*Exxon Valdez* Oil Spill Restoration Project Final Report

Monitoring, Habitat Use, and Trophic Interactions of Harbor Seals in Prince William Sound, Alaska

> Restoration Project 01064 Final Report

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August 2005

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**Study History:** Restoration Project 01064 began in 1993 as a continuation of the initial post-spill study effort conducted as Marine Mammal Study Number 5 (Assessment of Injury to Harbor Seals in Prince William Sound, Alaska, and Adjacent Areas) in 1989 through 1991 and reclassified as Restoration Study Number 73 (Harbor Seal Restoration Study) in 1992. A final report was issued in 1994 for the combined Marine Mammal Study Number 5 and Restoration Study Number 73, entitled <u>Assessment of Injury to Harbor Seals in Prince William Sound, Alaska, and Adjacent Areas Following the *Exxon Valdez* Oil Spill. Subsequently, annual reports were submitted entitled <u>Habitat Use, Behavior, and Monitoring of Harbor Seals in Prince William Sound: 1994 Annual Report, 1995 Annual Report, 1996 Annual Report, 1997 Annual Report, 1998 Annual Report, and 1999 Annual Report . Fatty acid studies funded under Restoration Project 94320-F (Trophic Interactions of Harbor Seals in Prince William Sound) were included in the 1994 annual report for 94064. Fatty acid studies were continued and reported as part of this study for the duration of the project.</u></u>

**The following in print, in press or submitted manuscripts resulted directly from this project:** Frost, K. F, L. F. Lowry and J. M. Ver Hoef. 1999. *Marine Mammal Science* 15: 494-506; Frost, K. J., M. A. Simpkins and L. F. Lowry. 2001. *Marine Mammal Science* 17:813-834; Frost, K. J., M. A. Simpkins, R. J. Small and L. F. Lowry. *Marine Mammal Science* (submitted, under revision); Hastings, K. K., K. J. Frost, M. A. Simpkins, G.W. Pendleton, U. G. Swain and R. J. Small. 2004. *Canadian Journal of Zoology* 82:1755-1773; Iverson, S. J., K. J. Frost and L. F. Lowry. 1997. *Marine Ecology Progress Series* 151: 255-271; Iverson, S. J., S. L. C. Lang and M. H. Cooper. 2001. *Lipids* 36:1283-1287; Iverson, S. J., K. J. Frost and S. L. C. Lang, 2002. *Marine Ecology Progress Series* 241:161-181; Iverson, S. J., C. Field, W. D. Bowen and W. Blanchard. 2004. *Ecological Monographs* 74: 211-235; Lowry, L. F., K. J. Frost, J. M. Ver Hoef and R. A. DeLong. 2001. *Marine Mammal Science* 17:835-861; Small, R. J., L. F. Lowry, J. M. Ver Hoef, K. J. Frost, R. A. DeLong, and M. J. Rehberg. 2005. *Marine Mammal Science* 21:671-694; and Ver Hoef, J. M. and K. J. Frost. 2003. *Environmental and Ecological Statistics* 10:201-209.

**Other in print, in press or submitted manuscripts using data and/or samples provided by this project include:** Adkison, M. D., T. J. Quinn II and R. J. Small. 2003. *Marine Mammal Science* 19:764-790; Boveng, P. L., J. L. Bengtson, D. E. Withrow, J. C. Cesarone, M. A. Simpkins, K. J. Frost and J. J. Burns. 2003. *Marine Mammal Science* 19:111-127; Burns, J.M., D.P. Costa, K. J. Frost and J.T. Harvey. 2005. *Physiological and Biochemical Zoology* (in press); Gotthardt, T. 2001. M.Sc. thesis, University of Alaska, Anchorage, Alaska; O'Corry-Crowe, G.M. and R.L. Westlake. 1997. *In* A.E. Dizon, S.J. Chivers and W.F. Perrin, eds. Molecular Genetics of Marine Mammals. *The Society of Marine Mammalogy Special Publication* 3:291-30; O'Corry-Crowe, G.M., K.K. Martien and B.L. Taylor. *Marine Mammal Science* (Submitted May 2005); Simpkins, M. A., K. L. Laidre and P. J. Heagerty. 2005. *Marine Mammal Science* 21: 243-259; Westlake, R.L. and G. M. O'Corry-Crowe. 2002. *Journal of Mammalogy* 83:1111– 1126; Zarnke, R. L., T. C. Harder, H. W. Vos, J. M. Ver Hoef and A. D. Osterhaus. 1997. *Journal of Wildlife Diseases* 33:459-465; and Zarnke, R. L., J. T. Saliki, A. P. Macmillan, S. D. Brew, C. E. Dawson, J. M. Ver Hoef and R. J. Small. 2005. *Journal of Wildlife Diseases* (submitted, under revision).

<u>Abstract:</u> This project investigated population status, habitat use and trophic interactions of harbor seals in Prince William Sound (PWS), Alaska. Aerial counts to monitor population trend indicated a 3.3% annual decline during 1990-1999. Counts were 20% lower in 1999 than in 1990, and >50% lower than in 1988. We sampled 390 seals during 1992-2000 and satellite-tagged 47 non-pups and 27 pups. Most tagged seals remained within PWS. Locations were almost all in water < 200 m deep. Subadults moved more, had larger home ranges, spent more time diving, dived more often, displayed a stronger diurnal

pattern, and dived deeper than adults. Harbor seal pups substantially increased their ability to dive during the first few months after weaning. Pups spent most of their time wet swimming at shallower than 30% of their maximum depth. Isotope dilution analyses of body composition indicated that PWS pups were consistently heavy and fat compared to other harbor seal populations. Our results suggest that lactating females were not nutritionally compromised, given the robust body condition of weaned pups. Body condition in combination with dive behavior suggest that food availability was not likely a major factor in the population decline in PWS during the period of this study.

**Key Words:** diet, diving behavior, *Exxon Valdez* oil spill, fatty acid signature analysis, habitat use, harbor seal, movements, *Phoca vitulina*, population monitoring, Prince William Sound, satellite telemetry.

**Project Data:** *Description of data* – Aerial survey count data for 1989-2000; morphometric measurements of all seals that were caught and handled; location and dive data for 78 seals that were satellite tagged during 1992-1999; results of disease assays conducted on harbor seal blood serum; and results of fatty acid signature analysis. *Format* – All data exist as computer databases, either as FoxPro, Excel, or text files. *Custodian* – All aerial survey, morphometric, and satellite tag data are maintained at the Alaska Department of Fish and Game, Division of Wildlife Conservation, P.O. Box 240020, Douglas, AK 99824-0020. Phone (907) 465-4345. Fax (907) 465-4272. E-mail:

gail\_blundell@fishgame.state.ak.us. Disease assay data are located at the Alaska Department of Fish and Game, Division of Wildlife Conservation, 1300 College Road, Fairbanks, AK 99701. Phone (907) 459-7257. Fax (907) 452-6410. E-mail: kimberlee\_beckmen@fishgame.state.ak.us. Fatty acids data are maintained by Dr. Sara Iverson at Dalhousie University, Department of Biology, Halifax, Nova Scotia B3H4J1. Phone (902) 494-2566. Fax (902) 494-3736. E-mail: Sara.Iverson@dal.ca.

#### **<u>Citation</u>:**

Frost, K.J., L.F. Lowry, J.M. Ver Hoef, S.J. Iverson, and M.A. Simpkins. 2005. Monitoring, habitat use, and trophic interactions of harbor seals in Prince William Sound, Alaska. *Exxon Valdez* Oil Spill Restoration Project Final Report (Restoration Project 01064), Alaska Department of Fish and Game, Habitat and Restoration Division, Anchorage, Alaska.

#### *Exxon Valdez* Oil Spill Restoration Project 01064 Final Report

#### Monitoring, Habitat Use, and Trophic Interactions of Harbor Seals in Prince William Sound, Alaska K. J. Frost, L. F. Lowry, J. M. Ver Hoef, S. J. Iverson and M. A. Simpkins.

#### <u>Abstracts for published, in press or submitted manuscripts</u> (PDFs for some of the papers are included at the end of this list):

# 1. Adkison, M. D., T. J. Quinn II, and R. J. Small. 2003. Evaluation of the Alaska harbor seal population (*Phoca vitulina*) population survey: a simulation study. *Marine Mammal Science* 19: 764-90.

Abstract: We used simulation to investigate robust designs and analyses for detecting trends from population surveys of Alaska harbor seals. We employed an operating model approach, creating simulated harbor seal population dynamics and haul-out behavior that incorporated factors thought to potentially affect the performance of aerial surveys. The factors included the number of years, the number of haul-out sites in an area, the number and timing of surveys within a year, known and unknown covariates affecting haul-out behavior, substrate effects, movement among substrates, and variability in survey and population parameters. We found estimates of population trend were robust to the majority of potentially confounding factors, and that adjusting counts for the effects of covariates was both possible and beneficial. The use of mean or maximum counts by site without covariate correction can lead to a substantial bias and low power in trend determination. For covariate-corrected trend estimates, there was minimal bias and loss of accuracy was negligible when surveys were conducted 20 d before or after peak haul-out attendance, survey date became progressively earlier across years, and peak attendance fluctuated across years. Trend estimates were severely biased when the effect of an unknown covariate resulted in a long-term trend in the fraction of the population hauled out. A key factor governing the robustness and power of harbor seal population surveys is intersite variability in trend. This factor is well understood for sites within the Prince William Sound and Kodiak trend routes for which at least 10 consecutive annual surveys have been conducted, but additional annual counts are needed for other areas. The operating model approach proved to be an effective means of evaluating these surveys and should be used to evaluate other marine mammal survey designs.

#### Boveng, P. L., J. L. Bengtson, D. E. Withrow, J. C. Cesarone, M. A. Simpkins, K. J. Frost, and J. J. Burns. 2003. The abundance of harbor seals in the Gulf of Alaska. *Marine Mammal Science* 19: 111-27.

Abstract: The abundance of harbor seals (*Phoca vitulina richardi*) has declined in recent decades at several Alaska locations. The causes of these declines are unknown, but there is concern about the status of the populations, especially in the Gulf of Alaska. To assess the status of harbor seals in the Gulf of Alaska, we conducted aerial surveys of seals on their haul-out sites in August - September 1996. Many factors influence the propensity of seals to haul out, including tides, weather, time of day, and time of year. Because these "covariates" cannot simultaneously be controlled through survey design, we used a regression model to adjust the counts to an estimate of the number of seals that would have been ashore during a hypothetical survey conducted under ideal conditions for hauling out. The regression, a generalized additive model, not only provided an adjustment for the covariates, but also confirmed the nature and shape of the covariate effects on haul-out

behavior. The number of seals hauled out was greatest at the beginning of the surveys (mid-August). There was a broad daily peak from about 11001400 local solar time. The greatest numbers were hauled out at low tide on terrestrial sites. Tidal state made little difference in the numbers hauled out on glacial ice, where the area available to seals did not fluctuate with the tide. Adjusting the survey counts to the ideal state for each covariate produced an estimate of 30,035 seals, about 1.8 times the total of the unadjusted counts (16,355 seals). To the adjusted count, we applied a correction factor of 1.198 from a separate study of two haul-out sites elsewhere in Alaska, to produce a total abundance estimate of 35,981 (SE 1,833). This estimate accounts both for the effect of covariates on survey counts and for the proportion of seals that remained in the water even under ideal conditions for hauling out.

# 3. Burns, J. M., D. P. Costa, K. J. Frost, and J. T. Harvey. 2005. Development of body oxygen stores in harbor seals: effects of age, mass, and body composition. *Physiological and Biochemical Zoology*.

Abstract: Harbor seal pups are highly precocial and can swim and dive at birth. Such behavioral maturity suggests that they may be born with mature body oxygen stores, or that stores develop quickly during the nursing period. To test this hypothesis, we compared the blood and muscle oxygen stores of harbor seal pups, yearlings, and adults. We found that pups had lower oxygen stores than adults (neonates 57%, weaned pups 75%, and yearlings 90% those of adults), largely because neonatal myoglobin concentrations were low (1.6  $\pm$  0.2 g% vs. 3.8  $\pm$  0.3 g% for adults), and changed little during the nursing period. In contrast, blood oxygen stores were relatively mature, with nursing pups having hematocrit (55  $\pm$  0.2%), hemoglobin (21.7  $\pm$  0.4 g%), and blood volumes (12.3  $\pm$  0.5 ml/kg) only slightly lower than adults (57  $\pm$  0.2%, 23.8  $\pm$  0.3 g%, and 15.0  $\pm$  0.5 ml/kg). As neonatal pups had relatively high metabolic rates (11.0 ml O<sub>2</sub>/kg·min) their calculated aerobic dive limit was less than 50% that of adults. These results suggest that harbor seals' early aquatic activity is primarily supported by rapid development of blood, with immature muscle oxygen stores and elevated use rates limiting aerobic diving ability.

#### 4. Frost, K. J., L. F. Lowry, and J. Ver Hoef. 1999. Monitoring the trend of harbor seals in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. *Marine Mammal Science* 15, no. 2: 494-506.

Abstract: We used aerial counts to monitor the trend in numbers of harbor seals, Phoca vitulina richardsi, in Prince William Sound, Alaska, following the 1989 Exxon Valdez oil spill. Repetitive counts were made at 25 haul-out sites during the annual molt period each year from 1990 through 1997. A generalized linear model indicated that time of day, date, and time relative to low tide significantly affected seal counts. When Poisson regression was ' used to adjust counts to a standardized set of survey conditions, results showed a highly significant decline of 4.6% per year. Unadjusted counts indicated a slight, but not statistically significant, decline in the number of seals. The number of harbor seals on the trend-count route in eastern and central PWS has been declining since at least 1984, with an overall population reduction of 63% through 1997.

Programs to monitor long-term changes in animal population sizes should account for factors that can cause short-term variations in indices of abundance. The inclusion of such factors as covariates in models can improve the accuracy of monitoring programs.

5. Frost, K. J., M. A. Simpkins, and L. F. Lowry. Submitted. Development of diving by harbor seal pups in two regions in Alaska: use of the water column. *Marine Mammal Science*. Abstract: Satellite-linked dive recorders were attached to 53 harbor seal pups in Prince William Sound (PWS) and at Tugidak Island, Alaska, during 1997-1999. We used generalized additive models and bootstrap techniques to describe pup diving behavior during their first year of life. Pups increased their ability to dive during the first few months, as indicated by increases in proportion of time wet and max-depth values. Timewet and/or max-depth later decreased, suggesting a seasonal component to diving behavior. Monthly time-wet varied from an overall minimum of 0.68 to a maximum of 0.89. Pups spent most of their time wet swimming at shallower than 30% of their max-depth, and < 5% of their time deeper than 70% of their max-depth. Average max-depths and deepest actual dives were similar for PWS and Tugidak pups (max-depth 50-100 m *vs.* 40-110 m; actual deepest dive 294 m *vs.* 308 m). PWS pups dove deeper sooner and spent less time wet than Tugidak pups during the first few months after tagging, probably as a result of regional bathymetric differences. Dive behavior and body condition suggest that food availability was not likely a major factor in the population decline in PWS during the period of this study.

6.

### -. 2001. Diving behavior of subadult and adult harbor seals in Prince William Sound, Alaska. *Marine Mammal Science* 17, no. 4: 813-34.

Abstract: Satellite-linked depth recorders (SDRs) were attached to 47 harbor seals in Prince William Sound, Alaska, during 1992–1996. Parameters describing diving effort, diving focus, and focal depth (depth bin to which diving was focused) were calculated from binned data on maximum dive depth and time spent at depth, and analyzed using repeatedmeasures mixed models. This analysis method accounted for individual variability, temporal autocorrelation, and the binned nature of SDR data, which are often ignored using standard statistical techniques. Results indicated that diving effort remained steady from September to April, when seals spent 68%–75% of their overall time in the water. Time spent in the water declined to 60% in May and to about 40% in July. Seals spent the most time in the water at night and the least in the morning. The diving of all seals in all months was highly focused. Overall, diving was focused to one depth bin approximately 75% of the time. Diving was more focused for females than for males and subadults. Focal depth and diving focus varied by region. Collinearity between month and region in the focal depth model suggested that seals move in winter to regions where prev are found deeper in the water column. Variations in diving behavior presumably result from combinations of regional bathymetry, seasonal cycles in type or depth distribution of prey, and seal lifecycle events such as reproduction and molting.

### 7. Gotthardt, T. 2001. "The foraging ecology of harbor seals in southcentral Prince William Sound, Alaska: 1994-1997." M.S. thesis, University of Alaska Anchorage.

Abstract: Fourteen harbor seals (*Phoca vitulina richardsi*) from southcentral Prince William Sound (PWS), Alaska, were outfitted with satellite-linked time depth recorders (SDRs) to monitor their movements and diving behavior. I subsequently examined available information on forage fish abundance, composition and distribution to evaluate whether the distribution and diving behaviors of seals corresponded to the seasonal and temporal distribution of their prey. A wide array of forage fishes were seasonally available to PWS harbor seals. Seasonal differences were apparent in depth of dives and distances moved by seals to foraging areas. It is likely that the two were inter-related, as the distant areas used by seals were also the deepest. Seasonal differences in diving depths and localities were likely due to seasonal changes in prey availability. Seals dove deeper and increased foraging ranges in winter, suggesting prey availability in winter may be greatly reduced compared to spring or summer.

#### 8. Hastings, K. K., K. J. Frost, A. Simpkins, G. W. Pendleton, U. G. Swain, and R. J. Small. 2004. Regional differences in diving behavior of harbor seals in the Gulf of Alaska. *Canadian Journal of Zoology* 82: 1755-73.

Abstract: Adult and subadult harbor seals (*Phoca vitulina richardi* (Gray, 1864); n = 108) from Southeast Alaska (SE), Kodiak Island (KO), and Prince William Sound (PWS) were instrumented with satellite data recorders to examine dive parameters for harbor seals in the Gulf of Alaska at regional and annual scales. Most dives (40%–80%) were <20 m in depth and <4 min in duration; however, dives from 50 to 150 m depth were not uncommon and dives to 508 m were recorded. PWS seals spent less time in the water during the prebreeding and breeding seasons than SE and KO seals. SE seals used a greater diversity of depths than KO and PWS seals. Only seals in PWS and SE (i) dived deeper and longer and spent more time diving in winter than during spring and summer and (ii) dived deepest during the day only in winter. Seals in all regions and seasons dived most frequently and spent the most time diving at night. Subadult seals spent more time diving, dived more often, displayed a stronger diurnal pattern with deepest dives during the day in the winter, and dived deeper than adults.

# 9. Iverson, S. J., C. Field, W. D. Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: a new method of estimating predator diets. *Ecological Monographs* 74, no. 2: 211-35.

Abstract: Accurate estimates of the diets of predators are required in many areas of ecology, but for many species current methods are imprecise, limited to the last meal, and often biased. The diversity of fatty acids and their patterns in organisms, coupled with the narrow limitations on their biosynthesis, properties of digestion in monogastric animals, and the prevalence of large storage reservoirs of lipid in many predators, led us to propose the use of quantitative fatty acid signature analysis (OFASA) to study predator diets. We present a statistical model that provides quantitative estimates of the proportions of prey species in the diets of individual predators using fatty acid signatures. We conducted simulation studies using a database of 28 prey species (n = 954 individuals) from the Scotian Shelf off eastern Canada to investigate properties of the model and to evaluate the reliability with which prev could be distinguished in the model. We then conducted experiments on grey seals (*Halichoerus grypus*, n = 25) and harp seals (*Phoca*) groenlandica, n = 5) to assess quantitative characteristics of fatty acid deposition and to develop calibration coefficients for individual fatty acids to account for predator lipid metabolism. We then tested the model and calibration coefficients by estimating the diets of experimentally fed captive grey seals (n = 6, switched from herring to a mackerel/capelin diet) and mink kits (Mustela vison, n = 46, switched from milk to one of three oilsupplemented diets). The diets of all experimentally fed animals were generally well estimated using QFASA and were consistent with qualitative and quantitative expectations, provided that appropriate calibration coefficients were used. In a final case, we compared video data of foraging by individual freeranging harbor seals (*Phoca vitulina*, n = 23) fitted with Crittercams and QFASA estimates of the diet of those same seals using a complex ecosystem-wide prey database. Among the 28 prey species in the database, QFASA estimated sandlance to be the dominant prey species in the diet of all seals (averaging 62%) of diet), followed primarily by flounders, but also capelin and minor amounts of other species, although there was also considerable individual variability among seals. These estimates were consistent with video data showing sandlance to be the predominant prey, followed by flatfish. We conclude that OFASA provides estimates of diets for individuals at time scales that are relevant to the ecological processes affecting survival, and can be used to study diet variability within individuals

over time, which will provide important opportunities rarely possible with other indirect

methods. We propose that the QFASA model we have set forth will be applicable to a wide range of predators and ecosystems.

10. Iverson, S. J., K. J. Frost, and S. L. C. Lang. 2002. Fat content and fatty acid composition of forage fish and invertebrates in Prince William Sound, Alaska: factors contributing to among and within species variability. Marine Ecology Progress Series 241: 161-81. Abstract: We determined the fat content and fatty acid composition of 26 species of fish and invertebrates (n = 1153) that are primary forage species of piscivorous seabirds and marine mammals in Prince William Sound (PWS), Alaska. Flatfish, shrimps and octopus had the lowest average fat contents (~1.0%), although some cods, as well as juvenile walleye pollock Theragra chalcogramma, Pacific herring Clupea harengus pallasi and pink salmon Oncorhynchus gorbuscha also ranged as low as 0.5 to 0.7% fat. The highest fat contents were found in eulachon Thaleichthys pacificus (25%), adult herring (21%) and the squid Berrytheuthis magister (5 to 13%). Within species, fat content varied mostly with season, but also with size. Fatty acid signatures generally distinguished forage species, with up to 95% of individuals correctly classified using either discriminant or classification and regression tree (CART) analyses. Discriminant plots provided insight into the relationships between fatty acid signatures of different species. Species with similar life histories and diets clustered closer together, while those with the greatest differences in ecology differed most in their fatty acid patterns. Within some species, changes in fatty acid signatures were apparent with increasing size and were consistent with known dietary shifts reported from stomach contents analyses. Furthermore, fatty acid signatures of Age 0 (vr) pollock and herring in PWS were consistent with previous stomach contents analysis that indicated annual differences in the timing of dietary changes from eating zooplankton to piscivory. Overall, when size/age classes were taken into account, species classification using fatty acid signatures was improved. Our findings have important implications for evaluating diets and food web interactions of fish stocks, as well as at higher trophic levels. Despite individual variation within species, our results indicate that fatty acid signatures accurately characterize forage species in this ecosystem, and consequently can be used to study and perhaps estimate the species composition of diets of their predators.

# 11. Iverson, S. J., K. J. Frost, and L. F. Lowry. 1997. Fatty acid signatures reveal fine scale structure of foraging distribution of harbor seals and their prey in Prince William Sound, Alaska. *Marine Ecology Progress Series* 151: 255-71.

Abstract: Fatty acid signature analysis was used to investigate the diet and the spatial scales of foraging in harbor seats Phoca vitulina richardsi in Prince William Sound (PWS) and elsewhere in the Gulf of Alaska. Blubber samples collected in 1994 and 1995 from 104 harbor seals from PWS, Kodiak Island, and southeast Alaska were analyzed for fatty acid composition. A total of 163 potential prey samples representing 10 taxa were collected and individually analyzed for total fat content and fatty acid composition. Approximately 70 fatty acids and isomers were found in both harbor seals and their prey. Classification and regression tree analysis was used to classify seals and prey according to their fatty acid signatures. Large differences were found in the fatty acid composition of blubber from seals sampled at Kodiak, southeast Alaska and PWS, over a broad geographical scale of 400 to 800 km, additionally, fatty acid signatures distinguished seals from different regions within PWS, as well as on finescale resolutions of specific haulout sites within 9 to 15 km of one another. These findings suggest that seals forage site - specifically. These conclusions are supported by prey fatty acid patterns, which also differed on similarly small spatial scales within PWS. Not only could prey species such as herring Clupea pallasi and pollock Theragra chalcogramma be differentiated from one another using fatty acid signatures, but they could also be distinguished by size-class and location within PWS,

reflecting differences in diet with age and as well as with fine-scale habitat. Results from this study are consistent with both satellite data from tagged harbor seals and stomach content analyses of forage fish species in PWS. Although preliminary, analyses suggest that large herring and pollock, as well as flatfish, may have dominated the diet of seals in southern PWS, whereas diets of seals in northern and eastern PWS may have been comprised more of small size classes of herring and pollock, and perhaps other items such as cephalopods, sandlance *Ammodytes hexapterus*, cod *Gadus macrocephalus*, and shrimp. We conclude that fatty acid signature analysis will be an important contribution to understanding marine food webs in estuarine and other marine environments

#### 12. Iverson, S. J., S. L. C. Lang, and M. H. Cooper. 2001. Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. *Lipids* 36, no. 11.

Abstract: For many studies, it is important to measure the total lipid content of biological samples accurately. The Bligh and Dyer method of extraction was developed as a rapid but effective method for determining total lipid content in fish muscle. However, it is also widely used in studies measuring total lipid content of whole fish and other tissues. Although some investigators may have used modified Bligh and Dyer procedures, rarely have modifications been specified nor has their effectiveness been quantitatively evaluated. Thus, we compared this method with that of the classic Folch extraction in determining total lipid content of fish samples ranging from 0.5 to 26.6% lipid. We performed both methods as originally specified; i.e., using the chloroform/methanol/water ratios of 1:2:0.8 and 2:2:1.8 (before and after dilution, respectively) for Bligh and Dyer and of 8:4:3 for Folch, and with the initial solvent/sample ratios of (3+1):1 (Bligh and Dyer) and 20:1 (Folch). We also compared these with several other solvent/sample ratios. In samples containing <2% lipid, the results of the two methods did not differ. However, for samples containing >2% lipid, the Bligh and Dyer method produced significantly lower estimates of lipid content, and this underestimation increased significantly with increasing lipid content of the sample. In the highest lipid samples, lipid content was underestimated by up to 50% using the Bligh and Dyer method. However, we found a highly significant linear relationship between the two methods, which will permit the correction of reported lipid levels in samples previously analyzed using an unmodified Bligh and Dyer extraction. In the future, modifications to procedures and solvent/sample ratios should be described.

#### 13. Lowry, L. F., K. J. Frost, J. M. Ver Hoef, and R. A. DeLong. 2001. Movements of satellitetagged subadult and adult harbor seals in Prince William Sound, Alaska. *Marine Mammal Science* 17, no. 4: 835-61.

Abstract: Satellite-linked tags were attached to 49 subadult and adult harbor seals captured in Prince William Sound (PWS), Alaska, and their movements were monitored during 1992-1997. Seals were tracked for a total of 5,517 seal-days and were located on about 80% of the days that tags transmitted. Most locations were in or near PWS, but some juvenile seals moved 300-500 km east and west into the Gulf of Alaska. While several seals traveled to 50-100 km offshore, virtually all locations were in water <200 m deep. Overall, juvenile seals moved more than adults and had larger home ranges. Movements were significantly affected by month, and age by month and sex by month interactions. In all months, mean distances between successively used haulouts were <10 km for adults and <20 km for juveniles. Mean monthly home ranges varied from <100 km2 to >1,500 km2, and were smallest during June-July. Mean haul-out to at-sea distance was 5-10 km for adults and generally 10-25 km for juveniles. Satellite-linked tags provided an effective means of monitoring and describing the full range of harbor seal movements in this region, with the exception of late summer when tags were shed during the molt.

# 14. O'Corry-Crowe, G. M., and R. L. Westlake. 1997. Molecular investigations of spotted seals (*Phoca largha*) and harbor seals (*P. vitulina*), and their relationship in areas of sympatry. In *Molecular Genetics of Marine Mammals*. Editors A. E. Dizon, S. J. Chivers, and W. F. Perrin, 291-330. Vol. 3. Society of Marine Mammalogy (Special Publication).

Abstract: The phylogenetic systematics of spotted and harbor seals (genus *Phoca*) and their relationship to other phocid seal species have not been satisfactorily resolved. Analysis of the mitochondrial DNA control region and adjacent proline transfer RNA gene supports the contention that populations of both forms constitute phylogenetically distinct clades, which can therefore constitute monophyletic species: *Phoca largha* and *Phoca vitulina* Linnaeus, 1758. Atlantic and Pacific harbor seals are phylogeographically distinguishable. Within the Pacific, however, samples corresponding to subspecies *P. v. stejnegeri* and *P. v. richardsi* do not occur as genetically distinct clades. Subspecies of spotted seals are likewise not genetically discernable across the geographic range studied. A single individual, identified as a harbor seal on the basis of gross morphology, location, and season of capture, possessed an mtDNA haplotype characteristic of spotted seals. This may be the result of misidentification, ancestral polymorphism leading to paraphyly, or hybridization between a female spotted seal and male harbor seal. The implications of hybridization for definitions of "species" and "subspecies," and concepts of appropriate units for management are briefly discussed.

#### 15. Simpkins, M. A., K. L. Laidre, and P. J. Heagerty. 2005. Multivariate regression of satellitelinked dive recorder data: simultaneous analysis of all bins. *Marine Mammal Science* 21: 243-59.

Abstract: Statistical analysis of diving behavior data collected from satellite-linked dive recorders (SDRs) can be challenging because: (1) the data are binned into several depth and time categories, (2) the data from individual animals are often temporally autocorrelated, (3) random variation between individuals is common, and (4) the number of dives can be correlated among depth bins. Previous analyses often have ignored one or more of these statistical issues. In addition, previous SDR studies have focused on univariate analyses of index variables, rather than multivariate analyses of data from all depth bins. We describe multivariate analysis of SDR data using generalized estimating equations (GEE) and demonstrate the method using SDR data from harbor seals (*Phoca vitulina*) monitored in Prince William Sound, Alaska between 1992 and 1997. Multivariate regression provides greater opportunities for scientific inference than univariate methods, particularly in terms of depth resolution. In addition, empirical variance estimation makes GEE models somewhat easier to implement than other techniques that explicitly model all of the relevant components of variance. However, valid use of empirical variance estimation requires an adequate sample size of individual animals.

#### 16. Small, R. J., L. F. Lowry, J. M. Ver Hoef, K. J. Frost, A. DeLong, and M. J. Rehberg. 2005. Differential movements by harbor seal pups in contrasting Alaska environments. *Marine Mammal Science* 21: 671-94.

Abstract: Movement patterns of Alaska harbor seal pups were studied using satellite telemetry during 1997-2000. Mean tracking duration was 277.3 d (SD = 105.8) for Tugidak Island pups (n = 26) and 171.2 d (108.3) for Prince William Sound (PWS) pups (n = 27). Movements were similar for males and females and were largely restricted to the continental shelf. Multiple return trips of >75 km from the natal area and up to ~3 weeks duration were most common, followed by movements restricted to <25 km from the natal area; one way movements from the natal site were rare. Distances moved and home range sizes remained relatively stable or increased gradually from July through winter, then

decreased markedly through spring. Monthly movements (maximum distance from tagging location, mean distance from haulouts to at-sea locations, and home range size) were significantly greater for Tugidak vs. PWS pups. Six of 7 pups from each region that traveled furthest and were tracked the longest had returned to their tagging site when their last location was recorded, indicating philopatry or limited dispersal during their first year of life. Seal pups exhibited similar movement patterns in the distinct habitats of the two regions but differed in the spatial extent of their movements.

#### 17. Ver Hoef, J. M., and K. J. Frost. 2003. A Bayesian hierarchical model for monitoring harbor seal changes in Prince William Sound, Alaska. *Environmental and Ecological Statistics* 10: 201-9.

Abstract: Bayesian hierarchical models were used to assess trends of harbor seals, *Phoca* vitulina richardsi, in Prince William Sound, Alaska, following the 1989 Exxon Valdez oil spill. Data consisted of 4-10 replicate observations per year at 25 sites over 10 years. We had multiple objectives, including estimating the effects of covariates on seal counts, and estimating trend and abundance, both per site and overall. We considered a Bayesian hierarchical model to meet our objectives. The model consists of a Poisson regression model for each site. For each observation the logarithm of the mean of the Poisson distribution was a linear model with the following factors: ( I ) intercept for each site and year, (2) time of year, (3) time of day, (4) time relative to low tide, and (5) tide height. The intercept for each site was then given a linear trend model for year. As part of the hierarchical model, parameters for each site were given a prior distribution to summarize overall effects. Results showed that at most sites, (1) trend is down; counts decreased yearly, (2) counts decrease throughout August, (3) counts decrease throughout the day, (4) counts are at a maximum very near to low tide, and (5) counts decrease as the height of the low tide increases; however, there was considerable variation among sites. To get overall trend we used a weighted average of the trend at each site, where the weights depended on the overall abundance of a site. Results indicate a 3.3% decrease per year over the time period.

#### Westlake, R. L., and G. M. O'Corry-Crowe. 2002. Macrogeographic structure and patterns of genetic diversity in harbor seals (*Phoca vitulina*) from Alaska to Japan. *Journal of Mammalogy* 83: 1111-26.

Abstract: We examined sequence variation in the control region of the mitochondrial genome from 778 seals sampled at 161 locations from northern Japan to southeastern Alaska to learn more about the evolutionary history and population structure of, and effects of recent declines on genetic diversity in, harbor seals (Phoca vitulina) in the northern Pacific Ocean. High haplotypic diversity (H 5 0.975) and a poorly resolved mitochondrial genome (mtDNA) phylogeny suggest that harbor seals in the Pacific underwent a rapid expansion in population size in their recent evolutionary past, possibly after the retreat of Pleistocene ice sheets. Weak phylogeographic partitioning of lineages attests to a complex evolutionary and demographic history of contemporary Pacific populations. Extensive macrogeographic subdivision was evident among a subset of grouped localities that represent centers of abundance along the distributional continuum. Heterogeneity was influenced by population size and correlated with geographic distance, suggesting that dispersal occurs primarily among neighboring subpopulations. The 2 currently recognized subspecies of harbor seal in the Pacific, P. v. richardi of North America and P. v. stejnegeri of Asia, do not represent phylogenetically discrete mtDNA assemblages. The greatest differentiation detected was along the Commander-Aleutian Island chain, the region of the presumed subspecies boundary and a likely contact zone for expanding refugial populations of a number of marine mammal species after retreat of ice sheets. Differentiation between the Kodiak Archipelago and Prince William Sound, and between Bristol Bay and the Pribilof Islands, indicates that current management stocks are inappropriate and highlights the need for a detailed analysis of population and stock structure in Alaska. A decline in population size in Prince William Sound over the past few decades was accompanied by a discernible reduction in mtDNA diversity, manifested as a loss of rare haplotypes through random drift. A continued population decline will erode genetic diversity further, with potentially adverse effects on evolutionary potential and individual fitness.

### 19. Zarnke, R. L., T. C. Harder, H. W. Vos, J. M. Ver Hoef, and A. D. Osterhaus. 1997. Serologic survey for phocid herpesvirus-1 and -2 in marine mammals from Alaska and Russia. *Journal of Wildlife Diseases* 33: 459-65.

Abstract: Blood samples were collected from 1,042 marine mammals off the coast of Alaska (USA) and Russia during the period 1978 to 1994. Eight species of pinnipeds were represented. Sera were tested for presence of neutralizing antibodies to both the PB84 isolate of phocid herpesvirus-1 (PhHV-1) and the 7848/Han90 strain of phocid herpesvirus-2 (PhHV-2). Species-specific antibody prevalences ranged from 22% to 77% for PhHV-1 and 11% to 50% for PhHV-2. Species-specific antibody prevalences for PhHV-1 were greater than or equal to prevalences for PhHV-2. For both viruses and each host species, differences in antibody prevalences were not related to: (1) sex, (2) location of capture, or (3) year of collection. Antibody prevalence of PhHV-1 in walruses (*Odobenus rosmarus*) could be quantitatively predicted as a function of age. These two viruses have distinct biological properties and based on current data the epizootiology of the two viruses is different, as well. No evidence of herpesvirus-induced mortality has been detected in areas included in this survey. Based on results of this survey, neither PhHV-1 nor PhHV-2 are considered significant mortality factors in mammals which inhabit the marine environment off the coast of Alaska or Russia.

# 20. Zarnke, R. L., J. T. Saliki, A. P. Macmillan, S. D. Brew, C. E. Dawson, J. M. Ver Hoef, and R. J. Small. 2005. Serologic survey for *Brucella* spp. bacteria, phocid herpesvirus-1, phocid herpesvirus-2, and phocine distemper virus in harbor seals (*Phoca vitulina richardsi*) from Alaska, 1976-1999. *Journal of Wildlife Disease*.

Abstract: Harbor seals (*Phoca vitulina richardsi*) were captured in the coastal regions of Southeast Alaska, Gulf of Alaska, Prince William Sound (PWS), and Kodiak Island during 1976-1999. Blood was collected from 286 seals. Sera were tested for evidence of exposure to *Brucella* spp. bacteria, phocid herpesvirus-1 (PhHV-1), phocid herpesvirus-2 (PhHV-2), and phocine distemper virus (PDV). Antibody prevalence rates were 46% (46/100) for *Brucella* spp., 93% (225/243) for PhHV-1, 0% (0/286) for PhHV-2, and 1% (2/160) for PDV. Antibody prevalence for *Brucella* spp. was directly related to age of the host. Antibody prevalence for PhHV-1 was higher in PWS as compared to the other three regions. No evidence of mortality due to these four agents was observed during the course of this study. Based on the results of this survey, none of these agents is considered a significant mortality factor in harbor seals from the four regions of coastal Alaska included in the study.

