Sexing American Bitterns, *Botaurus lentiginosus*, Using Morphometric Characteristics

DAVID A. AZURE[1,5], DAVID E. NAUGLE[2], JOHN E. TOEPFER[3], GARY HUSCHLE[4], and RICHARD D. CRAWFORD[1]

[1]Department of Biology, University of North Dakota, P.O. Box 9019, Grand Forks, North Dakota 58201, USA
[2]College of Natural Resources, University of Wisconsin-Stevens Point, Stevens Point, Wisconsin 54481, USA
[3]3755 Jackson Avenue, Plover, Wisconsin 54467, USA
[4]U.S. Fish and Wildlife Service, Agassiz National Wildlife Refuge, Route 1, Box 74, Middle River, Minnesota 56737, USA
[5]Current address: U.S. Fish and Wildlife Service, Windom Wetland Management District, Route 1, Box 273A, Windom, Minnesota 56101, USA

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Morphometric measures from 1995-1998 were used to develop a discriminant function that provides investigators with a practical, non-destructive technique for sexing American Bitterns (*Botaurus lentiginosus*). Thirty-two males were lured into mirror traps and mist nets using tape-recorded territorial vocalizations and 17 females were captured at nest-sites using long-handled dip nets. Sex of captured birds was known because only males respond aggressively to territorial vocalizations and only females incubate nests. Average morphometric measures were greater for male than female American Bitterns with overlap between the sexes. Tarsus length was the single most useful measurement in discriminating between sexes, correctly identifying 100% of individuals used to construct the function and 71.4% of birds that were not used in model development (hold-out test data set). The addition of short bill length measurements increased the proportion of correctly classified individuals in the hold-out test data set to 76.2% for males and 85.7% for females. This technique will enable field ecologists to separate population and behavioral data according to sex.

Key Words: American Bittern, *Botaurus lentiginosus*, discriminant function analysis, morphometric measurements, sex criteria, wetland birds, Minnesota.

Declines in American Bittern (*Botaurus lentiginosus*) populations over the last three decades led to its listing as a migratory nongame bird of management concern (USFWS 1995; Sauer et al. 1997). Conservation programs for American Bitterns have not been implemented because investigations of nearly every aspect of bittern ecology are lacking (Duebbert and Lokemoen 1977; Gibbs et al. 1992; Svedarsky 1992; Azure 1998). Studies into the most basic aspects of population or behavioral ecology require that investigators correctly sex study animals. Obtaining such information without sacrificing individuals for direct gonad examination is challenging because American Bitterns lack plumage characteristics by which sexes can be recognized. In this study, we use morphometric differences between known male and female birds to calculate a mathematical function that provides investigators with a practical, non-destructive technique for sexing American Bitterns.

Methods

Our principle study site, Agassiz National Wildlife Refuge (ANWR), is a 24846-ha area along the transition from prairie to coniferous forest in northwest Minnesota. Our secondary study site was a 1031-ha Wildrice (*Zizania aquatica*) farm of the Red Lake Band of Chippewa Indians, located 100 km southeast of ANWR. In the summers of 1995–1998, we broadcast tape-recorded territorial vocalizations (i.e., pumping calls) of American Bitterns to lure males into mirror traps and mist nets (Brininger 1996). Incubating female American Bitterns were captured on nests using long-handled dip nets. Sex of captured American Bitterns was positively determined because only males respond aggressively to territorial vocalizations (Brininger 1996) and only females incubate nests and feed young (Gibbs et al. 1992).

Upon capture, 32 male and 17 female American Bitterns were weighed to the nearest gram on an OHAUS balance scale and the following 13 morphometric measures were obtained using a digital caliper (nearest 0.1 cm): long and short bill lengths from the anterior and posterior margins of the nostril to the tip of the bill, exposed culmen length, bill widths anterior to the nostrils and dorsal to the cere, head width posterior to the eyes, tarsus length, length of the middle toe and width of the second phalange, length and width of the nail on middle toe, wing chord and tail length (Table 1). Seven of 14 measurements (Table 1) were used in analyses after eliminating weight, tail length, long bill length and width, short bill width, exposed culmen length and wing chord

TABLE 1. Means (± SE) and Coefficients of Variation (CV) of morphometric measurements obtained from male and female American Bitterns in northwest Minnesota, 1995–1998.

	Male (n = 31)			Female (n = 17)		
Morphometric measures[a]	$\bar{x}$	SE	CV	$\bar{x}$	SE	CV
Measures used in model construction						
Tarsus length	9.12	0.06	3.5	8.49	0.06	3.0
Short bill length	5.52	0.05	4.6	5.21	0.09	6.7
Head width	2.61	0.02	4.6	2.39	0.02	2.9
Middle toe length	7.84	0.07	4.8	7.28	0.06	3.4
Middle toe nail length	2.10	0.03	7.7	2.01	0.07	13.6
Second phalange width	0.40	0.01	11.3	0.35	0.01	10.7
Middle toe nail width	0.29	0.01	12.4	0.27	0.01	11.2
Measures excluded from model to reduce collinearity						
Weight (g)	928	17.26	10.4	569	8.81	6.2
Tail length	9.42	0.15	8.8	8.09	0.19	4.5
Long bill length	7.24	0.11	8.1	6.53	0.06	4.1
Long bill width	1.46	0.02	7.4	1.42	0.02	6.3
Short bill width	1.01	0.01	6.4	0.91	0.02	8.0
Exposed culmen length	7.58	0.07	4.7	7.00	0.10	4.7
Wing chord length	27.82	0.17	3.3	24.92	0.27	4.5

[a]Measurements other than weight (g) were measured to the nearest 0.1 cm.

length from the data set to reduce collinearity ($r > 0.5$).

