MECHANISMS OF IMPACT AND POTENTIAL RECOVERY OF PIGEON
GUILLEMOTS (*CEPHTHUS COLUMBA*) AFTER THE *EXXON VALDEZ* OIL SPILL

By

Pamela Edith Seiser

RECOMMENDED:

[Signatures and endorsements]

APPROVED:

[Signatures and endorsements]

Date

4-10-00
MECHANISM OF IMPACT AND POTENTIAL RECOVERY OF PIGEON GUILLEMOTS (*CEPPHUS COLUMBA*) AFTER THE *EXXON VALDEZ* OIL SPILL

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

By

Pamela Edith Seiser, B.S.

Fairbanks, Alaska

May 2000
The abundance of pigeon guillemots in oiled areas of Prince William Sound, Alaska, failed to increase after the 1989 Exxon Valdez oil spill. Population growth may be constrained by the physiological effects of oil exposure, food availability, and nest predation. I conducted a comparative study among unoiled, oiled, and pre-spill data sets, to provide insight on factors limiting population recovery in oiled areas. Blood samples from chicks in oiled and unoiled areas provided little evidence of physiological effects of exposure to oil. Pigeon guillemot diet, productivity, growth rates, and fledging weights in unoiled areas of southwestern Prince William Sound from 1994 to 1998 indicate oiled areas had a lower proportion of high-lipid fish in the chick diet and lower fledging weights, compared to unoiled and pre-spill studies. These results suggest that the lack of recovery in oiled areas is associated with a prey base that results in lower fledging weights, which may reduce juvenile survival.
# TABLE OF CONTENTS

**ABSTRACT** ................................................................................................................................................................................. iii

**LIST OF FIGURES** .................................................................................................................................................................. vi

**LIST OF TABLES** ................................................................................................................................................................... vii

**ACKNOWLEDGEMENTS** ................................................................................................................................................ viii

**CHAPTER ONE** OVERVIEW: THE RESPONSE OF PIGEON GUILLEMOTS TO THE 1989 EXXON VALDEZ OIL SPILL .......................................................................................................................... 1

**CHAPTER TWO** COMPARISON OF PIGEON GUILLEMOT, *CEPPHUS COLUMBA*, BLOOD PARAMETERS FROM OILED AND UNOILED AREAS OF ALASKA, EIGHT YEARS AFTER THE EXXON VALDEZ OIL SPILL .............................................................................................................. 6

  2.1 INTRODUCTION ................................................................................................................................................................. 6

  2.2 METHODS ........................................................................................................................................................................... 10

  2.3 RESULTS .......................................................................................................................................................................... 14

    2.3.1 Effects of age: nestlings ........................................................................................................................................ 14

    2.3.2 Effects of age: adults versus nestlings .................................................................................................................. 14

    2.3.3 Oiled vs. Unoiled Populations: nestlings ........................................................................................................... 15

    2.3.4 Oiled vs. Unoiled Populations: adults .................................................................................................................. 18

  2.4 DISCUSSION .................................................................................................................................................................. 20

    2.4.1 Effects of Development ........................................................................................................................................ 20

    2.4.2 Comparison between populations in oiled and unoiled areas ............................................................................. 24

**CHAPTER THREE** POPULATION TRENDS AND REPRODUCTIVE BIOLOGY OF PIGEON GUILLEMOTS IN UNOILED AREAS OF PRINCE WILLIAM SOUND, ALASKA, BETWEEN 1994 & 1998: INSIGHTS ON THE RECOVERY OF PIGEON GUILLEMOTS IN OILED AREAS .......... 34

  3.1 INTRODUCTION ................................................................................................................................................................. 34

  3.2 STUDY AREA .................................................................................................................................................................. 35

  3.3 METHODS ........................................................................................................................................................................... 37
3.4 RESULTS .......................................................................................................................................................................41

3.4.1 Population trends and nesting effort ............................................................................................................................41
3.4.2 Productivity ..................................................................................................................................................................43
3.4.3 Chick Diet .................................................................................................................................................................47
3.4.4 Delivery Rates ...........................................................................................................................................................48
3.4.5 Growth Rates and Fledging weights ..........................................................................................................................49
3.4.6 Fledgling Survival .......................................................................................................................................................51

3.5 DISCUSSION .................................................................................................................................................................52

3.5.1 Food Constraints .........................................................................................................................................................54
3.5.2 Demographic Limitations to Recovery .......................................................................................................................64

CONCLUSION .....................................................................................................................................................................66

CHAPTER FOUR  STATUS OF RECOVERY OF PRINCE WILLIAM SOUND'S PIGEON
GUILLEMOT POPULATION ...............................................................................................................................................69

LITERATURE CITED ..........................................................................................................................................................78
LIST OF FIGURES

Figure 2.1 Location of the oiled and unoiled reference areas in Prince William Sound and Kachemak Bay, Alaska. ................................................................. 11

Figure 3.1 Location of the Jackpot Island and Naked Island study areas in Prince William Sound, Alaska. ........................................................................................................36

Figure 3.2 The number of pigeon guillemots attending the Jackpot Island colony in Prince William Sound, Alaska, from 1994 to 1998. ................................................................. 42

Figure 3.3 The number of active pigeon guillemot nests found on Jackpot Island, Prince William Sound, Alaska, from 1994 to 1998. ........................................................................ 42

Figure 3.4 The mean clutch size, hatchlings per nest, fledglings per nest and productivity (fledglings egg$^{-1}$) for the pigeon guillemot colony at Jackpot Island, Prince William Sound, Alaska, from 1994 to 1998. ........................................................................ 45

Figure 3.5 Total fledglings (a), abandonment rate (b), and predation rate (c) for the pigeon guillemot colony at Jackpot Island, Prince William Sound, Alaska, from 1994 to 1998. ........................................................................ 46

Figure 3.6 Composition of the pigeon guillemot chick diet at Jackpot Island, Alaska, from 1994 to 1998...................................................................................................................... 47

Figure 3.7 Comparison of the mean delivery rates per chick for the pigeon guillemot colony at Jackpot Island, Alaska, among the 1995, 1997 and 1998 breeding seasons and between nests with one and two chicks......................................................... 48

Figure 3.8 The linear growth rate (a) and fledging weight (b) of pigeon guillemot chicks on Jackpot Island, Alaska, from 1994 to 1998................................. 51
LIST OF TABLES

Table 2.1  Mean, standard deviation (SD) and sample size (n) of the hematological and plasma chemistry of pigeon guillemot chicks sampled at 20 days of age. .................. 16

Table 2.2  Mean, standard deviation (SD) and sample size (n) of the hematological and plasma chemistry of pigeon guillemot chicks at 30 days of age................................. 17

Table 2.3  Mean, standard deviation (SD) and sample size (n) of the hematological and plasma chemistry of adult pigeon guillemots sampled in 1997 from oiled Naked Island, Prince William Sound and unoiled areas of Jackpot Island & Icy Bay, Prince William Sound and Kachemak Bay, Lower Cook Inlet in Alaska. .......................... 19

Table 3.1  June census counts for eight pigeon guillemot colonies in southwestern Prince William Sound for 1993, 1997 and 1998........................................................................ 43

Table 3.2  Estimates of median laying, hatching and fledging dates for the pigeon guillemot colony on Jackpot Island, Alaska, from 1994 to 1998................................. 44

Table 3.3  Mean clutch size, hatching success and nestling survival for pigeon guillemot nests found during the egg stage on Jackpot Island, Alaska, from 1994 to 1998. .... 44

Table 3.4  Comparison of clutch size, hatching success, fledgling success and productivity among unoiled Jackpot Island, oiled Naked Island, and pre-spill Naked Island pigeon guillemot studies. ................................................................. 58

Table 3.5  Comparison of growth rates and fledging weights among unoiled Jackpot Island, oiled Naked Island, and pre-spill Naked Island pigeon guillemot studies. ... 62
ACKNOWLEDGEMENTS

My research was a component of a larger study, the Nearshore Vertebrate Predator Project, funded by the Exxon Valdez Oil Spill Trustees Council. I have many individuals to thank because my thesis included data from three separate study areas, Jackpot Island, Naked Island and Kachemak Bay, which in turn were supported by three different agencies: the Alaska Cooperative Fish and Wildlife Research Unit, the office of Migratory Bird Management of the U.S. Fish & Wildlife Service and the Alaska Biological Science Center of the U. S. Geological Survey, in Anchorage, Alaska.

First, I would like to thank my committee members: my major advisor Dr. Dave McGuire for his patient instructions in the art of technical writing; Dr. Larry Duffy for his advice on biomarkers; and Dr. Alan Springer for his insights on seabird ecology. I thank the unofficial members of my committee Dr. Dan Roby and Dr. Scott Newman DMV, for contributing their expertise on avian nutrition and physiology. Dr. Greg Golet and Lindsey Hayes collected blood samples from Naked Island and shared their insight on the recovery of pigeon guillemots. I thank Cepphus experts George Divoky and Alex Prichard for their helpful discussions. I would also like to acknowledge the contribution of Jackpot Island's 1994 and 1995 data set by Lindsey Hayes and Gail Blundell, respectively.

My study includes data collected over a five-year project by many hard working individuals. At Jackpot Island, they were Adrian Gall, Mike Grene, Phil Joy, Cynthia Restrepo, Kelsey Sullivan, Mike Walgren and Darcie Zeil. Ted Spencer of the Naked Island crew provided assistance and humor, as well as numerous other 'Naked boys and girls': Laura Ballock, Mary Cody, Bryan Duggan, Amy Hahn, Jim Hamon, Melissa Luanglue, Aly McKnight, Angela Palmer, Mark Russell, Scott Schaffer, Bev Short, Oliver Sternicki, Dave Tessler, and Ed Vorisek. At Kachemak Bay, Mike Litzow, April Nielsen, and Sadie Wright generously contributed their time and energy to collect blood samples for my project. Maps were produced by GIS expert and friend Debbie Nigro. Last, but not least, I thank my family, friends, and office mates for the encouragement and supported they provide through my graduate work.
CHAPTER ONE
OVERVIEW: THE RESPONSE OF PIGEON GUILLEMOTS TO
THE 1989 EXXON VALDEZ OIL SPILL

OVERVIEW

In March of 1989, the oil tanker Exxon Valdez spilled 42 million L of crude oil into Prince William Sound (PWS). Between 100,000 to 375,000 birds were killed in the spill (Piatt et al., 1990; Ford et al., 1996; Piatt et al., 1996). Negative impacts of the Exxon Valdez oil spill (EVOS) on the abundance and subsequent return of PWS avian species back into heavily oiled areas after the spill was still evident in pigeon guillemots, Cepphus columba, two years after the spill (Murphy et al., 1997). The profound impact of EVOS on nearshore communities was related not only to the volume of the oil spill, but also to the fate of the spilled oil (Burger, 1993). Pigeon guillemots are tightly linked with the health of nearshore marine habitats (Ewins, 1993; Prichard, 1997). Among seabirds, pigeon guillemots are particularly vulnerable to contaminants in the vicinity of breeding colonies because of their limited foraging range during the chick rearing period (Ewins, 1993). Forty percent of EVOS oil was deposited on PWS shorelines in 1989 (Galt et al., 1991). Despite beach cleaning efforts and natural biodegradation processes, Wolfe et al. (1994) estimated that 15% of the EVOS oil remained in intertidal and subtidal areas two years after the spill. The majority of prey base of pigeon guillemots is associated with subtidal and intertidal substrates (Ewins, 1993). While the greater portion of PWS avian
communities demonstrated signs of population recovery 2 to 3 years after the spill (Wiens et al., 1996), pigeon guillemot populations in oiled areas, such as Naked Island, have continued to decline below 1990 levels (Hayes and Kuletz, 1997).

From 1979 to 1981, Oakely (1981) and Kuletz (1983) studied the breeding and foraging ecology of Naked Island's pigeon guillemots. The T/V Exxon Valdez ran aground in 1989 within 30 km of Naked Island. The degree of oiling along the convoluted shoreline of Naked Island varied from negligible to heavy. When Oakely and Kuletz (1996) returned to Naked Island in 1990, they found 43% fewer pigeon guillemots as compared to their pre-spill censuses (1978 to 1981). Many researchers investigating the impact of EVOS on the PWS ecosystem pointed out that natural changes in the marine environment between pre-spill and post-spill studies may be as significant as the impact of EVOS (Oakely and Kuletz, 1996; Piatt and Anderson, 1996; Wiens and Parker, 1995).

Broad scale changes in climate and oceanographic conditions in the Gulf of Alaska (GOA) between the late 1970's and late 1980's resulted in a shift in the relative abundance and size classes of fish species (Royer, 1993; Piatt and Anderson, 1996; Anderson et al., 1997; Fritz et al., 1993). The Alaska Coastal Current brings GOA waters into PWS resulting in ocean conditions similar to GOA in the southern parts of PWS (Niebauer et al., 1994). There is evidence that this shift in forage fish species negatively affected GOA seabird survival and productivity. Black-legged kittiwakes, Rissa tridactyla, of the outer GOA colonies of Middleton Island, Semidi Islands, and Kodiak Island suffered numerous breeding failures in the 1980's and colony populations
dropped in number (Hatch et al., 1992; Springer et al., 1993). As with the outer GOA colonies, kittiwake colonies in southern PWS also experienced low productivity from 1985 to 1989 (Irons et al., 1999; Hatch et al., 1992).

Pigeon guillemot abundance in PWS declined between the 1970's and 1980's in a manner parallel with wide scale declines in the abundance of surface schooling fish species and shrimp (Oakely and Kuletz, 1996; Agler et al., 1999). Similar population responses were observed in other PWS bird and marine mammal species that consumed forage fish (Kuletz et al., 1997; Piatt and Anderson, 1996). However, the declines observed at Naked Island and Knight Island between the eighties and the early nineties were greater along oiled shorelines than along unoiled shorelines (Oakley and Kuletz, 1996; Murphy et al., 1997).

Hayes and Kuletz (1997) reported that the proportion of surface schooling fish in the diet of pigeon guillemot chicks had declined between pre-spill (1979 to 1981) and post-spill studies (1990 to 1996). Surface schooling fish, Pacific herring, Clupea pallasi, and Pacific sand lance, Ammodytes hexapterus, are important food items for breeding seabirds. Because their summer lipid stores translate to high energy meals for chicks, and their schooling behavior represents a concentrated food source for foraging adults, surface schooling fish represent a potentially high provision rate for chicks (Golet et al., 2000).

Herring and sand lance spawn in the nearshore habitat. There is evidence of longer-term toxic effects of oil to fish populations when oil persists in their natal and spawning habitats (Murphy and Rice, 1999; Rice, 1999). Herring embryos exposed to oil
yielded more physically deformed larvae than unoiled embryos (Kocan et al., 1996; Hose et al., 1996). Several studies have reported that EVOS oil in sediments induce xenobiotic responses, induction of cytochrome P450, or liver lesions in intertidal fish species, including high cockcomb, Anoplarchus purpurescens, walleye pollock, Theragra chalcogramma, and kelp greenling, Hexagrammos decogrammus (Woodin et al., 1997; Collier et al., 1996; Jewett et al., 1995; Holland-Bartels, 1998). The two potential routes that pigeon guillemots may be exposed to residual oil are through ingestion of contaminated food items, or contact with oil sheens while foraging in oiled areas (King and Sanger, 1979; Piatt et al., 1990; Prichard, 1997).

