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Temporary Captivity as a Research Tool: Comprehensive Study of Wild Pinnipeds Under Controlled Conditions

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Abstract

A new approach to the study of free-ranging, endangered western stock Steller sea lions (*Eumetopias jubatus*) was implemented at the Alaska SeaLife Center in Seward, Alaska. Groups of up to four juvenile (n = 16) Steller sea lions were held in temporary quarantine for research periods of up to three months. Hematological and blood chemistry parameters were collected at the beginning and end of captivity and compared to free-ranging juvenile controls to determine if animals in temporary captivity can provide accurate physiological data representative of their wild counterparts. Free-ranging pups and juveniles were compared for hematological differences related to developmental stage. Overall, temporarily captive animals did not differ from free-ranging juveniles. Seven of 17 blood parameters measured changed significantly during captivity, likely as a function of a regular schedule and low-impact nutritional studies (e.g., increased mass, cholesterol, total protein, and globulins). A decrease in white blood cells during the study period (10.4 ± 0.59 to 7.9 ± 0.33 m/mm^3) to levels lower than that of free-ranging animals (10.7 ± 0.40 m/mm^3) indicated a drop in overall stress during captivity despite research and handling procedures. Calcium increased with captivity duration, suggesting that physiological changes can begin in even limited time frames. Eight parameters related to immune status and diet differed significantly between juveniles and pups from the same geographical region. A strategy that combines the benefits of an extended research design with temporary holding of free-ranging animals is proposed as an alternative to traditional field methods for some types of focused physiological studies.

Key Words: Steller sea lion, *Eumetopias jubatus*, temporary captivity, blood panel

Introduction

The study of physiology, endocrinology, and behavior of large mammals entails certain logistical boundaries that often severely limit the quantity and quality of research opportunities. Marine mammals are particularly challenging due to their predominantly inaccessible aquatic habitat. Increased human use of the world's oceans for commercial, strategic, and recreational use, however, has heightened the interest and need for research on these enigmatic and largely inaccessible animals.

Pinnipeds (seals, sea lions, and walruses) use both terrestrial and marine habitats, which makes them simultaneously versatile and vulnerable. They are dependent on aquatic prey, but tied to land or ice for breeding, resting, and nursing activities. Environmental change, and indirect and direct human impacts are thought to play substantial roles in the endangered status of four of North America's 18 pinniped species alone (*Monachus tropicalis*, *M. schauinslandi*, *Arctocephalus townsendi,* and *Eumetopias jubatus*) (Perrin et al., 2002).

Steller sea lions (*E. jubatus*) have been the focus of much research over the past decade due to rapid population declines in the endangered western stock of this species, coupled with uncertainty of the impacts of a substantial commercial fishery, which targets several of their major prey species (Pitcher, 1981; Merrick et al., 1997). These animals are large, aggressive, inhabit a remote sub-arctic region, and spend the majority of their lives at sea. Most studies are therefore based on cross-sectional sampling designs with single catch and release events. Recapture of these animals is not only extremely difficult, but it is also often logistically prohibitive. Alternatively, data have been collected from limited numbers of long-term captive individuals at aquaria or rehabilitation centers, which were then extrapolated to the free-ranging population. Potential variation in the physiology and behavior of these

individuals compared to the wild population can lead to serious complications with this approach.

Differences between long-term captive and wild animals have been experimentally verified for many taxa at the level of individual animals, as well as for populations in captive breeding programs. Significant differences have been reported for body mass of several lemur genera (*Hapalemur*, *Eulemar*, and *Varecia*) (Terranova & Coffman, 1998), in reproductive behavior of blue tits (*Parus caeruleus*) (Lambrechts et al., 1999) and nurse sharks (*Ginglymostoma cirratum*) (Carrier et al., 1994, 2003), blood parameters of Komodo dragons (*Varanus komodoensis*) (Gillespie et al., 2000), sex steroids in Hermann's tortoise (*Testudo hermanni hermanni*) (Huot-Daubremont et al., 2003), and thyroid hormone concentrations of manatees (*Trichechus manatus*) (Ortiz et al., 2000). Woodley et al. (1997) suggested that annual survival rates likely differ between captive and wild bottlenose dolphins (*Tursiops truncatus*), and killer whales (*Orcinus orca*). To address these known difficulties in applying results derived from captive animals to wild populations, an approach based on parallel studies of captive and wild populations has been suggested and applied in a few cases (Lambrechts et al., 1999; Jenssen et al., 2001); however, no specific mechanism has been proposed to adjust the interpretation of laboratory findings to make them representative of wild populations. Most such parallel approaches appear more suited for the comparative study of population-level genetic and phenotypic traits rather than implications of differences in physiological responses for population trends (Jenssen et al., 2001).

