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TEMPORAL ASSOCIATION BETWEEN LAND-BASED RUNOFF EVENTS AND CALIFORNIA SEA OTTER (*ENHYDRA LUTRIS NEREIS*) PROTOZOAL MORTALITIES

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ABSTRACT: *Toxoplasma gondii* and *Sarcocystis neurona* have caused significant morbidity and mortality in threatened Southern sea otters (*Enhydra lutris nereis*) along the central California coast. Because only terrestrial animals are known to serve as definitive hosts for *T. gondii* and *S. neurona*, infections in otters suggest a land to sea flow of these protozoan pathogens. To better characterize the role of overland runoff in delivery of terrestrially derived fecal pathogens to the near shore, we assessed the temporal association between indicators of runoff and the timing of sea otter deaths due to *T. gondii* and *S. neurona*. Sea otter stranding records 1998–2004, from Monterey and Estero bays were reviewed and cases identified for which *T. gondii* or *S. neurona* were determined to be a primary or contributing cause of death. Precipitation and stream flow data from both study sites were used as indicators of land-based runoff. Logistic regression was applied to determine if a temporal association could be detected between protozoal mortalities and runoff indicators that occur in the 2 mo preceding mortality events. A significant association was found between *S. neurona* otter deaths at Estero Bay and increased stream flow that occurred 30–60 days prior to mortality events. At this site, the cause of otter mortality following increased river flows was 12 times more likely to be *S. neurona* infection compared with nonprotozoal causes of death. There were no significant associations between the timing of *T. gondii* otter deaths and indicators of overland runoff. Our results indicate that the association between overland runoff and otter mortalities is affected by geography as well as parasite type, and highlight the complex mechanisms that influence transmission of terrestrially derived pathogens to marine wildlife. Policy and management practices that aim to mitigate discharges of contaminated overland runoff can aid conservation efforts by reducing pathogen pollution of coastal waters, which impacts the health of threatened marine wildlife and humans.

Key words: Protozoa, runoff, *Sarcocystis neurona*, sea otter, *Toxoplasma gondii*, water quality.

INTRODUCTION

After near extinction in the early 1900s, the Southern sea otter (*Enhydra lutris nereis*) population has failed to recover as well as other federally protected marine mammal populations (Estes et al., 1996). A major factor for the population's slow recovery has been mortality of prime-age adults from infectious disease (Cole, 1996). Infections with the two protozoan parasites *Toxoplasma gondii* and *Sarcocystis neurona*, are leading causes of deaths in sea otters. These pathogens can cause severe encephalitis and infections with *T. gondii* or *S. neurona* collectively listed as primary causes of death of 23% of beach-cast otter carcasses examined between 1998 and 2001 (Kreuder et al., 2003). In addition to causing mortality in sea otters, *T. gondii* is a zoonotic pathogen that infects up to one third of the human population (Hill and Dubey, 2002). Although infection is most often asymptomatic, death due to disseminated disease can occur in immunocompromised humans, and acute infection during pregnancy can result in abortion or congenital disease in the fetus (Jones et al., 2003). *Sarcocystis neurona* is not known to infect humans but is one of the main causes of equine protozoal myeloencephalitis (EPM), resulting in substantial morbidity and mortality of affected animals (Fenger, 1997).

Infection of sea otters with *T. gondii* and *S. neurona* has been puzzling because