### MONITORING THE TREND OF HARBOR SEALS IN PRINCE WILLIAM SOUND, ALASKA, AFTER THE EXXON VALDEZ OIL SPILL

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#### Abstract

We used aerial counts to monitor the trend in numbers of harbor seals, *Phoca vitulina richardsi*, in Prince William Sound, Alaska, following the 1989 *Exxon Valdez* oil spill. Repetitive counts were made at 25 haul-out sites during the annual molt period each year from 1990 through 1997. A generalized linear model indicated that time of day, date, and time relative to low tide significantly affected seal counts. When Poisson regression was used to adjust counts to a standardized set of survey conditions, results showed a highly significant decline of 4.6% per year. Unadjusted counts indicated a slight, but not statistically significant, decline in the number of seals. The number of harbor seals on the trend-count route in eastern and central PWS has been declining since at least 1984, with an overall population reduction of 63% through 1997.

Programs to monitor long-term changes in animal population sizes should account for factors that can cause short-term variations in indices of abundance. The inclusion of such factors as covariates in models can improve the accuracy of monitoring programs.

Key words: aerial surveys, *Exxon Valdez* oil spill, generalized linear model, harbor seal, *Phoca vitulina richardsi*, Poisson regression, population monitoring, Prince William Sound, trend analysis.

Monitoring programs to track long-term changes in population size are increasingly important in applied ecological studies. While indices of abundance have long been used in classical wildlife management, they have assumed additional importance in recent years as a means of measuring anthropogenic impacts on the natural world and the recovery, or lack thereof, from such impacts. Along with the realization of the importance of monitoring and environmental assessment programs has come increased attention to the design of such programs (Eberhardt and Thomas 1991, Taylor and Gerrodette 1993, Link et al. 1994) and their analysis (Mapstone 1995, Thomas and Martin 1996, Craig et al. 1997).

Harbor seals are one of the most common marine mammals in Prince William Sound (PWS), Alaska, and adjacent parts of the Gulf of Alaska. PWS has over 4,800 km of coastline, consisting of many fiords, bays, islands, and offshore rocks. The exact number of harbor seals inhabiting the region is unknown but is at least several thousand (T. R. Loughlin, unpublished report, National Marine Mammal Laboratory, NMFS, Seattle, WA.). Between 1984 and 1988 the number of seals counted at haul-out sites in eastern and central PWS declined by about 40% (Frost *et al.* 1994*a*).

On 24 March 1989, the T/V Exxon Valdez ran aground on Bligh Reef in northeastern PWS, spilling approximately 40 million liters of crude oil (Morris and Loughlin 1994). Studies conducted as part of a "Natural Resources Damage Assessment" program documented a substantial impact of the spill on harbor seals (Frost et al. 1994a,b; Lowry et al. 1994; Spraker et al. 1994). Approximately 300 seals were estimated to have died due to the spill, and pup production in 1989 was about 26% lower than normal (Frost et al. 1994a). Subsequent to the oil spill, as part of damage assessment and restoration science studies programs, monitoring of the harbor seal population was continued by flying aerial surveys during 1990–1997.

Many studies have demonstrated effects of time of day, date, and tide on the hauling-out behavior of harbor seals (Schneider and Payne 1983, Stewart 1984, Harvey 1987, Pauli and Terhune 1987, Yochem *et al.* 1987, Thompson and Harwood 1990, Moss 1992). The data to describe those behavioral patterns have usually come from continuous or repetitive visual observations of seal haul-outs or from telemetry studies. Information derived from those studies has been used in the design of harbor seal surveys, to the extent that survey programs are generally designed to occur on dates and at times when the greatest number of seals are expected to be out of the water and available for counting (Pitcher 1990, Harvey *et al.* 1990, Olesiuk *et al.* 1990, Huber 1995). However, once a "survey window" has been established, counts have usually been treated as replicates during analyses, and the possible effects of other factors on annual abundance estimates have been ignored.

This paper presents an analysis of aerial survey counts of harbor seals in PWS. The objectives are to (1) describe how covariates affected counts of harbor seals during the surveys, (2) use the covariates to adjust haul-out counts, and (3) determine whether or not significant population trends have occurred.

#### Methods

#### Aerial Surveys

We conducted aerial surveys along a trend-count route that covered 25 harbor seal haul-out sites in eastern and central PWS (Fig. 1). The route included seven sites that were substantially affected by the *Exxon Valdez* oil spill and 18 unoiled sites that were outside of the primary affected area (Frost



Figure 1. Map showing trend-count sites for aerial surveys of harbor seals in Prince William Sound, Alaska, 1984–1997. Sites 11–17 oiled by the Exxon Valdez oil spill.

et al. 1994a). Surveys were flown during the molting period (August-September) in 1984 and 1988-1997.

Visual counts of seals were conducted from a single-engine fixed-wing aircraft (Cessna 185) at altitudes of 200–300 m, usually with the aid of  $7 \times$  binoculars. Counts were usually conducted from two hours before low tide to two hours after low tide. A survey normally included counts at all 25 sites, but occasionally some sites could not be counted because of poor weather or a rapidly rising tide. For each survey the date, time and height of low tide, and time of sunrise and sunset were recorded. Each site was circled until the observer was confident that an accurate count had been made, and the time of the count was recorded. For larger groups of seals (generally those of 40 or more) color photographs were taken using a hand-held 35-mm camera, and seals were counted from images projected on a white surface. Each year several survey flights, usually 7–10, were made. The total number of counts for all sites and all years was 2,014.

#### Factors Affecting when Seals are Hauled Out

We used a generalized linear model (McCullagh and Nelder 1989) with a log link function and a Poisson distribution to analyze the factors that may affect the number of seals hauled out and available to be counted during surveys. The model may be written as:  $Pr(Z_{tij} = z) = \exp(-\lambda_{tij})\lambda_{tij}^{z}/z!$  with  $\ln(\lambda_{tij}) = \beta' x_{tij}$  where  $\beta$  is a parameter vector and  $x_{tij}$  is a vector containing information on the state of covariates: year, site, time of tide, height of tide, time of day, and date for the *j*th flight at site *i* in year *t*.

To estimate the average count at each site in any given year, we first used a model that contained site, year, and the interaction of site with year. These factors were used in all models. Then, effects for time of day, time of low tide, date, and tide height were entered into the model one at a time. If a factor with *m* parameters increased 2\*log-likelihood by more than a  $\chi^2$ -distribution with *m* degrees of freedom at  $\alpha = 0.05$ , we considered the factor to affect significantly the number of seals counted at haul-outs. The factor with the largest  $\chi^2$ -value was retained in the model, and then other factors were again entered into the model one at a time until any remaining factors were not significant. Time of day and time relative to low tide were analyzed as categorical data. Time increments before and after midday were placed in six separate categories and increments before and after low tide in eight categories. We combined some categories within a factor when preliminary analysis indicated that it could be done without changing the fit (again, if combining two categories decreased 2\*log-likelihood by more than a  $\chi^2$ -distribution with one degree of freedom, we considered that the fit was essentially unchanged). Date was a continuous variable entered into the model as a polynomial up to a quadratic power. Dates were numbered beginning 15 August and scaled so that each day was equal to 0.1 to keep parameter estimates from becoming too small (causing problems with significant digits in software packages). To construct the initial model, we used data from all surveys conducted during 1984–1997. The final model was checked using deviance residuals (McCullagh and Nelder 1989). The residuals were plotted against each factor, by year, to examine whether or not the effects were constant across years.

After obtaining a parsimonious model and fitting the parameters as described above, the count data were adjusted to a standardized set of covariates. The adjustment amounts to estimating counts at each site for each year as the expected count under optimal conditions.

#### Trend Analysis

A linear regression model was fitted to the adjusted yearly count estimates for 1990–1997. This model assumes constant amount of change per year. We also considered a model on the log-scale, where the rate of change is constant. Again, we used a generalized linear model (McCullagh and Nelder 1989) with a log link function and a Poisson distribution to model trend through time. This is also called Poisson regression. Linear and Poisson regressions were also fitted to the unadjusted counts.

This analysis was complicated because we first adjusted yearly counts for each site to a standardized date, time of day, and time relative to low tide, then summed over sites to get a yearly index, and then used the index in a trend regression analysis. Under these circumstances it is difficult to pass the uncertainty associated with adjusting the counts to the trend analysis. Therefore, we used bootstrap methods (Efron and Tibshirani 1993, Manly 1997) for the whole procedure. We resampled with replacement from the daily flights for each year, with the number of resamples equal to the actual number of flights for that year. After obtaining the bootstrap sample, we used the generalized linear model to re-estimate parameters, adjusted the counts based on the bootstrap parameter estimates, and then did both linear and Poisson regression trend estimation on the bootstrap samples. The trend parameters from the bootstrap appeared symmetrically distributed and centered on the original parameter estimate. Bootstrapping the whole procedure was quite computerintensive and only 200 resampled estimates were obtained, so we used the standard bootstrap method by taking,

#### estimate $\pm z_{\alpha/2}$ (Bootstrap Standard Deviation)

(Manly 1997) and if 0 was contained in the interval, there was little evidence of trend for the stated  $\alpha$ -level.

Bootstrapping was used to estimate variance of the unadjusted counts by resampling from the actual count values for each site in each year.

#### RESULTS

#### Factors Affecting when Seals are Hauled Out

Three primary factors significantly affected the counts of seals during aerial surveys (Table 1). Time of day was the most significant factor, followed by date, and time of count relative to low tide (P < 0.001 for all three). Tide height was not significant.

The model predicted that counts would have been highest in the period 2– 4 h before midday with 25% more seals expected than 2–4 h after midday (Fig. 2A). (These calculations are obtained from Table 1 by taking the expo-

Factor	Category	Parameter estimate
Time of day	before (midday $- 4$ hr) (midday $- 4$ hr) to (midday $- 2$ hr) (midday $- 2$ hr) to (midday) (midday) to (midday $+ 2$ hr) (midday $+ 2$ hr) to (midday $+ 4$ hr) after (midday $+ 4$ hr)	$\begin{array}{r} -0.0461 \\ -0.0000 \\ -0.1984 \\ -0.1594 \\ -0.2842 \\ -0.1594 \end{array}$
Date	day/10 since 15 August (day/10 since 15 August) <sup>2</sup>	-0.1239 -0.0192
Time relative to low tide	before (lowtide $-1.5$ hr) (lowtide $-1.5$ hr) to (lowtide $-1$ hr) (lowtide $-1$ hr) to (lowtide $-0.5$ hr) (lowtide $-0.5$ hr) to (lowtide) (lowtide) to (lowtide $+0.5$ hr) (lowtide $+0.5$ hr) to (lowtide $+1$ hr) (lowtide $+1$ hr) to (lowtide $+1.5$ hr) after (lowtide $+1.5$ hr)	$\begin{array}{r} -0.1602 \\ -0.0531 \\ 0.0000 \\ -0.0550 \\ 0.0000 \\ -0.0550 \\ 0.0000 \\ -0.3417 \end{array}$

Table 1. Parameter estimates for factors affecting counts of hauled-out harbor seals in Prince William Sound.

nent of the parameter estimates; e.g.,  $\exp(-0.2842) = 0.753$ , or 24.7% lower counts in the period 2-4 h after midday.) Relative to low tide, the model predicted the highest counts from 1.5 h before to 1.5 h after low tide, with substantially lower counts (about 29% lower) more than 1.5 h after low tide (Fig. 2B).

In Figure 3 we show summaries of raw count data along with the fitted model for date effects. We defined the deviations from raw counts as

$$r_{jk} = A_{jk} - B_j$$

where  $A_{jk}$  is the mean of sites for year j and date k, and  $B_j$  is the mean of sites and dates for year j.

This analysis did not correct for the influence of factors other than date, but nonetheless the decreasing trend in counts within year is apparent. The model predicted that the highest counts would have occurred on the earliest survey dates and that there would be an approximately linear decrease in counts throughout the survey period (Fig. 3). Relative to 15 August, counts would have been 22% lower on 31 August and 45% lower on 16 September.

The deviance residuals plotted for each factor by year showed no lack of fit, but some overdispersion. Overdispersion will not affect the fitted parameters significantly but will affect the standard errors (McCullagh and Nelder 1989). Effects of overdispersion on variance were accounted for in the bootstrap.

#### Trends in Seal Counts

Annual changes in unadjusted counts were substantial, ranging from 18% below to 17% above the previous year's counts, and regression analysis indicated no significant trend (Table 2; Fig. 4).





Parameter estimates from the generalized linear model (Table 1) were used to correct all unadjusted counts to "optimum" conditions, *i.e.*, 15 August, 4–2 h before midday, and 1.0–0.5 h before, 0–0.5 h after, or 1.0–1.5 h after low tide. Annual adjusted counts were 16%–40% higher than unadjusted counts (Table 2). The adjusted counts showed a significant decline in the number of seals in the trend area with both linear (P = 0.008) and loglinear (P < 0.001) regression analysis (Fig. 4).

#### DISCUSSION

#### Factors Affecting Harbor Seal Counts

We were concerned about the effects that date, time of day, and tide might have had on our aerial survey counts. There are several ways to deal with covariate effects in study design. The best approach that results in the least variability is to design the study so that the potential covariates are constant. For example, for harbor seals we would like to sample on consecutive days from 15-21 August, at 1000, and at slack low tide. However, the fact that



Figure 3. Effects of date on counts of harbor seals in Prince William Sound, Alaska. First eight panels show deviation of each individual daily count from mean count for that year. Final panel shows model fit for relationship of seal count *vs.* date for all years combined.

weather conditions and the time and height of low tide on a particular date vary from year to year precludes such an approach. Another approach is to randomize sampling relative to the covariate. For example, if survey dates are chosen randomly from within the general molt period, the effect of that covariate across years would "cancel out." This would result in more variability than keeping the covariates constant, but it is still design-unbiased, so simple linear or nonlinear models could be used to examine trend. However, it would only be possible to use this approach for one covariate such as date, and that would be logistically impractical. The third approach, the one we adopted, is to sample over a one- to two-week period as weather allows, and then use a model to adjust the counts to a standard set of conditions.

Aerial surveys are commonly used for assessing abundance of harbor seals. Most survey programs try to use a relatively narrow and standard "survey window" (*i.e.*, they attempt to hold covariates constant). Some investigators have used correction factors to adjust counts to account for certain measurable covariate effects. Olesiuk *et al.* (1990) used a correction factor to adjust for differences in dates of surveys relative to the pupping season. Thompson and Harwood (1990) used time-lapse photography to measure changes in the number of seals hauled out relative to time of day, then used that relationship to

Year	Unadjusted count	Adjusted count
1990	779	1,299
1991	920	1,215
1992	769	1,150
1993	774	1,140
1994	740	996
1995	869	1,131
1996	808	966
1997	751	935
linear regression		,
slope estimate	-5.885	-47.530
standard deviation	4.260	17.939
$Pr (H_0: slope = 0)$	0.167	0.008
loglinear regression		
slope estimate	-0.007	-0.043
standard deviation	0.005	0.011
$Pr (H_0: slope = 0)$	0.170	<0.001

Table 2. Unadjusted and adjusted mean counts and regression analyses, for harbor seal trend counts in Prince William Sound, 1990–1997. Adjusted counts derived using parameter estimates in Table 1. Standard deviations of slope estimates calculated by bootstrapping.



Figure 4. Trend in abundance of harbor seals in Prince William Sound based on unadjusted and adjusted counts, 1990-1997. Dashed line shows overall trend based on linear regression.

standardize aerial counts. Frequently, however, the assumption has been made that some or all potential covariate effects are unimportant and that ignoring them will have little effect on interpretation of results.

Our analysis showed that time of day, date, and time relative to low tide all significantly influenced harbor seal counts in PWS, and an assumption that covariate effects were negligible would have been erroneous. The model predicted counts to be highest before midday, and within 1.5 h of low tide. The model also predicted that peak counts would occur earlier in August than our surveys historically have begun, and that counts would decrease from the earliest survey date throughout the survey period. Our purpose in developing this model was to understand the factors affecting our counts, not to describe the behavior of harbor seals. Nonetheless, the results are consistent with those of investigators who have conducted behavioral studies of harbor seals in that the proportion of seals hauled out is related to date, time of day, and tide.

Many studies have shown that there are site-specific variations in harbor seal behavior patterns depending on habitat type, effects of disturbance, and other factors (e.g., Harvey 1987, Olesiuk et al. 1990, Moss 1992, Thompson et al. 1997), and therefore parameter values for covariate effects could vary greatly in different situations. If annual counts are to be used to monitor harbor seal trend in an area, studies should be done to assess factors that could influence seal behavior at that locale (Thompson et al. 1997). Results from those studies can be used for designing an initial survey protocol, as well as to select variables that should be recorded during surveys and used in subsequent data analyses.

#### Trend in Harbor Seal Numbers in PWS

Our analysis of PWS harbor seal counts showed that adjusting counts to consider variation in survey conditions greatly improved our ability to detect a trend. If we ignored the possible effects of covariates and looked only at unadjusted counts we would have concluded that, although there was a negative slope to the regression line, the trend in seal numbers during 1990-1997 was not significant. When we considered covariates, and counts from each year were "normalized" to standard conditions, the decline in seal numbers became highly significant. The adjusted count of seals on the trend route in 1997 was 28% lower than in 1990, and loglinear regression indicated that the population has been declining at an average rate of 4.6% per year. Because the model corrects each individual count for three covariates it is difficult to determine which aspects of survey design biased the interpretation of results from unadjusted counts. A partial explanation can be seen in the effect of date. During 1990–1994, the median dates for our surveys ranged from 27 August to 4 September, while the median dates during 1995–1997 were 21–23 August (see Fig. 3). Because a lower proportion of seals would be hauled out on later survey dates, counts made in earlier years were biased low, therefore masking the declining trend in abundance.

The number of harbor seals on the trend-count route in eastern and central

PWS has been declining since at least 1984 (Frost *et al.* 1994*a*). Using the parameter estimates derived in this study to correct the 1984 count data we estimate an adjusted trend-route count of 2,523 seals for that year. This indicates an overall population reduction of 63% during the period 1984–1997.

The Comprehensive Environmental Response, Compensation, and Liability Act requires the assessment of injury to natural resources caused by events such as oil spills, and that recovery objectives be established for injured species. The fact that the number of harbor seals in PWS was declining prior to the *Exxon Valdez* oil spill complicated both the assessment of injury due to the spill (Frost *et al.* 1994*a*), and the definition of recovery. The *Exxon Valdez* oil spill Trustee Council has determined that "harbor seals will have recovered from the spill when their population trend is stable or increasing." Based on the results of this study, as of 1997 harbor seals in PWS have not met the Trustee Council's recovery objective.

#### Significance to Monitoring Studies

Measurement of the trend in abundance of a population is an important tool for wildlife conservation. For example, as noted above, the legally required recovery objective for harbor seals impacted by the *Exxon Valdez* oil spill is based entirely on the population's trend.

In some cases it may be possible to use survey data to assess population trends without concern for covariate effects; for example, where changes are relatively large, data are collected over long periods of time, and study design holds covariates relatively constant. The conclusion that harbor seal numbers on Tugidak Island in the Gulf of Alaska underwent a major decline appears reliable, as counts were made under strict conditions, the decline was large (about 85%), and data were collected over a 12-yr period (Pitcher 1990). Confidence in the Tugidak situation is increased by the fact that similar trends were seen in both pupping and molting period counts. Conclusions that harbor seal numbers have increased in southern California (Stewart *et al.* 1988), Oregon (Harvey *et al.* 1990), and Washington (Huber 1995) also are likely to be correct, although in those studies counts were made in a relatively wide range of conditions and consideration of covariates in data analyses would likely improve the assessment of trends.

Where covariates have strong effects that cannot be avoided in study design they must be accounted for in the analysis. For example, Beaufort state and cloud cover have strong effects on counts of harbor porpoises (*Phocoena phocoena*), and therefore Forney *et al.* (1991) used those factors as covariates in their trend analysis. In an analysis of Florida manatee (*Trichechus manatus latirostris*) aerial survey data, Garrott *et al.* (1995) modeled the effects of survey conditions and air and water temperature on counts. About 50% of the variation in counts was explained by those variables, and when counts were adjusted for covariate effects a significant increase was seen in the number of manatees counted on the east coast of Florida during 1982–1991.

In many situations analyses of the kind we performed are not possible be-

cause data have been collected intermittently, inconsistently, or for only a few years. In the case of PWS harbor seals these analyses were possible, and useful, because there was a consistent, relatively long-term data set from which to develop models for use in adjusting data. The PWS example demonstrates the importance of long-term, cost-effective monitoring programs that allow the evaluation of population trends and can also provide a way to measure the impacts of human activities or accidents such as the *Exxon Valdez* oil spill.

#### ACKNOWLEDGMENTS

This study was conducted as part of the Exxon Valdez Oil Spill Restoration Program, funded by the Exxon Valdez Oil Spill Trustee Council. Funding for harbor seal surveys in PWS in 1992 was provided by the National Marine Fisheries Service, National Marine Mammal Laboratory. Ken Pitcher conceived the idea of harbor seal trend counts in PWS, and Dennis McAllister and Jon Lewis flew some of the earlier surveys. We thank Steve Ranney, the pilot for all of the aerial surveys, for his careful and conscientious support. Bob DeLong assisted with data analyses and presentation. Dean Hughes, Joe Sullivan, Sheila Westfall, Melanie Bosch, Melissa Johnson, and Diana Ground provided administrative support for this project. Grey Pendleton, Tim Gerrodette, Jeff Laake, and an anonymous reviewer provided helpful comments on drafts of the manuscript.

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> Received: 12 December 1996 Accepted: 18 May 1998

MARINE MAMMAL SCIENCE, 17(4):813–834 (October 2001) © 2001 by the Society for Marine Mammalogy

### DIVING BEHAVIOR OF SUBADULT AND ADULT HARBOR SEALS IN PRINCE WILLIAM SOUND, ALASKA

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#### Abstract

Satellite-linked depth recorders (SDRs) were attached to 47 harbor seals in Prince William Sound, Alaska, during 1992–1996. Parameters describing diving effort, diving focus, and focal depth (depth bin to which diving was focused) were calculated from binned data on maximum dive depth and time spent at depth, and analyzed using repeated-measures mixed models. This analysis method accounted for individual variability, temporal autocorrelation, and the binned nature of SDR data, which are often ignored using standard statistical techniques. Results indicated that diving effort remained steady from September to April, when seals spent 68%-75% of their overall time in the water. Time spent in the water declined to 60% in May and to about 40% in July. Seals spent the most time in the water at night and the least in the morning. The diving of all seals in all months was highly focused. Overall, diving was focused to one depth bin approximately 75% of the time. Diving was more focused for females than for males and subadults. Focal dive depth was deepest in winter and shallowest during May-July. Focal depth and diving focus varied by region. Collinearity between month and region in the focal depth model suggests that seals move in winter to regions where prey are found deeper in the water column. Variations in diving behavior presumably result from combinations of regional bathymetry, seasonal cycles

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in type or depth distribution of prey, and seal life-cycle events such as reproduction and molting.

Key words: harbor seal, *Phoca vitulina richardsi*, Prince William Sound, diving behavior, satellite telemetry, repeated-measures mixed models.

In many parts of the world pinniped populations have increased as predicted after protection from over-exploitation (e.g., Olesiuk et al. 1990). However, large declines in populations of harbor seals (*Phoca vitulina richardsi*) and Steller sea lions (*Eumetopias jubatus*) have been documented in parts of Alaska (Pitcher 1990, Loughlin et al. 1992). These declines occurred despite implementation of the 1972 Marine Mammal Protection Act, which stopped or limited several types of human-caused mortality. Likewise, since the 1970s some species of sea birds have also declined in the Gulf of Alaska and Bering Sea regions (Anderson and Piatt 1999). These unanticipated declines have prompted monitoring and assessment of marine mammal, sea bird, and fish population trends in these regions.

Harbor seals are one of the most abundant and widely distributed marine mammals in Prince William Sound, Alaska, hauling out and/or pupping at more than 50 sites. Since 1984, harbor seal numbers in Prince William Sound have declined by about 60%, with only part of this decline attributable to the 1989 *Exxon Valdez* oil spill (Frost *et al.* 1994, 1999). A change in the trophic structure of the ecosystem, and hence the availability of prey, is among the hypothesized causes for the harbor seal decline. Determining how harbor seals depend on seasonal or area-specific concentrations of prey may provide insight into the causes of the observed changes in abundance. In addition, harbor seals may act as important indicators of the status of other marine species.

To evaluate the food limitation hypothesis, information is needed not only about the diet of harbor seals, but also about seasonal or annual changes in feeding behavior and the habitats used for feeding. Satellite-linked telemetry can be used to gather the latter types of information (e.g., Stewart et al. 1989). Satellite-linked depth recorders (SDRs) have been deployed on a variety of marine mammals, providing insights into both large-scale horizontal movements and diving behavior in these animals (e.g., Heide-Jørgensen et al. 1992, Heide-Jørgensen and Dietz 1995, Nordøy et al. 1995, Stewart et al. 1996, Merrick and Loughlin 1997, Lowry et al. 1998). However, unlike time-depthrecorders (TDRs), which record and store information about individual dives, many SDRs sum dive information into bins over 6-h blocks of time. The binned nature of the SDR data, as well as substantial variability in diving behavior of individual seals, have made SDR data poorly suited to standard analysis techniques. These difficulties have often resulted in the application of simple summary statistics to SDR data and/or in the presentation of data for each individual, without a suitable means of combining data for groups of individuals (e.g., Mate et al. 1994, 1995; Davis et al. 1996; Stewart et al. 1996). The inferences about diving behavior that can be drawn from either



*Figure 1.* Map of Prince William Sound study area showing major harbor seal haulouts (solid triangles) and 200 m depth contour (dotted line).

summary statistics or individual descriptions are limited. Temporal autocorrelation in SDR data has also been largely ignored in these summary analyses.

In this paper we present a statistically robust method for analyzing SDR data that accounts for individual variability among animals, temporal autocorrelation, and the binned nature of the data. We use this method to analyze the diving behavior of harbor seals in Prince William Sound, Alaska, using a large SDR dataset collected during 1992–1997 (Lowry *et al.* 2001). We specifically address patterns in diving behavior related to sex and age of the seal, time of day, month, and region.

#### Methods

#### Data Collection

Harbor seals in Prince William Sound were captured in nets near haul-outs and outfitted with 0.5-w SDRs (Wildlife Computers, Redmond, WA; version 3.10 software) as described by Lowry *et al.* (2001). Seals weighing >50 kg were instrumented with tags that measured 14.8  $\times$  10.0  $\times$  3.8 cm and weighed about 750 g in air. For lighter seals we used tags that measured 11.9 cm  $\times$  5.1 cm  $\times$  4.5 cm and weighed 385 g. The larger tags had a projected capacity of about 100,000 transmissions, whereas the small tags were rated for approximately 30,000 transmissions. SDRs were equipped with a salt-water switch and transmitted only when a seal was at the surface.

Seals were tagged in spring (late April-May) and fall (late September). SDRs

attached in spring were not duty-cycled and transmitted continuously, because we expected that tags would be shed during the annual molt in August, long before the batteries failed. To conserve battery power, tags attached in the fall were programmed to not transmit during hours of poor satellite coverage (2200–0300 local time). In addition, small tags attached in the fall were dutycycled one day on and one day off, or one day on and two days off.

The SDRs sampled time and pressure (depth) every 10 sec and summarized and stored this information in bins representing four 6-h histogram periods per day: 2100-0259 (night), 0300-0859 (morning), 0900-1459 (midday), and 1500-2059 (evening), local time (GMT - 10 h). All 47 SDRs collected data about the maximum depth and the duration of each dive. The SDRs measured depth from 0 to 490 m, with depth resolution of 2 m. There is considerable instrument noise and inaccuracy in assigning depths to dives that are near the 2-m resolution of the pressure sensor. Thus, we chose a depth equal to twice the resolution of the instrument (4 m) as the minimum depth to be considered a dive. Maximum dive depths were accumulated in userdefined bins as follows: 4-20 m, 21-50 m, 51-100 m, 101-150 m, 151-200 m, and >200 m. Thirty-five of 47 SDRs also stored "time-at-depth" which recorded the amount of time a seal spent per 6-h period within these same depth bins. In addition, a 0-m bin recorded time spent at the surface and dry, and a 0-4-m bin recorded the time the seal was wet and swimming shallower than 4 m.

Dive and location data from SDRs were relayed *via* satellite receivers operated by Service ARGOS (Argos 1990). Location data were screened and erroneous records identified as described by Lowry *et al.* (2001). Dive data from SDRs were extracted using the software program SATPAK 3.0 (Wildlife Computers). This software used an error-checking algorithm to validate messages. Histogram messages were sorted by date, period, and type, and duplicate messages were removed.

#### Diving Behavior Analysis

We analyzed diving behavior of seals in Prince William Sound with respect to sex and age of the seal, month, time of day, and geographical region. Seal location data were initially assigned to eight regions as follows: eastern Prince William Sound, northern and western Prince William Sound, central Prince William Sound, southwestern Montague Island, Copper River Delta, Middleton Island, Yakutat, and Cook Inlet (Fig. 1). Tagged seals were rarely found within four of these regions (eastern PWS, southwestern Montague, Yakutat, and Cook Inlet). These data-poor regions were not used in the final analysis because they contained too few observations (<1.5% of seal locations were within the region) or data were available from only a few seals of a single age or sex class. Data were not analyzed for year effect because of unequal distribution of age and sex categories across years. Seals were classified as adults or subadults according to their weight. Males <55 kg and females <47 kg were considered to be subadults, based on historical age/weight data from the northern Gulf of Alaska (Pitcher and Calkins 1979).

Harbor seal SDR data were analyzed for diving effort, diving focus, and focal depth. SDRs provided several possible measures of diving effort, including number of dives, total duration of dives, and time spent in the water per 6-h data collection period. We chose "time-in-water" as the most representative effort variable. Data on number and duration of dives do not include any dives shallower than 4 m, yet seals spend considerable time in such shallow water. SDRs were programmed so that Bin 0 of the time-at-depth data recorded the proportion of time the sensor was dry during each period. Timein-water was therefore calculated as 6 h minus time in Bin 0 for each period. Time-in-water values ranged from 0 h, for periods during which a seal was continually hauled out, to 6 h, for periods during which a seal was always in the water.

Diving focus was defined as the dominance of one depth bin in the maximum dive-depth data for a 6-h period. Diving focus (F) was calculated as the inverse of Simpson's Diversity Index, (Simpson 1949, Washington 1984, Krebs 1999):

$$F = \sum \{ [n_i(n_i - 1)] / [N(N - 1)] \}$$

where  $n_i$  = number of dives to depth bin *i*, and N = total number of dives. The maximum value for focus, F = 1, indicated that all dives were to the same depth bin. A focus value of F > 0.5 indicated that dives in a period were primarily to one depth bin, while F = 0.167 indicated that dives were evenly distributed among the six depth bins. Because we used the "finite correction factor"  $\{n(n - 1) \text{ and } N(N - 1)\}$  in calculating dominance of bins, a smaller sample size required more relative focus to get the same value of F (*i.e.*, the analysis was more conservative for a small sample size). Also, Simpson's Diversity Index incorporates the distribution of bin use. When dives were allocated over several depth bins (instead of only two), proportionately more dives to the main depth bin were required to get a focus value of F > 0.5.

We defined focal depth as the dominant depth bin for a 6-h period, during which the seal's diving was primarily focused to that depth bin (F > 0.5). The term "focal depth", used in this context, has no relationship to "focal length" or other such optical terms. Seals were not considered to exhibit any depth preference when their diving was not focused to one depth bin, so focal depths were not determined for periods with F < 0.5.

Separate diving focus and focal depth analyses were conducted for "any timein-water" and "time-in-water > 3 h" data sets to explore the effect of timein-water on diving focus and focal depth. Bin data were summarized by standard 6-h periods, regardless of an individual's behavior, and some data represented periods when seals were diving less than half the time (time-in-water <3 h). These low time-in-water periods could have represented the beginning or ending of a diving bout, or sporadic diving around a haul-out which might differ from diving while foraging. It was our intent to investigate foraging behavior, so we compared diving focus and focal depth between high-effort (time-in-water > 3 h) periods and all periods. The "high time-in-water" and "any time-in-water" analyses produced consistent results, and we therefore used "any time-in-water" in subsequent analyses. This made it possible to include data from 12 additional seals in diving-focus and focal-depth analyses. SDRs from those seals provided maximum dive-depth data, but did not provide the time-at-depth data required to calculate time-in-water.

In addition to the time-in-water, focus, and focal-depth variables described above, a time series variable was created which combined the Julian date and time period for each record (time series = Julian date + time period/4). In cases where data from one seal spanned two years, the time series values in the second year were in sequence with those of the first year (e.g., 31 December 1995 period 3 = 365.75; 1 January 1996 period 2 = 366.5). This time-series variable was used for calculating, and correcting for, the effect of temporal autocorrelation on statistical models of diving behavior.

#### Statistical Analysis

Repeated-measures mixed models for time-in-water, diving focus, and focal depth were created using the MIXED procedure in SAS (version 6.12, SAS Institute Inc.; Littell *et al.* 1996). We selected random subsets of 100 records from the databases for each seal for inclusion in each analysis, where each record included data from one 6-h period. For seals with less than 100 records, all data were included in analyses. Subsetting the data greatly reduced computation time, and also balanced the impact on the model of seals with many or few records. This was particularly important since non-duty-cycled SDRs transmitted substantially more data than did the duty-cycled units, and without subsetting might have disproportionately influenced the analyses. An alternative approach would involve an analysis with data from each seal weighted differently, however such an approach would not reduce computation time, which was prohibitive without subsetting.

Since the repeated-measures analysis (which accounted for temporal autocorrelation in the data) was very computation-intensive, the best model for each analysis was first determined using forward stepwise procedures with variation between individual seals as a random effect but without repeatedmeasures analysis. Denoting individual variation as a random effect modeled the variation in behavior between individual seals as randomly distributed around a mean of zero for all seals. Thus, an "average" seal would have no impact on the model. Fixed effects (sex, age, month, period, and region) were added singly to each model, using Akaike's Information and Schwarz's Bayesian Criteria (Carlin and Louis 1996) to determine the order of entry into the model.

Models with the maximum number of significant fixed effects were chosen for further analysis by including repeated-measures within the MIXED procedure. A spherical spatial autocorrelation model was used with time series and a column of ones as the dimensions, and individual seals as subjects, in the model. Denoting seals as subjects in the model resulted in one global autocorrelation model being fit for all seals based on the autocorrelation found within the data for each seal (*i.e.*, data from seal X was not autocorrelated with data from seal Y). The random effect of variation among individual seals was maintained in the repeated-measures analysis. In several cases, parameters that had been significant in the mixed model were no longer significant in the repeated-measures mixed model. In those cases, non-significant fixed effects were removed one at a time to determine the final models that had the maximum number of significant fixed effects.

#### Results

We analyzed data from 47 seals (25 females, 22 males, 27 adults, and 20 subadults) captured between 1992 and 1996 (see Appendix 1 in Lowry *et al.* 2001). Forty-five were captured and tagged in central Prince William Sound, one in eastern Prince William Sound, and one in northern Prince William Sound (Fig. 1). Seals were tagged during spring (April or May) and fall (September). SDRs attached in spring (n = 21) operated for an average of 64 d (range 39–81), before being shed during the annual molt. Fall SDRs (n = 26), which were attached after the molt was completed, operated for an average of 179 d (range 40–312).

#### Time-in-Water

The subset of time-in-water data used in the statistical analysis contained 2,522 records, each of which represented one 6-h period, for a total of 15,132 h of diving by 35 seals. This subset excluded data from poorly represented regions and included  $\leq 100$  randomly selected records/seal, reducing the original database by 50% (from 4,995 records). Month and time period were significant fixed effects in the repeated-measures mixed model for time-inwater. Sex, age class (i.e., adult or subadult), and geographic region did not significantly affect time-in-water (Table 1). Time-in-water was similar throughout September-April (68%-75% of each 6-h period spent in the water), then declined steadily from 60% in May to 40% in July (Table 1, Fig. 2A). Seals spent the least time in the water diving in the morning (0300-0900) (Fig. 2B). Time-in-water increased throughout the day and was highest at night (2100-0300) (Table 1). At night seals spent approximately 80% of their time diving during September through April (range 77%-84%), compared to 50% in July. Seals spent about 19% less time diving in the early morning than they did at night.

#### Diving Focus

The subset of diving focus data used in the statistical analysis contained 3,163 records, for a total of 18,978 h of diving by 47 seals. This subset excluded data from poorly represented regions and included  $\leq$ 100 randomly

Table 1. Stepwise mixed models statistics and parameter estimates for time-inwater model for harbor seals in Prince William Sound, Alaska (n = 2,522 6-h periods, 35 seals). Covariance parameters for random effects and temporal autocorrelation are compared to the "total error variance" that would have been present in a simple fixedeffects model (total error variance = random effect variance + autocorrelation + residual variance).

