

Physiological predictors of long-term survival in juvenile Steller sea lions (*Eumetopias jubatus*)

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This study builds on a continued effort to document potential long-term research impacts on the individual, as well as to identify potential markers of survival for use in a field framework. The Transient Juvenile Steller sea lion (TJ) project was developed as a novel framework to gain access to wild individuals. We used three analyses to evaluate and predict long-term survival in temporarily captive sea lions ($n = 45$) through Cormack–Jolly–Seber open population modelling techniques. The first analysis investigated survival in relation to the observed responses to handling stress through changes in six principal blood parameters over the duration of captivity. The second analysis evaluated survival compared with body condition and mass at entry and exit from captivity. Finally, the third analysis 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 mass gains ($4.2 \pm 12\%$) over captivity and increases in leucocytes (WBC, $1.01 \pm 3.54 \times 10^3/\text{mm}^3$) resulted in a higher average survival rate (>3 years). Minor support was identified for the single-point measures of exit mass and entry WBC. A higher exit mass predicted a higher survival rate, whereas a higher WBC predicted a lower survival rate. While changes in mass and WBC appear to be the best predictors of survival when measured as a change over time, single-point sampling may still be an effective way to improve estimates of population health.

Key words: *Eumetopias jubatus*, physiology, Steller sea lion, survival

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Introduction

Population decline in the western distinct population segment of Steller sea lions (*Eumetopias jubatus*) has led to a substantial effort to understand the population dynamics and life-history strategies of this endangered species. Numerous studies have pointed to predation pressure (Springer *et al.*, 2003; Maniscalco *et al.*, 2007; Trites *et al.*, 2007; Horning and Mellish, 2009, 2012, 2014), decreased condition due to nutritional limitation (Fritz and Hinckley, 2005; Rosen and 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). Juvenile survival and natality have recently been at the forefront of research efforts, because they are believed to be important drivers of recent recovery trends (e.g. Pendleton *et al.*, 2006). Plasticity in maternal investment strategies may allow compensation for fluctuations in ecosystem dynamics (e.g. Maniscalco, 2014). While the cause of the decline will probably remain a mystery, there is a continued need for accurate predictions of population health and resiliency to ensure effective management strategies.

Health and survival

Breaking from conventional population study techniques, the Transient Juvenile Steller sea lion (TJ) project was developed to gain access to wild individuals (Mellish *et al.*, 2006). Juveniles held in temporary captivity, in highly controlled conditions, allowed researchers to manage data collection strategically while minimizing disturbance to the population at large. Over the last decade, this framework has allowed for studies across a broad range of topics, including behaviour, physiology and nutrition (Goldstein *et al.*, 2007; Mellish *et al.*, 2007b; Thomson and Mellish, 2007; Thomson *et al.*, 2008; Walker *et al.*, 2009, 2010, 2011a, b; Calkins *et al.*, 2013). The TJ project also facilitated the deployment of first-generation Life History Tags (LHX-1; Horning and Hill, 2005). LHX-1 allow 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 and Mellish, 2009). In order to monitor the health of these animals throughout various TJ project activities, individuals were handled periodically, allowing for detailed measures of morphometrics, ultrasound assessment of blubber depth (as per Mellish *et al.*, 2004) and blood collection for blood panels and serum chemistry. 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.

Physiological predictors

Comprehensive health screenings were completed on a regular basis. At a minimum, these examinations included complete blood counts and 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 are commonplace in the field (e.g. hot-iron branding, Mellish *et al.*, 2007a; 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 owing to baselines established for this species earlier in the TJ project (Mellish *et al.*, 2006).

Physiological responses from stressful events are commonly used as indicators of health and survival and can be tracked by changes in glucocorticoids and haptoglobins in the blood (Thomson and Mellish, 2007; Franceschini *et al.*, 2008; Petrauskas *et al.*, 2008; Bechshøft *et al.*, 2013). Skinner *et al.* (2015) recently identified six principal 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; in grams per decilitre), glucose (GLU; in milligrams per decilitre), total bilirubin (TBIL; in milligrams per decilitre), platelets (PLT; $\times 10^3$ per millimetre cubed), red blood cells (RBC; $\times 10^6$ per millimetre cubed) and leucocytes (WBC; $\times 10^3$ per millimetre cubed), fluctuated in response to food intake, time in captivity and recovery from LHX-1 implantation

(Mellish *et al.*, 2006; Skinner *et al.*, 2015). While a single-point measure would not be likely to have the ability to predict long-term survival, the time required for an individual to return baseline blood parameters after a perturbation event is a useful metric, as suggested by its common use in rehabilitation cases (Greig *et al.*, 2010; Witte *et al.*, 2014).

