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# SHORT COMMUNICATIONS

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## Haptoglobin Concentrations in Free-range and Temporarily Captive Juvenile Steller Sea Lions

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**ABSTRACT:** Haptoglobin (Hp) is an acute-phase protein synthesized in the liver that circulates at elevated concentrations in response to tissue damage caused by inflammation, infection, and trauma. As part of a larger study, sera Hp concentrations were measured in temporarily captive ( $n=21$ ) and free-range ( $n=38$ ) western stock juvenile Steller sea lions (*Eumetopias jubatus*) sampled from 2003 to 2006. Baseline Hp concentration at time of capture was  $133.3 \pm 17.4$  mg/dl. Temporarily captive animals exhibited a 3.2-fold increase in Hp concentrations during the first 4 wk of captivity, followed by a return to entry levels by week 5. Haptoglobin levels were not influenced by age, season, or parasite load. There was a significant positive correlation between Hp concentrations and white blood cell count ( $P < 0.001$ ) and globulin levels ( $P < 0.001$ ) and a negative correlation to red blood cell count and hematocrit ( $P < 0.001$  for both). There was no correlation between Hp levels and platelet count ( $P = 0.095$ ) or hemoglobin ( $P = 0.457$ ). Routine blubber biopsies collected under gas anesthesia did not produce a measurable Hp response. One animal with a large abscess had an Hp spike of 1,006.0 mg/dl that returned to entry levels after treatment. In conclusion, serum Hp levels correlate to the stable clinical health status observed during captivity, with moderate Hp response during capture and initial acclimation to captivity and acute response to inflammation and infection.

**Key words:** Acute-phase response, disease, *Eumetopias jubatus*, haptoglobin, Steller sea lion, stress.

Haptoglobin (Hp) is an acute-phase protein synthesized in response to tissue damage, such as inflammation, infection, and trauma (Gordon and Koj, 1985). Circulating Hp concentrations provide a sensitive indicator of disease in humans and animals (Gordon and Koj, 1985; Echersall et al., 1989); however, this diagnostic tool has found limited use with

marine and other aquatic mammals. In Steller sea lions (*Eumetopias jubatus*) and harbor seals (*Phoca vitulina*), Hp levels have been used to identify compromised populations (Zenteno-Savin et al., 1997). Haptoglobin concentrations were measured in healthy captive northern fur seals (*Callorhinus ursinus*) with a reported range of 35–114 mg/dl (Mazarro et al., 2004). River otters (*Lutra canadensis*) in the oiled waters of Prince William Sound had significantly elevated Hp concentrations 1 yr after the *Exxon Valdez* spill (Duffy et al., 1993), but concentrations returned to baseline levels the following year (Duffy et al., 1994).

We quantified Hp concentrations in endangered western stock juvenile Steller sea lions to assess the effects of research procedures on temporarily captive animals (Mellish et al., 2006) and other potential unknown stressors on free-ranging animals. Specific objectives were to establish baseline Hp levels immediately after capture, to monitor baseline Hp levels during captivity (up to 12 wk), to assess Hp levels during routine physiological studies (e.g., blubber biopsies), to assess Hp levels in relation to any preexisting condition (e.g., parasite load), and to compare Hp levels with other common indicators of health state (e.g., white blood cell count, platelets, globulins, red blood cell count, hematocrit, and hemoglobin).

Temporarily captive ( $n=21$ ) and free-ranging ( $n=38$ ) Steller sea lions were captured in Prince William Sound (60°N, 148°W) or Resurrection Bay (60°N, 149.3°W), Alaska and examined as part of a larger study conducted at the Alaska

SeaLife Center (ASLC), Seward, Alaska, USA (Mellish et al., 2006). Blood samples were collected from the caudal gluteal or flipper vein, and samples were stored until analysis at  $-80^{\circ}\text{C}$ . Animals held in temporary captivity were sampled under gas anesthesia up to weekly during a 10- to 12-wk captive research period. Various research procedures were conducted, including blood collection, ultrasound, blubber biopsy, diet manipulation, and routine physiologic examinations.