the only recognized definitive hosts of these parasites are felids (domestic and wild cats) and opossums (*Didelphis virginiana* and *D. albiventris*), respectively (Dubey et al., 1970; Fenger et al., 1995). These terrestrial mammals are the only species capable of shedding infective oocysts (*Toxoplasma*) or sporocysts (*Sarcocystis*) in their feces, which are the environmentally resistant stages of the parasites. Domestic cats and opossums were introduced to California in the early 1900s, and their populations have grown in association with coastal urbanization (Dijak, 2000; Clarke, 2002), increasing the likelihood of oocyst and sporocyst contamination of the nearshore marine environment. Because *T. gondii* and *S. neurona* require terrestrial hosts for completion of their life cycles, transmission of these parasites to sea otters indicates a land-to-sea movement of parasites. The route of infections with *T. gondii* and *S. neurona* in sea otters is uncertain. Ingestion of tissue cysts from an intermediate host is not likely to play a major role in disease transmission because sea otters do not typically prey on warm-blooded animals known to be involved in the parasites' life cycles (Estes et al., 2003). Accidental ingestion of oocysts from water or invertebrates that can filter and concentrate them in their tissues has been suggested as a likely route of infection for otters (Arkush et al., 2003). Miller et al. (2002a) implicated freshwater runoff as a risk factor for exposure of sea otters to *T. gondii*. The same authors also identified Elkhorn Slough and Estero Bay, two embayments along the central California coast that receive input of land-based runoff, as high-risk sites for sea otter infection and mortality from *T. gondii*. Johnson et al. (2009) further identified a stretch of coastline that encompasses Estero Bay (Cambria to San Simeon) as a high-risk site of otter infection with *T. gondii*, and southern Monterey Bay as a high-risk site for exposure to *S. neurona*.

The current understanding of *T. gondii* and *S. neurona* biology suggests that infection in sea otters is most likely to occur through exposure to oocysts (*T. gondii*) or sporocysts (*S. neurona*) that contaminate the nearshore environment through land-based runoff. Direct detection of *T. gondii* and *S. neurona* in runoff is hindered by a lack of available methods for recovering and visualizing oocysts and sporocysts in large volumes of water. Accepted methods for detection of related protozoan parasites such as *Cryptosporidium* and *Giardia* in environmental water samples utilize monoclonal antibodies to concentrate the parasites from water by immunomagnetic separation and for visualization of the oocysts and cysts microscopically with a direct florescence test (Environmental Protection Agency, 2001). In the absence of specific antibodies to the environmental stages of *T. gondii* and *S. neurona*, similar methods for concentration and detection of these parasites in contaminated water are not available. An alternative approach to assess the role of runoff in the influx of these parasites into coastal waters is to evaluate the association between runoff events and the timing of otter mortalities attributable to protozoal infection. We tested the hypothesis that temporal associations can be detected between land-based runoff events and sea otter deaths due to *T. gondii* and *S. neurona*. Our objectives were to characterize the relationship between runoff and protozoal-associated sea otter mortalities, and to provide additional insight into the possible transport route of terrestrially derived zoonotic pathogens to marine wildlife.

MATERIALS AND METHODS

Sea otter case selection

We studied sea otter carcasses recovered at two regions along the central California coast. The Monterey Bay stranding location included carcasses recovered from Santa Cruz (36°56′N, 122°3′W) to Pacific Grove (36°38′N, 121°56′W), and the Estero Bay stranding location included carcasses recovered

from San Simeon (35°35′N, 121°7′W) to Point Bichon (35°15′N, 120°53′W; Fig. 1). These study sites were selected based on previous work showing high otter mortality or exposure to *T. gondii* and *S. neurona* in these regions (Miller et al., 2002a; Kreuder et al., 2003; Kreuder et al., 2005; Miller et al., 2010). All fresh carcasses recovered in good postmortem condition (postmortem interval <72 hr) from the two study sites between January 1998 and December 2004, and that received a full necropsy by pathologists, were considered for inclusion in the study. Carcasses were necropsied at the California Department of Fish and Game Marine Wildlife Veterinary Care and Research Center, Santa Cruz, California. Protozoal mortality cases were selected if *T. gondii* or *S. neurona* encephalitis were identified as the primary or contributing cause of death according to the following case definition. A histopathologic diagnosis of "protozoal encephalitis" (PE) was defined when moderate to severe inflammation of brain tissue was observed in association with proliferating protozoa (Kreuder et al., 2003). *Toxoplasma gondii* mortality cases were defined based on presence of multifocal, lymphoplasmacytic protozoal encephalitis on histopathology, combined with *T. gondii* isolation in cell culture from brain or positive staining on immunohistochemistry (IHC; Miller et al., 2002b). *Sarcocystis neurona* cases were similarly defined based on presence of miliary and pleocellular pattern of protozoal encephalitis, combined with *S. neurona*-positive cell culture or IHC for brain tissue. Protozoal infections were classified as the primary cause of death if the *T. gondii* or *S. neurona*-associated lesions were considered extensive enough to have initiated the sequence of events leading directly to death, without evidence of other causes of death. Classification of these infections as a contributing cause of death occurred when the protozoal-associated pathology was severe enough to have increased the probability of death. Such cases include otters for which shark attacks or boat strikes were listed as a primary cause of death, but who also had moderate to severe protozoal encephalitis. This classification approach was used in a prior study that demonstrated statistical associations between moderate to severe brain inflammation and sea otter deaths due to trauma (Kreuder et al., 2003). Because current serologic assays for exposure to *T. gondii* or *S. neurona* in sea otters do not differentiate recent from chronic infections, we did not include this parameter in establishing the case definition for PE deaths. Antibody production against *T. gondii* is thought to be life-long, and

