Effects of oiling on exercise physiology and diving behavior of river otters: a captive study

M. Ben-David, T.M. Williams, and O.A. Ormseth

Abstract: Following the *Exxon Valdez* oil spill (EVOS), river otters (*Lontra canadensis*) on oiled shores had lower body mass, selected different habitat characters, and had larger home ranges and less diverse diets than did otters living in non-oiled areas. We explored the possibility that these changes were due to the effect of crude oil contamination on physiological and behavioral processes in otters. Fifteen otters were exposed to two levels of oil contamination under captive controlled conditions at the Alaska Sealife Center in Seward, Alaska, U.S.A. We collected blood samples for hematological examinations and measured oxygen consumption in otters exercising on a motorized treadmill. We also observed diving and foraging behavior of otters offered live fish. Otters exposed to oil became anemic relative to controls. While oxygen consumption of resting river otters was not related to changes in hemoglobin concentration, exercising river otters with decreased hemoglobin levels displayed significantly increased oxygen consumption (P = 0.042). Oiled otters also performed fewer dives when chasing fish (P = 0.04), representing a potential decrease of 64% in the capture rate of prey. Our data strongly support the hypothesis that changes in prey types and home-range utilization by oiled river otters following EVOS were influenced by hematological changes, associated increases in energetic costs, and reduced diving ability.

Résumé : Après le désastre causé par le naufrage de l'*Exxon Valdez* (EVOS), les Loutres de rivière (*Lontra canadensis*) des rives affectées avaient une masse corporelle plus faible que les loutres des régions non perturbées et elles ont choisi des habitats de caractères différents et adopté des domaines plus vastes et des régimes moins diversifiés. Nous avons examiné la possibilité que ces changements soient attribuables aux effets de la contamination de l'huile brute sur les processus physiologiques et comportementaux des loutres. Quinze loutres ont été exposées à deux degrés de contamination en captivité, dans des conditions contrôlées, au centre de biologie marine Alaska Sealife Center, à Seward, Alaska, É.-U. Nous avons prélevé des échantillons de sang pour fins d'examen hématologique et mesuré la consommation d'oxygène chez des loutres en pleine séance d'exercice sur un tapis roulant motorisé. Nous avons également observé les comportements de plongée et de recherche de nourriture chez des loutres à qui l'on a offert des poissons vivants. Les loutres exposées au pétrole sont devenues anémiques comparativement aux loutres témoins. Alors que la consommation d'oxygène des loutres au repos n'était pas reliée aux changements de leur concentration d'hémoglobine, les loutres à l'exercice avec des taux d'hémoglobine réduits affichaient une consommation d'oxygène significativement plus élevée (P = 0,042). Les loutres enduites de pétrole faisaient aussi significativement moins de plongées lors de leurs chasses aux poissons (P = 0,04), ce qui représente une diminution potentielle de 64 % du taux de capture des proies. Nos données appuient fortement l'hypothèse selon laquelle les changements dans les types de proies et l'utilisation du domaine vital chez les loutres enduites de pétrole à la suite du déversement de l'*Exxon Valdez* ont été influencés par des changements hématologiques, des augmentations des coûts énergétiques associés à ces changements et une diminution de la capacité de plonger.

[Traduit par la Rédaction]

Introduction

Investigations in Prince William Sound (PWS) following the *Exxon Valdez* oil spill (EVOS) revealed that river otters (*Lontra canadensis*) on oiled shores had lower body mass and elevated levels of biomarkers (i.e., blood proteins indicative of physiological damage) relative to otters living on non-oiled shores (Duffy et al. 1993, 1994*a*, 1994*b*, 1996; Blajeski et al. 1996; R.T. Bowyer, G.M. Blundell, M. Ben-David, S.C. Jewett, T.A. Dean, and L.K. Duffy[2]). In addition, otters from oiled areas selected different habitat characters, had larger home ranges, and less diverse diets than those in non-oiled areas (Bowyer et al. 1994, 1995). These observed differences between river otters from oiled shores and those from non-oiled areas strongly suggest that oil contamination had an effect on physiological and behavioral processes in otters.[2]

Studies initiated following EVOS indicated that several other mammalian and avian predators displayed physiological stress related to oil toxicity. Oiled sea otters (*Enhydra lutris*), collected for rehabilitation, suffered from emphysema, ulcers, anemia, lesions, and organ congestion (Williams et al. 1995). Similarly, free-ranging sea otters from oiled regions

Received September 21, 1999. Accepted March 20, 2000.

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[2]R.T. Bowyer, G.M. Blundell, M. Ben-David, S.C. Jewett, T.A. Dean, and L.K. Duffy. Effects of the *Exxon Valdez* oil spill on river otters: injury and recovery of a sentinel species. Submitted for publication.

had greater antigenic stimulation than those from non-oiled areas, and pups sampled in these regions had lower hemoglobin (Hb) levels than pups from non-oiled areas (Rebar et al. 1994). Pigeon guillemots (*Cepphus columba*) had elevated levels of haptoglobins and blood proteins in specific locations and years, although dosing experiments in the field failed to demonstrate the connection between oiling and these parameters (Prichard et al. 1997). Similar changes in plasma proteins, abnormalities in white blood cells (leukocytes), reduced numbers of red blood cells (erythrocytes), and electrolyte imbalance, were previously observed in mink (*Mustela vison*) and polar bears (*Ursus maritimus*) following exposure to hydrocarbons (Øritsland et al. 1981; J. Mazet, University of California Davis, personal communication).

Such physiological stress may have a marked effect on the ability of animals to perform in the wild. The foraging behavior of semi-aquatic mammals such as river otters relies, in part, on their capacity to support aerobic metabolism while submerged (Dunstone and O'Connor 1979*a*, 1979*b*; Nolet et al. 1993). Kruuk et al. (1990) demonstrated that the foraging success of European otters (*Lutra lutra*) in marine environments in the Shetland Islands was determined largely by the behavior of both prey and predators. Although river otters are well adapted to diving (e.g., large surface area of feet, high storage capacity for oxygen, and good propulsion capabilities; Tarasoff et al. 1972; Nolet et al. 1993; Fish 1994; Pfeiffer and Culik 1998), limitations on aerobic capacity could affect the duration and depth of the dives they perform (Nolet et al. 1993; Pfeiffer and Culik 1998) and their consequent foraging efficiency. Thus, exposure to oil, associated chronic physiological stress, and reduced numbers of red blood cells (i.e., lowered oxygen storage capacity; Øritsland et al. 1981) could have deleterious effects on the diving ability of coastal river otters.

In this study, we investigated the effects of exposure to oil on the exercise physiology and diving behavior of river otters under controlled conditions. We hypothesized that exposure to oil would result in hematological changes that could lower aerobic capacity. In river otters, such changes would limit terrestrial and aquatic performance and result in higher energetic costs, as well as altered diving behavior. Because most dives performed by marine mammals are shorter than the aerobic breath-holding limit (Kramer 1988; Kooyman 1989; Nolet et al. 1993), we predicted that, during the oiling period, foraging otters with reduced aerobic capacity would perform shorter dives than non-oiled controls. Alternatively, if dive duration is largely dependent on stored oxygen in the lungs, blood, and muscles (Kooyman 1989), then recovery times would be longer in oiled individuals as a consequence of a prolonged time for oxygen replenishment. Thus, diving efficiency (duration of dive divided by the sum of duration of dive and duration of following recovery) should be lower for oiled otters.