Haptoglobin concentrations were quantified in serum samples according to the manufacturer's instructions via colorimetric assay kit (Phase<sup>TM</sup> range haptoglobin assay, Tridelta Diagnostics, Morris Plains, New Jersey, USA). The manufacturer's published interassay coefficient of variation (CV) was 11%. Our internal control included in each assay resulted in a 19% CV. White blood cell count, platelet levels, red blood cell count, hematocrit, and hemoglobin were measured with a VetScan<sup>®</sup> HMTII analyzer (Abaxis, Union City, California, USA). Globulin levels were analyzed with a VetScan<sup>®</sup> Diagnostic Profile Plus rotor (Abaxis). Parasite load was analyzed via tracheal mucus cytology and direct fecal examination, and load was categorized as either present or absent. Summarized data are presented as means with standard error (SE). Data were analyzed with SigmaStat 3.11 (Systat Software Inc., Richmond, California, USA) and included analysis of variance (ANOVA), *t*-test, and linear regression.

Juvenile Steller sea lions were  $109.9 \pm 2.7$  kg in total body mass. Average age as determined by erupted canine length measurements (King, unpublished data) and adjusted to regional mean pupping date of 10 June (Maniscalco et al., 2006) was  $13.8 \pm 0.6$  mo (range 9–23 mo). Haptoglobin concentration collected within 4 hr of capture was  $133.3 \pm 17.4$  mg/dl ( $n=59$ ), there was no difference between animals immediately released ( $150.6 \pm 24.4$  mg/dl) and those retained for temporary captivity ( $101.9 \pm$

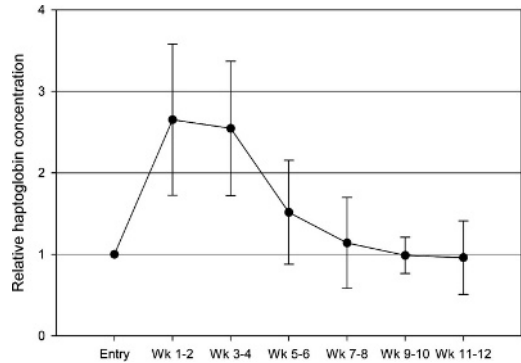


FIGURE 1. Mean haptoglobin concentrations in temporarily captive juvenile Steller sea lions (*E. jubatus*;  $n=15$ ), relative to individual entry levels.

$19.9$  mg/dl; *t*-test;  $P=0.388$ ). There was no difference between male ( $151.0 \pm 29.1$  mg/dl;  $n=31$ ) and female ( $113.8 \pm 17.5$  mg/dl;  $n=28$ ) animals (*t*-test;  $P=0.99$ ). No correlation between Hp levels and age (in months;  $r=0.208$ ,  $P=0.113$ ), season, or parasite load (two-way ANOVA: fecal  $P=0.482$ , tracheal  $P=0.526$ ) was evident. Haptoglobin levels increased 3.2-fold during the first 4 wk of captivity ( $n=15$ ), and then the levels decreased to entry levels during wk 5 and remained low until release (Fig. 1). One animal was omitted and is described separately below. In comparison, Hp concentrations in the endangered western stock of Steller sea lions were threefold (pups) and 1.8-fold (adults) higher than the mean values observed in the nonendangered eastern stock (Zenteno-Savin et al., 1997). There was a positive correlation between Hp concentrations and white blood cell count ( $r=0.563$ ,  $P<0.001$ ) and globulin levels ( $r=0.438$ ,  $P<0.001$ ) (Fig. 2). Red blood cell count ( $4.24 \pm 0.05 \times 10^{12}/l$ ) and hematocrit ( $46.3 \pm 0.5\%$ ) had a slightly negative correlation to Hp levels ( $r=0.498$  and  $r=0.483$ , respectively;  $P<0.001$  for both); however, both parameters were within the normal range for this species (Mellish et al., 2006). Haptoglobin levels were not correlated to platelet count ( $469.7 \pm 20.3 \times 10^9/l$ ;  $r=0.146$ ,  $P=0.095$ )

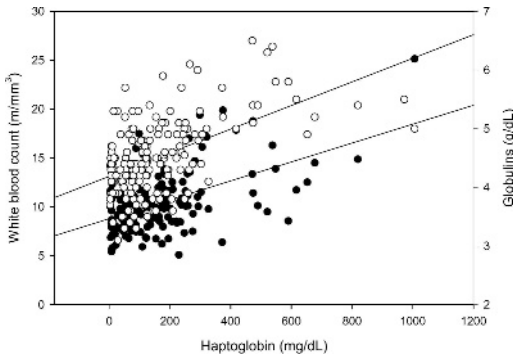


FIGURE 2. Relationship between haptoglobin concentration in temporarily captive juvenile Steller sea lions (*E. jubatus*;  $n=16$ ) to white blood cells (closed circles;  $y=0.00967x+8.790$ ,  $R^2=0.266$ ,  $P<0.001$ ) and globulin levels (open circles;  $y=0.00162x+4.193$ ,  $R^2=0.192$ ,  $P<0.001$ ).

or hemoglobin ( $15.6 \pm 0.8$  g/dl;  $r=0.067$ ,  $P=0.457$ ).

