*Exxon Valdez* Oil Spill
Restoration Project Final Report

# Pigeon Guillemot Restoration Research at the Alaska SeaLife Center

Restoration Project 01327-1

Final Report

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**Study History:** Restoration Project 01327-1 is similar to the research described in the original proposal submitted as 98327. The objectives were to (1) determine the feasibility of using direct techniques for restoration of Pigeon Guillemots (*Cepphus columba*), including providing artificial nest sites, use of social attraction (such as decoys and playbacks of vocalizations), and release of captive-reared young; (2) determine the effects of dietary variables on growth, development, and fledging condition of Pigeon Guillemots, including type of forage fish consumed, quantity of fish consumed, dietary lipid content, and energy intake rates; and (3) determine the dose-response of particular biomarkers of crude oil exposure (e.g., plasma chemistry, acute phase proteins, corticosterone, growth rates) to variables of exposure in nestling guillemots.

This research project is an outgrowth of the Alaska Predator Ecosystem Experiment (APEX) Project and the Nearshore Vertebrate Predators (NVP) Project. Both of these large ecosystem projects investigated potential factors limiting recovery of Pigeon Guillemot populations from the direct mortality suffered in the aftermath of the *Exxon Valdez* oil spill. Pigeon Guillemots are notable among seabird species injured by the spill in that there has been no detectable recovery in the 13 years since the spill. The present research project was conducted at the Alaska SeaLife Center under controlled laboratory conditions. This allowed us to examine the effects of diet composition and ingestion of weathered Prudhoe Bay crude oil on the growth, development, and survival of young Pigeon Guillemots that were hatched from eggs collected in the wild and raised in captivity.

During the 1998, 1999, and 2000 field seasons a total of 206 guillemot eggs and 37 guillemot hatchlings were collected from nests in the wild and transported to the SeaLife Center for incubation and/or captive rearing. A total of 144 eggs were hatched (70% hatching success) and a total of 156 chicks (86%) were successfully raised in captivity and fledged from the SeaLife Center. Chicks were raised on one of eight rations consisting of three types of forage fishes: juvenile Pacific herring, juvenile walleye pollock, or mixed blennies. Chicks were also assigned to either high-dose, low-dose, or control treatments for ingestion of weathered Prudhoe Bay crude oil. Growth rates, plasma chemistry markers, biomarkers of oil exposure, and survival were monitored for all chicks as indicators of diet quality and crude oil exposure.

The original proposal (98327) included three primary objectives. Objective 1, involving various direct restoration techniques for Pigeon Guillemots, will be addressed in a separate final report (01327-2) prepared by Dr. George Divoky, Research Associate at the Institute of Arctic Biology, University of Alaska Fairbanks. Dr. Divoky was the Co-principal Investigator on the original proposal. The Final Report presented here addresses Objectives 2 and 3 of the original proposal, and is prepared by the Principal Investigator

on the original proposal, Dr. Daniel Roby, and his Graduate Research Assistant, Dr. Andrew Hovey, both with the USGS-Oregon Cooperative Fish and Wildlife Research Unit at Oregon State University. Because Objective 1 included releasing captive-reared guillemots into the wild as a potential direct restoration technique, destructive sampling of captive-reared guillemots (e.g., for determination of total body composition or histological effects of oil ingestion) was precluded.

**Abstract:** The Pigeon Guillemot (*Cepphus columba*) population in Prince William Sound has failed to recover from declines that occurred both before and after the *Exxon Valdez* Oil Spill (EVOS). Post-spill studies of Pigeon Guillemot breeding biology have identified three potential factors limiting recovery: (1) predation on eggs and nestlings, (2) declines in the proportion of high-lipid, schooling forage fish (sand lance, herring, and capelin) in the diet, and (3) continued exposure to residual oil from the spill. This laboratory study with captive-reared Pigeon Guillemots at the Alaska SeaLife Center investigated two aspects of the species' biology that are relevant to restoration in the aftermath of EVOS. First, we investigated the role of dietary factors (prey type, quantity of food consumed, dietary fat content, and energy intake rate) in limiting the growth, development, survival, and fledging condition of nestling Pigeon Guillemots. The objective was to understand how changes in prey availability and prey quality might affect Pigeon Guillemot productivity. Second, we fed nestlings sublethal doses of weathered Prudhoe Bay Crude Oil (PBCO) and then measured several potential biomarkers of effects from this pollutant. These dose-response experiments were designed to (1) better understand the impact on nestling guillemots of petroleum hydrocarbons in food, (2) calibrate existing and potential biomarkers of exposure to PBCO in Pigeon Guillemots in a controlled, laboratory setting, and (3) develop better nondestructive biomarkers of exposure to PBCO in Pigeon Guillemots in particular, and seabirds in general.

Results of feeding experiments indicated that most variation in nestling growth rates could be explained by differences in daily energy intake. The type of forage fish consumed, the lipid or protein content of the forage fish, and even the quantity of food consumed daily did not have as strong an effect on nestling guillemot growth as did daily energy intake. The metabolic efficiency and growth performance of nestling guillemots was not enhanced on high-lipid diets, contrary to results with nestlings of some other seabird species. Instead, structural growth (wing length) in nestling guillemots was somewhat stunted on high-lipid diets. These attributes of guillemot nutritional requirements are associated with the guillemots' nearshore foraging niche and high food provisioning rates to nestlings. The average lipid content of sand lance (*Ammodytes hexapterus*), juvenile herring (*Clupea pallasi*), and capelin (*Mallotus villosus*) may represent the optimal dietary lipid content for nestling Pigeon Guillemots. This study supports the hypothesis that guillemot productivity is limited by the availability of these forage fishes through effects on energy provisioning rates to nestling guillemots. Consequently, recovery of Pigeon Guillemot populations injured by the *Exxon Valdez* oil spill is likely linked to recovery of these key forage fish stocks.

Results of the oil-dosing experiments indicated that nestling guillemots are surprisingly resistant to weathered PBCO in their food. No nestlings died or suffered noticeable health effects following dosing. The high dose in this study (0.5 ml $kg^{-1}$ $day^{-1}$) was sufficient to induce hepatic cytochrome P450A1 (a liver enzyme indicative of contaminant exposure), but growth rate, fledging mass, and plasma chemistry were largely unaffected. None of the 12 plasma or hematological markers examined responded in a dose-dependent manner to ingestion of weathered PBCO, except lactate dehydrogenase (LDH). Although baseline stress hormone (corticosterone) levels were not different between oil-dosed and control nestlings, a standardized acute stress protocol revealed that corticosterone was more elevated during stress for oiled nestlings compared to controls. Although we were not successful in identifying a noninvasive biomarker (e.g., growth) or a plasma biomarker (e.g., haptoglobin) of crude oil exposure in nestlings, we were able to confirm that levels of hepatic cytochrome P450A1 and corticosterone during stress were elevated by the sublethal doses administered during our experiments. Based on this and other studies, it is unlikely that the failure of Pigeon Guillemots to recover from the *Exxon Valdez* oil spill is due to effects on nestling health of residual oil in food.

**Key Words:** energetics, growth, *Exxon Valdez* oil spill, forage fish, lipid, diet composition, seabird, Pigeon Guillemot, oil pollution, biomarkers, cytochrome P-450.

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TABLE OF CONTENTS

Page

INTRODUCTION……1

EFFECTS OF DIET ON GROWTH AND DEVELOPMENT OF PIGEON GUILLEMOT NESTLINGS: CAPTIVE FEEDING EXPERIMENTS……...5

ABSTRACT…….5

INTRODUCTION……...5

METHODS……..….7

RESULTS……11

DISCUSSION……..16

CONCLUSIONS…….19

EFFECTS OF INGESTED PRUDHOE BAY CRUDE OIL ON BLOOD CHEMISTRY AND GROWTH OF NESTLING PIGEON GUILLEMOTS: DOSE–RESPONSE EXPERIMENTS IN THE LAB……..….21

ABSTRACT……21

INTRODUCTION……...21

METHODS……..….23

RESULTS……26

DISCUSSION……..29

CONCLUSIONS…….34

CONCLUSIONS AND SYNAPSIS…….36

ACKNOWLEDGMENTS……38

BIBLIOGRAPHY…….39

LIST OF TABLES


| Table | | Page |
|---|---|---|
| 2.1 | Numbers of pigeon guillemot eggs and hatchlings collected, hatching success, and fledging success by collection location during the years 1998-2000……..…….49 | |
| 2.2 | Experimental diets fed nestling pigeon guillemots raised in captivity at the Alaska SeaLife Center. ……..……..51 | |
| 2.3 | Proximate composition and energy density (kJ/g wet mass) of diet fishes fed to nestling pigeon guillemots in captivity (means ± standard errors). ……..……..52 | |
| 3.1 | Dosing and blood sampling regimes for captive-reared nestling pigeon guillemots fed weathered Prudhoe Bay crude oil……..…….53 | |
| 3.2a | Plasma chemistry values for pigeon guillemot chicks during a high-frequency dosing regime (daily from day 16 to day 25 post-hatch) with weathered Prudhoe Bay Crude Oil. …… 54 | |
| 3.2b | Plasma chemistry values for pigeon guillemot chicks during a low-frequency dosing regime (once on day 20 and again on day 25 post-hatch) with weathered Prudhoe Bay Crude Oil…… 56 | |
| 3.3 | Plasma chemistry markers in control pigeon guillemot chicks (not dosed with oil) comparing values among ages and between chicks on the low-intake (126 g/day) and high-intake (158 g/day) diets……58 | |

LIST OF FIGURES

Figure Page

2.1 Total daily energy intake for pigeon guillemot chicks on each experimental diet and proportion of energy in protein and lipid……60

2.2 Body mass of pigeon guillemots as a function of age for chicks fed three different diets……...…...61

2.3 Growth in body mass of pigeon guillemot chicks from day 15 to day 30 post-hatch as a function of prey type.…….……62

2.4 Growth in body mass of pigeon guillemot chicks from day 15 to day 30 post-hatch as a function of daily food biomass intake……....63

2.5 Scatterplot (jittered) of growth in body mass of pigeon guillemot chicks from day 15 to day 30 post-hatch as a function of daily energy intake…….……...64

2.6 Regression analysis of growth increment in body mass of pigeon guillemot chicks from day 15 to day 30 post-hatch as a function of daily energy intake…….…..65

2.7 Wing length of pigeon guillemots as a function of age for chicks fed three different diets……..……66

2.8 Scatterplot (jittered) of structural growth (growth increment in wing length from day 15 to day 30 post-hatch) in pigeon guillemot chicks as a function of daily energy intake…….……....67

2.9 Regression analysis of growth increment in wing length of pigeon guillemot chicks from day 15 to day 30 post-hatch as a function of daily energy intake……...68

2.10 Structural growth (growth increment in wing length from day 15 to day 30 post-hatch) for pigeon guillemot chicks as a function of daily intake of lipid energy……..….69

2.11 Metabolizable energy coefficients (MECs) of pigeon guillemot chicks fed three different diets..…….....70

LIST OF FIGURES (Continued)


Figure Page

3.1 Activity of hepatic cP450 from pigeon guillemot chicks dosed for 10 days with 0.50 ml $kg^{-1}$ $day^{-1}$ of weathered Prudhoe Bay crude oil (PBCO), compared with undosed controls……..71

3.2 Plasma lactate dehydrogenase (LDH) levels of pigeon guillemot chicks dosed with weathered Prudhoe Bay crude oil at high-frequency (once per day from days 16 to 25 days post-hatch) and at different dose levels……72

3.3 Plasma albumin levels (A) and creatine phosphokinase levels (B) of pigeon guillemot chicks dosed with weathered Prudhoe Bay crude oil at high-frequency (once per day from day 16 to day 25 post-hatch) and on low-intake diets..…… ……..…….….73

3.4 Haptoglobin levels of pigeon guillemot chicks dosed with weathered Prudhoe Bay crude oil at different dose levels and either high-frequency (once per day from day 16 to day 25 post-hatch) [A] or low-frequency (one dose at day 20 and another at day 25) [B]....……..….74

3.5 Body mass growth curves of pigeon guillemot chicks fed different doses of weathered Prudhoe Bay crude oil…….……..…75

3.6 Growth in wing length of pigeon guillemot chicks fed different doses of weathered Prudhoe Bay crude oil……..……..……76

3.7 Body mass increment for pigeon guillemot chicks as a function of dosing with weathered Prudhoe Bay crude oil over the time course of oil dosing (days 16-26 post-hatch)…… …….77

3.8 Wing length increment for pigeon guillemot chicks as a function of dosing with weathered Prudhoe Bay crude oil over the time course of oil dosing (days 16-26 post-hatch) …….78

3.9 Corticosterone levels of pigeon guillemot chicks dosed with weathered Prudhoe Bay crude oil. ……..……..……...79

Effects of Diet and Crude Oil Ingestion on Growth and Biochemistry of Captive-reared Pigeon Guillemots (*Cepphus columba*)

# CHAPTER 1: INTRODUCTION

The pigeon guillemot (*Cepphus columba*) population in Prince William Sound, Alaska, has failed to recover from declines that occurred both before and after the *Exxon Valdez* Oil Spill (EVOS) in 1989. Post-spill studies of pigeon guillemot reproductive success have identified three primary factors preventing recovery:

1. In Prince William Sound (Naked and Jackpot islands) and Kachemak Bay, predation on eggs and chicks has been a major source of nesting failure (Oakley and Kuletz 1996, Hayes and Kuletz 1997, Prichard 1997, G. H. Golet personal communication).

2. There has been a decline in the proportion of sand lance (*Ammodytes hexapterus*) in the diet at some guillemot colonies in Prince William Sound (e.g., Naked Island) and Kachemak Bay, and the proportion of high-lipid, schooling forage fish in the diet has been shown to be a key factor in guillemot reproductive success at both sites (Golet et al. 2000, Litzow et al. 2002). The Alaska Predator Ecosystem Experiment (APEX) Project components F ("Factors limiting pigeon guillemot recovery"), G ("Seabird energetics"), and M ("Seabird/forage fish studies in Lower Cook Inlet") investigated the relationship between lack of recovery in guillemot populations impacted by EVOS and the availability and quality of forage fish. A decline in availability of high-lipid forage fishes (sand lance, herring [*Clupea pallasi*], and capelin [*Mallotus villosus*]) during the 1980s and 1990s may have been responsible for lower growth rates, fledging weights, post-fledging survival, and adult recruitment in guillemot populations within the EVOS area.

3. The Nearshore Vertebrate Predator (NVP) Project (pigeon guillemot component) tested the hypothesis that exposure to residual oil from EVOS continues to limit recovery of pigeon guillemots. Pigeon guillemots feed on a diversity of nearshore demersal fishes and schooling forage fishes that use the substrate to avoid predators (e.g., sand lance), prey that were likely injured by EVOS (Jewett et al. 2002). The NVP study measured certain biomarkers in blood and compare biomarker levels in nestlings and adults from oiled and non-oiled areas. While adult guillemots from the oiled portion of Prince William Sound exhibited differences in plasma chemistry and hepatic cytochrome P4501A compared to adults from a non-oiled area, no differences were found between nestlings from the two areas (Seiser et al. 2000, Golet et al. 2002). Potential plasma biomarkers of crude oil exposure in pigeon guillemots need to be calibrated to known doses of weathered Prudhoe Bay Crude Oil (PBCO) in a controlled, laboratory setting.

The research described below was conducted at the Alaska SeaLife Center (ASLC) in Seward and addressed the latter two potential limiting factors described above. Experimental studies using captive subjects were integrated with raising pigeon guillemot nestlings in captivity in order to test the feasibility of captive rearing and release as a direct restoration technique for pigeon guillemots. Raising chicks in captivity provided an opportunity to conduct controlled experiments that are relevant to two major issues in pigeon guillemot restoration: (1) the effect of prey availability and quality on nestling growth rates and condition of young at fledging and (2) the utility of growth

performance and plasma biomarkers as indicators of exposure to crude oil and other environmental contaminants. Research on these two topics can best be conducted using captive subjects whose environment and diet can be carefully controlled to avoid confounding variables common in natural populations. However, because one study objective included releasing captive-reared guillemots into the wild as a potential direct restoration technique, destructive sampling of captive-reared guillemots as part of the diet or oil dosing experiments was precluded. Thus measurement of body fat deposits and total body composition or investigation of tissue damage associated with oil ingestion were not options for more in-depth examination of effects. The controlled feeding and dosing experiments described below were designed to complement field studies on the role of diet and residual oil in food on the productivity of pigeon guillemots in the EVOS area, studies that were part of the APEX and NVP projects.

The APEX Project identified a major shift in nearshore ecosystems of the northern Gulf of Alaska that apparently has resulted in fewer high-lipid schooling fish, particularly sand lance, being fed to nestling guillemots (Oakley and Kuletz 1996, Golet et al. 2000). Pre-spill studies found that sand lance, a nearshore schooling fish with relatively high average energy density, was the dominant prey returned to chicks. Post-spill studies have found that gadids and nearshore demersal fish (e.g., gunnels [Pholidae], pricklebacks [Stichaeidae], and sculpins [Cottidae]) comprised the majority of the diet. The NVP project explored the feasibility of using blood biomarkers to monitor exposure to residual oil in food and determined whether blood from individuals in wild populations indicated continued exposure to oil. Both of these projects examined wild populations exposed to numerous sources of variability that confounded the examination of factors affecting chick growth or blood biomarkers.

The pigeon guillemot is a seabird species that has completely failed to recover in the aftermath of EVOS. Experimental studies with captive-reared guillemots will provide a better understanding of how shifts in the diet of guillemots and other seabirds breeding in the EVOS area affect growth, development, fledging condition, and, ultimately, fitness. By monitoring the growth and development of nestlings raised on controlled rations, the relative nutritional quality of various prey can be assessed. Also, fitness tradeoffs between prey quantity and quality can be assessed by monitoring nestling growth. Dose-response experiments with nestling guillemots fed small, sublethal amounts of weathered PBCO also could provide crucial validation and calibration results for interpretation of on-going studies of biomarkers as indicators of crude oil exposure. Understanding the constraints imposed on recovery of guillemots by diet composition and oil exposure will be essential for designing management initiatives to enhance productivity in this and other seabird species that have failed to recover from EVOS.

There is a definite need for information on the relationship between diet and reproductive success for pigeon guillemots. Guillemots are the most neritic (nearshore) members of the marine bird family Alcidae (i.e., murres, puffins, and auks), and like the other members of the family, guillemots capture prey during pursuit-dives. Pigeon guillemots prey on a wide variety of fishes, including schooling forage fishes (e.g., sand lance, herring, pollock [*Theragra chalcogramma*]) and subtidal/nearshore demersal fish (e.g., gunnels, blennies [Clinidae], sculpins; Drent 1965, Kuletz 1983). There is strong evidence of a major shift in diet composition of guillemot pairs breeding at Naked Island in PWS. Sand lance was the predominant prey type fed to young in the late 1970s (Kuletz

1983), but sand lance was a minor component of the diet after the spill (Golet et al. 2000, Roby et al. 2000). In contrast, guillemots breeding in portions of Kachemak Bay continued to provision their young predominately with sand lance through the 1990s, and sand lance was particularly prevalent in the diet at sites that supported high densities of breeding pairs (Prichard 1997, Litzow et al. 2002). Also, young of breeding pairs that provisioned their nestlings with mostly sand lance had higher growth and survival rates (Prichard 1997, Golet et al. 2000, Litzow et al. 2002). Jackpot Island in southwestern Prince William Sound supported the highest nesting densities of guillemots anywhere in the Sound and growth rates of nestlings were correspondingly high (Roby et al. 2000). The high availability of juvenile herring to guillemots nesting at Jackpot Island may have been responsible for both the high nesting density and high growth rates. Thus, availability of high-quality schooling forage fishes (juvenile herring, sand lance) may be crucial for maintaining high nesting densities of pigeon guillemots in the EVOS area.

This study also was designed to evaluate and validate the use of nondestructive plasma biomarkers to assess the health status of nestling pigeon guillemots and potential exposure of nestlings to petroleum hydrocarbons in food. There is evidence that certain acute phase proteins (i.e., haptoglobin) in blood are induced by ingestion of sublethal doses of weathered crude oil (Prichard et al. 1997). Results of a dose-response experiment with wild guillemot nestlings in their natural nest sites, however, were ambiguous because of among-site variability in baseline values for biomarkers (Prichard et al. 1997). Also, guillemot nestlings were fed small doses (0.05-0.2 ml) of highly weathered PBCO in that study, and the dose levels were not sufficient to cause even a significant decline in growth rates of nestlings. Finally, blood samples for measuring biomarker levels were not collected until five days post-dosing, when any induction of an acute phase response likely had already peaked. Regardless of all these uncontrolled factors, the serum haptoglobin levels in guillemot chicks fed 0.2 ml of weathered PBCO were significantly different from that of controls. While the use of blood biomarkers for monitoring oil exposure and general population health of guillemots is promising, more research under controlled, captive conditions is required to validate the techniques and provide a sound basis for interpretation of results from wild guillemots.

The study design required that nestling pigeon guillemot be raised under controlled conditions in captivity. Guillemot eggs and hatchlings (<10 days post-hatch) were obtained from Alaskan source colonies on the Kenai Peninsula, Kodiak Island, non-oiled parts of Prince William Sound, and Southeast Alaska. All captive-reared chicks that survived to fledgling age in good health were banded and released at ASLC to assist in efforts to establish local breeding colonies of free-ranging guillemots. Information obtained from this project will benefit management of pigeon guillemot populations in the Gulf of Alaska, especially Prince William Sound. An understanding of the effect of prey type on chick growth will help explain the role of ecosystem shifts in continuing declines of pigeon guillemot populations. Assessing the utility of blood biomarkers for detecting and quantifying exposure to crude oil will benefit efforts to monitor the health status of pigeon guillemot populations throughout the spill zone without resorting to lethal sampling procedures.

The two primary objectives of this research project were:

1. Determine the effect of diet variables on growth performance, development, and fledging condition of pigeon guillemots, including:

a) type of forage fish consumed
b) quantity of forage fish consumed
c) lipid content of the diet
d) daily energy intake

2. Determine the response of particular biomarkers (plasma constituents, stress hormones, acute phase proteins, and growth performance) in nestling guillemots to exposure to weathered Prudhoe Bay crude oil (PBCO). Exposure variables that will be examined include:
   a) ingested dose of PBCO
   b) time post-dosing
   c) nestling age
   d) nestling food intake

The research was designed to test the following two basic hypotheses, which relate to each of the two primary objectives listed above:

Hypothesis 1: Differences in prey type, food intake rate, lipid content of prey, and energy intake rate have major effects on growth performance and fledgling condition of nestling pigeon guillemots.

Hypothesis 2: Biomarkers in plasma and growth performance of nestling pigeon guillemots can be used as indicators of exposure to weathered PBCO in the food supply.

# CHAPTER 2
# EFFECTS OF DIET ON GROWTH AND DEVELOPMENT OF PIGEON GUILLEMOT NESTLINGS: CAPTIVE FEEDING EXPERIMENTS


## ABSTRACT

Pigeon guillemot (*Cepphus columba*) populations in Prince William Sound, Alaska were injured by the *Exxon Valdez* Oil Spill (EVOS) and have failed to recover from declines occurring before and after the spill. Declines in certain schooling forage fishes (i.e., sand lance [*Ammodytes hexapterus*] and herring [*Clupea pallasi*]) have been implicated in declines in both numbers and productivity of guillemots in the EVOS area. It is not clear, however, whether the benefits of these key forage fishes are related to nutritional value or higher availability to nesting guillemots compared to alternative prey. We examined the effects of prey type and prey proximate composition (i.e., lipid and protein content) on growth and survival of guillemot nestlings by raising chicks in captivity on diet treatments that corresponded to various rates of energy intake within the range measured for nestling pigeon guillemots in the wild.

Growth in body mass of nestling guillemots did not vary with prey type or daily intake of either lipid or protein. Instead, growth in body mass was closely related to total daily energy intake. Structural growth (wing length) was not, however, related to daily energy intake, within the range of diet treatments used. Thus, growth in structural size (wing length) was considerably less sensitive to dietary differences compared to growth in body mass. Structural growth was stunted, however, in chicks fed a diet higher in lipid than is consumed in the wild, suggesting a nutritional optimum for guillemot chick diets in the range of 20-30% lipid (% dry mass of food).

Results of these feeding experiments support the hypothesis that parental energy provisioning rates to nestlings have a greater influence on growth performance and fledgling condition in pigeon guillemots than do prey type or lipid content *per se*. Consequently, general prey availability for breeding guillemots appears to have a greater bearing on guillemot productivity than availability of particular high-lipid prey. Enhanced forage fish availability, and the consequent higher energy provisioning rates to guillemot broods, is a likely requisite for recovery of guillemot populations that were injured by the EVOS. Prior to the spill, sand lance, juvenile herring, and perhaps capelin (*Mallotus villosus*) were the key forage fish resources that provided guillemots in the EVOS area with a readily available food supply. These forage fish species also have higher energy densities than most other prey consumed by guillemots in the EVOS area, and their lipid content is usually 20-30% of dry mass, the level that appears to support maximal growth rates in nestling guillemots.


## INTRODUCTION

The pigeon guillemot (*Cepphus columba*) population in Prince William Sound (PWS), Alaska declined from approximately 15,000 in the 1970's to about 1,800 most recently (Laing and Klosiewski 1993, Stephenson et al. 2001). While this decline apparently began prior to the *Exxon Valdez* oil spill (EVOS), an estimated 10-15% of the population in the spill area died as a direct result of the spill in 1989 (Piatt et al. 1990, Laing and Klosiewski 1993). Unlike most other avian species affected by the spill, pigeon guillemot populations in PWS have shown no evidence of recovery to pre-spill

numbers (Irons et al. 2000, Lance et al. 2001). Censuses at other breeding colonies in the northern Gulf of Alaska indicate that the decline in guillemots may have occurred over a wide region (U.S. Fish and Wildlife Service 2002). Post-spill studies have identified three potential factors preventing recovery: (1) declining availability of certain key prey types (Oakley and Kuletz 1996, Golet et al. 2000, Litzow et al. 2002), (2) increased nest predation (Oakley 1981, Hayes 1995), and (3) continued exposure to residual EVOS crude oil (Golet et al. 2002). This study focused on the potential effects of changes in the prey base on nesting productivity.

Pigeon guillemots are pursuit-diving seabirds that feed almost entirely on fishes found in nearshore habitats. They are semi-colonial, cavity-nesting alcids (family Alcidae) that utilize a wide range of sites found in shoreline habitats, such as rock crevices, cavities under tree roots, talus, burrows, and man-made structures (e.g., bridges, docks). Guillemots are the most neritic (nearshore) members of the Alcidae and parents commonly provision their brood at much higher rates than do other alcids (Golet et al. 2000**;** Roby et al. 2000). The proximity of guillemot foraging areas and nest sites is apparently responsible for a unique life history trait among the Alcidae: raising two-chick broods in the nest until they are full-grown. All other alcid species either lay one-egg clutches and raise a single chick per breeding attempt, or the young leave the nest shortly after hatching and accompany parents to foraging areas.

Pigeon guillemots prey on a wide variety of fishes over their breeding range, which in North America extends from the coast of central California to the northern Bering Sea (Gaston and Jones 1998). Guillemots are unusual among the piscivorous Alcidae in the prevalence of nearshore demersal fishes (i.e., blennies [Clinidae], gunnels [Pholidae], pricklebacks [Stichaeidae], sculpins [Cottidae], and ronquils [Bathymasteridae]) in the diet (Drent 1965, Kuletz 1983, Emms and Verbeek 1989, Roby et al. 2000). Some colonies or nesting aggregations rely more on schooling forage fishes (e.g., Pacific herring [*Clupea pallasi*], Pacific sand lance [*Ammodytes hexapterus*], capelin [*Mallotus villosus*], and walleye pollock [*Theragra chalcogramma*]; Kuletz 1983, Roby et al. 2000, Litzow et al. 2002), prey that generally are more prevalent in diets of seabirds that forage further offshore.

Certain forage fishes (e.g., sand lance, herring, and capelin) have declined in the EVOS area over the last two decades (Hatch et al. 1993, Piatt and Anderson 1996). Lower availability of these key prey species may be responsible for declines in chick growth rates, fledging success, post-fledging survival, and adult recruitment rates for various piscivorous seabirds. For example, the proportion of sand lance in the diet of breeding pigeon guillemots has declined dramatically at some PWS colonies, notably Naked Island (Oakley and Kuletz 1996). Golet et al. (2000) and Litzow et al. (2002) found that the percentage of high-lipid forage fishes, especially sand lance, in the diet is a key factor affecting guillemot breeding success. However, it is unclear whether the quality of prey (as defined by lipid content) or the availability of prey (as defined by biomass provisioning rates to nestlings) is the primary factor responsible for differences in breeding success (Golet et al. 2000, Litzow et al. 2002).