Methods

General

Fifteen wild male river otters were captured live in northwestern PWS using No. 11 Sleepy Creek® leg-hold traps (Blundell et al. 1999). Traps were placed on trails at latrine sites and monitored by trap transmitters (Telonics, Mesa, Ariz., U.S.A.) that signal when a trap is sprung (see footnote 2). Processing of the otters began within 1–2 h of capture. Otters were anesthetized with Telazol® (9 mg/kg; A.H. Robins, Richmond, Va., U.S.A.) administered using Telinject® darts (Telinject U.S.A. Inc., Saugus, Calif., U.S.A.) and a blowgun. Blood and tissues were sampled from each individual otter at this time.

The 15 wild-caught male river otters were flown, under sedation, to the Alaska Sealife Center (ASLC) in Seward, Alaska, U.S.A. The number of subjects in this experiment was determined by an a priori power analysis and the need to minimize the impact of removing individuals from the wild population of river otters in PWS. The otters were held in captivity at ASLC from May 1998 to March 1999. The animals were housed as one large group in a 90-m^2 area surrounding six pools (one large salt-water pool 4.5 m in diameter and 3 m deep; four small salt-water pools, each 2 × 1.5 × 1.5 m; and one small fresh-water tote, 1 × 1 × 1 m). Otters were fed frozen fish on a daily basis (for details see M. Ben-David, L.K. Duffy, and R.T. Bowyer)[3] and diet was supplemented with live prey, vitamins, and minerals (Robbins 1993).

Experiments began in August, allowing the animals 2.5 months to acclimate to the enclosure, feeding regimes, and handling. Following acclimation, otters were randomly assigned to three experimental groups of five individuals each: control group, which received no oil; low-dose group, which received 50 ppm of oil in the diet (i.e., 0.1 g of oil every other day, with an average individual daily consumption of 1 kg of food); and a high-dose group, which received 500 ppm of oil (i.e., 1.0 g every other day). The exposure level for the low-dose group was determined on the basis of levels of Prudhoe Bay crude oil (PBCO) found in mussel beds in PWS in 1995 (Short et al. 1996), in an attempt to simulate conditions of chronic exposure in the wild. The high-dose level was selected to simulate conditions in PWS immediately following EVOS. Weathered (comparable to 2 weeks weathering) Prudhoe Bay crude oil was administered to otters in gel capsules hidden in fish.[3] Oil feeding lasted 100 days from 21 August to 28 November 1998. Data collection continued for an additional 100 days of rehabilitation. Animals were then fitted with radio transmitters and released back at the site of capture in PWS. Animals are currently being monitored to determine post-release survival (M. Ben-David, unpublished data).

Hemoglobin levels

Prior to the exposure to oil (29–30 June and 15–16 August 1998), a series of blood and tissue samples were collected from each individual otter.[3] Blood and tissue samples were collected every 3 weeks from 15 August 1998 until 12 January 1999, with an additional sampling session on 24 February 1999.

Otters were anesthetized with a combination of ketamine hydrochloride (Ketaset® (Aveco Co., Fort Dodge, Iowa., U.S.A.), 100 mg/mL), at a dosage of 10 mg/kg, and midazolam hydrochloride (Versed® (Hoffman-LaRoche, Nutley, N.J., U.S.A.), 5 mg/mL), at a dosage of 0.25 mg/kg (Spelman et al. 1993). The dosage was mixed in the syringe and administered intramuscularly with Telinject® darts and a blowgun or hand-injected while the otter was immobilized in a squeeze box. We drew blood from the jugular vein of each otter with care, to keep samples sterile. A portion of the sample was preserved with EDTA (purple-top Vacutainer®; Becton-Dickinson, Franklin Lakes, N.J., U.S.A.) for complete blood counts (CBCs) and refrigerated until analysis (within 48 h). The remaining blood (approximately 10 mL) was collected in a red-top Vacutainer® and allowed to clot and serum was removed (within 8 h) following centrifugation at 3000 rpm for 10 min and

[3] M. Ben-David, L.K. Duffy, and R.T. Bowyer. Responses of river otters to oil contamination: an experimental study of biomarkers. Submitted for publication.

Table 1. List and definitions of data collected for each individual otter during foraging trials at the Alaska Sealife Center, Seward, Alaska.

Behavior	Description	Computer code	Analysis code	Comment
Dive	From head submerged to head emerged	Dive	Dive	
Surface swimming	Animal with head above water	Surf swim	Recovery	
Climb out	Animal is climbing out of the water	Climb out	Recovery	
Edge	Animal is either sitting, walking, or running around the edge	Edge	Recovery	If activity is immediately preceded and followed by a dive
			Non pool	If activity is preceded or followed by non-pool activity
Eat	Animal is feeding on fish	Eat	Non pool	
Non pool	All activities not related to pool (sleeping, scent marking, drinking, scratching door, etc.)	Non pool	Non pool	

Table 2. Fish types and session schedules for diving experiments of captive river otters at the Alaska Sealife Center, Seward, Alaska.

Fish type	Scientific name	Mass range (kg)	No. of fish	Session used
Schooling, fast				
Adult pink salmon	*Oncorhynchus gorbuscha*	2.0–3.0	220	Pre-oiling
Pacific cod	*Gadus macrocephalus*	3.0–4.0	8	Height of oiling
Sable fish (black cod)	*Anoplopoma fimbria*	2.0–3.0	28	Height of oiling
Juvenile pink salmon	*Oncorhynchus gorbuscha*	0.3–0.5	23	Rehabilitation
Nonschooling, slow				
Copper rockfish	*Sebastes caurinus*	0.75–1.5	11	All
Red Irish lord	*Hemilepidotus spinosus*	0.75–1.5	8	All
Yellowfin sole	*Limanda aspera*	0.75–1.5	16	All
Kelp greenling	*Hexagrammos decagrammus*	0.75–1.5	201	All

Note: Fish were obtained under permit from the Alaska Department of Fish and Game, Commercial Fish Division (No. CF-98-024), and were collected in south-central Alaska.

refrigerated until analyses of serum chemistry. Three blood smears were made for each river otter at the time blood was drawn. Serum-chemistry profiles were assayed with an Olympus 7000 (Olympus, Melville, N.Y., U.S.A.) and CBCs were performed with a Stack-S whole blood analyzer (Coulter, Miami, Fla., U.S.A.). Samples were analyzed at Quest Diagnostics Incorporated (Portland, Oreg., U.S.A.).

