

Exxon Valdez Oil Spill Restoration
Project Annual Report

**Pigeon Guillemot Restoration Research
at the Alaska SeaLife Center**

Restoration Project 98327
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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April 1999

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Study History: Post-spill (EVOS) studies (projects beginning in FY95 with APEX subproject 95163F and APEX subproject 95163M) have identified three potential factors preventing recovery of Pigeon Guillemots in Prince William Sound, including the reduction in high quality fishes in their diet, exposure to residual oil, and predation. EVOS Restoration Project 98327 was initiated in FY98 to study captively reared Pigeon Guillemots to determine viability of raising and fledging them in conjunction with a social attraction project at the Alaska SeaLife Center. During captive rearing, additional studies were to be performed on food and crude oil ingestion to help address the questions of recovery above. The project has been continued under EVOS Restoration Project 99327 in its second year and Restoration Project 00327 in its third year. This is the first annual report initiated under EVOS Restoration Project 98327.

Abstract: Pigeon Guillemot (*Cephus columba*) populations in Prince William Sound were injured by the Exxon Valdez Oil Spill and have failed to recover from declines occurring before and after the spill. Three factors have been proposed for the lack of recovery: (1) increased predation on eggs and chicks, (2) decreased availability of high-quality schooling forage fish prey (e.g., herring, sand lance), and (3) stress associated with exposure to residual oil. In 1998 we initiated studies on these three factors. We constructed nesting platforms with nest boxes and decoys at the Alaska SeaLife Center in Seward, Alaska and in Prince William Sound to examine the utility of man-made nest cavities in attracting guillemots to breed and in reducing nest predation. We also examined the effect of prey quality on chick growth and fledging success by hatching eggs in captivity and raising chicks on four restricted diet treatments ranging from low to high lipid content. Growth (body mass) of guillemot chicks fed equivalent biomass diets of different prey was positively correlated with the energy density of those prey. Chicks raised on lower lipid diets fledged at older ages. Captive-raised chicks (n = 23) were allowed to fledge into the wild from the Alaska SeaLife Center and subsequent resightings or recruitment at the nesting platforms could demonstrate the effect of pre-fledging diet on post-fledging survival, and the utility of captive rearing as a direct restoration technique. In 1999-2000, small numbers of chicks will be dosed with oil to allow identification of blood biomarkers of oil ingestion. A lack of prospectors at the nesting platforms and decreased breeding birds at colonies visited for egg procurement in 1998 could indicate Pigeon Guillemot populations are depleted throughout the northern Gulf of Alaska.

Key Words: pigeon guillemot, *Cephus columba*, social attraction, captive rearing, fledging, diet, lipid, hatching, proximate analysis, energy content, growth rate, fledging period, physiological ecology.

Project Data: Data collected to date include pigeon guillemot chick growth rates and fledging times for chicks raised on differing lipid diets. Data on collection and captive rearing success is also included. One of the most critical components derived in the first year of the study were the

requirements for successfully raising captive guillemots from egg collection to fledging back into the wild.

Citation: Hovey, A. K.; G. J. Divoky; and D. D. Roby. 1999. Pigeon guillemot restoration research at the Alaska SeaLife Center. *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 98327), Oregon Cooperative Fish and Wildlife Research Unit, Biological Resources Division-USGS and Department of Fisheries and Wildlife, Oregon State University, Corvallis, Oregon.

Introduction

The Pigeon Guillemot population in Prince William Sound (PWS) decreased from approximately 15,000 in the 1970's to less than 5,000 in the 1990's (Laing and Klosiewski 1993). While mortality from the 1989 Exxon Valdez Oil Spill contributed to the decline, the population was decreasing before the spill. Unlike some other species affected by the spill, Pigeon Guillemot populations in PWS have not recovered.

Post-spill studies have identified three potential factors preventing recovery:

- 1) The proportion of high-quality schooling forage fish in the diet has decreased at some PWS colonies (Oakley and Kuletz 1996). The percentage of high lipid forage fish in the diet is a pivotal factor affecting guillemot reproduction (Golet et al. in press). A decline in the availability of forage fishes (sand lance, herring, capelin) in the last two decades may be decreasing growth rates, fledging success, post-fledging survival, and adult recruitment rates.
- 2) Exposure to residual oil in nearshore demersal and schooling fish could increase adult mortality or decrease breeding success. The association of Pigeon Guillemots with nearshore benthic habitats would be expected to increase their exposure to residual oil associated with sediments in the Sound.
- 3) Predation on guillemot eggs and chicks is higher than prior to the spill and could be contributing to the decline or impairing recovery (Hayes 1995). Sub-optimal nest cavities that allowed successful reproduction in the past may no longer be able to do so due to increased accessibility of predators to some of these sites.

