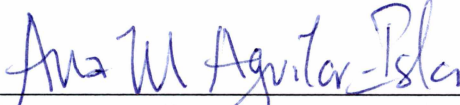


STELLER SEA LION SURVIVORS: A RETROSPECTIVE ON THE IMPACT OF  
ALTERNATIVE RESEARCH METHODS ON AN ENDANGERED SPECIES

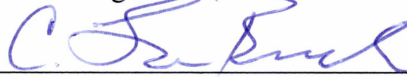
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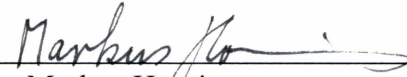
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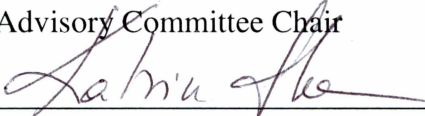
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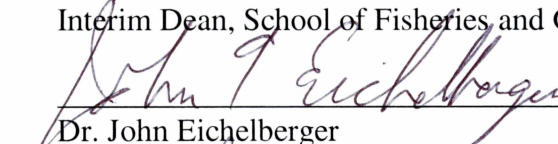
  
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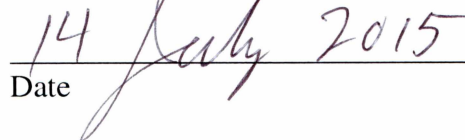
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STELLER SEA LION SURVIVORS: A RETROSPECTIVE ON THE IMPACT OF  
ALTERNATIVE RESEARCH METHODS ON AN ENDANGERED SPECIES

A  
THESIS

Presented to the Faculty  
of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements  
for the Degree of

MASTER OF SCIENCE

By

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Fairbanks, Alaska

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## ABSTRACT

Two novel research approaches were developed to facilitate access to wild juvenile Steller sea lions. First, the Transient Juvenile Steller sea lion Project (TJ) facilitated numerous studies of physiology, behavior, and nutrition through temporary captivity (branded TJs,  $n=45$ ) over the past decade. As a complement, a control group was sampled and released during capture events (FRs,  $n=35$ ). Second, the Life History Tag (LHX) project was implemented within the TJ project to implant individuals (LHX-1,  $n=35$ ) with internal transmitters to detect potential causes of mortality. Our goal was to evaluate the potential for long-term impacts of these two research programs on study individuals (Chapter 1) as well as identify potential metrics of survival for use in field efforts (Chapter 2). The first chapter used open-population Cormack-Jolly-Seber (CJS) mark-recapture models to project survival from resights of branded individuals in combination with demographic covariates in program MARK. TJ and FR groups were compared to identify the potential effect of temporary captivity on survival, while LHX-1 and non-implants were compared to examine a tagging effect on survival. Overall, our results mirror previous efforts to characterize survival in sea lions and indicate minimal long-term effects on mortality from research efforts, higher survival in females than males, and increasing survival rate with age. For the second chapter, a three-tiered approach to the decade of archived physiological data attempted to build links to survival in TJs through similar CJS modeling techniques. The first two levels looked at survival in relation to observed responses of handling stress through six *a priori* principle blood parameters measured at entry and exit. In addition, several condition indices were also incorporated into mark-recapture models, but separately considered when measured at entry and exit due to sampling inconsistencies. The third level evaluated the efficacy of single-point sampling to project similar trends for field use. Change in mass (kg) and white blood cell count (WBC,  $m/mm^3$ ) had the most support in predicting survival. Mass gains

over captivity and slight increases in WBC resulted in a higher averaged survival rate. Minor support was identified for exit mass and entry WBC. A higher exit mass predicted a higher survival rate, while a higher entry WBC predicted a lower survival rate and may demonstrate the efficacy of single-point sampling as a management tool.

## Dedication Page

This thesis is dedicated to my parents who instilled a love of marine science following their careers as oceanographers and always encouraged me to follow my passions to enrich and contribute to the world of science, knowing full well the struggle of a life in academia. I would also like to dedicate this to the many other mentors, be they fellow students or managing supervisors, who have advised me over the years from my first trip abroad in high school to study biodiversity in Costa Rica, to my first job as a teaching assistant helping fellow students delve into vertebrate anatomy and taxonomy, and on to my first real research experience at the Alaska SeaLife Center and everywhere in between. The names are too many, and the thanks will always be too few to these individuals who pushed me to be better and taught me everything they know about science and humanity.



# Table of Contents

	Page
Signature Page.....	i
Title Page.....	iii
Abstract.....	v
Dedication Page.....	vii
Table of Contents.....	ix
List of Figures.....	xiii
List of Tables.....	xv
Acknowledgements.....	xvii
General Introduction.....	1
Literature Cited.....	7
Chapter 1 Long-term Survival of Temporarily Captive Steller Sea Lions ( <i>Eumetopias jubatus</i> ) and the Implications of Novel Research Activities.....	11
1.1 Abstract.....	11
1.2 Introduction.....	12
1.2.1 General Overview.....	12
1.2.2 The Transient Juvenile Steller Sea Lion Project.....	12
1.2.3 Mark-recapture.....	13
1.2.4 Study Goals.....	15
1.3 Methods.....	15
1.3.1 Study Animals.....	15
1.3.2 Resights and Source Agencies.....	16



	Page
1.3.3 Model Development.....	17
1.4 Results.....	18
1.5 Discussion.....	20
1.5.1 Demographic Long-term Survival.....	20
1.5.2 Temporary Captivity.....	22
1.5.3 Life History Tag Implants.....	23
1.5.4 Other Considerations and Conclusions.....	25
Figures.....	27
Tables.....	28
Literature Cited.....	36
Chapter 2 Physiological Predictors of Long-term Survival in Steller sea lions ( <i>Eumetopias jubatus</i> ).....	41
2.1 Abstract.....	41
2.2 Introduction.....	42
2.2.1 General Overview.....	42
2.2.2 Health and Survival.....	42
2.2.3 Physiological Predictors.....	43
2.2.4 Study Goals.....	44
2.3 Methods.....	45
2.3.1 Study Animals.....	45
2.3.2 Blood Parameters.....	46
2.3.3 Body Condition.....	46

	Page
2.3.4 Survival Analysis.....	47
2.4 Results.....	49
2.5 Discussion.....	50
2.5.1 Changes in Mass.....	50
2.5.2 Leukocytes.....	51
2.5.3 Single-point Measures.....	52
2.5.4 Implications.....	53
Figures.....	55
Tables.....	56
Literature Cited.....	57
General Conclusions.....	65
Literature Cited.....	68



## List of Figures

	Page
Figure 1.1. Age-specific survival rates.....	27
Figure 2.1. Predicted model-averaged survival rates based on continuous covariates.....	55



## List of Tables

	Page
Table 1.1a. Model selection results for demographics only.....	28
Table 1.1b. Model selection results for temporary captivity.....	29
Table 1.1c. Model selection results for LHX implants.....	30
Table 1.2. Model-averaged apparent survival rates.....	31
Table 1.3a. Beta values for demographic survival models.....	32
Table 1.3b. Beta values for temporary captivity survival models.....	33
Table 1.3c. Beta values for LHX-1 survival models.....	34
Table 1.4. Model-averaged resighting probabilities.....	35
Table 2.1. Candidate physiological model set for predicting survival.....	56



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## GENERAL INTRODUCTION

Steller sea lions (*Eumetopias jubatus*), the largest and northernmost of the Otariids, have been the focus of a suite of intense studies due to the precipitous declines of certain population segments. Hypotheses as to the cause of the original decline are generally classified as either anthropogenic or naturally sourced, and broad ranging, including nutritional stress in relation to climate shifts, competition with fisheries, predation, infectious disease, contaminant exposure, and even as part of a sequential collapse from commercial whaling in the North Pacific (York 1994; Merrick *et al.* 1997; Bickham *et al.* 1998; Holmes & York 2003; Springer *et al.* 2003; Burek *et al.* 2005; Fritz and Hinckley 2005; Pendleton *et al.* 2006; Trites *et al.* 2007; Atkinson *et al.* 2008). While many combinations of stressors have been identified that may have contributed to the decline, the failure of this listed species to recover continues to receive attention. During the first decade of the 2000s, the primary focus of most research efforts was on understanding juvenile survival, or the lack thereof (York 1994; Raum-Suryan *et al.* 2004; Pendleton *et al.* 2006; Horning & Mellish 2009, 2012). With no obvious link to the observed population dynamics, a conceptual shift to the examination of natality as a primary impediment to population recovery in the western distinct population segment (wDPS) occurred towards 2010 (Holmes *et al.* 2007; Maniscalco *et al.* 2010; Horning & Mellish 2012; Maniscalco 2014). While the eDPS has recently been de-listed from the Endangered Species Act, the wDPS remains endangered (National Marine Fisheries Service 2013).

### *Novel Research Techniques for Wild Individuals*

Two novel approaches were developed to facilitate study of the endangered wDPS. These approaches were designed to overcome the inherent difficulty of data collection from a

species that spends the majority of their life at sea, through the use of temporary captivity and implanted data loggers.

The Transient Juvenile (TJ) project, initiated in 2001, used a temporary captivity model to allow for the accessibility and ability for multiple handling events on wild individuals (Mellish *et al.* 2006). Between 2003 and 2011, 65 juveniles ranging in age from one to three years were captured at haul-out sites in Prince William Sound and Resurrection Bay in south central Alaska. These TJ individuals were maintained at the quarantine South Beach facility at the Alaska SeaLife Center in Seward, Alaska, for up to a three month period. The health of these animals was closely monitored throughout their stay and compared to baseline health and condition parameters of wild conspecifics (Goldstein *et al.* 2007). Numerous studies included nutritional, physiological, and behavioral baseline parameters, in addition to specific dietary manipulations and monitoring of stress and immune responses to research procedures (Mellish *et al.* 2007a; Petrauskas *et al.* 2008; Walker *et al.* 2009, 2010, 2011a, 2011b).

Juvenile sea lions spend vast amounts of time at sea, as they do not yet gather predictably on breeding rookeries in the summer months. This has led to little information on their physiology and critical gaps in knowledge on their survival. The Life History Transmitter (LHX) was developed to detect mortality events specifically in Steller sea lions (Horning & Hill 2005). LHX are abdominally-implanted data-loggers that regularly measure and record temperature, light, and the surrounding medium (Horning *et al.* 2008). These tags transmit data via satellite only after they are extruded from the host body, post mortem. The history of these logged measures can give a strong indication as to the nature of the mortality, be it predation or otherwise (Horning *et al.* 2008). In contrast to an external satellite transmitter attachment, which

is limited by the annual molt, internal LHX tags yield absolute survival data (known-fate) for monitoring periods of 10 years and beyond (Horning & Mellish 2012). Phase 1 of this project (2001-2011) deployed dual LHX tags in 35 individuals participating in the TJ project (Horning *et al.* 2008; Horning & Mellish 2009). Previous evidence of mortality due to predation was primarily anecdotal; however, the LHX tags allow for unique evidence through end of life temperature curves. Patterns in these data provided the opportunity to speculate on the predator, such as the orca (*Orcinus orca*, Maniscalco *et al.* 2007; Horning & Mellish 2012). More recent results have also suggested predation by the Pacific sleeper shark (*Somniosus pacificus*, Horning & Mellish 2014). This finding gives new perspective on a mechanism that was not previously considered to contribute significantly to sea lion mortality rates.

Temporary captivity has not been attempted for many marine mammals, and it is particularly rare for an endangered species, due to the potential for habituation or disease transmission from temporarily captive to wild animals. Because of this, there was an unprecedented effort to document the physiological and behavioral impacts of the research protocols. Baselines for health and disease monitoring were established at the start of the program and monitored extensively for anomalies in every animal. Each sea lion had to pass an initial physical exam prior to transport to the quarantine facility and was sampled frequently (e.g., up to weekly) during their residence period. Ultimately, there was no significant difference in disease risk titer levels found between those animals that underwent temporary captivity and their free-ranging counterparts (Mellish *et al.* 2006). Some minor differences were noted during captivity, such as changes in inflammatory response as indicated by such things as white blood cell counts, likely due to the initial handling and transport events (Thomton & Mellish 2007). Behavioral and physiological responses to the protocol of hot-iron branding were studied over

longer monitoring periods than is possible with traditional field-based tagging (Mellish *et al.* 2007a; Walker *et al.* 2010, 2011b). A generalized inflammatory response was noted, and wound sensitivity behaviors were found to return to pre-brand baselines a few days after. Given the short duration of this inflammatory response, it appears that hot-iron branding does not have any apparent long-term or deleterious effects. Similar efforts to monitor responses to LHX surgical events were also undertaken, with similar returns to pre-surgical baseline levels for all physiological parameters measured and known to be indicative of wound healing response within a few weeks (Mellish *et al.* 2007b; Horning *et al.* 2008). Behavioral responses of individuals following surgery indicated signs of discomfort, but were found to dissipate and return to pre-surgery levels in the following weeks of captivity, independent of analgesic regime (Walker *et al.* 2009, 2011a). Post-release tracking of all sea lions through externally mounted satellite data transmitters showed no difference in post-treatment diving and ranging behavior compared to free-ranging controls (Mellish *et al.* 2007a; Thomson *et al.* 2008).

#### *Documenting Long-term Survival*

The effect of handling and experimental treatments on individual survival is typically only analyzed for short periods after a sampling event since it is generally assumed that the magnitude of response and its effect on survival will decrease thereafter. However, some studies have shown that this may not be the case and highlight the need for individual assessments. Flipper banding is a relatively common, non-invasive tagging technique for population studies in various species of penguins. Yet, it has been shown that penguin survival and breeding success is significantly reduced by banding, and that the overall energetic costs of wearing bands per individual increases (Gauthier-Clerc *et al.* 2004; Le Maho *et al.* 2011). Resight studies of Steller sea lions have been used to document population structure, size estimates, development, inter-

population movement, and survival (Raum-Suryan *et al.* 2002; Raum-Suryan *et al.* 2004; Pendleton *et al.* 2006; Hastings *et al.* 2009; Maniscalco *et al.* 2010; Jemison *et al.* 2013). These types of studies allow us to infer information of individual and population dynamics over the long-term utilizing the basic principles of a mark-recapture analysis (Seber 1986; White & Burnham 1999; Bonner *et al.* 2010). A set number of individuals are marked with a unique identifier, either a hot-iron brand on the left flank or a small flipper tag (Pitcher & Calkins 1981; Holmes *et al.* 2007; Hastings *et al.* 2009; Jemison *et al.* 2013). Unique scarring and fungal patch patterning may also be used to identify and track individuals over time (Maniscalco *et al.* 2006). However, hot-iron branding remains the ideal marking technique in sea lions as it allows for resights to take place at a greater distance and with greater accuracy than other techniques (Merrick *et al.* 1996; Wilkinson *et al.* 2011; Walker *et al.* 2012). In the particular case of the TJ and LHX projects, an extensive number of resights from branded animals exist with the addition of multiple pre-release sampling time points of health and physiological function. This allows not only for tracking long-term survival through a traditional mark-resight approach, but to also answer questions not possible with a single handling event. While short-term survival and behavior provided no indication of an effect, long-term survival must be evaluated in order to fully understand the impacts of our research activities.

### *Evaluating and Predicting Long-term Survival*

I evaluated the long-term effects of these research techniques on wild juvenile Steller sea lions and developed recommendations for future generations of work. First, I generated survival rates for Steller sea lions released from the TJ project using mark-recapture methods from resight data ( $n=45$ ). TJs were compared to a set of branded individuals that were sampled under restraint, but released immediately after capture ( $n=30$ , Free-ranger - FRs). A subset of the 45

branded TJs that received the LHX implants ( $n=35$ ) were also evaluated and compared to survival rates of the remaining 10 TJs. I compared these survival rates to those of other institutions monitoring the vital rates of the entire population. Second, I examined potential physiological predictors of long-term survival by modeling survival through health parameters collected from serology, serum clinical chemistry, morphometrics and body condition measures. Physiological indicators were integrated over captivity time to evaluate the ability of an individual to deal with stressful events (e.g., capture, LHX implantation). The overall goal was to investigate the possibility of constructing a predictive model of long-term survival using these physiological predictors and for their utility in field-based sampling constraints.

Results from this analysis may inform wildlife biologists and resource managers on the appropriate application of temporary captivity for Steller sea lions and to other species that present similar logistic challenges. The potential to identify predictors of survival from commonly measured physiological parameters may become an important tool for efficient monitoring and projecting of Steller sea lion population health from juvenile age classes. *In situ* assessments of population health will refine research approaches and facilitate informed management decisions.