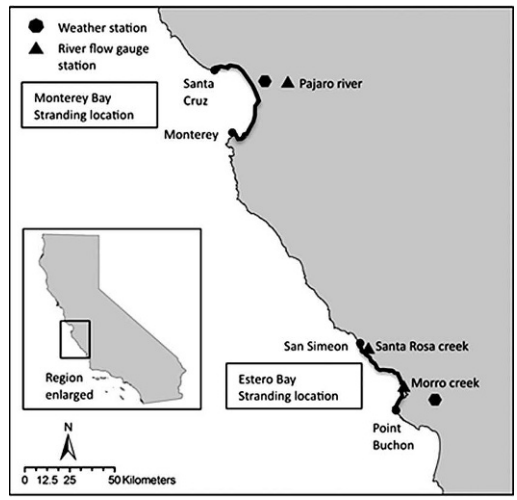

FIGURE 1. Map of central California, USA showing the two sea otter stranding (*Enhydra lutris nereis*) locations where mortalities were evaluated, 1998–2004. Weather and river-flow gauge stations from which precipitation data and river flow rates were obtained for the two stranding sites are displayed. The delineated Monterey Bay and Estero Bay stranding regions were previously determined to be high-risk sites of otter infections with *Toxoplasma gondii* and *Sarcocystis neurona*. River flow data from two creeks had to be combined for Estero Bay, because data from any single waterway for the entire 6-yr study were not available at this site.

the majority of stranded otters at the selected study sites were antibody-positive for this parasite (Miller et al., 2002a).

Otters that were determined to die from nonprotozoal causes were used as a comparison mortality group. Mortalities were classified into the comparison group if their primary or contributing cause of death was determined not to be associated with *T. gondii* or *S. neurona* infection, and if the otter was not exposed to either parasite based on serologic testing. Only otters with negative tissue culture, negative IHC, and titers <1:320 for both protozoal pathogens were included in the comparison mortality group (Miller et al., 2002b). Although antibody detection was not used in establishing PE death cases as mentioned above, antibody-positive cases were excluded from the comparison mortality group to reduce potential error due to misclassification bias (where pathologic lesions might have been missed during postmortem examination). Otters in the comparison group were collected during the same time period and from the same geographic range as cases.

Runoff data

Surface runoff refers to water that flows over land following rainfall events, eventually collecting in a receiving water body, such as a storm-water drainage, river, or ocean. Environmental indicators that can be associated with land-based runoff events, including precipitation and river flow rates, were evaluated for Monterey Bay and Estero Bay between 1998 and 2004. Daily precipitation data (millimeters) were obtained through the California Irrigation Management Information System (CIMIS). For Monterey Bay, precipitation data were obtained from the Pajaro station (Station 129; 36°54′N, 121°44′W), and for Estero Bay data were obtained from the San Luis Obispo station (Station 52; 35°18′N, 120°39′W). For the Monterey Bay region, daily stream flow measurements (m^3/sec) were obtained from the Pajaro River using data from the Pajaro Chittenden station (36°54′N, 121°35′W), which is monitored by the United States Geological Survey (USGS, 2011). Such online data from one stream for the time period investigated in this study were not available for the Estero Bay region. Instead, a combination of daily flow data from two streams (Santa Rosa Creek [35°34′N, 121°04′W] and Morro Creek [35°22′N, 120°51′W]) in the Estero Bay region were used, which were provided by the San Luis Obispo County Department of Public Works (Fig. 2).