Elements of body condition, 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 and Jonker, 2000; Hall *et al.*, 2002; Kovacs *et al.*, 2011; Maniscalco, 2014). Many efforts have been made to monitor these physiological markers in the short term as indicators of acute stress, but few have attempted to relate these in combination to long-term survival in wild individuals.

Study goals

Long-term tracking of TJs was achieved through visual resight of individual hot-iron brands applied prior to release to their left flank ($n = 45$). This practice is common for studying survival and behaviour of Steller sea lions through mark-recapture methods using the Cormack–Jolly–Seber open population model design (Cormack, 1964; Seber, 1986; Pendleton *et al.*, 2006; Hastings *et al.*, 2009; Jemison *et al.*, 2013) and was required by the research authorization (National Marine Fisheries Service Permit 881-1890, 14335).

The present 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 suit standard field practices. We used three analyses to evaluate research objectives from 10 years of archived data available. The first analysis investigated survival in relation to observed acute response to handling stress, through changes in selected blood parameters between entry and exit from temporary captivity. The second analysis evaluated common condition parameters in relation to the potential effects of captivity and their predictive ability on long-term survival. Finally, single-point sampling was evaluated as a technique to project similar survival trends for use in field sampling operations.

Methods

Study animals

Between 2005 and 2011, a total of 72 juvenile sea lions (45 males and 27 females) were captured via underwater lasso technique and branded (McAllister *et al.*, 2001). Forty-five of these individuals (TJs) were maintained for a maximum of 3 months (62 ± 17.1 days) in temporary captivity for research purposes (Mellish *et al.*, 2006). The remaining 27 sea lions were sampled, branded and released, experiencing no other handling events. All branded animals received a unique four-digit alphanumeric brand in the =900 series. Four animals were collected through a joint field effort with the Alaska Department of Fish & Game, with brands of =581 through

=584. Approximate age at capture was estimated as 1.56 ± 0.51 years (ranging between 14 and 36 months). 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 (King *et al.*, 2007). Initial health examinations and sample collection took place within 3 h of a capturing event (Mellish *et al.*, 2006). Specific details of the handling protocol and sample collection can be found in Mellish *et al.* (2004, 2006) and Goldstein *et al.* (2007). All work was carried out under permits NMFS nos 881-1668, 881-1890 and 14335. The consistency in collection of blood samples allowed for the inclusion of all individuals participating in captivity ($n = 44$) in this portion of the model study. One individual was dropped from the sample set because of clinical concerns outside of the scope of research activities. Morphometrics and condition parameters were not collected consistently across all years; therefore, the sample size for these measures differed at entry ($n = 36$) and exit ($n = 23$).

Blood parameters

Several blood analytes have been identified as being important indicators of physiological response in relation to research treatments (Skinner *et al.*, 2015). These analytes include serum chemistry and complete blood count values of GLOB, GLU, PLT, RBC, TBIL and WBC. The difference in these parameters measured at exit and entry (dGLOB, dGLU, dPLT, dRBC, dTBIL and dWBC) was used to assess their predictive power of long-term survival with the addition of an individual's change in mass (dMASS). Single time point measures of these blood parameters, measured at entry and exit (e.g. eGLOB and xGLOB, respectively), were also examined in their predictive ability for long-term survival and considered separately. Steller sea lions were restrained in a squeeze cage and administered isoflurane (AErrane; Baxter Healthcare Corp., Deerfield, IL, USA; 5% in oxygen) anaesthesia for the duration of sample collection. Blood samples were obtained from either the caudal plexus or hind flipper vein. All blood analytes were measured shortly after collection via VetScan HMII® and chemistry automated analysers (Abaxis, Union City, CA, USA). Serum aliquots were also archived and stored at -80°C . Specifics of collection are described by Mellish *et al.* (2006). Blood was collected consistently throughout the project at entry and exit, allowing us the use of all 44 individuals in this portion of the study.