## Literature Cited

- Atkinson, S., Demaster, D.P. & Calkins, D.G. (2008). Anthropogenic causes of the western Steller sea lion *Eumetopias jubatus* population decline and their threat to recovery. *Mammal Review*, **38**, 1–18.
- Bickham, J.W., Loughlin, T.R., Calkins, D.G., Wickliffe, J.K. & Patton, J.C. (1998). Genetic variability and population decline in Steller sea lions from the Gulf of Alaska. *Journal of Mammalogy*, **79**, 1390–1395.
- Bonner, S.J., Morgan, B.J.T. & King, R. (2010). Continuous covariates in mark-recapture-recovery analysis: A comparison of methods. *Biometrics*, **66**, 1256–1265.
- Burek, K.A., Gulland, F.M.D., Sheffield, G., Beckmen, K.B., Keyes, E., Spraker, T.R., Smith, A.W., Skilling, D.E., Evermann, J.F., Stott, J.L., Saliki, J.T. & Trites, A.W. (2005). Infectious disease and the decline of Steller sea lions (*Eumetopias jubatus*) in Alaska, USA: Insights from serologic data. *Journal of Wildlife Diseases*, **41**, 512–524.
- Fritz, L.W. & Hinckley, S. (2005). A critical review of the regime shift – “junk food” – nutritional stress hypothesis for the decline of the western stock of Steller sea lion. *Marine Mammal Science*, **21**, 476–518.
- Gauthier-Clerc, M., Gendner, J.P., Ribic, C.A., Fraser, W.R., Woehler, E.J., Descamps, S., Gilly, C., Le Bohec, C. & Le Maho, Y. (2004). Long-term effects of flipper bands on penguins. *Proceedings of the Royal Society of London, Series B*, **271**, S423–426.
- Goldstein, T., Stephens, C.A., Jang, S.S., Conrad, P.A., Field, C., Dunn, L.J. & Mellish, J.E. (2007). Longitudinal health and disease monitoring in juvenile Steller sea lions (*Eumetopias jubatus*) in temporary captivity in Alaska compared with a free-ranging cohort. *Aquatic Mammals*, **33**, 337–348.
- Hastings, K.K., Gelatt, T.S. & King, J.C. (2009). Postbranding survival of Steller sea lion pups at Lowrie Island in southeast Alaska. *Journal of Wildlife Management*, **73**, 1040–1051.
- Holmes, E.E., Fritz, L.W., York, A.E. & Sweeney, K. (2007). Age-structured modeling reveals long-term declines in the natality of western Steller sea lions. *Ecological Applications*, **17**, 2214–2232.
- Holmes, E.E. & York, A.E. (2003). Using age structure to detect impacts on threatened populations: A case study with Steller sea lions. *Conservation Biology*, **17**, 1794–1806.
- Horning, M., Haulena, M., Tuomi, P.A. & Mellish, J.-A.E. (2008). Intraperitoneal implantation of life-long telemetry transmitters in otariids. *BMC Veterinary Research*, **4**, 51.



- Horning, M. & Hill, R.D. (2005). Designing an archival satellite transmitter for life-long deployments on oceanic vertebrates: The Life History Transmitter. *IEEE Journal of Oceanic Engineering*, **30**, 807–817.
- Horning, M. & Mellish, J.-A.E. (2014). In cold blood: evidence of Pacific sleeper shark (*Somniosus pacificus*) predation on Steller sea lions (*Eumetopias jubatus*) in the Gulf of Alaska. *Fishery Bulletin*, **112**, 297–310.
- Horning, M. & Mellish, J.-A.E. (2012). Predation on an upper trophic marine predator, the Steller sea lion: evaluating high juvenile mortality in a density dependent conceptual framework. *PloS ONE*, **7**, e30173.
- Horning, M. & Mellish, J.-A.E. (2009). Spatially explicit detection of predation on individual pinnipeds from implanted post-mortem satellite data transmitters. *Endangered Species Research*, **10**, 135–143.
- Jemison, L.A., Pendleton, G.W., Fritz, L.W., Hastings, K.K., Maniscalco, J.M., Trites, A.W. & Gelatt, T.S. (2013). Inter-population movements of Steller sea lions in Alaska with implications for population separation. *PLoS ONE*, **8**, e70167.
- Le Maho, Y., Saraux, C., Durant, J.M., Viblanc, V.A., Gauthier-Clerc, M., Yoccoz, N.G., Stenseth, N.C. & Le Bohec, C. (2011). An ethical issue in biodiversity science: the monitoring of penguins with flipper bands. *Comptes Rendus Biologies*, **334**, 378–84.
- National Marine Fisheries Service (2013). Status Review of The Eastern Distinct Population Segment of Steller Sea Lion (*Eumetopias jubatus*). 144pp + Appendices. Protected Resources Division, Alaska Region, National Marine Fisheries Service, 709 West 9th St, Juneau, Alaska 99802.
- Maniscalco, J.M. (2014). The effects of birth weight and maternal care on survival of juvenile Steller sea lions (*Eumetopias jubatus*). *PloS ONE*, **9**, e96328.
- Maniscalco, J.M., Matkin, C.O., Maldini, D., Calkins, D.G. & Atkinson, S. (2007). Assessing killer whale predation on Steller sea lions from field observations in Kenai Fjords, Alaska. *Marine Mammal Science*, **23**, 306–321.
- Maniscalco, J.M., Parker, P. & Atkinson, S. (2006). Interseasonal and interannual measures of maternal care among individual Steller sea lions (*Eumetopias jubatus*). *Journal of Mammalogy*, **87**, 304–311.
- Maniscalco, J.M., Springer, A.M. & Parker, P. (2010). High natality rates of endangered Steller sea lions in Kenai Fjords, Alaska and perceptions of population status in the Gulf of Alaska. *PloS ONE*, **5**, e10076.

- Mellish, J.-A.E., Calkins, D.G., Christen, D.R., Horning, M., Rea, L.D. & Atkinson, S.K. (2006). Temporary captivity as a research tool: comprehensive study of wild pinnipeds under controlled conditions. *Aquatic Mammals*, **32**, 58–65.
- Mellish, J.-A., Hennen, D., Thomson, J., Petrauskas, L., Atkinson, S. & Calkins, D. (2007a). Permanent marking in an endangered species: physiological response to hot branding in Steller sea lions (*Eumetopias jubatus*). *Wildlife Research*, **34**, 43–47.
- Mellish, J.-A., Thomson, J. & Horning, M. (2007b). Physiological and behavioral response to intra-abdominal transmitter implantation in Steller sea lions. *Journal of Experimental Marine Biology and Ecology*, **351**, 283–293.
- Merrick, R.L., Chumbley, M.K. & Byrd, G.V. (1997). Diet diversity of Steller sea lions (*Eumetopias jubatus*) and their population decline in Alaska: a potential relationship. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 1342–1348.
- Merrick, R.L., Loughlin, T.R. & Calkins, D.G. (1996). Hot branding : a technique for long-term marking of pinnipeds. US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service [Alaska Fisheries Science Center], 1–21.
- Pendleton, G.W., Pitcher, K.W., Fritz, L.W., York, A.E., Raum-Suryan, K.L., Loughlin, T.R., Calkins, D.G., Hastings, K.K. & Gelatt, T.S. (2006). Survival of Steller sea lions in Alaska : a comparison of increasing and decreasing populations. *Canadian Journal of Zoology*, **84**, 1163–1172.
- Petrauskas, L., Atkinson, S., Gulland, F., Mellish, J.-A. & Horning, M. (2008). Monitoring glucocorticoid response to rehabilitation and research procedures in California and Steller sea lions. *Journal of Experimental Zoology*, **309A**, 73–82.
- Pitcher, K.W. & Calkins, D.G. (1981). Reproductive biology of Steller sea lions in the Gulf of Alaska. *Journal of Mammalogy*, **62**, 599–605.
- Raum-Suryan, K.L., Pitcher, K.W., Calkins, D.G., Sease, J.L. & Loughlin, T.R. (2002). Dispersal, rookery fidelity, and metapopulation structure of Steller sea lions (*Eumetopias jubatus*) in an increasing and decreasing population in Alaska. *Marine Mammal Science*, **18**, 746–764.
- Raum-Suryan, K.L., Rehberg, M.J., Pendleton, G.W., Pitcher, K.W. & Gelatt, T.S. (2004). Development of dispersal, movement patterns, and haul-out use by pup and juvenile Steller sea lions (*Eumetopias jubatus*) in Alaska. *Marine Mammal Science*, **20**, 823–850.
- Seber, G.A.F. (1986). A review of estimating animal abundance. *Biometrics*, **42**, 267–292.

- Springer, A.M., Estes, J.A., van Vliet, G.B., Williams, T.M., Doak, D.F., Danner, E.M., Forney, K.A. & Pfister, B. (2003). Sequential megafaunal collapse in the North Pacific Ocean: an ongoing legacy of industrial whaling? *Proceedings of the National Academy of Sciences*, **100**, 12223–12228.
- Thomton, J.D. & Mellish, J.-A.E. (2007). Haptoglobin concentrations in free-range and temporarily captive juvenile Steller sea lions. *Journal of Wildlife Diseases*, **43**, 258–261.
- Thomton, J.D., Mellish, J.-A.E., Hennen, D.R. & Horning, M. (2008). Juvenile Steller sea lion dive behavior following temporary captivity. *Endangered Species Research*, **4**, 195–203.
- Trites, A.W., Deecke, V.B., Gregr, E.J., Ford, J.K.B. & Olesiuk, P.F. (2007). Killer whales, whaling, and sequential megafaunal collapse in the North Pacific: a comparative analysis of the dynamics of marine mammals in Alaska and British Columbia following commercial whaling. *Marine Mammal Science*, **23**, 751–765.
- Walker, K.A., Horning, M., Mellish, J.-A.E. & Weary, D.M. (2009). Behavioural responses of juvenile Steller sea lions to abdominal surgery: Developing an assessment of post-operative pain. *Applied Animal Behaviour Science*, **120**, 201–207.
- Walker, K.A., Horning, M., Mellish, J.-A.E. & Weary, D.M. (2011a). The effects of two analgesic regimes on behavior after abdominal surgery in Steller sea lions. *The Veterinary Journal*, **190**, 160–4.
- Walker, K.A., Mellish, J.-A.E. & Weary, D.M. (2010). Behavioural responses of juvenile Steller sea lions to hot-iron branding. *Applied Animal Behaviour Science*, **122**, 58–62.
- Walker, K.A., Mellish, J.E. & Weary, D.M. (2011b). Effects of hot-iron branding on heart rate, breathing rate and behaviour of anaesthetised Steller sea lions. *The Veterinary Record*, **169**, 363.
- Walker, K.A., Trites, A.W., Haulena, M. & Weary, D.M. (2012). A review of the effects of different marking and tagging techniques on marine mammals. *Wildlife Research*, **39**, 15–30.
- White, G.C. & Burnham, K.P. (1999). Program MARK: survival estimation from populations of marked animals. *Bird Study*, **46**, S120–S139.
- Wilkinson, I.S., Chilvers, B.L., Duignan, P.J. & Pistorius, P.A. (2011). An evaluation of hot-iron branding as a permanent marking method for adult New Zealand sea lions, *Phocarctos hookeri*. *Wildlife Research*, **38**, 51–60.
- York, A.E. (1994). The population dynamics of northern sea lions, 1975 - 1985. *Marine Mammal Science*, **10**, 38–51.

## CHAPTER 1

# LONG-TERM SURVIVAL OF TEMPORARILY CAPTIVE STELLER SEA LIONS (*EUMETOPIAS JUBATUS*) AND THE IMPLICATIONS OF NOVEL RESEARCH ACTIVITIES<sup>1</sup>

### 1.1 Abstract

Two novel research approaches were developed in order to facilitate access to wild juvenile Steller sea lions for periods longer than is typically afforded by traditional fieldwork. The Transient Juvenile Steller sea lion Project (TJ) and the Alaska SeaLife Center (ASLC) facilitated nutritional, physiological, and behavioral studies on the platform of temporary captivity (branded TJs,  $n=45$ ). A control group of animals were sampled and released during capture events (FRs,  $n=35$ ). As part of the TJ project, the Life History Tag (LHX) project was implanted individuals ( $n=35$ ) with internal transmitters to detect potential causes of mortality post-release. My goal was to evaluate the potential for long-term impacts of these two research programs on study individuals as a unique retrospective analysis. This was achieved by using a simple open-population Cormack-Jolly-Seber mark-recapture model incorporating resightings of uniquely branded study individuals gathered by several contributing institutions. *A priori* models were developed to evaluate demographic comparisons of survival based around covariates of sex, age, capture age, cohort, and age class. I also compared TJ survival with FRs and LHX-implanted TJs to non-implanted TJs ( $n=10$ ) using program MARK. Top demographic models yielded sex (S), age at capture (Ac) and time-dependence to be most important for survival, sharing 62% of the overall model weight. Comparisons of TJ and FR survival resulted in three top models using S and Ac with the addition of the TJFR survival factor and shared 56%

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<sup>1</sup> Shuert, C., Horning, M., and Mellish, J. (*in review*). Long-term survival of temporarily captive Steller sea lions (*Eumetopias jubatus*) and the implications of novel research activities. *PLOS ONE*.

of the total model weight. The LHX analysis resulted in age class and sex as being most important and shared 70% of the overall model weight. Overall, our results are consistent with previous efforts to characterize survival rates in sea lions and indicate minimal long-term effects from our research efforts, higher survival in females than males, and increasing survival rate with age.

## **1.2 Introduction**

### *1.2.1 General Overview*

The two distinct population segments (DPS, east and west) of Steller sea lions (*Eumetopias jubatus*) have been the subject of extensive study in the past few decades due to substantial decline and changes in population dynamics, leading to their addition to the Endangered Species list (National Marine Fisheries Service 1990). While the eDPS has recently been de-listed, questions remain as to the slow or absent recovery of the wDPS (National Marine Fisheries Service 2013). Various hypotheses have been proposed as to the original cause of the decline, ranging from decreased nutritional status (Calkins *et al.* 1998; Fritz & Hinckley 2005; Rosen & Trites 2005; du Dot *et al.* 2008; du Dot *et al.* 2009; Rosen 2009) and natality (Holmes *et al.* 2007), to increased juvenile mortality (York 1994; Bickham *et al.* 1998) and predation pressure (Springer *et al.* 2003; Trites *et al.* 2007; Horning & Mellish 2009, 2012, 2014). In the absence of disease, a single factor was thought unlikely to be responsible for the decline. Similarly, there may be a combination of effects that are impeding population recovery in the wDPS.

### *1.2.2 The Transient Juvenile Steller Sea Lion Project*

As part of the intensive research effort to better understand the population dynamics of the wDPS, two novel approaches were developed in order to gain extended access to wild individuals and to infer potential causes of mortality. Temporary captivity of juvenile Steller sea

lions was implemented to study wild individuals under highly controlled conditions and with little disturbance to the population at large (Mellish *et al.* 2006). This approach, referred to as the Transient Juvenile Steller Sea Lion Project (TJ), facilitated numerous studies over the years with nutritional, physiological, and behavioral contributions (Castellini & Calkins 1993; Goldstein *et al.* 2007; Thomton & Mellish 2007; Thomton *et al.* 2008; Calkins *et al.* 2013). Long term, post-release tracking of these individuals was facilitated through mandated hot-iron brands on their left flank (n= 45). An additional cohort of sea lions (n=30, free-ranger FR) were branded to serve as a control group and released immediately after initial sampling, similar to those of other institutions using brand-resight methods (e.g., Hastings *et al.* 2011). Facilitated by the temporary captivity platform, 2005 saw the deployment of first generation intraperitoneally implanted Life History Tags (LHX-1, Horning & Hill 2005). In contrast to the relatively short lived external satellite transmitters, internal LHX-1 tags allow for life-long monitoring and generate known-fate mortality data (Horning *et al.* 2008; Horning & Mellish 2009). Through 2011, this tagging effort included the successful implantation and release of 35 sea lions, allowing for a more detailed evaluation of causes of mortalities (Horning & Mellish 2012, 2014). The remaining 10 branded TJs were not implanted and serve as a control for the implanted animals that underwent temporary captivity.

### *1.2.3 Mark-recapture*

Mark-recapture analysis was implemented to evaluate the effect on long-term survival of these two studies (TJ and LHX) on endangered Steller sea lions. Many efforts have been made to continue to study the survival and behavior of the wDPS utilizing external satellite and dive tags (Raum-Suryan *et al.* 2004; Thomton *et al.* 2008) as well as mark-resight studies of flipper tagged and hot-iron branded individuals (Raum-Suryan *et al.* 2002; Pendleton *et al.* 2006;

Jemison *et al.* 2013; Maniscalco 2014). However, the impact of these studies on survival is typically only documented in the short-term, with an assumption of decreasing impact beyond a certain time after a handling event. This is especially true of studies involving tagging of animals for tracking purposes, but has not been evaluated in many species due to logistical constraints (Gauthier-Clerc *et al.* 2004; Le Maho *et al.* 2011). This analysis was facilitated by a decade of shared data from multiple institutions, with a comprehensive survival analysis of three levels of treatment.

The Cormack-Jolly-Seber (CJS) model design is an open population mark-recapture model that includes only time-specific survival and recapture or resight probabilities, denoted as  $\Phi$  and  $p$ , respectively, existing as a multinomial product (Cormack 1964; White & Burnham 1999). This particular model type relies on live encounters in an open population that is subject to both emigration and immigration into the study area. The multinomial product of these two probabilities results in an overall return rate for tagged, or in this case branded, animals. The return rate is dependent on both the true survival of the animal between encounter or resight occasions and being resighted on said encounter occasion. This results in the return rate of an apparent survival rate, rather than a true known-fate design given that death, permanent emigration and mark loss cannot be distinguished (White & Burnham 1999). Encounter histories of individuals are then used as simple logistic data organizing each encounter occasion as presence and absence of an animal resight. The return rate of an individual encounter history then becomes the product of each survival rate between subsequent encounters ( $\Phi_1, \Phi_2 \dots \Phi_{n-1}$ ) and the resight rate, either seen or not seen, at each encounter event after the initial release ( $p_2, p_3 \dots p_n$ ). Program MARK offers a powerful tool for the analysis of data of this type (White & Burnham 1999; Cooch & White 2013). Encounter histories of each animal can be combined

with grouping variables, such as sex and age, along with individual covariates, such as age at capture, to allow for different levels of analysis depending on goals. Models predicting survival within these groups are then based around these covariates in various combination, both continuous and categorical (Bonner *et al.* 2010). Once these have been generated, top models are chosen from the Akaike Information Criterion (AIC) as derived from the Kullback-Liebler (K-L) discrepancy, which attempts to minimize the difference between an unknown true model and the data-based approximate model developed *a priori* (Anderson *et al.* 1998). In short, using Information Theory, the AIC attempts to derive the most parsimonious model by maximizing the expected log-likelihood of the model while at the same time decreasing the number of parameters (Anderson *et al.* 1994). In the current study, however, the corrected AIC (AICc) was used to compensate for small sample size bias and, therefore, has a much larger penalty term than AIC, but is still reasonable for CJS models (Hurvich & Tsai 1989).

