What has Toxoplasma in Sea Otters Taught Us about the Risks and the One Health Approach to Global Public Health

Clinician Outreach and Communication Activity (COCA) Webinar

Tuesday, February 6, 2018

At the end of this webinar, the participants will be able to:

- Describe the life cycle of *Toxoplasma gondii* and the importance of the oocyst in transmission.
- Explain the different mechanisms for oocyst accumulation in the ocean where sea otters become infected.
- List possible steps to reduce pathogen pollution in coastal habitats.
- Define a keystone species and discuss what we have learned about ecosystem health and human health risks from studying sea otter health.

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Planners have reviewed content to ensure there is no bias.

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Today's Presenter

Heather Fritz, DVM, PhD
University of California, Davis

What has Toxoplasma in sea otters taught us about risks and the One Health approach to Global Public Health

Heather Fritz DVM, PhD

University of California, Davis

UCDAVIS School of Veterinary Medicine

THE UNIVERSITY OF CALIFORNIA · LET THERE BE LIGHT · DAVIS

Global Health

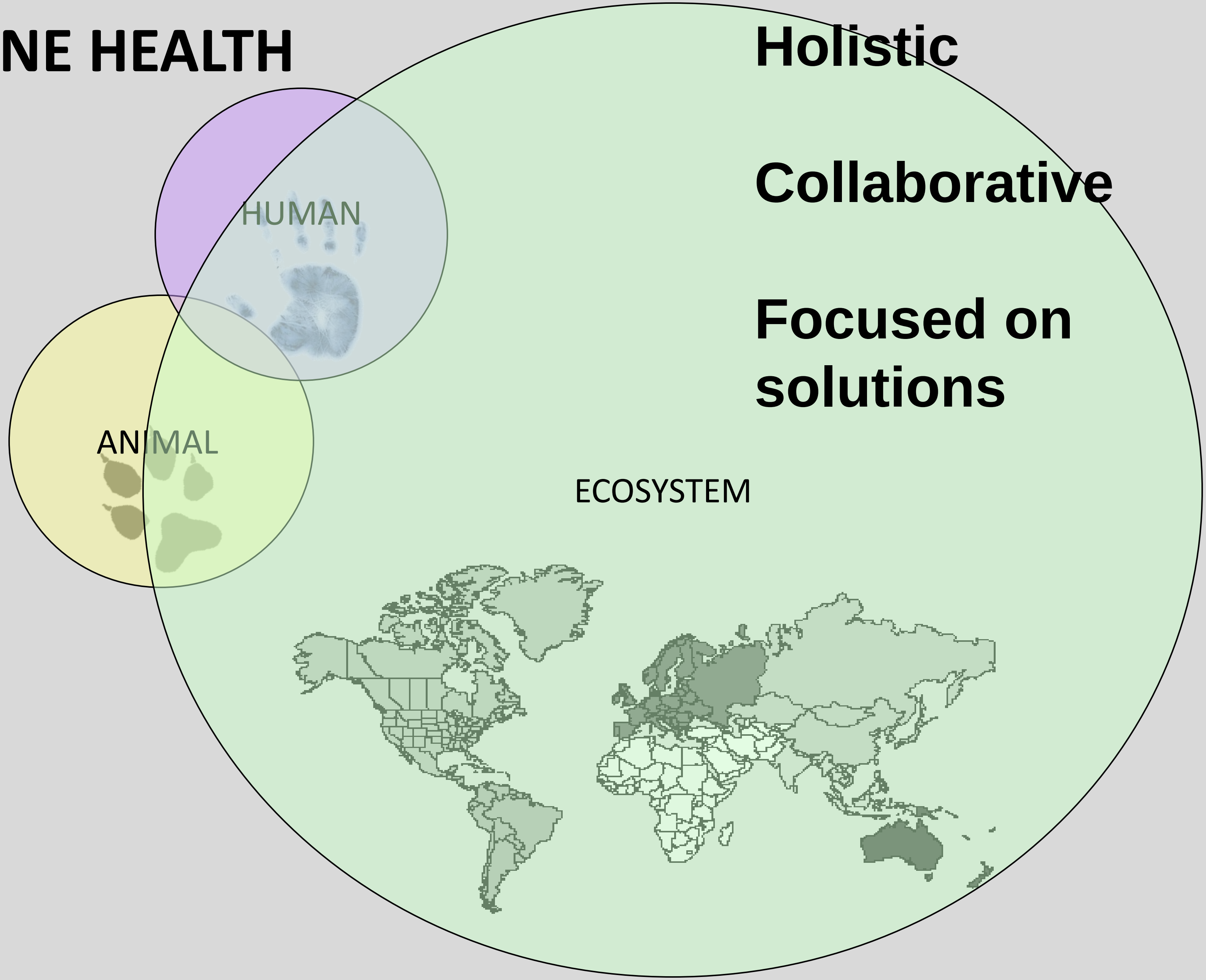
Area of study, research and practice with a priority on improving health and achieving health equity for people worldwide.

Transcend national borders
Protect against global threats

One Health

An interdisciplinary approach to solving specific, complex problems that arise at the interface of animals, humans and the environment.

ONE HEALTH



Holistic

Collaborative

Focused on solutions


SOUTHERN
SEA OTTER
(Enhydra lutris nereis)
Bryant Austin © 2001

The California Southern Sea Otter

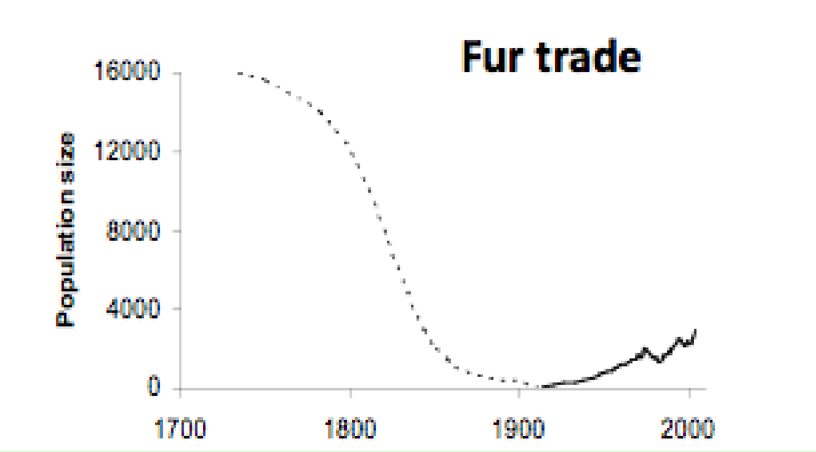
- Federally-listed threatened species
- Found only along the central coast of California
- Total population ~3,000 animals

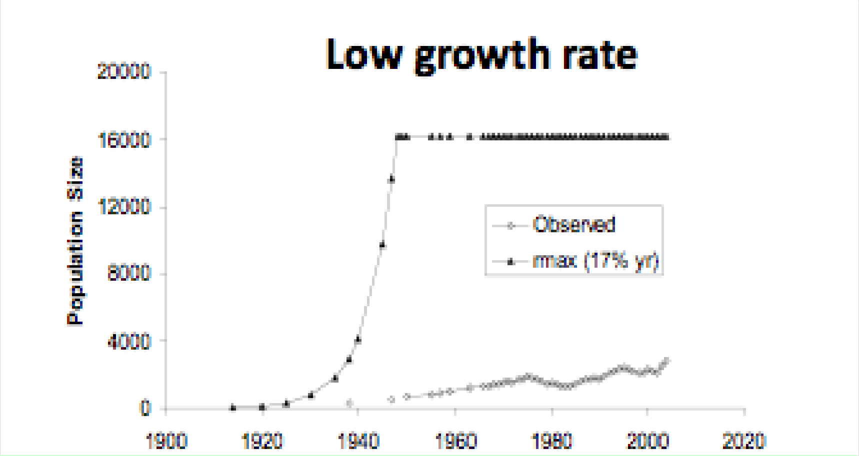
Otters are a Keystone Species

Importance of sea otters to coastal ecosystem

- **Kelp forests protect coast from erosion and provide habitat**
- **Sea urchins destroy kelp**
- **Sea otters prey on sea urchin**

Toxoplasma gondii





How is a terrestrial pathogen causing such profound disease in marine mammals?

What is killing California sea otters?

Most important: Infectious diseases

→ protozoal parasites

Seroprevalence of *T. gondii*:

~38% of 257 live otters

~52% of 305 dead otters

Based on IFAT - Miller et al 2002

What's Killing the Sea Otters

By DAN CRAY

Sund

A sea otter near
Alaska.

DAVID MCNEW / GET

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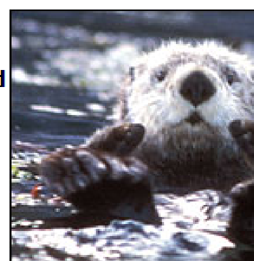
Cat parasite 'is killing otters'

By Paul Rincon
BBC News website science reporter, St Louis

A parasite carried by cats is killing off sea otters, a veterinary specialist has told a major US science conference.



Sea otters were hunted he
their fur in the 1800s

The Californian researcher has called for owners to keep their cats indoors.

Cat faeces carrying *Toxoplasma* parasites wash into US waterways and then into the sea where they can otters, causing brain disease.

The parasite is familiar to medical researchers, as it c damage human foetuses when expectant mothers be infected while changing cat litter.

The most likely source of infection for sea otters is th

javma News

California sea otters have been dying in alarming numbers for several years, raising concerns about the future of the species. The deaths have been blamed on pollution, disease, and human interference. A recent study suggests freshwater runoff containing *Toxoplasma gondii* may be partly to blame.

—*Could cat waste be killing sea otters?*

March 15, 2

Protozoal parasites important cause of death in sea otters

Toxoplasma gondii

- Definitive host = cat

Sarcocystis neurona

- Definitive host = Opossum

Terrestrial hosts. Terrestrial pathogens.

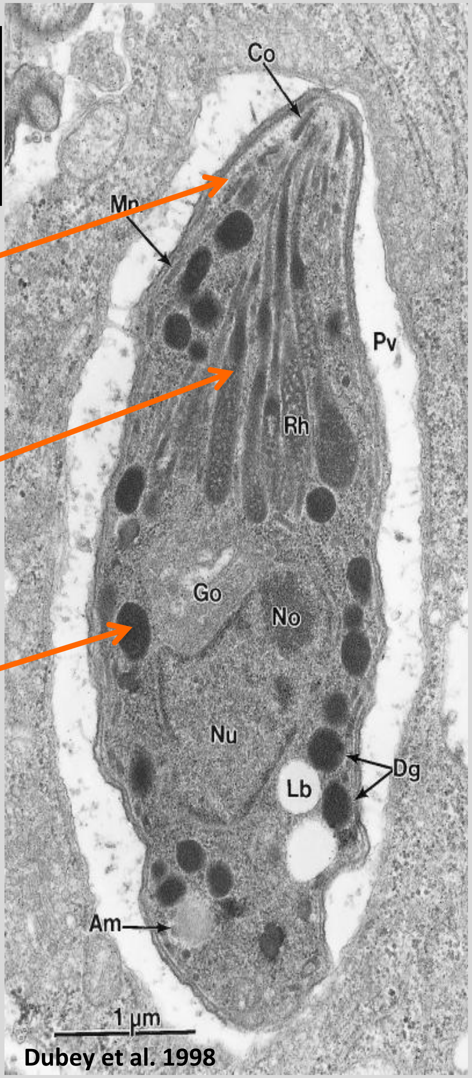
Toxoplasma gondii

***Toxoplasma* secretory organelles**

Micronemes (MICs)

Rhoptries (ROPs)

Dense granules (GRAs)



Discovered in 1908 in the North African 'gundi' rodent

***Toxo* = 'bow' *plasma* = 'form'**

- **Protozoan**
- **Apicomplexa**
- **Obligate intracellular parasite**
  - **Interacts with the host via secreted proteins**