The efficient capture of fish in foraging areas and transport of food energy to nestlings is critical to the breeding success of parent seabirds (Ricklefs 1983, Roby 1991). Low-lipid prey may have to be provisioned at a higher rate than high-lipid prey in order to achieve the same energy provisioning rates to young and support optimal

nestling growth rates. The neritic foraging strategy of guillemots is unique in the Alcidae and may allow such a high rate of chick provisioning that low-lipid fish may substitute for high-lipid prey, without subjecting nestlings to reduced energy provisioning rates. Chicks fed primarily low-lipid fish may, however, experience slower growth rates and lower fledging mass if low-lipid fish do not provide a nutritionally balanced diet (i.e., dietary lipid does not provide sufficient energy to meet requirements for maintenance metabolism; Roby 1991). Furthermore, diets of low-lipid fishes may translate to lower energy reserves in fledglings (Lance and Roby 2000, Romano 2000) and lower post-fledging survival (Perrins et al. 1973). If breeding success is directly related to the quality of available forage fish, then adult guillemots that rely on low-quality prey to provision their young may experience lower productivity (Golet et al. 2000, Litzow et al. 2002).

We investigated the relationship between diet and growth, development, and survival of captive pigeon guillemot nestlings raised at the Alaska SeaLife Center (ASLC). We collected pigeon guillemot eggs and hatchlings from the wild to use as subjects for captive rearing experiments. Hatchlings were assigned to several diet treatments that differed in both food quality and quantity in order to examine the effects of prey type, food biomass intake rate, dietary lipid content, and energy intake rates. These feeding trials were designed to distinguish among several competing hypotheses regarding the role of dietary constraints on growth, development, and survival of young guillemots.

Nestling growth and development is limited primarily by:

1. the type of prey fed to nestlings (prey types differ in nutritional quality)
2. the mass of food fed to nestlings (biomass provisioning rates matter most)
3. the lipid content of food fed to nestlings (high-lipid diets are optimal)
4. the energy intake rate of nestlings (growth performance is primarily energy-limited, regardless of prey type, biomass intake, or dietary lipid content)

This study of the effects of diet on chick growth performance using controlled laboratory conditions may, in conjunction with results from studies in the field, provide insight to how diet influences nesting success and productivity of pigeon guillemots in the EVOS area. However, because one study objective included releasing captive-reared guillemots into the wild as a potential direct restoration technique, destructive sampling of captive-reared guillemots as part of captive feeding trials was precluded. Thus, measurements of body fat deposits and total body composition associated with various diets were not possible. This study is relevant to restoration programs for pigeon guillemots and other piscivorous seabirds that have failed to fully recover in the aftermath of the EVOS (e.g., marbled murrelets [*Brachyramphus marmoratum*], common murres [*Uria aalge*]).

## METHODS

### Collection of eggs and chicks

Pigeon guillemot eggs were obtained from nests in the wild, hatched and raised in captivity, and used as subjects for this study. We collected guillemot eggs from active nests during late June and early July in all three years of the study (1998-2000). Nearly all eggs were collected from four areas in south-central and southeast Alaska (outside the EVOS area) where guillemot nesting densities are comparatively high and access to nests is comparatively easy: western PWS, the south shore of Kachemak Bay, Chiniak Bay on

Kodiak Island, and near Juneau (Table 2.1). In addition, four eggs were collected on Mummy Island in southeast PWS.

In 1998, collected eggs were placed in a vertical position in egg cartons for transport to the ASLC in Seward, Alaska. In 1999 and 2000, this protocol was modified and all collected eggs were placed in a horizontal position within foam rubber layers for transport to ASLC. Eggs were identified by collection location and date with pencil. In all three years**,** eggs were transported in a portable electric incubator ("The Brooder," Dean's Animal Supply, Inc., Orlando, FL). "The Brooder" is a modified insulated cooler with a thermostatically-controlled heater powered by a 12-volt battery when in transit. While in the portable incubator, eggs were kept at approximately 36°C and high humidity was maintained using wet sponges placed in the bottom of the incubator.

When guillemot nests were found in the wild, they occasionally contained nestlings instead of eggs. If the nestlings appeared to be less than five days old, we sometimes collected these hatchlings as well. Young chicks were transported in boxes or animal carriers with cardboard dividers, suitable ventilation, and, where appropriate, chemical hand warmers to provide heat. All guillemot eggs and hatchlings were collected under scientific collecting permits issued by the U.S. Fish and Wildlife Service and the Alaska Department of Fish and Game.

Egg incubation and hatching

Upon arrival at ASLC, we weighed each egg to the nearest 0.1 g and measured length and breadth to the nearest 0.1 mm with calipers. We candled eggs to determine if a viable embryo was present and to ascertain the stage of development before placing eggs in a Grumbach Incubator Model #1804. Eggs were maintained in the incubator at 37.2 ± 0.5°C and 50-60% humidity, and were turned by rollers every 6 hours. During incubation, we monitored egg mass every two days to ensure that the rate of mass loss would not exceed ca. 15% between laying and hatching (S. Rollins, ASLC, pers. comm.; Whittow 2002). In the latter stages of development, we examined eggs daily for signs of pipping (breaking of the shell by the emerging chick) or movement of the egg that indicated hatching was imminent. We then moved eggs to a second Grumbach incubator that served as a hatching unit with temperature and humidity the same as the first, but instead of on egg turners eggs were placed in a dish to hold the chick and shells after hatching. We checked hatching eggs every few hours to monitor progress, and after hatching, chicks were kept in the hatching unit until their down was dry (2-4 h).

Captive-rearing protocols

All protocols for raising guillemots in captivity and conducting the feeding experiments described below were reviewed and approved by the Institutional Animal Care and Use Committees for both the ASLC and Oregon State University. The portable incubators were used as brooders for hatchling guillemots until they were able to maintain their own body temperature (usually day 5 post-hatch). We reduced brooder temperatures approximately 5° C each progressive day so that chicks gradually became acclimated to room temperature (16°-18° C) over the first five days post-hatching. In the wild, pigeon guillemot chicks are brooded continuously by their parents for approximately four days post-hatching (Drent 1965), suggesting that homeothermy is poorly developed until day 5 post-hatch. On day 5-7 post-hatch, we color banded chicks

by collection location/date and moved them to individual indoor artificial nest sites without heat sources. These nest sites consisted of modified institutional 5-gallon plastic food buckets (with Dri-Dek flooring [Dri-Dek Corporation, Naples, FL] and a 6 inch long by 4 inch diameter ABS plastic pipe) without lids that were placed on their sides. Chicks directed their excreta outside the buckets through the opening. In 1998, we kept chicks in a cool (16°-18° C) indoor enclosure with a photoperiod similar to outdoor conditions for the remainder of the feeding trials. In 1999 and 2000, chicks were kept in a protected outdoor enclosure, where they were exposed to natural photoperiod and outdoor temperature fluctuations that averaged from daily lows of 9.0° C to highs of 16.9° C through the months of July and August (NCDC Cooperative Stations, www.worldclimate.com). We measured body mass (± 0.1 g) and wing length (± 1 mm) of chicks daily during the period from hatching until day 30 post-hatch.

Nestling feeding regimes

The diets for guillemot chicks were selected to provide feeding regimes that would test competing hypotheses for dietary constraints on growth and development. Forage fish types that were fed to captive guillemot chicks were chosen on the basis of their availability, whether they were typical prey of breeding guillemots in the EVOS area, and whether they represented a range of lipid contents and, therefore, prey quality. Age class 1+ juvenile Pacific herring (*Clupea pallasi*) is a primary food for some guillemots nesting in the EVOS area (e.g., Jackpot Island; Roby et al. 2000). This prey type generally is considered a high-lipid, high-quality forage fish for piscivorous seabirds in the EVOS area (Anthony et al. 2000), and it is readily available from commercial sources. Age class 1+ juvenile walleye pollock (*Theragra chalcogramma*) also is found frequently in guillemot diets within the EVOS area, is generally a low-lipid forage fish (Van Pelt et al. 1997, Anthony et al. 2000), and is therefore considered low quality compared to juvenile herring. National Marine Fisheries Service research trawls in the Bering Sea provided young pollock for our study. Finally, nearshore demersal fishes (i.e., blennies, gunnels, sculpins, ronquils) are important components of guillemot diets in the EVOS area (Roby et al. 2000) and generally are intermediate in lipid content between juvenile herring and juvenile pollock. However, they are not caught in any quantities either commercially or for research purposes, and were not readily available for feeding captive guillemots. Nevertheless, two species of nearshore demersal fishes, crescent gunnels (*Pholis laeta*) and high cockscomb (*Anoplarcus purpurescens*), commonly are found beneath larger rocks in rocky lower intertidal zones of PWS. These two nearshore demersal fishes were captured by hand during extremely low tides in Resurrection Bay near the ASLC in order to provide a third prey type on which to raise young guillemots. All fish were stored at –20 °C and within 12 hours of feeding thawed on ice.

The effects of daily food intake (g/day) on chick growth were investigated by raising guillemot chicks on restricted diets consisting of different biomass intake rates. Four biomass intake rates were chosen based on estimated food provisioning rates at guillemot nests in the wild (Roby et al. 2000): 125 g/day (low-intake), 160 g/day (moderate-intake), 180 g/day (high-intake), and *ad libitum* (unrestricted).

We fed hatchling pigeon guillemots a mixed, unrestricted diet of Atlantic silversides (*Menidia menidia*), juvenile walleye pollock, and juvenile Pacific herring eight times daily until day 11 post-hatch. All experimental diet assignments were based

on a stratified hatch date design (Table 2.2). On day 11, we switched chicks to their experimental diets (Table 2.2) and fish quantity provisioned was gradually increased by approximately 25 g/day to the final biomass/day ration of juvenile Pacific herring, juvenile walleye pollock, or blennies (mixed diet of high cockscomb and crescent gunnels). On day 11 we also switched all chicks to four feedings of equal biomass per day, except in 1998, when chicks were maintained on eight feedings per day for the duration of the feeding experiment. By day 15 post-hatch, all guillemot chicks were being fed the specified type and quantity of fish prescribed for their respective diet treatments (Table 2.2). In 1999, all chicks in the *ad libitum* herring treatment were fed only herring from day 1 post-hatch and were continually monitored continuously to ensure that fish were always present after day 11 post-hatch. In 2000, the chicks on the *ad libitum* diet were fed the same mixed diet as the chicks on restricted diets until day 11 post-hatch, when they were switched to an all herring diet.

In order to feed some guillemot chicks a diet higher in lipid content and energy density than any of the available forage fishes, we developed an artificial high-lipid diet by supplementing the low-intake herring diet (125 g/day) with 6 ml/day of fresh sockeye salmon oil (Kodiak Fishmeal Company). For chicks raised on the salmon oil-supplemented diet, about 1.5 ml of salmon oil was injected in one whole fish for each of four daily feedings.

Just prior to feeding chicks, we injected all fish with approximately 0.5-1.0 ml of water to ensure adequate water intake by chicks. A general vitamin supplement (Seatabs; Pacific Research Labs Inc.) was fed at a dose of one quarter to one half of a tablet per day, dependent upon chick age and size, as recommended by ASLC staff.

Energy and lipid content of diets

Lipid content of samples of fish fed to guillemot chicks was determined using proximate composition analysis. Samples of individual fish were dried to constant mass in a convection oven at 60° C to determine moisture content and then finely ground using a mortar and pestle. Lipid content of the dried, ground samples was determined by solvent extraction using a Soxhlet apparatus and a 7:2 hexane/isopropyl alcohol (v:v) solvent system (Radin 1981). Lean dry samples were transferred from cellulose extraction thimbles to pyrex beakers and ashed in a muffle furnace at 600°C for 12 hours to determine ash content, and ash-free lean dry mass (ca. 95% protein, Montevecchi et al. 1984) was measured by subtraction. Energy density was calculated from the results of the proximate composition analysis and the energy equivalents of lipid and protein fractions (39.3 kJ/g and 17.8 kJ/g, respectively, for uricotelic vertebrates; Schmidt-Nielsen 1997).

Metabolizable energy coefficients

The effect of diet treatment on metabolizable energy coefficients (MECs) of guillemot chicks was estimated by comparing energy intake rates and energy excretion rates. This is generally termed the “assimilation efficiency,” but strictly speaking is not. Excreted energy frequently includes significant amounts of assimilated energy that either can not be metabolized or represents an endogenous source of excreted energy. Thus, assimilation efficiency is always higher than the MEC. Assimilation efficiency can only be measured by separating assimilated and unassimilated material in excreta, and this is

especially difficult in birds where urine and feces are mixed in the cloaca prior to excretion.

We collected total excreta over a 24-hour period from each chick at day 29 post-hatch in year 2000. Excreta was collected in plastic containers that covered the front of each artificial nest site and was secured in place during the first morning feed and removed 24 hours later. Excreta samples were transferred to plastic bags and the container was rinsed with water and the rinse added to the collected excreta to ensure complete collection. Samples were frozen at –20° C until they could be dried in a convection oven at 60° C for dry mass determination. Dry samples were homogenized using a mortar and pestle. We determined total energy of each sample by combustion of one-gram subsamples from each bird in a Parr adiabatic bomb calorimeter, model 1341 (Parr Instrument Company, Moline, IL). We calculated MECs for chicks in three diet groups (herring low-intake, herring *ad libitum*, and herring plus salmon oil) using the formula:

$$MEC = [(EE_{in} - GE_{out}) / EE_{in}]$$

where $EE_{in}$ is the estimated energy intake (kJ) during 24 hours, based on the energy content of dietary fish as determined by proximate composition analysis, and $GE_{out}$ is the gross energy (kJ) excreted during 24 hours, based on bomb calorimetry of excreta. Every fifth subsample had a duplicate combusted for quality control. The average difference between duplicate MECs was ± 1.1%.

We used SAS (version 8.0) and Statgraphics Plus (version 4.0) for all statistical analyses.

## RESULTS

### Hatching and fledging success

A total of 206 pigeon guillemot eggs and 37 hatchlings were collected in the wild over the three years of the project (Table 2.1). Overall hatching success for eggs collected in the wild was 70%, and overall fledging success for eggs hatched in captivity plus hatchlings collected in the wild was 86%. Hatching success for eggs collected in 1998 was only 53.5%, and in that year hatching success was similar for eggs collected at the three primary collection areas (Table 2.1). Hatching success for eggs collected in 1999 and 2000 was 70.0% and 77.4%, respectively. The higher hatching success of eggs collected in the latter two years of the project was apparently related to handling during collection and transport to ASLC. During 1999 and 2000, guillemot eggs were wrapped in foam padding immediately after removal from the nest and transported to ASLC in the horizontal position, whereas in 1998 eggs were transported in the vertical position in standard poultry egg cartons. Guillemot eggs that experienced rough boat rides (e.g., eggs collected from Midway Island, southeast Alaska in 2000) also had lower hatching success. Of the four primary egg collection areas, eggs collected from islands in Chiniak Bay, eastern Kodiak Island had the highest hatching success (91.8%, n = 45 eggs). Variation in hatching success among the four main collection areas (all years combined) also seemed attributable to the differences in handling described above.

Survival of guillemot chicks in captivity until fledging age (day 30 post-hatch) was 92.0% in 1998 (n = 25), 77.9% in 1999 (n = 77), and 92.4% in 2000 (n = 79; Table 2.1). All chick mortality occurred prior to day 11 post-hatch, before chicks were switched

to experimental diets. Cause of death in captive guillemot chicks could not always be determined, but was not attributable to experimental procedures. Death of hatchlings usually followed the production of abnormal excreta and reluctance by the hatchling to take food. The lower chick survival rate in 1999, compared to 1998 and 2000 (Table 2.1), was largely attributable to a faulty thermostat in one of the portable incubators that was used as a brooder for young chicks. This mechanical failure apparently caused some hatchlings to be exposed to higher than normal brooder temperatures, from which some never recovered. Nevertheless, the results of our efforts to hatch guillemot eggs from the wild and raise chicks to fledging age in captivity demonstrate that fledging success as high as 90% is feasible for captive-reared chicks, much higher than for guillemots in the wild. Careful egg handling during collection and transport, plus maintaining hatchlings within their narrow thermal neutral zone while thermogenic capacity develops during the first week post-hatch, seem to be key factors for successfully raising pigeon guillemots in captivity.

Diet composition

The proximate composition and energy density of forage fish fed to guillemot chicks varied among diets (Table 2.3). Lipid content differed significantly among prey types ($F_{4,90} = 22.79$, $P < 0.0001$), as did protein content (ash-free lean dry mass, $F_{4,90} = 58.12$, $P < 0.0001$), and energy density (kJ/g wet mass; $F_{4,90} = 6.10$, $P = 0.0002$). The juvenile walleye pollock used in 1999 had significantly higher lipid content, ash-free lean dry mass (AFLDM), and energy density (kJ/g wet mass) than the pollock used in 1998 (Bonferonni Multiple Comparison, $P < 0.0001$; Table 2.3). The lipid content and energy density of the 1999 pollock (25.7% of dry mass and 5.0 kJ/g wet mass, respectively; Table 2.3) also were much higher than that of pollock collected in PWS during the guillemot nesting season (6.7% of dry mass and 3.2 kJ/g wet mass, respectively; Anthony et al. 2000). In fact, the pollock fed to guillemot chicks in 1999 had the highest lipid content and energy density of all the forage fishes used in this study (Table 2.3). The lipid content (12.3% of dry mass) and energy density (3.8 kJ/g wet mass) of juvenile pollock fed to guillemots in 1998 was, however, more similar to that of pollock caught in PWS. The high-lipid pollock used in 1999 were caught in the Bering Sea during July, whereas the low-lipid pollock used in 1998 were caught in the Bering Sea during winter. The juvenile pollock captured in the Bering Sea in July 1999 demonstrate that, while generally a low-lipid, low energy density diet for marine piscivores, under some circumstances juvenile pollock can be high in lipid content and energy density.

Juvenile herring used in this study were caught commercially during the late winters of 1998 and 1999 off the coast of British Columbia. Juvenile herring used in 2000 were from the same lot as those used in 1999. While juvenile herring caught during the guillemot nesting season are generally high in lipid and have a high energy density (Anthony et al. 2000), herring caught late in winter tend to have depleted energy reserves, low lipid content, and low energy density. Juvenile herring used in 1998 were significantly lower in lipid content, AFLDM content, and energy density than the juvenile herring used in 1999 (Bonferonni Multiple Comparison, $P < 0.0001$; Table 2.3). In fact, juvenile herring fed to guillemots in 1998 had the lowest lipid content and the lowest energy density of all forage fishes fed to guillemots in this study (Table 2.3). Juvenile herring used in this study in 1998 was much lower in lipid content (7.0% of dry mass)

and energy density (3.4 kJ/g; Table 2.3) compared to juvenile herring caught in PWS during the guillemot breeding season (26.8% of dry mass and 5.8 kJ/g, respectively; Anthony et al. 2000). Juvenile herring used in 1998 also were much lower in lipid content than juvenile pollock used in 1999 (Table 2.3). Herring used in 1999 had an average lipid content (21.2% of dry mass) and energy density (4.3 kJ/g wet mass) closer but still well below those of herring caught in PWS during the guillemot nesting season (Table 2.3).

We were able to collect sufficient quantities of blennies near ASLC to raise only two guillemot chicks at the high-intake rate (180 g/day; Table 2.2). This diet consisting of mixed crescent gunnels and high cockscomb was low in lipid but high in AFLDM (mostly protein) compared to other prey types; consequently, energy density tended to be intermediate between that of the low-lipid pollock or herring and the high-lipid pollock or herring (Table 2.3). The lipid content and energy density of crescent gunnels and high cockscomb fed to guillemots in this study were similar to those collected in PWS during the guillemot breeding season (Anthony et al. 2000).

The artificial high-lipid diet consisting of juvenile herring supplemented with salmon oil had a lipid content of 35% of dry mass, more than most juvenile herring caught in PWS during the guillemot nesting season (average lipid content = 27% of dry mass, SD = 10.6, n = 229; Anthony et al. 2000). Nevertheless, the energy density of the artificial diet (5.82 kJ/g wet mass) was similar to the average energy density of juvenile herring caught in PWS (mean = 5.84 kJ/g wet mass, SD = 1.66, n = 229; Anthony et al. 2000).

Energy intake rate associated with each experimental diet was calculated from the product of the daily biomass intake and the average energy density of the prey, based on proximate composition analysis (Table 2.2). Daily energy intake associated with the various experimental diets ranged from 544 to 819 kJ/day (Fig. 2.1). This range is similar to the range in average daily energy provisioning for pigeon guillemot broods in the wild in PWS (563 kJ/day at Naked Island in 1996; 1000 kJ/day at Jackpot Island in 1995; Roby et al. 2000). Guillemot broods in the wild, however, frequently consist of two nestlings, so energy provisioned to the nest must be shared between the two members of the brood and each will receive less than the total. The experimental diet with the lowest energy intake rate was the low-intake herring diet, while the diet with the highest energy intake rate was the *ad libitum* herring diet (Table 2.2, Fig. 2.1). Percent of daily energy intake that was in the form of lipid is also presented in Table 2.2, and is displayed graphically in Figure 2.1. Energy intake in lipid ranged from less than 18% of total calories in the blenny and low-lipid herring diets to nearly 60% of total calories in the salmon oil-supplemented herring diet.

Chick growth

There were no significant differences in either body mass ($F_{8,66} = 1.63$, $P = 0.133$) or wing length ($F_{8,49} = 1.46$, $P = 0.198$) among chicks assigned to the various diet groups at day 4 post-hatch (Fig. 2.2). On day 10 post-hatch, before experimental diet treatments were initiated, there was, however, a significant difference among diet treatment groups in body mass and wing length ($F_{8,74} = 4.07$, $P = 0.0005$; $F_{8,71} = 3.07$, $P = 0.005$, respectively; Fig. 2.2). It was not apparent why
these differences in body mass and wing length among the diet groups occurred prior to the initiation of the different diet treatments. In order to control for these initial

differences in body mass and wing length among treatment groups, we used the increments in body mass and wing length between day 15 and day 30 as the response variables (Fig. 2.2), rather than the final body mass and wing length at day 30. Growth increments in body mass and wing length between day 15 and day 30 were analyzed for differences among diet groups.

Growth in body mass

Average chick body mass at day 30 varied among diet groups, ranging from a low of ca. 370 g in the low-intake herring group to a high of ca. 460 g in the *ad libitum* herring group. Average fledging mass for wild nestling guillemots in the EVOS area varied from 390 g to 520 g, depending on the colony and year (Roby et al. 2000). Because the body mass of nestling guillemots was still increasing on day 30 (Fig. 2.2), and guillemot nestlings normally fledge at about 35 days post-hatch, the average body mass of chicks at day 30 in the present study appeared to be within the range of normal masses for pigeon guillemot nestlings in the wild.

The average body mass increment from day 15 to day 30 differed among diet groups; the *ad libitum* herring group exhibited the highest average body mass increment and the low-intake herring group had the lowest average body mass increment (Figs. 2.2 and 2.3). Differences among diet groups in body mass growth were not related to prey type *per se*; the three different prey types (herring, pollock, and blennies) supported a range of overlapping growth rates (Fig. 2.3).

The relationship between food biomass intake rate (g/day) and body mass increment from day 15 to day 30 was investigated using simple linear regression. Body mass increment was positively related to food biomass intake ($R^2 = 0.344$, $P < 0.00005$), as predicted, but food biomass intake explained only 34% of the variation in body mass increment and there was a significant lack-of-fit ($P < 0.00005$; Fig. 2.4). This suggests that other factors might be more responsible for differences in growth rate in body mass.

Average daily energy intake (kJ/day) explained a much higher percentage of the variation in body mass increment (62%, $R^2 = 0.621$, $P < 0.00005$, n = 80; Fig. 2.5) than did average daily food biomass intake. Not surprisingly, there was some variation within diet groups in body mass increment; when average body mass increment was calculated for each diet group (n = 8), 90% of the variation in average body mass increment was explained by average daily energy intake ($R^2 = 0.896$, $P = 0.0004$; Fig. 2.6).

Total daily energy intake for guillemots raised on fish diets consisted primarily of energy in the form of either lipid or protein, and the ratio of energy in lipid to energy in protein varied considerably among the experimental diets (Fig. 2.1). We calculated the proportion of total energy intake that was in the form of lipid as an index to the lipid content of the various diets (Table 2.2). We then regressed body mass increment against both total daily energy intake and the proportion of daily energy intake that was in lipid (arcsine square root transformed) using stepwise multiple regression. The percent energy in lipid did not explain a significant proportion of the residual variation from the regression of body mass increment on total daily energy intake ($t = -1.214$, $P = 0.228$). This result indicates that the lipid content of guillemot chick diets had little effect on chick growth rates, even after controlling for the effects of variation in total daily energy intake. The diet consisting of juvenile herring supplemented with salmon oil (Her/+ oil) had the highest lipid content of any experimental diet, yet the average body mass

increment for chicks fed this diet was below the regression line of body mass increment vs. average daily energy intake (Fig. 2.6). This result contradicts the hypothesis that high dietary lipid is associated with high growth rates in nestling guillemots.

Growth in wing length

Unlike the effects on body mass increment, the different diets had little effect on wing length increment from day 15 to day 30 (Fig. 2.7). There was no significant relationship between wing length increment of chicks and average daily energy intake over the range of diet treatments used in this study ($F_{1,74} = 0.26$, $R^2 = 0.0035$, $P = 0.614$; Fig. 2.8). Average wing length increment was nearly the same over the range of experimental diets (Fig. 2.9), indicating that wing growth is not limited in guillemot chicks when average daily energy intake exceeds about 540 kJ/day of fish. As with body mass increment, wing length increment from day 15 to day 30 varied among individuals within diet groups (Fig. 2.8). When the average wing length increment for each diet group was regressed on average daily energy intake, the relationship was still not significant ($F_{1,6} = 0.06$, $R^2 = 0.009$, $P = 0.821$; Fig. 2.9).

The wing length increment of chicks on the salmon oil-supplemented diet appeared to be lower than that of other diet groups (Fig. 2.7), and the average wing length increment for salmon oil-supplemented chicks was well below the regression line of wing length increment vs. average daily energy intake (Fig. 2.9). The Studentized residual for average wing length increment of salmon oil-supplemented chicks was –2.38, outside the 95% confidence interval, indicating that average wing length increment of oil-supplemented chicks was an outlier. Once this outlier was removed, the regression of average wing length increment on average daily energy intake was still not significant ($F_{1,5} = 0.68$, $R^2 = 0.120$, $P = 0.447$). These analyses confirmed that there was no linear relationship between
structural growth rates in guillemot nestlings and daily energy intake, within the range of daily energy intakes for nestlings in this experiment.

There was an apparent nonlinear trend, however, in the relationship between wing length increment and daily lipid intake (Fig. 2.10). Growth in wing length appeared to be maximized by diets with intermediate lipid levels, while the low-lipid and high-lipid (oil-supplemented) diets were associated with lower average rates of growth in wing length. A quadratic equation fitted to average wing length increment for each diet group was significant ($F_{2,5} = 8.31$, $R^2 = 0.769$, $P = 0.0257$; Fig. 2.10). This suggests that for guillemot nestlings there may exist an optimal level for dietary lipid, where structural growth is maximized. Our results suggest this level is about 20-30% lipid on a dry mass basis (Table 2.3).

Metabolizable energy coefficients

Metabolizable energy coefficients (MECs) of chicks in the low-intake herring, salmon oil-supplemented herring, and *ad libitum* herring diet groups were all high, 80% or above, indicating that these three diets were assimilated and metabolized efficiently. There were no significant differences in MECs among the three diet groups tested (ANOVA, $F_{2,59} = 1.34$, $P = 0.2702$; Fig. 2.11). These three diet treatments included the lowest daily energy intake, the highest daily energy intake, and the highest daily lipid intake of the eight diet treatments used. These results indicate that the lower wing length

increment (Fig. 2.9) and marginally lower body mass increment (Fig. 2.6) of chicks on the salmon oil-supplemented diet were not a consequence of lower energy assimilation on the high-lipid diet.

## DISCUSSION

### Factors affecting growth in body mass of nestling pigeon guillemots

Results of the captive feeding experiments provide no support for the hypothesis that the type of prey fed to nestling guillemots is the primary factor influencing rates of nestling growth and development. This suggests that there are no inherent differences in the nutritional quality of the three prey types fed to captive guillemot chicks (juvenile herring, juvenile pollock, blennies), and parent guillemots that select one of these prey types would not be expected to have higher productivity. (Note: If one of these prey types is deficient in an essential vitamin, the Seatabs that were fed to all captive chicks could have compensated for such a deficiency and masked the effect of prey type.)

