Temperature acclimatization in the black-billed magpie (Pica pica hudsonia, Sabine)
by Robert Eugene Stevenson

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Zoology
Montana State University

Abstract:
A study of temperature acclimatization of the black-billed magpie (Pica pica hudsonia) from southwestern Montana was done. One hundred eighteen birds were used in the study. The basal metabolic rate for newly captured birds in September and December was. 1.71±.52 cc02/g hr. A significant difference (P<.05) was found between April birds caged outside and birds taken in September and December with regard to metabolic rates. Thermal conductivities were higher in birds taken in late August and early September than in the December birds. Also respiratory water loss was significantly higher in late summer birds than at any other time.

The lipid indices was highest in the spring, correlating with reduced oxygen consumption rate at that time. There were no significant differences among birds with regard to water index, protein bound iodine, corticosterone or hematocrit.

It is proposed that black-billed magpies are limited in their eastward distribution in the mid-western United States by the lethal combination of high ambient temperatures and high relative humidities.

t Z

TEMPERATURE ACCLIMATIZATION IN THE BLACK-BILLED MAGPIE *(PICA PICA HUDSONIA*, SABINE)

by

ROBERT EUGENE STEVENSON

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Zoology

Approved:

Head, Major Department

Chairman, Examining Committee

Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

December, 1971

ACKNOWLEDGMENT

I am deeply indebted to my major advisor, Dr. Harold Picton, for suggesting this problem and providing needed advice and encouragement throughout the study. Dr. George Davis kindly furnished the space necessary for housing the birds. Mr. Allen Beeckler and his staff gave valuable advice and assistance in dealing with the care of birds and supplying equipment and feed whenever needed. Dr. Edward Moody was very generous with advice, time and equipment in dealing with the hormone assay, as was Dr. Larry Jackson with the lipid work. Many thanks are due Dr. Kenneth Tiahrt for help with the statistical analysis. Thanks are also due Dr. William Dorgan for providing cages and to Drs. Robert Eng, Lelyn Stadnyk and James Liebelt for assisting with the trapping of the birds. Finally, I want to express my sincere thanks to my wife, Pauline, for encouragement throughout my tenure as a graduate student. This work was financed in part by a National Science Foundation Traineeship.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

A study of temperature acclimatization of the black-billed magpie *(Pica pica hudsonia)* from southwestern Montana was done. One hundred eighteen birds were used in the study. The basal metabolic rate for newly captured birds in September and December was $1.71 \pm .52$ $cc^{0}{}_{2}/g$ hr. A significant difference ($P<.05$) was found between April birds caged outside and birds taken in September and December with regard to metabolic rates. Thermal conductivities were higher in birds taken in late August and early September than in the December birds. Also respiratory water loss was significantly higher in late summer birds than at any other time.

The lipid indices was highest in the spring, correlating with reduced oxygen consumption rate at that time. There were no significant differences among birds with regard to water index, protein bound iodine, corticosterone or hematocrit.

It is proposed that black-billed magpies are limited in their eastward distribution in the mid-western United States by the lethal combination of high ambient temperatures and high relative humidities.

INTRODUCTION

Avian adaptation to temperature has been studied by numerous investigators. Dawson and Hudson (1970) provide a comprehensive review of the available information on temperature regulation in various bird families.

My study involves a Corvid, the black-billed magpie *(Pica pica hudsonia* Sabine*)*. Black-billed magpies are permanent residents of many areas in Montana and they must withstand temperature extremes from 41 C to -46 C (105 F to -50 F), occasional winds of high velocity and varying snow depths. Prime habitat for these birds is brushy, tree filled coulees and large bushes and trees along stream bottoms bordering open agricultural lands. They are seldom found in mountainous terrain or taiga and tend to avoid the open treeless prairie. The black-billed magpie was chosen for this study because of its apparent hardiness, abundance and relative accessibility.

Black-billed magpies from southwestern Montana showed no weight loss during the winter months according to Todd, 1968. Erpino (1958), through thyroid histology of black-billed magpies from southern Wyoming, found no specific response to low temperatures.

The purpose of my study was to investigate the physiological and behavioral means whereby the black-billed magpie is able to accomodate itself to the temperature regime of this area. An effort was made to determine if photoperiod was important as a *Zeitgeber* for thermal acclimatization. Oxygen consumption, respiratory water loss, thermal conductivity, lipid and water indices, hematocrit, thyroxine and corticosterone levels were determined from birds taken during late summer and throughout the winter and from those birds maintained on the artificial photoperiods. Finally, a preliminary analysis was made of the eastward distribution of the black-billed magpie as correlated with the combination of high temperature and relative humidity.