Oxygen consumption

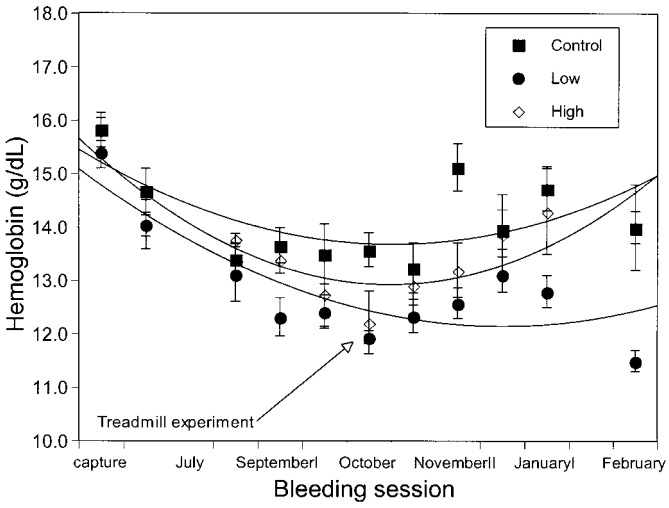
The rate of oxygen consumption was determined for 10 of the 15 male river otters (mean body mass = 11.1 ± 0.7 kg; three control, three low-dose, and four high-dose individuals) during rest and running in October 1998, which coincided with the height of the oiling period. The animals were trained to run in a Plexiglas chamber (54 cm high × 31 cm wide × 138 cm long) mounted on a variable-speed motorized treadmill. Resting measurements were taken for sedentary animals prior to each exercise test. Open-flow respirometry was used, with ambient air drawn in along the lower edge of the chamber, according to Williams (1983). Air was drawn through the chamber by a vacuum pump at flow rates averaging 61–64 $L \cdot min^{-1}$, to maintain the level of oxygen above 20% during the tests. Flow rates were monitored continuously with a dry gas meter (American Meter Co., DTM-325, San Leandro, Calif., U.S.A.) that had been calibrated against a constant volume pump (Calibringe, Vacumed, Ventura, Calif., U.S.A.). Expired air was removed through a port located on top of the box and samples were drawn through Drierite and Sodasorb columns to remove water and carbon dioxide, respectively. The percentage of oxygen in the air samples was continuously monitored with an oxygen analyzer (AEI Technologies, S3-A, Pittsburg, Pa., U.S.A.) connected to a personal computer. These values were converted to oxygen consumption ($\dot{V}_{O2}$), using Sable Systems (Salt Lake City, Utah, U.S.A.) respirometry software and equations modified from Fedak et al. (1981) and Withers (1977). All values were corrected to STPD (standard temperature and pressure, dry). The entire system was calibrated daily with dry ambient air (20.94% O_2) and nitrogen gas (100% N_2), using the nitrogen-dilution techniques of Fedak et al. (1981).

The experiments were conducted at a T_{air} of 2.9–9.4°C, which prevented the exercising otters from becoming overheated. On each experimental day, an otter was placed in the chamber and permitted to rest for approximately 10–15 min. The treadmill was turned on to the test speed and percentage of oxygen monitored. During the experiments, otters maintained a forward position at the front of the chamber. The animals ran for 10–20 min and were considered to be in a steady state when $\dot{V}_{O_2}$ varied by less than 4% over at least a 5-min period. Following the run, animals were released from the chamber and allowed to join the rest of the group in the enclosure; only one speed was tested on any experiment day. Test speeds were determined by the ability of the otters to keep up with the treadmill at high speed while maintaining steady gaits. Speeds included: fast walk, 0.72 $m \cdot s^{-1}$; mixed gait of walk and gallop, 1.05–1.2 $m \cdot s^{-1}$; and gallop, 1.4–1.6 $m \cdot s^{-1}$. Experiments were terminated if running performance was inconsistent or the animals turned around.

Diving behavior

Dive duration, recovery times, and capture success (Table 1) were recorded for each individual otter (n = 12; four control, three low-dose, and five high-dose individuals) during experimental foraging sessions, using a Newton MessagePad 2100 (Apple Inc., Austin, Tex., U.S.A.) and Ethoscribe® software (Tima Scientific,

Fig. 1. Levels of hemoglobin in blood of captive river otters at the Alaska Sealife Center exposed to three levels of hydrocarbons in their diet (n = 15; five in each treatment group). Blood was collected every 3 weeks between 15 August 1998 and 11 January 1999. Six weeks elapsed between the 29 June and 15 August sessions and between the 11 January and 24 February sessions. For both low- and high-dose groups, a significant decrease in hemoglobin occurred during the oiling period (SeptemberI–NovemberII sessions; Tukey's multiple comparison test, α = 0.05). No such decrease was detected in control animals. A significant decrease in hemoglobin levels occurred in the low-dose group in February, owing to iron deficiency.



N.B., Canada). Otters were offered two types of live fish in the large salt-water pool: schooling fast fish (salmon or sable fish) and nonschooling slow intertidal fish (greenling or rock fish; Table 2). The otters were allowed to forage for these fish for 30 min at a time (i.e., a trial). The pool contained a PVC structure that provided fish with places to hide from the chasing otter. In each trial, each otter was offered between three and five fish of the same type; fish were released into the pool together, prior to the introduction of the otter. Fish were allowed to explore the pool for several minutes before the beginning of the trial. Each session was videotaped, using an overhead remote camera and microwave-transmission system. Data on chase intensity were extracted with frame by frame analysis, using a Sony® SLV-998H VCR (video cassette recorder; Sony Corporation of America, Park Ridge, N.J.). Chase intensity was defined as the proportion of the dive time in which the otter actively chased the fish. Data were coded 4, if the otter spent 50–100% of the dive chasing the fish; 3, if the otter spent 10–50% of the time chasing the fish; 2, if the otter spent <10% of the time chasing the fish; and 1, if no chase occurred. Diving experiments were conducted prior to oiling (August 1998), at the height of oiling (October–November 1998), and at the end of the rehabilitation period (January–February 1999).

Statistical analysis

To determine the effects of oiling on Hb levels in river otters, we used repeated-measures ANOVA, with oiling group (i.e., control, low dose, and high dose) and bleed session as factors. Analysis was followed by Tukey's multiple comparison test to establish where significant differences occurred.

Data on oxygen consumption were analyzed using linear regression, with Hb as the independent and oxygen consumption as the dependent variable (Zar 1984). We conducted similar but separate analyses for animals at rest and during exercise.