If prey type does not exert a strong influence on nestling growth, then perhaps the total biomass of food fed to nestlings, irrespective of the taxonomic composition of the diet, is the primary factor influencing nestling growth rates. One prediction from this hypothesis is that parent guillemots should seek to maximize the biomass of food provisioned to nestlings, at least up to some level where nestlings can not increase rates of digestion and assimilation of nutrients. The low $R^2$ for the regression of body mass growth on biomass intake rate (0.344) and the significant lack of fit for the regression, however, provide only weak support for the hypothesis that provisioning rate of food biomass to nestlings is the primary factor influencing nestling growth rates.

Previous studies have suggested that certain high-lipid forage fishes (i.e., sand lance, herring, capelin) may constitute crucial prey resources for pigeon guillemots in the EVOS area (Golet et al. 2000, Roby et al. 2000, Litzow et al. 2002). The implication is that it is the lipid content of these prey types that make them superior to low-lipid prey, such as juvenile pollock and other gadids (Anthony et al. 2000). If lipid is a nutrient that limits growth in nestling guillemots, then the rate at which lipid is provided to nestlings by their parents should explain much of the variation in nestling growth. This hypothesis predicts that natural selection would favor parent guillemots that select high-lipid prey to provision their young.

The “high-lipid” prey type used in the present study (juvenile herring) had only a moderate to low lipid content compared to the juvenile herring typically fed to nestling guillemots (Anthony et al. 2000). Also, the “low-lipid” prey type used in this study (juvenile walleye pollock) had moderate to high lipid content compared to the juvenile pollock typically consumed by nestling guillemots in the wild (Van Pelt et al. 1997, Anthony et al. 2000). The lipid contents of the herring and pollock used in this study may have masked the benefits normally experienced by guillemots raised on diets dominated by juvenile herring, if lipid is the limiting nutrient. There was, however, no support for this hypothesis from our captive feeding experiments. Instead, the diet with the highest lipid content (salmon oil-supplemented herring) was associated with lower growth rates in body mass, after controlling for variation in daily energy intake (see Fig. 2.6).

If daily energy intake is the primary factor influencing growth and development of nestling guillemots, then we would expect a close relationship between growth rate

and average daily energy consumption. This hypothesis was strongly supported by the results of this study (Fig. 2.6), and predicts that parents that maximize the provisioning of food energy to their nestlings will be favored by natural selection. Consequently, our results support the conclusion that growth in body mass is primarily energy-limited, rather than being limited by the intake rate of biomass, lipid, protein, or some particular prey type. Both the availability (food biomass provisioning rate) and lipid content (energy density) of forage fish influence growth in body mass of nestling guillemots, because availability and lipid content are the two main factors that determine the rate at which food energy is provided to nestlings. Use of smaller or somewhat scarcer prey by parent guillemots could be favored by natural selection if these prey types have a higher lipid content and energy density that can compensate for lower biomass provisioning rates. Use of low-lipid prey by parents could be favored if these prey types are more available to foraging adults and/or are larger, thereby compensating for lower lipid content and energy density. Forage fish that are readily available to foraging adults near their nest and have high lipid contents should provide the optimal diet for breeding pigeon guillemots.

Factors affecting growth in wing length of nestling pigeon guillemots

Despite the close relationship between body mass growth and daily energy intake, there was no relationship between wing length (structural) growth and daily energy intake (Fig. 2.9). These results are in agreement with those from similar captive feeding trials conducted with two other species of fish-eating seabirds, tufted puffins (*Fratercula cirrhata*) and black-legged kittiwakes (*Rissa tridactyla*; Romano 2000), and suggest that structural growth in seabird nestlings is comparatively insensitive to constraints in parental energy provisioning. These results also suggest that (1) the higher growth rate in body mass associated with higher daily energy intake mostly represents higher deposition rates of body fat, and (2) even on restricted diets, young guillemots that preferentially allocate assimilated energy toward structural growth are favored by natural selection. The allocation of limited energy intake toward structural growth over a wide range of energy intake rates is adaptive for chicks to attain sufficient size to fledge and forage independently (there is no post-fledging parental care in pigeon guillemots). This assures that chicks are capable of flight at normal fledging age over a wide range of fledging body masses. Adequate wing development at fledging also may reflect the importance of pursuit-diving ability for foraging immediately after fledging.

The lack of variation in wing length growth across a range of daily energy intakes indicates that nestling wing length is a poor indicator of foraging conditions for parent guillemots during the nestling-rearing period. For nestling pigeon guillemots, rates of structural growth do not decline until daily energy intake is below 540 kJ/day (this study). The estimated parental energy provisioning rates to guillemot broods in the EVOS area always exceeded 540 kJ/day, regardless of the colony or year and associated differences in diet composition (Roby et al. 2000). These results suggest that pigeon guillemots nesting in the EVOS area generally are capable of provisioning energy to broods at a rate sufficient to support maximum rates of structural growth for at least one chick (singleton chicks or alpha chicks in two-chick broods).

The low growth rate in wing length for nestling guillemots fed salmon oil-supplemented diets (Figs. 1.9 and 1.10) was unexpected. We expected that chicks fed this

artificially high-lipid diet would have the highest MECs and would exhibit the highest growth rates in body mass and wing length. The stunted growth of chicks on the salmon oil-supplemented diet indicates that the level of lipid in this diet (35% of dry mass) exceeded the nutritional optimum for nestling guillemots. Nestling guillemots in the wild are fed juvenile herring by their parents that equal or even exceed this lipid content (Anthony et al. 2000). But the diet of nestling guillemots in the EVOS area tends to be diverse (Roby et al. 2000) and average lipid content of daily food intake is certainly less than 35% of dry mass. For example, the average lipid content of juvenile herring caught in PWS during the guillemot nesting season was only 26.8% of dry mass. Also, the average lipid content of adult sand lance, a prevalent, high-lipid guillemot food in the EVOS area (Golet et al. 2000, Roby et al. 2000, Litzow et al. 2002), was only 23.4% (Anthony et al. 2000). The average energy density of prey fed to nestling guillemots in the wild varied from 4.2 to 5.0 kJ/g wet mass, dependent on collection year and location (Roby et al. 2000), whereas the salmon oil supplemented diet had an energy density of 5.8 kJ/g wet mass (Table 2.3). There is some evidence that juvenile herring and adult sand lance are preferred prey types for parent guillemots feeding young, and dietary lipid content in the range of 20-30% of dry mass may be optimal for nestling guillemots.

Interspecific comparisons of factors affecting seabird nestling growth

Most nestling seabirds do not respond to high-lipid diets with stunted growth. Tufted puffins that were raised on diets consisting solely of juvenile herring averaging 36% lipid by dry mass had significantly higher growth rates than puffins raised on equal biomass rations of juvenile pollock that was 26% lipid by dry mass (Romano 2000). Black-legged kittiwakes that were raised on diets of the same high-lipid herring had significantly higher growth rates than kittiwakes raised on equal biomass rations of adult sand lance that was 23% lipid by dry mass (Romano 2000). Some nestling petrels (Order: Procellariiformes) are fed meals by their parents that contain considerable stomach oil, and lipid content frequently exceeds 50% of dry mass (Ricklefs et al. 1987). Thus nestling guillemots appear to be unusually intolerant of high-lipid diets, compared with other seabird nestlings.

Another contrast between the dietary requirements of nestling pigeon guillemots and nestling tufted puffins or black-legged kittiwakes relates to daily intake of food biomass. Nestling guillemots in the present study required at least 126 g/day of food to support growth rates in body mass similar to the lower range in growth rates observed in the wild (Roby et al. 2000), whereas, nestling guillemots that were fed *ad libitum* consumed 190 g/day of food, on average (Table 2.2). Nestling puffins and kittiwakes, on the other hand, grew normally on diets of just 100 g/day of high-lipid juvenile herring (Romano 2000). Most of this discrepancy is due to the higher lipid content and energy density of juvenile herring fed to nestling puffins and kittiwakes (36% of dry mass and 7.2 kJ/g wet mass, respectively). In comparison, the lipid content and energy density of juvenile herring fed to nestling guillemots was only 21% of dry mass and 4.3 kJ/g wet mass, respectively. But the present study indicates that the high level of dietary lipid in herring fed to nestling puffins and kittiwakes is not tolerated well by nestling guillemots. Consequently, the optimal diet for nestling guillemots appears to be higher biomass intake rates of lower lipid forage fishes, compared with other nestling piscivorous seabirds. These dietary requirements for nestling guillemots may have co-evolved with

the neritic foraging distribution and high rates of meal delivery to nestlings by parent guillemots. Nestlings of seabird species that forage further offshore and deliver chick meals at a lower rate evidently have evolved the ability to efficiently digest and assimilate higher lipid diets.

Digestive efficiency in relation to diet

Metabolizable energy coefficients (MECs) measured in the present study support the hypothesis that nestling pigeon guillemots are adapted to diets with a lower lipid content and lower energy density compared to nestlings of other seabird species. The MEC of nestling guillemots fed the salmon oil-supplemented diet (35% lipid of dry mass) was 0.813 ± 0.0076, whereas the MEC for nestling kittiwakes fed a high-lipid herring diet (36% lipid of dry mass) was 0.877 ± 0.0089 (Romano 2000). In contrast, the MEC of nestling guillemots fed the restricted herring diet (21% lipid of dry mass) was 0.822 ± 0.0061, whereas the MEC for nestling kittiwakes fed a restricted low-lipid pollock diet (10% lipid of dry mass) was only 0.732 ± 0.0202 (Romano 2000). The MECs of puffins on high-lipid and low-lipid diets were similar to those of kittiwakes; higher MECs were associated with high-lipid diets, and lower MECs were associated with low-lipid diets (Romano 2000). In nestling guillemots, however, MECs for high-lipid and low-lipid diets were similar and intermediate between the MECs of kittiwakes and puffins on high-lipid and low-lipid diets. This apparent digestive strategy of nestling guillemots would be optimal when average lipid content of food is normally moderate to low, and where meal frequency and biomass intake rates are normally high. As mentioned above, this is consistent with the neritic foraging distribution and high rates of meal delivery to nestlings by parent guillemots.

Previous studies of MECs and assimilation efficiencies in a variety of nestling and adult seabirds demonstrate interspecific and prey-dependent variation ranging from 0.54 to 0.90, with an average of approximately 0.75 (Dunn 1975, Furness 1978, Cooper 1980, Copestake et al. 1982, Heath and Randall 1985, Klassen et al. 1992, Brugger 1993, Brekke and Gabrielsen 1994). Consumption of high-lipid prey is associated with higher MECs than diets of low-lipid prey (Brekke and Gabrielsen 1994, Romano 2000). Furthermore, assimilation efficiencies in poultry have been enhanced by food supplemented with fat (Owings and Sell 1982, Maiorino et al. 1986, Hurwitz et al. 1988). The increased assimilation efficiency generally observed in birds on high-lipid diets may be due in part to the effect of dietary fat in reducing passage rates, thereby increasing retention time for digestion and absorption of nutrients (Brekke and Gabrielsen 1994). Thus, results from the present study on nestling guillemots do not agree with the general trend in birds of increased assimilation efficiency with increased fat content of the diet.

CONCLUSIONS

1. The different experimental fish diets used to raise young guillemots resulted in major differences in growth rate of body mass.
2. Total daily energy intake explained most of the variation in growth rate of body mass; daily food biomass intake, dietary lipid content, dietary protein content, and fish type explained little or none of the variation in body mass growth.

3. Growth rate in wing length (structural growth) varied little among the different experimental fish diets, and there was no relation between daily energy intake and structural growth.
4. Guillemots on a high-lipid diet were slightly stunted in structural growth, suggesting an optimal level of dietary lipid of 20-30% on a dry mass basis.
5. Metabolizable energy coefficients of nestling guillemots varied little with dietary lipid content, biomass intake rate, or energy intake rate.
6. Digestive strategies and growth patterns of nestling pigeon guillemots seem well-adapted for a seabird species that forages nearshore and provisions its nestlings frequently.
7. Productivity of nesting pigeon guillemots may be maximized when schooling forage fishes with lipid contents of 20-30% (dry mass) are abundant near the nest site.
8. Declines in stocks of sand lance, herring, and possibly capelin appear to be the primary factor limiting recovery of pigeon guillemot populations damaged by the *Exxon Valdez* oil spill.

# CHAPTER 3
# EFFECTS OF INGESTED PRUDHOE BAY CRUDE OIL ON BLOOD CHEMISTRY AND GROWTH OF NESTLING PIGEON GUILLEMOTS: DOSE–RESPONSE EXPERIMENTS IN THE LAB


## ABSTRACT

Pigeon guillemot (*Cepphus columba*) populations in Prince William Sound were injured by the *Exxon Valdez* Oil Spill (EVOS) and have failed to recover in the 13 years since the spill. One hypothesis for the lack of recovery is that residual oil from EVOS continues to contaminate guillemot prey (nearshore demersal fishes) and chronic ingestion of petroleum hydrocarbons in food has a negative effect on guillemot health. We fed nestling guillemots small, sublethal doses of weathered Prudhoe Bay crude oil (PBCO) in the lab and then measured several potential biomarkers of effects. These dose-response experiments were designed to (1) better understand the impact on nestling pigeon guillemots of petroleum hydrocarbons in food, (2) calibrate existing and potential biomarkers of exposure to PBCO for nestling guillemots in a controlled, laboratory setting, and (3) develop better nondestructive biomarkers of exposure to PBCO for pigeon guillemots in particular, and seabirds in general. Results of the oil-dosing experiments indicated that captive-reared nestling guillemots were resistant to the effects of small doses of weathered PBCO in food. No nestlings died or suffered noticeable health effects following dosing. The high dose in this study (0.5 ml $kg^{-1}$ $day^{-1}$ for 10 days) was sufficient to induce a fourfold increase in hepatic cytochrome P4501A (a liver enzyme indicative of contaminant exposure). Of the 12 plasma chemistry markers analyzed in oil dosed nestlings, only lactate dehydrogenase (LDH) exhibited a significant dose response compared to controls. However, changes in plasma LDH activity were small, and this biomarker also was influenced by diet and nestling age. There was a transitory, but significant, elevation of plasma haptoglobin, an acute phase protein, in nestlings fed the high dose of PBCO. Baseline corticosterone was not different in oil-dosed chicks compared to controls, but corticosterone was elevated in oil-dosed chicks during acute stress compared to controls. Despite these biochemical responses to ingestion of weathered PBCO, growth rate and fledging mass were not significantly affected, suggesting little or no impact of oil dosing on overall health of captive nestlings. Growth rate does not appear to be a sensitive noninvasive biomarker of chronic, low-level exposure to PBCO in nestling guillemots. Based on this lab study and other studies in the field, it is unlikely that the failure of pigeon guillemots to recover from the EVOS is due to effects on nestling health of residual oil in food.


## INTRODUCTION

Ten years after the *Exxon Valdez* oil spill (EVOS), levels of cytochrome P4501A (CYP1A) in pigeon guillemots (*Cepphus columba*) from Prince William Sound (PWS), Alaska were elevated in liver samples biopsied from adults breeding in oiled sites, compared to adults breeding in non-oiled sites (Golet et al. 2002). CYP1A is a xenobiotic-metabolizing enzyme that can be induced by components of crude oil, such as polycyclic aromatic hydrocarbons (PAHs). This suggests a link between long-term oil contamination from EVOS and biochemical effects on guillemots living in the EVOS

area. Exposure to residual oil by consuming nearshore demersal fish contaminated with petroleum hydrocarbons could negatively affect survival or reproductive success of adult pigeon guillemots. Pigeon guillemots forage primarily in lower intertidal and subtidal benthic habitats, sites that are known to harbor residual oil in sediments in oiled portions of PWS (Hayes and Michel 1999, Carls et al. 2001). Residual oil from EVOS also may affect survival and/or productivity of guillemot prey, thereby affecting guillemot populations indirectly through their food supply.

Exposure of seabirds to crude oil has been demonstrated to have various deleterious effects. External physical exposure has been shown to decrease buoyancy, inhibit flight, increase basal metabolic rate, and cause inflammation (Hartung and Hunt 1966, Lambert et al. 1982, Lee et al. 1985). In addition, oil can be ingested during preening of oiled plumage (Hartung 1963). Chronic ingestion or consumption of large quantities of crude oil can cause a myriad of problems for birds, including reduced rate of growth (Szaro et al. 1978, Peakall et al. 1982), lowered reproductive success (Grau et al. 1977, Trivelpiece et al. 1984), Heinz-body hemolytic anemia (Leighton et al. 1983), damage to internal organs, and death (Peakall et al. 1980, Fry and Lowenstine 1985, Khan and Ryan 1991). Longer-term effects of exposure to small, sublethal amounts of residual petroleum hydrocarbons several years after a spill are, however, poorly known. A particular challenge is detection of this type of exposure in free-ranging seabirds and quantification of health effects (Prichard et al. 1997, Seiser et al. 2000, Golet et al. 2002).

The focus of this study was on developing biomarkers of crude oil exposure in seabirds like pigeon guillemots, and the potential health effects of continued exposure in nestling guillemots to low-level residual oil. While hepatic cytochrome P450 has been used widely as an indicator of exposure in wild birds to crude oil and other xenobiotics (Holland-Bartels 2000, Golet et al. 2002), measurement of this mixed-function oxidase currently requires the collection of liver samples. This necessitates either sacrificing the subject or a potentially risky biopsy procedure that requires trained veterinary personnel (Degernes et al. In press). Hematological biomarkers offer the potential of easily and nondestructively assessing exposure to petroleum hydrocarbons, so that long-term exposure of bird species failing to recover from an oil spill can be monitored. Possible hematological biomarkers of oil exposure are changes that occur in response to ingestion of oil (e.g., decrease in packed cell volume or mean cell volume) and may be related to the health and fitness of the individual (Fry and Lowenstine 1985, Hugget et al. 1992, Peakall and Shugart 1993, Rattner et al. 1996, Newman et al. 1999, Seiser et al. 2000). Biomarkers may be affected by any of the individual constituents of oil (e.g., PAHs) or by any number of environmental or metabolic breakdown products of crude oil. However, the challenge is to associate the relatively nonspecific changes in some of these markers specifically with oil ingestion.

One potential plasma biomarker of oil exposure that has received considerable attention is haptoglobin, an acute phase protein (Johnson et al. 1993, Skinner and Roberts 1994). Field studies of river otters (*Lutra canadensis*) living in the EVOS area indicated that haptoglobin levels were elevated (Duffy et al. 1994). Prichard et al. (1997) dosed wild pigeon guillemot nestlings with weathered Prudhoe Bay crude oil (PBCO) and detected a significant effect on levels of serum haptoglobin. Recent work on pigeon guillemots living in PWS detected no differences in haptoglobin levels between nestlings from the oiled and non-oiled parts of the Sound nine years after EVOS (Seiser et al.

2000). Controlled dose-response experiments should help determine whether levels of haptoglobin in serum are a useful biomarker of exposure to petroleum hydrocarbons in nestling guillemots.

We dosed nestling pigeon guillemot with weathered PBCO in the lab to validate the use of certain potential biomarkers of crude oil exposure in free-ranging guillemots and other nearshore seabirds. We also investigated effects of oil ingestion on nestling growth rates, because growth may be a sensitive indicator of the health and condition of young birds. Various daily energy intake levels were used to help assess the role of diet on oil ingestion effects. Results of the oil dosing experiments allowed (1) examination of potential effects of residual oil ingestion on growth of nestling guillemots, and (2) identification of hematological and hepatic biomarkers for oil ingestion that can be used to assess exposure in wild populations of pigeon guillemots. Because captive-reared guillemots were to be released into the wild as a potential direct restoration technique, destructive sampling as part of the oil dosing experiments was precluded. Thus, total body composition measurements, as well as gross or histopathological investigations of tissue damage associated with oil ingestion, were not options.

## METHODS

### Collection, incubation, and hatching of eggs

Guillemot eggs and hatchlings were collected from nests in the wild in several coastal areas of Alaska (Table 2.1). See the Methods section of Chapter 2 for details on procedures used to collect eggs and hatchlings and transport them from the field to the ASLC, where eggs were hatched and hatchlings raised. Also, see Chapter 2 Methods for details of the protocol for incubating guillemot eggs and hatching them at the ASLC.

Procedures used to raise hatchling guillemots in captivity are described in detail in the Methods section of Chapter 2. Total body mass (± 0.1 g) and wing length (± 1 mm) of chicks were measured daily during the period from hatching until day 30 post-hatch. Newly hatched pigeon guillemots were fed an unrestricted diet of Atlantic silversides (*Menidia menidia*), juvenile walleye pollock (*Theragra chalcogramma*), and juvenile Pacific herring (*Clupea pallasi*) eight times per day until day 11 post-hatch.

### Oil dosing experiments

Beginning on day 11 post-hatch, all chicks that were subjects in the oil dosing experiments were fed diets consisting solely of juvenile herring. All subjects also were switched to four feedings per day on day 11, and daily food intake was increased gradually from day 11 to day 15. By day 15, all subjects were either on the low-intake ration of 126 g/day or the high-intake ration of 158 g/day of juvenile herring. Subjects were fed these daily rations throughout the experimental oil-dosing period, until day 30 post-hatch. Herring fed to both diet groups was from the same shipment and had an average energy density of 4.3 kJ/g wet mass. The low-intake and high-intake diets corresponded to average energy intake rates of 544 kJ/day and 683 kJ/day, respectively (Table 2.2).

The oil used in dosing experiments was weathered PBCO. Oil was weathered in the lab to remove the highly toxic volatile components by mixing a 1:1 ratio of oil and 3.5% NaCl solution together with a magnetic stirrer for one-week prior to the

experimental treatments (Fry and Lowenstine 1985). Weathered PBCO was administered via gel cap inserted in the mouth of a herring and offered as part of the same meal each day that dosing was scheduled for a subject. Controls were fed corn oil in gel caps that were inserted in a herring and fed to subjects in the same manner as the doses of weathered PBCO.

Subjects in the oil dosing experiments were divided into six groups, three groups were fed the high-intake diet in the first year of the study (1999) and three groups were fed the low-intake diet in the second year (2000). Chicks on the high-intake diet were fed either 0.5 ml of weathered PBCO on day 20 post-hatch and again on day 25 post-hatch (low-dose group), 1.0 ml of weathered PBCO on day 20 and again on day 25 (high-dose group), or 0.5 ml of corn oil on day 20 and again on day 25 (control group). The dose per mass ratio was roughly 2:1 for the high-dose:low-dose groups as chicks among the two groups were of similar mass when dosed. The quantity of oil ingested at one meal caused some chicks, especially those in the high-dose group, to regurgitate and/or defecate some oil soon after dosing. To avoid this problem and assure that subjects assimilated most or all the oil added to their food, we modified the oil-dosing protocol in the second year of the study (2000). Chicks on the low-intake ration were fed either a daily dose of 0.125 ml $kg^{-1}$ $day^{-1}$ weathered PBCO from day 16 post-hatch through day 25 post-hatch (low-dose group), a daily dose of 0.5 ml $kg^{-1}$ $day^{-1}$ weathered PBCO from day 16 through day 25 (high-dose group), or a daily dose of 0.125 ml $kg^{-1}$ $day^{-1}$ corn oil from day 16 through day 25 (control group). Thus, the chicks on the low-intake diet were fed less crude oil per meal compared to the chicks on the high-intake diet. The six oil-dosing regimes are shown in Table 3.1.

Blood sample collection

Blood was collected from chicks on the high-intake diet three times: (1) immediately before the first dose was administered on day 20 post-hatch, (2) immediately before the second dose was administered on day 25, and (3) three days after the second dose was administered, on day 28. Blood was collected from chicks on the low-intake diet (where chicks were dosed daily from day 16 through day 25) four times: (1) immediately before the first dose was administered on day 16 post-hatch, (2) after four days of dosing on day 20, (3) at the end of the dosing period on day 25, and (4) three days after the last oil dose, on day 28.

For each blood collection, ca. 0.8 ml of blood was drawn from the medial metatarsal or brachial vein with a 25- or 26-guage needle (needle size depended on chick age). Needles were pre-coated with sodium heparin. Blood samples were collected in 1.0 ml Microtainer brand plasma separator tubes with lithium heparin (Becton Dickinson, New Jersey) to prevent clotting, placed immediately on ice, and centrifuged for 10 min at 3000x *g* to collect plasma. Plasma samples were stored at -20°C or lower until analysis.

Standardized acute stress protocol

In order to assess general stress as measured by corticosterone levels, chicks in both the high-dose/low-intake group and the control/low-intake group underwent an acute stress test series. The acute stress protocol consisted of removing chicks from their containers on day 28 post-hatch, immediately collecting the first blood sample (baseline),

placing the chick in a suspended cloth bag, and collecting additional blood samples at 10 min, 30 min, and 50 min after placing the chick in the bag (Kitaysky et al. 1999). Each blood sample collected during the acute stress series consisted of 100-200 μl drawn via pricks to either the tarsal or basilic veins, with collection by heparinized capillary tubes. Blood samples were then centrifuged at 3000 x *g* for 10 min and the plasma collected. Plasma samples were stored at –20 °C until analysis by Dr. Alexander Kitaysky in the laboratory of Dr. John Wingfield at the University of Washington (Seattle). Corticosterone was quantified in plasma collected during the acute stress protocol, and in plasma collected from chicks prior to administering the first oil dose on day 16 post-hatch (initial baseline). Procedures for radioimmunoassay of corticosterone levels in 20-μl plasma samples are reported in Wingfield et al. (1992).

Liver biopsies for cytochrome P4501A assays

Liver biopsies were performed on six chicks from the high-dose/low-intake group and six chicks from the control/low-intake group. Liver biopsies were performed on day 28 post-hatch, on chicks that were not used in the acute stress test. First, gaseous isoflurane anesthetic was administered to chicks via a precision vaporizer (Machin and Caulkett 1998, 2000). Then feathers were plucked along an approximate 2-3 cm long region just caudal to the xyphoid process of the sternum. After preparing the plucked area with 1% providone iodine solution, a 1.5-2.0 cm incision was made. After applying a hemostat to the tip of one liver lobe, a 0.1-0.4 g slice of liver was excised from the tip with a scalpel blade and the liver sample immediately placed in a cryogenic vial at -80°C. If blood loss was minimal, the incision was closed with 3-0 Viacryl braided absorbable suture. Otherwise, the liver was cauterized, monitored for blood loss, and then the incision was sutured. Chicks were closely monitored for two days following the procedure to ensure that they had recovered from the surgery and were in good health.

Liver samples were analyzed for cytochrome P4501A (CYP1A) levels to determine whether oil dosing had induced this mixed-function oxidase. Liver samples from biopsies were stored at -80°C until analysis in the laboratory of Dr. John Stegeman at Wood's Hole Oceanographic Institution (Wood's Hole, Massachusetts). Liver samples were thawed, homogenized, and microsomal fractions were prepared by differential centrifugation (Stegeman et al. 1979). CYP1A activity was assayed by measuring the CYP1A-dependent catalytic activity of ethoxyresorufin *O*-deethylase (EROD), according to the modified method of Kennedy et al. (1993) using a Cytofluor 2300 fluorescent plate reader (Millipore). CYP1A activity was expressed as the catalyzed EROD activity in pmol $min^{-1}$ $mg^{-1}$.

Haptoglobin analysis

Plasma samples were stored between -20° and -80° C until analysis by Dr. Alexander Scheuerlein in the laboratory of Dr. Martin Wikelski at Princeton University (Princeton, New Jersey). Plasma was analyzed for the acute-phase protein haptoglobin by the method of Tarukoski (1966; modified by Makimura and Suzuki 1982) using the haptoglobin kit from Tridelta Diagnostics, Inc. (Morris Plains, New Jersey). Briefly, the assay is based on the principle that free haptoglobin binds with hemoglobin, and the bound complex maintains peroxidase activity at an acidic pH while incubated at 37 °C. Preservation of the peroxidase activity of hemoglobin is directly proportional to the

amount of haptoglobin present in the sample. A 7.5 μl sample of plasma, 100 μl of bovine hemoglobin, and kit reagent containing *O*-dianisidine were added to a total reaction volume of 200 μl in duplicate reactions. Spectrophotometric readings were taken at 630 nm following a 5-min reaction at 37 °C between the haptoglobin/hemoglobin complex and the chromagenic *O*-dianisidine reagent to quantify the peroxidase activity. Haptoglobin was quantified by fitting the experimental spectrophotometric readings to a standard curve of bovine haptoglobin. Duplicate values were within an average of 11% of one another.