MATERIALS AND METHODS

Trapping, Housing and Blood Removal

A total of 118 black-billed magpies were collected within a twenty mile radius of Bozeman, Montana. The trap used for capturing the birds was 1 2/3 m square with a cone-shaped entry 18 cm in length resting on the ground (Brown, 1958). Bait consisted of cow and pig viscera and dead Japanese quail *(Coturnix coturnix)*. Birds which were used in the photoperiod experiments were caged individually indoors in 70 cm x 70 cm x 45 cm wire cages. The light provided came from two 40 watt fluorescent lamps situated 1 2/3 m to 3 m from the cages. Light intensity at the nearest cage was 4 foot candles and at the most distant was 0.1 foot candle. The lights were turned on and off automatically.

Four birds caught on June 23, 1970 and eight birds taken on July 9, 1971 were maintained on a 16L 8D light schedule (16 hours of light and 8 hours of dark, beginning at 06:00 and ending at 22:00 daily) until late November and early December when they were killed. Control birds for all photoperiod experiments were kept individually outside in 25 cm x 40 cm x 18 cm wire cages. Nine birds captured on December 28 and 29, 1970 were used for the 9L 15D (08:00-17:00) light schedule. These birds were removed and killed at the end of March. Quail was used as food except during August, 1970 when chicken parts were substituted. Water was available *ad libitum.*

Blood was collected by lightly anesthesizing a bird and then severing the carotid artery about 2 cm above the base of the neck. The body of the bird was elevated above the head and a plastic tube placed at the site of the incision. Up to 6 ml of blood could be collected using this technique. Hematocrit values were obtained by centrifuging blood collected in heparinized micro-hematocrit tubes in a micro-hematocrit centrifuge for 20 minutes. Percentages were read on a mechanical hematocrit reader. Serum was collected by centrifuging whole blood at 3600 rpm for 20 minutes. The serum was stored in corked vials and kept frozen until used.

Oxygen Consumption and Respiratory Water Loss Measurements

Oxygen consuption was measured using an open circuit system. Two animal chambers were used. For temperatures above 10 C a cylindrical 18.9 liter plexiglass container surrounded by a 3 cm water jacket was used. One end of the chamber was sealed with plexiglass, the other end was sealed with a removable cork plug 4 cm thick. Temperatures in the chamber were reached and maintained by using water heated and circulated by a Brinkman ultra-thermostat. Chamber temperatures were measured using a mercury thermometer which was permanently installed in the cork end. The chamber was kept semi-darkened by covering it. An air pump was used to force air through the system. Air entering the chamber was dried by passing it first through Drierite. Outgoing air from the chamber passed through a copper coil maintained at room temperature, a flow meter, and a drying tube filled with silica gel. A Servomex Oxygen Analyzer Type OA 150, designed for direct readings of oxygen percentages, was used to determine oxygen consumption. For oxygen consumption determinations at ambient temperatures below 10 C the low temperature animal chamber was an 18.9 liter metal can. This can was fitted with copper tubing for entering and exciting air. This apparatus was placed in a Cole-Parmer Instrument Company low temperature cabinet. Temperatures of -41 C could be reached with this apparatus. In the chambers birds rested on a 1 cm^2 mesh screen fitted on a rectangular plastic pan. The pan was filled with mineral oil to a depth of 1.5 cm. The mineral oil prevented fecal moisture from being added to the respiratory water. Moisture-free air was pumped through a copper coil placed in a freezer in order to reduce the air temperature entering the chamber to that of the freezer. Outgoing air passed through another copper coil maintained at room temperature. A pump produced an air flow between 600 and 1010 cc/min, usually around 805 cc/min. Humidity in both chambers was estimated by the method of Lasiewski, Acosta and Bernstein (1966). The mean relative humidity was 51% (range 26%-82%) based on 95 determinations at a chamber temperature above 20 C. The birds were held undisturbed in the chambers for at least one hour before the first readings were taken. After this period oxygen consumption was recorded at five minute intervals for one half hour. These readings were averaged to determine the amount used by the bird at that ambient temperature. At least one hour was allowed to elapse before another set of oxygen consumption readings were taken. Temperatures were not changed by any set amount. All oxygen readings were converted to STP. Respiratory water loss was determined by weighing a drying tube filled with silica gel to 0.1 mg, passing outgoing air through the tube for 30 minutes and reweighing.