A series of blubber biopsies were performed on four animals as part of a nutritional stress and fatty acid turnover study, during which time animals were fasted up to 2 wk (Mellish and Horning, unpublished data). All blubber biopsies were conducted on anesthetized animals at three specific shaved and sterilized sites along the animals' flank. A 1-cm incision was created with a sterile surgical blade. Two 4-mm blubber cores were extracted from each site with a sterile, disposable UniPunch (Premier Medical Products, Plymouth Meeting, Pennsylvania, USA). Eight biopsies were performed: one biopsy at capture, three biopsies at wk 6 (prefast), three biopsies at wk 8 (end fast), and one final biopsy at wk 9 (Fig. 3). Haptoglobin levels in these four individuals increased during the first 4 wk in response to capture and captivity, levels returned to entry levels by wk 5, and levels remained low during the remaining biopsies. Baseline values represent the mean Hp concentration for temporarily captive animals postcapture ( $n=15$ ). Haptoglobin concentrations in these animals did not respond to the multiple minor tissue traumas associated with the biopsies. The biopsy incision wounds are similar in size

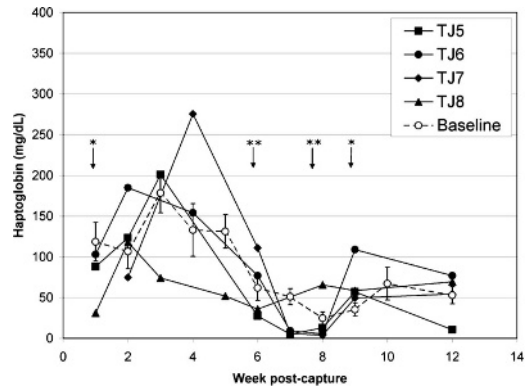


FIGURE 3. Haptoglobin response to multiple blubber biopsy events in four (TJ5–TJ8) juvenile Steller sea lions (*E. jubatus*) compared with baseline levels ( $n=15$ ). Timing of single (\*) and triple (\*\*) biopsies are noted.

and severity to the bite wounds from conspecific interactions frequently observed in captured free-range animals, and they likely explain why biopsies did not elicit an acute-phase response.

In one temporarily captive animal, a large abscess developed from a preexisting wound in the subdermal tissue on the neck. The abscess formation resulted in elevated white blood cell, platelet, globulin, and Hp levels (Fig. 4). White blood cells and Hp levels responded first, increasing from 7.9 to 25.1  $m/mm^3$  and from 9.0 to 1006.0 mg/dl, respectively, in a 14-day period. Globulins and platelets increased as well; however, the response was

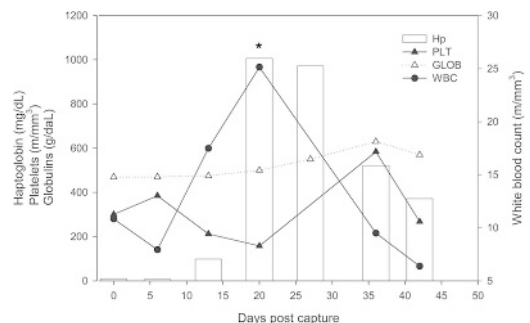


FIGURE 4. Changes in haptoglobin levels, white blood cell count, and platelet and globulin concentration. Day of treatment is noted (\*).

less marked, and it was delayed approximately 3 wk. Because there was no existing external drainage route, the abscess required irrigation and antibiotic administration. The individual responded well to treatment, and all parameters returned to baseline levels before release.

In summary, we have shown that Hp levels in juvenile Steller sea lions do not respond to parasite load or minor tissue trauma but do respond to acute insult (e.g., large abscess). Haptoglobin concentrations are positively correlated with some generalized measures of inflammation or infection (e.g., white blood cells and globins) but not others (e.g., platelets). Capture and temporary captivity did elicit a moderate short-term Hp increase with a distinct return to baseline levels. Further handling, anesthesia, and research procedures did not elevate Hp levels. These findings suggest rapid acclimation to the temporarily captive environment with no perceived sublethal stressors or threats.

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