Faced with the unexplained decline and continued recovery failure of the Steller sea lion, we implemented and tested an experimental design that combines access to free-ranging individuals with the facilities and controlled, repeated sample methodology of a captive population. Wild, juvenile (1 to 3 y) Steller sea lions (n = 16) were brought to a quarantine facility for up to three mo of collaborative research, after which they were released. Groups of up to four animals per cohort were rotated for year-round research accessibility. An additional subset of the population (n = 24) was sampled in the wild as controls. Given the typical weaning period of one year coupled with known changes associated with diving ability and hematology in very young sea lions (e.g., Richmond et al., 2005), animals less than 12 mo of age were considered separately and not eligible for temporary captivity. Particularly between the first and second years of life, exact age determination can be difficult and must be assessed with a combination of techniques, including time of year, mass, canine length (King et al., 2003), and potentially selected blood parameters.

Specifically, we compared nutritional and health indicators between captive and wild Steller sea lions at intake and exit. A secondary goal was the estimation of a time frame, within the time constraints of our project, during which phenotypic differences between captive and wild animals may appear. Our third goal was to determine if there were discernible differences in specific blood parameters between animals < 12 mo of age (pups) compared to those ≥ 12 mo (juveniles). While the specific design presented herein is applied to juvenile Steller sea lions, the concept is an adaptable template adjustable to the needs of many other marine mammal species.

Materials and Methods

Facilities

The specific holding needs of juvenile Steller sea lions (age 1 to 3 y; 75 to 250 kg) were identified in accordance with all applicable regulations under the Animal Welfare Act (Anonymous, 2002) and based on recommendations from multiple experts in the field developed during a dedicated workshop at the Alaska SeaLife Center (ASLC) in Seward, Alaska, in 2000. The resulting quarantine facility incorporates four adjoining primary enclosures, each with a fiberglass pool (1 x 4.0 m diameter and 3 x 5.0 m diameter, 1.5 m deep) encircled by approximately 122.0 m^2 of dry resting haul-out area. A 2.4-m chain link fence surrounded each enclosure in a configuration such that animals could be housed individually or share access to various configurations of multiple pools via 1.2-m sliding gates. A central walkway also could be subdivided into two smaller 29.0-m^2 dry holding areas and a 90.0-m^2 working and staging area. An adjacent support building (274.3 m^2) housed food preparation, laboratory, and sampling activities. The entire complex was surrounded by privacy fence, wind-rated to 109 km/h. A 40-hp pump provided up to 4,542 l/min of unfiltered sea water. Saltwater and adjustable temperature freshwater access points were located in the central walkway of the outdoor habitat. To ensure the isolated status of the wild animals (i.e., no contact with domestic or long-term captive animals), a two-way quarantine protocol was implemented.

Capture and Transport

Representative juvenile haul-out sites within Resurrection Bay and Prince William Sound, Alaska, were selected for animal acquisition between August 2003 and May 2005. Captures were performed using an underwater method pioneered by the Alaska Department of Fish and Game (ADF&G) (McAllister et al., 1997). Sixteen target animals between 1 to 3 y of age were chosen to be part of the temporary captivity program (denoted

as TJ or transient juvenile). Age of the animals was assessed in the field using a combination of mass, geographical location (i.e., juvenile haul-out versus breeding rookery), patterns of tooth eruption (i.e., milk teeth), and canine length (King et al., 2003). Measurement of canine length as an aging tool was not available for the first four animals and, therefore, was only applied to TJ-05 through TJ-16. Calendar month vs date of annual peak pupping was used to estimate age when canine length was not available for free-ranging animals. After a thorough health assessment on a larger support vessel (> 23 m), target animals were transported to the ASLC quarantine within 48 h of capture. Additional free-ranging pups (n = 15, 6 to 10 mo) and juveniles (n = 19, 12 to 20 mo) were captured, sampled, and released in the field during capture activities for a control comparison.

