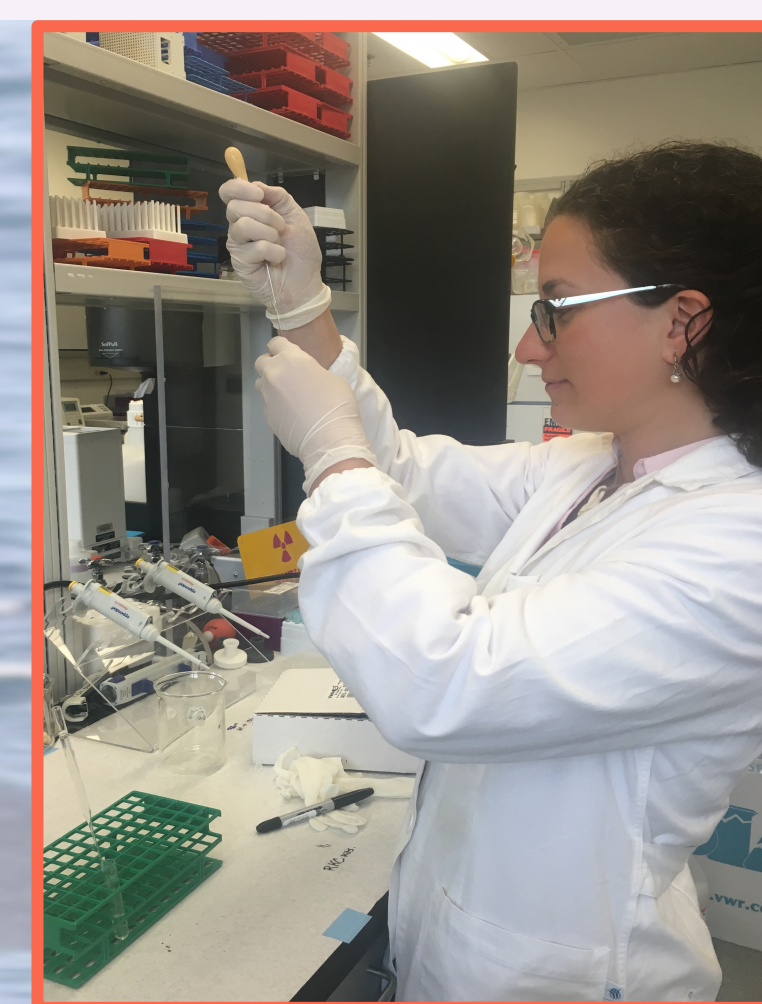


REPRODUCTIVE ENDOCRINE PROFILES IN BLUE WHALES FROM THE EASTERN NORTH PACIFIC OCEAN

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Introduction

- Blue whales in the eastern North Pacific Ocean are recovering from commercial whaling. Their habitat extends from central America (winter) to the US and Canada West Coast and the Gulf of Alaska (summer) (Calambokidis *et al.* 2009).
- Reproductive parameters are fundamental to understand population growth and dynamics. To date, crude birth rate and calving interval for blue whales are based only on observational studies (Sears *et al.* 2013).
- Steroid hormones, like progesterone and testosterone, are considered effective biomarkers for assessing reproductive processes (Mansour *et al.* 2002; Vu *et al.* 2015) and can be measured in a variety of tissue matrices (de Mello & de Oliveira 2016).

STUDY OBJECTIVES

1. Define **progesterone** (a) and **testosterone** (b) profiles across reproductive states and seasons in blubber of blue whales.
2. Assess the feasibility of **blubber preserved in DMSO** for endocrine analysis.

