish toxicity (Lane et al., 2010) and a time-series cross-correlation analysis showing that DA detection in shellfish was significantly correlated with DA detection in otters two months later ($r^2 = 0.218$). This model was used to estimate mean DA concentration and standard errors at monthly time steps for each of the 17 coastal zones (Fig. 3). Predicted DA values by month and coastal zone were related probabilistically to empirical data by assuming a zero-inflated gamma distribution for each observed variable, with estimated scaling factors used to convert shellfish and sea otter DA measurements to equivalent units as SPATT measurements. The Bayesian software package "STAN" (Carpenter et al., 2017) was used within the *R* modeling environment (R Core Team 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.) to develop and run this model.

To use the composite DA estimates as a predictor for fatal cardiomyopathy required defining an appropriate timescale for exposure risk. The actual timeline of DA exposure in marine mammals remains unknown and involves complex ecological dynamics from bloom-associated DA production to bioaccumulation in invertebrates to predator exposure through consumption of contaminated prey. The relevant exposure period likely differs among study animals, but for the purpose of modeling survival risk, a fixed period over which to define time-integrated exposure for all animals had to be selected. Several alternative temporal lags were evaluated and model performance was compared for each (i.e. convergence and posterior predictive checks), ultimately defining time-integrated DA exposure as the mean monthly DA value over the previous 12 months. The composite DA estimate was then log-transformed to normalize the variable. A DA exposure value was assigned for individual otters at each time step, based on their spatiotemporal overlap with modeled DA values over the previous 12 months, and this time-varying risk factor was included in the survival model.

2.6. Time-dependent Cox proportional hazards model

A null model was constructed to produce a baseline population survivorship function with unadjusted Nelson-Aalen survival estimates and respective 95% confidence intervals. To explore the effect of a single variable on time until fatal cardiomyopathy, Kaplan-Meier curves were developed (Fig. 4). Sea otters that died of cardiomyopathy sooner had consumed a higher proportion of crab and clam, were highly seropositive for *T. gondii*, and were older. As described above, DA exposure is a time-dependent variable, which does not lend itself to the Kaplan-Meier estimator, so for this analysis each individual's DA exposure over the study period was summarized as a time-weighted variable and the findings are presented separately for younger (subadult and adult) and older (aged adult) otters. Younger otters had lower survival if they were exposed to high DA, while this association became less important in older otters that were already at elevated risk of fatal cardiomyopathy.

A multivariate survival model was developed to examine risk factors that increased the hazard of fatal cardiomyopathy. A Cox proportional hazards model of cause-specific mortality was used to assess fatal cardiomyopathy in the presence of competing risks or death due to other causes. This approach separately estimates a hazard ratio for cardiomyopathy-specific mortality, treating other causes of death as censored categories, in addition to those who are censored from loss to follow-up or survival through the end of the study (Heisey and Patterson, 2006; Kleinbaum and Klein, 2012). Survival time was measured as the time since initial capture until fatal cardiomyopathy or censoring. The Efron approximation was used for estimating the partial likelihood to account for ties in mortality events.

Extended Cox regression models were fit based on the counting process formulation of the Cox proportional hazards model (Andersen and Gill, 1982; Therneau and Grambsch, 2013) that allows for time-varying covariates, such that multiple measurements over time can be accommodated. This model was well suited for time-dependent variables like DA exposure, age, and protozoal serology. Diet proportions were considered time-constant because individual sea otter diets are quite consistent, even over long temporal scales such as years, with relatively low within-individual diet variation (Tinker et al., 2012; Novak and Tinker, 2015). Multivariate models were developed based on *a-priori* knowledge of putative risk factors and confounders. Covariates were included in the multivariate models if they were biologically relevant, significantly associated in univariate models, and produced at least a 10% change in coefficients. The model was built using a forward stepwise approach. Variables that were significantly associated with survival and improved model fit, as assessed through Akaike information criteria (AIC), were included in the final model.

