Gross Morphology as an Indicator of Stress in Captive Common Grackles

By

David P. Scott
(Honors Research)

with Dr. T. W. Townsend (advisor)

School of Natural Resources
Division of Fisheries and Wildlife Management
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Animal laboratory studies are important for wildlife researchers in providing valuable data that would be difficult, if not impossible, to obtain under natural conditions. Caged wild animals are in a foreign environment. Stress that results from handling, dietary changes, confinement, banding or radio-tagging, and other laboratory practices could result in altered behavior, increased susceptibility to disease, or death. The goal of this study was to determine if changes in gross morphology of internal organs can be related to stress resulting from captivity and/or radio-tagging of common grackles (Quiscalus quiscula).

OBJECTIVES

1. To determine if differences exist between the mean organ weights of captive radioed, captive non-radioed, and naturally occurring grackles.
2. To determine if there were differences in the proportion of birds with enlarged adrenal glands, space occupying lesions (SOL's) or tumors, and gout.

BACKGROUND

Behavior and movements of the common grackle have been studied by several researchers (Ficken 1963, Bray et al. 1975, Wiley 1976, Stokes 1979). These researchers studied both radioed and non-radioed birds. In many telemetry studies on a variety of bird species, observed behavior was considered normal. Some studies found no difference in behavior, migration, and reproduction between radioed and non-radioed birds when the transmitters

were properly adjusted (Lance 1970, Gilmer et al. 1974, Bray et al. 1975, Lance and Watson 1977, Martin and Bider 1978, Erikstad 1979, Herzog 1979, Nenno and Healy 1979, Johnson and Berner 1980). Other studies have found significant differences in behavior that resulted from transmitter placement on birds. Differences in behavior include excessive weight loss (Boag 1972, Greenwood and Sargeant 1973, Perry 1981), decreased longevity (Gullion and Marshall 1968, Herzog 1979, Warner and Etter 1983), changes in previously observed migratory behavior (Cochran et al. 1967), and changes in daily maintenance behavior (Boag 1972, Ramakka 1972, Greenwood and Sargeant 1973, Perry 1981). Further study is needed before results of bird telemetry studies can be extrapolated to field conditions.

Evidence of crowding has been seen in changes in the weights of the adrenal gland, thymus, and spleen in female meadow voles (Microtus pennsylvanicus) (Christian and Davis 1966). Also, ruffed grouse (Bonasa umbellus) adrenal weights have been correlated with population densities (Neave and Wright 1968).

Radioed Franklin's spruce grouse (Canachites canadensis franklinii) showed increased mortality during periods of natural stress and several recovered dead females showed signs of renal gout (Herzog 1979). Gout is a little understood disease that is characterized by white powdery deposits of sodium urate in the kidneys, heart, liver, and joints of the feet and could be caused by low vitamin A in the diet, high protein in the diet, or stress (Hasholt 1969, Cooper 1979, Wobeser 1981).

METHODS

Three groups of birds were used for the study: (1) birds with transmitters that were observed in an environmental chamber for behavioral effects; (2) birds without transmitters that were similarly observed; and (3) birds

captured in the wild and not held in captivity or harnessed with transmitters. This group is the control group and was assumed to be representative of wild "normal" birds, however, the histories of these birds were unknown. Birds for groups 1 and 2 were captured between November and December 1983 in southeast Franklin Co., Ohio. These birds were then used in a behavioral study in an environmental chamber. Birds for group 3 were obtained in April 1984 from USFWS personnel at the Plumbrook Field Station in northwest Ohio. These birds had just been caught and were immediately transported to The Ohio State University for use in the study.

Dr. J. Donahoe, D.V.M. (pers. commun.) described and demonstrated the necropsy technique used. First, the bird to be necropsied was killed by using a combination of ether and cervical dislocation. This was the humanest method possible. The bird was then weighed on an electronic scale to the nearest 0.01 gm. After an external visual examination to note any abnormalities or parasites, the bird was opened up by removal of the chest muscles. Organs to be removed included the heart, liver, spleen, adrenal glands, gonads, and kidneys. Initially, the thymus was to be removed as well, but it could not be located in adult birds. In immature grackles the thymus is present and visible, but this organ atrophies and is hard to locate in adult birds. Other abnormalities such as SOL's, parasites, and gout were noted as the other organs were removed. Also, the general health of the bird was noted.