Dive duration and recovery times were plotted sequentially for each foraging trial and analyzed for fit to an exponential distribution. Only cases where r^2 was equal to or greater than 0.9 and the P value was equal to or less than 0.05 were included in subsequent analyses. For these sessions, mean dive duration and mean recovery times were calculated and used in future analyses, because in these cases the mean equals the variance (Haccou and Meelis 1992). In addition, number of dives in each trial and chase intensity during each dive were calculated. Mean dive duration, mean recovery times, mean chase intensity, and number of dives were tested for normality and were found to be normally distributed (W statistic > 0.9, SPSS for Windows). Differences in mean dive duration and mean recovery times for each trial were analyzed with a two-way ANOVA, with number of dives used as a covariate, to control for differences in number of dives between animals, trials, and sessions (Johnson and Wichern 1992; GLM procedure in SPSS for Windows). The two factors were oiling group (control, low dose, and high dose) and sampling session (prior to oiling, height of oiling, and rehabilitation). Data were blocked by individual to control for lack of independence among sessions. This corresponds to repeated-measures analysis. We also calculated dive efficiency by dividing dive duration by the sum of the dive duration and the following recovery time (Kooyman et al. 1980; Nolet et al. 1993), and then established the mean dive efficiency for each trial. We used a two-way ANOVA to investigate differences in dive efficiency between oiled and non-oiled otters, with number of dives and chase intensity as covariates, to control for differences in number of dives and chase intensities between animals, trials, and sessions (SPSS for Windows). The two factors were oiling group (control, low dose, and high dose) and sampling session (prior to oiling, height of oiling, and rehabilitation). We used a curve-fitting approach to establish a relation between Hb levels and dive efficiency, Hb levels and number of dives, and Hb levels and chase intensity (Zar 1984).

Results

Hemoglobin levels

Hb levels in our experimental otters were significantly reduced as a result of oiling (P < 0.01; group effect P = 0.001; session effect P < 0.001; Fig. 1). Hb levels decreased in a similar fashion for all animals over the 3 months from capture to sampling in August. No significant differences (P > 0.8) were detected between groups for that period (Fig. 1). During the oiling period, levels of Hb stabilized in the control group, while values continued to decline for the oiled animals. No significant differences (P > 0.2) were detected between the low- and high-dose groups during this period (Fig. 1). The lowest Hb levels were observed in the oiled otters in October, which coincided with both the treadmill and the diving experiments (i.e., height of oiling). After the administration of oil ended (28 November 1998), Hb levels increased, and no significant differences were detected between oiled and control animals in the December samples. Similarly, no differences in Hb levels were detected between the high-dose and the control groups in January and February, but the low-dose group experienced a significant decline in Hb levels during this time (Fig. 1). We were able to determine that this decline was caused by iron deficiency and reversed it with weekly iron injections before the animals were released.

Fig. 2. Oxygen consumption (mL $O_2 \cdot kg^{-1} \cdot min^{-1}$) plotted against hemoglobin concentration (g/dL) for captive river otters (n = 10 individuals) at rest and at a fast walk of 0.72 $m \cdot s^{-1}$. No significant relation was detected between oxygen consumption and hemoglobin levels at rest (P = 0.56), but oxygen consumption increased significantly with decreasing hemoglobin (P = 0.042). Experiments were conducted at the Alaska Sealife Center in Seward, Alaska.

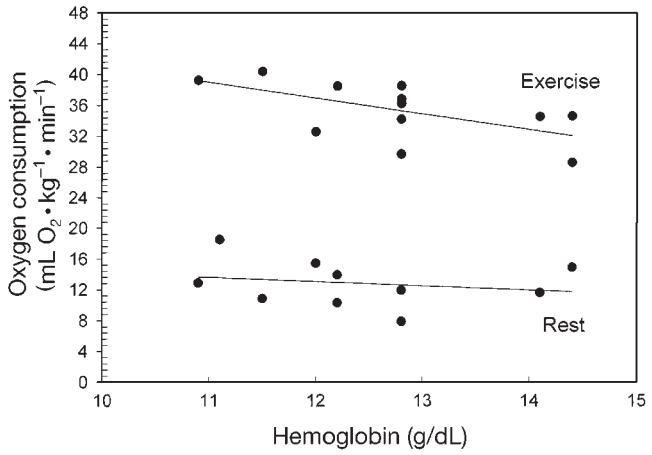
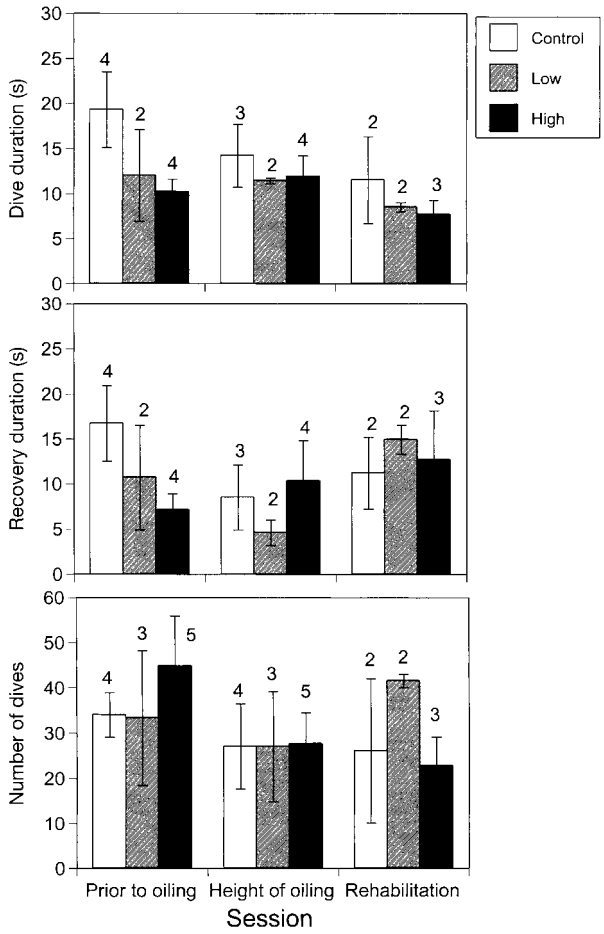


Fig. 3. Mean dive duration (s), mean recovery time (s), and number of dives for captive river otters diving after schooling fast fish. No significant differences were detected for these variables (ANOVA, $P > 0.1$). Experiments were conducted at the Alaska Sealife Center in Seward.



Running performance and oxygen consumption

When resting on the treadmill, the oxygen consumption of our river otters averaged 12.84 ± 0.95 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$ (mean ± SE) and did not significantly vary (P = 0.56) with changes in Hb concentration (Fig. 2). These values compare well with the resting values of 12.30 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$ (Pfeiffer and Culik 1998) and 9.60 mL $O_2.kg^{-1} \cdot min^{-1}$ (Kruuk 1995) for the smaller European otter.

Non-oiled and oiled otters from both the low- and high-dose groups exhibited different behavioral responses to running exercise. Oiled otters from both groups preferred to walk (0.72 $m \cdot s^{-1}$) and avoided speeds that required mixed walk–bound gaits (1.05–1.2 $m \cdot s^{-1}$) or bounding gaits (1.4–1.6 $m \cdot s^{-1}$). All three control animals tested in the experiment performed in all tests without added encouragement. Of the oiled animals, only one low-dose animal and one high-dose animal ran at the 1.4–1.6 $m \cdot s^{-1}$ speed; the low-dose animal only performed this trial once. The other five animals refused to run at 1.4–1.6 $m \cdot s^{-1}$, although they performed well at a speed of 0.72 $m \cdot s^{-1}$. Therefore, to compare performance of these individuals, we used only the data from walking-speed tests (0.72 $m \cdot s^{-1}$).

