Marine Mammal Pathology

How diseases are emerging and evolving in West Coast marine mammal populations in response to natural events, climate driven changes in the environment and human activities.

Pádraig Duignan

Director of Pathology, TMMC

The Marine Mammal Center

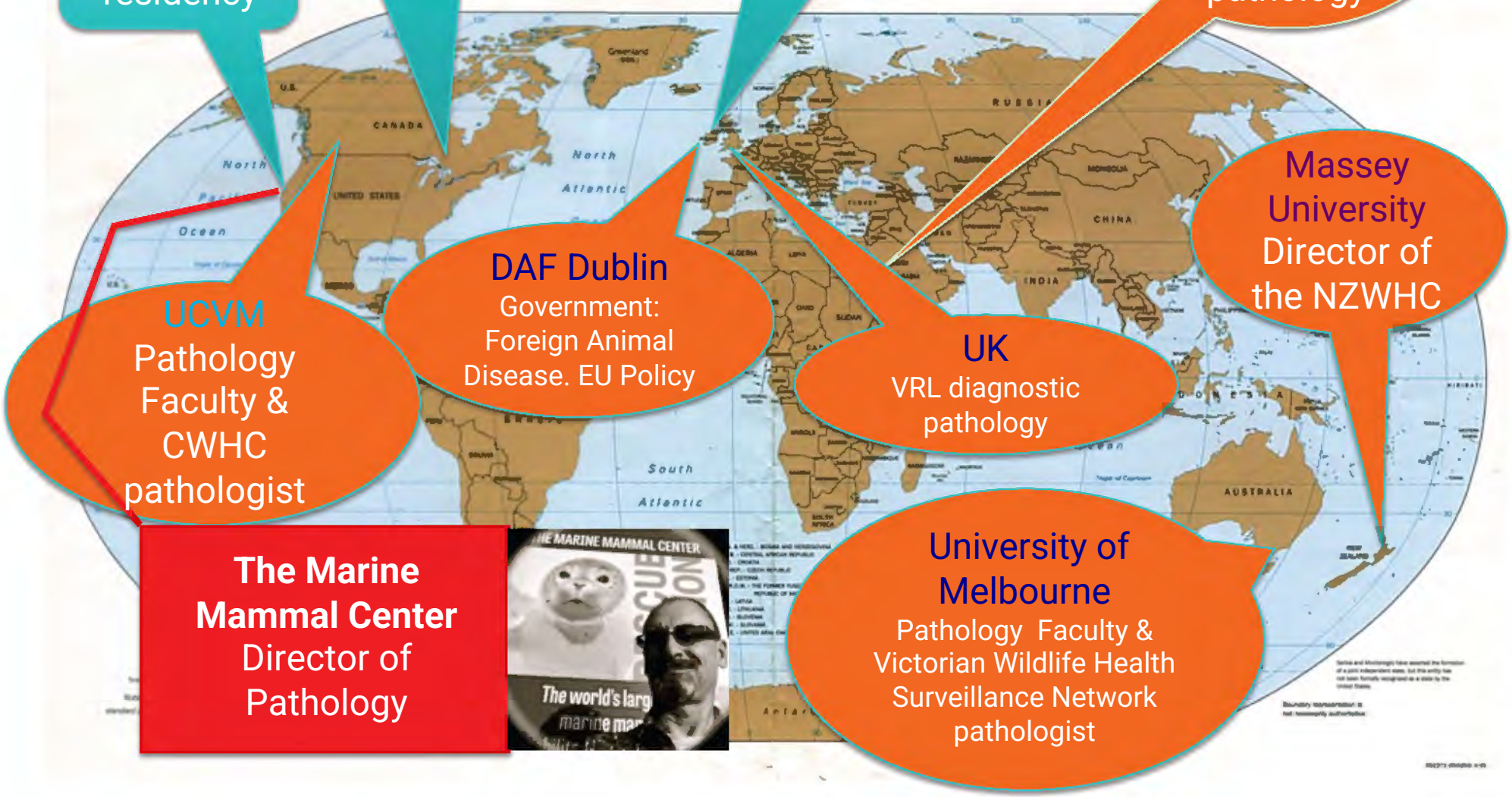
Why am I here to talk to you this evening?

UC Davis
Pathology residency

OVC, Canada
PhD in Marine Mammal Pathology & Anatomic Path Residency

UC Dublin
BSc Hons. Zoology MSc Biochemistry MVB (DVM)

Saudi Arabia
Avian and wildlife pathology



UCVM
Pathology Faculty & CWHC pathologist

DAF Dublin
Government: Foreign Animal Disease. EU Policy

UK
VRL diagnostic pathology

Massey University
Director of the NZWHC

The Marine Mammal Center
Director of Pathology

University of Melbourne
Pathology Faculty & Victorian Wildlife Health Surveillance Network pathologist

Acknowledgement

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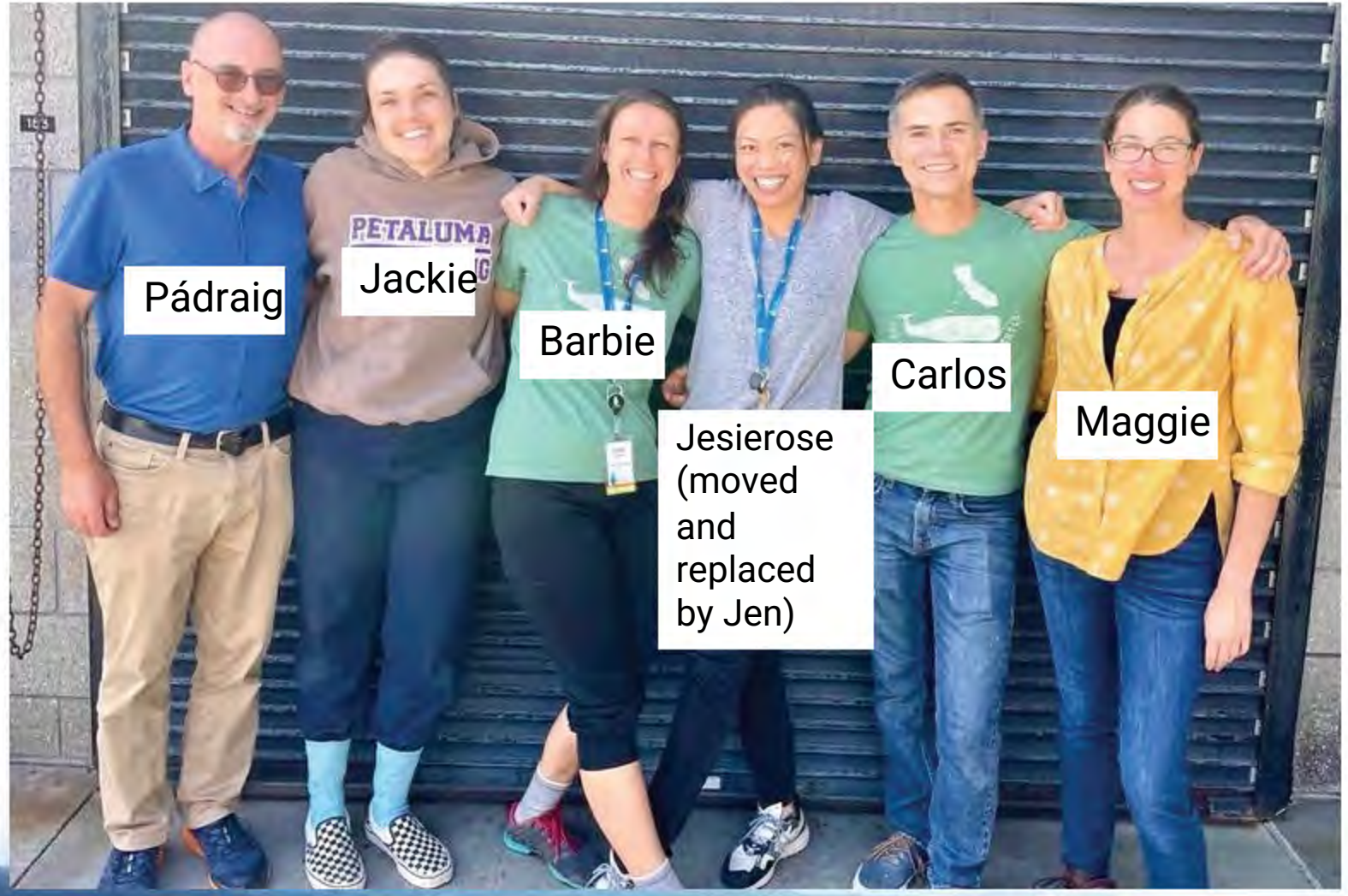
The Path & Diagnostics Team



Jen

2 Pathologists
2 Clinical Lab Technologists
2 Necropsy assistants

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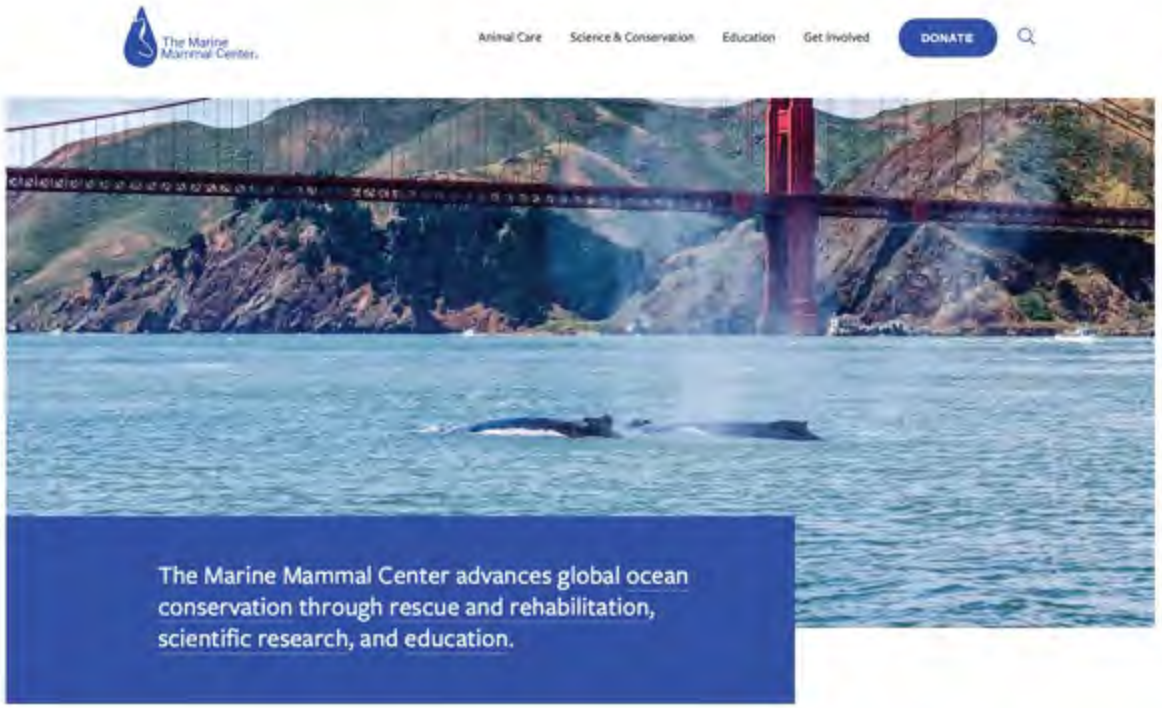

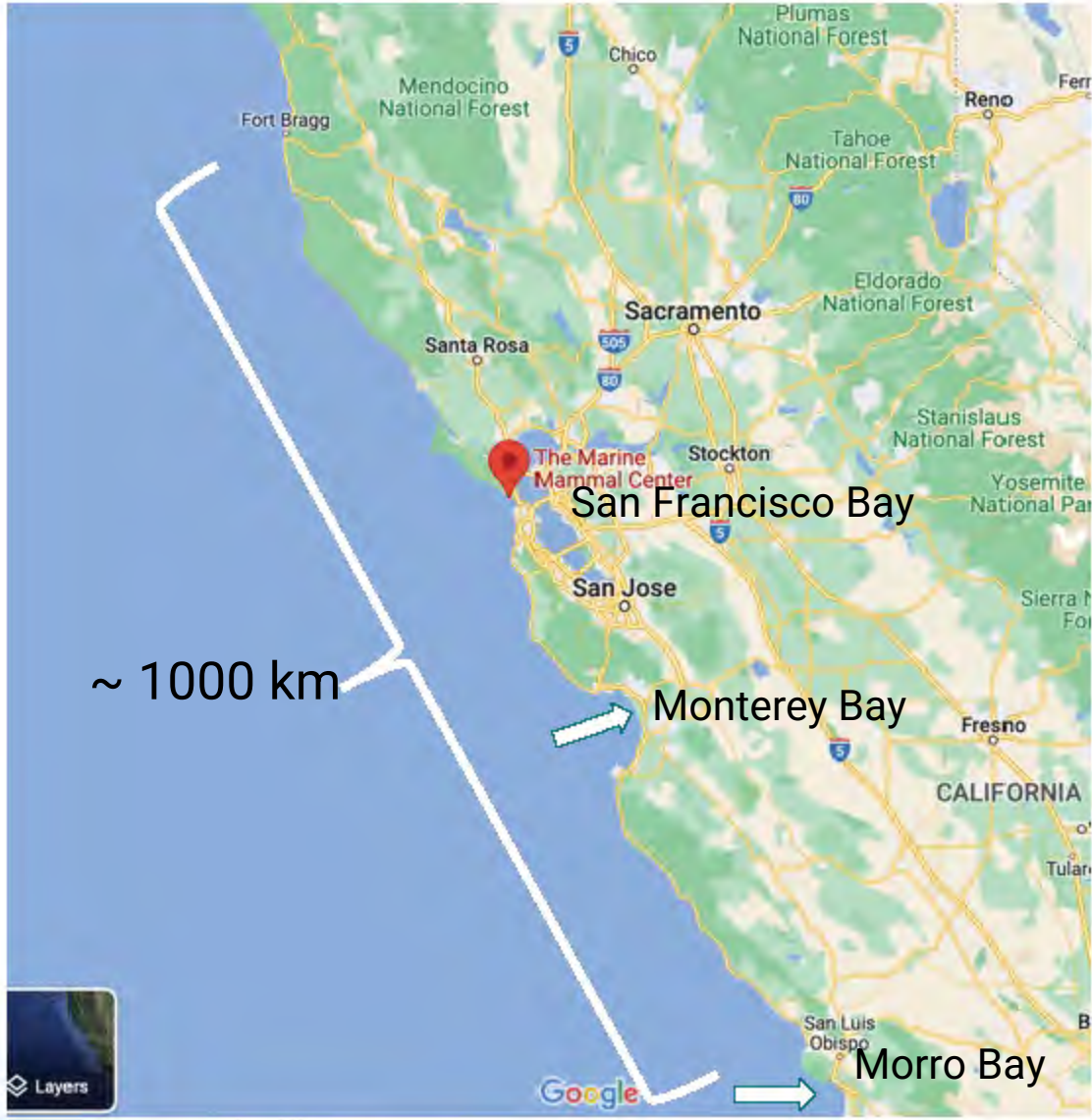

Commonalities between MMs and us

- Both are mammals and respond similarly to diseases, toxins or other insults
- We eat the same food
- Social interaction is similar – disease epidemiology is comparable
- Emerging diseases are appearing in MMs just as in the human population
- Climate change is impacting disease occurrence in both

TMMC's pathology research

- **Cancer Research.** ✓
  - Sea lion urogenital carcinoma
  - Other cancers eg northern elephant seal neonatal leukemia/lymphoma
- **Emerging/evolving infectious diseases**
  - Infectious diseases
    - Leptospirosis (Weil's disease) ✓
    - *Klebsiella pneumoniae* (HMV genetic variant) ✓
    - Pasteurellosis (avian cholera)
    - Brucellosis
    - Coccidioidomycosis (Valley Fever)
    - Coronaviruses
    - Morbilliviruses
    - Protozoa (Toxoplasmosis, Sarcocystosis) ✓
    - Parasites - Nematodes (eg Hepatic capillariasis, Otostrongyliasis)

TMMC's pathology research

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- **Toxins**
  - Harmful Algal Blooms (Domoic acid, saxitoxin and others) ✅
  - Fluorosis in CSLs ✅
- **Influence of environmental change on health**
  - Fresh Water Skin Disease in coastal cetaceans
  - Climate change and narwhals
- **Unusual Mortality Events**
  - Gray whales ✅
  - Guadalupe fur seals
  - Arctic phocid alopecia

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CANCER

Cancer

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- **Sea lion Urogenital Carcinoma (UGC)**
  - What is it?
  - How is it related to cancer in people?
  - What have we found?
  - What is new
  - Future directions
- **Other kinds of cancer**
  - Leukemia and lymphoma in elephant seal pups

UGC

Multifactorial Cancer similar to cervical cancer in humans

- **Herpesvirus** (OtHV-1)
- **Pollutants** (POPs - persistent organic pollutants)
- **Genetics** (inbreeding) may have a lesser role

Up to **25%** of adult CSLs necropsied at TMMC have it

- About 18% have metastatic disease that causes death directly or indirectly,
- The rest have subclinical cancer detected by microscopy when they die for another reason.

To put these numbers in context, according to WHO, cervical cancer in women which is regarded as having a high case fatality rate, causes 13.3 deaths per 100,000 globally each year. That would be an incidence rate of around 0.013%

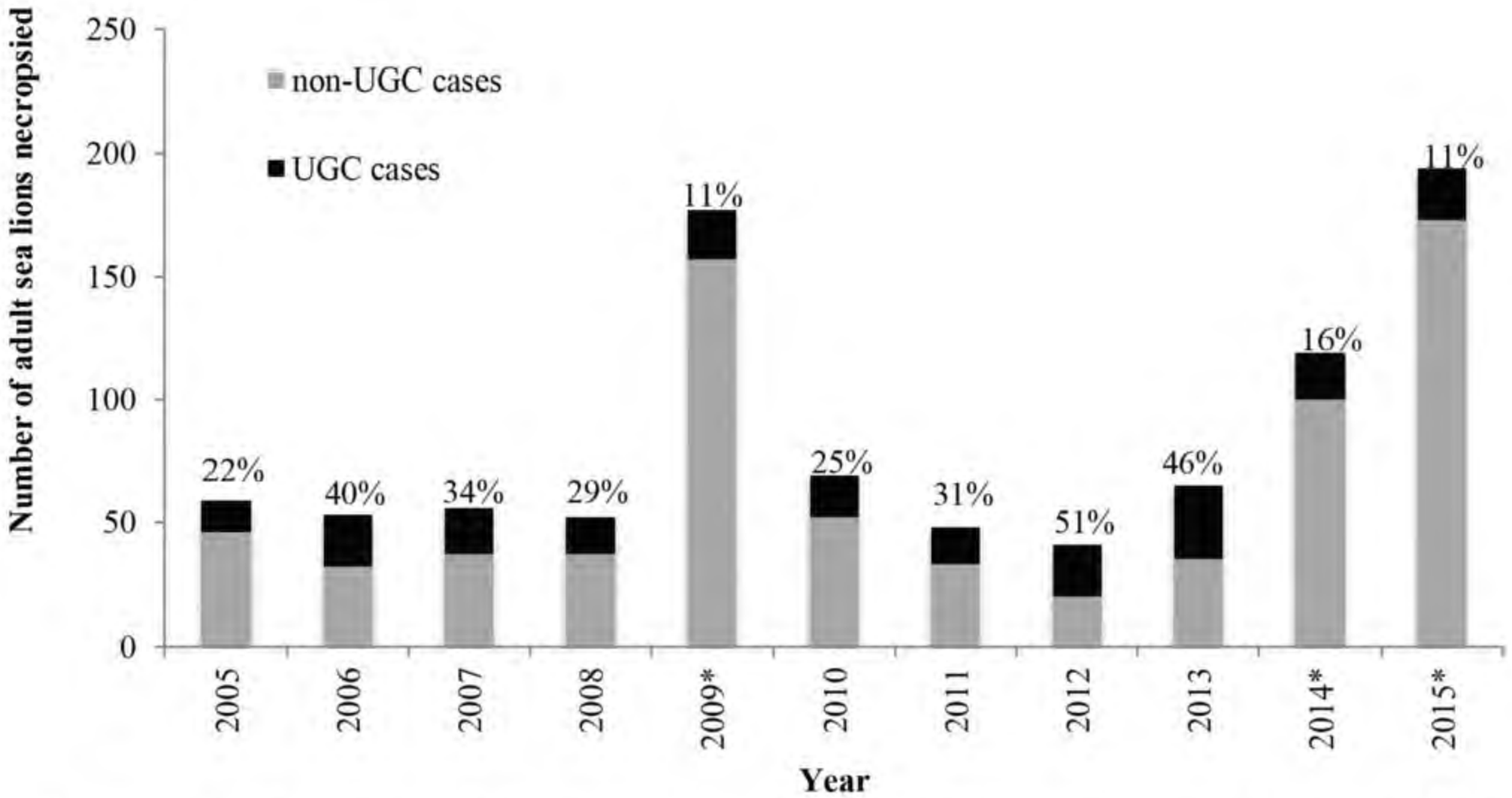

FIGURE 1. Total number of California sea lions (*Zalophus californianus*) necropsied (grey and black combined) and diagnosed histologically with urogenital carcinoma (black) and without urogenital carcinoma (grey) at The Marine Mammal Center, by year, from 2005 to 2015. Each year's prevalence is indicated by percentage above bar. *=2009 was an El Niño year.

In June and July this year, we saw an unprecedented number of cancer cases

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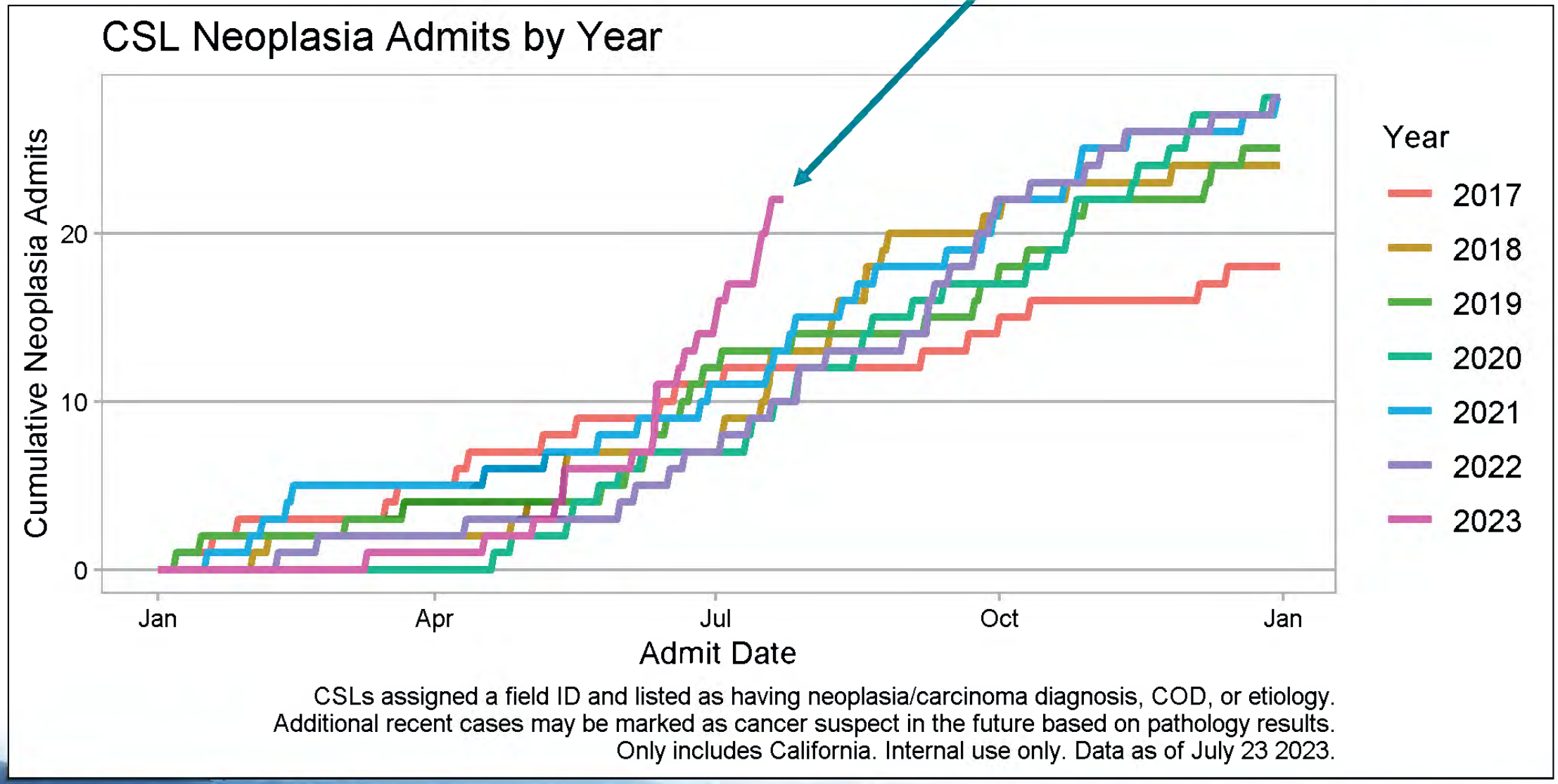


CSLs assigned a field ID and listed as having neoplasia/carcinoma diagnosis, COD, or etiology.
Additional recent cases may be marked as cancer suspect in the future based on pathology results.
Only includes California. Internal use only. Data as of July 23 2023.

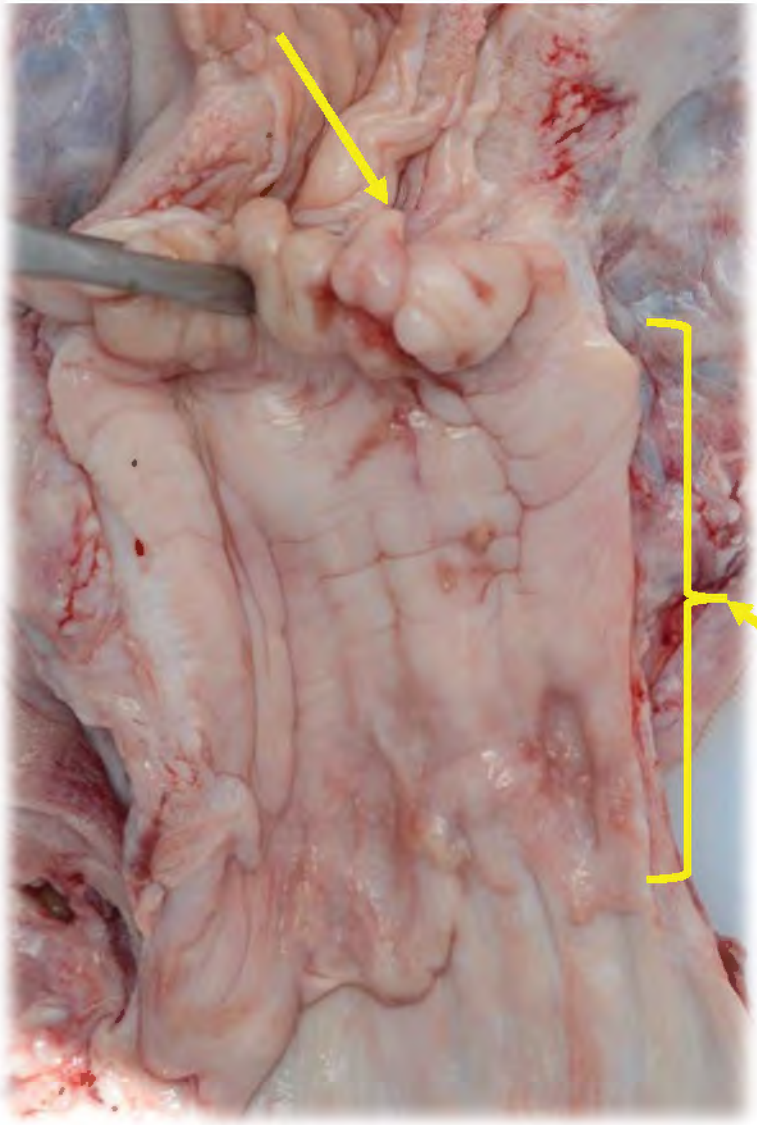
Freefly CSL 12736

Freefly was rescued in poor condition and with swelling (edema) of her vulva.

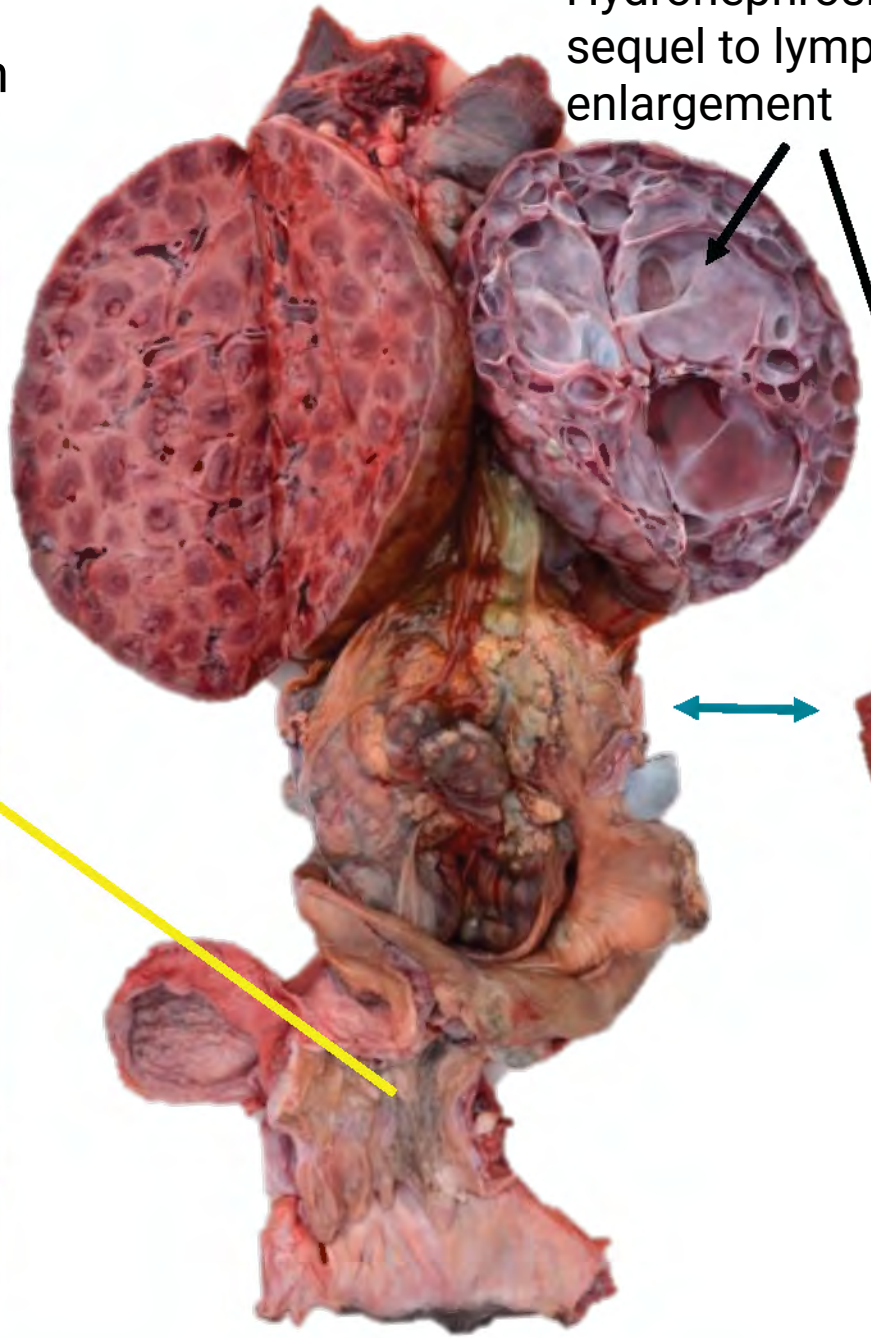
Birdman CSL 12778

Birdman, a subadult male, presented like Freefly with depression and a swollen hind end.

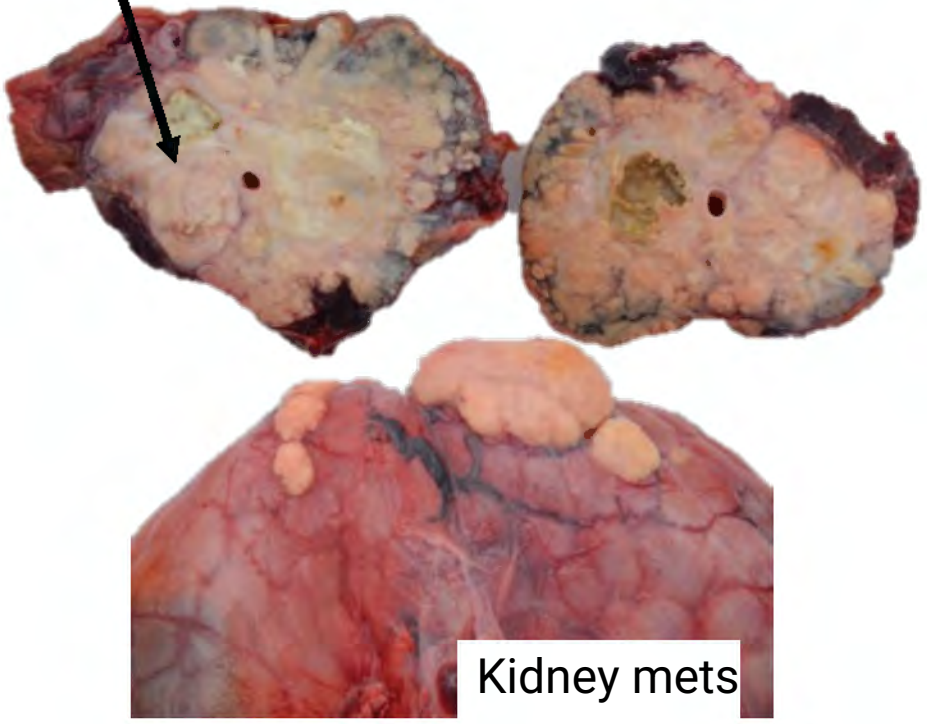
In females the primary site is usually the cervix as for women with cervical cancer



Hydronephrosis common sequel to lymph node enlargement



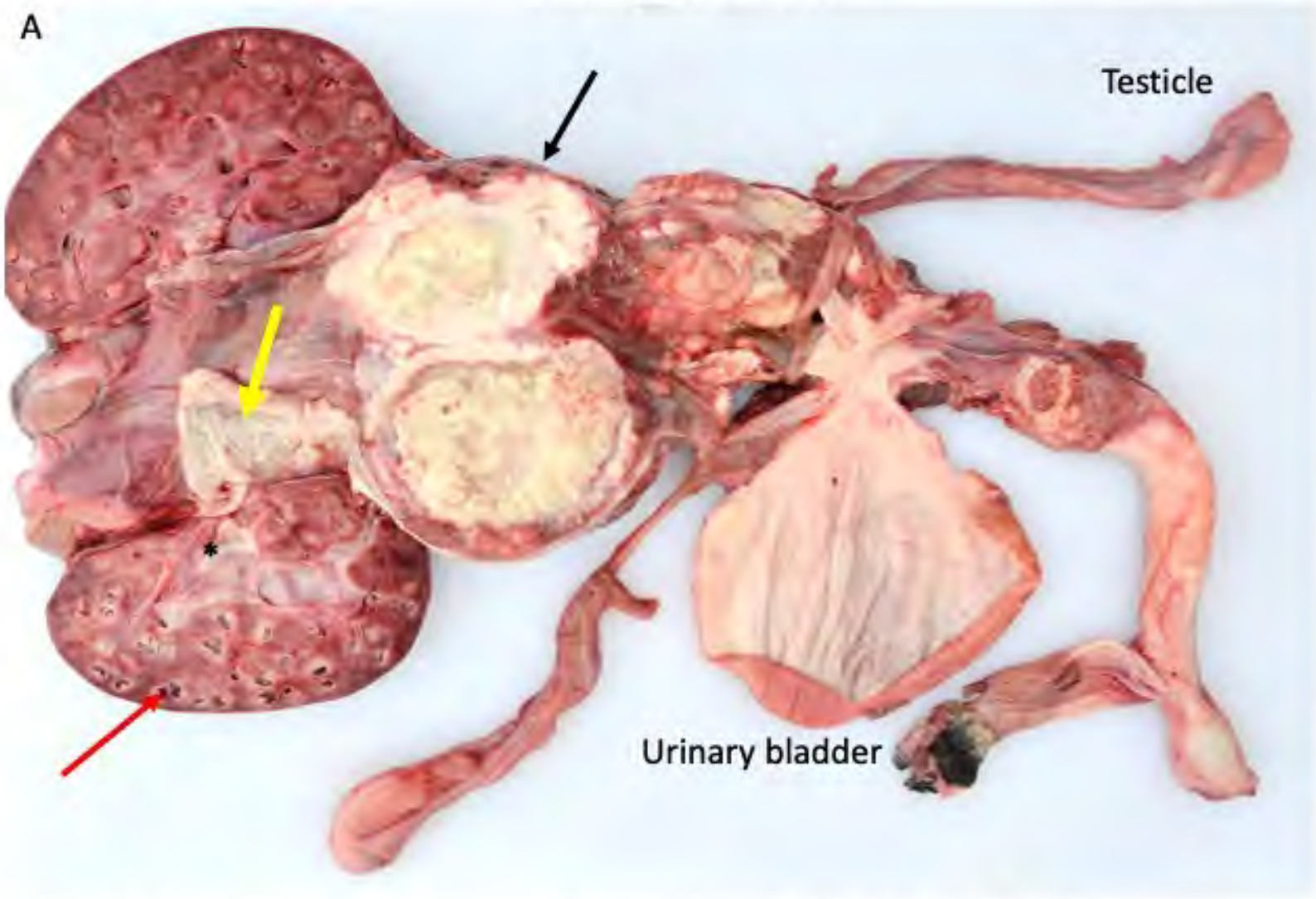
Liver metastasis



Kidney mets

Male CSL

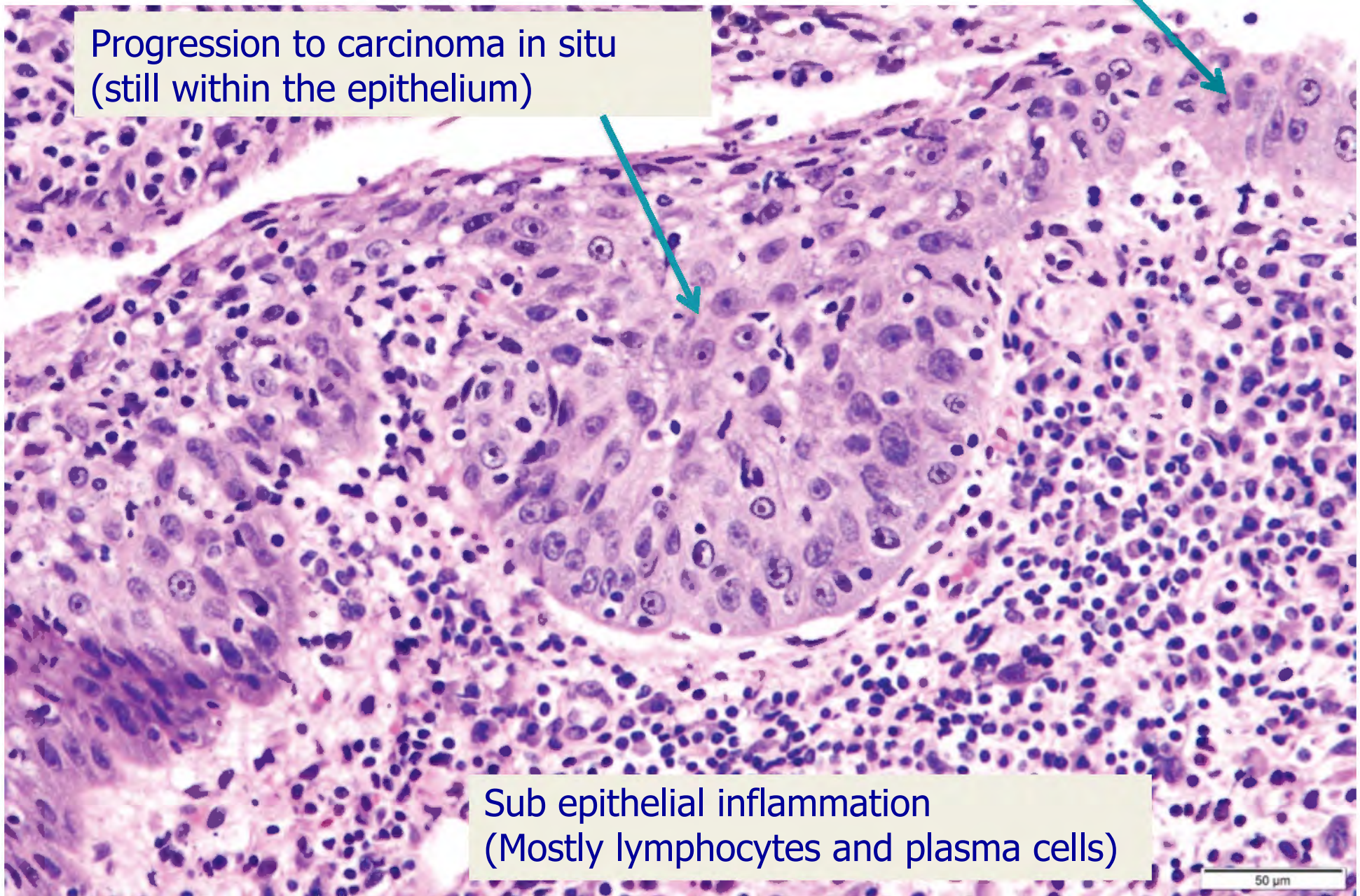
Sinha, CSL-14983



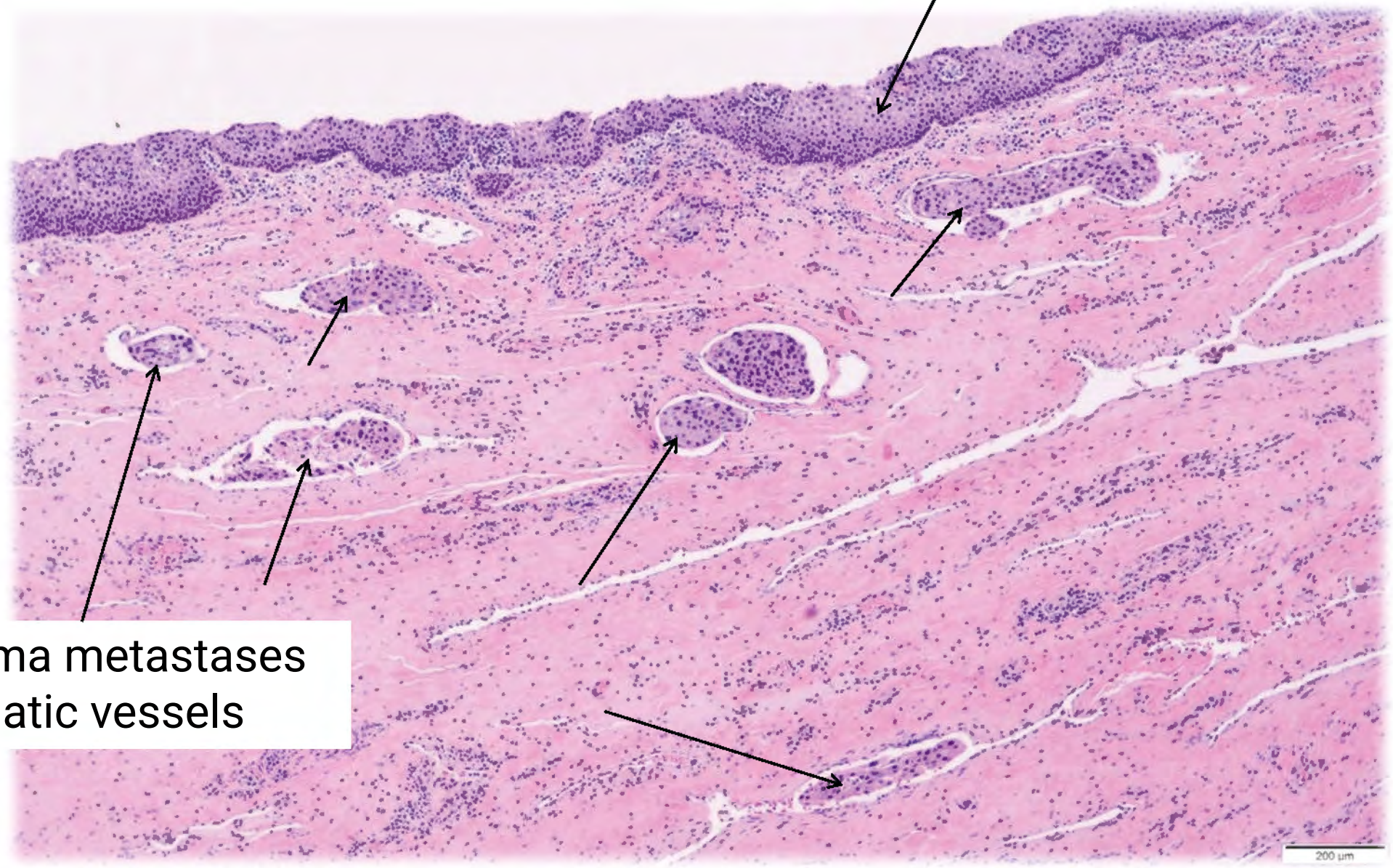
Metastatic urogenital carcinoma in the sublumbar lymph nodes (black arrow) with hydroureter (arrow) and hydronephrosis (red arrow).

