Distribution of the neurotoxin domoic acid (DA) among various tissues and bodily fluids of naturally-exposed, DA-positive southern sea otter (*Enhydra lutris nereis*).

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Introduction

Southern sea otters have been protected by the Endangered Species Act (ESA) since 1973 and are fully protected under state law (USFWS, 2016). As a federally-listed threatened species occupying only 13% of their historic range, it is pertinent to determine which factors are curtailing population growth of this protected species. An increasingly important threat to sea otters are harmful algal blooms that produce toxins such as domoic acid (DA). A mortality study determining causes of deaths for southern sea otters from 1998-2012 identified DA intoxication and cardiac disease as the third and fourth most common primary causes of death, respectively (Miller et al., 2017). Because the final cause of death coding was based on highly conservative criteria and cardiac disease is significantly associated with DA exposure (Kreuder et al., 2005; Miller et al., 2017), the actual impacts of DA on California's sea otters are likely to be even more substantial.

DA toxicity lies in its structural similarity to glutamate, binding excitatory receptors in the central nervous system and causing excitotoxic cell death (Gulland et al., 2002). Antemortem signs of DA toxicosis typically include neurologic abnormalities such as seizures and pathological findings often include lesions to the hippocampus, piriform lobe, and heart (Kreuder et al., 2005).

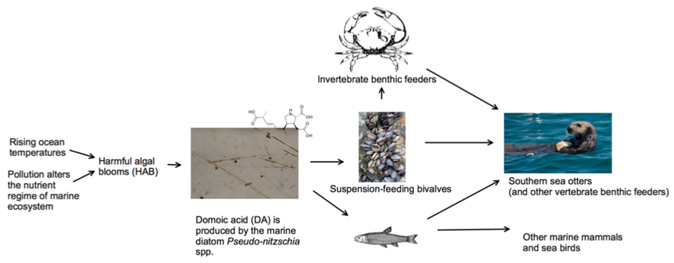

Figure 1. The primary route of DA exposure in southern sea otters comes from their prey items, which accumulate the toxin in their digestive tracts.

Objective

Determine if brain, pericardial fluid (PCF), cardiac muscle, kidney, liver, or bile samples can be informative for diagnosing DA exposure in sea otters.

Methods

In this retrospective study, we selected 11 southern sea otters with detailed postmortem examinations based on the following criteria:

- High to very high concentrations of domoic acid (≥ 300 ppb) in either postmortem urine or gastrointestinal (GI) samples
- More recent cases (2010-2017)
- At least 5/6 target tissues cryopreserved in sample archives

All necropsies were performed at the California Department of Fish & Wildlife – Marine Wildlife Veterinary Care & Research Center (CDFW-MWVCRC) in Santa Cruz. Tissues and fluids were routinely cryoarchived at the time of necropsy, accompanied by microscopic examination of all major tissues. Cryopreserved brain, PCF, cardiac muscle, kidney, liver, and bile were prepared and tested at the University of California, Santa Cruz (UCSC) using liquid chromatography/mass spectrometry (LC/MS).

This research project was designed as an exploratory assessment of novel biological tissues and fluids that may be used to detect domoic acid exposure through biochemical testing. Descriptive statistics and qualitative analyses were used to identify potential patterns that may warrant additional study

Results

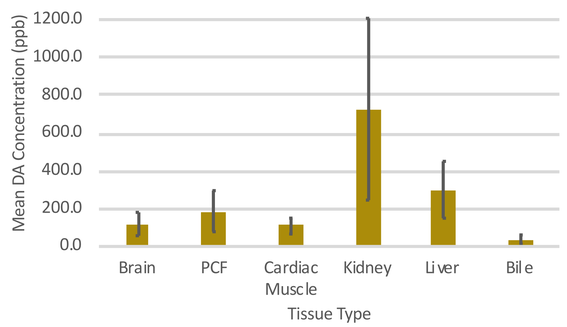

Figure 2. Mean domoic acid (DA) concentrations for brain, pericardial fluid (PCF), cardiac muscle, kidney, liver, and bile among 11* southern sea otters necropsied from 2010 – 2017. *9 sea otters had PCF, 6 sea otters had bile