The oxygen consumption of our river otters was 2.3–3.1 times higher when running on the treadmill at 0.72 $m \cdot s^{-1}$ than when at rest. In contrast with resting levels, the oxygen consumption of exercising river otters decreased significantly (P = 0.042) with increases in levels of blood Hb and was described by the equation:

$$[1] \qquad \dot{V}_{O_2} = 61.10 - 2.01[Hb] \quad (r^2 = 0.35,\ n = 12)$$

where $\dot{V}_{O_2}$ is in millilitres of oxygen per kilogram per minute and Hb is in grams per decilitre.

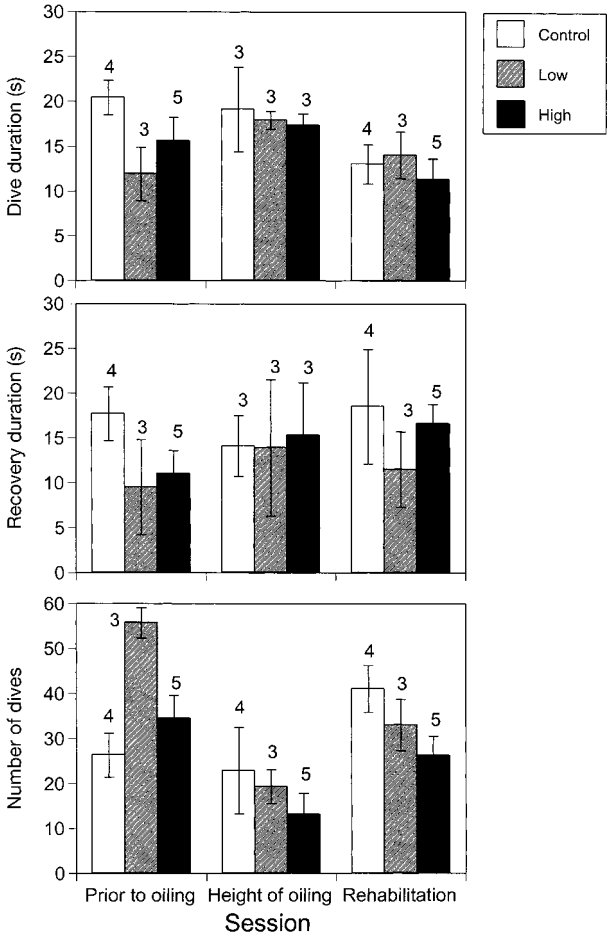
Diving performance and behavior

Dive duration and duration of recovery were significantly correlated for the entire data set (r = 0.501, P = 0.001), suggesting that long dives were usually followed by long recovery times. Nonetheless, there may be a problem with our estimation of recovery times. Recovery was defined as all activities conducted by the animal following dives that were immediately followed by another dive. This included surface swimming, climbing out of the pool, and sitting on the edge of the pool (Table 1). Several animals spent long periods of time sitting on the edge between two consecutive dives. These occurrences likely exceeded the time required for replenishing the stores of oxygen, and potentially reflect behavioral responses rather than physiological ones.

Dive duration was significantly lower for the otters chasing schooling fast fish than for chasing nonschooling slow fish (Wilcoxon's paired samples test, P = 0.02; Figs. 3 and 4), suggesting higher levels of oxygen consumption during these chases. For comparison, time to first capture (i.e., time elapsed from an otter's first dive to capture of the first fish) was significantly lower for otters foraging for slow fish (n = 12) than for foraging for fast fish (Wilcoxon's paired samples test, P = 0.013; Fig. 5).

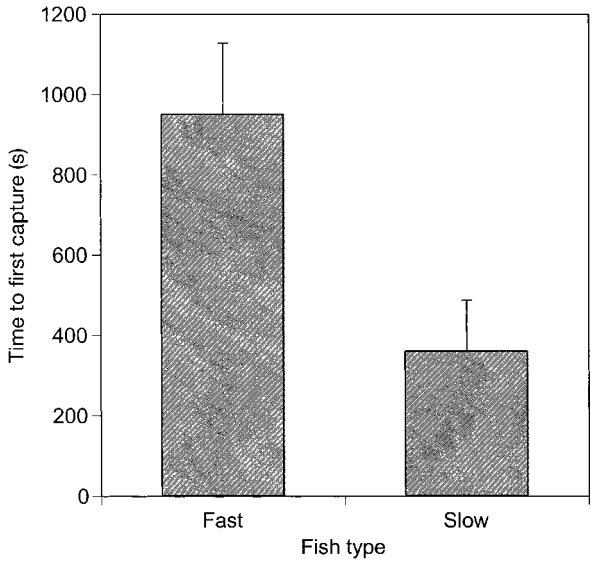
To reduce the potential effects of diving speeds on dive duration, we separated the two types of fish in subsequent analyses. With schooling fast fish, otter dive duration and recovery times did not differ significantly between the oiling-

Fig. 4. Mean dive duration (s), mean recovery time (s), and number of dives for captive river otters diving after nonschooling slow fish. No significant differences were detected for dive duration and recovery times (ANOVA, $P > 0.1$), but number of dives significantly differed between groups and sessions (ANOVA, $P = 0.04$). Number of dives during the height of oiling significantly differed between the control and highly oiled animals (Tukey's multiple comparison test, $P < 0.05$), but not between the control and low-dose animals (Tukey's multiple comparison test, $P > 0.05$). In addition, while the number of dives between sessions did not change significantly for the control animals, it did change significantly for both low-dose and high-dose animals (Tukey's multiple comparison test, $P < 0.05$).



treatment groups and sessions, when number of dives was introduced as a covariate ($P = 0.15$ and $P = 0.70$, respectively; Fig. 3). For nonschooling slow fish, otter dive duration differed between oiling groups and sessions ($P = 0.042$), but no difference was detected for recovery times ($P = 0.17$; Fig. 4). The difference in dive duration between groups and sessions was largely a result of the different numbers of dives ($P = 0.04$). Number of dives during the height of oiling differed significantly (Tukey's multiple comparison test, $P < 0.05$) between the control and highly oiled animals, but not between the control and low-dose animals (Tukey's multiple comparison test, $P > 0.05$). In addition, while the number of dives between sessions did not change significantly for the control animals, it did change significantly for both low-dose and high-dose animals

Fig. 5. Time to first capture (s) for captive river otters foraging for schooling fast and nonschooling slow fish (Wilcoxon's paired samples, $P = 0.013$; $n = 12$ trials by 12 individual otters for each fish type). Experiments were conducted at the Alaska Sealife Center in Seward.



(Tukey's multiple comparison test, $P < 0.05$; Fig. 4). When controlling for number of dives, the effect of oiling on mean dive duration became nonsignificant ($P = 0.40$).