Measurement of plasma chemistry markers

Approximately 100 μl of plasma was used to analyze for levels of the following 12 plasma chemistry markers: calcium, phosphorus, glucose, creatine phosphokinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), uric acid (UA), blood urea nitrogen (BUN), albumin, globulin, cholesterol, and total protein (TP). These plasma chemistry markers were selected for analysis because they are commonly used in clinical settings to detect health effects or tissue damage, especially in the liver where contaminant effects frequently manifest (S.H. Newman, DVM, University of California, Davis, personal communication). Plasma samples were stored between –20°and -80° C until analyses were performed at the Veterinary Medical Teaching Hospital at the University of California (Davis, California). For chicks on the low-intake diet, we also measured packed cell volume (PCV) for each blood sample. PCV frequently is used to screen for anemia, a condition previously described from birds exposed to crude oil (Leighton et al. 1983, Fry and Lowenstine 1985).

Collection protocols for plasma chemistry markers and subsequent analysis were modified between the first year (1999) high-intake diet and the second year (2000) low-intake diet studies (Table 3.1). Oil dosing effects on plasma chemistry markers were investigated for chicks on the high-intake diet by comparing levels of each plasma marker between day 20 (pre-dosing) and day 25 (five days after initial dosing), and between day 20 (pre-dosing) and day 28 (three days after second dosing) for each subject as a function of oil dose treatment (high-dose, low-dose, or control). Oil dosing effects for chicks on the low-intake diet were investigated by comparing levels of each plasma marker between day 16 (pre-dosing) and day 20 (four days after initial dosing), and between day 16 (pre-dosing period) and day 25 (end of dosing period) for each subject as a function of oil dose treatment (high-dose, low-dose, or control). Additional analysis of markers from all sampling ages and for all oil dose treatments was by repeated measures ANOVA.

All protocols for dosing guillemot chicks with weathered PBCO and conducting related experiments and sample collection were reviewed and approved by the Institutional Animal Care and Use Committees for both the ASLC and Oregon State University.

Statgraphics Plus (version 4.0) was used for all statistical analyses.

RESULTS

Cytochrome P4501A activity in response to oil ingestion

CYP1A levels were significantly higher for chicks in the high-dose/low-intake

group (n=6) three days after the last oil dose (day 28 post-hatch), compared to controls (n=6, $F_{1,9} = 52.12$, $P < 0.00005$; Fig. 3.1). Average CYP1A activity in the oil- dosed chicks ($20.18 \pm 2.19$ pmol $min^{-1}$ $mg^{-1}$) was nearly four times higher than that of controls ($5.67 \pm 0.34$ pmol $min^{-1}$ $mg^{-1}$). These results for a subsample of oil- dosed and control chicks indicated that oil dosing induced elevated levels of the mixed-function oxidase CYP1A, at least in chicks that were fed the high-dose of weathered PBCO. Measurement of this biomarker of exposure to crude oil, however, requires either a highly invasive biopsy procedure or necropsy in order to obtain liver samples. Collection of blood samples for analysis of potential blood and plasma biomarkers of exposure is, by comparison, minimally invasive and much easier.

### Plasma chemistry markers

The levels of three plasma chemistry markers (lactate dehydrogenase [LDH], creatine phosphokinase [CK], and albumin) in nestling guillemots were affected by ingesting weathered PBCO (Table 3.2). We used Kruskall-Wallis statistics to test for significant oil dose effects on plasma chemistry markers when there were significant differences among sample variances (Cochran's C test, $P < 0.05$). In the dose-response experiment where chicks were on the low-intake diet, there was a significant oil effect on plasma levels of LDH between day 16 (pre-dose) and day 20 (four days after dose initiation; Kruskall-Wallis = 6.65, $P = 0.036$), and between day 16 and day 25 (end of oil dosing; Kruskall-Wallis = 8.07, $P = 0.018$; Fig. 3.2). Because the levels of LDH in low- and high-dose chicks were statistically indistinguishable (repeated measures ANOVA, $F_{1,21} = 0.10$, $P = 0.75$), these doses were combined into an "oiled" response and compared with levels of LDH in control chicks. Control LDH levels were significantly different from the "oiled" levels over the entire time course (days 16-28; repeated measures ANOVA on log-transformed variables, $F_{1,21} = 4.42$, $P = 0.040$).

In the dose-response experiment where chicks were on the high-intake diet, there was a significant oil effect on plasma LDH between day 20 (pre-dose) and day 25 (five days after initial dose; $F_{2,17} = 3.89$, $P = 0.041$), and a marginally significant effect on LDH between day 20 and day 28 (three days after final dosing; $F_{2,18} = 3.18$, $P = 0.066$). Repeated measures ANOVA with all sampling ages, however, revealed no significant differences in LDH levels among chicks in the three dose treatments ($F_{2,19} = 0.49$, $P = 0.62$), or between oil-dosed and controls ($F_{1,14} = 0.66$, $P = 0.43$). In both experiments, LDH levels in oil-dosed chicks were lower than those of controls (Table 3.2).

Plasma albumin showed a significant oil effect between day 16 and day 25 for chicks on the low-intake diet (Kruskall-Wallis = 6.35, $P = 0.042$; Fig. 3.3a). The effect of ingested oil on plasma albumin was not, however, dose-dependent; plasma albumin increased more from day 16 to day 25 for chicks in the low-dose group than for either the high-dose or control group (Fig. 3.3a). Creatine phosphokinase (CK) was the only other plasma marker that exhibited a significant oil effect on the low-intake diet; specifically, by repeated measures ANOVA between the "oiled" (combined high- and low-doses) and control groups ($F_{1,21} =$
4.40, $P = 0.048$; Fig. 3.3b). Effects of ingested oil on CK showed no specific dose-dependent trends (Fig. 3.3b). The other nine plasma chemistry markers (calcium, phosphorus, glucose, cholesterol, blood urea nitrogen [BUN], uric acid [UA], globulin, aspartate aminotransferase [AST], and total protein [TP], plus packed cell volume [PCV])

exhibited no significant oil-dose effects for chicks on either the low-intake or high-intake diets (repeated measures MANOVA, $P > 0.05$; Table 3.2).

Several plasma chemistry markers exhibited significant age effects in control chicks. Globulin, albumin, TP, UA, AST, cholesterol (repeated measures ANOVA, $P < 0.005$; Table 3.3) and glucose (repeated measures ANOVA, $P = 0.03$; Table 3.3) all differed significantly from day 16 to day 28 among control chicks on the low-intake diet. LDH levels were significantly skewed for control chicks on the low-intake diet, so values were log-transformed before analysis for age effects. After log-transformation, the difference in LDH from day 16 to day 28 for control chicks was also significant (repeated measures ANOVA, $P = 0.020$). LDH, CK, globulin, albumin, and AST all differed significantly from day 20 to day 28 for control chicks on the high-intake diet (repeated measures ANOVA, $P < 0.05$).

We compared plasma chemistry markers in control chicks between those on the high-intake and low-intake diets to gain insight into the effects of diet on plasma markers. Cholesterol, CK, AST, and UA exhibited significant diet effects on days 20, 25, and 28 post-hatch (ANOVA, $P < 0.05$; Table 3.3). Log-transformed LDH levels for control chicks were significantly different between diet treatments on day 28 (ANOVA, $P = 0.048$). Levels of AST in plasma increased with age for chicks on both the low-intake and high-intake diets, but the level of AST and the rate of increase with age were higher for chicks on the low-intake diet (Table 3.3).

Haptoglobin

Plasma haptoglobin levels in chicks on the low-intake diet suggested a dose response (Fig. 3.4a). The difference between mean plasma haptoglobin on day 20 (four days after the initiation of oil dosing) and on day 16 (pre-dosing) was significantly greater for chicks on the high-dose oil treatment, compared to controls (Kruskall-Wallis statistic = 4.94, $P = 0.026$; Fig. 3.4a). This oil-dosing effect was transitory; by day 25 (last day of the 10-day dosing period) there was no indication of an oil dosing effect on plasma haptoglobin. Plasma haptoglobin in chicks on the high-intake diet exhibited no trend toward an oil dosing effect (Fig. 3.4b). Mean plasma haptoglobin on day 25 (five days after the initial dosing) was not different than on day 20 (pre-dosing) for chicks on the low- dose and high-dose oil treatments, compared to controls (Kruskall-Wallis statistic = 3.13, $P = 0.21$; Fig. 3.4b). The absence of an oil-dose effect on haptoglobin levels for chicks on the high-intake diet is likely due to the differences in oil dosing regime compared to chicks on the low-intake diet. Chicks on the high-intake diet were fed comparatively large volumes of weathered PBCO just twice, and several days elapsed between oil ingestion and plasma collection for analysis of haptoglobin levels. Based on the results from the dose-response experiment with chicks on the low-intake diet, where chicks were fed crude oil daily for 10 days, the induction of plasma haptoglobin following ingestion of PBCO is modest and short-lived. This suggests that haptoglobin induction could have been easily missed in chicks on the infrequent dosing and plasma-sampling regime (high-intake diet), if it occurred at all.

Effects of oil dose on chick growth

Growth rate potentially integrates a large number of factors that influence the health, condition, and fitness of nestling birds. It is also a potential non-specific

biomarker of contaminant effects that can be easily and non-invasively measured by periodically weighing and measuring chicks. Because growth rates are sensitive to nutritional constraints, we analyzed chicks on the high-intake and low-intake diets separately for effects of oil dosing on growth rates (Figs. 3.5, 3.6, 3.7, 3.8). No differences in body mass increment (growth rate) were exhibited over the oil-dosing period among the three oil dosing groups (high-dose, low-dose, control) on either the low-intake diet ($F_{2,27} = 0.16$, $P = 0.86$) or the high-intake diet ($F_{2,49} = 0.87$, $P = 0.43$; Figs. 3.5 and 3.7). Similarly, no differences in wing length increment (structural growth) were exhibited among the three oil dosing groups on either the low-intake diet ($F_{2,27} = 0.40$, $P = 0.67$) or the high-intake diet ($F_{2,49} = 1.03$, $P = 0.37$; Figs. 3.6 and 3.8). These results suggest that the oil doses administered in these experiments had no apparent effect on the health and body condition of captive-reared chicks.

Corticosterone as a stress marker for oil ingestion

Baseline levels of corticosterone for chicks on the low-intake diet were not significantly different among oil dosing groups prior to the onset of dosing on day 16 post-hatch ($F_{1,21} = 0.80$, $P = 0.38$; Fig. 3.9). Baseline levels of corticosterone also were not significantly different between oil-dosed and control chicks on day 28, three days after the end of the oil dosing experiment ($F_{1,21} = 1.88$, $P = 0.19$). Baseline corticosterone levels from chicks fed the low and high doses of weathered PBCO were combined because they were not statistically distinguishable ($F_{1,12} = 0.02$, $P = 0.90$; Fig. 3.9).

All chicks responded to a standardized acute stress with a rapid increase in secretion of corticosterone (Fig. 3.9). At all time points during the acute stress test, corticosterone levels from chicks on the low-dose and high-dose oil treatments were indistinguishable (e.g., at 30 min.: $F_{1,12} = 0.00$, $P = 0.96$; Fig. 3.9). Consequently, chicks subjected to the two different doses were combined into a single oil-dosed group for further analyses. In controls, corticosterone levels peaked at 10 min post-stress initiation, whereas in oil-dosed chicks corticosterone levels peaked at 30 min post-stress initiation (Fig. 3.9). Acute stress-induced levels of corticosterone were significantly higher in oil-dosed chicks than in control chicks at 30 min ($F_{1,21} = 5.92$, $P = 0.024$; Fig. 3.9), as well as over the duration of the entire stress series (repeated measures MANOVA, $F_{1,15} = 4.81$, $P = 0.045$).

## DISCUSSION

### Hepatic cytochrome P4501A

It is well documented that the family of cytochrome P450s is induced by various classes of xenobiotics (Hakkola et al. 1998). Specifically, cytochrome P4501A (CYP1A) is induced by planar halogenated aromatic hydrocarbons and the family of polycyclic aromatic hydrocarbons (PAHs), including many components found in crude oil (Hankinson 1995, Hahn and Stegeman 1994, Van Veld et al. 1997). Thus, CYP1A is considered a good biological indicator for exposure to crude oil, or other compounds with similar chemical composition. CYP1A levels are significantly elevated in livers of animals living in oiled environments (Trust et al. 2000, Holland-Bartels 2000), as well as other contaminated sites (Spies et al. 1982, Stegeman et al. 1987).

Ten years after the EVOS, adult pigeon guillemots from an oiled site in PWS showed significantly higher CYP1A activity than adults from a non-oiled site in PWS (Golet et al. 2002). CYP1A levels in nestlings from the same two sites, however, were not significantly different (Golet et al. 2002). Although adults from the oiled site appeared to still be exposed to PAHs, presumably from residual PBCO, nestlings were either avoiding exposure or were not as sensitive to PAHs in food (Golet et al. 2002).

Levels of CYP1A in control chicks from the present study ($5.67 \pm 0.34$ pmol $min^{-1}$ $mg^{-1}$) were similar to those in nestlings from both the non-oiled and oiled portions of PWS ($4.1 \pm 0.4$ pmol $min^{-1}$ $mg^{-1}$ and $4.7 \pm 0.5$ pmol $min^{-1}$ $mg^{-1}$, respectively; Golet et al. 2002). The induction of CYP1A in captive chicks dosed with crude oil ($20.18 \pm 2.19$ pmol $min^{-1}$ $mg^{-1}$) indicates that CYP1A levels in nestling guillemots may serve as a reliable marker of crude oil exposure. None of the wild nestling guillemots from PWS had such high CYP1A levels (Golet et al. 2002).

While levels of CYP1A appear to be a sensitive marker of exposure to weathered PBCO, current methods to measure CYP1A require tissue sampling by liver biopsy, a highly invasive surgical procedure that is particularly difficult in the field. As invasive as this procedure is, it can be accommodated in wild adult birds with minimal mortality when practiced by trained veterinary staff (Degernes et al. In press).

Responses of plasma chemistry markers to oil exposure

Attempts to identify biological markers for ingestion of weathered crude oil were motivated by the need for a simple, unequivocal method to assess exposure in wild vertebrates that is inexpensive, comparatively easy and straightforward, and involves little risk to subjects. Collection of blood samples meets these criteria and a whole suite of blood and plasma constituents and characteristics can be examined for potential responses to crude oil ingestion. However, plasma chemistry markers that are strongly and consistently associated with low-level, chronic exposure to crude oil have yet to be identified.

Only three of the 12 plasma chemistry and hematological markers measured in this study were significantly affected by ingestion of weathered PBCO, and two of these (albumin and CK) did not exhibit dose-response effects (Fig. 3.3). LDH, the only plasma marker that exhibited dose-response effects, was not strongly affected by oil dosing (Fig. 3.2). None of these three plasma markers has been noted as a potential marker for oil exposure in other studies of birds (Rattner 1981, Leighton 1993, Stubblefield et al. 1995, Prichard et al. 1997). Also, LDH levels in dosed chicks were lower than those of controls. A field study of pigeon guillemots in Prince William Sound found significant differences in LDH between adults from oiled and non-oiled portions of the Sound, but LDH in guillemots from the oiled site was higher than that of guillemots from the non-oiled site (Golet et al. 2002).

LDH is a general metabolic enzyme found in skeletal muscle, heart muscle, liver, kidney, bone, and red blood cells. Clinically elevated levels of LDH are generally reflective of liver damage, but because of the presence of LDH in such a wide variety of organs and tissues, changes in plasma LDH can reflect a variety of maladies (S. H. Newman, DVM, University of California, Davis, personal communication). The lower levels of LDH in oil-dosed guillemot chicks from the present study are difficult to explain, but may reflect impaired hepatic function.

Other experimental studies have shown no effects of oil dosing on plasma chemistry markers in mallards (*Anas platyrhynchos*; Rattner 1981, Stubblefield et al. 1995), alcid chicks (Leighton 1993), or, specifically, nestling pigeon guillemots (Prichard et al. 1997). A field investigation of blood biomarkers in nestling pigeon guillemots from Prince William Sound eight years after EVOS showed that nestlings from the oiled portion of the Sound had significantly lower plasma calcium levels compared to nestlings from the non-oiled portion of the Sound (Seiser et al. 2000). In the present study, however, plasma calcium was not significantly lower in oil-dosed chicks compared to controls. Studies of plasma chemistry markers in adult pigeon guillemots from PWS indicated that levels of AST were significantly elevated in adults from the oiled portion of the Sound eight years (Seiser et al. 2000) and nine years after EVOS (Golet et al. 2002). AST was not significantly elevated in oil-dosed chicks from the present study (Table 3.2). Our study suggests that chronic, low-level exposure of wild nestling guillemots to crude oil in food is unlikely to be detected using standard plasma chemistry markers.

Anemia has been associated with oil ingestion in some birds (Hartung and Hart 1966, Leighton et al. 1983, Fry and Lowenstine 1985). Experimental feeding of weathered PBCO to mallards (Stubblefield et al. 1995) and rhinoceros auklets (*Cerorhinca monocerata*; Newman et al. 1999), however, did not result in anemia. One of the clinical signs of anemia is a reduction in packed cell volume (PCV). The oil-dosed chicks in this study showed no indication of effects on PCV; PCV increased steadily during the oil dosing experiment (day 16 to day 25) in both control and oiled chicks (Table 3.2). Thus, loss of red blood cells was not detected for chicks fed either the low dose (0.125 ml $kg^{-1}$ $day^{-1}$) or high dose (0.50 ml $kg^{-1}$ $day^{-1}$) of weathered PBCO.

Hematological and plasma chemistry responses to age and diet

Age-related and dietary effects on many of these biomarkers confounded the use of plasma chemistry markers from chicks as indicators of exposure to weathered PBCO. In this study, most age-related trends in plasma and blood markers appear to also have been influenced by interactions with differences in energy intake between the low-intake (126 g/day; 543 kJ/day) and the high-intake diets (158 g/day; 683 kJ/day). There is evidence from a variety of bird species that many plasma chemistry and hematological parameters change during nestling development (Vinuela et al. 1991, Starck 1998). In addition, energy intake rates and nutritional status can affect various plasma chemistry markers (Lumeij and Remple 1991, Prichard et al. 1997).

Increasing levels of some plasma chemistry markers with age (AST, albumin, globulin, and TP) and declining levels of others (CK and UA) may be indicative of functional maturation of a number of organs that assist in the transport of assimilated nutrients to protein and fat deposits. The liver, in particular, is involved in the synthesis or processing of several of these plasma constituents (AST, LDH). CK is produced in muscle tissue and changing levels in plasma with age may reflect changes in the rate of muscle deposition.

Changes in plasma chemistry markers may also result from specific physiological or biochemical changes due to diet. The altered levels of some plasma constituents (AST, LDH, cholesterol, and UA) could reflect dietary differences that influence liver activity. Uric acid is a by-product of amino acid metabolism and may indicate increased

catabolism of proteins for energy in chicks on the low-intake diet. The lower levels of cholesterol in chicks on the low-intake diet may be a direct reflection of lower energy intake rates in these chicks, compared to those on the high-intake diet.

Comparison of plasma chemistry between wild and captive nestlings

Many differences were found between hematological and plasma chemistry markers of nestling guillemots raised in the lab (this study) and those in the wild (Seiser et al. 2000). Comparisons of plasma markers were made between captive chicks (low-intake diet) on day 20 post-hatch vs. wild chicks aged ca. 20 days, and between captive chicks on day 28 vs. wild chicks aged ca. 30 days. Levels of AST, CK, LDH, TP, and calcium were similar between captive and wild chicks at both ages. Plasma phosphorus levels were similar between captive and wild chicks at age 20 days post-hatch, but approximately 30% higher in captive chicks on day 28, compared to wild chicks at 30 days post-hatch. Uric acid and PCV levels were lower in captive chicks than in wild chicks at both ages (40-50 % and 17-18 % lower, respectively), which may indicate either a higher level of hydration or anemia in captive chicks compared to those in the wild. Plasma globulin levels of captive chicks were approximately one third that of wild chicks, which may be explained by the increased antigenic stimulation of wild chicks. Albumin levels declined with age in wild chicks (1.94 g/dL and 0.70 g/dL for age 20 days and 30 days post-hatch, respectively), but remained fairly stable with age in captive chicks at ca. 1.2 g/dL. These comparisons suggest that many factors may affect hematology and plasma chemistry markers in nestling guillemots. The largely controlled environment of the lab and the regular schedule of consistent feeding versus the variable environment in the wild may contribute to these differences.

Haptoglobin as a biomarker of oil exposure

The acute phase protein haptoglobin did not exhibit a pronounced dose-response effect in guillemot chicks dosed with weathered PBCO. Induction of haptoglobin was indicated by a significant elevation in plasma levels that was transitory and occurred only in the high-dose group. Based on the results from our laboratory experiments, it is not surprising that Seiser et al. (2000) detected no significant differences in plasma haptoglobin between oiled and non-oiled portions of PWS in either nestling or adult pigeon guillemots, regardless of whether exposure to residual PBCO was occurring.

Our results on haptoglobin induction were similar to those of dose-response experiments with captive river otters (Ben-David et al. 2001); in their study, plasma haptoglobin levels varied in complex and unpredictable ways to chronic low-level dosing with weathered PBCO. As Ben-David et al. (2001) point out, a whole suite of heme-related biomarkers can be used to investigate oiling effects in animals (e.g., haptoglobin, cytochrome P450, globulin, albumin) and these biomarkers may be involved in opposing processes *in vivo*. Although Prichard et al. (1997) detected a significant effect of oil dosing on serum haptoglobin in wild nestling guillemots, oil-dosed nestlings had lower levels of serum haptoglobin compared to controls. The lower levels of serum haptoglobin could have reflected a rebound and overshoot after induction of haptoglobin by oil ingestion five days earlier, or may have been due to the confounding effect of dietary differences between oil-dosed and control chicks in the wild (Prichard et al. 1997). Our results from oil dosing experiments in the laboratory do not support the hypothesis of a

relationship between diet and plasma haptoglobin, as control chicks on the high-intake and low-intake diets had similar levels of plasma haptoglobin (Fig. 4a and b). These results taken together indicate the difficulties inherent in using plasma haptoglobin as an indicator of exposure to crude oil in guillemot nestlings.

Oil exposure and chick growth rates

Growth rates are a noninvasive bioindicator of nestling health status and condition. Nestling growth rate and peak mass integrate a wide array of factors, including food intake, nutritional quality of the diet, parental care, thermostatic costs, parasite load, disease, and contaminant exposure (Massias and Becker 1990, Emms and Verbeek 1991, Butler and Lukasiewicz 1979**,** Golet et al. 2000). The feeding experiments described in Chapter 2 demonstrated the major role of daily energy intake in explaining variation in growth of nestling body mass. The growth rates in body mass of control chicks on the low-intake and high-intake diets used in the present study were significantly different (see Chapter 2), reflecting the differences in daily energy intake of chicks on the two diets. Nevertheless, oil dosing did not cause a reduction in body mass growth for chicks fed either the high-intake or low-intake diets. These results indicate that the oil dosing treatments did not cause a reallocation of assimilated energy and nutrients toward detoxifying, metabolizing, and excreting the ingested crude oil sufficient to limit deposition of new tissue in growing nestlings.

Other studies suggest that the growth of nestling guillemots is resistant to low levels of exposure to weathered PBCO. The overall growth rates in body mass of nestling guillemots from Naked Island (oiled portion of PWS) and Jackpot Island (non-oiled portion of PWS) were not significantly different (Golet et al. 2002). Controlled dose-response experiments with wild nestling pigeon guillemots in Kachemak Bay, Alaska resulted in a non-significant trend toward a reduction in body mass growth ($P = 0.0997$), but interpretation was confounded by the natural variability in nestling food intake (Prichard et al. 1997).

The lack of an oil dosing effect on growth rates of either body mass or wing length may be due to two principal factors: (1) the doses administered were too small to result in a physiological response or (2) the stage of nestling development when the dosing occurred was after the period of rapid growth (first half of the nestling period), when nestlings may be especially sensitive to contaminant exposure.

Corticosterone responses to ingestion of weathered crude oil

Corticosterone levels are a general measure of stress in chicks (Etches 1976). Elevated baseline levels of plasma corticosterone in birds have been linked to nutritional stress in particular (Axelrod and Reisine 1984, Cherel et al. 1988, 1992, Wingfield 1994). Previous studies have documented different responses in baseline corticosterone levels to crude oil exposure: (1) increases in externally-oiled penguins (Fowler et al. 1995) and oil-dosed nestling seabirds (Peakall et al. 1981), (2) declines in oil-dosed mallards (Gorsline and Holmes 1982), and (3) no difference in corticosterone levels in adult pigeon guillemots from oiled and non-oiled portions of PWS nine years after EVOS (Golet et al. 2002).

In this study, no differences were seen in baseline levels of corticosterone in nestling guillemots between pre-dosing and after a daily dosing regime for 10 days. This

suggests that baseline corticosterone levels may not be useful as indicators of low-level, chronic exposure to weathered PBCO. Wingfield (1994) points out, however, that the temporal secretion of corticosterone in subjects exposed to a standard stress protocol involving capture, handling, and restraint can be a sensitive indicator of stress. Oil-dosed guillemot chicks in this study showed a nearly two-fold greater response in corticosterone secretion following 30 min of a standardized acute stressor, compared to controls (16.3 ± 2.0 ng/ml and 8.7 ± 2.5 ng/ml, respectively).

The timing and magnitude of response in plasma corticosterone to application of an acute stress protocol differed between nutritionally stressed black-legged kittiwake (*Rissa tridactyla*) chicks and oil-dosed guillemot chicks in the present study. Peak corticosterone levels in food-deprived kittiwake chicks occurred 10 min after application of the acute stressor and peaked at approximately five times that of controls (Kitaysky et al. 1999), compared to 30 min and two times that of controls of the guillemot chicks in the present study. This implies that levels of plasma corticosterone in seabird nestlings are either more sensitive to nutritional stress than the stress of oil ingestion, or oil doses fed to guillemot chicks were less stressful than the food restriction applied to the kittiwake chicks. Regardless, the effects of crude oil ingestion on adrenocortical function and plasma corticosterone may be masked in the wild by a suite of environmental stressors, such as food, weather, predators, parasites, or other contaminants.

## CONCLUSIONS

1. Levels of hepatic cytochrome P4501A were the most sensitive biological marker of ingestion of weathered PBCO in nestling pigeon guillemots, among the markers examined in this study. Measurement of this biomarker, however, required surgery to obtain tissue samples from the liver.
2. Baseline levels of plasma corticosterone were not a useful biomarker of oil exposure in nestling guillemots, but levels of plasma corticosterone during a standardized acute stress were indicative of oil exposure.
3. Some standard plasma chemistry markers in nestling guillemots were significantly affected by oil dosing (i.e., LDH, CK, and albumin); these plasma chemistry markers also were affected by diet and chick age.
4. The dose response of the acute phase protein haptoglobin was only marginal and temporary during a 10-day dosing regime with weathered PBCO, suggesting that this potential biomarker of crude oil exposure is relatively insensitive.
5. Doses of weathered PBCO fed to captive-reared guillemot chicks did not affect growth in body mass or wing length; it is unlikely that growth parameters for nestling guillemots in the field could serve as a sensitive bioindicator of chronic, low-level exposure to petroleum hydrocarbons in food.
6. The biomarkers that were significantly affected by oil dosing in this study are not specific markers of crude oil ingestion. Detecting chronic exposure of nestling guillemots to crude oil in the environment will require a weight of evidence approach.
7. The results of this controlled laboratory experiment, field studies in the EVOS area (Seiser et al. 2000, Golet et al. 2002), and a dose-response experiment in the field (Prichard et al. 1997) suggest that, while pigeon guillemots in the EVOS area continue to be exposed to low levels of PBCO in food, recovery of guillemot

populations injured by EVOS is not limited by the health effects on nestling guillemots of ingesting residual oil.

# CHAPTER 4: CONCLUSIONS AND SYNOPSIS

Based on the captive growth study, total daily energy intake is the principle dietary factor that explains most of the variation in body mass growth of pigeon guillemot chicks. Dietary lipid content, protein content, daily biomass intake, and fish type explained little to none of the variation in body mass growth.

Structural (wing) growth was not affected by daily energy intake over the range used in our study (514-819 kJ/day). However, the diet with the highest lipid content (salmon oil-supplemented) resulted in a slight retardation of wing growth, suggesting that there may be an optimal level of dietary lipid intake (20-30% of dry mass) for structural growth in pigeon guillemot chicks.

The fact that metabolizable energy coefficients (MECs) of chicks did not vary significantly among chicks fed diets differing in biomass, dietary lipid content, and energy intake indicates that guillemot chicks may have a digestive strategy well adapted to a wide range of diets. This contrasts with studies on other seabirds that indicate a positive correlation between lipid content of prey and MECs (Brekke and Gabrielson 1994, Romano et al. 2000). Pigeon guillemot chicks appear to be adapted to diets with a lower lipid content and lower energy density than nestlings of other seabird species. This coupled with a high provisioning rate to broods (relative to other seabirds), indicates the predilection for guillemot chick diets to have higher biomass and lower energy density than those of other alcid chicks.