Body condition

Several measures of body condition were assessed in relation to their long-term effect on survival. Simple morphometric measures that were modelled included axial girth (AXGI; in centimetres) and standard length (STLE; in centimetres) via measuring tape to the nearest 10th of a centimetre, and mass (in kilograms) 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 ± 0.19 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 blood sample with two post-injection blood samples (2.0 and 2.25 h). All samples were analysed in triplicate for δD vs. Vienna-Standard Mean Ocean Water (V-SMOW; Scrimgeour *et al.*, 1993). Total body water (in kilograms) was calculated with correction for the overestimation of dilution space (Bowen and Iverson, 1998). Total body fat (%TBF) and total body protein (%TBP) were then calculated by equations empirically derived for grey seals (*Halichoerus grypus*; Reilly and Fedak, 1990; Rea *et al.*, 2007). The use of isotope dilution methods was discontinued after spring 2008 owing to logistic constraints. Instead, body composition was derived from a model predicting total body water from several morphometric measures (Reilly and Fedak, 1990; Shuert *et al.*, 2015). We also included the ratio of total body fat to total body protein (F:P; in kilograms), as a potentially important condition index (du Dot *et al.*, 2009). The %TBF, %TBP and F:P were all modelled with the addition of a seasonal component because variation of fat and protein content is heavily influenced by the time of year (Rea *et al.*, 2007).

Survival analysis

Branded animal resight information for the period of 15 May 2005 to 30 August 2013 was provided by the National Marine Mammal Laboratory (NMML, Seattle, WA, USA), Alaska Department of Fish & Game (ADFG, Juneau, AK, USA) and the Alaska SeaLife Center (ALSC, Seward, AK, USA). 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 only 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 the program MARK (White and 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 to November. Encounter histories were used for generating estimates of survival (Φ) and resight (P) probabilities through the Cormack–Jolly–Seber method for estimation. Resight effort was included in all P models as a covariate to scale yearly differences in institutional effort properly 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 1–10 for coding ease. Each model was then analysed through the program MARK via the RMark user interface with a corresponding set of encounter histories (Laake, 2013).

For the first analysis, each focal blood parameter was tested separately to measure its relative importance in predicting observed survival (Table 1A). Models included the interaction of sex and age for dGLOB, dRBC and dWBC because they are known to develop proportionally with age and sex (Horning and 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. The second analysis 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) at entry and exit. Sex and age were added as interactive terms. All continuous covariates were z-standardized to maintain normality assumptions in model selection (Cooch and White, 2013). Models were ranked based on Akaike information criterion model selection methods, corrected for small sample size (AICc; Anderson *et al.*, 1994, 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).

The potential for differences between sexes were evaluated for top model parameters via Student's paired *t*-tests.

Blood or condition parameters found to have extensive support in AICc rankings were used in the third analysis to investigate the use of single time point measures of long-term survival. This was achieved by using the same model structures as the previous analyses, but allowing for combinations of single entry and exit values (e.g. eMASS and xMASS) of top ranking parameters to compete against the changes in these parameters to assess their importance in predicting survival. In addition to these three main analyses, the effects of duration of captivity and LHX-1 implantation were also assessed for their potential explanatory power in a linear model framework of observed top blood and condition parameter trends from model ranking results.

