



An evaluation of the population biology, genetics and future viability of the breeding Wood Duck (*Aix sponsa*) population at Arrowwood National Wildlife Refuge  
by James Bruce Neill

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in  
Biological Sciences  
Montana State University  
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Abstract:

A breeding population of Wood Ducks (*Aix sponsa*), introduced to eastern-central North Dakota in 1968 was evaluated. This population occupies habitat outside the native range of Wood Ducks; numbers of breeding females have greatly declined over the past ten years. Two potential factors for this decline in population numbers were evaluated. These are competition from Hooded Mergansers (*Lophodytes cucullatus*), and reduced genetic variability of the Wood Duck population from the use of captive individuals to seed the population. Analysis of historical nesting data yielded no indication that Hooded Merganser's nesting activities have had any impact on the reproductive success of Wood Ducks. An analysis of 17 polymorphic allozymes was made for the Arrowwood population and a captive population similar to the one originally used to create the Arrowwood population. Using these data, mean heterozygosity, mean number of alleles/locus, and percent polymorphic loci were calculated. All of these indices indicate that the Arrowwood Wood Duck population is more genetically diverse than the captive population, and has levels of genetic variability similar to those reported for other native avian populations. The differences between the two populations were found not to be statistically different. Minisatellite DNA fingerprint analysis was carried out for the Arrowwood population, the captive population and a population from western-central Oregon. These analyses indicate that the Arrowwood population has significantly more variation than the captive population, and both of these exhibit more DNA polymorphism variability than the population in Oregon. It is suggested that the number of nesting females is being underestimated at Arrowwood National Wildlife Refuge because hens are nesting in natural nesting cavities and in areas outside the refuge boundaries. It is suggested that the Wood Duck population in North Dakota has high levels of genetic variability because of a constant influx of novel drakes. Unrecorded nesting and high genetic variability suggests the population of Wood Ducks in eastern-central North Dakota is in no danger of immediate extirpation. It appears that Wood Ducks in the Pacific Flyway have experienced long population bottle-necks causing a paucity of genetic variability; further analysis of Pacific Wood Duck populations is suggested.

AN EVALUATION OF THE POPULATION BIOLOGY, GENETICS AND FUTURE  
VIABILITY OF THE BREEDING WOOD DUCK (*AIX SPONSA*) POPULATION AT  
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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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## ABSTRACT

A breeding population of Wood Ducks (*Aix sponsa*), introduced to eastern-central North Dakota in 1968 was evaluated. This population occupies habitat outside the native range of Wood Ducks; numbers of breeding females have greatly declined over the past ten years. Two potential factors for this decline in population numbers were evaluated. These are competition from Hooded Mergansers (*Lophodytes cucullatus*), and reduced genetic variability of the Wood Duck population from the use of captive individuals to seed the population. Analysis of historical nesting data yielded no indication that Hooded Merganser's nesting activities have had any impact on the reproductive success of Wood Ducks. An analysis of 17 polymorphic allozymes was made for the Arrowwood population and a captive population similar to the one originally used to create the Arrowwood population. Using these data, mean heterozygosity, mean number of alleles/locus, and percent polymorphic loci were calculated. All of these indices indicate that the Arrowwood Wood Duck population is more genetically diverse than the captive population, and has levels of genetic variability similar to those reported for other native avian populations. The differences between the two populations were found not to be statistically different. Minisatellite DNA fingerprint analysis was carried out for the Arrowwood population, the captive population and a population from western-central Oregon. These analyses indicate that the Arrowwood population has significantly more variation than the captive population, and both of these exhibit more DNA polymorphism variability than the population in Oregon. It is suggested that the number of nesting females is being underestimated at Arrowwood National Wildlife Refuge because hens are nesting in natural nesting cavities and in areas outside the refuge boundaries. It is suggested that the Wood Duck population in North Dakota has high levels of genetic variability because of a constant influx of novel drakes. Unrecorded nesting and high genetic variability suggests the population of Wood Ducks in eastern-central North Dakota is in no danger of immediate extirpation. It appears that Wood Ducks in the Pacific Flyway have experienced long population bottle-necks causing a paucity of genetic variability; further analysis of Pacific Wood Duck populations is suggested.