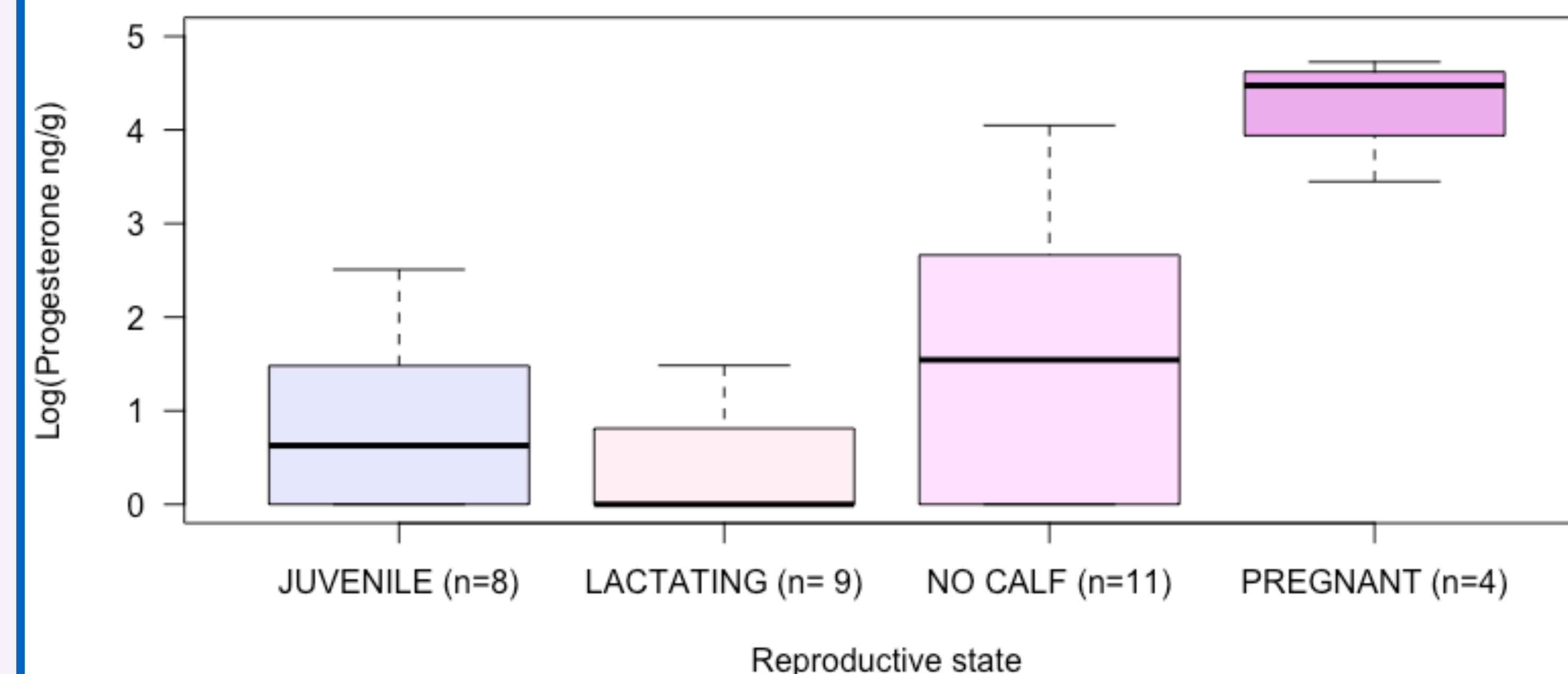


CRC ID 331 Photo credits: Don Gillies and Channel Islands National Marine Sanctuary

Methods

- Blubber samples collected from the US West Coast and the Sea of Cortez between 2002 and 2013 were analyzed for progesterone and testosterone using Enzyme Immunoassay (EIA) (Atkinson *et al.* in review).
- Frozen blubber of a known pregnant female was placed in Dimethyl sulfoxide (DMSO) and then analyzed after 2-, 4- and 8-weeks.
- Photo ID catalogs were used to determine age (based on year of first sighting) and reproductive states (i.e., if with calf) (Gendron & Ugalde de la Cruz, 2012; Calambokidis *et al.* 2009).