When an organ was removed from a bird to be weighed, all excess tissue and fat were carefully removed and the organ was dipped into a physiologically balanced saline solution (0.9% NaCl)(J. Donahoe, pers. commun.). This process helped to rinse blood and other body fluids from the organ that could bias the weight. Organs were then dried of excess liquid (saline) with a paper cloth and were weighed as above.

Weights of the various organs were then converted to ratios of the organ weight (gm) to body weight (kg) to allow for differences in individuals. Statistical analysis of these gm/km ratios involved 3 steps. First, homogeneity of the variances had to be checked using Cochran's test (J. Kasile, pers. commun.). Secondly, a one-way ANOVA was done for each of the organs between the 3 groups (Zar 1974, J. Kasile pers. commun., J. Scott pers. commun.). A two-way ANOVA to separate out the differences attributable to sex would have been more appropriate, however, the number of females in some of the groups was limited and the N would have been too small. Finally, Tukey's test of all possible pairwise comparisons was carried out to determine which groups were significantly different from the others (J. Kasile pers. commun., J. Scott pers. commun.). A statistical proportion test was used to analyze data like the presence or absence of enlarged adrenals, gout, or SOL's in the 3 groups (Spiegel 1961, J. Kasile pers. commun., J. Scott pers. commun.).

RESULTS AND DISCUSSION

Cochran's test for homogeneity of variance showed that there was no significant difference between the variances for any of the organs of the 3 groups at the $P \leq 0.01$ level. Therefore, one-way ANOVA (F-test) could be carried out without transformation of the data from the gm/kg ratio. ANOVA results indicated that there is a significant difference ($P \leq 0.05$) between the 3 groups in the mean weights of all organs except the spleen (Table 1). Tukey's test to determine which means were significantly different from each other ($P \leq 0.05$) showed a significant difference between the control group (highest means) and the non-radioed group (lowest means) for all these organs (Table 2). There was no difference between the mean weights of the 2 treatment groups, however, there was a difference between the radioed birds

and the control birds in mean heart weight.

Proportion analysis showed only a difference between the control birds and each of the treatment groups in the presence of enlarged adrenals (Table 3). There was no difference for gout among any of the groups; only 1 case was found and it was visceral gout in a control bird.

Holmes and Cronshaw (1980) reported that birds which experience an annual gonad enlargement and reduction often have a similar change in the weights of the adrenal glands. All birds in group 3 had slightly enlarged adrenals, whereas only 42.1% and 33.3% of groups 1 and 2, respectively, showed slightly enlarged adrenals. Group 1 and 2 birds were subject to light conditions unconducive to gonadal development for reproduction. Group 3 birds, caught in the spring, were in reproductive condition. Mean weights of reproductive organs were not statistically analyzed because of this difference among the groups. This reproductive state (group 3) explains why there was a significantly higher proportion of enlarged adrenals. Although stress could have caused adrenal enlargement in the treatment groups, they could not be fairly compared to the control group. A control group captured in the fall would provide a better comparison.

The occurrence of SOL's (tumors) in the treatment groups may indicate stress on these birds. However, the proportions were low (5.3% of radioed and 16.7% of non-radioed) and were not statistically significant from the controls (0%). The causes of SOL's are many, and they could have been present in the treatment birds when captured (J. Donahoe, pers. commun.). These birds may have died in the wild but were able to survive in captivity when plenty of food and water was available.

Only 1 case of gout (visceral) was discovered (group 3) and no significant

differences existed between the groups. Gout appears to be a poor stress indicator for grackles by the results of this study. As with the rest of these tests, a larger N for the various groups would help in cases of unclear or non-existent data.