## Chapter 1

### OVERVIEW OF DISSERTATION PROJECT

This thesis presents a study of breeding Wood Ducks (*Aix sponsa*), on Arrowwood National Wildlife Refuge, in east-central North Dakota. The principal focal points of this study are in the areas of conservation biology and wildlife management. It is a multi-disciplinary approach to a single problem of conservation and wildlife management. Information on the breeding biology, behavior, and genetics of this species is compiled to address the viability of an introduced population. In this manner, this study is representative of how successful conservation endeavors must utilize a spectrum of information in order to answer a single question concerning the relative health of populations.

Wood ducks have not traditionally occurred in this region of the United States, and they were purposely introduced to Arrowwood to determine whether or not a breeding population could survive there. Wood Ducks from captive populations were introduced to Arrowwood in 1968. The population flourished for several years and then appeared to decline rather sharply. It was unclear as to why the breeding population of wood ducks was declining at Arrowwood. This study attempts to understand better the apparent decline in breeding Wood Ducks at Arrowwood National Wildlife Refuge and to

analyze the genetics of that population to determine whether the decline could be due to genetic factors in the population arising from its origin as a captive population.

In this investigation, three hypotheses are addressed which could potentially explain the decrease in population of Wood Ducks at Arrowwood. The three hypotheses are : 1) Competition for nesting spaces from Hooded Mergansers is adversely affecting the reproduction of Wood Ducks, 2) Wood Ducks are exposed to environmental toxins at Arrowwood, and the success of Wood Duck reproduction is being adversely affected by exposure to these contaminants, and 3) There is low genetic variability among the Wood Ducks at Arrowwood and this reduced genetic variability is causing a decrease in reproductive success among the Wood Ducks at Arrowwood. By testing these different hypotheses, the overall health and viability of Wood Ducks at Arrowwood National Wildlife Refuge is evaluated.

The thesis is arranged into distinct chapters which cover different aspects of this investigation. The first chapter is an analysis of the population trends of breeding Wood Ducks at Arrowwood; in it an additional evaluation is made to determine whether another waterfowl species, the Hooded Merganser (*Lophodytes cucullatus*), appears to be a contributory factor to the apparent decline of nesting wood ducks. In this chapter, historical nesting data is used to infer whether environmental toxicants appear to be affecting the reproductive success of Wood Ducks. The second chapter is an analysis of the genetic composition of the Arrowwood population and a comparison of that population to a captive population similar to the population from which the Arrowwood population first originated. This genetic analysis uses protein (allozyme), variability to

infer levels of genetic variation within the Arrowwood and captive populations. The third chapter is a genetic investigation based on DNA fingerprinting analyses which measures amounts of genetic variability directly from DNA rather than indirect measurements of proteins. In this chapter, a third population is added to the analysis. This population is a naturally occurring, small population in Oregon. It was analyzed and compared to two populations analyzed in previous chapters.

Through these combined approaches, the three hypotheses proposed are tested and the health and viability of the Arrowwood breeding population of Wood Duck is assessed. This combined approach is indicative of how conservation studies and management practices must draw on a wide variety of techniques to ascertain the viability of natural populations and of management techniques used to alter and sustain populations of wild animals.

## Chapter 2

### POPULATION ANALYSIS OF NESTING WOOD DUCKS AND HOODED MERGANSERS AT ARROWWOOD NATIONAL WILDLIFE REFUGE.

#### Introduction

Arrowwood National Wildlife Refuge (ANWR) in eastern-central North Dakota was created in 1935 and contains 15,900 acres of prairie grassland along a 16-mile length of the James River. On the refuge there are four large impoundments of the river producing extensive shallow lakes and marshes; there is very little natural river channel remaining on the refuge. In these extensive limnetic zones there are communities of many aquatic and sub-aquatic plants which provide large areas suitable for waterfowl use.