The cause-specific hazard function for fatal cardiomyopathy was calculated as:

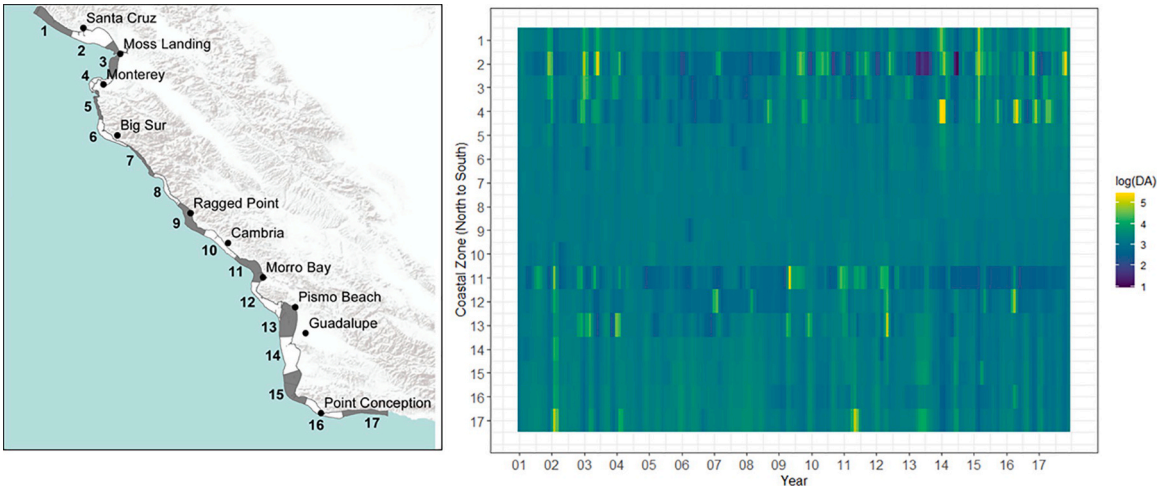

Fig. 3. Heatmap showing estimated composite domoic acid (DA) values (log mean DA) by coastal zone and year using a Bayesian spatiotemporal conditional autoregressive model. The model incorporated data from (1) Solid Phase Adsorption Toxin Tracking (SPATT) DA testing, (2) shellfish DA testing, and (3) postmortem sea otter DA testing.

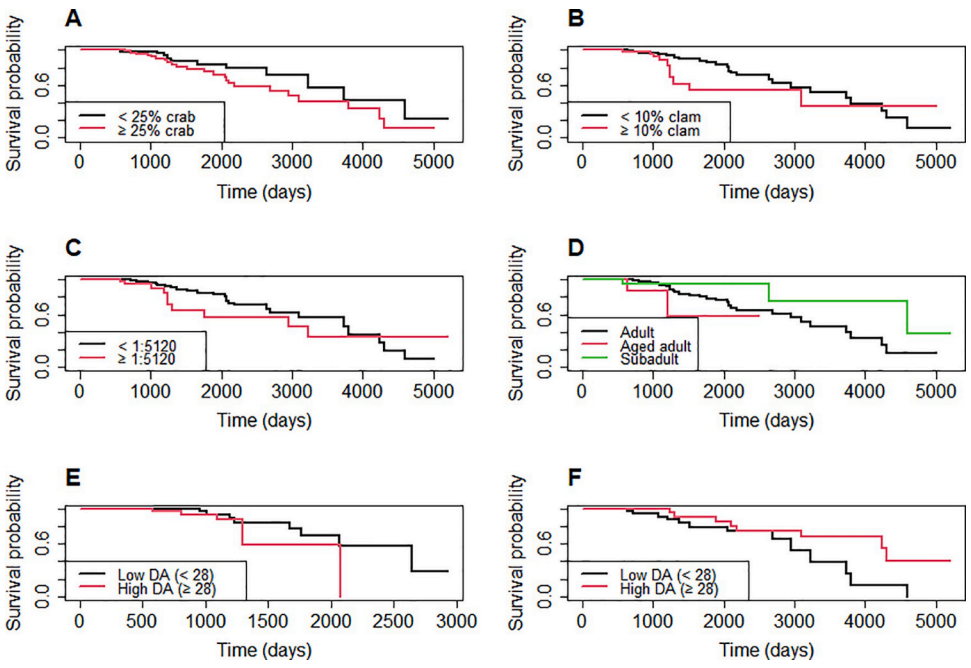

Fig. 4. Kaplan-Meier curves illustrating survival time until fatal cardiomyopathy as observed in 186 free-ranging sea otters. Each figure illustrates the effect of a single variable: **(A) Crab consumption**: high (≥ 25%) vs. low (< 25%) dietary proportion, **(B) Clam consumption:** high (≥ 10%) vs. low (< 10%) dietary proportion, **(C) *Toxoplasma gondii* exposure:** high positive (≥ 1:5120) vs. negative/low positive (< 1:5120) antemortem IgG serological titer, **(D) Age class:** subadults (< 4 years old), adults (4–10 years old), and aged adults (> 10 years old), **(E) Domoic acid (DA) exposure in younger otters:** high (≥ mean) vs. low (< mean) time-weighted DA exposure among younger otters (≤ 10 years old), and **(F) Domoic acid exposure in older otters:** high (≥ mean) vs. low (< mean) time-weighted DA exposure among older otters (> 10 years old). .