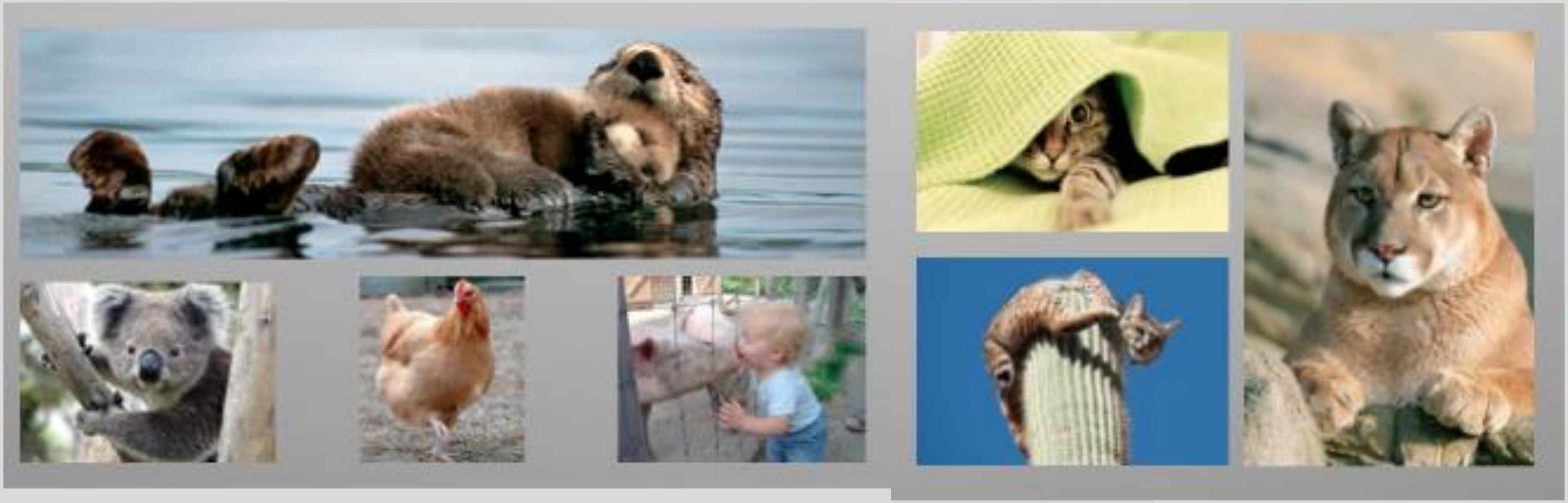
Toxoplasma gondii

Broad host range

Capable of infecting virtually any nucleated cell

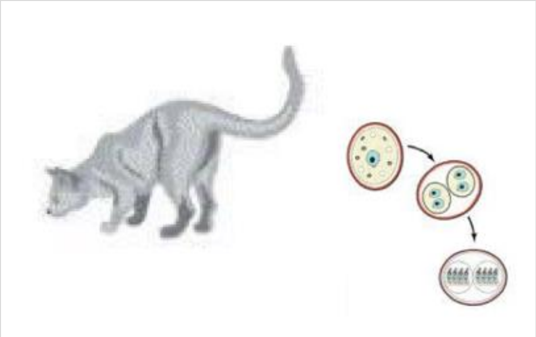
Able to cross several anatomical barriers

Only one *known* definitive host

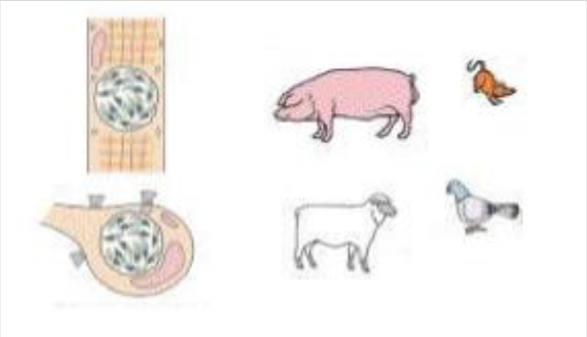


Three routes of infection

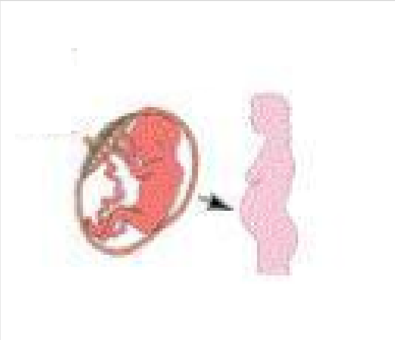
1. Ingestion of sporulated oocysts
2. Ingestion of tissue cysts in undercooked meat
3. Transplacental transmission



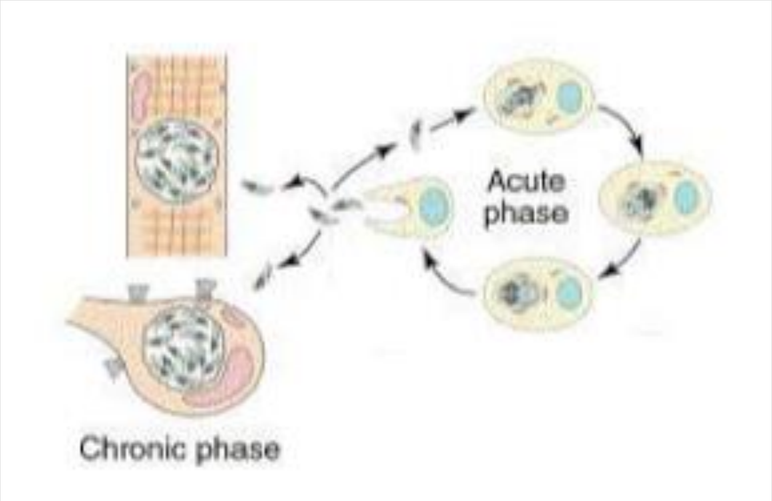
Sporozoites



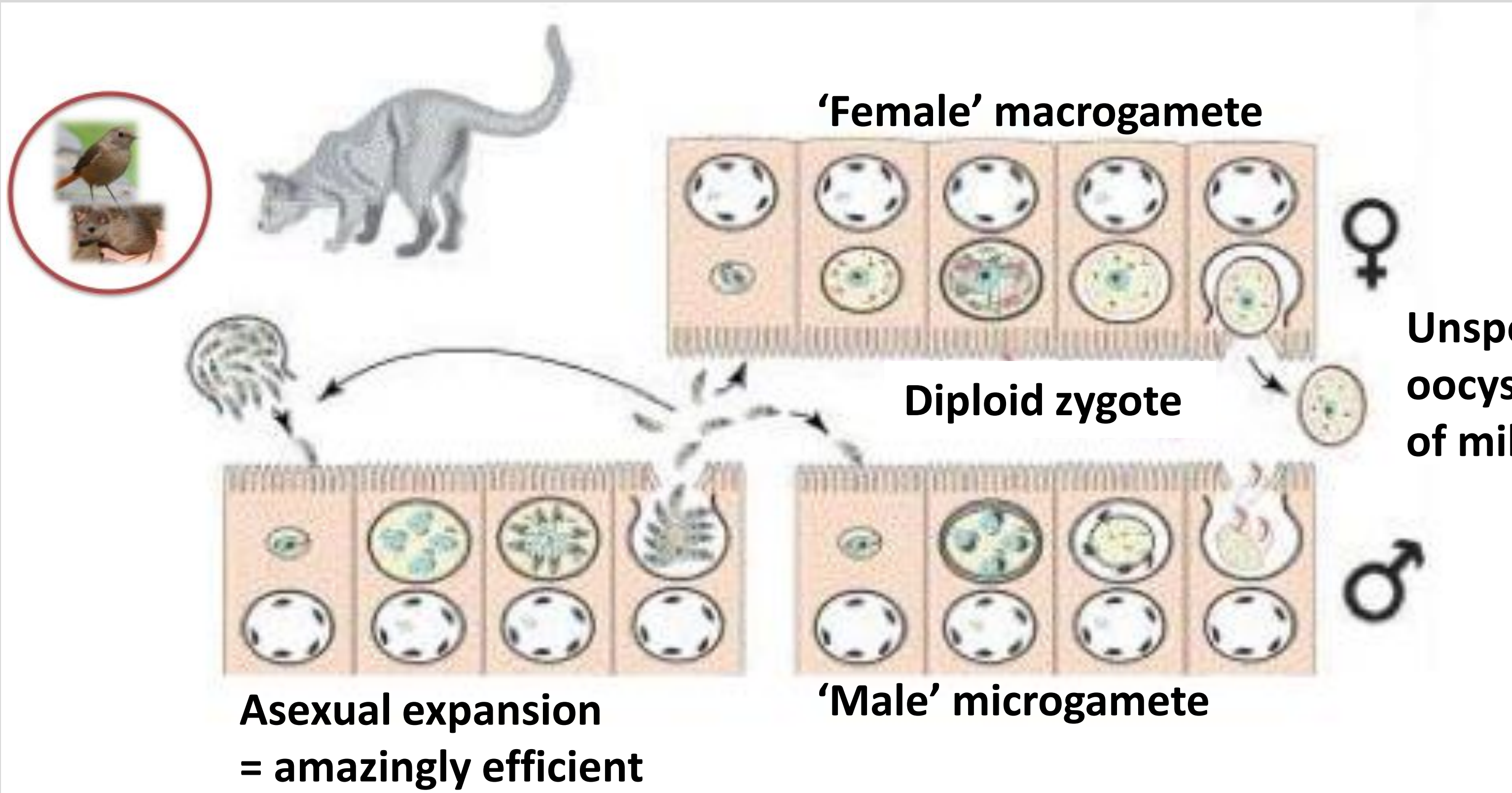
Bradyzoites



Tachyzoites



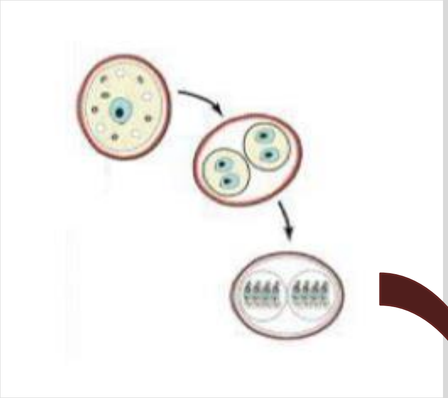
Lifecycle

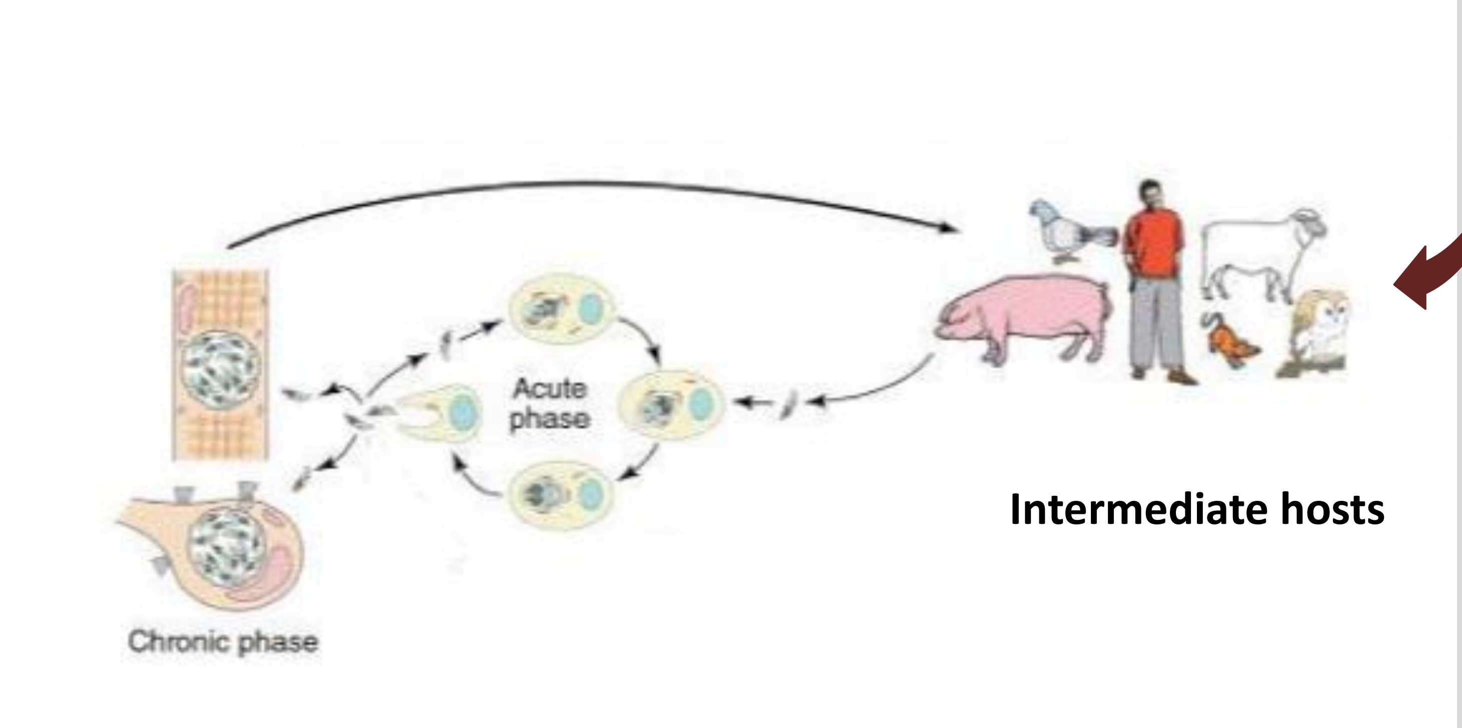


‘Female’ macrogamete
Unsporulated oocyst = hundreds of millions!
Diploid zygote
Asexual expansion = amazingly efficient
‘Male’ microgamete

