Development and Validation of a New Technique for Detection of Stress and Pregnancy

Shannon Atkinson, PI
17101 Point Lena Loop Road
Juneau, Alaska 99801
phone: (907) 796.5453 fax: (907) 796-5446 email: skatkinson@alaska.edu

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LONG-TERM GOALS

To promote the use of endocrine techniques to advance our knowledge of the physiology of cetaceans and their responses to change in their environment.

OBJECTIVES

Two objectives are being nested under the overall goal of developing techniques that can be used for the detection of stress and pregnancy in large whales.

1)The first objective is to develop and conduct analytical and preliminary biological validations of pregnancy and stress hormones for large whales (humpback whales, blue whales, and possibly insular false killer whales).

2) The second objective is to complete the biological validation using archived samples from whales with known life history or behaviors.

APPROACH

The project has a two year approach in which the first year will serve as the proof of concept and the second year will complete the validation and add in life history and behavior data. Details of each year's activities are separated below.

Year 1 - Commercially available radioimmunoassays (RIA) previously validated in other marine mammal species will be validated for corticosterone or cortisol and progesterone in humpback whale blubber. The validation will follow two forms, analytical and biological, described below.

Analytical Validation: The analytical validation will be conducted using standard methods of parallelism, accuracy and metabolite identification using high-pressure liquid chromatography (HPLC). Briefly, pooled blubber extract from animals of known gender will be serially diluted 1:2 and run as samples, in duplicate, in the assay to determine displacement by the pool. Accuracy of the assay will be determined by spiking the pool at the appropriate dilution with known amounts of hormone then performing the assays. Mass of the pool will be calculated and subtracted from the mass measured

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in the assay. The mean percent difference, standard error, and percent coefficient of variation, will be determined and taken as an index of accuracy of the assay. Mass added versus mass measured in the pool will be plotted in a standard scatter plot and simple regression analysis performed to determine slope, using the slope value as the index of precision of measurement of the assay. Final analytical validation of the assay will be determined through HPLC. All extracted blubber samples will be assayed in duplicate using the validated assays. Various forms of this analytical validation have been conducted over the years on a variety of biological media in our Endocrine Laboratory (Seltmann et.al., 2012; Trumble et al., 2012; Villegas-Amtmann et al., 2012; Verrier et al., 2012; Atkinson et al. 2011; Wang et al. 2010; Myers et al., 2009; Villegas-Amtmann et al., 2009; Nilsson et al., 2008; Atkinson et al., 2008a; Petrauskas et al., 2008; Mashburn and Atkinson, 2008; Mellish et al., 2007; Mashburn and Atkinson, 2007; Greig et al., 2007; Petrauskas and Atkinson, 2006; Petrauskas et al., 2006; Mashburn and Atkinson, 2004; Oki and Atkinson, 2004; West et al., 2000; Atkinson et al., 1999).

Biological Validation: The first part of the biological validation will begin with stranded whale samples from the National Marine Mammal Stranding Network or National Marine Fisheries Service (NMFS) from a humpback whale stranding (preferably in southeast Alaska). The preferred specimen will be an adult female, such that we can evaluate concentrations of corticoids and progesterone at different blubber depths as well as different locations on the body. Four locations along the axis of the whale will be assessed with both dorsal and ventrolateral samplings at each location. Each sampling will include a full depth core of blubber from the skin to the underlying muscle. Understanding the variation in concentrations at the various sites and in the various blubber depths will round out the validations so that we will have good confirmation (and confidence) that our measurements accurately reflect the biological variation that naturally occurs in any animal.

Year 2 - The second year of the proposal will round out the data sets and include samples from discreet seasons (and thus geography). The locations of primary interest are the winter breeding grounds in the Hawaiian Islands and the summer feeding grounds in southeast Alaska. These samples will allow us to assess the normal variations in endocrine profiles that can be expected at different times of year.

Samples from 4 main groups of whales will be sought with the following priority:

1) adult females with calves
2) adult females that may be pregnant
3) juvenile females
4) adult males

Samples with a maximum amount of life history and behavioral knowledge will be sought over samples with less corresponding information.

WORK COMPLETED

This project has started on time and is up to date in terms of our expectations. To date we have accomplishments in 3 major areas:

a) Sample identification, acquisition, and database compilation;

b) Initiation of the assay validations;

c) Communication with other ONR PIs.

Details are provided in the results section below.