where h_0 is the baseline hazard, β parameters reflect the coefficients for time-independent variables (clam and crab proportion in diet) and δ parameters represent the coefficients for time-dependent variables (DA exposure, age, high *T. gondii* titer, interaction between DA exposure and age). All predictor variables were centered by subtracting the mean (or median, in the case of age), to improve fitting and more easily interpret the effect of one variable on the hazard of fatal cardiomyopathy, while holding all other variables constant. Hazard ratios and 95% confidence intervals for significant risk factors were estimated. Model adequacy was assessed by inspecting residual plots (Schoenfeld, Cox-Snell, and Martingale residuals, dfbeta) to evaluate overall fit, check the functional form of covariates, identify influential data points, and assess the validity of the proportional hazards assumption. Goodness of fit was assessed using AIC. Concordance (the mean pairwise concordance between model-predicted relative survival for any pair of records and the observed relative survival) was used to describe the amount of variance explained by the Cox model. All statistical analyses were conducted using the survival library in *R* (R Core Team 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.) (Therneau et al., 2019).

$$h_c(t \mid X,\ X(t)) = h_0(t) * exp\left[\left(\beta_{clam} X_{clam} + \beta_{crab} X_{crab}\right) + \left(\delta_{DA} X_{DA}(t) + \delta_{age} X_{age}(t) + \delta_{T.gondii} X_{T.gondii}(t) + \delta_{DA*age} X_{DA}(t) X_{age}(t)\right)\right]$$

3. Results

Comprehensive necropsies were performed on 48 individuals: 34 had fatal cardiomyopathy, while 14 died of other causes. Of the remaining otters, 24 survived through the end of the study and 114 were lost to follow-up, either by unconfirmed mortality or after their transmitter batteries expired (expected battery life: 2–3 years). The study population included 143 females and 43 males; age at study entry ranged from 42 days old to > 12 years old (mean: 5.4 years, SD: 2.5 years); time of study ranged from 399 days to over 14 years (mean: 3.8 years, SD: 2.7 years); 43 otters had high *T. gondii* titers, while 21 had high *S. neurona* titers. For overall sea otter survivorship, the estimated median survival time (time until fatal cardiomyopathy) was 8.9 years (3231 days, 95% CI: 2687, NA).

3.1. Factors increasing the hazard of fatal cardiac disease

Using a time-dependent Cox proportional hazards model, behavioral, environmental, and demographic factors were found to increase the risk of fatal cardiac disease (Table 1). The best-fit survival model included time-integrated DA exposure (mean monthly estimates for the previous year), age (years), dietary proportion of crab, dietary proportion of clam, high *T. gondii* titer, and an interaction between DA exposure and age. This model had high predictive ability, with concordance = 0.79, indicating that it correctly predicted pairwise survival times for 79% of the data. Adjusted hazard ratios represent biologically relevant comparisons of otters in the 75th percentile of a given variable ("high") to otters in the 25th percentile ("low"), with all other covariates in the model held constant at mean values, and age held constant at the median (7 years old), unless otherwise noted.

Sea otters were found to be at greater risk of cardiomyopathy-associated death if they were exposed to high levels of environmental DA in the previous year. For example, a median-age animal with high DA exposure had a 1.7-fold increased hazard of fatal cardiomyopathy compared to one with low DA exposure (HR: 1.68, CI: 1.09 – 2.57, P = 0.02). Otters that consumed a high proportion of crab and clam had a 2.5- and 1.2-times greater hazard of death due to cardiomyopathy, respectively, than otters that consumed a low proportion of crab and clam (HR: 2.45, CI: 1.47 – 4.10, P<0.001; HR 1.19, CI: 1.17 – 1.55, P = 0.02). In addition to the spatiotemporally explicit DA exposure in the model, some of the effect of DA is likely accounted for by these dietary risk factors.

Increasing age was a substantial predictor of cardiomyopathy-related death, for example, a 10-year-old otter had 8.5-times higher risk of fatal cardiomyopathy than a 4-year-old otter (HR: 8.51, CI: 2.76 – 26.27, P<0.001). Cardiomyopathy is a progressive condition, with older

Table 1
Using a time-dependent Cox proportional hazards model, risk factors influencing time until fatal cardiomyopathy were identified among 186 free-ranging sea otters monitored in California from 2001 – 2017. Associations are reported as model coefficients (log hazard ratios) with standard errors (SE) and adjusted hazard ratios with 95% confidence intervals (CI) and *P*-values. All adjusted hazard ratios represent biologically relevant comparisons of otters in the 75th percentile vs. 25th percentile of a given variable, with covariates held constant at mean values and age at the median (7 years old), unless otherwise noted.