Primary site of the carcinoma (arrow) as a small plaque on the glans penis.

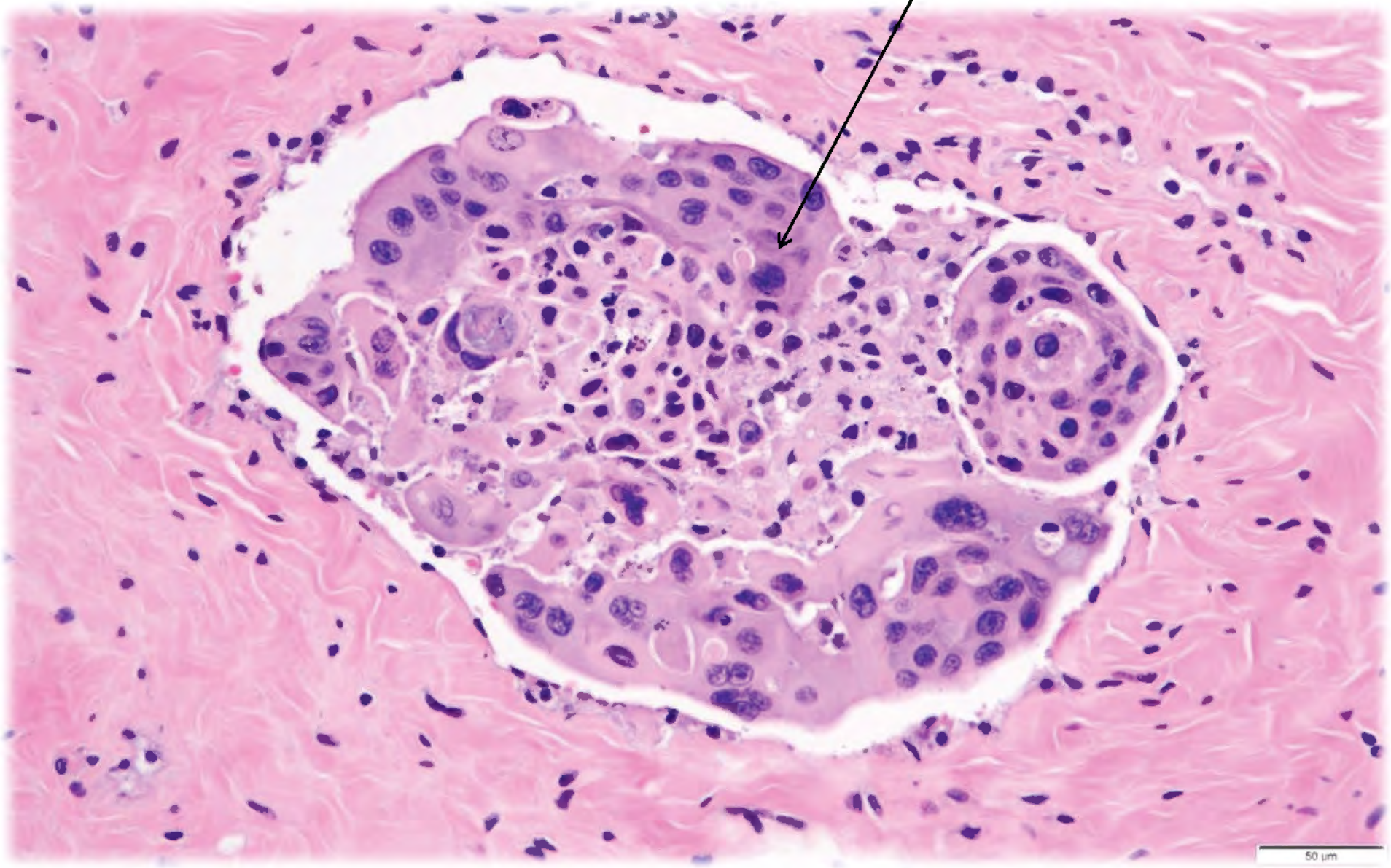
CSL Freefly: Cervix section



Birdman: Prepuce



Birdman: Prepuce



UGC: Recent research….

- Dr Alissa Deming recently defended her Ph.D. through UF on the role of herpesvirus in the pathogenesis of UGC
- 20 year case-control study led by TMMC to investigate the relative contribution of multiple factors published in 2020.

DOI: 10.7589/2017-08-208

Journal of Wildlife Diseases, 54(3), 2018, pp. 000–000

The Marine Mammal Center

Prevalence of Urogenital Carcinoma in Stranded California Sea Lions (*Zalophus Californianus*) from 2005–2015

Alissa C. Deming,[1,2,5] Kathleen M. Colegrove,[3] Padraig J. Duignan,[2] Ailsa J. Hall,[4] James F. X. Wellehan,[1] and Frances M. D. Gulland[2] [1]University of Florida, College of Veterinary Medicine, Department of Comparative, Diagnostic, and Population Medicine, 2015 SW 16th Avenue, Gainesville, Florida 32608, USA; [2]The Marine Mammal Center, 2000 Bunker Road, Sausalito, California 94965, USA; [3]Zoological Pathology Program, College of Veterinary Medicine, University of Illinois at Urbana–Champaign, 3300 Golf Road Brookfield, Illinois 60513, USA; [4]Sea Mammal Research Unit, Scottish Oceans Institute, University of St. Andrews, St. Andrews, UK; [5]Corresponding author: (email: ademing@ufl.edu)

Key Finding: The prevalence of UGC has not changed in the population over 30 years of monitoring. Approx. 15% have advanced metastatic disease that is the cause of stranding and death but up to 25% have microscopic lesions that will progress (These early cases are discovered when the animal dies for some other reason).

animals

MDPI

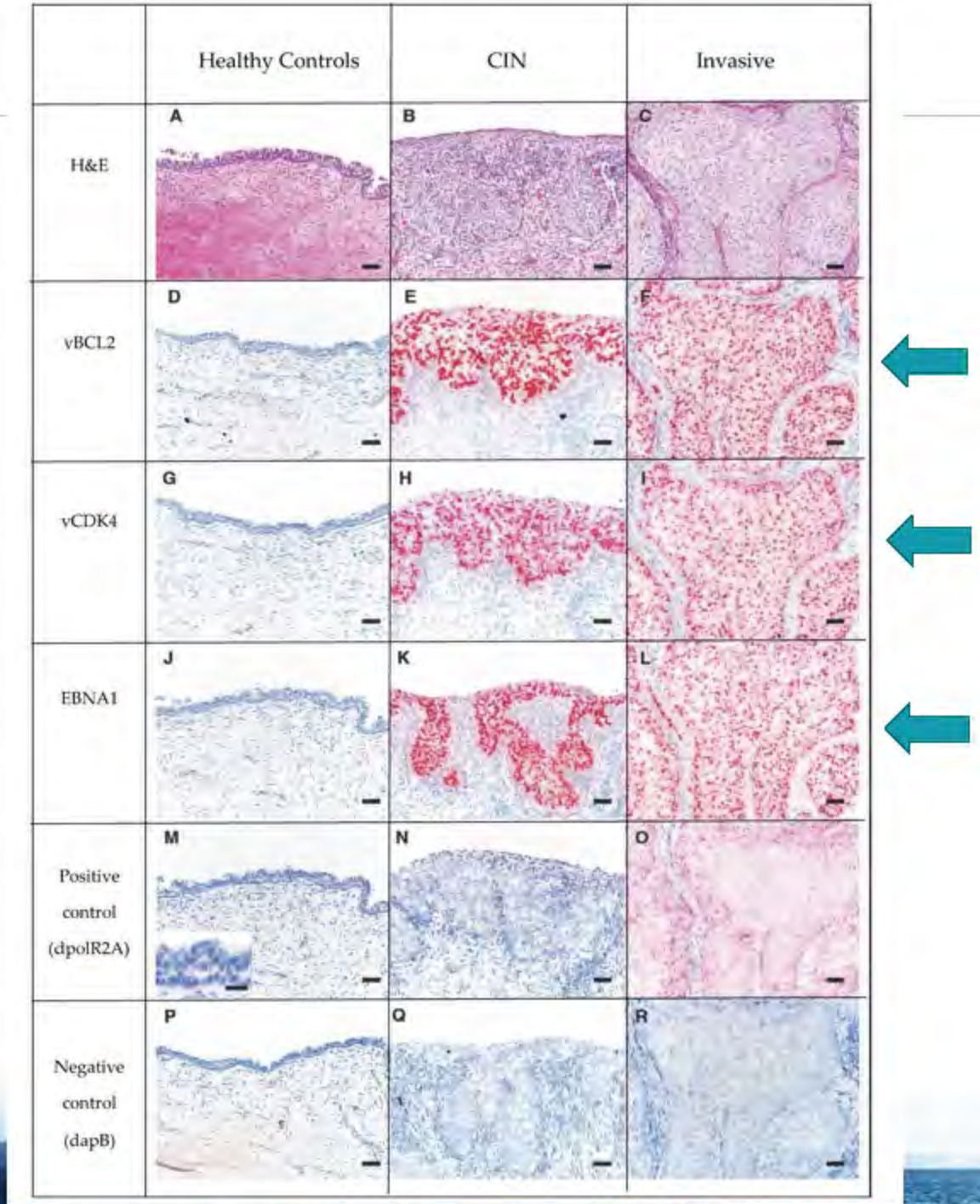
Article

Unlocking the Role of a Genital Herpesvirus, Otarine Herpesvirus 1, in California Sea Lion Cervical Cancer

Alissa C. Deming [1,2,3,*], James F. X. Wellehan [2], Kathleen M. Colegrove [4], Ailsa Hall [5], Jennifer Luff [6], Linda Lowenstine [7], Pádraig Duignan [3], Galaxia Cortés-Hinojosa [2,8] and Frances M. D. Gulland [3,7]

Key Finding: The herpesvirus found in all cases of UGC (OtHV-1) has the same cancer-causing genes (oncogenes) as the herpesvirus of people (HV-8) that causes Kaposi's sarcoma.

(Cervical cancer in women is also caused (95% of cases) by a virus but in that disease, it is Human papillomavirus, HPV)



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In-situ hybridization experiment showing OtHV-1 mRNA expression in carcinoma in situ (CIN) and invasive carcinoma in the cervix. Rows 2, 3 and 4 are oncogenes and all are positive (compared to bottom row) for CIN and invasive carcinoma.

Research Paper

frontiers in Marine Science

ORIGINAL RESEARCH
published: 10 December 2020
doi: 10.3389/fmars.2020.602586

Persistent Contaminants and Herpesvirus OtHV1 Are Positively Associated With Cancer in Wild California Sea Lions (*Zalophus californianus*)

Frances M. D. Gulland[1,2], Ailsa J. Hall[3], Gina M. Ylitalo[4], Kathleen M. Colegrove[5], Tenaya Norris[1], Pádraig J. Duignan[1], Barbie Halaska[1], Karina Acevedo Whitehouse[1,6], Linda J. Lowenstine[2], Alissa C. Deming[1,7] and Teresa K. Rowles[8]*

[1] The Marine Mammal Center Sausalito, Sausalito, CA, United States, [2] One Health Institute, University of California, Davis, Davis, CA, United States, [3] Sea Mammal Research Unit, Scottish Oceans Institute, University of St Andrews, St Andrews, United Kingdom, [4] Environmental and Fisheries Sciences Division, Northwest Fisheries Science Center, National Marine Fisheries Service/National Oceanic and Atmospheric Administration (NMFS/NOAA), Seattle, WA, United States, [5] Zoological Pathology Program, College of Veterinary Medicine University of Illinois at Urbana-Champaign, Brookfield, IL, United States, [6] Facultad de Ciencias Naturales, Autonomous University of Querétaro, Querétaro, Mexico, [7] College of Veterinary Medicine, University of Florida, Gainesville, FL, United States, [8] Office of Protected Resources, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Silver Spring, MD, United States

OPEN ACCESS

Edited by:
Alvin Cyril Camus, University of Georgia, United States

Reviewed by:
Gail Schofield,

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This multi-year case-control study involving hundreds of sea lions found a significant association with herpesvirus infection as expected but also with persistent organic pollutants from fish.

- Based on 394 sea lion necropsies conducted over 20 years, for each unit increase in the concentration of POPs in the body fat, the odds of developing cancer increased by 28%.
- With a low concentration of contaminants, the probability of cancer is approx. 30%.
- With a high concentration of contaminants, the probability of cancer increases to 80%.

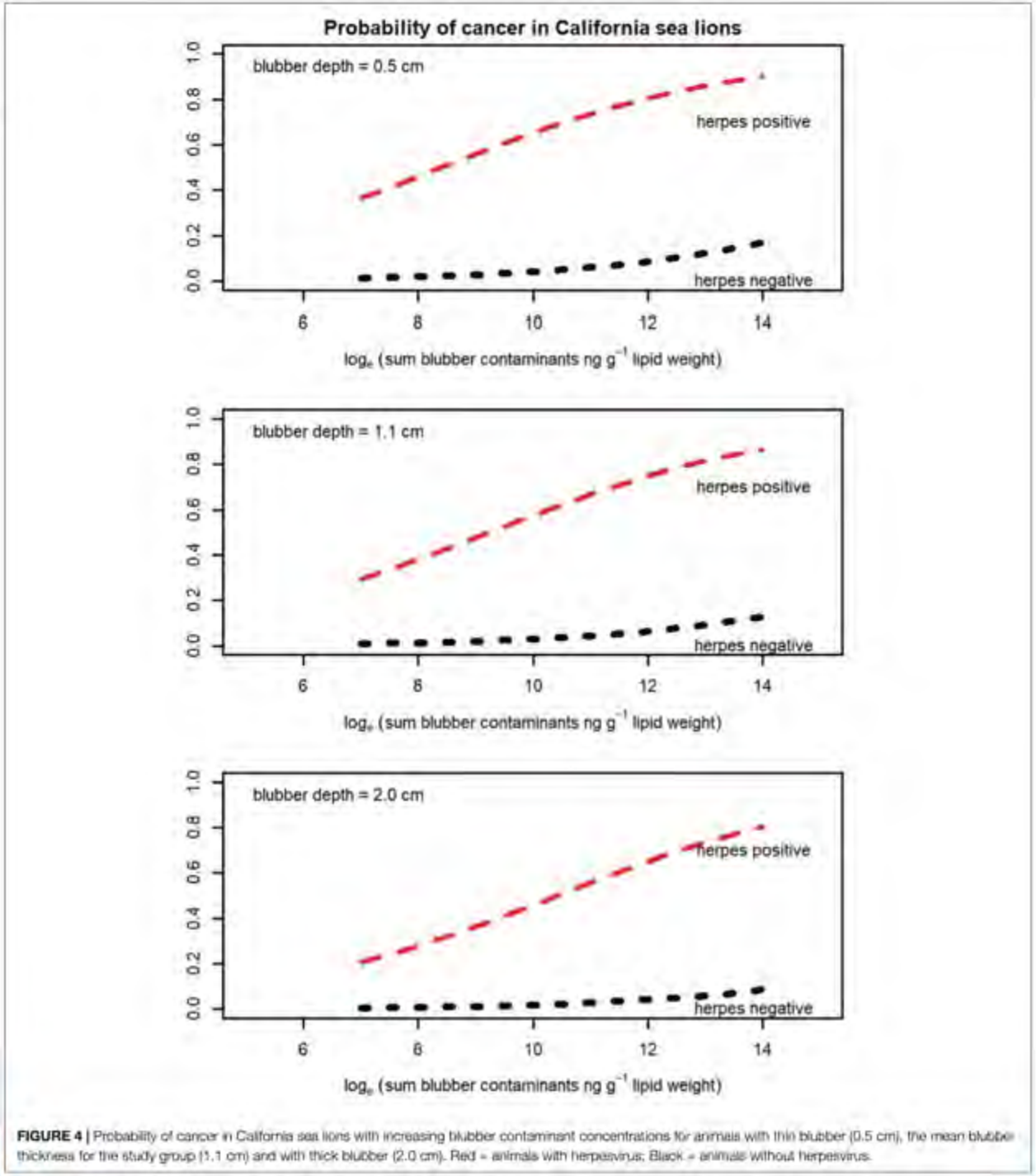

FIGURE 4 | Probability of cancer in California sea lions with increasing blubber contaminant concentrations for animals with thin blubber (0.5 cm), the mean blubber thickness for the study group (1.1 cm) and with thick blubber (2.0 cm). Red = animals with herpesvirus; Black = animals without herpesvirus.

CLIMATE & ENVIRONMENT

Sea lions are dying from a mysterious cancer. The culprits? Herpes and DDT

The Marine Mammal Center's chief pathologist Pádraig Duignan, right, and fellow Maggie Martinez perform a necropsy on a California sea lion that was euthanized due to untreatable cancer. (Bill Hunnewell / Marine Mammal Center)

BY ROSANNA XIA | STAFF WRITER

JAN. 31, 2021 6 AM PT

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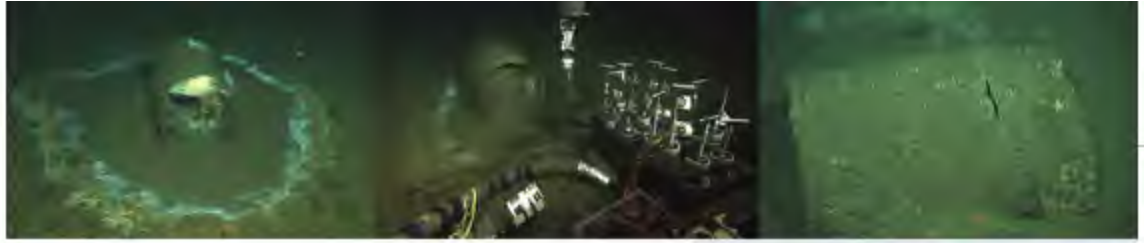
Health effects of DDX chemicals

- Reproduction
  - Premature parturition in CSL (De Long et al., 1973)
- Endocrine Disruption
  - Decrease in circulating thyroid hormones (Debrier et al., 2005)
- Immune Function
  - Altered NK and lymphocyte proliferation in vitro. PCB congener specific effects (Penin et al., 2018)
- Enhancement of seizures in domoic acid exposure cases?
  - zebrafish model (Tiedeken & Ramsdell 2009)
- Cancer



California Coastal Chloro-Contan Confere

UC SANTA BARBARA May 16 – 17, 2022

Sponsored by: UC SANTA BARBARA Office of Research | UC SANTA BARBARA Marine Science Institute

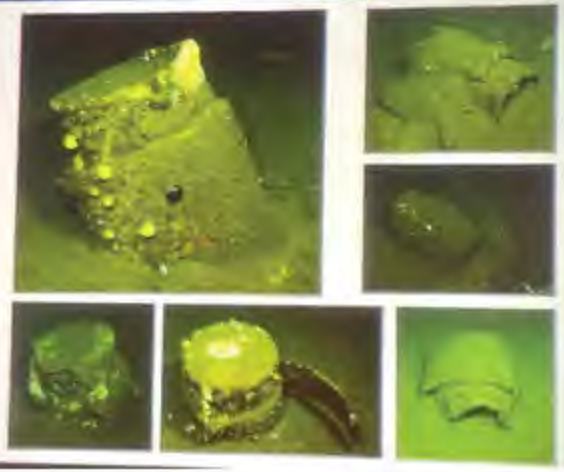
UC SANTA BARBARA

We Know...

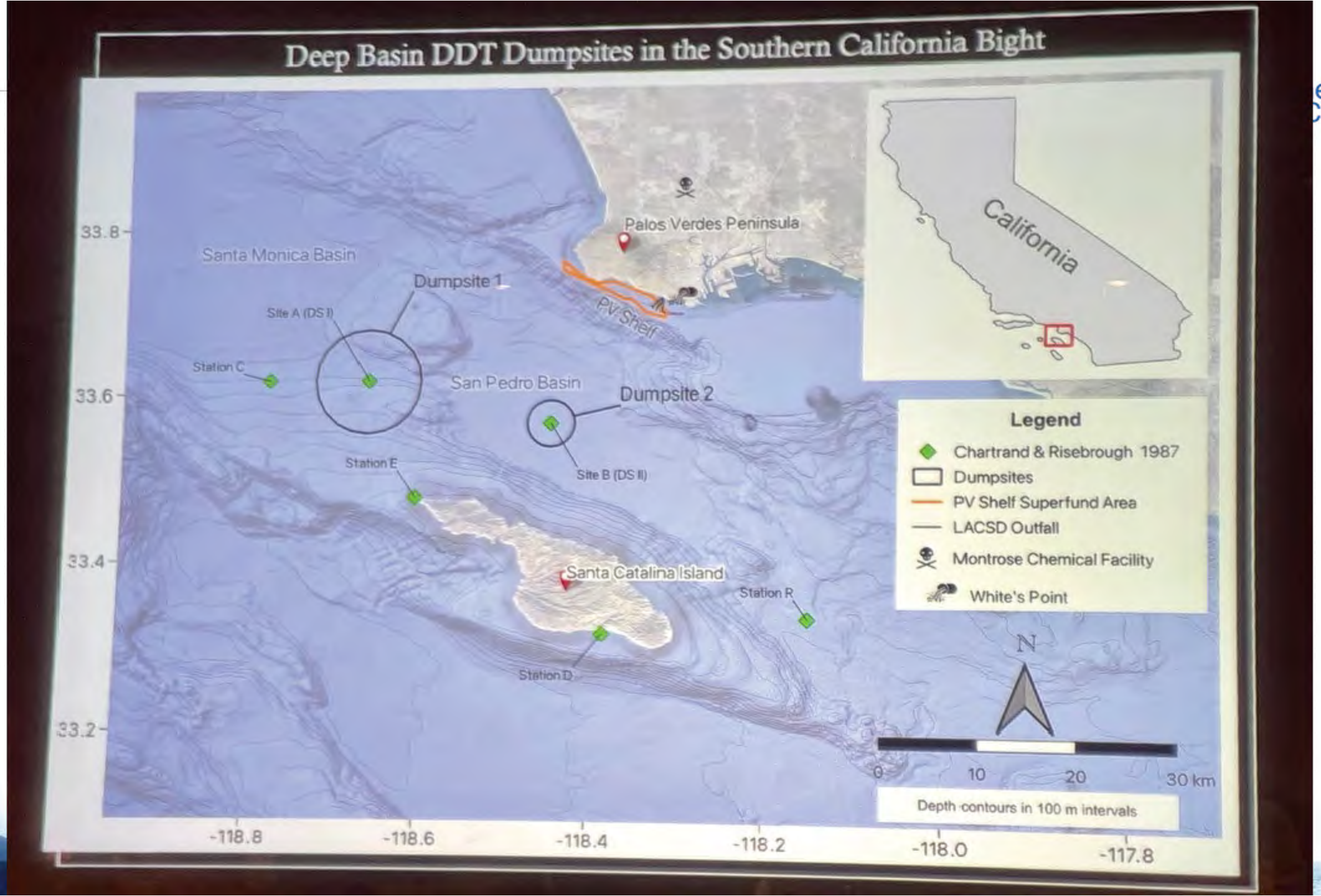
...How much technical grade DDT was manufactured by Montrose Chemical Company at thei

ROSE CHEMICAL RPORATION California TORRANCE PLANT

...Barrels of waste were systematically disposed of at Dumpsite 2.



1 million gallons of DDT waste generated annually by Montrose Chemical Corp.


Deep Basin DDT Dumpsites in the Southern California Bight
Palos Verdes Peninsula
Santa Monica Basin
Dumpsite 1
Site A (DS I)
PV Shelf
Station C
San Pedro Basin
Dumpsite 2
Site B (DS II)
Station E
Santa Catalina Island
Station R
Station D
California
Legend
Chartrand & Risebrough 1987
Dumpsites
PV Shelf Superfund Area
LACSD Outfall
Montrose Chemical Facility
White's Point
N
0
10
20
30 km
Depth contours in 100 m intervals
33.8
33.6
33.4
33.2
-118.8
-118.6
-118.4
-118.2
-118.0
-117.8

- Mackintosh et al. Environ. Sci. Technol. 2016, 50, 12129−12137
- http://dx.doi.org/10.1021/acs.est.6b03150

DDX Accumulation in Regional Marine Mammals and Wildlife and Their Health Impacts

Eunha Hoh, Ph.D.
Professor
School of Public Health
San Diego State University
San Diego, CA
ehoh@sdsu.edu

SAN DIEGO STA
UNIVERSITY

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Highest known DDT concentration in the world based on comparison of bottlenose dolphin monitoring data collected during 1995-2015 elsewhere the USA and globally. Higher concentration by one order of magnitude higher or more.

Table S4. Average concentrations of ∑DDT (*p,p'*- and *o,p'*- DDT, DDD, and DDE isomers) and ∑DDT (*p,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE, and *o,p'*-DDT) reported within the United States and globally.

Location	Year	Species	n	∑DDT Concentration (µg/g lipid)	Reference
Southern California Bight (off the coast of California)	1995-2010	*Tursiops truncatus*- Adult Male	8	∑DDT- 184 ∑DDT- 178	*This Study*
Southeastern US (off the coast of Florida)	2003-2005	*Tursiops truncatus*- Adult Male	33	∑DDT- 19	S2
Southeastern US(off the coast of Georgia)	2003-2005	*Tursiops truncatus*- Adult Male	36	∑DDT- 29	S2
Northern Gulf of Mexico	2010-2012	*Tursiops truncatus*- Male	108	∑DDT- 20	S3
Gulf of Mexico (off the coast of Louisiana and Florida)	2011	*Tursiops truncatus*- Male and Female mixed ages	22	∑DDT- 11	S4
East Coast of the US, the Gulf of Mexico, and Bermuda*	2000-2007	*Tursiops truncatus*- Male	261	∑DDT- 28	S5
Georgia*	2006-2008	*Tursiops truncatus*- Male	74	∑DDT- 28	S6
North Carolina, South Carolina, and Florida*	1995-2002	*Tursiops truncatus*- Male	18	∑DDT- 33	S7
Canary Islands Spain*	2003-2011	*Tursiops truncatus*- Male mixed ages	64	∑DDT- 105	S8
Brazil*	2000-2005	*Tursiops truncatus*- Male	2	∑DDT- 5	S9
Tanzania*	2000-2002	*Tursiops aduncus*- Adult Male	4	∑DDT- 43	S10

* Off the coast of the location

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Assessing Marine Endocrine-Disrupting Chemicals in the Critically Endangered California Condor: Implications for Reintroduction to Coastal Environments

Margaret E. Stack, Jennifer M. Cossaboon, Christopher W. Tubbs, L. Ignacio Vilchis, Rachel G. Felton, Jade L. Johnson, Kerri Danil, Gisela Heckel, Eunha Hoh, and Nathan G. Dodder*

Cite this: *Environ. Sci. Technol.* 2022, XXXX, XXX, XXX-XXX

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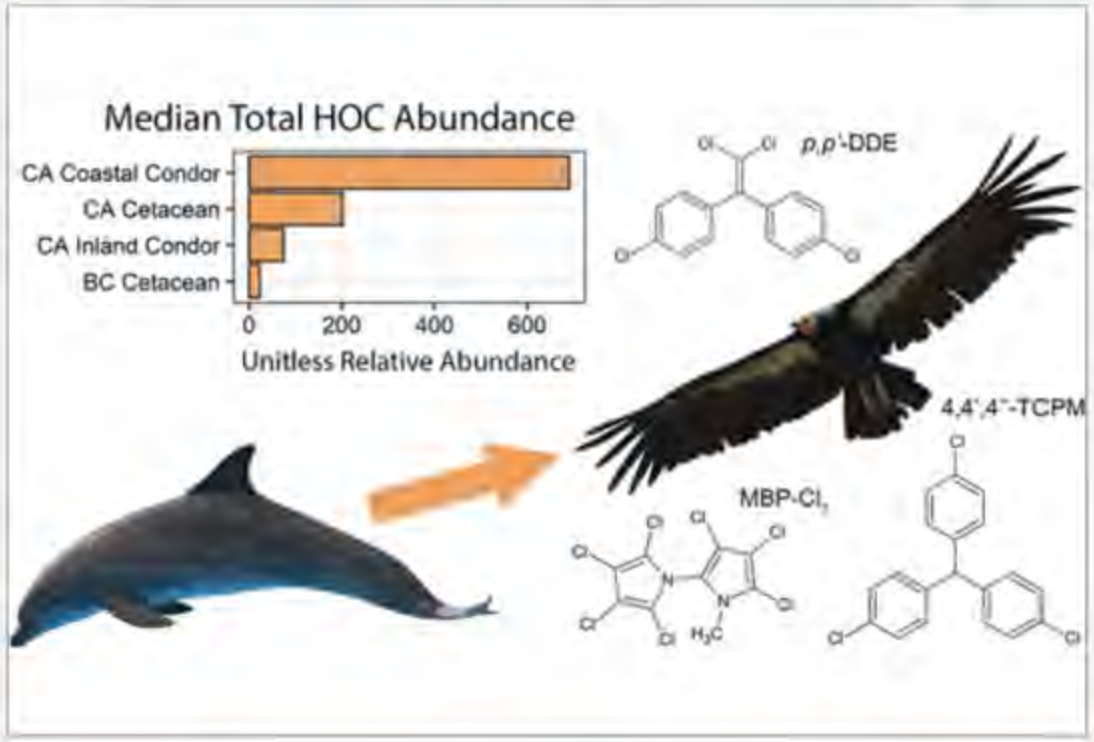

Los Angeles Times

Subscri

CLIMATE & ENVIRONMENT

Scientists find new and mysterious DDT chemicals accumulating in California condors

With a 9 1/2-foot wingspan, the California condor is a sight to behold in the wild. (San Diego Zoo Wildlife Alliance)

BY ROSANNA XIA | STAFF WRITER

MAY 19, 2022 5 AM PT

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Ongoing & Future Directions

- Developing a cytology-based method of detecting early cancer change in living animals (PAP smear for sea lions)
- On-going research into the genetics of carcinoma with the Breen Lab, North Carolina State University
- UGC as a model for studying the mechanisms of carcinoma metastasis
- Role of newly discovered DDT related compounds (~45) in causing adverse health effects.

California Sea Grant proposal – successfully funded 2023

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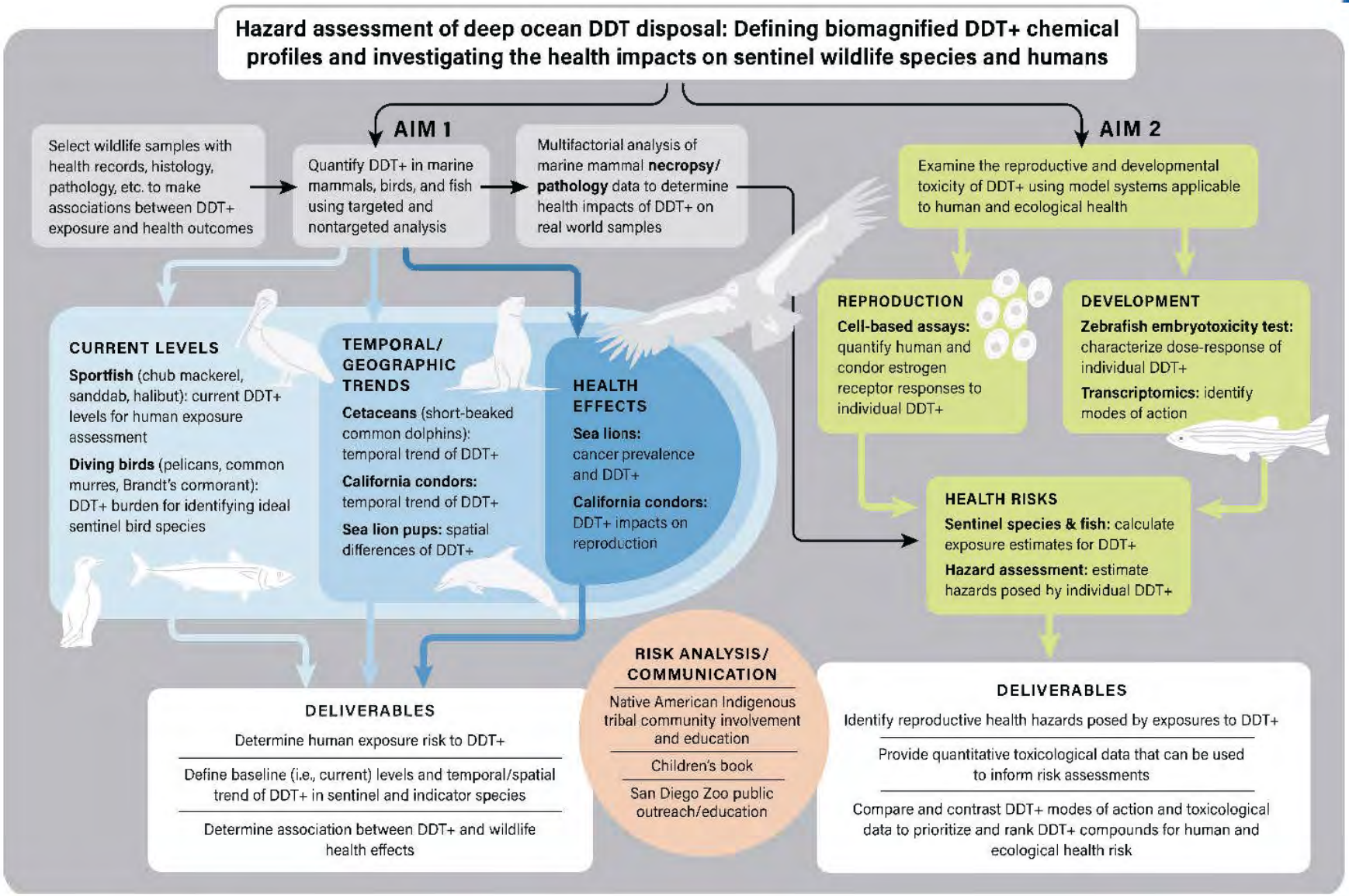

New Cancer: Lymphoma in NES pups

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Two male NES pups

- 2020 San Luis Obispo, CA
- 2021 Santa Cruz, CA

https://www.usgs.gov/centers/pcmsc/science/cosmos-31-central-california?qt-science_center_objects=0#qt-science_center_objects

Vol. 149: 59–70, 2022
https://doi.org/10.3354/dao03662

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published online May 24

Diffuse large B cell lymphoma and a novel gammaherpesvirus in northern elephant seals *Mirounga angustirostris*

Margaret E. Martinez[1,*], Nicole I. Stacy[2], James F. X. Wellehan Jr.[2], Linda L. Archer[2], Salvatore Frasca Jr.[3], Carlos Rios[1], Emily J. Trumbull[1,4], Michelle Rivard[1], Emily R. Whitmer[1], Cara L. Field[1], Pádraig J. Duignan[1]

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[2]College of Veterinary Medicine, University of Florida, Gainesville, Florida 32608, USA

[3]Connecticut Veterinary Medical Diagnostic Laboratory, Department of Pathobiology and Veterinary Science, University of Connecticut, Storrs, Connecticut 06269, USA

[4]SeaWorld, San Antonio, Texas 78251, USA

Pathology

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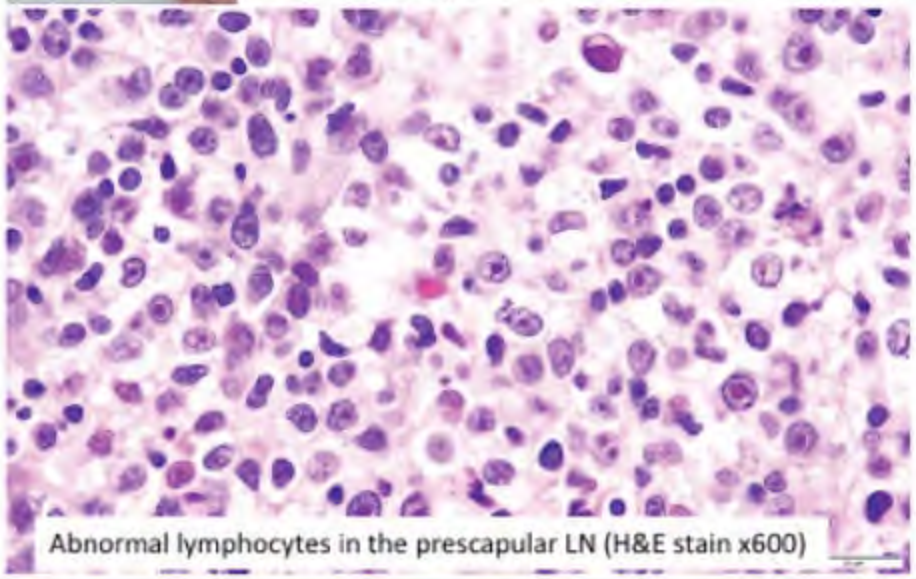
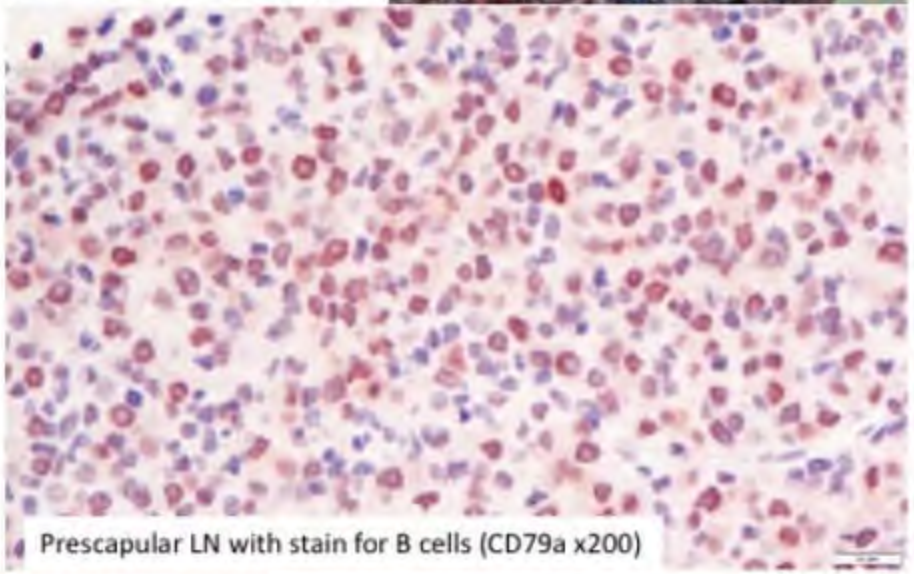
Fig. 2

Massively enlarged lymph nodes of the fore flippers (left) and abdominal cavity (right)



Abnormal lymphocytes in the prescapular LN (H&E stain x600)



Prescapular LN with stain for B cells (CD79a x200)

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Research Questions

- Does this new gamma herpesvirus (called miroungine herpesvirus 3 or MirGHV3) also carry oncogenes like OtHV1?
- Do contaminants play a role in the development of cancer?
- How prevalent is MirGHV3 in the elephant seal population?

DOI: 10.7589/JWD-D-22-00030

Journal of Wildlife Diseases, 59(1), 2023, pp. 000–000

The Marine Mammal Center.