Lifecycle

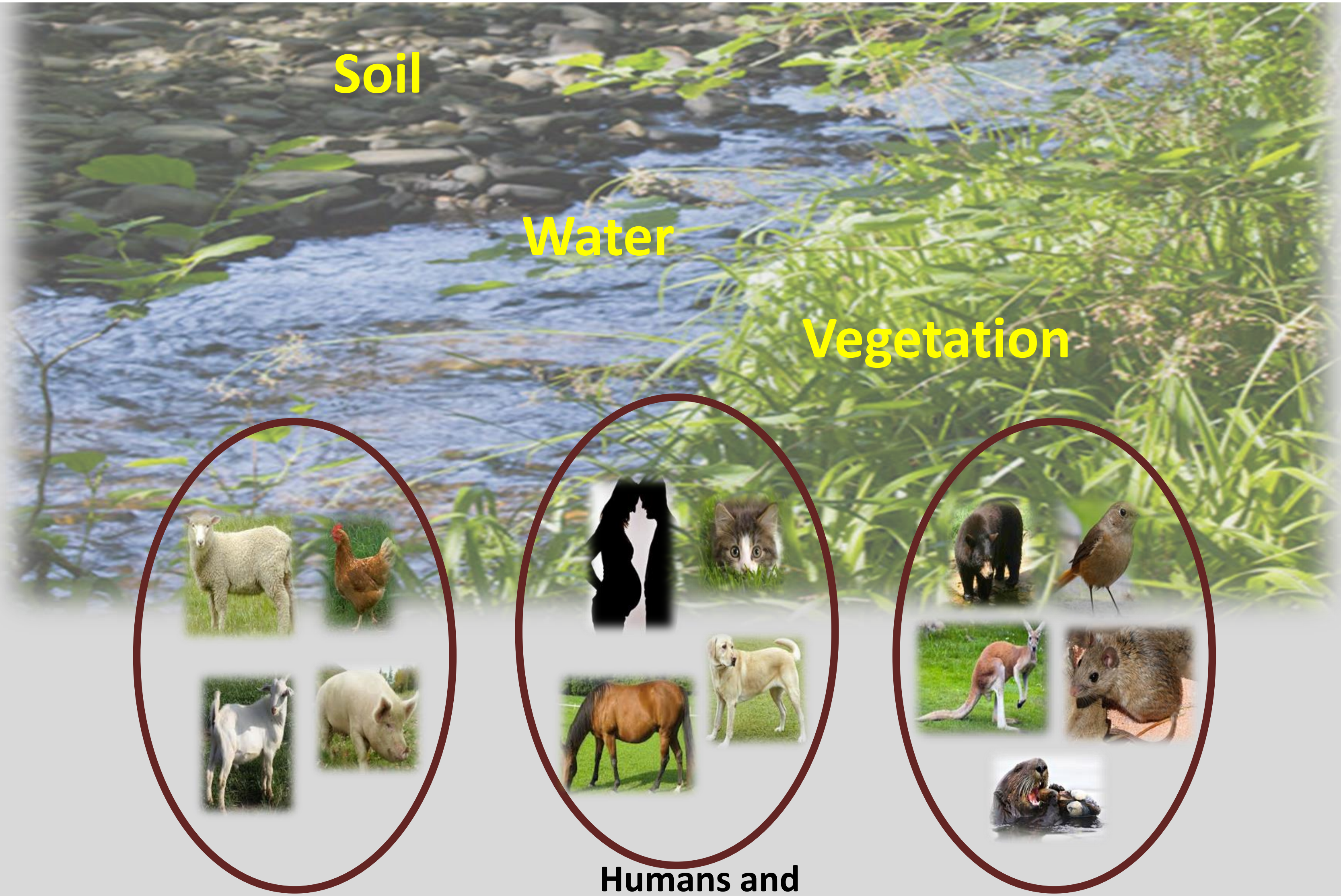
Sporulation occurs in the environment
- **2 sporocysts**
- **4 sporozoites in each sporocyst**





Intermediate hosts

Oocysts are extremely environmentally-resistant

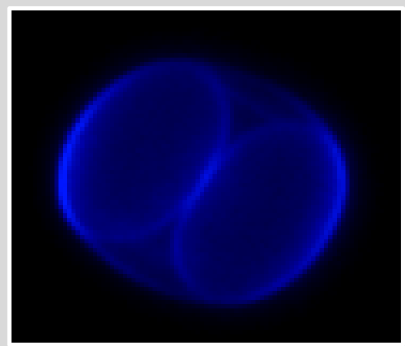


Oocysts are tough!

Chlorine

Ozone

Ultraviolet Radiation



Quaternary ammonium compounds

Ethanol

10% formalin

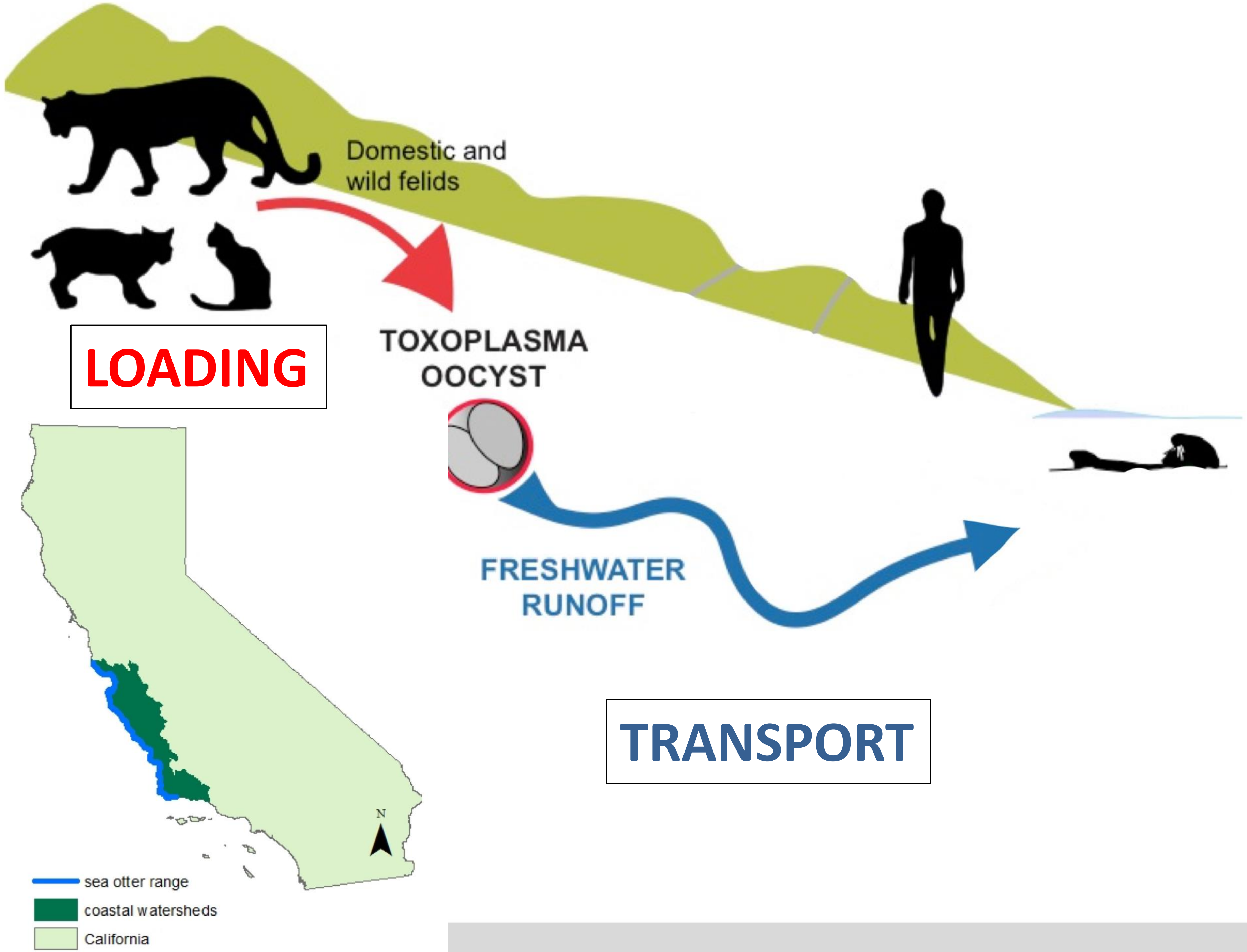
2% Sulfuric acid (H_2SO_4)

One Health Approach:
How is a terrestrial pathogen causing such profound disease in a marine mammal?

1. Develop a model for the transport of oocysts from land to sea.
2. How are oocysts encountered by otters in ocean.
3. What structures/factors are responsible for the remarkable environmental resistance of the oocyst?
4. How can we better identify where oocysts accumulate in the coastal environment to serve as a source of infection to otters?

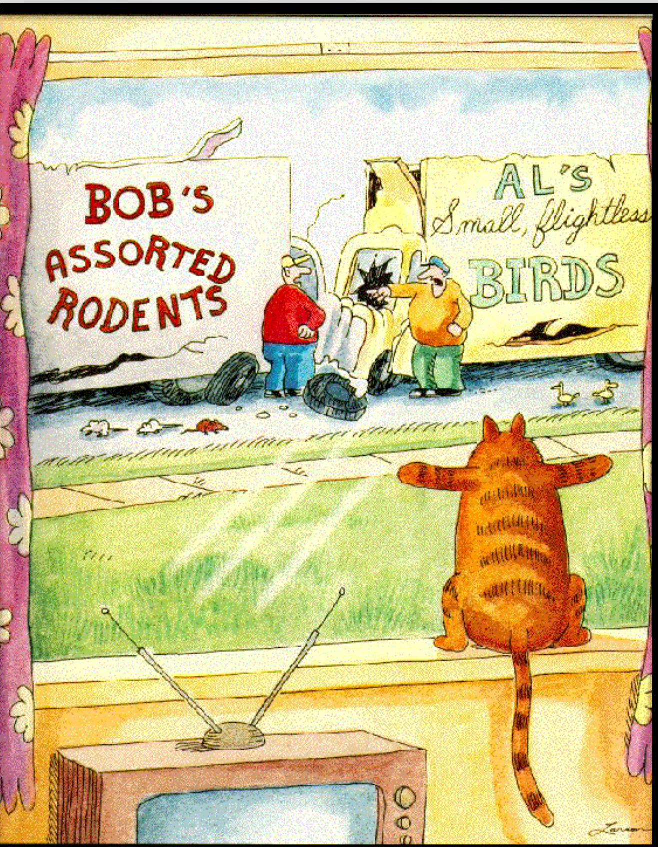
Modeling the transport of *Toxoplasma* oocysts from land to sea