Risk factor	Coefficient (+/- SE)	Hazard ratio (95% CI)	*P*
Domoic acid exposure	2.37 (+/- 1.15)	1.68 (1.090 – 2.57)	0.02
Age	0.36 (+/- 0.092)	8.51 (2.76 – 26.27)*	< 0.001
Crab	2.79 (+/- 0.91)	2.45 (1.47 – 4.10)	< 0.001
Clam	2.01 (+/- 0.83)	1.19 (1.17 – 1.55)	0.02
High *T. gondii* titer	0.86 (+/- 0.40)	2.36 (1.090 – 5.10)	0.03
Domoic acid exposure*Age	−0.45 (+/- 0.24)	2.25 (1.71 – 2.97)†	0.04
		1.25 (0.95 – 1.64)‡	0.04

* Compares the risk of fatal cardiomyopathy between 10-year-old vs. 4-year-old otters.
†,‡ Compares the risk of fatal cardiomyopathy between otters with high vs. low DA exposure among 4-year-old otters and 10-year-old otters, respectively.

animals at greater risk because they reflect this extended timeline of disease development (Kreuder et al., 2005; (Miller et al., 2020a)). Importantly, there was a biologically and statistically significant interaction with age in the effect of DA exposure on the development of fatal cardiomyopathy (Fig. 5). To examine this relationship, the effect of high DA exposure was evaluated separately for younger adult otters and older adult otters, at the lower and upper bounds of the adult age class (4 –10 years old). Among younger otters, DA exposure substantially increased the hazard of cardiomyopathy-associated death. For example, a 4-year--old otter with high DA exposure had 2.3 times greater risk of fatal cardiomyopathy than a similar age otter with low DA exposure (HR: 2.25, CI: 1.71 – 2.97, $P = 0.04$). Among older otters, DA exposure still increased the hazard of fatal cardiomyopathy, but not as markedly as in the younger cohort. For example, a 10-year-old otter with high DA exposure had just 1.2 times greater risk of fatal cardiomyopathy than a similar age otter with low DA exposure (HR: 1.25, CI: 0.95 – 1.64, $P = 0.04$).

High *T. gondii* titers also elevated the risk of death due to heart disease, with an estimated 2.4-fold (HR: 2.36, CI: 1.09 – 5.10, $P = 0.003$) increase among median-age otters. No relationship was found between high *S. neurona* titers and fatal cardiomyopathy, although only a small number of otters in this study had high antemortem titers, limiting the ability to detect an association with cardiomyopathy. Among the 21 otters with high antemortem *S. neurona* titers, only two had postmortem examinations and both were cardiomyopathy cases, five were still alive at the end of the study (observed for 3 months – 3 years after identifying a high *S. neurona* titer), and the remaining 14 were lost to follow-up with unknown outcomes.

4. Discussion

The findings presented here highlight the multifactorial nature of cardiac disease and identify DA as an ecological driver of fatal cardiomyopathy in sea otters. This research is the first to characterize the environmental threats underlying cardiomyopathy by integrating longitudinal environmental DA and wildlife monitoring datasets. The relationship between DA and cardiac disease in otters involves both behavioral and environmental processes. Incorporating prey preferences provided insight into individual-level risk factors, while utilizing spatiotemporally explicit DA data highlighted the environmental underpinnings of a complex disease.

Sea otters in central California exhibit diet specialization (Estes et al., 2003; Tinker et al., 2008), with individuals demonstrating preferences for particular prey types. Prey choice influences the risk of pathogen exposure in sea otters (Johnson et al., 2009) and findings from the present study suggest it also results in differential exposure to DA. Clams and crabs are particularly high risk prey because they are effective bioconcentrators of DA that also slowly excrete the toxin (Kizer et al., 1993; Wekell et al., 1994; Lund et al., 1997; Schultz et al., 2008, 2013). Sea otter postmortem examinations also indicate these prey may be associated with higher odds of fatal DA toxicosis (Miller et al., 2020a). Domoic acid toxicokinetics is highly variable among benthic invertebrates: the elimination half-life of DA is hours to days in mussels (Novaczek et al., 1992; Mafra et al., 2010), while it is on the order of months to years in razor clams, Dungeness crabs, and scallops (Wekell et al., 1994; Douglas et al., 1997; Lund et al., 1997). Sea otters specializing in these prey types may have sustained chronic DA exposure, potentially resulting in higher cumulative toxicity over time.