Statistical analysis

Univariable and multivariable logistic regressions were used to investigate associations between sea otter deaths due to *T. gondii* or *S. neurona,* and potential measures of land-based runoff events that could deliver terrestrial pathogens to coastal waters. Proxy variables for runoff included precipitation and river flow. Precipitation was used as a continuous variable that was log-transformed to normalize its distribution. River flow was included as a dichotomous variable, with low flow designated for each river as flow less than the maximum flow rate observed in that stream under dry summer conditions (as determined for the six dry periods included in the study), and high flow designated as flow rates greater than that value. For each otter (case or comparison), precipitation and river flow data were evaluated at the stranding location from which the carcass was recovered in the 30 days, 30–60 days, and 60 days prior to the date of stranding or carcass recovery. Lag intervals were selected based on the suspected incubation time (7–30 days) of *T. gondii* and *S. neurona*, which was derived from relevant literature on waterborne toxoplasmosis in humans (Benenson et al., 1982), and oocyst- or sporocyst-induced experimental infections in other species (Sofaly et al., 2002; Quirk and Dubey, 2008). An additional lag of up to 30 days was provided to examine and account for the estimated time needed for parasites to become entrained in runoff and deposited in seawater, and for an otter to encounter and ingest the parasites once they were deposited in coastal waters following runoff events. In dichotomizing river flow time intervals, high flow intervals were assigned if the flow rate exceeded dry conditions at any date within that interval. Thus, for each otter the risk of death due to protozoal infection following precipitation events (total rainfall) or river flow (high or low) was tested for three separate time intervals. Demographic variables including sea otter age, sex, and stranding location were included in multivariable analyses. Models for *T. gondii* and *S. neurona* were assessed separately because of differences in the parasites' life cycles and likely differences in disease pathogenesis.

Statistical analyses were performed using Stata 10.0 (Stata-Corp., College Station, Texas, USA). Univariable analyses were performed using the Fisher's exact test for dichotomous variables (river flow, age, sex, and location) or univariable logistic regression for continuous variables (precipitation). Variables with $P<0.25$ were considered in subsequent multivariable logistic models. Age and sex were forced into the multivariable model as confounders, based on prior research that demonstrated these demographic parameters to be significantly associated with protozoal-associated mortalities in sea otters (Miller et al., 2002a; Kreuder et al., 2003). A purposeful selection model-building strategy (Hosmer and Lemeshow, 2000) was used and variables were retained in the model when $P\leq0.05$. Potential confounding variables were evaluated in the model by assessing whether their removal resulted in a >10% change in the coefficient estimates of the main effect variables. The Hosmer and Lemeshow goodness-of-fit test statistic was used to evaluate overall fit of the final model.

RESULTS

From 1998–2004, 128 otter carcasses from the Monterey Bay stranding site and 73 carcasses from the Estero Bay stranding site were necropsied in good postmortem condition. Of these, 14 otters in each

