Toxoplasma transmission in marine ecosystems: Implications for public health

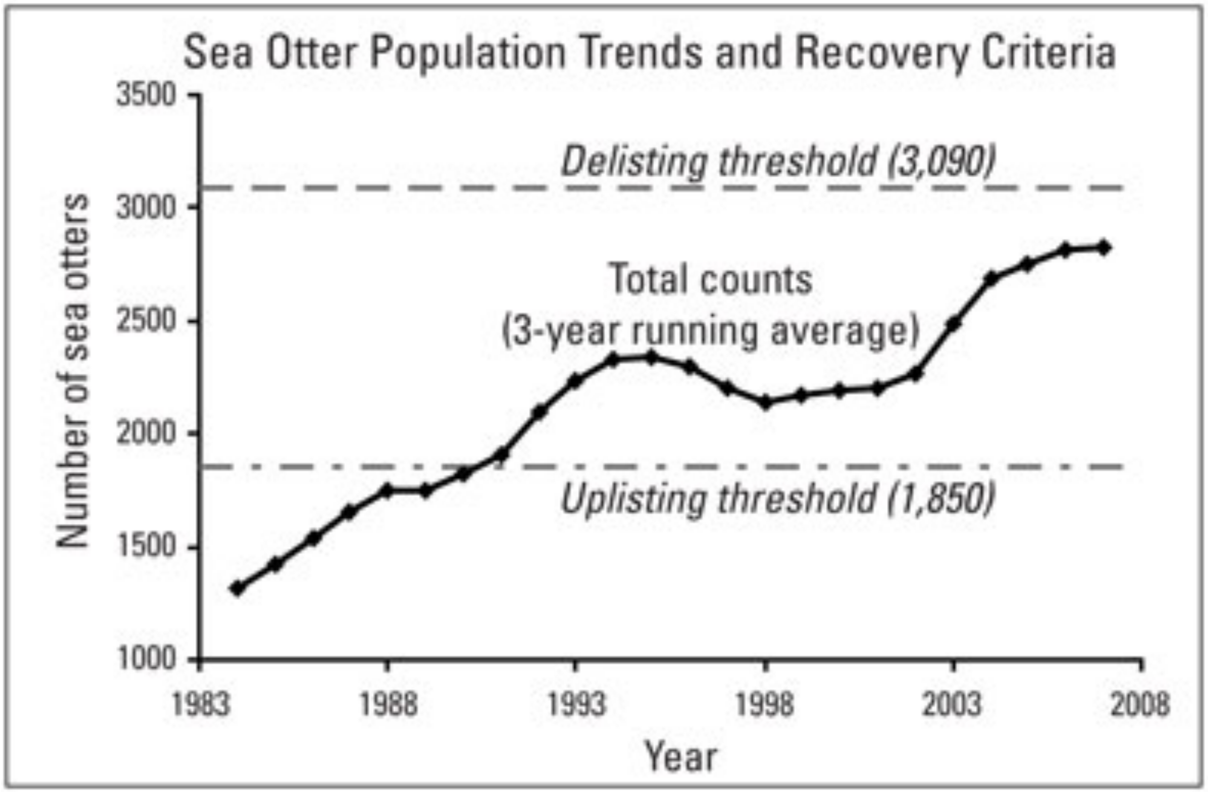
Karen Shapiro
University of California Davis
School of Veterinary Medicine
Pathology, Microbiology and Immunology

Introduction

- California sea otters
  - Exclusively coastal species
  - Closely linked with kelp forest ecology
  - Diet specialists - consume 1/3 body weight of marine invertebrates per day

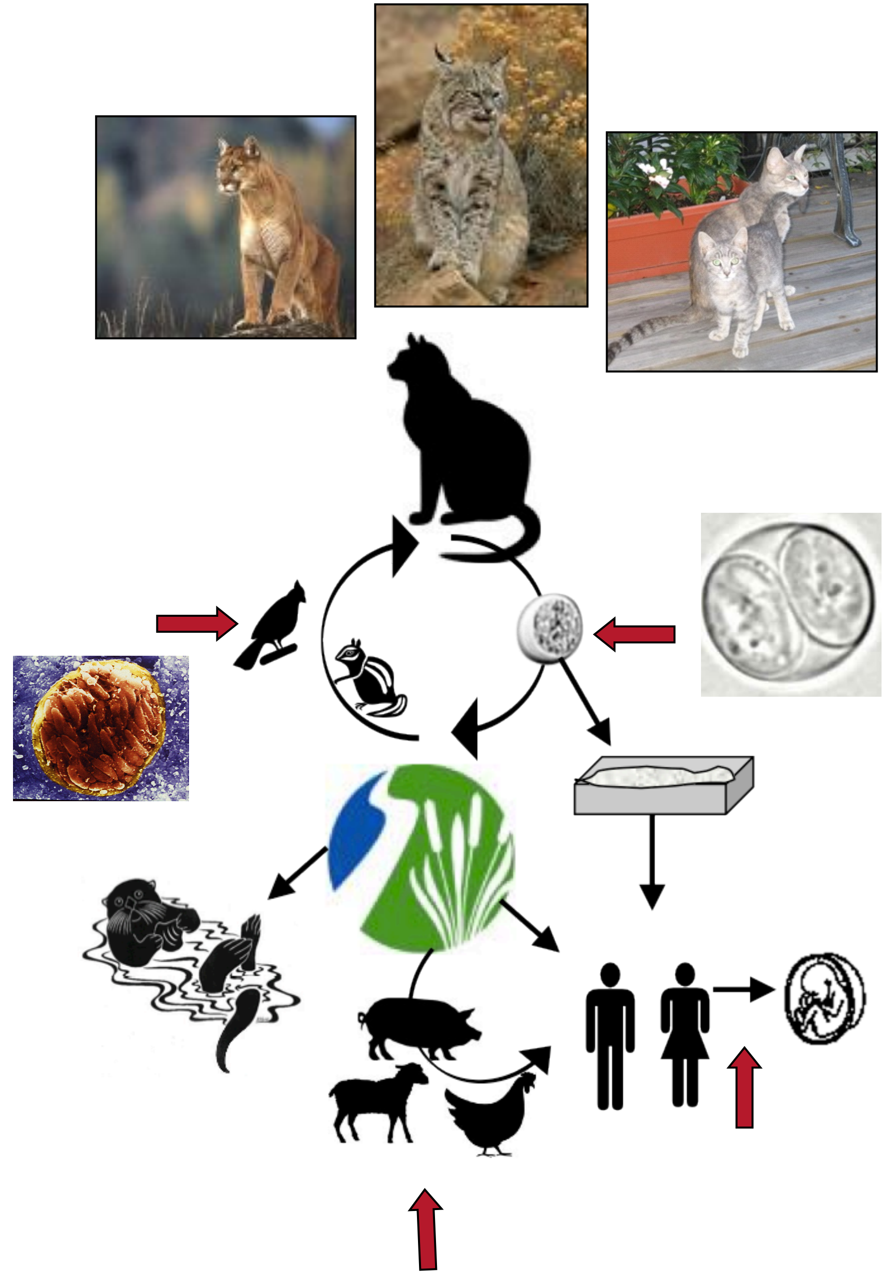
California sea otters: A threatened species

- Sea otter population nearly decimated due to fur trade
  - Slow population recovery following protection
  - Death of prime-age young adults due to infectious disease a primary cause
  - *Toxoplasma gondii* - Cause of mortality in 27% of otters



Introduction: *Toxoplasma gondii*

- Zoonotic protozoan parasite
- Life cycle
  - Felids definitive host
  - Many warm blooded animals as intermediate hosts
    - Humans
      - Global distribution ~ 1/3 humans infected
      - US: 15%
      - Inuit of Nunavik: 60%
- Transmission routes
  - Foodborne
  - Congenital
  - Oocyst ingestion
    - Route of sea otter infections
    - Humans
      - seafood-borne exposure
      - waterborne infections