Dive efficiency did not significantly differ between groups and sessions (ANOVA, $P = 0.07$) for otters chasing either slow or fast fish (Fig. 6). In addition, neither number of dives nor chase intensity had a significant effect on dive efficiency ($P > 0.2$). We were unable to detect a relation between Hb levels and dive efficiency ($P = 0.36$). In addition, we were unable to establish a relation between Hb levels and number of dives ($P = 0.45$; Fig. 7), but chase intensity exponentially decreased with decreasing Hb concentrations ($P = 0.027$; Fig. 7):

$$[2] \quad \text{chase-intensity score} = 2.4 + 0.3 \times \ln[\text{Hb}] \qquad (r^2 = 0.19,\ n = 49 \text{ trials})$$

where chase intensity is coded 1 (none) to 4 (high) and Hb is in grams per decilitre.

Discussion

Two factors seemed to cause a reduction in Hb levels in our experimental otters. The transfer of these otters from the wild to captivity could explain the initial reduction we observed in all otters (Fig. 1). Whether this reduction was a result of a change in diet, a change in the availability of vitamins and minerals, a reduction in levels of activity and exercise, or other environmental factors in this artificial setting is unclear. Nonetheless, the difference between the control and treatment groups throughout the oiling period suggests that oiling further aggravated the anemia. Similar results were previously observed in sea otters, mink, and polar bears, following exposure to hydrocarbons (Mohn and Nordstoga 1975; Øritsland et al. 1981; Williams et al. 1995). A reduction in aerobic capacity was previously suggested for oiled sea otters (Williams et al. 1995). That the two oil-treatment

Fig. 6. Mean diving efficiency (i.e., dive duration divided by the sum of dive duration and the following recovery time) for captive river otters diving after schooling fast (*a*) and nonschooling slow (*b*) fish. The error bars indicate standard error. No significant differences between groups and session were detected for dive efficiency (ANOVA, $P > 0.05$), regardless of fish type.

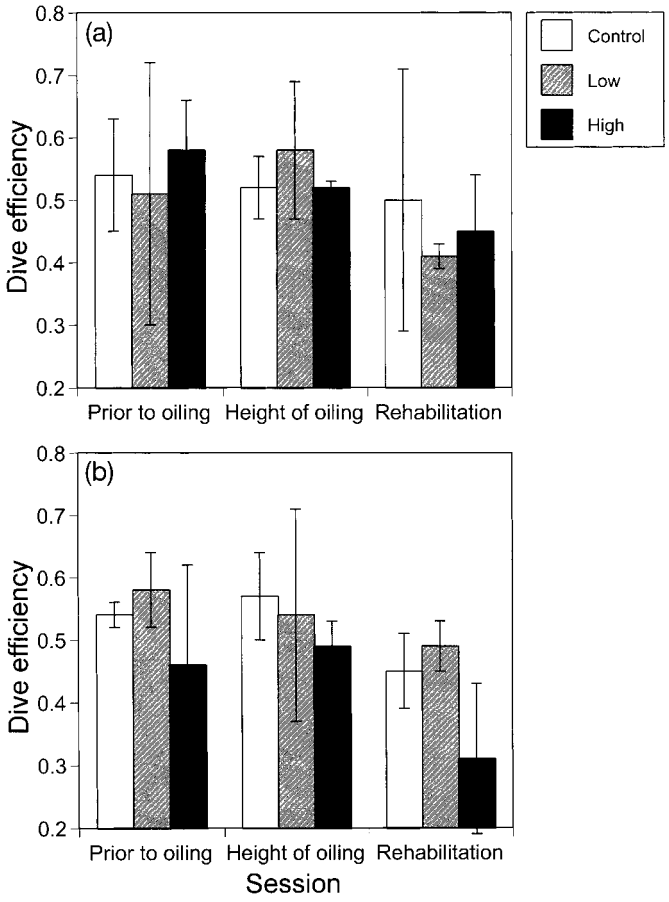
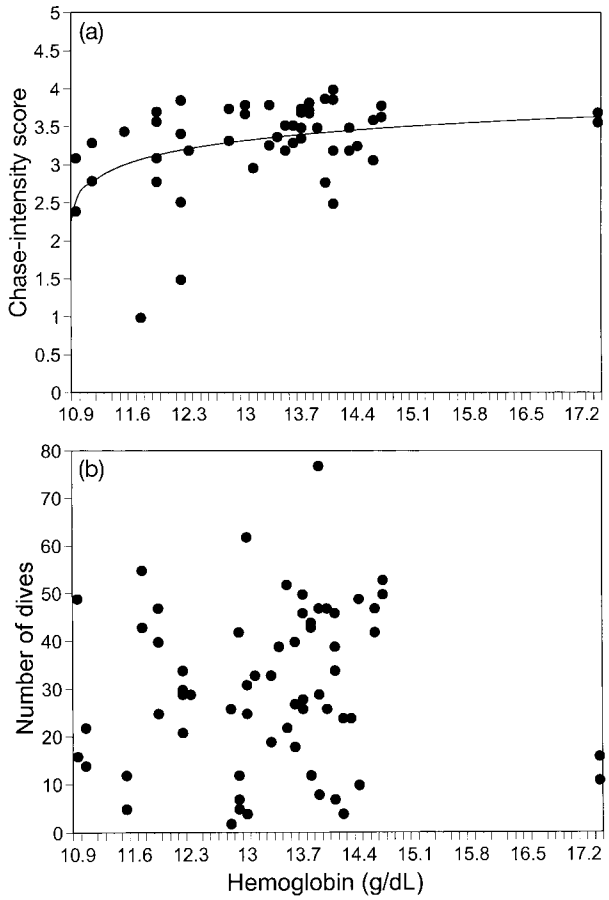


Fig. 7. Relation between hemoglobin levels (g/dL) and mean chase-intensity score (*a*) and number of dives (*b*). The mean chase-intensity score was coded from 1 (no chase) to 4 (high chase-intensity), with $n = 49$ trials. The number of dives had $n = 67$ trials. Chase intensity exponentially decreased with decreasing hemoglobin levels ($P = 0.027$), but no relation was detected between hemoglobin and number of dives ($P = 0.45$).



groups displayed comparable Hb levels was probably due to the effects of oil ingestion on intestinal absorption of hydrocarbons. Ormseth and Ben-David (2000) documented that the high-dose animals excreted much of the ingested hydrocarbons, resulting in similar physiological exposure levels in the two groups.

Changes in aerobic capacity associated with the decline in Hb concentration were apparent during activity both on land and in water for the river otters in the present study. Based on a normal Hb concentration of 15. 8 g/dL blood for wild river otters and extrapolation of the linear fit of the regression model (Fig. 2), the expected oxygen consumption for running at 0.72 $m \cdot s^{-1}$ is 29.3 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$ (from [1]). This compares with values measured in this study: 28.0–33.5 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$ for captive river otters with Hb levels of 14.4 g/dL blood and 40.4 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$ for river otters with the lowest Hb levels (10.9–11.1 g/dL blood). Consequently, the decrease in Hb levels and, presumably, the lower oxygen-carrying capacity of the blood, resulted in a 37.6% increase in the energetic cost of running in the severely anemic river otters. A potential mechanism for the observed elevation in costs may be increased respiratory intake of oxygen when energetic demands are high, to compensate for the reduced circulating oxygen. Because captivity had an effect on Hb levels, it would be difficult to directly assess the effects of oil contamination on wild river otters. Therefore, our discussion is limited to the relation between Hb levels and metabolic costs rather than dealing with the direct effects of oil contamination. Nonetheless, wild oiled sea otters exhibited reductions in Hb levels similar to those observed in our captive river otters (Williams et al. 1995), suggesting that our calculations are within reason.

