

# AN ATTEMPT TO FREEZE-BRAND MALLARD DUCKLINGS

BY RAYMOND J. GREENWOOD

## INTRODUCTION

Refinement of mammalian freeze-branding techniques (Farrell et al., 1966; Hadow, 1972) has stimulated interest in using cryogenic methods to mark waterfowl. Freeze-branding of mammals results in permanent depigmentation of pelage. If the technique could accomplish similar permanent depigmentation of feathers, it would have certain advantages over other types of bird markers. Identification could be affixed without attaching devices and would be more lasting than dyes and paints.

Literature on cryogenic treatment of birds is scant. Raspopova and Hvatov (1935) indicated the development of depigmented feathers on dark plumaged chickens, although their methods and results were not clear. Farrell (1966) reported discouraging results from experiments on chickens. The present paper presents the results of freeze-brand tests on Mallard ducklings (*Anas platyrhynchos*) conducted at the Northern Prairie Wildlife Research Center, Jamestown, North Dakota.

## MATERIALS AND METHODS

Three refrigerants were evaluated, ethyl alcohol ( $C_2H_5OH$ ) chilled with dry ice ( $CO_2$ ), chlorodifluoromethane ( $CClF_2$ ), and dichlorodifluoromethane ( $CCl_2F_2$ ). The compounds boiled at  $-72.0$ ,  $-42.5$ , and  $-33.5C$ , respectively. Treatment was by dermal contact with flat-faced, super-cooled brass instruments (31-325 mm<sup>2</sup> contact surfaces) or by application of liquid refrigerant (excluding  $C_2H_5OH$ ). Instruments were super-cooled by immersion in the selected liquid refrigerant until violent boiling ceased. Liquid refrigerants were applied with a dropper at an approximate rate of two drops per second.

Groups of 3-6 ducklings were treated when 5-10 days old. Down was clipped from treatment areas to expose dermal tissue. Exposed tissues were moistened with room temperature  $C_2H_5OH$  before contact with a branding instrument; room temperature  $C_2H_5OH$  was not applied before treatment with liquid refrigerants. Treatment times ranged from 2-24 seconds on one or more of the following areas on each bird: spinal tract, scapulohumeral tract, alar tract, and premaxilla. Treated birds were examined regularly until development of juvenal plumage was completed.

## RESULTS

Physical reaction to treatment was slight except in the case of  $C_2H_5OH/CO_2$ . Preliminary tests with an instrument chilled in  $C_2H_5OH/CO_2$  indicated excessive tissue damage (12-second maximum exposure); consequently, further use of that compound in this study was restricted to instrument treatment of the premaxilla

area. Growth after treatment appeared normal and the presence of bare areas did not stimulate picking among brooder mates. No mortality occurred. Tissue was usually frozen during treatment and remained so for several seconds thereafter; hyperemia developed within a few minutes of thawing. Edema generally developed on frozen tissues within 24 hours and was especially noticeable on the premaxilla. Tissues became scurfy a few days after treatment and severely frozen areas developed scabs; within 7-10 days surface epithelium sloughed off revealing smooth, pink tissue. Eight control birds, from which down was removed and skin moistened with  $C_2H_5OH$  and treatment was with an instrument at room temperature, showed normal plumage development.

Treatments (2-24 seconds) to feather tracts with instruments chilled in  $CCl_2F_2$  resulted in emergence of white and/or white-tipped down in 94 percent of 48 test birds; no follicle destruction was observed. Liquid treatments (2-24)seconds resulted in emergence of white and/or white-tipped down in 54 percent of 48 test birds; follicle destruction occurred on most birds treated for 12 seconds or longer.

Treatments (2-24 seconds) to feather tracts with instruments chilled in  $CCl_2F_2$  caused no noticeable follicle destruction and resulted in emergence of white and/or white-tipped down in 98 percent of 48 test birds. Treatments of 6 seconds or less did not freeze tissue. Liquid treatments resulted in emergence of white and/or white-tipped down in 40 percent of 48 test birds; follicle destruction occurred on approximately half of the birds treated for 12 seconds or longer. The most luxuriant growth of white down noted in this study occurred on each of three birds treated on the spinal tract for 16 seconds with a  $CCl_2F_2$  - chilled instrument. For unknown reasons that treatment did not appear to freeze tissue.

Only occasional traces of white down were evident 8 weeks after treatment. By that time contour feathers that emerged in or adjacent to treated areas obscured the remaining white down in most instances. Contour feathers that emerged were normally pigmented and only in a few of the most severe treatments did they fail to develop, although results were inconsistent.

All treatments to premaxillae (3-12 seconds,  $n = 126$ ) showed evidence of melanocyte destruction and the response was similar among refrigerants. Pink brands developed within 7-10 days after treatment and remained conspicuous for approximately 3 weeks. Within 5 weeks apparent migration of surrounding melanocytes into brand sites occurred on more than 75 percent of the test birds and made brands illegible. Little evidence of treatment remained 4 months after application.

#### DISCUSSION

Freeze-branding of feather tracts, as conducted in this study, appears to have little potential as a permanent marking technique for waterfowl. However, freeze-branding the premaxillae may have some potential as a temporary duckling marker. Treatment is

simple and, in Mallards, results remain conspicuous until dimorphism in bill color begins to become evident.

Inconsistent results obtained in treatment of feather tracts are possibly related to the nature of melanocyte formation and the timing of pigment deposition. According to Foulks (1943), melanocytes continually arise from undifferentiated melanoblasts in the dermis during feather growth. Apparently the presence of white-tipped down resulted from the destruction of melanocytes associated with the tip of the developing feather, which were later replaced with active melanocytes before the deposition process terminated. Although birds were treated at ages of 5-10 days, and at various early stages of feather development, no apparent effect on pigmentation of contour feathers occurred.

#### ACKNOWLEDGMENTS

Appreciation is extended to Forrest B. Lee who provided the experimental birds and arranged for their care. Alan B. Sargeant and Gary L. Pearson reviewed the manuscript.

#### LITERATURE CITED

- FARRELL, R. K. 1966. The freeze-branding technique. In Proceedings freeze brand seminar, p. 5-11. Pullman, Washington State University.
- FARRELL, R. K., L. M. KOGER, AND L. D. WINWARD. 1966. Freeze-branding of cattle, dogs and cats for identification. *J. Am. Vet. Med. Assoc.*, **149**: 745-752.
- FOULKS, J. G. 1943. An analysis of the source of melanophores in regenerating feathers. *Physiol. Zool.*, **16**: 351-382.
- HADOW, H. H. 1972. Freeze-branding: A permanent marking technique for pigmented mammals. *J. Wildl. Manage.*, **36**: 645-649.
- RASPOPOVA, N., AND B. HVATOV. 1935. The influence of different temperature factors on the pigmentation of the feather in birds. *Probl. Zootech. Exp. Endocrinol. (Vses. Akad. S-Kh. Nauk Im. Lenina)*, **1**: 328-334.

*Northern Prairie Wildlife Research Center, Jamestown, North Dakota 58401. Received 20 October 1974, accepted 24 February 1975.*