Results

A total of four model sets were run separately for each of the six *a priori* blood parameters and a total of seven model sets for both entry and exit body condition terms (Table 1). Goodness-of-fit testing through the program U-CARE resulted in \hat{c} values of ~ 1 , with no adjustments to grouping factors

Table 1: Candidate survival models predicted by blood parameters and body condition

	Survival models	Resighting models
(A)	Blood parameters	$*p(-\text{Sex} + \text{Age} + \text{effort})$
	$\Phi(-\text{Sex} + \text{Age})$	
	$\Phi(-\text{Sex} + \text{Age} + \text{dMASS})$	
	$\Phi(-\text{Sex} + \text{Age} + \text{d[B.P.]})$	
	$\Phi(-\text{Sex} + \text{Age} + \text{dMASS} + \text{d[B.P.]})$	
	OR	
	$\Phi(-\text{Sex} + \text{Age} + \text{Sex:Age})$	$*p(-\text{Sex} + \text{Age} + \text{Sex:Age} + \text{effort})$
	$\Phi(-\text{Sex} + \text{Age} + \text{Sex:Age} + \text{dMASS})$	
	$\Phi(-\text{Sex} + \text{Age} + \text{Sex:Age} + \text{d[B.P.]})$	
	$\Phi(-\text{Sex} + \text{Age} + \text{Sex:Age} + \text{dMASS} + \text{d[B.P.]})$	
(B)	Body condition parameters	$*p(-\text{Sex} + \text{Age} + \text{Sex:Age} + \text{effort})$
	$\Phi(-\text{Sex} + \text{Age} + \text{Sex:Age})$	
	$\Phi(-\text{Sex} + \text{Age} + \text{Sex:Age} + \text{AXGI})$	
	$\Phi(-\text{Sex} + \text{Age} + \text{Sex:Age} + \text{STLE})$	
	$\Phi(-\text{Sex} + \text{Age} + \text{Sex:Age} + \text{MASS})$	
	$\Phi(-\text{Sex} + \text{Age} + \text{Sex:Age} + \text{TBF} + \text{Season})$	
	$\Phi(-\text{Sex} + \text{Age} + \text{Sex:Age} + \text{TBP} + \text{Season})$	
$\Phi(-\text{Sex} + \text{Age} + \text{Sex:Age} + \text{F:P} + \text{Season})$		

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

required. 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 exit body condition models. The effects of dMASS and dWBC were considered to be the top models. dMASS was positively related to survival (Fig. 1A), whereas positive dWBC values were found to have only a minor increase in survival at best (Fig. 1B). On average, animals increased in mass by $4.2 \pm 12\%$, with no significant difference in mass change between the sexes (two-sample t -test, $P = 0.22$). This change in mass, along with the addition of sex and age, resulted in a model-averaged apparent survival rate (Φ) of 0.81 ± 0.15 (Fig. 1A). Leucocytes increased by $1.01 \pm 3.53 \times 10^3/\text{mm}^3$ during captivity (Fig. 1B). There was no evidence that dWBC differed between males and females (two-sample t -test, $P = 0.74$).

The addition of other non-covarying parameters (e.g. dGLU and dTBIL) did not improve the predictive ability of dMASS and dWBC. When investigated in a linear model framework, the duration of captivity (62 ± 17.1 days) was found to be a significant predictor of dMASS ($F = 6.56$, $P = 0.014$). LHX implant status was also found to be a significant predictor of

dMASS ($n = 34$ implanted, $F = 11.74$, $P = 0.001$). Length of stay in captivity was also a significant predictor of dWBC in a linear model framework ($F = 16.76$, $P < 0.001$), but LHX implant status was not ($F = 2.12$, $P = 0.14$).

For the final analysis, dMASS and dWBC were tested against combinations of their entry and exit values. Models including dMASS and dWBC still had the most support in the data set ($\leq 2 \Delta AICc$). However, models including xMASS (mean 131 ± 28.8 kg) and eWBC (mean $10.5 \pm 1.75 \times 10^3/\text{mm}^3$) and the additive of these two parameters still had support in our data for predicting long-term survival ($< 5 \Delta AICc$) and ranked above our null model. Animals with a greater xMASS had higher survival rates (Fig. 1C), whereas sea lions with higher eWBC had lower survival rates (Fig. 1D).

