Project Report SG344: Neuropeptide hormones as indicators of social and reproductive states and stress in the bottlenose dolphin

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Project Rationale

The objective of this project was to expand on a successful MASTS funded pilot study (SG153) which detected the hormone oxytocin in a cetacean species for the first time (bottlenose dolphins, *Tursiops truncatus*). We aimed to expand the preliminary dataset generated in the pilot study by analysing additional samples from wild bottlenose dolphins. The resulting larger dataset would contain essential data for evaluating the hormone as a potential indicator of stress, reproductive status and sociality in small cetaceans. The pilot work was conducted in 2014, and we expanded on the study by analysing plasma samples from the same population of wild bottlenose dolphins collected in 2015 and 2016.

Oxytocin is a neuropeptide hormone crucial for initiating and modulating maternal and social behaviour across mammalian species. The hormone is well known for promoting bonding and pro-social behaviours and additionally regulates the hypothalamo-pituitary-adrenal (HPA) axis by buffering physiological stress responses. Oxytocin is a biomarker for the presence and strength of social and maternal bonds and can be used to identify bond partners in a group. Our laboratory has previously succeeded in validating methods of detecting oxytocin in marine mammals samples (grey seals, *Halichoerus grypus*, harbour seals, *Phoca vitulina*, Weddell seals, *Leptonychotes weddellii* and bottlenose dolphins) and in linking concentrations of the hormone to maternal behaviour and bonding in wild seals. Studying variation in this hormone could generate methodologies to examine social cohesion, group structure, maternal investment behaviour and why these can break down. The hormone therefore has the potential to become a tool for identifying social states which previously have only been inferred from behavioural observations. Additionally, studying this hormone could offer a novel perspective on how different kinds of stressors affect the HPA axis and how a suitable social environment can mitigate stressful conditions.

Methodology

All samples used for this study were collected in 2015 and 2016 by project partners in Florida (Sarasota Dolphin Research Program, SDRP, Mote Marine Laboratory). All research was approved ethically by the University of St Andrews Animal Welfare and Ethics Committee. Plasma samples were collected from free-ranging bottlenose dolphins by the SDRP during annual routine health assessments. In 2015, 14 individuals were sampled and in 2016, 9 individuals were sampled. Plasma was frozen and transferred under CITES permit to the Sea Mammal Research Unit (SMRU) for analysis. A commercially available enzyme-linked immunosorbent assay (ELISA) (Enzo Life Sciences (formerly Assay Designs Inc), NY, USA) that had previously been validated for use on bottlenose dolphin plasma was then used to detect oxytocin in our samples. All samples were extracted prior to analysis, in accordance with the methodology established in the pilot study. Intra assay coefficient of variation (COV) for the plate was calculated, and inter assay COV was calculated across this plate and

the plates previously used to generate the basal dataset of plasma oxytocin concentrations for this species.

Outputs

It was possible to detect plasma oxytocin concentrations in all 25 individuals sampled in 2015 and 2016. Combined with the data from the 2014 pilot study, the basal dataset now contains 42 oxytocin concentrations from wild dolphins and 6 from captive dolphins. Intra-assay COV for the ELISA plate was 7% and inter-assay COV over the four ELISA plates to generate the dataset was 7.3%.

Analysis of the dataset shows significant differences in plasma oxytocin concentrations across different life history stages. We are therefore in the process of preparing a manuscript of our results, which will hopefully be published in the coming year (2018) in a journal such as the Journal of Comparative Physiology, Frontiers in Zoology or Physiological and Biochemical Zoology.

Expenditure Summary

The MASTS small grant (£500) was used to cover the following expenses towards this project:

Project expenses	Cost
Oxytocin ELISA plate (1)	£377.00
Postage of CITES import permit to the USA	£43.12
Shipment of samples from the USA to the UK on dry ice	£77.00
TOTAL	£497.12