#### *1.2.4 Study Goals*

The analysis of our research impact on Steller sea lion survival took place on several tiers. The initial analysis assessed survival based on the demographic effects of the individual, including sex, age, study cohort and time as well as covariates of age at capture and age class. The second tier investigated the potential influence of temporary captivity on survival by comparing TJs and FRs. The final tier included all individuals and demographic parameters to evaluate the potential effect of LHX-1 implants and surgeries on long-term survival of individuals.

### **1.3 Methods**

#### *1.3.1 Study Animals*

All work was carried out under permits NMFS #881-1668, 881-1890, 14335. This study included 72 juvenile Steller sea lions branded between 2005 and 2011. Forty-five of these



individuals (TJs) were retained for temporary captivity for research purposes, up to a maximum of three months (Mellish *et al.* 2006). Of the TJs, 33 were male and 12 were female. Thirty-five of these 45 TJs received dual LHX-1 implants, with the exception of the first two individuals, who only received a single implant. Twenty-seven animals were sampled, branded and immediately released (FRs, 12 male and 15 female). All individuals received a unique 4-digit alphanumeric brand. Age at capture was determined through canine length as per King *et al.* (2007). Approximate age in months of each individual was used to back calculate to the closest mean peak pupping date, June 10<sup>th</sup>, to get an estimated birth date (Maniscalco *et al.* 2006). Three individuals did not have canine measurements during sampling and were aged from a standard length-to-age correlation (King *et al.* 2007). Study animals were captured at a mean age of  $1.56 \pm 0.51$  years as estimated by canine length or extrapolation from standard length and categorized as either being in a 1 year (14-24 months) or 2 year (25-36 months) age group.

### *1.3.2 Resights and Source Agencies*

Branded animal resight information was provided for the period of May 15<sup>th</sup>, 2005 through August 30<sup>th</sup>, 2013 from the National Marine Mammal Laboratory (NMML, 7600 Sand Point Way, Seattle, Washington 98115), Alaska Department of Fish & Game (ADFG, 1255 W. 8th Street, Juneau, Alaska 99811), and the Chiswell Project at the Alaska SeaLife Center (ALSC, 301 Railway Ave, Seward, Alaska 99664). The first two sources provided resights from annual cruise efforts to survey haulouts and rookeries largely during the summer months in South Central and Southeastern Alaska (averaging 148 and 111 days per year, respectively, R. Towell and K. Hastings, pers. comm.). The third source focused on the Chiswell Island rookery and nearby haulouts, including Cape Resurrection, Grotto Island, and Seal Rocks, surveyed by remote video monitoring in the central Gulf of Alaska continuously through the summer

breeding season and periodically through the rest of the year (avg. 333 days per year, J. Maniscalco pers. comm.). All brand resights were compiled into a single database with at minimum the location, date, brand readability, and observer confidence in the accuracy of the resight. Supplementary information included the presence of offspring and additional notation of behavior or brand condition, but was not used in this analysis. Only those resights that had a “positive” or “strong likelihood” confidence level in resight accuracy were used in this analysis.

### *1.3.3 Model Development*

All resight data for each brand were reorganized into a simple binary code encounter history for use in program MARK (White & Burnham 1999). Individual encounter histories contained nine annual resight intervals between 2005 and 2013. Each year was set at a default ‘0’ for no resight events, and a ‘1’ if a resight occurred, regardless of the frequency of resights within an interval. The resight year included the months of March through November. Models were developed *a priori* and parameterized around demographic groups and covariates of sex (S), age (A), age at capture (Ac, 14-24 or 25-36 months bins), cohort (C), and time (t). Age class (A<sub>3</sub>) was also included with two juvenile classes of 14-24 months and 25-36 months of age as well as a single adult cohort, including animals older than 36 months, to evaluate the effect of differential juvenile and adult survival. These were used for generating estimates of survival ( $\Phi$ ) and resight (p) probabilities through the CJS method. Resight effort was included in all p models as a covariate in order to properly scale yearly differences in institutional effort and prevent inflation of resight probabilities. This was calculated by averaging the combined number of days each institution conducted resighting surveys in a given year on a scale of 1 to 10. Each model was then analyzed by MARK via RMark with a corresponding set of encounter histories (Laake 2013). Grouping variables for all animals included age first seen (AFS) to account for

differences in ages within resighting periods in addition to sex, and cohorts. These group characteristic variables simply allow MARK to properly interpret and index model parameters.

Three separate analyses were used to evaluate research questions. The first included all animals (n=72) with basic demographic covariates. The second analysis used the same model set with all animals (n=72), but with a secondary set of complementary  $\Phi$  models to include a categorical covariate term to distinguish TJs from FRs (TJFR). A third and final analysis included only TJs (n=45), with a model term (LHX) accounting for the presence of an LHX-1 implant, rather than a TJFR addition. Top models were determined by the lowest AICc values to account for small sample sizes (Anderson *et al.* 1998). Only those models that were within 2 delta AICc were considered. These were then used to generate comparison sets of  $\phi$  and  $p$  to assess important demographic factors as well as assessing the importance of difference between TJ and FR individuals and those with LHX-1 implants and those without on survival. Goodness-of-fit testing was also used for global models of each grouping factor to assess the potential for model overfitting through the program U-CARE (Choquet *et al.* 2009). All grouping variables had Chi-squared values that reflected non-significant P-values at an alpha value of 0.05. The derived  $\hat{c}$  values were all approximately equal to 1, so no adjustments were necessary for *a priori* models for overdispersion.

#### **1.4 Results**

A Fisher's exact test found that S was skewed within certain groups by comparing them to an equal sex ratio contingency table. Sex ratio comparisons were non-significant for FRs (p=0.81), while within TJs they were just not significant (p=0.051). Within these TJs, LHX implanted individuals had a significantly skewed sex ratio (p=0.018), while those without implants did not (p=0.73). The known effect of S on survival (e.g., Pendleton *et al.* 2006) along

with these results that ratios differ significantly between experimental groups lead to S being forced into all models. A Fisher's exact test also found that age at capture was significantly skewed between TJs and FRs ( $p=0.0013$ ), but not within the TJs alone ( $p=0.29$ ). Therefore, a reduction in the total number of models for each of the three analyses was made where only models that included S and  $A_c$  were considered for a demographic (9  $\Phi$ , 6 p models) and TJFR survival and resighting (18  $\Phi$ , 6 p models). Likewise, S was forced into all LHX survival models.  $A_c$  was not excluded, but not forced into each model. This resulted in a total of 26  $\Phi$  models and 6 p models.

The first demographic analysis found S and  $A_c$  to have considerable support for predicting both survival as well as resighting probability with the addition of a time-dependent factor. These two top models shared 62% of the overall model weight and were  $\leq 2 \Delta AIC_c$  (see Table 1.1*a*). Model results indicated that males appeared to have a slightly lower averaged survival than females. Individuals captured at an age of 25-36 months had an increased averaged annual survival over those captured at the age of 14-24 months (see Table 1.3*a*). The second tier of analysis found considerable support for S and  $A_c$  with the addition of the TJFR categorical survival factor and shared 56% of the total model weight (see Table 1.1*b*). Similarly, these models indicated that males, and those captured at 14-24 months, were found to have a lower survival rate than females and those captured at 25-36 months, respectively. TJs had lower averaged annual survival rates than FRs (see Table 1.3*b*). When considering TJs separately, LHX and  $A_3$  along with the inclusion of S were found to have considerable support in the data for predicting survival, while little support was found for covariate effects on resighting, except for S. These top two models shared 70% of the overall model weight (see Table 1.1*c*). Implanted females had a lower averaged annual survival than implanted males. Non-implanted

animals had slightly higher survival rates overall than those who were implanted (see Table 1.3c). Beta estimates for each of these top models are presented in Table 1.2. Cumulative survival rate to age 5 for the TJ group overall was calculated from annual age-specific survival rates to be 0.41 (Figure 1.1). Model averaged resighting probabilities for each of these three analyses are presented in Table 1.4.

## **1.5 Discussion**

### *1.5.1 Demographic Long-term Survival*

The use of branded animal resights for building mark-recapture models of survival and population size have been popular for many years because of their minimal disturbance to the population while still maintaining high quality data, especially in larger species of pinnipeds (Beck *et al.* 2007; Hastings *et al.* 2011; Fritz *et al.* 2014). This type of data has been extremely important in monitoring Steller sea lions (e.g., Holmes *et al.* 2007). While survival rates are well understood for wild animals where handling for tagging is minimal, the impact of more intensive sampling and temporary captivity had yet to be determined. In the current study, three levels of *post-hoc* analysis were used to mimic the study design of traditional mark-resight studies, as well as to investigate potential long-term effects of two novel research techniques on juvenile Steller sea lions.

The first tier of analysis investigated the long-term determinants of survival based solely around demographic parameters of sex, age, age class, experimental cohort, and age at capture. After the initial identification of sample size bias for sex and capture age, only those models that included these two parameters were considered. Results from this indicated that sex had extensive support in determining long-term survival. Averaged survival was found to be slightly higher for females (6.3%), a trend that has been demonstrated in Steller sea lions (e.g., Hastings

*et al.* 2011), as well as other pinnipeds such as grey seals (*Halichoerus grypus*, Hall *et al.* 2002), Galapagos and northern fur seals (*Arctocephalus galapagoensis* and *Callorhinus ursinus*, Trites 1990). Age at capture was important in predicting survival, with individuals captured at 24-36 months displaying a slightly higher (3.5%) survival rate than those captured at 14-24 months. Recent work by Maniscalco (2014) noted Steller sea lions weaned at a younger age have a lower survival rate than those weaned after 2 years of nursing. The collection of animals at age of 14 to 24 months may have led to a bias towards these early weaned animals. Age class was seen to have an effect on long-term survival with minor support in the data, though not considered in our top model criteria. Animals were found to have the lowest probability of survival in the first year age class (14-24 mo) with a marked increase in the second year age class (25-36 mo) and into adulthood (36+ mo). This supports the hypothesis that low juvenile survival may be affecting the recovery of the species (Raum-Suryan *et al.* 2004; Horning & Mellish 2012; Maniscalco 2014). Our results match trends of age-specific survival rates generated by contributing institutions, although ours appear slightly lower (Hastings *et al.* 2011; Fritz *et al.* 2014).

Resight probability was exclusively influenced by sex and age at capture with a time-specific covariate. Juvenile males tend to disperse farther than females (Raum-Suryan *et al.* 2002; Raum-Suryan *et al.* 2004), which may explain in part why males were found to most often have a lower resighting probability than female groups (see Table 1.4). Several of the study animals were resighted in the eDPS post-release, adding to recent evidence that these population segments may not maintain their distinct status (Jemison *et al.* 2013). Since the CJS model cannot separate permanent emigration from mortality, it is possible that individuals may have

simply emigrated out of the main resight effort area, both lowering their resighting probability as well as their apparent survival rates.

### *1.5.2 Temporary Captivity*

The second tier of this analysis evaluated the effect of temporary captivity on the long term survival of individuals. In order to do this, models were doubled, with half given an additional grouping variable to be included in each  $\Phi$  and  $p$  model that accounts for group membership to either TJ or FR. Similar to the demographic analysis, sex and capture age were found to be statistically biased and, therefore, only models that included these parameters were considered. The top three models contained similar information as the demographic analysis as expected. The TJFR grouping factor and the additive effect of sex and capture age, were found to be important in survival. Averaged apparent survival probability for FRs was higher than for TJs (by 2.5%). Males had an overall lower average survival rate than females between the two groups, with the FR male survival rate being higher than TJ males by 3%. The same pattern followed for females of both groups.

Post-release monitoring is typically associated with wildlife reintroduction for conservation management programs. These programs have encompassed a wide range of species from birds (e.g., Reynolds *et al.* 2008), rodents (e.g., Bright & Morris 1994), ungulates (e.g., Franceschini *et al.* 2008), to terrestrial carnivores (Bremner-Harrison *et al.* 2004; Jule *et al.* 2008). Each relies on several proxies for survival, stress management, and establishment success. While the goals of these programs differ, they are similarly focused on threatened or endangered species with a goal of minimal negative effects on health and survivability. These and other studies have greatly improved the research practices for release, yet very rarely are they able to directly evaluate the long-term (greater than 2 years) effects. Temporary captivity

has been shown to have little to no effect in the short-term for both behavior and acute health parameters in Steller sea lions (Mellish *et al.* 2006; Thomton *et al.* 2008). While we have shown temporary captivity to have a slight effect on survival in the longer term, it is considered to be statistically equivalent by our model standards to those only including demographic predictors such that the biological relevance of this effect is minimal at best. However, given that temporary captivity may have a slight negative effect on long-term survival, care should be taken in future to assess whether temporary captivity is necessary to accomplish study goals. Duration of the captivity period should be minimized, and balanced with the study requirements as closely as possible.

### *1.5.3 Life History Tag Implants*

Our final evaluation assessed the effects of LHX-1 implants in our TJ group. Two models were considered equal in importance: survival dependent of the age class of the animal and survival dependent on their LHX-1 implantation status. Sea lions that received LHX-1 implants were found to have a slightly lower survival rate than those without implants. When directly compared to the known-fate results as generated by the LHX-1 tags (Horning & Mellish 2012), CJS generated survival rates are slightly lower. This difference results in a discrepancy of roughly 2 deaths between mark-recapture analysis methods. LHX-1 mortality event detection probabilities are very high ( $P_{detect} > 0.98$ ; Horning & Mellish, 2012) as compared to our estimated resighting probabilities in Table 1.4. This appears to be a direct consequence of the CJS design assumptions that cannot separate out death from permanent emigration rather than a lack of LHX-1 reporting. It is likely that our models using opportunistic resighting underestimates survival, leading to discrepancies between our data and other efforts.



Despite the seemingly routine nature of wildlife tagging approaches, there is always room for evaluation and improvement. As part of ongoing refinement efforts, the overall sizes of the tag and the incision site have been reduced in association with the next generation of LHX-2 implants (Horning and Mellish, unpublished data). The goal of the present analysis was to avoid complacency and continually strive to refine methods, as even in routine procedures can result in unforeseen consequences. In the case of penguins, flipper banding was seen as a quick, easy, and non-invasive way to semi-permanently mark individuals. Retrospective analyses found that the additional energetic costs associated with drag reduced survival by half in the long-term (Gauthier-Clerc *et al.* 2004; Le Maho *et al.* 2011). A marked decrease in nestling success has also been noted in radio transmitter tagged tufted puffins in south central Alaska (*Fratercula cirrhata*, Whidden *et al.*, 2007).

Age class was found to be equivalent to the effect of LHX-1 implant status in long-term survival modeling. Age-specific cumulative survival was much lower for juveniles than adults, exhibiting a survivorship curve similar to non-study animals (Figure 1.1; e.g., Hastings *et al.* 2011; Maniscalco 2014). Cumulative survival to age 5 was calculated as 0.41 for the TJ group using the age-specific survival rates found in Figure 1.1, which is very close to results from parallel efforts in the same region to characterize 4 year survival based on similar methods to Maniscalco 2014. Sex was included with our top model as an additive effect with LHX implants. However, in this instance, females had a lower survival rate than males, which highlights the need to include sex as a covariate in our models due to sample size heterogeneity. LHX female to male ratios were skewed towards males, likely due to higher capture rates of juvenile males.

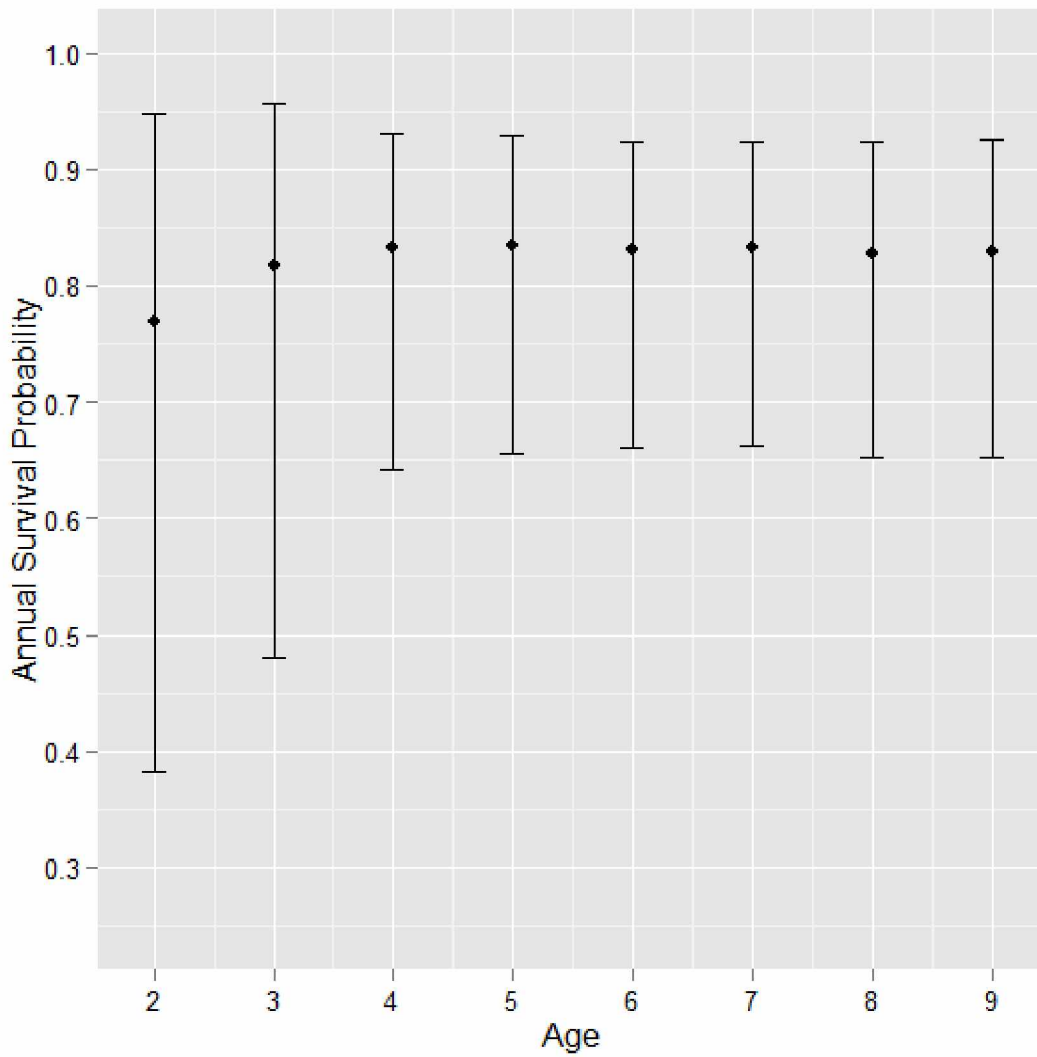
#### 1.5.4 Other Considerations and Conclusions

Due to the retrospective nature of this analysis, several statistical considerations should be noted. Every time an analysis is run in program MARK, a power analysis is run to demonstrate an effective sample size for the situation at hand. For this analysis, MARK returned an effective sample size of 211 animals. The complex nature of this study design as well as permitting restrictions prevent this from being an attainable quota. It may have contributed to our relatively low ability to detect small differences in survival. While we have also corrected for the small sample size by utilizing AICc, which by nature penalizes models with more complex structure much more harshly than AIC (Anderson *et al.* 1998), it may also prevent us from achieving a fine-scale analysis with these individuals.