Recovery of the pigeon guillemot population may be constrained by the physiological effects of oil exposure on chicks and adults, demographic limitations due to pigeon guillemot life history traits, food limitations, or other factors such as nest predation. In my thesis, I assess both the oil and non-oil related factors that may be constraining the post EVOS population growth of pigeon guillemots in PWS. In chapter two, I assess the impacts of residual oil on the clinical health of chicks and adults by comparing the hematological and plasma biochemical profiles of chicks and adults in oiled and unoiled areas. In chapter three, I examine demographic and food limitations. To gauge the current demographic limitations of populations in unoiled areas, I tracked trends in population, productivity, and fledgling survival, at unoiled Jackpot Island in southwestern PWS, from 1995 to 1998. I compared productivity parameters and the relative quality of the chick diet among unoiled Jackpot Island, pre-spill Naked Island, and post-spill Naked Island studies. To assess quality of the chick diet, I examined the
species composition of the chick diet and the frequency of meal deliveries. To evaluate food limitations, I compared the relative quality of the chick diet with chick survival rates, growth rates, and fledging weights. In my final chapter, I address the question 'Is it oil or is it food?' by providing a summary of my findings from chapters two and three. This eco-toxicology approach in assessing the health of post-spill pigeon guillemot populations is unique because few oil spill studies have tested for the lingering sub-lethal effects of residual oil, or gauged population response in accordance with available food resources.
CHAPTER TWO
COMPARISON OF PIGEON GUILLEMOT, CEPPHUS COLUMBA,
BLOOD PARAMETERS FROM OILED AND UNOILED AREAS
OF ALASKA, EIGHT YEARS AFTER
THE EXXON VALDEZ OIL SPILL

This chapter was published in Marine Pollution Bulletin and may be cited as follows:
Pamela E. Seiser, Lawrence K. Duffy, A. David McGuire, Daniel D. Roby, Gregory H.
columba, Blood Parameters from Oiled and Unoiled Areas of Alaska Eight Years after

2.1 INTRODUCTION

Population estimates of pigeon guillemots, Cepphus columba, in Prince William
Sound (PWS), Alaska, have declined from 15,000 individuals in 1972-73 to
approximately 3,000 individuals in the mid-1990's (Dwyer et al., 1976; Klosiewski and
Laing, 1994; Agler and Kendall, 1997; Sanger and Cody, 1994). A large-scale regime
shift in the Gulf of Alaska during the late 1970's (Piatt and Anderson, 1996) likely caused
much of this decline, as high-quality forage fish were more widely available in the 1970's
than in recent years (Hayes and Kuletz, 1997; Kuletz et al., 1997). Pigeon guillemot
populations in PWS were further impacted by the Exxon Valdez oil spill (EVOS; Murphy
et al., 1997), which occurred when the supertanker Exxon Valdez ran aground on 24 March 1989 and spilled 42 million L of crude oil into PWS. Approximately 40% of this oil was deposited on the shorelines of PWS (Galt et al., 1991). Between 100,000 to 375,000 birds died in the spill, of which 1,500 to 3,000 were pigeon guillemots (Piatt et al., 1990). Seven years after the spill, pigeon guillemots had not recovered to pre-spill numbers (Agler and Kendall, 1997; Oakley and Kuletz, 1996). It is not clear to what extent demography, food availability, or the physiological effects of lingering oil exposure may be constraining recovery of pigeon guillemots in PWS.

Pigeon guillemots are vulnerable to oil spills because they use the nearshore habitat (King and Sanger, 1979; Piatt et al., 1990). They breed in small colonies along rocky coastlines, and roost on intertidal rocks. Guillemots spend much of their time on the sea surface or diving for surface schooling fish, demersal fish, and invertebrates associated with the intertidal and subtidal zones.

The prey of pigeon guillemots are also susceptible to oil contamination. There is evidence of longer-term toxic effects of oil to fish populations when oil persists in their natal habitats (Murphy and Rice, 1999; Rice 1999). For example, Pacific herring, Clupea pallasii, embryos exposed to oil yielded more physically deformed larvae than unoiled embryos (Kocan et al., 1996; Hose et al., 1996). Biomarkers of oil ingestion were noted in PWS fish several years after EVOS. Walleye pollock, Theragra chalcogramma, collected from oiled Naked Island in 1990 and 1991, exhibited high levels of fluorescent aromatic compounds in their bile (Collier et al., 1996). Jewett et al. (1995) reported that demersal fish in the oiled eelgrass beds of Herring Bay, PWS, demonstrated a high

Acute toxic effects of petroleum hydrocarbons are well known (Leighton, 1993), but the lingering effects of chronic oil exposure have not been investigated fully in free ranging piscivorous birds (Fry and Lowenstine, 1985). Leighton (1993) provided an extensive review of avian studies of petroleum oil toxicity. Dosing experiments have shown that the effects of oil ingestion include: (1) lower hatch rate and altered yolk structure (Grau *et al.*, 1977; Szaro *et al*. 1978a); (2) reduced rate of growth (Szaro *et al.*, 1978b; Peakall *et al.*, 1982); (3) slower development and reduced survivorship of chicks (Trivelpiece *et al.*, 1984); (4) liver, kidney and intestine damage in long-term exposure (Khan and Ryan, 1991; Patton and Dieter, 1980; Fry and Lowenstine, 1985); and (5) Heinz-body hemolytic anemia associated with a substantial decrease in packed-cell volume (Leighton *et al.*, 1983).

Because guillemot chicks remain in their natal burrow until they fledge, oil contamination can occur through contact with the oiled feathers of an adult while in the egg or chick stage, or through ingestion of contaminated fish (Leighton, 1993; Peakall *et al.*, 1980). At nine days of incubation, avian embryos are extremely sensitive to oil
contacting the egg shell. As little as 5 µl of Prudhoe Bay crude oil has been reported to cause embryo death at this stage (Albers, 1977; Szaro et al., 1978a). Dosing studies of weathered crude oil on congeneric black guillemots, Cepphus grylle, suggest that oil ingestion may cause long-term physiological effects which could reduce a young bird’s ability to survive at sea (Peakall et al., 1980).

Payne et al. (1986) suggested that detecting simple changes in a biochemical or physiological response in a population may provide information on the presence of toxins. Hematological analyses (differential cell counts) may provide information about the immunological status of birds (Campbell, 1986a). Levels of plasma enzymes provide information on the function of organs, e.g. liver (Campbell, 1986a). Elevated levels of acute-phase protein haptoglobin indicate responses to exogenous toxins, bacterial or viral infections, and physical trauma (Silverman and LeGrys, 1987). Physiological changes occurring during the chick growth period have been suggested by many authors to influence blood parameters (Wolf et al., 1985; Hoffman et al., 1985; Kostelecka-Myrcha, 1987; Starck, 1998; Work, 1996; Prichard et al., 1997). To prevent age-dependent variation from biasing assessments, hematological and plasma biochemical profiles should be repeated on chicks at different stages of development.

To make an accurate assessment of clinical tests, reference values of healthy individuals are needed (Hawkey and Samour, 1988), but information on hematological and clinical chemistry on pigeon guillemots or other alcids is limited (Newman et al., 1997; Newman and Zinkl, 1998; Prichard et al., 1997; Kosteleck-Myrcha, 1987). We assume therefore that colonies in the unoiled areas represent healthy populations. If oil contamination is
limiting recovery of pigeon guillemots in PWS, we expected that blood chemistry and cell counts would differ between oiled and unoiled areas and these differences should be consistent with either toxic responses or lower fitness. In this study, we compare the hematological and plasma biochemical profiles between pigeon guillemot populations in an oiled area of PWS and in unoiled areas of PWS.

2.2 METHODS

During summer 1997, measurements of growth and blood samples from pigeon guillemot chicks were collected in areas oiled by the EVOS and in reference areas that were not oiled (Fig. 2.1). The oiled area we evaluated was Naked Island (60° 40' N, 147° 28' W) in central PWS. The prevailing winds and currents during spring of 1989 deposited oil predominately on the east and northwest shorelines of Naked Island (Galt et al., 1991; Oakley and Kuletz, 1996). The combined colonies of Jackpot Island (60° 19' N, 148° 11' W) and Icy Bay (60° 14' N, 148° 17' W) in southwestern PWS were not oiled and represent the reference areas in this study. For evaluating adults, we also included a third reference area located in Kachemak Bay (59° 35' N, 151° 19' W), which is located in lower Cook Inlet, Alaska.

For each chick, mass and length of wing-chord were measured every five days until the chick fledged. When possible, two blood samples were collected from each chick at approximately 20 and 30 days after hatch. The hatching date of the chick was determined from either direct observation or was estimated by comparing wing-chord
Figure 2.1 Location of the oiled and unoiled reference areas in Prince William Sound and Kachemak Bay, Alaska. The oiled area for this study included several pigeon guillemot colonies on Naked Island in central Prince William Sound. The unoiled reference areas for this study included pigeon guillemot colonies at Jackpot Island and Icy Bay in southwestern Prince William Sound, and pigeon guillemot colonies in Kachemak Bay.
length for chicks of unknown age to wing-chord length for chicks of known age. Adults were captured either by noose traps placed on roosting rocks or with a dip net.

One cc of blood was collected from the brachial vein of chicks using a one cc tuberculin syringe with a 25 or 26 gauge needle. Adults were bled from the medial metatarsal vein. Fresh blood was used to make blood smears on glass slides. Two heparinized micro-hematocrit tubes were filled with blood from the puncture site, capped with clay, and stored in coolers. Whole blood was placed in microtainer tubes treated with lithium heparin. These samples were centrifuged within two hours of collection. After centrifuging, plasma was removed with a disposable pipette and divided between two snap-top plastic vials. Vials were frozen in propane freezers. Blood smear slides, micro-hematocrit tubes and one vial of plasma were placed in chilled insulated boxes and shipped to the Avian and Exotic Laboratory of Redondo Beach, California, within 48 hours of collection. The following parameters were measured: red blood cell count (RBC), packed cell volume (PCV), mean cell volume (MCV), hemoglobin (Hp), mean cell hemoglobin content (MCHC), counts of white blood cells (WBC), heterophils, lymphocytes, eosinophils, basophils, activity of creatine phosphokinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alkaline phosphatase, gamma-glutamyl transferase (GGT), concentration of calcium, uric acid, plasma protein, total protein, alpha-1 macroglobulin, alpha-2 macroglobulin, beta globulin, gamma globulin, albumin, albumin to gamma globulin ratio, bile acid, phosphorus, and sodium. A second vial of frozen plasma was sent to the University of Alaska Fairbanks for measurement of haptoglobin concentration with electrophoresis kits (Helena Laboratories, Beaumont
Texas, USA). Plasma was applied to agarose gels and electrophoresed at 100 volts for one hour. Agarose plates were then fixed with 7.5% trichloroacetic acid and stained with o-dianisidine to detect the Hp-hemoglobin complex. The Hp-hemoglobin complex was quantified by densitometry and results are reported in mg hemoglobin binding capacity per 100 ml of plasma (Duffy et al., 1994). Enzyme immunoassay wipes were used to evaluate the presence of polyaromatic hydrocarbon molecules on the plumage of adults. The plumage of adults was wiped with a one-ply section of 5 by 5 cm gauze pad saturated with isopropanol. The gauze pad was then placed in aluminum foil and frozen until analysis. Levels of phenathrene, pentacosane, and hexacosane from the wipes were measured with the EnSysEnviroGard™ Polynuclear Aromatic Hydrocarbon test kit 70608, produced by Millpore Corporation (Bedford, Massachusetts, USA), or detected with gas chromatography-mass spectrometry (Duffy et al., 1999).

Data were tested for normality and equal variance with the Kolmogorov-Smirnov test with Littiefors correction and with the Levene median test, respectively. To test the hypothesis that there was no difference between samples collected at 20 days of age and 30 days of age, we used the paired t-test or the Friedmans test on ranks, a nonparametric test for a repeated measures design, on the samples collected in the reference area. Blood parameters with significantly different values between sampling ages are considered to be influenced by the development stage of the chicks. A t-test or Mann-Whitney test was used, when appropriate, to detect differences in blood parameters between oiled and unoiled areas, and between 30-day post hatch chicks and adults in the reference areas.
2.3 RESULTS

2.3.1 Effects of age: nestlings

We found several age-related differences in the blood samples. For chicks in southwestern PWS, significant differences between the blood samples of chicks 20 and 30 days after hatching included PCV (P = 0.014), RBC (P = 0.002) and alkaline phosphatase activity (P = 0.001). Differences in phosphorus concentrations were marginally non-significant (P = 0.063). The mean (± SD) wing-chord lengths of the 20-day and 30-day age groups were 92.8 ± 7.6 cm and 128.7 ± 6.3 cm, respectively. A multiple logistic regression model using variables RBC, PCV, and alkaline phosphatase activity correctly predicted the age group in 18 of 22 blood samples with a concordance of 82% (likelihood ratio test = 7.7, P = 0.051). Variables correlated with the nestling wing-chord length of chicks included PCV (r = 0.59, P = 0.001, n = 26), RBC (r = 0.58, P = 0.001, n = 24), alkaline phosphatase (r = 0.57, P = 0.003, n = 24), phosphorus (r = -0.39, P = 0.059, n = 24) and Hp (r = 0.56, P = 0.004, n = 24).

2.3.2 Effects of age: adults versus nestlings

The blood profiles of the adult birds from reference areas of Jackpot Island, Icy Bay and Kachemak Bay were distinct from the blood profile of the chicks from the reference area of Jackpot and Icy Bay. The age-related differences among chicks, which included PCV, RBC, alkaline phosphatase, and phosphorus, extended to our comparison between adults versus chicks. By the time a chick fledges, which occurs between 33 and
54 days of age, its weight is comparable to that of an adult, but its wing growth is not complete (Ewins, 1992; Ewins, 1993). For adults from southwestern PWS, the mean (± SD) for wing-chord length and body weight were 184 ± 4 cm and 508 ± 50 g, respectively. The wing-chord length at 20 days and 30 days after hatching was 49% and 70%, respectively, of wing-chord length in adults. The body mass at 20 days and 30 days after hatching was 66% and 86%, respectively, of the adult body mass. Because we had only samples from four adults in southwestern PWS, we incorporated adults from Kachemak Bay (n=3) into our sample of adults from unoiled areas. In the unoiled areas, adults had higher PCV (P = 0.001), RBC (P = 0.003), Hp (0.004), AST (P = 0.010), and albumin concentrations (P = 0.011), and lower alkaline phosphatase (P < 0.001) and lower phosphorus concentrations (P < 0.001) than 30-day old chicks in southwestern PWS. Adults also tended to have lower WBC (P=0.072), calcium concentration (P = 0.063), and bile acid concentration (P = 0.094) than chicks.

2.3.3 Oiled vs. Unoiled Populations: nestlings

In the 20-day age group, chicks sampled from the oiled population at Naked Island had lower calcium (P = 0.002), plasma protein (P = 0.008), and alkaline phosphatase activity (P = 0.025), and a higher lymphocyte count (P = 0.006) than chicks in the unoiled area of southwestern PWS (Table 2.1). In the 30-day age group, Naked Island chicks had significantly lower calcium (P = 0.043) and MCV (P = 0.015) than chicks from southwestern PWS (Table 2.2).
Table 2.1 Mean, standard deviation (SD) and sample size (n) of the hematological and plasma chemistry of pigeon guillemot chicks sampled at 20 days of age. Samples were collected in 1997 from oiled Naked Island colonies and unoiled Jackpot Island & Icy Bay colonies, in Prince William Sound, Alaska.