Stepwise mixed models statistics (with random seal effect but no repeated measures)			
Variables in model	Type III F-statistic	Р	
Sex	0.46	0.50	
Age	1.38	0.24	
Month	11.51	0.0001	
Period	14.67	0.0001	
Region	1.38	0.25	
Month, period	11.89, 15.72	0.0001, 0.0001	

Parameter estimates from best repeated-measures mixed model for time-in-water

	Time-in-water				
Fixed effect	(min)	SE	df	t	Р
Intercept	263.6614	11.053	34	23.85	0.0001
Month					
January	-10.7778	13.697	2474	-0.79	0.431
February	7.9979	14.541	2474	0.55	0.582
March	-14.9388	16.071	2474	-0.93	0.353
April	-5.0566	16.031	2474	-0.32	0.753
May	-44.2030	13.549	2474	-3.26	0.001
June	-74.4995	14.328	2474	-5.2	0.0001
July	-112.2920	19.937	2474	-5.63	0.0001
September	9.1771	15.089	2474	0.61	0.543
October	6.8952	11.972	2474	0.58	0.565
November	6.6206	12.332	2474	0.54	0.591
December	0				
Period					
2100-0300	28,3274	8.721	2474	3.25	0.001
0300-0900	-26.7608	5.218	2474	-5.13	0.0001
0900-1500	-14.06	5.240	2474	-2.68	0.007
1500-2100	0				
				Parameter	Proportion of total erro

Covariance parameter	Parameter estimate	of total error variance
Seal (random effect variance)	651.90	0.054
Sill-Nugget (repeated-measures/autocorrelation)	1,607.79	0.134
Residual variance	9,773.61	0.812
Total error variance	12,033.30	1.000
Range (spherical model, repeated-measures) = 15.0 d		



Figure 2. Modeled estimates of time-in-water for 35 satellite tagged harbor seals in Prince William Sound, Alaska, by month (A) and time of day (morning, midday, evening, night) (B). For ease of graphic presentation, data are adjusted from minutes to % time wet per 6-h period. Monthly estimates of time-in-water are average values for all periods of the day combined, and estimates for time of day are averages for all months combined (from Table 1).



selected records/seal, reducing the original database by 38% (from 5,133 records). Data were available from 12 seals that did not have time-at-depth data, in addition to the 35 seals included in the time-in-water analysis. Diving focus was significantly affected by time of day, region, and the interaction of sex and age (*i.e.*, sex-age class, Table 2). The diving of all seals was highly focused even before the effects of analysis variables were considered (model intercept F = 0.69, Table 2). The lowest focus predicted by the model was F= 0.54 for adult male seals in northwestern Prince William Sound, indicating that even the lowest focus values reflected a strong focus to one depth bin. Overall, diving was focused to one depth bin (F > 0.5) during approximately three quarters of the 6-h data periods recorded for all seals. Focus was not significantly affected by month.

Seal diving was most focused during midday (0900–1500) and secondarily at night (Fig. 3A). Adult female diving was the most focused of all demographic classes, and adult male diving was the least focused (Fig. 3B). Seal diving was most focused in the very shallow Copper River Delta and the least focused in Prince William Sound where bathymetry was highly variable (Fig. 3C).

#### Focal Depth Bin

The subset of focal depth data used in the statistical analysis contained 2,485 records, for a total of 14,910 h of diving by 47 seals. This subset excluded records with diving focus <0.5, as well as data from poorly represented regions including  $\leq 100$  records/seal, reducing the original diving focus database by 52% (from 5,133 records). Month and region were significant fixed effects in the model using data for any time-in-water (Table 3). However, collinearity between month and region, combined with lower sample size, resulted in month and region not being significant together in the model using only data with time-in-water >3 h. We used the "any time-in-water" model, since it overcame collinearity problems. This model indicated that the
Table 2. Stepwise mixed models statistics and parameter estimates for diving focus models of harbor seals in Prince William Sound. Estimate is for any time-in-water (n = 3,163 6-h periods, 47 seals). Covariance parameters for random effects and temporal autocorrelation are compared to the "total error variance" that would have been present in a simple fixed-effects model (total error variance = random effect variance + autocorrelation + residual variance).

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Stepwi	se mixed models statis	stics (with random seal	effect but n	o repeated measures)				
Variables in model Type III F-statistic				Р				
Sex	14.70			0.0001				
Age		1.24		0.266				
Month		2.21		0.015				
Period		20.30		0.0001				
Region		7.98		0.0001				
Sex, period		14.43, 20.23		0.0001, 0.000	)1			
Sex, period, region	13.	34, 20.10, 7.54		0.0003, 0.0001, 0	).0001			
Sex, month, period, region	12.56,	2.36, 19.29, 8.63		0.0004, 0.0090, 0.0001, 0.0001				
Sex, age, month, period, region	12.07, 1.44, 2.37, 19.24, 8.70			0.0005, 0.2304, 0.0085, 0.0001, 0.0001				
Parameter estimates from best repeated-measures mixed model for diving focus								
Fixed effect	Diving focus	SE	df	ţ	P			
Intercept	0.6872	0.041	43	16.57	0.0001			
Sex*Age								
Adult female	0.1599	0.044	3110	3.65	0.0001			
Subadult female	-0.0213	0.050	3110	-0.43	0.670			
Adult male	-0.0909	0.045	3110	-2	0.046			
Subadult male	0							
Period								
2100-0300	0.0446	0.016	3110	2.78	0.005			
0300-0900	0.0169	0.010	3110	1.7	0.089			
0900-1500	0.0731	0.010	3110	7.08	0.0001			
1500-2100	0							

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The 2. Continued.							
Fixed effect	Diving focus	SE	df :	t	Р		
Region							
Northwest PWS Central PWS	-0.0544 -0.0486	0.034 0.025	3110 3110	-1.61 - 1.98	$0.108 \\ 0.048$		
Copper River Delta Middleton	0.0933 0	0.037	3110	2.53	0.011		
Covariance parameter			Parameter estimate	Proportion	Proportion of total error variance		
Seal (random effect variance)			0.0084		0.132		
Sill-Nugget (repeated-measures/autocorrelation)			0.0091 0.144		0.144		
Residual variance		0.0459	0.724				
Total error variance		0.0634 1.0		1.000			
Range (spherical model, repeated-measures) = 24.2 d							

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Table 2. Continued.

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*Figure 3.* Modeled estimates of diving focus for 47 satellite-tagged harbor seals in Prince William Sound, Alaska, by time of day averaged across all regions and sex-age classes (A), sex-age class for all times of day and regions (B), and region for all times of day and all age-sex classes combined (C). Graphed estimates are average values of parameter estimates in Table 2.

focal depth bin was deepest during midwinter (February) and shallowest in spring (Fig. 4A). Focal depth was deepest in central Prince William Sound and shallowest in Copper River Delta (Fig. 4B).

## Random Effects and Temporal Autocorrelation

For each analysis a random effect for seal was included in the model. The model error terms included two parts: a temporal autocorrelation component for repeated measurements of a seal, and an independent component (residual error). Each seal was assumed to have the same autocorrelation parameters. This model fitted considerably better, as judged by likelihood equations, when compared to a simple fixed-effects model. The error variance of a simple fixed-effects model would include deviations from the model that we had accounted for by including temporal autocorrelation and the random effects of individual differences in seal behavior. Temporal autocorrelation accounted for 13%-26% of the total variance (random effect variance + autocorrelated error variance + independent error variance, Table 1–3). The estimated range of autocorrelation for the model errors was 9.9-24.2 d. The estimated variance of the random effects for seals for each analysis (normal distribution, mean = 0) accounted for 5%-30% of the total error variance (Table 1–3).

## DISCUSSION

The modeling and statistical approach we present here was developed to overcome some of the problems inherent in analysis of temporally autocorreTable 3. Stepwise mixed models statistics and parameter estimates for focal depth models of harbor seals in Prince William Sound. Estimate is for any time-in-water and for focused diving (F > 0.5, n = 2,485 6-h periods, 47 seals). Covariance parameters for random effects and temporal autocorrelation are compared to the "total error variance" that would have been present in a simple fixed-effects model (total error variance = random effect variance + autocorrelation + residual variance).

Stepwise mixed models	statistics (with	random seal e	ffect but i	no repeated	l measures)	
Variables in model	Type III F-statistic		P			
Sex	1.1	0.290				
Age	0.9	9		0.321		
Month	6.5	1		0.0001		
Period .	1.7	0	0.164			
Region	27.6	9	0.0001			
Month, region	4.77, 2	1.76	0.0001, 0.0001			
Parameter estimat	tes from best re	peated measur	res model	for focal d	epth	
	Focal					
Fixed effect	depth bin	SE	df	t	Р	
Intercept	1.3455	0.108746	46	12.37	0.0001	
Month						
January	0.1009	0.090	2425	1.13	0.260	
February	0.2425	0.097	2425	2.5	0.012	
March	-0.0265	0.110	2425	-0.24	0.810	
April	-0.1036	0.113	2425	-0.92	0.358	
May	-0.1934	0.101	2425	-1.92	0.055	
June	-0.1668	0.106	2425	-1.57	0.115	
July	-0.0366	0.127	2425	-0.29	0.772	
September	0.0339	0.101	2425	0.34	0.736	
October	-0.0614	0.080	2425	-0.77	0.443	
November	-0.0050	0.082	2425	-0.06	0.952	
December	0					
Region						
Northwest PWS	-0.0510	0.106	2425	-0.48	0.632	
Central PWS	0.1440	0.073	2425	1.98	0.048	
Copper River Delta	-0.3443	0.109	2425	-3.16	0.002 -	
Middleton	0			•		
				Pr	oportion of	
			Para	ameter (	otal error	
Covariano	estimate variance					

*		
Seal (random effect variance)	0.16	0.296
Sill-Nugget (repeated-measures/autocorrelation)	0.14	0.259
Residual variance	0.24	0.444
Total error variance	0.54	1.000
Range (spherical model, repeated-measures) = $9.9 \text{ d}$		



Figure 4. Modeled estimates of focal diving depth by month for all areas combined (A) and region averaged across all months (B), from parameter estimates in Table 3. Parameter estimates for this analysis are in units of depth bins where bins are numbered from 0 (4–20 m) to 5 (>200 m). In this figure, a depth index value of 1 corresponds to the 20–50-m bin, and a value of 2 to the 50–100-m bin.

lated SDR bin-type data with substantial individual variability among seals. Individual variability and temporal autocorrelation were significant factors in all three aspects of our analysis, accounting for a substantial part of the total error variance. Temporal autocorrelation in the data was detected over periods of many days. Individual variability, as measured by the random effects fit, encompassed a significant portion of the variation seen in the sample as a whole. Not surprisingly the focal-depth analysis demonstrated the strongest autocorrelation, as well as the greatest seal-to-seal variance. This is consistent with the facts that harbor seals often dive repeatedly to the bottom to feed, these feeding dives are often geographically clumped, and individual seals may use different areas and habitats for feeding (Boness *et al.* 1994, Ries *et al.* 1997, Tollit *et al.* 1998, Lesage *et al.* 1999, Lowry *et al.* 2001).

One problem with analyzing binned data is that the actual depth of any given dive is unknown. Some studies have analyzed data on a bin-by-bin basis, essentially studying diving behavior within each bin separately (Heide-Jørgensen and Dietz 1995, Heide-Jørgensen *et al.* 1998, Burns *et al.* 1999, Teilmann *et al.* 1999). Others have incorporated information from all bins by calculating a "mean depth" for each histogram period based on the assumption that the average depth of dives within each bin was equal to the bin midpoint (Mate *et al.* 1995, Merrick and Loughlin 1997, Burns and Castellini 1998, Folkow and Blix 1999). Results of comparisons between TDR and binned SDR data for Weddell seal pups suggest this assumption is reasonable (Burns and Castellini 1998). However, while foraging can be inferred from TDR dive profiles, foraging cannot be readily inferred from binned SDR data. For this reason, it is particularly important to explore ways of restricting analyses to subsets of the data that are more likely to represent foraging.

Many studies of harbor seal dive behavior have been conducted in regions where seals dive and forage in relatively shallow areas (<50 m, Boness *et al.* 1994, Coltman *et al.* 1997, Tollit *et al.* 1998, Lesage *et al.* 1999). In Prince William Sound the horizontal foraging ranges of seals are fairly similar to those for harbor seals in other areas (Lowry *et al.* 2001), but the bathymetry is highly variable. Depths of <50->200 m are available to seals within just a few kilometers of their haul-outs. Thus, seals using the same haul-out may

forage in very different water depths and habitats within a short period. When summary statistics from bin data (e.g., mean depth) are summed over periods without regard to diving focus (e.g., Merrick and Loughlin 1997, Folkow and Blix 1999), the results may be misleading. For example, summary histograms may imply non-selective use of the water column if seals usually dive to the bottom but water depth varies. In fact, diving in such an instance is highly focused but the habitat is variable. In contrast, the approach we used directly accounted for differences in focus and variability between individuals. We suggest that bin-type data can be more informative if an assessment of diving focus is conducted. Estimates of focal depth, together with information about bathymetry and prey availability, are likely to be more useful than summary statistics in determining when, where, and upon what animals are feeding.

Time-in-water measured for seals in this study is within the range of values reported for harbor seals in other areas, for example 61%-93% in Moray Firth, Scotland (Thompson *et al.* 1998), and 76\%-93% in the Dutch Wadden Sea (Ries *et al.* 1997), but somewhat lower than reported values of 90% or more for hooded seals (*Cystophora cristata*; Folkow and Blix 1999), northern elephant seals (*Mirounga angustirostris*; Le Boeuf *et al.* 1989), and southern elephant seals (*M. leonina*; Campagna *et al.* 1995). During September through April seals in this study spent more than two-thirds of their time in the water; then, time in the water decreased linearly to only 40% by July. The decline in time-in-water during May-July indicates that harbor seals spend more time hauled out as they become involved in activities such as pupping, breeding, and molting, a pattern also seen in other phocids (Lowry *et al.* 1980, Burns 1981, Thompson *et al.* 1989).

Merrick and Loughlin (1997) suggested that Steller sea lions in the Gulf of Alaska spent less time foraging and more time on land in spring and summer because prey were more abundant near haul-outs. In Prince William Sound some harbor seal prey are more abundant, and occur closer to shore, in summer than at other times of year. Energy-rich capelin (Mallotus villosus) and eulachon (Thaleichthys pacificus) winter offshore, but approach the coast to spawn in spring and early summer (Barraclough 1964, Anthony et al. 2000). Sand lance (Ammodytes hexapterus) swim above the sand in dense schools only during summer, when they are also highest in energy content (Robards et al. 1999). Salmon (Oncorhynchus spp.) smolt move offshore in spring, and adults return to nearshore areas to spawn in summer. In this study time-in-water decreased during May-July for seals of both sexes and a broad range of weights (28-105 kg). Pitcher (1986) showed that Prince William Sound harbor seal blubber thickness, and the percent of body weight made up by hide and blubber, increased during May-July. This suggests that in spring and summer harbor seals can obtain more energy with less time spent foraging than they can at other times of year.

Our analysis indicates that age and sex affect diving focus, with adult females showing greater focus than adult males or subadults (Fig. 3B). However, it is unclear whether adult females were really more focused in their diving, or whether regional bathymetry and the age and sex composition of our sample influenced these results. Ten of 15 adult females were tagged in the shallow Port Chalmers region of southcentral Prince William Sound. Two other adult females were tagged at haul-outs only a few kilometers away which were also surrounded by shallow water. Nonetheless, within a 25-km radius of these haul-outs, females had access to water depths exceeding 250 m. More than 90% of the at-sea locations for harbor seals departing from and returning to the same haul-out in Prince William Sound were within 25 km of that haulout (Lowry *et al.* 2001). Thus, it would appear that model results indicating high focus by adult females were not simply an artifact of sample distribution.

Harbor seals spend most of their time within 50 km of their haul-outs and are generally considered to feed in shallow, nearshore waters (Brown and Mate 1983, Thompson 1993, Suryan and Harvey 1998, Lowry et al. 2001). Studies in both North America (Boness et al. 1994, Coltman et al. 1997, Lesage et al. 1999) and Europe (Bjorge et al. 1995, Tollit et al. 1998) report modal dive depths of 60 m or less. Although some seals we tagged made dives to at least 480 m (Frost and Lowry, unpublished data), our analysis of seals diving in Prince William Sound and the nearby Gulf of Alaska indicated focal depths between 20 and 100 m (depth index 1-2, Fig. 4). This apparent preference for 20-50-m and 50-100-m depth bins was exhibited in all months, and all regions except the Copper River Delta where bottom depths rarely exceed 20 m and diving was consequently shallower. The varied bathymetry within central, northern, and western Prince William Sound made it difficult to determine when focal depths were limited by bathymetry in those regions, but seals diving around Middleton Island certainly had access to all six depth bins. Focal depth was somewhat greater in winter than in summer (Fig. 4A), suggesting that prey were less accessible in shallow nearshore waters at this time. The modal depth of Steller sea lions foraging in the northern Gulf of Alaska was also deeper in winter than in summer (Merrick and Loughlin 1997).

Diving effort as defined in this study included all time a seal was wet, even when it was near the surface in water <4 m. This is similar to VHF tagging studies where effort includes all time the transmitter is underwater (e.g., Rieset al. 1997), but in contrast to many SDR and TDR studies which have restricted analyses to dives greater than some minimum depth, usually 4-12 m (Boness et al. 1994, Le Boeuf et al. 1996, Coltman et al. 1997, Burns and Castellini 1998, Folkow and Blix 1999, Lesage et al. 1999). For large, deepdiving phocids such as elephant seals, it is unlikely that exclusion of time spent in such shallow water significantly biases interpretation of diving behavior, because more than 90% of their time is spent making prolonged deep dives (Le Boeuf et al. 1989). However, we suggest that exclusion of very shallow dives may greatly underestimate diving effort by harbor seals, and potentially bias conclusions about foraging. Fifty-four percent of the total dives of harbor seals in the St. Lawrence estuary in eastern Canada, and 20% of the dives by male harbor seals at Sable Island, Nova Scotia, were <4 m deep (Coltman et al. 1997, Lesage et al. 1999). In this study not all SDRs were programmed to record dives <4 m in a separate bin, but from the 13 that did, it is apparent that seals spent 40%-60% of their time during SeptemberMay in water <4 m (Frost and Lowry, unpublished data). Without additional sensors, SDRs provide no indication of what seals are doing in such shallow water. However, when Lesage *et al.* (1999) deployed stomach-temperature sensors on seals with TDRs, they found that 40% of the documented feeding events were at depths <4 m.

Like most other TDR and SDR-based studies, our analyses of diving focus and focal depth included only data for dives >4 m. This approach likely reduced inaccuracies due to dives near the resolution of pressure sensors and noise introduced by wave height, but it also quite clearly eliminated a substantial proportion of the total dives made by a seal (Lesage *et al.* 1999). Thus, it is likely that our focal depth analysis overestimates the preferred diving depths of harbor seals in the study area. While many of the dives made by seals in such shallow water may simply be associated with going to and from haul-outs, or with time spent near the surface between other dives, clearly some foraging may occur at this depth. Future studies of the diving behavior of species such as the harbor seal would be greatly facilitated by using instruments with pressure sensors that are more accurate at shallow water (*e.g.*, Tollit *et al.* 1998) from the simple absence of useful data.

Seasonal changes in focal depth, in combination with movements data for these same seals (see Lowry *et al.* 2001) suggest that deeper diving during winter coincided with movements to offshore areas of the Gulf of Alaska. We think it is likely these changes occurred as energy-rich prey such as eulachon, herring (*Clupea pallasi*), and salmon, which spawn nearshore but move to deeper water or offshore at other times of year, became less available. Recent and historical information on harbor seal diets in Prince William Sound and adjacent areas of the Gulf of Alaska indicate that pollock (*Theragra chalcogramma*) are a major dietary component in September–April (Pitcher 1980; Frost and Lowry, unpublished data). Small pollock of the size classes eaten by harbor seals are generally found in the Gulf of Alaska in near-bottom waters 150–200 m deep (Lowry *et al.* 1988, Muigwa 1989, Sample and Bakkala 1989).

Seasonal differences in time-in-water and focal depth were not reflected in diving focus, which showed no significant seasonal change. Seals concentrated their diving within only a few depth bins at all times of year, and dives were not distributed randomly among all available depth bins for any month. The focused nature of harbor seal diving is consistent with seals foraging on benthic prey or prey concentrated in layers within the water column. Seasonal changes in focal depth presumably reflect prey layers migrating vertically, and/or seals migrating horizontally and foraging in areas of different bathymetry.

Regional differences in diving focus reflect regional bathymetry. Seal diving was less focused in regions characterized predominantly by deeper water, such as central and northwestern Prince William Sound, and more focused in regions characterized by shallow water, such as Copper River Delta. When diving in shallow water, a seal can choose from only one or two depth bins, thus the focus variable is constrained to be greater than 0.5. In deep water, however, a seal can choose from all six depth bins, and the focus variable can range as low as 0.167. It is notable that the intercept for the diving focus model is 0.69, and the minimum diving focus predicted for any sex-age, period, or region is 0.54. Thus, even in regions where all six depth bins occur, there is a strong tendency for seals to focus their diving effort within one or two depth bins. Harbor seals in Scotland showed similarly high focus in their diving, with more than 90% of the telemetered seals exhibiting a relatively high use of one depth category (Tollit *et al.* 1998).

Harbor seal diving behavior was significantly linked to time of day, as reflected in significant changes in time-in-water and focus among the four 6-h time periods. Time-in-water increased steadily from a low in the morning (0300–0900) to a high at night (2100–0300). Seals spent 55 min more per 6-h period in the water at night than in early morning. Diving was more focused at night, and secondarily in midday, suggesting that seals were targeting prey at particular depths. Similar nocturnal foraging behavior has been observed for harbor seals in other areas of North America and in Europe (Thompson *et al.* 1989, Olesiuk *et al.* 1990, Boness *et al.* 1994). Such diurnal differences in diving behavior by many pinnipeds may reflect the behavior of diel migrating prey, which are more accessible at night (Le Boeuf *et al.* 1989, Hindell *et al.* 1991, Goebel *et al.* 1991, Folkow and Blix 1999). We did not detect a change in focal depth by time of day, as would be expected if seals were foraging on prey that have a diel vertical migration.

Our data on the diving behavior of harbor seals have significant implications for aerial surveys used to assess seal abundance. Many surveys are conducted during the molting period in August-September, and there may be considerable annual variation in survey dates due to weather and tides (Harvey *et al.* 1990, Olesiuk *et al.* 1990, Thompson and Harwood 1990, Frost *et al.* 1994). The abrupt increase in time-in-water between July and September (>30% increase) suggests that the timing of surveys may have a substantial effect on the number of seals counted. In fact, aerial survey data collected from mid-August to mid-September in Prince William Sound clearly demonstrate how large this effect can be. Frost *et al.* (1999) determined that counts made in mid-September would be 45% lower than counts in mid-August. In northeast Scotland harbor seal counts were also substantially lower in September than they were in June-August (Thompson *et al.* 1997). Thus, it is essential that surveys conducted to assess population trends be standardized for date, or the analysis must incorporate the effect of date (Frost *et al.* 1999).

## Acknowledgments

This study was conducted as part of the *Exxon Valdez* Oil Spill Restoration Program, funded by the *Exxon Valdez* Oil Spill Trustee Council. Many people assisted in the capture and tagging of seals. We especially thank Jay Ver Hoef, Rob DeLong, and Brian Fadely, and also Vladimir Burkanov, Jennifer Burns, Maggie and Mike Castellini, Tracey Gotthardt, Shane Kanatous, Dan Reed, Bob Small, Ken Taylor, Pam Tuomi, and Randy Zarnke. The Captain, Glen Hodge, and the crew of the research vessel *Pacific Star* provided excellent logistic support. Jay Ver Hoef assisted with data analyses. Ken Pitcher, Jennifer Burns, and Richard Merrick provided useful comments on a draft of the manuscript. This study was conducted under authorization of National Marine Fisheries Service Scientific Research Permits 770 and 1000 issued to the Alaska Department of Fish and Game.

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Received: 22 June 2000 Accepted: 26 January 2001

## J8731

# Comparison of the Bligh and Dyer and Folch Methods for Total Lipid Determination in a Broad Range of Marine Tissue

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**ABSTRACT:** For many studies, it is important to measure the total lipid content of biological samples accurately. The Bligh and Dyer method of extraction was developed as a rapid but effective method for determining total lipid content in fish muscle. However, it is also widely used in studies measuring total lipid content of whole fish and other tissues. Although some investigators may have used modified Bligh and Dyer procedures, rarely have modifications been specified nor has their effectiveness been quantitatively evaluated. Thus, we compared this method with that of the classic Folch extraction in determining total lipid content of fish samples ranging from 0.5 to 26.6% lipid. We performed both methods as originally specified; i.e., using the chloroform/methanol/water ratios of 1:2:0.8 and 2:2:1.8 (before and after dilution, respectively) for Bligh and Dyer and of 8:4:3 for Folch, and with the initial solvent/sample ratios of (3+1):1 (Bligh and Dyer) and 20:1 (Folch). We also compared these with several other solvent/sample ratios. In samples containing <2% lipid, the results of the two methods did not differ. However, for samples containing >2% lipid, the Bligth and Dyer method produced significantly lower estimates of lipid content, and this underestimation increased significantly with increasing lipid content of the sample. In the highest lipid samples, lipid content was underestimated by up to 50% using the Bligh and Dyer method. However, we found a highly significant linear relationship between the two methods, which will permit the correction of reported lipid levels in samples previously analyzed using an unmodified Bligh and Dyer extraction. In the future, modifications to procedures and solvent/sample ratios should be described.

Paper no. L8731 in *Lipids 36*, xxx–xxx (October 2001).

The total lipid content of biological samples is an important quantity used in many biochemical, physiological, and nutritional studies. Thus, reliable methods for the quantitative extraction of lipids from tissues are of critical importance. Natural lipids generally comprise mixtures of nonpolar components such as glycerides (primarily triacylglycerol) and cholesterol, as well as some free fatty acids and more polar lipids. Isolation, or extraction, of lipid from tissues is performed with the use of various organic solvents. In principle, the solvent or solvent mixture used must be adequately polar to remove lipids from their association with cell membranes and tissue constituents but also not so polar that the solvent does not readily dissolve all triacylglycerols and other nonpolar lipids (1). Folch *et al.* (2) were one of the first to recognize this and develop the chloroform/methanol/water phase system (the so-called "Folch" method), which, under various modifications, continues to be considered the classic and most reliable means for quantitatively extracting lipids. In the interest of economy, less exhaustive methods have been developed. By far the best known is the "Bligh and Dyer" method (3), which has become one of the most recommended methods for determining total lipid in biological tissues (4,5) and indeed has become the standard for lipid determination in many studies of marine fish (e.g., 1–12) as well as for other types of samples such as milks (e.g., 13,14).

The primary advantage of the Bligh and Dyer method is a reduction in the solvent/sample ratio (1 part sample to 3 parts 1:2 chloroform/methanol followed by 1 or 2 parts chloroform) (1,3). In contrast, the Folch method employs a ratio of 1 part sample to 20 parts 2:1 chloroform/methanol, followed by several washings of the crude extract (2). Despite this solvent reduction, the Bligh and Dyer method is nevertheless thought to yield recovery of  $\geq 95\%$  of total lipids (1). Although the procedure was developed using cod muscle, it states (1,3) that it can be applied to any tissue containing (or modified to contain) 80% water. Hence, it has been used ubiquitously. Although the Bligh and Dyer method has undergone rigorous and favorable evaluations (e.g., 5,9,16), virtually all of these evaluations have been performed on samples containing less than 1.5% total lipid. Some studies report using a modified Bligh and Dyer method for lipid-rich samples; however, the modifications are often unspecified (e.g., 15), making the evaluation and comparison of results difficult. In other cases, investigators report the use of the Bligh and Dyer method even with samples having high lipid contents, but do not indicate that any modifications have been made. In the course of recent studies in our laboratory, we discovered that samples of a known high lipid content were greatly underestimated using the Bligh and Dyer method compared to the Folch method, although we did not detect any difference in the fatty acid composition under either method. Since much of the data published on the lipid contents of whole fish and other samples have been derived using the Bligh and Dyer method, we undertook a study to evaluate the relationship between these methods in their estimation of total lipid content.

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#### MATERIALS AND METHODS

Fish and invertebrates were chosen to represent a wide range of lipid contents based on previous species estimates. A total of 36 individuals were used, which included pollock, herring, rock sole, rock fish, sculpin, octopus, and squid. Each whole animal was thoroughly ground and homogeneous subsamples were taken for extraction. To increase the range of lipid contents evaluated, we also used weighed aliquots (n = 9) of a homogeneous mixture of ground commercial fish (originally containing 2% fat) and commercial fish oil. Weighed quantities of oil were added to produce mixtures ranging from an estimated 21 to 26% lipid. Our primary interest was to evaluate the Bligh and Dyer method compared to the Folch method, but because of the high solvent volumes used in the Folch, we also evaluated the performance of a reduced-solvent Folch using a subset of these samples. Within each method, all samples were extracted and lipid contents were quantified in duplicate.

The Bligh and Dyer extraction was performed as originally outlined using the following ratios (1,3): Briefly, 100 g sample containing (or adjusted to contain) 80 g water (as determined by oven drying separate aliquots) was homogenized with 100 mL chloroform and 200 mL methanol (monophasic system). The solution was rehomogenized with 100 mL chloroform, following which 100 mL of either distilled water (3) or weak salt solution (e.g., 0.88% NaCl or KCl) (1,9) was added. After filtration was performed under suction, the final biphasic system was allowed to separate into two layers and the lower (chloroform) phase was collected. For quantitative lipid extraction (3), the tissue residue was then rehomogenized with 100 mL chloroform, filtered, and the filtrate added to the lower phase collected. Lipid content was then determined gravimetrically after evaporating a measured aliquot of the combined chloroform phase to dryness under nitrogen (see below). As Bligh and Dyer stated (3,16), the above volumes can be scaled down, as long as the critical ratios of chloroform, methanol, and water (1:2:0.8 and 2:2:1.8, before and after dilution, respectively) and of initial solvent to tissue [(3+1):1] are kept identical. Thus, we followed the above procedures but reduced the scale of all components (i.e., keeping all ratios the same) for use with a smaller sample amount (4 g sample in a 40 mL conical glass centrifuge tube), to allow both centrifugation of the final biphasic system and collection of the entire lower phase for evaporation and subsequent lipid estimation. Instead of applying manual pressure (3) to the small filter cake, we performed a second chloroform wash to improve removal of residual lipid during filtration.

The Folch extractions were performed as described, using the original extraction ratio of 20 parts 2:1 chloroform/ methanol to 1 part tissue, which can be done on any scale that is technically feasible (2). A weak salt solution (e.g., 0.58–0.88% NaCl or KCl) was then added to achieve a final ratio of 8:4:3 chloroform/methanol/water after including the water contained in the tissue (1,2). We also compared the original ratio against a modified version using 30 parts 2:1 chloroform/methanol to 1



**FIG. 1.** Estimates of total lipid content determined in replicate aliquots: (A) of samples (n = 27) extracted using both using a 20:1 and a 30:1 solvent/sample ratio Folch and (B) all samples (n = 45) using the Bligh and Dyer method in comparison with the original Folch method. The last nine samples on the x-axis represent the homogenates of commercial fish and oil, which were produced to contain a range of 21–26% lipid. All samples were analyzed in duplicate in each of the extraction methods and are presented in approximate order of increasing lipid content.

part tissue (1). After verifying that the 20:1 and 30:1 solvent/sample ratios produced similar results in our samples (n = 27, all <25% lipid; Fig. 1A), we analyzed the rest of the samples using only the 20:1 ratio as follows: 1.5 g tissue was homogenized with 30 mL 2:1 chloroform/methanol. Although Christie (1) reports improvement by first homogenizing with 10 mL methanol followed by 20 mL chloroform, we have tested both procedures without detecting differences (Iverson; S.J, Lang, S.L.C., and Cooper, M.H., unpublished results). The mixture was filtered and then washed several times with 2:1 chloroform/methanol, and 0.88% NaCl in water was added to the combined filtrate at a final ratio of 8:4:3 chloroform/methanol/water. Finally, we used a "reduced-solvent" Folch, where the ratios of solvent to sample were 7.5:1.0 (i.e., closer to that of the Bligh and Dyer method), but the chloroform/methanol/water ratio was kept the same (i.e., 8:4:3).

In all the above extractions (both Bligh and Dyer and Folch), the final biphasic system was centrifuged, and the entire lower phase (along with washings) was collected into a preweighed glass tube and evaporated to dryness in an analytical high-speed nitrogen evaporator (24-position N-EVAP 112, Organomation Associates, Inc., Shewsburg, MA) fitted with stainless steel 14-inch  $\times$  19-gauge needles and equipped with a thermostatically-controlled water bath maintained at 25–30°C. The nitrogen stream was continually moved so that it actively disturbed the evaporating surface of the sample until all detectable traces of solvent were gone. To remove all final traces of solvent and water, the sample tube was then wiped dry and placed in a sealed glass vacuum tube and flushed with nitrogen, and vacuum suction was applied for 5 min (Boc Edwards model RV3 vacuum pump, San Francisco, CA). Lipid content was then determined gravimetrically. Since results of the Folch method using 20:1 or 30:1 solvent/sample ratio did not differ, we used the results from the 20:1 Folch method as the basis for comparison with and evaluation of the other extraction methods.

## RESULTS

In general, duplicate analyses within each extraction method were very consistent, although more so for Folch extractions (n = 45, Fig. 1B). In samples containing <2% lipid (n = 11), results for the Bligh and Dyer method did not differ from those obtained by the Folch method (P = 0.150, paired *t*-test). However, for samples containing >2% lipid (n = 34), the Bligh and Dyer estimates of lipid content were significantly lower than those of Folch (P < 0.0001). In our nine samples of fish oil-supplemented homgenates, lipid content estimates (20.6–26.6%) using Folch extraction concurred with our estimated lipid contents (21-26%, as discussed in the Methods and Materials section), however, lipid content estimates using the Bligh and Dyer extraction were 50% lower (Fig. 1B). The next highest lipid contents were found in herring samples (n = 12, 10.7-18.6% lipid by Folch), which were estimated to be about 45% lower (6.1-11.6% lipid) using the Bligh and Dyer method.

The underestimation of lipid content by the Bligh and Dyer method increased significantly with increasing lipid content (Fig. 2A). From 0% to approximately 2% lipid, results of the two methods agreed well. However, with increasing lipid content, the deviation from the one-to-one reference line increased. We were interested in describing the predictive relationship between the two methods to allow correction of previous lipid content analyses that we had performed using the Bligh and Dyer method. Using a log-log plot, we found a highly significant linear relationship between lipid content determined by the Folch method and that determined by the Bligh and Dyer method (Fig. 2B).

The results of the reduced-solvent Folch (7.5:1.0 solvent/sample ratio) were highly correlated with both the 20:1 and 30:1 Folch (*r* = 0.999, *n* = 34 and *r* = 0.987, *n* = 27, respectively); however, the reduced-solvent method tended to underestimate lipid content as lipid content increased. In samples containing  $\leq 3\%$  lipid (*n* = 19), there was no significant difference between the Folch extractions using the 20:1 vs.

1.0 . 8 .6 .4 F = 0.047 + 1.211 (B&D)go r2 .2 = 0.980 < 0.0001 Ρ 0 -.2 .4 .6 .8 1.0 1.2 1.4 1.6 .4 -.2 0 .2 log % lipid (Bligh & Dyer) FIG. 2. (A) Correlation of the estimates of lipid content (duplicates aver-

aged) in 45 samples using the Folch (20:1) vs. Bligh and Dyer methods (r = 0.9834, P < 0.0001); the dashed line represents the one-to-one reference line. (B) The log-log predictive relationship between estimates of lipid content using the Folch vs. the Bligh and Dyer method.

the 7.5:1 solvent/sample ratios  $(1.9 \pm 0.16\% \text{ vs. } 1.9 \pm 0.18\%$ lipid, respectively; P = 0.9559, paired *t*-test), but in samples containing >3% lipid (n = 15), the reduced-solvent Folch significantly underestimated lipid content (10.7  $\pm$  1.18% vs. 12.0  $\pm$  1.30%, P < 0.0001). The lipid content estimates of these same 15 samples, using the Bligh and Dyer method, were even lower at  $7.2 \pm 0.65\%$  lipid. In the highest-lipid natural fish sample tested (herring), lipid content was estimated as 18.6, 16.4, and 11.6% using the 20:1 Folch, the 7.5:1.0 Folch, and the Bligh and Dyer methods, respectively.

## DISCUSSION

In the time since the Folch (2) and the Bligh and Dyer (3) methods for total lipid determination were published, there have undoubtedly been numerous modifications to both methods to improve the efficiency of lipid recovery from various tissues. However, in many publications where these methods have been used, modifications have been neither described nor validated. In other cases, investigators stated that lipids were quantified "according to" one or the other method, but they do not indicate whether any modifications were made,



implying that the methods were applied basically according to the original procedures, even though that may not have been the case. Given that many conclusions about tissue and whole-body lipid and energy values are based on published lipid contents, our purpose was to evaluate these two methods, basically as originally described, with the aim that investigators could evaluate previously published data and that appropriate modifications would be made and described in the future.

In numerous tests with samples containing < 2% lipid, the Bligh and Dyer method has been shown to be very effective and reliable (4,5,9,16). Like other investigators (5), we found that lipid extraction using the Bligh and Dyer method produced estimates of total lipid content identical to those of Folch in samples containing <2% lipid. We also did not detect any differences in the subsequent fatty acid composition of duplicate samples extracted under either method, although this may require further investigation in very low fat samples that contain a higher phospholipid/neutral lipid ratio (e.g., alkali hydrolysis followed by methylation and fatty acid quantitation could also be used to examine any biases in total fatty acid recovery). However, in contrast to low-lipid samples, in all samples containing >2% lipid, the Bligh and Dyer method produced significantly lower estimates of lipid content, and this underestimation increased with increasing lipid content of the sample.

We have several reasons to believe that the total lipid contents of all samples were accurately determined using the Folch extraction method. First, as stated above, in low-lipid samples both the Folch and Bligh and Dyer results were identical. Second, the estimates of percent lipid in the high-lipid fish oil-supplemented homogenates, using the basic Folch extraction, agreed with our calculated lipid contents; furthermore, an increased (30:1) solvent/sample ratio Folch produced the same values. Finally, these homogenates were also analyzed for protein content (by macro-Kjeldahl), as well as dry matter (M.H. Cooper, unpublished data). The amount of dry matter not accounted for by protein and lipid in these samples was reasonably consistent with the expectation at 2–4% using the lipid values obtained by Folch extractions, but was quite high (14-20%) using the lipid values obtained by the Bligh and Dyer extractions.