DEVELOPMENT AND VALIDATION OF A NOVEL DUPLEX PROBE–HYBRIDIZATION QUANTITATIVE PCR FOR LYMPHOMA-ASSOCIATED MIROUNGINE GAMMAHERPESVIRUS 3 IN NORTHERN ELEPHANT SEALS (*MIROUNGA ANGUSTIROSTRIS*)

Molly Horgan,[1] Margaret E. Martinez,[2] Linda L. Archer,[1] Pádraig J. Duignan,[2] and James F. X. Wellehan Jr.[1,3]

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ABSTRACT: Recently, a novel gammaherpesvirus, miroungine gammaherpesvirus 3 (MirGHV3), was described in two juvenile elephant seals (*Mirounga angustirostris*) with diffuse large B-cell lymphoma. We developed and validated a quantitative (q)PCR for rapid detection of MirGHV3 and investigated its potential association with lymphoma. We developed a duplex probe–hybridization qPCR with MirGHV3 DNA polymerase (*pol*) as the target gene. Each primer–probe combination was cross-validated against the others. Interference was not seen when they were run in the same well as a duplex assay. Twenty-three samples from seven northern elephant seals were tested using the duplex assay. Viral DNA was detected by the assay in 9 of 9 (100%) tissues affected by lymphoma and in 6 of 14 (43%) samples from tissues unaffected by lymphoma. There was a strong correlation between viral copies detected with each of the assays ($P=0.0002$). Viral load was significantly higher in tissues affected by lymphoma than in those unaffected ($P<0.0001$). Excluding the virus-negative samples, viral load was still significantly higher in tissues affected by lymphoma than in those unaffected ($P=0.0004$). This is consistent with a potential role of MirGHV3 in oncogenesis in northern elephant seals, although more studies are needed to determine this definitively. The qPCR developed has utility for further investigations of MirGHV3.

Key words: Elephant seal, gammaherpesvirus, lymphoma, *Mirounga angustirostris*, oncogenesis.

Lab test will be used to determine the prevalence of the virus in the NES population

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LEPTOSPIROSIS

Leptospirosis in California sea lions

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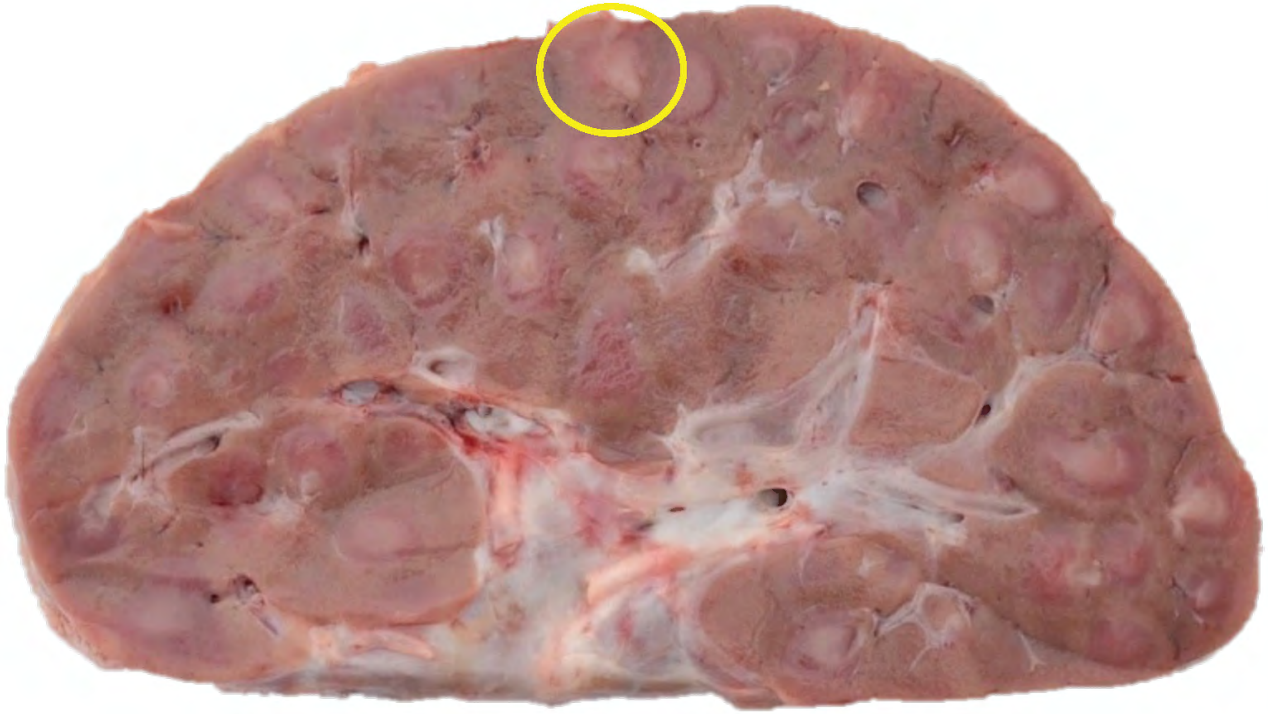
- ***Leptospira interrogans* Pomona** has been causing periodic epidemics in sea lions since at least the early 1970s
- In past decade, a ”**fade-out**” phenomenon was observed in which infection disappeared completely from the population.
- This has been shown to be linked to changed in the NE Pacific ecosystem that appear to be climate driven.
- When the disease reappeared, there were some difference in the pathology observed

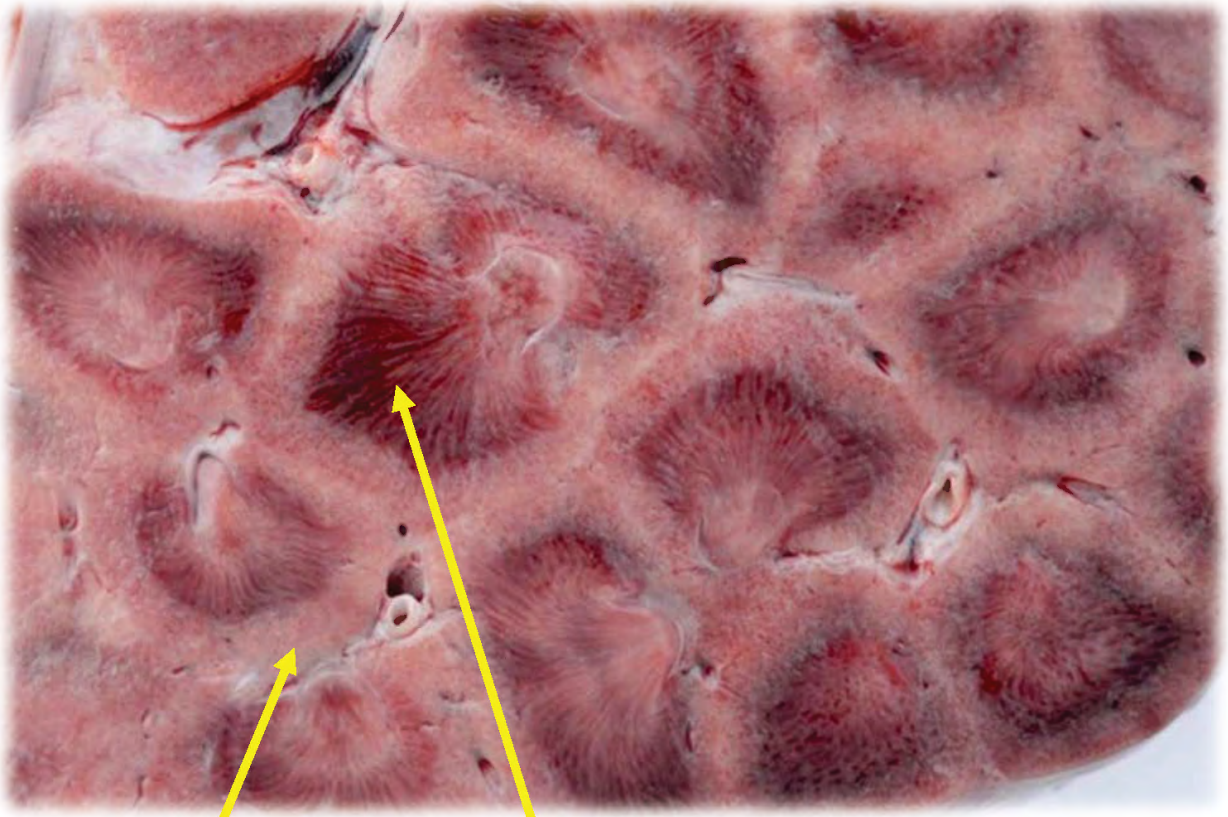
Pathology

Marine mammal kidneys have a ”reniculate” structure like a bunch of grapes and each “grape” is an individual kidney unit with its own cortex, medulla and pelvis.



Normal sea lion kidney

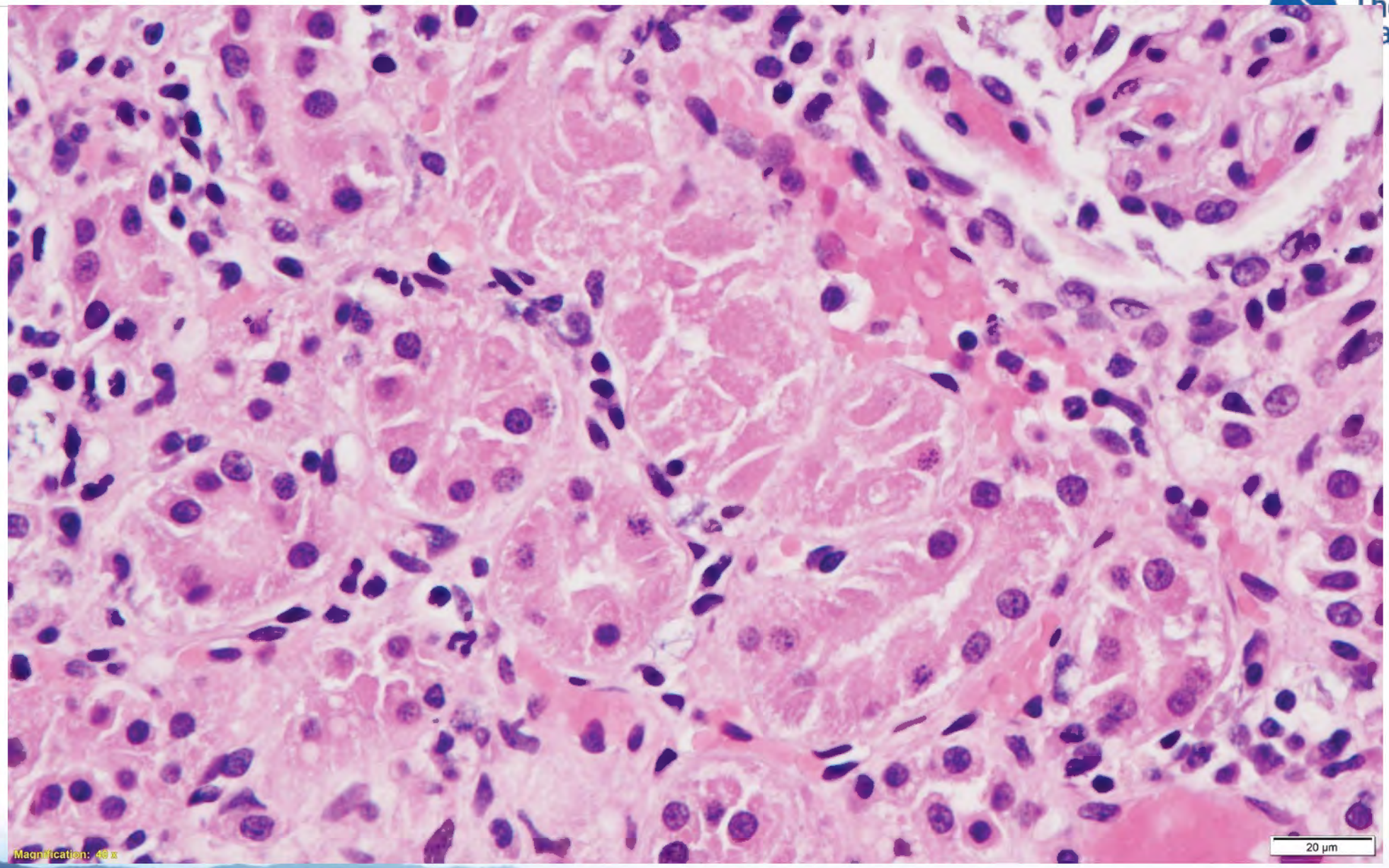
Leptospirosis kidney



Expanded pale cortices (Inflammation)

Hemorrhage at the cortico-medullary junction

Acute Tubular Necrosis



Special stains to reveal leptospires

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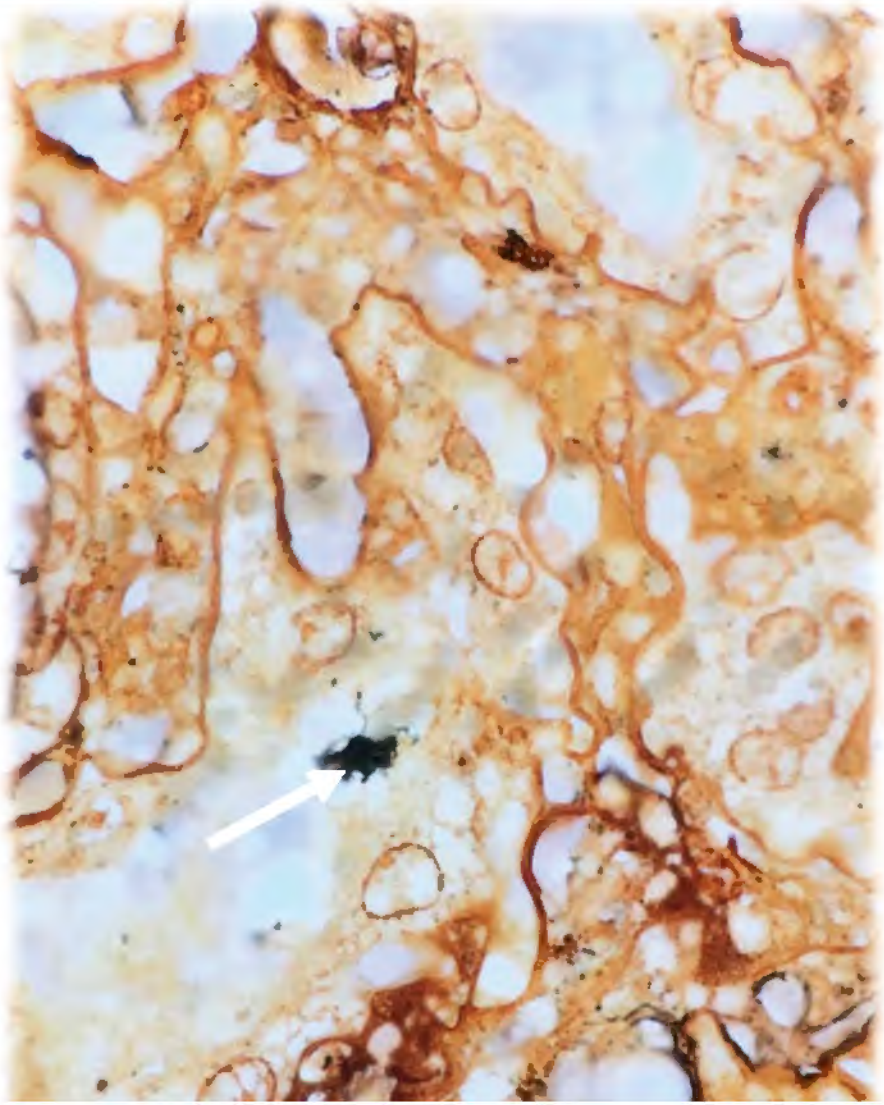
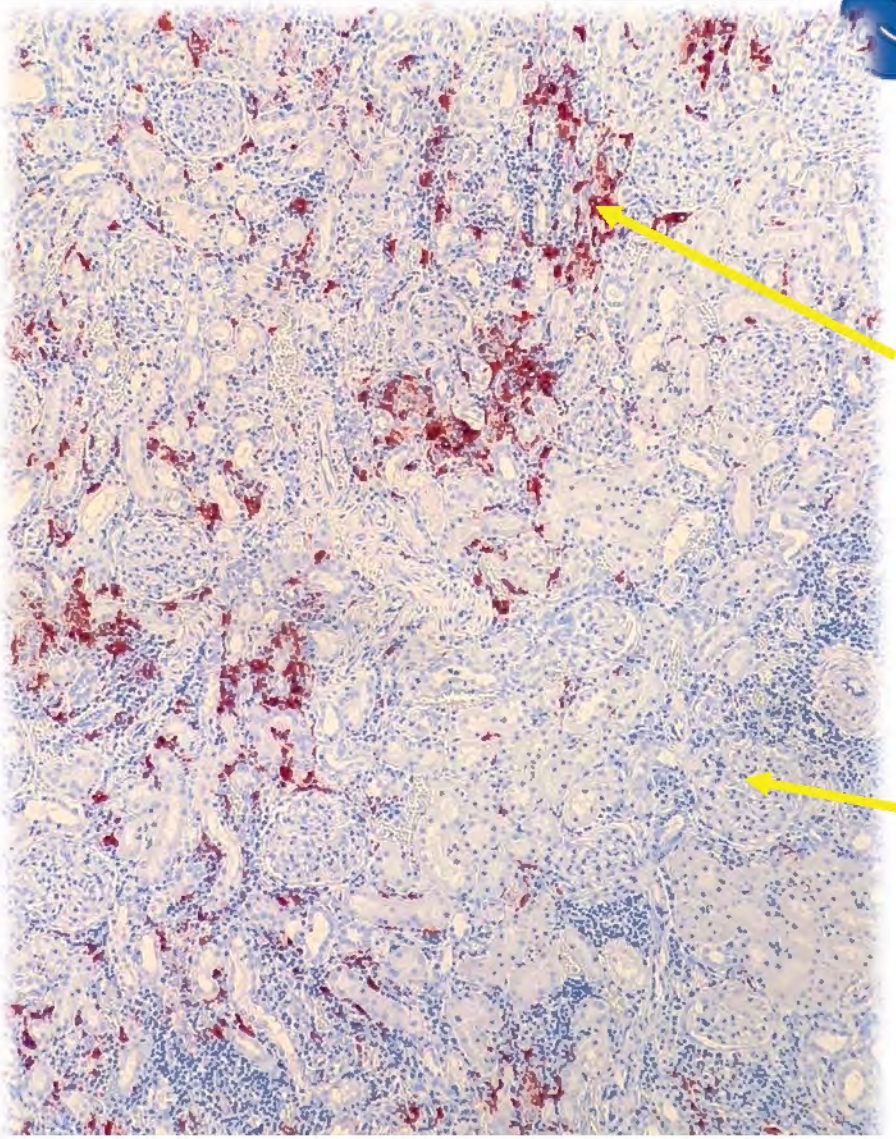


Silver Stain: Non specific for leptospires (Black)



Immunohistochemistry specifically detects *Leptospira* antigen (Red)

Pathologic Presentation

- **Tubulointerstitial nephritis**

  Acute tubular necrosis with sloughing of necrotic epithelial cells and replacement by re-epithelization

  Leptospires demonstrable in the lumen

  Pleocellular (mixed) leukocytic infiltration into the interstitium with over-representation of lymphocytes and plasma cells.

- **Uremic syndrome**

  The above plus mucosal and epithelial ulceration: buccal cavity, esophagus, stomach, urogenital tract.

  Interstitial pneumonia with alveolitis and edema.

  Dermatitis – erosive and ulcerative.

  Cerebral edema

Previously Described

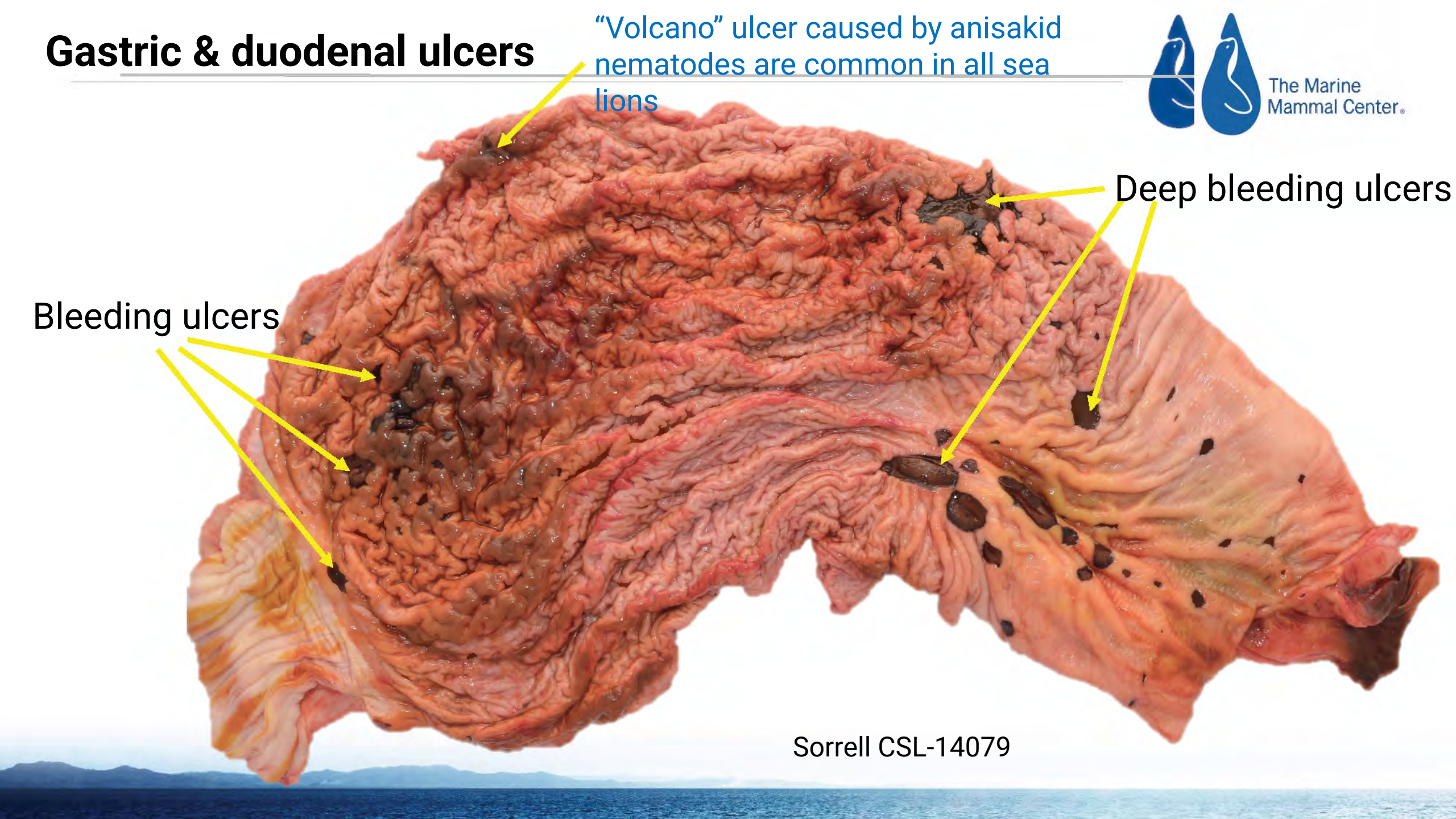
First seen in 2017/'18

Buccal ulcers

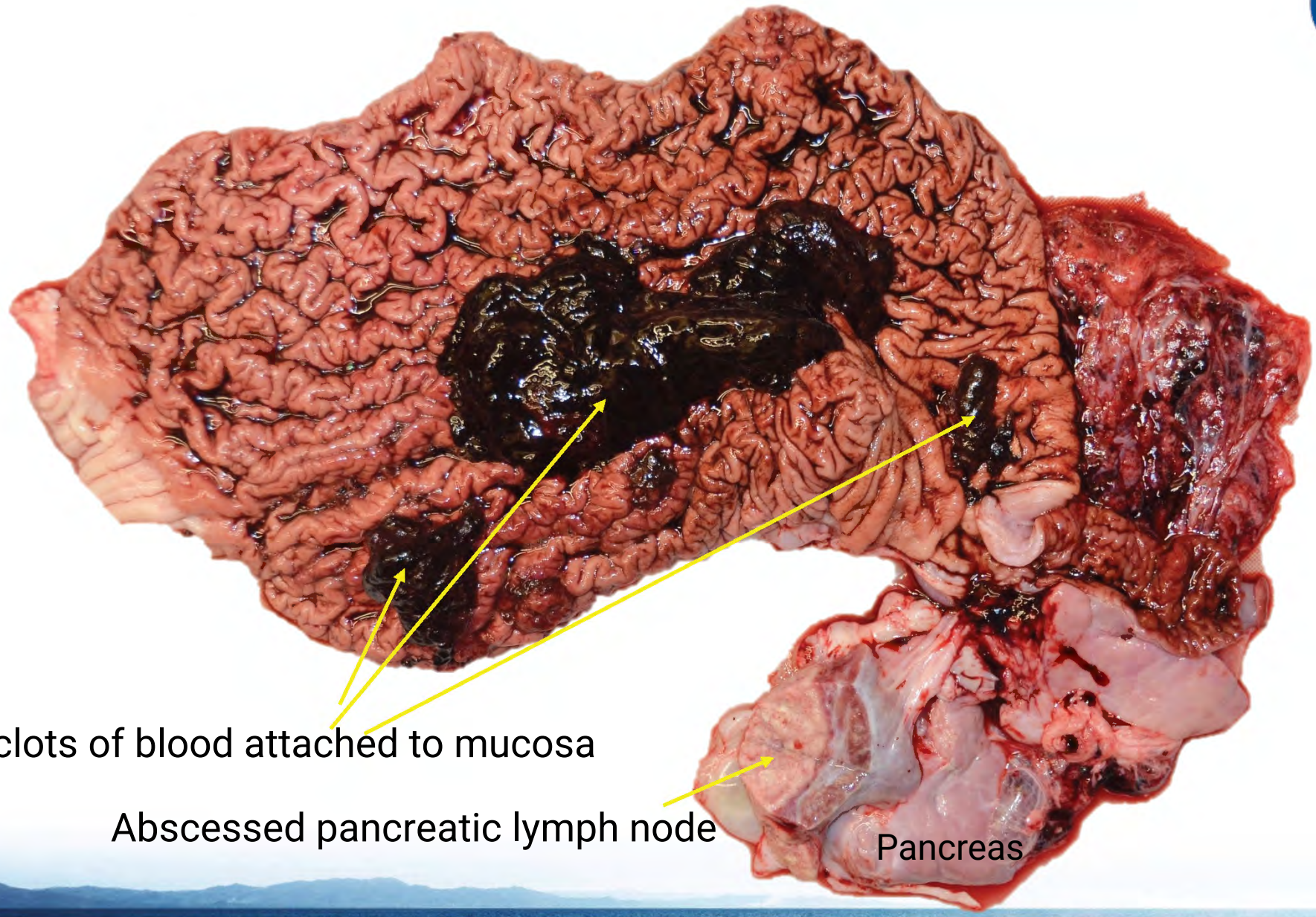
Sorrell CSL-14079 a subadult male with severe gingival and labial ulceration

Systemic Lesions
“Uremic Syndrome”

Gastric & duodenal ulcers



Gastric ulcers



CSL-14086 Ocelia

Skin ulcers and erosions

Frequently seen on the ventral surfaces at pressure points. The hind flippers are the most common site affected as in this case: CSL-13966 Valerie.

Interstitial Pulmonary Edema

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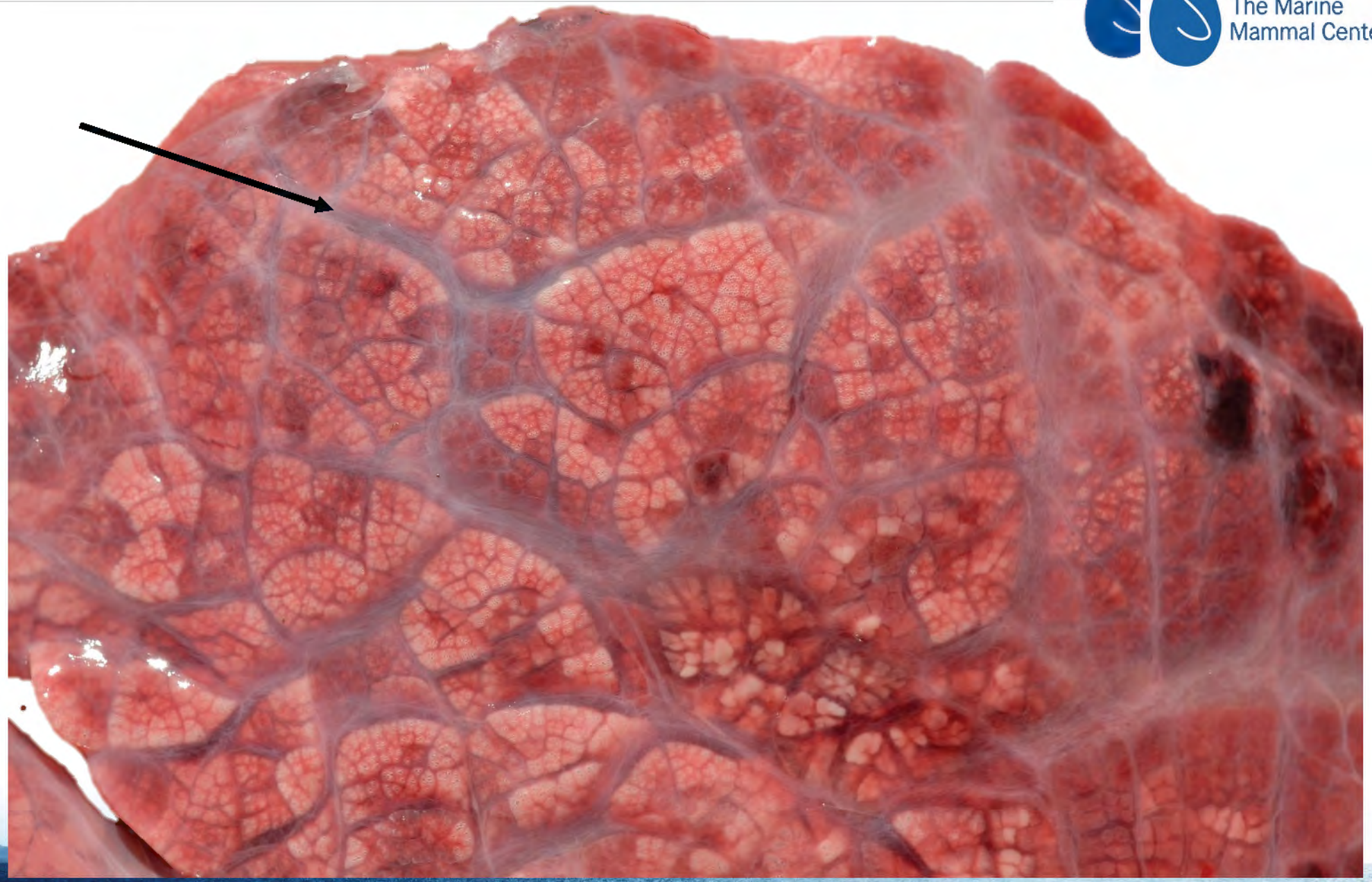

Casita CSL-14047

Interstitial Pulmonary Edema

The Marine Mammal Center

Delcarlo CSL-14050

Fade-out & Re-emergence

- Since 1984 yearly seasonal outbreaks with periodic (3 to 5yr) epidemics.
- Serology data from free-ranging (prevalence) and stranded CSLs (incidence) showed absence of cases between 2013 & 2017.
- All pups born between 2013 and 2016 were seronegative.
- Fade-out was followed by a small outbreak in autumn 2017 and a major epidemic in 2018.

Leptospirosis: An endemic disease with periodic epidemics

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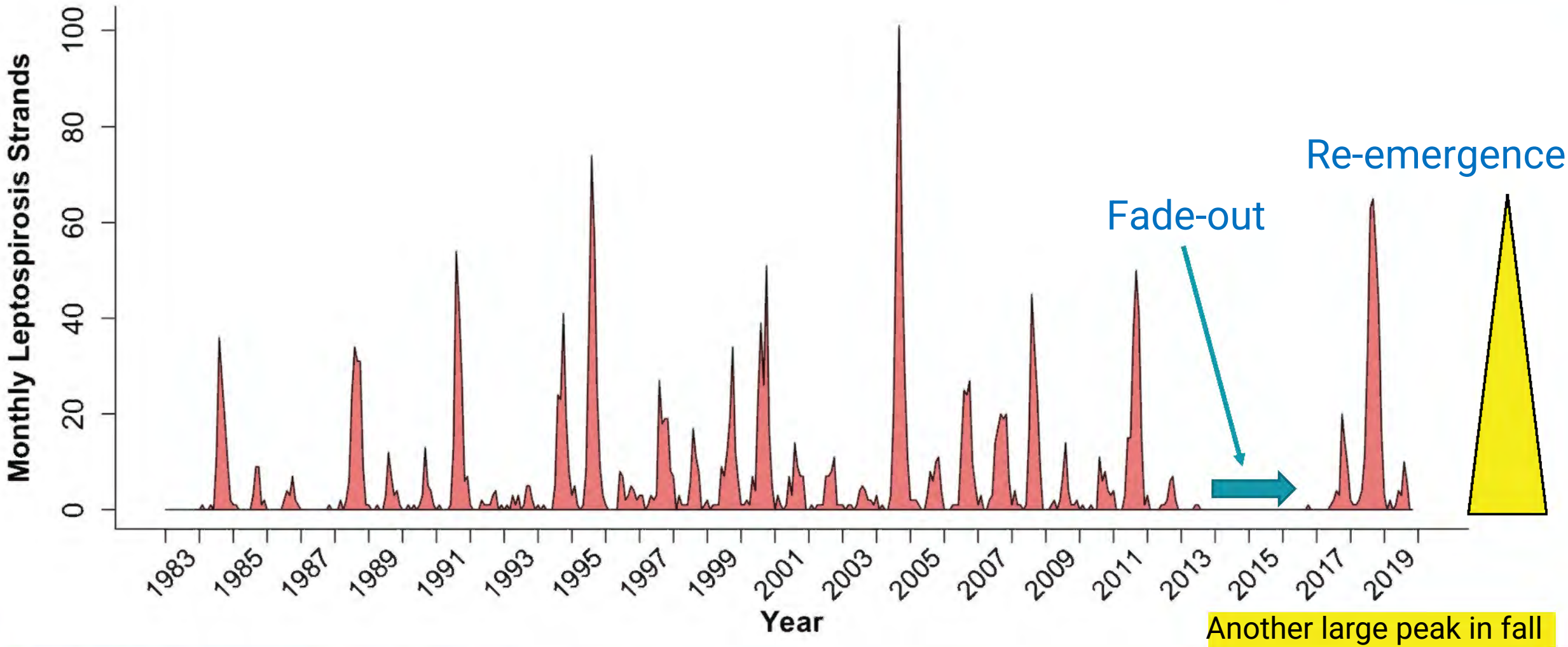


Another large peak in fall 2022 that has continued through summer 2023

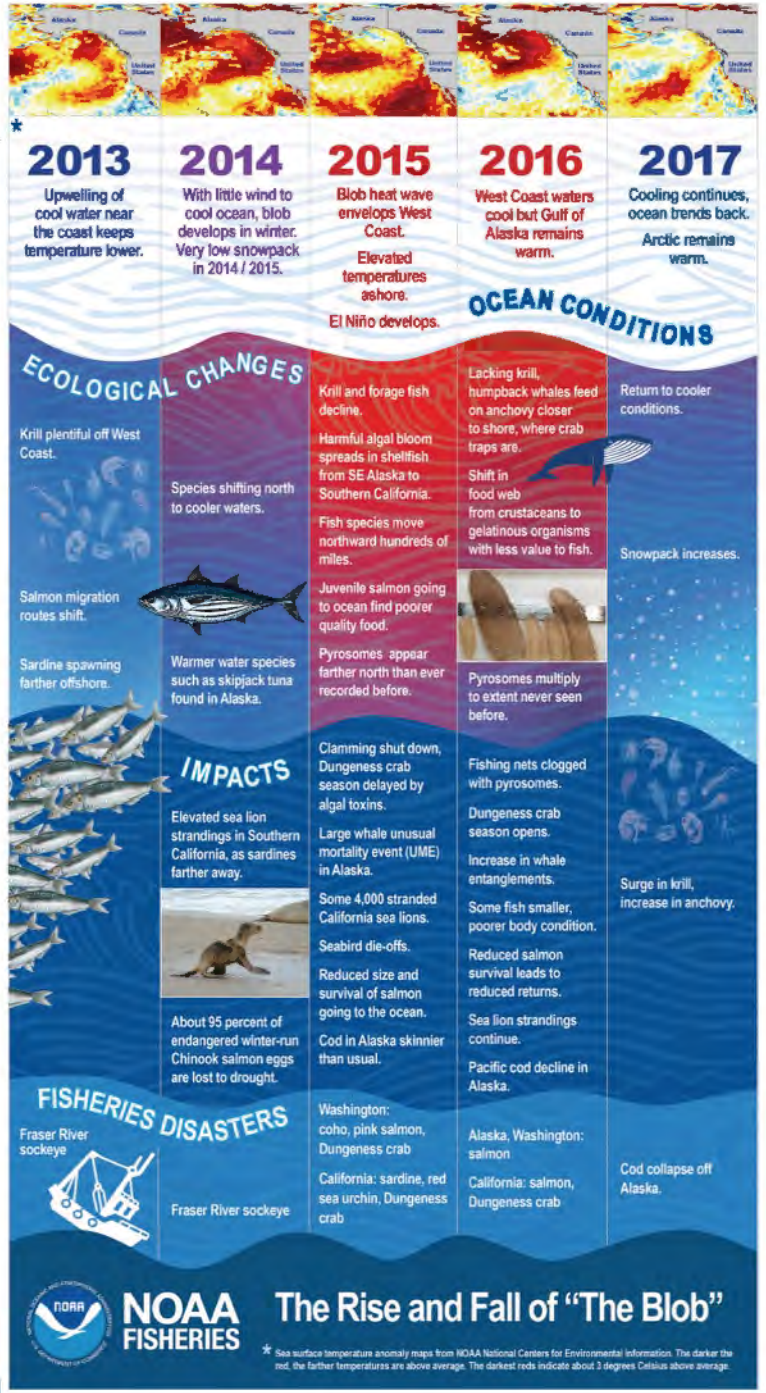
Hypothesis

Fade out and re-emergence driven by recent oceanographic thermal anomalies (Marine Heatwave “The Blob”) by 2 possible mechanisms:

(1) Reduced buildup of susceptible hosts due to reduced pup production and increased pup mortality,

(2) Changes in age- and sex-specific movement patterns (prey driven?) which altered population mixing and hence transmission dynamics.

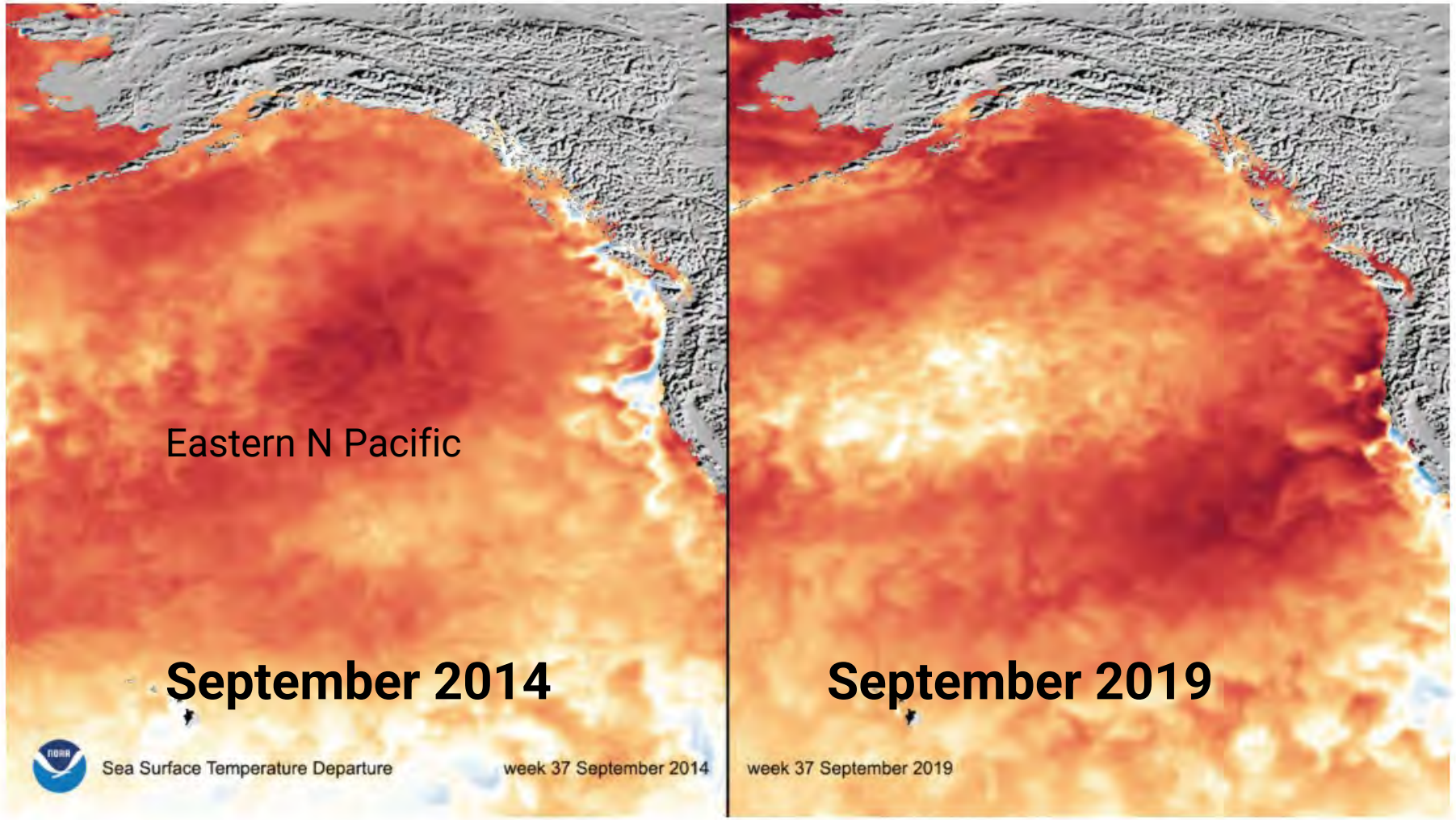
(For more, follow the work of Katie Prager, Benny Borremans and Jamie Lloyd-Smith, Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA, USA)



Infographic via NOAA Fisheries

The Marine Mammal Center.