We have presented a direct comparison of two mark-recapture model techniques. LHX-1 tags allow for known-fate survival modeling and have been used to determine juvenile survival and the specifics of predation pressures (Horning and Mellish 2014). All animals that received implants were also branded and monitored through mark-resight analysis under CJS and other related model approaches. Obviously, known-fate data through LHX-1 tags have a much higher detection probability as compared to the current data as previously discussed. The logistical constraints of executing such an endeavor, however, will likely prevent it on a large scale from replacing brand-resight modeling.

Overall, the effects of two novel research techniques, temporary captivity and implantation of LHX-1, appear to be minimal in Steller sea lions. This study uniquely evaluated the assumption that recovery of individuals after a handling event is only critical during the short-term, post-release period. This assumption may be valid for these two research techniques. Animals appear to survive in the long-term at similar rates and by similar demographic patterns

compared to those not exposed to either of these techniques. This study suggests that older juveniles may be preferred candidates for both temporary captivity and LHX implantation, despite the inherent difficulty in preventing this bias with underwater capture techniques. In more general terms, continued refinement and assessment of study techniques allows us to evaluate the efficacy of vital rates.



**Figure 1.1. Age-specific survival rates.** Model averaged age-specific annual survival rates of juvenile Steller sea lions (*Eumetopias jubatus*) participating in temporary captivity.

**Table 1.1a. Model selection results for demographics only.** Model selection results for demographics only assessing the long-term survival of juvenile Steller sea lions (*Eumetopias jubatus*) concerning covariates of sex (S), age class (A<sub>3</sub>), capture age (Ac), age (A), cohort (C), time (t) and effort (Ef). Models were built under Cormack-Jolly-Seber assumptions with selection based on corrected Akaike Information Criterion (AICc). Results presented here include only the top 10 models for brevity. Best models were considered to be within 2 delta AICc and are highlighted in **bold**.

Model	<i>k</i>	AICc	Delta AICc	Model Weight	Deviance
<b>Phi(~S + Ac)*p(~S + Ac + Ef)</b>	<b>7</b>	<b>425.7909</b>	<b>0</b>	<b>0.4305</b>	<b>293.6898</b>
<b>Phi(~S + Ac)*p(~S + Ac + t + Ef)</b>	<b>14</b>	<b>427.3667</b>	<b>1.5757</b>	<b>0.1958</b>	<b>279.6745</b>
Phi(~S + Ac + A <sub>3</sub> )*p(~S + Ac + Ef)	9	428.2697	2.4787	0.1246	291.8247
Phi(~S + Ac)*p(~Ac + A + S + Ef)	15	429.2919	3.5009	0.0747	279.2810
Phi(~S + Ac)*p(~Ac + S + A <sub>3</sub> + Ef)	9	429.8593	4.0683	0.0563	293.4143
Phi(~C + S + Ac)*p(~S + Ac + Ef)	17	430.9313	5.1403	0.0329	276.2109
Phi(~S + Ac + A <sub>3</sub> )*p(~S + Ac + t + Ef)	16	431.6272	5.8362	0.0232	279.6745
Phi(~S + Ac)*p(~S + Ac + A <sub>3</sub> + t + Ef)	16	432.0280	6.2370	0.0190	291.6697
Phi(~S + Ac + A <sub>3</sub> )*p(~S + Ac + A <sub>3</sub> + Ef)	11	432.5458	6.7548	0.0146	278.8916
....	...	...	...	...	...
Phi(~Ac + S + A + C + t)p(~Ac + S + A + t + Ef)	47	484.5323	58.7413	0	245.3019

**Table 1.1b. Model selection results for temporary captivity.** Model selection results for assessing the impact of temporary captivity on long-term survival of juvenile Steller sea lions (*Eumetopias jubatus*) concerning covariates of sex (S), age class ( $A_3$ ), capture age (Ac), age (A), cohort (C), time (t) and effort (Ef). Models were built under Cormack-Jolly-Seber assumptions with selection based on corrected Akaike Information Criterion (AICc). Results presented here include only the top 10 models for brevity. Best models were considered to be within 2 delta AICc and are highlighted in **bold**.

Model	$k$	AICc	Delta AICc	Model Weight	Deviance
<b>Phi(~S + Ac)*p(~S + Ac + Ef)</b>	<b>7</b>	<b>425.7909</b>	<b>0</b>	<b>0.2742</b>	<b>312.4183</b>
<b>Phi(~S + Ac + TJFR)*p(~S + Ac + Ef)</b>	<b>8</b>	<b>426.8142</b>	<b>1.0232</b>	<b>0.1644</b>	<b>311.2804</b>
<b>Phi(~S + Ac)*p(~S + Ac + t + Ef)</b>	<b>14</b>	<b>427.3667</b>	<b>1.5757</b>	<b>0.1247</b>	<b>298.4030</b>
Phi(~S + Ac + $A_3$ )*p(~S + Ac + Ef)	9	428.2697	2.4787	0.0794	310.5533
Phi(~S + Ac + TJFR)*p(~S + Ac + t + Ef)	15	428.4812	2.6902	0.0714	297.1987
Phi(~S + Ac)*p(~S + Ac + A + Ef)	15	429.2919	3.5009	0.0476	298.0095
Phi(~S + Ac + $A_3$ + TJFR)*p(~S + Ac + Ef)	10	429.4159	3.6249	0.0447	309.4950
Phi(~S + Ac)*p(~S + Ac + $A_3$ + Ef)	9	429.8593	4.0683	0.0358	312.1429
Phi(~S + Ac + TJFR)*p(~S + Ac + A + Ef)	16	430.4313	4.6403	0.0269	296.8063
.....	.....	...	...	...	...
Phi(~Ac + S + A + C + t + TJFR)*p(~S + Ac + A + t + Ef)	48	487.7670	61.9760	0	263.9091

**Table 1.1c. Model selection results for LHX implants.** Model selection results for assessing the impact of LHX implants on the long-term survival of juvenile Steller sea lions (*Eumetopias jubatus*) concerning covariates of sex (S), age class (A<sub>3</sub>), capture age (A<sub>c</sub>), age (A), cohort (C), time (t) and effort (Ef). Models were built under Cormack-Jolly-Seber assumptions with selection based on corrected Akaike Information Criterion (AICc). Results presented here include only the top 10 models for brevity. Best models were considered to be within 2 delta AICc and are highlighted in **bold**.

(a)	Model	<i>k</i>	AICc	Delta AICc	Model Weight	Deviance
	<b>Phi(~LHX + S)*p(~S + Ef)</b>	<b>6</b>	<b>252.0855</b>	<b>0</b>	<b>0.4644</b>	<b>172.8612</b>
	<b>Phi(~A3 + S)*p(~S + Ef)</b>	<b>7</b>	<b>253.3629</b>	<b>1.2774</b>	<b>0.2452</b>	<b>171.8911</b>
	Phi(~A3 + LHX + S)*p(~S + Ef)	8	254.7807	2.6952	0.1206	171.0222
	Phi(~LHX + S)*p(~S + A3 + Ef)	8	256.5502	4.4646	0.0498	172.7917
	Phi(~C + S)*p(~S + Ef)	14	257.2185	5.1330	0.0356	158.8590
	Phi(~A3 + S)*p(~A3 + S + Ef)	9	257.9745	5.8889	0.0244	171.8892
	Phi(~A3 + LHX + S)*p(~A3 + S + Ef)	10	259.4694	7.3839	0.0115	171.0162
	Phi(~C + A3 + S)*p(~S + Ef)	16	259.4796	7.3940	0.0115	155.8891
	Phi(~C + LHX + S)*p(~S + Ef)	15	259.7956	7.7101	0.0098	158.8449
	.....	...	...	...	...	...
	Phi(~C + A + t + LHX + S)p(~A + t + S + Ef)	43	346.5888	94.5032	0.0000	146.7825

**Table 1.2. Model-averaged apparent survival rates.** Model-averaged apparent survival rates from top Cormack-Jolly-Seber models predicting long-term survival in juvenile Steller sea lions (*Eumetopias jubatus*). Tables include the influence of demographics in all animals (*a*), of captivity by comparing temporarily captive animals (TJs) to those that were not (FRs; *b*), and of LHX-1 implants on TJs (*c*). Tables represent the averaged mean survival over the resighting period.

(a)	Estimate	Standard Error	95% Confidence	
			Lower	Upper
Male, Captured 12-24mo	0.7928	0.0644	0.6397	0.8915
Male, Captured 24-36mo	0.8333	0.0533	0.7018	0.9134
Female, Captured 12-24mo	0.8618	0.0575	0.7073	0.9407
Female, Captured 24-36mo	0.8910	0.0441	0.7695	0.9517

(b)	Estimate	Standard Error	95% Confidence	
			Lower	Upper
Male TJ, Captured 12-24mo	0.7687	0.0825	0.5727	0.8917
Male TJ, Captured 24-36mo	0.8307	0.0524	0.7026	0.9102
Female TJ, Captured 12-24mo	0.8349	0.0851	0.6017	0.9442
Female TJ, Captured 24-36mo	0.8840	0.0480	0.7524	0.9499
Male FR, Captured 12-24mo	0.8075	0.0626	0.6558	0.9022
Male FR, Captured 24-36 mo	0.8465	0.0659	0.6712	0.9371
Female FR, Captured 12-24mo	0.8660	0.0528	0.7253	0.9400
Female FR, Captured 24-36 mo	0.8996	0.0436	0.7759	0.9581

(c)	Estimate	Standard Error	95% Confidence	
			Lower	Upper
Male, age 12-24 mo	0.6846	0.1686	0.3194	0.9094
Male, age 24-36 mo	0.7964	0.1523	0.3828	0.9610
Male, age 36+ mo	0.8362	0.0422	0.7362	0.9033
Female, age 12-24 mo	0.6874	0.1671	0.3237	0.9100
Female, age 24-36 mo	0.7986	0.1572	0.3686	0.9642
Female, age 36+ mo	0.8380	0.0615	0.6802	0.9264
Male LHX	0.8237	0.0519	0.6983	0.9034
Male nLHX	0.8306	0.0629	0.6711	0.9212
Female LHX	0.8093	0.0748	0.6214	0.9162
Female nLHX	0.8172	0.0799	0.6110	0.9270



**Table 1.3a. Beta values for demographic survival models.** Top model beta parameters for survival probability ( $\Phi$ , Phi) and resight probability (p) for demographics-only analysis of long-term survival in juvenile Steller sea lions (*Eumetopias jubatus*) as determined by being within 2 delta AICc model rankings of a Cormack-Jolly-Seber mark-recapture format. Top model grouping covariates included sex (S) and age at capture (Ac), and time-dependence (t). All models included a term to scale the heterogeneous nature of opportunistic resights (Effort).

Model	Parameter	Beta	Standard Error	95% Confidence Interval	
				Lower	Upper
Phi(~ S + Ac) p(~S + Ac + Effort)	Phi(Intercept)	1.8365	0.3675	1.1162	2.5568
	Phi(S)	-0.4926	0.3833	-1.2439	0.2586
	Phi(Ac)	0.2802	0.3703	-0.4455	1.006
	p(Intercept)	5.5586	1.9937	1.6509	9.4663
	p(S)	-1.0249	0.3846	-1.7789	-0.2709
	p(Ac)	-0.4749	0.3891	-1.2376	0.2877
	p(Effort)	-0.6986	0.3591	-1.4026	0.0053
	Phi(~S + Ac) p(~S + Ac + t + Effort)	Phi(Intercept)	1.8407	0.3603	1.1343
Phi(S)		-0.5107	0.3759	-1.2476	0.2261
Phi(Ac)		0.2279	0.3620	-0.4817	0.9377
p(Intercept)		1.0802	444.45	-870.04	872.20
p(S)		-1.2369	0.4119	-2.0442	-0.4295
p(Ac)		-0.4704	0.4036	-1.2616	0.3207
p(t2007)		-1.5047	88.895	-175.73	172.72
p(t2008)		-0.1488	0.8425	-1.8001	1.5025
p(t2009)		0.6971	0.8640	-0.9962	2.3906
p(t2010)		0.5372	88.890	-173.69	174.77
p(t2011)		0.8595	0.8817	-0.8686	2.5877
p(t2012)		0.6492	0.8797	-1.0750	2.3735
p(t2013)		0.9748	1.0392	-1.0620	3.0117
p(Effort)		0.1259	88.890	-174.09	174.35

**Table 1.3b. Beta values for temporary captivity survival models.** Top model beta parameters for survival probability ( $\Phi$ , Phi) and resight probability (p) for models assessing the effect of temporary captivity on long-term survival in juvenile Steller sea lions (*Eumetopias jubatus*) as determined by being within 2 delta AICc model rankings of a Cormack-Jolly-Seber mark-recapture format. Top model grouping covariates included sex (S), age at capture (Ac), temporary captivity (TJFR), and time-dependence (t). All models included a term to scale the heterogeneous nature of opportunistic resights (Effort).

Model	Parameter	Beta	Standard Error	95% Confidence Interval	
				Lower	Upper
Phi(~S + Ac) p(~S + Ac + Effort)	Phi(Intercept)	1.8365	0.3675	1.1162	2.5568
	Phi(S)	-0.4926	0.3833	-1.2439	0.2586
	Phi(Ac)	0.2802	0.3703	-0.4455	1.0060
	p(Intercept)	5.5586	1.9937	1.6509	9.4663
	p(S)	-1.0249	0.3846	-1.7789	-0.2709
	p(Ac)	-0.4749	0.3891	-1.2376	0.2877
	p(Effort)	-0.6986	0.3591	-1.4026	0.0053
Phi(~S + Ac + TJFR) p(~S + Ac + Effort)	Phi(Intercept)	1.4154	0.5278	0.3808	2.4500
	Phi(S)	-0.3270	0.4121	-1.1348	0.4808
	Phi(Ac)	0.4989	0.4235	-0.3311	1.3290
	Phi(TJFR)	0.4763	0.4498	-0.4052	1.3580
	p(Intercept)	5.6082	1.9893	1.7092	9.5073
	p(S)	-1.0445	0.3858	-1.8008	-0.2881
	p(Ac)	-0.4802	0.3887	-1.2422	0.2817
p(Effort)	-0.7046	0.3587	-1.4078	-0.0014	
Phi(~S + Ac) p(~S + Ac + t + Effort)	Phi(Intercept)	1.8407	0.3603	1.1343	2.5470
	Phi(S)	-0.5107	0.3759	-1.2476	0.2261
	Phi(Ac)	0.2279	0.3620	-0.4817	0.9377
	p(Intercept)	1.0802	444.45	-870.04	872.20
	p(S)	-1.2369	0.4119	-2.0442	-0.4295
	p(Ac)	-0.4704	0.4036	-1.2616	0.3207
	p(t2007)	-1.5047	88.895	-175.73	172.72
	p(t2008)	-0.1488	0.8425	-1.8001	1.5025
	p(t2009)	0.6971	0.8640	-0.9962	2.3906
	p(t2010)	0.5372	88.890	-173.69	174.77
	p(t2011)	0.8595	0.8817	-0.8686	2.5877
	p(t2012)	0.6492	0.8797	-1.0750	2.3735
	p(t2013)	0.9748	1.0392	-1.0620	3.0117
p(Effort)	0.1259	88.890	-174.09	174.35	

**Table 1.3c. Beta values for LHX-1 survival models.** Top model beta parameters for survival probability ( $\Phi$ , Phi) and resight probability ( $p$ ) for models assessing the effect of LHX-1 implants on long-term survival in temporarily captive juvenile Steller sea lions (*Eumetopias jubatus*) as determined by being within 2 delta AICc model rankings of a Cormack-Jolly-Seber mark-recapture format. Top model grouping covariates included LHX-1 implant status (LHX) and age class ( $A_3$ ) along with the inclusion of sex (S) to compensate for a male-biased sample. All models included a term to scale the heterogeneous nature of opportunistic resights (Effort).

Model	Parameter	Beta	Standard Error	95% Confidence Interval		
				Lower	Upper	
Phi( $\sim$ LHX + S)	p( $\sim$ S + Effort)	Phi(Intercept)	1.6012	0.5174	0.5870	2.6154
		Phi(LHX)	-0.1943	0.5076	-1.1893	0.8007
		Phi(S)	0.0900	0.5026	-0.8950	1.0751
		p(Int)	2.1878	2.8441	-3.3866	7.7623
		p(S)	-1.7674	0.7838	-3.3038	-0.2310
		p(Effort)	0.0395	0.5293	-0.9979	1.0770
Phi( $\sim$ A <sub>3</sub> + S)	p( $\sim$ S + Effort)	Phi(Intercept)	0.7884	0.7781	-0.7367	2.3137
		Phi(Juv)	0.5892	1.2779	-1.9154	3.0939
		Phi(Adult)	0.8556	0.7891	-0.6910	2.4024
		Phi(S)	-0.0133	0.5123	-1.0174	0.9908
		p(Int)	2.0124	2.8741	-3.6209	7.6457
		p(S)	-1.7527	0.7834	-0.9783	-0.2171
	p(Effort)	0.0699	0.5348	-3.2883	1.1182	

**Table 1.4. Model-averaged resighting probabilities.** Model averaged resighting probabilities for top predictors of Cormack-Jolly-Seber models predicting return rate and long-term survival in juvenile Steller sea lions (*Eumetopias jubatus*).

