Glutathione-*s*-transferase protein and activity in epidermal tissue of humpback whales

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1. Introduction

Cetaceans (whales dolphins and porpoises) are particularly susceptible to accumulation of toxic burdens of lipophilic organochlorine compounds (OCs) due to their longevity and position at the top of marine food chains. Despite frequently reported elevated levels of blubber OC burdens in these species, the performance of chemical risk assessments is precluded by a lack of anciliary individual and toxicological information. Recently the International Whaling Commission (IWC) steering group on contaminants flagged the importance of driving research towards facilitation of cetacean chemical risk assessment[1].

Currently OC toxicology on mysticetes (filter-feeding whales) relies primarily on skin and blubber biopsies due to the rarity of stranding events of these large, often migratory species. As such advancements in mysticete toxicology must be underpinned by utilisation of these tissues for further molecular assessments.

Glutathione-*s*-tranferase (GST) catalyses the conjugation of glutathione with various xenobitics and therefore plays a major role in preventing oxidative stress[2]. GST has been detected across the animal kingdom and like many detoxification enzymes is substrate inducible and therefore a potential candidate for biomarker applications. To date no studies have reported the presence of GST in the skin, the most accessible tissue, of cetaceans.

Here we investigate the presence and activity of GST in the epidermal tissue of southern hemisphere humpback whales (*Megaptera novaeangliae*) and make a preliminary assessment of its applicability as a biomarker tool.

2. Materials and methods

Whales were biopsied off north Stradbroke Island, south east Queensland, Australia, at two time points on their annual migration; 1) northward (post-summer feeding) and 2) southward (end of fasting). Whale skin was stored at -80^{o}C upon collection and the cytosolic fraction, with the microsomal fraction removed, was extracted and applied to the subsequent tests.

Whale skin cytosol was probed for GST protein by western blotting with a goat polyclonal GST primary antibody (Abcam) and anti-goat IgG secondary antibody (Sigma-Aldrich). Bands were detected by the ECL kit as per manufacturer instructions (GE Healthcare). GST activity was measured fluorometrically by the production of the GST-CDNB (1-chloro-2,4-dinitrobenzene) conjugation product via the method of Habig et al. (1974)[3].

3. Results and discussion

Western blotting verified for the first time the presence of GST protein in the skin of humpback whales (Fig 1). Following verification of the GST protein in a sub-set of animals, GST activity was measured in skin extracts of 33 individual animals. 22 of the animals were biopsied on the northward leg of their migration (19 male, 3 female) and 11 on the southward leg (6 males and 5 females). All extracts were analysed in triplicate and datasets showing a coefficient of variation of ≤35% were further compared.

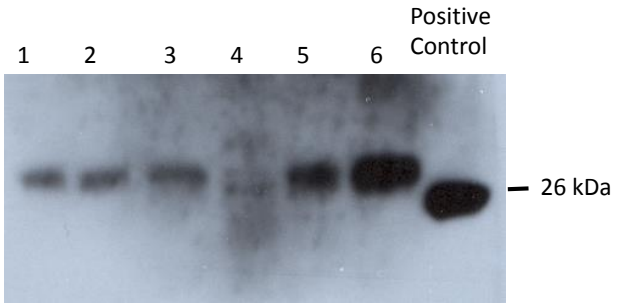

Figure 1: Western blot of five individual male whales (#4 is the microsomal fraction of #1). The positive control employed was rat liver cytosol.

The average measured activity in extracts was 1.44 U (1 unit = the amount of enzyme producing 1 µmol GST-CDNB product). No significant differences in activity were observed between the sexes or between migration cohorts, although expressed activity was observed to be lower in southward migrating (fasted) cohorts of both sexes (Fig. 2). This is in contrast to the expected increase in lipophilic OC exposure occuring at this time due to remobilisation of contaminant burdens along with stored lipid reserves. The production of reactive oxygen species is however a consequence of all metabolic processes and it is possible that at this late stage of the migration, following extended fasting, metabolism has been significantly depressed masking the expected increase in lipophilic contaminant exposure.

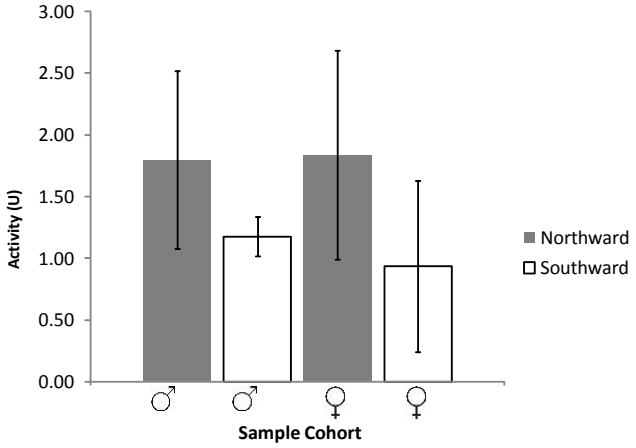

Figure 2: Measured GST activity in humpback whale cytosol. Error bars depict the standard deviation of sample cohorts.

4. Conclusions

This study verified for the first time the presence of an active GST system in the skin of humpback whales. GST activities expressed were generally low and this combined with a relatively small sample number prevented quantitative examination of variation between sample cohorts. Future work will investigate the relationship between activity and OC contaminant burdens of the whales to further assess the suitability of the enzyme as a biomarker of OC exposure.

5. References

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