Liz VanWormer
DVM, PhD

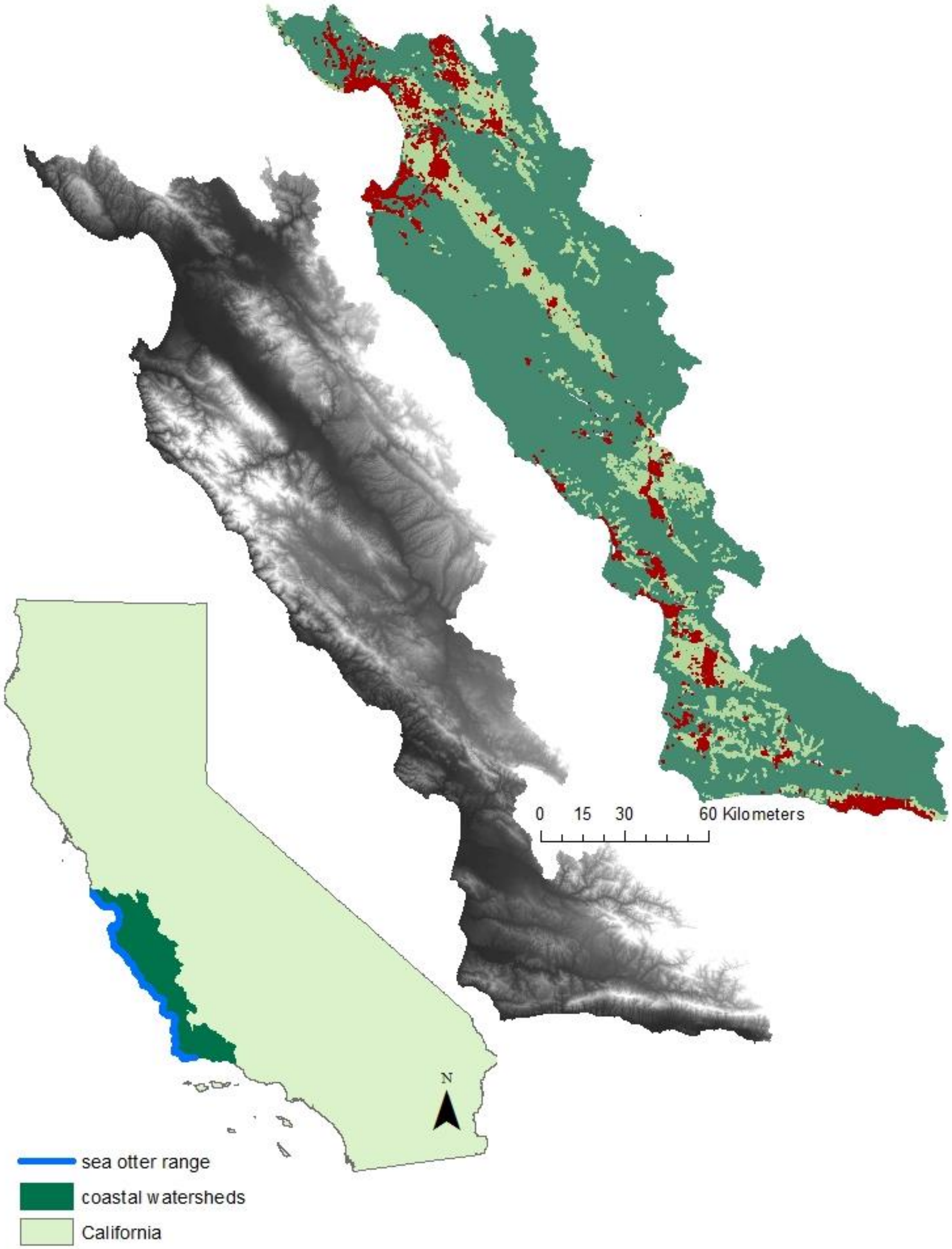

Domestic and
wild felids
LOADING
TOXOPLASMA
OOCYST
FRESHWATER
RUNOFF
TRANSPORT
N
sea otter range
coastal watersheds
California

Oocysts per cell = number of cats * infection prevalence * oocysts shed

Differences in infection and shedding

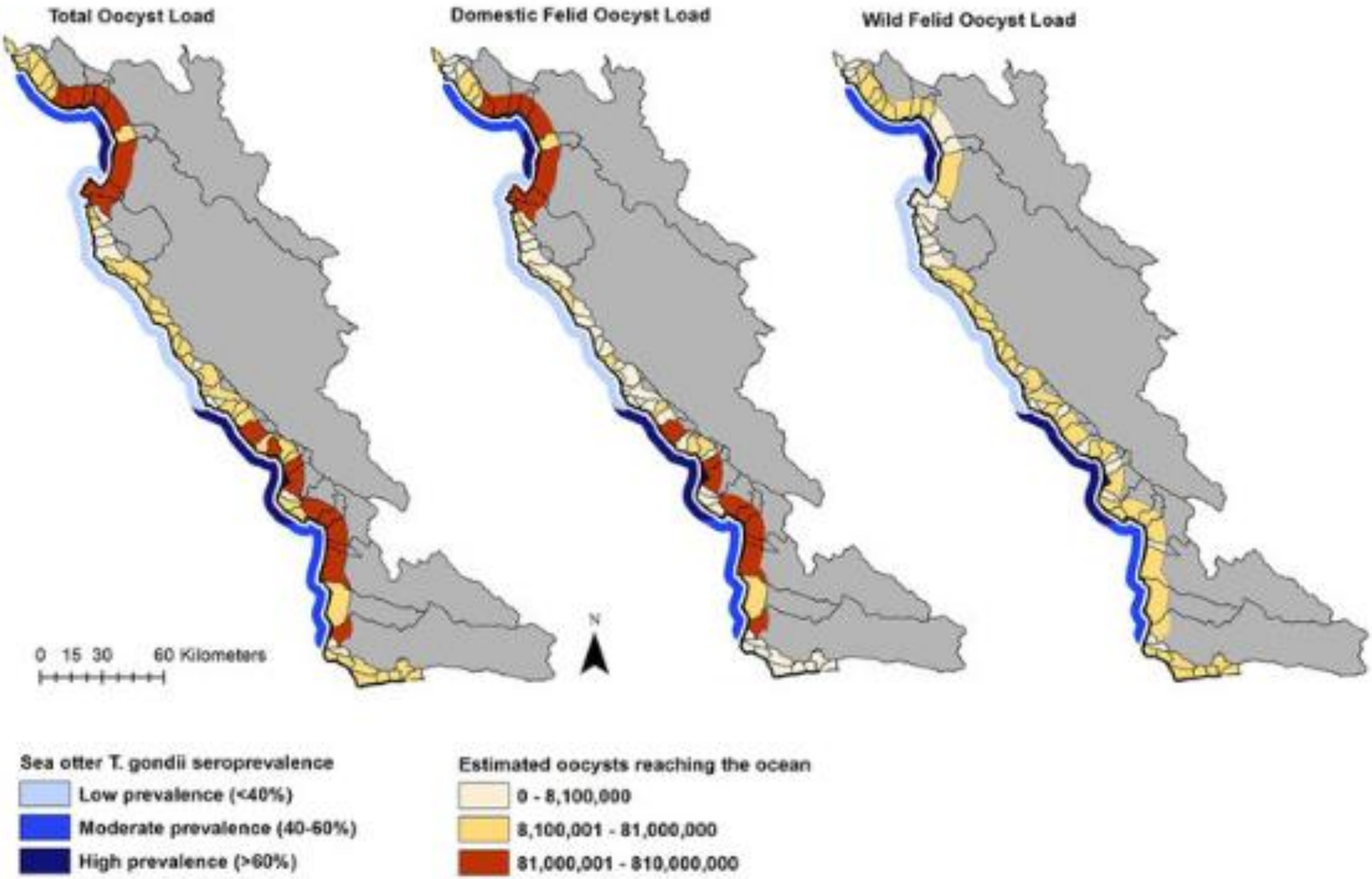



Both domestic and wild felids contribute *Toxoplasma* oocysts to terrestrial coastal environments

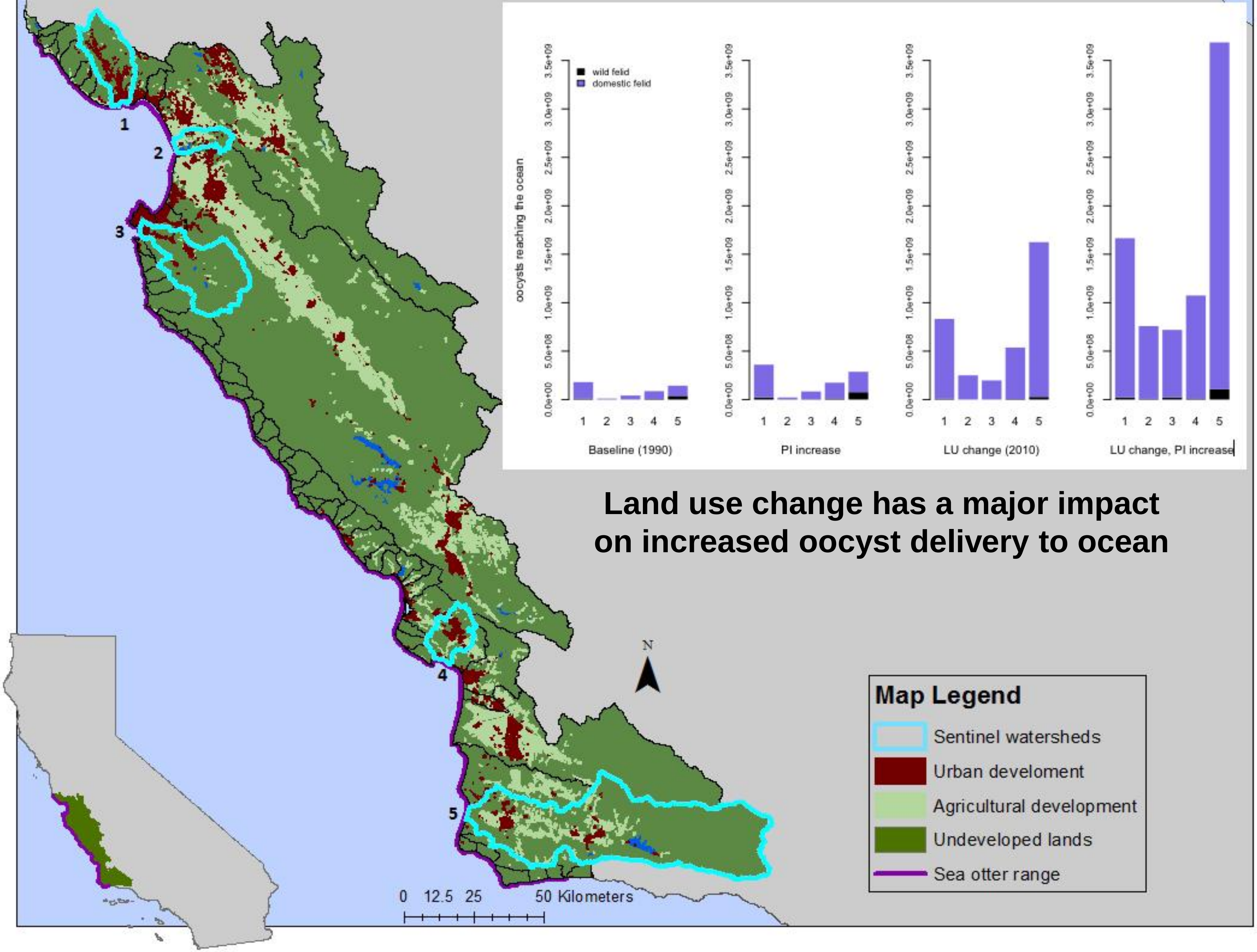


Modeling oocyst transport in freshwater runoff

Figure 2: Spatial distribution of *Toxoplasma gondii* oocysts carried to the ocean via freshwater runoff (light yellow to red shading).



How do coastal development and precipitation influence pathogen flow from terrestrial to aquatic environments?



Land use change has a major impact on increased oocyst delivery to ocean

Article | OPEN

Coastal development and precipitation drive pathogen flow from land to sea: evidence from a *Toxoplasma gondii* and felid host system

Elizabeth VanWormer ✉, Tim E Carpenter, Purnendu Singh, Karen Shapiro, Wesley W. Wallender, Patricia A. Conrad, John L. Largier, Marco P. Maneta & Jonna A. K. Mazet ✉

Scientific Reports **6**, Article number: 29252 (2016)
doi:10.1038/srep29252
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Ecological epidemiology
Infectious diseases

Received: 11 March 2016
Accepted: 14 June 2016
Published online: 26 July 2016

How are oocysts encountered by otters in the ocean

Karen Shapiro
DVM, MPVM, PhD

The puzzle: Toxoplasmosis in California sea otters

Terrestrial definitive hosts

?

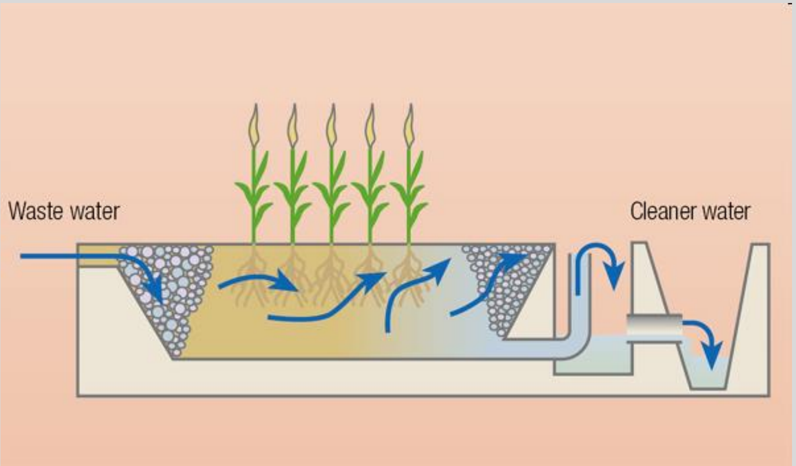
Marine mammals infected

It’s a big ocean out there…

How can so many otters become infected with a terrestrial parasite?