Tracking Marine Heat Waves



Thermal anomalies may become the “new norm” with Climate Change

Something new in 2022

- Seven confirmed field-acquired cases in northern elephant seal pups stranded in WA, OR and CA
- *Leptospira interrogans* Pomona positive serology and on PCR
- All presented with azotemia and either died or were euthanized
- Gross appearance of kidneys was not remarkable
- Renal tubular necrosis and interstitial nephritis on histopathology

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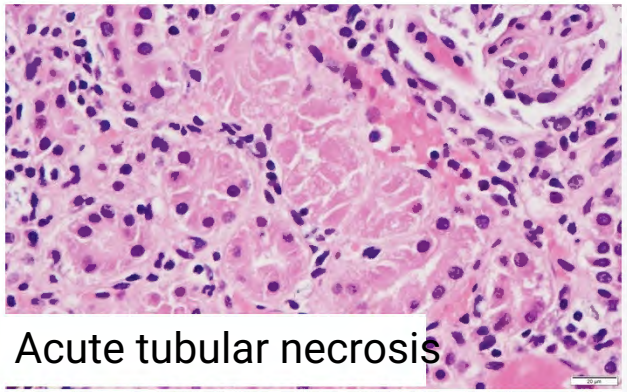
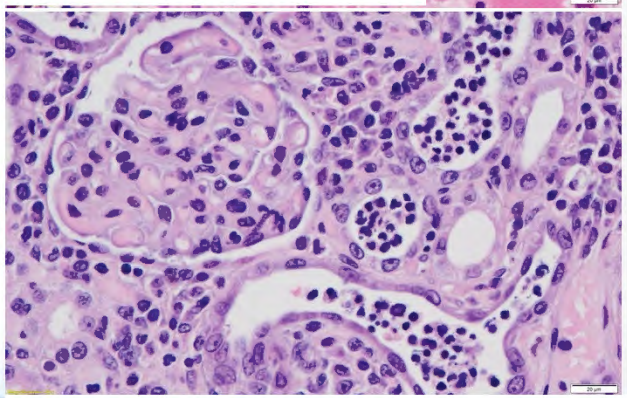
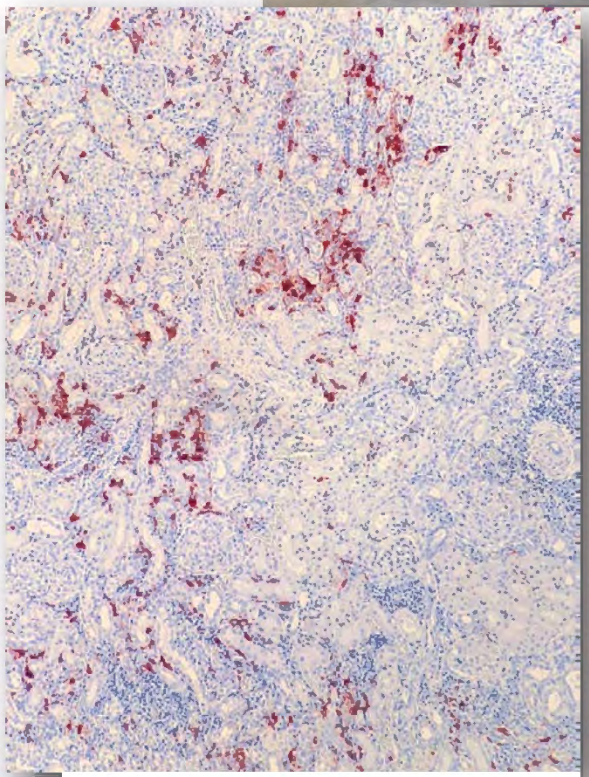
Comp Metabolic Panel		
Iron	31 - 179	130.
GGT	53 - 249	270.
ALT SGPT	20 - 94	51.
AST SGOT	0 - 87	102.
Alk Phosph	15 - 111	55.
Glucose	87 - 141	156.
Bilirubin	0.0 - 1.1	0.6
Phosphorus	4.3 - 6.7	10.9
Total Protein	7.1 - 8.9	7.6
BUN	17 - 41	221.
Creatinine	0.0 - 1.0	9.11
Calcium	8.3 - 9.7	8.4
Sodium	144 - 154	170.4
Potassium	4.1 - 5.1	2.94
Chlorine	91 - 123	141.5
Albumin	2.4 - 3.4	2.9
Creat Kin	80 - 1058	1321.
B/Creat Ratio		24.26
Globulin		4.7



Acute tubular necrosis



Lymphoplasmacytic inflammation



Positive Pomona IHC

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KLEBSIELLLOSIS

Klebsiella pneumoniae (HMV variant)

New Zealand sea lion
rāpoka / whakahao

Phocarctos hookeri
Endemic to NZ
Threatened and declining (~12,000)

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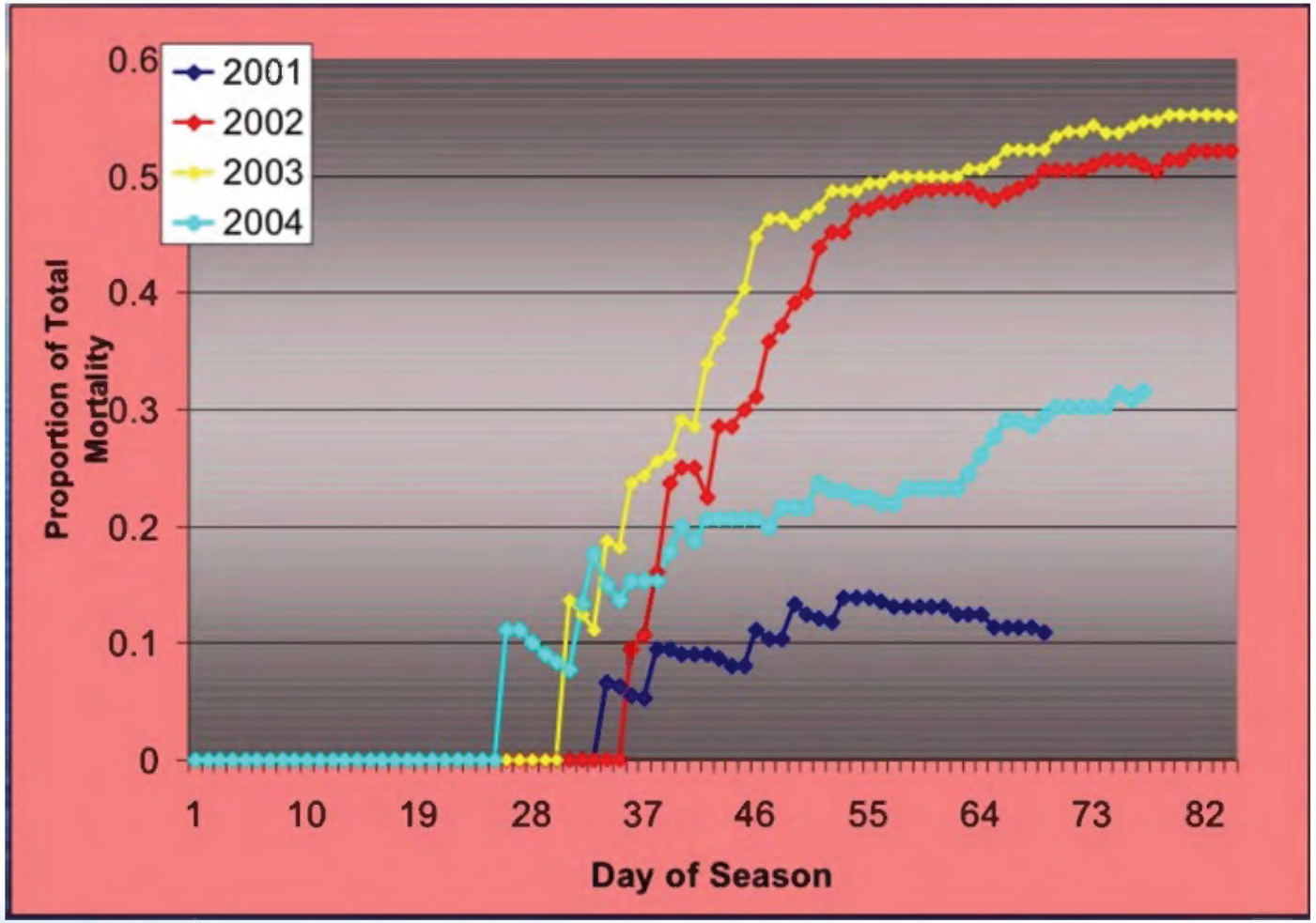
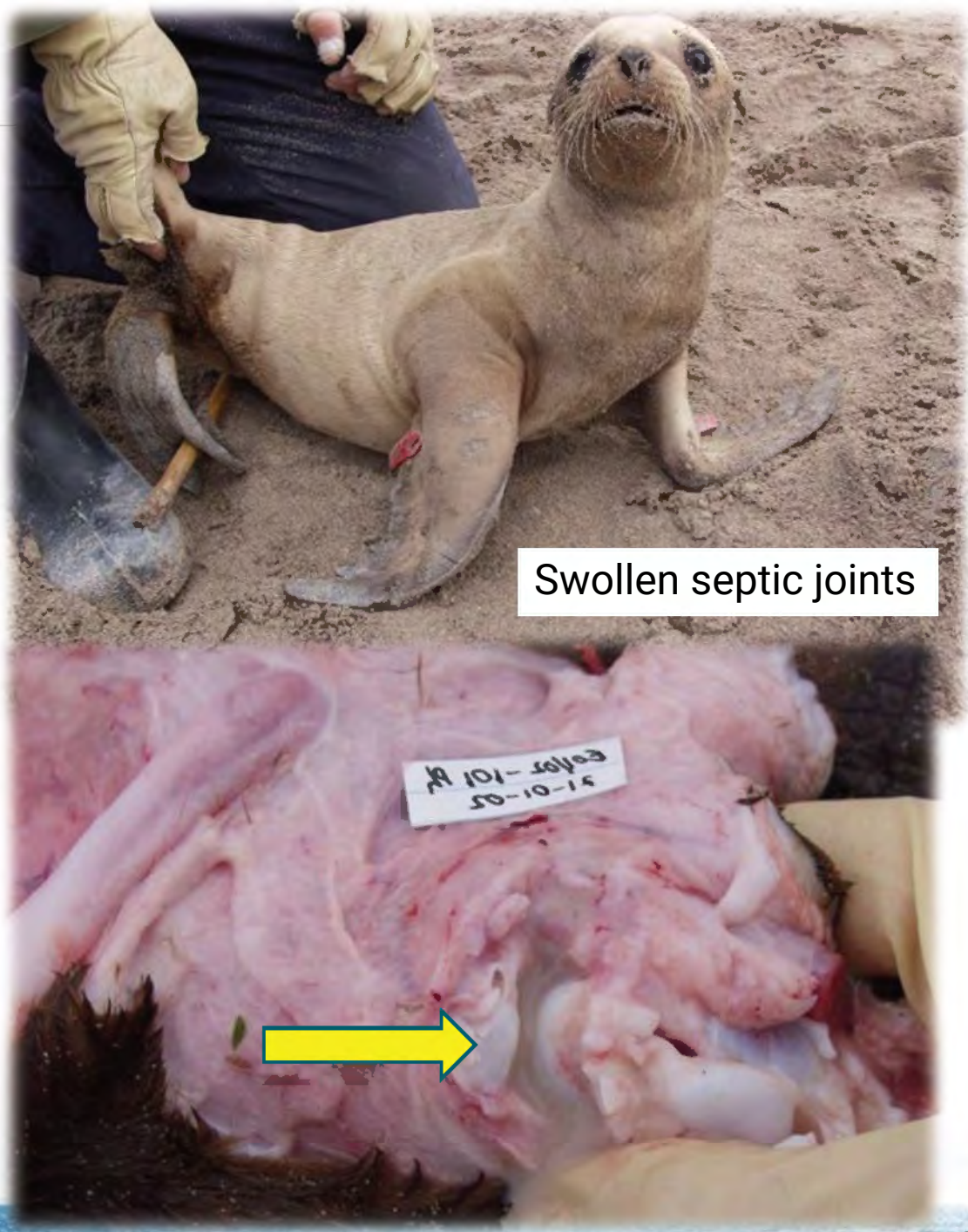
In 2002 AND 2003 pupping seasons unprecedented epidemics occurred that killed 50% of all pups





Severe acute suppurative and necrotizing polyarthritis and myositis

Klebsiella pneumoniae epidemics in NZ Sea Lions, Auckland Islands, 2002 & 2003

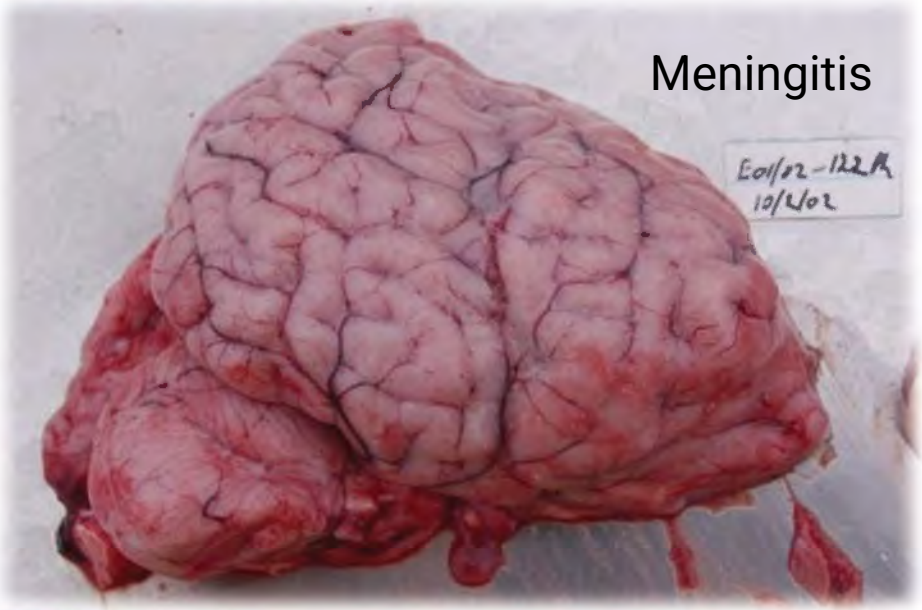
Pádraig Duignan,Aurelie Castinel, Alex Grinberg, Ian Wilkinson

Department of Conservation
Te Papa Atawhai

Te Kunenga ki Pūrehuroa

ttp://wildlife.massey.ac.nz

Necrotizing dermatitis

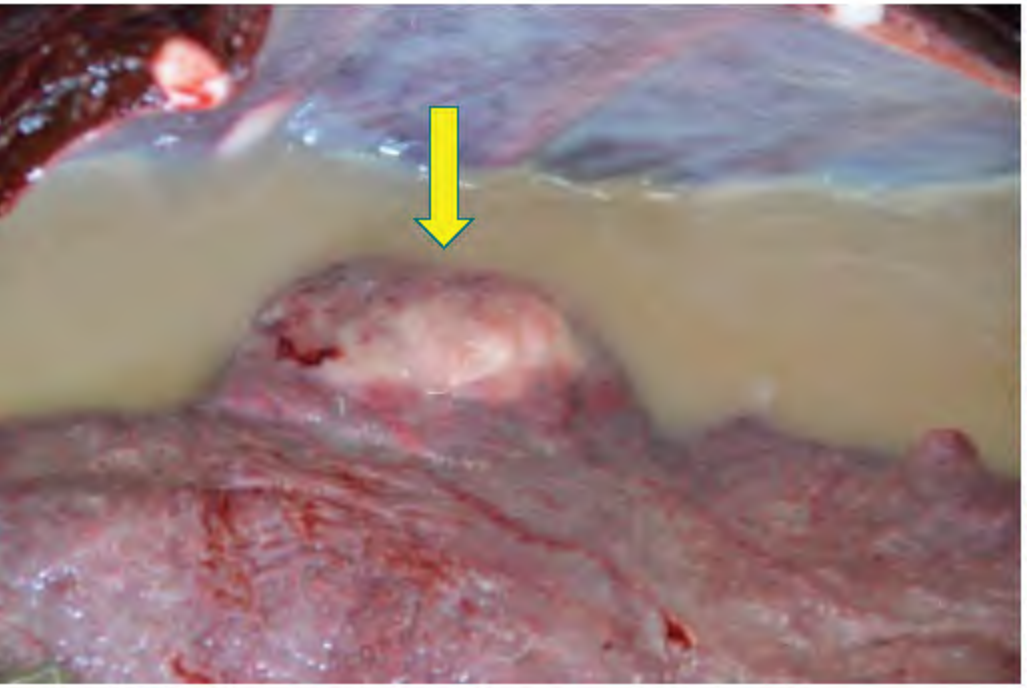


Meningitis

Around the same time (2001/02) it appeared in CSL pups on San Miguel but the outbreak was not fully documented. Further outbreaks occurred in NZ through 2009 and it is now endemic. Sporadic cases seen in older CSLs at TMMC over past 20 years.

Sporadic cases seen at TMMC over the years

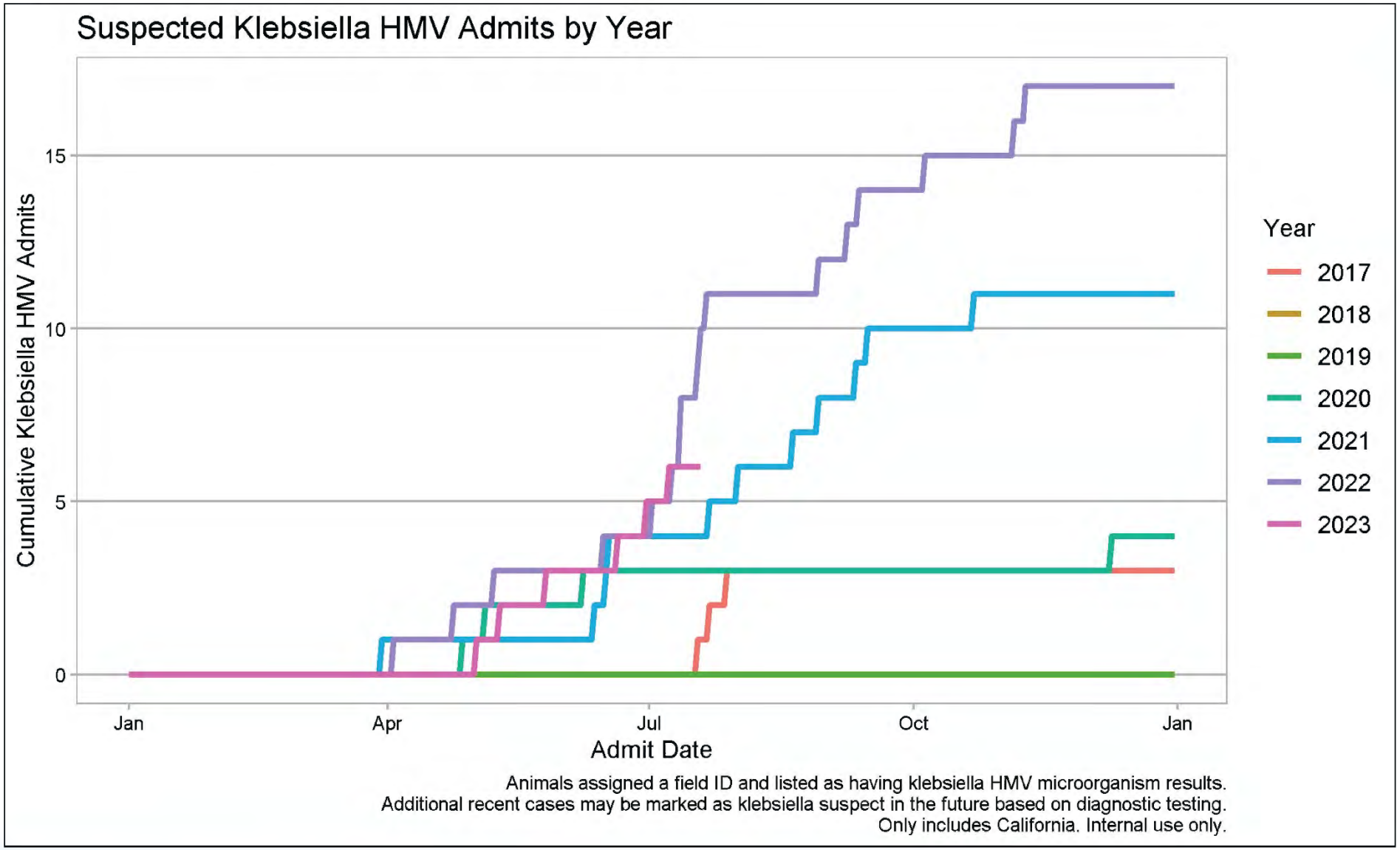
- Most present as lung abscesses that rupture causing pyothorax (pus in chest).
- This is a typical case - CSL Jamelia from 2020



Large abscess on the lung which ruptured causing huge accumulation of pus in the chest cavity

Over past 3 years we have seen a marked increase in Klebsiella cases

The Marine Mammal Center.



So where did HMV *Klebeiella pneumoniae* come from?

- Kp is very common in people as cause of pneumonia
- HMV Kp arose as a nosocomial (hospital acquired infection) in east Asia in the late 1980s and spread globally. It as acquired unique morphologic features and often carries antimicrobial resistance genes.
- Surveillance work in NZ prior to 2002 showed that it was **not present** in NZ sea lions until 2002 and it likely appeared in CA around same time.
- Our research showed that NZSL isolates over 2 years of epidemics were clonal (ie likely all from a single source of infection).
- Most likely introduced by an infected person: fishing fleet, eco tourists, researchers, conservation field staff (there are no permanent residents on the Auckland Islands).
- Our current research with Dr Esteban Soto, UC Davis, is looking at the genetic diversity in TMMC isolates over recent years.

Virulence and genetic diversity of *Klebsiella pneumoniae* from California sea lions (*Zalophus californianus*)

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[1] Aquatic Animal Health Laboratory, Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA
[2] The Marine Mammal Center, Sausalito, CA

INTRODUCTION

Klebsiella pneumoniae

- Gram-negative, aerobic, non-motile, encapsulated bacillus within the *Enterobacteriaceae* family
- Opportunistic pathogen with a broad host range
- In humans a hypervirulent strain has been associated with a hypermucoviscous (HMV) phenotype, but this has not yet been established in animal hosts[2]
- Multiple capsular serotypes exist within the HMV phenotype, including K1, K2, and K5 serotypes
- Sudden unexplained increase in the prevalence of HMV K2 *K. pneumoniae* in marine mammals, but especially in California sea lions (*Zalophus californianus*) (CSLs) – from 0.92% in 2017 to 7.2% in 2022 (Fig. 1.)[3]
- Causes klebsiellosis, contributing to disease and standings in marine mammals [4] (Fig. 2. and 3.)

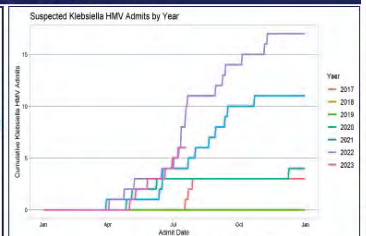

Fig. 1. Suspected HMV klebsiellosis admits to The Marine Mammal Center (TMMC)[3]
Incidence of suspected HMV *K. pneumoniae* admits to The Marine Mammal Center, a marine mammal hospital and rehabilitation facility in Sausalito, CA, from 2017 to 2023. There has been an increase in the number of HMV cases, as well as an admittance of cases earlier in the year, in recent years compared to 2017. Courtesy of Dr. Pádraig Duignan and Carlos Rios.

AIMS

1. Investigate the genetic diversity and virulence gene profiles of HMV K2 *K. pneumaniae* isolates recovered from diseased CSLs in California between 2020 to 2023.

   Hypothesis: HMV K2 strains isolated from CSLs are genetically diverse following housekeeping genes and virulence factor typing schemes.

2. Investigate the virulence of representative HMV K2 *K. pneumaniae* genotypes through *in-vitro* challenge models of kidney epithelial cells extracted from an African green monkey (*Chlorocebus sabaeus*) (Vero cell line).

   Hypothesis: HMV K2 strains isolated from CSLs are significantly more cytotoxic to mammalian cells (Vero cells) than non-HMV strains recovered from marine mammals.

METHODS

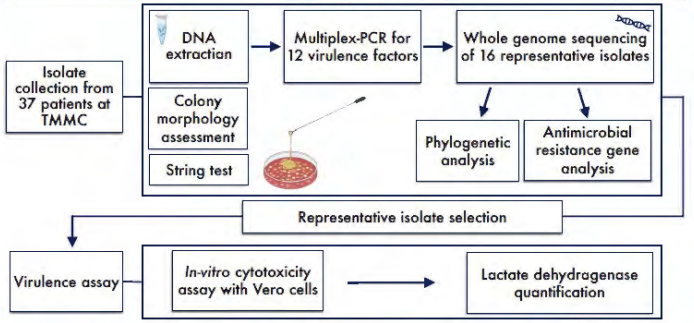

Fig. 5. In-vitro cytotoxicity
Vero cells grown for 24 hours at 37°C to confluence, inoculated with an MOI of 1:1 for 1hr following the Cytotox96® Non-Radioactive Cytotoxicity Assay protocol by Promega.

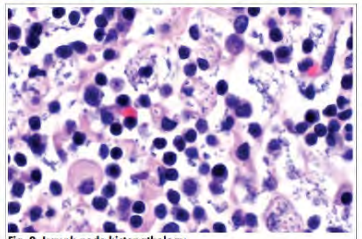

Fig. 2. Lymph node histopathology
Encapsulated bacterial rods within the cytoplasm of inflammatory cells in the lymph node of an affected Southern sea otter (SSO).
Photo courtesy of Dr. Melissa Miller

Fig. 3. Perilaryngeal abscess
K. pneumoniae causes widespread infection in marine mammals, resulting in conditions such as pleuritis, suppurative pneumonia, and abscesses.
Photo courtesy of Dr. Pádraig Duignan

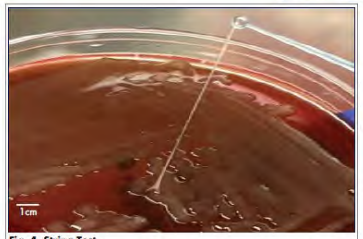

Fig. 4. String Test
31/37 isolates were positive for the string test, indicating the HMV phenotype. All HMV isolates were of the K2 serotype. Image shows a positive string test performed on an HMV K2 Clade I isolate.

RESULTS

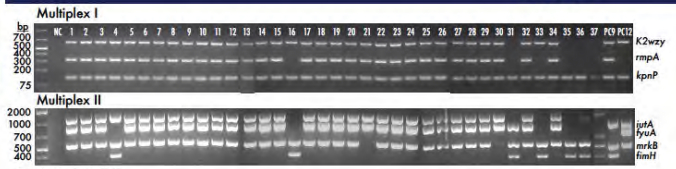

Fig. 6. Multiplex-PCR
Multiplex-PCR showing 37/37 isolates positive for the *kpnP* gene, confirming all isolates were *K. pneumoniae*. 31/37 isolates were positive for the *rmpA* gene, a regulator gene for the mucoid phenotype. 33/37 isolates were positive for the K2*wzy* gene, indicating the K2 capsular serotype. Within the K2 serotype, four different virulence profiles were observed and characterized based on the presence of virulence genes: *iutA* (siderophore aerobactin), *fyuA* (siderophore yersiniabactin), *mrkB* (type 3 fimbriae promoting biofilm formation), and *fimH* (type 1 fimbriae promoting adhesion to host epithelial cells)[5]. 28 K2 isolates belong to Clade I (*iutA*$^+$/*fyuA*$^+$/*mrkB*$^+$/*fimH*$^-$), 1 K2 isolate (#4) belongs to Clade II (*iutA*$^+$/*fyuA*$^-$/*mrkB*$^+$/*fimH*$^+$), 2 K2 isolates (#16, #33) belong to Clade III (*iutA*$^-$/*fyuA*$^-$/*mrkB*$^+$/*fimH*$^+$), and 2 K2 isolates (#21, #30) belong to Clade IV (*iutA*$^+$/*fyuA*$^+$/*mrkB*$^-$/*fimH*$^+$). The remaining 4 isolates (#31, #35, #36, #37) were not HMV and did not have capsular serotype K1, K2, or K5. Positive control isolate PC9 belongs to Clade II; positive control isolate PC12 belongs to Clade I.

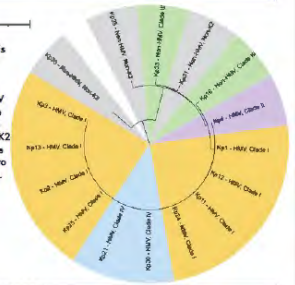

Fig. 7. Phylogenetic Analysis
Phylogenetic analysis showing homogeneity within Clade I (yellow) and between Clades I and IV (blue). Clade III non-HMV K2 isolates (green) appear more closely related to non-HMV non-K2 isolates (grey) than to other K2 isolates. Clade II HMV K2 isolate (purple) is more closely related to Clade I isolates than to Clade III.

Isolate	Host	HMV	K2 Clade	Virulence Related Genes: *kpnP*	*rmpA*	*K2wzy*	*K5wzy*	*iroD*	*iutA*	*fyuA*	*rmpA*	*ddB*	*mrkB*	*fimH*	Antimicrobial Resistance: Macrolides	Aminocoumarins	Tetracyclines	Phosphonic acids	Fluoroquinolones	Peptides	Monobactams	Carbapenems	Cephalosporins	Aminoglycosides	Diaminopyrimidine	Sulfonamides	Disinfectants & antiseptics
1	CSL	+	I	+		+			+	+	+		+		+++++		+	+	+++++	+++++		++	++	++			
4	CSL	+	II	+		+			+		+		+	+	+++++	++	+	+	+++++	+++++		++	++	++			
16	CSL		III	+		+							+	+	+++++	++	+	++	+++++++	+++++		++	++	+++			
33	CSL		III	+		+							+	+	+++++++	++	+	++	+++++++	+++++++	+	++++	+++	+++	+	+	+
21	CSL	+	IV	+		+			+	+	+				+++++	++	+	+	+++++	+++++		++	++	++			
31	CSL			+						+			+	+	++++	++	++	+	++++++++	+++++	++	++++	++++	+++++	+	+	
35	SSO			+									+	+	+++++		+	++	++++++++	+++++		++	++	++			
36	CSL			+					+				+	+	+++++	++	+	++	+++++++	++++	+	++	+	+			

Fig. 8. Isolate Characterization
All sequenced Clade I isolates had identical virulence and antimicrobial resistance gene profiles and are represented here by isolate #1. Both Clade IV isolates had identical antimicrobial resistance gene profiles to Clade I isolates and are represented here by isolate #21. All sequenced isolates had identical antimicrobial resistance gene profiles to the following antimicrobial classes: glycopeptides (*vanG*), nitroimidazoles (*msbA*), elfamycins (*E. coli* EF-Tu mutants), and cephamycins (*H. influenzae* PBP3). A (+) represents the presence of a single gene, with multiple (+) indicating the presence of multiple antimicrobial resistance genes.

CONCLUSION

- Multiple clades exist within the HMV K2 serotype of *K. pneumoniae*, with genetic diversity in both virulence factor profiles and antimicrobial resistance gene profiles.
- *In-vitro* virulence of both HMV and non-HMV isolates can provide a model of infection to expand our knowledge on the pathogenesis of this emerging pathogen.

ACKNOWLEDGEMENT + REFERENCES

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SARCOCYSTOSIS

Sarcocystis neurona-associated myositis in California Sea Lions

- In 2010 an adult female sea lion (*Zalophus californianus*) was rescued because of lethargy and difficulty with ambulation.
- She had elevated creatine kinase suggesting muscle damage.
- Biopsies of skeletal muscle showed *S. neurona*-like cysts in myocytes and qPCR for Sarcocystis was positive.
- She was treated with anti-protozoal medication and released.
- Long term survival is not known.

This disease did not appear again until 2016

Diagnosis and treatment of *Sarcocystis neurona*–induced myositis in a free-ranging California sea lion

Daphne P. Carlson-Bremer, DVM, MPVM; Frances M. D. Gulland, VetMB, PhD; Christine K. Johnson, VMD, PhD; Kathleen M. Colegrove, DVM, PhD, DACVP; William G. Van Bonn, DVM

Case Description—An underweight, lethargic adult female California sea lion (*Zalophus californianus*) became stranded along the California shore and was captured and transported to a rehabilitation hospital for assessment and care.

Clinical Findings—Initial physical assessment revealed the sea lion was lethargic and in poor body condition. Active myositis was diagnosed on the basis of concurrent elevations in activities of alanine aminotransferase and creatine kinase detected during serum biochemical analysis. Infection with *Sarcocystis neurona* was diagnosed after serologic titers increased 4-fold over a 3-week period. Diagnosis was confirmed on the basis of histopathologic findings, positive results on immunohistochemical staining, and results of quantitative PCR assay on biopsy specimens obtained from the diaphragm and muscles of the dorsal cervical region.

Treatment and Outcome—Anticoccidial treatment was instituted with ponazuril (10 mg/kg [4.5 mg/lb], PO, q 24 h) and continued for 28 days. Prednisone (0.2 mg/kg [0.09 mg/lb], PO, q 12 h) was administered for 2 days and then every 24 hours for 5 days to treat associated inflammation. At the end of treatment, the sea lion was clinically normal, alanine aminotransferase and creatine kinase values were within reference limits, and antibody titers against *S neurona* had decreased 6-fold. The sea lion was released approximately 3 months after becoming stranded.

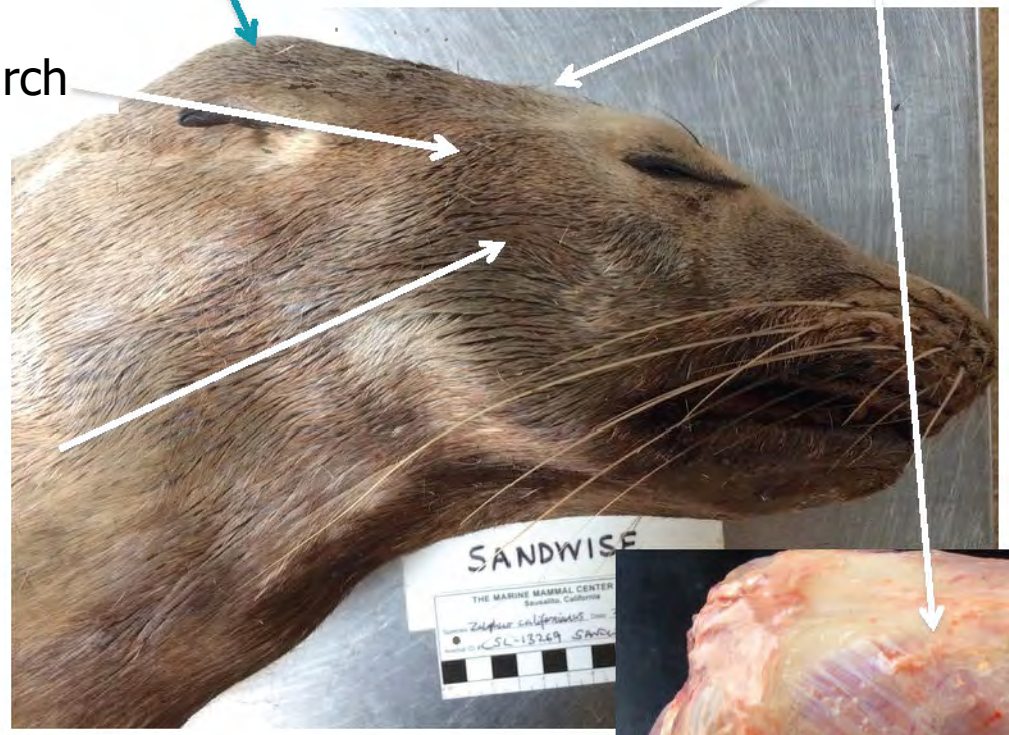
Clinical Relevance—*S neurona*–induced myositis was diagnosed in a free-ranging California sea lion. On the basis of the successful treatment and release of this sea lion, anticoccidial treatment should be considered for marine mammals in which protozoal disease is diagnosed. (*J Am Vet Med Assoc* 2012;240:324–328)

Carlson_Bremmer et al., JAVMA, 2012

Prominent occipital crest

Atrophied temporalis muscle

Prominent zygomatic arch



Atrophied masseter muscle

CSL-Sandwise

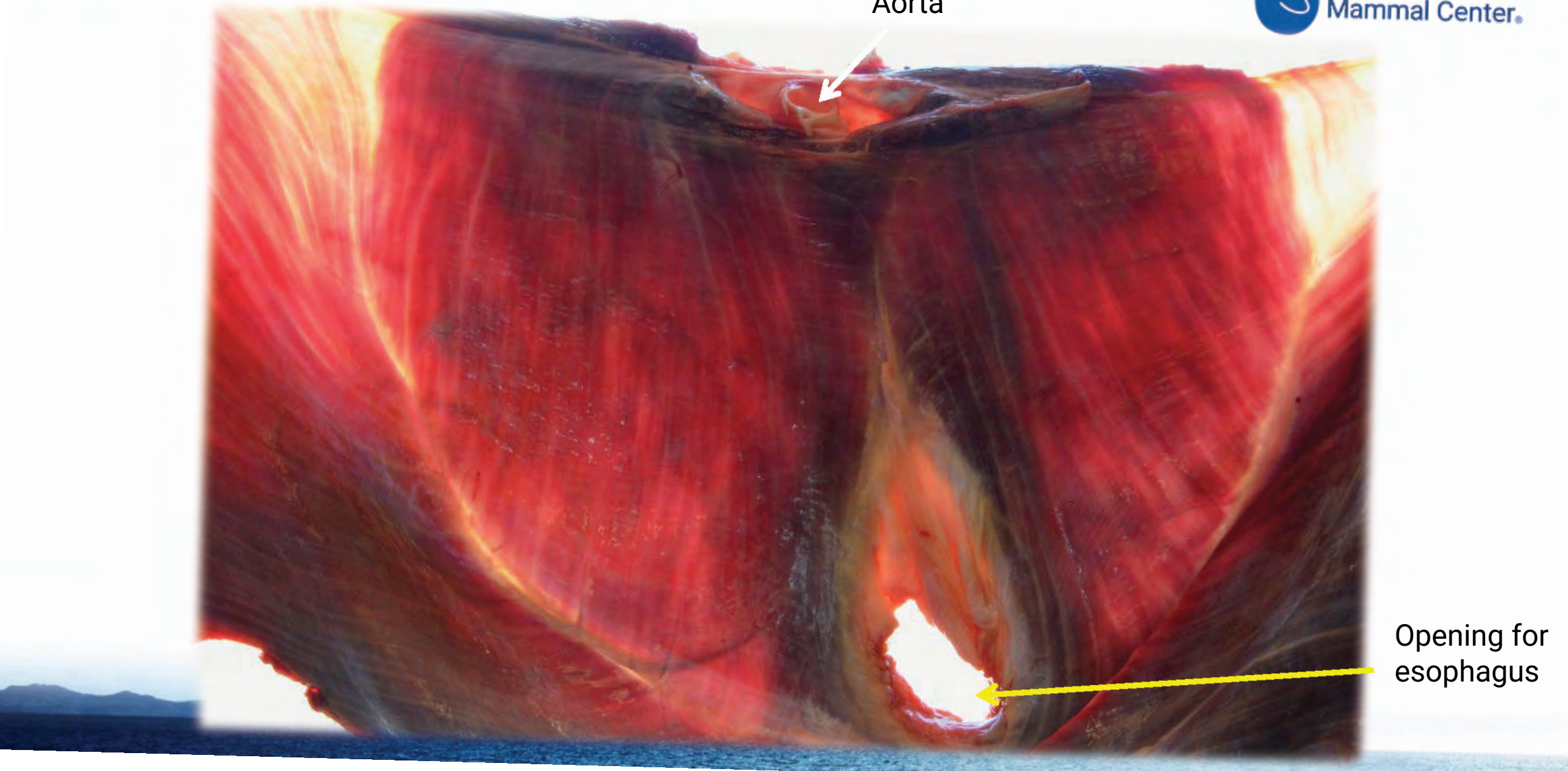
Pale streaky atrophied
Laryngeal muscles
(M. cricoarytenoideus)