That this relation between Hb levels and oxygen consumption during running ([1]) explained only 35% of the variability indicates that other factors influenced oxygen consumption in the otters running on the treadmill. Such factors may be associated with the habituation of animals to handlers, or alternatively to other effects of oiling. M. Ben-David, L.K. Duffy, and R.T. Bowyer (see footnote 3) and M. Ben-David, P.W. Snyder, and J.J. Stegeman[4] documented an elevation in the levels of several liver enzymes, a reduc-

[4] M. Ben-David, P.W. Snyder, and J.J. Stegeman. Expression of P450-1A1 in captive oiled and non-oiled river otters: evaluating two methods. Submitted for publication.

tion in white-cell counts, and an elevation in the level of endothelial cytochrome P450-1A associated with oiling in these otters. These biomarker responses may indicate additional physiological damage, which could have affected the exercise performance of these otters independent of the reduction in Hb levels.

An increase in aerobic cost of 37.6% in running oiled otters represents an additional metabolic demand on the naturally elevated energetic costs characteristic of aquatic or semi-aquatic mustelids. Both the resting (Iverson 1972) and active (Dunstone and O'Connor 1979*a*, 1979*b*; Williams 1983, 1989; Stephenson et al. 1988; Pfeiffer and Culik 1998) metabolic rates of many species of mustelids (i.e., minks, river otters, sea otters) are higher than rates predicted for terrestrial mammals. In this study, the cost of transport, defined as the amount of fuel required to transport one unit of body mass over a unit distance (Schmidt-Nielsen 1972), for running river otters with normal Hb levels was 13.6 $J \cdot kg^{-1} \cdot m^{-1}$. This value is nearly 2.7 times the predicted value for minimum transport costs for terrestrial mammals (Taylor et al. 1982). The difference between these values may be attributed, in part, to the unusual gait patterns of semi-aquatic mustelids, as described for running mink (Williams 1983). Alternatively, the otters in the present study may not have been tested at a speed that offered minimum transport cost (T.M Williams, unpublished data). Regardless, the extraordinarily high costs of running for this mammal appear to be exacerbated by the decrease in Hb concentration. As a result, the cost of transport for running river otters with the lowest Hb levels in this study was 18.8 $J \cdot kg^{-1} \cdot m^{-1}$, 3.8 times the predicted level for running terrestrial mammals.

Similar decreases in aerobic capacity were apparent for aquatic activity in our river otters displaying decreased Hb levels. Although a significant difference in number of dives existed between groups and sessions for otters chasing slow fish (Fig. 4), such a difference could not be detected for otters chasing fast fish (Fig. 3). That the number of dives did not differ between sessions and groups for otters chasing fast fish may be attributed to the behavior of the fish. These fish were more visible and may have induced the chase instinct in the otters more readily. Alternatively, the data for otters chasing fast fish are less reliable than those for otters chasing slow fish, because we were forced to use different types of fish in each session (Table 2). Adult pink salmon were used for the pre-oiling session, sable fish for the height of oiling session, and juvenile pink salmon for the rehabilitation period. In addition, we did not have enough juvenile pink salmon to conduct the experiments on all 12 individuals. This greatly reduced our sample size and the power to detect differences, because of missing values.

In contrast with our predictions, dive duration did not decrease, recovery times did not increase, and dive efficiency remained unchanged between sessions and treatment groups. Instead, during the height of oiling, the otters foraging for slow fish apparently corrected for impairment in diving ability by reducing the number of dives (i.e., submergence time) and chase intensity rather than by modifying dive characteristics. This is further supported by the observation that, during this session, only one control animal was reluctant to dive after fish (an animal that performed poorly in all sessions and perished of starvation soon after release; M. Ben-David, unpublished data). In comparison, three of the oiled animals (one low-dose and two high-dose individuals, all of which are currently thriving in the wild) were reluctant to do so (Figs. 3 and 4). Although the number of dives performed by the oiled otters during the height of oiling did not significantly differ from that of the controls, it is important to note that only in the oiled groups did the same individuals significantly alter their behavior between the three sessions, as indicated by the repeated-measures analysis. Nonetheless, number of dives was not directly related to reduction in Hb levels (Fig. 7). Similarly, although chase intensity (or the work performance of the diving otters) was related to Hb levels, this variable accounted for only 19% of the variation, suggesting that other factors, including those associated with oiling, may have affected the otters motivation to dive.

We attempted to evaluate the effects of the reduction in Hb levels on oxygen stores by using a theoretical calculation of aerobic dive limit (ADL). The ADL, which is defined as the maximum breath hold possible without an increase in blood lactate concentration during or after a dive (Kooyman 1989), was likely reduced in these animals as a result of a decreased oxygen-carrying capacity of the blood. For this theoretical consideration, we chose to compare Hb levels of wild free-ranging otters with a value representative of the most severely anemic otter in our experiment. For example, 1.34 mL of oxygen is carried with each gram of Hb in the blood of healthy mammals (Guyton 1986). At capture, Hb content for river otters in this study was 15.8 g Hb/dL blood, which corresponds to an oxygen content of 21.1 mL O_2/100 mL blood (15.8 g Hb/dL blood × 1.34 mL O_2/g Hb). This compares with an oxygen content of only 14.9 mL O_2/100 mL blood for a river otter with a Hb content of 11.1 g Hb/dL blood (Fig. 2). Using the calculations of Kooyman (1989), we find that the total blood oxygen stores of a wild free-ranging river otter is 110 mL of oxygen, while a river otter with low Hb content (i.e., 11.1 g Hb/dL blood) has a blood oxygen store of 68 mL of oxygen.

The effect of these lower blood oxygen stores is a reduction in the total oxygen reserve available to the animal during submergence. When diving, aquatic mammals rely on oxygen stored in the blood, lungs, and muscles to support aerobic metabolic pathways (for a review see Butler and Jones 1997). Calculated oxygen stores for wild river otters, based on Kooyman (1989), are 27.2 mL $O_2 \cdot kg^{-1}$ body mass divided between the lungs (30.2%), blood (40.4%), and skeletal muscle (29.4%). This store is reduced to 23.0 mL $O_2 \cdot kg^{-1}$ body mass for river otters with a Hb content of 11.1 g Hb/dL blood. The ADL of the river otter may be determined by dividing these oxygen stores by the metabolic rate of the submerged animal. In a study by Pfeiffer and Culik (1998), the average metabolic rate of submerged swimming European otters (body mass = 6.0 kg) was 0.51 mL $O_2 \cdot kg^{-1} \cdot s^{-1}$. Because the river otters in the present study were larger (body mass = 11.1 kg), we corrected this metabolic rate for body-size differences, using body mass raised to the 0.75 power (Robbins 1993) and obtained an approximation sufficient for this discussion. This calculation predicts that the ADL would be 54.1 s for river otters with a normal Hb level and 45.7 s for river otters with a Hb level of 11.1 g Hb/dL blood. Such a reduction in the Hb content of the blood could potentially reduce submergence time in a single dive by as much as

8.35 s or 15.4%. While these predictions are based on estimates of average metabolic rates and should be interpreted with caution, they provide a general framework for discussing the effects of decreased Hb levels on the diving ability of river otters.