Wetlands and Water Quality

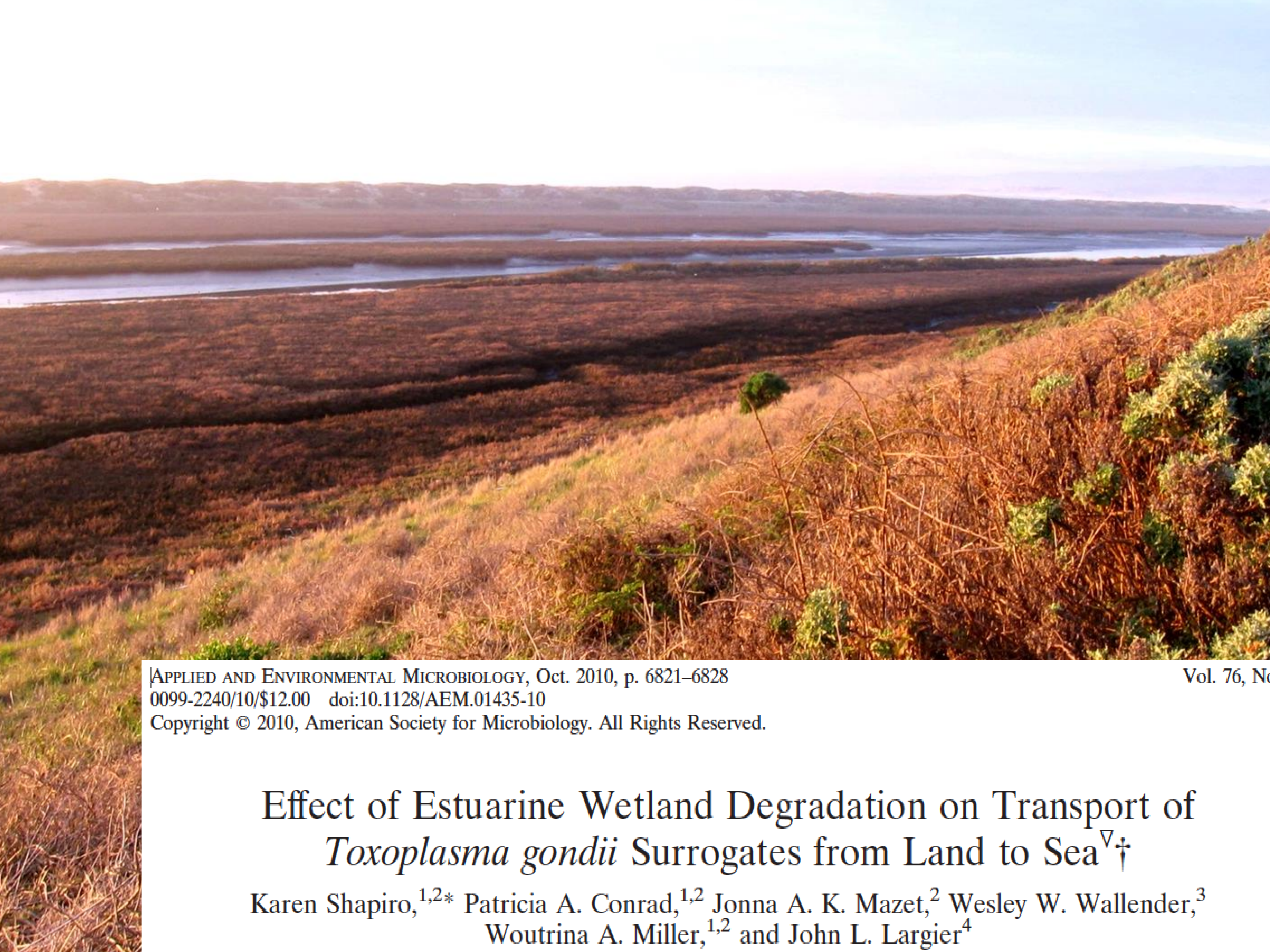
- **Wetland water effluents have reduced contaminants**
- **Physical processes**
  Sedimentation
  Adsorption and straining
- **Biological processes**
  Flora and fauna
  Metabolism and predation



↓ Pesticides

↓ Heavy metals

↓ Pathogens

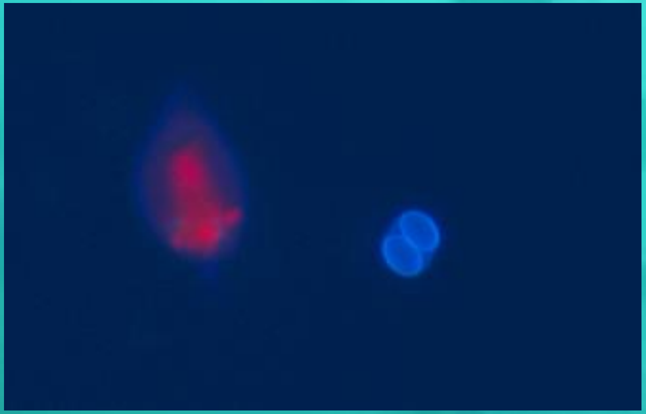


APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 2010, p. 6821–6828 Vol. 76, No
0099-2240/10/$12.00 doi:10.1128/AEM.01435-10

Effect of Estuarine Wetland Degradation on Transport of *Toxoplasma gondii* Surrogates from Land to Sea[∇]†

Karen Shapiro,[1,2]* Patricia A. Conrad,[1,2] Jonna A. K. Mazet,[2] Wesley W. Wallender,[3] Woutrina A. Miller,[1,2] and John L. Largier[4]

A mechanism for pathogen concentration in the ocean: marine snow



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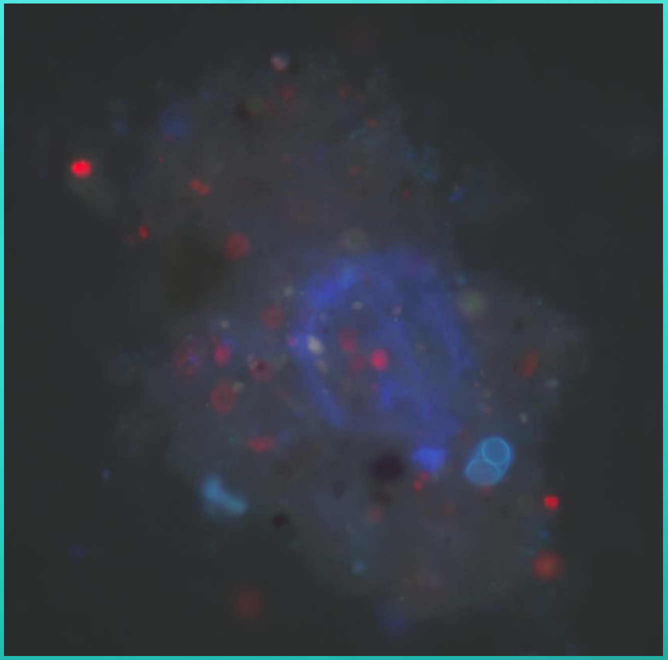
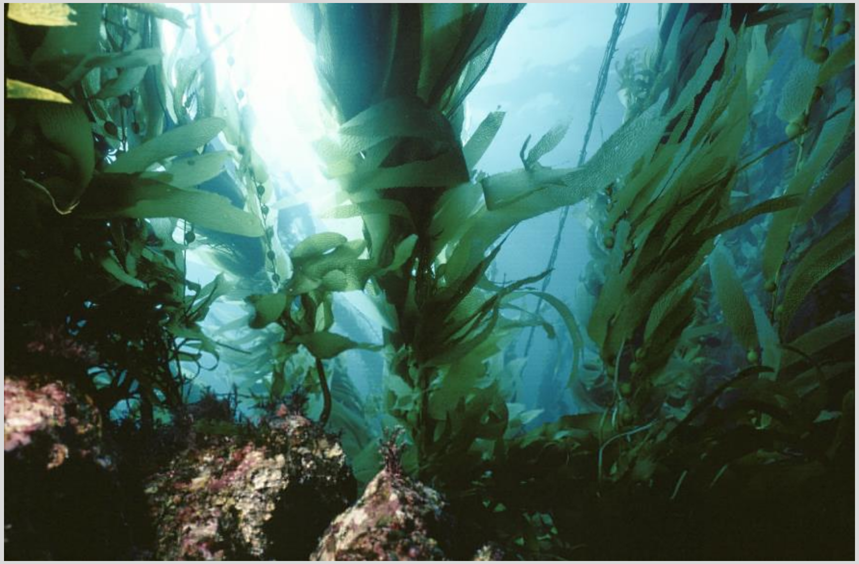


- Clumps of organic and inorganic material
- Snow tends to sink – accumulation zones may determine risk
- Food for invertebrates = entry into marine food webs

Where, when and how does marine snow form?

- Water salinity, currents, particle size and…
  - Transparent Exopolymer Particles (TEP) - Invisible, sticky, gel-like particles – the glue matrix of snow
  - Produced by phytoplankton, cyanobacteria, and…kelp

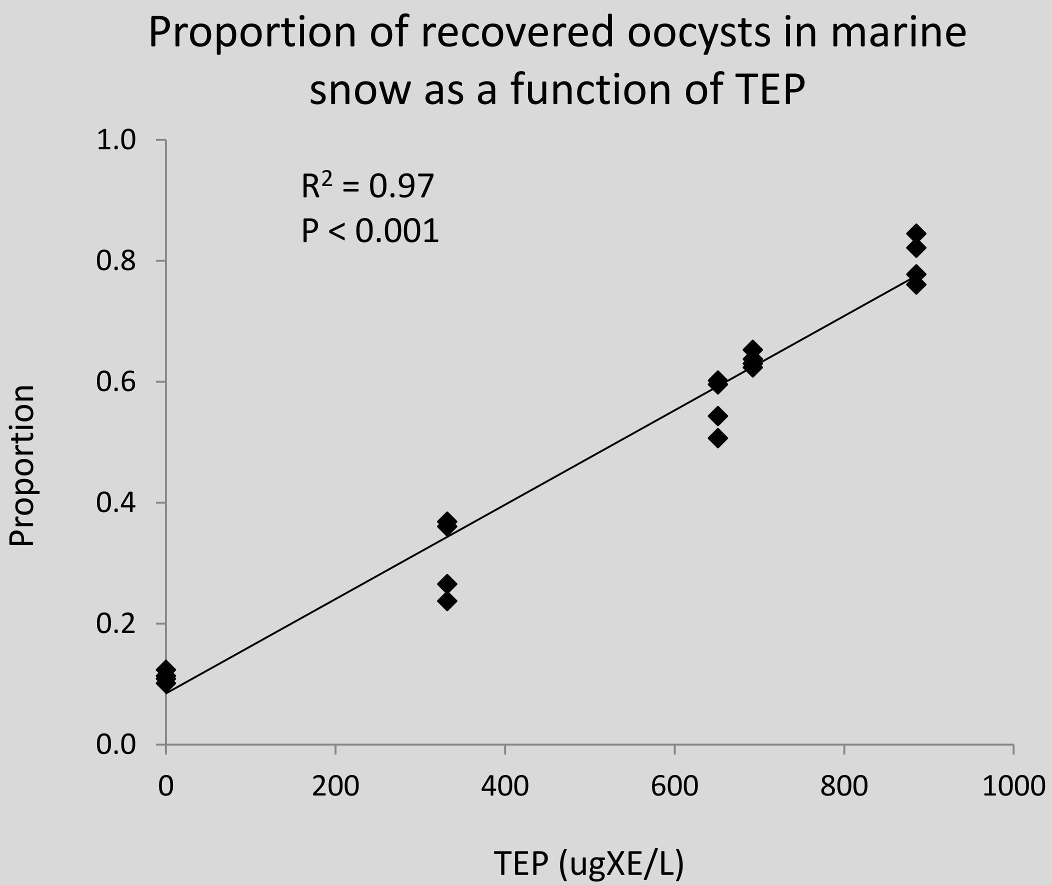


- Hypothesis:

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

Aggregation in TEP-spiked seawater

Objective: Test for the association of *T. gondii* with marine snow in seawater spiked with increasing concentrations of alginic acid => TEP produced by kelp



Findings: Increased concentrations of TEP typically present in sea otter habitat enhance the association of *T. gondii* oocysts with marine snow