Objective 1a: Progesterone as indicators of reproductive status in females

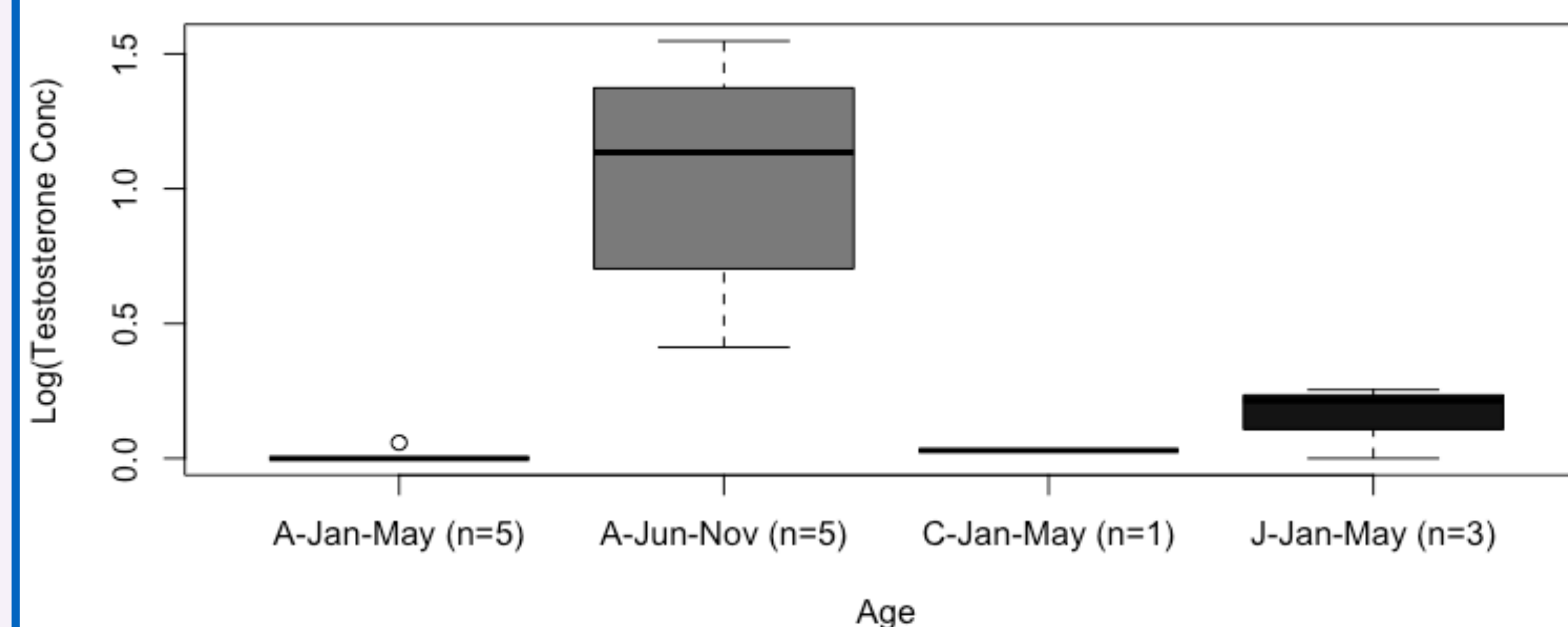


Progesterone concentrations in blubber (log-transformed) were significantly different (ANOVA p-value <0.05) among reproductive states in female blue whales. Values below the limit of detection (avg 25 pg/ml) were considered 0.