	Estimate	Standard Error	95% Confidence	
			Lower	Upper
Male, Capture Age 12-24mo	0.7059	0.0912	0.5070	0.8514
Male, Capture Age 24-36mo	0.6050	0.0982	0.4093	0.7758
Female, Capture Age 12-24mo	0.8685	0.0632	0.6938	0.9515
Female, Capture Age 24-36mo	0.8096	0.0681	0.6447	0.9106

## Literature Cited

- Anderson, D.R., Burnham, K.P. & White, G.C. (1994). AIC model selection in overdispersed capture-recapture data. *Ecology*, **75**, 1780–1793.
- Anderson, D.R., Burnham, K.P. & White, G.C. (1998). Comparison of Akaike information criterion and consistent Akaike information criterion for model selection and statistical inference from capture  $\pm$  recapture studies. *Journal of Applied Statistics*, **25**, 263–283.
- Beck, C.A., Iverson, S.J., Bowen, W.D. & Blanchard, W. (2007). Sex differences in grey seal diet reflect seasonal variation in foraging behaviour and reproductive expenditure: evidence from quantitative fatty acid signature analysis. *The Journal of Animal Ecology*, **76**, 490–502.
- Bickham, J.W., Loughlin, T.R., Calkins, D.G., Wickliffe, J.K. & Patton, J.C. (1998). Genetic variability and population decline in Steller sea lions from the Gulf of Alaska. *Journal of Mammalogy*, **79**, 1390–1395.
- Bonner, S.J., Morgan, B.J.T. & King, R. (2010). Continuous covariates in mark-recapture-recovery analysis: A comparison of methods. *Biometrics*, **66**, 1256–65.
- Bremner-Harrison, S., Prodohl, P.A. & Elwood, R.W. (2004). Behavioural trait assessment as a release criterion: boldness predicts early death in a reintroduction programme of captive-bred swift fox (*Vulpes velox*). *Animal Conservation*, **7**, 313–320.
- Bright, P.W. & Morris, P.A. (1994). Animal translocation for conservation: performance of dormice in relation to release methods, origin and season. *Journal of Applied Ecology*, **31**, 699–708.
- Calkins, D.G., Atkinson, S., Mellish, J.-A., Waite, J.N. & Carpenter, J.R. (2013). The pollock paradox: juvenile Steller sea lions experience rapid growth on pollock diets in fall and spring. *Journal of Experimental Marine Biology and Ecology*, **441**, 55–61.
- Calkins, D.G., Becker, E.F. & Pitcher, K.W. (1998). Reduced body size of female Steller sea lions from a declining population in the Gulf of Alaska. *Marine Mammal Science*, **14**, 232–244.
- Castellini, M.A. & Calkins, D.G. (1993). Mass estimates using body morphology in Steller sea lions. *Marine Mammal Science*, **9**, 48–54.
- Choquet, R., Lebreton, J.D., Gimenez, O., Reboulet, A.M. & Pradel, R. (2009). U-CARE: Utilities for performing goodness of fit tests and manipulating CAPture-REcapture data. *Ecography*, **32**, 1071–1074.
- Cooch, E.G. & White, G.C. (2013). Program MARK - A Gentle Introduction, 12th edn. (E.G. Cooch & G.C. White, Eds.).
- Cormack, R.M. (1964). Estimates of survival from the sighting of marked animals. *Biometrika*, **51**, 429–438.

- du Dot, T.J., Rosen, D.A.S. & Trites, A.W. (2009). Energy reallocation during and after periods of nutritional stress in Steller sea lions: Low-quality diet reduces capacity for physiological adjustments. *Physiological and Biochemical Zoology*, **82**, 516–530.
- du Dot, T.J., Rosen, D.A.S. & Trites, A.W. (2008). Steller sea lions show diet-dependent changes in body composition during nutritional stress and recover more easily from mass loss in winter than in summer. *Journal of Experimental Marine Biology and Ecology*, **367**, 1–10.
- Franceschini, M.D., Rubenstein, D.I., Low, B. & Romero, L.M. (2008). Fecal glucocorticoid metabolite analysis as an indicator of stress during translocation and acclimation in an endangered large mammal, the Grevy's zebra. *Animal Conservation*, **11**, 263–269.
- Fritz, L.W. & Hinckley, S. (2005). A critical review of the regime shift –“junk food”– nutritional stress hypothesis for the decline of the western stock of Steller sea lion. *Marine Mammal Science*, **21**, 476–518.
- Fritz, L.W., Towell, R., Gelatt, T.S., Johnson, D.S. & Loughlin, T.R. (2014). Recent increases in survival of western Steller sea lions in Alaska and implications for recovery. *Endangered Species Research*, **26**, 13–24.
- Gauthier-Clerc, M., Gendner, J.P., Ribic, C.A., Fraser, W.R., Woehler, E.J., Descamps, S., Gilly, C., Le Bohec, C. & Le Maho, Y. (2004). Long-term effects of flipper bands on penguins. *Proceedings of the Royal Society of London, Series B*, **271**, S423–426.
- Goldstein, T., Stephens, C.A., Jang, S.S., Conrad, P.A., Field, C., Dunn, L.J. & Mellish, J.-A.E. (2007). Longitudinal health and disease monitoring in juvenile Steller sea lions (*Eumetopias jubatus*) in temporary captivity in Alaska compared with a free-ranging cohort. *Aquatic Mammals*, **33**, 337–348.
- Hall, A.J., Connell, B.J.M.C. & Barker, R.J. (2002). The effect of total immunoglobulin levels, mass and condition on the first-year survival of grey seal pups. *Functional Ecology*, **16**, 462–474.
- Hastings, K.K., Jemison, L.A., Gelatt, T.S., Laake, J.L., Pendleton, G.W., King, J.C., Trites, A.W. & Pitcher, K.W. (2011). Cohort effects and spatial variation in age-specific survival of Steller sea lions from southeastern Alaska. *Ecosphere*, **2**, art111.
- Holmes, E.E., Fritz, L.W., York, A.E. & Sweeney, K. (2007). Age-structured modeling reveals long-term declines in the natality of western Steller sea lions. *Ecological Applications*, **17**, 2214–32.
- Horning, M., Haulena, M., Tuomi, P.A. & Mellish, J.-A.E. (2008). Intraperitoneal implantation of life-long telemetry transmitters in otariids. *BMC Veterinary Research*, **4**, 51.
- Horning, M. & Hill, R.D. (2005). Designing an archival satellite transmitter for life-long deployments on oceanic vertebrates: The Life History Transmitter. *IEEE Journal of Oceanic Engineering*, **30**, 807–817.

- Horning, M. & Mellish, J.-A.E. (2014). In cold blood: evidence of Pacific sleeper shark (*Somniosus pacificus*) predation on Steller sea lions (*Eumetopias jubatus*) in the Gulf of Alaska. *Fishery Bulletin*, **112**, 297–310.
- Horning, M. & Mellish, J.-A.E. (2012). Predation on an upper trophic marine predator, the Steller sea lion: evaluating high juvenile mortality in a density dependent conceptual framework. *PLoS ONE*, **7**, e30173.
- Horning, M. & Mellish, J.-A.E. (2009). Spatially explicit detection of predation on individual pinnipeds from implanted post-mortem satellite data transmitters. *Endangered Species Research*, **10**, 135–143.
- Hurvich, C.M. & Tsai, C.-L. (1989). Regression and time series model selection in small samples. *Biometrika*, **76**, 297–307.
- Jemison, L.A., Pendleton, G.W., Fritz, L.W., Hastings, K.K., Maniscalco, J.M., Trites, A.W. & Gelatt, T.S. (2013). Inter-population movements of Steller sea lions in Alaska with implications for population separation. *PLoS ONE*, **8**, e70167.
- Jule, K.R., Leaver, L.A. & Lea, S.E.G. (2008). The effects of captive experience on reintroduction survival in carnivores: a review and analysis. *Biological Conservation*, **141**, 355–363.
- King, J.C., Gelatt, T.S., Pitcher, K.W. & Pendleton, G.W. (2007). A field-based method for estimating age in free-ranging Steller sea lions (*Eumetopias jubatus*) less than twenty-four months of age. *Marine Mammal Science*, **23**, 262–271.
- Laake, J.L. (2013). RMark: An R interface for analysis of capture-recapture data with MARK. AFSC Processed Report, 2013-01, 1–25.
- Le Maho, Y., Saraux, C., Durant, J.M., Viblanc, V.A., Gauthier-Clerc, M., Yoccoz, N.G., Stenseth, N.C. & Le Bohec, C. (2011). An ethical issue in biodiversity science: the monitoring of penguins with flipper bands. *Comptes Rendus Biologies*, **334**, 378–84.
- Maniscalco, J.M. (2014). The effects of birth weight and maternal care on survival of juvenile Steller sea lions (*Eumetopias jubatus*). *PLoS ONE*, **9**, e96328.
- Maniscalco, J.M., Parker, P. & Atkinson, S. (2006). Interseasonal and interannual measures of maternal care among individual Steller sea lions (*Eumetopias jubatus*). *Journal of Mammalogy*, **87**, 304–311.
- Mellish, J.-A.E., Calkins, D.G., Christen, D.R., Horning, M., Rea, L.D. & Atkinson, S.K. (2006). Temporary captivity as a research tool: comprehensive study of wild pinnipeds under controlled conditions. *Aquatic Mammals*, **32**, 58–65.
- National Marine Fisheries Service (2013). Status Review of The Eastern Distinct Population Segment of Steller Sea Lion (*Eumetopias jubatus*). 144pp + Appendices. Protected Resources Division, Alaska Region, National Marine Fisheries Service, 709 West 9th St, Juneau, Alaska 99802.

- National Marine Fisheries Service (1990). Listing of Steller sea lions as threatened under the Endangered Species Act. *Federal Register, Rules and Regulations*, **55**, 49204-49241.
- Pendleton, G.W., Pitcher, K.W., Fritz, L.W., York, A.E., Raum-Suryan, K.L., Loughlin, T.R., Calkins, D.G., Hastings, K.K. & Gelatt, T.S. (2006). Survival of Steller sea lions in Alaska : a comparison of increasing and decreasing populations. *Canadian Journal of Zoology*, **84**, 1163–1172.
- Raum-Suryan, K.L., Pitcher, K.W., Calkins, D.G., Sease, J.L. & Loughlin, T.R. (2002). Dispersal, rookery fidelity, and metapopulation structure of Steller sea lions (*Eumetopias jubatus*) in an increasing and decreasing population in Alaska. *Marine Mammal Science*, **18**, 746–764.
- Raum-Suryan, K.L., Rehberg, M.J., Pendleton, G.W., Pitcher, K.W. & Gelatt, T.S. (2004). Development of dispersal, movement patterns, and haul-out use by pup and juvenile Steller sea lions (*Eumetopias jubatus*) in Alaska. *Marine Mammal Science*, **20**, 823–850.
- Reynolds, M.H., Seavy, N.E., Vekasy, M.S., Klavitter, J.L. & Laniawe, L.P. (2008). Translocation and early post-release demography of endangered Laysan teal. *Animal Conservation*, **11**, 160–168.
- Rosen, D.A.S. (2009). Steller sea lions *Eumetopias jubatus* and nutritional stress: evidence from captive studies. *Mammal Review*, **39**, 284–306.
- Rosen, D.A.S. & Trites, A.W. (2005). Examining the potential for nutritional stress in young Steller sea lions: physiological effects of prey composition. *Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology*, **175**, 265–73.
- Springer, A.M., Estes, J.A., van Vliet, G.B., Williams, T.M., Doak, D.F., Danner, E.M., Forney, K.A. & Pfister, B. (2003). Sequential megafaunal collapse in the North Pacific Ocean: an ongoing legacy of industrial whaling? *Proceedings of the National Academy of Sciences*, **100**, 12223–12228.
- Thomton, J.D. & Mellish, J.-A.E. (2007). Haptoglobin concentrations in free-range and temporarily captive juvenile Steller sea lions. *Journal of Wildlife Diseases*, **43**, 258–261.
- Thomton, J.D., Mellish, J.-A.E., Hennen, D.R. & Horning, M. (2008). Juvenile Steller sea lion dive behavior following temporary captivity. *Endangered Species Research*, **4**, 195–203.
- Trites, A.W. (1990). Thermal budgets and climate spaces: the impact of weather on the survival of Galapagos (*Arctocephalus galapagoensis* Heller) and Northern fur seal pups (*Callorhinus ursinus* L.). *Functional Ecology*, **4**, 753–768.
- Trites, A.W., Deecke, V.B., Gregr, E.J., Ford, J.K.B. & Olesiuk, P.F. (2007). Killer whales, whaling, and sequential megafaunal collapse in the North Pacific: a comparative analysis of the dynamics of marine mammals in Alaska and British Columbia following commercial whaling. *Marine Mammal Science*, **23**, 751–765.



- Whidden, S.E., Williams, C.T., Breton, A.R. & Buck, C.L. (2007). Effects of transmitters on the reproductive success of Tufted Puffins. *Journal of Field Ornithology*, **78**, 206–212.
- White, G.C. & Burnham, K.P. (1999). Program MARK: survival estimation from populations of marked animals. *Bird Study*, **46**, S120–S139.
- York, A.E. (1994). The population dynamics of northern sea lions, 1975 - 1985. *Marine Mammal Science*, **10**, 38–51.

## CHAPTER 2

### PHYSIOLOGICAL PREDICTORS OF LONG-TERM SURVIVAL IN STELLER SEA LIONS (*EUMETOPIAS JUBATUS*)<sup>2</sup>

#### 2.1 Abstract

This study builds on a continued effort to document potential longer-term research impacts on the individual, as well as to identify protocols for potential markers of survival for use in field efforts. The Transient Juvenile Steller sea lion (TJ) project was developed as a novel framework to gain access to wild individuals. I took a three-tiered approach to evaluate and predict long-term survival in temporarily captive sea lions ( $n=45$ ) through a Cormack-Jolly-Seber open population modeling techniques. The first two levels investigated survival in relation to the observed responses to handling stress through changes in six principle blood parameters over the duration of captivity in addition to values of various condition indices at entry and exit from the project. I also sought to evaluate the efficacy of single-point sampling to project similar survival trends for use in field sampling operations. Results from *a priori* models ranked through Akaike Information Criterion model selection methods indicated that the change in mass (kg) and white blood cell count (WBC,  $10^3/\text{mm}^3$ ) had the most support in predicting long-term survival. Mass gains ( $4.2 \pm 12\%$ ) over captivity and slight increases in WBC ( $1.01 \pm 3.54$   $10^3/\text{mm}^3$ ) resulted in a higher averaged survival rate. These two terms ranked higher than single-point measures; however, minor support was identified for exit mass and entry WBC. A higher exit mass predicted a higher survival rate, while a higher entry WBC predicted a lower survival rate. While changes in mass and WBC appear to best predict survival when measured as a

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<sup>2</sup> Shuert, C., Mellish, J., and Horning, M. (*in review*). Physiological predictors of long-term survival in juvenile Steller sea lions (*Eumetopias jubatus*). *Conservation Physiology*.

change over time, single-point sampling may still be an effective way to improve estimates of population health.

## **2.2 Introduction**

### *2.2.1 General Overview*

Population decline in the western distinct population segment (wDPS) of Steller sea lions (*Eumetopias jubatus*) has led to a substantial effort to understand the population dynamics and life-history of this endangered species. Following the decline, numerous studies have pointed to predation pressure (Springer *et al.* 2003; Maniscalco *et al.* 2007; Trites *et al.* 2007; Horning & Mellish 2009, 2012, 2014), decreased condition due to nutritional limitation (Fritz & Hinckley 2005; Rosen & Trites 2005; du Dot *et al.* 2008, 2009; Rosen 2009; Calkins *et al.* 2013), and changes in natality (Holmes *et al.* 2007; Maniscalco *et al.* 2010) as potential primary drivers of population change. Juvenile survival and natality have been at the forefront of recent research efforts as they are believed to be important vital rates in recent recovery trends (e.g., Pendleton *et al.* 2006). Maternal investment strategies may fluctuate in response to ecosystem dynamics, with effects on offspring size and survival (e.g., Maniscalco 2014). While we may never find a clear cause to the decline, effective management relies on accurate predictions of population health and physiological resiliency.

### *2.2.2 Health and survival*

The Transient Juvenile Steller sea lion (TJ) project was developed as a framework to access wild individuals under highly controlled conditions (Mellish *et al.* 2006). This allowed researchers to carefully and strategically manage data collection while minimizing disturbance to the population at large. Over the last decade, this project included studies of behavior,

physiology, and nutrition (Goldstein *et al.* 2007; Mellish *et al.* 2007a; Thomton & Mellish 2007; Thomton *et al.* 2008; Walker *et al.* 2009, 2010, 2011a, 2011b; Calkins *et al.* 2013). The TJ project also facilitated the deployment of first generation Life History Tags (LHX-1, Horning & Hill 2005). LHX-1 allows for life-long monitoring of an individual sea lion resulting in previously unattainable, high resolution, mortality detection (known-fate) data (Horning *et al.* 2008; Horning & Mellish 2009). Multiple handling events for a given individual allowed for an extensive database across a longer time frame than traditionally available with a single sampling event.