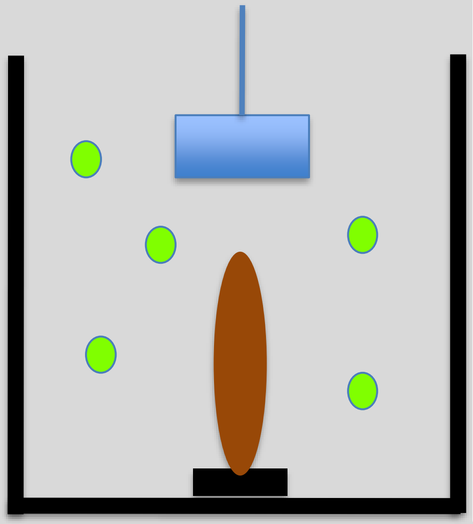
Unraveling the puzzle – Beyond snow

- Many invertebrate species that serve as prey for otters eat snow…
- But only snails identified as a risk factor for sea otter exposure to *T. gondii*
  - 12 X odds of *T. gondii* infection
  - Turban snails are kelp grazers

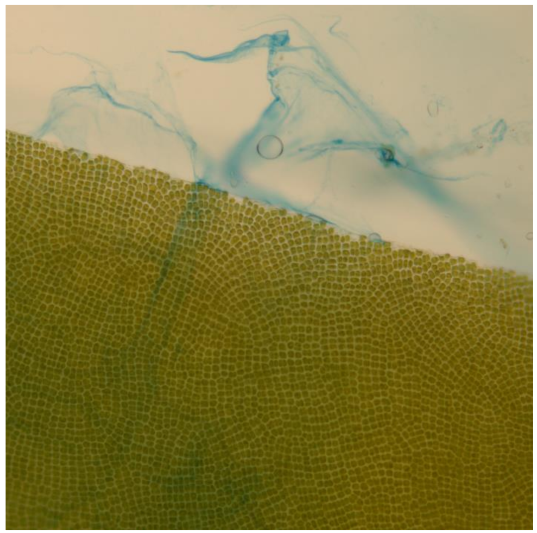


Association of *T. gondii* with kelp

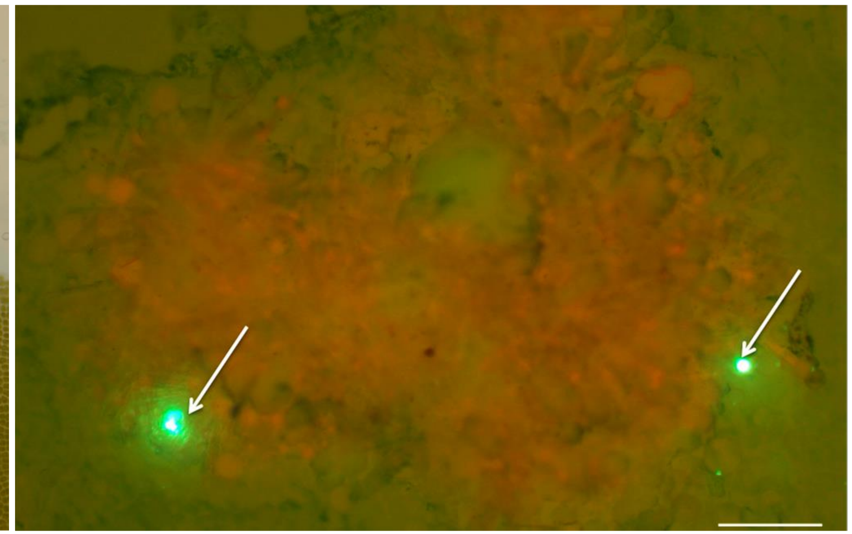
- **Objective**: Can *T. gondii* oocyst surrogates adhere to kelp surfaces?



Tank kelp experiments using *T. gondii* surrogates



TEP oozing from kelp surface

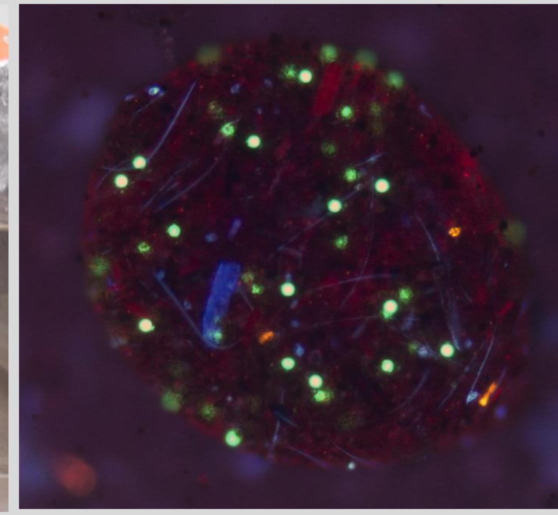


T. gondii surrogates associated with kelp biofilm

- **Findings**: Up to 30% of *T. gondii* surrogates attach to kelp blades due to TEP coating on kelp

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

- **Findings**:
  - Retention of oocysts up to 11 days
  - Concentration 2-3 orders of magnitude greater than seawater

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

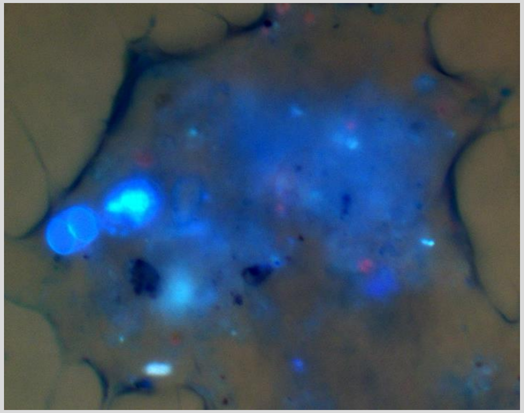
- Prolong exposure period
- Bio concentrator

Unraveling the puzzle

How can a land parasite infect so many otters?

T. gondii **oocysts can concentrate in coastal ecosystems through two mechanisms:**

1) Enrichment in marine snow

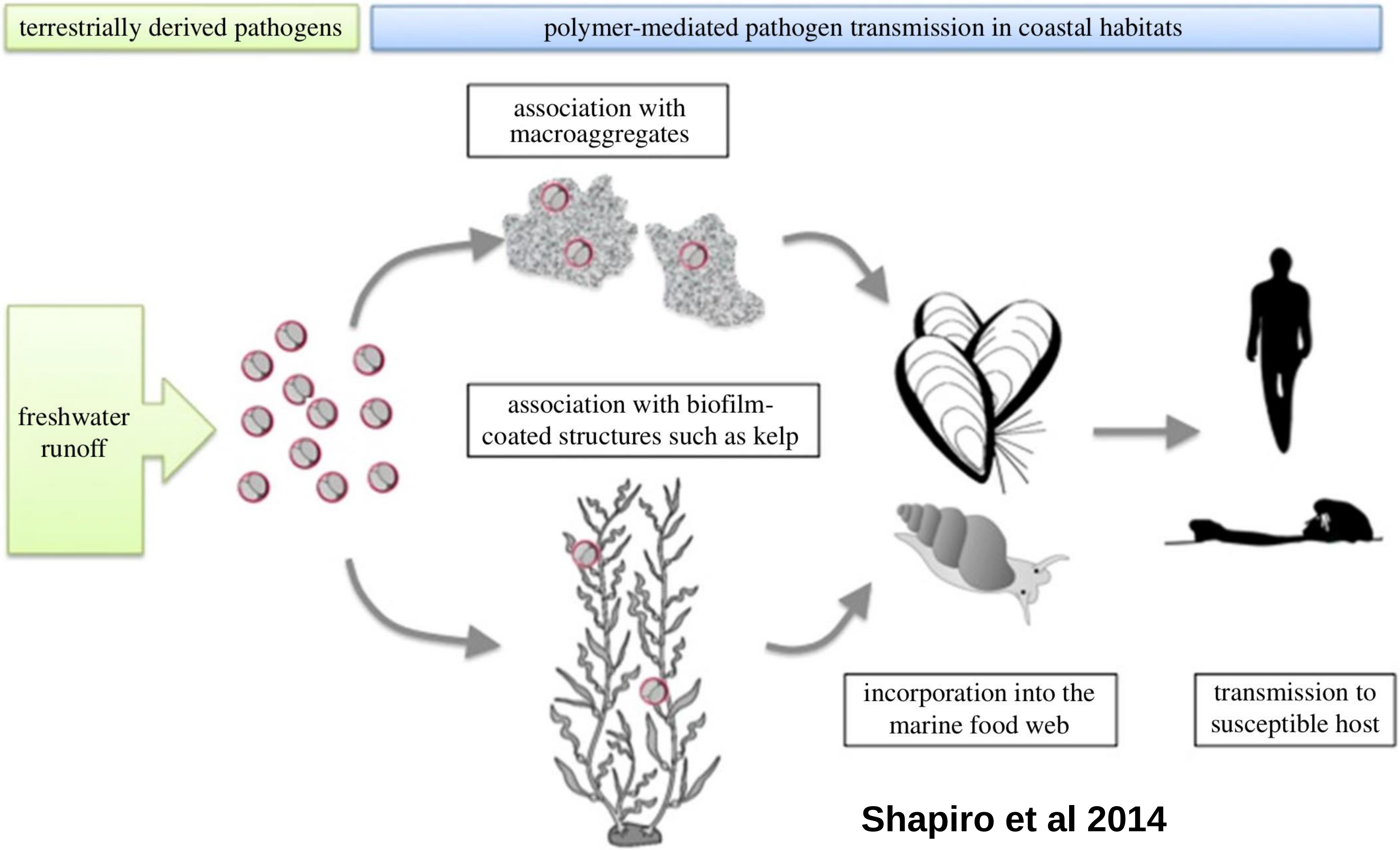


2) Association with kelp surfaces

Snails as mechanical hosts

Snail-specializing otters at greater odds of infection

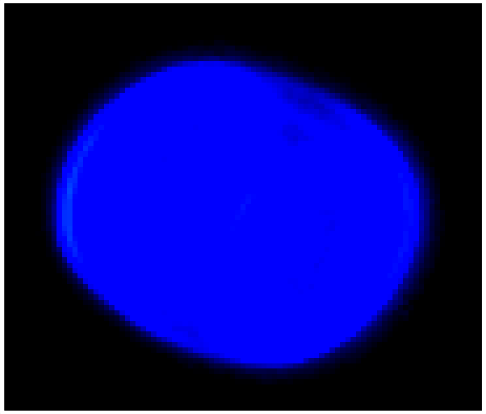
Terrestrial to Marine System Oocyst Transport



Shapiro et al 2014

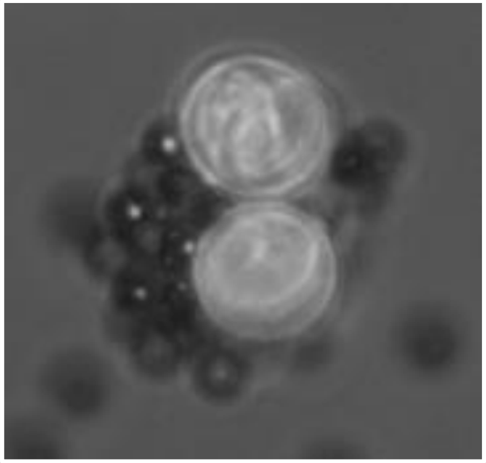
Questions

1. **What structures/factors are responsible for the remarkable environmental resistance of the oocyst?**



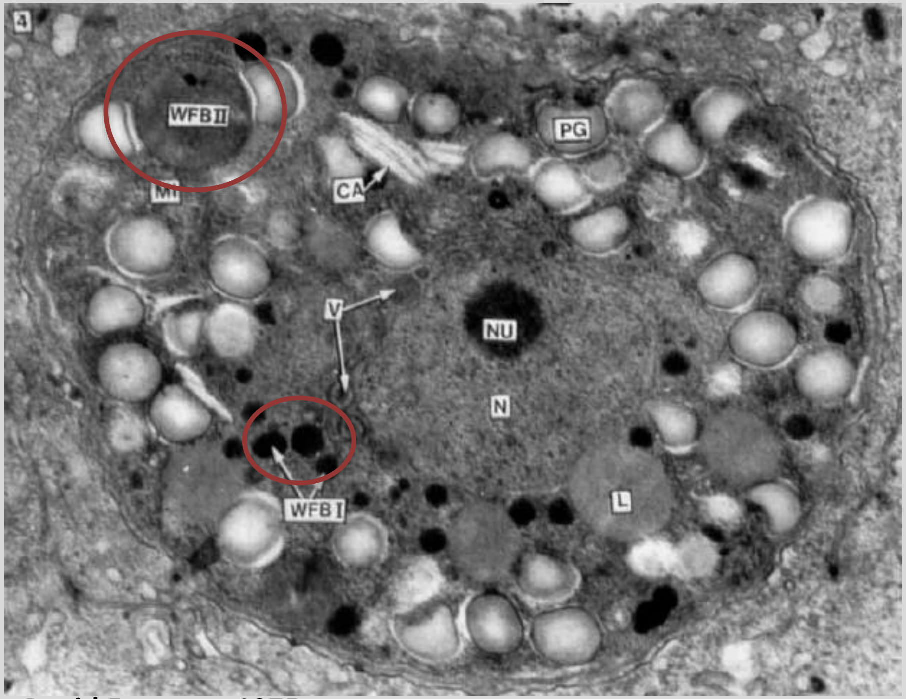
Hypothesis: Proteins present in one or both layers of the oocyst wall confer environmental resistance.