An important contribution of this study is the recognition that DA exposure affects the risk of fatal cardiomyopathy in different ways, depending on sea otter age. It has been well-established that aged adult otters are at considerable risk of dying from cardiomyopathy (Kreuder et al., 2005; Miller et al., 2020a), yet this study highlights the risk among prime-age adults (4 – 10 years old) for the first time through the discovery of an interaction with age in the effect of DA exposure on heart disease risk. Among prime-age adults, otters with high vs. low DA exposure were compared separately from otters at the lower and upper bounds of this age class. Four-year-old otters with high DA exposure had a more substantially increased risk of cardiomyopathy-associated death than 10-year-old otters with high DA exposure. Southern sea otter

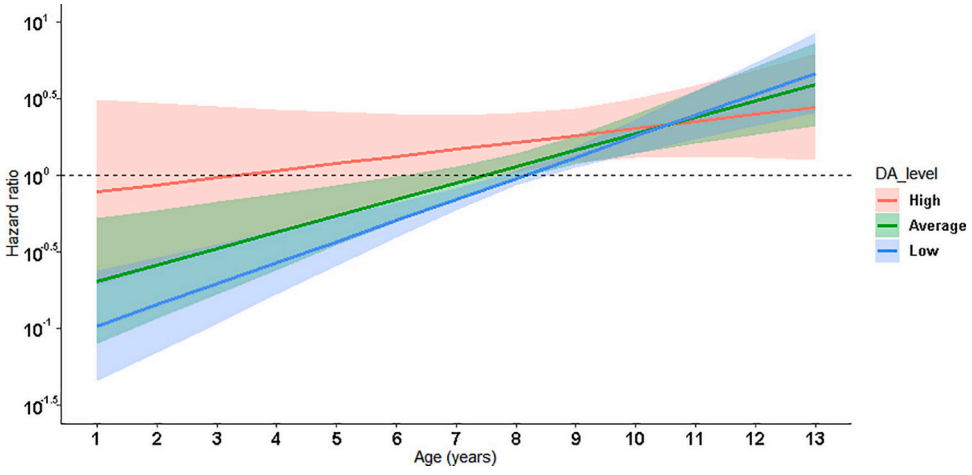

Fig. 5. Risk of fatal cardiomyopathy in sea otters as a function of age and domoic acid (DA) exposure in the time-dependent Cox proportional hazards model. To visualize this interaction, the effect of low (25th percentile; blue line), average (50th percentile; green line), and high (75th percentile; red line) DA exposure on the hazard of fatal cardiomyopathy for otters of different ages are compared. Hazard ratios are presented on the log scale, with the baseline of 10° (dashed line) representing the risk of fatal cardiomyopathy among median-age (7-year-old) otters with average DA exposure, clam and crab consumption, and a negative/low positive *T. gondii* titer. Uncertainty around each hazard ratio (80% confidence interval) is shown as a shaded band for each level of DA exposure. . (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

generation time is approximately 7 – 8 years (Gagne et al., 2018), with prime-age adult otters contributing to the next generation through reproduction and pup care. Survival of prime-age adults, particularly females, is the primary determinant of population growth (Tinker et al., 2006), and thus increased cardiomyopathy mortality within this age class has important implications for future sea otter recovery.

Using data from telemetry-based field studies of radio-tagged sea otters, the Cox proportional hazards model demonstrates that previous exposure to DA impacted individual survivorship by increasing the hazard of fatal cardiomyopathy. To assess the population-level impact of this threat, the findings from this study are being incorporated into an integrated population model, a population projection matrix model that brings together multiple independent data sources related to animal health, population trends, and survival (Tinker et al., 2020). This model is used to project future population estimates of growth and determine the impacts of specific threats, and will be an important aspect of the southern sea otter status review as a threatened species under the Federal Endangered Species Act (USFWS, 2003). The results from both models consistently demonstrate that the risk of cardiomyopathy increases with high DA exposure and this effect disproportionately impacts prime-age adult survival. Quantitative risk assessment models such as these are important management tools for informing conservation efforts aimed at protecting southern sea otters.