Results indicate that low levels of crude oil ingestion by guillemot chicks are unlikely to cause significant effects on growth parameters. Furthermore, the only biochemical factor apparently affected solely by oil ingestion was hepatic CYP1A. Other biochemical parameters that showed strong correlations to oil dosing (lactate dehydrogenase, creatine kinase, and albumin) were affected by other factors in this study, such as age and diet, or by diet alone in other seabird studies (corticosterone; Kitaysky et al. 1999).

Although residual oil continues to be present in PWS over a decade after the *Exxon Valdez* Oil Spill (Carls et al. 2001), this would appear to have a minimal impact on growth rates of wild guillemot chicks based on our captive oil dosing trials. Thus, a direct effect of residual oil on chick health and nest productivity appears unlikely. This does not rule out indirect effects of residual oil on guillemot populations due to the potential effects of crude oil in sediments on forage fish populations. Instead, chick growth rates appear to be more sensitive to daily energy intake by chicks. As long as forage fish are available to provide adequate energy intake, wild guillemot chicks should be able to maintain growth parameters sufficient for fledging. This supports the hypothesis that a regime shift in the northern Gulf of Alaska may have negatively affected wild guillemot populations primarily by decreasing availability of prey fishes (Hare and Mantua 2000).

Evidence from this study indicates that although the focus on residual oil in Prince William Sound is justified, it probably is not directly responsible for the failure of pigeon guillemot populations to recover to pre-spill numbers in the Sound. Shifts in the species, numbers, and availability of forage fishes to guillemot populations, coincident with the timing of EVOS (Hare and Mantua 2000), are likely responsible for the failure of the PWS guillemot populations to recover during the post-spill years.

Attempts to find biomarkers specific to petroleum ingestion have so far been unsuccessful in any species studied. Some biomarkers are positively and directly linked to the ingestion of oil (or other PAHs), such as hepatic CYP1A; some are linked positively and indirectly to oil ingestion, such as corticosterone and lactate dehydrogenase. Based on this study, no conclusive biomarker or suite of biomarkers are available to indicate specific exposure to oil ingestion. The best evidence available for ingestion of low levels of crude oil ingestion is elevated hepatic CYP1A levels, which could also be present due to exposure to a number of other toxins in the environment (i.e., PAHs and halogenated hydrocarbons). So many other factors (e.g., diet and age) can potentially affect most of the plasma constituents that their response in the field would be very difficult to interpret.

A number of heme-related molecules have received attention as potential biomarkers for oil ingestion or PAH exposure in different animal species (Miranda et al. 1987, Stegeman et al. 1992, Duffy et al. 1994). These molecules include hemoglobin, haptoglobin, cytochrome P450, porphyrins, globulins, and albumin. Complex biochemical interactions between these molecules *in vivo* may easily conceal any specific oil-induced effects on their levels (Ben-David et al. 2001). Haptoglobin specifically has received attention in the last ten years as a potential biomarker of oil ingestion. Any level of hemolysis, as potentially triggered by the presence of PAHs in the bloodstream, can trigger alterations in the levels of hemoglobin-binding haptoglobin. Levels of free haptoglobin in plasma depend upon the levels of haptoglobin-hemoglobin complex, the 'recycling' rate of the haptoglobin-hemoglobin complex, and the induction level of new haptoglobin. These interactions, and others, make interpretation of the levels of haptoglobins, as well as the other heme-related molecules, difficult. Small and transitory changes in haptoglobin levels were found in the guillemot chicks dosed with crude oil. This could be due to a lack of hemolysis at the oil doses tested or to the complex biochemistry involved.

Further studies are needed to identify adequate biomarkers of oil ingestion. The development of an assay for lymphocytic CYP1A using quantitative reverse transcriptase polymerase chain reaction (RT-PCR) methods may be one of the most promising methods being developed. Problems with this method stem from difficulties stabilizing lymphocyte RNA under field conditions by immediate ultracold storage, maintenance of sterile conditions required for high-quality RNA isolation, followed by subsequent problems with quantitation inherent in the technique (Nakamoto et al. 2000, Roy et al. 2002). Other promising markers may be the PAHs themselves in bile, serum, or in the form of intracellular protein-PAH adducts (Downs et al. 2002, Jewett et al. 2002, Ziccardi et al. 2002). Problems with these techniques include 1) each geographically distinct crude oil has different biophysical and chemical profiles that could lead to problems interpreting *in vivo* PAH levels (Roussis and Fitzgerald 2000); 2) detection of biliary fluorescent activated compounds involves a highly invasive procedure; and 3) limited application or reliability of application of techniques to birds.

The most effective methods of determining low-level chronic exposure to oil likely will involve the use of a suite of biological markers, including cytochrome P4501A. More studies are needed to ferret out appropriate markers and how they could best be used.

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Table 2.1. Numbers of pigeon guillemot eggs and hatchlings collected, hatching success, and fledging success by collection location during the years 1998-2000.

| YEAR | LOCATION | EGGS COLLECTED | EGGS HATCHED (% Success) | CHICKS COLLECTED | CHICKS RAISED (% Success)* |
|---|---|---|---|---|---|
| **1998** | **PRINCE WILLIAM SOUND** | **10** | **5 (50)** | | **5 (100)** |
| | Fool Island | 6 | 1 (16.7) | | 1 (100) |
| | Mummy Island | 4 | 4 (100) | | 4 (100) |
| | **KACHEMAK BAY** | **11** | **6 (54.5)** | | **6 (100)** |
| | **SOUTHEAST ALASKA** | **22** | **12 (54.5)** | **2** | **12 (85.7)** |
| | Bird Island | 22 | 12 (54.5) | 2 | 12 (85.7) |
| | **TOTAL** | 43 | 23 (53.5) | 2 | 23 (92) |
| **1999** | **PRINCE WILLIAM SOUND** | **36** | **27 (75.0)** | | **19 (70.3)** |
| | Fool Island | 12 | 11 (91.7) | | 4 (36.4) |
| | Naked Island | 13 | 8 (61.5) | | 7 (87.5) |
| | Icy Bay | 11 | 8 (72.7) | | 8 (100) |
| | **KACHEMAK BAY** | **9** | **1 (11.1)** | **12** | **11 (84.6)** |
| | **SOUTHEAST ALASKA** | **13** | **11 (84.6)** | **7** | **14 (77.8)** |
| | Bird Island | 13 | 11 (84.6) | 7 | 14 (77.8) |
| | **KODIAK ISLAND** | **12** | **10 (83.3)** | **9** | **16 (84.2)** |
| | Mary Island | 5 | 3 (60) | 9 | 11 (91.7) |
| | Popoff Island | 6 | 6 (100) | | 4 (66.7) |
| | Blodgett Island | 1 | 1 (100) | | 1 (100) |
| | **TOTAL** | **70** | **49 (70)** | **28** | **60 (77.9)** |

Table 2.1 (continued)

| | | | | | |
|---|---|---|---|---|---|
| **2000** | **PRINCE WILLIAM SOUND** | **22** | **16 (72.7)** | | **16 (100)** |
| | Fool Island | 6 | 6 (100) | | 6 (100) |
| | Naked Island | 4 | 2 (50) | | 2 (100) |
| | Icy Bay | 8 | 7 (87.5) | | 7 (100) |
| | Jackpot Island | 4 | 1 (25) | | 1 (100) |
| | **SOUTHEAST ALASKA** | **34** | **21 (61.8)** | | **20 (95.2)** |
| | Couverdon Island | 8 | 6 (75) | | 5 (83.3) |
| | Midway Island | 26 | 15 (57.7) | | 15 (100) |
| | **KODIAK ISLAND** | **37** | **35 (94.6)** | **7** | **37 (88.1)** |
| | Mary Island | 24 | 23 (95.8) | 5 | 25 (89.2) |
| | Blodgett Island | 8 | 8 (100) | | 7 (87.5) |
| | Popof Island | 5 | 4 (80) | 2 | 5 (83.3) |
| | **TOTAL** | **93** | **72 (77.4)** | **7** | **73 (92.4)** |
| | **PRINCE WILLIAM SOUND** | **68** | **48 (70.6)** | **0** | **40 (83.3)** |
| | **KACHEMAK BAY** | **20** | **7 (35)** | **12** | **17 (89.4)** |
| | **SOUTHEAST ALASKA** | **69** | **44 (63.8)** | **9** | **46 (86.8)** |
| | **KODIAK ISLAND** | **49** | **45 (91.8)** | **16** | **53 (86.9)** |
| | **GRAND TOTAL** | **206** | **144 (69.9)** | **37** | **156 (86.2)** |

*Total number of hatched eggs and collected hatchlings that were successfully raised to fledging age (day 30 post-hatch).

Table 2.2. Experimental diets fed nestling pigeon guillemots raised in captivity at the Alaska SeaLife Center.

| Diet | No. of Chicks | Year | Daily intake (g) [1] | Energy Density (kJ/g wet mass) | Energy Intake Rate (kJ/day) | Energy Intake in Lipid (%) |
|---|---|---|---|---|---|---|
| Herring/low-lipid | 7 | 1998 | 181.0 ± 0.62 | 3.37 ± 0.16 | 610 | 17.6 |
| Herring/high-lipid | 8 | 1999 | 158.4 ± 0.46 | 4.31 ± 0.12 | 683 | 43.1 |
| Herring/*ad libitum* | 21 | 1999/2000 | *Ad lib.* (190 ± 2.52)[1] | 4.31 ± 0.12 | 819 | 43.1 |
| Herring/low-intake | 23 | 2000 | 126.2 ± 0.16 | 4.31 ± 0.12 | 544 | 43.1 |
| Herring/+ salmon oil | 10 | 2000 | 123.5 ± 0.58 (+ 6 ml) | 5.82 ± 0.12 | 751 | 58.7 |
| Pollock/low-lipid | 7 | 1998 | 176.3 ± 1.20 | 3.76 ± 0.23 | 663 | 28.6 |
| Pollock/high-lipid | 10 | 1999 | 161.1 ± 0.43 | 5.01 ± 0.11 | 807 | 47.5 |
| Blennies[2] | 2 | 1998 | 177.4 ± 1.56 | 4.06 ± 0.13 | 720 | 17.5 |

[1]Mean ± SE during period from day 15 to day 30 post-hatch.

[2]Crescent gunnels (*Pholis laeta*) and high cockscomb (*Anoplarcus purpurescens*).

Table 2.3. Proximate composition and energy density (kJ/g wet mass) of diet fishes fed to nestling pigeon guillemots in captivity (means ± standard errors).

| Fish Taxa | Year | Water (% wet mass) | Lipid (% dry mass) | AFLDM[1] (% lean dry mass) | Gross Energy Density (kJ/g wet mass) |
|---|---|---|---|---|---|
| Juvenile Pacific herring | 1998 | 78.5 ± 0.74 | 7.0 ± 1.43 | 81.7 ± 0.24 | 3.37 ± 0.16 |
| Juvenile walleye pollock | 1998 | 78.4 ± 0.66 | 12.3 ± 2.25 | 83.1 ± 0.22 | 3.76 ± 0.23 |
| Blennies[2] | 1998 | 75.0 ± 0.34 | 7.1 ± 1.45 | 84.1 ± 0.51 | 4.06 ± 0.13 |
| Juvenile Pacific herring[3] | 1999/2000 | 78.5 ± 0.36 | 21.2 ± 1.15 | 83.1 ± 0.12 | 4.31 ± 0.12 |
| Juvenile walleye pollock | 1999 | 76.6 ± 0.25 | 25.7 ± 1.05 | 86.6 ± 0.21 | 5.01 ± 0.11 |
| Juvenile Pacific herring + salmon oil | 2000 | 75.1 ± 0.36 | 34.8 ± 1.15 | 83.1 ± 0.12 | 5.82 ± 0.12 |

[1] Ash-free lean dry mass, which is approximately 95% protein (Montevecchi et al. 1984).
[2] Crescent gunnels (*Pholis laeta*) and high cockscomb (*Anoplarcus purpurescens*).
[3] These fish were used in the following diets: Her/Low Intake, Her/High Lipid, Her/ Ad Lib, and Her/+ Oil.

Table 3.1. Dosing and blood sampling regimes for captive-reared nestling pigeon guillemots fed weathered Prudhoe Bay crude oil.

| Dosing Group Name | N | Daily Food Intake (g/day) | Crude Oil Dose | Dosing Schedule (days post-hatch) | Total Oil Ingested | Blood Samples (days post-hatch) |
|---|---|---|---|---|---|---|
| Control/High-Intake | 8 | 158 | None | 20, 25 | 0.0 ml | 20, 25, 28 |
| Low-Dose/High-Intake | 12 | 158 | 0.5 ml/day | 20, 25 | 1.0 ml | 20, 25, 28 |
| High-Dose/High-Intake | 9 | 158 | 1.0 ml/day | 20, 25 | 2.0 ml | 20, 25, 28 |
| Control/Low-Intake | 14 | 126 | None | 16 - 25 | 0.0 ml/kg | 16, 20, 25, 28 |
| Low-Dose/Low-Intake | 16 | 126 | 0.125 ml $kg^{-1}day^{-1}$ | 16 - 25 | 1.25 ml/kg | 16, 20, 25, 28 |
| High-Dose/Low-Intake | 15 | 126 | 0.5 ml $kg^{-1}day^{-1}$ | 16 - 25 | 5.0 ml/kg | 16, 20, 25, 28 |

Table 3.2a. Plasma chemistry values for pigeon guillemot chicks during a high-frequency dosing regime (daily from day 16 to day 25 post-hatch) with weathered Prudhoe Bay crude oil.

| | Chick Age (days) | Control Dose | SE | n | Low Oil Dose | SE | n | High Oil Dose | SE | n |
|---|---|---|---|---|---|---|---|---|---|---|
| **Albumin (g/dL)** | 16 | 1.01 | 0.03 | 11 | 0.95 | 0.03 | 11 | 0.98 | 0.03 | 12 |
| | 20 | 1.22 | 0.04 | 10 | 1.14 | 0.03 | 10 | 1.18 | 0.02 | 12 |
| | 25 | 1.15 | 0.03 | 11 | 1.18 | 0.03 | 11 | 1.10 | 0.03 | 12 |
| | 28 | 1.22 | 0.02 | 11 | 1.14 | 0.04 | 10 | 1.15 | 0.04 | 11 |
| **AST (U/L)** | 16 | 131.4 | 11.1 | 11 | 142.2 | 9.0 | 11 | 146.0 | 7.4 | 12 |
| | 20 | 157.6 | 18.7 | 10 | 148.4 | 15.3 | 10 | 156.0 | 10.2 | 12 |
| | 25 | 212.7 | 26.5 | 11 | 260.7 | 51.1 | 11 | 202.5 | 19.7 | 12 |
| | 28 | 264.7 | 33.6 | 11 | 300.8 | 52.9 | 10 | 270.0 | 21.2 | 11 |
| **BUN (mg/dL)** | 16 | 5.6 | 0.4 | 11 | 5.5 | 0.4 | 11 | 5.0 | 0.5 | 12 |
| | 20 | 4.8 | 0.4 | 10 | 4.4 | 0.5 | 10 | 3.7 | 0.3 | 12 |
| | 25 | 4.9 | 0.5 | 11 | 5.3 | 0.4 | 11 | 3.8 | 0.5 | 12 |
| | 28 | 6.2 | 0.6 | 11 | 6.2 | 0.4 | 10 | 5.0 | 0.7 | 11 |
| **Calcium (mg/dL)** | 16 | 10.18 | 0.15 | 11 | 10.16 | 0.16 | 11 | 9.90 | 0.14 | 12 |
| | 20 | 9.96 | 0.20 | 10 | 9.84 | 0.17 | 10 | 9.88 | 0.18 | 12 |
| | 25 | 10.38 | 0.17 | 11 | 10.34 | 0.14 | 11 | 10.33 | 0.12 | 12 |
| | 28 | 10.03 | 0.17 | 11 | 10.34 | 0.23 | 10 | 10.24 | 0.14 | 11 |
| **Cholesterol (mg/dL)** | 16 | 379.9 | 22.0 | 11 | 360.7 | 12.6 | 11 | 334.8 | 13.0 | 12 |
| | 20 | 427.6 | 23.6 | 10 | 368.8 | 17.2 | 10 | 360.3 | 10.7 | 12 |
| | 25 | 411.8 | 24.9 | 11 | 413.3 | 18.8 | 11 | 362.3 | 10.9 | 12 |
| | 28 | 394.7 | 21.2 | 11 | 365.4 | 18.9 | 10 | 345.9 | 19.5 | 11 |
| **Creatine Kinase (U/L)** | 16 | 491.2 | 28.3 | 11 | 436.0 | 42.1 | 11 | 435.8 | 26.1 | 12 |
| | 20 | 589.6 | 40.5 | 10 | 519.1 | 34.2 | 10 | 466.7 | 21.1 | 12 |
| | 25 | 626.0 | 70.4 | 11 | 540.2 | 32.7 | 10 | 530.5 | 49.5 | 12 |
| | 28 | 564.6 | 32.7 | 11 | 506.6 | 48.1 | 10 | 577.8 | 42.8 | 11 |
| **Glucose (mg/dL)** | 16 | 277.4 | 6.5 | 11 | 279.3 | 7.5 | 11 | 281.8 | 5.9 | 12 |
| | 20 | 274.4 | 7.7 | 10 | 274.8 | 6.0 | 10 | 274.2 | 8.0 | 12 |
| | 25 | 281.3 | 6.4 | 11 | 273.6 | 5.2 | 11 | 286.3 | 5.1 | 12 |
| | 28 | 298.9 | 6.5 | 11 | 286.2 | 6.6 | 10 | 293.1 | 9.7 | 11 |
| **Globulin (g/dL)** | 16 | 2.15 | 0.07 | 11 | 2.08 | 0.05 | 23 | 2.03 | 2.03 | 12 |
| | 20 | 2.30 | 0.07 | 10 | 2.26 | 0.05 | 22 | 2.25 | 0.04 | 12 |
| | 25 | 2.60 | 0.07 | 11 | 2.5 | 0.05 | 23 | 2.42 | 0.05 | 12 |
| | 28 | 2.49 | 0.07 | 11 | 2.45 | 0.05 | 21 | 2.41 | 0.07 | 11 |

Table 3.2a (continued)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **LDH (U/L)** | 16 | 895.2 | 53.4 | 11 | 854.5 | 43.7 | 11 | 924.0 | 60.9 | 12 |
| | 20 | 897.4 | 48.9 | 10 | 809.2 | 49.5 | 10 | 822.8 | 49.4 | 12 |
| | 25 | 1050.2 | 81.7 | 11 | 917.1 | 89.2 | 11 | 843.0 | 54.3 | 12 |
| | 28 | 1056.6 | 72.5 | 11 | 908.4 | 48.9 | 9 | 966.6 | 64.5 | 11 |
| **Total Plasma Protein (g/dL)** | 16 | 3.15 | 0.08 | 11 | 3.07 | 0.06 | 11 | 3.02 | 0.05 | 12 |
| | 20 | 3.52 | 0.07 | 10 | 3.42 | 0.09 | 10 | 3.43 | 0.04 | 12 |
| | 25 | 3.74 | 0.08 | 11 | 3.76 | 0.08 | 11 | 3.52 | 0.05 | 12 |
| | 28 | 3.71 | 0.09 | 11 | 3.64 | 0.13 | 10 | 3.56 | 0.09 | 11 |
| **Phosphorus (mg/dL)** | 16 | 9.63 | 0.25 | 11 | 9.36 | 0.28 | 11 | 9.10 | 0.18 | 12 |
| | 20 | 9.44 | 0.28 | 10 | 8.78 | 0.24 | 10 | 8.60 | 0.13 | 12 |
| | 25 | 9.20 | 0.29 | 11 | 9.11 | 0.29 | 11 | 8.83 | 0.19 | 12 |
| | 28 | 8.98 | 0.32 | 11 | 10.04 | 0.22 | 10 | 8.76 | 0.45 | 11 |
| **Uric acid (mg/dL)** | 16 | 15.54 | 0.87 | 11 | 16.85 | 3.01 | 11 | 13.67 | 1.13 | 12 |
| | 20 | 12.82 | 1.66 | 10 | 15.30 | 2.35 | 10 | 11.37 | 1.06 | 12 |
| | 25 | 12.71 | 0.94 | 11 | 12.47 | 1.54 | 11 | 11.18 | 0.65 | 12 |
| | 28 | 8.73 | 1.60 | 11 | 13.18 | 1.71 | 11 | 8.97 | 1.87 | 11 |
| **PCV (%)** | 16 | 31.7 | 1.1 | 10 | 31.7 | 1.2 | 11 | 31.8 | 1.2 | 12 |
| | 20 | 35.5 | 0.6 | 10 | 35.2 | 0.6 | 9 | 35.0 | 0.7 | 12 |
| | 25 | 39.6 | 0.4 | 11 | 39.1 | 0.4 | 11 | 37.8 | 0.5 | 12 |
| | 28 | 39.3 | 0.9 | 11 | 38.0 | 0.7 | 11 | 38.8 | 0.8 | 12 |

Table 3.2b. Plasma chemistry values for pigeon guillemot chicks during a low-frequency dosing regime (once on day 20 and again on day 25 post-hatch) with weathered Prudhoe Bay crude oil.

| | Chick Age (days) | Control | SE | n | Low Oil Dose | SE | n | High Oil Dose | SE | n |
|---|---|---|---|---|---|---|---|---|---|---|
| **Albumin (g/dL)** | 20 | 1.12 | 0.05 | 5 | 1.13 | 0.05 | 9 | 1.13 | 0.04 | 6 |
| | 25 | 1.00 | 0.05 | 6 | 1.11 | 0.04 | 9 | 1.13 | 0.04 | 6 |
| | 28 | 1.20 | 0.04 | 7 | 1.11 | 0.04 | 9 | 1.17 | 0.06 | 6 |
| **AST (U/L)** | 20 | 95.0 | 9.9 | 6 | 92.0 | 4.7 | 9 | 82.0 | 10.7 | 6 |
| | 25 | 105.7 | 10.6 | 6 | 96.0 | 7.0 | 9 | 79.3 | 11.5 | 6 |
| | 28 | 115.4 | 8.4 | 7 | 97.1 | 10.0 | 9 | 90.0 | 13.7 | 6 |
| **BUN (mg/dL)** | 20 | 6.7 | 0.4 | 6 | 5.1 | 0.4 | 9 | 5.7 | 0.8 | 6 |
| | 25 | 5.3 | 1.1 | 6 | 4.9 | 0.5 | 9 | 5.0 | 1.0 | 6 |
| | 28 | 4.9 | 1.0 | 7 | 5.1 | 0.4 | 9 | 6.7 | 0.8 | 6 |
| **Calcium (mg/dL)** | 20 | 9.97 | 0.29 | 6 | 10.16 | 0.23 | 9 | 10.60 | 0.41 | 6 |
| | 25 | 10.43 | 0.41 | 6 | 10.11 | 0.20 | 9 | 9.87 | 0.18 | 6 |
| | 28 | 10.14 | 0.12 | 7 | 9.73 | 0.14 | 9 | 10.07 | 0.30 | 6 |
| **Cholesterol (mg/dL)** | 20 | 306.0 | 18.8 | 6 | 316.9 | 19.9 | 9 | 322.7 | 15.4 | 6 |
| | 25 | 297.3 | 22.2 | 6 | 316.9 | 22.0 | 9 | 324.7 | 25.1 | 6 |
| | 28 | 305.1 | 13.2 | 7 | 323.3 | 19.7 | 9 | 326.3 | 18.1 | 6 |
| **Creatine Kinase (U/L)** | 20 | 463.3 | 46.4 | 6 | 613.1 | 74.9 | 9 | 434.3 | 53.2 | 6 |
| | 25 | 397.0 | 60.9 | 6 | 363.3 | 39.5 | 9 | 290.3 | 35.0 | 6 |
| | 28 | 243.7 | 48.3 | 7 | 238.7 | 32.7 | 9 | 281.0 | 75.8 | 6 |
| **Glucose (mg/dL)** | 20 | 288.0 | 10.0 | 6 | 278.4 | 5.7 | 9 | 292.0 | 9.8 | 6 |
| | 25 | 300.0 | 2.3 | 6 | 292.9 | 5.6 | 9 | 296.3 | 9.2 | 6 |
| | 28 | 310.9 | 7.4 | 7 | 294.0 | 7.5 | 9 | 293.3 | 5.1 | 6 |
| **Globulin (g/dL)** | 20 | 2.23 | 0.10 | 6 | 2.31 | 0.11 | 9 | 2.23 | 0.10 | 6 |
| | 25 | 2.40 | 0.07 | 6 | 2.47 | 0.09 | 9 | 2.37 | 0.08 | 6 |
| | 28 | 2.31 | 0.13 | 7 | 2.44 | 0.09 | 9 | 2.40 | 0.05 | 6 |

Table 3.2b (continued)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **LDH (U/L)** | 20 | 931.0 | 106.1 | 6 | 986.7 | 85.6 | 9 | 888.3 | 47.0 | 6 |
| | 25 | 943.7 | 100.5 | 6 | 887.6 | 76.7 | 9 | 813.3 | 55.4 | 6 |
| | 28 | 843.4 | 70.2 | 7 | 765.3 | 64.9 | 9 | 769.7 | 47.1 | 6 |
| **Total Plasma Protein (g/dL)** | 20 | 3.32 | 0.15 | 5 | 3.44 | 0.14 | 9 | 3.37 | 0.11 | 6 |
| | 25 | 3.40 | 0.09 | 6 | 3.58 | 0.11 | 9 | 3.50 | 0.09 | 6 |
| | 28 | 3.51 | 0.17 | 7 | 3.56 | 0.11 | 9 | 3.57 | 0.10 | 6 |
| **Phosphorus (mg/dL)** | 20 | 9.10 | 0.35 | 6 | 8.80 | 0.28 | 9 | 9.53 | 0.61 | 6 |
| | 25 | 8.97 | 0.66 | 6 | 8.40 | 0.45 | 9 | 9.70 | 0.41 | 6 |
| | 28 | 9.29 | 0.35 | 7 | 8.91 | 0.38 | 9 | 9.43 | 0.52 | 6 |
| **Uric acid (mg/dL)** | 20 | 19.44 | 1.64 | 6 | 18.51 | 1.21 | 9 | 14.80 | 1.21 | 6 |
| | 25 | 16.97 | 1.29 | 6 | 17.42 | 1.43 | 9 | 16.73 | 1.43 | 6 |
| | 28 | 17.00 | 2.34 | 7 | 16.29 | 2.61 | 9 | 14.07 | 2.61 | 6 |

Table 3.3. Plasma chemistry markers in control pigeon guillemot chicks (not dosed with oil) comparing values among ages and between chicks on the low-intake (126 g/day) and high-intake (158 g/day) diets.

| | Chick Age (days) | Low-Intake Diet | SE | n | High-Intake Diet | SE | n | Food Intake Effects[1] (P-value) | Age Effects, Low-Intake Diet[2] (P-value) | Age Effects, High-Intake Diet[3] (P-value) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Albumin (g/dL)** | All | 1.19 | 0.02 | 32 | 1.11 | 0.03 | 18 | | <0.00005 | 0.008 |
| | 16 | 1.01 | 0.03 | 11 | | | | | 0.0003 | |
| | 20 | 1.22 | 0.04 | 10 | 1.12 | 0.05 | 5 | | | |
| | 25 | 1.15 | 0.03 | 11 | 1 | 0.05 | 6 | 0.016 | | |
| | 28 | 1.22 | 0.02 | 11 | 1.2 | 0.04 | 7 | | | |
| **AST (U/L)** | All | 213.4 | 17.2 | 32 | 105.9 | 5.6 | 19 | | <0.00005 | 0.0021 |
| | 16 | 131.4 | 11.1 | 11 | | | | | 0.0015 | |
| | 20 | 157.6 | 18.7 | 10 | 95 | 9.9 | 6 | 0.0284 | | 0.0039 |
| | 25 | 212.7 | 26.5 | 11 | 105.7 | 10.6 | 6 | 0.0162 | | |
| | 28 | 264.7 | 33.6 | 11 | 115.4 | 8.4 | 7 | 0.0032 | | |
| **BUN (mg/dL)** | All | 5.3 | 0.3 | 32 | 5.6 | 0.5 | 64 | | | |
| | 16 | 5.6 | 0.4 | 11 | | | | | | |
| | 20 | 4.8 | 0.4 | 10 | 6.7 | 0.4 | 6 | 0.0135 | | |
| | 25 | 4.9 | 0.5 | 11 | 5.3 | 1.1 | 6 | | | |
| | 28 | 6.2 | 0.6 | 11 | 4.9 | 1 | 7 | | | |
| **Calcium (mg/dL)** | All | 10.13 | 0.11 | 32 | 10.18 | 0.16 | 19 | | | |
| | 16 | 10.18 | 0.15 | 11 | | | | | | |
| | 20 | 9.96 | 0.2 | 10 | 9.97 | 0.29 | 6 | | | |
| | 25 | 10.38 | 0.17 | 11 | 10.43 | 0.41 | 6 | | | |
| | 28 | 10.04 | 0.17 | 11 | 10.14 | 0.12 | 7 | | | |
| **Cholesterol (mg/dL)** | All | 410.9 | 13.2 | 32 | 302.9 | 9.83 | 19 | | 0.0013 | |
| | 16 | 379.9 | 22 | 11 | | | | | | |
| | 20 | 427.6 | 23.6 | 10 | 306 | 18.8 | 6 | 0.0030 | | |
| | 25 | 411.8 | 24.9 | 11 | 297.3 | 22.2 | 6 | 0.0084 | | |
| | 28 | 394.7 | 21.2 | 11 | 305.1 | 13.2 | 7 | 0.0068 | | |
| **Creatine Kinase (U/L)** | All | 592.5 | 28 | 31 | 361.5 | 35.9 | 19 | | | 0.0045 |
| | 16 | 491.2 | 28.3 | 11 | | | | | | |
| | 20 | 589.6 | 40.5 | 10 | 463.3 | 46.4 | 6 | 0.0421 | | 0.0018 |
| | 25 | 626 | 70.4 | 10 | 397 | 60.9 | 6 | 0.0431 | | |
| | 28 | 564.5 | 32.7 | 11 | 243.7 | 48.3 | 7 | < 0.0001 | | |