<table>
<thead>
<tr>
<th></th>
<th>Oiled Area Naked Island</th>
<th></th>
<th></th>
<th>Oiled Area Jackpot Island &amp; Icy Bay</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SD</td>
<td>n</td>
<td>mean</td>
<td>SD</td>
<td>n</td>
</tr>
<tr>
<td>Red Blood Cells (cu mm$^{-3}$)</td>
<td>2.6</td>
<td>0.4</td>
<td>14</td>
<td>2.58</td>
<td>0.4</td>
<td>17</td>
</tr>
<tr>
<td>Packed Cell Volume (%)</td>
<td>44</td>
<td>15</td>
<td>14</td>
<td>43</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>Mean cell volume (cu mm$^{-3}$)</td>
<td>159</td>
<td>16</td>
<td>14</td>
<td>160</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>Hemoglobin (g dl$^{-1}$)</td>
<td>12.7</td>
<td>1.6</td>
<td>14</td>
<td>11.6</td>
<td>1.6</td>
<td>16</td>
</tr>
<tr>
<td>MCHC (g dl$^{-1}$)</td>
<td>30.7</td>
<td>5.4</td>
<td>14</td>
<td>28</td>
<td>4.3</td>
<td>16</td>
</tr>
<tr>
<td>White Blood Cells (10$^3$ mm$^{-3}$)</td>
<td>13</td>
<td>5</td>
<td>14</td>
<td>16</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>Heterophil *</td>
<td>49</td>
<td>12</td>
<td>14</td>
<td>61</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>Lymphocytes *</td>
<td>49</td>
<td>12</td>
<td>14</td>
<td>37</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>0.6</td>
<td>1.3</td>
<td>14</td>
<td>0.7</td>
<td>1.3</td>
<td>16</td>
</tr>
<tr>
<td>Basophil</td>
<td>1.4</td>
<td>1.3</td>
<td>14</td>
<td>1.1</td>
<td>1.4</td>
<td>18</td>
</tr>
<tr>
<td>Calcium (mg dl$^{-1}$)*</td>
<td>8.9</td>
<td>1.9</td>
<td>14</td>
<td>11.0</td>
<td>1.2</td>
<td>18</td>
</tr>
<tr>
<td>CK (u l$^{-1}$)</td>
<td>530</td>
<td>233</td>
<td>14</td>
<td>776</td>
<td>541</td>
<td>17</td>
</tr>
<tr>
<td>LDH (u l$^{-1}$)</td>
<td>937</td>
<td>234</td>
<td>14</td>
<td>897</td>
<td>471</td>
<td>18</td>
</tr>
<tr>
<td>AST (u l$^{-1}$)</td>
<td>277</td>
<td>106</td>
<td>14</td>
<td>221</td>
<td>119</td>
<td>16</td>
</tr>
<tr>
<td>Uric Acid (mg dl$^{-1}$)</td>
<td>18.3</td>
<td>8.7</td>
<td>14</td>
<td>20.0</td>
<td>11.2</td>
<td>16</td>
</tr>
<tr>
<td>Plasma Protein (g dl$^{-1}$)*</td>
<td>3.1</td>
<td>0.5</td>
<td>14</td>
<td>3.8</td>
<td>0.6</td>
<td>18</td>
</tr>
<tr>
<td>Total Protein (g dl$^{-1}$)</td>
<td>4.5</td>
<td>0.6</td>
<td>14</td>
<td>4.8</td>
<td>0.8</td>
<td>18</td>
</tr>
<tr>
<td>Alpha-1 (g dl$^{-1}$)</td>
<td>0.39</td>
<td>0.11</td>
<td>14</td>
<td>0.44</td>
<td>0.18</td>
<td>18</td>
</tr>
<tr>
<td>Alpha-2 (g dl$^{-1}$)</td>
<td>0.70</td>
<td>0.31</td>
<td>14</td>
<td>0.75</td>
<td>0.32</td>
<td>18</td>
</tr>
<tr>
<td>Beta (g dl$^{-1}$)</td>
<td>0.88</td>
<td>0.21</td>
<td>14</td>
<td>0.91</td>
<td>0.35</td>
<td>18</td>
</tr>
<tr>
<td>Gamma Globulin (g dl$^{-1}$)</td>
<td>0.70</td>
<td>0.16</td>
<td>14</td>
<td>0.75</td>
<td>0.15</td>
<td>18</td>
</tr>
<tr>
<td>Albumin (g dl$^{-1}$)</td>
<td>1.86</td>
<td>0.33</td>
<td>14</td>
<td>1.94</td>
<td>0.51</td>
<td>18</td>
</tr>
<tr>
<td>Albumin/Gamma Globulin (g dl$^{-1}$)</td>
<td>0.72</td>
<td>0.17</td>
<td>14</td>
<td>0.68</td>
<td>0.15</td>
<td>18</td>
</tr>
<tr>
<td>Bile Acid Assay (umol l$^{-1}$)</td>
<td>38.8</td>
<td>35.6</td>
<td>14</td>
<td>61.9</td>
<td>105</td>
<td>14</td>
</tr>
<tr>
<td>Alkaline phosphatase (u l$^{-1}$)*</td>
<td>372</td>
<td>151</td>
<td>14</td>
<td>279</td>
<td>82</td>
<td>17</td>
</tr>
<tr>
<td>GGT (u l$^{-1}$)</td>
<td>25.2</td>
<td>12.5</td>
<td>14</td>
<td>20.6</td>
<td>14.8</td>
<td>13</td>
</tr>
<tr>
<td>Phosphorus (mg dl$^{-1}$)</td>
<td>9.6</td>
<td>4.8</td>
<td>13</td>
<td>6.2</td>
<td>1.7</td>
<td>17</td>
</tr>
<tr>
<td>Sodium (mmol l$^{-1}$)</td>
<td>129</td>
<td>17</td>
<td>11</td>
<td>141</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>Haptoglobin (Hp binding dl$^{-1}$)</td>
<td>109</td>
<td>40</td>
<td>15</td>
<td>124</td>
<td>51</td>
<td>16</td>
</tr>
</tbody>
</table>

*Means significantly different (P < 0.050) between chicks sampled at Naked Island and Jackpot-Icy Bay.
Table 2.2 Mean, standard deviation (SD) and sample size (n) of the hematological and plasma chemistry of pigeon guillemot chicks at 30 days of age. Samples were collected in 1997 from oiled Naked Island colonies and unoiled Jackpot Island & Icy Bay colonies, in Prince William Sound, Alaska.

|                               | Red Blood Cells (mm\(^3\)) | Packed Cell Volume (%) | Mean cell volume (cu mm\(^3\))\* | Hemoglobin (g dl\(^{-1}\)) | MCHC (g dl\(^{-1}\)) | White Blood Cells (10\(^3\) mm\(^{-3}\)) | Heterophil | Lymphocytes | Eosinophil | Basophil | Calcium (mg dl\(^{-1}\))\* | CK (u l\(^{-1}\)) | LDH (u l\(^{-1}\)) | AST (u l\(^{-1}\)) | Uric Acid (mg dl\(^{-1}\)) | Plasma Protein (g dl\(^{-1}\)) | Total Protein (g dl\(^{-1}\)) | Alpha-1 (g dl\(^{-1}\)) | Alpha-2 (g dl\(^{-1}\)) | Beta (g dl\(^{-1}\)) | Gamma Globulin (g dl\(^{-1}\)) | Albumin (g dl\(^{-1}\)) | Albumin/Gamma Globulin (g dl\(^{-1}\)) | Bile Acid Assay (umol l\(^{-1}\)) | Alkaline phosphatase (u l\(^{-1}\)) | GGT (u l\(^{-1}\)) | Phosphorus (mg dl\(^{-1}\)) | Sodium (mmol l\(^{-1}\)) | Haptoglobin (Hp binding dl\(^{-1}\)) |
|-------------------------------|-------------------------------|------------------------|----------------------------------|----------------------------|------------------------|---------------------------------------------|------------|-------------|------------|----------|-------------------------------|----------------------|----------------------|----------------------|-----------------------------|----------------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|---------------------------------|----------------------|------------------------|------------------------|------------------------|----------------------|
| **Oiled Area**                |                               |                        |                                  |                            |                        |                                             |            |             |            |          |                                |                       |                      |                      |                             |                             |                             |                            |                            |                             |                             |                            |                             |                             |                      |                      |
| Naked Island                  | 3.16                          | 0.40                   | 24                               | 2.95                       | 0.42                   | 13                           |            |             |            |          |                                |                       |                      |                      |                             |                             |                             |                            |                            |                             |                             |                            |                             |                             |                      |                      |
| Jackpot Island & Icy Bay      | 2.95                          | 0.42                   | 13                               | 1.8                        | 0.4                     | 14                           |            |             |            |          |                                |                       |                      |                      |                             |                             |                             |                            |                            |                             |                             |                            |                             |                             |                      |                      |
| **Unoiled Area**              |                               |                        |                                  |                            |                        |                                             |            |             |            |          |                                |                       |                      |                      |                             |                             |                             |                            |                            |                             |                             |                            |                             |                             |                      |                      |
| Naked Island                  | 48                            | 4                      | 25                               | 47                         | 6                       | 15                           |            |             |            |          |                                |                       |                      |                      |                             |                             |                             |                            |                            |                             |                             |                            |                             |                             |                      |                      |
| Jackpot Island & Icy Bay      | 47                            | 6                      | 15                               | 1.8                        | 0.4                     | 14                           |            |             |            |          |                                |                       |                      |                      |                             |                             |                             |                            |                            |                             |                             |                            |                             |                             |                      |                      |

*Means significantly different (P < 0.050) between chicks sampled at Naked Island and Jackpot-Icy Bay.
2.3.4 Oiled vs. Unoiled Populations: adults

Our sample size of adults was small. The number of adult blood samples from Naked Island, southwestern PWS, and Kachemak Bay were 10, 4 and 3, respectively. Adults at Naked Island were captured between 29 July and 3 August. Three of the adults in the reference areas were captured in June and two in August. Adults captured in the oiled area had significantly higher AST activity ($P = 0.017$), lower RBC ($P = 0.006$), Hp ($P = 0.004$) and GGT ($P = 0.015$) than adults in the reference areas (Table 2.3). The AST activity for the adults in the oiled area was nearly double the levels for the adults in the reference areas. The plumage wipes from adults at Naked Island ($n = 10$) indicated low levels of phenathrene, pentacosane and hexacosane (mean ± SD: 0.004 ppm ± 0.002, 0.178 ppm ± 0.059, and 0.202 ppm ± 0.047, respectively).
Table 2.3 Mean, standard deviation (SD) and sample size (n) of the hematological and plasma chemistry of adult pigeon guillemots sampled in 1997 from oiled Naked Island, Prince William Sound and unoiled areas of Jackpot Island & Icy Bay, Prince William Sound and Kachemak Bay, Lower Cook Inlet in Alaska.

<table>
<thead>
<tr>
<th>Oiled Area</th>
<th>Unoiled Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naked Island</td>
<td>Jackpot Island, Icy Bay and Kachemak Bay</td>
</tr>
<tr>
<td><strong>Red Blood Cells (cu mm³)</strong></td>
<td>mean SD n</td>
</tr>
<tr>
<td>3.01</td>
<td>0.35</td>
</tr>
<tr>
<td><strong>Packed Cell Volume (%)</strong></td>
<td>53</td>
</tr>
<tr>
<td><strong>Mean cell volume (cu mm³)</strong></td>
<td>168</td>
</tr>
<tr>
<td><strong>Hemoglobin (g dl⁻¹)</strong></td>
<td>18.3</td>
</tr>
<tr>
<td><strong>MCHC (g dl⁻¹)</strong></td>
<td>34.3</td>
</tr>
<tr>
<td><strong>White Blood Cells (10³ mm⁻³)</strong></td>
<td>8</td>
</tr>
<tr>
<td><strong>Heterophil</strong></td>
<td>58</td>
</tr>
<tr>
<td><strong>Lymphocytes</strong></td>
<td>37.9</td>
</tr>
<tr>
<td><strong>Eosinophil</strong></td>
<td>0</td>
</tr>
<tr>
<td><strong>Basophil</strong></td>
<td>4</td>
</tr>
<tr>
<td><strong>Calcium (mg dl⁻¹)</strong></td>
<td>8.6</td>
</tr>
<tr>
<td><strong>CK (u l⁻¹)</strong></td>
<td>244</td>
</tr>
<tr>
<td><strong>LDH (u l⁻¹)</strong></td>
<td>892</td>
</tr>
<tr>
<td><strong>AST (u l⁻¹)</strong></td>
<td>979</td>
</tr>
<tr>
<td><strong>Uric Acid (mg dl⁻¹)</strong></td>
<td>14.85</td>
</tr>
<tr>
<td><strong>Plasma Protein (g dl⁻¹)</strong></td>
<td>4.7</td>
</tr>
<tr>
<td><strong>Total Protein (g dl⁻¹)</strong></td>
<td>5.5</td>
</tr>
<tr>
<td><strong>Alpha-1 (g dl⁻¹)</strong></td>
<td>0.45</td>
</tr>
<tr>
<td><strong>Alpha-2 (g dl⁻¹)</strong></td>
<td>0.67</td>
</tr>
<tr>
<td><strong>Beta (g dl⁻¹)</strong></td>
<td>0.90</td>
</tr>
<tr>
<td><strong>Gamma Globulin (g dl⁻¹)</strong></td>
<td>0.69</td>
</tr>
<tr>
<td><strong>Albumin (g dl⁻¹)</strong></td>
<td>2.75</td>
</tr>
<tr>
<td><strong>Albumin/Gamma Globulin (g dl⁻¹)</strong></td>
<td>1.03</td>
</tr>
<tr>
<td><strong>Bile Acid Assay (umol l⁻¹)</strong></td>
<td>40.3</td>
</tr>
<tr>
<td><strong>Alkaline phosphatase (u l⁻¹)</strong></td>
<td>93</td>
</tr>
<tr>
<td><strong>GGT (u l⁻¹)</strong></td>
<td>3</td>
</tr>
<tr>
<td><strong>Phosphorus (mg dl⁻¹)</strong></td>
<td>2.2</td>
</tr>
<tr>
<td><strong>Sodium (mmol l⁻¹)</strong></td>
<td>138.6</td>
</tr>
<tr>
<td><strong>Haptoglobin (Hp binding dl⁻¹)</strong></td>
<td>122</td>
</tr>
</tbody>
</table>

*Means significantly different (P < 0.050) between adults sampled at oiled areas and unoiled areas.
2.4 DISCUSSION

The clinical hematology and biochemistry of seabirds is not as well known as for waterfowl, poultry or pet species (Newman and Zinkl, 1998). Blood parameters vary among species according to life history patterns, diet, and activity level. Pigeon guillemots differ from more commonly studied birds in that they have rapidly growing semi-precocial chicks, their diet is composed of marine fish, and they are adapted to diving to depths greater than 20 m (Ewins, 1993). Interpreting our results is also made difficult because of the paucity of biochemical studies on this species. The few reference values for this species are from studies with sample sizes of less than ten individuals (Newman and Zinkl, 1998; Newman et al., 1997; Prichard et al., 1997; Haggblom et al., 1988; Bradley and Trefall, 1974). Our study extends the biochemical information for chicks of this species by providing reference values for different stages of development that are based on larger sample sizes.