2. **Where do oocysts accumulate in the environment to serve as a source of infection to humans and animals?**



Hypothesis: Immunomagnetic separation can be used to concentrate Toxoplasma oocysts in water to identify sources of oocyst accumulation.

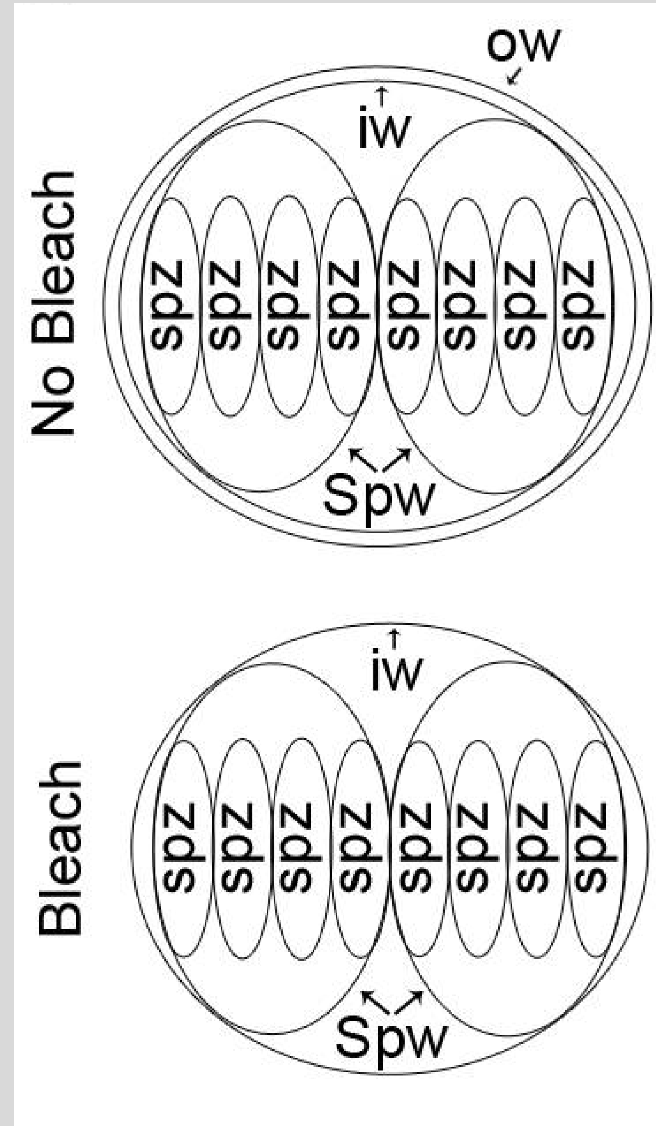
Oocyst wall formation



David Ferguson 1975

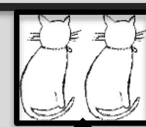
- WFBI – Outer layer

- WFBII – Inner layer



<u>**Bleach strips the outer layer of the oocyst wall**</u>

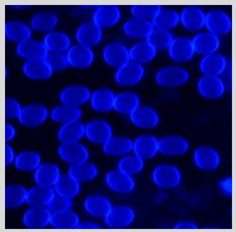
Approach



Microarray

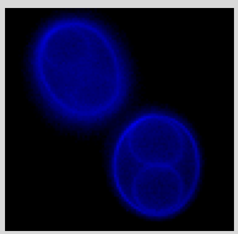
Day 0

Unsporulated



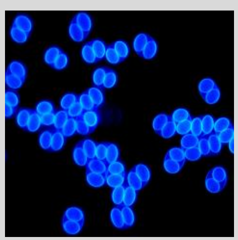
Day 4

Mid sporulation



Day 10

Sporulated



Compare same strain (M4):

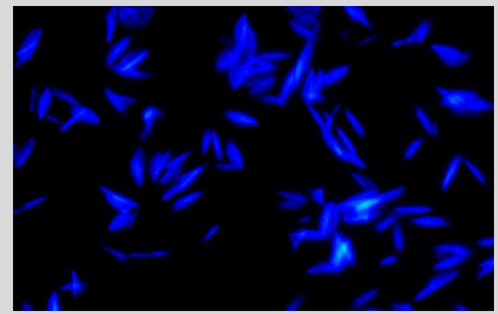
***in vitro* tachyzoites**

***in vivo* bradyzoite cysts**

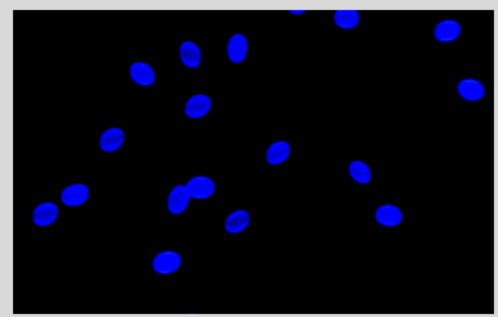
Mass spectrometry

Day 10 oocysts

± bleach treatment



Walls



Sporocysts (sporozoites)

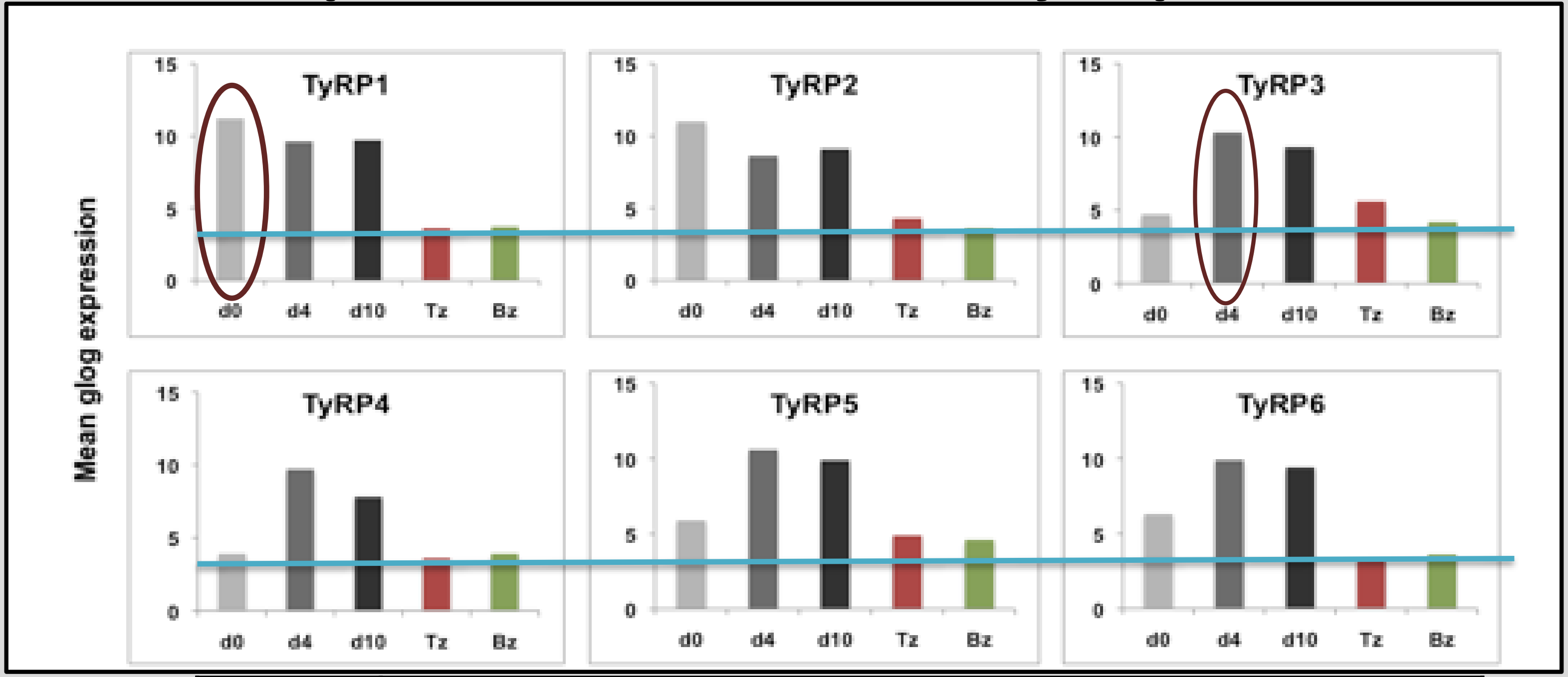
Results: Top 15 oocyst-specific transcripts

➡ Tyrosine rich protein (TyRP)

Product	Fold-change d10 vs. Tz	Fold-change d10 vs. Bz	Proteomic Oo	Proteomic Tz
hypothetical protein	111.1	103.3	Y	N
hypothetical protein (TyRP 6.2%)	90.9	84.6	Y	N
hypothetical protein (TyRP 5.6%)	76.9	69.7	Y	N
hypothetical protein	76.9	63.8	N	N
late embryogenesis abundant domain-containing protein (TgERP)	71.4	55.4	Y	N
late embryogenesis abundant domain-containing protein	66.7	60	Y	N
hypothetical protein (TyRP 15.5%)	66.7	77.6	Y	N
hypothetical protein	52.6	51.7	N	N
hypothetical protein	58.8	48	N	N
hypothetical protein, conserved	55.6	47.5	Y	Y
SRS28 (SporoSAG)	55.6	63.2	Y	N
hypothetical protein	52.6	43.9	Y	N
glutaredoxin, putative	62.5	45.7	Y	N
hypothetical protein (TyRP 5.5%)	26.3	51.2	Y	N
hypothetical protein (TyRP 13.5%)	41.7	50.7	Y	N

Results

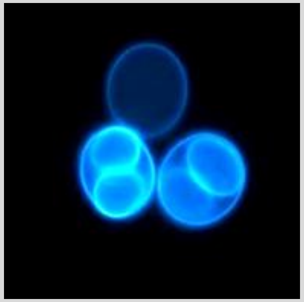
Tyrosine-rich proteins are abundantly expressed in oocysts



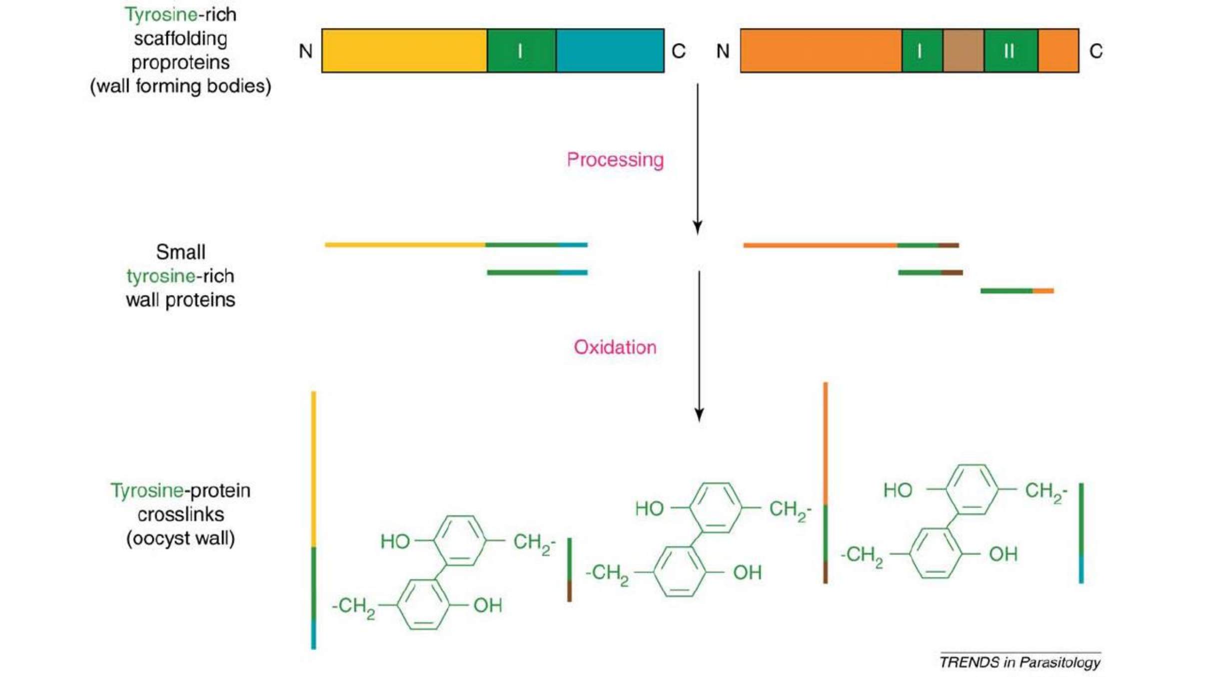
ID	Mass Spectrometry Spectral Counts					
	Experiment One		Experiment Two			
	Not Treated		Bleach Treated		Not Treated	
	Walls	Sp	Walls	Sp	Walls	Sp
TyRP1	27	3	2	5	2	9
TyRP2	13	3	0	3	3	6
TyRP3	9	50	10	70	22	47
TyRP4	2	6	0	2	0	7
TyRP5	12	6	0	13	10	31
TyRP6	0	2	0	0	0	3

