

# NON-INVASIVE SEX RECOGNITION IN THE WHITE-FACED WHISTLING DUCK

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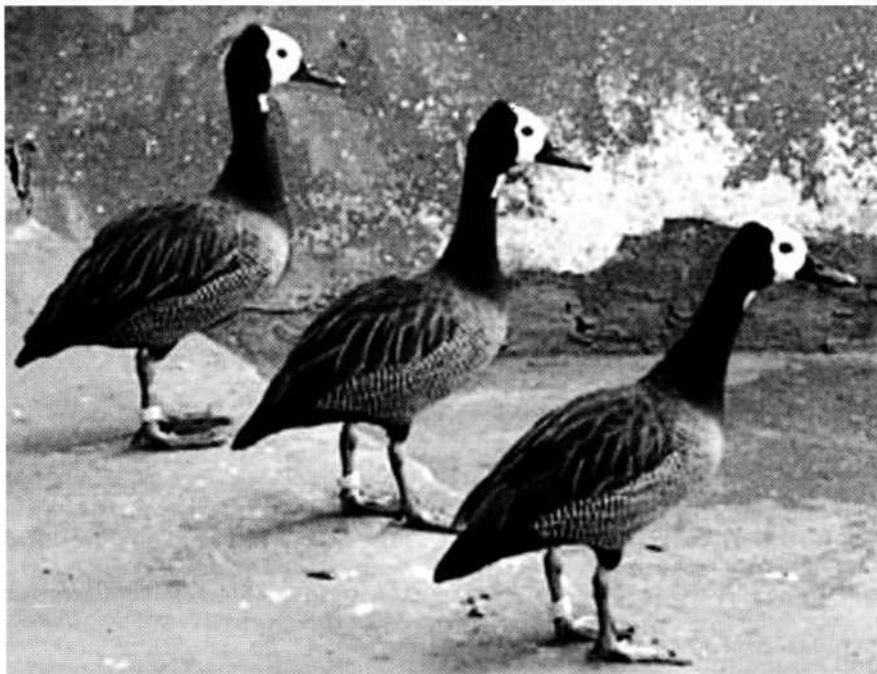
## Introduction

The problem of sex determination in birds without external sexual differences arises repeatedly both in captive management and during field observations (Volodina and Volodin, 1999). Behavioural data do not always provide reliable indicators, because many species without sexual dimorphism readily form homosexual pairs, and the behaviour of the 'mates' in these pairs is often indistinguishable from that seen in heterosexual pairs (Lorenz, 1966; Fabricius, 1981; Conover and Hunt, 1984; Hunt *et al.*, 1984; Conover, 1989; Volodin, 1990). Sex determination based on laparoscopy, cloacal inspection or DNA analysis demands that the bird is captured and subjected to relatively unpleasant treatments, making it undesirable (especially for rare species) or impossible (in the wild). So recent decades have seen the development of an alternative approach, based on call structure, which is non-invasive and non-traumatic (Tikhonov *et al.*, 1988; Volodina and Volodin, 1999). The background to intersexual differences in bird vocalisations lies in the complexity of avian vocal organs and in the sex-specific occurrence of extensions to the vocal tract (Fitch, 1999). Data on different bird taxa have shown that the reliability of sex determination by this method is no lower than that obtained by using the traumatic procedures (Tikhonov *et al.*, 1988; Eakle *et al.*, 1989; Carlson and Trost, 1992; Venuto *et al.*, 2001).

The white-faced whistling duck (*Dendrocygna viduata*) is a beautifully ornamental species and one of the favourite waterfowl kept in zoos (Bolen, 1973). It is one of eight species of the tribe Dendrocygnini - a compact group with entire absence of any sexual dimorphism in size, coloration or behaviour (Johnsgard, 1965). Male and female share the incubation of eggs and the care of ducklings. After hatching, the parents together lead the ducklings to the nearest pool. One adult will often fly ahead of the brood to scout for possible danger (C. Wintle and P. Ginn, pers. comm.). There are no peculiarities, in either adult or young individuals, which allow sex to be identified from a distance, except observations of copulation (Clark, 1978). Some differences in mates before egg-laying - in comfort behaviour (higher in females) and alertness (higher in males) - may not serve as fast and reliable indicators of sex (Petrie and Rogers, 1997).