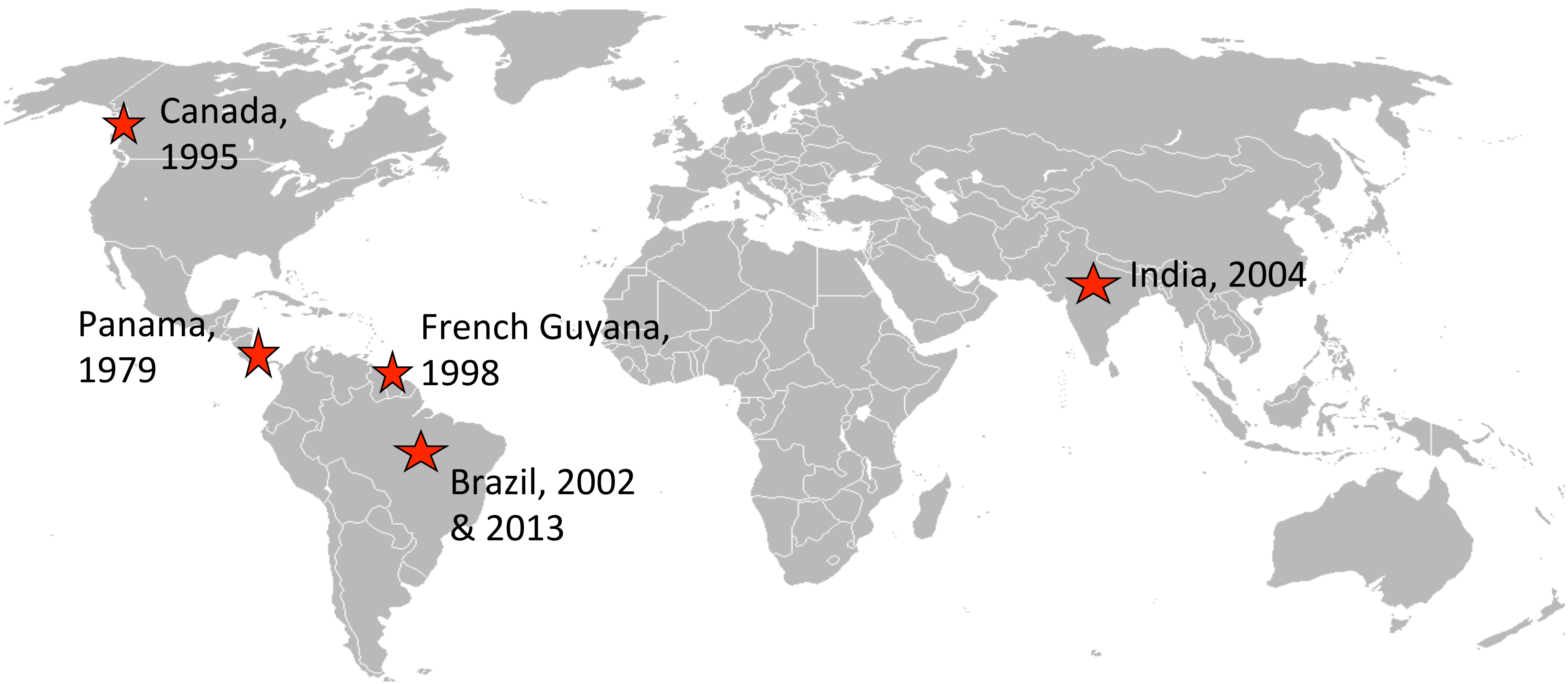


Human Toxoplasmosis

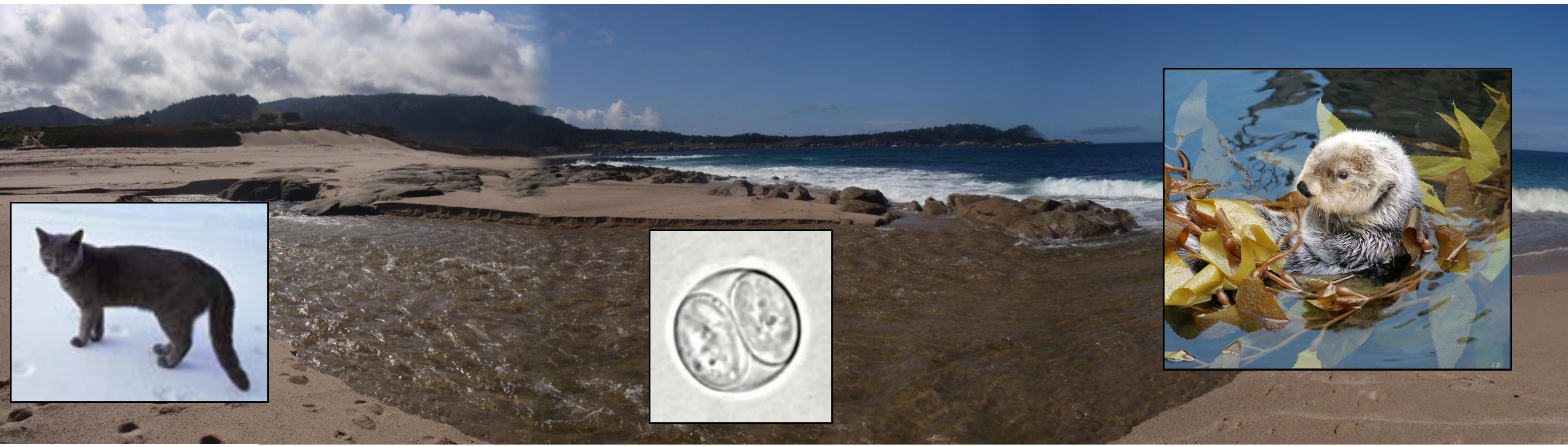
- Healthy adults – 90% subclinical
- Fatal disease in Immunocompromised
- Children of women infected during pregnancy
    - Myeloencephalitis
    - Birth defects
    - Retardation
    - Pneumonia
    - Blindness
    - Schizophrenia

Healthy adults at risk from waterborne infection

Toxoplasmosis: Waterborne disease outbreaks in people



The puzzle: Toxoplasmosis in California sea otters



Terrestrial definitive host

?

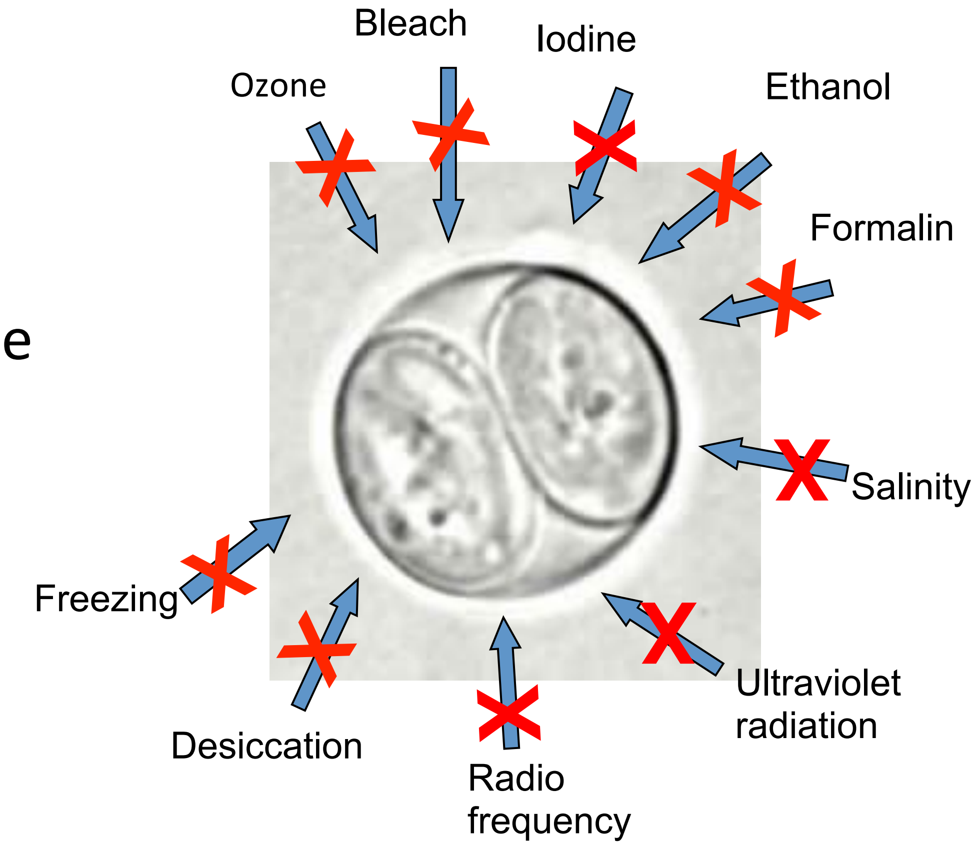
Marine mammals infected

Question 1: How can so many oocysts reach the ocean?

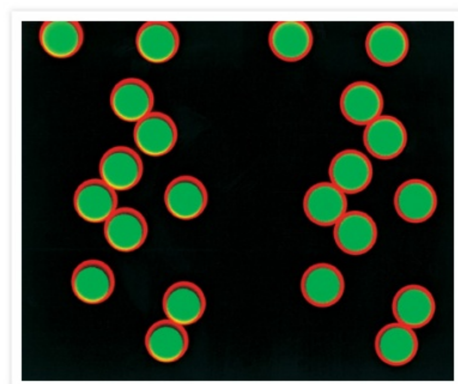
Question 2: How can so many otters become infected?

Investigating oocyst transport

- Challenges
  - Biohazard
  - Environmental resistance
  - Production
  - Detection



- Surrogate approach: Synthetic microspheres
  - Safe
  - Mimic oocyst behavior
  - Detectable

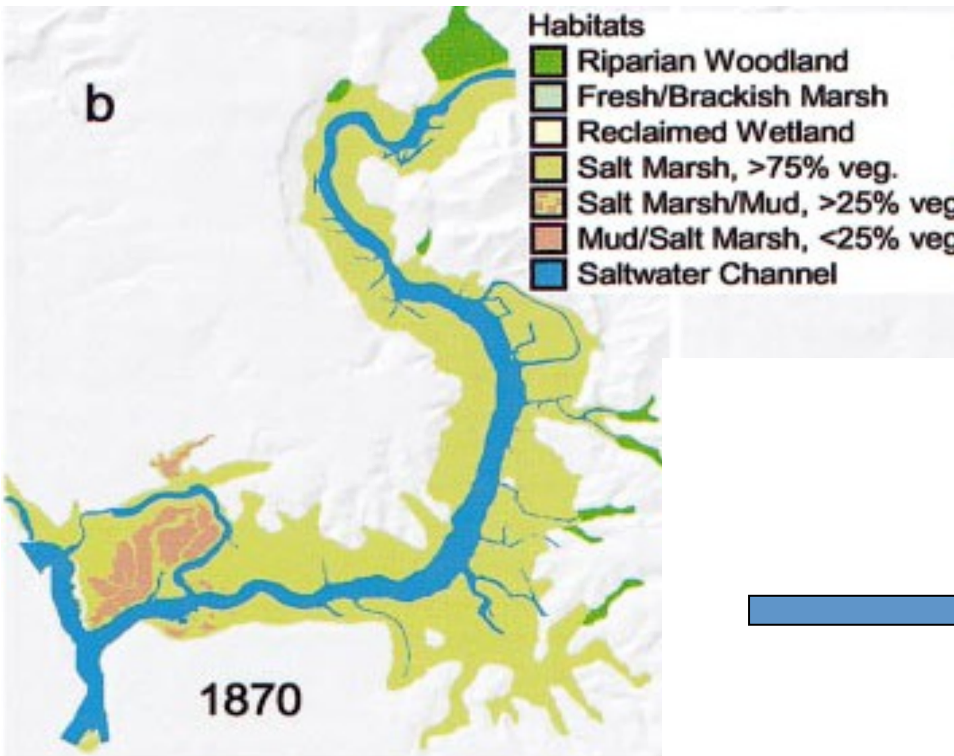


Addressing Question 1:
How can so many *T. gondii* oocysts reach the ocean?