2.4.1 Effects of Development

Physiological changes occurring during post hatch development of chicks affect many hematological and biochemical parameters (Starck, 1998; Vinuela et al., 1991; Kostlecka-Myrcha, 1987). Age-related variation in blood parameters is an important consideration when collecting samples from pigeon guillemot colonies, because the range in chick ages may be as great 42 days (Drent, 1965). This is caused by asynchronous nesting or the laying of replacement clutches (Ewins, 1993; Drent, 1965). It has been
well documented in many avian species that adults have higher PCV, RBC, and Hp than immature birds (Work, 1996; Wolf et al., 1985; Kostlecka-Myrcha, 1987; Fairbrother et al., 1990), but there is little documentation of the changes in these parameters within the nestling period for free-living species (Kostelecka-Myrcha, 1987). Anemia has been associated with oil contamination (Hartung and Hunt, 1966; Szaro et al., 1978b; Pattee and Franson, 1982; Fry and Lowenstein, 1985; Leighton et al., 1983). Clinical signs of anemia are low PCV, RBC, MCV or MCHC. Therefore it was critical for us to identify these age-specific differences in red blood cell parameters before evaluating the health of immature birds. During the nestling period, there are dramatic changes in the profile of the red blood cells as embryonic forms, natal forms, and adult forms replace one another (Schenk et al., 1978). Kostelecka-Myrcha (1987) documented PCV increases and MCV decreases during the nestling period of the little auk, Plautus alle, as smaller sized adult red blood cells replaced the red blood cells after hatching. The greatest increases in RBC occurred during the first 10 days after hatch (Kostelecka-Myrcha, 1987; Hoffman et al., 1985). Post-hatch development of erythropoietic tissue is closely related to growth of body mass. As the chick approaches adult size or asymptotic body mass, bones are ossifying in preparation for flight and erythropoietic tissue decreases to adult levels (Starck, 1998). Pigeon guillemot chicks reach asymptotic growth between 30 and 40 days of age (Ewins, 1993). Kostelecka-Myrcha (1987) noted a non-significant increase in Hp level during the latter half of the nestling period. Our study and the study of Haggblom et al. (1988) confirm that similar age-related changes in Hp occur in pigeon
guillemots chicks. We expect subtle changes in red blood cells and Hp to continue after chicks fledge.

Elevated alkaline phosphatase (AP) activity in birds is associated with increased osteoblastic activity such as skeletal growth and repair, egg production, or nutritional deficiencies (Lumeij, 1994). Therefore the normal range of AP activity in rapidly growing chicks is higher than in adults (Wolf et al., 1985; Hoffman et al., 1985; Vinuela et al., 1991; Work, 1996). We found AP activity nearly doubled between the samples for chicks 20 days and 30 days after hatching. The activity of AP reported by Newman and Zinkl (1998) for fledglings were similar to the AP activity for 20-day old chicks in our study. In red kites, Milvus milvus, Vinuela et al. (1991) reported that AP activity peaked at 38 days after hatch, when the growth of long bones were near completion. Pigeon guillemot chicks also had higher phosphorus and marginally higher calcium levels than adults. Vinuela et al. (1991) noted that increases in calcium and phosphorus levels correlated with increases in AP activity during the nestling period of red kites. In brown pelicans, Pelecanus occidentalis, Wolf et al. (1985) found that AP activity and phosphorus concentration were highest during the first 10 months of development and remained moderately elevated through the first two years of life. Pigeon guillemots are smaller than pelicans, but their skeletal growth continues after fledging for at least two months (Ewins, 1992). These patterns suggest that AP activity, phosphorus and calcium concentrations of guillemot chicks will peak prior to fledging then gradually drop to adult range within the first six months of life.
Elevated WBC is a symptom of infection. Interpretation of elevated WBC in juvenile birds is difficult because their normal range is variable and higher than adults (Fudge, 1996). For terns, shearwaters, and petrels, Work (1996) reported that older chicks tend to have higher WBC than adults. Puerta et al. (1990) reported similar results for common cranes. We could not detect differences in WBC between 20-day and 30-day old chicks, but these chicks had higher WBC than adults.

Similar to our results, Prichard et al. (1997) and Work (1996) reported that chicks had lower AST activity than adults. Newman and Zinkl (1998) found that young pigeon guillemots between five and ten weeks old have AST activity greater than or equal to the activity in adults. Elevated AST activity is associated with hepatocellular damage, septicemia and muscle injury. Bollinger et al. (1989) studied the effect of different capture methods on waterfowl AST activity and reported that AST activity becomes elevated with physical exertion. We suggest that chicks have lower AST activity than adults because they are sedentary and their muscles are less developed. Compared to adults, chicks offer little resistance to capture and are less likely to experience muscular exertion and injury.

Age-related differences in Hp concentration have been documented in mammals. Stellar sea lion, *Eumetopias jubatus*, pups that are less than 15 days old have significantly lower haptoglobin (Hp) levels than adults (Zenteno-Savin et al., 1997). In humans, neonates do not have detectable levels of Hp until two months of age (Henry, 1991). Prichard and co-workers (1997) reported that pigeon guillemot chicks had significantly lower Hp levels than adults. Adults in our study had lower mean Hp levels than reported
by Prichard (1997), which may explain why we did not find similar age-related differences. Prichard (1997) noted that Hp was correlated with the rate at which adults deliver meals to the nest. In our study Hp was significantly correlated with the rate of weight gain immediately prior to the drawing of blood from chicks. This relationship supports Prichard’s speculation that Hp is sensitive to the nutrition of chicks. We also documented a positive correlation between Hp and RBC, which suggests that Hp levels may be linked to the development of erythropoietic tissue during chick development.

2.4.2 Comparison between populations in oiled and unoiled areas

Various oil-dosing studies have been conducted on birds, but the symptoms of toxicity of oil ingestion have varied with species, age, the chemical composition of the oil, the dosing levels, and the presence of additional stress factors (Hartung, 1995; Leighton, 1993). Ingestion of sublethal levels of crude oil may constitute a nonspecific stressor for birds and render them more vulnerable to stress factors such as persistent cold temperatures and bacterial diseases (Holmes et al., 1979). To evaluate the presence of injury at the oiled colonies in this study, we measured blood parameters that were indicators of physiological health of organ systems that involve the liver function, kidney function, the haematopoietic system, immune function, and electrolyte balance.

The avian liver responds to oil ingestion with hypotrophic activity (Szaro et al., 1978b; Patton and Dieter, 1980; Stubblefield et al., 1995) and induction of hepatic cytochrome P-450 (Peakall et al., 1989; Lee et al., 1985). Enlargement of the liver may be a compensatory response to metabolize the high burden of toxic material introduced in
experimental diets (Patton and Dieter, 1980; Stubblefield et al., 1995) or an inflammation response to cell injury. Hepatocellular damage and necrosis are associated with elevation in the activity of plasma liver enzymes (Lewandowski et al., 1986). In Leighton’s (1993) review of oil toxicity research, he found that the evidence of injury to the liver was inconsistent among studies, which may be associated with enzyme responses that are specific to species (Franson et al., 1985). Our indicators of liver injury were elevated bile acid, AST and LDH activity in the plasma. In pigeons, *Columba livia*, elevated levels of bile acid (Lumeij, 1988) and AST are the most sensitive indicator of experimentally induced liver injury (Lumeij, 1988; Campbell, 1986b). Ingestion stimulates the release of bile acid. Fasted peregrine falcons, *Falco peregrinus*, experienced a three-fold increase in plasma bile acid concentration after ingestion of meat (Lumeij and Remple, 1992). During our study adults fed their nestlings at a rate of 0.4 to 1.0 fish h\(^{-1}\). We did not control the food intake of chicks and this would explain some of variation in bile acid concentrations between individuals. Post-prandial increases in bile acid concentration represent 1-fold to 2-fold increases, while hepatobiliary disease results in 5-fold to 10-fold increases relative to the reference range (Lumeij, 1991). Elevated levels of bile acid concentration (exceeding 200 micro mol l\(^{-1}\)) indicate persistent loss of hepatic function (Fudge, 1996). The bile acid concentrations of chicks at Naked Island were in the ranges reported for pigeons and peregrine falcons (Lumeij, 1988; Lumeij and Remple, 1992). While AST and LDH are considered non-specific because they occur in many tissues, Campbell (1986b) found that AST and LDH were sensitive indicators of liver disease in carnivorous birds including red tail hawks, *Buteo jamicensis*, and great horned owls,
*Bubo virginianus*. Elevated BA, AST or LDH concentrations were uncommon among chicks in both the oiled and unoiled areas, and we did not observe a significant difference in mean activity of BA, AST or LDH between chicks of Naked Island and southwestern PWS. Other researchers working with weathered Prudhoe Bay crude oil found no effect of oil dosing on liver enzyme responses of alcid chicks (Leighton, 1993; Prichard, 1997) and mallards, *Anas platyrhynchos* (Rattner, 1981; Stubblefield *et al.*, 1995). The blood variables associated with liver function and hepatocellular damage do not indicate deleterious effects on livers of chicks at Naked Island.

Renal tubular necrosis was documented in Cassin's auklets, *Prychoramphus aleuticus*, after oil was applied to their breast feathers (Fry and Lowenstine, 1985). Increases in uric acid in the plasma may indicate adverse effects on renal function (Allen, 1988; Fudge, 1996). In veterinary practices uric acid levels greater than 20 mg dl⁻¹ are abnormal (Allen, 1988; Fudge, 1996). Newman and coworkers (1997) noted that uric acid levels in adult piscivorous marine birds are typically higher than in other avian species. They suggest that high protein diets combined with the osmoregulation demands of living in a marine environment causes higher concentrations of serum uric acid. In our study, both chicks and adults had uric acid levels that were below 20 mg dl⁻¹, which is within the reference range previously reported for adult pigeon guillemots (Newman and Zinkl, 1998; Newman *et al.*, 1997). Therefore, the uric acid levels of chicks in the oiled area of our study do not appear to indicate the presence of impaired renal function or damage.
Anemia was documented in several species of birds following exposure to oil (Hartung and Hunt, 1966; Szaro et al., 1978b; Pattee and Franson, 1982; Fry and Lowenstein, 1985; Fry and Addiego, 1987; Leighton et al., 1983). Reduced PCV and Heinz-body hemolytic anemia was documented in young herring gulls, *Larus argentatus*, and Atlantic puffins, *Fratercula arctica*, after experimental ingestion of crude oil (Leighton et al., 1983). Yet, ingestion of high doses of Prudhoe Bay crude oil did not result in anemia in both adult rhinoceros auklets, *Cerorhinca monocerata*, (Newman, personal communication) and mallards (Stubblefield et al., 1995). Hemolytic anemia was documented in adult white-winged scoters, *Melanitta fusca*, rescued from an oil spill, but blood samples were taken several days after the birds were captured (Yamato et al., 1996). The decrease in physical activity, the stress of handling, and the change in diet associated with captivity may influence erythropoiesis in adult alcids (Newman, personal communication). Anemia is the result of reduced erythropoiesis, accelerated erythrocyte destruction (hemolytic anemia), or blood loss. Clinical signs of anemia are low PCV, RBC, MCHC or MCV. There is little variation in PCV among species, and values below 32% are considered diagnostic of anemia (Hawkey and Samour, 1988). In our study, the values for PCV, MCHC and hemoglobin were within the ranges that are normal for immature birds, which indicates that there was probably no anemia for chicks in the oiled area of our study. The MCV values for 30-day old chicks at Naked Island were significantly less than the MCV for chicks in southwestern PWS and in Kachemak Bay, Alaska (Seiser, unpublished data). It is not clear why MCV values are lower in the oiled area.
Immunosuppression has been noted in various oil dosing studies (Leighton, 1993). Reduced lymphocytes and reduced resistance to bacterial pathogens have been recorded in mallards (Holmes et al., 1979; Rocke et al., 1984). In adult rhinoceros auklets, ingestion of crude oil elicited no inflammatory response in WBC or differential cell counts, but young alcids may respond differently (Newman, personal communication). Leighton (1986) reported morphological changes to the lymphoid glands of young Atlantic puffins and herring gulls. In our study, WBC and differential cell counts (lymphocytes, heterophils, eosinophils and basophils) were our indicators of the state of the immune system. The ratio of lymphocytes to heterophils for the 20-day old chicks at Naked Island was significantly different from the ratio for chicks in southwestern PWS, but this pattern did not persist for the 30-day old chicks. We found that Naked Island did not have significantly lower values of WBC or differential cell counts than the unoiled area in southwestern PWS, which suggests that the immune system was not stressed or impaired in a way that would influence cell production.

Hypertrophy of salt glands has been documented in marine birds dosed with crude oil (Peakall et al., 1980, 1982, 1983; Miller et al., 1978). Osmoregulatory impairment can be accompanied by increases in plasma sodium levels. Peakall et al. (1980) noted a transient rise in plasma sodium levels in black guillemot chicks dosed with 0.1 ml and 0.2 ml of Prudhoe Bay crude oil. Similar results have been found in herring gulls (Miller et al., 1978) and mallards (Eastin and Rattner, 1982). In contrast, Prichard (1997) found sodium levels of pigeon guillemot chicks did not respond to dosing with 0.2 ml of weathered Prudhoe Bay crude oil. The sodium levels for chicks in the unoiled area of our
study were similar to levels for the control chicks in the study by Prichard et al. (1997). Because the sodium levels for the chicks at Naked Island were not significantly different from the levels for chicks in southwestern PWS, we conclude that there is no evidence for hypertrophy of salt glands.

The results reported here also extend the data base for Hp levels in pigeon guillemots. Haptoglobin is an acute phase protein that has been widely used in human and other mammal medical practices as an indicator of inflammatory diseases, infectious diseases, trauma or stress. Gevaert and co-workers (1991) demonstrated that Hp concentrations increased after the pigeons were infected with salmonellosis. Although Hp has been employed to assess potential stressors in compromised wildlife populations (Duffy et al., 1993; Duffy et al., 1994; Zenteno-Savin et al., 1997; Prichard et al., 1997), it has not been widely used for assessing health in free-ranging birds. The recovery of river otters from the initial impact of the EVOS was documented with the use of Hp (Duffy et al., 1993; Duffy et al., 1994). In comparisons between declining and stable populations of pinnipeds, significantly higher Hp concentrations were associated with the declining populations of harbor seals, Phoca vitulina, and sea lions (Zenteno-Savin et al., 1997). Prichard et al. (1997) examined the use of Hp as a potential biomarker of oil ingestion in pigeon guillemot chicks, but found that variation in growth rates and feeding rates among chicks from different colonies confounded their interpretation of Hp response to the ingestion of weathered crude oil. In our study, there was no evidence of poor health identified by our suite of health indicators, which is consistent with the similar Hp levels we observed in chicks from oiled and unoiled areas.
Because nearly all the chicks that were sampled for blood in our study ultimately fledged, we conclude that our handling and blood sampling did not affect survival. This observation also supports our diagnosis of clinically healthy chicks. In contrast, the overall fledging success (fledglings per hatchling) for Naked Island and Jackpot Island was 46% and 68%, respectively. In Kachemak Bay, Prichard (1997) also noted that the majority of nestling mortality occurred in the first 12 days after hatch. Predators or food shortages are the most common sources of mortality of young chicks (Hayes and Kuletz 1997, Nelson, 1987). Mink, a major predator of nestlings in PWS, was not present on Jackpot Island in 1997, but was at Naked Island. The shoreline of Naked Island suffered both oil contamination and physical disturbance from efforts to clean beaches after the spill. Both events tend to have negative effects on the prey base of pigeon guillemots. Therefore, we limit our conclusions on the health of chicks to the latter half of the nesting period. Currently, hematological and biochemical variables of the pigeon guillemots we studied provide little evidence of oil-related injury for chicks that hatched in 1997, eight years after the Exxon Valdez oil spill. In contrast to chicks, the pilot study we conducted on adult health suggests that the issue of oil-related injury in pigeon guillemot adults cannot be dismissed without further study.