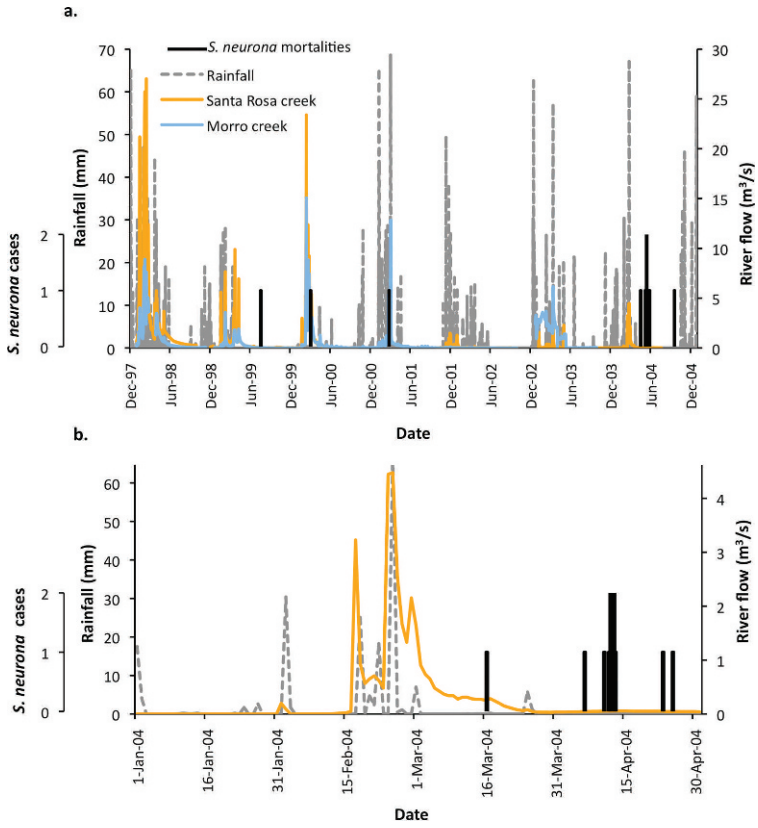

FIGURE 2. Rainfall, river flow, and temporal distribution of *Sarcocystis neurona*-associated sea otter (*Enhydra lutris nereis*) deaths in Estero Bay, California, USA displayed for the entire study period 1998–2004 (a), and a higher resolution view highlighting the Spring, 2004 *S. neurona* outbreak (b).

location were determined to have died due to *T. gondii* (11% and 19% in Monterey and Estero Bays, respectively). *Sarcocystis neurona* was implicated as the cause of death in 18 otters (14%) from Monterey Bay and 15 otters (21%) from the Estero Bay region. In each stranding location, three otters were determined to have died from concurrent *T. gondii* and *S. neurona* infection, characterized by the presence of moderate to severe brain lesions associated with both parasites. Because statistical analyses were performed separately for the two parasites, dual cases were included with each model (i.e., six of the 33 *S. neurona* cases were dually infected with *T. gondii*, and six of the 28 *T. gondii* cases were dually infected with *S. neurona*). Otters that matched the comparison mortality group definition totaled 30 (23%) for Monterey and 14 (19%) for Estero Bay.

Results from the univariable evaluation of risk factors for protozoal deaths in sea otters are displayed in Table 1 for *T. gondii* and Table 2 for *S. neurona.* When otters from both stranding locations were compared with nonprotozoal mortalities, the odds of otters dying due to *T. gondii* infection were four times greater for adults than juveniles ($P=0.01$). In Monterey Bay, adult age was positively associated with *T. gondii* mortality (odds ratio, 6.3; $P=0.02$), and total rainfall 60 days prior to stranding was negatively associated with *T. gondii* mortality. None of the predictor variables were significantly associated with *T. gondii* death for otters stranding at Estero Bay. For *S. neurona* mortalities, demographic or environmental

TABLE 1. Univariable odds ratios and 95% confidence intervals (CI) of *Toxoplasma gondii* mortality associated with demographic and environmental parameters in sea otters (*Enhydra lutris nereis*) for both stranding locations combined, and for Monterey and Estero Bays (California, USA) separately, 1998–2004.

Location	Variable	Reference	Odds ratio	95% CI	*P* value[a]
Combined ($n=72$)	Sex (Female)	Male	0.62	0.20–1.86	0.45
	Age (Adult)	Juvenile	4.08	1.34–12.77	0.01*
	Location (Estero)	Monterey	2.14	0.72–6.35	0.14*
Time prior to mortality	Riverflow (High)	Low			
	30 days		0.68	0.22–2.05	0.62
	30–60 days		1.05	0.37–3.03	1.00
	60 days		0.77	0.24–2.41	0.80
Time prior to mortality	Rainfall (Total mm)	Continuous			
	30 days		0.58	0.31–1.10	0.09*
	30–60 days		0.90	0.50–1.63	0.74
	60 days		0.68	0.40–1.19	0.18*
Monterey Bay ($n=44$)	Sex (Female)	Male	0.52	0.10–2.40	0.51
	Age (Adult)	Juvenile	6.33	1.24–41.22	0.02*
Time prior to mortality	Riverflow (High)	Low			
	30 days		0.63	0.13–2.77	0.53
	30–60 days		0.58	0.13–2.54	0.52
	60 days		0.52	0.10–2.40	0.51
Time prior to mortality	Rainfall (Total mm)	Continuous			
	30 days		0.48	0.20–1.14	0.10*
	30–60 days		0.52	0.21–1.24	0.14*
	60 days		0.41	0.19–0.91	0.03*
Estero Bay ($n=28$)	Sex (Female)	Male	0.74	0.12–4.33	1.00
	Age (Adult)	Juvenile	3.33	0.55–21.66	0.25
Time prior to mortality	Riverflow (High)	Low			
	30 days		1.00	0.14–7.07	1.00
	30–60 days		3.33	0.55–21.66	0.25
	60 days		2.40	0.27–30.85	0.65
Time prior to mortality	Rainfall (Total mm)	Continuous			
	30 days		0.48	0.26–1.90	0.48
	30–60 days		1.44	0.58–3.58	0.43
	60 days		1.14	0.47–2.79	0.77