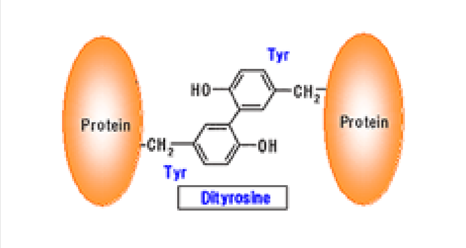
Further investigation of a tyrosine-rich protein

Oocyst walls are autofluorescent



Oocyst walls believed to be composed of tyrosine cross-linked proteins

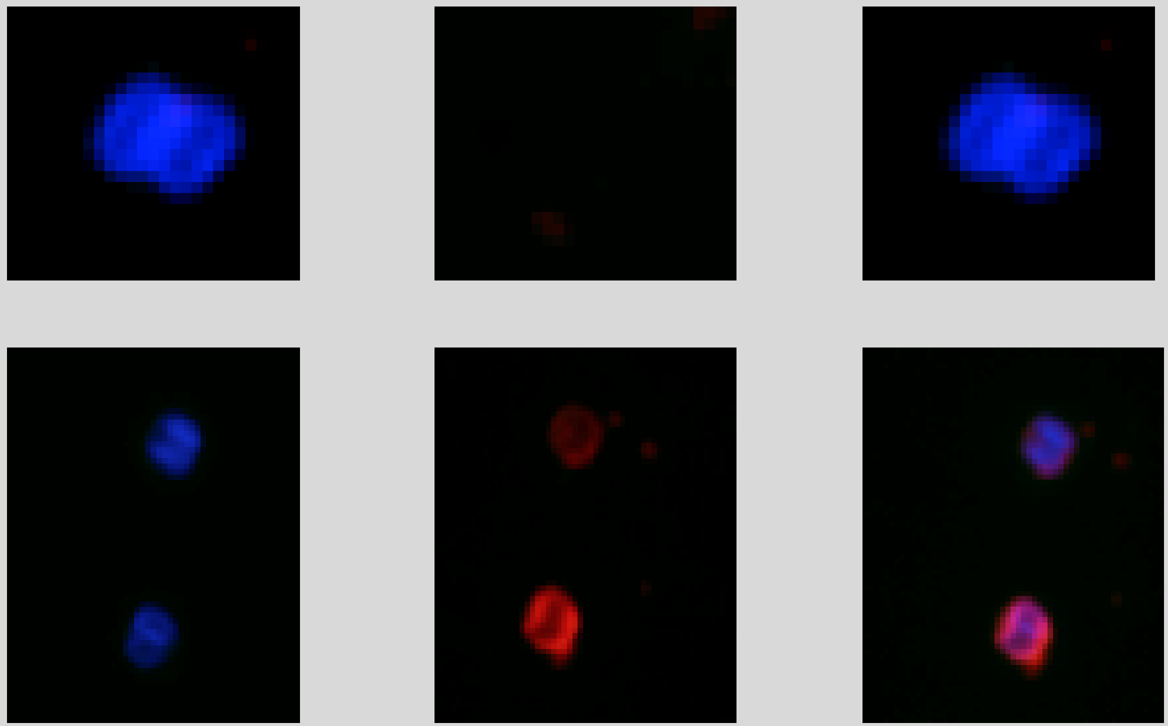




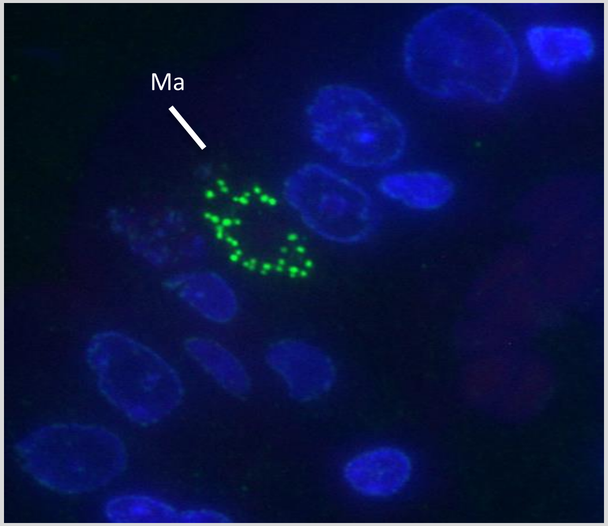
Dityrosine:

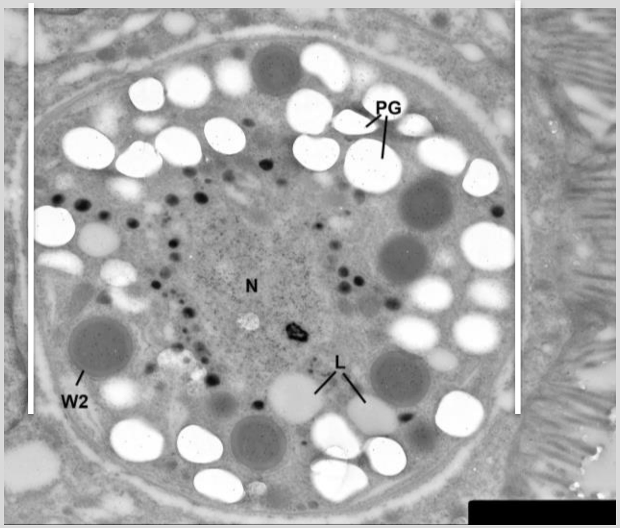
- Autofluorescence
- Sclerotization (hardening)
  - -Sea urchin eggs
  - -Insect resilin
  - -Yeast cell walls
  - -Coccidian oocysts

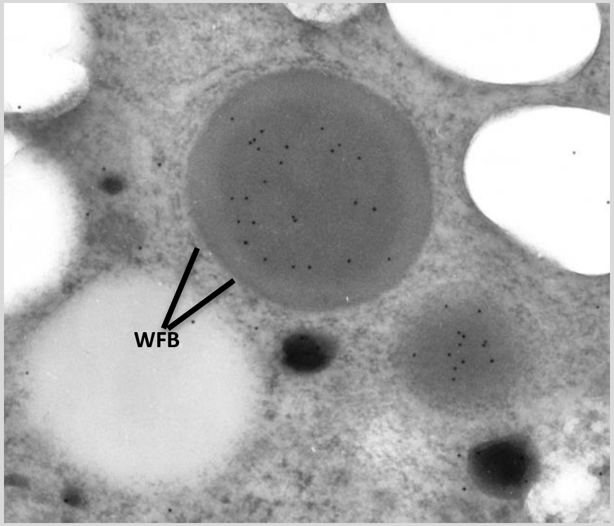
First identification of a tyrosine-rich protein in the oocyst wall



And also in the in macrogamete!





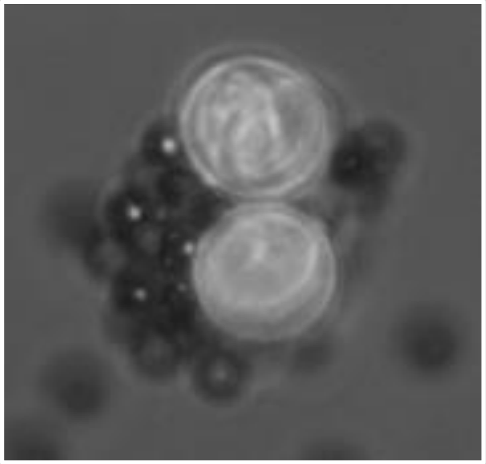


TyRP1 localizes to the macrogametes – role in oocyst wall formation?

David Ferguson
University of Oxford

Questions

2. **Where do oocysts accumulate in the environment to serve as a source of infection to humans and animals?**



Hypothesis: Immunomagnetic separation can be used to concentrate Toxoplasma oocysts in water to identify sources of oocyst accumulation.

Oocyst detection in water

EPA-Approved method to test water for *Cryptosporidium* and *Giardia:* Immunomagnetic Separation and Immunofluorescence Assay

1. Develop mAb(s) to oocyst wall

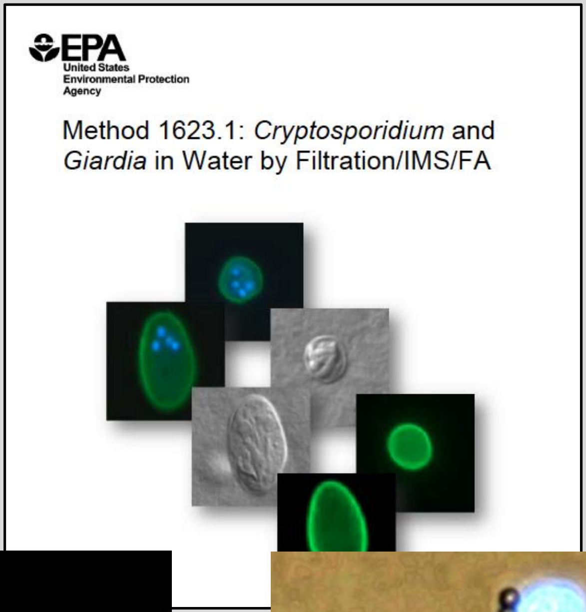
1. Couple mAb to paramagnetic beads

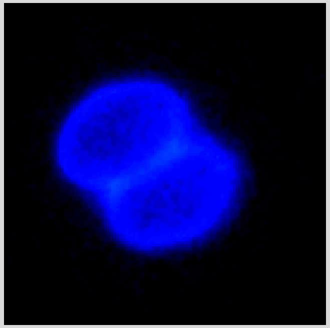
2. Co-incubate mAb-coupled beads with concentrated water sample containing oocysts

3. Retain beads + oocysts on magnet

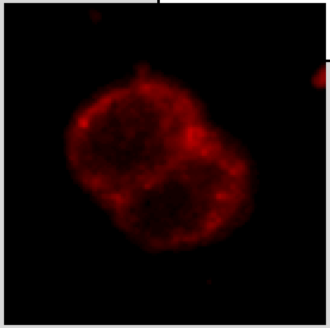
4. Elute oocysts off of beads

5. Detect oocysts by DFA





DAPI



Ms-α-oocyst

Oocysts + beads

Acknowledgements

UC Davis

Pat Conrad
Conrad Lab
Peter Barry
Division of Biostatistics/SOM

University of South Florida

Michael White

Funding:

- NIH - KO1 1K01RR031487
- NIH - EuPathDB (Boothroyd)
- CA Ocean Protection Council
- NIH - T32-RR07038

Stanford University

John Boothroyd
Kerry Buchholz
Paul Bowyer
Matthew Bogyo

University of Liverpool

Jonathan Wastling
Dong Xia

Oxford University

David Ferguson

ToxoDB

David Roos
Omar Harb
Brian Brunk
Brian Gregory

What we've learned . . . . .

Studies are more powerful when we integrate information across disciplines

ecosystem-level studies

+

population health and laboratory studies

=

Tackle complex problems

Sea otters are sentinels of environmental contamination

“pathogen pollution”

One Health Approach

Human

Ecosystem

Domestic Animals

Wildlife




Thank You

Funding: NSF EID, NOAA OHHI, CA Sea grant

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Woutrina Miller, Ann Melli,
Andrea Packham, Heather Fritz , Aiko Adell

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UCD Environmental Engineering
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UCD Hydrology; University of Montana Hydrology
Wes Wallender, Purnendu Singh; Marco Maneta

UCSC Marine Sciences
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Joe Tomoleoni, Ben Weitzman, Zach Randell

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