Project Report SG153: Quantifying a novel endocrinological indicator of stress and sociality in a large marine predator.

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Project Rationale

Cetacean species are vulnerable to disturbance from acoustic and chemical pollution from shipping, oil and gas operations, military activities and marine renewable energy developments. Accurately analysing and understanding biological indicators of stress, reproductive and social status are vital in determining our impact on any species potentially affected by anthropogenic activities in the UK. However, information on the underlying physiology acting on these processes in cetaceans, and on the best practices for analysing them, are poorly represented in the published literature. Oxytocin is a neuropeptide hormone crucial for initiating and modulating maternal and social behaviour across vertebrate animals and is involved in regulating the hypothalamo-pituitary-adrenal (HPA) stress response. The hormone could act as biomarkers for stress, reproductive and social statuses in small cetaceans such as the bottlenose dolphin (*Tursiops truncates)*. The objective of the research project was therefore to validate a protocol for the detection of the hormone oxytocin in bottlenose dolphins and to generate a basal dataset of plasma oxytocin concentrations in this species across different sexes, ages and reproductive states.

Methodology

All samples used for this study were collected in 2014 by project partners in Europe (Zoo Duisburg, ZD) and Florida (Sarasota Dolphin Research Program, SDRP). All research was approved ethically by the University of St Andrews Animal Welfare and Ethics Committee. Plasma samples were collected from 6 captive (ZD) and 19 free-ranging (SDRP) bottlenose dolphins during routine health assessments (Figures 1 and 2) conducted by the two organisations. Plasma was then frozen and transferred under CITES permit to the Sea Mammal Research Unit (SMRU) for analysis. A commercially available enzyme-linked immunosorbent assay (ELISA) for detecting plasma oxytocin in domestic mammal species (Assay Designs Inc, Ann Arbor, MI, USA) was then validated to determine its suitability for detecting oxytocin in bottlenose dolphin plasma. To validate the ELISA, samples of dolphin plasma was spiked with know quantities of oxytocin (n=10). They were then extracted and run on the plate to calculate recovery rates. Both raw and extracted dolphin plasma was run on the plate in serial dilution to evaluate parallelism with the standard curve. Intra assay coefficient of variation (COV) for the plate was calculated, and inter assay COV was calculated across this plate and the subsequent plate analysing all samples collected to generate a basal dataset of plasma oxytocin concentrations for this species.

Outputs

The validation of the ELISA was a success, with both raw and extracted dolphin plasma dilutions showing excellent parallelism with the standard curve (Figure 3). Recovery rates for the extraction and ELISA procedure were 112.8% (n=10), intra-assay COV for this assay was 1% and inter-assay COV over the validation plate and the plate to generate the basal dataset was 2.8%.

It was possible to detect plasma oxytocin concentrations in all 25 individuals sampled, generating the first data on oxytocin concentrations for not only bottlenose dolphins, but any cetacean species. It was possible to sample both sexes (females: n=17, males: n=8), a variety of age (adults: n=16, juveniles: n=3, calves: n=6) and reproductive classes (females without calves: n=6, females with calves: n=6, adult males: n=7, calves: n=6), giving the basal dataset good coverage of oxytocin values throughout potentially important stages of an individual's life. This dataset will hopefully be expanded in the coming years, and once a larger number of individuals have been included in the dataset, our findings will be submitted for publication in a peer reviewed journal.

Expenditure Summary

The MASTS small grant was used to cover the following expenses towards this project:

Project expenses	Cost
Disposable lab equipment (ELISA plates, chemicals etc)	£1489.45
Postage and permits to send samples from the USA and Europe to the UK	£432.75
Lab Technician Salary	£1014.75
TOTAL	£2936.95

Figure 1. Health assessments of free-ranging bottlenose dolphins conducted by the SDRP. Individual dolphins are held by a team of experienced handlers while vets collect plasma samples from the underside of the tail fluke while the individual is still in the water (see figure 2). The boat to the left of the photograph has laboratory equipment for processing and freezing samples while on the water. Photo taken under U.S. National Marine Fisheries Scientific Research Permit No. 15543.

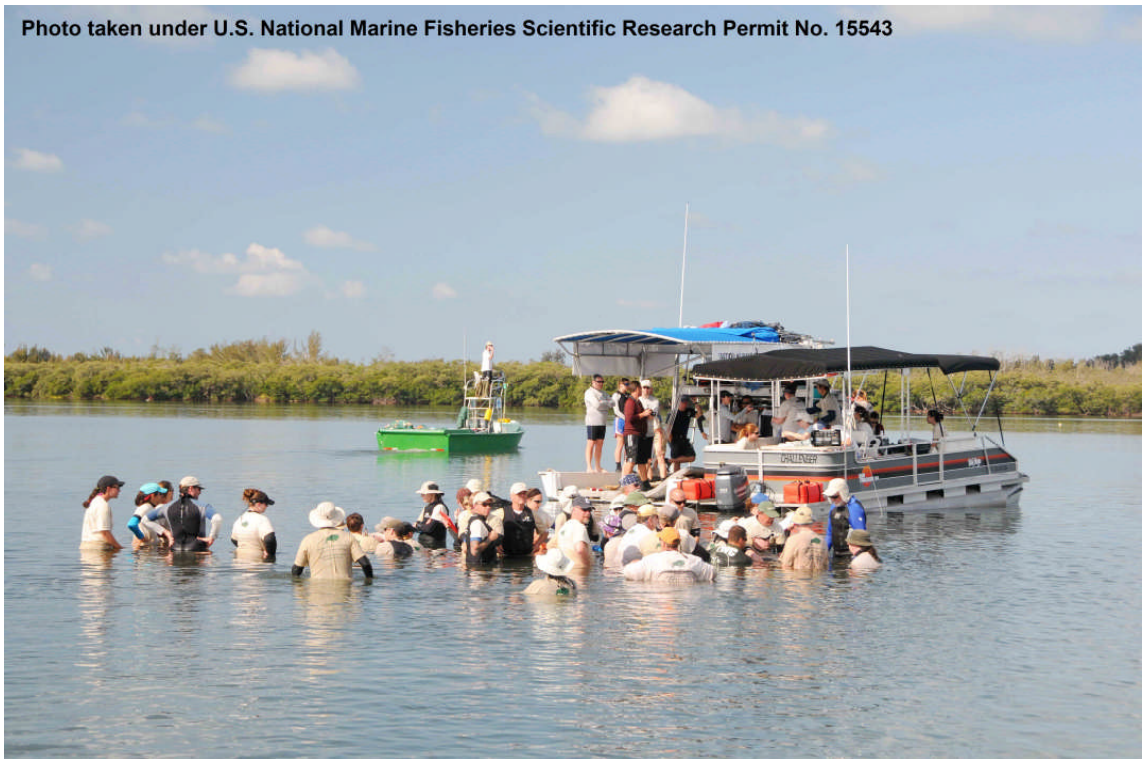

Figure 2. Plasma sampling from the underside of the tail fluke during a health assessment. Photo taken under U.S. National Marine Fisheries Scientific Research Permit No. 15543.

Figure 3. The percentage bound oxytocin in the ELISA standards from the two plates used for this study and those from serially diluted bottlenose dolphin plasma after extraction.