Sea otter exposure to environmental DA is challenging to evaluate because toxin testing of biological samples only detects acute exposure, but cardiomyopathy is a progressive disease with acute, subacute, and chronic DA exposure hypothesized as risk factors for various stages of disease expression (Miller et al., 2020b). The first epidemiologic study to propose an association between DA and cardiomyopathy in sea otters classified dead otters as having previous DA exposure if they stranded within a temporal and spatial vicinity of one of four otters that died of acute DA toxicosis (Kreuder et al., 2005). Recent advances in sea otter postmortem evaluations have revealed that sea otters with DA-associated pathology in the central nervous system often have cardiovascular lesions that are progressive, with dilated cardiomyopathy at the far-end of the lesion continuum (Miller et al., 2020b). Chronic DA toxicity based on clinical and pathologic findings has been well-described in sea otters (Miller et al., 2020a,b) and California sea lions (Silvagni et al., 2005; Goldstein et al., 2008; Zabka et al., 2009), yet there has been a lack of research to characterize and evaluate long-term, repeated environmental DA exposure in marine mammals. Biochemical testing has been the standard approach for documenting marine mammal DA exposure, but this method is designed to detect acute exposure and is insufficient for assessing repeated sublethal exposure (Lefebvre et al., 2012), likely under-representing the true extent of environmental toxin exposure. *Pseudo-nitzschia* abundance and shellfish DA levels have been used to assess toxin exposure in association with mortality events (Scholin et al., 2000; Goldstein et al., 2008; de la Riva et al., 2009; Bargu et al., 2010), but this data only describes a 'snapshot' of potential exposure at a particular location and point in time (Lane et al., 2010).

In the present study, these knowledge gaps were addressed using a variety of novel methods. A composite metric for DA exposure was developed by integrating several sources of environmental monitoring data using a novel Bayesian spatiotemporal model. This powerful approach increased sample size, improved the spatial and temporal scope, and leveraged the strengths of each dataset. The broad spatial distribution of sea otter and shellfish data contributed to the spatial autocorrelation of the model, while the fine temporal scale and time-integrative nature of SPATT data improved the temporal autocorrelation and provided crucial information on cumulative toxin levels. For the purposes of modeling survival risk, DA exposure was defined as the mean monthly value over the previous 12 months, which was an important consideration for cardiomyopathy, which is a progressive condition. The inherent structure of a time-dependent Cox proportional hazards model allowed DA exposure to vary over time for each animal in the survival model, rather than being a fixed value. This is the first time these methods have been used to evaluate health impacts of environmental DA and could be useful for evaluating repeated exposure in other wildlife species.

Sea otters have relatively small home ranges, but are mobile between coastal zones (Tarjan and Tinker, 2016), emphasizing the importance of capturing spatial variation in DA exposure in our analyses. Direct observations documented sea otter locations at various time points and a DA exposure value was assigned to individuals at each time-step of the survival model, based on their spatiotemporal overlap with modeled DA values over the previous year. This time-varying risk factor was included in the Cox proportional hazards model, with the counting process formulation allowing DA exposure to change based on the movement patterns of individuals during the study. Both the Bayesian hierarchical and time-dependent Cox models account for variation in DA exposure at the level of the individual and over space and time. For example, sea otter exposure to a short-lived toxic bloom in one location vs. a more prolonged bloom over a larger geographic area is reflected in that individual's DA exposure value at each time-step of the survival model. \.

The results of the Bayesian model for estimating DA exposure showed spatial and temporal variation in monthly DA composite values, although zones 2 – 4 (Monterey Bay) and 11 (Morro Bay) were the most heterogeneous, with both high and low values (Fig. 3). These zones are also the most data-rich, with DA testing information from shellfish and sea otter carcasses (and SPATT in zone 2) (Fig. 2) contributing to more robust estimates of DA composite values. This is in contrast to more rural, rugged sections of the California coastline like zones 6 – 10 (Big Sur), where limited DA surveillance resulted in more homogeneous, average DA estimates. This likely introduced some spatial sampling bias. In the future, additional environmental monitoring in these areas could improve our estimation of coastwide DA exposure.

There are additional DA monitoring datasets that could provide more spatial coverage, such as *Pseudo-nitzschia* abundance; however, phytoplankton data was not used in the DA composite metric because it is less useful for signaling biotoxicity in animals due to the fact that it describes phytoplankton assemblages at a single point in time and only gives circumstantial evidence for toxin accumulation (Lane et al., 2010). Furthermore, a study in California sea lions found that moderate to high numbers of toxic *Pseudo-nitzschia* did not always coincide with DA-related stranding events (Bargu et al., 2010). Sea otters are exposed to DA through consumption of benthic invertebrates, which have variable rates of toxin retention, and cardiomyopathy takes time to develop, indicating that a direct measurement of phytoplankton would not accurately represent exposure. In this study, SPATT provided a time-integrative assessment of cumulative DA, shellfish testing documented DA levels in prey, and sea otter carcass testing demonstrated that otters were directly exposed to DA.