Larynx

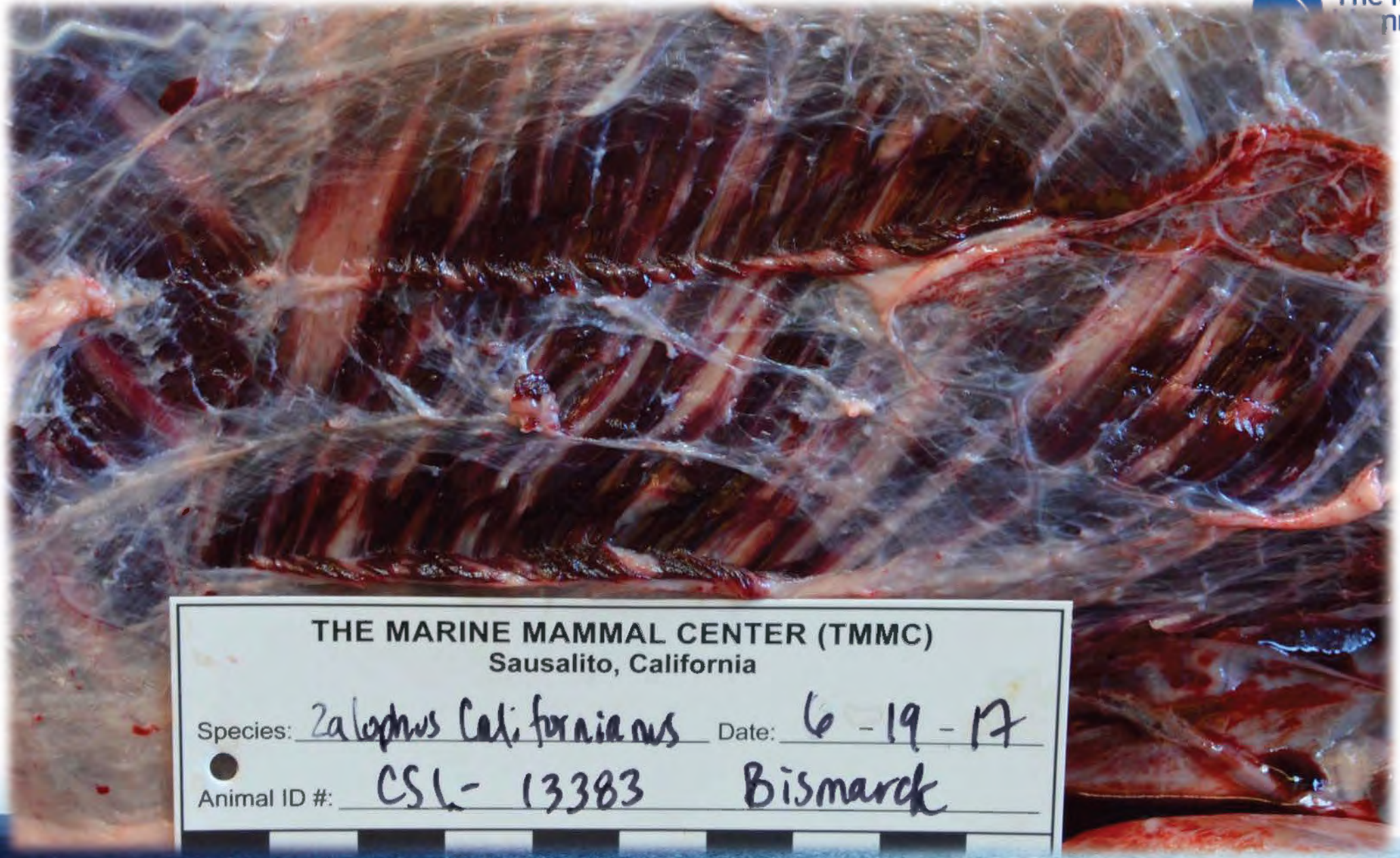
Trachea

Base of tongue

Severely atrophied diaphragm (thin and streaky)



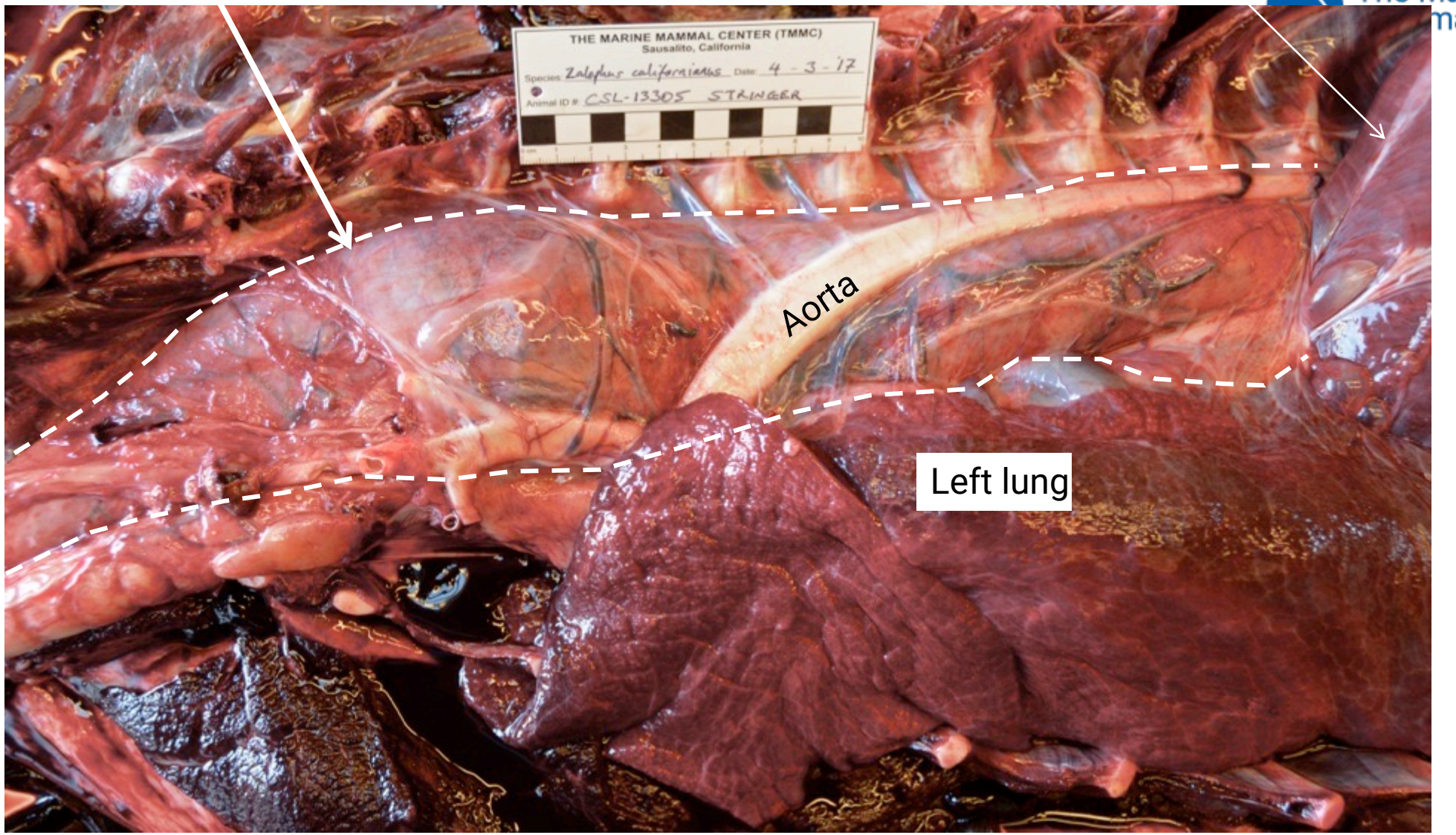
Pectoral muscles with severe atrophy and streaking



Greatly distended esophagus (dashed lines)

Diaphragm (pale and streaky)

The Marine Mammal Center



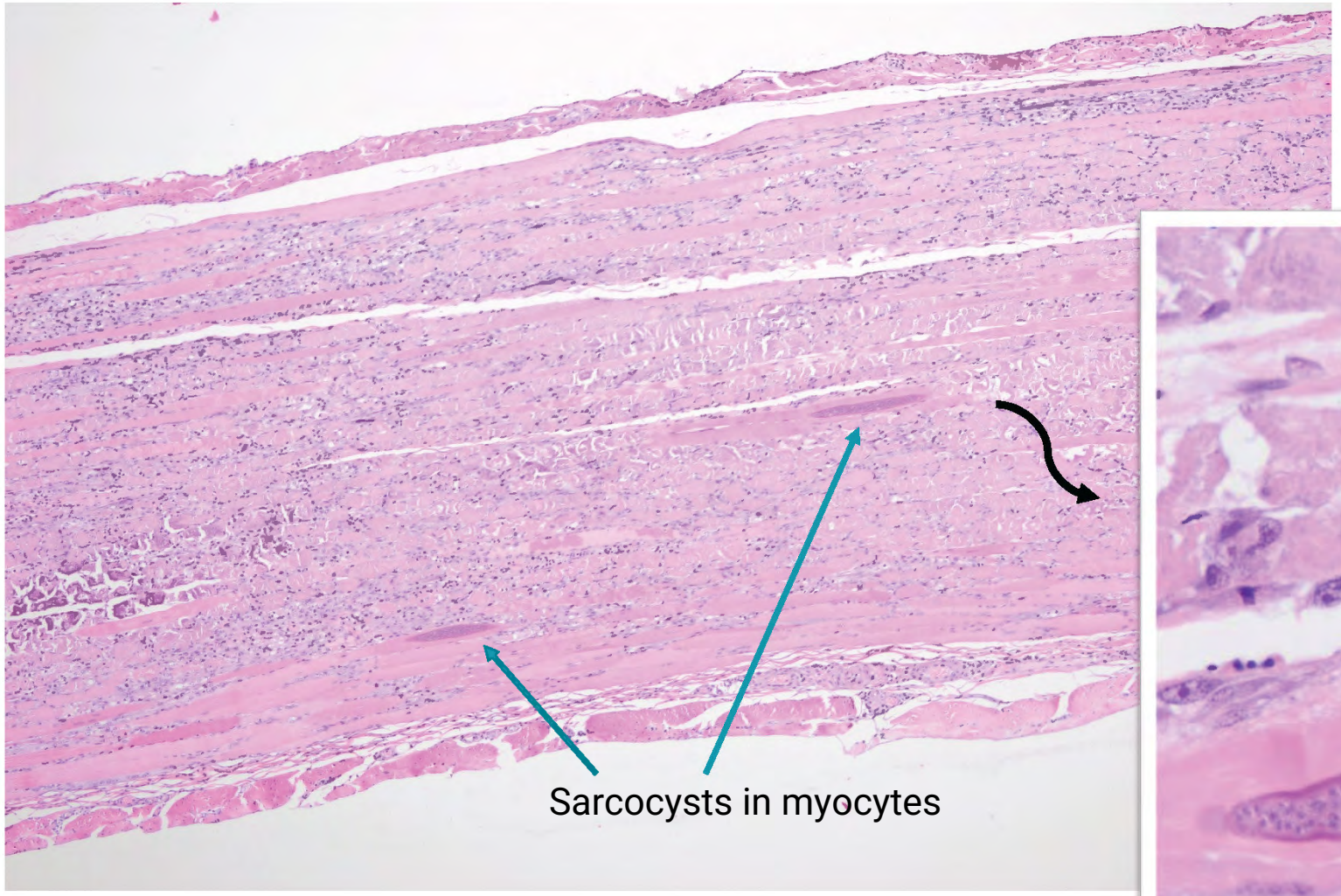
Opened esophagus packed with fish bones and flesh

Pale streaky diaphragm

The Marine Mammal Center.

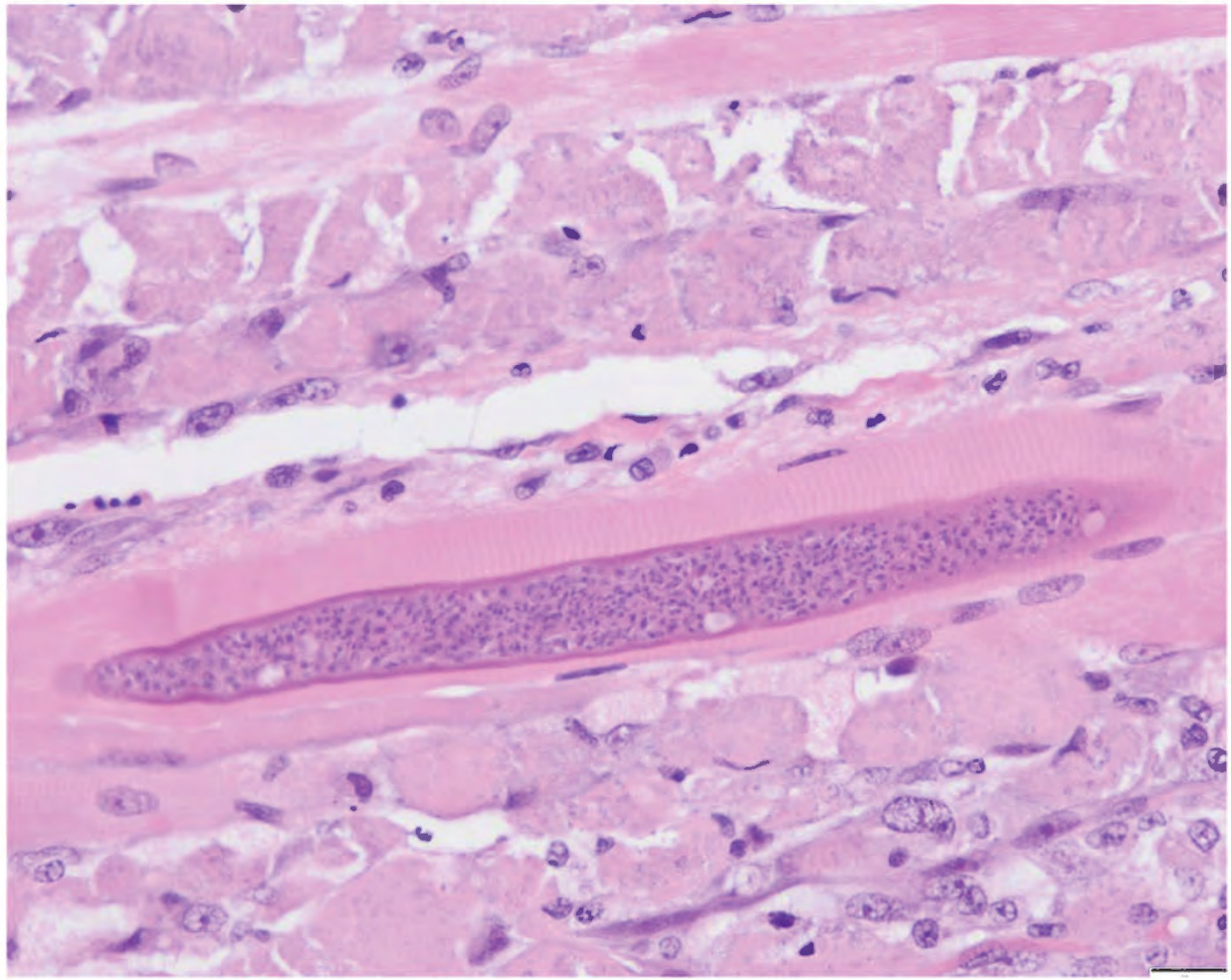
Histologic section from the diaphragm of CSL Hillard

The Marine Mammal Center



The predominantly blue tissue is all damaged muscle

Sarcocyst in a myocyte surrounded by necrotic (dead) myocytes, inflammatory cells and satellite (stem) cells.



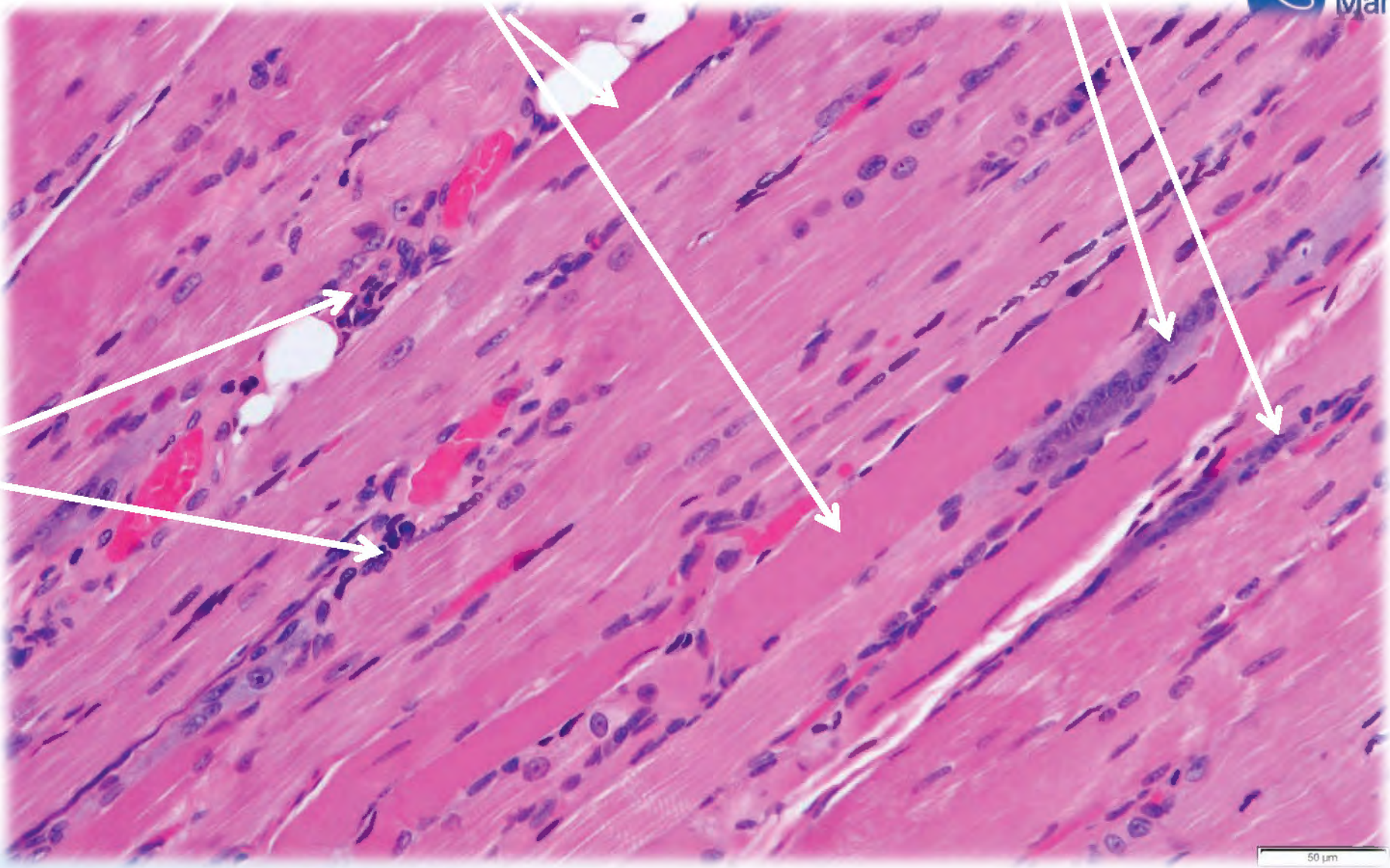
Temporalis Muscle

Hyper-eosinophilic myofibers (necrosis)

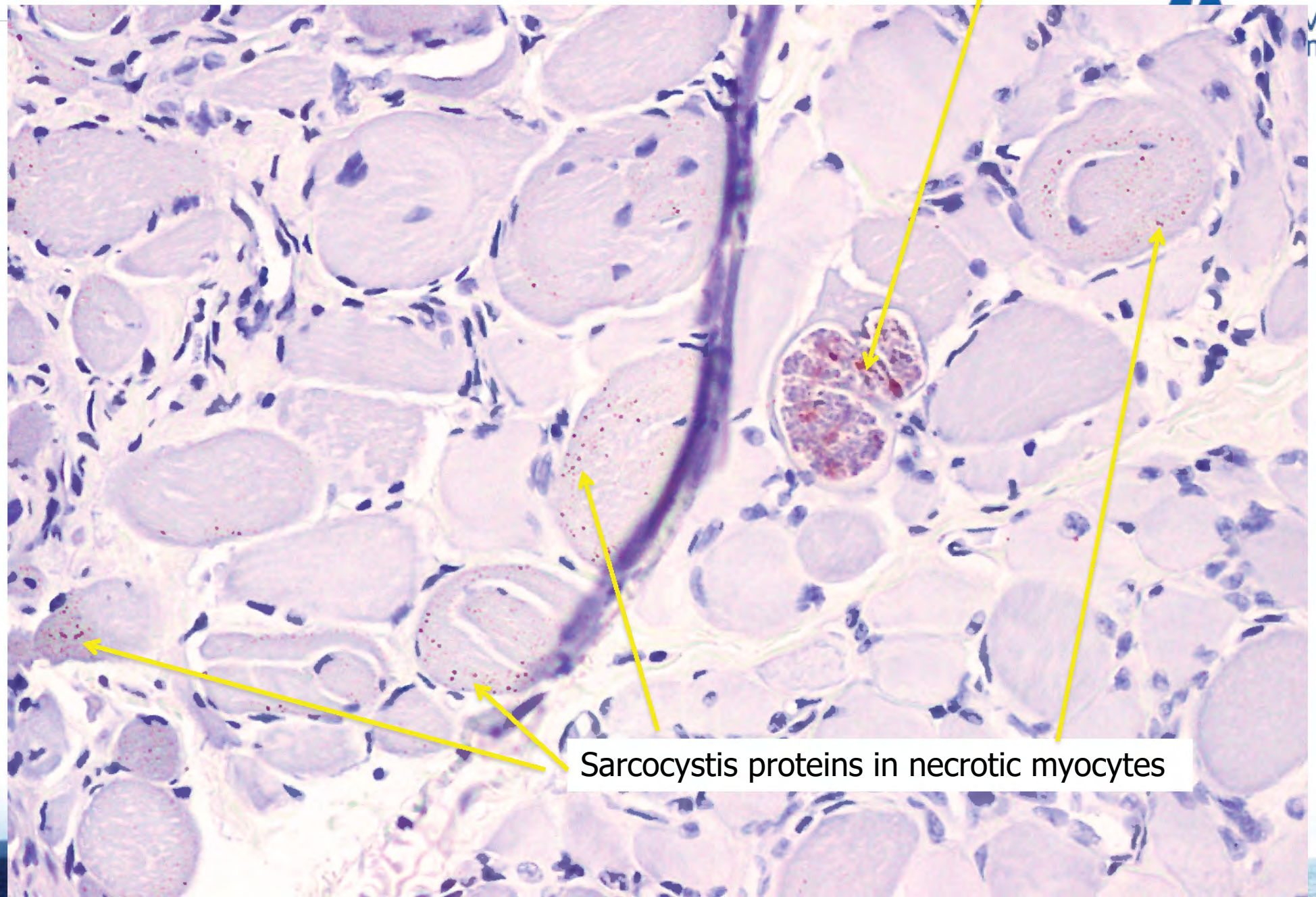
Nucelar rowing (regeneration)

The Marine Mammal Center.

Infiltrating lymphocytes

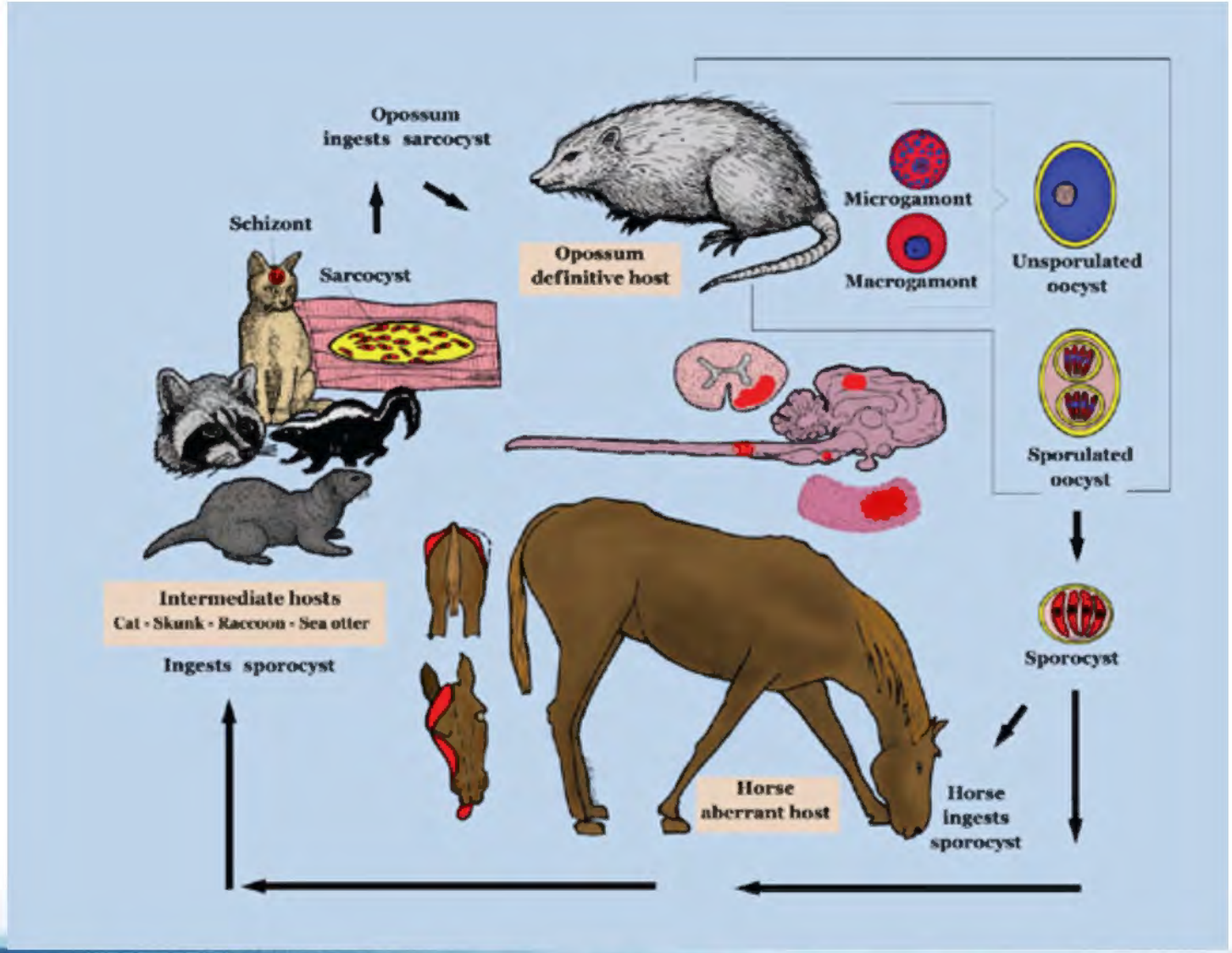


Immunohistochemistry for Sarcocystis

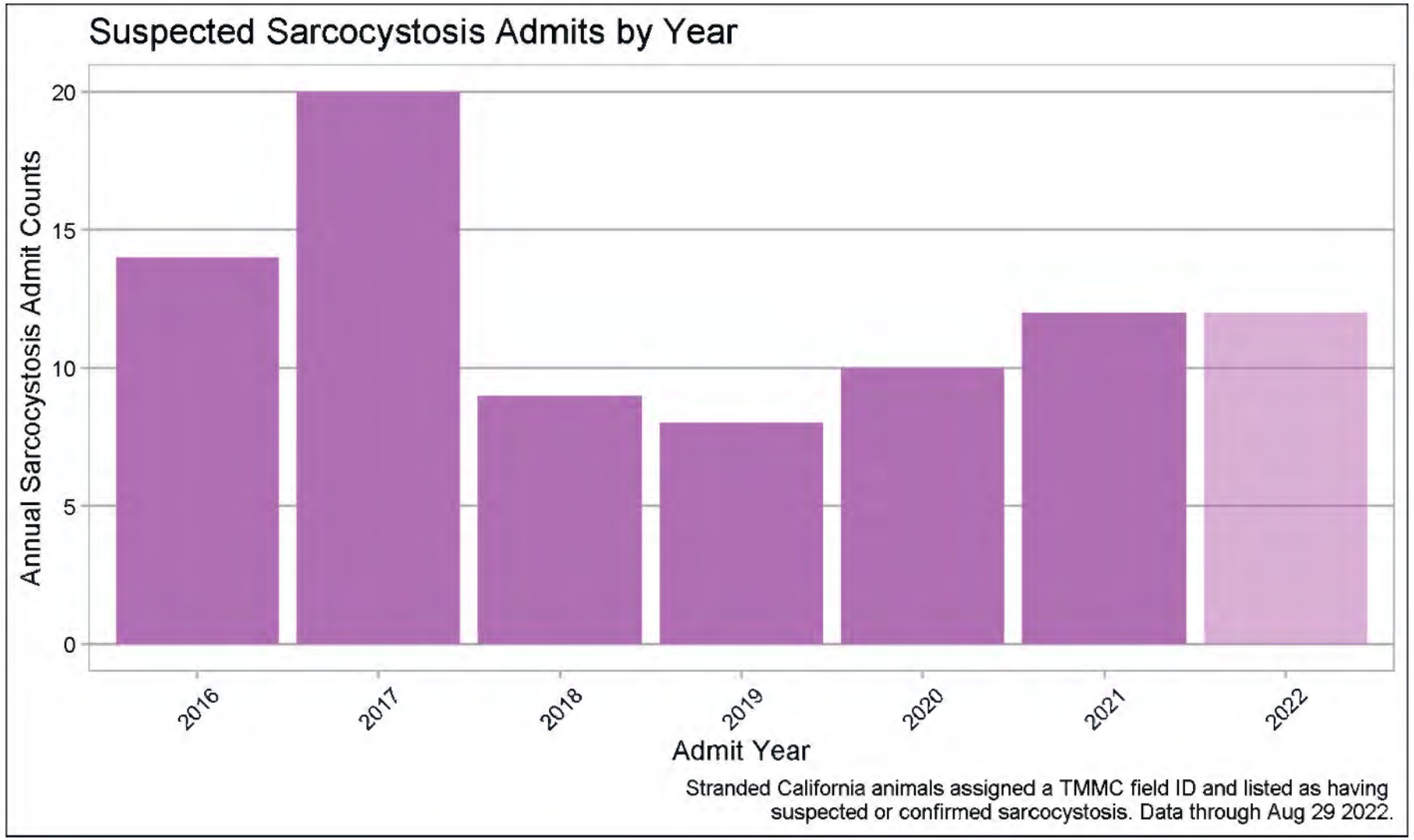


Life Cycle of *Sarcocystis neurona*

- Apicomplexan protozoan parasite that is very similar to *Toxoplasma gondii*.
- The definitive host is the Virginia opossum (*Didelphis virginiana*) not the cat (*T. gondii*).
- It is a well known cause of spinal cord damage in horses (Equine potozoal myelitis).
- In marine mammals causes encephalitis in harbor seals and sea otters in the eastern North Pacific.
- Land-to-Sea mode of transmission.



Sea Lions with Polyphasic Rhabdomyositis



Just one case in 2010 prior to this outbreak

Polyphasic Rhabdomyositis in California Sea Lions (*Zalophus Californianus*): Pathology and Potential Causes

Mauricio Seguel[1], Kathleen M. Colegrove[2], Cara Fi
Sophie Whoriskey[3], Tenaya Norris[3], and Padraig Du

Abstract

A myositis syndrome has been recognized for more than a decade in C
detailed description of the lesions and potential causes of this conditi
rhabdomyositis were examined. Rhabdomyositis was considered incident
to the animal stranding (significant rhabdomyositis) in 33% (45/136). O
consisted of a few small foci of lymphohistiocytic inflammation. Of the
presented with major comorbidities such as leptospirosis (2 animals) and
severe polyphasic rhabdomyositis as the only major disease process ass
muscles had multiple white streaks and diffuse atrophy. Microscopically, th
and histiocytes admixed with areas of myofiber regeneration, and/or mode
to intact *Sarcocystis neurona* cysts. At the interface of affected and norma
coplasm of intact myocytes, and occasional myofibers expressed MHCII p
cyst burden were higher in animals with significant polymyositis antibody ti
active myonecrosis than animals with incidental rhabdomyositis antibody
The presented findings suggest that *S. neurona* infection and immune-me
polyphasic rhabdomyositis in CSLs.

Veterinary Pathology
1-11

The Marine Mammal Center.

Aquatic Animals

Clinical signs, treatment, and outcome for California sea lions (*Zalophus californianus*) with *Sarcocystis*-associated polyphasic rhabdomyositis

Sophie T. Whoriskey DVM
Pádraig J. Duignan DVM, PhD
Abby M. McClain DVM
Mauricio Seguel DVM, PhD
Frances M. D. Gulland VetMB, PhD
Shawn P. Johnson DVM, MPVM
Cara L. Field DVM, PhD

From the Department of Veterinary Medicine and Science, The Marine Mammal Center, Sausalito, CA 94965 (Whoriskey, Duignan, McClain, Gulland, Johnson, Field); and Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1, Canada (Seguel).

Address correspondence to Dr. Whoriskey (whoriskeys@tmmc.org).

OBJECTIVE

To describe clinical signs, treatment, and outcome for California sea lions (*Zalophus californianus*) with *Sarcocystis*-associated polyphasic rhabdomyositis.

ANIMALS

38 free-ranging juvenile to adult California sea lions examined at a rehabilitation center in California between September 2015 and December 2017.

PROCEDURES

Medical records at The Marine Mammal Center were reviewed to identify sea lions in which sarcocystosis had been diagnosed.

RESULTS

Clinical signs were highly variable and associated with polyphasic rhabdomyositis attributed to *Sarcocystis neurona* infection. Generalized severe muscle wasting, respiratory compromise, and regurgitation secondary to megaesophagus were the most profound clinical findings. Respiratory compromise and megaesophagus were associated with a poor prognosis. Eight of the 38 sea lions were treated and released to the wild, and 2 subsequently restranded and were euthanized. Two additional animals received no targeted treatment and were released. The remaining 28 animals were either euthanized or died during treatment.

CONCLUSIONS AND CLINICAL RELEVANCE

Results suggested that unlike other marine mammals, which typically develop encephalitis, California sea lions with sarcocystosis often have polyphasic rhabdomyositis with highly variable clinical signs and that extensive diagnostic testing may be required to confirm the diagnosis. Treatment with an antiprotozoal drug in combination with corticosteroids may resolve clinical disease, but the prognosis is guarded.

Hypothesis: This is a new strain of Sarcocystis that sea lions are highly susceptible to

International Journal for Parasitology 45 (2015) 595–603

Contents lists available at ScienceDirect

International Journal for Parasitology

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

A novel *Sarcocystis neurona* genotype XIII is associated with severe encephalitis in an unexpectedly broad range of marine mammals from the northeastern Pacific Ocean

Lorraine Barbosa [a,b], Christine K. Johnson [a], Dyanna M. Lambourn [c], Amanda K. Gibson [b], Katherine H. Haman [b,d], Jessica L. Huggins [e], Amy R. Sweeny [b], Natarajan Sundar [b], Stephen A. Raverty [d,f], Michael E. Grigg [b,d,*]

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[b] Molecular Parasitology Section, Laboratory of Parasitic Diseases, National Institutes of Health, NIAID, Bethesda, MD 20892, USA
[c] Department of Fish and Wildlife, Olympia, WA 98501, USA
[d] University of British Columbia, Department of Zoology, Fisheries Centre, Marine Mammal Research Unit, Vancouver, British Columbia V6T 1Z4, Canada
[e] Cascadia Research Collective, Olympia, WA 98501, USA
[f] Animal Health Center, Ministry of Agriculture, Abbotsford, British Columbia V3G 2M3, Canada

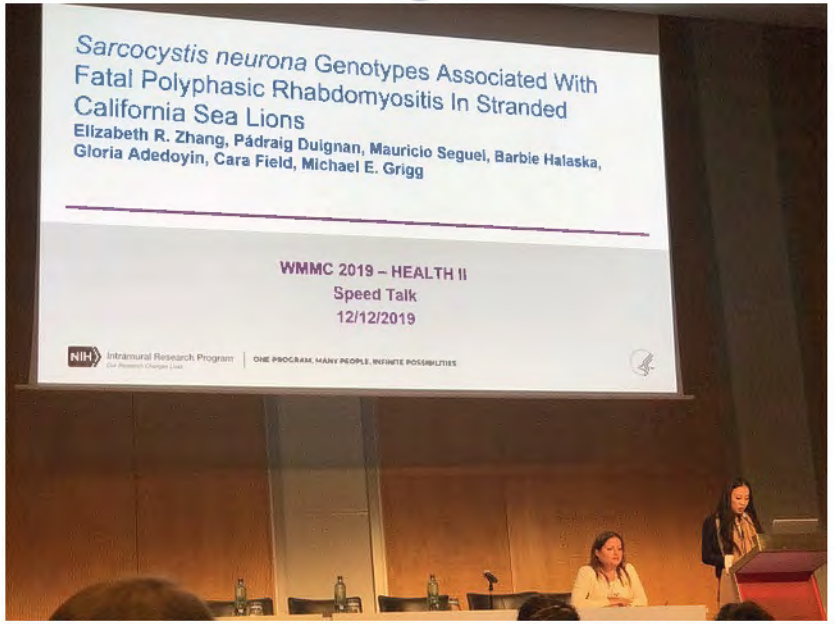
ARTICLE INFO

ABSTRACT

However….

In collaboration with DR Mike Grigg and Elizabeth Zhang, NIH, Multilocus genotyping of these *S. neurona* isolates at surface antigen and microsatellite biomarkers revealed a preponderance of **Type II and Type VI strains**.

Both strains were already circulating in marine mammal populations found along the Eastern Pacific coast of the United States and previously associated only with neurologic disease.



Elizabeth Zhang presenting at the World Marine Mammal Conference, Barcelona, December 2019

How are sea lions exposed to Sarcocystis and why has the prevalence of disease apparently increased since 2016?

PATTERNS OF MORTALITY IN SOUTHERN SEA OTTERS (*ENHYDRA LUTRIS NEREIS*) FROM 1998–2001

Author(s): C. Kreuder, M. A. Miller, D. A. Jessup, L. J. Lowenstine, M. D. Harris, J. A. Ames, T. E. Carpenter, P. A. Conrad, and J. A. K. Mazet

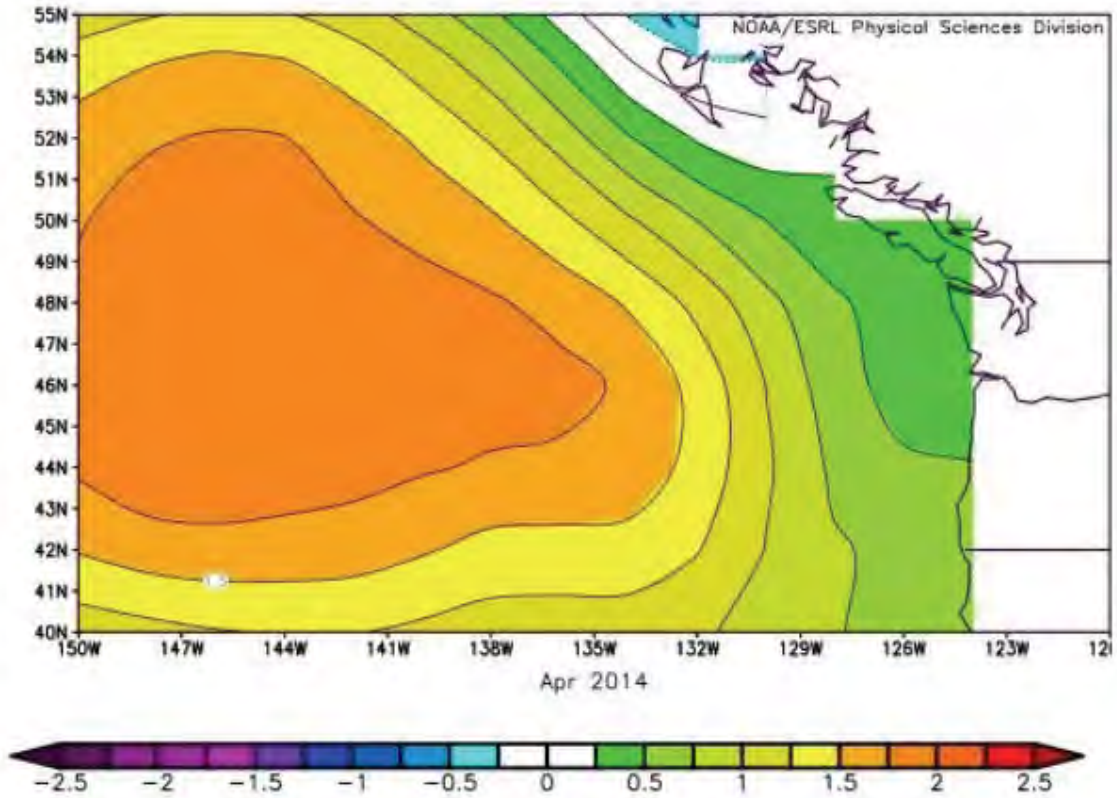
Source: Journal of Wildlife Diseases, 39(3):495-509.
Published By: Wildlife Disease Association
DOI: http://dx.doi.org/10.7589/0090-3558-39.3.495
URL: http://www.bioone.org/doi/full/10.7589/0090-3558-39.3.495

We know that in sea otters, infection is seasonal, and related to run-off from winter rain

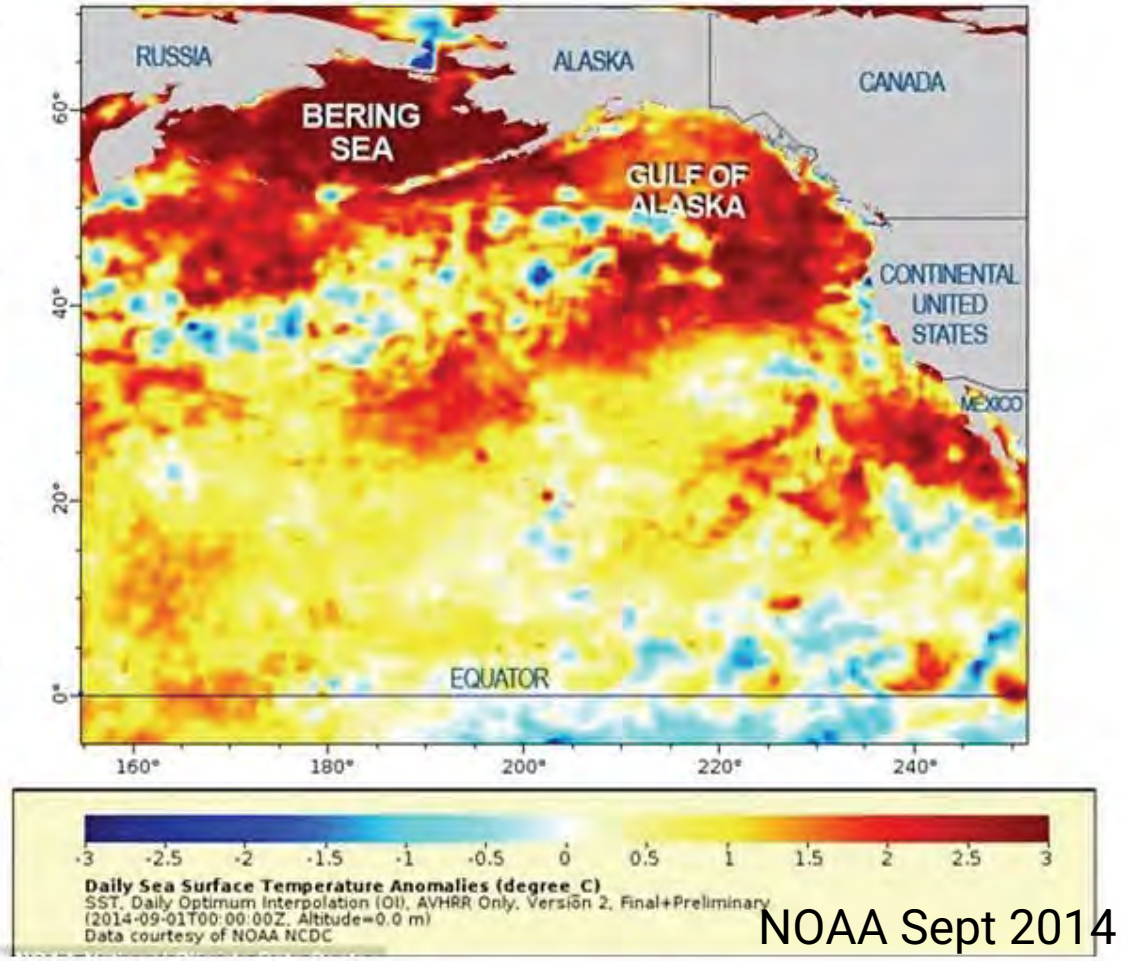
The increase in cases coincided with the North Pacific Thermal Anomaly.