### *2.2.3 Physiological predictors*

Comprehensive health screenings were completed on TJ animals on a regular schedule while individuals were in captivity. At a minimum, these exams included complete blood counts, serum chemistry panels, standard morphometrics and an assessment of body condition. This information allowed for an in-depth evaluation of the physiological responses to the various research activities, including procedures that were commonplace in the field (e.g., hot-iron branding, Mellish *et al.* 2007b; Walker *et al.* 2010), as well as those that were novel (e.g., LHX-1 implantation, Mellish *et al.* 2007b). Comparisons of this level were only possible due to baselines established for this species earlier in the TJ project (Mellish *et al.* 2006).

Physiological responses from stressful events are commonly used as indicator of health and survival and can be tracked by changes in glucocorticoids and haptoglobins in the blood (Thomton & Mellish 2007; Franceschini *et al.* 2008; Petrauskas *et al.* 2008; Bechshøft *et al.* 2013). Skinner *et al.* (2015) recently pointed to six principle analytes as important indicators of physiological responses to various research activities specific to this group of sea lions. Several commonly measured blood parameters including serum globulins (GLOB, g/dL), glucose (GLU,

mg/dL), total bilirubin (TBIL, mg/dL), platelets (PLT,  $10^3/\text{mm}^3$ ), red blood cells (RBC,  $10^6/\text{mm}^3$ ), and white blood cells (WBC,  $10^3/\text{mm}^3$ ) fluctuated in response to food intake, time in captivity, and recovery from LHX-1 implantation (Mellish *et al.* 2006; Skinner *et al.* 2015). While it would not be expected that a single point measure would predict long-term survival, the ability of an individual to return to baseline blood parameters may indicate resiliency. This approach has been applied in rehabilitation cases for other pinniped species with varying success (Greig *et al.* 2010; Witte *et al.* 2014). Elements of body condition on the other hand, such as mass and body fat, have long been established as proxies for potential survival in a variety of marine mammal species (Davis *et al.* 1988; Beck *et al.* 1993; Trites & Jonker 2000; Hall *et al.* 2002; Kovacs *et al.* 2011; Maniscalco 2014). While many efforts have been made to monitor these physiological markers in the short term as indicators of acute stress, few have attempted to relate these in combination to long-term survival in wild individuals.

#### 2.2.4 Study Goals

Long-term tracking of TJs was achieved through visual resight of individual hot-iron brands on their left flank ( $n=45$ ) prior to release. This practice is common in this species for studying survival and behavior through mark-recapture methods using the Cormack-Jolly Seber (CJS) open population model design (Cormack 1964; Seber 1986; Pendleton *et al.* 2006; Hastings *et al.* 2009; Jemison *et al.* 2013), and it was a mandated procedure in accordance with the research authorization (National Marine Fisheries Service Permit 881-1890, 14335).

The current analysis builds on a continued effort to document potential longer-term research impacts on the individual, as well as to identify potential markers of survival that may be modified to suit standard field practices. We took a three-tiered approach to evaluate research objectives. The first tier investigated survival in relation to observed acute response to handling

stress through changes in selected blood parameters between exit and exit from temporary captivity. The second tier evaluated common condition parameters in their predictive ability on long-term survival and the potential effects of captivity. The final tier evaluated single time point sampling in its efficacy as a simplified technique to project similar survival trends for use in field sampling operations.

## **2.3 Methods**

### *2.3.1 Study Animals*

A total of 72 juvenile sea lions were captured via underwater lasso technique and branded between 2005 and 2011 (McAllister *et al.* 2001). Forty-five of these individuals (TJs) were maintained for a maximum of three months in temporary captivity for research purposes (Mellish *et al.* 2006). The remaining twenty-seven sea lions were sampled, branded and released, experiencing no other handling events. All branded animals received a unique 4-digit alphanumeric brand in the =900 series. Four animals were collected through a joint field effort with the Alaska Department of Fish & Game, and as a result have brands of =581 through =584. Age at capture was  $1.56 \pm 0.51$  years. Initial age estimates were made via canine length (King *et al.* 2007), and further refined to the closest mean pupping date for that cohort birth year (Maniscalco *et al.* 2006). Three individuals were aged from a standard length at age correlation as canine lengths were not coincidentally measured (King *et al.* 2007). Health exams and sample collection took place within three hours of a capturing event (Mellish *et al.* 2006). Specific details of handling protocol and sample collection can be found in Mellish *et al.* (2004), Mellish *et al.* (2006), and Goldstein *et al.* (2007). The consistency in collection of blood samples allowed for the inclusion of all individuals participating in captivity (n=45) in this portion of the model study. However, morphometrics and condition parameters were not collected consistently

across all years; therefore, sample size for these measures differed at entry (n=36) and exit (n=23). All work was carried out under permits NMFS #881-1668, 881-1890, 14335.

### *2.3.2 Blood Parameters*

Several blood analytes have been previously identified as indicators of physiological response in relation to research treatments (Skinner *et al.* 2015). These analytes include serum chemistry and complete blood count values of globulins (GLOB), glucose (GLU), platelets (PLT), erythrocytes (RBC), total bilirubin (TBIL), and leukocytes (WBC). The difference in these parameters measured at exit and entry (dGLOB, dGLU, dPLT, dRBC, dTBIL, dWBC) was used to assess their predictive power of long-term survival with the addition of their change in mass (dMASS). One individual was dropped from the sample set due to clinical concerns outside of the scope of research activities during captivity. Blood samples were obtained while animals were under isoflurane anesthesia from either the caudal plexus or hind flipper vein. All blood analytes were measured within 5 minutes of collection via VetScan HMII<sup>®</sup> and chemistry automated analyzers (Abaxis, Union City, CA). Serum aliquots were also archived and stored at -80°C. Specifics of collection can be found in Mellish *et al.* (2006). Blood was collected consistently throughout the study at entry and exit allowing us the use of all 44 individuals in this portion of the study.

### *2.3.3 Body Condition*

Several measures of body condition were assessed in relation to their long-term effect on survival. Simple morphometric measures that were modeled included axial girth (AXGI, cm) and standard length (STLE, cm) via measuring tape to the nearest tenth of a centimeter, and mass (kg) rounded to the nearest half kilogram. Deuterium (D) isotope dilution, a commonly used

non-lethal proxy for components of body composition, was based on an intramuscular injection of deuterium oxide (syringe containing on average  $10.4 \pm 0.19\text{g}$  of 99.9% D weighed before and after injection, Sigma-Aldrich, St. Louis, MO, USA). Each equilibration was calculated by comparing a pre-injection with two post-injection blood samples (2.00 and 2.25 hr). All samples were analyzed in triplicate for  $\delta\text{D}$  versus Vienna-Standard Mean Ocean Water (V-SMOW) (Scrimgeour *et al.* 1993). Total body water (kg) was calculated with correction for the overestimation of dilution space (Bowen & Iverson 1998). Total body fat (%TBF) and total body protein (%TBP) were then calculated by equations empirically derived for grey seals (*Halichoerus grypus*; Reilly & Fedak 1990; Rea *et al.* 2007). The use of isotope dilution methods was discontinued after spring 2008 due to logistic constraints. Instead, body composition was derived from a model predicting total body water from several morphometric measures (Reilly & Fedak 1990; Shuert *et al.* 2015). We also included the ratio of total body fat to total body protein (F:P, kg), as a potentially important condition index (du Dot *et al.* 2009). %TBF, %TBP, and F:P were all modeled with the addition of a seasonal component as variation of fat and protein content is heavily influenced by the time of year (Rea *et al.* 2007).

#### 2.3.4 Survival Analysis

Branded animal resight information for the period of May 15<sup>th</sup>, 2005 through August 30<sup>th</sup>, 2013, was provided by the National Marine Mammal Laboratory (NMML, 7600 Sand Point Way, Seattle, Washington 98115), Alaska Department of Fish & Game (ADFG, 1255 W. 8th Street, Juneau, Alaska 99811), and the Alaska SeaLife Center (ALSC, 301 Railway Ave, Seward, Alaska 99664). The first two sources provided resights as conducted by yearly cruise efforts to survey haulouts and rookeries largely during the summer months in South Central and Southeastern Alaska. The third source focused on the Chiswell Island rookery and nearby



haulouts surveyed by remote video monitoring in the central Gulf of Alaska continuously through the summer breeding season and periodically through the rest of the year.

Resight data for each brand were organized into a simple binary code encounter history for use in program MARK (White & Burnham 1999). Individual encounter histories contained nine resight intervals between 2005 and 2013. Each year was set at a default '0' for no resight events, and a '1' if a resight occurred, regardless of the frequency of resights within an interval. The resight year included the months of March through November. Encounter histories were used for generating estimates of survival ( $\Phi$ ) and resight ( $p$ ) probabilities through the CJS method for estimation. Resight effort was included in all  $p$  models as a covariate to properly scale yearly differences in institutional effort and prevent inflation of resight probabilities. This was done by scaling down the average days of effort per year to a proportion of the year where effort took place. This proportion was then simply translated to whole numbers on a scale of 1 to 10 for coding ease. Each model was then analyzed through program MARK via the RMark user interface with a corresponding set of encounter histories (Laake 2013).

Each focal blood parameter was tested separately to measure its relative importance in predicting observed survival (see Table 2.1). Models included the interaction of sex and age for dGLOB, dRBC, and dWBC as they are known to develop proportionally with age and sex (Horning & Trillmich 1997; Richmond *et al.* 2005; Keogh *et al.* 2010). Other models testing dGLU, dPLT, and dTBIL included both sex and age as separate additive parameters, but not as an interaction term. Other models included morphometric values (AXGI, STLE, and MASS), body condition (%TBF and %TBP with the addition of a seasonal term), and body composition ratios (F:P with additive effect of season) with the addition of the interaction of sex and age to account for age and sex specific changes in these parameters. All continuous covariates were z-

standardized to maintain normality assumptions in model selection (Cooch & White 2013). Models were ranked based on Akaike Information Criterion model selection methods, corrected for small sample size (AICc, Anderson *et al.* 1994, Anderson *et al.* 1998). Models with  $\leq 2$   $\Delta$ AICc were deemed to have extensive support, while models with  $\leq 5$   $\Delta$ AICc were allotted minor support in the data set. Models with  $\geq 5$   $\Delta$ AICc were not considered to have support. Goodness-of-fit testing was also used for global models of each grouping factor to assess the potential for model overfitting through the program U-CARE (Choquet *et al.* 2009).

## 2.4 Results

A total of 4 model sets were run separately for each of the six *a priori* blood parameters and a total of 7 model sets for both entry and exit body condition terms (see Table 2.1). Goodness-of-fit testing through program U-CARE resulted in  $\hat{c}$  values of approximately 1, so no adjustments to grouping factors were needed. Extensive support in the data ( $\leq 2$   $\Delta$ AICc) was identified for dMASS and the additive effect of dMASS and dWBC for predicting the apparent survival of temporarily captive sea lions, with the addition of sex and age interactions. Minor support ( $\leq 5$   $\Delta$ AICc) was identified for the importance of the additive effects of dMASS with dGLOB, dGLU, dPLT, dRBC, and dTBIL as well as all entry body condition parameters. Little to no support was identified for any exit body condition models. The effects of dMASS and dWBC were considered to be the top models. On average, animals saw an increase in mass of  $4.2 \pm 12\%$ , with no significant difference between the sexes (two sample t-test,  $p=0.22$ ). Change in mass, with the addition of sex and age, resulted in a model-averaged apparent survival rate ( $\Phi$ ) of  $0.81 \pm 0.15$  (see Figure 2.1). Leukocytes increased by  $1.01 \pm 3.53$   $m/mm^3$  over the period of captivity, with no evidence that dWBC was significantly different for males and females in our age classes (two sample t-test,  $p=0.74$ ). The addition of other parameters that did not covary

(e.g., dGLU and dTBIL) was done in an attempt to improve predictive ability of dMASS and dWBC, but yielded none. For the final tier, dMASS and dWBC were tested against combinations of their entry and exit values, and dMASS and dWBC still had the most support in the data set. However, exit MASS, entry WBC, and the sum of the two still had minor support in the data ( $\leq 5 \Delta AICc$ ). Animals with a greater exit mass had higher survival rates whereas sea lions with higher WBC at entry had lower survival rates (see Figure 2.1).

## **2.5 Discussion**

### *2.5.1 Changes in mass*

The changes over captivity of several potentially important blood parameters were assessed for their importance in the long-term survival of Steller sea lions. When tested separately, the change in mass (dMASS) ranked higher in AICc model selection than most blood parameters and, when modeled with sex and age, was found to have the most support in the data overall. Increased mass gains showed a strong relationship to an increasing survival rate, similar to first year survival in grey seals (*Halichoerus grypus*, Hall *et al.* 2002; Bowen *et al.* 2015). Mass has long been used as a key vital rate for assessments of population health in a variety of marine mammal species (Castellini & Calkins 1993; Hall *et al.* 2002; Greig *et al.* 2010; Rutishauser *et al.* 2010; Mellish *et al.* 2011), and age classes (Castellini *et al.* 1993; Hastings *et al.* 2011; Maniscalco 2014). Changes in mass have been identified as important predictors of the survival of animals admitted into rehabilitation programs (Greig *et al.* 2010). Results presented here suggest that clinically healthy reference ranges contain fine-scale heterogeneity in their projected effects on long-term survival rates. Mass at the population level are usually only of concern when animals are below a minimum threshold (e.g., starveling pups) or when population

level mass means are tracked over decades (e.g., York, 1994; Trites and Jonker, 2000; Holmes *et al.*, 2007).

The length of captivity (average 62, range 22-82 days) was a significant predictor of dMASS when run in a linear model framework ( $F=6.56$ ,  $p=0.014$ ). While mass gains could be a direct result of the modified *ad libitum* feeding regime necessary in the holding facility, some individuals may have lost mass as part of a behavioral response to a contained environment. Most animals were within the expected mass reference ranges for this population, regardless of gains or losses associated with captivity and research procedures. Individual mass changes may be an indicator of resiliency to new environments or forage opportunities (Mellish *et al.* 2006). LHX implant status ( $n=34$  implanted) was also a significant predictor of dMASS ( $F=11.74$ ,  $p=0.001$ ). Previous work documenting implantation responses found that food consumption dropped and rebounded within a week of LHX implantation (Skinner *et al.* 2015). This may also be a reason for the significance of LHX status in overall survival as found in Chapter 1 of this thesis.

### 2.5.2 – *Leukocytes*

The change in leukocytes (dWBC) was found to have extensive support in the model with the additive effect of dMASS for predicting long-term survival. Our model predictions on survival were not as strong as and more variable than dMASS (Figure 2.1), and it does appear that there is a small increase in survival with increasing dWBC. This may be explained by several mechanisms. Increases in WBC might reflect an individual's ability to regulate their immune system, in spite of research procedure stress (e.g., Fair *et al.* 2014). Since the animals studied here were weaned within a year or two of capture, the observed change in WBC may be related to developmental status. The critical development period for leukocytes is during the pup

and pre-weaning juvenile stages, when immunity is passed from mother to offspring (e.g., Keogh *et al.* 2010). WBC have been found to be important indicators of survival in rehabilitation settings, and have been repeatedly correlated with importance to the sub-adult age class (Greig *et al.* 2010).

Length of stay in captivity was a significant predictor of dWBC in a linear model framework ( $F=16.76$ ,  $p<0.001$ ). It has been found that WBC count tended to decrease over the time of captivity, but increased at a 15 and 30 day response for LHX implantation and branding, respectively (Skinner *et al.* 2015). Changes in captivity duration with experimental procedure shifts have influenced our finding of dWBC as being predicted by length of captivity, rather than it being a direct link to captivity stress. Conversely, LHX implant status was not a significant predictor of dWBC ( $F=2.12$ ,  $p=0.14$ ), suggesting that the observed response here was de-coupled from implantation stress. It is possible that the positive influence of increased WBC values in sea lions may be reflecting similar results in humans that demonstrated that post-surgery survival greatly increased when an increase in WBC was observed (Lu *et al.* 2014).