Objectives

This research project has three primary objectives listed below. During the first year of the project (FY 98), the primary focus was on objectives 1 and 2.

Objective 1. Determine the feasibility of using direct techniques for restoration of Pigeon Guillemots including:

- a) providing artificial nest sites;
- b) use of social attraction, such as decoys and playbacks of vocalizations;
- c) release of captive-reared young.

Objective 2. Determine the effect of diet variables on growth performance, development, fledging condition, and post-fledging survival of Pigeon Guillemots, including:

- a) types of forage fish consumed, with emphasis on schooling forage fishes vs. nearshore demersal fishes;
- b) lipid content of the diet;
- c) size of prey items.

Objective 3. Determine the response of specific biomarkers for crude oil exposure to variables of exposure in Pigeon Guillemot nestlings, and the survival of exposed nestlings post-fledging. Exposure variables that will be examined include:

- a) dose of ingested oil;
- b) degree of weathering of ingested oil;
- c) time since ingestion of dose;
- d) frequency of exposure.

Methods

In 1998 we initiated a study of the above three factors at the Alaska SeaLife Center (ASLC) in Seward, Alaska.

Social attraction. To assess the utility of nest site provisioning as a direct restoration option we erected nesting platforms with Pigeon Guillemot decoys and nest boxes in Resurrection Bay, adjacent to the ASLC, and in Prince William Sound at Naked Island. All platforms had a similar design: a 1.2 m x 2.4 m plywood platform, which supported four nesting boxes, covered by another 1.2 m x 2.4 m piece of plywood with four to six decoys bolted to the top of the structure. The nest boxes consisted of 30 cm x 60 cm boxes with entrances at both ends and two interior baffles obstructing viewing of the central nesting chamber from either entrance. Additionally, audio playbacks of Pigeon Guillemot vocalizations were played near the nesting platforms at the ASLC. Nesting platforms were regularly monitored for visits by prospecting guillemots.

Egg and Chick Collection. Eggs and chicks were obtained from wild populations and hatched and raised in captivity. Lack of suitable sources of eggs in close proximity to Seward forced us to prospect for potential sources in other areas. Eggs were collected from five areas: Kachemak Bay, Eastern Prince William Sound, Northwestern Prince William Sound, Shumagin Islands, and Deception Bay (Southeast Alaska). The number of eggs collected at each location is presented in Table 1.

Eggs were placed within commercial egg containers and transported from the location of collection inside a portable "Brooder" manufactured by Dean's Animal Supply, Inc. and provided by ASLC. The "Brooder" is a modified insulated container with a thermostatically-controlled heater powered by a 12-volt battery when in transit. While in the "Brooder," eggs were kept at 36°C with humidity maintained by two sponges next to the egg containers. Eggs were collected primarily from two islands in Prince William Sound (Fool Island, Little Mummy Island), one island in Kachemak Bay (Hesketh Island), and two islands in Deception Bay (near Juneau). Eggs were transported in the portable brooder to the ASLC in Seward, Alaska, where all laboratory work was done.

Egg incubation and hatching. Upon arrival at ASLC the eggs were weighed to the nearest 0.1 gram and the length and breadth measured to the nearest 0.1 mm. Eggs were candled to determine if a viable embryo was present and the stage of development before being placed in a Grumbach Incubator Model #1804. Additionally, the buoyancy and orientation of eggs in fresh water was recorded to provide additional information on stage of development. Eggs were

maintained in an incubator at 37.2°C, 85% humidity, and were automatically turned by rollers every 6 hours. During incubation, eggs were weighed every two days to monitor weight loss. Alcid eggs average a 15% mass loss from laying to hatching (S. Rollins, pers. com.); consequently we attempted to achieve this level of mass loss. In the latter stages of development, eggs were examined daily for signs of pipping (breaking of the shell by the emerging chick) or movement of the egg that indicated hatching was imminent. Eggs were then moved to a second Grumbach incubator with temperature and humidity the same as the first, but with eggs placed in a dish to hold the chick and shells after hatching. Eggs were checked every few hours to monitor progress of hatching. After hatching, chicks were kept in the incubator until their down was dry.