Health Assessments

All animals underwent an initial health screening that consisted of mass, complete blood counts (CBC), selected clinical chemistry parameters, and a manual physical assessment (e.g., lesions, broken bones, abrasions, etc.). Hematology (white blood cell counts, hematocrit, hemoglobin, platelet counts) was assessed using a VetScan® HMT analyzer, and clinical chemistry parameters were analyzed with a VetScan® Diagnostic Profile Plus analysis rotor while the animal was still under isoflurane anesthesia. Cholesterol levels were not available for 10 of the 19 free-ranging juveniles.

Additional samples collected, but not reported herein, included a viral serology panel (e.g., *Leptospirosis*, *Brucella*, phocid herpesvirus, *Toxoplasmosis*) (Stephens et al., 2005), epidemiology survey (e.g., bacterial and viral) (Stephens et al., 2005), and body composition via deuterium dilution (Mellish et al., unpubl. data, 2005). While we were not specifically selecting for healthy individuals, we were selectively excluding any animals that might have pre-existing conditions that precluded a successful temporary captivity period (e.g., major parasite load, overt systemic infection, severe physical trauma).

During the temporary captivity period, mass measurements, basic blood collections, and superficial health assessments were made on a weekly to biweekly schedule basis to ensure that the animals were closely monitored while still minimizing the amount of handling and human interaction. All assessments took place under isoflurane anesthesia to minimize handling time and stress. No antibiotics or other veterinary treatments were used unless deemed necessary by the attending wildlife veterinarian to preserve the animal's initial health state.

A final comprehensive health assessment (e.g., same as entry exam) was performed on all animals two weeks prior to release. An abbreviated anesthesia and health screen was used on the day prior to or on the day of release, which consisted of blood panel, flipper tag insertion (Rototag, Dalton, UK), and external satellite tag attachment for post-release monitoring purposes (Schrader et al., 2005).

Husbandry of Temporarily Captive Animals

Animals were maintained in primary enclosures that exceeded the minimum standards outlined in the federal animal welfare regulations for the care of captive marine mammals. Freshly thawed fish were offered several times per day, based on animal mass and appetite. Diet items included a variety of species that naturally occur in wild Steller sea lion diets such as herring (*Clupea harengus*), pollock (*Theragra chalcogramma*), and capelin (*Mallotus villosus*). Remote methods for food delivery to minimize association with the husbandry personnel included integrated feeders via influent saltwater plumbing, casting food from behind a barrier, and planting fish into unoccupied pools prior to allowing access.

Whenever possible, animals were maintained with conspecifics in varying numbers and enclosure configurations. Animals were conditioned to move through remotely operated gates into other pool enclosures, holding runs, and cages by associating access to food as a direct consequence of moving through a remotely operated, controlled gate. This context-specific conditioning facilitated the management, feeding, sanitation, and maintenance required to husband these animals with a greatly decreased level of human contact than that necessary to physically manipulate the animals.

Data Analysis

Differences between free-ranging and transient animals were performed with Student's t-test, whereas changes within the captivity period were analyzed with Student's paired t-tests. Parameters without a normal distribution or variance were tested with a Mann-Whitney sum rank test. All statistical analyses were performed using *SigmaStat 2.03*.