- Risk of sea otter exposure to *T. gondii* not uniform
  - High risk sites in Monterey and Estero Bays

Can human development and coastal habitat change impact oocyst transport from land to sea?

Wetland loss at Elkhorn Slough

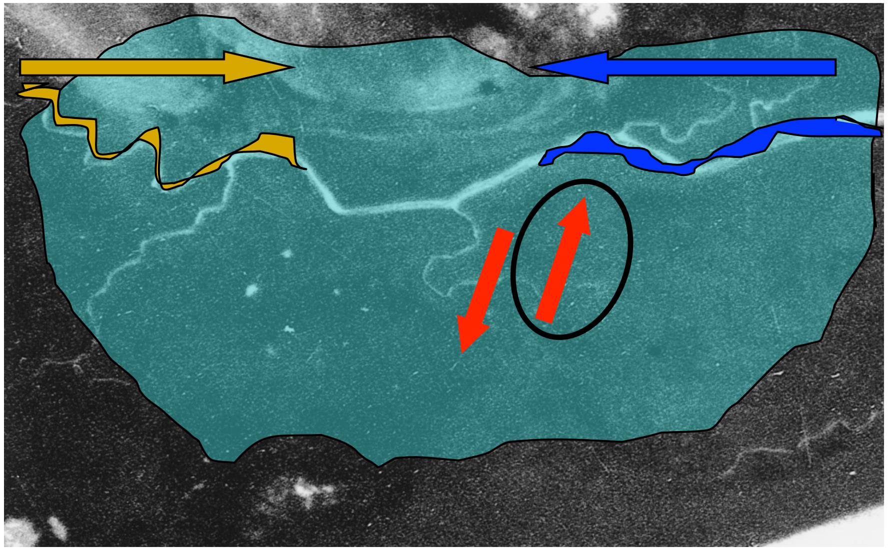


- Elkhorn Slough: High risk site for otters
- Historically rich in wetlands
- 1947: Moss Landing Harbor created
    - Tidal scour leads to marsh erosion

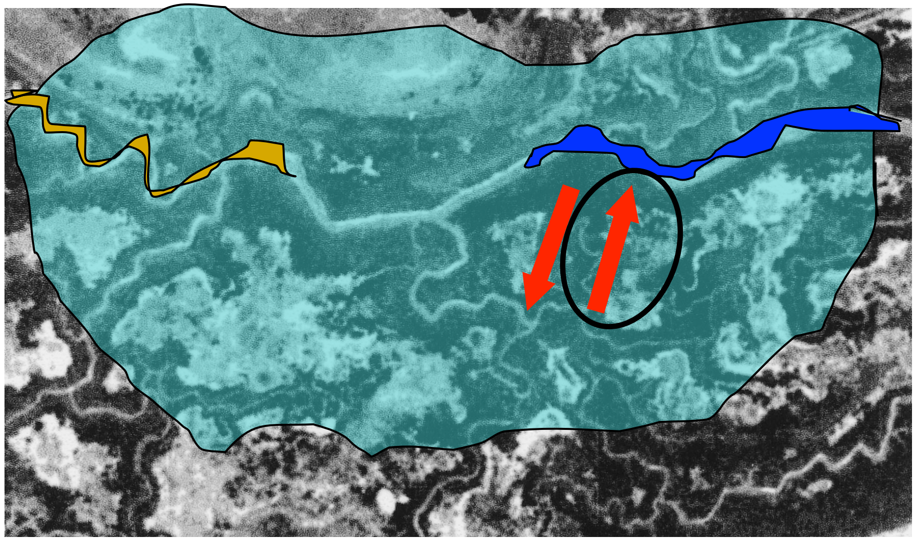
Eric Van Dyke and Kerstin Wasson, 2005. "Historical Ecology of a Central California Estuary: 150 Years of Habitat Change"

Estuarine wetlands: Role in water quality

LAND **SEA**



1937



1956

Specific study question: *Is there a difference in oocyst delivery from contaminated runoff to the ocean before and after wetland degradation?*

Effect of wetland degradation on transport of *T. gondii* surrogates

Experimental design

- Bottomless frame sunk in mud flats and vegetated marsh
- Water pumped to simulate flow as measured in field conditions
- Surrogate microspheres and tracer dye released and sampled 1.6 m downstream for 60 min post release

Results

Vegetated:

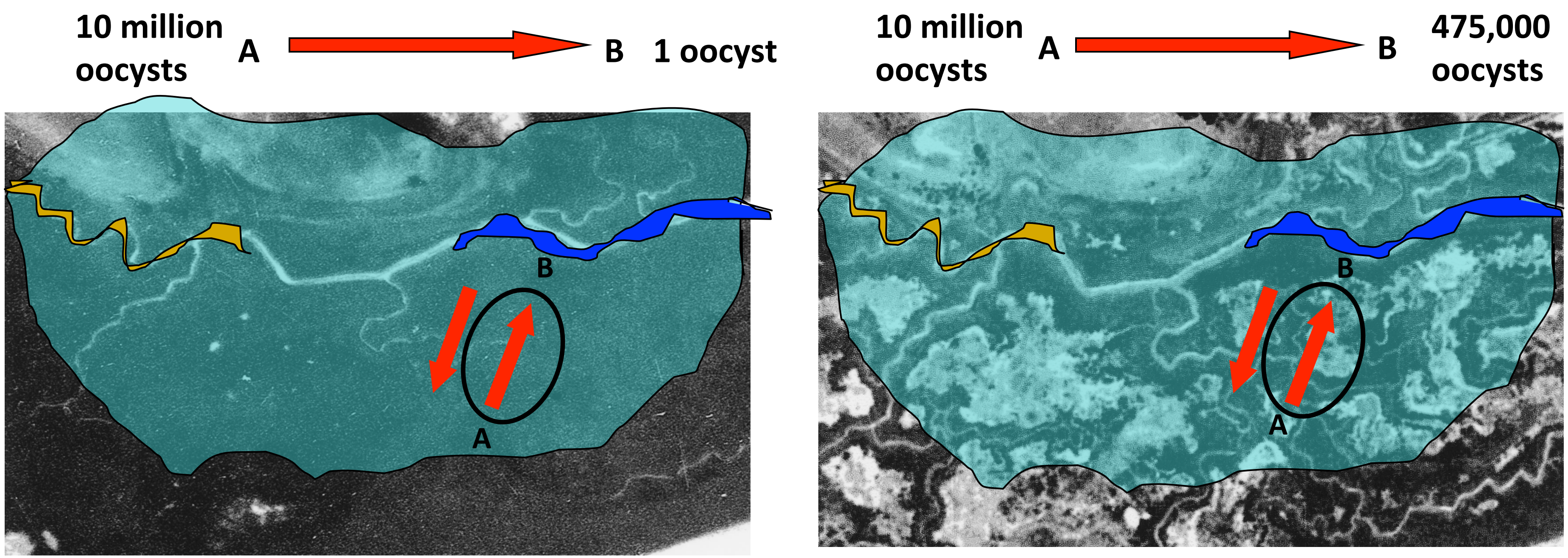
Recovered 43% of microspheres

Mudflat:

Recovered 85% of microspheres

Impact of wetland loss on oocyst transport

- Elkhorn Slough: 36% of wetland marsh eroded to mudflats
- Effect on transport of *T. gondii* oocysts:
    - Surrogate recovery: 43% through marsh and 85% through mud every 1.6 meters
    - Example: 30m of previously vegetated marsh now eroded



Unraveling the puzzle

Question 1: How can so many oocysts reach the ocean?

Transport of *T. gondii* oocysts to the sea is exacerbated by anthropogenic activities

Land → **Sea**

↑ Oocyst source + ↑ Impervious surfaces + ↑ Wetland degradation = ↑ Oocysts reaching coastal waters

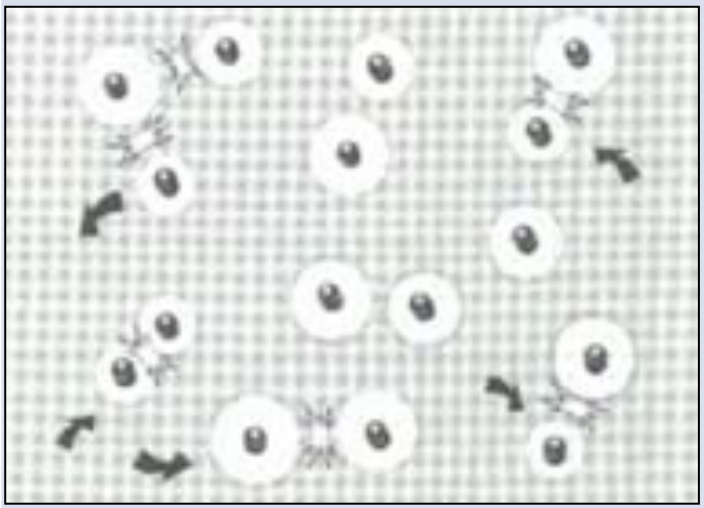
Unraveling the puzzle

Question 1: How can so many oocysts reach the ocean?