Could that have affected the spread of a land pathogen to the ocean?

Or, could a shift in prey during these years have exposed sea lions to the parasite?



"The Blob" warm water anomaly 2013-'16



NOAA Sept 2014

A current project is looking at developing a serologic test for exposure to the parasite

Validation of an Indirect Fluorescent Antibody Test for *Sarcocystis neurona* infection in California sea lions

Amalie Luneng Solli[1], Cara Field[2], Pádraig Duignan[2], Andrea Packham[1], Magdalena Plancarte[1], Karen Shapiro[1], Devinn Sinnott[1], Woutrina A. Smith[1]
[1]School of Veterinary Medicine University of California, Davis [2]The Marine Mammal Center

Objective

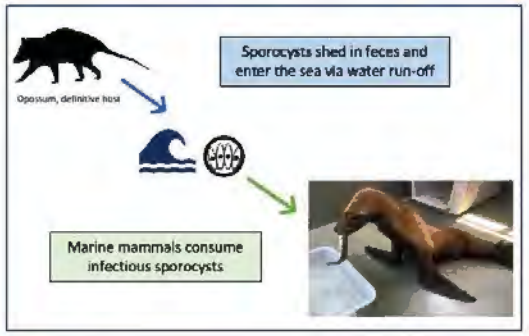
To evaluate IFAT test performance, identify cases of *Sarcocystis neurona* infections in stranded California sea lions (CSL) and control animals followed by analysis of the correlation between the gold-standard testing approach of histopathology and molecular sequencing against the SarcoFlour IFAT.

Rationale

- Seroprevalence in CSL has increased from 5-6% (1998-2009) to 14% (2010-2019)

- Infection can result in polyphasic rhabdomyositis and death; prognosis is guarded even with treatment in CSL
- IFAT diagnostic test advantages: low-cost, quick, better informs treatment plans but not yet validated in CSL

Land-to-Sea Transmission



Methods

Indirect Fluorescent Antibody Test

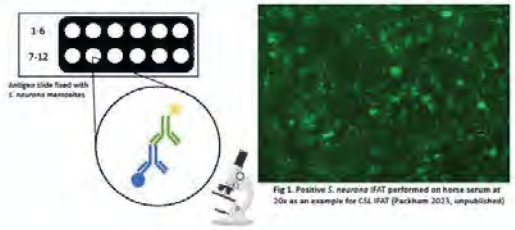


Fig 1. Positive *S. neurona* IFAT performed on horse serum at 20x as an example for CSL IFAT (Packham 2023, unpublished)

Histopathology

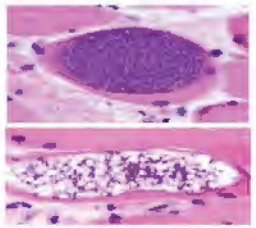


Fig 2. Mature and immature *S. neurona* cyst within a CSL myocyte (Seguel et al. 2016)

Molecular Sequencing

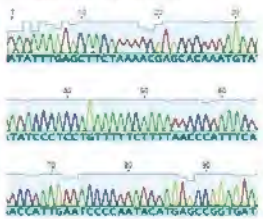


Fig 3. Chromatogram for *S. neurona* ITS1 sequence from CSL (Sinnott 2023, unpublished)

Results

Progress to date

- **20 sea lion cases** and **31 sea lion controls** have been identified. The target sample size is 40 cases and 80 control animals.
- **Cases** are animals with suspected myopathy confirmed by necropsy and evidence of *S. neurona* cysts on histopathology; and will be confirmed with molecular identification.
- **Controls** are animals with a non-protozoal cause of death and no evidence on histopathology of *S. neurona* infection.
- The Kappa statistic on comparing test performance is **0.92**.

IFAT titer to call a sample positive

- **1:320 titer** maximizes sensitivity and specificity at 95 and 96.8%, respectively, with area under the receiver-operating curve of 0.98.

Discussion

Key Findings

- Kappa statistic compares agreement between histopathology and IFAT in classifying a case or control as positive or negative. A value of 0.92 suggests that there is good agreement between the two.
- Preliminary data suggest that an IFAT titer of 1:320 is likely an appropriate threshold for calling a sample positive.

Discussion

- Validation of the low-cost, ante-mortem IFAT diagnostic tool is important due to the increase of sarcocystosis cases in stranded CSL at TMMC and other stranded marine mammal facilities.
- Postulated factors contributing to increased *S. neurona* infections in CSL:
  - Increase in parasite prevalence associated with changing environmental conditions
  - Shift in prey consumption based on altered historic prey availability

Limitations

- Serologic antibody titer does not equal active disease
- Time constraints on TMMC and UCD labs with high-volume case loads
- Financial restraints in studying wildlife diseases

Future Directions

- Risk factors associated with clinical disease and outcome
- Environmental source of infection (e.g. food source, microplastics, etc.) and time until symptom onset in CSL

Acknowledgements

I would like to express immense gratitude for the continued collaboration between The Marine Mammal Center and One Health Institute in addition to Lian Hortensius for providing a patient database. Financial support was provided by Students Training in Advanced Research (STAR) Program through a UC Davis School of Veterinary Medicine Endowment Fund.
Image Credit © The Marine Mammal Center, Smith & Shapiro Lab.

The Marine Mammal Center operates under NOAA permit # 24359

Miller, M.A. et al. 2002. Evaluation of an Indirect Fluorescent Antibody Test (IFAT) for demonstration of antibodies to *Toxoplasma gondii* in the Sea otter (*Enhydra lutris*). J. of Parasitology. 88(3):549-599.

Seguel et al. 2019. Polyphasic Rhabdomyositis in California Sea Lions (*Zalophus Californianus*): Pathology and Potential Causes. Veterinary Pathology. 56(4):619-629.

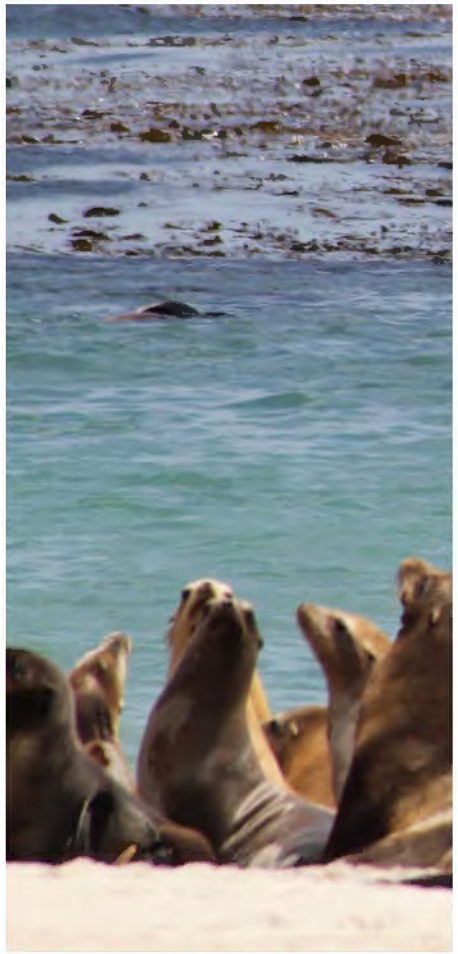
Taylor, A. et al. 2023. Epidemiology and risk factors for *Sarcocystis neurona* and *Toxoplasma gondii* exposure in California sea lions (*Zalophus californianus*) from 2010-2019.

This will help with disease ecology studies: Who is infected and are there hot spots for infection.

The Marine Mammal Center®

HARMFUL ALGAL BLOOMS

Domoic acid intoxication



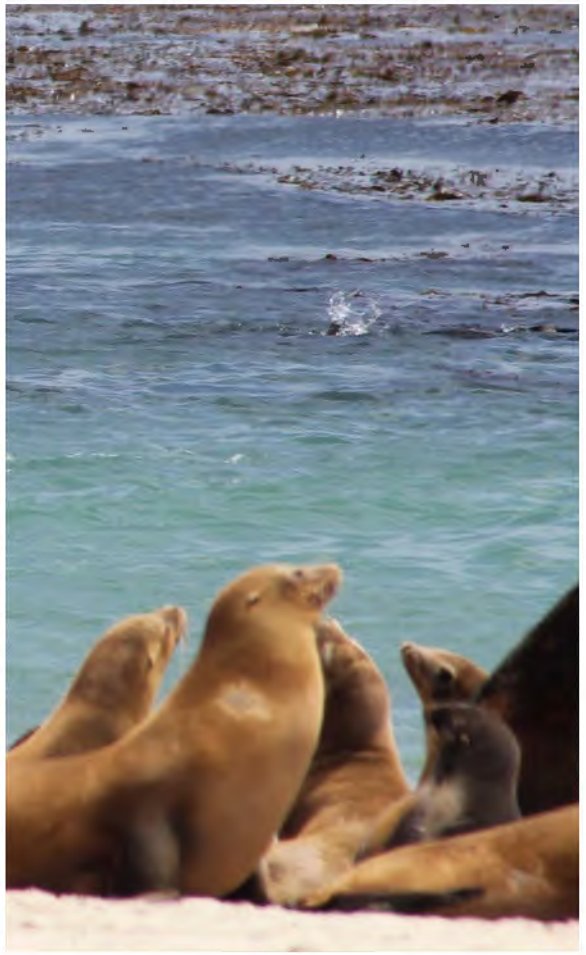


Photo Credit Tony Orr; NOAA Permit 16807

kpbs

KPBS
All Things Considered
NEXT UP:

Local

Toxic algae hitting sea lions hard along Southern California coast

By Erik Anderson / Environment Reporter
Contributors: Carlos Castillo / Video Journalist
Published July 6, 2023 at 6:16 PM PDT

LISTEN • 0:56

The Marine Mammal Center.

Los Angeles Times

SUBSCRIBE

CALIFORNIA

What's killing hundreds of sea lions and dozens of dolphins along the Southern California coast?

Sea lions recuperate at the Marine Mammal Care Center in San Pedro on Tuesday. More than 1,000 marine mammals have become ill or died this month because of toxic algae blooms along the coast of Southern California, according to the National Oceanic and Atmospheric Administration. (Luis Sinco / Los Angeles Times)

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DA intoxication evolving epidemiology

- First diagnosed as a cause of mass mortality in California sea lions in 1998 (Scholin et al. 2000).
- Retrospective research identified 2 likely earlier events in sea lions in1991 and ‘92 (Greig et al 2005).
- For 2 decades (90s & 2000’s) Harmful Algal Bloom (HAB) events were sporadic and usually in summer & early autumn.
- Since 2014, toxic blooms causing mortality have occurred annually and over extended geographic and temporal scale.
- Related to climate and marine thermal anomalies?

Domoic Acid Intoxication

The Marine Mammal Center

A Decade of Live California Sea Lion (*Zalophus californianus*) Strandings Along the Central California Coast: Causes and Trends, 1991-2000

Denise J. Greig,[1] Frances M. D. Gulland,[1] and Christine Kreuder[2]

[1]*The Marine Mammal Center, Marin Headlands, 1065 Fort Cronkhite, Sausalito, CA 94965, USA*
[2]*The Wildlife Health Center, School of Veterinary Medicine, University of California, Davis, CA 95616, USA*

Aquatic Mammals 2005, *31*(1), 11-22, DOI 10.1578/AM.31.1.2005.11

Vet Pathol 42:184–191 (2005)

Harmful Algae 9 (2010) 374–383

Contents lists available at ScienceDirect

Harmful Algae

journal home page: www.elsevier.com/locate/hal

Clinical signs and histopathology associated with domoic acid poisoning in northern fur seals (*Callorhinus ursinus*) and comparison of toxin detection methods

Kathi A. Lefebvre[a,*], Alison Robertson[a], Elizabeth R. Frame[a], Kathleen M. Colegrove[b], Shelly Nance[a], Keri A. Baugh[a], Heather Wiedenhoft[a], Frances M.D. Gulland[c]

[a] NOAA Fisheries, Northwest Fisheries Science Center, Marine Biotoxins Program, 2725 Montlake Blvd. East, Seattle, WA 98112, USA
[b] Zoological Pathology Program, University of Illinois College of Veterinary Medicine, LUMC, Bld. 101, Rm 0745, 2160 S. First Ave., Maywood, IL 60153, USA
[c] The Marine Mammal Center, Marin Headlands, 2000 Bunker Road, Fort Cronkhite, Sausalito, CA 94965, USA

Vet Pathol 46:105–119 (2009)

Pathology of Domoic Acid Toxicity in California Sea Lions (*Zalophus californianus*)

P. A. Silvagni, L. J. Lowenstine, T. Spraker, T. P. Lipscomb, and F. M. D. Gulland

Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California Davis, Davis, CA (PAS,[1] LJL); Colle
Department of Veterinary Pathology, ,
Mam

frontiers in Marine Science

ORIGINAL RESEARCH
published: 26 May 2021
doi: 10.3389/fmars.2021.585501

Characterization of a Degenerative Cardiomyopathy Associated with Domoic Acid Toxicity in California Sea Lions (*Zalophus californianus*)

Zabka, T. Goldstein, C. Cross, R. W. Mueller, C. Kreuder-Johnson, S. Gill, and F. M. D. Gulland

rine Mammal Center, GGNRA, Sausalito, CA (FMDG, TG, TSZ); The Wildlife Health Center, sity of California-Davis, Davis, CA (TSZ, TG, CK); Center for Wildlife Health, University of ssee, Knoxville, TN (CC); and Banting Research Center, Tunney's Pasture, Health Canada, Ottawa, Ontario, Canada (RWM, SG)

ELSEVIER

Domoic acid exposure a Pacific harbor seals (*Phc*

Elizabeth A. McHuron[a], Denise
Terry R. Spraker[e], Frances M.D

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[b] The Marine Mammal Center, 2000 Bunker Road, Fo
[c] Zoological Pathology Program, College of Veterinary
[d] New Hampshire Veterinary Diagnostic Lab, Univers
[e] Department of Microbiology, Immunology and Path
[f] Environmental Conservation Division, Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Blvd. East, Seattle, WA 98112, USA

Clinical Signs and Pathology Associated With Domoic Acid Toxicosis in Southern Sea Otters (*Enhydra lutris nereis*)

OPEN ACCESS

Edited by:
Debra Lee Miller,
The University of Tennessee,

Melissa A. Miller[1,2], Megan E. Moriarty[1,2], Pádraig J. Duignan[2,3], Tanja S. Zabka[4], Erin Dodd[1], Francesca I. Batac[1], Colleen Young[1], Angelina Reed[1], Michael D. Harris[1], Katherine Greenwald[1], Raphael M. Kudela[5], Michael J. Murray[6], Frances M. D. Gulland[2], Peter E. Miller[7†], Kendra Hayashi[5], Catherine T. Gunther-Harrington[8], Martin T. Tinker[9] and Sharon Toy-Choutka[1]

pal Neuropathology of Domoic Acid–pilepsy in California Sea Lions (*Zalophus us*)

1,2* Xiling Wen,[1] Izumi Toyoda,[1] Frances M.D. Gulland,[3] and William Van Bonn[3]

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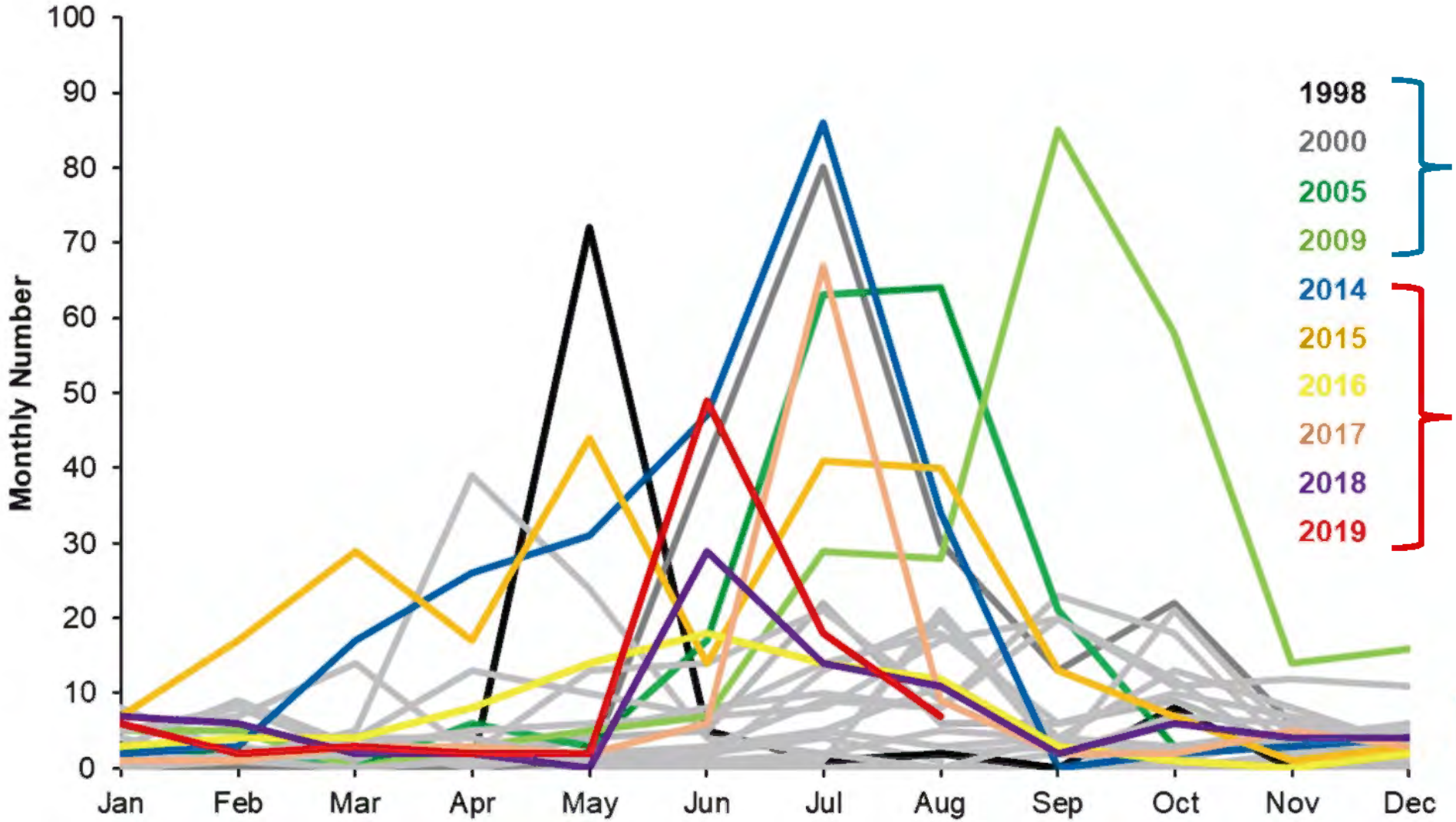
The Journal of Comparative Neurology | Research in Systems Neuroscience 522:1691–1706 (2014)

DA diagnosed in CSLs at TMMC 1998-2019

The Marine Mammal Center



When first recognized, outbreaks occurred irregularly and not every year. Usually summer or autumn

Outbreaks now occur every year and may be seen in any month. Particularly common in Blob years (2014-'19) and El Niño years (2023).

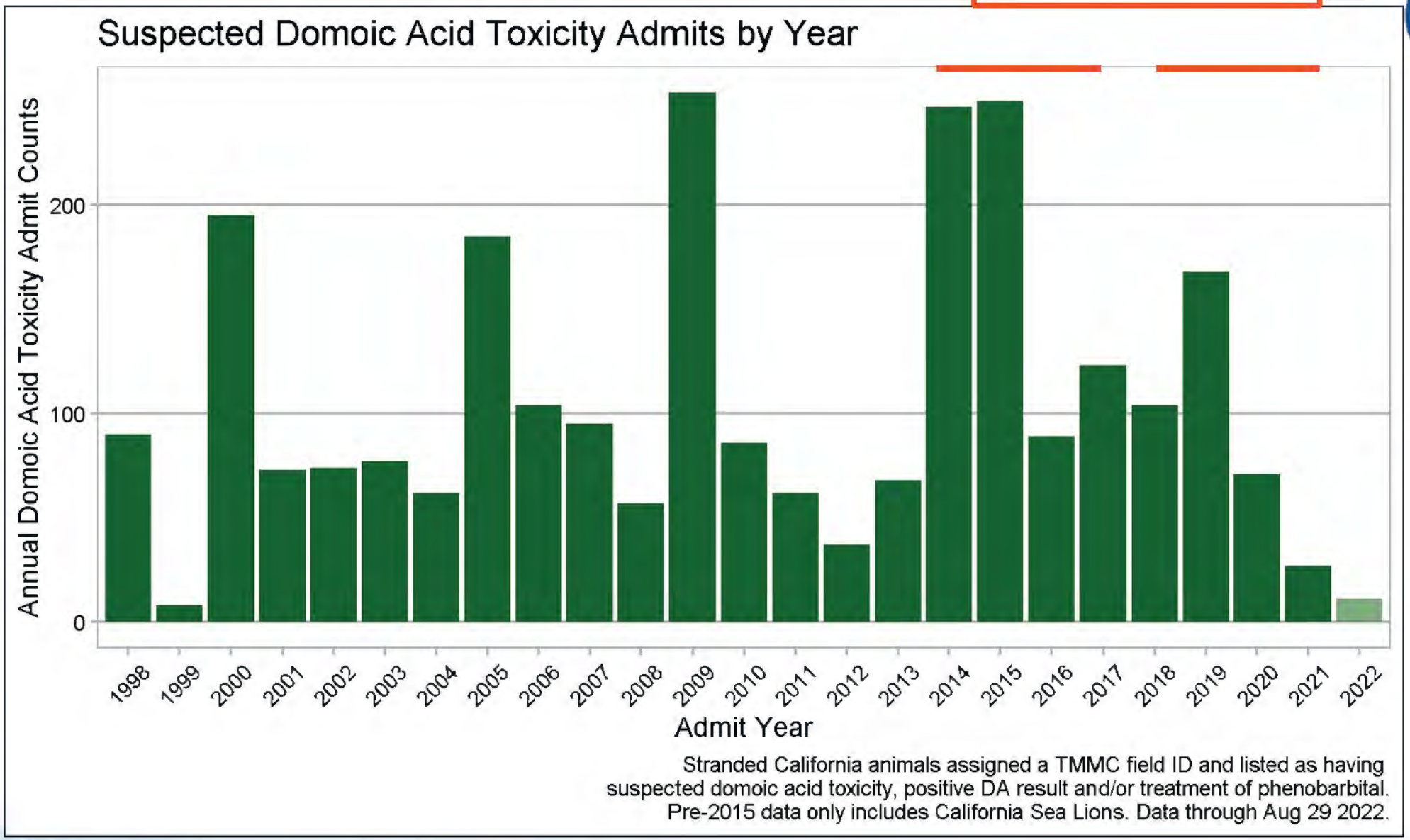
SCCOOS

New SCCOOS & CeNCOOS Harmful Algal Bloom Monitoring page!

Check out the new and improved Harmful Algal Bloom Monitoring Alert Program page that reviews SCCOOS and CeNCOOS weekly monitoring effort at nine university-run or municipal pier stations along California's coast for a suite of HAB species and for the neurotoxin domoic acid (DA) caused by the Harmful Algal Bloom (HAB) producing diatom *Pseudo-nitzschia*. The new page also includes an interactive plotting tool for the recorded observations at each site.

Thermal Anomalies

The Marine Mammal Center.

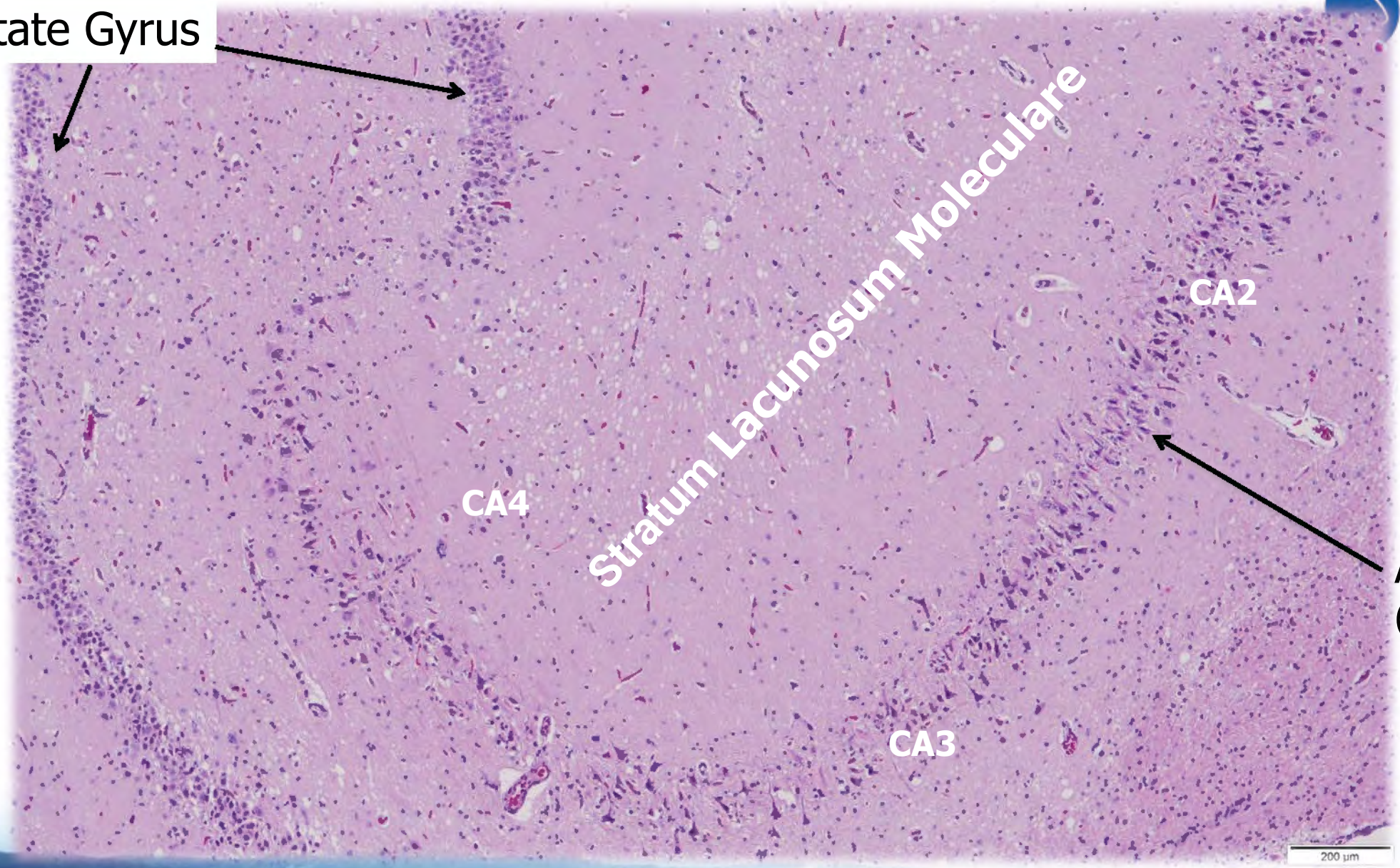


DA Pathology

- Biotoxin produced by *Pseudo-nitzschia* sp. diatoms
- Analog of the neurotransmitter glutamate that exerts potent excitatory activity in the brain, heart, and other tissues
- Causes neuronal necrosis and vacuolation in the limbic system: Olfactory tracts, amygdala, hippocampus, parahippocampus.
- Conduction system of the heart – cardiomyopathy.

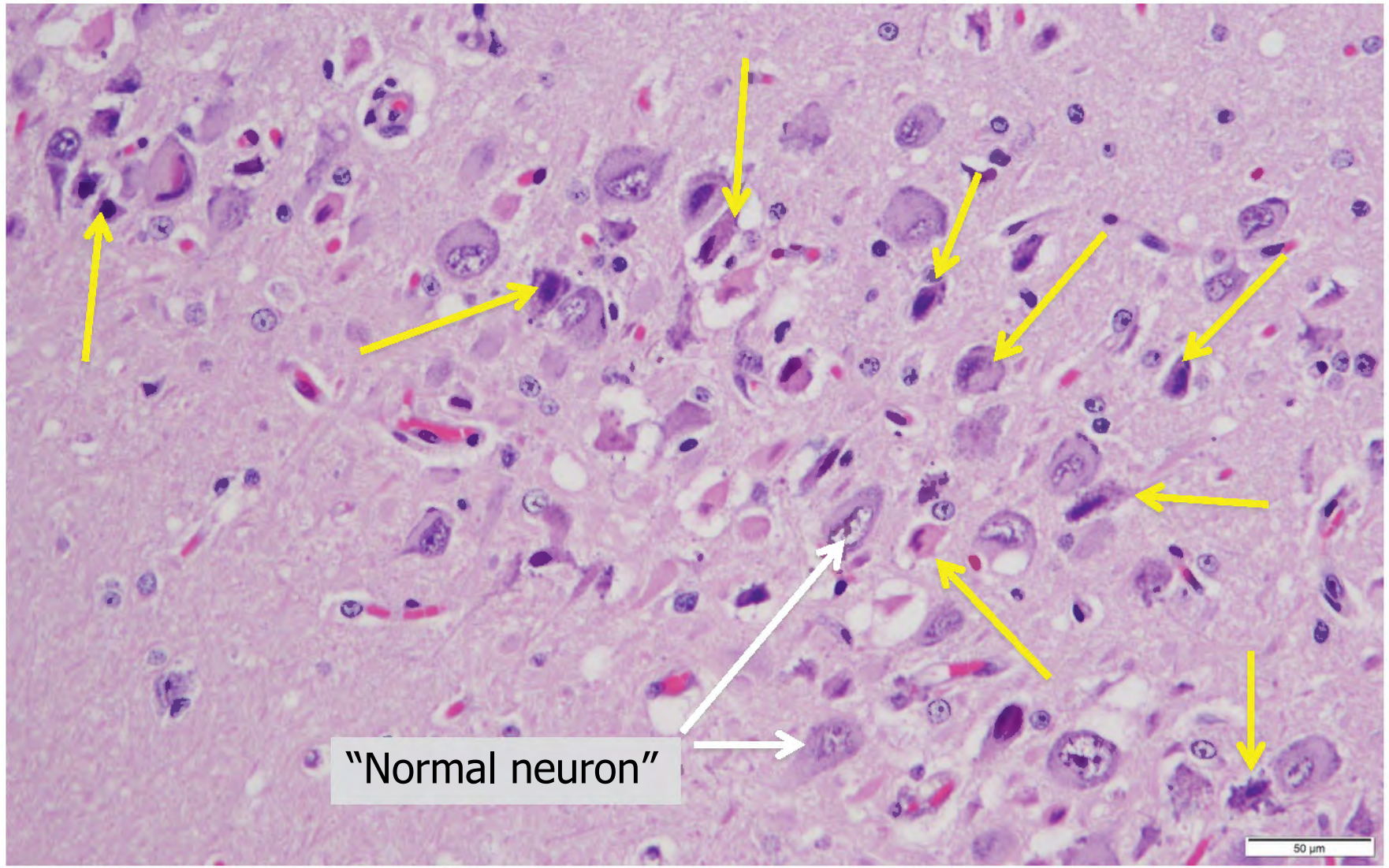
Sea Otter: Brain (hippocampus) “Acute DA”

The Marine Mammal Center.



Ammon’s Horn
(Cornu Ammonis)

Sea Otter: Brain - hippocampus



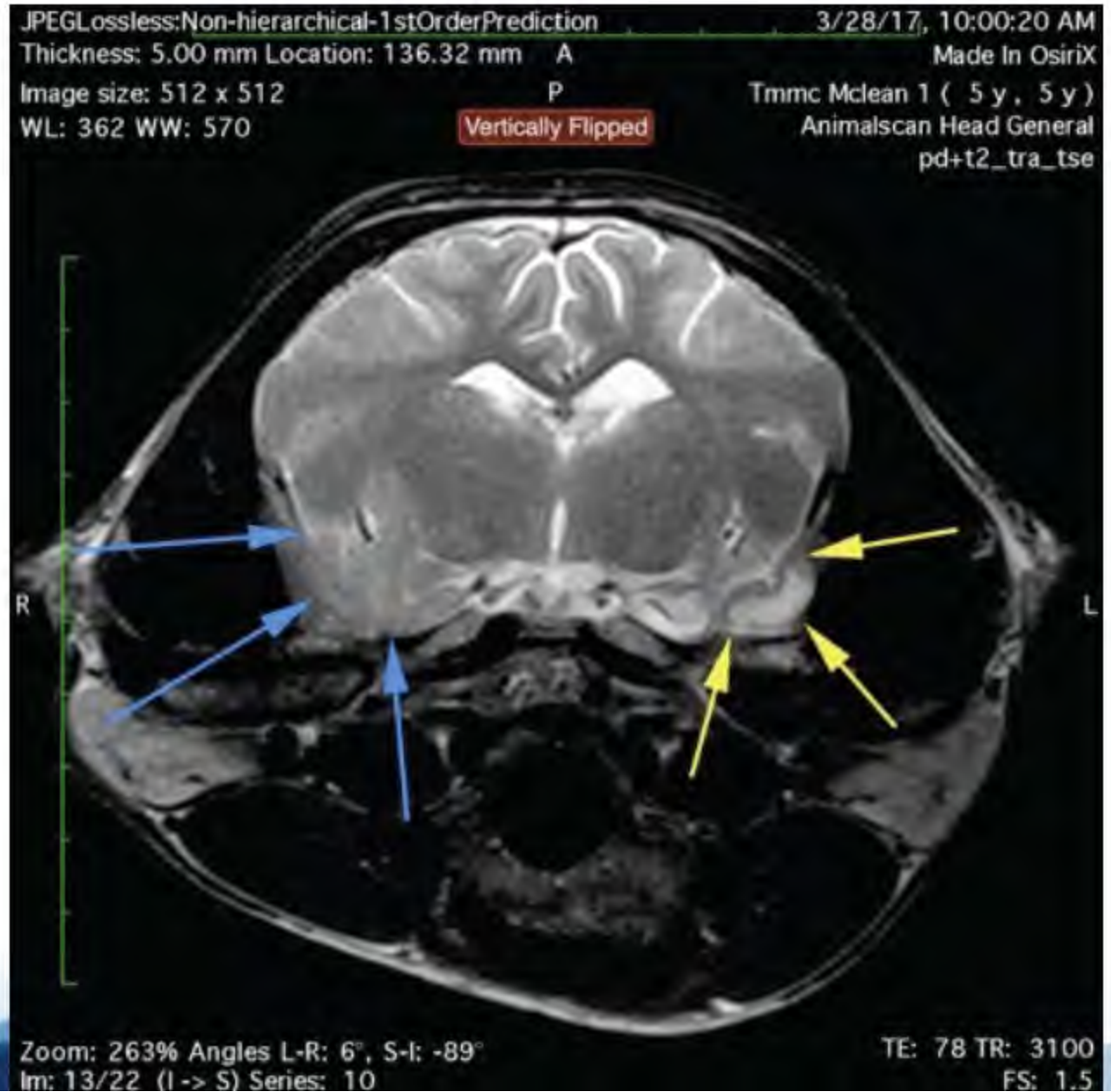
High power view of necrotic neurons in Ammon's Horn CA4

Adult Female Guadalupe Furseal (Chronic DA)

The Marine Mammal Center

Right

Normal

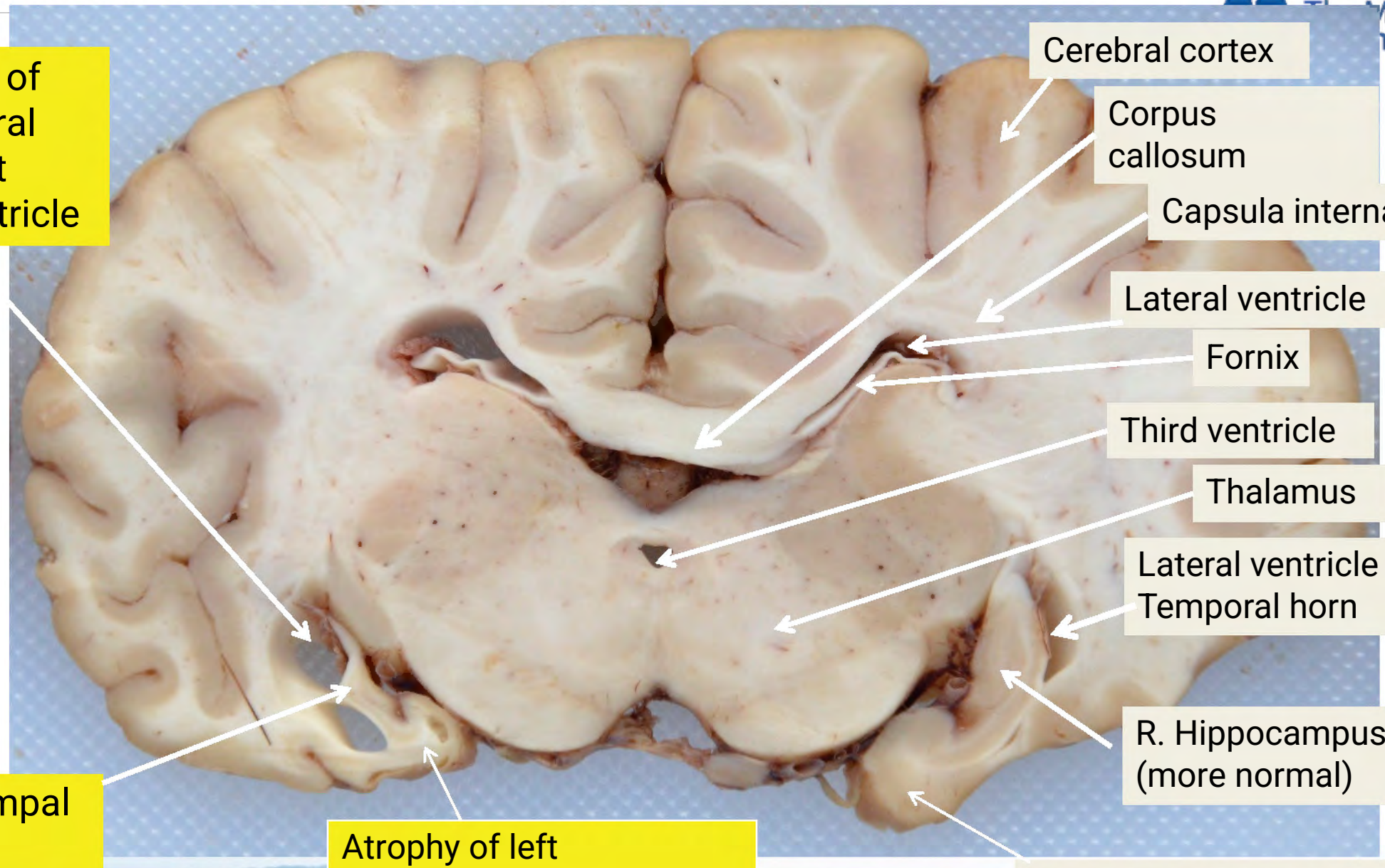


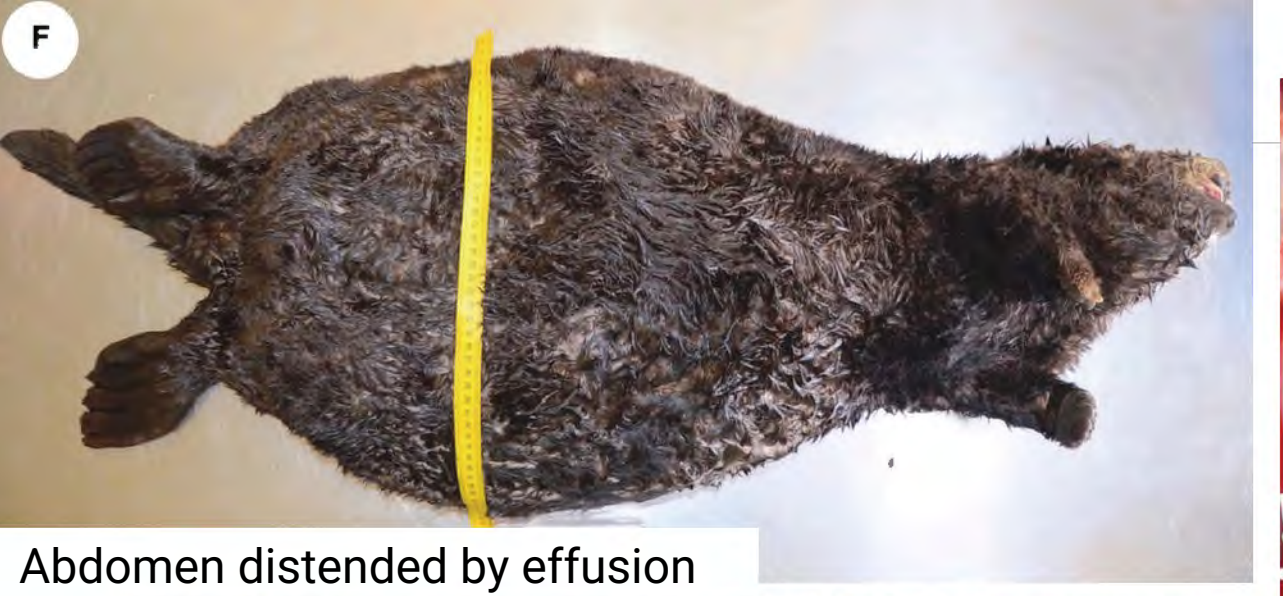
Left

Yellow arrows show atrophy of the left temporal lobe relative to the right (blue arrows)