An important study limitation is that DA exposure was assigned based on spatiotemporal overlap of individual sea otter space use patterns and estimated DA levels for a given month and coastal zone. This simplifying assumption indicates that all otters present at a given place and time were similarly exposed, which disregards variation in foraging ecology, reproductive status, and toxin uptake and retention in prey. Ascertainment bias is a potential concern because estimated DA exposure may not be completely accurate. In the Bayesian model, a two-month biological time-lag between DA datasets was selected, but it is recognized that this is just a starting place; realistically, lags are not fixed, but variable in time and space. It is biologically plausible that the intensity of a bloom may predict the length of this time lag. For example, a severe toxic bloom may result in swift and substantial DA bioaccumulation in benthic invertebrates and rapid succession of sea otter exposures or death, while a low-level bloom may take longer to concentrate in prey and impact sea otter health. Additional tools are needed to trace the path of DA through the food web, such as stable isotope analyses, additional monitoring of non-commercial benthic invertebrates, and more high-quality time-series data in regions with toxic

blooms.

The prevalence of antemortem *T. gondii* infection is widespread in free-ranging otters, with 13.3% seropositivity among otters captured from Elkhorn Slough to the Santa Barbara Channel between 1998 – 2013 (Burgess et al., 2018), and is associated with a spectrum of diseases from incidental infection to disseminated toxoplasmosis (Kreuder et al., 2003; Miller et al., 2018, Miller et al., 2020a). Infection with *T. gondii* and *S. neurona* (based on histopathology and/or seropositivity) have previously been identified as risk factors for cardiomyopathy (Kreuder et al., 2005; Miller et al., 2020a), but there was no association between high *S. neurona* titers and fatal cardiomyopathy in this study. High *S. neurona* titers indicate severe or active infection, which is often rapidly fatal due to meningoencephalitis, such that there is no time to develop chronic heart disease; this explanation is supported by previous research that found *S. neurona* seropositivity was a significant predictor of myocarditis, but not dilated cardiomyopathy (Kreuder et al., 2005).

Along the Pacific Coast of North America, elevated levels of DA have been documented in Dungeness crab, rock crab, and razor clams, resulting in extensive and prolonged closures of commercial and recreational fisheries (Wekell et al., 1994; McCabe et al., 2016). Sea otters are at risk of substantial dietary exposure to DA over their lifetimes because they eat large amounts of benthic invertebrates that contain high toxin levels, consuming 20 – 25% of their body mass in prey daily to meet their metabolic demands (Kenyon, 1969). Sea otters eat many of the same marine foods as humans and serve as valuable bioindicators of the potential long-term health effects of repeated DA exposure through the consumption of seafood considered "safe" based on current regulatory limits, which were developed to protect against high-level exposure and acute poisoning (Mariën K, 1996; Ferriss et al., 2017). The findings in sea otters support the recommendation that chronic and repetitive sublethal DA exposure also need to be considered when developing guidelines for seafood consumption in people (Lefebvre et al., 2019).

The systemic and lasting effects of DA are widely recognized in marine mammals, including cardiomyopathy, temporal lobe epilepsy, spatial memory deficits and impaired navigation, reproductive failure, fetal toxicity, and DA transfer through the placenta and milk (Kreuder et al., 2005; Brodie et al., 2006; Goldstein et al., 2008; Ramsdell and Zabka, 2008; Zabka et al., 2009; Rust et al., 2014; Cook et al., 2015; Miller et al., 2020b). Taken together, these findings indicate that DA toxicosis likely has widespread effects that warrant additional investigation. In particular, these chronic syndromes may be associated with recurrent, low-level DA exposure. Recent studies of human health risks associated with repeated DA exposure have focused on chronically-exposed tribal and recreational shellfish harvesters in the U. S. Pacific Northwest, where DA is frequently detected at low levels in razor clams year-round (Grattan et al., 2016; Ferriss et al., 2017; Lefebvre et al., 2019). Adverse effects of repetitive low-dose exposure have been described in epidemiological and laboratory studies, including memory deficits (Grattan et al., 2016), increased neurological sensitivity to the toxin (Lefebvre et al., 2012), and impaired mitochondrial function (Hiolski et al., 2014). Most research has emphasized effects on the central nervous system, without assessing potential cardiovascular impacts. There have been reports of arrhythmia, tachycardia, unstable blood pressure, and acute myocardial infarction in amnesic shellfish poisoning cases (Perl et al., 1990; Teitelbaum et al., 1990; Pulido, 2008); however, cardiac damage may be missed by health care providers and public health agencies because the broader health impacts of DA have not been fully explored. High-frequency shellfish consumers, pregnant women, the elderly, and people with pre-existing cardiovascular and renal disease are uniquely susceptible to DA toxicity (Ramsdell and Zabka, 2008; Grattan et al., 2016; Ferriss et al., 2017; Lefebvre et al., 2019; Burbacher et al., 2019). Future studies investigating the impact of repeated low-level DA exposure in humans could include metrics of cardiovascular health, such as serological biomarkers of cardiac disease and long-term outcomes in high-risk groups.