Results

Transient Juveniles

We maintained 16 Steller sea lions (6 female and 10 male) for an average of 64 ± 4.4 d (range 28 to 84 d) of temporary captivity between August 2003 and May 2005. This included two initial cohorts of two animals each, followed by three cohorts of four animals. Estimated admission age was 15.2 ± 0.31 mo. Including the day of arrival at the quarantine habitat as day 1, animals accepted food after day 5 ± 0.99 d of temporary captivity. Food was accepted

by 31% of animals as early as their second day of captivity, and 88% within the first week.

Mass significantly increased on average by 10 ± 2.2% over entry values during captivity (t = -3.52, DF = 11, p = 0.005; Table 1). White blood cell (WBC) count dropped significantly from entry to exit (t = 3.35, DF = 11, p = 0.006), whereas platelet levels conversely increased (t = -3.77, DF = 11, p = 0.001). Hematocrit and hemoglobin levels did not differ through the captivity period, nor did most clinical chemistry parameters (Table 2). Albumin (t = 2.98, DF = 10, p = 0.013), amylase (t = 0.84, DF = 10, p = 0.048), calcium (t = -2.33, DF = 10, p = 0.010), cholesterol (t = -2.68, DF = 10, p = 0.023), total protein (t = -3.42, DF = 10, p = 0.006), and globulins (t = -1.64, DF = 10, p = 0.031) increased from entry to exit. Only calcium was influenced on an absolute (p = 0.001) or relative (r^2 = 0.544, p = 0.002) level by length of stay (Figure 1).

Transient Versus Free-Ranging Juveniles

Nineteen juveniles were sampled during capture activities as control individuals (8 female and 11 male). Free-ranging juveniles were slightly smaller (t = -3.11, DF = 29, p = 0.001) than transient animals. WBC were comparable among entry and free-ranging juveniles, but lower in exiting transient animals (t = 4.49, DF = 29, p = 0.001). Hematocrit levels were higher in exiting transients than free-ranging juveniles (t = -2.65, DF = 29, p = 0.013), while platelet counts were elevated in transients only at entry compared to free-ranging juveniles (t = 2.86, DF = 29, p = 0.012).

Only calcium levels differed at entry for transient vs free-ranging juveniles (t = 3.13, DF = 28, p = 0.004). In contrast, several blood chemistry parameters differed between exiting juveniles and free-ranging animals, including elevated levels of albumin (t = -4.48, DF = 29, p = 0.001), cholesterol (t = -2.30, DF = 19, p = 0.033), creatinine (t = -2.34, DF = 29, p = 0.026), total protein (t = -5.19, DF = 29, p = 0.001), and globulins (t = -1.71, DF = 29, p = 0.027), with decreased amylase (t = 2.75, DF = 29, p = 0.010) and glucose (t = 2.10, DF = 29, p = 0.044). All other parameters did not differ between free-ranging juveniles and transients at either entry or exit (Table 2).

Free-Ranging Juveniles and Pups

Given that there were no overall significant differences between free-ranging juveniles and entry transients, these animals were grouped for comparison to free-ranging pups (Tables 1 & 2). Free-ranging pups were smaller (t = -2.16, DF = 48, p = 0.034) and displayed lower WBC levels than older animals (t = -2.13, DF = 48, p = 0.039).

Seven blood chemistry parameters differed between the two age groups (Table 2). Pups had lower alanine amino transferase (t = -3.64, DF = 47, p = 0.001), blood urea nitrogen (t = 2.16, DF = 47, p = 0.036), calcium (t = -2.55, DF = 47, p = 0.014), potassium t = -2.70, DF = 47, p = 0.009), total protein (t = -4.40, DF = 47, p = 0.001), and globulin counts (t = -5.11, DF = 47, p = 0.001) than older animals; however, alkaline phosphatase was higher in pups than in juveniles (t = -3.17, DF = 47, p = 0.003).