Discussion

After almost a decade of work, the TJ and LHX projects have amassed extensive data on the behaviour, physiology and mortality of the juvenile Steller sea lion. Combined with extensive long-term tracking, this study was in a unique position to evaluate the role of several commonly measured parameters in their predictive ability of long-term survival trends. The LHX project has become a novel way to detect mortalities directly by returning known-fate survival estimates with a high detection probability (Hornung and Mellish,

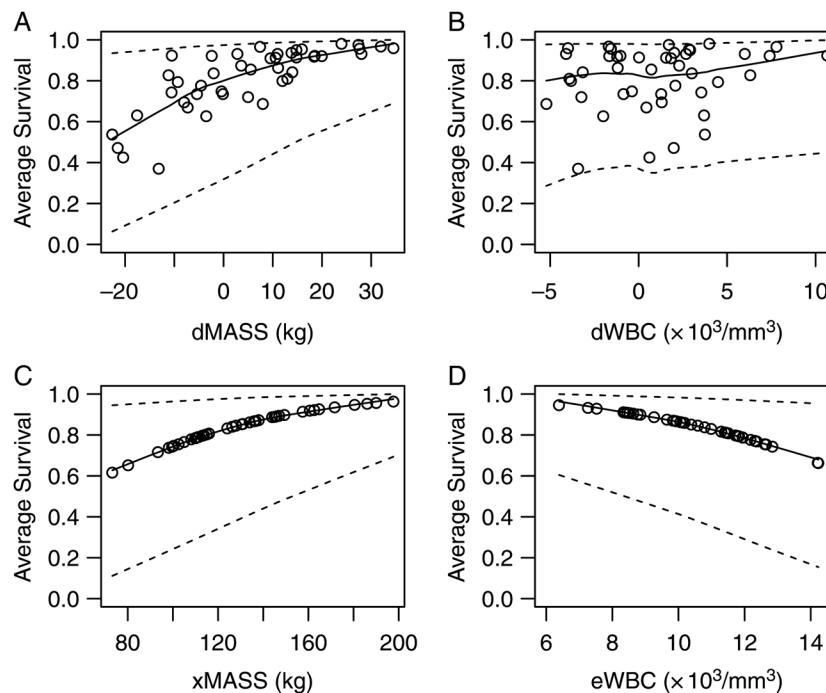


Figure 1: Predicted model-averaged survival rates based on continuous covariates. The change in mass (dMASS, in kilograms; **A**) and white blood cell counts (dWBC, $\times 10^3$ per millimetre cubed; **B**) had the most support in the data on modelling the apparent survival rates in juvenile temporarily captive Steller sea lions (*Eumetopias jubatus*). Single-point measures yielded exit mass (xMASS, in kilograms; **C**) and entry white blood cell counts (eWBC, $\times 10^3$ per millimetre cubed; **D**) to be slightly less supported, but important in predictive survival. Continuous lines represent the general trend through loess smoothing. Dashed lines indicate 95% confidence intervals in predicted averaged survival.

2009, 2012, 2014). While this may reveal predation as the main source of mortality, little work has attempted to predict long-term survivability of this critical age class at a physiological level. It is imperative to explore more than one avenue in order to obtain a clearer insight into the mechanisms driving population fluctuations in this species at risk.

Effect of mass

The changes of several blood parameters throughout captivity were assessed for their importance in the long-term survival of Steller sea lions. When tested separately, dMASS ranked higher in AICc model selection than most blood parameters. When modelled with sex and age, dMASS was found to have the most support in the data overall. Increased mass gains displayed a relation to increased survival, similar to first year survival in grey seals (*H. grypus*, Hall *et al.*, 2002; Bowen *et al.*, 2015). Mass is a standard metric for population health in a variety of marine mammal species (Castellini and Calkins, 1993; Hall *et al.*, 2002; Greig *et al.*, 2010; Rutishauser *et al.*, 2010; Mellish *et al.*, 2011). Mass changes in pinnipeds are typically assessed by gross population body size means over decades (e.g. York, 1994; Trites and Jonker, 2000; Holmes *et al.*, 2007). While mass can act as a proxy for adult health, it has also been used to predict pup and juvenile survival in Steller sea lions (Castellini *et al.*, 1993; Hastings *et al.*, 2011; Maniscalco, 2014). Larger animals at weaning tend to have much higher survival rates through their first winter and into the next year (Craig and Ragen, 1999; McMahon *et al.*, 2000; Harding *et al.*, 2005). This positive effect of mass has not been demonstrated into the adult years, with the exception of maternal investment and lactation performance (Bowen *et al.*, 2001).