[a] Univariable analyses were conducted using Fisher's exact test for categorical variables, and logistic regression for continuous variables.

* Variables with $P<0.25$ were considered in the multivariable logistic model.

predictor factors were not significantly associated with death in the analysis that combined cases from both stranding locations, as well as for otters from Monterey Bay. However, considering otter deaths from Estero Bay only, otters were 10 times ($P=0.01$) more likely to die from *S. neurona* infection compared with nonprotozoal mortalities following high river flows that occurred in the 1–2 mo preceding death.

After age was incorporated into the multivariable logistic model, none of the environmental predictor variables were significantly associated with *T. gondii* mortality. However, after accounting for otter age, sex, and location, multivariable analysis on animals from both stranding locations showed that high river flow in the preceding 30–60 days was associated with nearly four times higher odds of *S. neurona*-associated otter death, when compared to nonprotozoal mortalities ($P=0.02$). When stratified by stranding location, this association was only significant for Estero Bay, where high river flow was associated with a about a 12-fold

TABLE 2. Univariable odds ratios and 95% confidence intervals (CI) of *Sarcocystis neurona* mortality associated with demographic and environmental parameters in sea otters (*Enhydra lutris nereis*) for both stranding locations combined, and for Monterey and Estero Bays (California, USA) separately, 1998–2004.

Location	Variable	Reference	Odds ratio	95% CI	*P* value[a]
Combined ($n=77$)	Sex (Female)	Male	1.10	0.40–2.99	1.00
	Age (Adult)	Juvenile	2.32	0.83–6.50	0.10*
	Location (Estero)	Monterey	1.79	0.63–5.04	0.24*
Time prior to mortality	Riverflow (High)	Low			
	30 days		1.20	0.44–3.30	0.82
	30–60 days		2.85	0.96–8.88	0.06*
	60 days		1.10	0.38–3.14	1.00
Time prior to mortality	Rainfall (Total mm)	Continuous			
	30 days		0.77	0.41–1.45	0.42
	30–60 days		1.82	0.99–3.35	0.06*
	60 days		1.31	0.72–2.38	0.37
Monterey Bay ($n=48$)	Sex (Female)	Male	1.31	0.35–4.95	0.77
	Age (Adult)	Juvenile	3.45	0.87–14.37	0.07*
Time prior to mortality	River flow (High)	Low			
	30 days		2.29	0.59–9.40	0.24*
	30–60 days		1.50	0.36–6.84	0.75
	60 days		1.63	0.43–6.26	0.55
Time prior to mortality	Rainfall (Total mm)	Continuous			
	30 days		0.74	0.33–1.65	0.47
	30–60 days		1.31	0.57–2.99	0.52
	60 days		0.94	0.43–2.05	0.87
Estero Bay ($n=29$)	Sex (Female)	Male	0.89	0.16–4.96	1.00
	Age (Adult)	Juvenile	1.67	0.28–10.73	0.70
Time prior to mortality	River flow (High)	Low			
	30 days		0.63	0.07–4.77	0.68
	30–60 days		10.00	1.42–80.36	0.01*
	60 days		0.92	0.06–14.67	1.00
Time prior to mortality	Rainfall (Total mm)	Continuous			
	30 days		0.80	0.27–2.34	0.68
	30–60 days		2.45	0.94–6.41	0.07*
	60 days		1.92	0.73–5.02	0.18

[a] Univariable analyses were conducted using Fisher's exact test for categorical variables, and logistic regression for continuous variables.