Discussion

Experimental Design

The ability to study large, free-ranging marine mammals is severely limited by their habitat, size, and behavior; however, repeated measures and manipulative approaches under reasonably controlled conditions (or experimental designs) are necessary for many physiological and ecological studies to characterize the degree of phenotypic variation observed in the wild. Long-term captive and rehabilitation animals are not ideal subjects for any model of a wild population because significant differences in many aspects of the biology of these animals have been

Table 1. Mean ± SE mass and hematology of temporarily captive Steller sea lion (*Eumetopias jubatus*) juveniles (1 to 3 y) with comparison to free-ranging pups (< 12 mo) and juveniles

	Transient entry n = 16	Transient exit n = 16	Free-ranging juveniles n = 19	Free-ranging pups[c] n = 15
Mass (kg)	129.0 ± 5.5	142.0 ± 6.4[a]	104.0 ± 3.9[a, b]	101.2 ± 4.9[c]
WBC (m/mm^3)	10.4 ± 0.59	7.9 ± 0.33[a]	10.7 ± 0.40[b]	9.2 ± 0.61[c]
Hematocrit (%)	44.5 ± 1.00	48.0 ± 1.35	42.3 ± 1.12[b]	42.3 ± 0.62
Hemoglobin (g/dl)	15.0 ± 0.37	14.8 ± 0.30	14.7 ± 0.35	14.9 ± 0.16
Platelets (m/mm^3)	316.0 ± 29.4	429.0 ± 30.3[a]	425.0 ± 28.1[a]	344.0 ± 20.8

[a]Significantly different than entry values

[b]Significantly different than exit values

[c]Significantly different than combined free-ranging and entry juveniles

Table 2. Mean ± SE serum chemistry of temporarily captive Steller sea lion (*Eumetopias jubatus*) juveniles (1 to 3 y) with comparison to free-ranging pups (< 12 mo) and juveniles

	Transient entry $n = 16$	Transient exit $n = 16$	Free-ranging juveniles $n = 19$	Free-ranging pups $n = 15$
Albumin (g/dl)	3.8 ± 0.06	4.1 ± 0.08	3.6 ± 0.09[b]	3.7 ± 0.06
Alkaline phosphatase (U/l)	100.0 ± 10.3	90.0 ± 13.1	87.0 ± 4.4	119.0 ± 4.8[c]
Alanine amino transferase (U/l)	54.0 ± 7.6	58.0 ± 5.4	51.0 ± 4.4	30.0 ± 1.2[c]
Amylase (U/l)	108.0 ± 16.8	75.0 ± 12.4[a]	145.0 ± 19.0[b]	132.0 ± 13.0
Total bilirubin (mg/dl)	0.3 ± 0.03	0.3 ± 0.02	0.3 ± 0.01	0.3 ± 0.03
Blood urea nitrogen (mg/dl)	24.0 ± 4.4	21.0 ± 0.7	22.0 ± 4.7	13.0 ± 0.7[c]
Calcium (mg/dl)	9.2 ± 0.11	9.7 ± 0.10[a]	9.9 ± 0.14[a]	9.2 ± 0.10[c]
Cholesterol (mg/dl)	176.0 ± 9.7	223.0 ± 10.0[a]	183.0 ± 13.0[b]	169.0 ± 9.1
Creatinine (mg/dl)	0.9 ± 0.09	1.0 ± 0.08	0.7 ± 0.11[b]	0.8 ± 0.07
Glucose (mg/dl)	142.0 ± 7.3	133.0 ± 3.1	144.0 ± 3.8[b]	135.0 ± 3.1
Potassium (mmol/l)	4.0 ± 0.21	3.9 ± 0.10	3.8 ± 0.16	3.4 ± 0.08[c]
Total protein (g/dl)	7.8 ± 0.11	8.4 ± 0.12[a]	7.5 ± 0.10[b]	7.0 ± 0.10[c]
Globulins (g/dl)	4.0 ± 0.11	4.3 ± 0.15[a]	3.9 ± 0.11[b]	3.3 ± 0.07[c]

[a]Significantly different than entry values
[b]Significantly different than exit values
[c]Significantly different than combined free-ranging and entry juveniles