MRI by Dr Sophie Dennison

GFS: Chronic DA, brain section through hippocampus

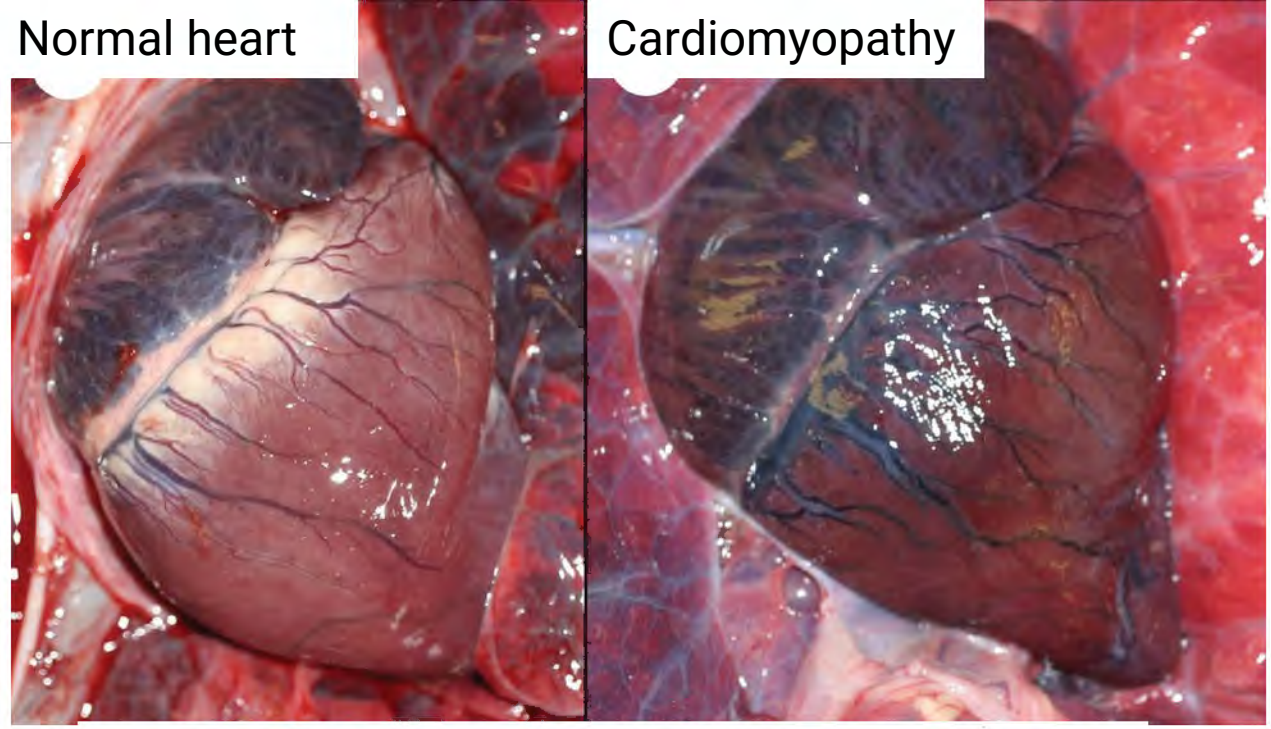




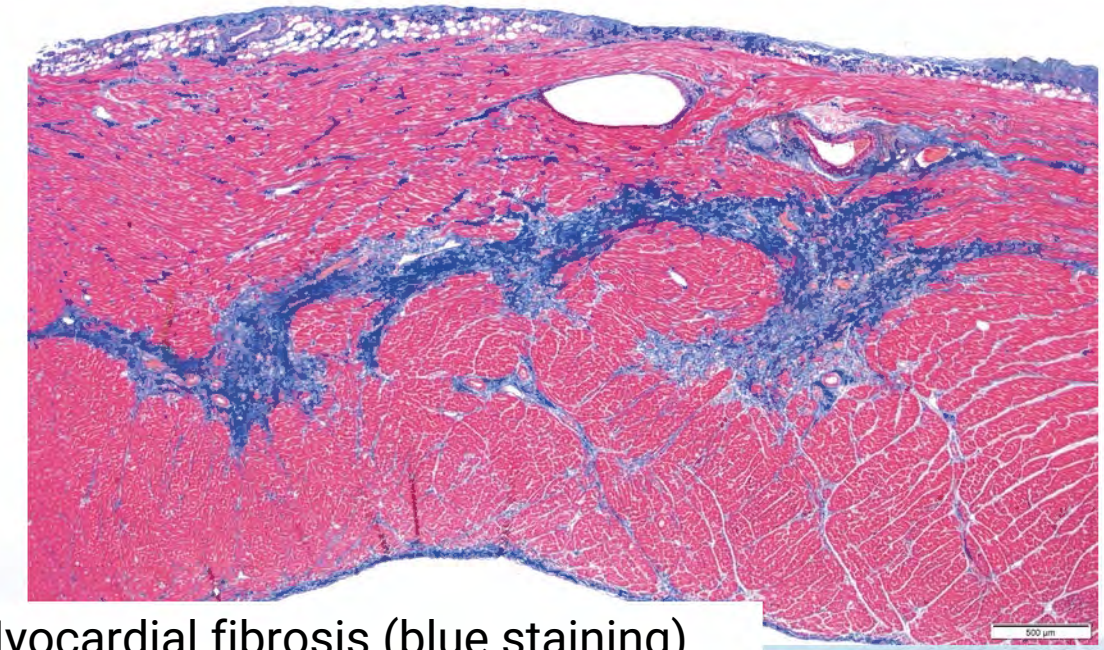
Abdomen distended by effusion

Normal heart

Cardiomyopathy



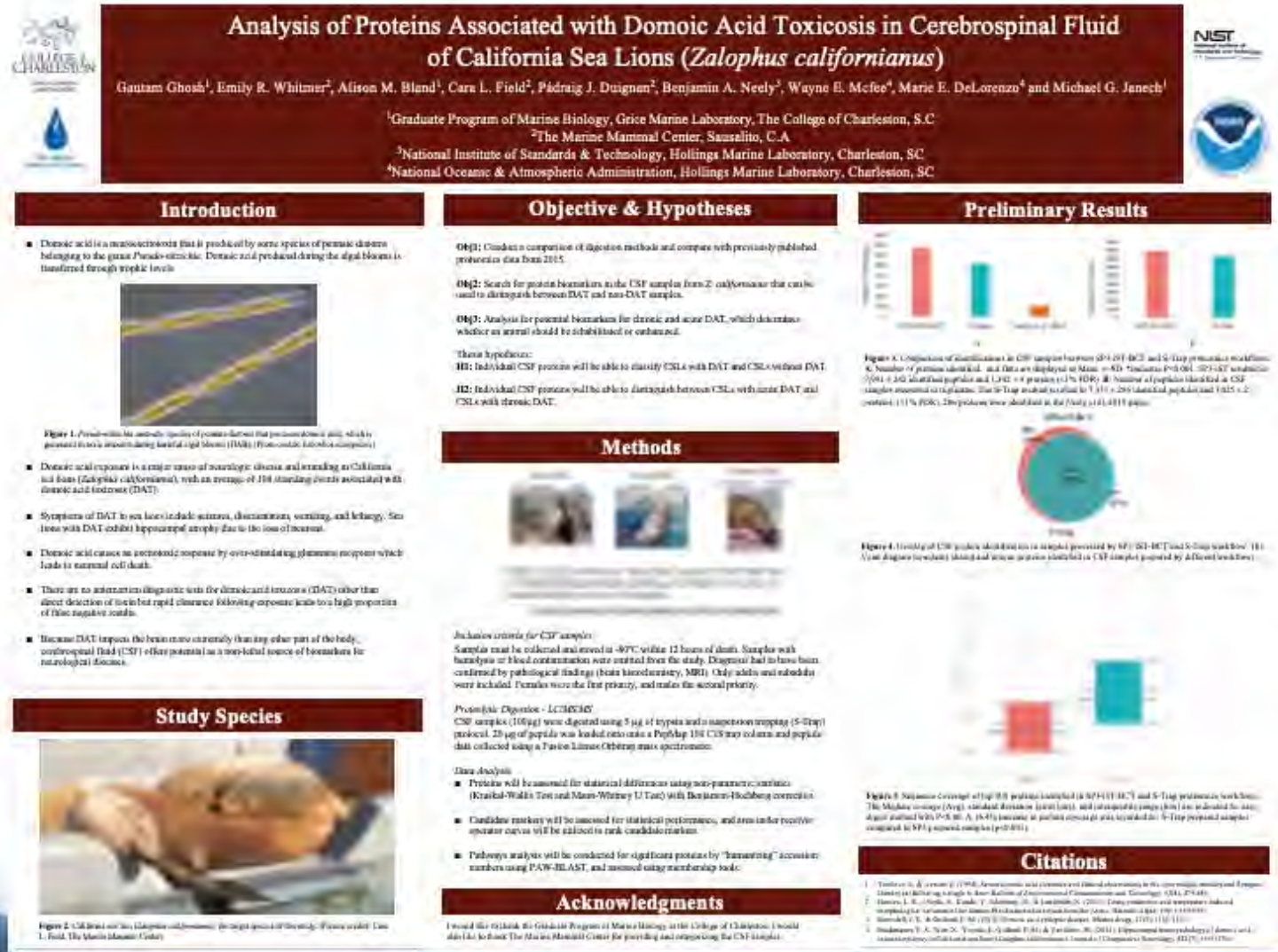
Pale rounded heart and edematous lungs



Myocardial fibrosis (blue staining)

Recent Project:Can proteomics be used to diagnose acute toxicosis?

Collaborative study with Grice Marine Laboratory, NIST, NOAA and TMMC.
Gautam Ghosh recently defended MSc thesis



Analysis of Proteins Associated with Domoic Acid Toxicosis in Cerebrospinal Fluid of California Sea Lions (*Zalophus californianus*)

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Introduction

Objective & Hypotheses

Preliminary Results

Study Species

Methods

Citations

Acknowledgments

Selection criteria based on CSL brain histopathology

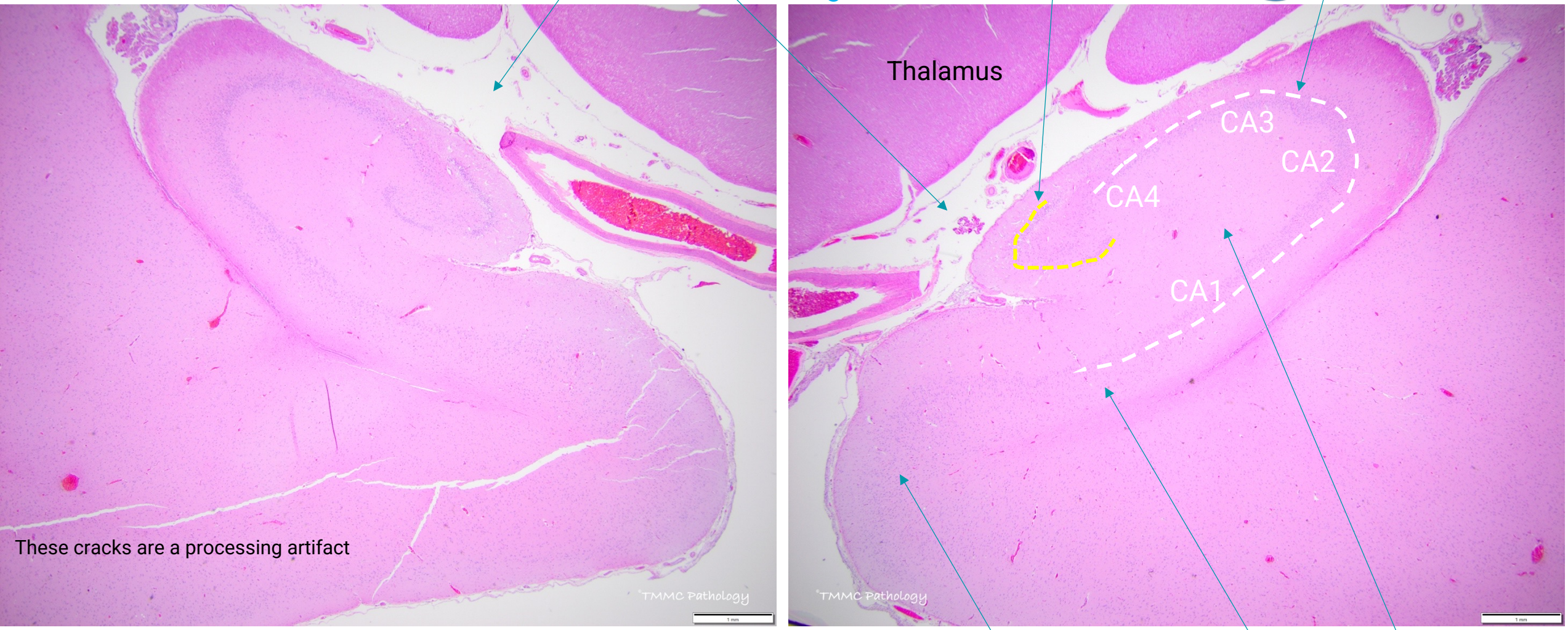
- Acute neuronal necrosis without evidence of past exposure “acute DA”
- “Chronic DA” – atrophied hippocampal complex with sclerosis but no ongoing necrosis or inflammation.
- “Acute on chronic” pre-existing hippocampal atrophy with acute neuronal necrosis.

CSL-14884 Cringles

Normal hippocampal complex

Left side

Right side

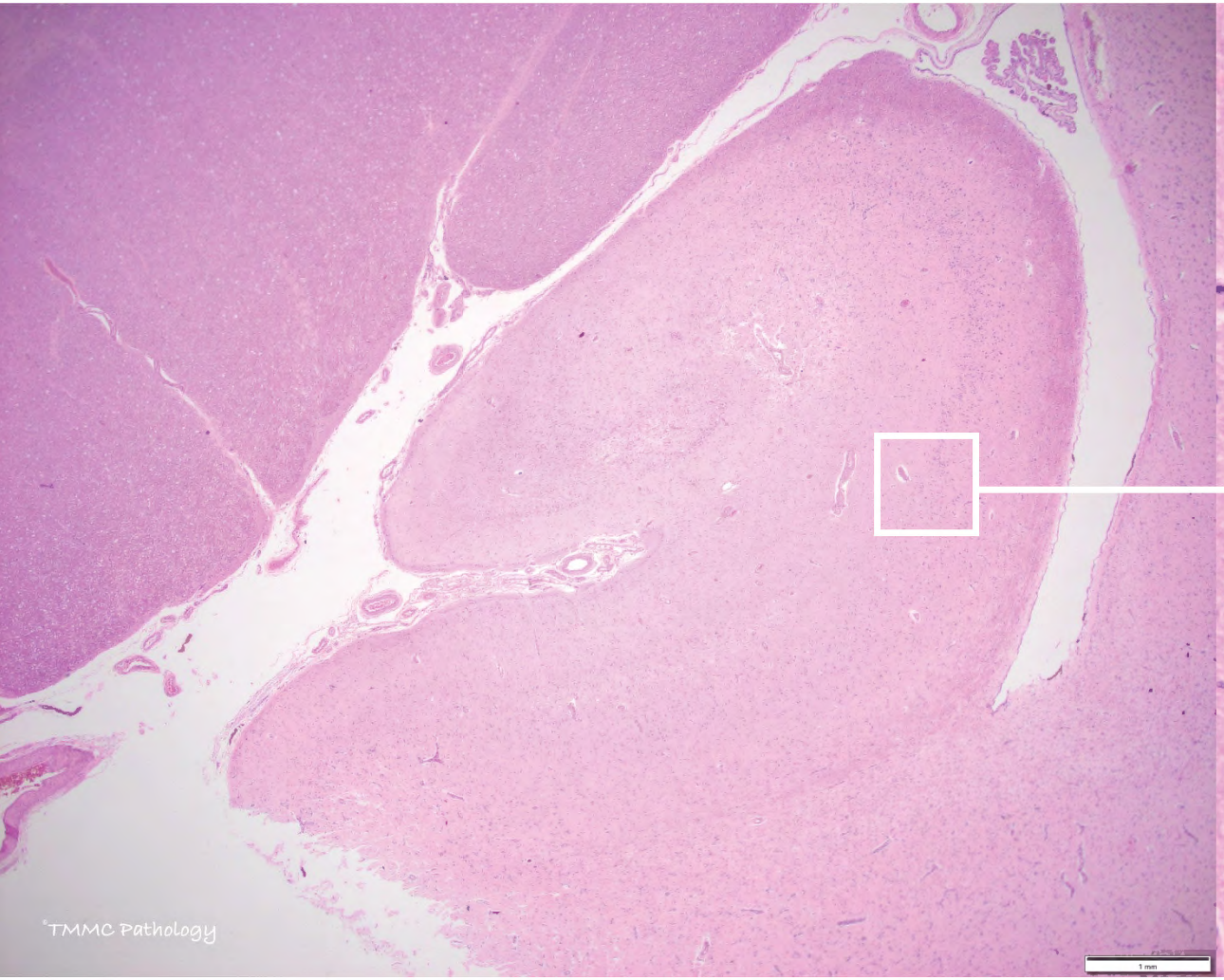


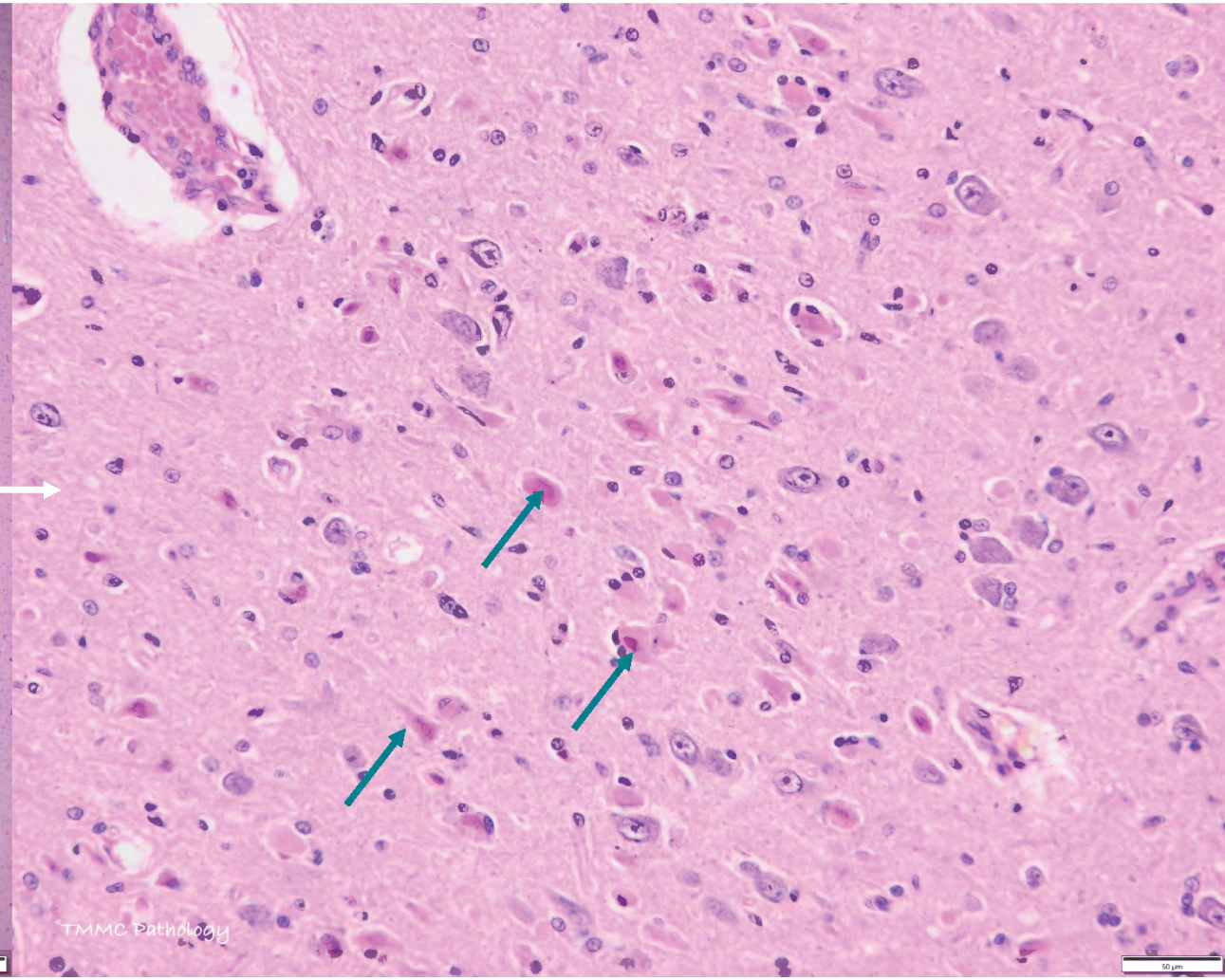
Note the clearly defined S shape to the hippocampal complex.
The size of the lateral ventricle surrounding it.
The clearly visible neurons of the CA and DG

CSL-14435 Cowbell

Acute DA toxicosis

Right hippocampal complex





At low power (12.5x) there is very little change from the normal structure.

High power (200x) of the CA1 segment of Ammon's horn shows large number of acutely necrotic neurons (bright pink staining cells).

CSL- 14827 Antares

Chronic DA hippocampal atrophy: “mild” lesion

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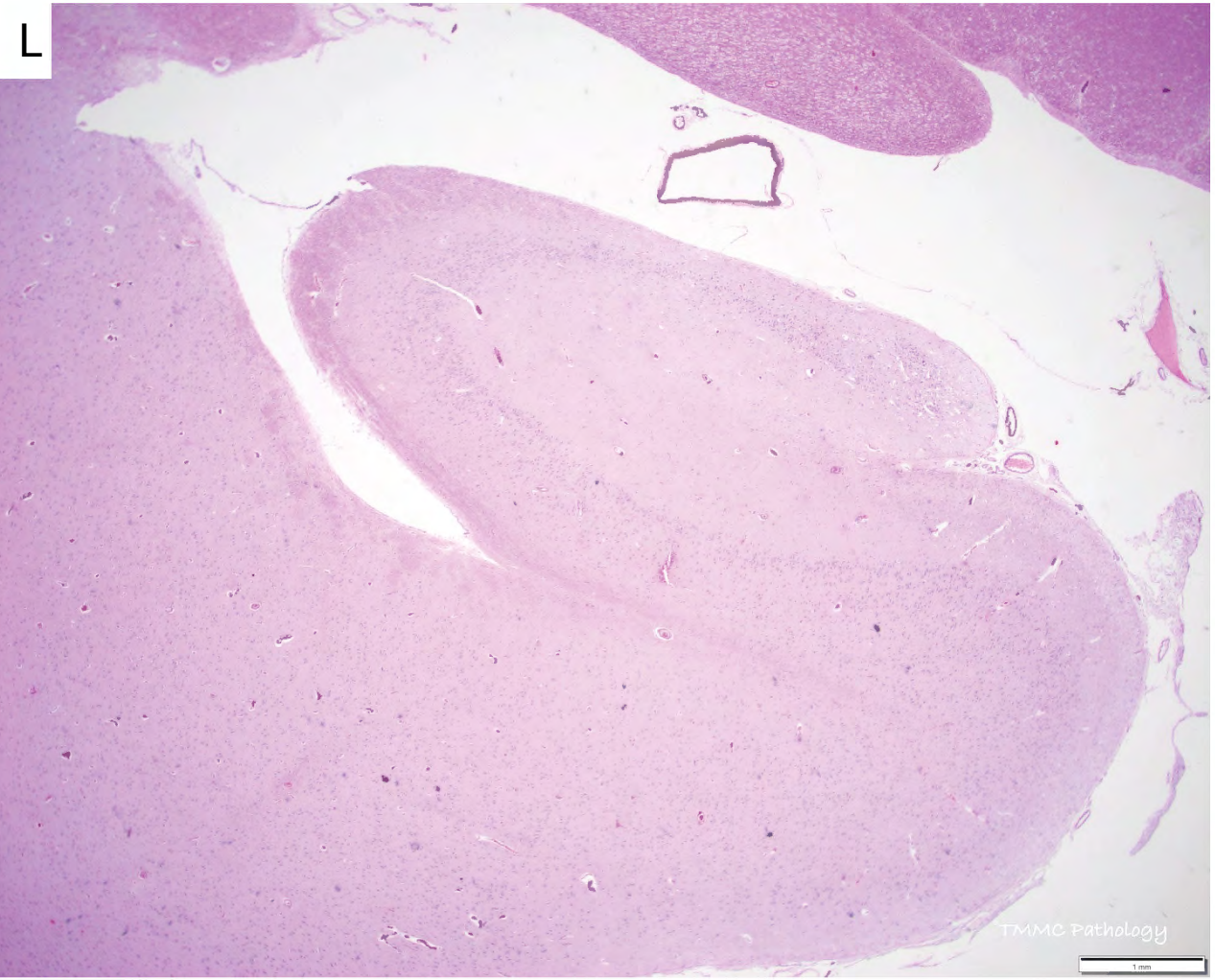
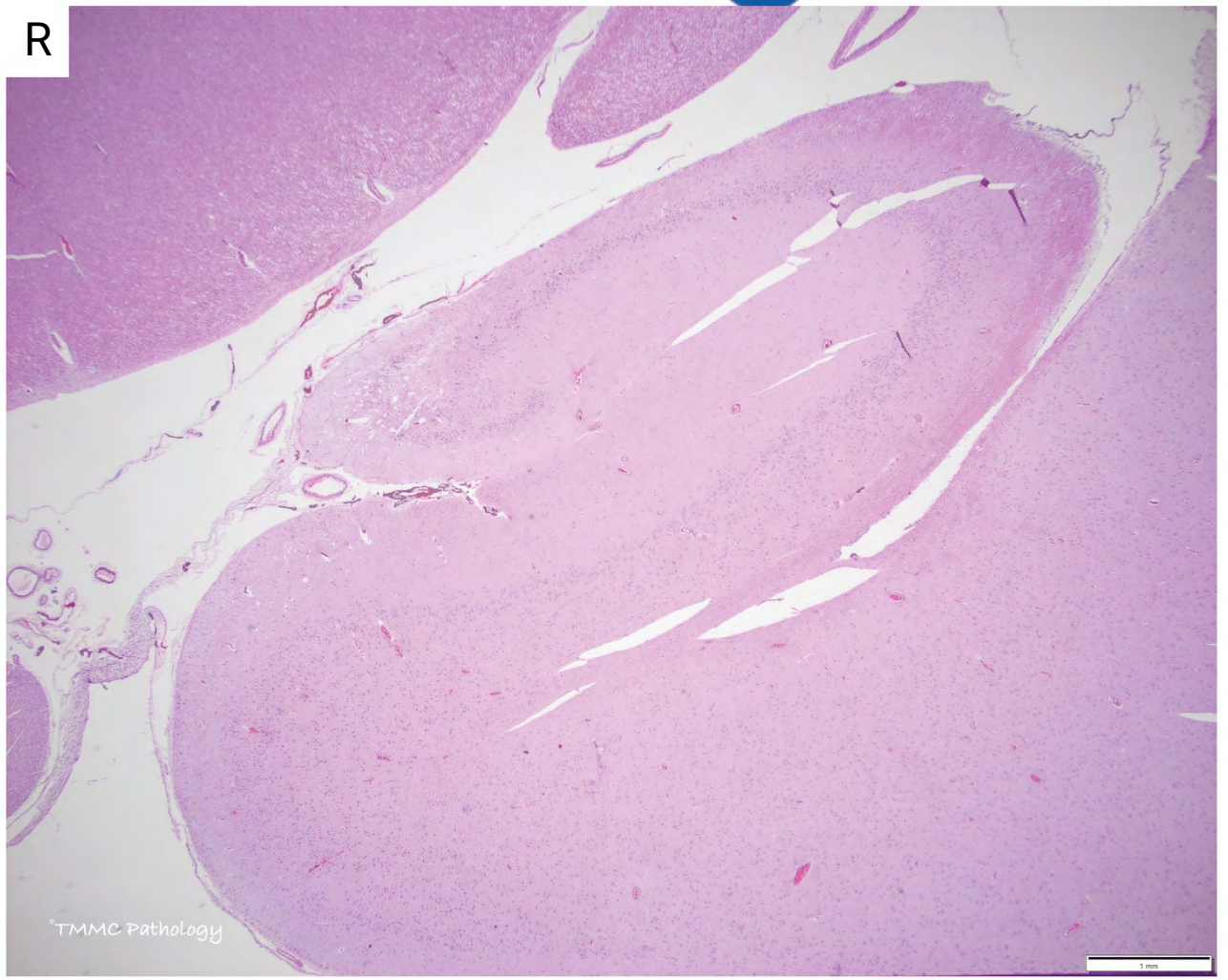


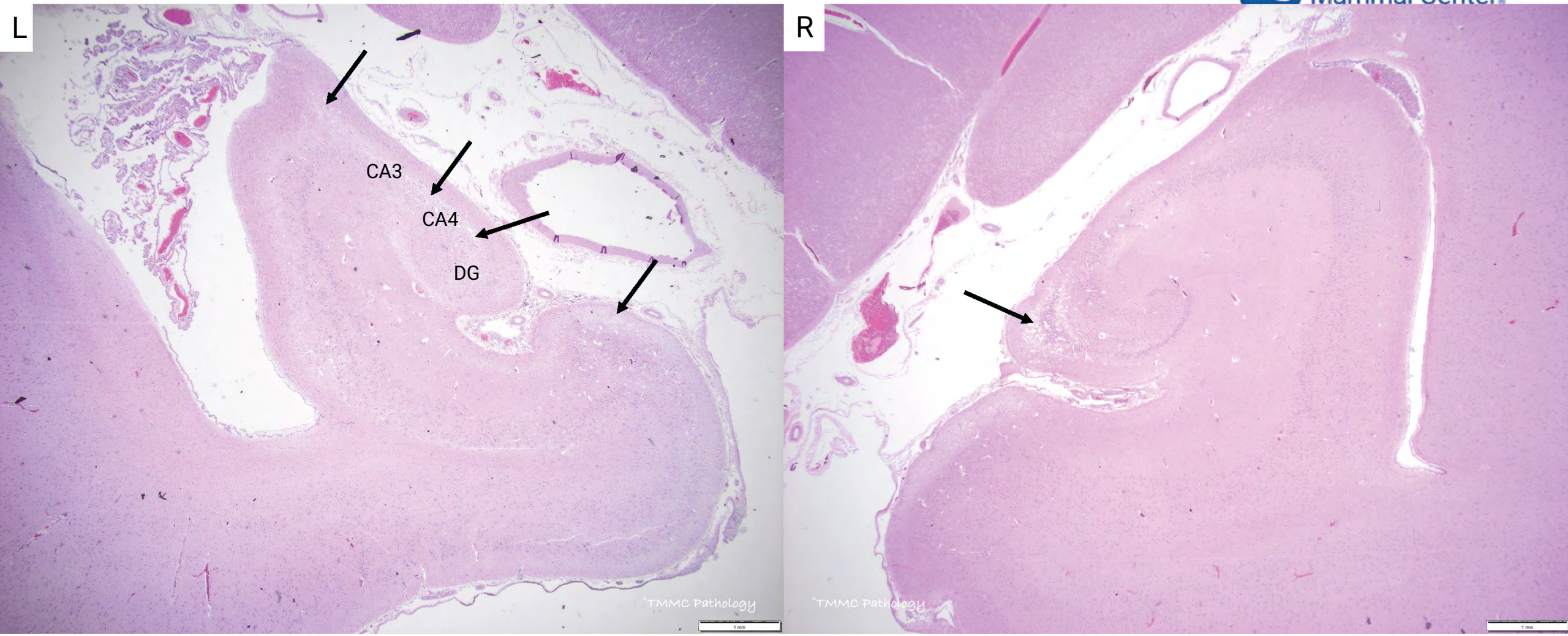


Note that the hippocampal complex is contracted and the lateral ventricle space is relatively expanded
The lesion is asymmetric and slightly more marked on the left
It is less apparent at this low magnification but there are fewer neurons in the CA and DG

CSL-14861 Lamora

Chronic DA hippocampal atrophy: “Marked” lesion

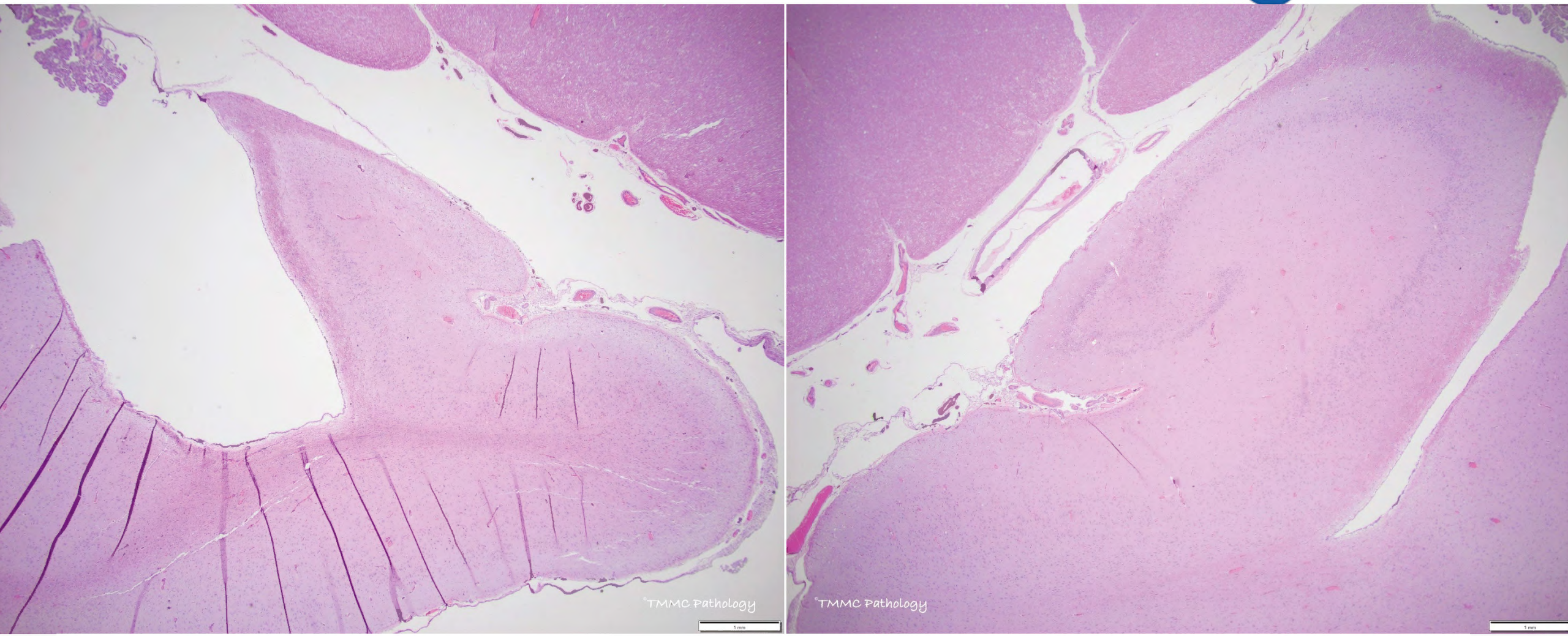
The Marine Mammal Center



Asymmetric atrophy is much more pronounced on the left with contraction and distortion of the hippocampal complex
Note the laminar pale areas of vacuolation (arrows) and the loss of neurons from CA3, CA4 and the DG particularly on the left

CSL-14855 Protector

Chronic DA hippocampal atrophy: “severe” lesion



Asymmetric hippocampal atrophy with severe lesion on the left but only a mild/insignificant change on the right.
On the left there is complete loss of CA2, CA3, CA4 and the dentate gyrus. On the right, just mild loss of neurons from CA3 & CA4.
The remaining structure on the left is severely contracted with relative expansion of the lateral ventricle

CSL-14657 Melli

Acute on chronic DA toxicosis

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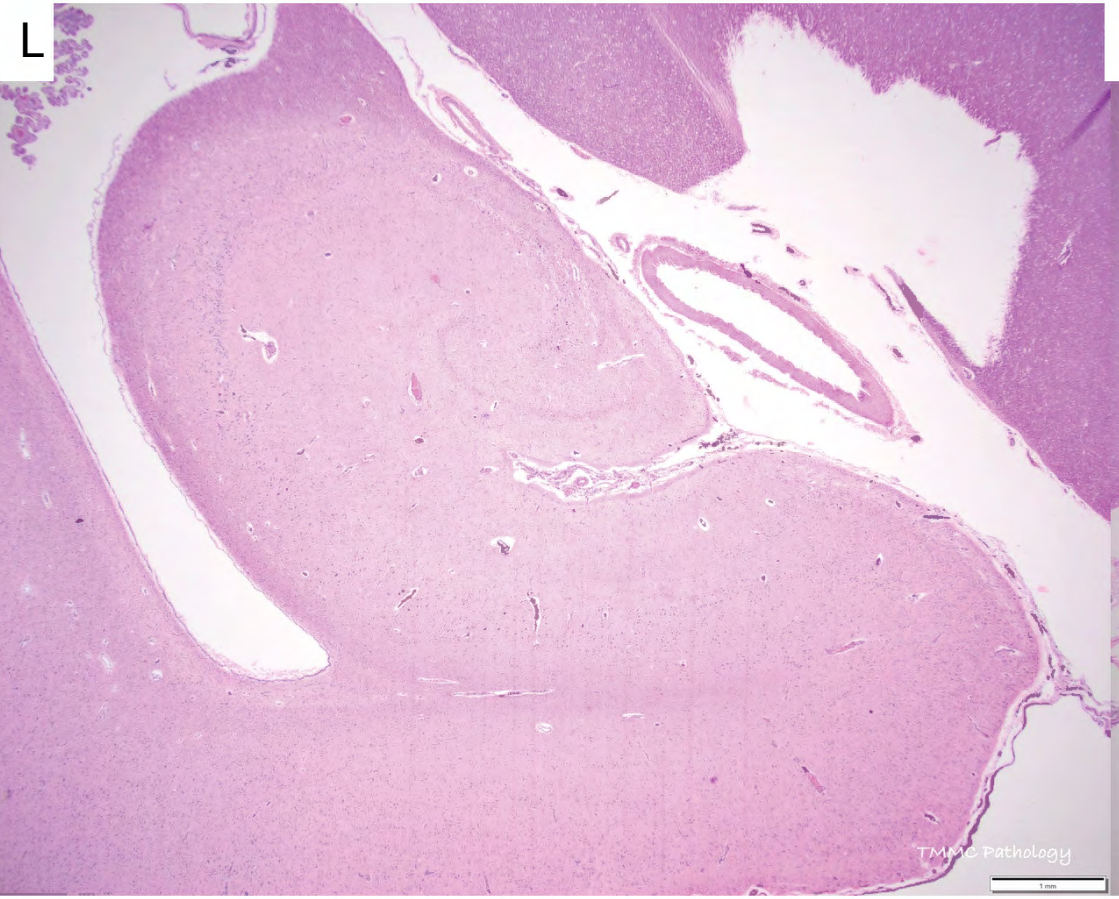
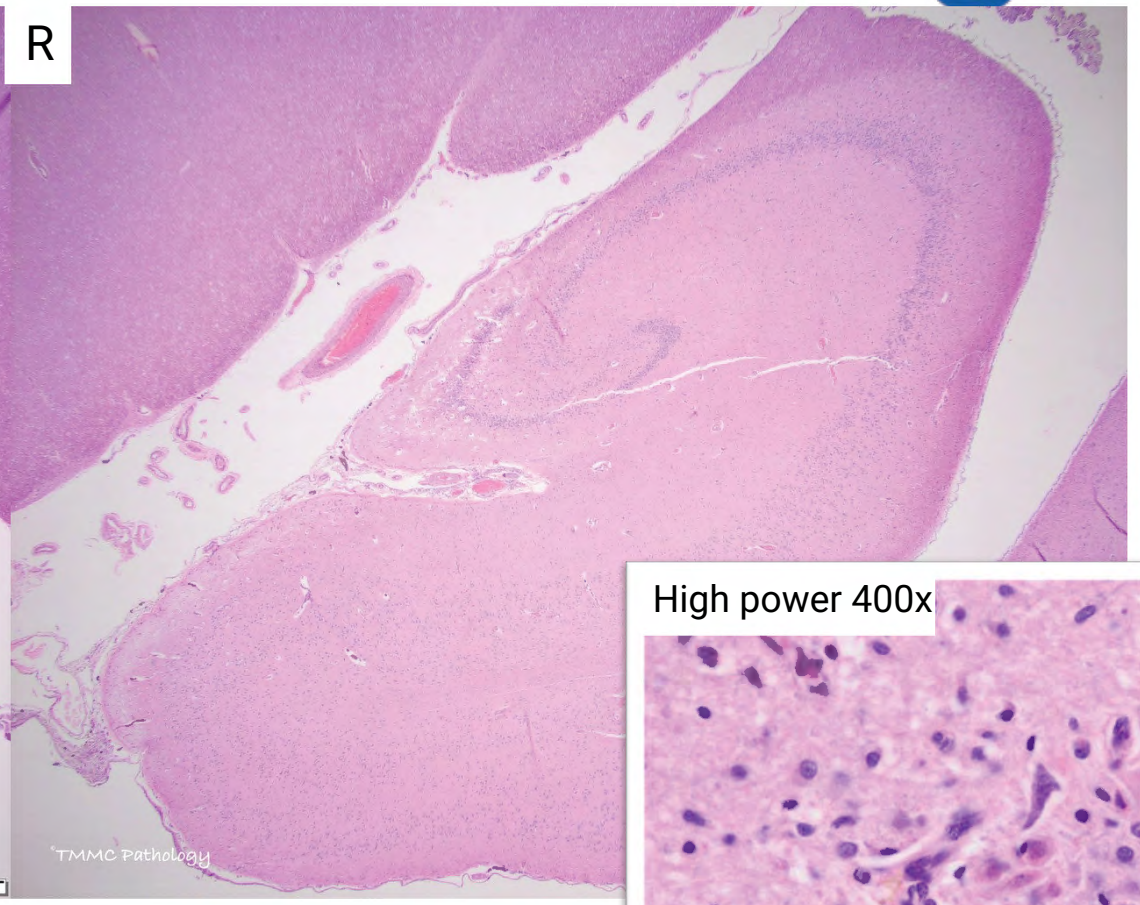
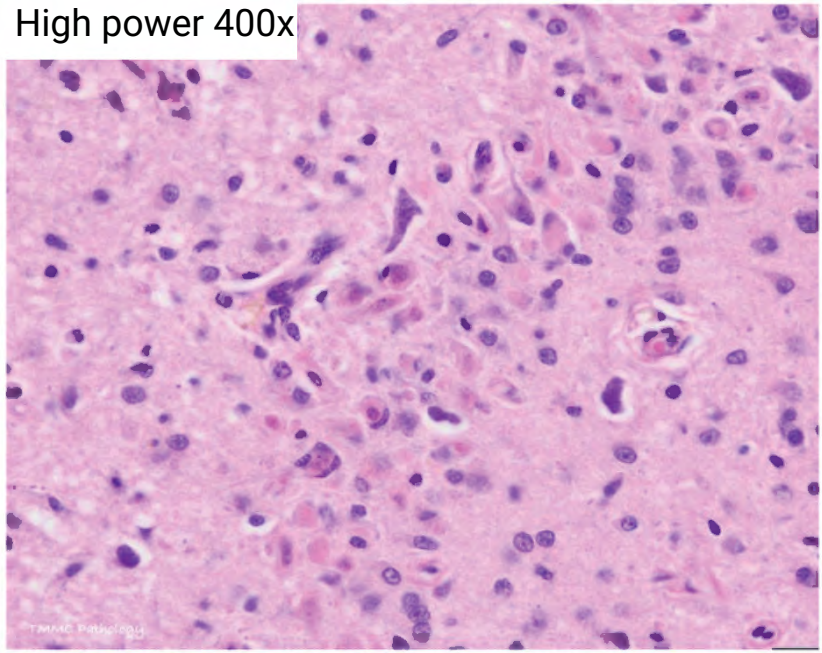
L



R



High power 400x



Note the symmetric atrophy of the hippocampus (expanded clear space around it). This is the chronic component.