Significant differences in the mean organ weights [heart, liver, kidneys (whole and in part)] between the control group and non-radioed birds was consistently evident. In every case, the highest mean weight was for the control birds, then the radioed birds, and non-radioed birds were the lowest. Control birds had the least amount of fat in their bodies (visual estimate). This was probably due to the recent spring migration and reproductive activities. The ratio (gm/kg) for each organ in these birds should be higher since less fat is contributing to total weight. Treatment birds often had large fat reserves, contributing to lower ratios for the organs. These birds also had less exercise and easier access to food and water. Radioed birds may have had to expend additional energy to carry the extra 5% body weight radio package, and even though they still got enough food and water, less fat was stored. Therefore, the gm/kg ratio was not as low as that of the non-radioed birds. Radioed birds may actually have stayed more "fit" while in captivity due to the extra weight and exercise.

Differences between the 2 treatment groups and the control group in mean heart weight could be from decreased exercise (little flight) resulting in atrophy or "softening" of this muscle. To actually be able to explain this result would take detailed tests (histological and structural) on the hearts of grackles in captivity and in the wild (J. Donahoe, pers. commun.). Such tests are beyond the scope of this paper.

CONCLUSIONS

Treatment birds in this study had a higher incidence of SOL's, although not significant; these birds could have been predisposed if left to overwinter in the wild. Continued collection of data on the occurrence of SOL's with larger sample sizes for all groups may prove significant in the future. The same may be true for gout, however, it appears to be rare in grackles.

Observation of adrenal glands from control birds captured in the fall could yield valuable data as to the significance of these organs as stress indicators. Adrenals probably have the highest potential for indicating stress in birds, but the timing of capture of the control birds for this study caused their use to be inconclusive.

Mean weight of organs proved to be significantly different, but differences were probably a result of good diet and lack of exercise in the treatment groups.

Overall, exceptional stress indicators could not be found at this time in any of the groups or organs. Potential indicators exist and may be useful in the future.

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Table 1. One-way ANOVA table for heart, liver, spleen, and kidney weights (gm/kg) with critical value, $\hat{F}$-value, and sample sizes listed.

ANOVA information	Heart	Liver	Spleen	Left kidney	Right kidney	Total kidney weight
Critical value	3.97	3.97	3.97	3.97	3.97	3.97
$\hat{F}$-value	9.96*	4.78*	0.21	6.58*	4.25*	5.78*
Sample size:						
Total	N=54	N=54	N=53	N=54	N=54	N=54
Radioed	19	19	19	19	19	19
Non-radioed	18	18	17	18	18	18
Control	17	17	17	17	17	17

* significant at the $P \leq 0.05$ level

Table 2. Results from Tukey's test for all possible pairwise comparisons for radioed (R), non-radioed (NR), and control (C) groups showing which means are significantly different at the $P \leq 0.05$ level for heart, liver, and kidney values.

Group comparison	Heart	Liver	Left kidney	Right kidney	Total kidney weight
Critical value	0.864	3.884	0.587	0.603	1.146
R vs. NR	0.33	1.57	0.43	0.40	0.84
R vs. C	1.21*	3.37	0.46	0.34	0.80
C vs. NR	1.54*	4.94*	0.89*	0.74*	1.64*

* significant at the $P \leq 0.05$ level

Table 3. Proportion analysis results showing radioed (R), non-radioed (NR), and control (C) groups. Abnormalities tested and Z-values are listed. The critical range ($P \leq 0.05$) is -1.96 to 1.96.

Abnormality Tested	P_r vs. P_{nr}	P_r vs. P_c	P_c vs. P_{nr}	$P_{treat.total}$ vs. P_c
P(adrenal enlargement)	0.552	-3.759*	4.141*	NA
P(SOL's)	-1.119	0.956	-1.750	1.409
P(gout)	NA	NA	NA	-1.470

* significant at the $P \leq 0.05$ level

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We, the undersigned, certify that David P. Scott has been examined in accordance with the regulations of the Natural Resources Honors Program and we recommend his degree be granted (with) ~~without~~ distinction.

The student is a major in Wildlife Management.

The adviser certifies that he has a 3.5 or higher point hour in his area of specialization.

Date 5/24/84

Examination Committee Signatures:

T. W. Townsend
Faculty Adviser

John P Donahoe

Thomas J. Stoddard

Submit in triplicate on or before:

TO: Secretary
School of
Natural Resources
Agr. Adm. Bldg.
2120 Fyffe Road

Approved Robert E. Henne
Robert E. Henne
Secretary