Bligh and Dyer (3) developed their method using fish fillets (i.e., muscle) that generally contained low levels of lipid and a high proportion of phospholipid. In whole animals and in tissue, an increase in total lipid content is due predominantly to increases in triacylglycerol. Indeed, subsets of our isolated lipid subjected to thin-layer chromatography (17) showed that the primary component in the extract was triacylglycerol (especially as lipid content increases), followed by minor amounts of more polar lipid classes. Although Bligh and Dyer (3) stated that their method could readily be applied to other biological tissues, they, as well as others, acknowledged that lipid-rich samples may require modifications. For instance, Christie (1) suggested that very lipid-rich tissues such as adipose tissue and oil seeds should be extracted first with a nonpolar solvent such as diethyl-ether or chloroform, after which the remaining lipid could be recovered effectively using Bligh and Dyer methods. However, this appears to have often gone unrecognized. The total yield of lipids may be more reduced than most investigators have suspected, especially given the wide-scale use of apparently unmodified Bligh and Dyer extractions for whole fish and other tissues. Even in samples containing 2–10% lipid (which is common for many marine fish and invertebrates), underestimation will still be a significant problem (e.g., Fig. 1), and this has likely been neglected.

The reduced efficiency of the Bligh and Dyer method with increasing tissue lipid contents might be explained from several standpoints. One cause of reduced lipid yield at high lipid concentrations could be the limited solubility of the predominantly nonpolar lipids, such as triacylglycerols, in the seemingly relatively polar solvent solution (1:2 vol/vol chloroform/methanol) employed in the Bligh and Dyer method, which was designed chiefly to extract phospholipid efficiently. However, although the initial solvent ratios are different in the Bligh and Dyer vs. the Folch methods, they do not result in measurably different contents of methanol in the final organic (chloroform) phase (e.g., 16). Hence, this is not likely to be a significant factor. Smedes and Thomasen (16) found that the absorption of the organic phase by the tissue was one of the main causes of incomplete lipid yield. Relatively constant amounts of the organic phase are absorbed by the tissue such that using greater volumes of organic-phase solvents reduces the fraction of the organic phase that is lost in this manner (16). When tissues with increasing lipid contents are extracted (using the same volumes of solvents), the lipid concentration in the organic phase should also increase, assuming that limits of solubility are not reached. This would result in increased loss of lipid in the fraction of organic phase absorbed by the tissue, causing a reduction in final lipid yield. Thus, in addition to maintaining critical solvent and water ratios, perhaps the most important consideration is simply the ratio of solvent to dry-weight sample (and expected fat content), as even with the Folch method, a reduced ratio produced significant underestimates of lipid content.

Our results do indicate that all methods used to estimate lipid contents were highly correlated. Fortunately, there is a highly predictable relationship between the Bligh and Dyer and Folch methods (Fig. 2B), potentially allowing correction of reported values from previous analyses that used an unmodified Bligh and Dyer extraction. It may also be the case that investigators have used a modified Bligh and Dyer extraction employing an increased solvent/sample ratio that produced reliable results and have simply not stated this. It will be important in the future that investigators specify modifications to any of these procedures, especially the precise solvent/sample ratio used. For instance, although an increase in the solvent/sample ratio (i.e., to 30:1) from the original Folch did not appear to alter the estimated lipid content significantly (Fig. 1A), we would not recommend making this assumption for tissues containing greater than 25% lipid (i.e. adipose tissue, milks of many species) unless verified. In such samples, a further increase in the solvent/sample ratio and/or further multiple extractions may be necessary for quantitative lipid evaluation (e.g., 1), as we have found for marine mammal milks (personal communication).

## ACKNOWLEDGMENTS

This study was supported by grants to Sara J. Iverson from the *Exxon Valdez* Oil Spill Trustee Council, Alaska, and by the Natural Sciences and Engineering Research Council (NSERC, Canada). We thank L. Smith for assistance with some sample analyses and S. Budge for helpful comments. We are also grateful for the suggested improvements to the manuscript by the editor and three anonymous reviewers.

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[Received January 19, 2001; in revised form October 10, 2001; accepted October 12, 2001]

1.msp. 4, lines 64, 65: May I change "homogenous" to "homogeneous"?

2.msp. 5, line 82: This reference is somewhat confusing. Should it read "As Bligh and Dyer (3) and Smedes and Thomasen (16) stated?

3.msp. 6, line 101: Are the names I have inserted for these unpublished results the same as for this paper?

4.msp. 6, line 109: Is the location of Organomation Associates, Inc. correct as Shewsbury, MA?

5.msp 7, lines 114-115: Correct that the company name of Edwards is BOC Edwards, located in San Francisco, CA?

6.msp. 12, line 231: The first initials and last name(s) of the recipients of the personal communication will need to be added.

7.msp. 12, line 241: Would you please provide the full first name of L Smith and S. Budge.

8.msp. 13, line 248 in Ref. 2: Is the spelling "Lipides" correct as shown?

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Thank you, Susan Krusemark Copy editor

## QUANTITATIVE FATTY ACID SIGNATURE ANALYSIS: A NEW METHOD OF ESTIMATING PREDATOR DIETS

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Abstract. Accurate estimates of the diets of predators are required in many areas of ecology, but for many species current methods are imprecise, limited to the last meal, and often biased. The diversity of fatty acids and their patterns in organisms, coupled with the narrow limitations on their biosynthesis, properties of digestion in monogastric animals, and the prevalence of large storage reservoirs of lipid in many predators, led us to propose the use of quantitative fatty acid signature analysis (QFASA) to study predator diets. We present a statistical model that provides quantitative estimates of the proportions of prey species in the diets of individual predators using fatty acid signatures. We conducted simulation studies using a database of 28 prey species (n = 954 individuals) from the Scotian Shelf off eastern Canada to investigate properties of the model and to evaluate the reliability with which prey could be distinguished in the model. We then conducted experiments on grey seals (*Halichoerus grypus*, n = 25) and harp seals (*Phoca groenlandica*, n = 5) to assess quantitative characteristics of fatty acid deposition and to develop calibration coefficients for individual fatty acids to account for predator lipid metabolism. We then tested the model and calibration coefficients by estimating the diets of experimentally fed captive grey seals (n = 6, switched from herring to a mackerel/capelin diet) and mink kits (Mustela vison, n = 46, switched from milk to one of three oil-supplemented diets). The diets of all experimentally fed animals were generally well estimated using QFASA and were consistent with qualitative and quantitative expectations, provided that appropriate calibration coefficients were used. In a final case, we compared video data of foraging by individual freeranging harbor seals (*Phoca vitulina*, n = 23) fitted with Crittercams and QFASA estimates of the diet of those same seals using a complex ecosystem-wide prey database. Among the 28 prey species in the database, QFASA estimated sandlance to be the dominant prey species in the diet of all seals (averaging 62% of diet), followed primarily by flounders, but also capelin and minor amounts of other species, although there was also considerable individual variability among seals. These estimates were consistent with video data showing sandlance to be the predominant prey, followed by flatfish. We conclude that QFASA provides estimates of diets for individuals at time scales that are relevant to the ecological processes affecting survival, and can be used to study diet variability within individuals over time, which will provide important opportunities rarely possible with other indirect methods. We propose that the QFASA model we have set forth will be applicable to a wide range of predators and ecosystems.

*Key words:* feeding ecology; food webs; marine carnivores; pinnipeds; predator diets; predatorprey relationships; prey fatty acid composition and signatures; statistical model.

#### INTRODUCTION

The dynamics of predator-prey relationships, the structure of food webs, and the foraging behavior of individuals are central themes in ecology (e.g., Schoener 1971, Paine 1980, Stephens and Krebs 1986, Pimm et al. 1991, Sih et al. 1998). Accurate estimates of predator diets are required to understand these areas of ecology. For some carnivores (e.g., lions [*Panthera leo*]; wolves [*Canis lupis*]; sea otters [*Enhydra lutris*])

Manuscript received 9 December 2002; revised 25 July 2003; accepted 27 July 2003; final version received 17 September 2003. Corresponding Editor: J. T. Wooten.

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direct observation of feeding can be used to estimate diet. However, for many carnivores, including cetaceans, pinnipeds, mustelids, and ursids, as well as for nonbreeding seabirds, direct observation of feeding is rarely possible and indirect methods must be used to reconstruct the diet. These indirect methods are based on the recovery of digestion-resistant prey structures from feces, stomach contents, or from spewings such as owl pellets (Gaston and Noble 1985, Pierce and Boyle 1991). While there are some differences in the way such methods are used across taxa, the principles are the same (e.g., Carss and Parkinson 1996).

Although much of our current understanding of predator diets is derived from these methods, such estimates can be biased (e.g., Jobling and Breiby 1986, Jobling 1987, Carss and Parkinson 1996). Most soft-bodied prey are difficult to identify given their rapid digestion. The diagnostic hard parts of some prey (e.g., shells of crustaceans, heads of large fish) may not be consumed by the predator or may be eroded during digestion, such that the size of prey consumed may be underestimated or the identification of prey may not be possible. Furthermore, the degree of erosion of hard parts is speciesspecific and often a function of prey size within species (Bowen 2000). Thus, differential rates of digestion among prey species may seriously bias estimates in favor of species with large and robust hard parts. Finally, these methods provide only a snapshot of the most recent meal and may not be representative of the longer term diet.

These limitations have led to the development of techniques that do not depend on the recovery of digestion-resistant hard parts (e.g., antisera to Atlantic salmon, *Salmo salar*, with limited success [Boyle et al. 1990]; stable isotope ratios of carbon and nitrogen [Rau et al. 1992, Gannes et al. 1997, Kelly 1999]). Although stable isotope ratios are useful in estimating the trophic level of a predator, they usually cannot determine the species composition of the diet (e.g., Hobson 1993, Gilmore et al. 1995, Koch et al. 1995).

A third method involves the use of fatty acid signatures (Iverson 1993). Fatty acids are the main constituent of most lipids, and unlike other nutrients, such as proteins that are readily broken down during digestion, fatty acids are released from ingested lipid molecules (e.g., triacylglycerols) during digestion, but are not degraded. The fatty acids of carbon chain-length 14 or greater pass into the circulation intact and are generally taken up by tissues the same way. Since a relatively limited number of fatty acids can be biosynthesized by animals (Cook 1991), it is possible to distinguish dietary vs. nondietary components. Once taken up by tissues, fatty acids are either used for energy or re-esterified, primarily to triacylglycerols, and stored in adipose tissue. Although some metabolism of fatty acids occurs within the predator, such that the composition of predator tissue will not exactly match that of their prey, fatty acids can be deposited in adipose tissue with little modification and in a predictable way.

Fatty acids in marine organisms are extremely diverse and have high levels of long-chain, polyunsaturated fatty acids that originate from various unicellular phytoplankton and seaweeds (Ackman 1980). Numerous studies have demonstrated that specific fatty acid patterns are passed from prey to predator near the bottom of the food web (e.g., Sargent et al. 1988, Fraser et al. 1989, Graeve et al. 1994, Navarro et al. 1995, St. John and Lund 1996, Kirsch et al. 1998) and that the fatty acid composition of zooplankton directly influences the fatty acid composition of blubber lipids of baleen whales (e.g., Klem 1935, Ackman and Eaton 1966, Hooper et al. 1973). Fatty acids have also indicated the presence of fish or other prey in the diets of terrestrial and aquatic carnivores (e.g., Johnson and West 1973, Rouvinen and Kiiskinen 1989, Wamberg et al. 1992, Colby et al. 1993, Pond et al. 1995, Raclot et al. 1998), the degree to which plants have been consumed by terrestrial carnivores (Iverson and Oftedal 1992, Iverson et al. 2001*b*), and changes in the diets of pinnipeds (Iverson 1993, Iverson et al. 1997*a*, Kirsch et al. 2000).

To date, fatty acid signatures have been used qualitatively to infer trophic levels and spatial and temporal differences in diets both within and among species (e.g., Kakela et al. 1993, R. J. Smith et al. 1996, S. Smith et al. 1997, Iverson et al. 1997a, b). However, since the pattern of fatty acids found in some plants and in many fish and invertebrates can be used to accurately identify individual species (Iverson et al. 1997b, 2001b, 2002, Budge et al. 2002), prey fatty acid signatures might provide quantitative estimates of predator diets. To do this requires an understanding of the characteristics of prey fatty acid signatures and the extent to which they differ in a given ecosystem, an understanding of how ingested fatty acids are metabolized and deposited in various tissues of the predator, appropriate sampling of predator tissue, and a statistical model that relates the predator signature to a mixture of possible prey signatures. Here we present a statistical model that provides quantitative estimates of the proportions of prey species in the diets of individual predators using fatty acid signatures. We use simulation studies to investigate the properties of the model, and controlled feeding studies of grey seals (Halichoerus grypus) and harp seals (Phoca groenlandica) to assess quantitative characteristics of fatty acid deposition. We then test the model by estimating the diets of experimentally fed captive grey seals and mink (Mustela vison), and the diets of individual free-ranging harbor seals (Phoca vitulina) filmed during natural feeding events. We used each of these systems to represent increasing complexity of diet estimation.

#### METHODS

#### The model

We refer to the quantitative distribution of all fatty acids measured in a predator or prey sample as its fatty acid signature. To estimate the composition of the predator's diet based on these signatures, we take a weighted mixture of the fatty acid signatures of the potential prey types and choose the weighting that minimizes a statistical distance from that of the predator. Each prey type (typically species, but potentially subsets of species or groupings of similar species; e.g., Iverson et al. 2002) is summarized by its mean fatty acid signature, and we estimate its proportional contribution to the predator's diet.

We proceed by first defining how close the predicted diet (i.e., the quantitative mixture of signatures) is from the true diet. We then develop the concept of "calibration coefficients," which are required to account for predator lipid metabolism and the fact that the fatty acid signature of the prey will not be laid down exactly in the predator (i.e., for some fatty acids the values observed in the predator may be always higher, or always lower, than that found in the diet; e.g., Kirsch et al. 2000). Related to the concept of calibration, is whether to estimate the diet using all fatty acids identified or a subset that might better reflect diet. Lastly, the estimated signature contribution from prey must be corrected to account for differences in fat content (and thus fatty acid contribution) among prey types. All else being equal, species with a higher fat content will contribute proportionately more to the predator signature than those with a lower fat content. However, given that we know the fat content of each prey, it is straightforward to translate the estimated signature contribution to the proportion of each prey type eaten.

*Model notation.*—To set the basic model notation, let  $y_{ij}$  denote the proportion of the *j*th fatty acid of the *i*th predator. The *i* notation will be dropped when it is clear we are referring to a single predator. Let  $x_{klj}$  denote the proportion of the *j*th fatty acid from the *l*th prey of the *k*th prey type (in this case species) and  $n_k$  the number of individual prey of type *k*. The mean  $\bar{x}_{kj}$  is the mean of the prey of type *k* for fatty acid *j*. The problem is to estimate  $\pi_k$ , the true proportion of the *k*th prey type found in the predator's diet with the estimate denoted by  $p_k$ . The estimated proportion of each prey in the diet,  $\hat{y}$ , over all fatty acids, is formed as follows:

$$\hat{y} = \sum_{k} p_k \bar{x}_k.$$

Distance measures and estimation of  $\pi_k$ .—The estimation problem is to choose  $p_k$  such that  $\hat{y}$  is "close" to y. Both y and  $\hat{y}$  sum to 1 and can be thought of as distributions over the fatty acids. In this context, the Kulback-Liebler (KL) distance (*Encyclopedia of Statistics* 1983), defined as

$$KL = \sum_{j} (y_j - \hat{y}_j) \log(y_j / \hat{y}_j)$$

is a natural choice, as it was developed to compare distributions. There are several other possible distances including the more usual squared error (SQ) distance,  $\sum_j (y_j - \hat{y}_j)^2$ , the squared relative error (REL),  $\sum ((y_j - \hat{y}_j)/y_j)^2$  and the squared error distance of the logs (LSQ),  $\sum_j (\log(y_j) - \log(\hat{y}_j))^2$ . To understand the relative behavior of these distances, we considered an absolute difference of 0.01 between the true (y) and predicted ( $\hat{y}$ ) proportion for a common, an intermediate, and a rarer fatty acid, respectively (i.e., true proportions: 0.20, 0.05, and 0.01; predicted proportions: 0.21, 0.06, and 0.02, respectively). The SQ distance attributes the same weight for all true values. However, an absolute error of 0.01 should be more serious in the rare as opposed to the common fatty acid. Hence, the other three distances, which give more weight to the differences in the rare fatty acids, are preferable; of these three distances, the KL distance does so most conservatively and proportionately.

To then estimate the  $p_k$ , we carried out an optimization over the number of prey types, k, with the  $p_k$ 's constrained to be positive and sum to 1. The starting values for the optimization have the  $p_k$ 's all equal. The optimization was carried out in S-Plus (S-Plus 2000) using the function nlminb, which is a local minimizer for smooth nonlinear functions subject to bound-constrained parameters, and uses a quasi-Newton method. However, to efficiently conduct the simulations on large, complex data sets, we used a FORTRAN optimizer from Netlib.

Standard errors of estimates.—A major source of variability comes from variation in fatty acid signatures among individuals of a particular prey type (e.g., Iverson et al. 1997b, 2002, Budge et al. 2002). To capture this variability, we carried out the following bootstrapping procedure in which we repeatedly create new prey means by sampling with replacement from the prey database.

For b = 1, ..., B, steps 1 and 2 below are carried out:

- For each prey type k, randomly select n<sub>k</sub> individuals with replacement and create a new mean x
   <sup>kb</sup>.
- 2) Carry out the estimation procedure for the bootstrap prey means and compute  $p_k^{*b}$ . The estimate of the standard error (SE) is computed as

$$SE(p_k) = \sqrt{\frac{\sum_{b} [p_k^{*b} - mean(p_k^{*b})]^2}{B - 1}}.$$

Calibration coefficients.—Calibration coefficients,  $c_j$ , were computed as follows: for a particular fatty acid,  $c_j$  is computed as the 10% trimmed mean of the following  $r_{l_i}^i$ 's:

$$r_{li}^{j} = \text{seal}_{ij}/\text{diet}_{lj}$$

for all *l* and *i*. For example, to estimate the "grey seal" calibration coefficients, we had eight seals and 30 herring. Since we could not analyze the actual herring that individual seals ate, *i* (1 to 8) indexes the seals and *j* (1 to 30) indexes the herring. This gives 240 calibration coefficients for each fatty acid, for which the 10% trimmed mean is then computed. These coefficients are then included in the distance measures by replacing the predator's observed proportion of fatty acid of type *j* by

$$z_j = \frac{y_j/c_j}{\sum\limits_s y_s/c_s}.$$

Although we used the trimmed mean across all individuals in modeling, we also estimated the 10%

trimmed mean within each individual to estimate a within-study sE for coefficients.

Fatty acid subsets.—We refer to fatty acids by the standard nomenclature of carbon chain length:number of double bonds, and the location (n-x) of the double bond nearest the terminal methyl group. In analyses of marine lipids, over 70 fatty acids can be identified and quantified, depending on the analytical methods and gas chromatograph (GC) column used (Fig. 1). However, not all fatty acids provide equal information about diet due to predator metabolism (Iverson 1993). For instance, if short- or medium-chain fatty acids (i.e., <14 carbons; also including *iso5*:0 in some cetaceans) are found in predator adipose tissue, these could arise only from biosynthesis, since any of these consumed in the diet would be immediately oxidized (Jackson 1974). In contrast, fatty acids with n-6 or n-3 double bonds or components such as 22:1n-11 generally arise only from diet; however, 22:1n-11 may exhibit reduced deposition (Bremer and Norum 1982). Other fatty acids arise from a combination of diet and biosynthesis. For instance, although both are found in prey, in predators 14:1n-5 is produced predominantly from biosynthesis, while some 22:5n-3 arises from modification (Ackman et al. 1988, Iverson 1993, Iverson et al. 1995). Fatty acids such as 16:0, 16:1n-7, 18:0 and 18:1n-9, may arise to some extent from biosynthesis in the predator, but are also highly indicative of differences in various prey (e.g., Fig. 1; Iverson 1993, Iverson et al. 2001b). Thus, for both of these latter types of fatty acids (i.e., those that always occur at predictably higher or lower levels in the predator than in prey due to some biosynthesis or some reduced deposition, respectively), calibration coefficients can be used to reduce the influence of systematic deviations on diet estimation.

Finally, some fatty acids found at low or trace levels may not be correctly identified and separated from abundant nearby peaks (e.g., 18:1n-11 from 18:1n-9; Fig. 1) depending upon the nature of the chromatographic equipment used. Therefore their detection in chromatograms can be problematic or inconsistent. Since most such fatty acids occur at low levels in carnivore tissue, these can be removed from further analysis if necessary.

In the present study, we did not use the fatty acids that could only be present in the predator primarily from biosynthesis, nor any fatty acids that were inconsistently identified (Appendix A). Of the remaining fatty acids, we used two subsets for modeling: (1) "dietary," which includes only those 33 fatty acids that could arise from dietary origin, and (2) "extendeddietary" (41 fatty acids), which includes all "dietary" fatty acids as well as eight fatty acids that could be biosynthesized by predators, but whose levels in a predator are also influenced by consumption of specific prey (Appendix A). The subsets of fatty acids used were renormalized to sum to 1 (after application of calibration coefficients if used) prior to modeling. Conversion from proportions in fatty acid signature to those in diet.—Given the estimated proportions of each prey type in the predator's fatty acid signature, the  $p_k$ 's, and the average fat content of each prey type, the  $f_k$ 's, one can then express the proportion of the actual diet derived from the kth prey type, denoted by  $a_k$ , as follows:

$$a_k = \frac{p_k/f_k}{\sum_k p_k/f_k}$$

#### The data

The data used in the present study represent hundreds of samples analyzed and 67 fatty acids identified per sample, and cannot be presented in detail. Thus, where possible we show representative examples.

*Prey fatty acid signatures.*—Simulation studies of the estimation model were based on a prey database of 954 fatty acid signatures (e.g., Fig. 1) of 28 marine fish and invertebrate species collected on the Scotian Shelf off eastern Canada (from Budge et al. 2002).

Calibration coefficients.-To determine the extent to which specific fatty acids undergo selective deposition or metabolism, we conducted three feeding experiments. The aim of these experiments was to develop calibration coefficients to weight individual fatty acids according to how directly they were deposited from diet. The first two studies used eight juvenile (2-3 yr old) grey seals ("grey calibration") and five juvenile harp seals ("harp calibration"), which were housed temporarily in large indoor seawater tanks at Dalhousie University's Aquatron facilities. The grey seals were maintained for at least five months on a diet consisting solely of Atlantic herring (Clupea harengus, 6.2 ± 0.30% fat). The harp seals were maintained for up to five months on the same herring, but these animals had been in captivity for less time than the grey seals. All herring fed during the five-month period had been collected from a single lot and, although variable in fat and fatty acid composition, were considered to be the most uniform diet we could feed. At the end of the five-month period, a full-depth ( $\sim$ 5 cm) blubber biopsy was taken from the pelvic region of each seal using a sterile biopsy punch according to Kirsch et al. (2000). The blubber biopsy was placed in a glass vial containing chloroform with 0.01% BHT and stored frozen until analysis. Thirty herring were randomly collected throughout the feeding period and kept frozen until analysis (<six months). In these two studies, we used the initial assumption that in the approximate fivemonth period, the fatty acid composition of blubber would resemble that of the seal's diet as much as it ever would.

In the third calibration study, we examined the degree to which blubber fatty acid composition resembled the diet after a period of complete and rapid fattening on a high-fat diet. Grey seal pups are born with neg-



FIG. 1. Fatty acid chromatogram of one individual of each of two prey species, (a) pollock (*Pollachius virens*) and (b) sandlance (*Ammodytes dubius*), from the Scotian Shelf, illustrating relative differences between species. Here 67 fatty acids are identified and quantified in each chromatogram; however, only selected peaks are labeled on this plot. Fatty acids are eluted ("retention time") in order of carbon chain length, number of double bonds, and position of double bonds on a polar capillary column (see *Methods*). The integrated area under each peak represents the relative mass percentage of each component.

ligible blubber, but at weaning (about 16 days postpartum [dpp]) they have deposited  $\sim 24$  kg of fat in blubber from a milk-only diet, which is in turn produced completely from the blubber stores of the fasting mother (Iverson et al. 1993). Thus, virtually all blubber fatty acids in suckling pups arise from milk intake, permitting accurate estimation of calibration factors for individual fatty acids from a completely homogenous diet. Full-depth blubber biopsies were collected as described above from 17 grey seal pups at 15 dpp (i.e., immediately prior to weaning) on Sable Island, Nova Scotia, Canada (43°55' N, 60°00' W). Milk samples (40-60% fat; Iverson et al. 1993) were collected from each of these pups' mothers (n = 17) at 0, 5, 10, and 15 dpp, and the average milk fatty acid signature for each mother (i.e., here used as the "prey") was compared with that of her single pup ("pup calibration"). All samples were stored frozen in glass vials containing chloroform with 0.01% BHT until analysis.

Experimental diet studies.-We investigated the performance of the model using data from two captive feeding experiments (Kirsch 1997, Layton 1998). Both of these studies were designed to evaluate the effect of a known change in diet on the fatty acid signature of a predator. In one study, a second group of juvenile grey seals (n = 6, age 1–3 yr), housed temporarily in a seawater tank at the Aquatron facilities, had previously been maintained on a diet of Atlantic herring (averaging  $5.1 \pm 0.46\%$  fat, from various lots) for up to five months. At the start of the diet trial, each seal was weighed, body composition was measured using isotope dilution (Oftedal and Iverson 1987, Bowen and Iverson 1998) and a full-depth blubber biopsy was taken and stored as described above. Seals were then fed an experimental diet, consisting of Atlantic mackerel (Scomber scombrus) and capelin (Mallotus villosus) for a period of 20 days. Atlantic herring, mackerel, and capelin share some similarities in fatty acid signatures (e.g., Budge et al. 2002), thus allowing evaluation of model performance when species in the diet do not differ markedly from one another. Due to the large size of the mackerel (averaging 38.1 cm, 0.5 kg), we removed the heads and cut the remainder of each into 5cm thick cross-sections (i.e., including the viscera) for feeding. Seals were fed to satiation (or until they lost interest) twice daily; however, due to the constraints of this captive situation, it was not possible to determine individual intakes. As a result, some individuals undoubtedly consumed more and also different proportions of the prey species than others. Capelin (averaging  $1.8 \pm 0.23\%$  fat) was offered only in the mornings and mackerel (averaging  $18.3 \pm 0.56\%$  fat) only in the afternoons, in an attempt to get seals to eat the less-preferred capelin. The approximate daily ration offered averaged 5.4 kg·d<sup>-1</sup>·seal<sup>-1</sup>, comprising about three parts capelin to one part mackerel. At this daily ration, approximate fat intake would be 0.32 kg·d<sup>-1</sup>·seal<sup>-1</sup> (Kirsch 1997). On days 12 and 20 of the experimental diet, seals were again weighed and a blubber biopsy was taken as described above; on day 20, body composition was again measured. Throughout the experiment, individual herring (n = 15), mackerel (n = 25), and capelin (n = 25) were randomly collected and stored frozen in airtight containers for analysis (<6 months).

In the second study, we used fatty acid data from fattening mink kits as an example of a terrestrial carnivore (Layton 1998). Briefly, until 21 dpp, 17 lactating females were fed primarily a wet diet (6.6% fat) along with some pelleted diet (17.3% fat), while kits consumed solely their mother's milk. Both the wet and pelleted diets consisted of primarily poultry offal (Layton 1998). Prior to feeding the experimental diets at 21 dpp, perirenal adipose tissue was sampled from 10 mink kits, euthanized in the course of other studies. The remainder of kits and their mothers were then fed one of three experimental wet diets. Each diet (6.6% fat) was composed of primarily poultry offal and fish meal, supplemented with either poultry fat, aquaculture herring oil, or seal oil (purchased from commercial sources) as 70% of the dietary fat source. Perirenal adipose tissue was sampled from six kits on each of the three wet diets at both 28 and 42 dpp (i.e., n = 36total). Since diets were completely homogenous, a single sample of each was analyzed in duplicate for fat content and fatty acid composition. We were not able to obtain milk samples from the mothers. All samples were stored as described above.

Free-ranging harbor seals filmed during foraging.— In a final case, we studied 23 free-ranging adult male harbor seals during the breeding season of May-June 1997 on Sable Island. Throughout this period, males make routine foraging trips on the Scotian Shelf in the vicinity of Sable and reliably return to the island every few days (Walker and Bowen 1993, Coltman et al. 1997). Each male was fitted with an animal-borne video system ("Crittercam," [National Geographic Television, Washington, D.C., USA] Marshall 1998) for  $\geq 3$ d. The camera was positioned such that the animal's head was visible in the camera's field of view and programmed to film 10-min segments every 45 min during daylight, thus permitting the prey species that were eaten to be recorded (Bowen et al. 2002). At each deployment/recapture, a full-depth blubber biopsy was taken and diets were estimated using the model and the Scotian Shelf prey database. Since adult males remain in the vicinity of Sable for several months prior to reproduction, we assumed that prey eaten during these short-term studies would reflect the somewhat longer term diet inferred through blubber fatty acids. Thus, in one sense this was a validation experiment under freeranging conditions, which employed a much more complex prey base than would have been possible in captive experiments.



FIG. 2. Hierarchical cluster analysis on the mean fatty acid signatures (extended dietary subset) of 28 prey species (n = 954 individuals) from the Scotian Shelf (Budge et al. 2002). Scientific names of all species not previously described in the text are as follows (in alphabetic order of teleosts, crustaceans): argentine (*Argentina silus*), butterfish (*Peprilus triacanthus*), gaspereau (*Alosa pseudoharengus*), halibut (*Hippoglossus hippoglossus*), ocean pout (*Macrozoarces americanus*), red hake (*Urophycis chuss*), redfish (*Sebastes* sp.), sculpin (*Myoxocephalus octodecemspinosus*), sea raven (*Hemitripterus americanus*), smooth skate (*Raja senta*), thorny skate (*Raja radiata*), white hake (*Urophycis tenuis*), winter skate (*Raja ocellata*), lobster (*Homarus americanus*), red crab (*Geryon quinquedens*), rock crab (*Cancer irroratus*), shrimp (*Pandalus borealis*). The Kulback-Liebler (KL) distance measure was used to determine how similar any two taxa were with respect to their fatty acid signatures. The average linkage method was used, which tends to identify spherical clusters.

#### Laboratory analyses

Lipid was quantitatively extracted from all samples (Folch et al. 1957, Iverson et al. 2001a). Each whole prey was individually ground and homogenized prior to extraction. Milk and blubber samples were also homogenized prior to extraction. Fatty acid methyl esters were prepared using 1.5 mL of 8% boron trifluoride in methanol (Iverson et al. 1997b); this method in our laboratory produces identical results to that using Hilditch reagent (0.25 mol/L H<sub>2</sub>SO<sub>4</sub> in methanol). Duplicate analyses of fatty acid composition were performed on all samples using temperature-programmed gas chromatography as described previously (Iverson et al. 1992, 1997b, Budge et al. 2002), on a Perkin Elmer Autosystem II Capillary FID (Perkin Elmer, Boston, Massachusetts, USA) gas chromatograph (GC) fitted with a 30 m  $\times$  0.25 mm ID column coated with 50% cyanopropyl polysiloxane (0.25 µm film thickness; J&W DB-23; Folsom, California, USA) and linked to a computerized integration system (Turbochrom 4 software, PE Nelson, San Jose, California, USA). Fatty acids and isomers were identified using the following methods: known standard mixtures (Nu Check Prep., Elysian, Minnesota, USA), silver-nitrate (argentation) chromatography, and GC-mass spectrometry (Hewlett Packard 6890 GC, 1:20 split injection, Micromass Autospec oa-TOF mass spectrometer, operated at 1000 resolution, scanning masses 120 to 450 [Hewlett Packard, Palo Alto, California, USA]). Fatty acid identifications on all chromatograms were checked, and corrected and reintegrated as necessary. Fatty acids are expressed as mass percent of total fatty acids.

### Simulation studies

Simulation with no calibration coefficients.—To investigate the properties of the estimation procedures and the robustness of the model in determining a given diet, we performed a number of simulation studies using the Scotian Shelf prey database. The first simulations were performed without calibration coefficients to assess the ability to estimate true diet based solely on differentiating and quantifying prey species by their fatty acid signatures. We used hierarchical cluster analysis to determine the relative similarity of prey species'

TABLE 1. Species composition of diets constructed for simulation studies.

	Nonzero elements of the composition vector, $\pi$ (proportion of diet)							
Diet	Cod	Haddock	Pollock	Silver hake	Plaice	Winter flounder	Yellowtail flounder	Sandlance
1	0.333	0.333	0.167	0.167				
2	0.200		0.800					
3		0.200		0.800				
4	0.100			0.100	0.100	0.100	0.100	0.500

*Notes:* Prey species used were based on 954 fatty acid signatures of 28 marine fish and invertebrate species collected on the Scotian Shelf off eastern Canada (Budge et al. 2002). Sample sizes of the above prey species were as follows: cod (*Gadus morhua*; n = 84), haddock (*Melanogrammus aeglefinus*; n = 54), pollock (*Pollachius virens*; n = 25), silver hake (*Merluccius bilinearis*; n = 38), plaice (*Hippoglossoides platessoides*; n = 99), winter flounder (*Pseudopleuronectes americanus*; n = 25), yellowtail flounder (*Limanda furruginea*; n = 92), and sandlance (*Ammodytes dubius*; n = 71).

signatures (Fig. 2). We then constructed four diets (Table 1): Diets 1–3 each contained two or four prey species that were more similar to one another than to all other species in the fatty acid database. These three diets represented difficult or, in some sense, "worst case" estimation scenarios. Diet 4 contained six species, some of which again were similar in fatty acid composition, and was constructed to represent the diet of a free-ranging grey seal based on results of fecal analysis (Bowen and Harrison 1994).

Simulations were used to evaluate how the accuracy of our estimates was affected by five factors: diet (four diets), fatty acid subset (dietary and extended-dietary), distance measure (KL, LSQ, SQ, REL), amount of "noise" in the simulated seal (0, 10%, 20%), and the number of individual prey (n = 30, 60, or 90) used in constructing the "pseudo-seal" fatty acid signature. Noise was meant to represent the proportion of the diet made up of incidental consumption of prey species that were not included in the assumed diet. The pseudoseal fatty acid signature was constructed by sampling the prey database in the proportions specified by our simulated diet, with additional random prey added in to create the noise. Details of the simulation procedures are provided in Appendix B.

We calculated the relative mean squared error (RMSE) to measure how well simulations estimated the assumed diet. The RMSE was constructed by summing the relative squared deviations of the true diet from the estimated diet, ([true – estimate]/true)<sup>2</sup>, for each simulation run and then averaging over the 1000 simulation runs for each factor setting.

Simulation with calibration coefficients.—To estimate the diet of a real predator, the effect of predator lipid metabolism on the deposition of dietary fatty acids must be included. Therefore, we also performed simulations using the three sets of calibration coefficients to examine how model estimates of diets were affected by the use of calibration coefficients and to test whether all sets of coefficients produced similar results. We used the grey seal calibration coefficients as the standard with which to compare the other two sets, as these should be applicable to the other experimental seal diet studies and to the free-ranging harbor seals and arose from the longer of the two seal feeding trials. The procedures for these simulations are described in Appendix B. We used the sum of the RMSEs of predicted diet from true diet (i.e., Table 1) of each pseudo-seal for the 1000 simulation runs and for the two fatty acid subsets to evaluate performance. These RMSEs were then compared to the RMSEs of predicted diet from true diet of the same pseudo (grey) seal using no calibration coefficients, and using harp and pup calibration coefficients in the fitting process.

#### RESULTS

#### Calibration coefficients

Despite large differences in fat content and homogeneity of the diet fed, in the known dietary history of the animals, and in the degree to which they fattened during the study, overall there was a reasonable degree of correspondence between the three sets of calibration coefficients and low within-study variability (Fig. 3). Calibration coefficients for most fatty acids were close to one, particularly in the case of suckling pups; however, there were notable exceptions. In general, the coefficients for the grey and harp seals fed herring were more similar to one another and deviated more from 1.0 than did the pup coefficients, but the pattern of deviations (Fig. 3) was similar in all three studies, suggesting that the underlying metabolic processes were common among animals and diets. The fatty acids with the 10 highest and 10 lowest calibration coefficients in both grey and harp seals, were also mostly among the highest and lowest in pups, although again the magnitude of deviation from 1.0 was smaller in pups (Fig. 3, Appendix A). Fatty acids such as 14:1n-5, 16:1n-11, 16:1n-9, 17:1 and 18:1n-11, with generally high coefficients, are predominantly biosynthesized by the predator and/or occur at low levels (generally occurring at <0.8% of total fatty acids in seals and/or prey). Because small errors in minor or trace fatty acids with large calibration coefficients might have large ef-



FIG. 3. Calibration coefficients (mean  $\pm$  1 sE) of the 10% trimmed means calculated within each individual (note that in most cases the standard error is too small to see) estimated for all 67 fatty acids quantified, using three different feeding studies: juvenile grey and harp seals maintained for five months on a diet of herring (6.2  $\pm$  0.30% fat), and suckling grey seal pups at weaning having consumed only their mothers' milk (40–60% fat) and in which virtually all blubber fatty acids have arisen from milk intake. The 1:1 line is presented, which denotes the deviation of a given fatty acid in a predator from that consumed in its diet. Stars ( $\star$ ) indicate examples of fatty acids with large deviations from 1:1 but which usually occur at minor amounts (<0.5%) in seals and their prey. Arrows indicate common fatty acids that would be expected to have additional contribution from biosynthesis in predators, especially if on lower fat diets. See Appendix A for fatty acids used in modeling sets.

fects on estimates from the model, we removed these fatty acids from modeling subsets at the outset (see Appendix A). Relatively high coefficients of other fatty acids, such as 16:1n-7 and 18:1n-9 or 22:5n-3, are also consistent with the expected contribution from biosynthesis or metabolic modification, respectively, in the predator. However, these major fatty acids are good indicators of prey species (e.g., Fig. 1), and calibration coefficients provide a means of using them in the model.