Care and fledging of Pigeon Guillemots. The schedule of brooder temperatures used for chicks from the time they were dry (typically 2-4 hours after hatching) until 96 hours post-hatch is presented in Table 2. Dean's "Brooders" were used for all chicks until they were able to maintain their own body temperature. In the wild, guillemot chicks are typically brooded continuously for approximately four days (Drent 1965), indicating that homeothermy is not completely developed until after that age. At day 4-5, the chicks were moved to individual artificial nest sites at a temperature of approximately 13°-16°C. Mass and wing chord lengths of chicks were obtained daily during the period from their hatch until fledging.

Pigeon Guillemots, in the wild, fledge at night as early as 30 days after hatching, with most fledging after 35 days (Hayes 1995). After day 30 post-hatch, captive-reared chicks were moved each evening to a roof location at the ASLC adjacent to Resurrection Bay. Birds were observed and given the opportunity to fledge during the night hours. All fledglings were banded with a stainless steel U.S. Fish and Wildlife Service leg band and a unique combination of colored polyvinyl chloride bands to allow individual identification at a distance.

Acquisition of fish prey. The dates of harvest, harvest location, standard length ranges, and the range of masses of fish used for chick diets are reported in Table 3.

Analysis of energy and lipid content of fish prey. Bomb calorimetry analysis was performed to compute the whole body energy content (WBEC) of fishes. Multiple fish samples were dried in a 60°C convection oven until constant mass was achieved. Individual wet and dry masses were used to compute the moisture content of each fish sample. Dried fishes were ground by mortar and pestle and measurements of caloric content were made using a Parr adiabatic bomb calorimeter in the laboratory of Dr. A.J. Paul at the Institute for Marine Science, University of Alaska-Fairbanks in Seward.

Lipid content of fish samples was determined by proximate analysis. Individual fish samples were dried to constant mass in a convection oven at 60°C to determine moisture content. Lipid content of the dried samples was determined by solvent extraction using a soxhlet apparatus and a 7:2 hexane/isopropyl alcohol (v:v) solvent system. Lean dry samples were transferred from cellulose extraction thimbles to pyrex beakers and ashed in a muffle furnace at 600°C to calculate ash-free lean dry mass (protein) by subtraction. WBEC was calculated from the results

of the proximate analysis and the energy equivalents of the lipid and protein-containing fractions (Schmidt-Nielsen 1997).

Restricted diet experiments. Newly hatched Pigeon Guillemots were fed on an unrestricted diet of 0+ sand lance eight times per day until an age of 11 days post-hatch. On day 11, chicks were switched to one of four restricted diet treatments: juvenile Pacific herring (*Clupea harengus pallasii*), juvenile walleye pollock (*Theragra chalcogramma*), Pacific sand lance (*Ammodytes hexapterus*), or nearshore demersal fishes (crescent gunnel [*Pholis laeta*] and high cockscomb [*Anoplarchus purpurescens*]). At day 11, birds were fed 100 g; at day 12: 125 g; at day 13: 150 g; at days 14-30: 180 g. After day 30, birds were fed *ad libitum* at each of their 8 daily feedings until they fledged.

Blood and excreta sample collection. Blood and excreta samples were collected this year to obtain some baseline levels for the biomarkers to be assayed. Blood was drawn from 20, 25, and 30 day-old chicks. Approximately 1 ml was collected from a brachial vein at each blood draw using 25 or 26 gauge syringe needles. All blood samples were collected in sodium heparin-containing tubes to prevent clotting, placed immediately on ice, and centrifuged for 10 minutes at 1000x g to collect plasma and stored at -70°C until ready for analysis.

Excreta samples were obtained from the bottom of each chick's artificial nest site on days 20, 25, and 30 post-hatch. Samples were stored in plastic bags at -20°C until ready for analysis.