RESULTS

a) Sample identification, acquisition and database compilation

To date the humpback whale sample database from Co-PI Adam Pack has be obtained. A preliminary list of humpaback whale samples was also obtained from Co-PI Jan Straley. Likewise, the list of blue whale samples to transport from Mexico (Co-PI Diane Gendron) has been obtained. Samples to conduct the depth study were obtained from NMFS (collaborator John Moran).

b) Assay validation

The assay validation procedures were intitiated with assay recovery and blubber depth studies. Samples were pooled using blubber, skin, and muscle tissue from large blubber chunks from 3 individual humpback whales. Four individual pools were created for blubber and two individual pools were created for both skin and muscle. In the case of muscle tissue, only two of the individuals had muscle tissue attached. Therefore, pools were created using the muscle tissues of those two individuals. Pools were both created and extracted at the same time individual samples for the depth study were created to minimize handling differences. Figure 1 is a photograph depicting the depth study core samplings. All samples were extracted using the extraction procedure outlined by Keller et al (2006).

Figure 1. Depth study core samplings.

All pools received 3H progesterone tracer prior to extraction procedures, and duplicate vials containing the same volume of tracer as samples, as well as blanks containing no tracer, were created at the same time. Each residue remaining after each extraction step was completely dried and brought up in 1ml 100% ethanol. From each 1 ml residue tube, 100 ul was removed in duplicate and aliquoted into scintillation vials and allowed to equilibrate overnight. The final extract, 2ml acetonitrile, also had 100 ul duplicate aliquots removed and placed into scintillation vials. After overnight equilibration, all scintillation vials were counted using a beta counter. Resultant cpm for each sample were corrected for total volume of sample, background and the cpm for the duplicates were averaged. Percent recoveries are expressed as a percentage of cpm recovered versus cpm added to the original sample (Table 1).

Table 1. Extraction recoveries for blubber, skin and muscle from humpback whales.

Sample ID	Mean Individual recovery %	Pools Mean recovery %	Pools Std Dev	Pools % CV
Blubber Pool 1	75.3			
Blubber Pool 2	81.5			
Blubber Pool 3	83.5			
Blubber Pool 4	82.5			
Mean Blubber		**80.7**	**3.7**	**4.6**
Skin Pool 1	80.6			
Skin Pool 2	66.5			
Mean Skin		**73.6**	**10.0**	**13.6**
Muscle Pool 1	76.8			
Muscle Pool 2	74.6			
Mean Muscle		**75.7**	**1.5**	**2.0**

c) Communication with other ONR PIs

The ONR Marine Mammal Program Review that was held in Arlingotn VA in April provided an excellent venue to learn details of the bigger program that our study is fitting into. Several subsequest interactions have taken place with other project PIs, including a draft preproposal to conduct an inter-laboratory calibration.

IMPACT/APPLICATIONS

The validated assays will be used to analyze stress and reproductive hormones to compare physiological function relative to proximity to anthropogenic disturbances such as noise.

RELATED PROJECTS

A new master's candidate, Ms Kelly Cates, has been recruited to the University of Alaska Fairbanks, School of Fisheries and Ocean Sciences to study physiology of male humpback whales. Her project will either focus on male reproduction or on stress responses in male humpback whales. She started about 4 weeks ago and over the next few months her project and its relation to our ONR project will be better defined.

REFERENCES

Atkinson S, Combelles, C., Vincent, D., Nachtigall, P. and Pawloski, J. 1999. Monitoring of progesterone in captive female false killer whales, pseudorca crassidens. Gen. Compar. Endocrinol. 115: 323-332.

Atkinson, S. and Yoshioka, M. 2007. Endocrinology of Reproduction. In "Reproductive Biolo gy and Phylogeny of Cetacea" (B.G.M. Jamieson Ed.) Science Publishers, Inc. Enfield, New Hampshire 7. 171-192.

Atkinson, S., Arnould, J. P. Y., Mashburn, K. 2011. Plasma cortisol and thyroid hormone concentrations in pre-weaning Australian fur seal pups. Gen. Comp. Endocrinol. 172: 277-281.

Greig, D.J., Mashburn, K.L., Rutishauser, M., Gulland, F.M.D., Willliams, T.M., and Atkinson, S. 2007. Seasonal changes in circulating progesterone and estrogen concentrations in the California sea lion (Zalophus californianus) J. Mammal. 88(1): 67-72.

Kellar NM, Trego, M.L., Marks, C.I., Dizon, A.E. 2006. Determining pregnancy from blubber in three species of delphinids. Mar. Mammal Sci. 22(1): 1-16.

Mashburn K.L, Atkinson, S. 2004. Evaluation of adrenal function in serum and feces of Steller sea lions (Eumetopias jubatus): influences of molt, gender, sample storage, and age on glucocorticoid metabolism. Gen. Compar. Endocrinol. 136: 371-381.