In all three studies, some of the lowest calibration coefficients were found for 20:0 (except in pups), 22:1n-11, 22:1n-9, 22:1n-7, and 24:1 (Fig. 3). Of these, 20:0 and 24:1 are either rare and not indicative of diet or inconsistently detectable (Appendix A) and thus were eliminated from use in the model at the outset. In contrast, 22:1n-11, 22:1n-9 and 22:1n-7 are important dietary indicators (e.g., Fig. 1; Iverson 1993, Iverson et al. 1997b). Again, for these and most other fatty acids with deviations from 1.0, calibration coefficients allow their use in the model.

#### Simulations with no calibration coefficients

Our aim here was to determine the relative importance of diet complexity, fatty acid subset, distance measure, amount of "noise" in the simulated seal, and prey sample size in minimizing the RMSE of the estimated diet. Variation in RMSE due to sample size of individual prey (30, 60 or 90) was obtained by averaging over all the other factors. The RMSE decreased with increasing sample size by  $\sim 20\%$  and 5% for the extended-dietary and dietary fatty acid subsets, respectively, indicating that a sample size of 30 individual prey would provide representative results. Variation in the average RMSE due to the level of "noise" used (0%, 10%, or 20%) did not exceed 10%. Thus, to assess the effect of the other three factors on the performance of the estimation model, we used a sample size of 30 and 10% noise in the other simulations.

We next considered the effects of the distance measure, fatty acid subset, and the complexity of the simulated diet on model performance. Significant effects were found for fatty acid subset, and diet, with a distance measure by diet interaction (P < 0.05, three-way ANOVA on the medians across the 1000 simulations), but not for distance measure alone. For the dietary fatty acid subset, SQ tended to perform somewhat worse than the other distance measures, whereas for the extended-dietary fatty acid subset, the KL distance generally performed best. Overall, the RMSEs were lowest



FIG. 4. Results of the simulation study for Diet 1 as defined in Table 1 with 10% error (noise) added, using the 28 Scotian Shelf prey species (n = 954), the extended-dietary fatty acid subset, and no calibration coefficients, and with 30 individual prey used in constructing the pseudo-seal. Species are listed in alphabetic order (teleosts, crustaceans). In plots, "a" denotes the value (proportion) specified for each of the four prey species chosen for the diet. The simulation was run 1000 times, and estimated diet results are represented in box plots, as the median (middle horizontal bar), the 25th percentile (lower bar), and the 75th percentile (top bar) of the data distribution (i.e., the box contains 50% of the data). Dots represent outliers defined as being any value greater (or less) than 1.5 times the interquartile range (75th percentile–25th percentile) above the 75th (or below the 25th) percentile.

for the extended-dietary subset and KL distance, and highest for SQ. On the basis of these results, we concluded that any of the three distances, KL, REL, or LSQ, would generally give reasonable results. However, we have chosen to use the KL distance as this is a natural distance between two distributions, and arises in a number of statistical settings including the bootstrap (DiCiccio and Romano 1989).

Next we examined how well the model estimated each component of the simulated diets. As the noise was set at 10% for these simulations, accurate estimation would give a total of 10% other prey. Hence for Diet 1, we should estimate 30% each of cod and haddock and 15% each of pollock and silver hake, obtained by multiplying Diet 1 levels in Table 1 by 0.9. Using the extended-dietary fatty acid subset, the model estimated the true diet rather well (Fig. 4), with the major species in the diet distinguished from others in the prey database. Nevertheless, there was some misidentification (7%) of the diet composition to other prey types above the added noise. The results of simulations for all four diets and both fatty acid subsets are summarized in Table 2. Using the dietary fatty acid subset, although some species in each diet were reasonably estimated, others were not, resulting in a consistent overestimate of the other prey category. In contrast, using the extended-dietary subset, estimates of individual species within each diet were generally closer to the true values, but the other prey category still tended to be somewhat overestimated. When simulations of the same four diets were performed with no noise included, in all cases components of the diet were more accurately predicted and a lower proportion of the diet was attributed to other prey.

Patterns of values across these simulations provide insight into how the model performed within each diet (Fig. 5). For Diet 1, while the best fits corresponded closely to the specified diet, as the fit worsened the estimates became low for cod and high for pollock, suggesting that these two species may be difficult to distinguish. We also underestimated silver hake as the estimates deteriorated. In Diet 2, the estimates of pollock decreased as RMSE increased, with the balance going either to cod or other prey. In Diet 3, the estimates of haddock and silver hake decreased as the fit worsened, with the proportion attributed to other prey becoming very large in the worst fits. In contrast to the other diets, estimates did not change notably for Diet 4 as the fit worsened, except that yellowtail flounder became somewhat overestimated. In summary, especially for diets

 TABLE 2. Mean estimated diets of pseudo-seals over the 1000 simulation runs for each of the four diets and two fatty acid subsets using the Kulback-Liebler (KL) distance and with noise set at 10%.

		Specified	Dietary fatty acids		Extended-dietary fatty acids	
Diet	Species	diet	Estimate	1 sd	Estimate	1 sd
1	Cod Haddock Pollock Silver hake Other	$\begin{array}{c} 0.30 \\ 0.30 \\ 0.15 \\ 0.15 \\ 0.10 \end{array}$	0.37 0.14 0.18 0.08 0.23	0.150 0.130 0.085 0.070 0.085	0.29 0.26 0.16 0.12 0.17	0.129 0.127 0.085 0.072 0.070
2	Cod Pollock Other	0.18 0.72 0.10	0.14 0.60 0.25	0.134 0.151 0.123	0.14 0.58 0.28	0.116 0.154 0.128
3	Haddock Silver hake Other	0.18 0.72 0.10	$0.01 \\ 0.49 \\ 0.50$	0.034 0.127 0.134	0.12 0.59 0.29	0.089 0.096 0.114
4	Cod Silver hake Plaice Winter flounder Yellowtail flounder Sandlance Other	$\begin{array}{c} 0.09 \\ 0.09 \\ 0.09 \\ 0.09 \\ 0.09 \\ 0.45 \\ 0.10 \end{array}$	$\begin{array}{c} 0.09 \\ 0.02 \\ 0.05 \\ 0.06 \\ 0.12 \\ 0.39 \\ 0.27 \end{array}$	$\begin{array}{c} 0.098\\ 0.036\\ 0.064\\ 0.048\\ 0.088\\ 0.090\\ 0.106\\ \end{array}$	$\begin{array}{c} 0.05 \\ 0.03 \\ 0.06 \\ 0.07 \\ 0.11 \\ 0.42 \\ 0.26 \end{array}$	$\begin{array}{c} 0.074 \\ 0.039 \\ 0.066 \\ 0.047 \\ 0.085 \\ 0.079 \\ 0.100 \end{array}$

*Note:* Pseudo-seals were created using 30 individual prey in the specified diet (no calibration) and modeled with the 28 Scotian Shelf prey species (n = 954, see Fig. 2).

that were specifically chosen to have species with similar signatures, in the worst cases it may be difficult always to separate these species, with an increasingly large percentage being attributed to other prey (i.e., Diets 1–3; Fig. 5a–c). However, as would be expected, fits are better and more consistent when diet items are more easily distinguished (i.e., Diet 4; Fig. 5d).

#### Simulations with calibration coefficients

In the second set of simulation studies, calibration coefficients were used in the construction of pseudoseals to mimic predator lipid biochemistry. Our primary interest in performing these simulations was to determine whether the three sets of calibration coefficients were comparable and whether they differed significantly from using no calibration. As before, we used a sample size of 30 prey and 10% noise. When pseudoseals were created using calibration coefficients from a random grey seal and compared with pseudo-seals fitted using each of the four calibration scenarios (i.e., including no calibration, Appendix B), the RMSEs differed significantly among calibration coefficients and diets (P < 0.01, three-way ANOVA on the medians across the 1000 simulations), but not for fatty acid subset; there were no significant interactions. Overall, the RMSEs were lowest for the grey seal calibration coefficients and extended-dietary subset. Estimates of the simulated diets using no calibration coefficients differed most dramatically from those based on any of the three sets of coefficients, but differed less with pup coefficients. Although the grey and harp seal coefficients tended to give similar results, in a few cases the harp seal coefficients performed poorly compared to

the grey seal ones. Simulations using the pup coefficients typically performed worse (higher RMSEs) compared to either grey or harp seal coefficients.

These results suggest that if differential lipid metabolism/deposition occurs in the predator (e.g., Fig. 3), calibration coefficients are needed to get accurate estimates of diets from the model. Different calibration coefficients produced similar, but not identical, results. Therefore, we assessed which coefficients were most applicable to the predator in question in modeling the diets of animals in the controlled feeding experiments.

#### Experimental diet studies and model application

*Captive grey seals.*—Juvenile grey seals were fed a diet of herring prior to the start of this experiment. They were then offered a diet of  $\sim$ 3.4:1 mackerel/capelin, on a fat content (i.e., fatty acids) basis. Seals ate the mackerel readily and consumed all that was offered. However, they did not consume all the capelin that was offered. Thus, we assumed seals ate approximately half of the capelin, resulting in a ratio of mackerel to capelin fatty acids of 6.9:1.

Seals averaged 55.4  $\pm$  4.31 kg and 33.0  $\pm$  2.98% body fat (mean  $\pm$  1 sE) at the start of the experiment and gained 4.5  $\pm$  0.67 kg over the 20-d feeding trial. Although all seals gained mass, they lost body fat (Kirsch 1997). Nevertheless, the fatty acid composition of blubber changed significantly (P < 0.05, MANOVA) over the course of the feeding trial (Fig. 6a) in the expected direction of the fatty acid patterns in the experimental diet. For example, the mackerel/capelin diet was somewhat lower in levels of 14:0, 16:0, and 22:1n-11 and higher in 18:1n-9, 20:5n-3, and 22:6n-3



FIG. 5. Line plots of the simulation results for Diets 1-4 (a-d, respectively) yielding the best fits (minimum relative mean squared error, RMSE), the median fits (median RMSE) and the worst fits (maximum RMSE), as well as the runs at the 25th (first quartile) and 75th (third quartile) percentiles of the RMSE. Each plot represents the RMSE for mean diet for the 2% of the runs around the particular quartile. The quartiles of the RMSE are computed for the 1000 simulation runs using no calibration, 30 prey, 10% error, and the KL distance measure.

compared to the pre-experimental herring diet (Kirsch 1997). This corresponded to relative decreases and increases, respectively, in these components in blubber over the 20 days (Fig. 6a).

Using these data, we estimated the possible contributions of the experimental diet to the overall blubber fatty acid signature for comparison with model estimates. The average seal started this experiment with  $\sim$ 18.3 kg blubber and consumed a total of 5–6 kg of new dietary fat in 20 days. Turnover of blubber fatty acids occurs even in a nonfattening animal (Kirsch et al. 2000). However, the actual turnover in our study animals was unknown. Thus, we used several scenarios to bracket the probable response of seal blubber fatty acids to the experimental diet. In one scenario, we assumed that all the new fatty acids consumed were deposited with existing fat and then used by the animal as a single pool. In this case,  $\sim 24\%$  of the experimental diet signature ( $\sim 21\%$  mackerel and  $\sim 3\%$  capelin) would have been represented in the seal's blubber signature, with  $\sim$ 76% of the pre-experimental herring signature remaining. If we assumed that some fraction of the fatty acids consumed were immediately oxidized and not deposited, this generates correspondingly lower estimated contributions of the experimental diet. A simpler scenario assumed that seals consumed similar daily rations before and during the feeding trial, and thus that the experimental diet represents a proportion of days fed. Assuming blubber represented an integration of diet over the previous 3–5 months (i.e., 91–152 days), we predicted the experimental diet would constitute 13–22% of total diet at day 20.

We modeled the grey seals using the two fatty acid subsets and six options of calibration (no calibration, grey, harp, and pup coefficients alone, the grey/harp average, and the grey/harp/pup average). At each blubber sampling (0, 12, and 20 days), the estimated contributions of each prey to the fatty acid signature of seals were significantly affected by the set of calibration coefficients used (P < 0.001), but not consistently by fatty acid subset (P > 0.135), as there was a significant interaction of the two effects (P < 0.001, twoway repeated measures ANOVA on arcsin-transformed data). Grey or grey/harp average coefficients tended to give similar results, as did those of harp or grey/harp/ pup average, but all other sets differed significantly from one another (P < 0.05, Tukey-Kramer multiple comparisons). Nevertheless, the experimental diet was



FIG. 6. Selected abundant fatty acids (15 of the 67 quantified) in blubber or adipose stores of the three case-study species: (a) captive juvenile grey seals previously fed herring and switched to a diet of mackerel and capelin for 20 days; (b) 42-dayold mink kits that had been raised until 21 days postpartum (dpp) on their mothers' milk and then switched to one of three diets supplemented with either poultry fat, aquaculture herring oil, or seal oil as the primary dietary fat sources; and (c) freeranging adult male harbor seals filmed during natural feeding events. Bars are means, and vertical lines show  $\pm 1$  SE except for harbor seals (c), where vertical lines show minimum and maximum values measured among individuals.

always better predicted using any of the sets of calibration coefficients than when no calibration was used.

Overall, the diet was best predicted using either the grey or the grey/harp average coefficients and the ex-

tended-dietary fatty acid subset. Using extended-dietary fatty acids and the grey seal calibration coefficients, the seals' fatty acid signatures were estimated to be composed of >95% herring at the start of the



Days of Grey Seal Feeding Trial

FIG. 7. Model estimates of the contribution of prey species to blubber fatty acid signatures (left) and to diets (right; i.e., after taking into account relative fat contents of prey) of captive grey seals previously fed herring and switched to a diet of mackerel and capelin for 20 days. Results are presented as the mean  $\pm$  the average within-seal standard error (from boot-strapping) for percentage of signature estimates (left) and as the mean  $\pm$  the average among-seal standard error for diet estimates. Results from three different model inputs are presented: (a) grey seal calibration coefficients (Fig. 3) and the extended-dietary fatty acid subset, (b) the average of the grey/harp/pup calibration coefficients and the extended-dietary fatty acid subset, and (c) no calibration coefficients and the dietary fatty acid subset. Vertical rectangles (with symbols enclosed) in each graph bracket the expected values for each prey item at the start and end of the experiment assuming deposition of 100% or 50% of dietary fatty acids at a ratio of mackerel to capelin fat of 6.9:1, or assuming blubber represents an integration of diet over 3–5 months (see text); the range of all three scenarios is included in vertical rectangles.

experiment (Fig. 7a). By day 20, herring had declined to 79%, with mackerel accounting for 21% of the signature. Although capelin was detected among the 1000 bootstrap estimates, especially by day 20, the average estimate of capelin in signatures was 0% at these times.

After taking into account the relative fat contents of the prey fed, these signature values corresponded to average diet estimates of 98% herring and 2% mackerel initially, and 91% herring and 9% mackerel at 20 d. Using the grey/harp average coefficients, signatures also were estimated to have changed gradually over the experiment to 69% herring and 31% mackerel at 20 d. In this case, diets were estimated to be composed of 96% herring and 4% mackerel initially and of 87% herring and 13% mackerel at 20 d.

Estimates of diet using the same extended-dietary fatty acids and the average of grey/harp/pup coefficients (Fig. 7b) differed significantly from the grey or grey/harp results, although overall patterns were similar. Herring and mackerel accounted for 90% and 10%, respectively, of the estimated diet at day 0 and 74% and 26%, respectively, at day 20. Capelin was again not detected as a significant component of the diet. Overall, estimates using grey, grey/harp, or grey/harp/ pup coefficients corresponded well with the range of expected responses (e.g., Fig. 7a,b). While capelin did not appear in average estimates, we did not know the amount of capelin actually consumed by the seals; the maximum that could have been represented in signatures by day 20 even if seals had consumed all capelin offered was only 3-6%.

In contrast to any model using calibration, when the seals were modeled using no calibration coefficients and either fatty acid subset, estimates of the percent contribution to signatures or to diets did not correspond to either known or expected diet contributions at any time (Fig. 7c).

Captive mink kits.-Until 21 dpp, all mink kits had consumed only mothers' milk, while their mothers consumed a mixture of "lactating pellets" and "wet diet." Milk fatty acids in carnivores, including mink, are largely derived from direct dietary intake (Wamberg et al. 1992, Iverson and Oftedal 1995). Since we were unable to sample milk for input into our model estimates, we assumed that the adipose tissue fatty acid composition of mink kits would resemble that of their mothers' diet (lactating pellets/wet diet) through "indirect" consumption. From 21 to 28 dpp, kits directly consumed one of three different oil-supplemented diets, in addition to milk from their mothers fed on these same oil-supplemented diets. By 42 dpp kits consumed primarily the oil-supplemented diets alone (Layton 1998). As expected, the fatty acid composition of adipose tissue of mink kits changed significantly over time (P < 0.001, MANOVA). The fatty acid composition also differed significantly among the kits fed the three different diets at both 28 and 42 dpp (P < 0.001, MANOVA, e.g., Fig. 6b).

Mink kits in this study contained an average of 7 g body fat at 21 dpp; by 42 dpp, after being switched to the oil-supplemented diets, kits had increased to an average of 27 g body fat (Layton et al. 2000). If fat deposited from new intake was roughly additive, the new oil-supplemented diet could comprise a maximum of 74% (i.e., (27 - 7)/27) of the overall dietary signature at 42 dpp, without accounting for milk also consumed or poultry and fish meal still in diets. We used this as an expected value to compare our results from modeling

diets. Given that all oil-supplemented diets contained the same fat content and assuming all kits consumed similar quantities of milk and direct feeds, estimated signatures can be taken as the diet in this case.

Mink kits were modeled using the two fatty acid subsets and the six calibration sets described for grey seals. The estimated contributions of each diet type to the overall fatty acid signatures of mink were again significantly affected by the calibration coefficient set used, but also by the fatty acid subset, as well as an interaction of the two effects (P < 0.001, two-way repeated measures ANOVA on arcsine-transformed data). However, unlike grey seals, the major diet types were generally well estimated, as judged against our maximum estimated values, for all calibration sets and the two fatty acid subsets (e.g., Fig. 8). The largest errors occurred in differentiating the lactating pelletswet diet from the poultry oil-supplemented diet. This was expected, as the lactating pellets and wet diets both were composed primarily of poultry offal and therefore had a similar signature to the poultry oil-supplemented diet. Signatures of kits were least accurately predicted at 21 dpp, both because of this similarity and because kits had only consumed the lactating pellets/wet diet "indirectly" though their mothers' milk, which was likely not identical to the diet. Since some fish meal was also contained in all diets, both before and after 21 dpp, minor amounts of seal oil and herring oilsupplemented diets (i.e., similar to a fish meal signature) appeared in modeled diets as expected.

Using the extended-dietary fatty acid subset and the average of the grey/harp/pup calibration coefficients (Fig. 8a), signatures of kits at 21 dpp were estimated to be composed of  $\sim 31\%$  of the mix of lactating pellets/ wet diet and 57% of the poultry oil-supplemented diet, or a total of ~88% of poultry-based diet. By 28-42 dpp, the poultry oil-supplemented diet accounted for 87–90% of the estimated signatures. Similarly, by 28– 42 dpp, diet signatures of kits fed the herring oil and seal oil-supplemented diets, were estimated to be composed of 54-78% and 82-88% of each of these diets, respectively (Fig. 8a). In contrast to the grey seals, when the signatures of mink kits were modeled using no calibration (Fig. 8b), estimates of the percent contribution of the various formulated diets to the overall fatty acid signatures remained relatively consistent with expectation, and the indirect diets of kits at 21 dpp were actually better predicted. In each case, only the fed experimental diet appeared in the kit signatures at 28 and 42 dpp (Fig. 8b).

*Free-ranging harbor seals filmed while foraging.*— The fatty acid signatures of the 23 free-ranging adult male harbor seals were similar among individuals (Fig. 6c), suggesting similar diets, but also exhibited some variability. The diets of these individuals were modeled using the entire Scotian Shelf prey database of 954 individuals representing 28 species (e.g., Fig. 2). As in the captive grey seal feeding experiment, the estimated





FIG. 8. Model estimates (mean  $\pm$  1 sE) of the relative contribution of experimental diets to the fatty acid signatures of adipose tissue in mink kits. Mink kits were raised until 21 days postpartum (dpp) on milk from their mothers (fed in turn on a mixture of "lactating pellets" and "wet" diet), and thereafter both mothers and kits were switched to one of three different oil-supplemented diets. Results are presented for the three different experimental diet groups (left, poultry fat; center, aquaculture herring oil; right, seal oil) and using two different model inputs: (a) the average of the grey/harp/pup calibration coefficients (Fig. 3) and the extended-dietary fatty acid subset, and (b) no calibration coefficients and the dietary fatty acid subset. Symbols within boxes at 21 and 42 dpp represent the maximum estimated contribution that oil-supplemented diets could be represented in signatures. Because lactating pellets and the wet diet were composed primarily of poultry offal, these are listed together with poultry oil-supplemented symbols due to similar signatures and thus overlap in model estimates. At 21 dpp, the results for the same 10 kits are presented in each graph for comparison with the latter treatment groups. At 28 and 42 dpp, data points represent results from six different mink kits in each graph (i.e., an additional 36 individuals). Note that bootstrapping of estimates was not possible as the diets were completely homogeneous.

proportional contributions of each prey type to the overall fatty acid signature of seals were significantly affected by the calibration coefficient set used, but not by fatty acid subset, and again there was an interaction of the two effects (P < 0.001, two-way repeated measures ANOVA on arcsine-transformed data).

Using the extended-dietary fatty acid subset and the grey seal calibration coefficients, all individuals were estimated to have consumed primarily sandlance (Fig. 9). Sandlance accounted for 37–90% of individuals' diets, averaging 62% of diets overall. This was followed by an average of 12% flounders (primarily yellowtail flounder) and 10% capelin. However, there was clearly variability among individuals; other prey items estimated for some individuals included varying amounts of cod, halibut, herring, skate, crab, and

shrimp. Using the average of grey/harp seal calibration, sandlance was similarly estimated to comprise 63% of diets, followed by flounders, capelin, skate, halibut, and cod. Using the average of grey/harp/pup calibration resulted in the same predominant species, but lower estimates of sandlance and higher estimates of flounders and skate.

These results were consistent with qualitative expectations from filming these same individuals while foraging (Fig. 9, inset). Video recordings from 30 seals (including the 23 above), filmed intermittently over an average of three days each, showed all but one male foraging on sandlance. Of 223 10-minute video-sampling units filming identifiable prey captured, 91% were on sandlance, 7% on flatfish, and the remainder on gadoids and other prey (Bowen et al. 2002). In contrast



FIG. 9. Individual model estimates of the contribution of prey species to diets of 23 free-ranging adult male harbor seals deployed with an animal-borne video system ("Crittercam") and filmed during feeding events. Seal signatures were modeled using the entire Scotian Shelf data base of 954 prey representing 28 species (e.g., Fig. 2), and proportions were converted to diet estimates after taking into account relative fat contents of each species. Results are presented using the extended-dietary fatty acid subset and the grey calibration coefficients (see Fig. 3). Inset: prey types consumed in video recordings of these seals, expressed as the percentage of all 10-minute video-sampling units (VSU) that filmed prey captures and that contained identifiable items (n = 223, from Bowen et al. 2002).

to these results, completely different diets were estimated when no calibration coefficients were used: using either fatty acid set, sculpin dominated the diet, followed predominantly by gaspereau and skate, with <1% flounder and no sandlance.

#### DISCUSSION

The use of quantitative fatty acid signature analysis (QFASA) to study predator diets relies upon the diversity of fatty acids and characteristic patterns among prey species, coupled with the narrow limitations on their biosynthesis in animals and the prevalence of storage depots of lipid in many predators. Our results demonstrate that QFASA is an effective tool for estimating pinniped and mink diets, and suggest that this approach could be widely applied to other predators. QFASA will enable us to study the foraging behavior of individuals and the structure of food webs in greater detail than has previously been possible in many ecosystems. We present here the first generation of this method, along with the underlying requirements that are essential to its use.

Fatty acids have previously been used to examine qualitative aspects of food webs. However, this is the first attempt to use fatty acids to provide quantitative estimates of predator diets. The problem is to match

weighted patterns of possibly hundreds of individual prey samples with those of the predator, using up to 67 fatty acids in each sample of predator and prey (e.g., Fig. 1). Given this complexity, it is generally not possible to interpret fatty acid patterns in predators by visual inspection, especially when the number of potential prey choices is large, when significant withinspecies variability exists, and when aspects of lipid metabolism of the predator must be taken into account. Our approach has been to develop a mixture model of prey species signatures that most closely resembles that of the predator and thereby estimate its diet. The use of QFASA to accurately estimate predator diets has four fundamental requirements: (1) a quantitative model and an appropriate measure of its performance; (2) appropriate sampling, analysis, and evaluation of potential prey species; (3) appropriate sampling and analysis of predator tissue; and (4) an understanding of, and accounting for, lipid metabolism and deposition in the predator.

#### Statistical model parameters

There are a number of ways to determine how close the predicted fatty acid signature is to the observed predator signature. We have used the KL distance, as it gives more weight to relatively larger errors from
the true value, does so more conservatively and proportionately than the other measures, and because it generally performed better than the other three distance measures in our simulations.

Our model currently uses the mean of each prey species to estimate its contribution to the predator's signature. However, free-ranging animals do not consume homogenous species, but rather individuals of various prey species. Although it may be possible to accurately distinguish species within an ecosystem by their fatty acid signatures, there can be considerable variability within species (Budge et al. 2002, Iverson et al. 2002). In some cases, this variability may correspond to predictable changes with age or size of the prey (e.g., Iverson et al. 1997b, 2002), such that species subgroups could be incorporated into the model to provide additional detail about the diet. However, even in these cases, we must find a way to incorporate the variability in prey into model estimates of diet. We have used a bootstrapping procedure to compute standard errors of individual estimates. At present, this seems the most appropriate way in which to calculate confidence limits on estimates of prey composition in the diet. Another approach would be to use each individual prey in the database (e.g., n = 954 for the harbor seal example). However, this would lead to computational problems and statistical issues, since we would be modeling on more prey than fatty acids.

Another source of variability arises from within-species differences in fat content. Prey species with higher fat contents will contribute proportionately more per unit intake to a predator's signature than species with a lower fat content; hence this must be factored into estimation of diet from signatures (e.g., see Fig. 7). For illustration, we have used prey species averages in these diet estimates. However, it would be straightforward to incorporate within-species variability in fat content into the standard errors of estimated diets using a similar bootstrapping procedure.

#### The prey database and simulation studies

A prerequisite of QFASA is a database of potential prey species and an understanding of whether those species can be distinguished by their fatty acid signatures. Obviously, QFASA cannot detect a prey species in the diet of a predator if that species is not represented in the database. Rather, the model will produce an estimated diet that best matches that of the predator, given the available prey. The "best" fit will be found even if this fit is poor and key prey are missing. Thus, the onus is on the investigator to sample the appropriate species and to understand within-species variability. Nevertheless, sampling every species in the ecosystem is neither possible nor warranted. QFASA will not likely detect the occasional consumption of a prey species. Thus, species that are probably rare in the diet, either because they occur at low numbers or are not available to the predator (e.g., outside its foraging range or depth), need not be included in the fatty acid database. Although such decisions will ultimately depend on the complexity of the ecosystem and the question being asked, in general the potential loss of rare species in the estimated diet may be more than compensated by the ability to determine those prey that the predator depends upon for survival.

OFASA also requires that the potential prey can be reliably distinguished on the basis of their fatty acid patterns. Multivariate techniques such as discriminant analysis and classification trees are useful for this purpose (e.g., Smith et al. 1997). For instance, in two different ecosystems (the Scotian Shelf, the Gulf of Alaska), multivariate analyses revealed that >26 species could be distinguished by their fatty acid signatures with >95% accuracy. Nevertheless, some species with similar ecology and diets, such as certain flatfishes, can be somewhat difficult to distinguish from one another (Budge et al. 2002, Iverson et al. 2002). Although other multivariate methods, including hierarchical cluster analysis (Fig. 2), provide insight into overall relationships among species fatty acid signatures, model simulations provide a more powerful means for assessing which prey may be too similar to be reliably distinguished in the estimation model (e.g., Figs. 4 and 5). We also have found that sequentially removing prey species that arise in diet estimates and then rerunning the model can be quite informative. The newly estimated diet can then be used to determine which species are substituted for the missing species and therefore allow a deeper understanding of model diet estimates.

#### Biological issues: calibration coefficients, fatty acid subsets, and predator sampling

Dietary fatty acids are directly incorporated into the lipid stores of predators across all trophic levels (see Introduction). But while many of the fatty acids in a predator's tissue provide information about diet, some fatty acids provide information less directly than others, as a consequence of their deposition characteristics and their ability to be biosynthesized. Thus, the fatty acid composition of a predator's lipid stores will never exactly match that of its prey. Our conception and use of calibration coefficients and fatty acid subsets recognizes this and assumes that, if physiological and biochemical processes are shared among animals, similar animals consuming similar diets should share similar characteristics of fatty acid deposition and biosynthesis. Understanding these characteristics, and which predator tissues to sample, are critical in using QFASA.

Several factors affect the deposition and biosynthesis of fatty acids. Fatty acid synthesis in animals is greatly reduced or absent, and dietary fatty acids tend to be stored directly in adipose tissue, when animals consume a high fat diet in excess of energy requirements (Nelson 1992). For instance, in seals consuming a diet in which fat comprised >95% of calories, blubber fatty

acid composition was not significantly different from that of the diet (Iverson et al. 1995). However, most animals do not eat a diet of almost pure fat, but instead consume a complex mixture of fat, protein, and carbohydrate. Although preformed dietary fatty acids are less likely to enter typical lipid synthetic pathways (Nelson 1992), carbohydrates or amino acids (protein) consumed in excess of requirements are used to synthesize fatty acids in the liver or adipose tissue. Thus, in carnivores, excess dietary amino acids are used to synthesize certain fatty acids, which will augment those directly deposited from diet, hence influencing tissue fatty acid signatures. These synthesized fatty acids are usually restricted to those with 16 or 18 carbon atoms and usually, at most, one double bond in specific positions (i.e., 16:0, 16:1n-7, 18:0, and 18:1n-9) (Volpe and Vagelos 1973, Wakil et al. 1983, Cook 1991, Nelson 1992). These fatty acids are also common in prey, and thus the proportions found in predators may reflect both differences among prey (e.g., Fig. 1) and biosynthesis. Therefore, proportions of some of these fatty acids found in predators will always be absolutely higher than those found in the prey (e.g., Kirsch et al. 2000). Other fatty acids may have reduced deposition in the predator (e.g., Bremer and Norum 1982, Lin and Connor 1990, Jandacek et al. 1991), but will still be reflective of differences among prev. In this case, the proportion in the predator will always be absolutely lower than that in prey (e.g., isomers of 22:1, Fig. 3).

The calibration coefficients we have developed to account for predator lipid metabolism are clearly an important component of estimating the diets of predators using QFASA (Figs. 7-9), but current estimates of these coefficients are by no means definitive. Each of our calibration experiments had limitations concerning our ability to sample the experimental diet, the type of diet that was fed, or the duration of the experiment. For example, we are not necessarily convinced that five months was long enough to eliminate the influence of the pre-experimental diet on the fatty acid pattern in blubber of grey and harp seals. In addition, the diet fed to seals was not homogeneous, and we could only sample a subset of individual herring not actually fed to seals, with the assumption that these were representative of the entire lot fed over the five months. In contrast, the pup calibration coefficients are based on a well-sampled homogeneous diet (i.e., milk) from birth. However, the potential problem of applying these coefficients more generally is that the diet was exceedingly high fat (i.e., 60% fat milk), which likely suppressed fatty acid biosynthesis fully. This, in addition to the high digestibility of milk in general, may explain why a larger number of the pup calibration coefficients were close to 1.0 compared to those measured in the seals fed fish.

Despite these limitations, we are confident that these sets of calibration coefficients are a good starting point in accounting for the effects of predator metabolism on fatty acid deposition, given the results of applying them in our model. Furthermore, the three sets of coefficients reveal some similarities among animals in patterns of fatty acid deposition and biosynthesis and, in general, are comparable to those recently calculated from feeding studies of other captive phocid and otarrid pinnipeds and seabirds (Iverson and Springer 2002; S. J. Iverson, unpublished data). Nevertheless, it will be important for the investigator to determine the most applicable set of coefficients for a given predator. For instance, most pup coefficients were closer to 1.0 than were those of grey and harp seals, and were generally more similar to those obtained from seabird adipose tissue. It may be that calibration coefficients in the more structural blubber of pinnipeds are characterized by generally greater deviations from 1.0 than are those in newly suckling pups or in the less structured adipose tissue of seabirds, and those of other mammals. Characteristics of calibration would also require investigation before they can be applied to modeling very different types of predators such as ectothermic fish.

In addition to calibration, there is also the possibility of further refining the subset of fatty acids used in the model (Appendix A). We have currently evaluated two subsets. Both in simulations and in modeling the diets of experimental animals, the model generally performed better with the extended-dietary subset. We expect that the extended fatty acid subset performed better simply because we are using more information (fatty acids) in the estimation of diet. Nevertheless, we believe there is room for fine-tuning the fatty acid subset(s) used to model diets. The most appropriate fatty acid subset may also vary with the type of predator (e.g., mammal vs. bird) and the ecosystem (i.e., temperate or tropical marine, freshwater, terrestrial) under study. We see this as an important area for further research.

A final issue in the use of QFASA, and somewhat related to the issue of calibration coefficients, is appropriate sampling of predator tissue and an understanding of the basic properties of the tissue sampled. Although fatty acids are stored in a number of tissues, the primary site of fat storage in most vertebrates is adipose tissue (Pond 1998). Adipose tissue is composed of numerous specialized cells called adipocytes, which are capable of storing massive amounts of triacylglycerols and thus, fatty acids. Adipose tissue is also extremely dynamic, as adipocytes alternately store or mobilize triacylglycerols largely depending on energy balance. Hence, adipose tissue will be most directly influenced by dietary fat intake and is the tissue that should generally be sampled for QFASA. However, not all adipose tissue behaves in the same way, and only those sites that represent the most metabolically active fat energy reservoir should be sampled. For example, in mammals, very small adipose depots are scattered throughout the body, many of which may have specialized functions (e.g., Pond 2000). In contrast, the fewer large adipose depots (e.g., visceral or subcutaneous fat, blubber) are likely to serve mainly

to store lipid and should be targeted for QFASA. Among three such large sites sampled in individual seabirds and polar bears (*Ursus maritimus*), the fatty acid composition did not differ significantly, indicating that any of these sites could be used (Iverson and Springer 2002; G. Thiemann, S. J. Iverson, and I. Stirling, *unpublished data*). Such verification may be important in other predators.

The blubber of marine mammals represents a specialized form of adipose tissue, whose function and fatty acid composition may differ over the body and by tissue depth (Iverson 2002). In particular, blubber taken near the skin is more structural in nature, and thus is less rapidly influenced by changes in diet. In pinnipeds, although sampling the full depth of the blubber layer provides accurate estimates of overall diet (e.g., this study), splitting blubber into inner and outer halves can reveal temporal change in diet, as the inner and outer halves provide estimates of more recent and less recent diet, respectively, even with use of currently available "full-depth" calibration coefficients (Cooper et al. 2001). However, in cetaceans, because their blubber is much more structured and stratified (e.g., Koopman et al. 1996, Iverson 2002), blubber samples taken only from the metabolically active inner layer (i.e., near the body core) are appropriate, as this is where dietary fatty acids are primarily deposited and mobilized (Koopman et al. 2002).

Finally, it is important to recognize that many animals undergo extended periods of fasting and depletion of fat stores, followed by replenishment. Some studies have indicated selective mobilization of fatty acids from adipose tissue during induced fasting (e.g., in rats, Raclot and Groscolas 1995), but studies of weaned grey seals showed no overall change in blubber fatty acid signatures after three weeks of natural fasting (S. J. Iverson and L. Rea, *unpublished data*). Although any such issues may be in part accounted for by calibration coefficients, the precise effects of modeling diets in species after extended fasting requires further research.

#### Experimental studies

Our grey seal and mink experimental feeding studies, along with the filmed free-ranging harbor seals modeled with a complex ecosystem-wide prey database, provided validations of the QFASA model. In captive grey seals fed for some time on herring and then switched to a short-term diet of mackerel and capelin, diets were generally well predicted as long as appropriate calibration coefficients were used (Fig. 7). However, capelin were not eaten readily and seals did not consume all that was offered. This may account for the fact that the proportions of mackerel and herring predicted in QFASA diets were consistent with expectations, but capelin was not. Although we estimated that  $\sim 3\%$  of capelin should have appeared in signatures (assuming seals ate half the capelin offered), it is also possible that not enough capelin was eaten to be reliably estimated by the model. As indicated previously, QFASA may not be able to detect trace levels of a prey in the diet. Further research is needed to determine the detection limit of QFASA and how this might vary among prey.

The diets of mink kits were remarkably well estimated, especially at the time when we could quantify direct consumption of oil-supplemented diets by kits. By 42 dpp, when kits had fed directly on diets for two weeks, the experimental diets were all well predicted by the model (Fig. 8). All oil-supplemented diets were estimated to comprise  $\sim 80\%$  of diets, consistent with our calculations from total body fat deposition ( $\sim$ 74%) during that period. Given that the initial lactating pellets and wet diet comprised primarily poultry offal, it is not surprising that this diet overlapped with estimates of the poultry oil-supplemented diet. Likewise, since all oil-supplemented diets contained some fish meal, small amounts of both seal- and fish-oil diets also appeared in signature estimates. In contrast to the seals, the estimates of mink diets without using calibration coefficients were still reasonable (Fig. 8b). This is likely due to the fact that true calibration coefficients for mink adipose tissue are generally closer to 1.0, as is the case for seabird adipose tissue (Iverson and Springer 2002) and suckling seal pups (Fig. 3).