Results and Discussion

Social attraction. Artificial nesting platforms with a total of 12 nesting cavities were set up on a seawall and pier pilings at the head of Resurrection Bay. Decoys and audio playbacks were used in an attempt to attract prospecting Pigeon Guillemots. These platforms were observed from the ASLC for any signs of the presence of Pigeon Guillemots. Four distinct observations were made of Pigeon Guillemots at the head of Resurrection Bay, near the platforms' location. Observations are summarized in Table 4. No guillemots were seen prospecting for nest sites in the immediate vicinity of the nesting platforms during the 1998 season.

Hatching and fledging success. Success in hatching the 44 eggs collected was 52% (Fig.1). Hatching success in wild alcid populations is typically > 70% with most loss coming from parental inattentiveness, predation or other loss of eggs that would be expected in natural situations. No clear indications could be found for the cause of low hatching success. Candling techniques showed that the vast majority of eggs had gone through some embryonic development during the incubation period and were thus not infertile. Our 52% success rate for eggs in an incubator was apparently due to: 1) vibration or other unintentional mishandling of the eggs after collection leading to damage to the developing embryo and/or 2) temperature or humidity settings in the incubator resulting in stress to the developing embryo.

Of the 25 nestlings that we attempted to raise (2 collected as chicks), 92% were successfully raised and fledged (Fig. 1).

Diet composition. A variety of dietary treatments were used to assess diet quality in the growth of Pigeon Guillemot chicks. Fish of higher energy density (i.e., higher lipid content) and fish of lower energy density (i.e., lower lipid content) were used to feed chicks. Sand lance and herring are typically considered “higher energy density” fishes, juvenile walleye pollock are considered “lower energy density” fishes. Gunnels are considered intermediate in lipid content and energy density. Bomb calorimetry techniques were used to assess the whole body energy content (WBEC) of three of the fish species used in the dietary treatments (Fig. 2). The juvenile Pacific herring had the lowest WBEC of the fish tested, contrary to expectation. The apparent reason was that these fish were harvested in March 1998 and were at a seasonal low for energy density (Paul et al 1998a). The WBEC of the juvenile pollock was somewhat higher than expected, and the apparent reason was that the pollock were harvested during a seasonal high for energy density (Table 3; Paul et al 1998b). As a result, the observed order of WBECs of the fish species used for guillemot diet treatments was different than expected. Bomb calorimetry analysis indicated that the juvenile herring had the lowest WBEC, juvenile pollock the next lowest, and sand lance the highest WBEC of the fishes analyzed by this method (Fig. 2).

Proximate composition analysis of these fishes provided an alternative calculation (to bomb calorimetry) of WBEC and the contribution of lipid to WBEC. The final WBECs obtained through calculations by this method revealed the same rank order, from least to greatest, as the values from bomb calorimetry analysis (Fig. 3), but some of the values differed significantly between the two methods. The values for sand lance and juvenile herring were significantly different (respectively, 0.96 and 0.46 kJ/g wet mass more for the bomb calorimetry data). The values obtained for juvenile pollock whether determined from bomb calorimetry or proximate composition analysis were the same, and the values for gunnel species were similar to the average for crescent gunnels (4.69 ± 0.08 kJ/g wet mass) reported previously (Anthony et al. in press; Van Pelt et al. 1997). Because the bomb calorimetry data are a more direct measure of WBEC, these data were used for estimates of dietary energy intake. The proximate composition analysis data did provide a WBEC value for gunnels, which was used for estimates of energy intake of gunnel-fed birds. Also obtained in the composition analysis data was a breakdown of caloric content of fish as by component: the pollock and sand lance contained 23.6% and 23.2% of total energy as lipid, respectively (Fig. 3). The herring and gunnels both contained 15.9% of total energy as lipid (Fig. 3).

Chick growth rates. After day 13 post-hatch, chicks were fed daily on 180 g of one of the four diet treatments: 1) juvenile herring, 2) juvenile pollock, 3) sand lance, or 4) gunnels. Daily energy intake on these diets varied from 587 kJ/day for the herring diet to 805 kJ/day for the sand lance diet (Fig. 4). We expected that the sand lance diet would be even higher, if the chicks on these diets had consumed a constant 180 g of food per day. The average intake of sand lance by chicks, however, was 142.7 ± 18.6 (SD) g/day for chicks fed sand lance *ad libitum*.