Stepwise discriminant function analysis (SYSTAT 8.0; Wilkinson 1998) was performed on measurements from 10 randomly selected individuals of each sex (analysis data set), entering at each step the measurement that added the most separation between the sexes. Wilks' lambda was used to test significance of our classification functions (Wilkinson 1998). We used a jack-knife statistical procedure (also called the leave-one-out analysis; Lachenbruch and Mickey 1968) where each individual was classified using a function derived from the sample less the individual being classified as one method of cross-validating our ability to discriminate between sexes. This method produces unbiased classification rates of discriminant functions (e.g., Seber 1984). We also cross-validated our predictive capability by classifying 21 male and 7 female American Bitterns that were not used in model construction (i.e., hold-out test data set). One male with a bill length >2 standard deviations from the mean was excluded from analyses.

Results

Average morphometric measurements were greater for male than female American Bitterns with overlap between the sexes (Table 1). Tarsus length (Figure 1) was the single most useful measurement in discriminating between sexes, correctly identifying all individuals used to construct the function (i.e., analysis data set) (Table 2). Equations using tarsus length (Wilks' Lambda = 0.37, F = 30.05, df = 1, 18, P < 0.001; Table 2), where individuals are classified according to the largest value of classification functions, correctly classified 71.4% of both male and female American Bitterns that were not used in model construction (i.e., hold-out test data

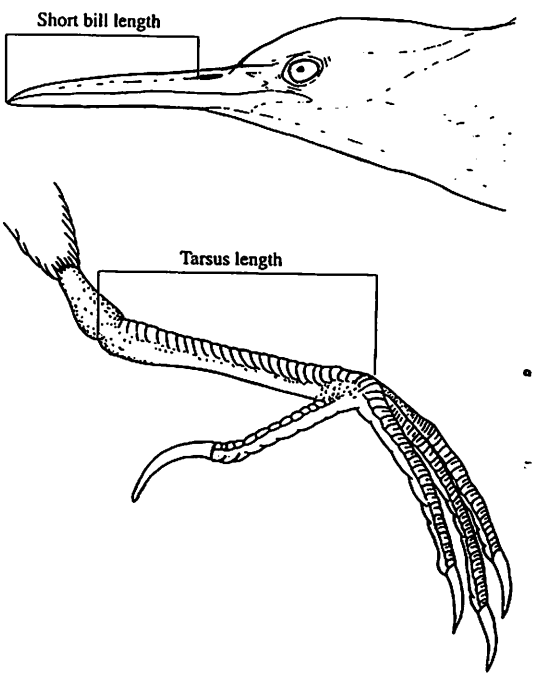

Figure 1. The two "best" morphometric measurements used in discriminant functions to sex American Bitterns were short bill length and tarsus length.

TABLE 2. Classification functions for discriminating between sexes of American Bitterns in northwest Minnesota, 1995–1998.

Variable name	Classification Function Coefficients	
	Male	Female
Tarsus length only[a]		
Constant	-742.88	-651.43
Tarsus length	162.85	152.49
Apparent classification rate	100%	100%
Jack-knifed classification rate	100%	100%
True classification rate	71.4%	71.4%
Tarsus and short bill lengths[b]		
Constant	-893.33	-783.82
Tarsus length	162.34	152.01
Short bill length	55.03	51.62
Apparent classification rate	100%	100%
Jack-knifed classification rate	100%	100%
True classification rate	76.2%	85.7%

[a]Wilks' Lambda = 0.37, F = 30.05, df = 1, 18, P < 0.001.
[b]Wilks' Lambda = 0.33, F = 16.92, df = 2, 17, P < 0.001.

set). The addition of short bill length (Wilks' Lambda = 0.33, F = 16.92, df = 2, 17, P < 0.001; Figure 1), increased the proportion of correctly classified individuals in the hold-out test data set to 76.2% for males and 85.7% for females (Table 2). On the basis of Wilks' Lambda values and number of correctly classified cases, inclusion of additional variables into functions was unwarranted.

Discussion

Tarsus and bill length measures provide a practical, non-destructive method for sexing American Bitterns. Advantages of using tarsus and bill lengths outweigh minor increases in predictive capability (<5%) that results with inclusion of different or additional measurements. Tarsus and bill length measures (Figure 1), do not vary with fluctuations in body condition that occur seasonally due to breeding (e.g., egg laying), molting and diet. Although male American Bitterns typically weigh more than females (Gibbs et al. 1992), we reduced collinearity by excluding body weight (Table 1) because weights of male American Bitterns radio-collared in May and recaptured in August declined 200–250 g (D. Azure, unpublished data); making body weight a potentially poor predictor of sex. Similarly, Zavalaga and Paredes (1997), in developing a function for classifying sex of Humboldt Penguins (*Spheniscus humboldti*), excluded body mass from their discriminant function because penguin weights varied significantly between seasons (CV = 13.6%). In this study, body weight of male American Bitterns was the most variable measure (CV = 10.4%) of those excluded from analyses (Table 1).

We were unable to correctly sex all individuals because morphometric measures of 8 males in the hold-out test data set were consistently low compared to average measurements for all male bitterns. Each incorrectly classified male had a tarsus length < 9 cm and no male correctly classified in the hold-out data set had a tarsus length < 9 cm. Although females captured while incubating nests or feeding young were known to be adults, we were unable to verify age of male American Bitterns because subadults as well as adults respond to territorial vocalizations. We suspect discrepancies in morphometric measures of male American Bitterns are age-related. Males with tarsus lengths < 9 cm were likely subadults captured while performing mating rituals and courtship displays. While this technique has its limitations, it will enable field ecologists to separate population and behavioral data according to sex.

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