Pigeon guillemot adults have greater opportunities for exposure to oil than nestlings. Adults feed on invertebrates including crabs, shrimps, and bivalves (Oakley 1981; Kuletz, 1983; Sanger, 1987), but rarely provision their chicks with invertebrates (Oakley, 1981; Ewin, 1993). In the winter, invertebrate consumption may increase because of seasonal changes in distribution of prey fish. Pacific sand lance, *Ammodytes*
*hexapterus*, are inaccessible because they are burrowed in the sediment, and young cod move to deeper waters (Oakley, 1981; Sanger, 1987). Bioaccumulation of polynuclear aromatic hydrocarbons (PAH) is greater in invertebrates than fish. Invertebrates cannot metabolize PAH as efficiently as fish, because of differences in the activity of mixed function oxigenase enzymes and metabolic rate between invertebrates and fish (Gibson, 1977). Therefore, adults potentially have a greater dietary source of PAH's than nestlings (Bolger *et al.*, 1996; Baumard *et al.*, 1998).

It is important to recognize that our sample of adults is small and was obtained opportunistically. The majority of the samples from the unoiled areas were obtained in June, while the samples from the oiled area were collected in late July and early August. Also, we do not know the sex of the birds we sampled. Sex and reproductive condition have been documented to affect plasma biochemistry (Wolf *et al.*, 1985; Fairbrother *et al.*, 1990; Gee *et al.*, 1981). Because interpretation of differences between blood parameters for adults from the oiled and unoiled areas in our study is complicated by sampling issues, the interpretation we present is preliminary and should be viewed with some caution.

In comparison to adults in the unoiled area of our study, GGT activity was significantly lower for adults in the oiled area. GGT activity is commonly measured in mammal clinical practices to detect cholestatic diseases of the liver or the consumption of drugs and other toxic substances that induce the microsomal enzyme system (Henry, 1991). For example, fungi infested feed produces elevated plasma GGT activity in domestic chickens (Espada *et al.*, 1994). GGT activity is not a sensitive indicator of avian
hepatocellular injury (Campbell, 1986b). Egg laying also appears to elevate serum GGT activity. In domestic mallard hens, Fairbrother et al. (1990) observed that serum GGT activity was 10-fold higher during the egg-laying period compared to the incubation period. Newman and Zinkl (1998) measured the mean serum GGT activity for several seabird species, and reported a mean GGT activity of 16.5 IU l⁻¹ with a range of 0 to 60 IU l⁻¹ for five pigeon guillemot adults captured during the egg laying period. These values were slightly higher than the values we observed for adults in the unoiled areas of our study, which were also sampled early in the breeding season. For adults at Naked Island, which were sampled late in the breeding season, the GGT activity was within the range previously reported for adult rhinoceros auklets, Cerorhinca monocerata, common murre, Uria aalge, incubating western gull, Larus occidentalis, and non-breeding white pelicans, Pelecanus onocrotalus (Newman and Zinkl, 1998; Puerta et al., 1991). It is not clear if the lower GGT activity we observed for adults in the oiled area represents a normal seasonal trend in GGT activity for adult pigeon guillemots.

The AST activity of adults in the oiled area was significantly higher and nearly double the AST activity of adults in the unoiled areas of our study and double the AST activity of adult pigeon guillemots observed in other studies (Newman et al., 1997; Newman and Zinkl, 1998). Elevated AST activity is associated with both hepatocellular damage and muscle injury (Bollinger et al., 1989). Muscle injury associated with capture causes elevated CK or LDH activity in waterfowl species (Bollinger et al., 1989; Franson et al., 1985; Fudge, 1996). We did not observe significant differences in CK or LDH between adults in oiled and unoiled areas of our study. Because similar capture methods
were used in the oiled and unoiled areas of our study, we suggest that the elevated AST concentrations in the adults from the oiled area are more consistent with hepatocellular injury than muscle injury. Confirmation of hepatocellular injury requires histological examination of liver tissue. Because adults have greater opportunities for exposure to residual oil than nestlings, we recommend additional studies to fully evaluate the health of adults residing in oiled areas.
3.1 INTRODUCTION

Pigeon guillemots were impacted by the *Exxon Valdez* oil spill (EVOS). Piatt *et al.* (1990) estimated between 1,500 to 3,000 pigeon guillemots were killed in 1989. When the pigeon guillemot population of oiled Naked Island began to decline in 1993, concern arose that it was a symptom of sub-lethal effects of chronic exposure to residual oil. However, several factors may cause the population to decline and limit growth, such as physiological effects of oil on chicks and adults, food shortages, and nest predators. At Naked Island, Hayes and Kuletz (1997) correlated population declines between pre- and post-spill periods with declines in the abundance of sand lance, an important prey species of pigeon guillemots.

The decline in sand lance abundance may be attributed to either localized oiling or broad scale changes in climatic-oceanographic conditions. In the Gulf of Alaska, many piscivorous seabird and marine mammal populations dependent on high-quality forage species declined during the 1970's and 1980's (Agler *et al.*, 1999; Piatt and Anderson,
Declines in high-quality forage species, such as herring, sand lance and capelin were associated with climatic and oceanographic changes in the Gulf of Alaska in the late 1970's (Piatt and Anderson, 1996). To provide insight concerning the role of oil and food in constraining recruitment at oiled areas in PWS, I conducted a five-year study to document population trends, breeding success, and chick diet at an unoiled area in southwestern PWS.

3.2 STUDY AREA

Jackpot Island (60° 19' N, 148° 11' W) is located near the mouth of Jackpot Bay in southwestern PWS, Alaska (Fig. 3.1). The oiled area study site, Naked Island (60° 40' N, 147° 28' W) is in central PWS, approximately 55 km NE of Jackpot Island. Descriptions of Naked Island are found in Oakely and Kuletz (1996), Golet et al. (2000) and Galt et al. (1991).

In 1993, Sanger and Cody (1994) noted that the density of breeding pigeon guillemots at Jackpot Island (103 birds km$^{-1}$) was the highest in PWS. Jackpot Island is separated from the mainland by 0.9 km of water. Deep waters (> 120 m) surround Jackpot Island, but to the north, Dangerous Passage and its associated bays offer shallower water (< 40 m) and to the south, guillemots forage at the submarine moraine at the mouth of Icy Bay. The large land mass of Chenega Island shielded Jackpot Island and its adjacent guillemot foraging areas from direct oiling in the aftermath of the EVOS (Galt et al., 1991).
Figure 3.1 Location of the Jackpot Island and Naked Island study areas in Prince William Sound, Alaska. The inserted map of Prince William Sound shows the area oiled by the 1989 Exxon Valdez oil spill.

### 3.3 METHODS

In this chapter, I compare productivity parameters and diets among three PWS pigeon guillemot studies: post-spill unoiled Jackpot Island, post-spill oiled Naked Island and pre-spill Naked Island. I collected the Jackpot Island (1994-1998) data using methods described below. Similar methods were used at the Naked Island study site. The pre-spill Naked Island (1979-1981) data were obtained from Oakely and Kuletz (1996) and post-spill oiled Naked Island data (1994-1997) from Hayes and Kuletz (1997) and Golet *et al.* (2000).

From 1994 to 1998, I documented the number of the birds attending the Jackpot Island colony, the phenology of the nesting season, the survival of eggs and chicks, the
composition of the chick diet, the delivery rate of food to chicks, and the growth rate and fledging weight of chicks. In early June, during the morning high tide cycle, I conducted boat-based counts of the number of pigeon guillemots attending the Jackpot Island colony following census methods commonly used for guillemots (Ewins 1985; Drent, 1965; Kuletz, 1983). In 1997 and 1998, I also censused colonies at the Pleiades Islands, Gage Island, Flemming Point, Point Countess, West Arm of Whale Bay, and two locations in Icy Bay (denoted Icy Bay 2040 and Icy Bay 2035). The locations of all seven colonies are listed in the Beringian Seabird Colony Catalog (USFWS, 1999).

My estimates of productivity were restricted to nests found during the egg stage. Hatching dates of chicks were determined from direct observations or were estimated by comparing the wing length for chicks of unknown age to the wing length recorded for chicks of known age (Thoresen and Booth, 1958; Oakely, 1981). I calculated laying date by subtracting 32 days (Drent, 1965) from my estimated hatching dates. For the majority of fledglings, my estimated fledge date was within 2 days of the actual date that the fledglings evacuated the burrow.

I began inspecting nest sites in late June, coinciding with hatch dates reported by Oakely and Kuletz (1996). Because incubating guillemots are sensitive to human disturbance (Drent et al., 1964; Vermeer et al., 1993; Cairns, 1980), I restricted the number of visits during the incubation period. Active nests were visited every third day in 1995 and every fifth day in the other years. To determine fledging weight and date, I increased my visitation rate to every other day after chicks reached the age of 30 days or when wing length became greater than 120 mm. All previously used nest sites were
checked for re-occupation. During the incubation period nests are cryptic, but during the chick rearing period we could easily detect nests because of fecal stains at the burrow entrance, vocalization of the chick, or delivery of fish by adults. There were very few nest sites on Jackpot Island for which I could not physically or visually assess the presence of chicks.

On each visit to a nest, I measured the body mass of each chick to the nearest 1 g using a hand-held spring scale and measured the maximum flattened wing-length to the nearest 1 mm. I found the linear phase of growth for PWS chicks was 8 to 20 days post hatch (~ 40 to 90 mm wind length), similar to Emms and Verbeek (1991) and Koelink (1972). I conducted regression analyses between mass and age during the linear growth phase and used the slope of the relationship for my estimate of growth rates (g day⁻¹). To examine the effects of brood size and sibling competition on growth rate and fledging weight, I classified chicks as singleton chicks (chicks in one-chick broods), alpha chicks (first hatching chicks of two-chick broods) or beta chicks (second hatching chicks of two-chick broods).

I conducted provisioning watches to determine composition of the chick diet and the rate that adults provision their chicks with food (delivery rate). My observation platform was a boat anchored approximately 30 m offshore. During these 16-hour provisioning watches I recorded the time that the adult brought a prey item to the nest and identified the prey item to the lowest possible taxon. My hourly delivery rates were based on the 16-hour observation periods (0600 h to 2200 h). Annual mean delivery rates were computed from the mean delivery rate of individual nests.
Prey items were also classified into 4 groups: surface schooling fish (herring, sand lance, smelt, or salmon), gadids (Pacific cod, Pacific tomcod, or walleye pollock), non-schooling fish (such as gunnels, ronquil, and sculpins) or other species (uncommon species not included in the 3 previous groups, such as flatfish, lingcod, *Ophiodon elongatus*, and greenling, *Hexogrammos* spp.). The composition of the chick diet was based on the total sum of identified chick meals recorded during all provisioning watches.

All the chicks I handled were marked for future identification with a unique combination of two colored bands on the right leg and a single colored band on the left leg. The color of the left leg band represented the hatch year of the chick. Pigeon guillemots first breed at the age of 3 or 4 years (Drent, 1965; Nelson, 1991). In 1998, when I expected to observe the 1994 and 1995 cohorts of fledglings return to the colony to breed, I dedicated five days to observing banded birds. Observations of banded birds were also noted during provisioning watches. I estimated the percentage of the 1997 and 1998 fledglings surviving to their third year based on the number of banded fledglings observed at Jackpot Island during the 1997 and 1998 breeding season.

I tested data for normality and equal variance with the Kolmogorov-Smirnov goodness of fit test and the Levene median test, respectively. I tested for significant differences among years using analysis of variance (ANOVA) or the non-parametric Kruskal-Wallis test, as appropriate. When these tests resulted in significant differences, I identified significant differences among groups by conducting multiple pair-wise comparisons with the Bonferroni t-test or the non-parametric Dunn’s method. I assumed statistical significance if $P < 0.05$. Means are reported with standard deviation.
3.4 RESULTS

3.4.1 Population trends and nesting effort

The number of pigeon guillemots attending the Jackpot Island colony increased 36% from 74 to 101 birds over the five-year study period (Fig. 3.2). For seven colonies in southwestern PWS, comparison between my 1998 census and the 1993 census conducted by Sanger and Cody (1994) indicates population increases at six colonies and no change at one colony (Table 3.1). Based on the report of 78 birds at Jackpot Island in 1993 by Sanger and Cody (1994), the annual changes in the population at Jackpot Island between 1993 and 1998 were –5%, 7%, 9%, 0% and 17%, respectively.

There was little variation in number of nesting attempts among the five years (Fig. 3.3); nesting attempts varied between 36 in 1996 and 40 in 1995. Nesting effort, which is defined as active nests per number of pairs in the June census, was higher during the first two years (97% in 1994, 100% in 1995) than nesting effort observed in the last three years (81% in 1996, 88% in 1997, 72% in 1998). Over the five-year study period, we found 184 clutches dispersed over 72 different nest sites. New nest sites were discovered each year, but more commonly, nests from the previous year were re-occupied. Between 1995 and 1998, the percentage of current active nest sites occupied in the previous year ranged from 55% to 72%. The three nests where I found adults killed by mink were not occupied the following year. Of the 36 nest sites discovered in 1994, eleven were not occupied the following year, six were occupied for five consecutive years, five for four consecutive years, nine sites for three consecutive years, and five nest sites for two consecutive years.
Figure 3.2 The number of pigeon guillemots attending the Jackpot Island colony in Prince William Sound, Alaska, from 1994 to 1998. Multiple census were conducted in 1996, 1997 and 1998.

Figure 3.3 The number of active pigeon guillemot nests found on Jackpot Island, Prince William Sound, Alaska, from 1994 to 1998.
Table 3.1 June census counts for eight pigeon guillemot colonies in southwestern Prince William Sound for 1993, 1997 and 1998.

<table>
<thead>
<tr>
<th>Year</th>
<th>Jackpot Island</th>
<th>Pleiades Islands</th>
<th>Gage Island</th>
<th>Flemming Point</th>
<th>Point Countess</th>
<th>Whale Bay West arm</th>
<th>Icy Bay 2040</th>
<th>Icy Bay 2035</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>78</td>
<td>48</td>
<td>16</td>
<td>8</td>
<td>6</td>
<td>8</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>1997</td>
<td>86</td>
<td>48</td>
<td></td>
<td>7</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>1998</td>
<td>100</td>
<td>76</td>
<td>22</td>
<td>15</td>
<td>9</td>
<td>11</td>
<td>9</td>
<td>6</td>
</tr>
</tbody>
</table>

* Source: Sanger and Cody (1994)

3.4.2 Productivity

Estimates of median laying and hatch dates varied 15 days over the course of this study, and the 1995 and 1996 dates were earlier than the median dates for the other 3 years (Table 3.2). The 1995 median fledging date was significantly earlier than the median fledging date in each of the other three years (Table 3.2; Kruskal-Wallis test, $H = 19.7$, $P < 0.001$, df = 3).