This area is outside the traditional breeding range of the Wood Duck (*Aix sponsa*). Wood Ducks were introduced to (ANWR) in 1968 (Doty & Kruse 1972). The initial introduction was accompanied by the placement of approximately 300 nest boxes within the refuge boundaries. These combined activities were parts of an experiment designed to evaluate the effectiveness of establishing Wood Duck populations in novel geographic locations (Doty & Kruse 1972). Since 1969, nest-box utilization has been monitored and recorded on an annual basis by the staff of ANWR as a part of the waterfowl management program, and these data have been used to monitor the status of the breeding Wood Duck population on the refuge.

Wood Ducks faced near extinction in the early parts of this century; it is estimated that in 1915 there were more Wood Ducks in captive flocks in Europe than in the wild in North America (Ripley 1973). This species was saved from extinction by strict legislation imposing a moratorium on hunting (Bellrose & Heister 1987, Baldassarre & Bolen 1994) and considerable re-introduction efforts from European captive populations (Ripley 1973). Since that time, Wood Ducks have made a very successful recovery and are now fairly common throughout their original range even though this species experienced population bottlenecks and perhaps extensive inbreeding for a period of 10-20 generations.

The traditional range of Wood Ducks occupied the Atlantic, Pacific and Mississippi waterfowl Flyways of North America. Within these flyways, Wood Ducks are most abundant in southern regions during both the breeding and winter seasons. Since the 1970's, Wood Ducks have experienced a range expansion and they are now known to breed and winter in the Central Flyway (Ladd 1990); this expansion is attributed to anthropogenic introductions and a natural colonization of the southeastern portions of that area.

Population densities of Wood Ducks are extremely difficult to obtain, and accurate population densities are not available for much of their range. The principal method by which population sizes are estimated is from harvest statistics gathered from hunters. This method does not provide accurate information on actual population numbers, but does provide insight with regard to general population trends. It is perhaps useful to compare numbers between different flyways, although changing harvest regulations and the

dynamic nature of hunting efforts can be confounding factors. Nonetheless, population densities of Wood Ducks appear generally much higher in the Atlantic and Mississippi Flyways than in the Central or Pacific Flyways (Bellrose 1980, Baldassarre & Bolen 1994). An estimate of the number of breeding Wood Ducks for the Central Flyway (Bellrose 1980), was around 50,000 individuals, although this estimate is probably very conservative and could be off by up to 30-50% (May 1986; Ladd 1990). An estimate of the numbers of individuals in the Pacific Flyway is 60,000 (Bartonek *et al.* 1990). Although estimates of absolute numbers of individuals are not available for Atlantic and Mississippi Flyways, harvests in those flyways combined is in excess of 1.2 million individuals per year (Baldassarre & Bolen 1994). From these data, it appears that populations in the Pacific Flyway are the lowest in North America and could be up to two orders of magnitude lower than those of the eastern United States. The origin of Wood Ducks in the Pacific Flyway is unclear, but there has been little or no population mixing between the Pacific Flyway and the flyways east of the Rocky Mountains; this isolation has caused the Pacific populations to remain genetically isolated with respect to other populations.

During the last 20-30 years, Wood Ducks have been successfully introduced to regions outside their native breeding ranges (Doty & Kruse 1973, Baldassarre & Bolen, 1994). Most of the introductions to novel or peripheral environments have been accomplished by using individuals from captive breeding stocks to propagate new breeding populations. One notable introduction was accomplished in 1968, when a

breeding population of Wood Ducks was established in eastern-central North Dakota on Arrowwood National Wildlife Refuge using stocks from captive populations in North Dakota (Doty & Kruse 1972).