Table 3.3 (continued)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Glucose (mg/dL)** | All | 285.2 | 4.2 | 32 | 300.2 | 4.6 | 19 | | 0.030 | |
| | 16 | 277.4 | 6.5 | 11 | | | | | 0.064 | |
| | 20 | 274.4 | 7.7 | 10 | 288 | 10 | 6 | | | |
| | 25 | 281.3 | 6.4 | 11 | 300 | 2.3 | 6 | 0.055 | | |
| | 28 | 298.9 | 6.5 | 11 | 310.9 | 7.4 | 7 | | | |
| **Globulin (g/dL)** | All | 2.47 | 0.05 | 32 | 2.32 | 0.06 | 19 | | <0.00005 | 0.0403 |
| | 16 | 2.15 | 0.07 | 11 | | | | | 0.0032 | |
| | 20 | 2.3 | 0.07 | 10 | 2.23 | 0.09 | 6 | | | |
| | 25 | 2.6 | 0.07 | 11 | 2.4 | 0.07 | 6 | 0.076 | | |
| | 28 | 2.49 | 0.09 | 11 | 2.31 | 0.13 | 7 | | | |
| **LDH (U/L)** | All | 1003 | 41.3 | 31 | 902.7 | 50.9 | 19 | | 0.047 | 0.0298 |
| | 16 | 895.2 | 53.4 | 11 | | | | | 0.057* | |
| | 20 | 897.4 | 48.9 | 10 | 931 | 106.1 | 6 | | | 0.018 |
| | 25 | 1050.2 | 81.7 | 11 | 943.7 | 100.6 | 6 | | | |
| | 28 | 1056.6 | 72.5 | 11 | 843.4 | 70.2 | 7 | 0.048* | | |
| **Total Plasma Protein (g/dL)** | All | 3.66 | 0.05 | 32 | 3.42 | 0.08 | 18 | | <0.00005 | |
| | 16 | 3.15 | 0.08 | 11 | | | | | 0.0002 | |
| | 20 | 3.52 | 0.07 | 10 | 3.32 | 0.15 | 5 | | | |
| | 25 | 3.75 | 0.08 | 11 | 3.4 | 0.09 | 6 | 0.0135 | | |
| | 28 | 3.71 | 0.09 | 11 | 3.51 | 0.17 | 7 | | | |
| **Phosphorus (mg/dL)** | All | 9.2 | 0.17 | 32 | 9.13 | 0.25 | 19 | | | |
| | 16 | 9.63 | 0.25 | | | | | | | |
| | 20 | 9.44 | 0.28 | 10 | 9.1 | 0.35 | 6 | | | |
| | 25 | 9.2 | 0.29 | 11 | 8.97 | 0.66 | 6 | | | |
| | 28 | 8.98 | 0.32 | 11 | 9.29 | 0.35 | 7 | | | |
| **Uric acid (mg/dL)** | All | 11.38 | 0.87 | 32 | 17.76 | 1.06 | 19 | | 0.0037 | |
| | 16 | 15.54 | 0.87 | 11 | | | | | 0.0046 | |
| | 20 | 12.82 | 1.66 | 10 | 19.43 | 1.64 | 6 | 0.0195 | | |
| | 25 | 12.71 | 0.94 | 11 | 16.97 | 1.29 | 6 | 0.0171 | | |
| | 28 | 8.73 | 1.6 | 11 | 17 | 2.34 | 7 | 0.0079 | | |

[1]All ages analyzed independently between diet groups with ANOVA. $P < 0.1$ reported.
[2]Paired t-test analyses between age 16- and 28- day post-hatch sample results. Analysis of all ages with repeated measures ANOVA. $P < 0.1$ reported.
[3]Paired t-test analyses between age 20- and 28-day post-hatch sample results. Analysis of all ages with repeated measures ANOVA. $P < 0.1$ reported.
*Log-transformed variables used for analysis.

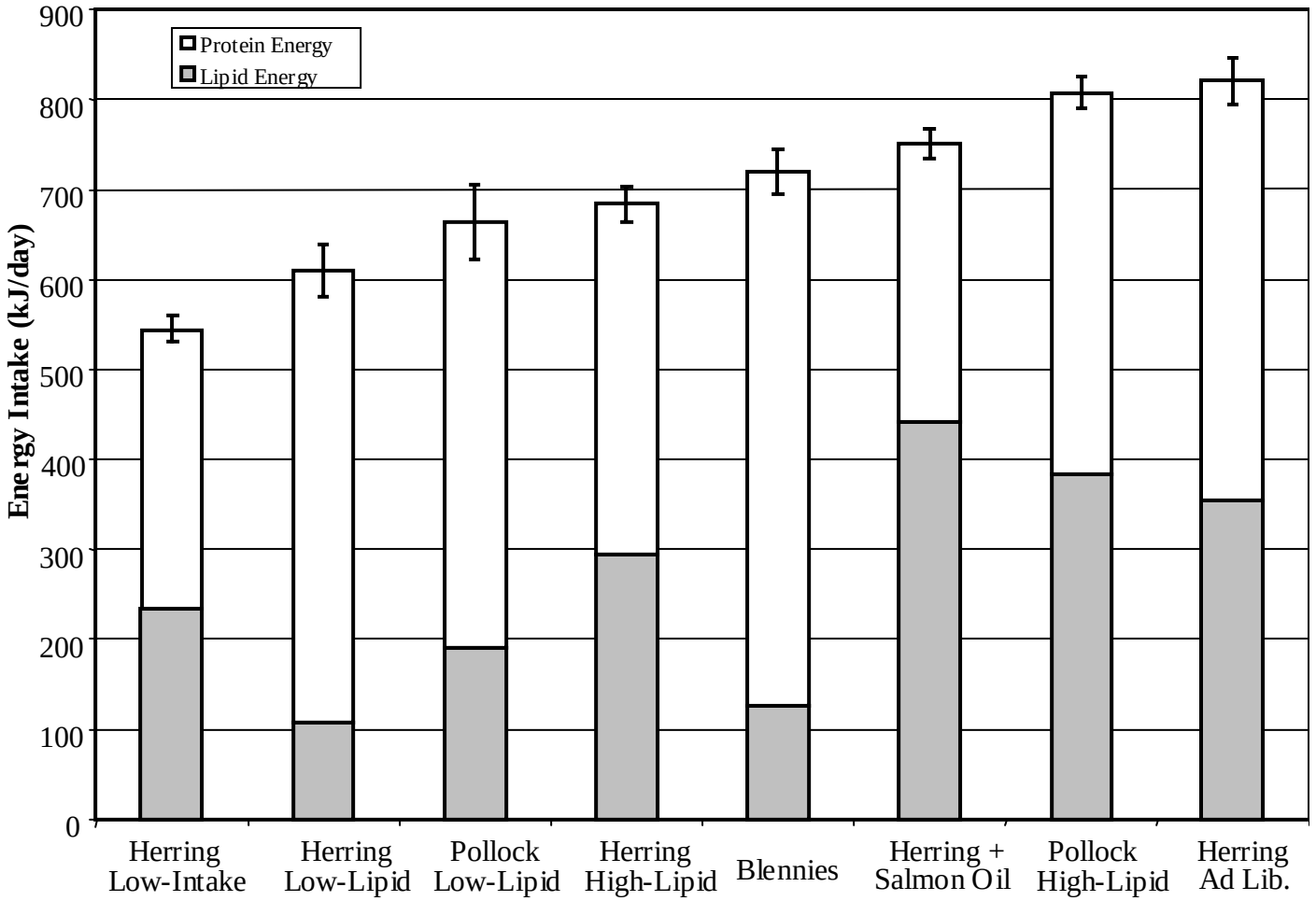


Figure 2.1. Total daily energy intake for pigeon guillemot chicks on each experimental diet and proportion of energy in protein and lipid. Standard error bars are shown for total daily energy intake values.

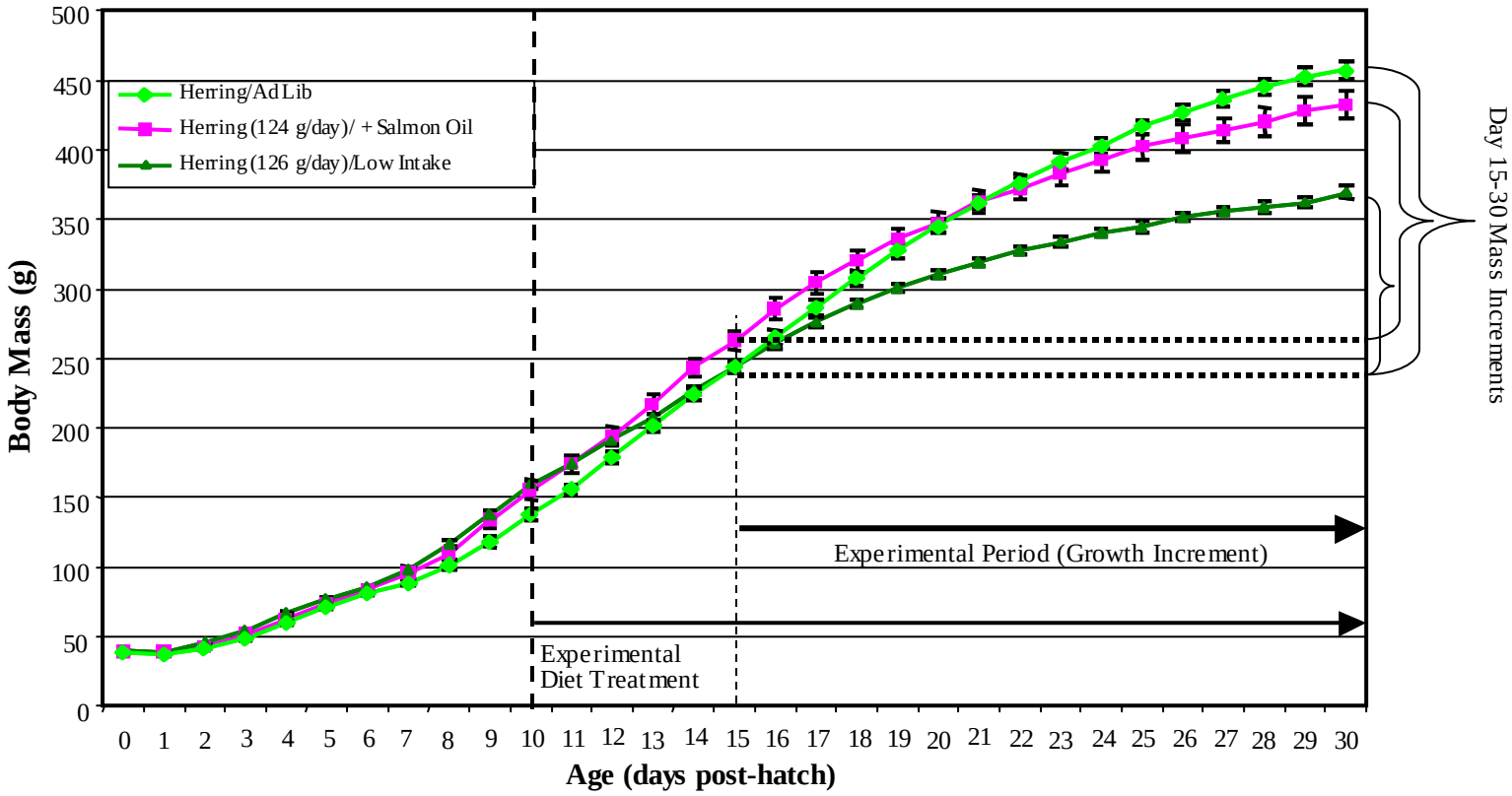


Figure 2.2. Body mass of pigeon guillemots as a function of age for chicks fed three different diets. Each point represents average body mass at age ± 1 SE.

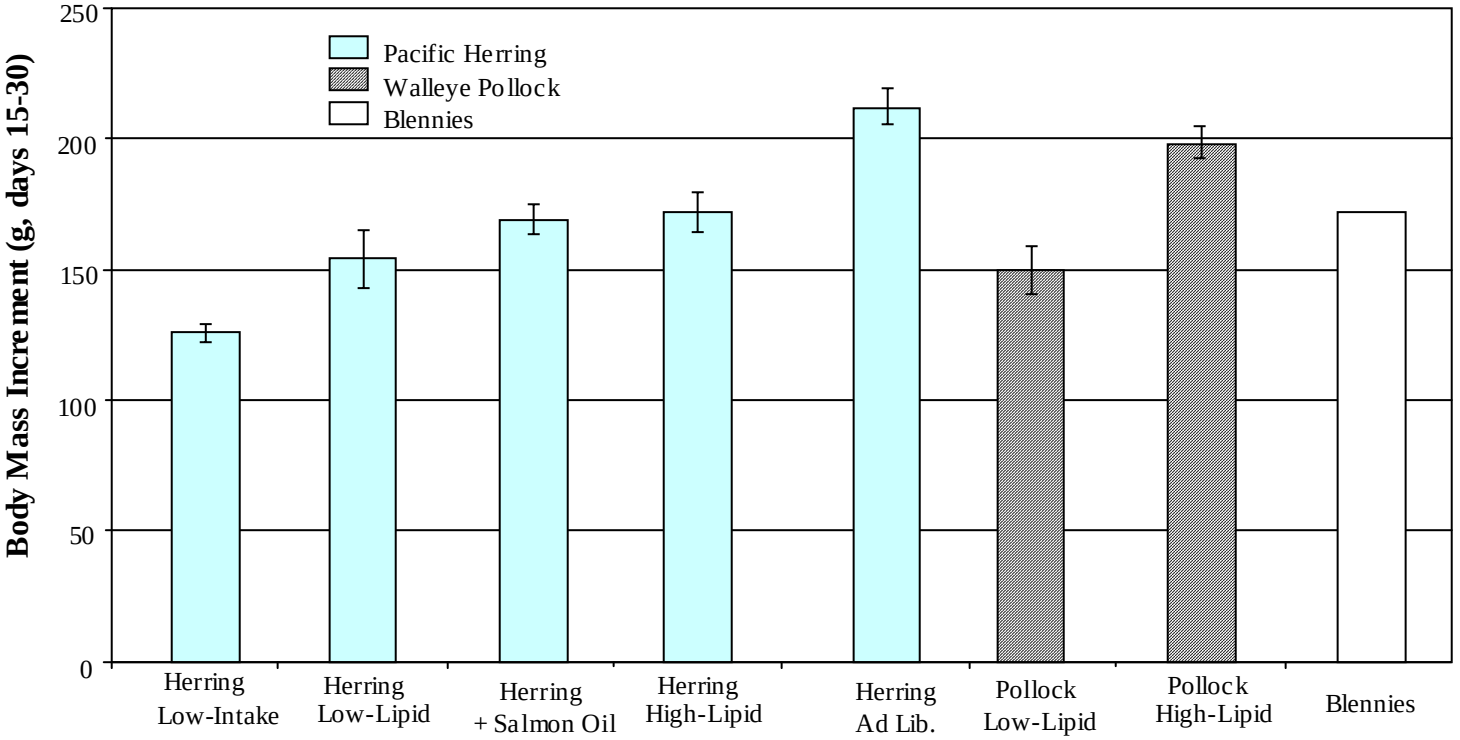


Figure 2.3. Growth in body mass of pigeon guillemot chicks from day 15 to day 30 post-hatch as a function of prey type. Error bars represent ± 1 SE. The X-axis labels represent the various diet treatments: Herring/Low-Intake = 126 g/day of high-lipid juvenile Pacific herring (*Clupea pallasi*), Herring/Low-Lipid = 181 g/day of low-lipid juvenile Pacific herring, Herring/+ Salmon Oil = 123.5 g/day of high-lipid juvenile Pacific herring supplemented with 6 mls/day of sockeye salmon oil, Herring/High-Lipid = 158 g/day of high-lipid juvenile Pacific herring, Herring/Ad Lib = *Ad libitum* high-lipid juvenile Pacific herring, Pollock/Low-Lipid = 176 g/day of low-lipid juvenile walleye pollock (*Theragra chalcogramma*), Pollock/High-Lipid = 161 g/day of high-lipid juvenile walleye pollock, Blennies = 177 g/day of crescent gunnels (*Pholis laeta*) and high cockscomb (*Anoplarcus purpurescens*).

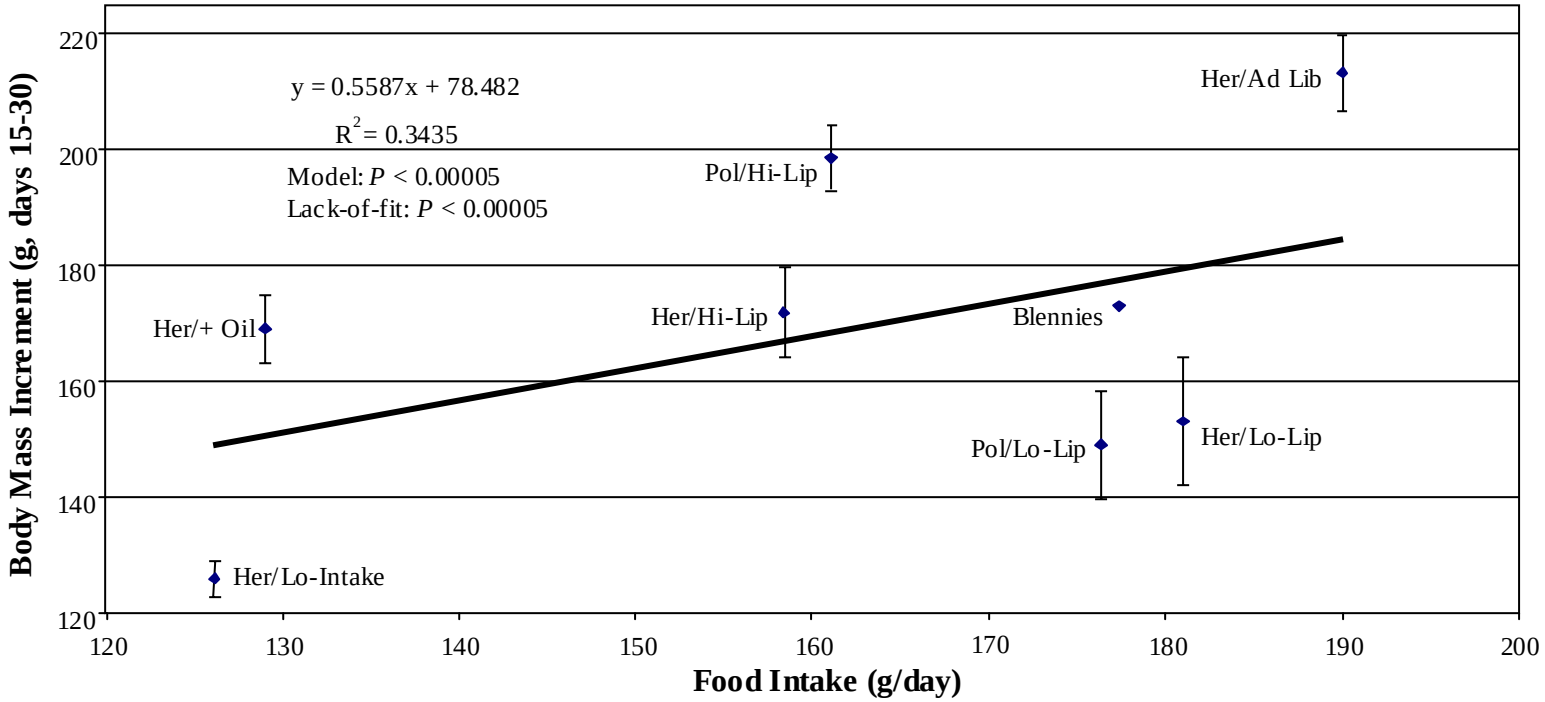


Figure 2.4. Growth in body mass of pigeon guillemot chicks from day 15 to day 30 post-hatch as a function of daily food biomass intake. Error bars represent ± 1 SE. Food intake values are calculated as the mean food intake from day 15 to day 30 post-hatch for all chicks in each diet group. Data point labels are abbreviations for the various diet treatments: Her/Lo-Intake = 126 g/day of high-lipid juvenile Pacific herring, Her/Lo-Lip = 181 g/day of low-lipid juvenile Pacific herring, Pol/Lo-Lip = 176 g/day of low-lipid juvenile walleye pollock, Her/Hi-Lip = 158 g/day of high-lipid juvenile Pacific herring, Blennies = 177 g/day of crescent gunnels and high cockscomb, Pol/Hi-Lip = 161 g/day of high-lipid juvenile walleye pollock, Her/+ Oil = 123.5 g/day of high-lipid juvenile Pacific herring supplemented with 6 mls/day of sockeye salmon oil, Her/Ad Lib = *Ad libitum* high-lipid juvenile Pacific herring.

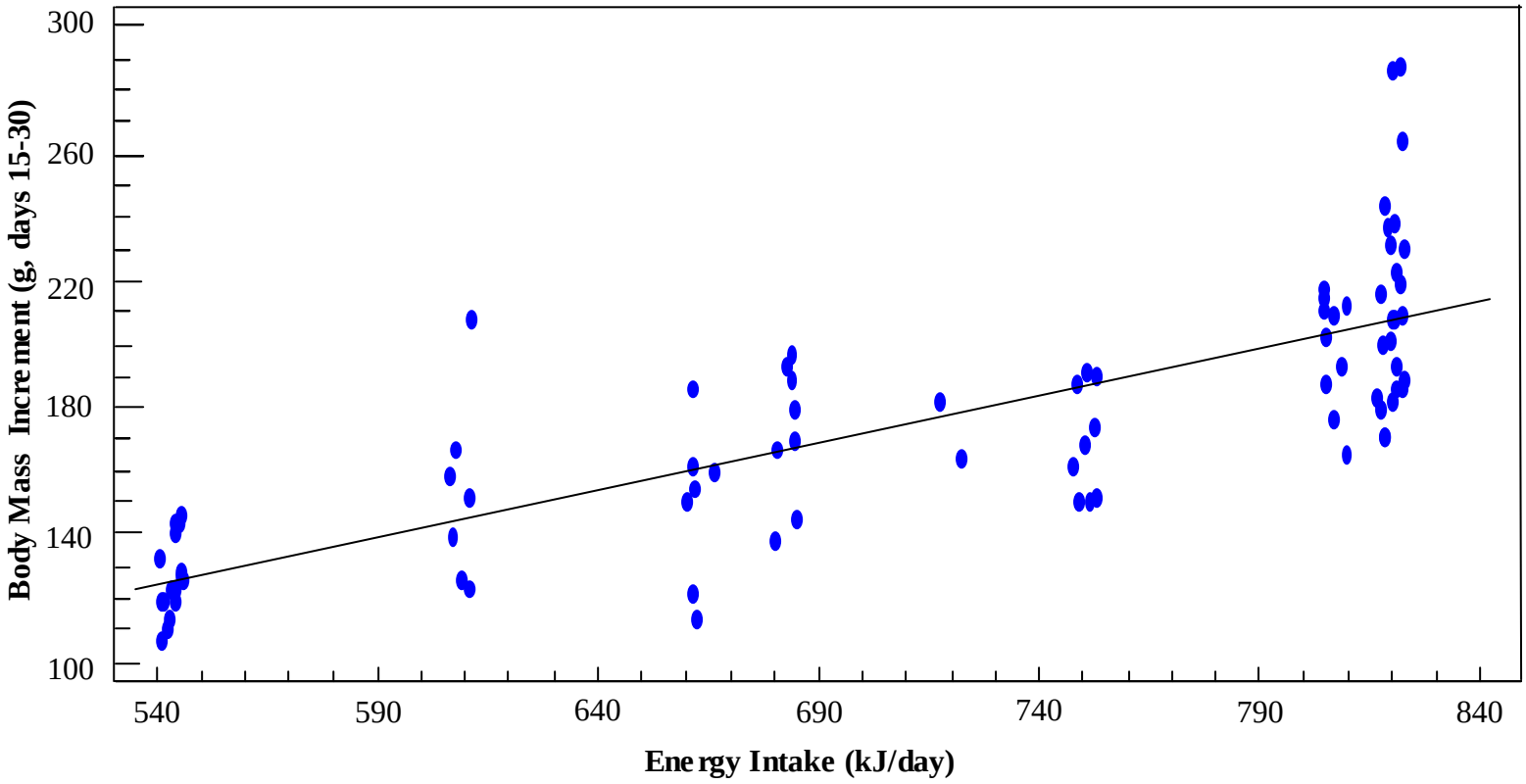


Figure 2.5. Scatterplot (jittered) with regression line for growth in body mass of pigeon guillemot chicks from day 15 to day 30 post-hatch as a function of daily energy intake.

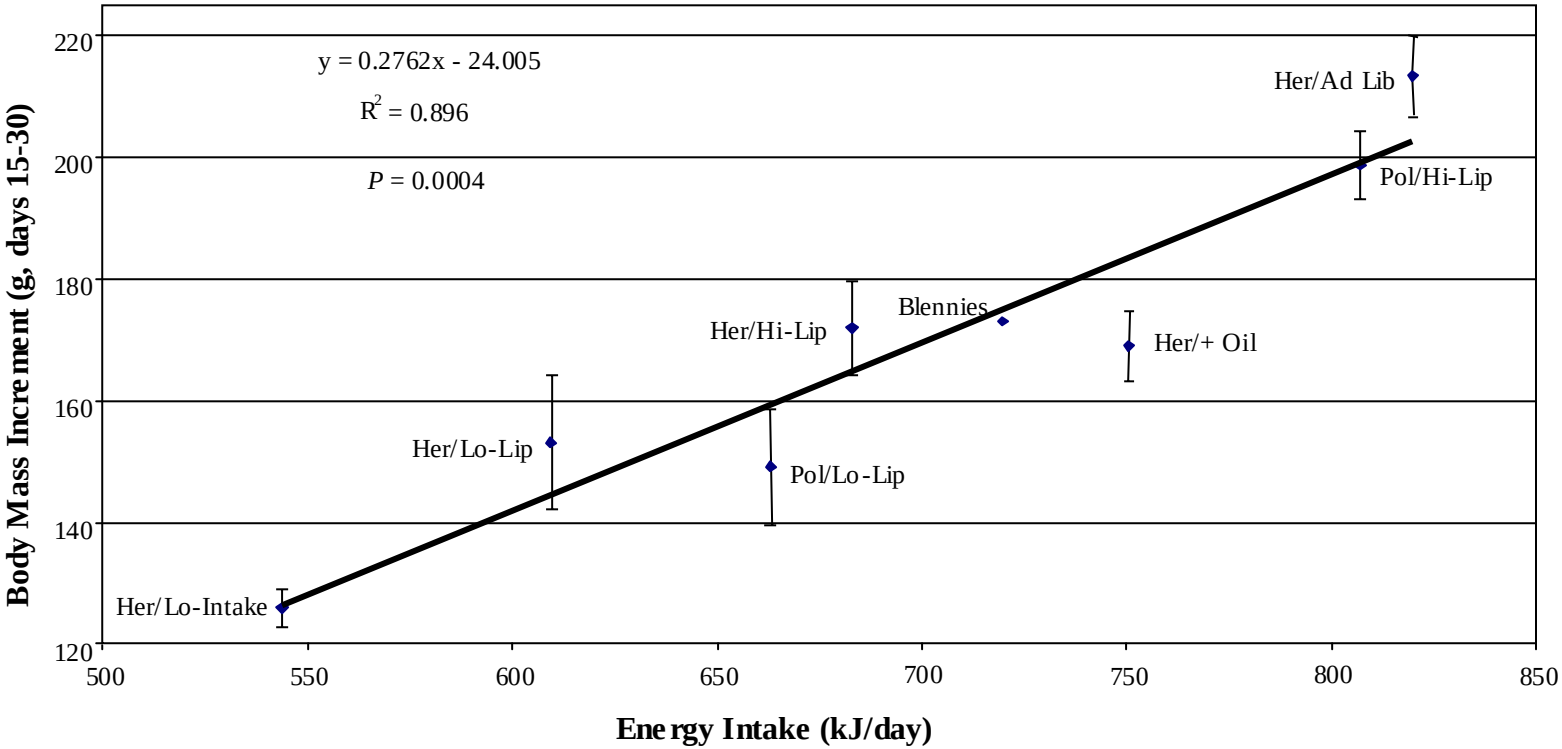


Figure 2.6. Regression analysis of growth increment in body mass of pigeon guillemot chicks from day 15 to day 30 post-hatch as a function of daily energy intake. Error bars represent ± 1 SE. Data point labels are abbreviations for the various diet treatments: Her/Lo-Intake = 126 g/day of high-lipid juvenile Pacific herring, Her/Lo-Lip = 181 g/day of low-lipid juvenile Pacific herring, Pol/Lo-Lip = 176 g/day of low-lipid juvenile walleye pollock, Her/Hi-Lip = 158 g/day of high-lipid juvenile Pacific herring, Blennies = 177 g/day of crescent gunnels and high cockscomb, Pol/Hi-Lip = 161 g/day of high-lipid juvenile walleye pollock, Her/+ Oil = 123.5 g/day of high-lipid juvenile Pacific herring supplemented with 6 mls of sockeye salmon oil, Her/Ad Lib = *Ad libitum* high-lipid juvenile Pacific herring.