Among the five years, mean clutch size, hatching success, fledging success and productivity were $1.82 \pm 0.09$ eggs nest$^{-1}$, $0.55 \pm 0.18$ chicks egg$^{-1}$, $0.48 \pm 0.30$ fledglings chick$^{-1}$, and $0.27 \pm 0.22$ fledglings egg$^{-1}$, respectively (Table 3.3). The 1994 breeding season was the most productive of the five years, with the highest number of hatchlings per nest, fledglings per nest, fledglings per egg laid, and total fledglings produced (Fig 3.4 and Fig. 3.5a).
Table 3.2 Estimates of median laying, hatching and fledging dates for the pigeon guillemot colony on Jackpot Island, Alaska, from 1994 to 1998.

<table>
<thead>
<tr>
<th>Year</th>
<th>Median Laying Date</th>
<th>Median Hatch Date</th>
<th>Median Fledging Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SD</td>
<td>nests</td>
</tr>
<tr>
<td>1994</td>
<td>1 June</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>1995</td>
<td>24 May</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>1996</td>
<td>26 May</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>1997</td>
<td>2 June</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>1998</td>
<td>6 June</td>
<td>6</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 3.3 Mean clutch size, hatching success and nestling survival for pigeon guillemot nests found during the egg stage on Jackpot Island, Alaska, from 1994 to 1998.

<table>
<thead>
<tr>
<th>Year</th>
<th>Nests (eggs/nest)</th>
<th>Clutch Size (chicks/egg)</th>
<th>Hatching Success</th>
<th>Nestling Survival (fledglings/chick)</th>
<th>Productivity (fledglings/egg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td>24</td>
<td>1.92</td>
<td>0.80</td>
<td>0.76</td>
<td>0.61</td>
</tr>
<tr>
<td>1995</td>
<td>29</td>
<td>1.90</td>
<td>0.56</td>
<td>0.45</td>
<td>0.25</td>
</tr>
<tr>
<td>1996</td>
<td>21</td>
<td>1.73</td>
<td>0.61</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>1997</td>
<td>31</td>
<td>1.74</td>
<td>0.44</td>
<td>0.68</td>
<td>0.31</td>
</tr>
<tr>
<td>1998</td>
<td>28</td>
<td>1.79</td>
<td>0.33</td>
<td>0.53</td>
<td>0.18</td>
</tr>
<tr>
<td>5-year mean</td>
<td>1.82 ± 0.09</td>
<td>0.55 ± 0.18</td>
<td>0.48 ± 0.30</td>
<td>0.27 ± 0.22</td>
<td></td>
</tr>
</tbody>
</table>
Between 1994 and 1998, the percentage of nests with at least one fledgling was 75%, 41%, 0%, 26%, and 22%, respectively. Thus, Jackpot Island supported twice as many successful breeding pairs during the first two years than the last two years. The 1995 breeding season was less productive than the 1994 breeding season, the 1996 breeding season was a failure because of predation, and few fledglings were produced during 1997 and 1998 because of low hatching success. Hatching success was lower in the last two years because of nest abandonment during the incubation stage (Fig. 3.5b). With the exception of 1996, losses to predators were low on Jackpot Island (Fig. 3.5c). Predation losses in 1995 were attributed to a pair of northwestern crows nesting on the island, and the catastrophic losses in 1996 were caused by the presence of mink on the island. In 1997 and 1998, there was little evidence of nest predation as abandoned eggs and dead chicks remained in the burrows the entire breeding season.

Figure 3.4 The mean clutch size, hatchlings per nest, fledglings per nest and productivity (fledglings egg$^{-1}$) for the pigeon guillemot colony at Jackpot Island, Prince William Sound, Alaska, from 1994 to 1998. Means are based on nests that were found during the egg stage.
Figure 3.5 Total fledglings (a), abandonment rate (b), and predation rate (c) for the pigeon guillemot colony at Jackpot Island, Prince William Sound, Alaska, from 1994 to 1998. The total number of pigeon guillemot fledglings included all nesting attempts; the percentage of pigeon guillemot nests abandoned during the incubation stage is based on all nesting attempts; and the percentage of pigeon guillemot nests experiencing predation is based on nests found during the egg stage.
3.4.3 Chick Diet

Surface schooling fish, which include herring and sand lance, formed at least one third of the diet of Jackpot Island chicks in four out of the five years (Fig. 3.6). Herring was the dominant species of schooling fish in the diet, and composed 42%, 29%, 20%, 0.4% and 41% of the number of fish delivered between 1994 and 1998, respectively.

Figure 3.6 Composition of the pigeon guillemot chick diet at Jackpot Island, Alaska, from 1994 to 1998. Surface schooling fish include Pacific herring (*Clupea pallasi*) and Pacific sand lance (*Ammodytes hexapterus*). Non-schooling fish include pricklebacks (Stichaeidae), gunnels (Pholidae), ronquils (Bathymasteridae) and sculpins (Cottidae). Gadidae include Pacific cod (*Gadus macrocephalus*), Pacific tomcod (*Microgadus proximus*) and walleye pollock (*Theragra chalacogramma*). Other species represent food items not included in the three previous groups, such as flatfish (Bothidae and Pleuronectidae) and greenling (Hexagrammidae).
Over the five year period, sand lance ranged from 0.5% to 13% of the diet. Non-schooling demersal fish, which include gunnels, pricklebacks, and sculpins, were as common as surface schooling fish in chick diet. In 1997 there was a major shift in the composition of the chick diet, in which schooling fish were rare and adults provided chicks with higher numbers of non-schooling demersal fish (Fig. 3.6). During 1994 gadids formed nearly one quarter of the chick diet, but in the following years gadids were less frequent. Other prey fish species, which include flatfish and greenlings, were uncommon and comprised no more than 3% of the chick diet in any given year.

3.4.4 Delivery Rates

The sample of delivery rates in 1994 included only one observation period, and I have no data on delivery rates for chicks older than 8 days in 1996 because of mink predation during that year. Therefore, I eliminated both the 1994 and 1996 data from the following analyses of delivery rate variability among years and between brood size.

Although delivery rates per chick were not significantly different among the 1995, 1997, and 1998 breeding seasons (Two-way ANOVA, year effect $F = 2.379, P = 0.114, df = 2$), delivery rates tended to be higher in 1995 (Fig. 3.7). Delivery rates per chick were significantly higher for nests with one chick than nests with two chicks (Fig. 3.7; Two-way ANOVA, brood size effect $F = 15.707, P < 0.001, df = 1$). The interaction between year and brood size was not significant ($P = 0.559$). Similar to analysis for delivery rates per chick, delivery rate per nest for 1995 ($0.86 \pm 0.28$ fish nest$^{-1}$ hr$^{-1}$, $n = 23$ nests), 1997 ($0.79 \pm 0.25$ fish nest$^{-1}$ hr$^{-1}$, $n = 16$ nests), and 1998 ($0.75 \pm 0.20$ fish nest$^{-1}$ hr$^{-1}$, $n = 7$
nests) was not significantly different among years (One-way ANOVA, $F = 1.270$, $P = 0.296$, $df = 3, 46$).

![Box plot showing delivery rates per chick for the pigeon guillemot colony at Jackpot Island, Alaska, among the 1995, 1997, and 1998 breeding seasons and between nests with one and two chicks.](image)

Figure 3.7 Comparison of the mean delivery rates per chick for the pigeon guillemot colony at Jackpot Island, Alaska, among the 1995, 1997, and 1998 breeding seasons and between nests with one and two chicks. Lines in the box plots indicate the median, and the 5th, 25th, 75th, and 95th percentiles. Letters identify significantly different groups ($P < 0.05$).

### 3.4.5 Growth Rates and Fledging weights

The growth rate of chicks was not significantly different among years (Fig. 3.8a; One-way ANOVA; $F = 0.619$, $P = 0.651$, $df = 4, 58$). Growth rates between nest mates were significantly different (Paired t-test, $t=3.12$, $P=0.008$, $df = 12$): single chicks grew significantly faster than the alpha and beta chicks of 13 pairs of siblings (One-way ANOVA; $F = 3.087$, $P = 0.53$, $df = 2, 60$). Beta chicks grew at slower rates ($14.7 \pm 3.5$ g...
d⁻¹, n = 13) than their alpha siblings (16.4 ± 2.8 g d⁻¹, n = 13) or singleton chicks (17.6 ± 2.7 g d⁻¹, n = 21). Fledging weight in 1994 (500 ± 37 g) tended to be high in comparison with 1995 (467 ± 46 g), 1997 (463 ± 41 g), and 1998 (482 ± 42 g) (Fig. 3.8b; One-way ANOVA, F = 2.021, P = 0.121, df = 3, 56). Fledging wing-length in 1994 (141 ± 6 mm) tended to be smaller than 1995 (145 ± 7 mm), 1997 (144 ± 7 mm), and 1998 (145 ± 3 mm) (One-way ANOVA, F = 2.336, P = 0.083, df = 3, 57). Although fledging weight was significantly correlated with growth rate (r = 0.336, P= 0.032, n = 41), growth rate explains only 11% of the variation in fledging weight. Compared to their nest mate, beta chicks spent more days in the nest and tended to have slightly lower fledging weights (Paired t-test, t=1.974, P = 0.072, df = 12) and wing lengths (Paired t-test, t = 1.818, P = 0.092, df = 13).
3.4.6 Fledgling Survival

I banded 28, 22, 0, 16 and 15 fledglings, in 1994, 1995, 1996, 1997 and 1998, respectively. In 1997, I located eight of the birds banded in 1994. Six of the birds were observed at Jackpot Island, one bird was observed at the Pleiades Islands and one bird was observed at a colony in Icy Bay. The following year, the searched area was limited to Jackpot Island. In 1998, I recorded two additional birds from the 1994 hatch year and five birds from the 1995 hatch year. My observations indicate that at least 36% of
fledglings banded in 1994 survived to their third year. My conservative estimates of the proportion of fledglings returning to their natal colony in their third year is 21% for the 1994 cohort and 23% for the 1995 cohort. I documented four banded birds breeding at 3 years of age.

3.5 DISCUSSION

Recent increasing population trends at colonies in southwestern PWS suggest that favorable environmental conditions exist in Prince William Sound for the expansion of pigeon guillemot populations. However, at the end of this 5-year study, the abundance of pigeon guillemots at oiled Naked Island remained below their 1994 levels (G. Golet personal communication). These contrasting population trends suggest one or more demographic parameters, such as productivity, fledgling survival or adult survival, varies between the oiled and unoiled areas. Population growth at Naked Island may be limited by physiological effects of oil exposure, quality of diet, or other factors such as predators.

I could find no evidence that recovery was constrained by the physiological effects of oil exposure on chicks, although this hypothesis still needs to be rigorously evaluated for adults (Seiser et al., 2000). The abundance of high-quality fish during the breeding season is a factor of particular interest because Hayes and Kuletz (1997) observed pre-spill and post-spill differences in the proportion of high-quality fish delivered to chicks at Naked Island. Sand lance and herring are surface schooling fish and are noted for their rich lipid stores and relative high energy density (kJ g\(^{-1}\)) (Anthony et al., In Press). Temporal or regional differences in diet may represent limitation to
population development if associated with lower productivity or survival rates (Carins, 1987). Declines in the relative abundance of these surface schooling fish in the chick diet has been associated with lower breeding success for Atlantic puffin, *Fratercula arctica,* (Lid, 1981; Anker-Nilseen, 1987) and arctic terns, *Sternaparadisaea* (Montevecchi, 1993).

I evaluated the hypothesis that availability of high-quality food is playing a role in constraining population growth in oiled areas by comparing diet and survival of chicks from unoiled Jackpot Island and pre-spill Naked Island to that of oiled Naked Island. In my discussion, I restricted the post-spill Naked Island data set to the five-year period coinciding with my Jackpot Island study. My pre- and post-spill comparison of Naked Island differs from Hayes and Kuletz (1997), because I do not include the two breeding seasons immediately following the grounding of Exxon Valdez oil tanker. Oakley and Kuletz (1996) examined the acute effects of EVOS on pigeon guillemot breeding success and diet, whereas I focus on a period when pigeon guillemot breeding success and diet may be influenced by chronic effects of EVOS to the nearshore community.

I ranked the relative quality of diet of chicks based on the proportion of high-quality fish in the diet and the rate that chicks received fish. I then examined nestling survival rate, growth rates and fledgling weight of the various colonies to determine if the observed difference in diet of chicks may have affected demographic parameters of productivity and fledgling survival rates.
3.5.1 Food Constraints

Diet of Chicks

Herring and sand lance accumulate lipid stores during summer months to sustain themselves during winter fasting periods (Blaxter and Holiday, 1963). Because of these substantial lipid stores, herring and sand lance tend to have higher energy density (kJ g⁻¹) than the other food items in the chick diet (Van Pelt et al., 1997; Hislop et al., 1991; Paul et al., 1998; Anthony et al., In press). I compare the proportion of surface schooling fish, demersal fish, gadids and other fish delivered to chicks at Jackpot Island to the proportions delivered to pre- and post-spill chicks at Naked Island to evaluate whether differences in diet composition have the potential to constrain recovery of pigeon guillemots in oiled areas of PWS.

Based on data in the literature, I have ranked the whole-body energy content among four categories of fish from high to low, as follows: surface schooling fish, demersal fish, gadids, and other fish. Paul and Paul (1998) reported significant regional, seasonal and annual variation in whole body energy content of PWS forage fish. Other researchers are currently addressing these diet issues for PWS pigeon guillemots (D. Roby, personal communication). In my evaluation of the quality of chick diet, I assume whole body energy of fish is constant over time and region. The validity of this assumption will be addressed in other studies.

The composition of the diet varied among the unoiled Jackpot Island colony reported here and oiled and pre-spill colonies at Naked Island (Chi-square; \( \chi^2 = 31.8, P < 0.001, \text{df} = 8 \)). Compared to the diet of pre-spill chicks at Naked Island, I documented a
lower abundance of sand lance and greater abundance of gadids in the diet of Jackpot Island chicks, which was similar to Hayes and Kuletz (1997) report on the diet of post-spill chicks at Naked Island. However, I found the abundance of herring in the diet of Jackpot Island chicks was significantly higher than the abundance of herring in the diet of both pre-spill and post-spill chicks at Naked Island (One-way ANOVA: F = 5.568, P = 0.024, df = 2, 10). In contrast to the Naked Island studies, the majority of surface schooling fish delivered to Jackpot Island chicks were herring rather than sand lance. Surface schooling fish represented 33 ± 16% of the Jackpot Island chick diet, which is intermediate between the proportion at Naked Island during the pre-spill period (48 ± 11%) and post-spill period (21 ± 7%) (One-way ANOVA: F = 4.704, P = 0.036, df = 2, 10). During the warm-water year of 1997, herring sharply declined in the diet of Jackpot Island chicks, while the Naked Island chicks experienced a modest gain in the abundance of surface schooling fish in their diet. With the exception of 1997, chicks at Jackpot Island had a greater proportion of schooling fish in their diet (40 ± 5%) than the chicks at post-spill Naked Island (T-test, t = 6.277, P < 0.001, df = 6).