Transport of *T. gondii* oocysts to the sea is exacerbated by anthropogenic activities

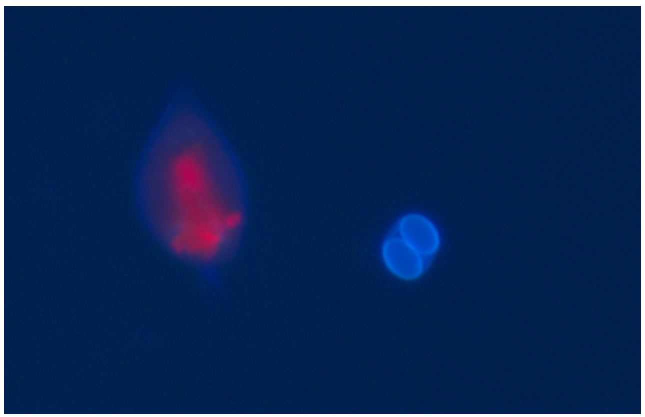
Question 2: It's a big ocean out there… how can so many sea otters become infected with a terrestrial parasite?

Oocyst surface properties: *T. gondii* aggregation

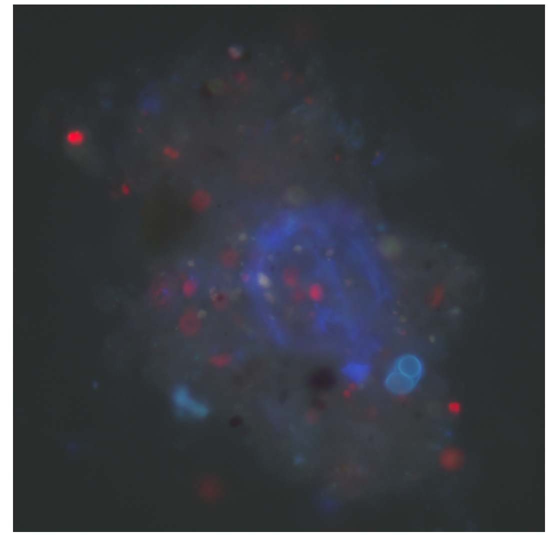
Water Type	
Fresh	Saline
Oocysts are negatively charged	Oocysts' charge is neutralized
	
Discrete suspended particles	Aggregated particles

Surface charge measurements suggest oocyst aggregation is enhanced in saline waters

Why is aggregation relevant?



≠

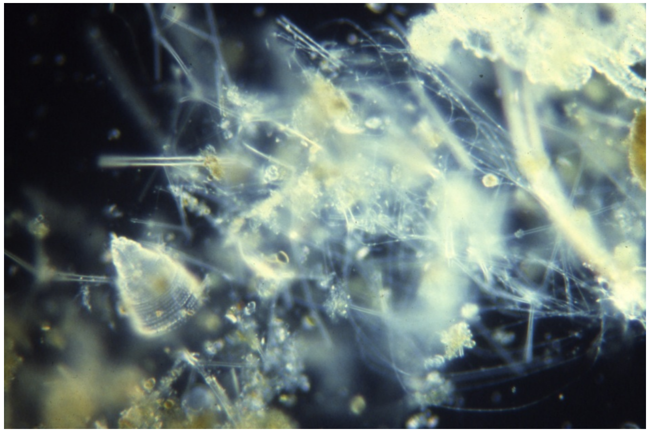


- Understanding *T. gondii* aggregation:
  - Predict spatial distribution = Risk
  - Transmission route clues: Bioavailability for invertebrates including seafoods consumed by humans or otters
  - To date – estimating waterborne pathogen distribution largely assumes individual particles

Aggregates in the marine environment = Marine Snow

Aquatic aggregates: Marine snow

- Particles > 0.5 mm
- Composition: 0rganic detritus, phytoplankton, fecal pellets, inorganic material, microorganisms, mucous



- Significance:
  - The ocean's biological pump: Primary means of vertical transport for matter and energy
  - Role in disease transmission largely unexplored…

Association of *T. gondii* with marine snow

- Hypothesis:

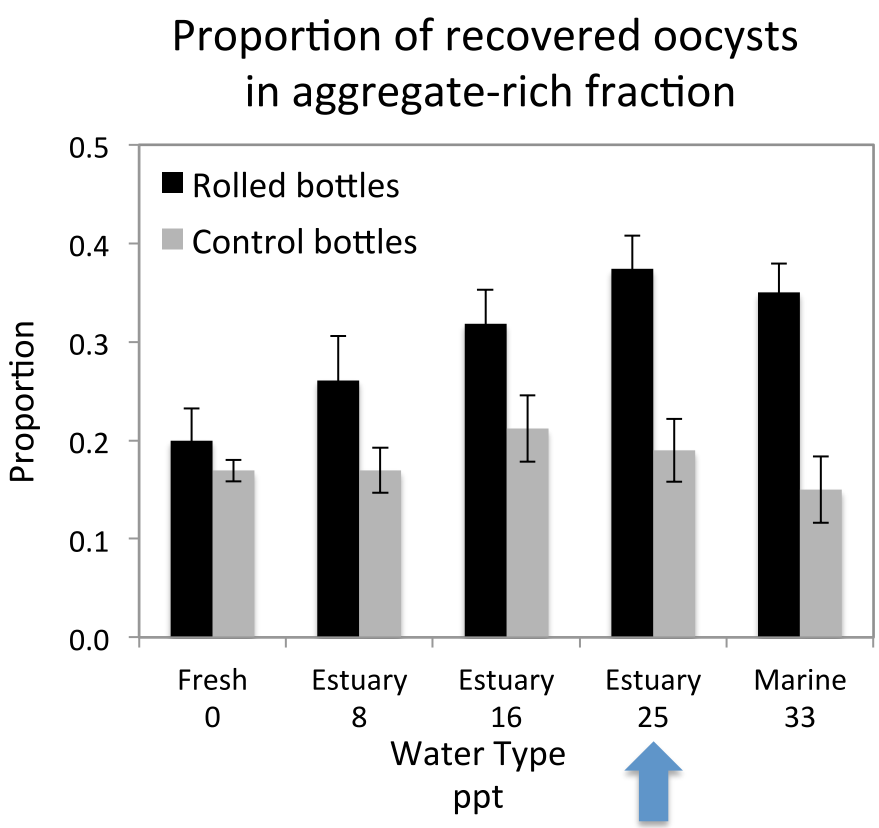
  <u>Association of *T. gondii* with marine snow is enhanced in saline waters</u>

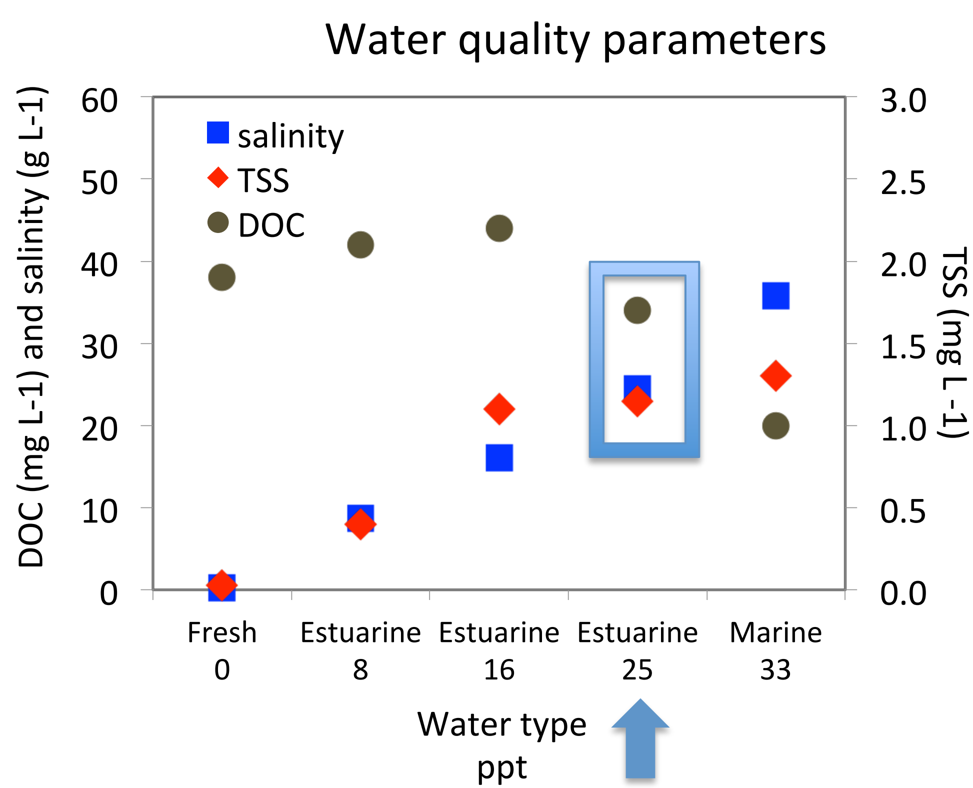
- Experimental approach:
  - **Expt 1:** River water spiked with sea salt: identical water constituents except for salinity
  - **Expt 2:** Natural river, estuarine, and sea water

1. Aggregate production: Water spiked with *T. gondii* oocysts and surrogate microspheres - rolled for 24 hrs
2. Aggregate separation
3. Oocysts enumerated in aggregate-rich and poor fractions

