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ORIGINAL ARTICLE

Morphological and genetic sex identification of white-tailed eagle *Haliaeetus albicilla* nestlings

Björn Helander · Frank Hailer · Carles Vilà

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Abstract Identifying the sex of bird nestlings is relevant to studies of behaviour and ecology and is often a central issue in the management of endangered or captive populations. The white-tailed eagle *Haliaeetus albicilla* is a formerly threatened Eurasian raptor which is closely monitored in many countries due to its high exposure to environmental pollutants in the food chain. The aim of this study was to evaluate the reliability of sex identification methods for white-tailed eagle nestlings based on morphological measurements that can be recorded at the nest by a single person and with minimum disturbance. The sex of each bird was independently determined using molecular (genetic) methods. One measure of tarsus width allowed the correct identification of sex for 96% of the nestlings from southern and central Sweden. However, we found that the criteria for sex identification were not directly applicable to the population in Swedish Lapland, where nestlings are typically thinner, probably due to a limited food supply. These results show that sexing in the field of white-tailed eagle nestlings can be feasible with high accuracy based on a limited number of measurements. However, the criteria employed to separate sexes may have to be adjusted for each population.

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B. Helander (✉)
Department of Contaminant Research,
Swedish Museum of Natural History, Box 50007,
104 05 Stockholm, Sweden
e-mail: bjorn.helander@nrm.se

F. Hailer · C. Vilà
Department of Evolutionary Biology,
Evolutionary Biology Centre, Uppsala University,
Norbyvägen 18d, 752 36 Uppsala, Sweden

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Introduction

Sex identification can provide researchers with important information regarding the ecology and behaviour of bird species (e.g. Ellegren and Sheldon 1997) and also yields valuable insights into their conservation and management (Morris and Doak 2002; Sutherland 2002). A knowledge of the sex of individuals is relevant, for example, for studies of demography, sex ratios, population viability analyses (Gilpin and Soulé 1986), territorial behaviour, life history or species management through the translocation of nestlings. Despite the lack of external sexual organs, the sex of adult individuals of many bird species can be ascertained by their plumage (colouration or form). The sex of juveniles, however, is generally hard to discern. Many raptor species exhibit sexual size dimorphism even at the nestling or subadult stages (e.g. Bortolotti 1984a, b, c; Masterov 2000; Shephard et al. 2004), potentially enabling sex identification based on morphometry. However, if the traits used for sex discrimination also show geographical variation (see, for example, Bent 1961; Salomonsen 1979; Merz and Merz 2004; Nebel 2006), their accuracy may vary from population to population. Moreover, problems can arise when the degree of dimorphism varies or when individuals are sampled at different times during their nestling period (Bortolotti 1984b; Setiawan et al. 2004). We present here an assessment of the accuracy of one such sex identification method in two populations of white-tailed eagle *Haliaeetus albicilla*.

White-tailed eagles are protected and classified as vulnerable or threatened over most of their distribution range.

Although ringing of nestlings is taking place in many countries, there is a mandate to reduce disturbance at nest sites as much as possible. Since nests are often built in tree tops, simple and quick methods are preferable to make the handling of nestlings on the tree more comfortable and reduce both risk and stress to the nestlings. This precludes the development of multivariate methods for sex determination (Shephard et al. 2004). A method to sex white-tailed eagles nestlings based on two tarsal measurements has been suggested (Helander 1981). This approach takes into account the relatively larger size of the tarsus in females than in males and is applicable to field work conditions with only one person handling the eagle nestlings in the treetop. Nevertheless, no formal assessment of the value of this method to identify the sex of the birds has been carried out. An accurate assessment of the validity of this method will require that it be compared to an independent and more precise approach.

Molecular genetic methods offer the possibility for sexing of birds based on the fact that females are heterogametic (carry two different sex chromosomes, ZW), whereas males are homogametic (with two Z chromosomes, ZZ). Several methods based on the polymerase chain reaction (PCR) have been suggested (Fridolfsson and Ellegren 1999; Griffiths et al. 1998). One commonly applied method is based on the PCR amplification of an intron within the *CHD1* gene, which is present on both sex chromosomes, using primers located in conserved exonic regions flanking the intron. If the amplified intron shows a pronounced length difference between the Z and W chromosome gene copies, males and females can be discriminated on the basis of a simple agarose gel electrophoresis. The product of a PCR amplification will then produce two bands in females and just one in males.

In the present study, we evaluate the reliability of sex identification methods for white-tailed eagle nestlings based on morphological measurements. We also assess if morphology-based methods to identify sex in one population also can be used in other populations with different ecological conditions.