The video-recorded harbor seals provided an opportunity to assess how QFASA performs in a free-ranging predator foraging in an ecosystem with many potential prey species. We also had the advantage that harbor seals in this population have been extensively studied, allowing us to carefully evaluate results of the model estimates. The video records from these males showed that their major prey was sandlance, and this has also been shown for animals in this population from both gastric lavage data (Bowen et al. 2001) and fecal analyses (W. D. Bowen, unpublished data). Sandlance is abundant near Sable Island and is the major prey of grey seals foraging in that area as well (Bowen and Harrison 1994). The fact that our model also estimated sandlance to be the major prey component of harbor seal diets (Fig. 9) provided a unique type of validation of QFASA. Other diet items estimated by QFASA included yellowtail flounder and other flatfish, capelin, gadoids, and crustaceans, all of which are known to be consumed by these harbor seals (Bowen et al. 2001, 2002; W. D. Bowen, unpublished data). The OFASA results also revealed considerable variation in diet among individuals. Although this variability was implied from earlier fecal or stomach contents analysis, these methods had previously provided only a snapshot of the last meal, limiting the ecological interpretation of individual variability.

#### Comparison with other methods

Both direct and indirect methods are used to determine the diets of predators. Each of these methods has advantages and disadvantages, and some methods are more applicable to some species. The diets of terrestrial and marine carnivores and seabirds are most often estimated from the identification of prey structures that are resistant to digestion. The obvious disadvantage of these methods is that not all prey may have such structures (or they are not consumed by the predator), and there may be differential digestion of structures among prey species, both leading to biased estimates. In addition, only the last meal is represented. Nevertheless, these methods have contributed greatly to our understanding of diets of many taxa. In many cases, results from these methods also provide an opportunity for both qualitative validation of, as well as useful comparison with, results from QFASA. However, QFASA offers several advantages over these methods, one being that prey without hard parts, or with easily digested parts, can be detected. QFASA also provides quantitative estimates of proportions of prey in diets, which is a more meaningful measure than the frequency-ofoccurrence measure commonly obtained from the recovery of hard parts. But perhaps one of the more striking advantages of QFASA is that it provides estimates of diets for individual animals and at time scales (i.e., integrated over longer periods) that are relevant to the ecological processes affecting survival. Because sampling is nonlethal, QFASA can be used to study diet variability within individuals over time, providing opportunities rarely possible with other indirect methods. While we believe QFASA offers a number of such advantages, it is important to remember that wise application of QFASA requires a rather considerable investment in prey sampling and a recognition that some prey species may have fatty acid signatures that are too similar to permit their separate identification in the diet.

# Conclusions and future directions

We have presented a statistical model that provides quantitative estimates of the proportions of prey species in the diets of individual predators using fatty acid signatures. We have shown that predator fatty acid signatures respond rapidly to changes in diet, and that these changes are well estimated using QFASA. Nevertheless, we need to better understand how predator fatty acid signatures respond to changes in diet over longer time scales. For some animals, such as many bears and marine mammals, which go through annual periods of extensive depletion of fat stores during fasting followed by intensive fattening prior to the next breeding season, we currently have some insight into the likely time frame over which the fatty acid signatures are integrating the diet. However, for many other animals this may be less obvious. We suggest that the current QFASA model can be applied to a number of predators and ecosystems. However, as with any new method, additional experimental studies are needed to better understand aspects of the turnover and deposition of fatty acids (in both the blubber of marine mammals and in the adipose tissue of other predators), in order to provide robust quantitative estimates of predator diets.

#### ACKNOWLEDGMENTS

We thank P. Kirsch and H. Layton for use of their M.Sc. data in some of the modeling exercises. We also thank J. Eddington and D. Pedipaw for assisting with the captive seal experiments, and D. Boness, B. Bulhier, D. Coltman, S. Lang, J. McMillan, J. Mellish, and D. Tully for assisting in field studies on Sable Island; S. Lang and J. Mellish also assisted in laboratory analyses. We thank K. Frost for useful feedback and interest in early stages of model development and R. Myers for help in formulating the initial approach to the model. We also thank T. Wootton, I. L. Boyd, J. Estes, B. Smith, C. A. Beck, S. Budge, M. Cooper, H. Koopman, and two anonymous reviewers for providing valuable comments on previous drafts of the manuscript. This research was supported by grants to S. J. Iverson from the Exxon Valdez Oil Spill Trustee Council, Alaska, the Natural Sciences and Engineering Research Council (NSERC, Canada) and the U.S. National Marine Fisheries Service (NMFS, Honolulu Lab). Additional financial support to W. D. Bowen was provided by the Department of Fisheries and Oceans, Canada, and NSERC.

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APPENDIX A Fatty acids routinely quantified in the current study, their predominant source in predator adipose tissue, those currently used in the two modeling sets, and their calibration coefficients estimated from three experimental studies.

	Average in Scotian Shelf	Predominant source in	"Dietary"	"Extended- dietary"	Calibration coefficients				
Fatty Acid†	ecosystem‡ (%)	predator§	fatty acids	fatty acids	Grey	Harp	Pup		
12:0	0.10	В			0.97	0.86	0.92		
13:0	0.03	b			1.00	1.00	1.00		
<i>iso</i> -14:0	0.03	b/?		v	1.00	1.00	1.00		
14:1n-9	0.15	b		Λ	0.30	1.06	0.95		
14:1n-7	0.05	b			1.14	1.03	1.26		
14:1n-5	0.46	B			10.92	8.83	1.54		
150-15:0 anteiso-15:0	0.20	b/? b/?			1.12	1.11	0.91		
15:0	0.39	b			1.09	0.97	0.97		
15:1n-8	0.03	b			1.00	1.00	1.00		
15:1n-6	0.04	b			1.24	1.00	1.20		
<i>150</i> -16:0	0.19	b/ ?		x	1.16	0.82	0.96		
16:1n-11	0.55	b		71	2.51	2.24	0.85		
16:1n-9	0.34	b			3.37	2.64	1.11		
16:1n-7	9.44	b		Х	1.52	1.61	1.30		
16:1n-5	0.25	b			1.10	1.08	1.04		
16:2n-6	0.12	Ď	Х	Х	0.76	0.74	0.81		
iso-17:0	0.18	b/?			1.09	1.05	0.96		
16:2n-4	0.46	D	X	X	1.50	0.95	0.89		
10:3n-0 17:0	0.37	D b	А	X	0.86	0.91	0.78		
16:3n-4	0.24	Ď	Х	X	0.68	0.87	0.98		
17:1¶	0.36	b			2.67	2.04	1.27		
16:3n-1	0.08	D	X	X	0.85	0.57	1.14		
16:4n-1	0.12	D	X	X	0.59	0.77	0.90		
18:0	2.17	b		X	0.84	0.79	0.64		
18:1n-13	0.10	D			0.95	0.74	0.89		
18:1n-11 18:1n 0	1.63	B		v	15.04	10.40	1.04		
18:1n-7	3.69	b		X	1.41	1.44	1.13		
18:1n-5	0.46	b			1.04	1.00	0.99		
18:2 $\Delta$ 5,11	0.07	D			1.04	1.00	0.87		
18:2n-7 18:2n-6	0.06	D	x	x	1.13	1.00	1.26		
18:2n-4	0.13	D	X	X	0.98	0.86	0.94		
18:3n-6	0.10	D	Х	Х	1.08	0.94	0.78		
18:3n-4	0.12	D	X	X	2.32	2.59	1.01		
18:3n-1	0.37	D	X	X	0.95	0.95	0.88		
18:4n-3	1.15	D	X	X	0.96	0.99	0.96		
18:4n-1	0.16	D	Х	Х	1.10	1.39	1.01		
20:0 20:1p 11	0.09	b	v	v	0.50	0.50	1.00		
20:1n-9	6.30	D	X	X	0.81	1.00	0.97		
20:1n-7	0.70	D	Х	Х	0.71	1.05	0.82		
20:2n-9	0.05	b	V	N/	1.00	2.93	1.00		
20:2n-6	0.27	D	X X	X	1.65	1.39	1.02		
20:311-0 20:4n-6	1.15	D	X	X	0.82	1.00	0.91		
20:3n-3	0.11	D	X	X	1.16	0.98	0.98		
20:4n-3	0.48	D	X	X	2.11	1.50	1.00		
20:5n-3	9.51	D	X X	X	0.65	0.80	0.82		
22:1n-9	0.62	D	X	X	0.20	0.59	0.49		
22:1n-7	0.16	D	Х	Х	0.18	0.26	0.90		
22:2n-6	0.02	D	X	X	1.00	1.00	1.00		
21:5n-3 22:4n-6	0.36	ע ח	X X	X X	1.37	1.45	1.02		
22:5n-6	0.29	D	X	X	1.00	0.76	0.96		
22:4n-3	0.09	D	Х	Х	2.58	1.55	1.01		
22:5n-3	3.53	b	V	X	4.64	3.91	1.09		
∠2:0n-3 24:1¶	0.50	D	А	А	0.13	0.93	0.32		

#### APPENDIX A. Continued.

	Average in Scotian Shelf	Predominant source in predator§	"Dietary"	"Extended- dietary" fatty acids	Calibration coefficients					
Fatty Acid†	ecosystem‡ (%)		fatty acids		Grey	Harp	Pup			
Total us	sed in current model	ing sets:#	33	41						

† Fatty acids are listed in order of elution on a polar capillary column. Although not detected in samples in the current study, shorter chain fatty acids routinely identified in other samples in our laboratory include *iso*-4:0, 4:0, *iso*-5:0, 6:0, 8:0, *iso*-10:0, 10:0, and *iso*-12:0. However, any of these present in a predator arise solely from biosynthesis, since fatty acids of chain length  $\leq$  12:0 consumed in the diet are immediately oxidized (e.g., Jackson 1974). Thus, these could not be used in modeling. Although very long chain fatty acids (>24 C) do exist, their occurrence in blubber or adipose tissue is rare and at trace levels only; *trans*-fatty acids also measured in other samples in our laboratory are equally rare in the animals used in this study.

‡ Levels of individual fatty acids, averaged across all prey and seals from the Scotian Shelf (SS) database in the current study, to provide an idea of relative abundance or rarity of fatty acids in this marine ecosystem.

§ Predominant source in a monogastric predator: B = all or primarily from biosynthesis; D = all or primarily from direct dietary intake; b = relatively large contributions from both biosynthesis and diet; ? = not fully understood. For instance, *iso-* and *anteiso-* fatty acids are produced primarily by bacterial biosynthesis from branched-chain amino acids; thus in mammals they are produced largely de novo (e.g., from gut bacteria and possibly other sources; Ackman et al. 1975, Gurr and James 1980); varying degrees for this capacity have also been demonstrated in cetacean blubber and melon (e.g., Morii and Kaneda 1982, Koopman et al. 1996, 2003).

|| Calibration coefficients determined from three studies (see *Methods: The model*) using fish-fed grey seals ("Grey") and harp seals ("Harp") and suckling grey seal pups ("Pup"). Values are the 10% trimmed mean across all individuals as used in modeling. Values are absent for a fatty acid if it was not detected in either predator or prey in a given study.

¶ This fatty acid category represents several isomers combined, as their detection occurred with varying degrees of reliable separation on some individual GC columns due to slight stationary phase shifts in production.

# Fatty acids that arise in the predator largely from biosynthesis, or those that were generally found at trace levels or were inconsistently detected, were not used in modeling, since minor errors in fatty acids with large calibration coefficients that are present in small amounts would have large effects on the consistent performance of the model.

#### **APPENDIX B**

#### PROCEDURES USED IN MODEL SIMULATIONS

#### Simulation with no calibration coefficients

We construct a pseudo-seal from our specified diets (Table 1) as follows:

1) Choose the diet composition vector  $(\pi)$ , the amount of noise (*e*), and the number of prey to be sampled  $(n^{s})$ .

2) For each prey type, split the samples into two sets: a simulation set  $x_{kj}^s$  and a modeling set  $x_{kj}^{M}$  with sample sizes  $n_k^s$  and  $n_k^M$ , respectively. (The splitting process is only carried out for species with  $n_k > 5$ ).

3) From the *k*th prey type, sample with replacement  $n^{s} \times \pi_{k}$  from the  $n_{k}^{s}$ . Call the selected sample  $x_{kli}^{s*}$ .

4) To simulate noise, sample with replacement,  $n^s \times e$ , prey from prey types which are not part of the diet composition vector,  $\pi$ . Call this sample,  $e_{li}^{**}$ .

5) Adding each sampled prey from the prey types in the diet and the simulated noise from step 4 forms a pseudo-seal. We then divide by the total number of prey sampled:

$$y_j^* = \frac{\sum_{k} \sum_{l} x_{klj}^{S*} + \sum_{l} e_{lj}^{S*}}{(1+e)n^S}.$$

6) Next using the modeling data set from step 2 plus all other species samples in the Scotian Shelf database, compute the composite prey mean for each of the k prey types by averaging the  $n_k^M$  prey of each type. This is expressed in the following formula:

$$\bar{x}_{kj}^{M} = rac{1}{n_{k}^{M}} \sum_{l=1}^{n_{k}^{M}} x_{klj}^{M}.$$

7) Perform the estimation procedure using the simulated seal  $y^*$  and  $\bar{x}_k^M$  to get an estimated diet  $p_k^r$ .

8) Repeat steps 1–7 1000 times.

#### Simulation with calibration coefficients

 A) Choose a seal at random from the eight available seals in the grey seal calibration study and compute its calibration coefficient as described previously.

- B) Perform Steps 1 through 4, as detailed above.
- C) Modify Step 5 as follows:

$$y_{j}^{*} = c_{j} \frac{\sum_{k} \sum_{l} x_{klj}^{s*} + \sum_{k} e_{lj}^{s*}}{(1 + e)n^{s}},$$

with re-normalization performed as follows:

$$y_j^* = \frac{y_j^*}{\sum_j y_j^*}.$$

D) Since the pseudo-seal now has simulated metabolism effects, we take this into account in the fitted procedure described in Step 7: since we used one of the eight grey seals to form the pseudo-seal, the average of the other seven grey seals is used in the fitting process.

E) Both the pseudo-seal and the other seven seals are randomly chosen in each of the 1000 repeated simulations.

May 2004

MARINE MAMMAL SCIENCE, 17(4):835–861 (October 2001) © 2001 by the Society for Marine Mammalogy

# MOVEMENTS OF SATELLITE-TAGGED SUBADULT AND ADULT HARBOR SEALS IN PRINCE WILLIAM SOUND, ALASKA

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# Abstract

Satellite-linked tags were attached to 49 subadult and adult harbor seals captured in Prince William Sound (PWS), Alaska, and their movements were monitored during 1992-1997. Seals were tracked for a total of 5,517 sealdays and were located on about 80% of the days that tags transmitted. Most locations were in or near PWS, but some juvenile seals moved 300-500 km east and west into the Gulf of Alaska. While several seals travelled to 50-100 km offshore, virtually all locations were in water <200 m deep. Overall, juvenile seals moved more than adults and had larger home ranges. Movements were significantly affected by month, and age by month and sex by month interactions. In all months, mean distances between successively used haulouts were <10 km for adults and <20 km for juveniles. Mean monthly home ranges varied from  $<100 \text{ km}^2$  to  $>1,500 \text{ km}^2$ , and were smallest during June-July. Mean haul-out to at-sea distance was 5-10 km for adults and generally 10-25 km for juveniles. Satellite-linked tags provided an effective means of monitoring and describing the full range of harbor seal movements in this region, with the exception of late summer when tags were shed during the molt.

Key words: harbor seal, *Phoca vitulina richardsi*, Alaska, satellite tags, distribution, movements, foraging range, home range.

Harbor seals (*Phoca vitulina richardsi*) are one of the most common and widely distributed pinnipeds in coastal waters of southern Alaska. They occur in a variety of habitats, from rocky intertidal areas to river estuaries and glacial fords (Hoover-Miller 1994). Despite their ubiquitous nature, few biological

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studies of Alaskan harbor seals were done prior to the 1990s, perhaps because of the relatively remote areas they inhabit and a perceived lack of acute conservation problems. Some studies were done to describe basic biology (e.g., Pitcher 1980, Pitcher and McAllister 1981) and to track abundance in selected areas using "trend counts" (Pitcher 1990, Frost et al. 1994a).

Two things happened to focus research on Alaskan harbor seals during the 1990s. Between the late 1970s and late 1980s, declines of 60% to more than 80% were documented for harbor seals in some trend count areas in the northern Gulf of Alaska (Pitcher 1990, Frost *et al.* 1994*a*). Then, on 24 March 1989, the *T/V Exxon Valdez* ran aground on Bligh Reef in northeastern Prince William Sound (PWS), spilling approximately 40 million liters of crude oil (Morris and Loughlin 1994). Spilled-oil spread to harbor seal habitats in PWS and adjacent parts of the Gulf of Alaska. Studies conducted as part of a Natural Resources Damage Assessment program showed that seals contacted the oil (Lowry *et al.* 1994*b*), and they developed lesions and showed behavioral anomalies that were likely the result of hydrocarbon toxicity (Spraker *et al.* 1994, Lowry *et al.* 1994). Approximately 300 seals were estimated to have died in PWS due to the spill (Frost *et al.* 1994*a*).

Monitoring of harbor seal numbers in PWS following the oil spill indicated that the population continued to decline during 1990–1997, both within and outside of the oiled areas (Frost *et al.* 1999). For this reason, and because harbor seals are an important resource to Alaska Native subsistence hunters in PWS as well as an important apex predator in this region, the *Exxon Valdez* Oil Spill Trustee Council funded a Restoration Science Study to identify important seal habitat and investigate movements and diving behavior through the use of satellite-linked depth recorders (SDRs).

Prior to the development of satellite-linked transmitters, investigations of the movements of seals relied primarily on re-sightings of individuals (e.g., Finley 1979, Jeffries et al. 1993), or the use of very high frequency (VHF) radio tags (e.g., Pitcher and McAllister 1981, Brown and Mate 1983, Thompson 1989, Thompson and Miller 1990). Harbor seals are considered to haul out and feed locally, rarely travelling more than 25–50 km from their haulouts (Brown and Mate 1983, Suryan and Harvey 1998, Thompson 1923). However, because most previous studies have relied on VHF technology, longer distance movements may have gone undetected.

The development of satellite linked transmitters, with on-board environmental sensors and micro-processors that store data for later transmission, has greatly increased our ability to gather data on the activities of marine mammals when they are underwater and off shore (e.g., Heide-Jørgensen et al. 1992, Nordøy et al. 1995, Stewart et al. 1996, Lowry et al. 1998). Such information is essential to develop a complete understanding of foraging ecology, population genetics and geographic interchange, interactions with fisheries, and wildlife epidemiology.

Seasonal, age and sex-related differences in movements, diving, and hauling out behavior have been reported for harbor seals as well as for a variety of other pinnipeds (Thompson 1989, DeLong and Stewart 1991, Merrick and Loughlin 1997, Thompson et al. 1998, Härkönen et. al 1999, Le Boeuf et al. 2000, Frost et al. 2001). Most such studies have examined age or sex-related differences in characteristics such as depth and duration of dives and time spent foraging. A few have noted seasonal or sex-related differences in movement patterns and foraging ranges (e.g., Merrick and Loughlin 1997, Thompson et al. 1998, Le Boeuf et al. 2000).

In this paper we describe the distribution and movements of harbor seals that were satellite-tagged and tracked in PWS during 1992–1997. We were interested in determining if movement patterns of PWS seals were similar to those described from other areas, and whether there were differences in movements among age and sex classes. A description and analysis of diving behavior of these instrumented seals is presented separately (Frost *et al.* 2001).

#### Methods

# Capture and Tagging

Field work was conducted primarily in southern PWS (Fig. 1). Seals were caught by entanglement in nets (30-cm stretch mesh, 7.5 m deep, 90 m long) deployed near their haul-outs. Entangled seals were brought into small boats, transferred to hoop nets, and taken to the research vessel for processing. Most animals older than pups were sedated with ketamine (2–4 mg/kg body mass) and/or diazepam (0.6–1.5 mg/kg body mass). Each seal was weighed (to the nearest 0.1 kg), measured (standard length, curvilinear length, and girth to the nearest 1.0 cm), and tagged in the hindflippers with individually numbered plastic tags. Males <55 kg and females <47 kg were considered to be subadults, based on historical age/weight data from the northern Gulf of Alaska (Pitcher and Calkins 1979).

SDRs were glued to the mid-dorsal surface of the seal using quick-setting epoxy (Fedak *et al.* 1984, Stewart *et al.* 1989). We used two versions of 0.5 watt power output SDRs (Wildlife Computers, Redmond, WA; version 3.10 software). The "large tag" measured  $14.8 \times 10.0 \times 3.8$  cm, and weighed about 750 g in air, whereas the "small tag" measured 11.9 cm  $\times 5.1$  cm  $\times$ 4.5 cm, and weighed 385 g. In general, large tags were attached to seals weighing >50 kg, and small tags were attached to lighter seals. The large tags had a projected capacity of about 100,000 transmissions, while the small tags were rated for approximately 30,000 transmissions.

Tags were shed when the animals molted in July-September (Ashwell-Erickson *et al.* 1986). We therefore attached tags to seals in spring (late April-May) and fall (late September) to achieve the most complete seasonal coverage possible. Because they would be shed after three to four months, SDRs attached in spring were not duty-cycled and transmitted continuously. To conserve battery power, tags attached in the fall were programmed to stop transmitting during hours of poor satellite coverage (2200-0300 local time). In



Figure 1. Map of Prince William Sound study area showing locations mentioned in this paper, and major harbor seal haul-outs (solid triangles).

addition, small tags attached in the fall had duty cycles of one day on and one day off, or one day on and two days off.

# SDR Data

SDRs transmitted on 401.65 MHz to receivers operated by Service Argos on board National Oceanic and Atmospheric Administration polar orbiting satellites. In the area covered by this study there were on average 19 satellite passes per day and satellites were above the horizon for about 15% of the time (Fancy *et al.* 1988).

The Argos data collection and location system recorded the date and time of each signal received by the satellite (termed an "uplink") and calculated a location for the tag based on Doppler shift whenever sufficient uplinks were received during a satellite pass. Argos assigns a quality ranking of 3, 2, 1, or 0 to each location, with quality 3 predicted to be the most accurate. Locations that are based on few uplinks or have other potential problems are assigned quality 0. Stewart et al. (1989) and Fancy et al. (1988) provide additional description and analysis of the Argos system.

A multi-stage process was used to screen out erroneous location records. First, records that failed validation tests performed by Argos were deleted from the database. Then, an error index value was calculated for each remaining record according to the equation described in Keating (1994), and all location records that had a value greater than 25 were deleted. Finally, the time, distance, and speed between sequential locations were calculated for all remaining records. Records that indicated apparent speeds of >10 km/h (which is slightly more than the likely sustained swim speed of harbor seals (Williams and Kooyman 1985)) were reviewed, and the locations that were most distant from adjacent records were deleted. Numbers of location records referred to in this paper include only those records that remained after the complete screening process.

SDRs included a conductivity sensor that indicated whether the tag was dry or submerged. If the tag had been submerged, the microprocessor did not change the reported status to dry until the conductivity sensor had been dry for 10 consecutive transmission intervals (450 sec total). We assumed that when the sensor indicated wet the seal was at sea, and when it indicated dry the seal was on land.

Land-sea sensor data were merged with location records to produce a datafile that included SDR number, date, time (converted from Greenwich mean time to local time by subtracting 10 hours), latitude, longitude, location quality, and whether the seal was on land or at sea. A computer program calculated from this datafile the average daily positions of each seal which were plotted using an ArcInfo geographic information system (GIS) and ArcView. Another datafile was created that included for each seal the average position for each haul-out bout (*i.e.*, one or more consecutive on-land locations) using only onland records with location quality greater than zero, and all individual at-sea records, and this datafile was used to calculate distances from haul-outs to atsea locations.

# Data Analysis

We used two methods for data analysis. In the first analysis to investigate seal movement patterns, we created binary variables that indicated whether a seal (1) stayed near the tagging location, (2) moved to glacial fjords, or (3) moved into the Gulf of Alaska. A generalized linear model with a logit link function and a binomial distribution (McCullagh and Nelder 1989) was used to determine if these seal movement patterns were related to age and sex of the seal. Age (adult or juvenile) and sex were treated as categorical variables. All main and pair-wise interactions were considered in the model. Effects that were not significant ( $\alpha > 0.05$ ) were removed until the most parsimonious model was obtained. The final model contained only those effects, and possible interactions, which were significant with a log-likelihood ratio statistic at  $\alpha \leq 0.05$ . In the second analysis, to investigate distances moved and home ranges of seals, we measured the following variables:

- 1. maximum distance moved—the largest distance from the first location received to any other location
- 2. mean haul-out distance—the average distance between successively used different haul-out sites
- 3. maximum haul-out distance—the largest distance between any two haul-outs used
- 4. mean haul-out to at-sea distance—the average distance from a haul-out site to the subsequent at-sea locations up until the start of the next haulout bout
- 5. home range-minimum convex polygon home range using all points, with land area excluded.

Variables 1–5 were calculated monthly as well as for the entire duration of tracking.

We used a repeated-measures model (e.g., Lindsey 1993) to investigate effects of sex, age class, and month on these variables for all data from each seal combined. The model can be stated as:

 $Y_i(t) = \beta_0 + \beta_1 \times \text{OPDAYS}_i(t) + \text{SEX}(Y_i) + \text{AGE}(Y_i) + \text{MONTH}(t)$ 

+ · · · all 2-way interactions between SEX, AGE, and MONTH · · ·

 $+ \epsilon_i(t),$ 

where

 $Y_i(t)$  is a random dependent variable (maximum distance moved, mean haulout distance, maximum haul-out distance, mean haulout to at-sea distance,

or home range) for the *i*th animal during the *t*th month,

 $\beta_0$  is an intercept,

 $\beta_1$  is a regression parameter for OPDAYS<sub>i</sub>(t), which is a continuous variable of the number of operational days for the tth month,

 $SEX(Y_i)$  is a parameter for the sex of the *i*th animal,

 $AGE(Y_i)$  is a parameter for the age of the *i*th animal,

MONTH(t) is a parameter for the *t*th month (a categorical variable) and month 1 is January and month 12 is December, and

 $\epsilon_i(t)$  is the random error for the *i*th animal during the *t*th month.

The number of operational days (the total number of days from when the SDR was attached until the day the last transmission was received) varied among seals, so OPDAYS was included in the model as an independent variable to correct for this. The random errors are assumed to follow a time-series model for repeated measurements on each animal, and the random errors are assumed independent among animals. We chose to use a first-order autoregressive model (AR(1)) for  $\epsilon_i(t)$  because it is a natural yet simple model for many types of data (Lindsey 1993). Starting with a basic model with all terms and 2-way interactions, terms with statistical significance P > 0.10 were re-

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moved from the model, and then the model was fitted again. After that, only terms with significance P < 0.05 were retained. After fitting the model, residuals were checked for normality using a QQ plot and the method of Shapiro and Wilk (1965). Because the residuals are not independent, these methods are only approximate. When appropriate, transformations were used to obtain normal-looking residuals.

When transformations were used, the fitted models can later be back transformed to the original scale. When back transforming, we need to make a correction for bias using a Taylor series expansion,

$$g(\hat{\mu}) = g(\mu) + (\hat{\mu} - \mu)g'(\mu) + (\hat{\mu} - \mu)^2 g''(\mu)/2 + \cdots$$

Taking expectations of both sides gives,

$$E[g(\hat{\mu})] = g(\mu) + \sigma^2 g''(\mu)/2 \dots$$

where  $\sigma^2$  is the variance on the transformed scale and  $g''(\mu)$  is the second derivative of the back transforming function evaluated at  $\hat{\mu}$ . Therefore, to reduce bias, we take as our estimate,

$$g(\hat{\mu}) - \hat{\sigma}^2 g''(\hat{\mu})/2,$$

where  $\hat{\mu}$  is the fitted value on the transformed scale. For example, if a log transformation is used, then  $g(x) = \exp(x)$ . The fitted model is,

 $\hat{\mu} = \hat{\beta}_0 + \hat{\beta}_1 \times \text{OPDAYS} + \text{other fitted parameters in model,}$ 

and a particular instance of this model can be obtained by standardizing on 25 OPDAYS (per month) and specifying the other effects by choosing their parameter estimates. Then the nearly unbiased back transformation is,

$$\tilde{\mu} = \exp[\hat{\mu}] - \hat{\sigma}^2 \exp(\hat{\mu})/2$$

where  $\hat{\sigma}^2$  is the estimated variance of  $\hat{\mu}$ . We can back transform the estimates, but we can also use the same formulas for back transforming the confidence interval endpoints. That is, from the confidence interval on the transformed scale, and then let the endpoints be  $\hat{\mu}$  in the formula above. This will give the same coverage on the original scale as on the transformed scale.

All of our models included a variable of the number of operational days (OPDAYS) to correct for this effect on the response variable. For specific models, we had variations on the basic model. When the response variable was a mean, then the number of observations per mean was used as a weight to get the proper variances. That is, a mean value computed with 20 values is more precise than one computed with five values. Also, at times we added covariates in addition to OPDAYS to make further corrections for specific effects. For example, because we used minimum convex polygons for home range analysis, we expect that the home range estimate will be influenced by the number of locations used to calculate the home ranges. By including the number of locations as a covariate, we correct for that effect. All analyses used the mixed procedure in the SAS statistical software package.

Table 1. Summary of performance of satellite-linked depth recorders attached to 49 juvenile and adult harbor seals in Prince William Sound, Alaska. Values given are means for all seals within the age sex/class, with standard deviations in parentheses.

Age/sex class (n)	Tracking days	% days located	Total locations	# locations/ dayª
Juvenile female (11)	112.7 (75.31)	83.7 (12.43)	230.1 (94.79)	3.8 (0.96)
Juvenile male (11)	132.0 (82.59)	76.0 (19.12)	180.5 (92.97)	2.5 (1.91)
Adult female (15)	122.9 (84.41)	85.0 (13.38)	453.1 (241.59)	4.4 (2.30)
Adult male (12)	140.0 (83.66)	81.0 (11.46)	381.1 (240.94)	2.8 (0.68)

<sup>a</sup> Calculated based on number of days of transmission, *i.e.*, off days of duty cycle were not included.

#### RESULTS

### Capture and Tagging of Seals

During 1992–1996 we captured 160 seals at several locations in PWS (Fig. 1) and attached SDRs to 49 (Appendix 1). We had initially planned to spread our sample of tagged seals broadly within PWS, but it turned out that relatively few locations were physically suitable for capturing seals. As a result our effort was concentrated in south-central PWS, with most seals tagged at Port Chalmers (12), Seal Island (9), Channel Island (8), Applegate Rocks (8), Little Green Island (4), and Stockdale Harbor (3). The distribution of SDRs by age/sex class was: juvenile females (JF)—11; juvenile males (JM)—11, adult females (AF)—15; and adult males (AM)—12. Tagged juvenile seals averaged 112.7 cm standard length and on average weighed 41.0 kg while adults averaged 136.4 cm and 75.5 kg. Within age categories, males and females were of similar sizes (JF *vs.* JM weight t = 1.72, df = 20, P = 0.10; JF *vs.* JM length t = 0.59, df = 20, P = 0.56; AF *vs.* AM weight t = 0.85, df = 25, P = 0.40; AF *vs.* AM length t = 0.90, df = 25, P = 0.37).

# Satellite-linked Depth Recorder Performance

The 49 SDR-tagged seals were tracked for a total of 5,517 seal-days. During that time 24,949 locations were received of which 15,885 (64%) passed the screening criteria. Fifty-two percent of the locations were received while seals were at sea. As expected due to the timing of the molt, durations of tracking for seals tagged in April-May were much shorter (mean = 64 d, range 39-83 d) than for seals tagged in September (mean = 178 d, range 40-312 d). The overall mean duration of tracking was 113 days. Because of the molt, we obtained no location information during the period from early August through mid-September.

On average, seals were located on about 80% of the days that their SDRs were transmitting, with similar location frequencies for all age/sex classes (Table 1; JF vs. JM t = 1.12, df = 20, P = 0.27; JF vs. AF t = 0.24, df = 24, P = 0.81; JM vs. AM t = 0.77, df = 21, P = 0.45; AF vs. AM t = 0.82,

df = 25, P = 0.42). Although more total locations were received for adult seals than for juveniles, when duty-cycling was accounted for the number of locations per day was similar (JF vs. AF t = 0.84, df = 24, P = 0.0.20; JM vs. AM t = 0.60, df = 21, P = 0.56). There was, however, a difference in the number of locations per day by sex, with both juvenile and adult females providing more locations per day than males (JF vs. JM t = 2.67, df = 20, P = 0.01; AF vs. AM t = 2.35, df = 25, P = 0.03).

### Distribution and Movements of Seals

For adult seals, all average daily locations were in PWS, the Copper River Delta, or near Middleton Island (Fig. 2a, b). For juveniles, most locations were also in or near PWS (Fig. 2c, d), but some individuals moved west as far as the west side of Cook Inlet (350 km), east to Icy Bay (300 km), and past Yakutat Bay (500 km) (Fig. 3). Each of those four seals was still at the distant location when the last locations were received in March, June, and February. Nine seals (two juvenile females, four juvenile males, and three adult females) moved out of PWS to the Copper River Delta, 125 km to the east of the main capture area, and spent a minimum of 9-233 d there. Six of them returned to PWS at some time during the tracking period, and four were near the Copper River Delta when transmissions ended. Five seals (one juvenile female, one juvenile male, one adult female, and two adult males) moved south 120 km from the main capture area to the Middleton Island region. Two of them went there once, while the other three made two to four trips, staying in the Middleton area for minimum periods of 3-197 d and returning to PWS in between. Four of those seals were in PWS when the last transmission was received, and one was at the Copper River Delta.

While most locations were relatively near shore, some seals within each age/ sex class spent time at sea over the outer part of the continental shelf, 50-100 km offshore (Fig. 2, 3). The only two locations that were significantly beyond the continental shelf were for an adult male that on two days was located southwest of Middleton Island, 140 km offshore from the mainland, in water 600 m and 2,400 m deep.

By following sequential locations of individual seals using a GIS, we distinguished three general movement patterns, as follows: movement restricted to near the tagging location (27 seals); movement to glacial fiords (College Fiord, Unakwik Inlet, Icy Bay, Columbia Bay) in northern or western PWS (9 seals); and movement out of PWS into the Gulf of Alaska (17 seals). Four seals moved both to glacial fiords and into the Gulf of Alaska. The logit model we used to investigate the effects of age, sex, sex + age, and sex × age interaction on these movement patterns indicated there was no significant effect of age or sex on how commonly seals moved to glaciers and/or into the Gulf of Alaska (Table 2). Adult females showed the greatest tendency to make local movements only for the entire tracking period, but the model was not significant at P < 0.05. The best model fit was for an effect of age and sex



Figure 2a. Average daily locations of 15 adult female harbor seals satellite-tagged in Prince William Sound, 1992–1997. Dotted line shows 200-m depth contour.

Figure 2b. Average daily locations of 12 adult male harbor seals satellite-tagged in Prince William Sound, 1993–1995. Dotted line shows 200-m depth contour.



Figure 2c. Average daily locations of 11 juvenile female harbor seals satellite-tagged in Prince William Sound, 1994–1997. Dotted line shows the 200-m depth contour.

Figure 2d. Average daily locations of 11 juvenile male harbor seals satellite-tagged in Prince William Sound, 1992–1997. Dotted line shows the 200-m depth contour.



Figure 3. Average daily locations two juvenile female (A and K) and two juvenile male (G and J) harbor seals in Prince William Sound and Gulf of Alaska. Dotted line shows the 200-m depth contour.

combined on where seals were located when signals ended, with adult males always near the tagging location, and juvenile females usually somewhere else.

We then examined possible effects of age and sex on home range size and movements between haulouts and at-sea locations using data for the entire tracking period of each seal. As expected the repeated-measures model showed that the number of days that SDRs were operational had a significant effect (P < 0.01) on home range size and most movement variables, except mean

Table 2. General movement patterns of juvenile and adult harbor seals satellite tagged in Prince William Sound, Alaska, and probability (P) values for effects due to age, sex, age + sex, and age\*sex interaction from a logit model.

Age/sex class (n)	Local movement only	Moved to glaciers	Moved to Gulf of Alaska	At/near tagging location at end
Juvenile female (11)	36%	18%	55%	36%
Juvenile male (11)	45%	36%	36%	73%
Adult female (15)	80%	7%	20%	87%
Adult male (12)	50%	17%	33%	100%
Age effect P	0.070	0.146	0.153	0.006
Sex effect P	0.332	0.188	0.999	0.040
Sex + age effect $P$	0.130	0.156	0.359	0.001
Sex*age effect P	0.103	0.293	0.335	0.327

distance moved between haulouts (P = 0.09). For those variables with an operational days effect, the number of days was scaled to 25 (per month) for presentation of results. Sex alone did not have a significant effect on any of the variables (P > 0.31) nor were there significant age\*sex interactions (P > 0.24). However, there were significant differences between juveniles and adults for all variables except cumulative distance moved between all haul-outs used (Table 3). Juvenile seals moved more than adults: their maximum distance moved from the initial location was 58% greater; the mean and maximum distances between successively used haul-outs were 152% and 141% greater; the mean distance from haul-outs to at-sea locations was 96% greater; and mean home range size was 81% larger. Successively used haul-out locations were 7.8 km apart for juveniles and 3.1 km apart for adults.

When the movement data were examined by month there were significant effects of age and month, as well as age\*month and sex\*month interactions (Table 4). In most months the maximum distance from the tagging location was greater for juveniles than for adults, but adults were substantially farther away than juveniles in September and in April (Fig. 4). Overall, juveniles tended to be farthest from the initial tagging location during fall and winter, whereas adults were farthest away in spring (March-May).

The distances between haul-outs used were greatest in February and March for females, and in September and April for males (Fig. 5). Periods of greatest or least movement between haul-outs were similar for seals of the same sex. In general, haul-outs used by males were closer together than haul-outs used by females during October-March and farther apart during April-September.