Results: *T. gondii* aggregation

- **Expt 1:** Increased salinity associated with increasing numbers of *T. gondii* oocysts recovered from the aggregate-rich fraction (P<0.01)
- **Expt 2:** Natural river, estuarine, and marine waters

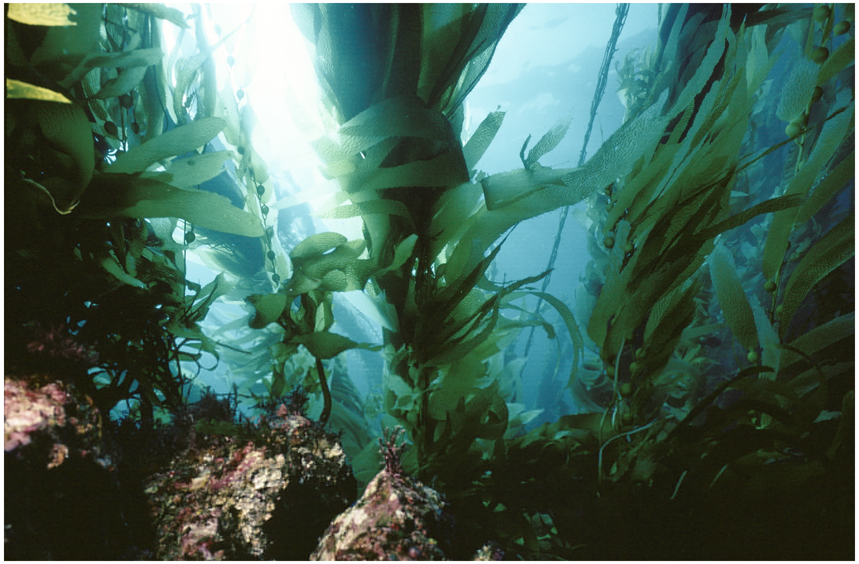




- Water quality parameters other than salinity impact magnitude of pathogen aggregation, for example…. **Transparent Exopolymer Particles (TEP)**

TEP and Marine Snow

- Transparent Exopolymer Particles = TEP
- Invisible, sticky, gel-like particles – the glue matrix of snow
- Produced by phytoplankton, cyanobacteria, and …. kelp



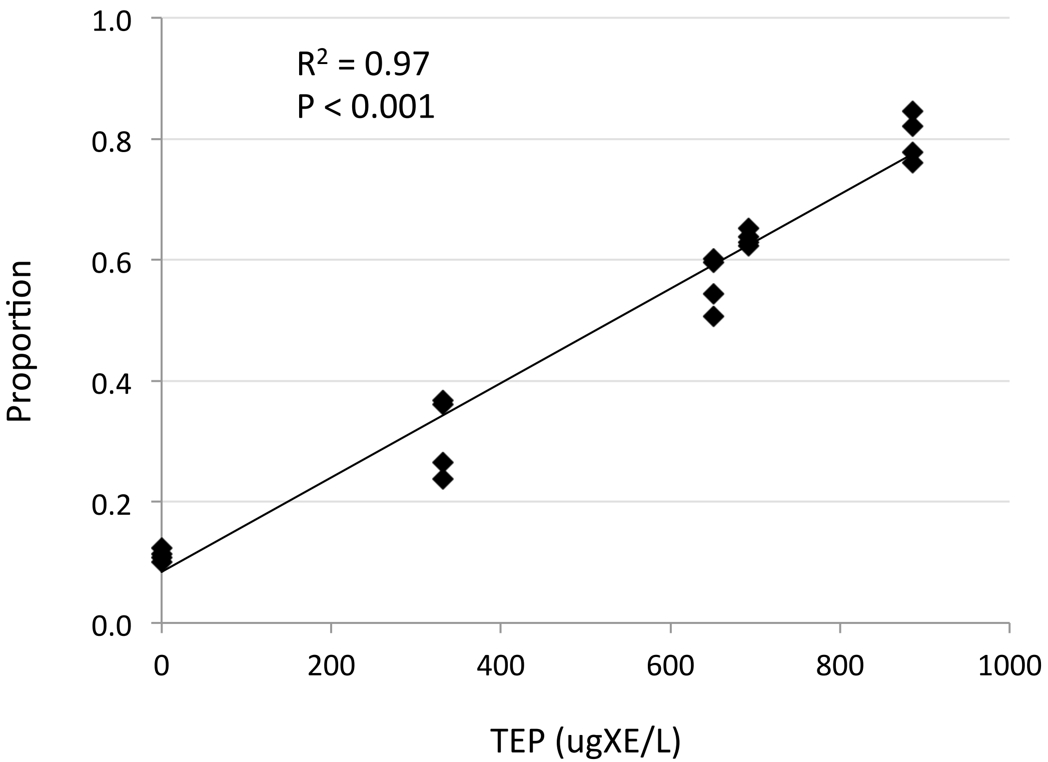
- Hypothesis:

Association of *T. gondii* oocysts with marine snow will increase as a function of TEP

Aggregation in TEP-spiked seawater

Identical seawater samples spiked with increasing concentrations of alginic acid => TEP produced by kelp

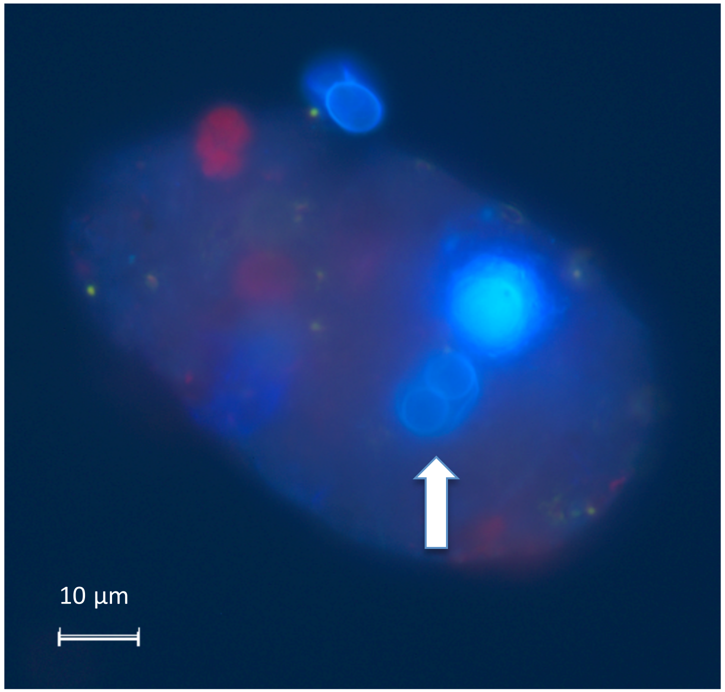
Proportion of recovered oocysts in aggregate-rich fraction as a function of TEP

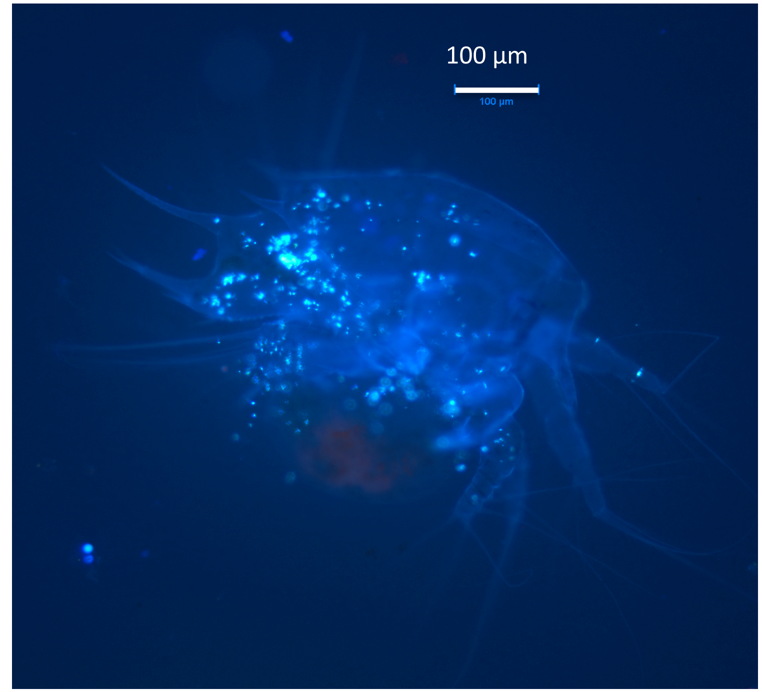


Increased concentrations of TEP enhance the association of *T. gondii* oocysts with marine snow