Mashburn K.L., Atkinson, S. 2007. Seasonal and predator influences on adrenal function in adult Steller sea lions: Gender matters. Gen. Com. Endocrinol. 150: 246-252.

Mashburn, K.L., and Atkinson, S. 2008.. Variability in leptin and adrenal response in juvenile Steller sea lions (Eumetopias jubatus) to adrenocorticotropic hormone (ACTH) in different seasons. Gen. Comp. Endocrinol. 155(2): 352-385.

Mellish, J., Hennen, D., Thomton, J., Petrauskas, L., Atkinson, S. and Calkins, D. 2007. Permanent marking in an endangered species: Physiological response to hot-branding in juvenile Steller sea lions (Eumetopias jubatus). Wildl. Res. 34:43-47.

Myers, M. Litz, B. and Atkinson, S. 2009. The effects of age, season and geographic region on circulating serum cortisol concentrations in threatened and endangered Steller sea lions (Eumetopias jubatus). Gen. Comp. Endocrinol. 165: 72-77.

Nilsson, P., Hollmén, T., Atkinson, S., Mashburn, K., Tuomi, P., Esler, D., Mulcahy, D. and Rizzolo, D. 2008.. Effects of ACTH, capture, and short term confinement on glucocorticoid concentrations in harlequin ducks (Histrionicus histrionicus). Comp. Biochem. Physiol. 149(1): 275-283.

Oki, C. and Atkinson, S. 2004. Diurnal patterns of cortisol and thyroid hormones in the harbor seal (Phoca vitulina) during summer and winter seasons. Gen. Comp. Endocrinol. 136:289-297.

Petrauskas, L., Atkinson, S., Gulland, F., Mellish, J.A. and Horning, M. 2008. Monitoring glucocorticoid response to rehabilitation and research procedures in California and Steller sea lions. J. Exper. Biol. 309A: 73-82.

Petrauskas, L., Tuomi, P., Atkinson, S. 2006. Noninvasive monitoring of stress hormone levels in a female Steller sea lion (Eumetopias jubatus) pup undergoing rehabilitation. J. Zoo and Wildl. Med.37 (1): 75-78.

Petrauskas, L.R. and Atkinson, S. 2006. Variation of fecal corticosterone concentrations in captive Steller sea lions (Eumetopias jubatus) in relation to season and behavior. Aqua. Mamm. 32(2) 168-174.

Pietraszek J, Atkinson, S. 1994. Concentrations of estrone sulfate and progesterone in plasma and saliva, vaginal cytology, and bioelectric impedance during the estrous cycle of the Hawaiian monk seal (Monachus schauinslandi) Mar. Mammal Sci. 10(4): 430-441.

Seltmann, M.W., Ost, M., Jaatinen, K., Atkinson, S., Mashburn, K., Hollmen, T. 2012. Stress responsiveness, age and condition interactively affect risk taking in female eiders. Anim. Behav.84: 889-896.

Trumble, S.J., O'Neil, D.O., Cornick, L.A., Gulland, F., Castellini, M.A., Atkinson, S. 2012. Changes during the rehabilitation process elicit endocrine responses in developing harbor seal (Phoca vitulina) pups. Zoo Biol. 32: 134-141.

Verrier, D., Atkinson, S., Guinet, C., Groscolas, R., Arnould, J.P.Y. 2012. Hormonal responses to extreme fasting in subantarctic fur seal (Arctocephalus tropicalis) pups. Am. J. Physiol-Reg I. 302: R929-R940.

Villegas-Amtmann, S., Atkinson, S. and Costa, D.P. 2009. Low synchrony in the breeding cycle in Galapagos sea lions revealed by seasonal progesterone concentrations. J. Mammal. 90(5): 1232-1237.

Villegas-Amtmann, S., Atkinson, S. Costa, D.P. 2012. Seasonal variation in muscle oxygen stores attributed to diving behavior, environmental temperature and pregnancy in a marine predator the California sea lion. Comp. Biochem. Physiol. Part A.162: 413-420.

Wang, D., Shelver, W.L., Atkinson, S., Mellish, J.A., and Li, Q.X. 2010. Tissue distribution of polychlorinated biphenyls and organochlorine pesticides and potential toxicity to Alaskan northern fur seals assessed using PCBs congener specific mode of action schemes. Arch. Environ. Contam. Toxicol. 58: 478-488.

West, K.L., Atkinson, S., Carmichael, M.J., Sweeney, J.C., Krames, B., and Krames, J. 2000. Concentrations of progesterone in milk from bottlenose dolphin during different reproductive status. Gen. Comp. Endocrinol.117: 218-224.