During the warm-water year of 1997, adults delivered more non-schooling demersal fish to chicks at Jackpot Island compared to other years. Among the other years, the proportion of non-schooling demersal fish delivered to chicks at Jackpot Island (39 ± 8%) was similar to the proportion delivered to Naked Island chicks during the pre-spill years (38 ± 5%), but less than during the post-spill years (58 ± 9%, n = 3). Gadids occurred in similar frequency in the diet of chicks at Jackpot Island (15 ± 7%, n = 5) and post-spill Naked Island (16 ± 13%, n = 4). In both areas, the abundance of gadids
declined after 1994. However, the post-spill abundance of gadids in the chick diet remained greater than pre-spill diets at Naked Island (4 ± 4%). Other fish species comprised a minor proportion of diets of post-spill chicks: Jackpot Island (2 ± 2%) and Naked Island (5 ± 2%). The proportion of other fish species (10 ± 9%) was slightly higher for pre-spill chicks at Naked Island.

The lower abundance of high-quality fish in chick diets in oiled areas of PWS has the potential to constrain growth rates and survival of juvenile pigeon guillemots if delivery rates do not compensate for the lower energy content of chick meals. During periods of food shortages, brood reduction will offset the effect of low delivery rates. Single chicks received a significantly higher number of meals than individuals in two chick nests at Jackpot Island as well as in other studies (Prichard, 1997). I did not test the effect of brood size on delivery rates because information on brood size was not available for Naked Island delivery observations. The mean delivery rates I observed at Jackpot Island (0.86 ± 0.18 fish nest\(^{-1}\) hr\(^{-1}\), n = 4) and pre-spill Naked Island (0.90 ± 0.20 fish nest\(^{-1}\) hr\(^{-1}\)) were not significantly higher than the post-spill Naked Island (0.74 ± 0.13 fish nest\(^{-1}\) hr\(^{-1}\); One-way ANOVA; F = 1.256, P = 0.330, df = 2, 9).

The observation of lower abundance of high-quality fish at post-spill Naked Island without a significant change in rate of fish delivered to chicks to compensate for the lower quality suggests lower energy content in the diet of Naked Island chicks during the post-spill years. The lower quality of the chick diet found at Naked Island can not be interpreted as a population limitation unless it is associated with lower productivity or fledgling survival (Cairns, 1988).
**Productivity**

Food limitations at the egg laying, incubation and chick rearing periods occur at different temporal scales or at different levels of prey supply (Carins, 1988). Therefore I examined the three components of productivity individually for evidence of food limitation (Table 3.4). The mean clutch size at Jackpot Island over the five-year study period (1.82 ± 0.09 eggs nest\(^{-1}\)) is only slightly higher than the mean clutch sizes observed at Naked Island during the pre-spill period (1.69 ± 0.14 eggs nest\(^{-1}\)) and during the post-spill period (1.72 ± 0.07 eggs nest\(^{-1}\)). Because there are no significant differences in the clutch size between these studies (One-way ANOVA; F = 1.766, P = 0.216, df = 2, 11), egg production is not impeding post-spill population growth.

During this study, I documented high abandonment rates at the Jackpot Island colony in two out of five years. Kuletz (1983) reported unusually low hatching rates at
Table 3.4 Comparison of clutch size, hatching success, fledgling success and productivity among unoiled Jackpot Island, oiled Naked Island, and pre-spill Naked Island pigeon guillemot studies. Means are based on pigeon guillemot nests found during the egg stage.

<table>
<thead>
<tr>
<th>Period</th>
<th>Study Area</th>
<th>Clutch Size (eggs/nest)</th>
<th>Hatching Success (chicks/egg)</th>
<th>Fledgling success (fledglings/chick)</th>
<th>Productivity (fledglings/egg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-spill</td>
<td>Jackpot Island</td>
<td>1.82 ± 0.09</td>
<td>0.55 ± 0.18</td>
<td>0.48 ± 0.30 n = 5</td>
<td>0.27 ± 0.22 n = 5</td>
</tr>
<tr>
<td>(1994-1998)</td>
<td>(unoiled)</td>
<td></td>
<td></td>
<td>0.61 ± 0.14 n = 4</td>
<td>0.35 ± 0.21 n = 4</td>
</tr>
<tr>
<td></td>
<td>Naked Island</td>
<td>1.72 ± 0.07</td>
<td>0.62 ± 0.13</td>
<td>0.42 ± 0.17 n = 5</td>
<td>0.35 ± 0.15</td>
</tr>
<tr>
<td>(1994-1998)</td>
<td>(oiled)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-spill</td>
<td>Naked Island</td>
<td>1.69 ± 0.14</td>
<td>0.78 ± 0.07</td>
<td>0.77 ± 0.19d</td>
<td>0.47 ± 0.15</td>
</tr>
<tr>
<td>(1979-1981)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ANOVA

| All          | P = 0.216          | P = 0.056               | P = 0.041                     | P = 0.530                          |


*bSource: Oakley and Kuletz 1996, Golet et al. 2000

*cFour year mean excludes 1996 breeding season at Jackpot due to high mink predation.

*dBonferroni t-test pair-wise comparisons (P < 0.05)
Naked Island in one out of three years. However, during the post-spill years Naked Island colonies experienced little interannual variation in hatching rates. Therefore, mean hatching success at Jackpot Island (0.55 ± 0.18 chicks egg\(^{-1}\)) was lower than the mean hatching success observed at Naked Island during both the pre-spill years (0.62 ± 0.13 chicks egg\(^{-1}\)) and the post-spill years (0.78 ± 0.07 chicks egg\(^{-1}\); One-way ANOVA; \(F = 3.790, P = 0.056, df = 2, 11\)). Low hatching rates for guillemots has been associated with food shortages, presence of mammalian predators or frequent disturbance by humans (Ainley \textit{et al.}, 1990; Drent \textit{et al.}, 1964; Drent 1965; Hodder and Graybill, 1983; Emms and Morgan, 1987).

At Naked Island, Kuletz (1983) captured adults in their burrows during the 1980 nesting season and reported the high abandonment rate for birds she disturbed. At Jackpot Island, I avoided capturing incubating adults. The pattern of high nest abandonment in the last two years of the 5-year study suggests that factors besides our presence on the island prompted the birds to abandon their nests. There is indirect evidence that availability of prey during the incubation period may have declined in the last two years of this study. During the 1997 nesting season I observed substantial abandonment of nests coupled with a scarcity of herring among the fish delivered to chicks. The scarcity of juvenile herring in 1997 was associated with higher than average sea surface temperatures in the Gulf of Alaska that lasted from May 1997 to March 1998 (calculated from records of the National Data Buoy Center, NOAA). However, birds nesting at the Naked Island colonies did not experience similar food limitations. Sand lance in the Naked Island areas responded to warm waters by forming surface schools
earlier in 1997 than observed in the two previous years (Brown, 1997).

In contrast, the abandonment I observed in 1998 occurred when herring were abundant in the chick diet. However, precipitation was twice as high in June of 1998 compared to the four other years of the study, and the second highest recorded in 16 years at Main Bay weather station (WRCC, 1999). High rainfall in June of 1998 may have represented poor foraging conditions for the birds at Jackpot Island. Kuletz (1983) documented that the rate adults provisioned chicks declined during periods of poor weather.

Thus, the abandonment I observed in both 1997 and 1998 may have been caused by an overall scarcity of food or poor foraging conditions. This interpretation is consistent with observations at the Farallon Islands by Ainley et al. (1990), who noted that low hatching success of pigeon guillemots was associated with warm-water years and low abundance of primary prey species, rockfish, *Sebastes* spp., and that high hatching success was associated with cold water years that resulted in exceptional food availability.

Evaluating the role of food in the lower post-spill nestling survival at Naked Island is confounded by reports of increased nest predation (Oakley and Kuletz 1996). Mink are a major nest predator in PWS. The failure of the 1996 breeding season at Jackpot Island was caused by mink predation. The colonies at Naked Island suffered losses to mink predation on an annual basis and poor nesting success in 1998 was attributed to mink predation. Of the three pair-wise comparisons of nestling survival, only the comparison between pre-spill Naked Island and post-spill Naked Island was
significantly different (One-way ANOVA; $F = 4.471$, $P = 0.041$, df = 2, 10; Bonferroni t-test, $t = 2.978$, $P = 0.042$).

Productivity was similar between Jackpot Island ($0.35 \pm 0.21$ fledgling egg$^{-1}$, $n = 4$ years) and post-spill Naked Island ($0.35 \pm 0.15$ fledgling egg$^{-1}$) because the lower hatch rates at Jackpot Island were balanced by lower survival of nestlings at Naked Island. The productivity of pre-spill Naked Island birds ($0.47 \pm 0.15$ fledgling egg$^{-1}$) was not significantly greater than that of post-spill Naked Island birds (One-way ANOVA; $F = 0.678$, $P = 0.530$, df = 2, 10). Because of the small range observed between pre-spill and post-spill productivity, I suggest that other demographic factors, such as juvenile survival to breeding age or adult survival rates, are responsible for the post-spill populations trends at Naked Island.

**Growth Rates, and Fledging Weight**

In comparison to the measurement of fledglings per egg, growth rates and fledgling weights (Table 3.5) may be better measurements of overall reproductive performance because of their influence on post-fledgling survival (Greenwood et al., 1993). From 1994 to 1998, the mean linear growth rate of chicks at Jackpot Island ($18.6 \pm 1.1$ g$^{-1}$ day$^{-1}$) was not significantly different from the pre-spill growth rate at Naked Island ($20.4 \pm 2.3$ g$^{-1}$ day$^{-1}$), or the post-spill growth rate at Naked Island ($18.2 \pm 2.6$ g$^{-1}$ day$^{-1}$) (One-way ANOVA; $F = 1.123$, $P = 0.363$, df = 2, 10). In contrast, the mean fledging weight of chicks at Jackpot Island ($482 \pm 18$ g chick$^{-1}$) was similar to the pre-spill fledging weight at Naked Island ($480 \pm 40$ g;) and significantly greater than the post-
Table 3.5 Comparison of growth rates and fledging weights among unoiled Jackpot Island, oiled Naked Island, and pre-spill Naked Island pigeon guillemot studies.

<table>
<thead>
<tr>
<th>Period (years)</th>
<th>Study Area</th>
<th>Growth Rate (grams/day)</th>
<th>Fledging Weight (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-spill 1994-1998</td>
<td>Jackpot Island (unoiled)</td>
<td>18.6 ± 1.1</td>
<td>482 ± 18</td>
</tr>
<tr>
<td>Post-spill 1994-1998</td>
<td>Naked Island(^a) (oiled)</td>
<td>18.2 ± 2.6</td>
<td>446 ± 14(^c)</td>
</tr>
<tr>
<td>Pre-spill 1979-1981</td>
<td>Naked Island(^b)</td>
<td>20.4 ± 2.3</td>
<td>480 ± 40</td>
</tr>
<tr>
<td>ANOVA</td>
<td>All</td>
<td>P = 0.363</td>
<td>P = 0.001</td>
</tr>
</tbody>
</table>

\(^b\)Source: Oakley and Kulet (1996) and Golet et al. (2000).  
\(^c\)Bonferroni t-test pair-wise comparisons (P < 0.05)

spill fledging weight at Naked Island (446 ± 14 g) (One-way ANOVA; F = 9.788, P < 0.001, df = 2, 10). Post spill chicks at Naked Island fledged with similar wing length but at lighter weights than Jackpot Island and pre-spill chicks.

Guillemot chicks that are fed predominantly sand lance (>50%) have higher growth rates (Prichard, 1997, Golet et al., 2000) and peak fledging weights (Golet et al., 2000) than chicks that are fed predominantly gadids or non-schooling demersal fish. Similar results have been noted for rhinoceros auklets, Cerorhinca monocerata, (Bertram and Kaiser, 1993; Wilson and Manuswal, 1986) and Atlantic puffins, Fratercula arctica, (Harris and Hislop, 1978). Romano et al. (1999) reported that tufted puffins, Fratercula cirrhata, raised on schooling fish diets had greater fat reserves than birds raised on
walleye pollock diets. In field studies at the Farallon Islands, Shultz and Sydeman (1997) reported that low fledging weights were associated with years of low food abundance. During the post-spill period, the combination of similar linear growth rates at Jackpot Island and Naked Island with the greater fledging weight at Jackpot Island suggests that food constraints were primarily realized late in the chick-rearing period and most likely associated with development of fat reserves.

Food limitations for fledglings are different than those of nestlings because fledglings do not receive food from their parents and fledglings are less experienced at capturing prey than adults. Fledgling survival may be increased through greater energy reserves and advanced development (Thompson and Flux, 1988). Fledging weight has been positively related to the survival of juveniles in many species (Manx shearwaters, *Puffinus puffinus*, Perrins *et al.*, 1973; South African gannet, *Sula capensis*, Jarvis, 1974; blue tit, *Parus caeruleus*, Nur, 1984; black-legged kittiwake, *Rissa tridactyla*, Coulsen and Porter, 1985; blackbird, *Turdus merula*, Magrath 1991; but see Harris and Rothery, 1985 on Atlantic puffins, *Fratercula arctica*.) For the 1994 and 1995 year-classes of guillemots, I found no significant difference in fledging weights between birds I observed in later years and birds I assumed dead. However, these fledglings were produced during a summer of high food abundance as indicated by delivery rates and abundance of schooling fish in the chick diet. Harris and Rothery (1984) suggested fledging weight is not critical to survival of puffins when post-fledging food resources are abundant. Although fledging weight has been suggested as an index of fledgling survival, this has not yet demonstrated for pigeon guillemots.
3.5.2 Demographic Limitations to Recovery

During my study, I observed a 36% increase in the number of adults attending the Jackpot Island colony, but annual population growth was not consistent among years. The 1998 increases in populations observed for several other southwestern and central PWS colonies suggest that conditions favorable for breeding success and fledgling survival existed throughout PWS in the mid-1990's. This pattern of colony growth may represent recruitment of a strong year-class after several years of lower recruitment.

Demographic factors that could contribute to lack of recovery at Naked Island compared to Jackpot Island, include lower production, higher net emigration, higher post-fledging mortality, or a combination of these factors. Because the production at Naked Island and Jackpot Island are similar, production does not appear to be the factor responsible for the lack of recovery at Naked Island, which is in agreement with the observations of Hayes and Kuletz (1997). With respect to emigration, we have one documented case of a chick banded at Naked Island that subsequently nested at Jackpot Island in 1997 and 1998. Although this observation suggests the potential for emigration, we do not know the relative difference in emigration rates of pigeon guillemots between Jackpot Island and Naked Island. The higher fledging weights I observed at Jackpot Island in comparison to Naked Island suggest that recovery at Naked Island may be constrained through reduced fledging survival. It is also possible that recovery at Naked Island may be constrained through reduced adult survival.

Factors that contribute to the mortality of adult seabirds include predators, entanglement in fishing nets, food shortages, the long-term effects of oil exposure and
disease. I observed mortality of nesting pigeon guillemot caused by mink predation, similar to that reported by others working in guillemot colonies (Petersen, 1981; Folkestad, 1982; Barrett and Vader, 1984,), however we do not know if adult predation is higher at Naked Island than colonies in unoiled areas. Similarly, we have no reason to believe that there are differences between Naked Island and unoiled populations in adult mortality caused by gillnet fisheries; gillnets are known to be a significant source of mortality for seabirds (DeGange et al., 1993; Carter and Sealy, 1982; Takekawa et al., 1990). The solitary foraging habits and moderate diving depth of guillemots may make them less susceptible to gillnet losses unless the nets are in the vicinity of colonies (Evans and Nettleship, 1985). Gillnets are used in PWS herring and salmon fisheries. During this study the herring fisheries was closed for three years. Wynne (1990, 1991) reported no mortality of pigeon guillemots associated with the PWS Copper River salmon gillnet fisheries.

