First results on paretic syndrome research in gulls from Málaga (S Spain) reveals that they did not bear BMAA, paralytic and amnesic shellfish toxins.

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Introduction

In the last decade, common gulls from the Iberian Peninsula have been observed to suffer from a paralyzing syndrome of unknown origin, affecting mostly adults in the west and south regions. This disease has been attributed to different causes, such as nutritional deficiencies, avian botulism, and exposure to algal toxins. In the quest for the causative agent, we selected twelve individuals of *Larus michahellis, L. fuscus*, *L. ridibundus* and *Ichthyaetus melanocephalus* that succumbed to this illness concomitantly with marine algal blooms in the nearby coasts of Málaga (Spain).

Fig 1. Seagulls with paretyc syndrom at the recovery facilities

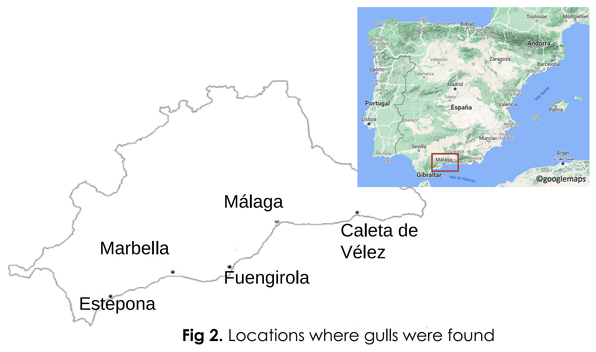


Fig 2. Locations where gulls were found

Objectives

- Detect Domoic Acid (DA), Paralytic Shellfish Toxins (PSTs) and β-methylamino-L-alanine (BMAA) and related compounds in tissues of gulls dying showing neurological symptoms (paretic syndrome).
- Improve extraction and detection protocols for BMAA in seabird tissues.
- Link the presence of toxins with trophic position of gulls by stable isotopes analyse (SIA).

Methods

DA and PSTs analysis

- **Domoic Acid**

Homogenization → methanol extraction (50%)→ centrifugation → Clean-up with HLB-SPE cartridges → LC-MS/MS (Wang et al, 2012 with modifications)

- **PSP toxins**

Homogenization → acidic extraction (heat)→ centrifugation → clean-up with C18-SPE cartridges → Peroxide/periodate oxidations → LC-FLD (DeGrasse et al, 2014 with modifications)

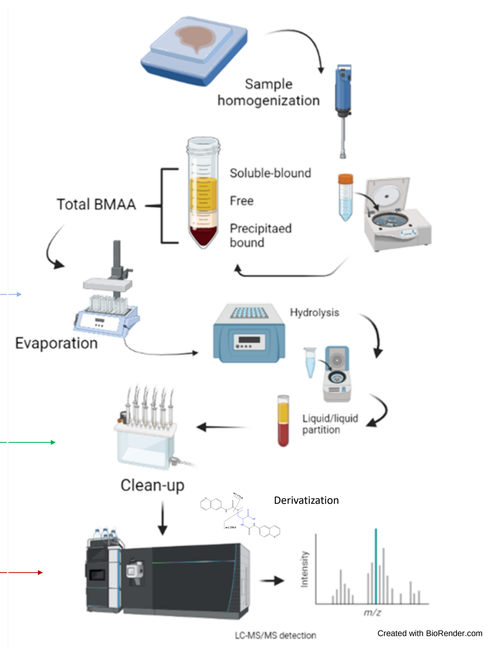
BMAA analysis

- Evaporation test to check toxin losses due to evaporation
- SPE test to check the posibiity of avoiding the cleaning-up steps.
- Method validation and toxin recovery

Table 1. Sample information: Samples shaded in grey were analysed for the whole set of toxins (DSP, PSP in serum viscera and dregs and BMAA in serum, brain and liver); for those in blue BMAA and SIA were performed (liver and brain) .