The conditions that drive harmful algal blooms are complex and the underlying mechanisms remain largely elusive, but there is consensus that large-scale climatic changes will likely favor the production of more geographically extensive and longer-lasting toxic algal blooms (Moore et al., 2008; Wells et al., 2015; McKibben et al., 2017). Predicting exactly how climate drivers will affect spatial and temporal patterns of harmful algal blooms remains challenging (Moore et al., 2008; Wells et al., 2020), but there is evidence of climatic regulation of DA in the west coast of the USA, with the timing of elevated DA in shellfish strongly associated with indices of warm ocean conditions and climate variability (McKibben et al., 2017). The health impacts of DA on marine mammals and humans will also likely intensify if harmful algal blooms become more pervasive with climate change as predicted (McKibben et al., 2017; Moore et al., 2008; Van Dolah, 2000; Wells et al., 2020).

5. Conclusions

These results demonstrate that DA exposure is an important environmental threat underlying fatal cardiac disease in southern sea otters and is most detrimental for prime-age adults, which has important implications for future recovery of this threatened species. Sea otters that specialized in crab and clam also had an elevated risk of death due to cardiomyopathy; these prey types bioconcentrate and slowly excrete DA, potentially resulting in sustained and chronic DA exposure over time. Trophic transfer of DA and its accumulation throughout marine food webs (Lefebvre et al., 1999, 2002; Scholin et al., 2000) emphasize the pervasiveness of this environmental toxin and its potential for detrimental effects on many species. This study developed a novel approach for characterizing toxin exposure by combining several DA surveillance datasets and integrating this with detailed life history data from free-ranging sea otters, including survival, diet preferences, reproduction, movements, protozoal serology, and causes of death. The findings in this study highlight the value of large, long-term environmental and wildlife monitoring datasets to detect chronic health effects of DA. With changing ocean conditions and increasing threats from harmful algal blooms (Moore et al., 2008; Wells et al., 2015; McKibben et al., 2017), there is an urgent need for reliable information on the wide-reaching health impacts of DA exposure on humans and wildlife. The findings presented here suggest that toxin bioaccumulation in the food web may contribute to the development of progressive conditions like cardiac disease in sea otters, raising awareness of under-recognized chronic health effects of recurrent DA exposure.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported by a Morris Animal Foundation Fellowship Training Grant (D17ZO-413) awarded to Megan Moriarty and the National Science Foundation Grant (OCE-1538582). We are grateful to all the collaborating investigators and volunteers who have assisted in sea otter captures, field efforts, and postmortem examinations, but in particular we would like to thank Jim Estes, Brain Hatfield, Ben Weitzman, Gena Bentall, Nicole LaRoche, George Esslinger, Dan Monson, Jim Bodkin, Keith Miles, Liz Bowen, Sarah Espinosa, Sarah Chinn, Alisha Kage, Dave Jessup, Laird Henkel, Mike Harris, Jack Ames, Colleen Young, Eva Berberich, Sharon Toy-Choutka, Frank Wilhelm, RC Mitchell, Jessica Kunz, Katie Greenwald, Angie Reed, Mike Murray, Teri Nicholson, Marissa Young, Erin Lenihan, Anne Tewksbury, Julie Stewart, Mike Kenner, Zach Randell, Laura Yeates, Robin Duncan, Nancy Anderson, Chris Fiorello, Emily Whitmer, Claire Simeone, Heather Harris, Lesanna Lahner, Jen Coffey, and Cara Field. Support for SPATT samplers was provided in part by the Central and Northern California

Ocean Observing System through NOAA award NA16NOS0120021 and Kendra Hayashi compiled the data. Tim Tinker and Joe Tomoleoni were supported by the USGS Ecosystem Mission Area. We appreciate the epidemiological and statistical input of Dan Tancredi, David Rocke, Pranav Pandit, Julie Rushmore, Diego Montecino-Latorre, Karen Holcomb, Nistara Randhawa, Jessica Sanchez, and Peter Sebastian. Special thanks goes to Eunah Cho Preston for help creating the Fig. 1 diagram. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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