SSO ID	Brain	PCF	Cardiac Muscle	Kidney	Liver	Bile	Tissue with highest [DA]
5856-10	26.6	NA	33.7	20.2	18.8	NA	cardiac muscle
6132-11	33.6	24.1	23.2	18.9	10.2	NA	brain
6178-11	656.3	38.8	21.4	201.7	297.7	NA	brain
6510-12	68.1	61.4	214.0	164.1	388.2	NA	liver
6565-12	359.4	17.1	0.0	613.9	1739.3	127.7	liver
7064-14	68.9	686.6	362.7	1490.3	296.4	0.0	kidney
7588-15	26.2	828.7	331.7	5344.6	53.1	NA	kidney
7806-16	0.0	0.0	11.8	21.0	330.7	0.0	liver
8381-17	55.3	0.0	178.1	72.3	101.2	0.0	cardiac muscle
8410-17	0.0	NA	0.0	0.0	48.3	95.2	bile
8481-17	9.8	22.8	9.9	40.2	14.3	0.0	kidney
Total Samples with [DA] ≥ 300 ppb	2	2	2	3	3	0	

Negative | Low (0-99) | Moderate (100-299) | High (300-499) | Very high (≥500)

Table 1. Summary DA concentrations (ppb) for brain, pericardial fluid (PCF), cardiac muscle, kidney, liver, and bile among 11 southern sea otters necropsied from 2010 – 2017. DA concentration was determined using Liquid chromatography-tandem mass spectrometry (LC-MS/MS). Particular samples were unavailable for individual otters and are represented with NA.

Discussion

This pilot study provides preliminary data concerning the distribution of DA in southern sea otters among various tissues and body fluids. Univariate analyses reveal that the tissues with the most samples in the high-very high concentration ranges for domoic acid are liver and kidney (Table 1). These two tissues also produced the fewest number of negative results (Table 1). This is consistent with current understanding of this toxin's mechanism and excretion, as DA is known to be eliminated in both urine and feces (Gulland et al., 2002).

Experimental studies with fish have found that DA initially accumulates in the kidney and bile of these species an order of magnitude more than any other tissue (Lefebvre et al., 2007). The DA concentrations found in our kidney samples support this; however, it is interesting that the bile samples from our otters displayed the highest frequency of negative results. This may be the result of a small sample size (6) or it may indicate a species difference in DA accumulation.

A cross-sectional study investigating DA exposure in Alaskan marine mammals tested a variety of sample types, including stomach content, feces, bile, and pericardial fluid (Lefebvre et al., 2016). Researchers tested bile in Steller sea lions and harbor seals and reported the tissues with the highest DA concentrations: stomach content (7 ppb) and feces (8 ppb) This suggests that if DA was detected in bile, it would have been less than 8 ppb. Two of the six bile samples in our sea otter project were found to be positive for DA at much higher levels (95.2 and 127.7 ppb).

Future studies will include control groups of otters with no detectable DA in urine or GI content and larger sample sizes to determine how the distribution of this toxin may differ in sea otters with and without known domoic acid exposure. Furthermore, as the case definition for DA toxicosis in southern sea otters is developed and chronicity is better characterized (e.g. acute, subacute, and chronic), it will be interesting to revisit these cases to determine if there is a relationship between the chronicity of an animal's exposure and the tissue distribution of DA.

Acknowledgements and references

We wish to thank the dedicated volunteers and staff of the CDFW-MWVCRC, Monterey Bay Aquarium, U.S. Geological Survey, and The Marine Mammal Center (TMMC) who have facilitated sea otter carcass recovery and necropsy efforts over many years. We would specifically like to thank Erin Dodd, Francesca Batac, and Laird Henkel of the CDFW-MWVCRC as well as Kendra Negrey (UCSC) and Padraig Duignan from TMMC for all of their help and support throughout this project.

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