Wood Ducks often inhabit densely forested aquatic habitats. These aquatic habitats are extremely difficult ones in which to conduct direct population surveys of this species (Hein 1966, Bellrose 1980, Parr & Scott 1978, Brakhage 1990, Moser & Graber 1990, Robb & Bookhout 1990). Many methods have been employed to make population estimates of Wood Duck abundance, but no feasible method exists to date. Wood Ducks are also very secretive nesters, most commonly nesting in naturally occurring cavities in trees. Such arboreal nesting locations are often difficult to find, and if found they are commonly placed such that access to nests and their contents is impossible to obtain. Wood Duck hens will readily nest in artificial nesting structures, and when nesting in these structures their nesting behaviors are much easier to monitor. Much of the information regarding nesting densities, clutch size, reproductive success, and other reproductive components of Wood Duck biology has been obtained from analysis of nesting activities in artificial nesting structures. Such nest-box monitoring has proved valid to monitor cavity-nesting breeding waterfowl populations, and it is nearly the only method useful for studies of Wood Duck nesting activities (Zicus & Hennes 1987, Ladd 1990, Robb & Bookhout 1988).

The breeding biology of the ANWR Wood Duck population has been evaluated through nest-box data analysis and was presented by Doty *et al.* (1984). During the 13



years following their introduction, the number of nesting Wood Duck females at ANWR has fluctuated widely and appeared to be declining in 1982 (Doty *et al.* 1984). The nesting population experienced a marked increase during the initial years, but in the early 1980's it appeared to be steadily declining, and extirpation was imminent in the near future.

The initial release of Wood Ducks at ANWR consisted of 280 ducklings that had been incubated and reared in the Northern Prairie Wildlife Research Center (Doty & Kruse 1972). The sources for eggs from which these individuals hatched were from captive flocks at the Center and a captive population in Minnesota. After hatching, the ducklings were maintained in the Center for 9 to 16 days and then transported to an open-topped release pen on ANWR; when the ducklings were between 19 and 26 days of age they were sexed, banded and released (Doty & Kruse 1972). Of the 280 initial ducklings released, approximately 253 (132 females and 121 males), survived to flight stages, and approximately 193 survived until all Wood Ducks departed in the fall of 1968 (Doty & Kruse 1972). Band recovery from the first post-release winter indicated that the ducks migrated along the western Central flyway to the western portion of normal Wood Duck winter range (Doty & Kruse 1972). During the following breeding season, 12 of the banded Wood Duck hens from the original release returned to ANWR and nested in nest-box structures on the refuge (Doty *et al.* 1984). For the following six years the number of nesting females continued to increase.

Since the introduction of Wood Ducks to ANWR, Hooded Mergansers (*Lophodytes cucullatus*), have naturally expanded their breeding range to include the aquatic habitats there; in 1973 Hooded Mergansers were first observed to nest at the refuge (Doty *et al.* 1984). Hooded Mergansers are cavity nesters, and it is hypothesized that the two species might compete for nesting cavities and influence one-another's nesting success. Intra- and interspecific cases of nest parasitism are known for each of the species (Morse & White 1969, Clawson *et al.* 1979, Doty *et al.* 1984, Haramis & Thompson 1985, Sherman & Semel 1989). Hooded Mergansers have been reported to initiate nesting activities earlier in the year than Wood Ducks (Fitzner & Fitzner, 1973). This temporal difference in nest-initiation times could allow Hooded Mergansers to exclude Wood Duck hens from nesting structures through a mechanism of exploitative competition. The potential negative influence of Hooded Mergansers to the decline of nesting Wood Ducks at ANWR has been hypothesized by scientists (Doty *et al.* 1984) and by refuge personnel.