* Variables with $P<0.25$ were considered in the multivariable logistic model.

increase in risk of otter death due to *S. neurona* ($P=0.01$; Table 3).

DISCUSSION

The significant association between increased river flows in Estero Bay and sea otter deaths attributable to *S. neurona* suggests that otters were exposed to environmentally persistent sporocysts through contaminated land-based runoff at this stranding site. A seasonal pattern for *S. neurona*-associated deaths in sea otters has been described to occur during late spring and early summer (Kreuder et al., 2003). In addition, a mortality outbreak caused by *S. neurona* occurred near the town of Morro Bay (located within Estero Bay) in April, 2004 (Fig. 2b; Miller et al., 2010). In the outbreak, 15 of 16 examined otters were determined to have died from *S. neurona* infection within a period of several weeks. Our finding that *S. neurona*-associated sea otter deaths are significantly associated with increased river flows in Estero Bay but not in Monterey Bay suggests that unique condi-

TABLE 3. Final multiple variable logistic model of *Sarcocystis neurona* mortality associated with demographic and environmental parameters in sea otters (*Enhydra lutris nereis*). Results are shown for mortalities stratified by stranding location in California, USA, 1998–2004.

Risk factor	Odds ratio	95% confidence interval	*P* value	GOF[a]
Estero Bay stranding location ($n=29$)				0.74
Sex (female)	1.29	0.22–7.77	0.78	
Adult age	2.77	0.39–19.65	0.31	
River Flow 30–60 days	12.40	1.92–80.21	0.01	
Monterey Bay stranding location ($n=48$)				0.69
Sex (female)	1.09	0.31–3.88	0.89	
Adult age	3.49	0.98–12.37	0.11	
River Flow 30–60 days	1.65	0.43–6.33	0.47	

[a] Hosmer and Lemeshow (2000) goodness-of-fit *P* value.

tions between the sea otter hosts, environmental factors, geography, and *S. neurona* strain pathogenicity or ecology might be involved in the epidemiology of this pathogen in the nearshore.

Diet has can play a key role in exposure of sea otters to both *S. neurona* and *T. gondii* (Johnson et al., 2009) and the distribution of different prey items at the two stranding locations might alter transmission likelihood of protozoan pathogens following a contamination event. Razor clams in Estero Bay were proposed as possible dietary source of *S. neurona* in the Spring 2004 outbreak (Miller et al., 2010). Bivalves can concentrate infective *T. gondii* oocysts in their tissues (Arkush et al., 2003), and a similar food-borne transmission mechanism of parasites from contaminated water to otters is likely to play a role in *S. neurona* epidemiology. A recent study indicated that foraging on clams was linked with exposure to *S. neurona* (Johnson et al., 2009). In the same study a diet consisting of >10% marine snails (but not bivalves) was highly correlated with *T. gondii* exposure in sea otters. Thus, the spatial combination of contaminated land-based runoff sources with distribution of prey species that are able to concentrate infective protozoal stages in their tissues might contribute to explicit geographic zones where otters are at greater risk of infection from terrestrial pathogens.

In addition to environmental factors, host characteristics can contribute to likelihood of disease. Site fidelity at high-risk sites would further facilitate infection and potential pathology from land-based pathogens. The Estero Bay stranding region encompasses a stretch of coastline from Cambria to San Simeon, which has a high dependent pup-to-adult ratio, indicating a predominance of breeding adults (USGS, 2010). In contrast, otter demographics within the majority of Monterey Bay are characterized by a low pup-to-adult ratio, suggesting otters in this location encompass a migratory, non-breeding population. Site-fidelic otters defending a breeding territory and foraging in a smaller area might in fact be more likely to develop disease and die if their smaller home range is located near a highly contaminated stretch of coastline that puts them at risk of repeated exposure to high pathogen loads.