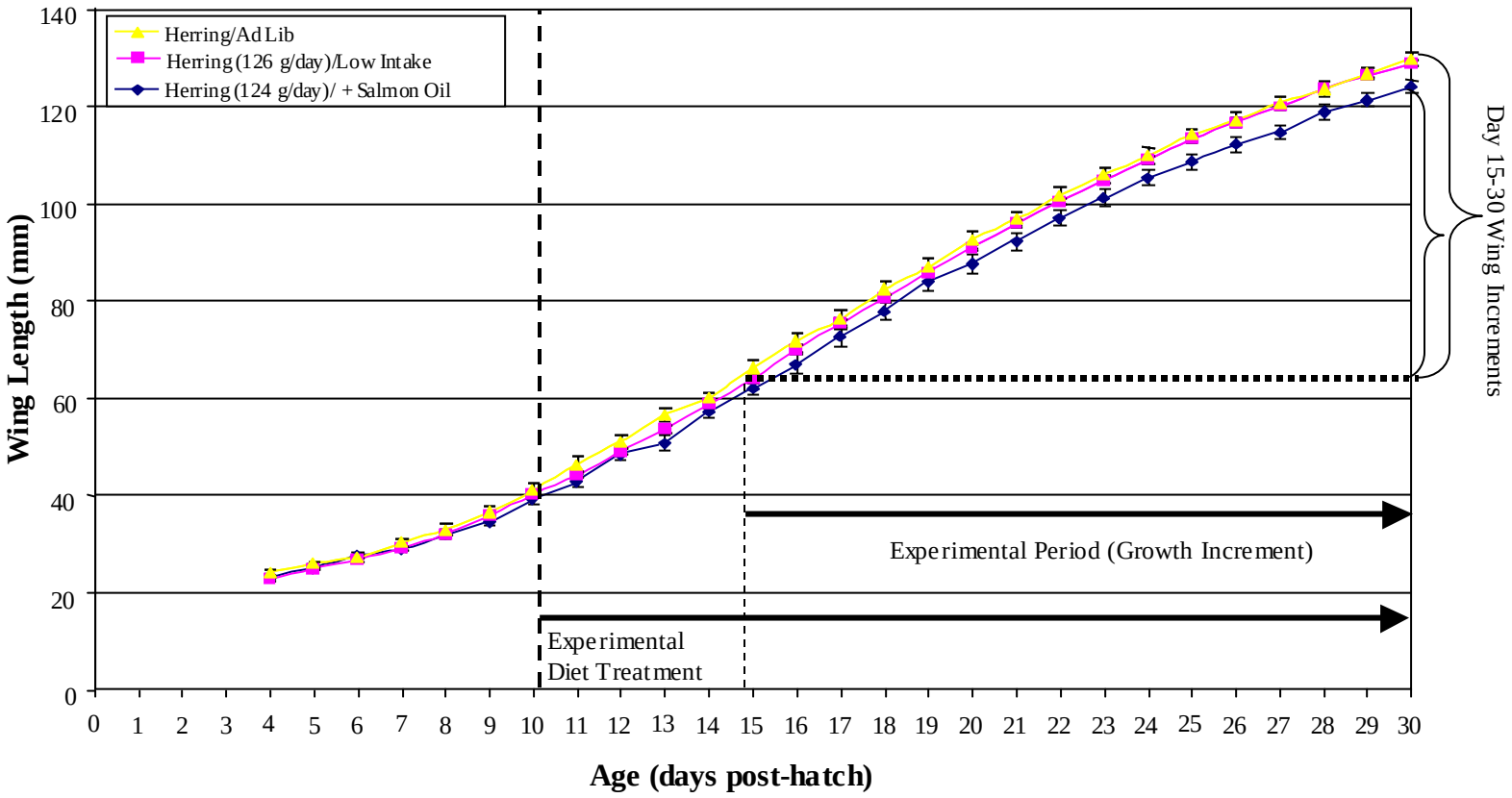


Figure 2.7. Wing length of pigeon guillemots as a function of age for chicks fed three different diets. Each point represents the average wing length ± 1 SE.

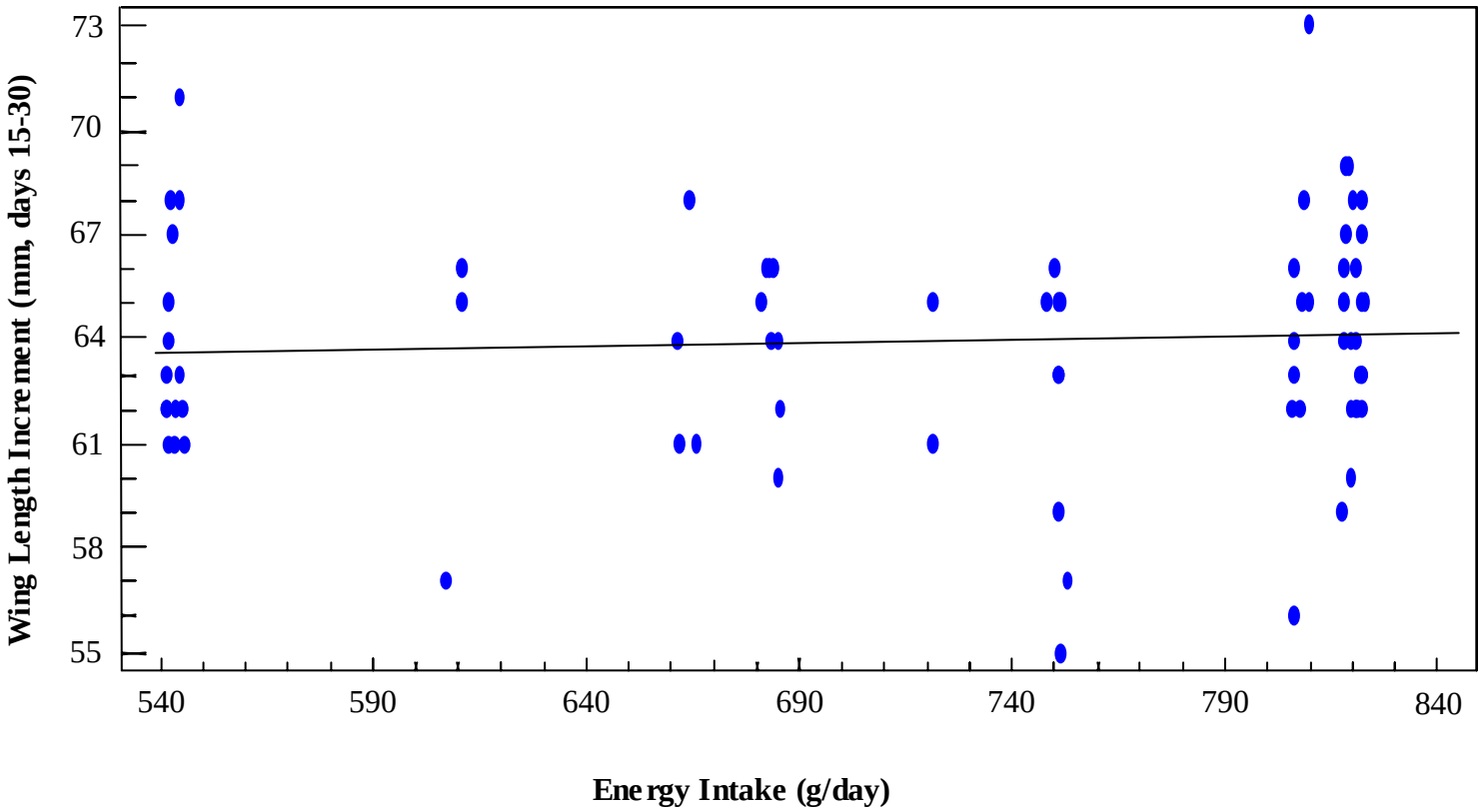


Figure 2.8. Scatterplot (jittered) and regression line for structural growth (growth increment in wing length from day 15 to day 30 post-hatch) in pigeon guillemot chicks as a function of daily energy intake.

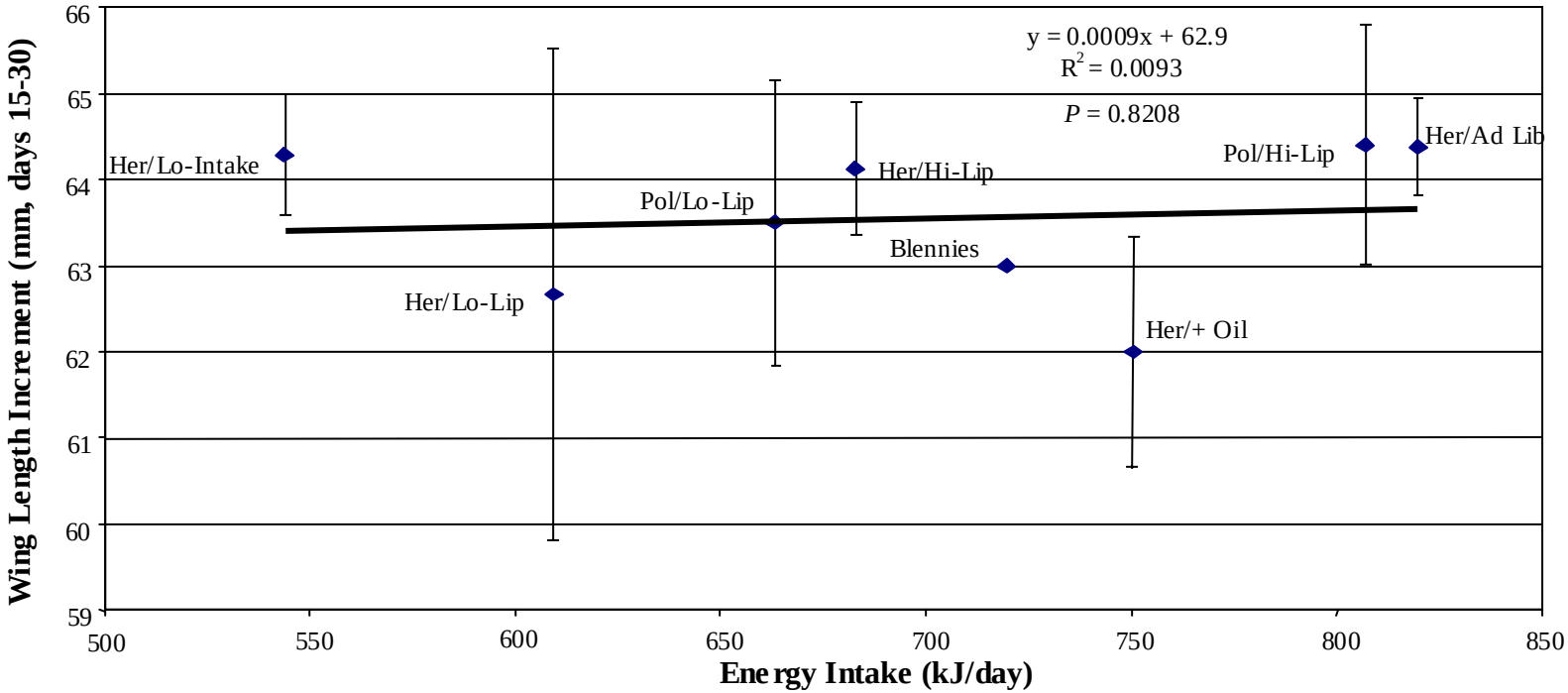


Figure 2.9. Regression analysis of growth increment in wing length of pigeon guillemot chicks from day 15 to day 30 post-hatch as a function of daily energy intake. Error bars represent ± 1 SE. Energy intake values are calculated as the mean energy intake from day 15 to day 30 post-hatch of all chicks in each diet group. Data point labels are abbreviations for the various diet treatments: Her/Lo-Intake = 126 g/day of high-lipid juvenile Pacific herring, Her/Lo-Lip = 181 g/day of low-lipid juvenile Pacific herring, Pol/Lo-Lip = 176 g/day of low-lipid juvenile walleye pollock, Her/Hi-Lip = 158 g/day of high-lipid juvenile Pacific herring, Blennies = 177 g/day of crescent gunnels and high cockscomb, Pol/Hi-Lip = 161 g/day of high-lipid juvenile walleye pollock, Her/+ Oil = 123.5 g/day of high-lipid juvenile Pacific herring supplemented with 6 mls of sockeye salmon oil, Her/Ad Lib = *Ad libitum* high-lipid juvenile Pacific herring.

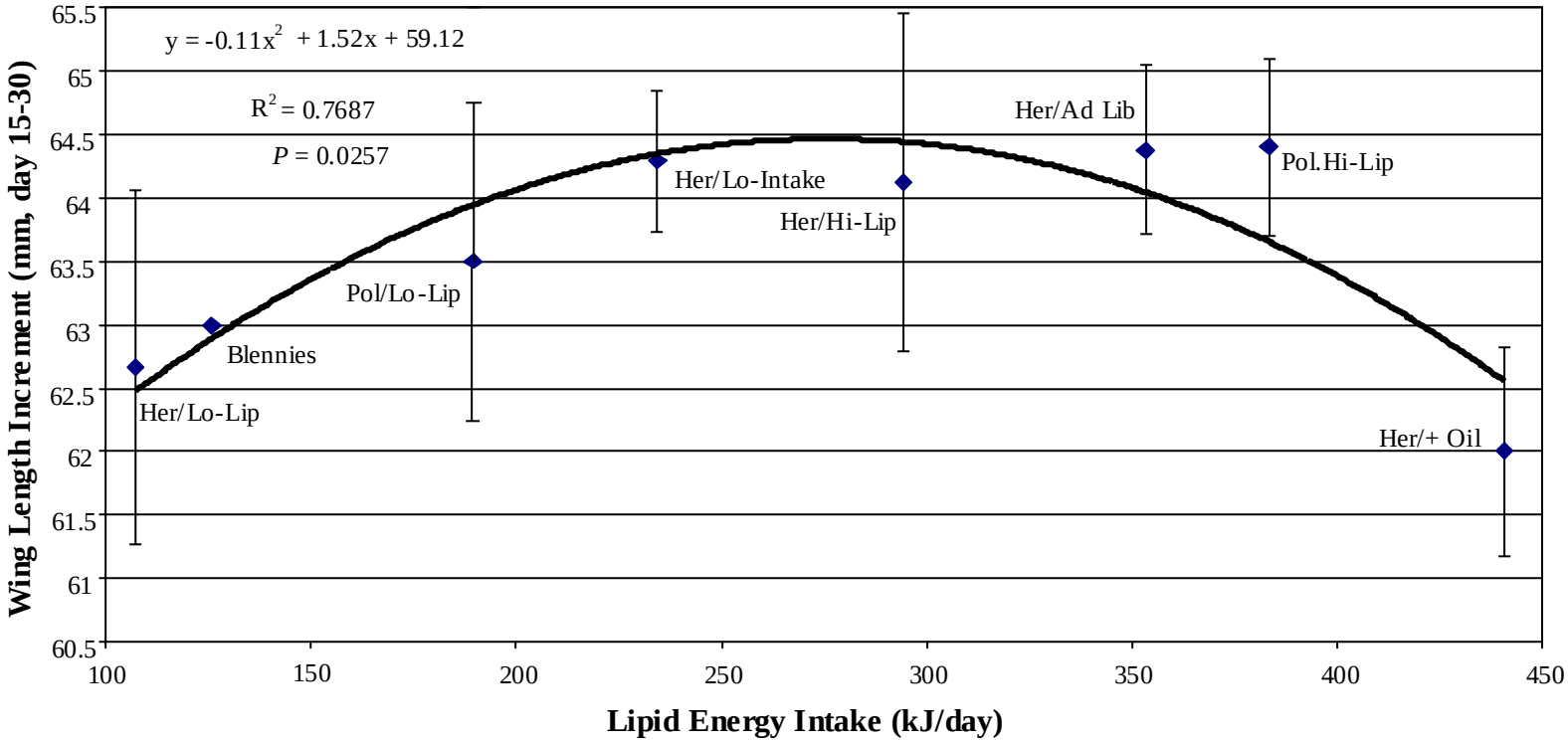


Figure 2.10. Structural growth (growth increment in wing length from day 15 to day 30 post-hatch) for pigeon guillemot chicks as a function of daily intake of lipid energy. The curve represents the quadratic model fitted to the means. Error bars represent ± 1 SE.

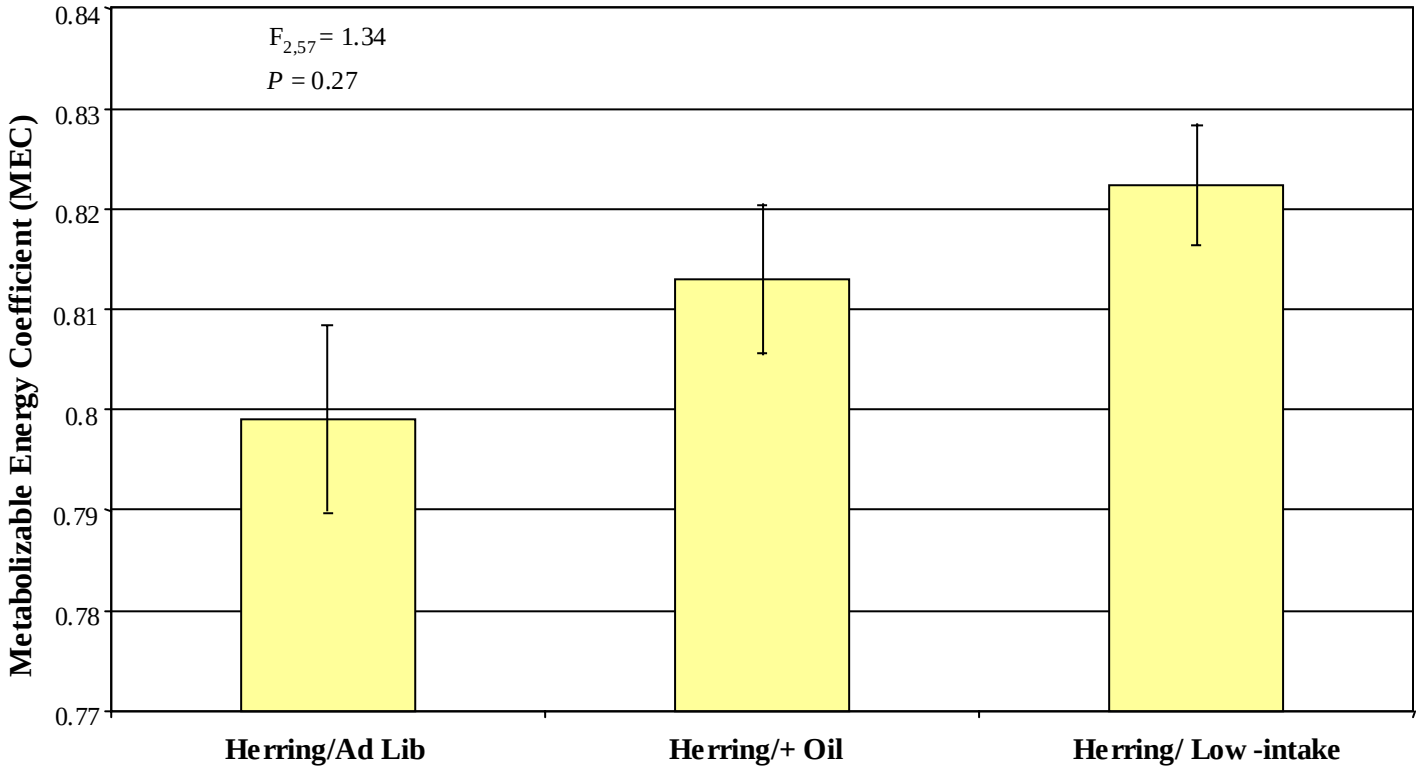


Figure 2.11. Metabolizable energy coefficients (MECs) of pigeon guillemot chicks fed three different diets. Error bars represent ± 1 SE. Diets are: Herring/Ad Lib = *Ad libitum* high-lipid juvenile Pacific herring, Herring/+ Oil = 123.5 g/day high-lipid juvenile Pacific herring supplemented with 6 mls of sockeye salmon oil, Herring/Low-intake = 126 g/day high-lipid juvenile Pacific herring.

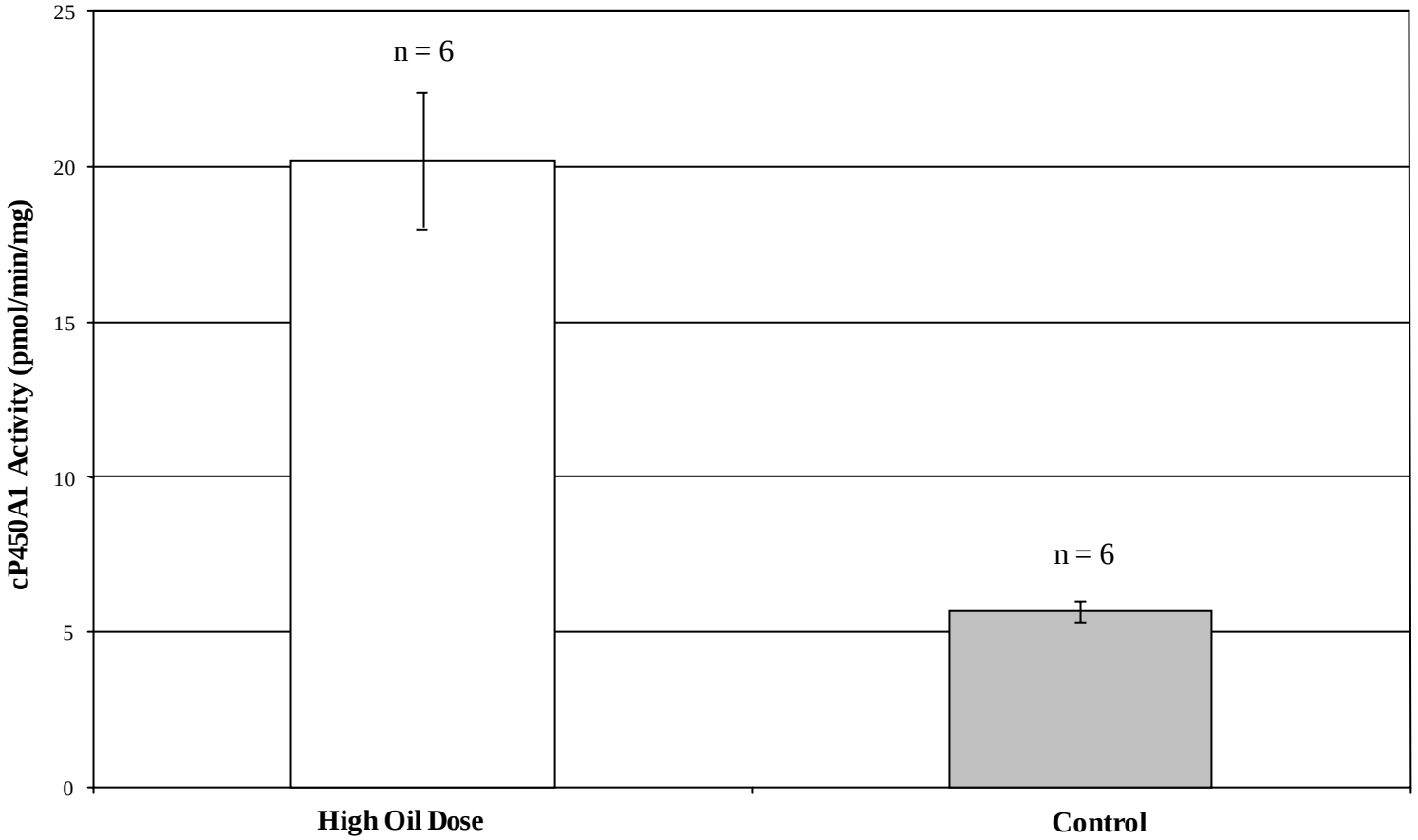


Figure 3.1. Activity of hepatic cP450 from pigeon guillemot chicks dosed for 10 days with 0.50 ml $kg^{-1}$ $day^{-1}$ of weathered Prudhoe Bay crude oil (PBCO), compared with un-dosed controls. Liver samples were biopsied from chicks on day 28 post-hatch (two days after dosing ended).

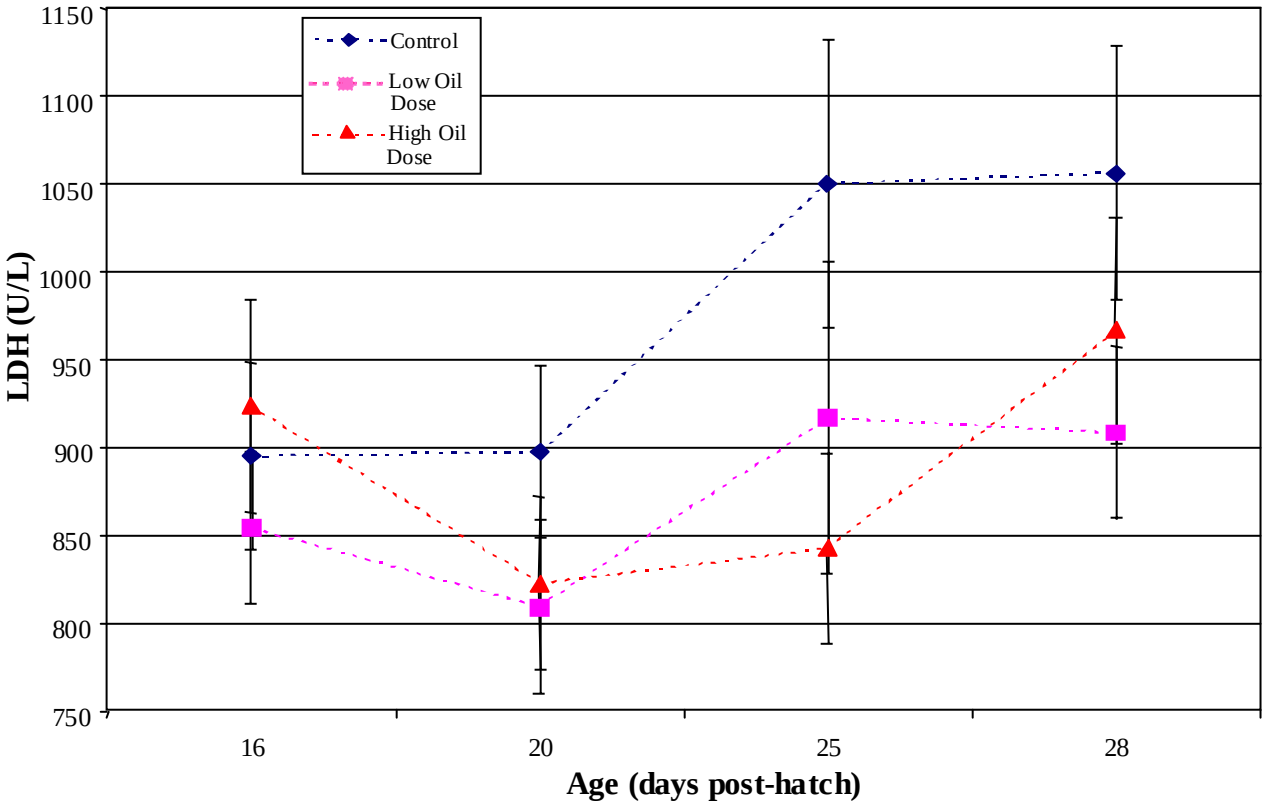


Figure 3.2. Plasma lactate dehydrogenase (LDH) levels of pigeon guillemot chicks dosed with weathered Prudhoe Bay crude oil at high-frequency (once per day from day 16 to day 25 post-hatch) and at different dose levels. Average LDH levels shown ± SE. High-dose = 0.50 ml $kg^{-1}$ $day^{-1}$; Low-dose = 0.125 ml $kg^{-1}$ $day^{-1}$; Control = 0.125 ml corn oil $kg^{-1}$ $day^{-1}$.