Juvenile seals moved farther to sea from their haul-outs than adults in all months except April (Fig. 6). Distances moved to sea by adults varied little with month, although the shortest distances were in June-September. Juveniles moved greatest distances in February-March and September-November. These were also the months when differences between juveniles and adults were greatest.

Mean home range size varied greatly by month (Fig. 7). Home range sizes were small for all age/sex categories in June and July, and for juventles in September. During most months, home ranges were of similar size for adults and juveniles of the same sex. The exceptions were September and May, when adult home ranges were much larger than juvenile home ranges, and October when the reverse was true. Females of any age almost always had larger home ranges than males during September–March (January was the only exception), while home ranges for females and males within the same age group were of similar size during April–July.

#### DISCUSSION

The use of satellite-linked telemetry proved to be an effective means of studying the distribution and movements of harbor seals in PWS and adjacent parts of the Gulf of Alaska. On average, seals were located on four out of every five days that tags transmitted, and many locations were received both on land

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Table 3.	Movements	(km) :	and h	ome r	range	(km <sup>2</sup>	) of	juvenil	e and	adul	t harb	or seal	s satel	lite	tagged	in	Prin	ce V	Willian	n Sound	, Ala	iska.
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Age class (n)	Maximum distance from initial location	Mean distance between successive haul- outs	Maximum distance between any haul-outs	Cumulative distance between all haul- outs used	Mean distance from haul-outs to at-sea locations	Home range		
Juveniles (22)								
Fitted mean <sup>a</sup> Range <sup>b</sup>	96.6 20–525	7.8 1–68	51.6 3–436	246.5 16–854	15.5 5–63	3,167 485–54,976		
Adults (27)								
Fitted mean <sup>a</sup> Range <sup>b</sup>	61.3 22–189	3.1 1–14	21.4 3–178	233.5 8–1266	7.9 4–19	1,749 311–16,970		
P value for age effect	0.016	< 0.001	0.004	0.910	<0.001	0.028		

<sup>a</sup> For cases where the number of operational days was a significant factor in the model, values are scaled to an operational period of 1 <sup>b</sup> Figures shown are the range in overall values for the individual seals within the age class.

Table 4.	P-values	for effects	of age,	sex, a	nd month	on	movements	of	juvenile a	nd adı	ilt harboi	: seals	satellite	tagged	in	Prince	William
Sound, Alask	a.																

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Effect	Maximum distance from initial location	Mean distance between successive haul-outs	Maximum distance between any haul-outs	Cumulative distance between all haul-outs used	Mean distance from haul-outs to at-sea locations	Home range
Age	0.280	0.001	0.009	0.700	0.001	0.435
Sex	0.234	0.775	0.902	0.862	0.886	0.306
Month	0.001	0.144	0.482	0.407	0.014	0.001
Age*sex	0.686	0.566	0.329	0.522	0.628	0.485
Age*month	0.001	0.372	0.674	0.487	0.052	0.001
Sex*month	0.533	0.011	0.020	0.123	0.081	0.011

1.5

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Figure 4. Maximum distance from initial location by month for 49 satellite-tagged harbor seals in Prince William Sound, Alaska, 1992–1997.

and at sea. They were tracked to locations hundreds of km distant from where they were tagged, places where they would never have been found using VHF tags. Most tags provided data for 2-10 mo, which resulted in good seasonal coverage for all months of the year except during the molt.

SDR-tagged adult harbor seals were relatively sedentary, with 18 of 27 animals remaining near the tagging location for the duration of tracking. The maximum recorded distance away from the tagging location for adults was 189 km. Seven adult seals did move from PWS into the Gulf of Alaska, but four of those had returned to the tagging location when signals stopped.

Overall, juvenile harbor seals moved more than adults. They moved greater distances from the initial tagging location, the haul-outs they used were more spread out, and they ranged farther from haul-outs on trips to sea. This tendency for juveniles to travel more than adults has also been documented in other areas (Thompson 1993, Stewart and Yochem 1994). Some of this movement was likely associated with foraging, but some may have been dispersal. A tendency for juvenile seals to disperse may be why they moved greater distances from the tagging location.

There are some limitations to using our data to examine fidelity to regions. One is the possibility that the location where a seal was captured was not its "home site." Alternatively, seals may simply have been on trips when their transmitters failed. All adult seals relocated in June–July during breeding and the start of the molting season were in the area where they were initially tagged. Only two adult seals were not near the capture site when transmitters failed. Both of those were females tagged in Port Chalmers in September that were located in the Copper River delta the following May when their tags failed. Both had made one or more trips away from, and returned to, Port Chalmers over the winter. Large runs of eulachon, (*Thaleichthys pacificus*), a high-fat forage fish eaten by PWS harbor seals (Pitcher 1980) are present in





the Copper River delta in May, and those females may have been feeding on eulachon prior to parturition. Taken in aggregate, we think the movement data from the 27 adult seals we tracked over periods of 1-10 mo indicate strong site fidelity by adult seals in PWS, despite occasional forays away from their home sites.

Juvenile seals were much more likely to be away from the capture site when their tags stopped transmitting. Final locations for almost half of the juveniles we tagged were not at the site where they were captured. As indicated by the logit model, juvenile females demonstrated the least site fidelity, with 7 of 11 last located away from capture site. Seals caught in spring were less likely than seals caught in fall to be far from the capture site when the last signals were received. Even though 3 of 10 spring-tagged juveniles were away from the capture site when transmissions ceased, all final locations were within 20





km of that site. In contrast, 7 of the 12 fall-tagged juveniles were 100-500 km away from the capture site when their signals were last received. Although satellite tags enabled us to monitor harbor seal movements over periods of 1-10 mo they provided no information about longer-term movements. Thus, using SDR data we can only speculate about whether traveling seals might eventually return to original capture locations, or the degree to which dispersal might occur. However, as part of this study we also attached flipper tags to every seal that we handled. Eleven of these flipper-tagged seals have been recaptured by us, or harvested and reported by Alaska Native subsistence hunters. Seven of them, recovered 12-45 mo after tagging, were at the exact location where they were initially captured. Four others, recovered 12-49 mo after tagging, were at locations 8–43 km distant from the capture location. These results show long-term fidelity to the PWS region for at least some seals. Results from genetics studies of Alaska harbor seals also support long term site fidelity. Analyses of mitochondrial DNA indicate substantial regional genetic differentiation within PWS harbor seals, as well as between seals from PWS and adjacent areas.<sup>2</sup>

Tracking seals with SDRs or other telemetry does not unequivocally identify places where they feed without additional information such as from stomach temperature sensors or underwater cameras. In this study we used the mean distances from haul-outs to subsequent at-sea locations as a measure of how far seals moved when they went to sea. We recognize that this is not a direct measure of the size of harbor seal foraging ranges, but rather an index of atsea movement. For seals tagged in PWS, 90% of the mean distances from haul-outs to subsequent at-sea locations were 25 km or less, and 97% were 50 km or less (Fig. 8). These results correspond well with other information

<sup>2</sup> Personal communication from Barbara Taylor, Southwest Fisheries Science Center, National Marine Fisheries Service, La Jolla, CA 92038, 15 April 1999.





that suggests that harbor seals in PWS often feed near their haul-outs. Iverson *et al.*-(1997) found differences in blubber fatty acid signatures, and therefore diets, in seals sampled at haul-outs 9-15 km apart in PWS, and concluded that the seals may depend on a very localized prey base.

The at-sea movements of PWS harbor seals were generally consistent with foraging behavior described in other areas. In Washington and California, most at-sea locations of tagged harbor seals were within 5 km of haul-outs although some seals ranged as far as 75 km (Brown and Mate 1983, Suryan and Harvey 1998, Stewart *et al.* 1989). In Moray Firth, Scotland, mean foraging ranges for 37 VHF tagged seals extended 4–55 km from their haul-out sites (Thompson *et al.* 1998). Thompson *et al.* (1998) found that the mean foraging range for males was larger than that for females, but we found no sex-related differences in at-sea movement patterns in PWS.

Thompson (1993) and Thompson et al. (1998) distinguished between for-



Figure 8. Distribution of mean distances from haul-outs to subsequent at sea locations for 49 harbor seals satellite-tagged in Prince William Sound, Alaska, 1992– 1997.

aging trips and seasonal movements or dispersal, and suggested that when foraging areas were more than 50-60 km from haul-outs, harbor seals changed their haul-out location. While some PWS seals did change haul-out locations during the time they were tagged, not all long trips could be attributed to seasonal movements. For example, a juvenile female tagged in PWS moved more than 500 km to Yakutat Bay in October (see Fig. 3). While this was likely a seasonal movement (or dispersal), six subsequent trips to sea were not. Over the next four months, this seal alternated 3-10-d periods in Yakutat Bay with 11-20-d trips to the Gulf of Alaska more than 150 km away. Two males moved more than 100 km south in winter into the Gulf of Alaska, where they hauled out at Middleton Island and made trips seaward from there to areas more than 100 km away. An adult female made an 18-d trip from Seal Island to an area more than 130 km away in the Gulf, with no intermediate hauling-out stops, and then returned directly to Seal Island. Clearly although PWS harbor seals frequently stay within 25 km of a haul-out when they go to sea, some may travel to areas much farther away. We presume that feeding is the main purpose of most of those trips to sea.

The average distance from haul-outs to at-sea locations for PWS seals was almost twice as far for juveniles as it was for adults. For adults in this study, the mean haul-out to at-sea distances were 5-10 km, and were similar throughout the year. Monthly mean haul-out to at-sea distances for juveniles generally exceeded 10 km (Fig. 6). This differs from the results of Thompson *et al.* (1998) who found a positive relationship between body size and foraging range for harbor seals in northeast Scotland, and suggested that larger animals spend more time at sea and travel farther to foraging areas because they are energetically able to do so. In fact, the smallest seal we instrumented (28 kg) made some of the longest trips to sea of 150 km or more.

On an annual basis we found no significant differences in the movement

patterns of harbor seals based on sex alone, but there were sex by month interactions for several variables. Mean and maximum distances between haulouts used were generally larger for females in fall and winter, and larger for males in spring and summer (Fig. 5). Mean home ranges were also larger for females than for males during all months of the fall and winter except January, and were two to four times larger during October and February–March (Fig. 7). This tendency for females to move more during fall-winter was also apparent in harbor seals from California (Allen 1988). In contrast, during spring and summer the home ranges of adult males and females in PWS were similar.

In May, just prior to breeding, the mean home ranges of adult harbor seals in PWS were much larger than for juveniles, and were also larger than at any other time of year (except March for females). Such large home ranges may represent redistribution as the breeding season approaches, with females moving to haul-outs preferred for pupping, and males seeking access to females. Alternatively, adults may be spending more time away from haul-outs to maximize energy intake prior to periods of reduced feeding associated with breeding. However, this alternative is not substantiated by an analysis of diving effort for these same seals, which indicated that diving effort decreased rather than increased in May following eight months of high and fairly constant effort (Frost et al. 2001). Van Parijs et al. (1997) found that male harbor seals in Moray Firth, Scotland, traveled widely in June, then restricted their ranges in early July when females began foraging in late lactation. In that study, mean 7-d home ranges decreased from 65-480 km<sup>2</sup> in June to 4-70 km<sup>2</sup> in July. Home ranges of females in Moray Firth decreased in size about two weeks earlier than males, with the onset of pupping (Thompson et al. 1994). We calculated mean home ranges by month rather than by week, but also saw a marked decline in both males and females from about  $1,000 \text{ km}^2$  in May to 200-300 km<sup>2</sup> in June and July. Pupping begins in mid-May in PWS, somewhat earlier than in Moray Firth, and extends through early to mid-June (Lowry and Frost, unpublished data), thus some females would be entering late lactation in June, about the time male home ranges became smaller.

Home ranges of juvenile males and juvenile females were similar during spring through early fall, likely because of molt chronology and similar physiological requirements of non-breeding animals. During winter, however, mean home ranges were more similar for animals of the same sex than for those of the same age. For example, mean home ranges for females (adults and juveniles) were more than 600 km<sup>2</sup> in February and 1,200 km<sup>2</sup> in March, compared to 200–300 km<sup>2</sup> for males. While it would seem straightforward to attribute age or sex-related differences in movements and home ranges during spring and summer to changes associated with reproduction and molting, we have no obvious explanation for similarities in movements during winter. Others have observed differences in movement patterns similar to the ones we report here for adult males and females during winter, with females ranging farther than males (Thompson 1993). However, we know of no other studies reporting sex-based differences for juvenile harbor seals. While Thompson *et al.* (1998) discussed differences between males and females of all sizes, and size-related differences for both sexes combined, they did not address whether there might be sex-related differences within juvenile and adult age groups. Overall, they concluded that larger animals spent more time at sea and had larger foraging ranges than smaller animals. Tagged harbor seals in this study did not follow this pattern. Mean distances moved to sea and the mean distance between haul-outs used were greater for juveniles than for adult seals. Furthermore, females of both sizes had larger home ranges than males, which was the opposite of what was reported by Thompson *et al.* (1998). Additional geographic comparisons of seal movement patterns will be useful to better understand how seal physiology and environmental characteristics interact to affect such parameters as home range and foraging range.

The fact that more locations per day were obtained for females of all ages than for males introduces some possibility for bias in interpreting data about movements and home range size. The additional locations generated by females might produce a more detailed record of movements, and thus reflect larger home ranges and longer distances. If this occurred, we might expect the data to indicate consistently larger home ranges for females, as well as longer distances moved to at-sea locations. This was not the case. While females had larger home ranges in most months, the home ranges of males were sometimes larger. Similarly, the model indicated no sex-related differences in distance traveled from haul-outs to at-sea locations. It is unclear what might cause such sex-related differences in the number of locations obtained. Transmissions to the satellite, and thus number of locations obtained, could be affected by consistent differences in the amount of time spent diving, or a tendency for males to dive at a time of day when satellite coverage was poor. However, analysis of dive data for these seals indicated no such sex-related differences (Frost et al. 2001).

Only about 10% of the locations in our data set were obtained during the nighttime period (2100–0300), which might constitute a source of bias in estimating both home range size and distances traveled. This was the time of day with the least satellite coverage. Also, seals in this study spent more time in the water at night than at other times of day (Frost *et al.* 2001). More time spent diving at night, in combination with infrequent satellite coverage, resulted in limited information on nighttime movements. Fewer locations may have resulted in underestimation of both home range size and distance traveled. If seals are foraging extensively at night, when few locations are received, it will be difficult to accurately determine foraging areas using existing SDR technology. However, even though the absolute values for movements may be somewhat underestimated, the dive data presented by Frost *et al.* (2001) suggest that any biases should be consistent across sex and age groups.

#### ACKNOWLEDGMENTS

This study was conducted as part of the Exxon Valdez Oil Spill Restoration Program, funded by the Exxon Valdez oil spill Trustee Council. We thank Brian Fadely, Vladimir Burkanov, Jennifer Burns, Mike and Maggie Castellini, Shane Kanatous, Dan Reed, Ken Taylor, Pam Tuomi, and Randy Zarnke for help in the capture, sampling, and tagging of seals. We also thank the several Alaska Native subsistence hunters who returned flipper tags to us. The Captain, Glen Hodge, and the crew of the research vessel *Pacific Star* provided excellent logistic support. Dean Hughes, Joe Sullivan, Bill Hauser, Melanie Bosch, Celia Rozen and Diana Ground provided administrative support for this project. Paul Thompson and Bernie McConnell provided helpful reviews of the draft manuscript. This study was conducted under authorization of National Marine Fisheries Service Scientific Research Permits 770 and 1000 issued to the Alaska Department of Fish and Game.

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Received: 13 September 2000 Accepted: 9 April 2001 Environmental and Ecological Statistics 10, 201–219, 2003

# A Bayesian hierarchical model for monitoring harbor seal changes in Prince William Sound, Alaska

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Received March 2001; Accepted March 2002

Bayesian hierarchical models were used to assess trends of harbor seals, Phoca vitulina richardsi, in Prince William Sound, Alaska, following the 1989 Exxon Valdez oil spill. Data consisted of 4-10 replicate observations per year at 25 sites over 10 years. We had multiple objectives, including estimating the effects of covariates on seal counts, and estimating trend and abundance, both per site and overall. We considered a Bayesian hierarchical model to meet our objectives. The model consists of a Poisson regression model for each site. For each observation the logarithm of the mean of the Poisson distribution was a linear model with the following factors: (1) intercept for each site and year, (2) time of year, (3) time of day, (4) time relative to low tide, and (5) tide height. The intercept for each site was then given a linear trend model for year. As part of the hierarchical model, parameters for each site were given a prior distribution to summarize overall effects. Results showed that at most sites, (1) trend is down; counts decreased yearly, (2) counts decrease throughout August, (3) counts decrease throughout the day, (4) counts are at a maximum very near to low tide, and (5) counts decrease as the height of the low tide increases; however, there was considerable variation among sites. To get overall trend we used a weighted average of the trend at each site, where the weights depended on the overall abundance of a site. Results indicate a 3.3% decrease per year over the time period.

*Keywords*: trend analysis, abundance estimation, population monitoring, Markov Chain Monte Carlo, Poisson regression, aerial surveys, *Exxon Valdez* oil spill, harbor seal, *Phoca vitulina richardsi*, Prince William Sound

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# 1. Introduction

Monitoring programs to track long-term changes in population size are important for applied ecological studies. Such monitoring programs often have multiple objectives that include monitoring trends, estimating abundance, and estimating the effects of covariates, both for large areas and smaller areas that comprise the larger area. In this paper we develop a Bayesian hierarchical model for analyzing trend, abundance, and the effects of covariates for monitoring programs of multiple sites, and we apply it to counts of harbor seals following the *Exxon/Valdez* oil spill of 1989 in the Prince William Sound, Alaska.

Harbor seals are one of the most common marine mammal species in Prince William Sound (PWS), Alaska, and adjacent parts of the Gulf of Alaska. PWS has over 4800 km of coastline, consisting of many fiords, bays, islands, and offshore rocks. The exact number of harbor seals inhabiting the region is unknown, but is at least several thousand (T. R. Loughlin, unpublished report, National Marine Mammal Laboratory, NMFS, Seattle, WA.). Between 1984 and 1988 the number of seals counted at haulout sites in eastern and central PWS declined by about 40% (Frost *et al.*, 1994). The harbor seal population was monitored by flying aerial surveys during 1989–1999 subsequent to the *Exxon/Valdez* oil spill as part of damage assessment and restoration programs.

Many studies have demonstrated effects of time of day, date, and tide on the hauling out behavior of harbor seals (Schneider and Payne, 1983; Stewart, 1984; Harvey, 1987; Pauli and Terhune, 1987; Yochem et al., 1987; Thompson and Harwood, 1990; Moss, 1992; Frost, et al., 1999). The data to describe those behavioral patterns has usually come from continuous or repetitive visual observations of seal haulouts, or from telemetry studies. Information derived from those studies has been used in the design of harbor seal surveys, to the extent that survey programs are generally designed to occur on dates and at times when the greatest number of seals is expected to be out of the water and available for counting (Pitcher, 1990; Harvey et al., 1990; Olesiuk et al., 1990; Huber, 1995). However, once a "survey window" has been established counts have usually been treated as replicates during analyses, and the possible effects of other factors on annual abundance estimates have been ignored. In fact there are generally two ways to account for the effects of covariates. One is to use a design that "standardizes" for all of the effects, such as picking a narrow range of dates, having a particular weather condition, a particular time of day, a particular time in the tide cycle, etc. While desirable, the problem with the standardized design approach, for our study, is that date, weather and tide cycles rarely cooperate to provide standardized conditions year after year. We adopt an alternative where we pick a relatively broad range of dates and count seals when weather allows. We then make adjustments to counts based on data collected on covariates that are known to have an effect on counts. Of course, the estimation of the effects of the covariates themselves is also of interest.

There are often several statistical methods to analyze such data. One of the most fundamental differences among statistical methods occurs when making a choice between Bayesian and classical (frequentist) methods. While there are strong philosophical differences, in practice results can be quite similar, and the choice can be made on practical considerations. In this study, we consider models of trend and abundance that include the effects of covariates for twenty-five sites individually. Then it is natural to give the parameters of all 25 sites a common distribution, thus developing a hierarchical model. The advantage of this approach is that the problems of estimating trend, abundance, and the effects of covariates are given a single unified probability framework. The hierarchical model also helps stabilize estimates in cases where sample sizes for individual components are small.

This paper presents an analysis of aerial survey counts of harbor seals in PWS. The objectives are to develop a Bayesian hierarchical model to (1) estimate trends at individual sites, (2) estimate trends in the study area as a whole, (3) estimate yearly abundance at each site, (4) estimate yearly abundance for all sites combined, and (5) study the effects of covariates: date, time of day, time relative to low tide, and tide height, on seal counts.

# Bayesian hierarchical model

While we developed this model for harbor seal data, we believe it has broader application in many other monitoring situations.

# 2. Methods

# 2.1 Aerial surveys

Harbor seals generally have high fidelity to a haulout site during the molting period. They haul out near low tide, which allows them to be counted on multiple occasions. We conducted aerial surveys along a trend count route that covered 25 harbor seal haulout sites in eastern and central PWS (Fig. 1). The route included 7 sites that were substantially affected by the *Exxon Valdez* oil spill and 18 unoiled sites that were outside of the primary affected area (Frost *et al.*, 1994). Surveys were flown during the molting period (August–September) in 1984 and 1988–1999.

Visual counts of seals were conducted from a single-engine fixed-wing aircraft (Cessna 185) at altitudes of 200–300 m, usually with the aid of 7-power binoculars. Counts were usually conducted from two hours before low tide to two hours after low tide. A survey normally included counts at all 25 sites, but occasionally some sites could not be counted because of poor weather or a rapidly rising tide. For each survey the date, time and height of low tide, and time of sunrise and sunset were recorded for each site. Each site was circled until the observer was confident that an accurate count had been made, and the time of the count was recorded. For larger groups of seals (generally those of 40 or more) color photographs were taken using a hand-held 35-mm camera, and seals were counted from images projected on a white surface. Several survey flights, usually 7–10, were made each year. The effects of the oil spill on harbor seal numbers has been extensively described (e.g., Frost *et al.*, 1994; Lowry *et al.*, 1994; Morris and Loughlin, 1994). In this paper, we only consider data after the 1989 oil spill, from 1990 to 1999. The total number of counts for all sites for the time period was 1739.

Prior to further data analysis, the covariates: date, time-of-day, time-relative-to-low-tide, and tide-height were rescaled to prevent computer overflows during estimation. The effect of year was rescaled by setting 1994 as year 0. Specifically, the covariates were adjusted as follows:

$$j = \text{Year}-1994,$$

$$x_{1ijk} = \frac{(\text{Date with August 1 as day 1-28})}{100},$$

$$x_{2ijk} = \frac{(\text{Time-of-day from midnight [in minutes]}-720)}{1000},$$

$$x_{3ijk} = \frac{(\text{Time-relative-to-low-tide [in minutes]})}{100},$$

$$x_{4ijk} = \frac{(\text{Tide-height of low tide [in feet]})}{10},$$

for the *k*-th flight at site *i* in year *j*.
#### Ver Hoef, Frost



Figure 1. Map showing trend-count sites for aerial surveys of harbor seals in Prince William Sound, Alaska, 1990–1999.

### 2.2 Previous methods-Poisson regression for all sites combined

Frost *et al.* (1999) used a generalized linear model (McCullagh and Nelder, 1989) with a log link function and a Poisson distribution to analyze the factors that may affect the number of seals hauled out and available to be counted during surveys. The model may be written as:  $\Pr(Z_{ijk} = z) = \exp(-\lambda_{ijk})\lambda_{ijk}^z/z!$  with  $\ln(\lambda_{ijk}) = \beta' x_{ijk}$  where  $\beta$  is a parameter vector and  $x_{ijk}$  is a vector containing information on the state of covariates: site, year, date, time of day, time relative to low tide, and tide height, for the *k*-th flight at site *i* in year *j*. Loglikelihood ratios were used to obtain a parsimonious model. Then the count data were

adjusted to a standardized set of covariates. The adjustment amounted to the expected count at each site for each year under optimal conditions. Next, to assess overall trend, linear regression and Poisson regression models were fitted to the adjusted yearly count estimates. The analysis of Frost *et al.* (1999) was complicated because they first adjusted yearly counts for each site to a standardized date, time of day, and time relative to low tide, then summed over sites to get a yearly index, and then used the index in a trend regression analysis. Under these circumstances, it is difficult to take all of the uncertainty associated with adjusting the counts and then using trend analysis on the adjusted counts. Therefore, they used bootstrap methods (Efron and Tibshirani, 1993; Manly, 1997) for the whole procedure.

### 2.3 Bayesian hierarchical model

The Bayesian hierarchical model begins with Poisson regression for each observation. Let  $Z_{ijk}$  be a random variable of the number of seals counted for the *k*-th replicate flight in the *j*-th year at the *i*-th site. Write

$$f(z_{ijk}) = \exp(-\lambda_{ijk})\lambda_{ijk}^{z_{ijk}}/z_{ijk}!$$

with

$$\ln(\lambda_{ijk}) = \theta_{ij} + x_{1ijk}\beta_{1i} - x_{1ijk}^2b_{2i} + x_{2ijk}\beta_{3i} - x_{2ijk}^2b_{4i} + x_{3iik}\beta_{5i} - x_{3iik}^2b_{6i} + x_{4iik}\beta_{7i} + \varepsilon_{iik},$$
(1)

where  $\theta_{ij}$  is an intercept,  $\varepsilon_{ijk}$  is an overdispersion parameter, and  $x_{pijk}$  is the *p*-th explanatory variable containing observed values of the covariates:  $x_{1ijk} = \text{date}$ ,  $x_{2ijk} = \text{time of day}$ ,  $x_{3ijk} = \text{time relative to low tide}$ , and  $x_{4ijk} = \text{height of tide}$ , for the *k*-th flight at site *i* in year *j*. For the effects of date, time of day, and time relative to low tide, we wanted a model that would be unimodal with a single peak value, so we forced  $b_{2i}$ ,  $b_{4i}$ , and  $b_{6i}$  to be positive by reparameterizing; e.g.,  $b_{2i} = \exp(\beta_{2i})$ , where  $-\infty \le \beta_{2i} \le \infty$ . Thus, the two terms  $x_{1ijk}\beta_{1i} - x_{1ijk}^2b_{2i}$  form a Gaussian curve when exponentiated. We assume that conditional on the covariates, all observations are independent, so the joint density is,

$$f(\boldsymbol{z}|\boldsymbol{\theta},\boldsymbol{\beta}) \equiv \Pi f(z_{ijk})$$

In the next level of the hierarchy, we develop a separate trend model for each site,  $f(\theta_{ij}|\tau_{0i}, \tau_{1i}, \delta^2) = N(\tau_{0i} + \tau_{1i} \times j, \delta^2)$ , where N(m, V) is a normal distribution with mean *m* and variance *V*, and jointly,

$$f(\theta|\tau,\delta^2) = \prod_{i=1}^{25} \prod_{j=-4}^{5} f(\theta_{ij}|\tau_{0i},\tau_{1i},\delta^2).$$

Next, we group the site-specific covariate parameters and give them a distribution;  $f(\beta_{pi}|\mu_p, \sigma_p^2) = N(\mu_p, \sigma_p^2)$ , where jointly,

$$f(\boldsymbol{\beta}|\boldsymbol{\mu},\boldsymbol{\sigma}) = \prod_{p=1}^{7} \prod_{i=1}^{25} f(\beta_{pi}|\boldsymbol{\mu}_p,\sigma_p^2).$$

For the trend parameters, we will also group the site-specific covariate parameters and give them a distribution;  $f(\tau_{qi}|\eta_q, \gamma_q^2) = N(\eta_q, \gamma_q^2)$ , where jointly,

$$f(\boldsymbol{\tau}|\boldsymbol{\eta},\boldsymbol{\gamma}) = \prod_{q=0}^{1} \prod_{i=1}^{25} f(\tau_{qi}|\boldsymbol{\eta}_{q},\boldsymbol{\gamma}_{q}^{2}).$$

We also group the overdispersion parameters and give them a distribution;  $f(\varepsilon_{iik}|\mathbf{0}, \xi_i^2) = N(0, \xi_i^2)$ , where jointly,

$$f(\boldsymbol{\varepsilon}|\mathbf{0},\boldsymbol{\zeta}) = \prod_{i=1}^{25} \prod_{j=-4}^{5} \prod_{k=1}^{n_{ij}} f(\varepsilon_{ijk}|\mathbf{0},\boldsymbol{\zeta}_{i}^{2}),$$

and  $f(\xi_i|\nu_a,\nu_b) = GAM(\nu_a,\nu_b)$ , where GAM(a,b) is a gamma distribution with parameters *a* and *b*, where jointly,

$$f(\boldsymbol{\xi}|\nu_{a},\nu_{b}) = \prod_{i=1}^{25} f(\xi_{i}|\nu_{a},\nu_{b}).$$

In the fourth and final level of the hierarchy, we give diffuse prior distributions,  $f(\mu_p)$  and  $f(\eta_q)$  are N(0, 1,000,000); and  $f(\delta^2), f(\sigma_p^2), f(\gamma_q^2), f(\nu_a)$  and  $f(\nu_b)$  are GAM(0.001, 0.001). Jointly,

$$f(\boldsymbol{\mu}) = \prod_{p=1}^{7} f(\mu_p), f(\eta) = \prod_{q=0}^{1} f(\eta_q), f(\boldsymbol{\sigma}) = \prod_{p=1}^{7} f(\sigma_p^2), \text{ and } f(\boldsymbol{\gamma}) = \prod_{q=0}^{1} f(\gamma_q^2).$$

The hierarchical model is shown diagrammatically in Fig. 2. Using the hierarchical setup, Bayes theorem allows us to write the posterior distribution:

$$f(\boldsymbol{\theta},\boldsymbol{\beta},\boldsymbol{\tau},\boldsymbol{\varepsilon},\delta^{2},\boldsymbol{\xi},\boldsymbol{\mu},\boldsymbol{\sigma},\boldsymbol{\eta},\boldsymbol{\gamma},\nu_{a},\nu_{b}|\boldsymbol{z}) \propto f(\boldsymbol{z}|\boldsymbol{\beta},\boldsymbol{\theta})f(\boldsymbol{\theta}|\boldsymbol{\tau},\delta^{2})f(\boldsymbol{\beta}|\boldsymbol{\mu},\boldsymbol{\sigma})f(\boldsymbol{\varepsilon}|\boldsymbol{0},\boldsymbol{\xi})f(\boldsymbol{\tau}|\boldsymbol{\eta},\boldsymbol{\gamma})f(\delta^{2})f(\boldsymbol{\xi}|\nu_{a},\nu_{b})f(\boldsymbol{\mu})f(\boldsymbol{\sigma})f(\boldsymbol{\eta})f(\boldsymbol{\gamma})f(\nu_{a})f(\nu_{b}).$$
(2)



Figure 2. Diagrammatic scheme of hierarchical model.

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It is difficult to obtain an analytical solution to the above equation; however the modern techniques of Markov Chain Monte Carlo (MCMC, see, for example, Gilks *et al.*, 1996) allow us to obtain samples from the posterior distribution. From these samples we can compute functions and summaries of the posterior distribution, such as expectation, standard errors, quantiles, etc. The resulting tables use covariates on their standardized scale, but the figures show the effects back on the original scale. Rescaling the covariates helped to stabilize the MCMC methods.

From the posterior distribution, several parameters have particular interest. The parameter  $\tau_{1i}$  is the slope parameter for the *i*-th site, and  $\eta_1$  is the mean of all 25 sites, which is an overall indication of trend among all sites. However,  $\eta_1$  is not entirely satisfactory because it weights all sites equally (actually, it depends on their sample sizes—in this study, they are all relatively equal). In order to give sites with greater abundance more weight, we can consider the following:

$$\alpha_{1} = \frac{\sum_{i=1}^{25} \exp(\tau_{0i}) \tau_{1i}}{\sum_{i=1}^{25} \exp(\tau_{0i})},$$
(3)

as an indicator of overall trend. Other weighting schemes are possible, such as weighting by the last year, or the average of all years. The hierarchical Bayes method using MCMC makes it easy to obtain inference on  $\alpha_1$ —we simply use the samples from the posterior distributions of  $\tau_{0i}$  and  $\tau_{1i}$  to compute the posterior distribution of  $\alpha_1$ . Another function of the parameters that has particular interest is an indication of overall abundance for each year, which we compute as,

$$\phi_j = \sum_{i=1}^{25} \exp(\theta_{ij} + x_{1s}\beta_{1i} - x_{1s}^2b_{2i} + x_{2s}\beta_{3i} - x_{2s}^2b_{4i} + x_{3s}\beta_{5i} - x_{3s}^2b_{6i} + x_{4s}\beta_{7i}).$$
(4)

where  $x_{ks}$ ; k = 1, ..., 4, are specified values for the covariates.

We performed some model diagnostics. A common measure for the fit of the model is to compute a Chi square discrepancy (see, for example, Gelman *et al.*, page 172). In general, it is defined as,  $[y - E(Y)]^2/var(Y)$ . For our application, we computed the posterior distribution of the Chi square discrepancy for each site,

$$R_{i} = \frac{1}{N_{i}} \sum_{j=-4}^{5} \sum_{k=1}^{n_{ij}} \frac{(Z_{ijk} - \lambda_{ijk})^{2}}{\lambda_{ijk}}$$

where  $N_i$  is the total number of observations over all replicates and years for the *i*th site. If the model is fitting well, we expect  $R_i$  to be near one for each site. We compute  $R_i$  by site to highlight whether lack of fit occurs locally or globally.

The statistical package *WinBUGS* was used for the Bayesian hierarchical model. For the MCMC, we let the chain "burn in" for 4000 samples, and then computed the means, standard errors, and percentiles based on the next 10,000 simulations. We started the chain from several different points and obtained very similar results, and examination of the trace of the chain did not reveal any irregularities. Typically the autocorrelation in the chain for each parameter dropped to near zero well before 30 iterations.

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### 3. Results

### 3.1 Covariates

Four primary factors were considered that might affect the counts of seals during aerial surveys. Figs 3 to 6 show the effects of date, time of day, time relative to low tide, and tide height for each site. There graphs were developed by first transforming the covariates as described in Section 2, call them  $x_{ks}$ ; k = 1, ..., 4. Then each panel is a graph of

$$\exp\left(x_{ks}\hat{\beta}_{pi} - x_{ks}^2\hat{b}_{qi}\right),\tag{5}$$

for the *i*-th site, where  $\hat{\beta}_{pi}$  is the mean of the MCMC sample from the posterior distribution for that  $\beta$  associated with  $x_{ks}$ , and  $\hat{b}_{qi}$  is the mean of the MCMC sample from the posterior distribution for  $b_{qi} = \exp(\beta_{qi})$ . Notice that Fig. 6 only contains the term  $x_{ks}\hat{\beta}_{pi}$ . Also notice that another estimate that easily allows credibility intervals can be obtained for the graphs by using,



Figure 3. Effect of date on counts of harbor seals for each of the 25 haul-out locations in Prince William Sound, Alaska.



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Figure 4. Effect of time of day on counts of harbor seals for each of the 25 haul-out locations in Prince William Sound, Alaska.

$$\frac{1}{N}\sum_{L=1}^{N}\exp(x_{ks}\beta_{pi}^{(L)}-x_{ks}^{2}\beta_{qi}^{(L)}),$$

rather than (5), where L indexes the MCMC iteration. However, all iterations must be stored for various x values, so it requires more storage.

Note that  $\hat{b}_{qi}$  is enforced to be positive, which forces all curves in Figs 3 to 5 to be Gaussian with a single maximum (which may be off the range of the abscissa). We chose to do this because, from a biological viewpoint, we expect seals to spend most of their time hauled out during the molting period, which is around mid to late August. Thus, there should be a well-defined maximum during these molting dates for Fig. 3. Likewise, we expect a peak time of day for haulout, and a peak time relative to low tide. However, we expect only a linear trend (on the log scale) for tide height.

Finally, note that for standardized states of the covariates, Equation (1) can be written as

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Figure 5. Effect of time relative to low tide on counts of harbor seals for each of the 25 haul-out locations in Prince William Sound, Alaska.

$$\lambda_{ij} = \exp(\theta_{ij}) \exp(x_{1s}\beta_{1i} - x_{1s}^2 b_{2i}) \exp(x_{2s}\beta_{3i} - x_{2s}^2 b_{4i}) \exp(x_{3s}\beta_{5i} - x_{3s}^2 b_{6i}) \exp(x_{4s}\beta_{7i}),$$
(6)

so (5) can be seen as a multiplicative factor for each effect that controls the proportional change in the expected counts for the *i*-th site in the *j*-th year.

The overall effects of covariates are given in Fig. 7. The model predicted that, overall, maximum counts occur near the 15th of August, after which counts decrease. Counts are about 10% lower on the 21st of August compared to the 15th, and about 20% lower by the beginning of September (Fig. 7(a)). The model predicted that overall counts would decrease throughout the day, with counts 10% lower at noon than at 7:00 am, and another 10% lower at 5:00 pm than at noon (Fig. 7(b)). Relative to low tide, the model predicted the highest counts near low tide, with lower counts (about 10% lower) at  $\pm$  2 hrs from low tide (Fig. 7(c)). There is a small effect due to the height of the low tide (Fig. 7(d)), with slightly higher counts at lower tides.



Figure 6. Effect of the height of the low tide on counts of harbor seals for each of the 25 haul-out locations in Prince William Sound, Alaska.