Body Temperature, Hormone and Lipid Analyses

Daily body temperature fluctuations were obtained using a small animal thermistor coupled to a two channel Rustrak automatic chart recorder. The thermistor was inserted two to three centimeters into the cloaca and held in place with pinch clamps attached to the rectricies. The second channel measured ambient temperature.

Birds were prepared for lipid analysis by plucking and then removing the manus, head and viscera. The carcass was weighed, homogenized in a blender or meat grinder and oven dried at 70 C or lower. The dried material was reweighed. A fraction of the dried material was placed in a Soxhlet extractor using petroleum ether (30-60 C boiling point) as the solvent. Extractions lasted for at least eight hours. The majority of the solvent was evaporated by flash evaporation and the remainder removed by drying under nitrogen. The lipids were then weighed. Thyroxine was assayed by the Hycel cuvette method for the determination of protein bound iodine (PBI). Serum corticosterone was determined by the radio protein binding assay method of Murphy (1967) as modified by Adams and Wagner (1970).

Values for thermal conductivities were determined for all birds from the relationship (Murish, 1969):

$$\text{conductance} = \frac{(\text{heat produced} - \text{loss by evaporation})}{(\text{body temperature} - \text{ambient temperature})}$$

The caloric equivalent of O_2 was assumed to be 4.8 cal/ml O_2 and the latent heat of evaporation of water = 0.58 cal/mg H_2O.

RESULTS

Physiological Acclimatization

Thermoneutral ranges and the mean oxygen consumption values within the thermoneutral ranges for magpies tested at various times throughout the year and for those subjected to altered photoperiods are shown in Table I. The upper critical temperatures (T_c) were similar in all groups tested (35 C) except for the March birds (40 C) maintained on a 9L 15D photoperiod. The lower T_c varied with the season, being lowest (-10 C) in the December natural (those birds captured and immediately tested) and the December birds (-9 C) caged outside. It was intermediate (0 C) in the September natural, December inside birds (16L 8D) and the April outside caged birds. The highest lower T_c (10 C) was from March inside birds. The widest span enclosed by the thermoneutral range was 45° in December natural birds and the narrowest was 30° in March inside birds. Mean monthly temperatures recorded inside the unheated building which housed the experimental birds and outdoor temperatures from the Montana State University campus weather station are shown in Fig. 1.

Comparison of the mean oxygen consumptions from the thermoneutral ranges showed a significantly smaller difference ($P<.05$) between March birds and all other birds tested except for the April birds caged outside. The oxygen consumption of April birds was also significantly smaller ($P<.05$) than the December outside and December inside caged

Table I. Thermoneutral ranges, means of oxygen consumption rates within the thermoneutral ranges and regression coefficients of lines fitted by method of least squares.

	Sept. (12)[a]	Dec. (6)	April (3) Outside	Dec. (7) Outside	Dec. (9) Inside 16L 8D	March (9) Inside 9L 15D
Thermo-neutral range (°C)	0 to 35	-10 to 35	0 to 35	-9 to 35	0 to 35	10 to 40
Means and sd (ccO_2/gmhr)	1.68±.48	1.74±.56	1.37±.45	1.98±.50	2.00±.80	1.19±.45
Regression coefficients	-.07870	-.02127	-.05825	-.04492	-.00730	-.06899

[a] sample size
computed average regression coefficient $\bar{b}$ = -.055

Table II. Hematocrit, protein bound iodine and corticosterone mean values for black-billed magpies.

	Sept.	Oct.	Dec.	Jan.	Dec. 16L 8D Inside	Dec. Outside	March 9L 15D Inside
Hematocrit (%)	33.2±15.1[a] (10)[b]	37.2±12.6 (10)	42.5±8.2 (10)	43.7±8.3 (10)	41.4±5.2 (10)	39.6±5.2 (6)	43.2±7.7 (3)
Corticosterone mg/ml	66.91±18.1 (11)	51.7±47.7 (10)	69.1±38.1 (11)	65.22±63.3 (8)	57.2±28.3 (9)	51.2±28.3 (6)	10.8±7.5 (5)
Protein bound iodine µg/100 ml	3.7±1.1 (11)	4.3±1.3 (10)	4.4±1.8 (7)	5.1±2.7 (9)	5.9±3.6 (8)	3.3±1.8 (4)	4.0±0.8 (5)