Multidecade studies on large ungulates have revealed that there may be a positive link not only between mass and juvenile survival, but also into adult lifespan (Festa-Bianchet *et al.*, 1997, 2000; Loison *et al.*, 1999; Gaillard *et al.*, 2000). In this case, longer life did not translate to increased individual female fitness as a result of greater body mass. A longer lifespan and an earlier age at first reproduction may translate to more opportunities to breed, flexibility in life-history strategies and a robust response to environmental change (Jorgenson *et al.*, 1993; Loison *et al.*, 1999; Festa-Bianchet *et al.*, 2000; Gaillard *et al.*, 2000). Female Steller sea lions are known to exhibit differential life-history strategies to handle environmental fluctuations, in relation to individual health on a regional basis (Maniscalco *et al.*, 2006; Maniscalco, 2014). Further work should be done to investigate the potential relation between body mass at various life stages and lifespan as well as reproductive success. This may serve a useful basis for the prediction of the response of this population to future environmental change.

The length of captivity (mean 62 days, ranging 22–82 days) was a significant predictor of dMASS. Notably, most animals in this study fell within the expected mass reference ranges, despite gains or losses associated with captivity and research procedures (Mellish *et al.*, 2006). While mass gains could be a

direct result of an *ad libitum* feeding regime, some individuals may have lost mass as part of a behavioural response to an unnatural environment. Mass changes could instead be a potential indicator of resiliency to new environments or forage opportunities (Mellish *et al.*, 2006). Changes in mass have been identified as important predictors of the survival of animals admitted into rehabilitation programmes and are a direct indication of resiliency to a traumatic event (Greig *et al.*, 2010). LHX implant status ($n = 34$ implanted) was a predictor of dMASS. Previous work documenting implantation responses found that food consumption dropped and rebounded within a week of LHX implantation, but neither mass nor body condition changed significantly throughout captivity or with other associated research activities (Mellish *et al.*, 2007b; Skinner *et al.*, 2015). Mass therefore appears to be a much more robust indicator of health on a longer term scale, rather than fluctuating quickly in response to acute trauma. This may explain the significance of LHX status with survival.

Effect of leucocytes

The dWBC was found to have extensive support, with the additive effect of dMASS, for predicting long-term survival. While our model predictions on survival do not have as strong a trend as dMASS (Fig. 1), it does appear that there is a small increase in survival with increasing dWBC throughout captivity, although variability in predicted survival is much greater. While somewhat puzzling, this may be explained by several mechanisms. A slight increase might reflect an individual's ability to regulate their immune system, in spite of research procedure stress (e.g. Fair *et al.*, 2014). Given that the animals studied here were weaned a year or two before capture, the observed change in WBC may be related to developmental status. The critical development period for leucocytes is during the pup and pre-weaning juvenile stages, when immunity is passed from mother to offspring (e.g. Keogh *et al.*, 2010). This link of WBC response may be an indication of maternal care and immune status in this age class of sea lions, translating to their long-term survivability.

As with dMASS, length of stay in captivity was a significant predictor of dWBC. 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). Shortening captivity as research objectives changed may have coincided with these peaks and is likely to be the reason that we see dWBC being significantly predicted by duration of captivity, rather than as a direct link to captivity stress. Conversely, LHX implant status was not a predictor of the observed trends in dWBC, suggesting that the observed survival response was de-coupled from implantation stress. Unlike sea lions, an increase in human WBC values has been shown to increase post-surgery survival greatly (Lu *et al.*, 2014). Leucocytes are strong indicators of survival in rehabilitation settings and have been repeatedly correlated with survival at the sub-adult stage (Greig *et al.*, 2010). Elevated WBC counts may be an indicator of

exposure to novel pathogens (Goldstein *et al.*, 2011). They are also known to fluctuate with prey switching in harbour seals, where a reduction in total WBC was observed with poorer quality diets (Thompson *et al.*, 1997). The variety of stressors that trigger a leucocyte response demonstrates its utility as a diagnostic tool on both an individual and a population level.