Results: *T. gondii* aggregation

Microscopy findings





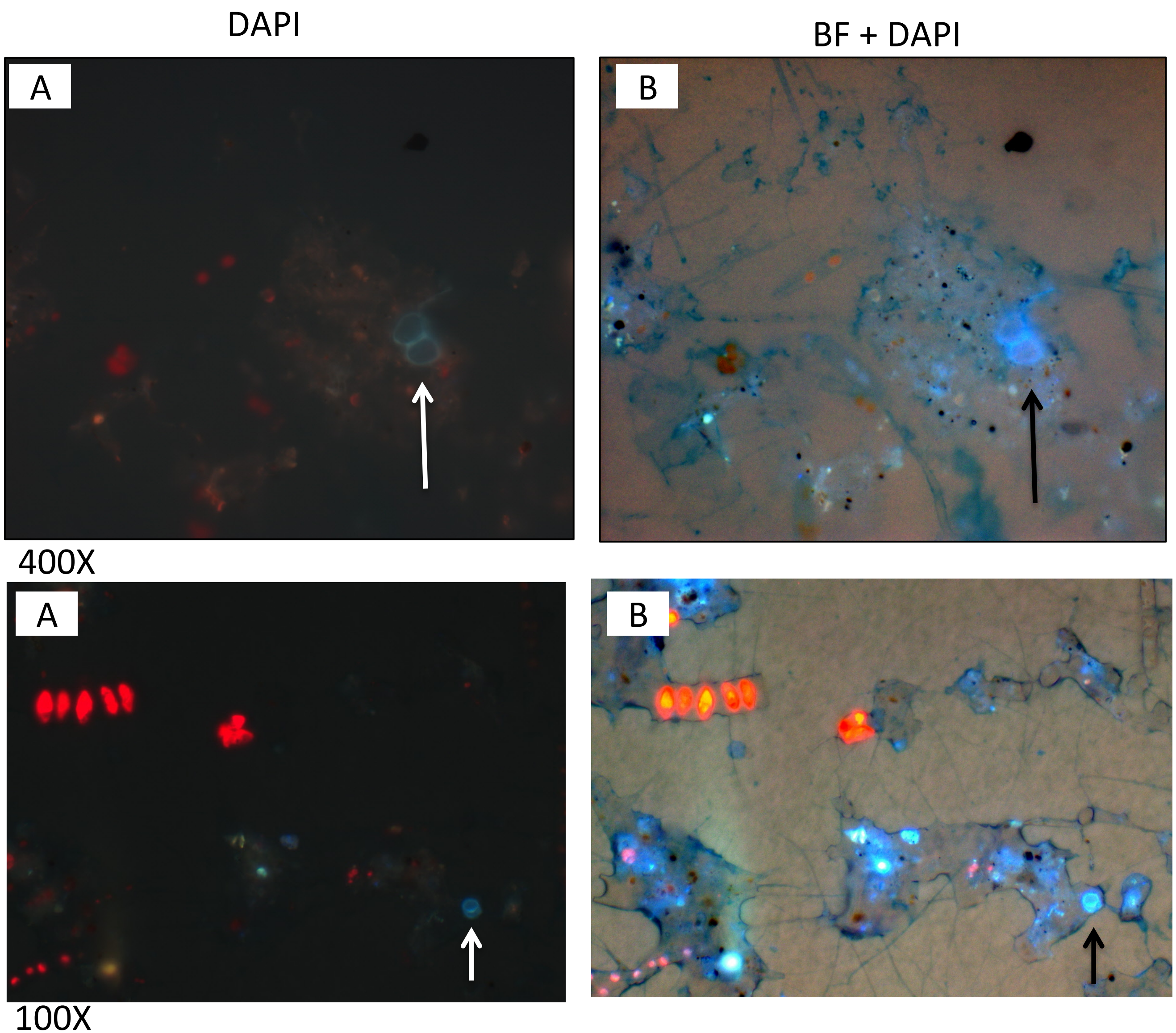
First visual evidence for incorporation of *T. gondii* in invertebrate fecal pellets

=

Entry into marine food web

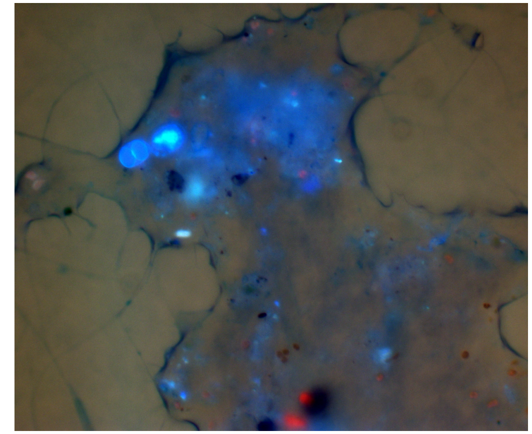
Visualizing *T. gondii* entrapment by TEP

Using alcian blue stain



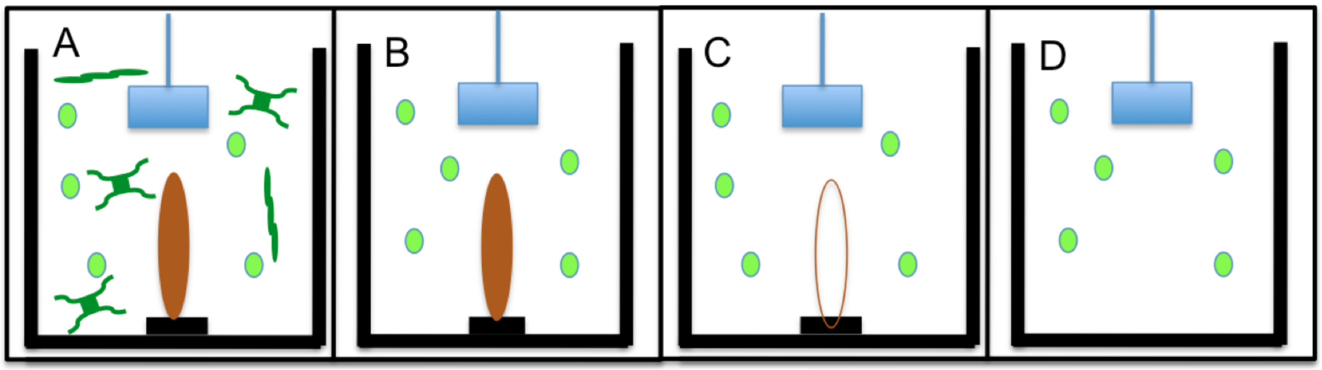
Beyond Snow – Impact of TEP on *T. gondii* transmission

- Snow experiments support enhanced association of *T. gondii* with marine snow in high TEP waters
  - Kelp forests
- Many kelp dwelling invertebrates eat snow…
- But only snails identified as a dietary risk factor for sea otter exposure to *T. gondii*
  - 12 X odds of *T. gondii* infection
  - Turban snails are kelp grazers



Association of *T. gondii* with kelp

- **Objective**: Can *T. gondii* oocysts adhere to kelp surfaces?

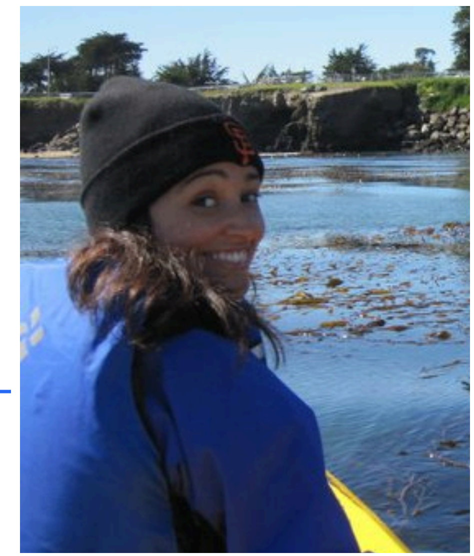


Kelp
Seawater
(TEP in water and kelp)

Kelp
Filtered SW
(TEP on kelp)

Model kelp
Filtered SW
(no TEP)

Filtered SW
only



Fernanda Mazzillo

- **Findings**: Up to 30% of *T. gondii* surrogates attach to kelp blades due to TEP coating on kelp
- **Conclusions**: TEP-mediated adhesion of *T. gondii* to kelp suggests possible transmission route through kelp-grazing snails

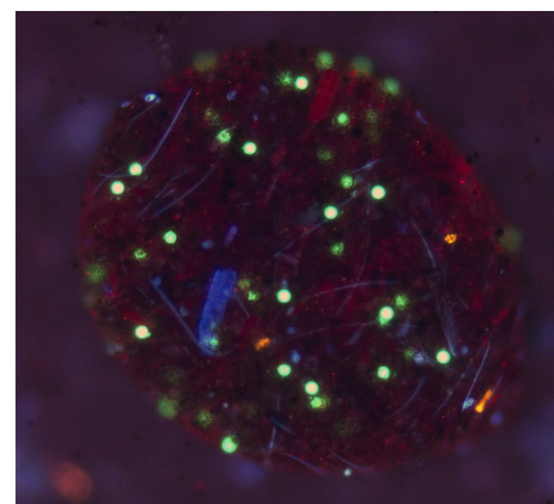
From kelp to otters – The snail connection

- **Objective**: Can marine snails serve as mechanical hosts for *T. gondii*?