CODE	Species	Age (cy)	Date found (dd/md/yy)	Symptom. Scale (1-3)	Location	Sample type				Observations
LARFUS-22	*Larus fuscus*	1	16/11/18	1	Fuengirola	serum	viscera	brain	dregs	Died after 2 days
LARMIC-M3	*Larus michaellis*	1	29/10/18	3	Málaga	serum	viscera	brain	dregs	Died on the same day
LARMIC-67	*Larus michaellis*	1	24/09/18	1	Caleta de Vélez	serum	viscera	brain	dregs	Died after 2 days
LARMIC-23	*Larus michaellis*	1	25/09/18	2	Fuengirola	serum	viscera	brain	dregs	Died the next day
LARRID-101	*Larus ridibundus*	Adult	13/12/18	1	Caleta de Vélez	serum	0	brain	dregs	Died on the 18/12/18
LARMEL-1	*Larus melanocephalus*	Adult	27/02/19	3	Caleta de Vélez	serum	0	brain	dregs	Can stand up, torticollis. Died on the 2/03/19
LARFUS MARCH-2	*Larus fuscus*	Adult	07/03/2019	Dead	Málaga	0	viscera	brain	0	Bloom of cyanobacteria
LARFUS MARCH-5	*Larus fuscus*	2	10/03/2019	Dead	Fuengirola	0	viscera	brain	0	Bloom of cyanobacteria
LARFUS MARCH-4	*Larus fuscus*	Adult	15/03/2019	Dead	Estepona	0	viscera	brain	0	Bloom of cyanobacteria
LARUFUS-11/12/2019	*Larus fuscus*	4	12/11/2019	Dead	Málaga	0	viscera	brain	0	-
LARMIC MARCH-1	*Larus michaellis*	3	13/03/2019	Dead	Marbella	0	viscera	brain	0	Bloom of cyanobacteria
LARRID MARCH-3	*Larus ridibundus*	Adult	15/03/2019	Dead	Estepona	0	viscera	brain	0	-

BMAA extraction & detection



Stable Isotopes

- SIA was conducted in the liver and brain samples of *L. michahellis* and *L. fuscus*.
- Stable isotope ratios of δ13C used to identify the food sources and δ15N for inferring the relative trophic position of a consumer within the food web.
- Liver represents the fastest turnover (weeks) and brain the intermediate turnover (months).

Results

- All the samples analysed from all the organs had negative results.
- Limit of detection/quantification for liver, brain and serum 25 ng/mL.
- Evaporation test → evaporation caused losses of BMAA, mainly in liver and brain, and BMAA could no be detected at low concentrations.
- SPE test → the cleaning-up procedure may cause toxin losses but skyping this step reduce reproducibility and accuracy leading to matrix interferences.
- Recovery → 15% liver, 10% brain, 18% serum.
- The lower 13C and 15N in *L. fuscus* indicates more offshore pelagic feeding grounds and less predatory diet (in agreement with field observations).
- The brain signatures are different from liver signatures, and the latter indicates a recent shift to more marine sources in both species (*L. fuscus* is a wintering species in Málaga).

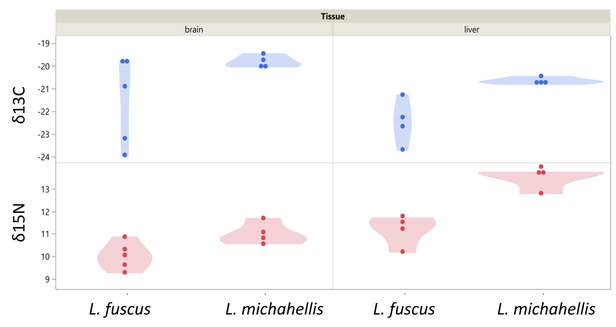


Fig 3. Diagram showing δ 13C and δ 15N levels (in ‰) for liver and brain in the species *L. fuscus* and *L. michahellis*.

Conclusions

- The absence of BMAA was not related to species-specific feeding habits.
- The causative agent of paralysis may not be linked to the occurrence of DA, PSP and BMAA toxins.
- The method for BMAA extraction and detection in seabird tissues were optimized, although requires improvements (toxin losses).
- Despite negative results this work provides a baseline for future studies.

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