When populations are maintained in captivity, there is often a loss of genetic variability due to inbreeding, genetic drift due to low  $N_e/N$  ratios and other factors which tend to degrade genetic diversity (Hedrick *et al.* 1986, Briscoe *et al.* 1992). The maintenance of genetic variability is widely believed important because the long-term survival and viability of populations is likely related to levels of genetic variation among members of a population (Soulé 1980, Frankel & Soulé 1981, Barrett & Vyse 1982, Beardmore 1983, Lande 1993). Although the exact mechanisms of this relationship have

been recently questioned (Caro & Laurenson 1994), it is nonetheless widely accepted that populations having small amounts of genetic variability are more extinction-prone than populations with higher levels of genetic variability. In captive breeding programs designed to release individuals into the wild, efforts need to be made to insure that genetic variability is not degraded during captivity so that the individuals released will represent a significant portion of the genetic variability naturally present in the species under management (Hederick *et al.* 1986, Soulé 1987). Many different strategies exist in breeding programs that attempt to minimize degradation of genetic variance in captive populations. This crucial need to manage captive populations for genetic diversity is widely accepted among breeders and game managers now, but it was not recognized at the time of the introduction of Wood Ducks to ANWR in 1968. Consequently, no attention was paid to the genetic composition of the Wood Ducks released at ANWR.

The long-term viability of the Wood Duck population at ANWR is uncertain because: 1) ANWR is outside the traditional nesting range of Wood Ducks, 2) The nesting population is in decline, 3) a strong potential for interspecific nest competition exists, and it originated from captive breeding stocks whose genetic variability is unknown but likely to be below that of natural populations.

In this analysis, nesting data are presented and analyzed to evaluate further population trends of nesting Wood Ducks at ANWR. Nesting success data for both Wood Ducks and Hooded Mergansers are examined to investigate the role of interspecific competition that might adversely affect Wood Duck nesting success.

### Materials and Methods

Nesting-box structures on ANWR are monitored every fall (after all nesting activity has ceased) by refuge personnel as part of the waterfowl management program. During this monitoring, each nest-box is located, its condition noted, and the contents of the box recorded. The presence of eggs, egg shells, and/or egg membranes is recorded, quantified, and identified as to the species of origin. The contents of each nest box is then removed, and any repairs and/or modifications needed to the structure or nesting materials are made in preparation for the next nesting season. Hooded Merganser and Wood Duck eggs and egg shells can be readily distinguished from one-another (Soulliere 1985), making it possible to determine which of these two waterfowl species used a particular nest during the past nesting season. From this information, the number of active nests, number of successful nests, and hatching success of eggs is obtained for species.

The original waterfowl nesting data for ANWR was obtained for the years 1968-1988 from the nest-box survey records maintained by the refuge personnel. In 1989, I collected the data for 320 nest box structures at two different times during the year. The first data collection period was during late May when nesting activity was ongoing and Wood Duck hens could be captured on the nests. The second monitoring was made during September after the cessation of nesting activities. The methods I employed were identical to those used by refuge personnel during the previous years.

## Results

The numbers of nesting females of both species are presented in Figure 1. There was an increase in the Wood Duck population for the first six years followed by a sharp decline over the next seven years. Since 1982, there has been some variation in the number of Wood Duck nests, but the population is essentially stationary at approximately 20-30 nests per year (Mean =  $29 \pm 9$ ). The numbers of Hooded Merganser and Wood Duck nests indicate a very general trend of Wood Duck nests decreasing and Hooded Merganser nests increasing (Figure 1). The number of Wood Duck nests does not rapidly decline with the advent of Hooded Mergansers at the refuge, and there is no apparent pattern indicating the size of the breeding population of one species closely correlates with the number of nests of the other species. In 1982, the number of Hooded Merganser nests surpassed the number of Wood Duck nests; and the number of nesting Hooded Mergansers has always been greater than the number of nesting Wood Ducks since 1984. In 1987 both species showed an increased number of nests over the past 3 years.

The number of successful egg-hatches per species per nesting season was used as an index of reproductive success (Figure 2). A regression of egg hatching success over time as expressed as the number of years since the initial nesting of Hooded Mergansers at ANWR revealed that neither species has experienced a significant change in reproductive success over the period of 1979-1989. For Wood Ducks,  $r^2 = 0.03$ ,  $F = 0.44$ ,  $p(F) = 0.52$ ; for Hooded Mergansers  $r^2 = 0.02$ ,  $F = 1.13$ ,  $p(F) = 0.29$ .

























































































































































































