Naked Island could also be experiencing higher adult mortality because of the long-term effects of oil exposure. In chapter two I presented preliminary evidence that adults from oiled areas have elevated aspartate aminotransferase concentrations, which is consistent with hepatocellular injury. Confirmation of hepatocellular injury requires histological examination of liver tissue. Additional studies to fully evaluate the health of adults residing in oiled areas would help to evaluate the issue of whether adult mortality caused by the long-term effects of oil exposure plays a role in the lack of recovery at Naked Island.

Several studies have found declines in seabird populations associated with
declining abundance of herring or sand lance (Atlantic puffins: Lid, 1981; Harris and Wanless, 1991; black-legged kittiwakes: Heubeck and Mellor, 1994). The higher fledging weights I observed at Jackpot Island compared to Naked Island, suggest that food limitation may be expressed in the later part of the breeding season. If late-season food shortages affect body condition of adults, then these shortages may affect survival. I recommend that adult survival and late-season body condition of adults be monitored for breeding birds at Naked Island and Jackpot Island to determine whether late-season food shortages have the potential to cause higher adult mortality.

For an injured population to return to their initial levels after a major mortality event, such as an oil spill, environmental conditions must favor breeding success, survival of juveniles to breeding age and survival of breeding adults. My analysis indicates that lack of recovery of pigeon guillemot populations in oiled areas of PWS is likely associated with lower quality prey, which results in lower fledging weight, and which may constrain recovery through reduced fledgling survival. Food shortages and the long-term effects of oil exposure may also constrain recovery if they result in lower adult survival.

**CONCLUSION**

1. From 1994 to 1998, I observed a positive trend in the Jackpot Island pigeon guillemot population. For four consecutive years, the number of birds at Jackpot Island met or exceeded the previous year's counts.
2. Jackpot Island experienced high rates of nest abandonment in 1997 and 1998. High sea-surface temperatures in June of 1997 and high rainfall in June of 1998 may have caused poor foraging conditions during the incubation period. These factors may have contributed to the high abandonment rates observed during those two years.

3. Productivity losses to mink predation occurred only in one out of five breeding seasons at Jackpot Island. The presence of mink on the island in 1996 resulted in higher morality rates for both adults and nestlings. The relative isolation of Jackpot Island from mink predation may explain the difference in nesting density between Jackpot Island and the shoreline of the mainland.

4. The proportion of high-lipid fish in the diet of Jackpot Island chicks was higher than the post-spill Naked Island, but lower than pre-spill Naked Island. The abundance of herring was significantly higher in the diet of Jackpot Island chicks compared to pre- and post-spill Naked Island chicks. Delivery rates were not significantly different among the three studies. Therefore, the quality of chick diet was higher at Jackpot Island than post-spill Naked Island, but not pre-spill Naked Island.

5. Mean fledgling success, productivity rates and growth rates at Jackpot Island and post-spill Naked Island were similar. However, fledging weights were significantly higher at Jackpot Island. This observation suggests that food limitation at Naked Island was experienced in the later stages of the nesting period. Lower fledging weights at Naked Island may lead to a lower post-fledgling survival rate.
6. Population trends at unoiled Jackpot Island and oiled Naked Island did not exhibit similar temporal patterns. Because mean productivity levels did not differ between the two areas, the disparity in fledging weight may partially account for these varying population trends. It is unknown if food limitation at Naked Island extends to breeding adults. I recommend comparative studies on adult survival rates and late summer body condition.
Pigeon guillemots and their foraging areas were impacted by the 1989 Exxon Valdez oil Spill (EVOS) (Piatt et al., 1990; Spies et al., 1996). In the years immediately following EVOS, no significant increase in pigeon guillemot abundance were reported at several spatial scales within Prince William Sound (Oakely and Kuletz, 1993; Murphy et al., 1995; Agler and Kendall, 1996). The pigeon guillemot population had not recovered according to the conventional measurement of recovery, the numeric replacement of individuals directly killed by oiling. This definition assumes that current environmental conditions support recruitment. Oakley and Kuletz (1996) pointed out that survey estimates prior to the spill (1972, 1984-85) indicated that the abundance of pigeon guillemots in PWS were declining. Present oceanic conditions may not support population growth. Therefore, comparing population trends in oiled areas to adjacent unoiled areas might be a more suitable indicator of recovery than comparing post-spill populations to pre-spill abundance levels. My observations at unoiled Jackpot Island and several other colonies in southwestern PWS indicate that it was possible for pigeon guillemot populations to substantially expand in the post-spill period, between 1994 and 1998. However, during the same period, the abundance of pigeon guillemots at oiled Naked Island dropped below the population level measured in 1994. Several factors may
constrain population growth in oiled areas: the physiological effects of oil exposure on guillemots, food limitations, predation, and other factors, such as disease. I assessed the role of oil exposure and food limitations in the recovery of pigeon guillemot populations affected by the EVOS.

Before this study, information on the breeding success and diet of pigeon guillemots in PWS was only available for Naked Island colonies in central PWS. In 1979, ten years prior to the spill, Naked Island supported 1,200 pigeon guillemots (Oakely and Kuletz, 1996). Since the spill, the annual counts of pigeon guillemots along Naked Island shorelines have oscillated between 400 to 700 birds (Hayes and Kuletz, 1997). The pre-spill (1979 to 1981) and post-spill studies (1989 to 1991, 1994 to 1998) of Naked Island pigeon guillemots represent 12 years of data on breeding success and diet. Because of the time laps between studies and oiling of Naked Island, researchers did not have the information necessary to determine whether the lack of population growth in the nineties would have occurred in the absence of EVOS or not (Oakley and Kuletz, 1996; Hayes and Kuletz, 1997; Golet et al., 2000).

To provide insight on mechanisms behind the population trend in oiled areas, I collected information on the health, food habits and population dynamics of pigeon guillemots at Jackpot Island. This unoiled reference site is located in southwestern PWS. To make comparisons between an oiled area and a reference site, the reference site must meet three criteria: (1) the foraging habitats of the two areas must be similar except for oiling; (2) the movement of birds between the reference site and the oiled site must be
limited, and (3) the reference site must have an adequate number of accessible nests for logistical and statistical purposes.

Jackpot Island was selected as a reference site because it offered a large concentration of nests located at a fair distance (55 km) from oiled Naked Island. Selecting reference sites with similar oceanographic conditions to oiled study sites is difficult because oil was not randomly distributed in PWS, nor was the degree of oiling consistent along shorelines. Wind and current patterns responsible for the distribution of oiling along PWS shorelines may also be correlated with other less obvious habitat variables (Laur and Haldorson, 1996). I found that birds in the two areas feed on similar prey species, but other measurements of foraging habitat varied. The shoreline density of nests in the greater Jackpot area was low, compared to Naked Island's. Breeding densities may be related to the quality of the forage area, availability of nesting sites, or predator densities. The lower shoreline density of pigeon guillemots in unoiled areas is an unavoidable weakness in my study. Despite these limitations for a direct comparison to Naked Island, the diet of Jackpot Island birds typifies the oil exposure levels and foraging habits of breeding birds in the unoiled area of Prince William Sound.

To evaluate the health of birds in 1997, I compared the hematological and plasma biochemical profiles among populations of pigeon guillemots in oiled and unoiled areas. If the effect of chronic exposure to residual oil is significant enough to limit the recovery of pigeon guillemots in PWS, then I expected the blood parameters to differ between populations in oiled and unoiled areas in a pattern that would be consistent with toxic responses. I examined chicks and adults separately, because adults have greater
opportunities for exposure to residual oil than nestlings residing in burrows. With the 30-day old chick, I found calcium and mean cell volume were significantly different between populations in oiled and unoiled areas. However, these blood biomarkers provided little evidence of continuing oil injury to chicks. Preliminary data from adults indicated elevated aspartate aminotransferase activity (AST) for adults in the oiled area, which is consistent with hepatocellular injury. These findings indicate that exposure to residual oil elicited a physiological response in pigeon guillemots. The consumption of invertebrates by adults (Oakely, 1981; Ewins, 1993) may contribute to difference in biomarker responses between adults and chicks. Bioaccumulation of polynuclear aromatic hydrocarbons is greater in invertebrates than fish (Gibson, 1977). The energetic costs of this physiological response and its influence on adult survival and productivity are unknown. I recommend studies that fully evaluate health and survival rates of adults residing in oiled areas.

Alcid productivity is affected by availability and abundance of food in the vicinity of their nests (Evans and Nettleship, 1985). To evaluate food resources and limitations during the breeding season, I examined the diet, survival, growth performance and fledging weight of chicks. Hayes and Kuletz (1997) suggest that the availability of high-lipid fish was limiting the growth of the population at Naked Island. They reported a decline in the abundance of high-lipid fish in the diet of chicks between the pre-spill (1979-81) and post-spill (1989-90, 1994-96) studies. In PWS, distribution of pigeon guillemot colonies in non-glaciated waters, overlap with the summer distribution of juvenile herring and sand lance documented by Brown (1997, 1998). Thus populations in
both oiled and unoiled areas of PWS would be influenced with changes in abundance of high-lipid fish. Little is know about the abundance of fish in areas with tidewater glaciers, because silt and ice prevent aerial and sonar detection of surface schooling fish. I expanded on the pre-and post-spill comparisons of Hayes and Kuletz (1997) to include post-spill data for an unoiled area. I also truncated the post-spill Naked Island data set to the 5-year period corresponding to the data from the unoiled area.

I evaluated the quality of chick diet based on the proportion of the meal that were high-lipid fish and the frequency at which meals were delivered to the chicks. I found that the proportion of high-lipid fish in the diet of Jackpot Island chicks was higher than post-spill Naked Island chicks, but not pre-spill Naked Island chicks. Yet, delivery rates were not significantly different among the three studies. Similar to the observations by Hayes and Kuletz (1997), I concluded that the quality of post-spill diet was lower than the pre-spill diet. However, the quality of the post-spill diet was higher for chicks at Jackpot Island than for the chicks at Naked Island. The abundance of herring at Jackpot contributed to post-spill regional differences in diet quality. Although food limitation was not expressed in the linear growth rates of chicks, the difference in diet quality between Jackpot Island and Naked Island was expressed later in the chick rearing period and translated into lower fledging weights for Naked Island chicks. Fledging weight has been suggested as an index of fledgling survival (Perrins et al., 1993; Jarvis 1974). Consequently, the lower fledging weights at Naked Island suggest that recovery at Naked Island may be constrained through reduced juvenile survival.
Populations increase only by recruitment of natal juveniles and immigration. I noted that mean productivity rates over the five-year period were not significantly different between Jackpot and Naked Island, but lower fledging weights at Naked Island may lead to regional differences in recruitment rates. Martin (1987) suggested that productivity be defined by both the number of fledglings that survive to breed and by the negative effects of the breeding effort on the parent. Tinbergen et al. (1984) reported that the cost of reduced survival for adults was expressed in years when winter food abundance was low. Guillemot species experience seasonal shifts in their diet from a fish-dominated diet in the summer to a mixed diet of fish and invertebrates in the winter (Vermeer et al., 1987). I recommend that winter food limitations be examined for pigeon guillemots in oiled and unoiled areas.

Cairnes and Elliot (1987) theorize that the rate of population recovery from a large mortality event such as an oil spill depends on the size and location of neighboring colonies. The spatial distribution of breeding pigeon guillemots in PWS does not present a high immigration potential for Naked Island. According to the colony surveys of Sanger and Cody (1994), the density of pigeon guillemots in the area surrounding Naked Island and its associated islands in central PWS, is much lower than the Naked Island complex. The observation by Murphy et al. (1997) that the abundance of pigeon guillemots occupying oiled shorelines did not increase the first two years after the spill supports the notion that immigration potential in PWS is low. The failure of the Naked Island population to maintain levels above the 1990 census level (723 birds) suggests that
recruitment as well as immigration are insufficient to compensate for adult morality and emigration losses.

To maintain a stable population, breeding pairs must produce at least two fledglings over the course of their lifetime. Based on present production (0.6 fledgling per nest) and fledgling survival (36%) rates at Jackpot Island, a breeding pair would have nest 9 years to insure two of their fledglings survived to breeding age, an annual survival rate of 90%. However, lower adult survival rates lower reported in the literature for pigeon guillemot (80%; Nelson, 1981) and black guillemot (85%; Asbirk 1979; 89%; Frederiksen, 1998). Lower abundance of high-lipid fish in the oiled area and the long-term effects of oil exposure are potential mechanisms to create regional differences in adult survival rates. Again, I recommend future research on adult survival rates.

Herring was the major contributing factor to the high-lipid diet at Jackpot Island. The herring population in PWS peaked in 1989 and crashed in 1993, just prior to this study. In 1989 herring composed 25% of the diet of Naked Island chicks (Oakely and Kuletz, 1996). After 1993, only 5% fish in the Naked Island chick diet was herring (G. Golet personal communication). In contrast, a third of fish delivered to chicks at Jackpot Island were herring. The local abundance of juvenile herring is attributed to the size of local spawning biomass as well as favorable spring wind and current patterns for retention of planktonic larva (Stokesbury et al., 1997). Few herring spawn in Southwest PWS and the source of Jackpot Bay's juvenile herring population has not been traced (Stokesbury et al., 1997). Naked Island has both an intermittent spawning population (Funk, 1995) and a favorable location for receiving larva from other spawning areas (B.
Norcross, personal communication). Differences in the abundance of herring may be attributed to physical differences in nearshore habitats, natural cycles in herring populations, or to the lingering effects of EVOS.

The difference in abundance of herring and sand lance at Jackpot Island and Naked Island was also noted in the diet of marble murrelets (Kuletz and Kendall, 1999), and in Brown's (1997, 1998) aerial surveys of surface schooling fish. Little is known about historic population trends in the abundance of sand lance because lack of commercial interest in the species. The abundance of high-lipid fish in the diet of oiled Naked Island chicks continued to remain low from 1990 to 1998. Declines in high-lipid fish abundance between the pre-spill and post-spill studies may be related to declines in primary productivity (Bertram et. al., 1991), diseases and oiling (Hose et al., 1996, Carls et al., In press).

If the effect of EVOS on the pigeon guillemot populations was limited to the 1989 mortality event, then I would expect by 1994, that the population dynamics in oiled and unoiled areas would follow similar trends. In fact population trends differed between oiled Naked Island and unoiled Jackpot Island, which suggests regional differences in one or more demographic parameters. Productivity was similar between the two areas but indirect measurements suggest potential for lower juvenile and adult survival rates for populations residing in oiled areas. Blood biomarkers provided evidence that residual oil in the nearshore environment is a potential health risk to adult birds. But, I lack information to determine at what scale oil toxicity currently inhibits adult survival or productivity. I presented evidence that food limitations at Naked Island may lead to
lower fledgling survival. The recovery of pigeon guillemots from EVOS depends on the status of food resources in the nearshore habitat. The low abundance of high-lipid fish during the breeding season was one mechanism limiting population expansion in oiled areas. Current environmental conditions in the oiled areas of PWS do not appear to support growth and recovery of pigeon guillemot populations.
LITERATURE CITED