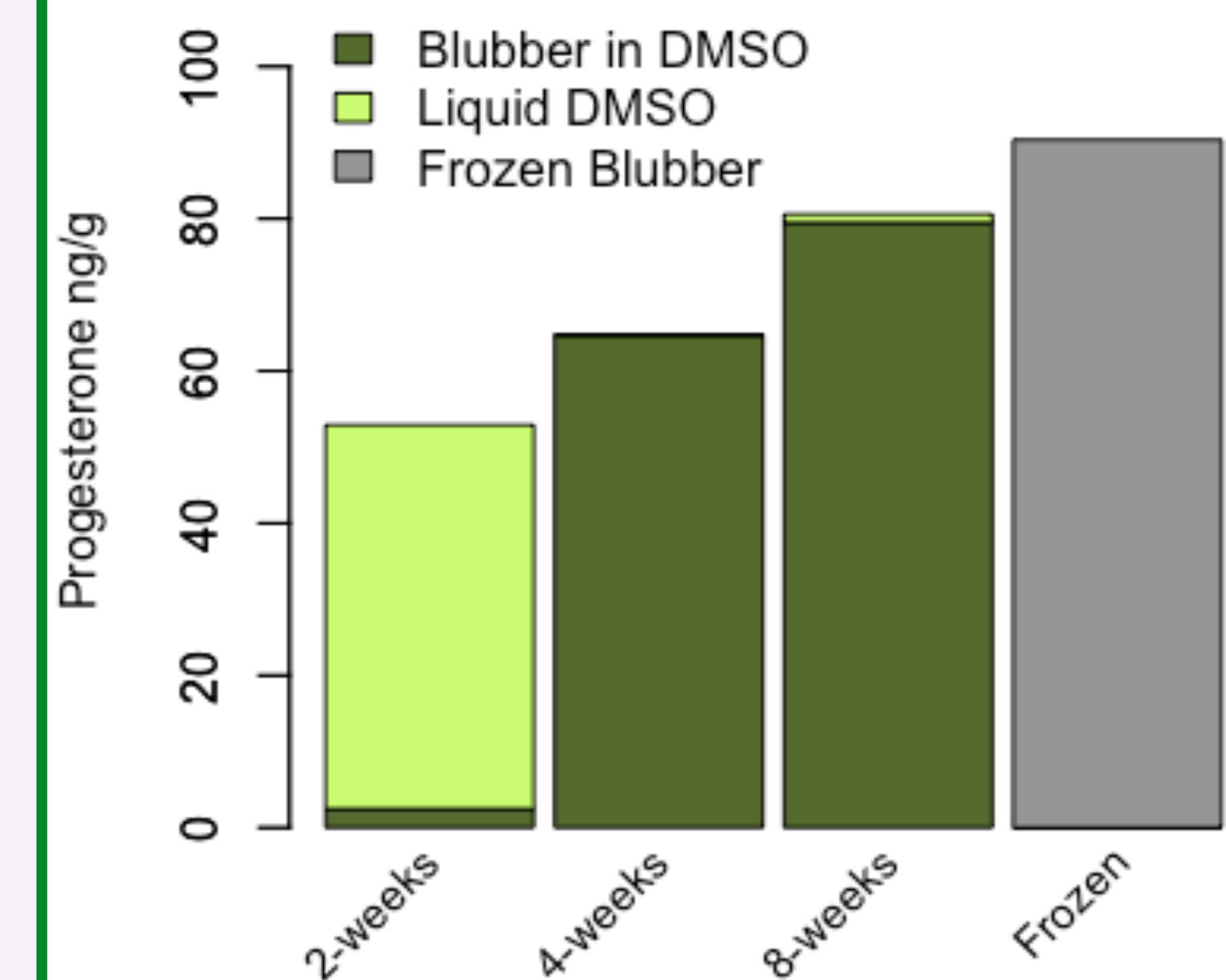
Photo credit : CICIMAR

Objective 1b: Testosterone profiles in males



Testosterone concentrations in blubber (log-transformed) varies across age (A=Adult, C= Calf, J= Juvenile) and season. Values below the calculated assay limit of detection (3.2 pg/ml) were considered 0.

Objective 2: Detection of progesterone in blubber preserved in DMSO



Validation for blubber in DMSO and liquid DMSO:

- **Parallelism test:** serial dilutions of extracts show parallelism to the standards of progesterone.
- **Accuracy check:**
 - Blubber in DMSO: $y = 0.83x + 0.17$; $R^2=0.99$
 - Liquid DMSO: $y = 0.9x - 38.51$; $R^2=0.98$

Results and Discussion

- Progesterone concentrations can be used as indicator of reproductive state. Pregnant whales have high progesterone, while juvenile and lactating females show low concentrations.
- Testosterone does change between season and is lower in males from the Gulf of California.
- Progesterone in blubber preserved in DMSO can be accurately detected and measured with EIA, in quantities comparable with those detected in frozen blubber.