Whistling ducks received their name because of their characteristic species-specific loud whistling calls (Johnsgard, 1965). Birds usually produce these calls during foraging and flight in the flocks that they form after breeding. Individuals stimulate each other to call, and sometimes all the members of a flock begin to call together (Clark, 1978). Besides the loud whistles, the vocal

repertoire of whistling ducks involves a few other types of call, significantly less intensive and audible only at close range (Clark, 1978; Volodina and Volodin, 2003). A comparison between the calls of individually marked fulvous whistling ducks (*D. bicolor*) has shown that the structure of these quiet calls did not differ between the sexes, and that only the loud whistles have the potential to be indicators of sex (Volodina and Volodin, 2003). An aim of this research was to reveal sexual differences in the structure of the loud whistles in the white-faced whistling duck.



*White-faced whistling ducks show entire absence of sexual dimorphism: left - two males, right - female. (Photo: I. Volodin)*

## **Methods**

Eleven (9.2) adult, sexually mature white-faced whistling ducks born in 1998 were used as study animals. All of them were individually marked with coloured leg rings. Sex was determined before the beginning of the study using cloacal inspection. The ducks were housed in a flock, together with other waterfowl, in an outdoor enclosure of about 100 m<sup>2</sup> at Moscow Zoo. The enclosure has a small pond with running water, bushes and large stones. All the white-faced ducks used all the enclosure territory, without preferences for particular parts; no individual or pair home ranges were identified.

The ducks' loud whistles were recorded from 10 June to 17 September 2001 in the evening after the zoo was closed to visitors. We used an Agidel-302C tape recorder with a Tesla-AMD-411N dynamic microphone attached to the encl-

sure netting. In total we had 14 recording sessions of from 25 to 60 minutes each (total - 615 min.). During the sessions two observers, who stood outside the enclosure, determined the individuals responsible for the whistles. The distance from bird to microphone varied from two to ten metres.

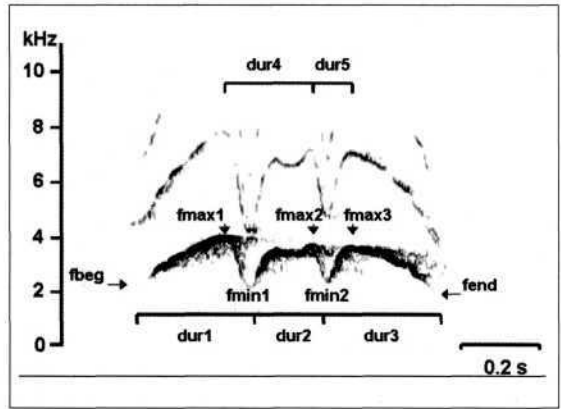
The loud whistles were often produced by birds who had lost visual contact with their conspecifics, and this in turn evoked loud whistles in response from other birds. The calls were usually produced in series. We did not find any other factors influencing loud whistle production in this species.

For sonographic analysis we used Avisoft-SAS Lab Pro version 3.4e (© R. Specht). We used sampling rate 22 kHz, Hamming window, FFT-length 512 points, frame 50%, overlap 87.5%. Correspondingly, these settings provided bandwidth 111 Hz, time resolution 2.9 ms and frequency resolution 43 Hz.

We selected for analysis only calls of good quality from individual birds who were identified by both observers. For each bird we randomly selected from 18 to 22 calls, except with two males who provided only five and seven calls with precise individual identification. In total, we analysed 194 calls.

The loud whistles of white-faced ducks have a specific three-part structure (Fig. 1). In the course of a call three maxima and two minima of fundamental frequency are clearly distinguishable. For each call we measured seven frequency and five temporal parameters (Fig. 1).

We used stepwise discriminate analysis on seven frequency and five temporal parameters to assign individuals to a certain sex. The analysis was made in STATISTICA, version 5.0.



**Figure 1.** Measured parameters in loud whistles of white-faced whistling ducks. On the sonogram, points of measurements for initial frequency (*fbeg*), end frequency (*fend*), three maxima (*fmax1*, *fmax2*, *fmax3*) and two minima (*fmin1*, *fmin2*) of fundamental frequency are shown. Sections illustrate measurements of temporal parameters: durations of first, second and third parts of a call (*dur1*, *dur2* and *dur3* respectively), and durations between first and second (*dur4*) and second and third (*dur5*) frequency maxima.

## Results

Figure 2 shows differences in values of frequency and temporal parameters in loud whistles of white-faced whistling duck males and females. It is evident that values for all fundamental frequency parameters are much higher in females. Moreover, six out of seven frequency parameters (Fig. 2, two upper rows) did not show any overlapping between male and female samples, that is, for these parameters differences were perfect, not statistical. Initial frequencies (*fbeg*) were below 2.65 kHz in males, and above this magnitude in females.