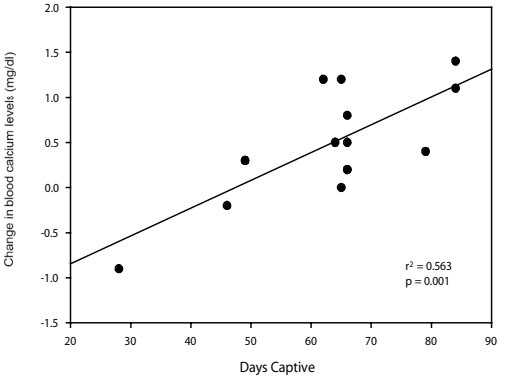

Figure 1. Change in blood calcium concentration (mg/dl) in temporarily captive juvenile Steller sea lions (*Eumetopias jubatus*) as a function of time (y = 0.031x -1.459)

experimentally verified. Lambrechts et al. (1999) and Jenssen et al. (2001) showed that models developed on captive populations may lead to erroneous conclusions about the degree of phenotypic plasticity or adaptive value of certain behavioral traits in wild populations. Movement and exercise, feeding behavior, body mass, body condition, reproductive behavior, endocrinology of stress, health, and reproduction, as well as clinical chemistry panels may differ substantially between captive and wild populations. While some of these differences are thought to relate to genotypic changes that occur in captive breeding programs, others of a phenotypic nature may occur within the generation of animals first brought to captivity. No study to date has examined the rapidity with which such phenotypic changes occur.

To address these concerns about the applicability of results derived from captive animals, we aimed to combine repeated access over time to a subset of wild individuals in a temporary captive setting. This method would provide extended study opportunity with the ability to conduct research manipulations while maintaining the integrity of the wild individuals and data collected. The primary goal of the first two years of activity was to ensure the validity of the method through physiological (e.g., health assessments) and behavioral methods (e.g., post-release monitoring) (Schrader et al., 2005).

Influence of Temporary Captivity

Mass increased during the captivity period, likely as a function of continued growth in young animals as well as several low-impact nutritional studies that incorporated daily feeding regimes based on satiation, which may be atypical of wild foraging behavior (Calkins et al., 2005; Mellish & Horning, 2005). Several animals were admitted to the program in various stages of the annual molt during which animals are not anticipated to forage frequently. Given that the captured animals were juveniles in a phase of ontogeny during which growth continues, the additional mass gain observed was not considered a detrimental effect of temporary captivity.

Of the 17 hematological and clinical chemistry parameters measured, seven changed during the period of temporary captivity (Tables 1 & 2). WBCs are a common indicator of inflammation

and general stress (Latimer & Prasse, 2003), and therefore, the decrease noted during captivity and in comparison to free-ranging animals validated that these animals were not overtly stressed by repeated handling and/or the artificial habitat. Increased platelet levels were likely due to the mild, but nonsignificant changes in hematocrit levels. While comparable data do not exist for Steller sea lions, platelet levels in captive, adult California sea lions (*Zalophus californianus*) were found to be slightly lower (158 to 355) (Bossart et al., 2001).

Amylase typically is used as an indicator of pancreatic function; however, there is no currently known clinical pathology for a drop in levels in marine mammals. Cholesterol and total protein increased during captivity, both of which are influenced by dietary intake similar to captive *Zalophus* (34 to 179 mg/dl). Normal hematocrit levels with an increased protein level can be associated with increases in globulins, albumin, and calcium levels (Bossart et al., 2001), all of which were exhibited over time, with the exception of albumin which was not significant.

Free-Ranging Versus Transient Juveniles

Intake values of captive animals in general did not differ from free-ranging control animals such that animals selected for the temporary captivity and research period were considered representative of the free-ranging juvenile population. Of parameters differing between free-ranging juveniles and exiting transients, most can be accounted for by the influence of a research-dictated diet (e.g., albumin, cholesterol, creatinine, glucose, total protein, and globulins). As mentioned previously, the cue for a change in amylase is at this point unknown, but there is no currently detrimental effect of a lowered activity of this enzyme in marine mammals.

