Supporting Information

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SI Text

Mechanisms of Dietary Exposure. Strong correlations between diet composition and protozoal infection in southern sea otters suggest a mechanism linking foraging behaviors with the probability of pathogen infection. In terrestrial systems, the only intermediate hosts propagating *T. gondii* and *S. neurona* infections are endothermic vertebrates (1–3). Sea otters in California feed almost exclusively on ectothermic invertebrates and do not prey on any currently recognized intermediate hosts for either pathogen. Direct ingestion of oocysts in seawater or filter-feeding bivalves has been proposed as a biologically plausible source of sea otter infections with *T. gondii* (4–6). Both field and laboratory studies have demonstrated the ability of filter-feeding marine invertebrates to concentrate other protozoa, such as *Giardia* and *Cryptosporidium* (7–10), in their gills and gastrointestinal tract. In the laboratory, California mussels (*Mytilus californianus*) and eastern oysters (*Crassostrea virginica*) have also been shown to remove *T. gondii* oocysts from experimentally contaminated water tanks (11, 12). New methods that use antibodies to concentrate sporocysts hold promise for detection of *T. gondii* in water (13), but these methods require further development to detect oocysts at exceedingly low concentrations in naturally contaminated water samples or ectothermic transport hosts in which the pathogen does not multiply. A recent field study by Miller *et al.* (14) screened 1,396 marine and estuarine invertebrates (mussels, clams, and sand crabs) collected predominantly during the rainy season near heavy freshwater outflows in central California with large populations of domestic cats, and type X *T. gondii* was detected in the digestive tract of just one sentinel mussel (*Mytilus californianus*) outplanted in an estuary draining into Monterey Bay. Our preliminary efforts to detect *T. gondii* in marine snails with known spiked quantities of oocysts were mostly unsuccessful at environmentally relevant concentrations, suggesting that these mucous-secreting benthic invertebrates may further inhibit the diagnostic sensitivity of tests available at commercial and research laboratories that have been successful in screening bivalves. These findings indicate that current methods to detect *T. gondii* (and likely *S. neurona*) may not be useful for epidemiologic studies until technology to effectively concentrate and detect oocysts in sea water, sediment, and invertebrate prey is further developed.

Because available diagnostic tests preclude the direct testing of marine snails as potential transport hosts for *T. gondii* infection in sea otters, it is important to explore alternate explanations. Although we controlled statistically for some factors that might alternatively explain trophic patterns in pathogen exposure, we cannot rule out the possibility that specialization on marine snails is linked to accompanying high-risk behaviors or microhabitat use at a scale that could not be measured. The feeding ecology of the prey themselves may be associated with differential risk of encountering oocysts. Although both snails and abalone are herbivorous gastropods that graze primarily on macroalgae, snails are mobile and move along the kelp stipe or blades while grazing (15), whereas abalone reside in crevices and wait for drifting kelp to minimize risk of predation in areas with high sea otter density. Marine snail species tend to segregate vertically along kelp (16), but observations of otter foraging events are consistent with otters preying on any one of the three subtidal *Tegula* spp common along the central coast (*T. brunnea, T. montereyi,* or *T. pulligo*). Our direct observations of sea otter foraging activities and data retrieved from time-depth recorders indicate that snail-feeding otters dove to a mean depth of 8 m before returning to the surface with snails (17), suggesting that otters were feeding primarily on *Tegula* spp. gathered at the base of the kelp stipe or along the kelp forest floor. Otters feeding on abalone dove to similar (or slightly deeper) depths, but prey capture rates were much lower (17). In fact, correlations between diet-type and pathogen exposure might be explained simply as a function of prey size, because sea otters must eat hundreds of individual small prey, such as snails, but eat relatively few abalones to fulfill their daily caloric requirements (18). If each prey item was equally likely to be harboring an infective oocyst, smaller prey consumed by the hundreds would effectively pose a higher risk. Therefore, although marine snails warrant further investigation with improved laboratory detection techniques, they are not likely the only prey source exposing sea otters to *T. gondii*, nor could they be a source of infection for other marine mammals, such as sea lions, seals, and dolphins, that forage predominantly on fish.

Most otters were infected with protozoal pathogens by the time of first capture, so it is also possible that *T. gondii*-infected otters switched prey and specialized on marine snails because they were compromised by illness and unable to forage effectively or capture other prey. Brain infections with *T. gondii* have been shown to alter host behavior, specifically in mice and rats by blocking their innate aversion to cat urine (19). Likewise, sea otters with moderate or severe encephalitis caused by *T. gondii* were more vulnerable to attack by sharks (20). Although behavioral modification in parasitized otters cannot be ruled out, sea otters involved in this study were observed closely in the field and none exhibited signs consistent with encephalitis (muscle tremors, abnormal motor function, recurrent seizures). In fact, marine snail specialists in this study survived the entire follow-up period and we did not find evidence of deteriorating health during examinations at subsequent captures. Rather, sea otters specializing on marine snails maintained their territories, bred effectively and, based on analyses that combined observed prey capture rates with prey biomass and calorific density measurements (21), experienced rates of energy gain while foraging that were comparable to otters using alternate prey specializations.

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Table S1. Demographic, behavioral, and environmental risk factors evaluated for their association with protozoal pathogen infection in 118 radio-tagged sea otters

Risk factor	Categories	*N*	*T. gondii* prevalence, %	*S. neurona* prevalence, %
Dependent variables				
T. gondii infection	Positive	56	-	-
	Negative	62	-	-
S. neurona infection	Positive	39	-	-
	Negative	79	-	-
Independent variables				
Age class	Immature	17	24	18
	Adults	101	52	36
Sex	Males	48	46	40
	Females	70	49	29
Mean 90-day move	<1 km	18	50	28
	1–2 km	24	38	46
	3–4 km	21	57	29
	5–15 km	19	53	37
	16–50 km	13	31	38
	51–190 km	16	44	31
Abalone specialists*	Abalone <10% of diet	53	59	40
	Abalone >10% of diet	8	13	0
Clam specialists*	Clams <10% of diet	46	54	33
	Clams >10% of diet	15	47	40
Snail specialists*	Snails <10% of diet	51	45	35
	Snails >10% of diet	10	90	30
Offshore habitat use*	<20% feeding offshore	40	43	33
	20–50% feeding offshore	12	58	33
	>50% feeding offshore	9	89	44
Exposed rocky cliff habitat use*	<20% feeding along rocky cliff	55	49	31
	>20% feeding along rocky cliff	6	83	67
Rocky bench habitat use*	<20% feeding in rocky bench	28	61	39
	>20% feeding in rocky bench	33	46	30
Developed shore habitat use*	Feeding elsewhere in range	51	55	28
(soft substrate)	Feeding in developed shore	10	40	70
Capitola to Seaside	Never in Capitola to Seaside	79	53	23
	Observed in Capitola to Seaside	39	36	54
Monterey Peninsula	Never in Monterey Peninsula	71	58	21
	Observed in Monterey Peninsula	47	32	51
San Simeon/Cambria	Never in San Simeon/Cambria	58	35	40
	Observed San Simeon/Cambria	60	60	27

*Based on feeding observations, data only available for subset of 61 otters with >300 observed foraging dives.