Comparisons of the overall growth rates, by mass, of chicks during the linear phase of growth (defined here as days 10 to 30 post-hatch), indicated that the gunnel-fed chicks had higher growth rates than the sand lance- and pollock-fed chicks (Fig. 5). Herring-fed chicks had lower growth rates than sand lance- and pollock-fed chicks during this same period (Fig. 5). Masses of chicks on the four different diets were compared at the end of the restricted diet treatments (Fig.

6). Significant differences in mass were noted between the herring-fed group and the pollock-, sand lance-, and gunnel-fed groups (paired t-test, p-values=0.0008, 0.007, and <0.0005, respectively; Fig. 6). There was also a significant difference in mass between the sand lance-fed group and the gunnel diet group (paired t-test, p-value=0.021; Fig. 6).

Examination of wing chord lengths at the end of the restricted diet treatments showed that the sand lance-fed group had significantly lower wing length than the other three groups (Fig. 7). The other three groups had wing lengths that were statistically indistinguishable (Fig. 7).

Fledging analyses. Examination of the mass, wing chord length, and age of chicks on the day of fledging was performed (Fig. 8). The chicks fed low energy herring fledged at an older age than those from the other three diet groups (Fig. 8).

Analysis of baseline blood parameters in growing chicks. Measurement of specific biomarkers in blood or excreta may be useful in determining the direct exposure of wild Pigeon Guillemots to oil. In the upcoming years of this study, dosing with weathered Prudhoe Bay crude oil (PBCO) will be performed on some of the captive-reared chicks in an attempt to see if the presence or lack of certain biomarkers are associated with oil ingestion in a controlled laboratory environment. Currently we are evaluating options for biomarker assays as we try to target the best markers for the upcoming oil dosing work.

One of the biomarkers we may use in our work is haptoglobin. Work with river otters living in the Exxon Valdez oil spill area indicated that haptoglobin levels were elevated (Duffy et al 1994). Pritchard et al. (1997) dosed wild Pigeon Guillemot chicks with weathered PBCO and showed that it had a significant effect in lowering serum haptoglobin levels. Recent work on Pigeon Guillemots living in the Exxon Valdez oil spill area in Prince William Sound has detected no differences in haptoglobin levels between birds living in oiled and non-oiled areas (Seizer In prep; G. Golet, pers. com.). Looking at haptoglobin levels in Pigeon Guillemots in a controlled environment may help define whether haptoglobin can be a useful biomarker for petrochemical ingestion.

However, current work being done by others, including Dr. Scott Neuman at UC-Davis, may help us focus our attention on other plasma proteins that may serve as better potential biomarkers for oil exposure.

Base line plasma samples and excreta samples from 1998 are in -70°C storage as we decide which biomarkers to assay.

Conclusions

- Nesting platforms with artificial nest cavities and decoys were set up in 1998 but not used by guillemots.

- Guillemot eggs and chicks (n=46) were collected from wild populations and hatched and raised at the Alaska SeaLife Center to provide chicks for the examination of the effects of prey quality on growth and fledging.
- Hatching success of the 44 eggs was 52%. Ninety-two percent of the captive-reared chicks (n=25) were successfully fledged into the wild.
- Herring harvested in late winter 1998 had a WBEC and lipid content below what was expected because they were at seasonal lows for energy density.
- Pollock were harvested during the spring zooplankton bloom in 1998 at a seasonal high for WBEC, resulting in higher than expected energy density.
- Chicks fed on sand lance diets had more health problems than chicks on the other diets. Two chicks died and two more underwent antibiotic therapy while on this diet treatment. This suggests that the commercially-provided sand lance was of poor quality.
- Growth (measured by mass) of guillemots on restricted diets was positively correlated with daily energy intake, excluding the sand lance-fed group.
- The growth results in the sand lance-fed group were confounded by health effects or fish quality unrelated to energy content resulting in excessive variation in daily energy assimilation among individuals
- Age at fledging was negatively correlated with daily energy intake.

Acknowledgments

Sadie Wright and Lisa Thomas provided invaluable assistance in the field and laboratory. The staff and volunteers of the Alaska SeaLife Center, especially Susan Inglis, provided logistical support and encouragement throughout. Greg Golet, Mike Litzow, John Piatt and Bruce Wright assisted with acquisition of eggs and chicks. Denise McKelvey supplied pollock collected on NMFS surveys.

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