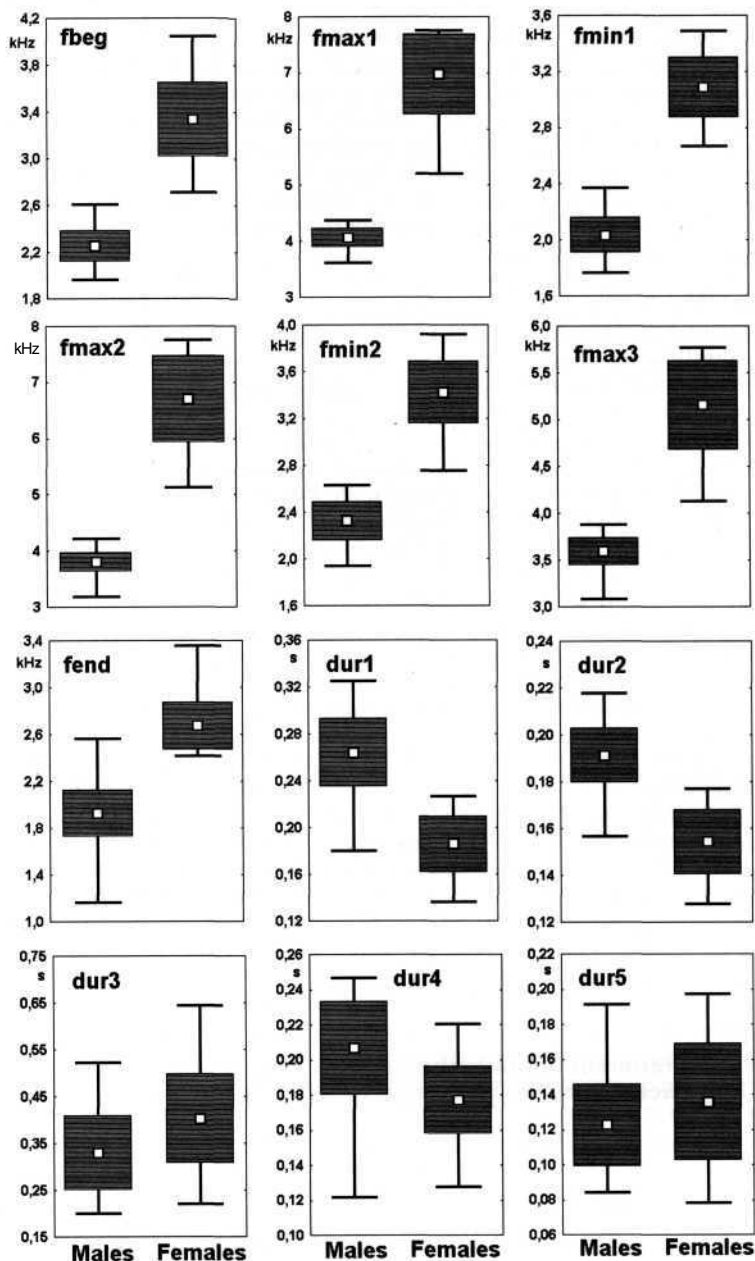
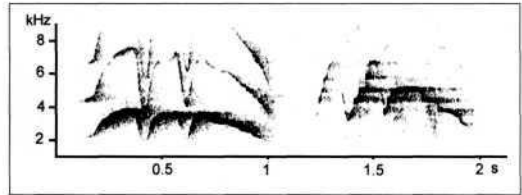


Figure 2. Values of frequency and temporal parameters of male and female loud whistles in white-faced whistling duck. Light square = mean, dark rectangle = mean  $\pm$  SD, vertical lines illustrate range from minimum to maximum value.

Similarly, the first maximum frequency (*fmax1*) was below 4.5 kHz in males and above 5.0 kHz in females; the first minimum (*fmin1*) below 2.4 kHz in males and above 2.6 kHz in females; *fmax2* below 4.2 kHz in males and above 5.0 kHz in females; *fmin2* below 2.7 kHz in males and above 2.7 kHz in females; *fmax3* below 3.9 kHz in males and above 4.1 kHz in females (Fig. 2). There is some overlapping of final frequency (*fend*) values among males and females. However, *fend* was also significantly lower in males than in females (Mann-Whitney U-test,  $U = 13$ ,  $p < 0.001$ ). Therefore, male loud whistles were significantly lower in frequency in comparison with female ones (Fig. 3).