Interestingly, mean dive duration for our captive river otters was well below the expected ADL even before the exposure to oil, regardless of fish type chased (Figs. 3 and 4). The longest foraging dive recorded was 88 s, which exceeds the aerobic limit we calculated for wild otters, but such long dives were rare (0.3% of 2293 dives performed by 12 individual otters). In addition, only 1.1% of all dives performed in our experiments were longer than the 45.7-s limit we calculated for otters with a Hb level of 11.1 g/dL blood. This suggests that, like other diving mammals, river otters are capable of performing anaerobic dives, although most dives will be within aerobic limits. These data correspond well with lengths of dives observed in a companion study on wild otters in PWS (mean ± SE = 21 ± 1 s, n = 441 dives; M. Ben-David, unpublished data), as well as with those of dives performed by European otters in the Shetland Islands (Kruuk 1995). Similar observations were made for other marine mammals. Kooyman (1989) reported that 90% of all dives performed by free-ranging marine mammals were below the ADL of the species. That total submergence time and chase intensity were the only significant responses observed in this study and the fact that most dives were well below our calculated ADL suggest that the otters altered their behavior to avoid the cumulative depletion of oxygen stores that may result from multiple dives.

Whether wild otters will respond in a manner similar to captive well-fed animals is unclear. Nonetheless, if a reduction in oxygen stores is translated into decreased submergence time within each specific foraging bout, then body condition, survival, and reproduction in coastal river otters may be compromised. For example, the average number of dives for oiled otters chasing slow fish in 30-min trials decreased from 42 to 15. When multiplied by the average dive duration for otters chasing slow fish (15 s), the result is a reduction from 10.5 to 3.75 min in the total time of submergence in a 30-min foraging bout. Thus, if total submergence time dictates the potential rate of capture of fish, this potential may be reduced by nearly 64% for an oiled otter. Kruuk (1995) demonstrated that, in European otters, the amount of time required for fishing increases exponentially with decreasing potential capture rate of prey. Using the model developed by Kruuk (1995) and a potential capture rate of 600 fish in grams per hour for non-oiled otters, we calculated that a 64% decrease in the potential capture rate would result in an increase from an estimated 1.5 to 5.2 h of foraging for oiled otters. This, in turn, would result in additional energetic costs, such as those associated with thermoregulation in water. Kruuk (1995) demonstrated that the exponential relation between the amount of time required for fishing and the potential capture rate of prey is dependent on water temperature, which represents the costs of thermoregulation.

The increase in foraging time (potentially 64%) and the increase in the energetic costs associated with thermoregulation, as well as the potential increase of 37.6% in the energetic costs of terrestrial locomotion (recorded in our treadmill experiment), are likely to cause a significant decrease in body condition in oiled free-ranging river otters. Indeed, Duffy et al. (1993) documented a significant reduction in body mass (controlled for age and sex classes) for otters captured live in oiled areas in PWS. Furthermore, the constraints imposed on diving behavior of otters by oiling will likely alter the diet of otters. We would expect otters to concentrate on prey that are easy to capture and have high capture rate potential. Bowyer et al. (1994) documented that changes in the diet of otters from oiled shores resulted mostly from a reduction in prey species. Between 1989 and 1990, perciform fish (sand lances, gunnels, and ronquils) declined in the diets of otters in oiled areas, whereas these groups increased in the diets of otters from non-oiled sites during the same period. Conversely, Malacostraca (crustaceans) increased in the diet of otters from an oiled area but declined in the diet of those from a non-oiled area (Bowyer et al. 1994; see footnote 2). Yet, surveys of intertidal and subtidal organisms in PWS suggested that species composition and biomass did not differ between oiled and non-oiled areas (Thomas A. Dean, Coastal Resources Associates, Inc., personal communication). This suggests that these diet differences were a result of changes in the foraging strategies of otters inhabiting oiled areas rather than from differences in prey availability.

To our knowledge, ours is the first study to document a relation between hematological changes due to oiling and an increase in energetic costs for wild animals under controlled conditions. We were able to establish that even low doses of weathered oil caused a significant reduction in the aerobic capacity of river otters through anemia. This reduction, in turn, led to an increase in the energetic costs of terrestrial locomotion, a potential decrease in ADL, and a potential increase in foraging time, owing to a decrease in the total time of submergence during each foraging bout. These responses to oiling could have major implications for the maintenance of body condition, survival, and reproduction in coastal river otters, as well as in other diving mammals and birds. In addition, our findings highlight the controversy associated with rehabilitation of oiled wildlife. Professionals should consider the relations between hematological profiles, aerobic capacity, and foraging ability, to increase the likelihood of post-release survival of their subjects. Although recovery from anemia by oiled animals may require several months (Williams et al. 1995), detaining animals in captivity for long periods can be detrimental to their subsequent survival (M. Ben-David, unpublished data). Further studies investigating the potential effects of reduction in Hb levels on the aerobic capacity of diving animals would greatly enhance our understanding of the long-term effects of exposure to oil, and may assist professionals in deciding on the course of action to be taken following future oil spills.

Acknowledgements

The assistance of S. Noren and M. Rutishauser was vital to the success of the treadmill experiments. A.K. Norin, C. Smith, M. Thompson, and S. Trillhose assisted in all aspects of the project. We thank all ASLC employees, interns, and volunteers, in particular, S. Inglis, P. Tuomi (Doctor of Veteri-

nary Medicine), M. Gray, and J. Moreland. Live fish were obtained with the help of many volunteers, H. Golden (Alaska Department of Fish and Game), the crew of the *F/V Florette C*, R. Cabana, and the employees of the Trail Lakes Hatchery. G.M. Blundell, K. Beckett, B. Kellog, and C. Taylor helped during the blood-collection sessions. D. Naber spent long hours conducting the frame by frame analyses, and M.N. Rosing and E. Debevec provided assistance with statistical analyses. We are indebted to R.T. Bowyer, H. Kruuk, M.A. Castellini, and R.W. Davis for thoughtful discussions that improved this study. This project was supported by the EVOS Trustees Council (project No. 348) and the California Department of Fish and Game's Oil Spill Response Trust Fund through the Oiled Wildlife Care Network at the University of California, Davis (UOA97119). All methods used in this research were approved by the Institutional Animal Care and Use Committees at the University of Alaska Fairbanks and ASLC (Nos. 97-14 and 98-002, respectively); trapping permits were issued by the Alaska Department of Fish and Game (98-001). All procedures adhere to the guidelines for animal care and use adopted by the American Society of Mammalogists (Animal Care and Use Committee 1998).

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