Juvenile Versus Pup Blood Panels

Steller sea lion pups typically nurse through the first year of life and go through multiple physiological changes that enable them to become proficient divers and foragers (Richmond et al., 2005). Therefore, animals within the first year of life, regardless of weaning status, were not considered eligible for temporary captivity as they were not truly representative of the slightly older animals that were the research focus. During some months of the year (i.e., winter), it can be difficult to immediately discern sea lions in their first year of life vs older animals based on haul-out location and mass alone. This situation has led to the development of additional tools, such as patterns of tooth eruption and canine length (e.g., King et al., 2003), as measures of age. This and previous studies also displayed that there are measurable differences in blood parameters that can be discerned between animals less than or greater than 12 mo of age. Some parameters, particularly relating to an underdeveloped immune status, could potentially be used to rule out individuals for the captivity program as they may be at a greater risk.

WBCs and globulins, as immune status indicators, are also typically found in lesser quantities in younger animals, including Steller sea lions (5.4 to 28.8 m/mm^3 and 1.7 to 2.6 g/dl, respectively) (Bossart et al., 2001).

Alkaline phosphatase is a liver enzyme that is known to be higher in young mammals—cetaceans and pinnipeds included (Bossart et al., 2001). Conversely, lower alanine amino transferase in pups compared to juveniles may be related to incidence of parasitism more common in animals foraging on fish and not necessarily found in smaller, younger sea lions. Most juveniles sampled exhibited some form of external and/or internal parasite loads (e.g., skin mites, lungworms). Diet may also play a role between the younger, free-ranging animals and older juveniles as evidenced by lower BUN value, similar to those previously reported for pups (8 to 29 mg/dl [Bossart et al., 2001]; 12 to 15 mg/dl [Rea et al., 1998]). Younger mammals in general also tend to exhibit a greater hydration state than larger, older animals, resulting in lower total proteins and associated globulin and calcium levels. Interestingly, total protein values for pups were intermediate between juvenile animals and those previously reported for younger pups, possibly as a reflection of a fish diet vs milk diet (5.6 to 6.9 g/dl) (Bossart et al., 2001).

Application of Experimental Design

We see the absence of significant changes over the period of captivity in most monitored parameters outside of body mass, combined with the lack of difference between intake values of captives and wild animals, as a validation of our experimental approach; however, it is suggested that the effects of captivity and a moderate research schedule, particularly those studies that include nutritional components, may become apparent in selected parameters within a few months. In addition, animals must be selected within the constraints of the life history of the species in context with the goals of the research—in this case, greater than one year. Within the limits of our small sample size distributed over a range of seasons, we conclude that wild animals are not distinguishable from animals of the same cohort at the time of capture, but they may become progressively more distinguishable as captivity extends. The most vulnerable aspects of this change likely include body

condition, which is strongly influenced by a regular captive diet and limited activity level compared to wild conditions.

During the initial two-year study period, we supported the research efforts of 11 biologists from seven different institutions. Data were produced for 17 studies, including general health and body condition, parasitology, epidemiology, nutrition, vitamin status, stress response, immune function, reproductive status, and many more. In addition to the study of phenotypic variation in behavior and physiology, and the subsequent development of models applicable to wild populations, our approach will permit the development of further novel experimental designs. For example, new implantable telemetry devices have been developed to specifically study long-term behavior and survival of individual wild sea lions (Horning & Hill, 2005). These and other new telemetry devices may require implantation or other invasive procedures. The validation of the absence of complications resulting from surgery and implants will become important for such new research approaches. While such validations should first occur on surrogate, non-endangered species, a second step prior to a large-scale application of such technology involves tests on wild subjects of the target species under highly controlled conditions.

Our new approach promises to be a highly successful means to study a species in need of a more comprehensive research technique than is currently available with traditional methods. The temporary captivity period allows for a repeated sampling design, while maintaining a wild animal's status. Furthermore, the benefits of on-site research allow for a vast amount of collaboration among widespread institutions and researchers. While the base infrastructure and logistical needs are not insignificant, the cumulative information and inter-researcher effort far exceeds the initial input as well as the potential impact of each researcher acting independently. The collaborative nature of the design also fosters positive dialogue among concerned parties, enhancing a broad discussion of knowledge and ideas potentially critical for management and recovery teams.

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