24 hr exposure → 14 day follow-up

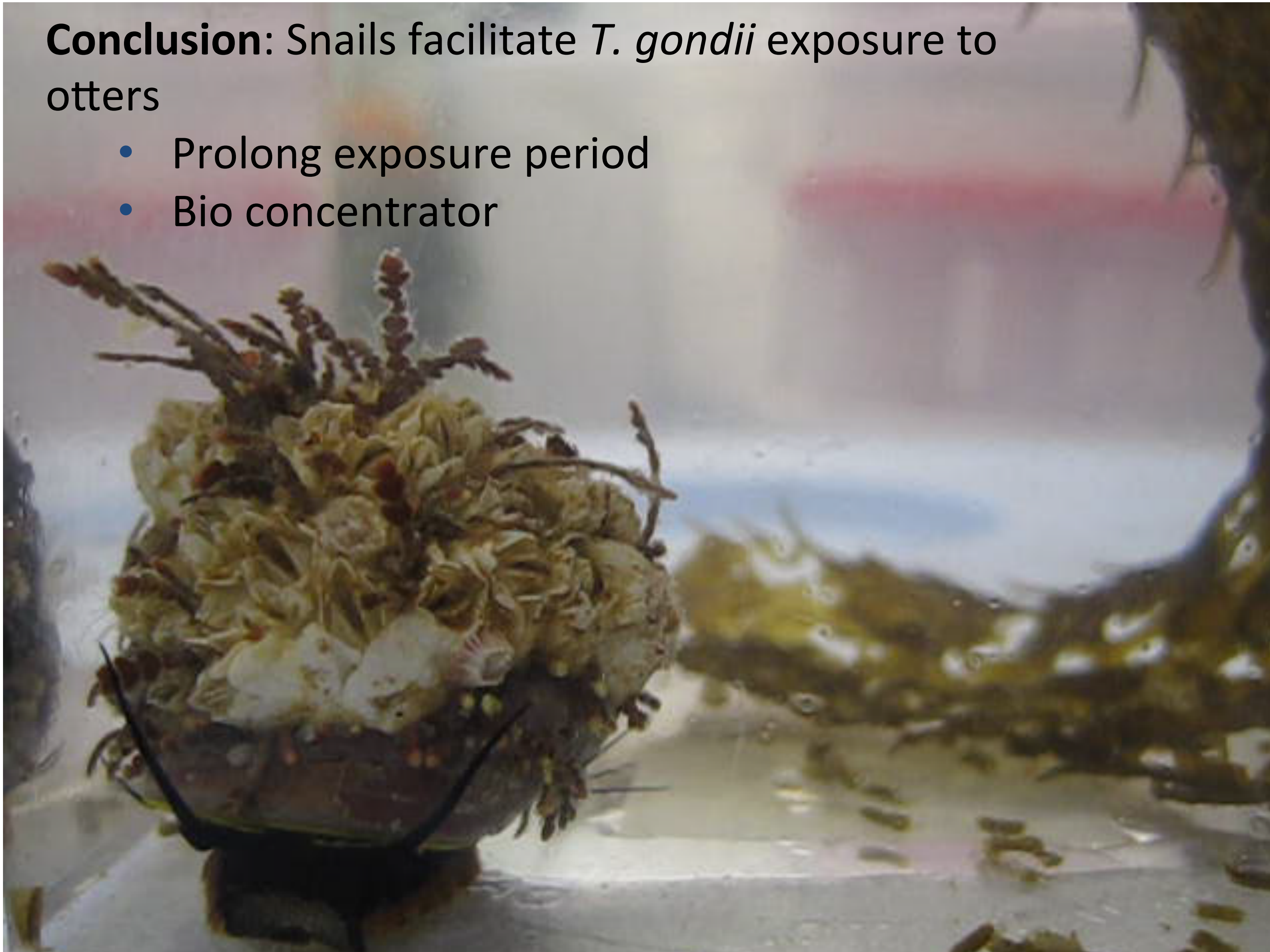
Colin Krusor

- **Findings**:
  - Retention and excretion of oocysts up to 11 days following exposure
  - Concentrations in feces 2-3 orders of magnitude greater than seawater



Conclusion: Snails facilitate *T. gondii* exposure to otters

- Prolong exposure period
- Bio concentrator

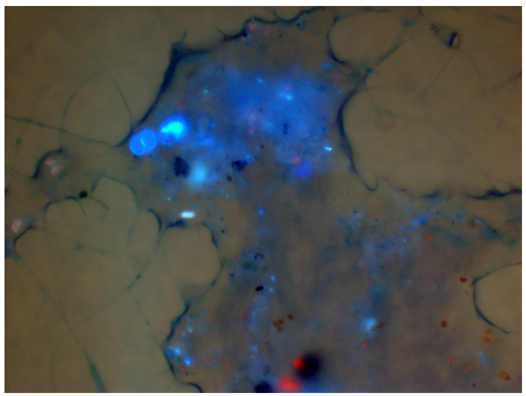


Unraveling the puzzle

Question 2: How can so many otters become infected?

T. gondii **oocysts can concentrate in coastal ecosystems where sea otters live through two mechanisms:**

1) Enrichment in marine snow



?

Harbor seals

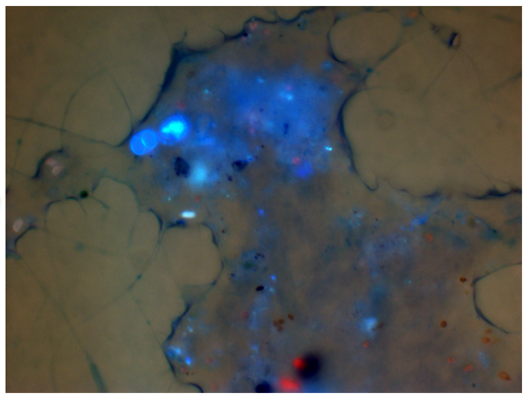
2) Association with kelp surfaces

Snails as mechanical hosts

Snail-specializing otters at greater odds of infection

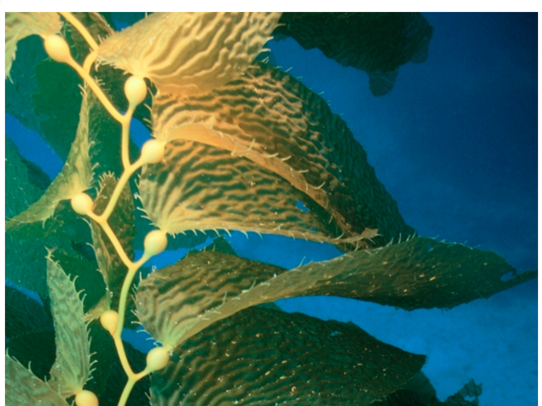
New insight on *T. gondii* transmission: Human public health implications

1) Enrichment in marine snow



Contaminated seafood

2) Association with kelp surfaces



Recreation

Kelp Krawlers Monterey Bay open swimming

Toxoplasma in marine ecosystems: Field investigations

Challenge: Detection methods for *T. gondii* in environmental matrices are not standardized, insensitive, and laborious.

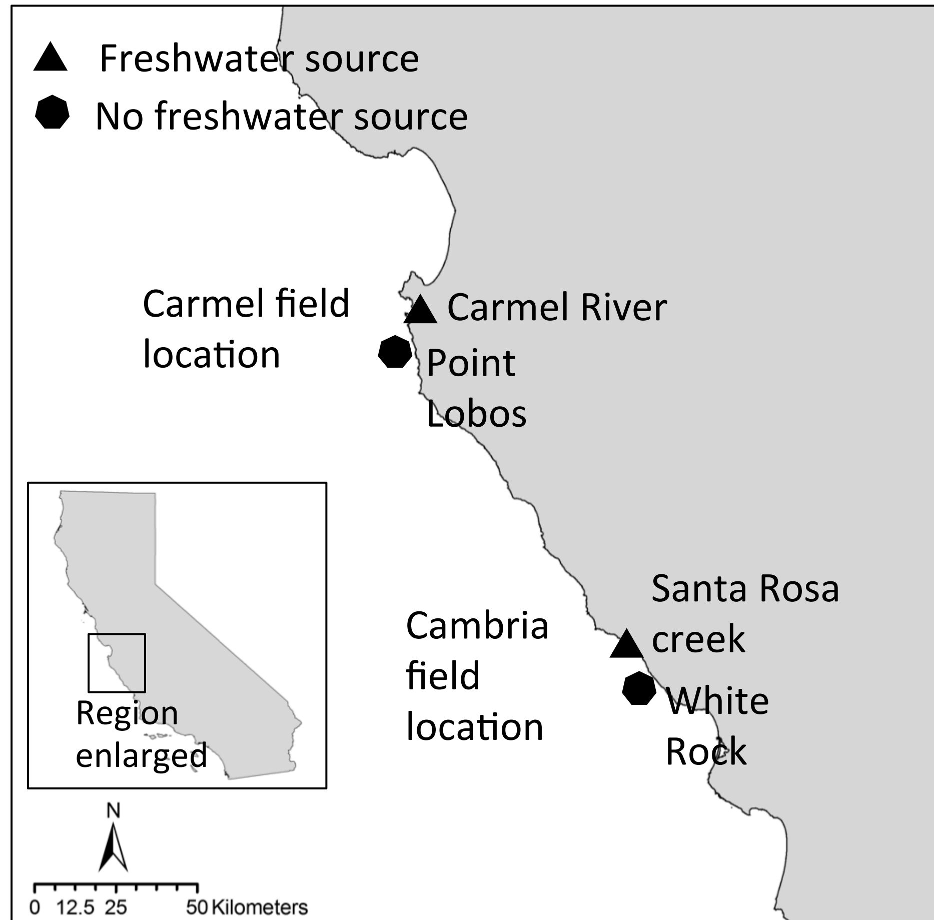
Approach: Use mussels as sentinels for coastal pathogen contamination

Objectives:

1) Determine prevalence of *T. gondii*-contaminated mussels in coastal CA

2) Determine spatial and seasonal risk factors for mussel *T. gondii* contamination:

- Proximity to runoff
- Wet. vs. dry season



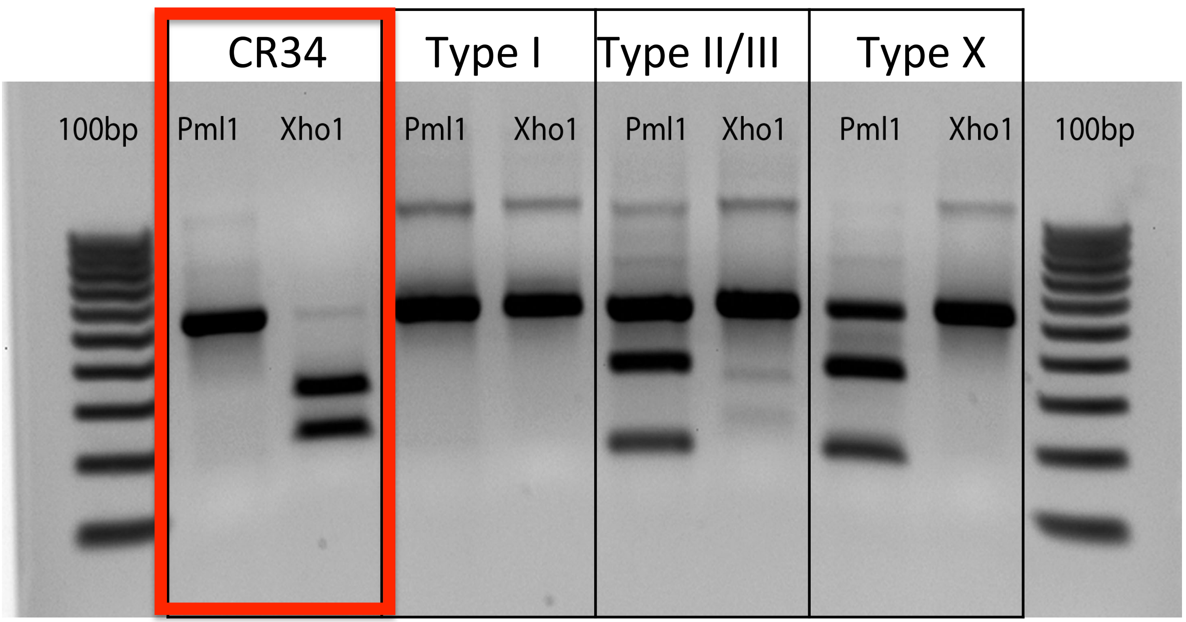
Prevalence of *T. gondii* in mussels: Screening assays

Region	Site	# positive / # tested	Prevalence (%)
Carmel	Runoff present	4/271	1.5
	Runoff absent	2/209	1.0
Cambria	Runoff present	7/241	2.9
	Runoff absent	0/238	0
	Total	13/959	1.4

- Significant association with:
  - Proximity to runoff (OR=5)
  - Open river mouth (wet season) (OR=12)
- Further molecular characterization:
  - Sequencing
  - Multi-locus Restriction Fragment Length Polymorphism (RFLP)

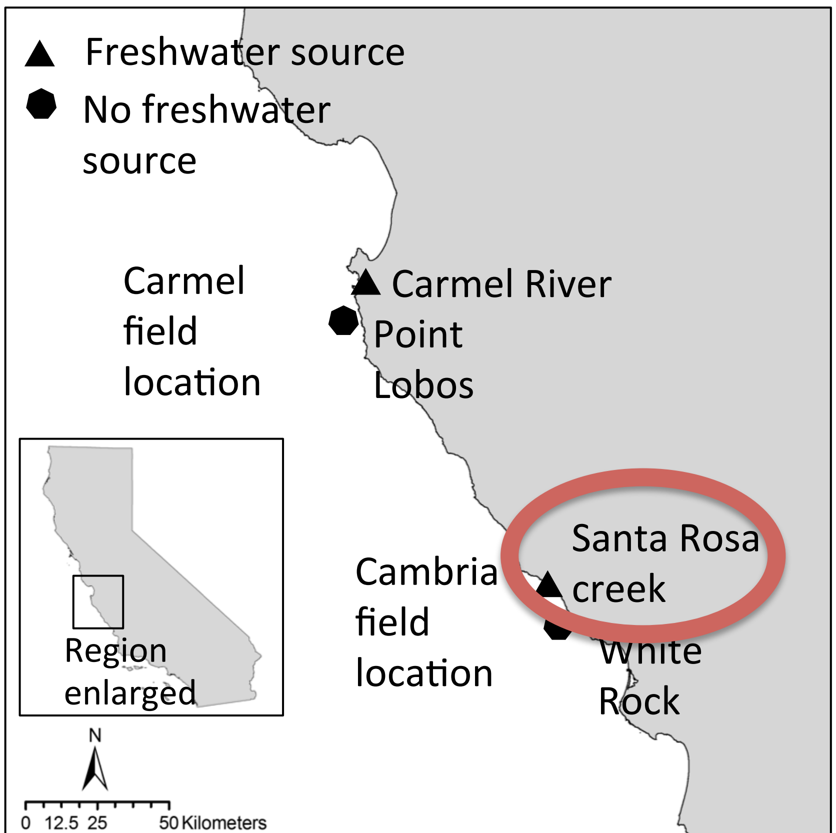
Molecular characterization of *T. gondii* in mussels: Genotyping assays

- Carmel River mussel: Surprising RFLP results
  - SAG1: Type II
  - B1: New RFLP pattern
  - Identical RFLP pattern from 1 mt lion and 2 fox samples
    - New atypical strain in central CA?



T. gondii contamination 'outbreak' in Cambria?

- In April 2013 - 7 of 30 mussels tested at Santa Rosa creek were positive for *T. gondii*
  - 23% prevalence
- Real coastal contamination event or lab contamination?



Sequencing results of *T. gondii* isolates (B1 locus)

	B1 gene nucleotide position			
Isolate	225	366	378	504
Control	**C**	**T**	**G**	**G**
1	C	C	G	C
2	T	T	A	G
3	C	T	G	C
4	C	T	A	C
5	C	C	G	G
6	C	T	G	G

All 6 SR *T. gondii* samples differ from each other:
True pollution event - NOT lab contamination

Toxoplasma – special considerations in Alaska

The good news…

- Terrestrial load of oocysts may be lower
  - Only Lynx endemic definitive host
- Oocyst survival may be reduced
  - Unsporulated oocysts inactivated at -20°C within one day
  - Sporulated oocysts viability diminished after 4 weeks at -20°C

The bad news…

- Marine transmission of *T. gondii* may be a significant source of infections to humans in arctic climates:
  - Oocysts in coastal waters may survive through freezing temperatures – whereas oocysts on land would likely not be viable after an Alaskan winter
  - Consumption of shellfish a significant risk for *T. g*ondii exposure
  - Consumption of undercooked marine mammal meat poses unique health risk for Alaska Natives

Thank You

Funding: NSF EID, NOAA OHHI, CA Sea grant

Our team: **UCD**

Veterinary Medicine

Patricia Conrad, Jonna Mazet, Colin Krusor, Terra Berardi, Beatriz Aguilar, Liz VanWormer,, Tim Carpenter, Woutrina Miller, Ann Melli, Andrea Packham, Heather Fritz , Aiko Adell

Bodega Marine Laboratory

John Largier, David Dunn, Matt Robart

Environmental Engineering

Stefan Wuertz, Alexander Schriewer,

Hydrology

Wes Wallender, Purnendu Singh

UCSC Marine Sciences

Mary Silver, Tim Tinker, Fernanda Mazzillo, Alexis Walker, Joe Tomoleoni, Ben Weitzman, Zach Randell

University of Maryland Coastal Oceanography

Nicholas Nidzieko

For more information…

- Dubey JP et al. Toxoplasma gondii, Neospora caninum, Sarcocystis neurona, and Sarcocystis canis-like infections in marine mammals. Vet Parasitol. 2003 Oct 30; 116(4):275-96.)
- Frenkel, J. K., and J. P. Dubey. Effects of freezing on viability of Toxoplasma oocysts. Journal of Parasitology 1973 59:587-588.
- Karsten Hueffer et al., Zoonotic infections in Alaska: disease prevalence, potential impact of climate change and recommended actions for earlier disease detection, research, prevention and control. Int J Circumpolar Health. 2013; 72
- Mazzillo F., et al. A new pathogen transmission mechanism in the ocean: the case of sea otter exposure to the land-parasite Toxoplasma gondii.2013 PLOS ONE. 8 (12): e82477
- Messier V et al., Seroprevalence of *Toxoplasma gondii* among Nunavik Inuit (Canada). Zoonoses Public Health. 2009 May; 56(4):188-97.
- Shapiro, K. et al. Surveillance for Toxoplasma gondii in California mussels (Mytilus californianus) reveals transmission of atypical genotypes from land to sea. Environmental Microbiology. 2014 doi: 10.1111/1462-2920.12685
- Shapiro, K. et al. Aquatic polymers can drive pathogen transmission in coastal ecosystems. Proceedings of the Royal Society B. 2013 281: 1795 20141287. doi: 10.1098/rspb.2014.1287

For more information…continued

- Shapiro, K. Climate and coastal habitat change: A recipe for a dirtier ocean. Marine Pollution Bulletin.2012 64(6) 1079–1080
- Shapiro, K. et al. Association of Toxoplasma gondii oocysts with fresh, estuarine, and marine macroaggregates. Limnology & Oceanography. 2012 57(2) 449-456
- Shapiro, K. et al. Temporal association between land-based runoff events and California sea otter (Enhydra lutris nereis) protozoal mortalities. Journal of Wildlife Disease. 2012 48(2) 394-404
- Shapiro, K. et al. Effect of estuarine wetland degradation on transport of Toxoplasma gondii surrogates from land to sea. Journal of Applied and Environmental Microbiology, 2010 76(20) 6821-8.
- Shapiro, K. et al. Detection of Toxoplasma gondii oocysts and surrogate microspheres in water using capsule and ultrafiltration and capsule filtration. Water Research 2010 44(3) 893-903.
- Shapiro, K. et al. Surface Properties of Toxoplasma gondii oocysts and surrogate microspheres. Applied and Environmental Microbiology, 2009 75(4) 1185-91.
- Stieve E., et al., Neospora caninum and Toxoplasma gondii antibody prevalence in Alaska wildlife. J Wildl Dis. 2010 Apr;46(2):348-55.
- VanWormer, E.et al. Molecules to modeling: Toxoplasma gondii oocysts at the human-animal-environment interface. Comparative Immunology, Microbiology & Infectious Diseases. 2013 36: 217-231