The difference in association between *S. neurona* deaths and runoff events at the two stranding locations also might be due to spatial variability in pathogen loading and strain type present within the two distinct coastal locations. *Sarcocystis neurona* sporocyst shedding by opossums varies significantly by location (Rejmanek et al., 2009), and the two coastal sites might harbor opossums with different *S. neurona* shedding rates. Variability of *S. neurona* strain virulence also could influ-

ence likelihood of otter death following exposure to the parasite. Distinct *S. neurona* strains were isolated from stranded otters from the Monterey and Estero bay regions, suggesting that clonal expansion of a virulent *S. neurona* strain might have contributed to the Spring 2004 sea otter epizootic in Estero Bay (Wendte et al., 2010). Once shed in feces, transport of protozoal parasites from land to sea also depends on local topography and watershed characteristics (Shapiro et al., 2009). Depending on regional weather patterns and *S. neurona* fecal shedding rates, the time when terrestrial loading with the parasite is highest might or might not coincide with rainfall-driven runoff events. Highest concentrations of contaminants in surface runoff typically occur during a "first flush" event following a long, dry summer (Lee et al., 2002). After the ground has been saturated by precipitation, overland runoff flushes contaminants that have accumulated on land during the dry season into waterways and through estuaries to the ocean (Asaf et al., 2004). Thus, the highest potential for coastal water contamination with *S. neurona* may occur when first flush events coincide with peak seasonal shedding of *S. neurona* by opossums. Seasonal sporocyst shedding by opossums may peak during spring months (Rejmanek et al., 2009). A wet spring weather pattern that potentially overlapped with the peak timing for sporocyst shedding by opossums occurred in Estero Bay during Spring 2004, when as many as 40 sea otter deaths were attributed to *S. neurona* infection (Miller et al., 2010; Fig. 2).

The lack of association between land-based runoff and sea otter deaths attributable to *T. gondii* infection might be due to study design factors, alternate mechanisms or yet undiscovered cycles of pathogen exposure and transmission, or the complex pathophysiology of *T. gondii* in otters, where death might not occur within a short time after exposure to the parasite. One study variable that might limit our ability to detect relationships between runoff events and sea otter death is the nature of the data from which information on river flow could be obtained. In Estero Bay flow data were obtained from Santa Rosa and Morro creeks, highly seasonal streams where flow is driven by rainfall events. In contrast, irrigation and small dams influence flow rate of Pajaro River into Monterey Bay. Fluctuations in the Pajaro river dataset are therefore not as intimately associated with rainfall events as the two streams monitored in Estero Bay.

Although we found no statistical associations between sea otter deaths due to *T. gondii* infection and runoff events, other studies support the hypothesis of a land to sea flow of this zoonotic parasite (Miller et al., 2002a; Conrad et al., 2005). Unlike *S. neurona, T. gondii* might have a more chronic onset of disease in sea otters, similar to the pathophysiology of toxoplasmosis in humans (Hill and Dubey, 2002). Current data suggest that otters can be subclinically infected with *T. gondii*, but can develop clinical signs and die from parasite recrudescence at a later time (Miller, 2008). Establishing relationships between the timing or location of pathogen exposure and subsequent otter mortality is especially challenging for pathogens with a chronic onset of disease due to otter movement along the central California coastline. In addition, our study is based on carcasses that are preferentially recovered from easily accessible sandy beaches (Kreuder et al., 2003). However, carcasses can drift to locations away from the initial site of pathogen exposure as a result of weather patterns, currents, and coastal topography.

The association between increased river flow and *S. neurona* mortalities in sea otters provides further evidence that the likely route of transmission of this parasite into the coastal environment is through contaminated overland runoff. The lack of association between river flow and *T. gondii* mortalities, as well as the finding

that exposure to increased river flow is not associated with *S. neurona* deaths at Monterey Bay, highlight the complex epidemiology of these pathogens in marine wildlife. Watershed characteristics, prey availability, pathophysiology, pathogen virulence, and host behavior are some of the factors that collectively determine whether a marine animal becomes infected with, and eventually dies from, terrestrially derived pathogens. Sea otter deaths due to *T. gondii* and *S. neurona* highlight the potentially fatal outcome of coastal pathogen pollution in threatened wildlife. In addition to marine animals, human well-being is also intimately linked to adequate water quality of coastal habitats. Human populations worldwide depend on marine resources for food and gain economic and social value from the seas through recreation. Insight into pathogen transmission mechanisms from land to sea is essential for implementing monitoring strategies, management practices, and policy decisions for protecting wildlife and human health that depend on clean nearshore waters.

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