### 2.5.3 – *Single-point measures*

Models containing measures of our top competing parameters (MASS and WBC) at entry and exit were pitted against the original models of the change in these parameters. While dMASS and dWBC were the top models, models including exit mass (xMASS, mean  $131 \pm 28.8$  kg) and entry leukocytes (eWBC, mean  $10.5 \pm 1.75$  m/mm<sup>3</sup>) still had support in our models for predicting long-term survival ( $< 5 \Delta AICc$ ), and ranked above our null model. When compared to reference values derived from Mellish *et al.* (2006), all data fit within a 95% confidence interval for both xMASS and eWBC, with only a few exceptions. While xMASS followed the same pattern in predicting survival, eWBC values indicated that a slightly elevated entry count may

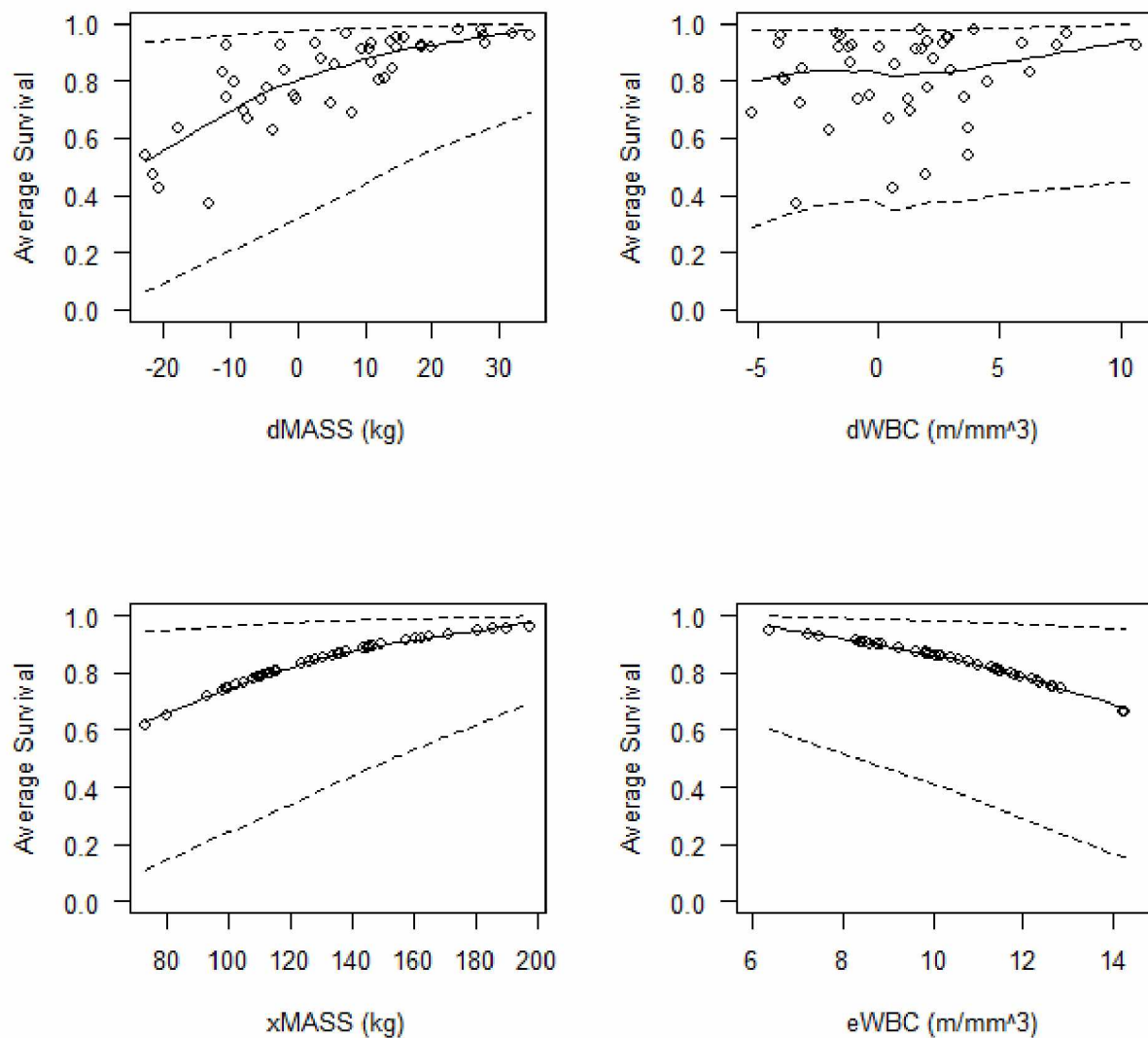
reflect a decreased average survival rate of about 10-20% (see Fig. 1). The predicted survival rates for by dWBC and eWBC may reflect a trade off in leukocyte count, where our mean may indicate the ideal. Single time point measures, while not ideal in our model rankings, may be useful in predicting long-term survival trends in field sampling where only one handling event is feasible.

#### 2.5.4 - Implications

Predictions of survival and mortality have always been a goal of health research both in at-risk animal populations as well as in humans. Most often, this is approached through stress hormones and their associated serum proteins. Broad ranging efforts to monitor stress as a proxy for survival have been attempted in terrestrial translocation programs (e.g., Grevy's zebra *Equus grevyi*, Franceschini *et al.* 2008), conservation monitoring efforts (e.g., polar bear *Ursus maritimus*, Bechshøft *et al.* 2013), and in retrospect to sampling procedures in this cohort of sea lions (e.g., Thomson & Mellish 2007) as well as other marine species (e.g., bottlenose dolphin *Tursiops truncatus*, Fair *et al.* 2014). Human studies represent the greatest effort in research and risk identification for factors of disease and links to survival (e.g., Yudkin *et al.* 2000; Hackman & Anand 2003). A recent retrospective analysis of a human biological database found that elevated levels of various blood parameters, including glycoproteins, cholesterol, albumin, and citrate, in presumed healthy individuals were indicative of mortality a few years following sampling (Fischer *et al.* 2014).

With the findings in the current study, we can comment about the potential factors leading to mortality through known-fate data returns from the LHX-1 implants. The majority of mortality events reported by implanted tags has likely been due to predation as based on temperature curves at end of life (Horning & Mellish 2014). Our results indicated mass gain and

mass at exit are strong indicators of survival, with smaller animals experiencing a lower average survival. This could be a direct indication that outside of any measure of health status, smaller animals might simply be less developed in their diving ability and efficiency for predator avoidance (e.g., Le Boeuf *et al.* 1996; Burns 1999; van den Hoff & Morrice 2008). Smaller animals that are weaned early may be spending more time at sea foraging due to this inefficiency in diving, thus spending more time at risk to predation. Dive ability is tightly linked to mass through the ability to store oxygen, which greatly increases with mass in pinnipeds (e.g., Noren *et al.*, 2005). Our results indicate that mass in general may be a good predictor of survival in the long term. Finer scale indicators, such as WBC, may be useful in more detailed prediction models. Despite the existence of “healthy” reference ranges for this species, it appears that variability still exists within projected survival rates.



**Figure 2.1. Predicted model-averaged survival rates based on continuous covariates.** The change in mass (dMASS, kg) and white blood cell counts (dWBC, m/mm<sup>3</sup>) had the most support in the data on modeling the apparent survival rates in juvenile temporarily captive Steller sea lions (*Eumetopias jubatus*). Single point measures yielded exit mass (xMASS, kg) and entry white blood cell counts (eWBC, m/mm<sup>3</sup>) to be slightly less supported, but important in predictive survival. Solid lines represent the general trend through loess smoothing. Broken lines indicate 95% confidence intervals in predicted averaged survival.



**Table 2.1. Candidate physiological model set for predicting survival.** Model structures designed *a priori* for predicting long-term survival ( $\phi$ ) and resighting probability ( $p$ ) in juvenile Steller sea lions (*Eumetopias jubatus*). Blood parameters (a, B.P.) were modeled with (dGLOB, dRBC, dWBC) and without (dGLU, dPLT, dTBIL) interaction terms when they were found to trend with sex and age or not, respectively. Body condition parameters were always modeled with an interaction of sex and age as well as a seasonal component (b). Overall return rates were modeled using the Cormack-Jolly-Seber population approach as the multinomial product of  $\phi$  and  $p$  models.

(a)	Survival Models	Resighting Models
	Phi(~Sex + Age)	
	Phi(~Sex + Age + dMASS)	
	Phi(~Sex + Age + d[B.P.])	* p(~Sex + Age + effort)
	Phi(~Sex + Age + dMASS + d[B.P.])	
	- OR -	
	Phi(~Sex + Age + Sex:Age)	
	Phi(~Sex + Age + Sex:Age + dMASS)	
	Phi(~Sex + Age + Sex:Age + d[B.P.])	* p(~Sex + Age + Sex:Age + effort)
	Phi(~Sex + Age + Sex:Age + dMASS + d[B.P.])	
(b)		
	Phi(~Sex + Age + Sex:Age)	
	Phi(~Sex + Age + Sex:Age + AXGI)	
	Phi(~Sex + Age + Sex:Age + STLE)	
	Phi(~Sex + Age + Sex:Age + MASS)	* p(~Sex + Age + Sex:Age + effort)
	Phi(~Sex + Age + Sex:Age + TBF + Season)	
	Phi(~Sex + Age + Sex:Age + TBP + Season)	
	Phi(~Sex + Age + Sex:Age + F:P + Season)	

## Literature Cited

- Anderson, D.R., Burnham, K.P. & White, G.C. (1994). AIC model selection in overdispersed capture-recapture data. *Ecology*, **75**, 1780–1793.
- Anderson, D.R., Burnham, K.P. & White, G.C. (1998). Comparison of Akaike information criterion and consistent Akaike information criterion for model selection and statistical inference from capture  $\pm$  recapture studies. *Journal of Applied Statistics*, **25**, 263–283.
- Bechshøft, T.Ø., Sonne, C., Rigét, F.F., Letcher, R.J., Novak, M.A., Henchey, E., Meyer, J.S., Eulaers, I., Jaspers, V.L.B., Covaci, A. & Dietz, R. (2013). Polar bear stress hormone cortisol fluctuates with the North Atlantic Oscillation climate index. *Polar Biology*, **36**, 1525–1529.
- Beck, C.G., Smith, T.G. & Hammill, M.O. (1993). Evaluation of body condition in the Northwest Atlantic harp seal (*Phoca groenlandica*). *Canadian Journal of Fisheries and Aquatic Sciences*, **50**, 1372–1381.
- Bowen, W.D., den Heyer, C.E., McMillan, J.I. & Iverson, S.J. (2015). Offspring size at weaning affects survival to recruitment and reproductive performance of primiparous gray seals. *Ecology and Evolution*, **7**, 1412–1424.
- Bowen, W.D. & Iverson, S.J. (1998). Estimation of total body water in pinnipeds using hydrogen-isotope dilution. *Physiological Zoology*, **71**, 329–332.
- Burns, J.M. (1999). The development of diving behavior in juvenile Weddell seals: pushing physiological limits in order to survive. *Canadian Journal of Zoology*, **77**, 737–747.
- Calkins, D.G., Atkinson, S., Mellish, J.-A., Waite, J.N. & Carpenter, J.R. (2013). The pollock paradox: Juvenile Steller sea lions experience rapid growth on pollock diets in fall and spring. *Journal of Experimental Marine Biology and Ecology*, **441**, 55–61.
- Castellini, M.A. & Calkins, D.G. (1993). Mass estimates using body morphology in Steller sea lions. *Marine Mammal Science*, **9**, 48–54.
- Castellini, M.A., Davis, R.W., Loughlin, T.R. & William, T.M. (1993). Blood chemistries and body condition of Steller sea lion pups at Marmot Island, Alaska. *Marine Mammal Science*, **9**, 202–208.
- Choquet, R., Lebreton, J.D., Gimenez, O., Reboulet, A.M. & Pradel, R. (2009). U-CARE: Utilities for performing goodness of fit tests and manipulating CAPture-REcapture data. *Ecography*, **32**, 1071–1074.
- Cooch, E.G. & White, G.C. (2013). Program MARK - A Gentle Introduction, 12th edn. (E.G. Cooch & G.C. White, Eds.).

- Cormack, R.M. (1964). Estimates of survival from the sighting of marked animals. *Biometrika*, **51**, 429–438.
- Davis, R.W., Williams, T.M., Thomas, J.A., Kastelein, R.A. & Cornell, L.H. (1988). The effects of oil contamination and cleaning on sea otters (*Enhydra lutris*): Metabolism, thermoregulation, and behavior. *Canadian Journal of Zoology*, **66**, 2782–2790.
- du Dot, T.J., Rosen, D.A.S. & Trites, A.W. (2009). Energy reallocation during and after periods of nutritional stress in Steller sea lions: Low-quality diet reduces capacity for physiological adjustments. *Physiological and Biochemical Zoology*, **82**, 516–530.
- du Dot, T.J., Rosen, D.A.S. & Trites, A.W. (2008). Steller sea lions show diet-dependent changes in body composition during nutritional stress and recover more easily from mass loss in winter than in summer. *Journal of Experimental Marine Biology and Ecology*, **367**, 1–10.
- Fair, P.A., Schaefer, A.M., Romano, T.A., Bossart, G.D., Lamb, S. V. & Reif, J.S. (2014). Stress response of wild bottlenose dolphins (*Tursiops truncatus*) during capture-release health assessment studies. *General and Comparative Endocrinology*, **206C**, 203–212.
- Fischer, K., Kettunen, J., Würtz, P., Haller, T., Havulinna, A.S., Kangas, A.J., Soininen, P., Esko, T., Tammesoo, M.L., Mägi, R., Smit, S., Palotie, A., Ripatti, S., Salomaa, V., Ala-Korpela, M., Perola, M. & Metspalu, A. (2014). Biomarker profiling by nuclear magnetic resonance spectroscopy for the prediction of all-cause mortality: An observational study of 17,345 persons. *PLoS Medicine*, **11**.
- Franceschini, M.D., Rubenstein, D.I., Low, B. & Romero, L.M. (2008). Fecal glucocorticoid metabolite analysis as an indicator of stress during translocation and acclimation in an endangered large mammal, the Grevy's zebra. *Animal Conservation*, **11**, 263–269.
- Fritz, L.W. & Hinckley, S. (2005). A critical review of the regime shift –“junk food”– nutritional stress hypothesis for the decline of the western stock of Steller sea lion. *Marine Mammal Science*, **21**, 476–518.
- Goldstein, T., Stephens, C.A., Jang, S.S., Conrad, P.A., Field, C., Dunn, L.J. & Mellish, J.-A.E. (2007). Longitudinal health and disease monitoring in juvenile Steller sea lions (*Eumetopias jubatus*) in temporary captivity in Alaska compared with a free-ranging cohort. *Aquatic Mammals*, **33**, 337–348.
- Greig, D.J., Gulland, F.M.D., Rios, C.A. & Hall, A.J. (2010). Hematology and serum chemistry in stranded and wild-caught harbor seals in central California: reference intervals, predictors of survival, and parameters affecting blood variables. *Journal of Wildlife Diseases*, **46**, 1172–84.
- Hackman, D.G. & Anand, S.S. (2003). Emerging risk factors for atherosclerotic vascular disease: a critical review of the evidence. *Journal of the American Medical Association*, **290**, 932–940.

- Hall, A.J., Connell, B.J.M.C. & Barker, R.J. (2002). The effect of total immunoglobulin levels, mass and condition on the first-year survival of grey seal pups. *Functional Ecology*, **16**, 462–474.
- Hastings, K.K., Gelatt, T.S. & King, J.C. (2009). Postbranding survival of Steller sea lion pups at Lowrie Island in southeast Alaska. *Journal of Wildlife Management*, **73**, 1040–1051.
- Hastings, K.K., Jemison, L.A., Gelatt, T.S., Laake, J.L., Pendleton, G.W., King, J.C., Trites, A.W. & Pitcher, K.W. (2011). Cohort effects and spatial variation in age-specific survival of Steller sea lions from southeastern Alaska. *Ecosphere*, **2**, art111.
- van den Hoff, J. & Morrice, M.G. (2008). Sleeper shark (*Somniosus antarcticus*) and other bite wounds observed on southern elephant seals (*Mirounga leonina*) at Macquarie Island. *Marine Mammal Science*, **24**, 239–247.
- Holmes, E.E., Fritz, L.W., York, A.E. & Sweeney, K. (2007). Age-structured modeling reveals long-term declines in the natality of western Steller sea lions. *Ecological Applications*, **17**, 2214–32.
- Horning, M., Haulena, M., Tuomi, P.A. & Mellish, J.-A.E. (2008). Intraperitoneal implantation of life-long telemetry transmitters in otariids. *BMC Veterinary Research*, **4**, 51.
- Horning, M. & Hill, R.D. (2005). Designing an archival satellite transmitter for life-long deployments on oceanic vertebrates: The Life History Transmitter. *IEEE Journal of Oceanic Engineering*, **30**, 807–817.
- Horning, M. & Mellish, J.-A.E. (2014). In cold blood: evidence of Pacific sleeper shark (*Somniosus pacificus*) predation on Steller sea lions (*Eumetopias jubatus*) in the Gulf of Alaska. *Fishery Bulletin*, **112**, 297–310.
- Horning, M. & Mellish, J.-A.E. (2012). Predation on an upper trophic marine predator, the Steller sea lion: evaluating high juvenile mortality in a density dependent conceptual framework. *PLoS ONE*, **7**, e30173.
- Horning, M. & Mellish, J.-A.E. (2009). Spatially explicit detection of predation on individual pinnipeds from implanted post-mortem satellite data transmitters. *Endangered Species Research*, **10**, 135–143.
- Horning, M. & Trillmich, F. (1997). Development of hemoglobin, hematocrit, and erythrocyte values in Galapagos fur seals. *Marine Mammal Science*, **13**, 100–113.
- Jemison, L.A., Pendleton, G.W., Fritz, L.W., Hastings, K.K., Maniscalco, J.M., Trites, A.W. & Gelatt, T.S. (2013). Inter-population movements of Steller sea lions in Alaska with implications for population separation. *PLoS ONE*, **8**, e70167.
- Keogh, M.J., Maniscalco, J.M. & Atkinson, S. (2010). Steller sea lion (*Eumetopias jubatus*) pups undergo a decrease in circulating white blood cells and the ability of T cells to proliferate

- during early postnatal development. *Veterinary Immunology and Immunopathology*, **137**, 298–304.
- King, J.C., Gelatt, T.S., Pitcher, K.W. & Pendleton, G.W. (2007). A field-based method for estimating age in free-ranging Steller sea lions (*Eumetopias jubatus*) less than twenty-four months of age. *Marine Mammal Science*, **23**, 262–271.
- Kovacs, K.M., Lydersen, C., Overland, J.E. & Moore, S.E. (2011). Impacts of changing sea-ice conditions on Arctic marine mammals. *Marine Biodiversity*, **41**, 181–194.
- Laake, J.L. (2013). RMark: An R interface for analysis of capture-recapture data with MARK. *AFSC Processed Report*, 2013-01, 1–25.
- Le Boeuf, B.J., Morris, P. a, Blackwell, S.B., Crocker, D.E. & Costa, D.P. (1996). Diving behavior of juvenile northern elephant seals. *Canadian Journal of Zoology*, **74**, 1632–1644.
- Lu, J., Chen, Y., Zhang, L., Wang, X., Li, Y. & Wang, C. (2014). Laboratory nutritional parameters can predict one-year mortality in elderly patients with intertrochanteric fracture. *Journal of Nutritional Health & Food Science*, **2**, 1–6.
- Maniscalco, J.M. (2014). The effects of birth weight and maternal care on survival of juvenile Steller sea lions (*Eumetopias jubatus*). *PloS ONE*, **9**, e96328.
- Maniscalco, J.M., Matkin, C.O., Maldini, D., Calkins, D.G. & Atkinson, S. (2007). Assessing killer whale predation on Steller sea lions from field observations in Kenai Fjords, Alaska. *Marine Mammal Science*, **23**, 306–321.
- Maniscalco, J.M., Parker, P. & Atkinson, S. (2006). Interseasonal and interannual measures of maternal care among individual Steller sea lions (*Eumetopias jubatus*). *Journal of Mammalogy*, **87**, 304–311.
- Maniscalco, J.M., Springer, A.M. & Parker, P. (2010). High natality rates of endangered Steller sea lions in Kenai Fjords, Alaska and perceptions of population status in the Gulf of Alaska. *PloS ONE*, **5**, e10076.
- McAllister, D.C., Calkins, D.G. & Pitcher, K.W. (2001). Underwater capture of juvenile Steller sea lions with Scuba: a narrated video presentation. S.C. Jewett, ed, Cold water diving for science. *Proceedings of the 21st Annual Scientific Diving Symposium*, Seward, Alaska, September 19-23, p. 98.
- Mellish, J.-A.E., Calkins, D.G., Christen, D.R., Horning, M., Rea, L.D. & Atkinson, S.K. (2006). Temporary captivity as a research tool: comprehensive study of wild pinnipeds under controlled conditions. *Aquatic Mammals*, **32**, 58–65.
- Mellish, J.-A., Thomson, J. & Horning, M. (2007a). Physiological and behavioral response to intra-abdominal transmitter implantation in Steller sea lions. *Journal of Experimental Marine Biology and Ecology*, **351**, 283–293.