Single-point measures

Single-point measures were investigated because of their potential utility in a field sampling environment. Models containing measures of our top 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 still the top models, models including xMASS and eWBC also had support in our data for predicting long-term survival. When compared with reference values derived from Mellish *et al.* (2006), all data appeared to fit within a 95% confidence interval for both xMASS and eWBC. There were only a few exceptions (See Fig. 1) to our confidence intervals for dMASS, but they did not exhibit any other blood or condition parameters outside the normal reference ranges for this study group (Mellish *et al.*, 2006). While xMASS followed the same pattern in predicting survival as dMASS, elevated eWBC may reflect a decreased average survival rate of about 10–20%. Predicted survival rates based on dWBC and eWBC may reflect an ideal leucocyte count as represented by our mean for each parameter. Similar results have been identified in juvenile wood storks (*Mycteria americana*), where poor survival was associated with both high and low WBC single-measure counts (Hylton *et al.*, 2006). It appears from this that single-point measures, while not ideal in our model rankings, may be useful in predicting long-term survival trends.

Implications

Predictions of survival and mortality have always been a goal of both biologists studying species at risk and human-related health research. Most often, this is approached through monitoring stress hormones and their associated serum proteins. Broad-ranging efforts to monitor stress as a proxy for survival have been attempted in terrestrial translocation programmes (e.g. *Equus grevyi*, Franceschini *et al.*, 2008), conservation monitoring efforts (e.g. *Ursus maritimus*, Bechshøft *et al.*, 2013) and as a retrospective to sampling procedures in this cohort of sea lions (e.g. Thomson and Mellish, 2007) and other marine species (e.g. *Tursiops truncatus*, Fair *et al.*, 2014). Mirroring efforts by wildlife biologists, varying levels of success have been achieved in predicting human survival based on simple physiological parameters (e.g. Yudkin *et al.*, 2000; Hackman and Anand, 2003). A recent retrospective analysis of a human biological database found that elevated levels of certain blood parameters were indicative of mortality a few years after sampling, despite healthy status individuals at the time of sample collection (Fischer *et al.*, 2014). Fischer *et al.* (2014) found that human mortality was greatly associated with indicators of cardiovascular health and cancer risk (alpha-1-acid glycoprotein, albumin, very-low-density lipoprotein and

citrate). Unfortunately, none of these parameters overlaps those contained in the present study or the typical suite collected from sea lions. However, the suggestion remains that such a simplified procedure could be of merit in wildlife health assessments if the appropriate indicators can be found.

The present findings allow us to comment on potential mortality factors through known-fate data returns from the LHX-1 implants. While these juvenile animals appear to have comparable survival rates to those not experiencing captivity (C. Shuert, M. Horning, and J. Mellish, unpublished data), the majority of mortalities reported by LHX-1 implanted tags have probably been due to predation, as inferred by end-of-life temperature curves (Horning and Mellish, 2014). Our results suggest that smaller animals experience a lower average survival. Apart from their immediate health status, smaller animals might simply be less developed in their diving ability, efficiency and predator avoidance skills, because diving ontogeny is tightly linked to mass in pinnipeds (e.g. Burns, 1999; Noren *et al.*, 2005). Leucocytes present a far more puzzling case, given that those at either end of the normal range were equally prone to lower survival estimates. The role of leucocytes to respond to a number of physiological conditions may result in a generic physical compromise that cannot be pinpointed on a finer scale, but can suggest that any animal outside of the centre of the distribution is at a higher risk level for predation.

Conclusions

This study provides a unique perspective for the evaluation of a wide range of potential indicators of long-term survival, by using a decade of extensive physiological and resighting data. Our results demonstrate that mass and leucocytes show promise as diagnostics of long-term survival in Steller sea lions. Despite the existence of reference ranges of ‘normal’ mass, body condition and complete blood counts for this species, heterogeneity exists within that ‘normal’ range to produce variable projected survival rates. A changing climate, with the potential for increased contaminant exposure, changes in food availability and warming, particularly in higher latitudes, may shift the centre of what is considered normal for these health indicators (Burek *et al.*, 2008; Ozgul *et al.*, 2010). This baseline identification will allow management entities to provide better monitoring and identification of sensitive or vulnerable populations of Steller sea lions.

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