CA3, CA4 and DG on the left are also pale from loss of neurons.

On high power examination of Ammon's Horn (right image) note all the red dead neurons (acute lesion)

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FLUOROSIS

New Toxicosis in California Sea Lions (as if they needed it!)

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CSL Cringles

- Subadult (4-8 years) male California sea lion (*Zalophus californianus*).
- Stranded at Ft Bragg with in poor body condition and ambulatory difficulties.
    - Bone proliferation detected on humerus by radiography
- Despite 3 weeks of care, he became anorexic, lethargic and reluctant to move so euthanasia carried out. DDX *Sarcocystis* polyphasic rhabdomyositis
- Three more carcasses recovered from same area of Mendocino County.

Necropsy Findings- Humerus

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Necropsy Findings

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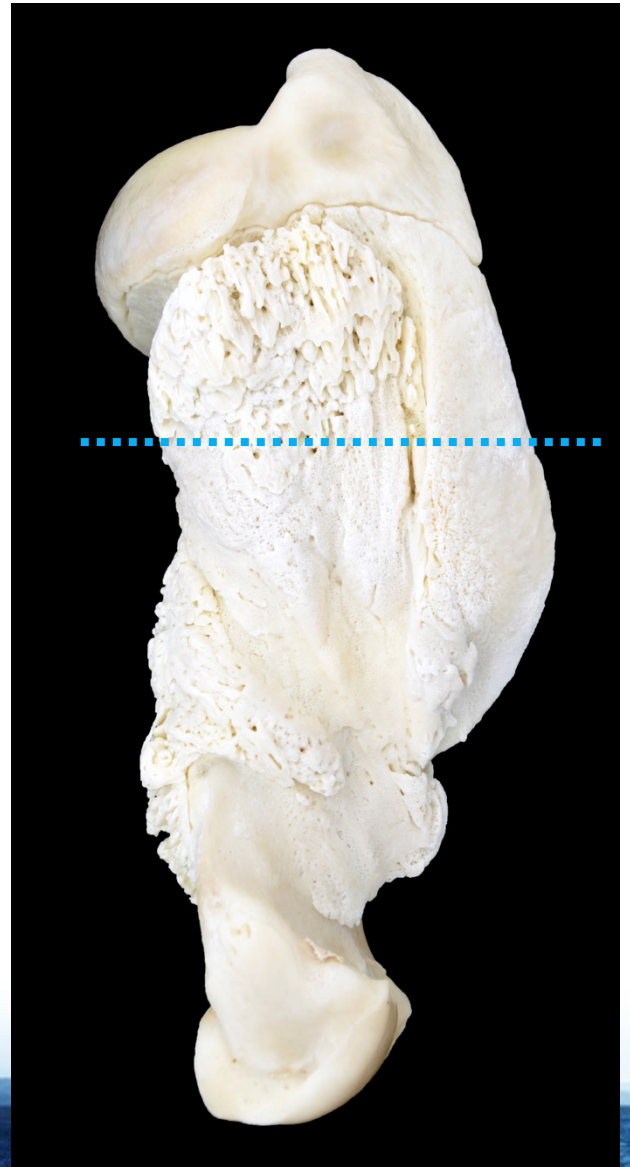
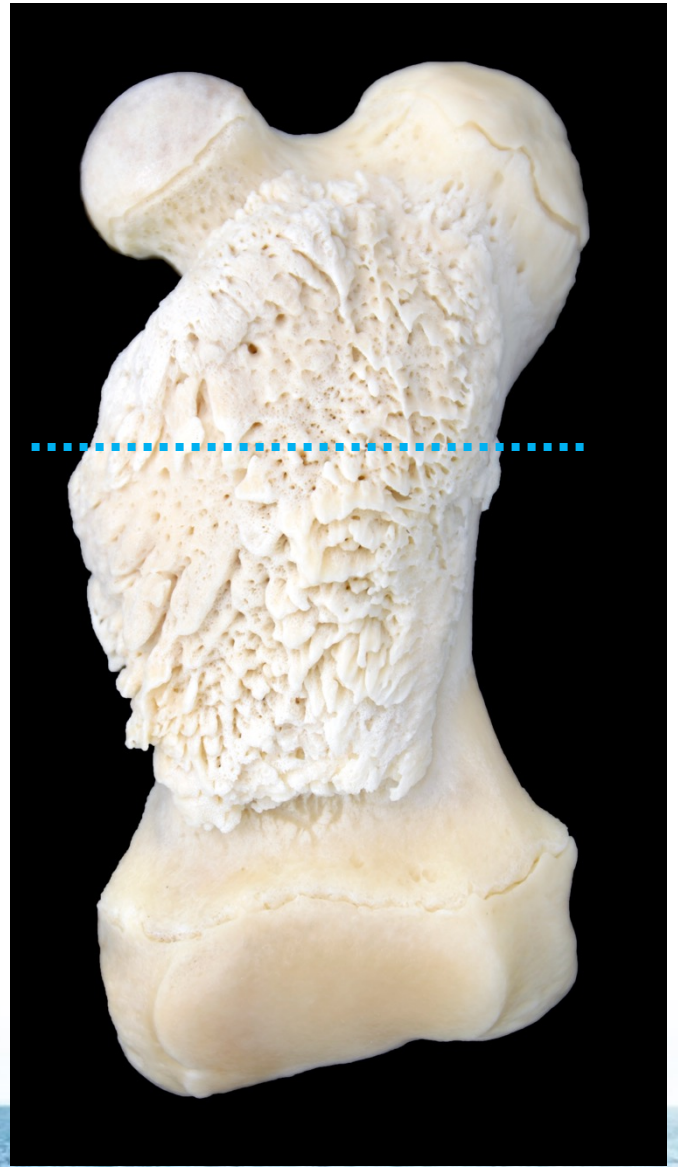


Humerus



Femur

Necropsy Findings

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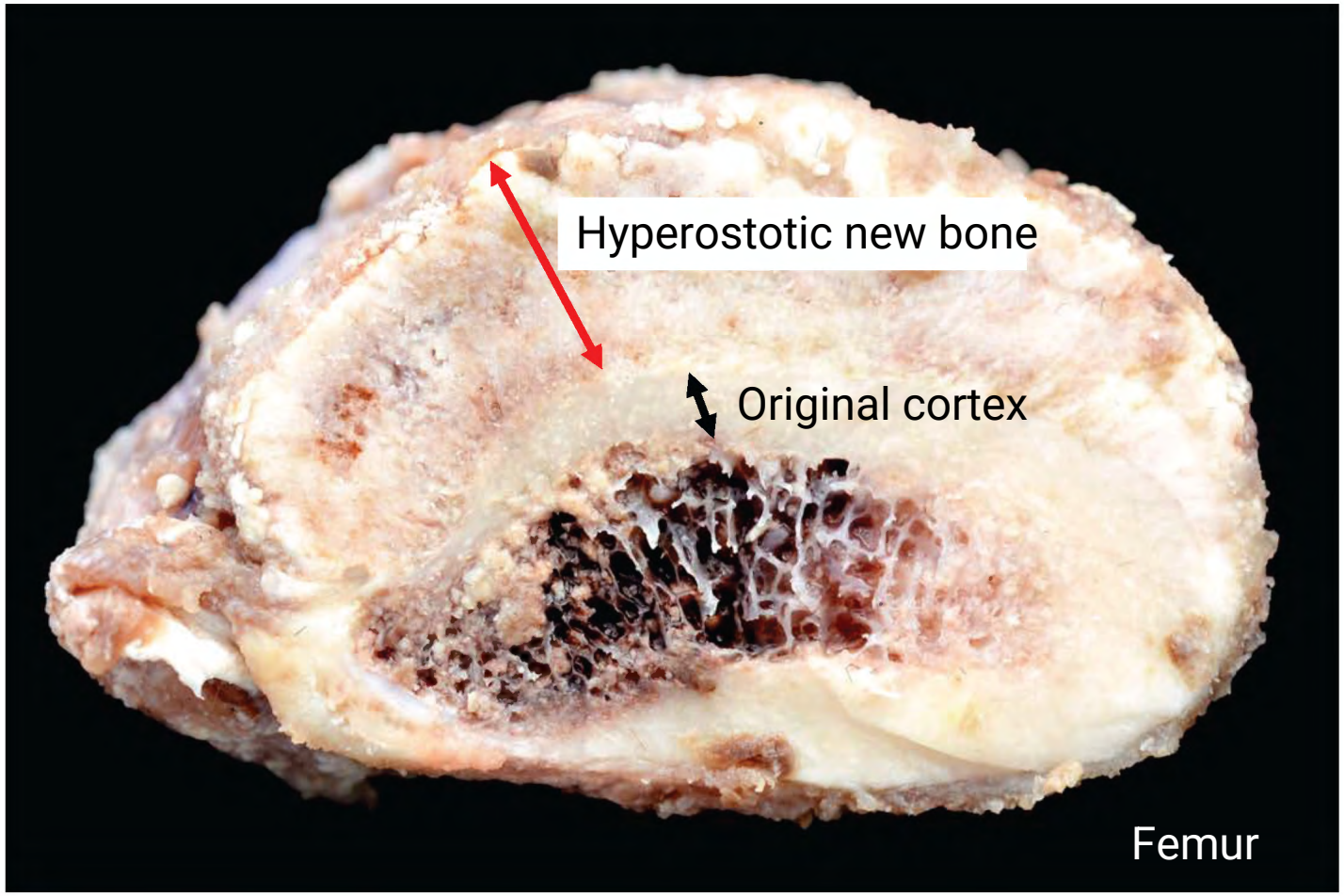

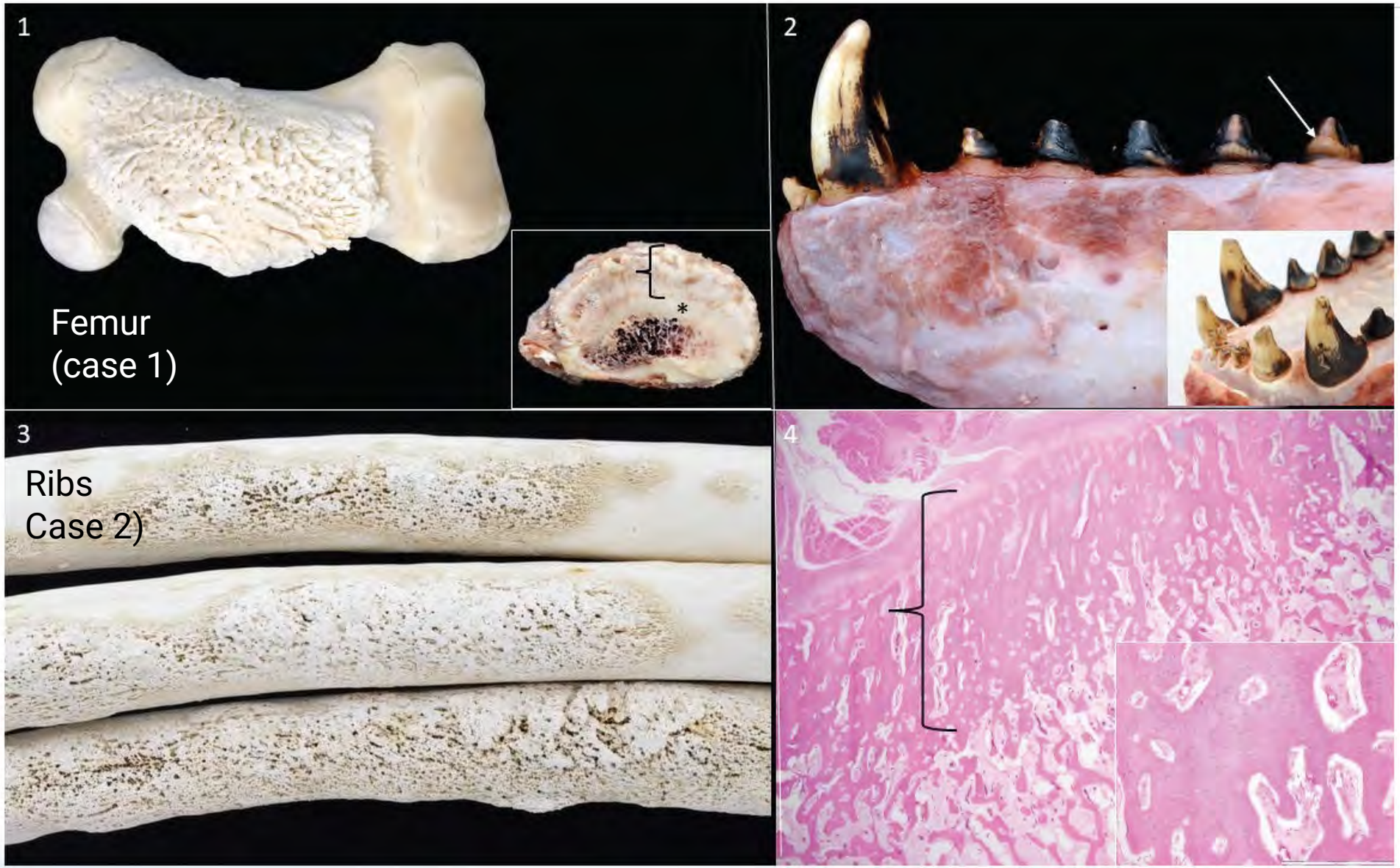

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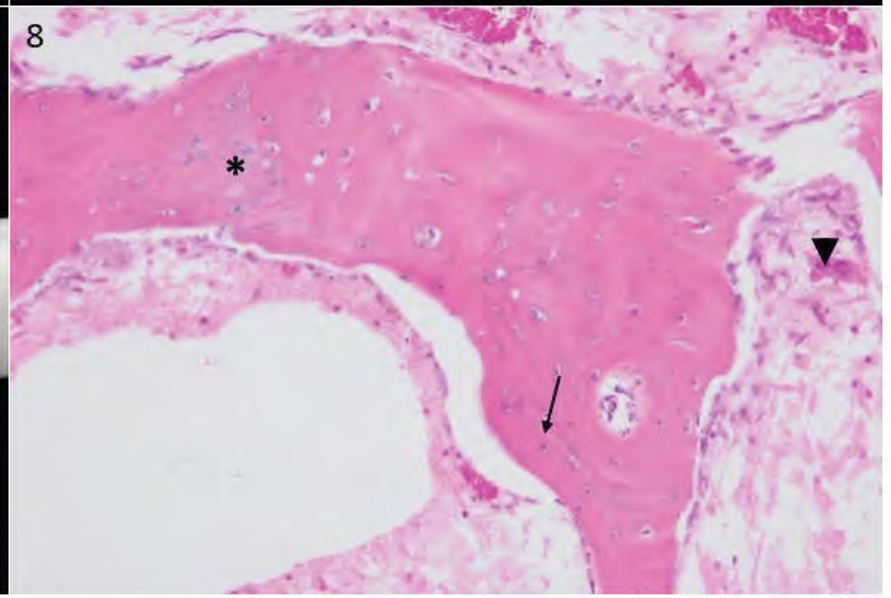
Case 1 mandible (uncleaned) with dull brown enamel (arrow) and pitting on canine. Inset- Case 1 maxillary incisors and canines with variable and increased wear

Histology (H&E) of case 1 humerus with thick radiating and proliferating periosteal new bone formation (bracket) with thinner similar inner trabeculae with intertrabecular fibrous tissue. Inset- Higher magnification of bracket area comprised of boney trabeculae of endochondral ossification comprised of chondrocytes and cartilaginous matrix transitioning into poorly mineralized osteoid matrix.

Control Mandible
(not cleaned)

Case Mandible with severe dental attrition and mandibular hyperostosis



Histology (H&E) of thinner inner trabeculae comprised of poorly mineralized osteoid (asterisk), reversal lines (arrow), lined by osteoblasts and osteoclasts (arrowhead). Intertrabecular spaces comprised of fine connective tissue and capillaries.

Case os penis with hyperostosis

Differentials for Hyperostosis

Genetic/congenital

- Congenital hyperostosis of pigs
- Craniomandibular osteopathy of West Highland White and Scottish Terriers
- Calvarial hyperostosis of Bullmastiffs
- Caffey disease of infants

Inflammatory

- Hypertrophic osteopathy (Marie's disease)
- *Hepatozoon americanum* in canids

Toxic

- Fluoride
- Vitamin A

Fluoride Bone Measurements

Bone	CSL Case (ppm dw)	CSL Control (ppm dw)	Older Cattle with fluorosis (ppm)	Control Cattle (ppm)
Humerus	8,1000	3,800	2,500 – 3,000	600-900
Femur	6,100	1,500		
Rib	9,700	2,000		

Fluoride levels in PPM/dry weight

	Case 1	Case 2	Case 3	Control 1	Control 2	Control 3	Control 4
Age	SA	SA	U	SA	Y	J	SA
Sex	M	M	U	M	M	M	F
Femur	6100			1500			
Humerus	8100		4500				
Rib	9700	5000	4500	2000	1400	1400	1500
Proximal Humerus	9000						
Tooth	4000	3800		2400			
Mandible		3700		3200			
Tibia	4700			1700			
Humeral cortical bone				1700			
Humeral medullary trabeculae				3800			
Calvarium	6500						
Vertebra		4200	4300				
Metacarpus		4200					
Scapula			4600				
Mean	6871	4180	4475	2329	1400	1400	1500

Fluorosis Lesions are well described in marsupials in Victoria downwind from an aluminium smelter

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Osteofluorosis

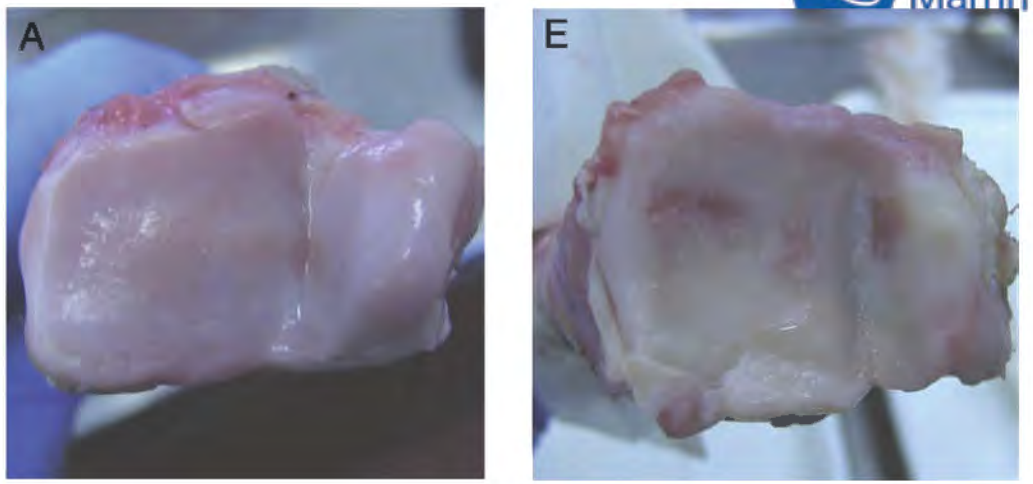
- Hyperostosis
- Osteosclerosis, osteoporosis, osteophytosis and/or osteomalacia

Growth plates and joints

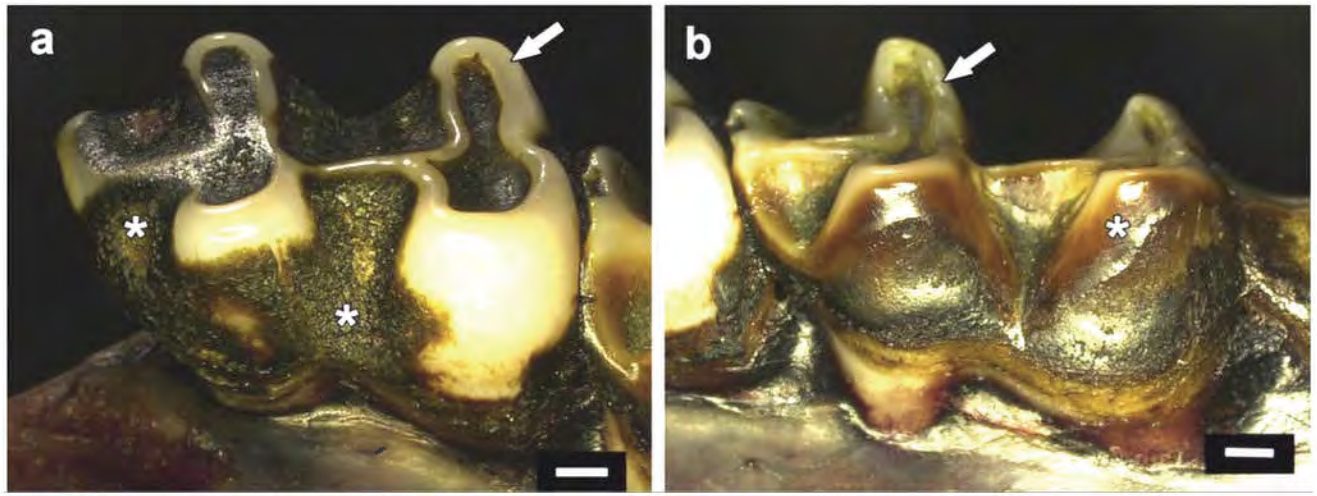
- Rickets-like physeal lesions
- DJD-like lesions

Dental Fluorosis

- Poorly mineralized and hypoplastic enamel



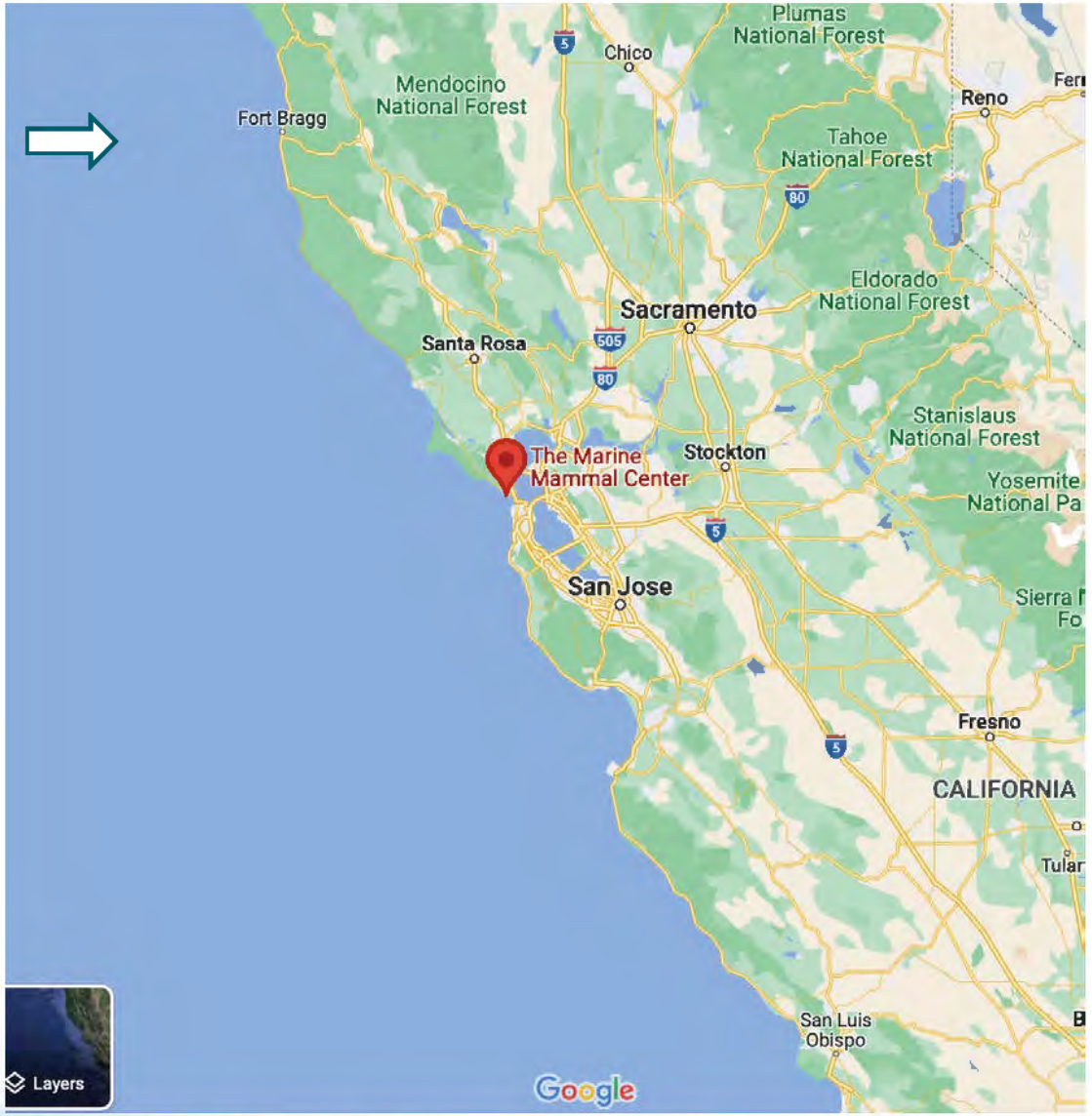
Hufschmid J, et. al. Skeletal Pathology of Eastern Grey Kangaroos (Macropus giganteus) Exposed to High Environmental Fluoride Levels in South-Eastern Australia**.** ***Journal of comparative pathology*****. 2015**;153(2-3):167-184. doi:10.1016/j.jcpa.2015.06.002



Death C*, et. al.* Dental fluorosis and skeletal fluoride content as biomarkers of excess fluoride exposure in marsupials. *The Science of the total environment*. 2015;533:528-541.

Exposure for sea lions?

- Ongoing collaborative research project with Dr Pobert Poppenga of The California Animal Health Laboratory and toxicology resident, Dr Chelsea Sykes to determine the prevalence of intoxication.
- Also working with NOAA sea lion biologists and the California Academy of sciences (historic samples).
- All cases stranded in Ft Bragg area of Mendocino County.
- Sampling all age classes from multiple locations through time.
- Future work to look at exposure (prey species from different areas).



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GRAY WHALE UNUSUAL MORTALITY EVENT

Gray whale (*Eschrichtius robustus*) mortality events

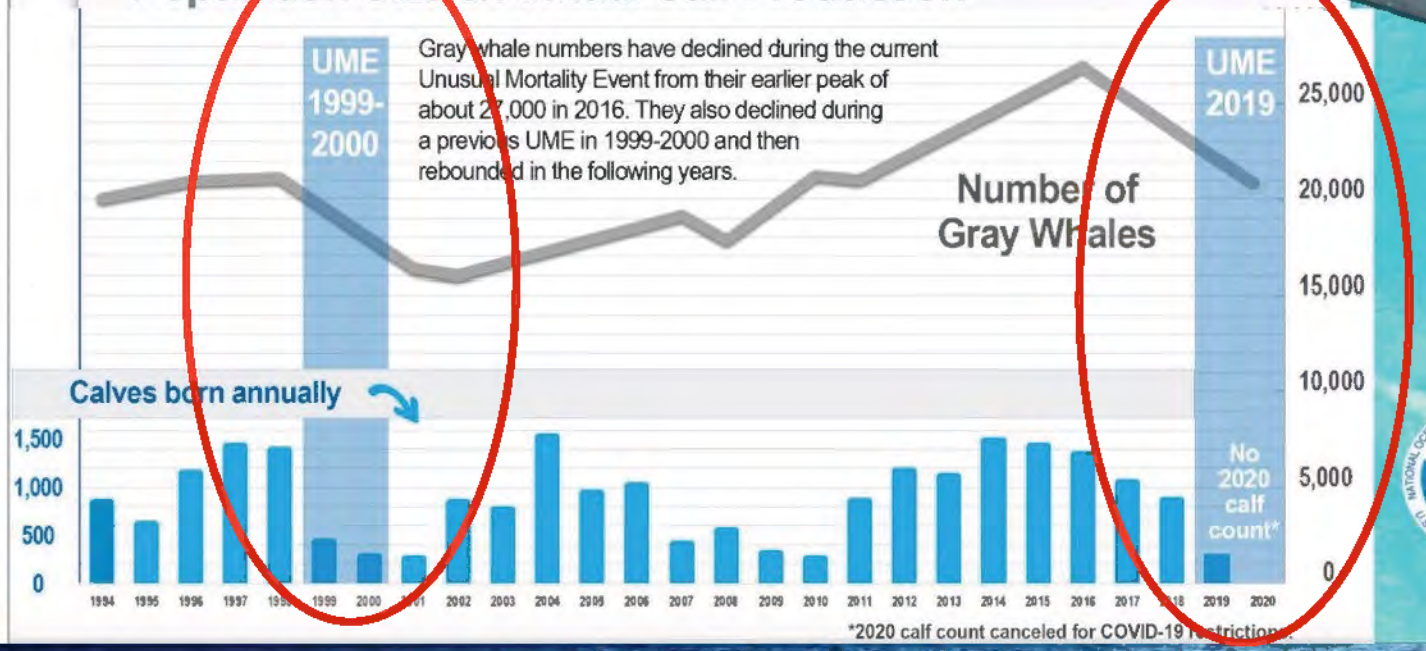
The Marine Mammal Center.

Eastern North Pacific Gray Whale Population

1999-2000

2019-2022

Population Size & Annual Calf Production



*2020 calf count canceled for COVID-19 restrictions.

Summer feeding in the Arctic:
May to November
Gray whales feed in the Arctic in summer, consuming sea-bottom amphipods and other organisms living in and above the sediment in the Bering, Chukchi, and Beaufort Seas, building fat for their long migration. Gray whales eat primarily in summer.

Chukchi Sea

Alaska

Bering Sea

Gulf of Alaska

CANADA

UNITED STATES

MEXICO

Baja Peninsula

Scammon's Lagoon

San Ignacio Lagoon

Magdalena Bay Lagoon

Long Migration

Eastern North Pacific gray whales have the longest migration of any mammal. They travel 10,000 to 12,000 miles every year.

Southward migration:
November to February
The whales usually travel within 2.5 miles of the shore. Pregnant females go first, followed by adult males and other adult females. Juveniles migrate last, and some may not reach Mexico before turning back north again.

Northward migration:
February to May
Newly pregnant females go first followed by adult males, other females, and immature whales. Mothers with calves remain a month or two longer while calves gain blubber and strength before their long journey north.

Winter in Mexico:
January to March
Gray whales frequent the Mexican coast including the Baja Peninsula in winter. Calves are born in the warm shallow lagoons.

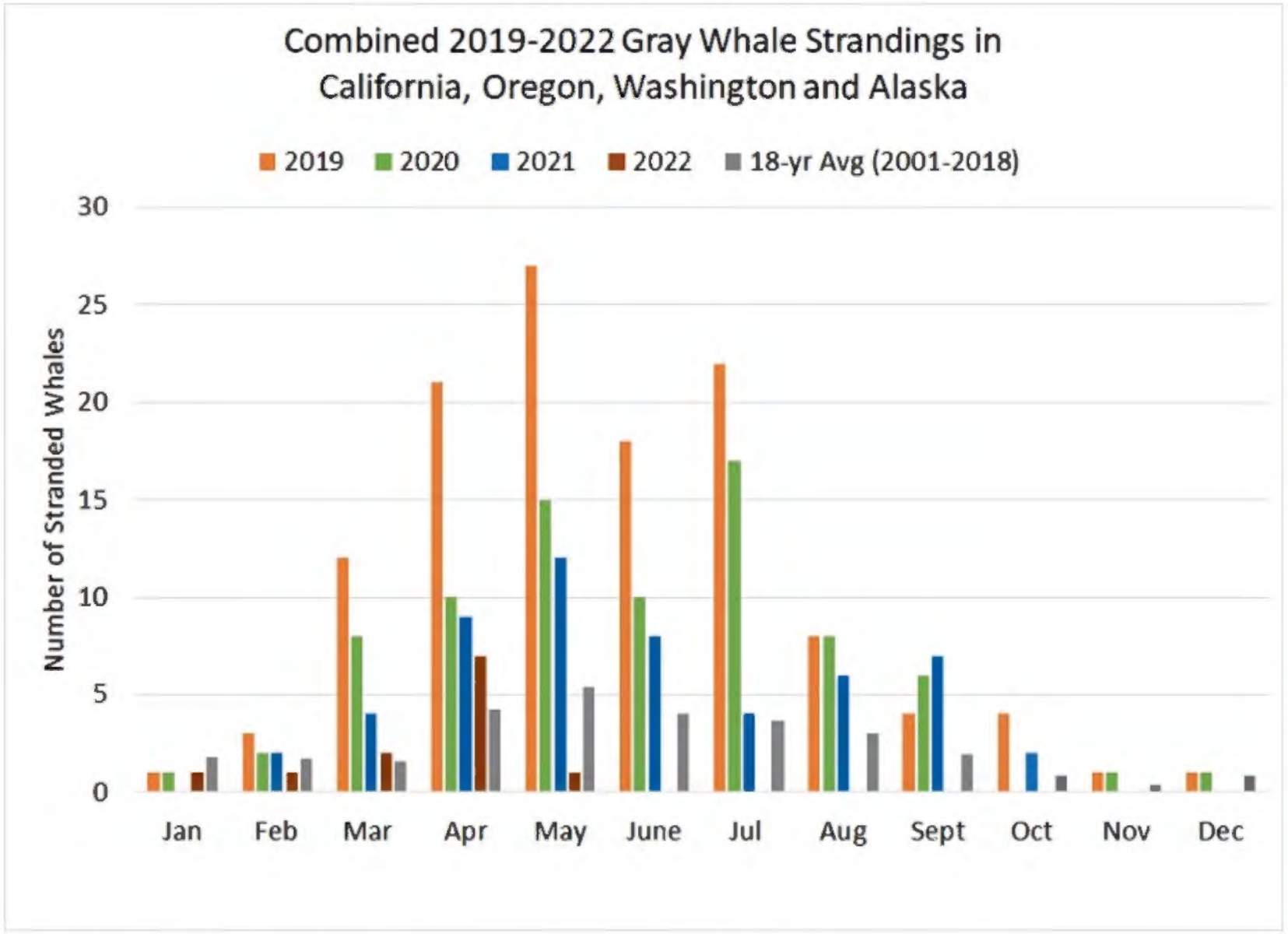
NOAA FISHERIES

Gray whale illustration by Uko Gorter

Unusual Mortality Event

- 590 dead gray whales (Canada, Mexico, and U.S.) documented from 2019 through June 30, 2022.
- Recent abundance estimates found a **~24% decrease in the population between 2016 and 2020** (Stewart and Weller, 2021).

The Marine Mammal Center.



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Combined 2019-2022 Gray Whale strandings in California, Oregon, Washington, and Alaska.

C-552 Gray Whale (*Eschrichtius robustus)*

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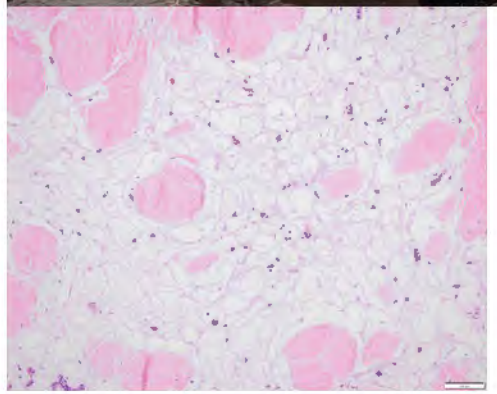
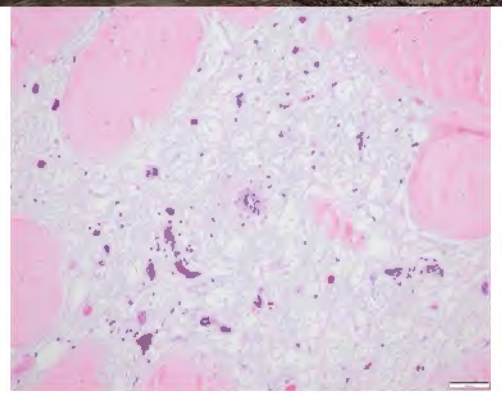
Juvenile male found floating in the Bay and beached on Angel Island, 8th March 2018

Massive infestation with whale lice (Cyamids)

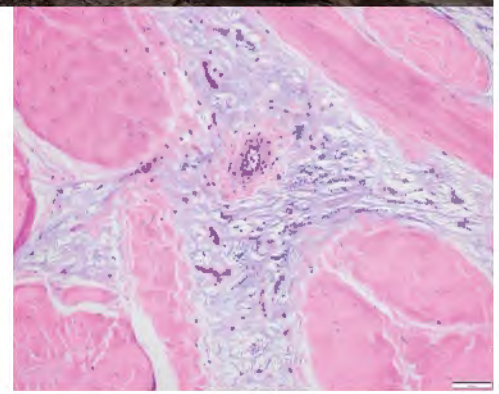
C-673 Sub-adult male



Outer blubber (60% adipose)



Mid blubber (50% adipose)



Deep blubber (25% adipose)

Stranded 12 May 2021, Cause of death – ship strike (fractured vertebrae)
However, he was also in poor body condition based on blubber depth and histology

Findings

- **No single cause of death**
- Deaths occurred because of trauma (ship strike), malnutrition, predation (killer whales), or combinations of these.
- For many, decomposition prevented determination of cause of death.
- No pathogens identified
- Biotoxin levels (Saxitoxin, DA) were undetectable, low or moderate but the significance of even the highest levels are unknown.
- However, a common factor in these years was poor body condition.
- This was also corroborated by other methods such as drone photogrammetry of live whales on migration and in Mexico

- Sea ice cover in the Bering and Chukchi Seas has declined dramatically in the past two decades
- The distribution of benthic prey species has shifted northward
- Gray whale distribution has also changed in response to this (Moore et al., 2022)
- The 1999-2000 UME was preceded by the 1997/98 El Nino event
- The 2019-22 UME was preceded by the N Pacific Thermal Anomalies

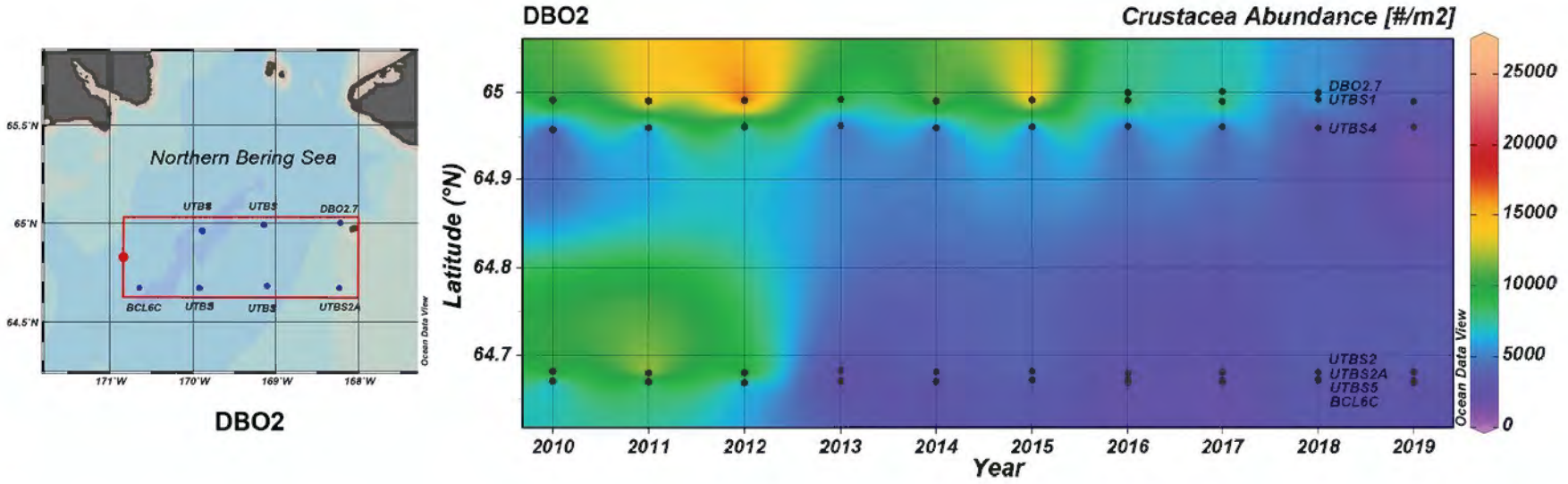


Figure from Moore et al. (2022) showing a marked decline in benthic amphipods in the northern Bering Sea from 2010 to 2019

"Whether or not gray whales were nutritionally stressed due to a change or reduction in prey availability related to environmental forcing, and/or to increased competition for food resulting from their burgeoning population size, remains a pivotal question with regard to both UME events" Moore et al. 2022

- **In other words, we don't whether the UMEs were caused by Climate Change (too few groceries) or because of Carrying Capacity (K) of the environment (too many whales for the resources)?**
- **Perhaps a combination of both?**

Thank You.

The Marine Mammal Center advances global ocean conservation through marine mammal rescue and rehabilitation, scientific research, and education.

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