[a] standard deviation

[b] sample size

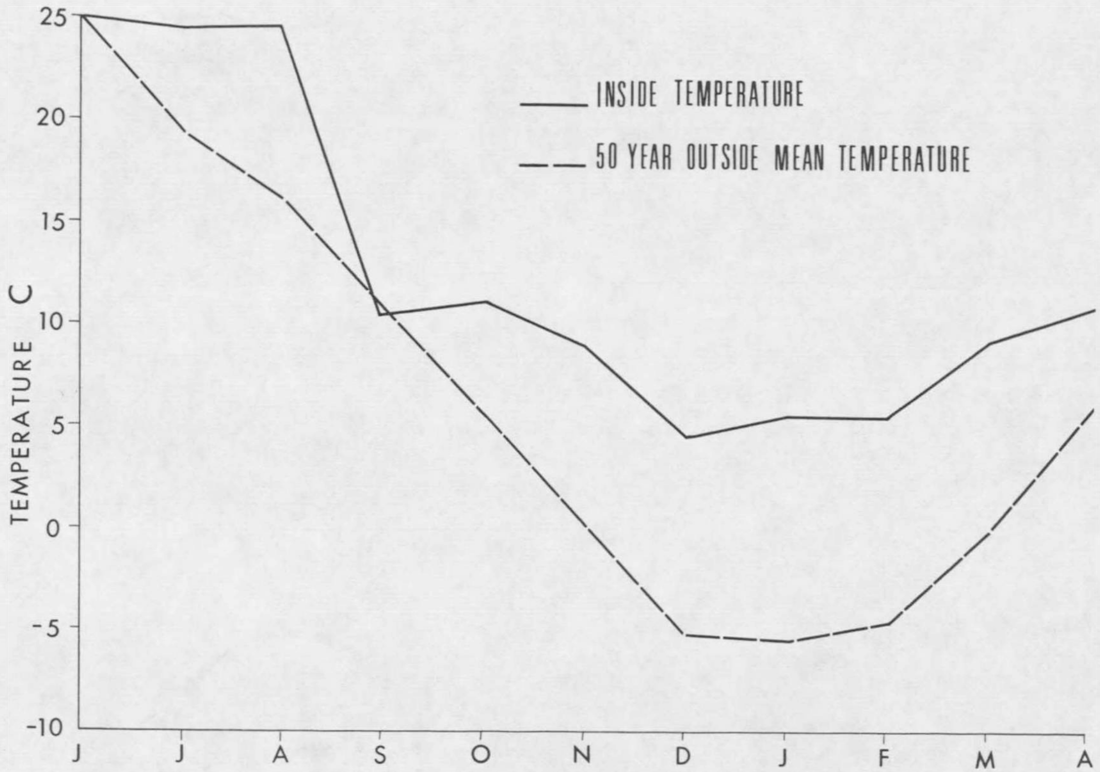

Figure 1. Mean monthly temperature inside barn (1970-71) and mean monthly outside temperature at Montana State University campus, Bozeman, Montana based on 50 year record (1909-1958). Mont. Agric. Expt. Sta. (1967).

birds. Fig. 2 shows six oxygen consumption curves. The regression equations for the points below the lower T_C are included for each curve. The equality of the slopes of the regression lines (Sokal and Rohlf, 1969) was tested. Regression coefficients were found to be homogenous at the 95% level. The average computed regression coefficient was $\overline{b} = -.055$.

Data from each experiment on respiratory water loss (RWL) were fitted to a regression curve containing linear and quadratic terms (Fig. 3). The derived equations were then compared to see if significant differences existed between any group of determinations for the months or photoperiod treatments. RWL of September birds was significantly greater from all other groups ($P<.05$).

The thermal conductivity of black-billed magpies as related to T_a is shown in Fig. 4. In order to adequately compare groups of birds, the means of points above 20 C did not lend themselves to ordinary statistical analysis because no satisfactory linear or quadratic equations were found which truely represented these points. Thermal conductivity is most important during winter so analysis of points below 20 C should show changes due to season or photoperiod. An analysis of variance and a test for homogenity of the means were conducted. The thermal conductivity of December inside caged birds was found to be significantly higher ($P<.05$) than all other groups (Table III).