### 3.2 Trend and abundance

In the model (Equation (1)), the intercept term  $\theta_{ij}$  contains information on the expected counts. The value  $\exp(\theta_{ij})$  can be interpreted as the abundance for the *i*-th site in the *j*-th year, for some standardized values of the covariates where  $x_{ks} = 0$  for each k, k = 1, ..., 4. This can be seen in Equation (1), which was given in multiplicative form in Equation (6). The mean value of  $\exp(\theta_{ij})$  from the posterior distribution, for all years j = 1, 2, ..., 10, for each of the sites i = 1, 2, ..., 25, is given in Fig. 8. The actual counts are also given in Fig. 8. Notice that the value of  $\exp(\theta_{ij})$  from the posterior distribution may be quite far from the actual counts because  $\exp(\theta_{ij})$  is standardized for certain values of the covariates, while the actual counts may have occurred under a different set of values for the covariates.

The estimated trend is also given in Fig. 8, which is the posterior distribution of  $\exp(\tau_{0i} + \tau_{1i} \times j)$  for  $j = -4, -3, \dots, 5$  where j = -4 is the re-indexed value for year

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Figure 7. Overall effects of date, time of day, time relative to low tide, and height of low tide, on counts of harbor seals for all of the 25 haul-out locations in Prince William Sound, Alaska.

1990. Fig. 8 shows that most sites have a decreasing trend. The credibility intervals, which are not shown, often do not contain zero.

An example of the full range of inference on trend and abundance for a specific site is given in Fig. 9 for site 4. Notice that we give estimates of abundance for each year, along with the 2.5% and 97.5% credibility limits of the parameter estimates from the posterior distribution. The estimated trend curve is also given, along with 2.5% and 97.5% bounds for the curve from the posterior distribution. Notice that the actual counts show a slight increase over the years but the estimated abundance and trend is downward. This is explained by the fact that counts in earlier years were generally obtained later in the season (often in September). The effect of decreasing counts with date for site 4 can be seen in Fig. 3. Because of scaling to the standardized date (August 28), the abundance estimates show a pattern different than the observed data.

Using a sample from the posterior distribution (2), Fig. 10 shows the posterior distribution of both the mean trend parameter estimate  $\eta_1$  and the weighted trend estimate  $\alpha_1$ , given by Equation (3). The mean of the posterior distribution of  $\eta_1$  is -18.5% change per year with a standard deviation of 6.08% and a 95% credibility interval of -30.6% to -6.5%; the mean of the posterior distribution of  $\alpha_1$  is -2.5% change per year with a standard deviation of 30.6% credibility interval of -5.21% to 0.14%. The contrast in the results is interesting, and due to the fact that several small sites dropped to zero, creating several steep negative trends that had a large effect on  $\eta_1$  but having little effect on  $\alpha_1$ . Nevertheless, both results indicate that over the 10 years from 1990 to 1999, there has been a significant overall declining trend in harbor seal numbers. Frost *et al.* (1999) estimated a 4.6% yearly decline from the period 1990 to 1997.

The overall abundance estimates for each year, given by Equation (4), were also determined using a sample from the posterior distribution (2). The results for two different



Figure 8. Trend and abundance for each of the 25 haul-out locations in Prince William Sound, Alaska.

sets of covariate values are shown in Fig. 11. In Fig. 11, the results of Frost *et al.* (1999) are also shown, where the abundance estimates were standardized to optimum conditions under their model. Although absolute estimates of abundance vary (due mostly to differing covariate adjustments), the temporal patterns are very similar.

Fig. 12 shows the model assessment, using Chi squared discrepancy,  $R_i$ , for each site. We initially tried a model without the overdispersion parameter. As Fig. 12 shows, most

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Figure 9. Trend and abundance for site 4 in Prince William Sound, Alaska.





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Figure 11. Overall abundance for all 25 sites in Prince William Sound, Alaska.



Figure 12. Chi squared discrepancy for all 25 sites in Prince William Sound, Alaska. The vertical bars indicate the 95% credibility interval from the posterior distribution.

sites had more variability than explained with only covariates in (1). The addition of the overdispersion parameter  $\varepsilon_{ijk}$  in (1) gives a good model fit for these data.

Other parameters from the posterior distribution have less interest and are not given.

### 4. Discussion

The goals for monitoring ecological populations, even within a single study, are varied. We may often be interested in population estimates at a given time and/or trend estimates for each location or a collection of sites. We may also be interested in functions of population estimates, trends, or their combination. Finally, we may have information on covariates, and we may be interested in the effect of covariates on population trends and abundance. In this paper, we considered a general setup where we have repeated samples within years, at several sites, across several years. In this setup, there are four sources of variability due to: (1) effect of covariates on observations, (2) sampling to estimate the population at some site at some time, (3) the error of the true population at some time about the hypothetical trend curve for that site, and (4) differences in trend among sites. For this setup, we considered the Bayesian treatment of hierarchical models to be the ideal method of statistical inference for several reasons: (1) the 4 sources of variability described earlier could be put into one unified probability framework, (2) estimates of populations or trend "borrowed strength" from the unified probability framework, (3) using MCMC methods, it was relatively easy to make a wide range of inferences on functions of population estimates and trends for collections of locations, and (4) we could make inferences on the effect of covariates.

There is some need to discuss the modeling of trend with a simple linear model for each site. True populations are fluctuating according to a model that we have no hope of ever knowing completely. A linear trend component for the model is useful because a single parameter, the slope, captures the essence of how we visualize "trend." We realize that the linear model is smoothing over true population fluctuations. Our view is that this is desirable; for our application, and many others, we want to smooth over the small variations in time and look at trend over longer time frames. Also, we could add quadratic and higher terms in the model. This might be desirable in order to assess whether a population has "bottomed out." Bayes factors (see, for example, Gelman *et al.*, 1995, page. 175) could be used to make decisions on competing models. It was not our goal to make such a decision, but rather to model trend, so our linear model is appropriate.

Other enhancements to the model could be considered. For example, it is possible that the date for peak haulout has been trending through time, getting either later or earlier in the season. In that case, (6), which is equal to (1), can be reparameterized so that it contains  $\exp((x_{1s} - \beta_{1ij})^2/b_{2i})$ , and then  $\beta_{1ij}$  can be given a linear trend per site (call it a date trend). The date trend parameters can be given a distribution, just as the abundance trend parameters. Once again, Bayes factors could be used to decide if this model provided an improvement.

The Bayesian hierarchical model was used to assess trends of harbor seals, *Phoca vitulina richardsi*, in Prince William Sound, Alaska, following the 1989 *Exxon Valdez* oil spill. With respect to covariates, results showed that overall, (1) counts decreased with date, (2) counts decreased throughout the day, (3) counts were at a maximum near low tide, and (4) there was very little effect of tide height; however, there was considerable

variation among sites. To get the overall trend we used a weighted average of the trend at each site, where the weights depended on the overall abundance of a site. The overall trend indicated a continued significant decrease in the harbor seal population. To get overall abundance for each year, we summed the abundance estimates for each site. We used MCMC methods to obtain a sample from the posterior distribution of the parameters, which also yields a sample from the posterior distribution of the overall trend and abundance. Other studies have shown site-specific trends and patterns of behavior in harbor seals (Thompson *et al.*, 1997). Other researchers have also used Bayesian hierarchical models to assess trends in wildlife populations (e.g., Craig *et al.*, 1997), although the models vary depending on the wildlife species and sampling method. Here, the use of a Bayesian hierarchical model allowed assessment of trend, abundance, and effects of covariates both at specific sites and overall.

### Acknowledgments

This study was conducted as part of the *Exxon Valdez* Oil Spill Restoration Program, funded by the *Exxon Valdez* Oil Spill Trustee Council. Funding for harbor seal surveys in PWS in 1992 was provided by the National Marine Fisheries Service, National Marine Mammal Laboratory. Ken Pitcher conceived the idea of harbor seal trend counts in PWS, and Dennis McAllister and Jon Lewis flew some of the earlier surveys. We thank Steve Ranney, the pilot for all of the aerial surveys, for his careful and conscientious support. Rob DeLong assisted with data analyses and presentation. Three anonymous reviews helped improve the manuscript.

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### Biographical sketches

Jay M. Ver Hoef obtained a B.S. in Botany from Colorado State University in 1979, an M.S. in Botany from the University of Alaska, Fairbanks in 1985, and a co-major Ph.D. in both Statistics and EEB (Ecology and Evolutionary Biology) from Iowa State University in 1991. Since then he has been a biometrician with the Alaska Department of Fish and Game in Fairbanks. He is also an adjunct professor with the Department of Mathematical Sciences at the University of Alaska, Fairbanks. He acts as a consulting statistician on a variety of wildlife research and management projects. His research interests are in spatial statistics and Bayesian methods, and he applies them to ecological, environmental, and wildlife data.

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### Abstracts for other manuscripts prepared using samples and/or data from this study:

### 1. Adkison, M. D., T. J. Quinn II, and R. J. Small. 2003. Evaluation of the Alaska harbor seal population (*Phoca vitulina*) population survey: a simulation study. *Marine Mammal Science* 19: 764-90.

Abstract: We used simulation to investigate robust designs and analyses for detecting trends from population surveys of Alaska harbor seals. We employed an operating model approach, creating simulated harbor seal population dynamics and haul-out behavior that incorporated factors thought to potentially affect the performance of aerial surveys. The factors included the number of years, the number of haul-out sites in an area, the number and timing of surveys within a year, known and unknown covariates affecting haul-out behavior, substrate effects, movement among substrates, and variability in survey and population parameters. We found estimates of population trend were robust to the majority of potentially confounding factors, and that adjusting counts for the effects of covariates was both possible and beneficial. The use of mean or maximum counts by site without covariate correction can lead to a substantial bias and low power in trend determination. For covariate-corrected trend estimates, there was minimal bias and loss of accuracy was negligible when surveys were conducted 20 d before or after peak haul-out attendance, survey date became progressively earlier across years, and peak attendance fluctuated across years. Trend estimates were severely biased when the effect of an unknown covariate resulted in a long-term trend in the fraction of the population hauled out. A key factor governing the robustness and power of harbor seal population surveys is intersite variability in trend. This factor is well understood for sites within the Prince William Sound and Kodiak trend routes for which at least 10 consecutive annual surveys have been conducted, but additional annual counts are needed for other areas. The operating model approach proved to be an effective means of evaluating these surveys and should be used to evaluate other marine mammal survey designs.

### Boveng, P. L., J. L. Bengtson, D. E. Withrow, J. C. Cesarone, M. A. Simpkins, K. J. Frost, and J. J. Burns. 2003. The abundance of harbor seals in the Gulf of Alaska. *Marine Mammal Science* 19: 111-27.

Abstract: The abundance of harbor seals (Phoca vitulina richardi) has declined in recent decades at several Alaska locations. The causes of these declines are unknown, but there is concern about the status of the populations, especially in the Gulf of Alaska. To assess the status of harbor seals in the Gulf of Alaska, we conducted aerial surveys of seals on their haul-out sites in August - September 1996. Many factors influence the propensity of seals to haul out, including tides, weather, time of day, and time of year. Because these "covariates" cannot simultaneously be controlled through survey design, we used a regression model to adjust the counts to an estimate of the number of seals that would have been ashore during a hypothetical survey conducted under ideal conditions for hauling out. The regression, a generalized additive model, not only provided an adjustment for the covariates, but also confirmed the nature and shape of the covariate effects on haul-out behavior. The number of seals hauled out was greatest at the beginning of the surveys (mid-August). There was a broad daily peak from about 11001400 local solar time. The greatest numbers were hauled out at low tide on terrestrial sites. Tidal state made little difference in the numbers hauled out on glacial ice, where the area available to seals did not fluctuate with the tide. Adjusting the survey counts to the ideal state for each covariate produced an estimate of 30,035 seals, about 1.8 times the total of the unadjusted counts (16,355 seals). To the adjusted count, we applied a correction factor of 1.198 from a separate study of two haul-out sites elsewhere in Alaska, to produce a total abundance estimate of 35,981 (SE

1,833). This estimate accounts both for the effect of covariates on survey counts and for the proportion of seals that remained in the water even under ideal conditions for hauling out.

### 3. Burns, J. M., D. P. Costa, K. J. Frost, and J. T. Harvey. 2005. Development of body oxygen stores in harbor seals: effects of age, mass, and body composition. *Physiological and Biochemical Zoology*.

Abstract: Harbor seal pups are highly precocial and can swim and dive at birth. Such behavioral maturity suggests that they may be born with mature body oxygen stores, or that stores develop quickly during the nursing period. To test this hypothesis, we compared the blood and muscle oxygen stores of harbor seal pups, yearlings, and adults. We found that pups had lower oxygen stores than adults (neonates 57%, weaned pups 75%, and yearlings 90% those of adults), largely because neonatal myoglobin concentrations were low (1.6  $\pm$  0.2 g% vs. 3.8  $\pm$  0.3 g% for adults), and changed little during the nursing period. In contrast, blood oxygen stores were relatively mature, with nursing pups having hematocrit (55  $\pm$  0.2%), hemoglobin (21.7  $\pm$  0.4 g%), and blood volumes (12.3  $\pm$  0.5 ml/kg) only slightly lower than adults (57  $\pm$  0.2%, 23.8  $\pm$  0.3 g%, and 15.0  $\pm$  0.5 ml/kg). As neonatal pups had relatively high metabolic rates (11.0 ml O<sub>2</sub>/kg·min) their calculated aerobic dive limit was less than 50% that of adults. These results suggest that harbor seals' early aquatic activity is primarily supported by rapid development of blood, with immature muscle oxygen stores and elevated use rates limiting aerobic diving ability.

### 4. Frost, K. J., L. F. Lowry, and J. Ver Hoef. 1999. Monitoring the trend of harbor seals in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. *Marine Mammal Science* 15, no. 2: 494-506.

Abstract: We used aerial counts to monitor the trend in numbers of harbor seals, Phoca vitulina richardsi, in Prince William Sound, Alaska, following the 1989 Exxon Valdez oil spill. Repetitive counts were made at 25 haul-out sites during the annual molt period each year from 1990 through 1997. A generalized linear model indicated that time of day, date, and time relative to low tide significantly affected seal counts. When Poisson regression was ' used to adjust counts to a standardized set of survey conditions, results showed a highly significant decline of 4.6% per year. Unadjusted counts indicated a slight, but not statistically significant, decline in the number of seals. The number of harbor seals on the trend-count route in eastern and central PWS has been declining since at least 1984, with an overall population reduction of 63% through 1997.

Programs to monitor long-term changes in animal population sizes should account for factors that can cause short-term variations in indices of abundance. The inclusion of such factors as covariates in models can improve the accuracy of monitoring programs.

5. Frost, K. J., M. A. Simpkins, and L. F. Lowry. Submitted. Development of diving by harbor seal pups in two regions in Alaska: use of the water column. *Marine Mammal Science*. Abstract: Satellite-linked dive recorders were attached to 53 harbor seal pups in Prince William Sound (PWS) and at Tugidak Island, Alaska, during 1997-1999. We used generalized additive models and bootstrap techniques to describe pup diving behavior during their first year of life. Pups increased their ability to dive during the first few months, as indicated by increases in proportion of time wet and max-depth values. Timewet and/or max-depth later decreased, suggesting a seasonal component to diving behavior. Monthly time-wet varied from an overall minimum of 0.68 to a maximum of 0.89. Pups spent most of their time wet swimming at shallower than 30% of their max-depth, and < 5% of their time deeper than 70% of their max-depth. Average max-depths and deepest actual dives were similar for PWS and Tugidak pups (max-depth 50-100 m *vs.* 40-110 m;

actual deepest dive 294 m vs. 308 m). PWS pups dove deeper sooner and spent less time wet than Tugidak pups during the first few months after tagging, probably as a result of regional bathymetric differences. Dive behavior and body condition suggest that food availability was not likely a major factor in the population decline in PWS during the period of this study.

6. — . 2001. Diving behavior of subadult and adult harbor seals in Prince William Sound, Alaska. *Marine Mammal Science* 17, no. 4: 813-34.

Abstract: Satellite-linked depth recorders (SDRs) were attached to 47 harbor seals in Prince William Sound, Alaska, during 1992–1996. Parameters describing diving effort, diving focus, and focal depth (depth bin to which diving was focused) were calculated from binned data on maximum dive depth and time spent at depth, and analyzed using repeatedmeasures mixed models. This analysis method accounted for individual variability, temporal autocorrelation, and the binned nature of SDR data, which are often ignored using standard statistical techniques. Results indicated that diving effort remained steady from September to April, when seals spent 68%–75% of their overall time in the water. Time spent in the water declined to 60% in May and to about 40% in July. Seals spent the most time in the water at night and the least in the morning. The diving of all seals in all months was highly focused. Overall, diving was focused to one depth bin approximately 75% of the time. Diving was more focused for females than for males and subadults. Focal depth and diving focus varied by region. Collinearity between month and region in the focal depth model suggested that seals move in winter to regions where prev are found deeper in the water column. Variations in diving behavior presumably result from combinations of regional bathymetry, seasonal cycles in type or depth distribution of prey, and seal lifecycle events such as reproduction and molting.

7. Gotthardt, T. 2001. "The foraging ecology of harbor seals in southcentral Prince William Sound, Alaska: 1994-1997." M.S. thesis, University of Alaska Anchorage. Abstract: Fourteen harbor seals (*Phoca vitulina richardsi*) from southcentral Prince William Sound (PWS), Alaska, were outfitted with satellite-linked time depth recorders (SDRs) to monitor their movements and diving behavior. I subsequently examined available information on forage fish abundance, composition and distribution to evaluate whether the distribution and diving behaviors of seals corresponded to the seasonal and temporal distribution of their prey. A wide array of forage fishes were seasonally available to PWS harbor seals. Seasonal differences were apparent in depth of dives and distances moved by seals to foraging areas. It is likely that the two were inter-related, as the distant areas used by seals were also the deepest. Seasonal differences in diving depths and localities were likely due to seasonal changes in prey availability. Seals dove deeper and increased foraging ranges in winter, suggesting prey availability in winter may be greatly reduced compared to spring or summer.

### 8. Hastings, K. K., K. J. Frost, A. Simpkins, G. W. Pendleton, U. G. Swain, and R. J. Small. 2004. Regional differences in diving behavior of harbor seals in the Gulf of Alaska. *Canadian Journal of Zoology* 82: 1755-73.

Abstract: Adult and subadult harbor seals (*Phoca vitulina richardi* (Gray, 1864); n = 108) from Southeast Alaska (SE), Kodiak Island (KO), and Prince William Sound (PWS) were instrumented with satellite data recorders to examine dive parameters for harbor seals in the Gulf of Alaska at regional and annual scales. Most dives (40%–80%) were <20 m in depth and <4 min in duration; however, dives from 50 to 150 m depth were not uncommon and dives to 508 m were recorded. PWS seals spent less time in the water during the prebreeding and breeding seasons than SE and KO seals. SE seals used a greater diversity

of depths than KO and PWS seals. Only seals in PWS and SE (i) dived deeper and longer and spent more time diving in winter than during spring and summer and (ii) dived deepest during the day only in winter. Seals in all regions and seasons dived most frequently and spent the most time diving at night. Subadult seals spent more time diving, dived more often, displayed a stronger diurnal pattern with deepest dives during the day in the winter, and dived deeper than adults.

### 9. Iverson, S. J., C. Field, W. D. Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: a new method of estimating predator diets. *Ecological Monographs* 74, no. 2: 211-35.

Abstract: Accurate estimates of the diets of predators are required in many areas of ecology, but for many species current methods are imprecise, limited to the last meal, and often biased. The diversity of fatty acids and their patterns in organisms, coupled with the narrow limitations on their biosynthesis, properties of digestion in monogastric animals, and the prevalence of large storage reservoirs of lipid in many predators, led us to propose the use of quantitative fatty acid signature analysis (QFASA) to study predator diets. We present a statistical model that provides quantitative estimates of the proportions of prey species in the diets of individual predators using fatty acid signatures. We conducted simulation studies using a database of 28 prey species (n = 954 individuals) from the Scotian Shelf off eastern Canada to investigate properties of the model and to evaluate the reliability with which prey could be distinguished in the model. We then conducted experiments on grey seals (*Halichoerus grypus*, n = 25) and harp seals (*Phoca* groenlandica, n = 5) to assess quantitative characteristics of fatty acid deposition and to develop calibration coefficients for individual fatty acids to account for predator lipid metabolism. We then tested the model and calibration coefficients by estimating the diets of experimentally fed captive grey seals (n = 6, switched from herring to a mackerel/capelin diet) and mink kits (*Mustela vison*, n = 46, switched from milk to one of three oilsupplemented diets). The diets of all experimentally fed animals were generally well estimated using QFASA and were consistent with qualitative and quantitative expectations, provided that appropriate calibration coefficients were used. In a final case, we compared video data of foraging by individual freeranging harbor seals (*Phoca vitulina*, n = 23) fitted with Crittercams and OFASA estimates of the diet of those same seals using a complex ecosystem-wide prey database. Among the 28 prey species in the database, OFASA estimated sandlance to be the dominant prey species in the diet of all seals (averaging 62% of diet), followed primarily by flounders, but also capelin and minor amounts of other species, although there was also considerable individual variability among seals. These estimates were consistent with video data showing sandlance to be the predominant prey, followed by flatfish. We conclude that OFASA provides estimates of diets for individuals at time scales that are relevant to the ecological processes affecting survival, and can be used to study diet variability within individuals

over time, which will provide important opportunities rarely possible with other indirect methods. We propose that the QFASA model we have set forth will be applicable to a wide range of predators and ecosystems.

10. Iverson, S. J., K. J. Frost, and S. L. C. Lang. 2002. Fat content and fatty acid composition of forage fish and invertebrates in Prince William Sound, Alaska: factors contributing to among and within species variability. *Marine Ecology Progress Series* 241: 161-81. Abstract: We determined the fat content and fatty acid composition of 26 species of fish and invertebrates (n = 1153) that are primary forage species of piscivorous seabirds and marine mammals in Prince William Sound (PWS), Alaska. Flatfish, shrimps and octopus had the lowest average fat contents (~1.0%), although some cods, as well as juvenile

walleye pollock Theragra chalcogramma, Pacific herring Clupea harengus pallasi and pink salmon Oncorhynchus gorbuscha also ranged as low as 0.5 to 0.7% fat. The highest fat contents were found in eulachon Thaleichthys pacificus (25%), adult herring (21%) and the squid Berrytheuthis magister (5 to 13%). Within species, fat content varied mostly with season, but also with size. Fatty acid signatures generally distinguished forage species, with up to 95% of individuals correctly classified using either discriminant or classification and regression tree (CART) analyses. Discriminant plots provided insight into the relationships between fatty acid signatures of different species. Species with similar life histories and diets clustered closer together, while those with the greatest differences in ecology differed most in their fatty acid patterns. Within some species, changes in fatty acid signatures were apparent with increasing size and were consistent with known dietary shifts reported from stomach contents analyses. Furthermore, fatty acid signatures of Age 0 (yr) pollock and herring in PWS were consistent with previous stomach contents analysis that indicated annual differences in the timing of dietary changes from eating zooplankton to piscivory. Overall, when size/age classes were taken into account, species classification using fatty acid signatures was improved. Our findings have important implications for evaluating diets and food web interactions of fish stocks, as well as at higher trophic levels. Despite individual variation within species, our results indicate that fatty acid signatures accurately characterize forage species in this ecosystem, and consequently can be used to study and perhaps estimate the species composition of diets of their predators.

### 11. Iverson, S. J., K. J. Frost, and L. F. Lowry. 1997. Fatty acid signatures reveal fine scale structure of foraging distribution of harbor seals and their prey in Prince William Sound, Alaska. *Marine Ecology Progress Series* 151: 255-71.

Abstract: Fatty acid signature analysis was used to investigate the diet and the spatial scales of foraging in harbor seats Phoca vitulina richardsi in Prince William Sound (PWS) and elsewhere in the Gulf of Alaska. Blubber samples collected in 1994 and 1995 from 104 harbor seals from PWS. Kodiak Island, and southeast Alaska were analyzed for fatty acid composition. A total of 163 potential prey samples representing 10 taxa were collected and individually analyzed for total fat content and fatty acid composition. Approximately 70 fatty acids and isomers were found in both harbor seals and their prey. Classification and regression tree analysis was used to classify seals and prey according to their fatty acid signatures. Large differences were found in the fatty acid composition of blubber from seals sampled at Kodiak, southeast Alaska and PWS, over a broad geographical scale of 400 to 800 km. Additionally, fatty acid signatures distinguished seals from different regions within PWS, as well as on finescale resolutions of specific haulout sites within 9 to 15 km of one another. These findings suggest that seals forage site - specifically. These conclusions are supported by prey fatty acid patterns, which also differed on similarly small spatial scales within PWS. Not only could prey species such as herring Clupea pallasi and pollock Theragra chalcogramma be differentiated from one another using fatty acid signatures, but they could also be distinguished by size-class and location within PWS, reflecting differences in diet with age and as well as with fine-scale habitat. Results from this study are consistent with both satellite data from tagged harbor seals and stomach content analyses of forage fish species in PWS. Although preliminary, analyses suggest that large herring and pollock, as well as flatfish, may have dominated the diet of seals in southern PWS, whereas diets of seals in northern and eastern PWS may have been comprised more of small size classes of herring and pollock, and perhaps other items such as cephalopods, sandlance Ammodytes hexapterus, cod Gadus macrocephalus, and shrimp. We conclude that fatty acid signature analysis will be an important contribution to understanding marine food webs in estuarine and other marine environments

### 12. Iverson, S. J., S. L. C. Lang, and M. H. Cooper. 2001. Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. *Lipids* 36, no. 11.

Abstract: For many studies, it is important to measure the total lipid content of biological samples accurately. The Bligh and Dyer method of extraction was developed as a rapid but effective method for determining total lipid content in fish muscle. However, it is also widely used in studies measuring total lipid content of whole fish and other tissues. Although some investigators may have used modified Bligh and Dyer procedures, rarely have modifications been specified nor has their effectiveness been quantitatively evaluated. Thus, we compared this method with that of the classic Folch extraction in determining total lipid content of fish samples ranging from 0.5 to 26.6% lipid. We performed both methods as originally specified; i.e., using the chloroform/methanol/water ratios of 1:2:0.8 and 2:2:1.8 (before and after dilution, respectively) for Bligh and Dyer and of 8:4:3 for Folch, and with the initial solvent/sample ratios of (3+1):1 (Bligh and Dyer) and 20:1 (Folch). We also compared these with several other solvent/sample ratios. In samples containing <2% lipid, the results of the two methods did not differ. However, for samples containing >2% lipid, the Bligh and Dyer method produced significantly lower estimates of lipid content, and this underestimation increased significantly with increasing lipid content of the sample. In the highest lipid samples, lipid content was underestimated by up to 50% using the Bligh and Dyer method. However, we found a highly significant linear relationship between the two methods, which will permit the correction of reported lipid levels in samples previously analyzed using an unmodified Bligh and Dyer extraction. In the future, modifications to procedures and solvent/sample ratios should be described.

### 13. Lowry, L. F., K. J. Frost, J. M. Ver Hoef, and R. A. DeLong. 2001. Movements of satellitetagged subadult and adult harbor seals in Prince William Sound, Alaska. *Marine Mammal Science* 17, no. 4: 835-61.

Abstract: Satellite-linked tags were attached to 49 subadult and adult harbor seals captured in Prince William Sound (PWS), Alaska, and their movements were monitored during 1992-1997. Seals were tracked for a total of 5,517 seal-days and were located on about 80% of the days that tags transmitted. Most locations were in or near PWS, but some juvenile seals moved 300-500 km east and west into the Gulf of Alaska. While several seals traveled to 50-100 km offshore, virtually all locations were in water <200 m deep. Overall, juvenile seals moved more than adults and had larger home ranges. Movements were significantly affected by month, and age by month and sex by month interactions. In all months, mean distances between successively used haulouts were <10 km for adults and <20 km for juveniles. Mean monthly home ranges varied from <100 km2 to >1,500 km2, and were smallest during June-July. Mean haul-out to at-sea distance was 5-10 km for adults and generally 10-25 km for juveniles. Satellite-linked tags provided an effective means of monitoring and describing the full range of harbor seal movements in this region, with the exception of late summer when tags were shed during the molt.

# 14. O'Corry-Crowe, G. M., and R. L. Westlake. 1997. Molecular investigations of spotted seals (*Phoca largha*) and harbor seals (*P. vitulina*), and their relationship in areas of sympatry. In *Molecular Genetics of Marine Mammals*. Editors A. E. Dizon, S. J. Chivers, and W. F. Perrin, 291-330. Vol. 3. Society of Marine Mammalogy (Special Publication).

Abstract: The phylogenetic systematics of spotted and harbor seals (genus *Phoca*) and their relationship to other phocid seal species have not been satisfactorily resolved. Analysis of the mitochondrial DNA control region and adjacent proline transfer RNA gene supports the contention that populations of both forms constitute phylogenetically distinct clades, which

can therefore constitute monophyletic species: *Phoca largha* and *Phoca vitulina* Linnaeus, 1758. Atlantic and Pacific harbor seals are phylogeographically distinguishable. Within the Pacific, however, samples corresponding to subspecies *P. v. stejnegeri* and *P. v. richardsi* do not occur as genetically distinct clades. Subspecies of spotted seals are likewise not genetically discernable across the geographic range studied. A single individual, identified as a harbor seal on the basis of gross morphology, location, and season of capture, possessed an mtDNA haplotype characteristic of spotted seals. This may be the result of misidentification, ancestral polymorphism leading to paraphyly, or hybridization between a female spotted seal and male harbor seal. The implications of hybridization for definitions of "species" and "subspecies," and concepts of appropriate units for management are briefly discussed.

### 15. Simpkins, M. A., K. L. Laidre, and P. J. Heagerty. 2005. Multivariate regression of satellitelinked dive recorder data: simultaneous analysis of all bins. *Marine Mammal Science* 21: 243-59.

Abstract: Statistical analysis of diving behavior data collected from satellite-linked dive recorders (SDRs) can be challenging because: (1) the data are binned into several depth and time categories, (2) the data from individual animals are often temporally autocorrelated, (3) random variation between individuals is common, and (4) the number of dives can be correlated among depth bins. Previous analyses often have ignored one or more of these statistical issues. In addition, previous SDR studies have focused on univariate analyses of index variables, rather than multivariate analyses of data from all depth bins. We describe multivariate analysis of SDR data using generalized estimating equations (GEE) and demonstrate the method using SDR data from harbor seals (*Phoca vitulina*) monitored in Prince William Sound, Alaska between 1992 and 1997. Multivariate regression provides greater opportunities for scientific inference than univariate methods, particularly in terms of depth resolution. In addition, empirical variance estimation makes GEE models somewhat easier to implement than other techniques that explicitly model all of the relevant components of variance. However, valid use of empirical variance estimation requires an adequate sample size of individual animals.

### 16. Small, R. J., L. F. Lowry, J. M. Ver Hoef, K. J. Frost, A. DeLong, and M. J. Rehberg. 2005. Differential movements by harbor seal pups in contrasting Alaska environments. *Marine Mammal Science* 21: 671-94.

Abstract: Movement patterns of Alaska harbor seal pups were studied using satellite telemetry during 1997-2000. Mean tracking duration was 277.3 d (SD = 105.8) for Tugidak Island pups (n = 26) and 171.2 d (108.3) for Prince William Sound (PWS) pups (n = 27). Movements were similar for males and females and were largely restricted to the continental shelf. Multiple return trips of >75 km from the natal area and up to ~3 weeks duration were most common, followed by movements restricted to <25 km from the natal area; one way movements from the natal site were rare. Distances moved and home range sizes remained relatively stable or increased gradually from July through winter, then decreased markedly through spring. Monthly movements (maximum distance from tagging location, mean distance from haulouts to at-sea locations, and home range size) were significantly greater for Tugidak vs. PWS pups. Six of 7 pups from each region that traveled furthest and were tracked the longest had returned to their tagging site when their last location was recorded, indicating philopatry or limited dispersal during their first year of life. Seal pups exhibited similar movement patterns in the distinct habitats of the two regions but differed in the spatial extent of their movements.

### 17. Ver Hoef, J. M., and K. J. Frost. 2003. A Bayesian hierarchical model for monitoring harbor seal changes in Prince William Sound, Alaska. *Environmental and Ecological Statistics* 10: 201-9.

Abstract: Bayesian hierarchical models were used to assess trends of harbor seals, *Phoca* vituling richardsi, in Prince William Sound, Alaska, following the 1989 Exxon Valdez oil spill. Data consisted of 4-10 replicate observations per year at 25 sites over 10 years. We had multiple objectives, including estimating the effects of covariates on seal counts, and estimating trend and abundance, both per site and overall. We considered a Bayesian hierarchical model to meet our objectives. The model consists of a Poisson regression model for each site. For each observation the logarithm of the mean of the Poisson distribution was a linear model with the following factors: (I) intercept for each site and year, (2) time of year, (3) time of day, (4) time relative to low tide, and (5) tide height. The intercept for each site was then given a linear trend model for year. As part of the hierarchical model, parameters for each site were given a prior distribution to summarize overall effects. Results showed that at most sites, (1) trend is down; counts decreased yearly, (2) counts decrease throughout August, (3) counts decrease throughout the day, (4) counts are at a maximum very near to low tide, and (5) counts decrease as the height of the low tide increases; however, there was considerable variation among sites. To get overall trend we used a weighted average of the trend at each site, where the weights depended on the overall abundance of a site. Results indicate a 3.3% decrease per year over the time period.

### Westlake, R. L., and G. M. O'Corry-Crowe. 2002. Macrogeographic structure and patterns of genetic diversity in harbor seals (*Phoca vitulina*) from Alaska to Japan. *Journal of Mammalogy* 83: 1111-26.

Abstract: We examined sequence variation in the control region of the mitochondrial genome from 778 seals sampled at 161 locations from northern Japan to southeastern Alaska to learn more about the evolutionary history and population structure of, and effects of recent declines on genetic diversity in, harbor seals (Phoca vitulina) in the northern Pacific Ocean. High haplotypic diversity (H 5 0.975) and a poorly resolved mitochondrial genome (mtDNA) phylogeny suggest that harbor seals in the Pacific underwent a rapid expansion in population size in their recent evolutionary past, possibly after the retreat of Pleistocene ice sheets. Weak phylogeographic partitioning of lineages attests to a complex evolutionary and demographic history of contemporary Pacific populations. Extensive macrogeographic subdivision was evident among a subset of grouped localities that represent centers of abundance along the distributional continuum. Heterogeneity was influenced by population size and correlated with geographic distance, suggesting that dispersal occurs primarily among neighboring subpopulations. The 2 currently recognized subspecies of harbor seal in the Pacific, P. v. richardi of North America and P. v. stejnegeri of Asia, do not represent phylogenetically discrete mtDNA assemblages. The greatest differentiation detected was along the Commander-Aleutian Island chain, the region of the presumed subspecies boundary and a likely contact zone for expanding refugial populations of a number of marine mammal species after retreat of ice sheets. Differentiation between the Kodiak Archipelago and Prince William Sound, and between Bristol Bay and the Pribilof Islands, indicates that current management stocks are inappropriate and highlights the need for a detailed analysis of population and stock structure in Alaska. A decline in population size in Prince William Sound over the past few decades was accompanied by a discernible reduction in mtDNA diversity, manifested as a loss of rare haplotypes through random drift. A continued population decline will erode genetic diversity further, with potentially adverse effects on evolutionary potential and individual fitness.

### 19. Zarnke, R. L., T. C. Harder, H. W. Vos, J. M. Ver Hoef, and A. D. Osterhaus. 1997. Serologic survey for phocid herpesvirus-1 and -2 in marine mammals from Alaska and Russia. *Journal of Wildlife Diseases* 33 : 459-65.

Abstract: Blood samples were collected from 1,042 marine mammals off the coast of Alaska (USA) and Russia during the period 1978 to 1994. Eight species of pinnipeds were represented. Sera were tested for presence of neutralizing antibodies to both the PB84 isolate of phocid herpesvirus-1 (PhHV-1) and the 7848/Han90 strain of phocid herpesvirus-2 (PhHV-2). Species-specific antibody prevalences ranged from 22% to 77% for PhHV-1 and 11% to 50% for PhHV-2. Species-specific antibody prevalences for PhHV-1 were greater than or equal to prevalences for PhHV-2. For both viruses and each host species, differences in antibody prevalences were not related to: (1) sex, (2) location of capture, or (3) year of collection. Antibody prevalence of PhHV-1 in walruses (*Odobenus rosmarus*) could be quantitatively predicted as a function of age. These two viruses have distinct biological properties and based on current data the epizootiology of the two viruses is different, as well. No evidence of herpesvirus-induced mortality has been detected in areas included in this survey. Based on results of this survey, neither PhHV-1 nor PhHV-2 are considered significant mortality factors in mammals which inhabit the marine environment off the coast of Alaska or Russia.

## 20. Zarnke, R. L., J. T. Saliki, A. P. Macmillan, S. D. Brew, C. E. Dawson, J. M. Ver Hoef, and R. J. Small. 2005. Serologic survey for *Brucella* spp. bacteria, phocid herpesvirus-1, phocid herpesvirus-2, and phocine distemper virus in harbor seals (*Phoca vitulina richardsi*) from Alaska, 1976-1999. *Journal of Wildlife Diseases*.

Abstract: Harbor seals (*Phoca vitulina richardsi*) were captured in the coastal regions of Southeast Alaska, Gulf of Alaska, Prince William Sound (PWS), and Kodiak Island during 1976-1999. Blood was collected from 286 seals. Sera were tested for evidence of exposure to *Brucella* spp. bacteria, phocid herpesvirus-1 (PhHV-1), phocid herpesvirus-2 (PhHV-2), and phocine distemper virus (PDV). Antibody prevalence rates were 46% (46/100) for *Brucella* spp., 93% (225/243) for PhHV-1, 0% (0/286) for PhHV-2, and 1% (2/160) for PDV. Antibody prevalence for *Brucella* spp. was directly related to age of the host. Antibody prevalence for PhHV-1 was higher in PWS as compared to the other three regions. No evidence of mortality due to these four agents was observed during the course of this study. Based on the results of this survey, none of these agents is considered a significant mortality factor in harbor seals from the four regions of coastal Alaska included in the study.