Sexual differences in temporal parameters were more complex (Fig. 2). First (*dur1*) and second (*dur2*) call parts were significantly longer in males than in females ( $U = 121$ ,  $p < 0.001$  and  $U = 127.5$ ,  $p < 0.001$  respectively). Third call part (*dur3*), in contrast, was significantly longer in females ( $U = 1798$ ,  $p < 0.001$ ). Duration between first and second frequency maxima (*dur4*) was significantly longer in males ( $U = 811.5$ ,  $p < 0.001$ ), whereas duration between second and third maxima (*dur5*) did not differ between the sexes ( $U = 2601.5$ , ns).



**Figure 3. Sonograms of male (left) and female (right) loud whistles of white-faced whistling duck. Note the meaningful differences in fundamental frequency of the whistles.**

Percentages of correct assignment of calls to a particular sex, counted using discriminate function analysis, are shown in Table 1. All calls without exception were correctly assigned to a sex. Stepwise discriminate analysis has shown that the primary contribution in distinguishing between the sexes was provided by the frequency of first maximum (*fmax1*) and duration of second call part (*dur2*), and these two parameters were able on their own to guarantee 100% of correct assignment to a sex. In general, frequency parameters contribute more to discrimination between the sexes. However, taken separately, both frequency and temporal parameters provided 100% correct assignment to a certain sex.

**Table 1. Assignment of loud whistles to a certain sex in the white-faced whistling duck on the basis of stepwise discriminate function analysis.**

Sex	Assignment to a predicted group		Total	Percentage of correct assignment
	Males	Females		
Males	152		152	100.0
Females		42	42	100.0
Total	152	42	194	100.0

## Discussion

The data obtained in this study show that in white-faced whistling ducks — a species in which the sexes cannot be distinguished either by coloration or by behaviour — sex may be accurately determined even by a single loud whistle produced by a bird. Male calls are much lower in frequency than female calls, and these differences are great enough to allow sex estimation by ear alone after brief training, without the use of tape recording equipment.

Such significant differences in male and female call structures are most probably related to sexual differences in vocal tract morphology. The male trachea in white-faced whistling ducks has a symmetrical extension in its lower part. Females do not have such an extension, but they do have a membranous area at the point where the bronchi diverge (Johnsgard, 1965; 1971). However, more research will be needed to determine the role of each of these morphological structures in the production of the fairly different loud whistles in this species, especially since in a morphologically related species - the fulvous whistling duck - sexual differences appear in the duration, not the frequency, of the loud whistles (Volodina and Volodin, 2003). However, the practical use of vocal cues for sex determination, both in captive management and in observations in the wild, is already possible.

The call-based method of sex determination is not yet widespread, but there is already much evidence of its usefulness for a few bird taxa. First of all, intersexual differences in calls may be found in species with morphological differences in the vocal tract between the sexes. These differences are present in some Gruiformes (limpkin, *Aramus guarauna*), Ciconiformes (yellow-billed stork, *Mycteria ibis*), Galliformes (nocturnal curassow, *Nothocrax urumutum*, and all nine species of *Ortalis*) and Passeriformes (trumpet bird, *Phonygammus keraudrenii*, and three species of *Manucodia*). Males and females of all the three *Manucodia* species are similar in their coloration, but only males have a tracheal elongation, which results in significant voice lowering in males in comparison with females (Fitch, 1999). Sexual differences in call structure may also occur also in species which do not differ in their tracheal anatomy. For example, in adult whooping cranes (*Grus americana*) the accuracy of sex identification by guard call frequency reaches 98.8% (Carlson and Trost, 1992). Over a number of years visual analysis of spectral differences in the calls of common cranes (*Grus grus*) from the European population was used for testing of sexual and individual identity (Wessling, 2000). Besides this, there are preliminary data on the existence of sexual differences in stress calls of six species of *Poicephalus* parrots (Venuto *et al.*, 2001), noisy calls of eastern screech owl (*Otus asio*) (Cavanagh and Ritchison, 1987) and alarm calls of black-headed gull (*Larus ridibundus*) chicks (Koschmianova *et al.*, 1984).

Call-based sex identification has potential practical value in the domestic poultry industry as a method of sexing day-old chicks and goslings (Tikhonov *et al.*, 1988). The technique has also been tested on game species, for example Canada goose (*Branta canadensis*) (Phokin, 1985). However, most of these data were based on limited samples and further testing will be necessary.

Modern computer technology allows us not only to listen to sounds, but also to look at them. Cheaply (or even freely) available software for sound analysis and playing provides a good basis for progress in research into call-based sex recognition in sexually monomorphic bird species and for applying this approach in zoos' and breeders' practice.

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