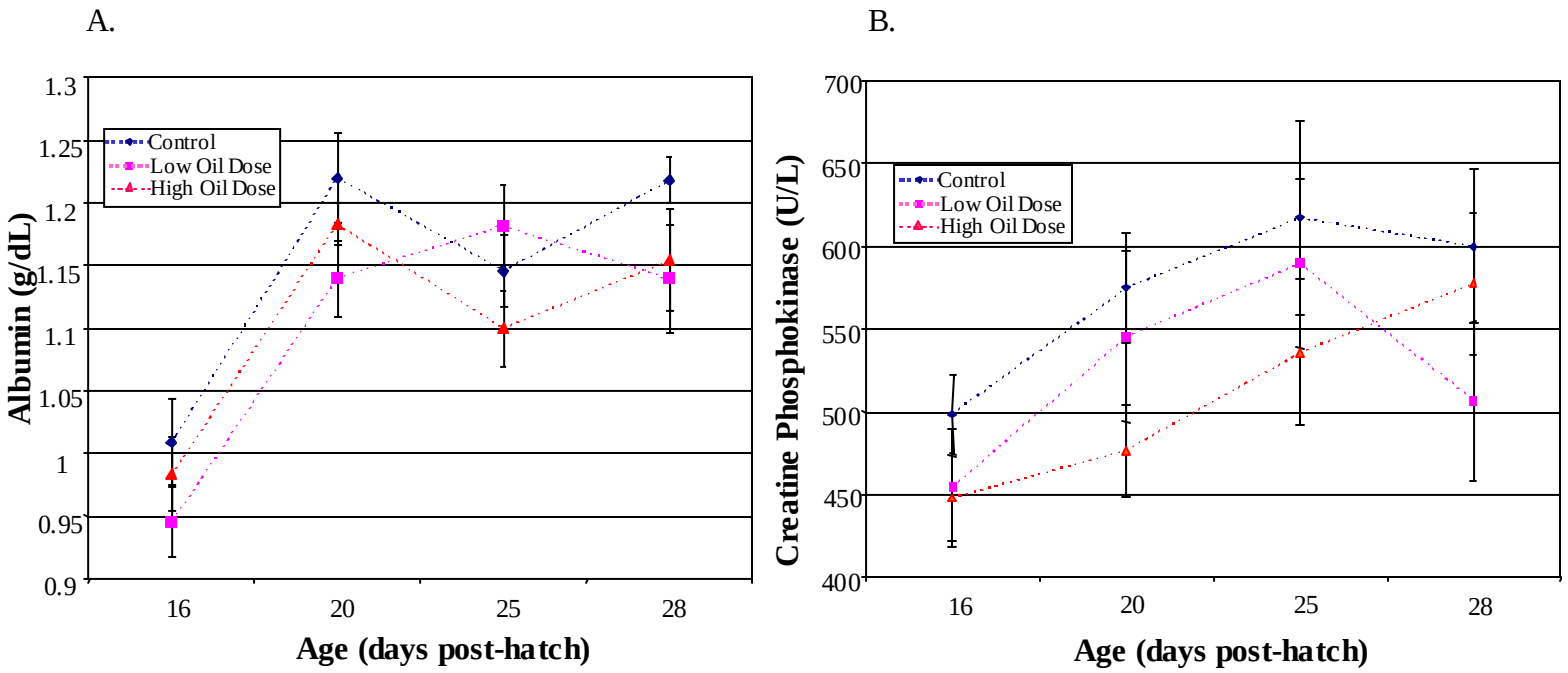


Figure 3.3. Plasma albumin levels (A) and creatine phosphokinase levels (B) of pigeon guillemot chicks dosed with weathered Prudhoe Bay crude oil at high-frequency (once per day from day 16 to day 25 post-hatch) and on low-intake diets. Average levels shown ± SE. High-dose = 0.50 ml $kg^{-1}$ $day^{-1}$; Low-dose = 0.125 ml $kg^{-1}$ $day^{-1}$; Control = 0.125 ml corn oil $kg^{-1}$ $day^{-1}$.

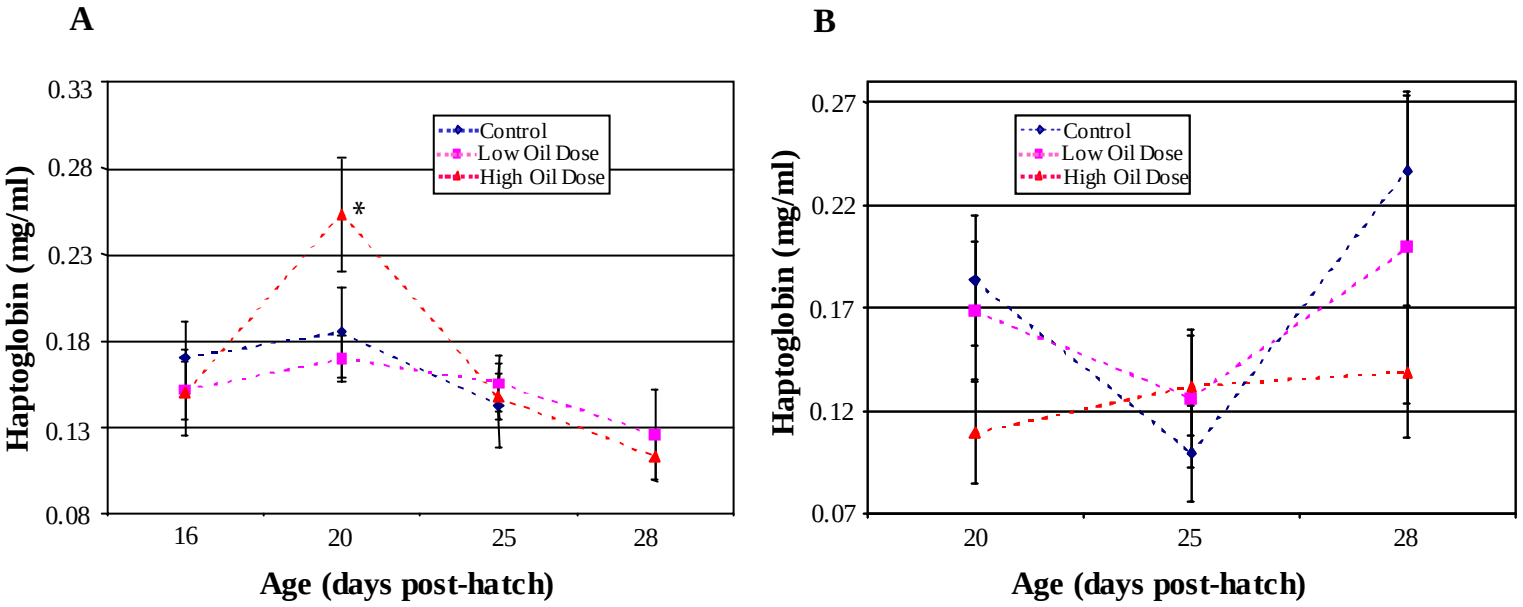


Figure 3.4. Haptoglobin levels of pigeon guillemtot chicks dosed with weathered Prudhoe Bay crude oil at different dose levels and either high-frequency (once per day from day 16 to day 25 post-hatch) [A] or low-frequency (one dose at day 20 and another at day 25) [B]. Average haptoglobin levels shown ± SE. High-frequency doses were as follows: high dose = 0.50 ml $kg^{-1}$ $day^{-1}$; low dose = 0.125 ml $kg^{-1}$ $day^{-1}$; control = 0.125 ml corn oil $kg^{-1}$ $day^{-1}$. Low-frequency doses were as follows: high-dose = 1.0 ml per dose; low-dose = 0.5 ml per dose; control = 0.5 ml corn oil per dose. Significant oil-dosing effect is denoted with an asterisk (*).

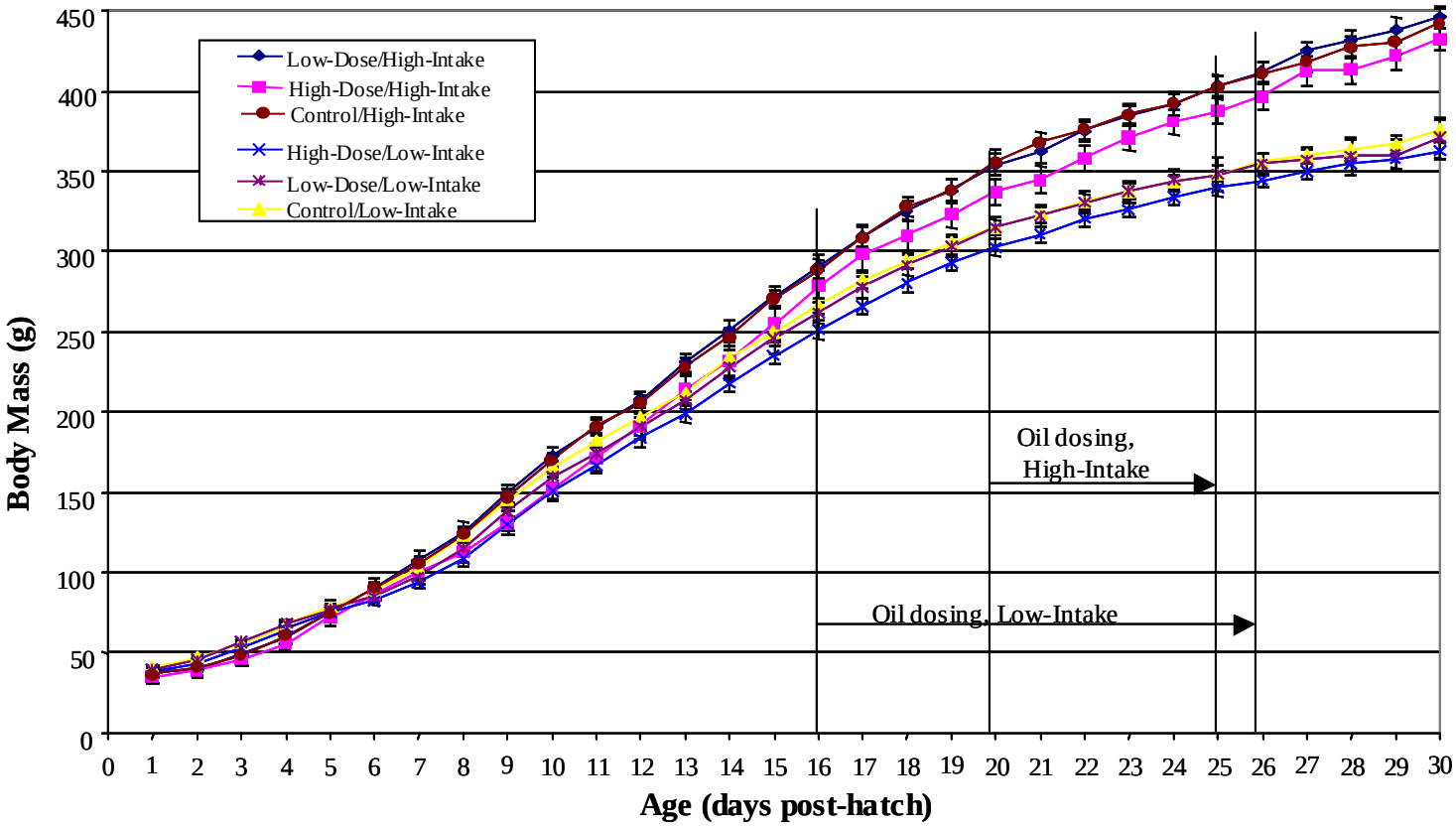


Figure 3.5. Body mass growth curves of pigeon guillemot chicks fed different doses of weathered Prudhoe Bay crude oil. Oil was administered under different feeding regimes (low-intake = 126 g/day; high-intake = 158 g/day). See Methods section for details on dose amount and frequency.

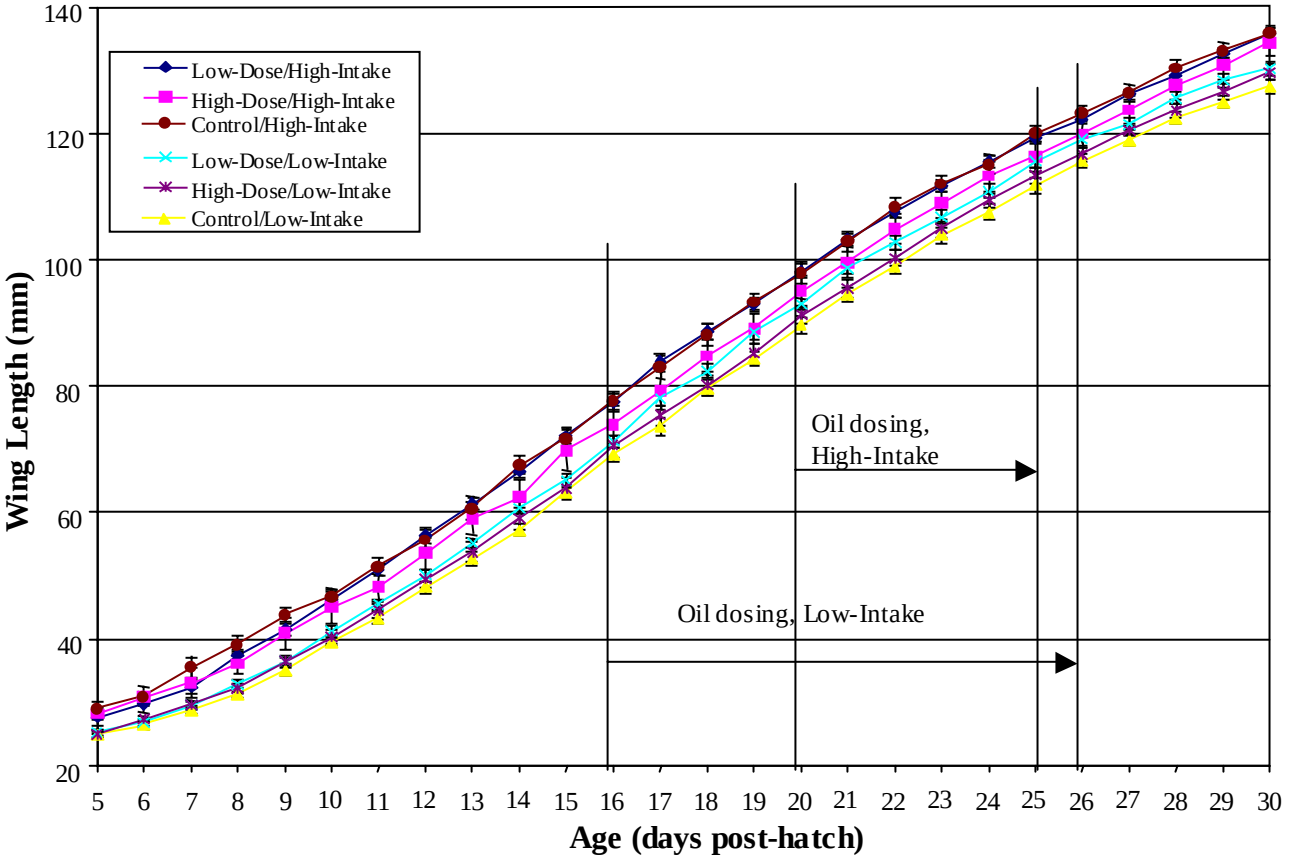


Figure 3.6. Growth in wing length of pigeon guillemot chicks fed different doses of weathered Prudhoe Bay crude oil. Oil was administered with different frequencies and doses under different feeding regimes (low-intake = 126 g/day; high-intake = 158 g/day). See Methods for details on the dosing amount and frequency.

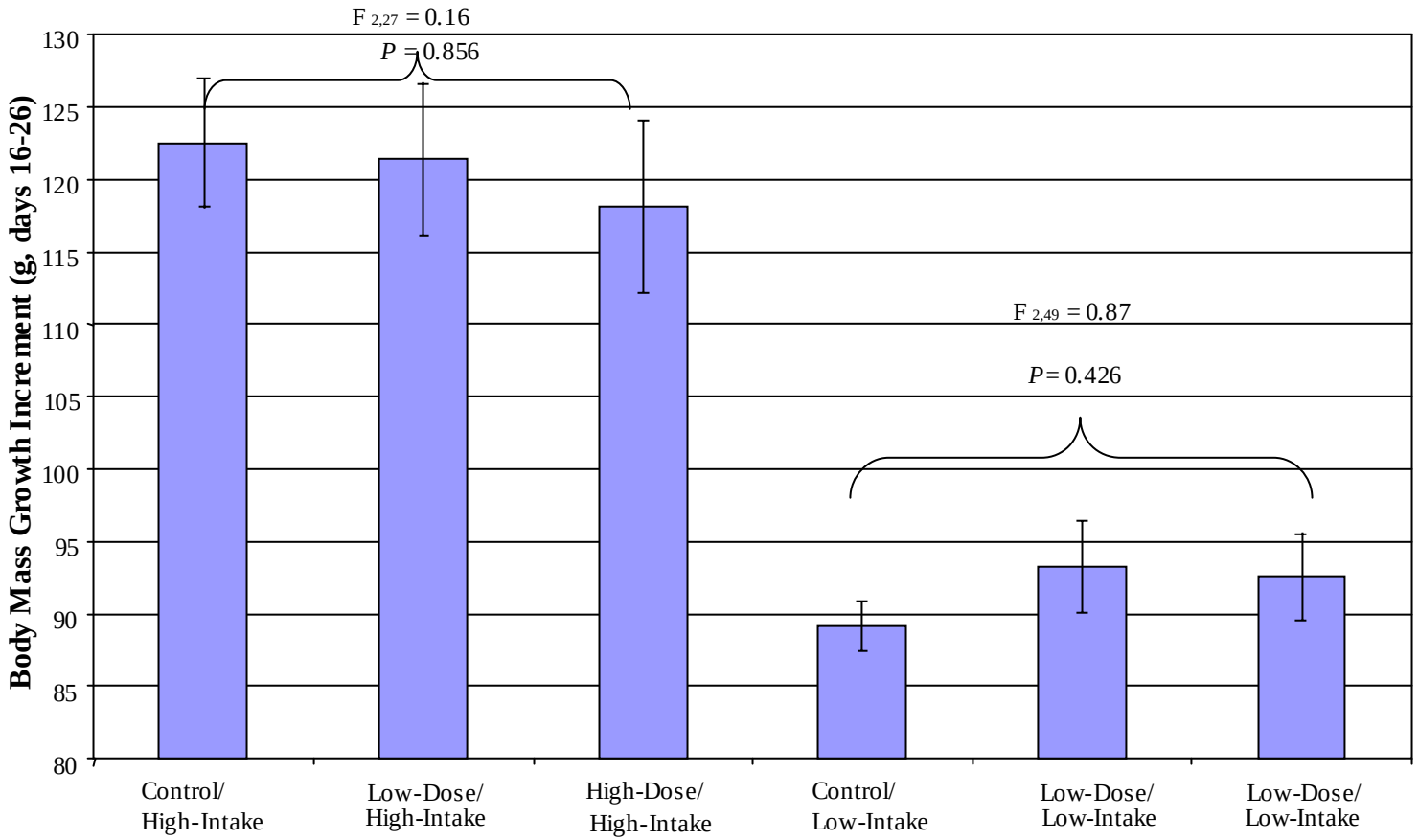


Figure 3.7. Body mass increment for pigeon guillemot chicks as a function of dosing with weathered Prudhoe Bay crude oil over the time course of oil dosing (days 16-26 post-hatch).

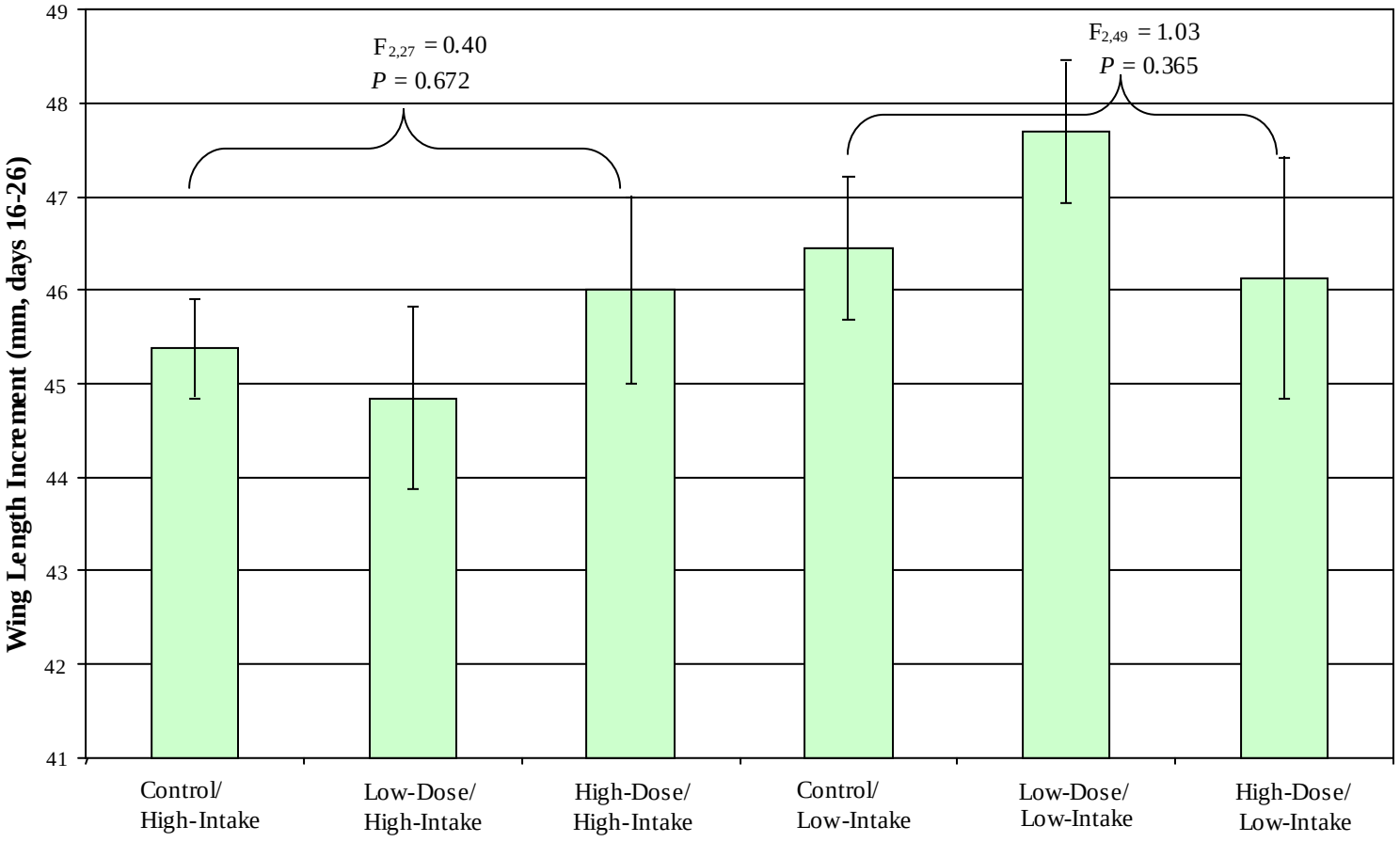


Figure 3.8. Wing length increment for pigeon guillemot chicks as a function of dosing with weathered Prudhoe Bay crude oil over the time course of oil dosing (days 16-26 post-hatch).

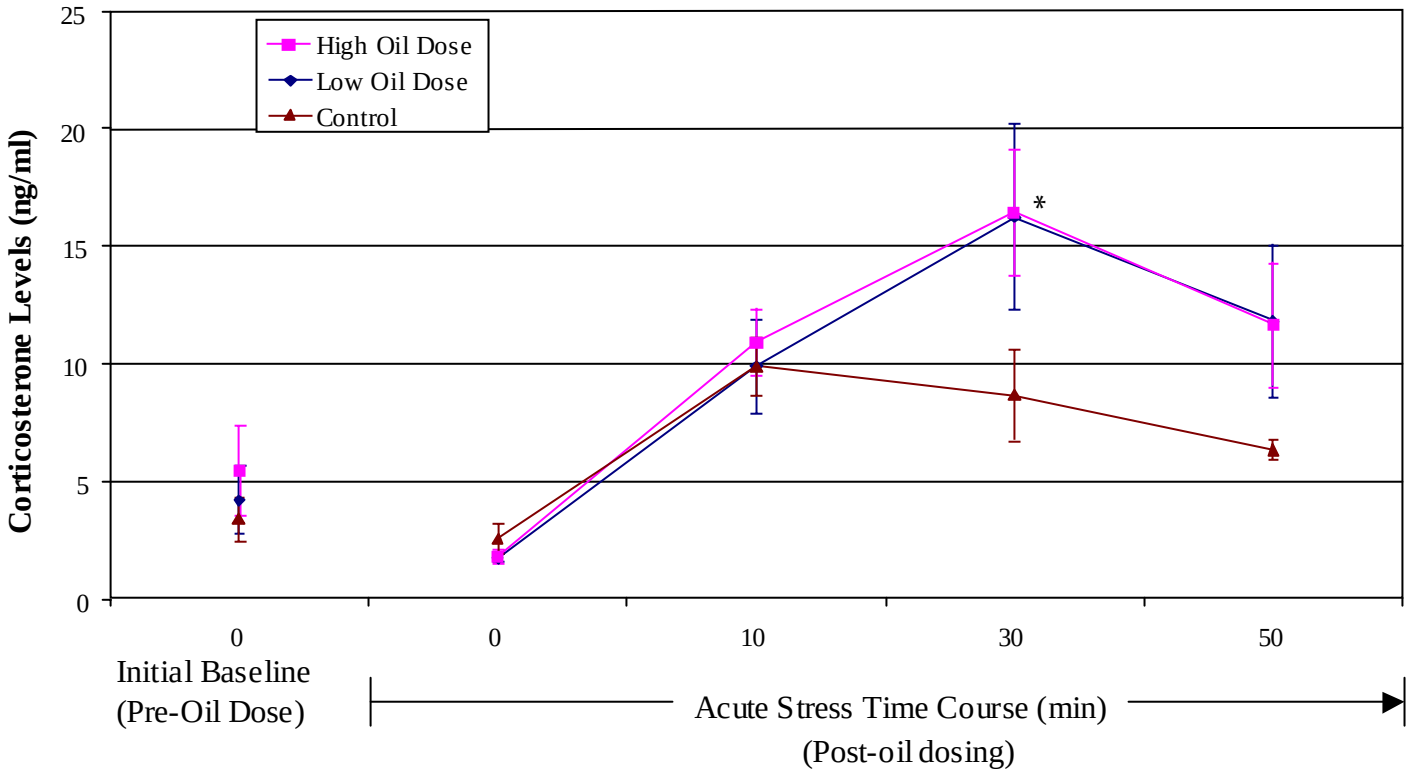


Figure 3.9. Corticosterone levels of pigeon guillemot chicks dosed with weathered Prudhoe Bay crude oil. Initial baseline levels were collected on day 16 post-hatch (pre-dosing). The acute stress time course was applied on day 28 post-hatch for all chick groups (3 days after oil dosing ended). Doses of weathered PBCO were as follows: Low-dose = 0.125 ml $kg^{-1}$ $day^{-1}$; High-dose = 0.50 ml $kg^{-1}$ $day^{-1}$; Control = 0.125ml $kg^{-1}$ $day^{-1}$ corn oil. Average corticosterone values shown ± SE. Significant oil-dosing effect denoted with an asterisk (*).