- Mellish, J.-A.E., Hindle, A.G. & Horning, M. (2011). Health and condition in the adult Weddell seal of McMurdo Sound, Antarctica. *Zoology*, **114**, 177–83.
- Mellish, J.-A., Hennen, D., Thomson, J., Petrauskas, L., Atkinson, S. & Calkins, D. (2007b). Permanent marking in an endangered species: physiological response to hot branding in Steller sea lions (*Eumetopias jubatus*). *Wildlife Research*, **34**, 43–47.
- Mellish, J.-A.E., Tuomi, P.A. & Horning, M. (2004). Assessment of ultrasound imaging as a noninvasive measure of blubber thickness in pinnipeds. *Journal of Zoo and Wildlife Medicine*, **35**, 116–8.
- Noren, S.R., Iverson, S.J. & Boness, D.J. (2005). Development of the blood and muscle oxygen stores in gray seals (*Halichoerus grypus*): implications for juvenile diving capacity and the necessity of a terrestrial postweaning fast. *Physiological and Biochemical Zoology: PBZ*, **78**, 482–490.
- Pendleton, G.W., Pitcher, K.W., Fritz, L.W., York, A.E., Raum-Suryan, K.L., Loughlin, T.R., Calkins, D.G., Hastings, K.K. & Gelatt, T.S. (2006). Survival of Steller sea lions in Alaska : a comparison of increasing and decreasing populations. *Canadian Journal of Zoology*, **84**, 1163–1172.
- Petrauskas, L., Atkinson, S., Gulland, F., Mellish, J.-A. & Horning, M. (2008). Monitoring glucocorticoid response to rehabilitation and research procedures in California and Steller sea lions. *Journal of Experimental Zoology*, **309A**, 73–82.
- Rea, L.D., Rosen, D.A.S. & Trites, A.W. (2007). Utilization of stored energy reserves during fasting varies by age and season in Steller sea lions. *Canadian Journal of Zoology*, **85**, 190–200.
- Reilly, J.J. & Fedak, M.A. (1990). Measurement of the body composition of living gray seals by hydrogen isotope dilution. *Journal of Applied Physiology*, **69**, 885–891.
- Richmond, J.P., Burns, J.M., Rea, L.D. & Mashburn, K.L. (2005). Postnatal ontogeny of erythropoietin and hematology in free-ranging Steller sea lions (*Eumetopias jubatus*). *General and Comparative Endocrinology*, **141**, 240–247.
- Rosen, D.A.S. (2009). Steller sea lions *Eumetopias jubatus* and nutritional stress: evidence from captive studies. *Mammal Review*, **39**, 284–306.
- Rosen, D.A.S. & Trites, A.W. (2005). Examining the potential for nutritional stress in young Steller sea lions: physiological effects of prey composition. *Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology*, **175**, 265–73.
- Rutishauser, M.R., Costa, D.P., Goebel, M.E. & Williams, T.M. (2010). Ecological implications of body composition and thermal capabilities in young antarctic fur seals (*Arctocephalus gazella*). *Physiological and Biochemical Zoology: PBZ*, **77**, 669–81.

- Scrimgeour, C.M., Rollo, M.M., Mudambo, S.M.K.T., Handley, L.L. & Prosser, S.J. (1993). A simplified method for deuterium/hydrogen isotope ratio measurements on water samples of biological origin. *Biological Mass Spectrometry*, **22**, 383–387.
- Seber, G.A.F. (1986). A review of estimating animal abundance. *Biometrics*, **42**, 267–292.
- Shuert, C.R., Skinner, J.P. & Mellish, J.E. (2015). Weighing our measures: approach-appropriate modeling of body composition in juvenile Steller sea lions (*Eumetopias jubatus*). *Canadian Journal of Zoology*, **180**, 177–180.
- Skinner, J.P., Tuomi, P.A. & Mellish, J.E. (2015). The influence of time in captivity, food intake and acute trauma on blood analytes of juvenile Steller sea lions, *Eumetopias jubatus*. *Conservation Physiology*, **3**, 1–12.
- Springer, A.M., Estes, J.A., van Vliet, G.B., Williams, T.M., Doak, D.F., Danner, E.M., Forney, K.A. & Pfister, B. (2003). Sequential megafaunal collapse in the North Pacific Ocean: an ongoing legacy of industrial whaling? *Proceedings of the National Academy of Sciences*, **100**, 12223–12228.
- Thomton, J.D. & Mellish, J.-A.E. (2007). Haptoglobin concentrations in free-range and temporarily captive juvenile Steller sea lions. *Journal of Wildlife Diseases*, **43**, 258–261.
- Thomton, J.D., Mellish, J.-A.E., Hennen, D.R. & Horning, M. (2008). Juvenile Steller sea lion dive behavior following temporary captivity. *Endangered Species Research*, **4**, 195–203.
- Trites, A.W., Deecke, V.B., Gregr, E.J., Ford, J.K.B. & Olesiuk, P.F. (2007). Killer whales, whaling, and sequential megafaunal collapse in the North Pacific: a comparative analysis of the dynamics of marine mammals in Alaska and British Columbia following commercial whaling. *Marine Mammal Science*, **23**, 751–765.
- Trites, A.W. & Jonker, R.A.H. (2000). Morphometric measurements and body condition of healthy and starveling Steller sea lion pups (*Eumetopias jubatus*). *Aquatic Mammals*, **26**, 151–157.
- Walker, K.A., Horning, M., Mellish, J.-A.E. & Weary, D.M. (2009). Behavioural responses of juvenile Steller sea lions to abdominal surgery: Developing an assessment of post-operative pain. *Applied Animal Behaviour Science*, **120**, 201–207.
- Walker, K.A., Horning, M., Mellish, J.-A.E. & Weary, D.M. (2011a). The effects of two analgesic regimes on behavior after abdominal surgery in Steller sea lions. *The Veterinary Journal*, **190**, 160–4.
- Walker, K.A., Mellish, J.-A.E. & Weary, D.M. (2010). Behavioural responses of juvenile Steller sea lions to hot-iron branding. *Applied Animal Behaviour Science*, **122**, 58–62.
- Walker, K.A., Mellish, J.E. & Weary, D.M. (2011b). Effects of hot-iron branding on heart rate, breathing rate and behaviour of anaesthetised Steller sea lions. *The Veterinary Record*, **169**, 363.

- White, G.C. & Burnham, K.P. (1999). Program MARK: survival estimation from populations of marked animals. *Bird Study*, **46**, S120–S139.
- Witte, K.A., Driver, J., Rosenberger, T., Adler, S. & Siebert, U. (2014). Analysis of blood gases, serum fat and serum protein: a new approach to estimate survival chances of stranded Harbor seal (*Phoca vitulina*) pups from the German North Sea. *Acta Veterinaria Scandinavica*, **56**, 10.
- York, A.E. (1994). The population dynamics of northern sea lions, 1975 - 1985. *Marine Mammal Science*, **10**, 38–51.
- Yudkin, J.S., Kumari, M., Humphries, S.E. & Mohamed-Ali, V. (2000). Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis*, **148**, 209–14.





## GENERAL CONCLUSIONS

This thesis has built on over a decade of data collection facilitated by the Transient Juvenile (TJ) Steller sea lion project, in a continued effort to monitor and evaluate the long-term consequences of research investigations on an endangered species (Mellish *et al.* 2006). Taking the extensive dataset from the TJ project one step further, this thesis modeled and projected the long-term impact of research activities in the form of a survival analysis. This self-evaluation was further used to build recommendations for future applications of temporary captivity, implantation protocols, and adaptation for more common field sampling practices for Steller sea lions.

Temporary captivity has provided an extensive look into aspects of the physiology of the Steller sea lion. In Chapter 1, I evaluated the survival of these individuals across three comparison levels. Sex and capture age were found to be most important characteristics overall, as females and those captured at 25-36 months had higher survival rates than males and those captured at 14-24 months of age, regardless of experimental treatment (e.g., captivity or no captivity). These results are comparable to previous survival findings for the species as part of their life history and maternal investment trade-offs (Raum-Suryan *et al.* 2002; Raum-Suryan *et al.* 2004; Maniscalco 2014). The differential survival between capture ages leads to a recommendation for future use of a temporary captivity program to focus whenever possible on individuals who are aged at 25 months or greater at the time of capture. Results from the second level of analysis indicated that sea lions in temporary captivity also appeared to have lower survival rates than those immediately released after capture. While it is hard to pinpoint the exact cause of this difference, care should be taken in assessing research goals to minimize the length of captivity as much as possible. The final portion of analysis in Chapter 1 focused on the

impact of LHX-1 implantation procedures. The Life History Tag project (LHX-1) has allowed us to confirm and infer causes of Steller sea lion mortality (Horning & Mellish 2012, 2014).

Animals implanted with LHX-1 tags were found to have a lower overall survival rate than those who were not, but the effect was minimal at best and most likely due to modelling design methods rather than true biological relevance. However, efforts to continually refine protocols are in place, such as the newly reduced the size of the second generation LHX-2 tags (Horning & Mellish, pers. comm.).

The TJ project also provided baseline data on a variety of commonly measured blood and condition parameters, as well as behavioral and physiological responses to research procedures (Mellish *et al.* 2006; Goldstein *et al.* 2007; Mellish *et al.* 2007a, 2007b; Thomson & Mellish 2007; Thomson *et al.* 2008; Walker *et al.* 2009, 2010, 2011a, 2011b). Building on the efforts of Skinner *et al.* (2015), Chapter 2 investigated the efficacy of using blood and condition parameters to predict long-term survival. The identified importance of changes in mass (kg) and white blood cell counts (WBC,  $m/mm^3$ ) over captivity demonstrates fine-scale changes in commonly measured parameters as potential long-term indicators of juvenile health. Individuals increasing mass over captivity time were found to exhibit higher survival than those that lost mass. Likewise, individuals with increasing WBC over captivity correlated to a slightly higher survival than those that did not. Both of these measures match previous findings of their importance as individual and population vital rates, as well as their diagnostic role in rehabilitation situations (Hall *et al.* 2002; Rutishauser *et al.* 2010; Greig *et al.* 2010; Keogh *et al.* 2010; Mellish *et al.* 2011; Fair *et al.* 2014). Chapter 2 also investigated the use of a single time point measure of mass or WBC to predict survival. Minimum support was identified for the use of entry values of WBC and exit values of mass from a handling event for predicting long-term

survival. Survival increased with higher exit mass values, and decreased with higher WBC values measured at entry. These models, however, only indicate potential importance in long-term survival and must be validated further before they could be applied as a useful tool for population sampling predictions.

Survival is the ultimate measure of health and resiliency into adulthood, on both individual and population scales. This analysis has evaluated the long-term effects of research treatments in context of their physiological development for juvenile age classes of Steller sea lions. These findings indicate that both the TJ and LHX-1 approaches should be considered as a template for future work with other pinniped species at risk. While research on marine mammals requires a significant level of mandated scrutiny, it is recommended that researchers maintain an elevated level of vigilance, such as retrospective self-evaluation, in order to uphold credible science endeavors in the eyes of peers and public alike.

## Literature Cited

- Fair, P.A., Schaefer, A.M., Romano, T.A., Bossart, G.D., Lamb, S. V. & Reif, J.S. (2014). Stress response of wild bottlenose dolphins (*Tursiops truncatus*) during capture-release health assessment studies. *General and Comparative Endocrinology*, **206C**, 203–212.
- Goldstein, T., Stephens, C.A., Jang, S.S., Conrad, P.A., Field, C., Dunn, L.J. & Mellish, J.-A.E. (2007). Longitudinal health and disease monitoring in juvenile Steller sea lions (*Eumetopias jubatus*) in temporary captivity in Alaska compared with a free-ranging cohort. *Aquatic Mammals*, **33**, 337–348.
- Greig, D.J., Gulland, F.M.D., Rios, C.A. & Hall, A.J. (2010). Hematology and serum chemistry in stranded and wild-caught harbor seals in central California: reference intervals, predictors of survival, and parameters affecting blood variables. *Journal of Wildlife Diseases*, **46**, 1172–84.
- Hall, A.J., Connell, B.J.M.C. & Barker, R.J. (2002). The effect of total immunoglobulin levels, mass and condition on the first-year survival of grey seal pups. *Functional Ecology*, **16**, 462–474.
- Horning, M. & Mellish, J.-A.E. (2014). In cold blood: evidence of Pacific sleeper shark (*Somniosus pacificus*) predation on Steller sea lions (*Eumetopias jubatus*) in the Gulf of Alaska. *Fishery Bulletin*, **112**, 297–310.
- Horning, M. & Mellish, J.-A.E. (2012). Predation on an upper trophic marine predator, the Steller sea lion: evaluating high juvenile mortality in a density dependent conceptual framework. *PloS ONE*, **7**, e30173.
- Keogh, M.J., Maniscalco, J.M. & Atkinson, S. (2010). Steller sea lion (*Eumetopias jubatus*) pups undergo a decrease in circulating white blood cells and the ability of T cells to proliferate during early postnatal development. *Veterinary Immunology and Immunopathology*, **137**, 298–304.
- Maniscalco, J.M. (2014). The effects of birth weight and maternal care on survival of juvenile Steller sea lions (*Eumetopias jubatus*). *PloS ONE*, **9**, e96328.
- Mellish, J.-A.E., Calkins, D.G., Christen, D.R., Horning, M., Rea, L.D. & Atkinson, S.K. (2006). Temporary captivity as a research tool: comprehensive study of wild pinnipeds under controlled conditions. *Aquatic Mammals*, **32**, 58–65.
- Mellish, J.-A., Hennen, D., Thomson, J., Petrauskas, L., Atkinson, S. & Calkins, D. (2007a). Permanent marking in an endangered species: physiological response to hot branding in Steller sea lions (*Eumetopias jubatus*). *Wildlife Research*, **34**, 43–47.
- Mellish, J.-A.E., Hindle, A.G. & Horning, M. (2011). Health and condition in the adult Weddell seal of McMurdo Sound, Antarctica. *Zoology*, **114**, 177–83.

- Mellish, J.-A., Thomson, J. & Horning, M. (2007b). Physiological and behavioral response to intra-abdominal transmitter implantation in Steller sea lions. *Journal of Experimental Marine Biology and Ecology*, **351**, 283–293.
- Raum-Suryan, K.L., Pitcher, K.W., Calkins, D.G., Sease, J.L. & Loughlin, T.R. (2002). Dispersal, rookery fidelity, and metapopulation structure of Steller sea lions (*Eumetopias jubatus*) in an increasing and decreasing population in Alaska. *Marine Mammal Science*, **18**, 746–764.
- Raum-Suryan, K.L., Rehberg, M.J., Pendleton, G.W., Pitcher, K.W. & Gelatt, T.S. (2004). Development of dispersal, movement patterns, and haul-out use by pup and juvenile Steller sea lions (*Eumetopias jubatus*) in Alaska. *Marine Mammal Science*, **20**, 823–850.
- Rutishauser, M.R., Costa, D.P., Goebel, M.E. & Williams, T.M. (2010). Ecological implications of body composition and thermal capabilities in young antarctic fur seals (*Arctocephalus gazella*). *Physiological and biochemical zoology: PBZ*, **77**, 669–681.
- Skinner, J.P., Tuomi, P.A. & Mellish, J.E. (2015). The influence of time in captivity, food intake and acute trauma on blood analytes of juvenile Steller sea lions, *Eumetopias jubatus*. *Conservation Physiology*, **3**, 1–12.
- Thomson, J.D. & Mellish, J.-A.E. (2007). Haptoglobin concentrations in free-range and temporarily captive juvenile Steller sea lions. *Journal of Wildlife Diseases*, **43**, 258–261.
- Thomson, J.D., Mellish, J.-A.E., Hennen, D.R. & Horning, M. (2008). Juvenile Steller sea lion dive behavior following temporary captivity. *Endangered Species Research*, **4**, 195–203.
- Walker, K.A., Horning, M., Mellish, J.-A.E. & Weary, D.M. (2009). Behavioural responses of juvenile Steller sea lions to abdominal surgery: Developing an assessment of post-operative pain. *Applied Animal Behaviour Science*, **120**, 201–207.
- Walker, K.A., Horning, M., Mellish, J.-A.E. & Weary, D.M. (2011a). The effects of two analgesic regimes on behavior after abdominal surgery in Steller sea lions. *The Veterinary Journal*, **190**, 160–4.
- Walker, K.A., Mellish, J.-A.E. & Weary, D.M. (2010). Behavioural responses of juvenile Steller sea lions to hot-iron branding. *Applied Animal Behaviour Science*, **122**, 58–62.
- Walker, K.A., Mellish, J.E. & Weary, D.M. (2011b). Effects of hot-iron branding on heart rate, breathing rate and behaviour of anaesthetised Steller sea lions. *The Veterinary Record*, **169**, 363.