Methods

Study period and populations

The 211 samples used for this study were collected between 1999 and 2003 across the range of the white-tailed eagle (*Haliaeetus albicilla*) in Sweden. Its present distribution comprises two separate areas: a population along the Baltic coast and also extends into freshwaters in central and southern Sweden, and an inland population in Lapland (northern Sweden), mainly north of latitude 66°N. In the year 2000 the number of territorial pairs was estimated at 250 in the southern and central areas and 50 in Lapland (Helander 2003a). These two populations have likely been demographically independent from other populations in northern Europe during the past decades (Helander 2003b) and are genetically differentiated from each other (Cederberg et al. 2003; Hailer et al. 2006). The present study includes samples from throughout the species range in Lapland and from a major part of its range in southern and central Sweden (nests south of latitude 61°N).

Nestling measurement and sampling

Nest trees were climbed when nestlings were expected to be between 4 and 8 weeks old. Before taking measurements, each nestling was assigned an estimated age based on its stage of development and feathering (Heinroth and Heinroth 1967; Helander 1981, 1982). Nestlings were ringed lying flat on their belly with the feet stretched backwards and held with one hand. Before the rings were attached, a calliper was used to measure the thickness of the tarsus to the nearest 0.5 mm at the thinnest point (located approximately mid-way between the toes and the joint of the heel; tars1). At the same location, the thickest tarsus diameter was also measured (tars2), at an approximately right angle to the first measurement. With the nestling still lying on its belly after ringing, a metal-coil ruler was used to measure the length of the folded wing to the nearest 5 mm from the carpal joint to the tip of the longest primary (wing). The nestling was then put into a plastic micromesh bag and weighed on a 10 kg Pesola spring balance (Pesola AG, Baar, Switzerland). Weight was recorded to the nearest 0.05 kg and was then corrected for the weight of the bag and for crop contents (weight). The estimated volume of the crop was used as a proxy for the weight of its contents (Helander 1981).

Blood (0.5 ml) was sampled from the brachial vein using sterile techniques and was buffered in 1 ml EDTA/SSC and kept frozen until treatment in the laboratory. From broods containing more than one chick in the southern population, the larger chick was often chosen for sampling (potentially implying a bias towards the – larger – females; see Discussion). In the northern (Lapland) population, nests commonly contained only one chick (Helander 2003a), but when broods of two occurred, both nestlings were generally sampled to increase the number of samples from this smaller population.

Molecular sex identification

DNA was extracted from the blood samples using a standard proteinase K and phenol-chloroform procedure

(Sambrook et al. 1989). To identify the sex of each nestling, we used the primers 2550F and 2718R (Fridolfsson and Ellegren 1999) to amplify an intron within the *CHD1* genes on the Z and W chromosomes. For the PCR amplification, we used approximately 10 ng of genomic DNA, 3 pmol of each primer, 2 nmol of each dNTP, 0.3 U of HotStar *Taq* (Qiagen, Hilden, Germany) and 1× of HotStar *Taq* reaction buffer (Qiagen) containing Tris–Cl, KCl, $(NH_4)_2SO_4$ and a final concentration of 1.5 mM $MgCl_2$, in a total volume of 10 µl. We used the following PCR programme on a PTC-225 machine (MJ Research, Watertown, Mass.): 38 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s and elongation at 72°C for 30 s. A prolonged denaturation step (15 min at 95°C) was carried out prior to the first denaturation cycle, and the last cycle was followed by an additional annealing step for 1 min and a final elongation step for 15 min at 72°C. The amplified PCR product was run on 2% agarose gels in 0.5× TAE buffer at 75 W for 40 min. DNA was visualized using ethidium bromide staining and cross-illumination with UV light. The PCR produced one band (approximately 750 bp) in birds identified as males and two bands (approximately 750 and 450 bp) in birds identified as females, which is consistent with their ZZ and ZW genotypes, respectively.

Statistical analysis

Two-sample *t*-tests were used to analyse sexual dimorphism in the following measurements: tars1, tars2, wing, weight, the product tars1 × tars2 (as proposed for sex determination by Helander 1981), the ratio tars1/tars2 (shape index) and tars1 × tars2/wing (standardized index of overall tarsus size). To ascertain the power of these measurements and their combinations for the classification of individuals as males and females in a consistent manner across comparisons, we used stepwise discriminant analysis. Our goal was to identify morphological characteristics (or combinations of them) that would enable sex identification across a range of nestling ages that correspond to the suitable period for ringing and blood sampling. To investigate which measures do not change greatly with age, we studied the correlations between different morphometric characters and wing length, which is tightly correlated with age (Helander 1981; see below). We also attempted to statistically control for nestling growth by standardizing various measurements by wing length. We chose not to try weight to standardize for age, since an individual's weight can vary extensively depending on its nutritional status and because starvation has been found to be a common cause of death for second-hatched chicks in Lapland (Helander 1983, 2003a). Tests and analyses were performed with the software SYSTAT v.9 (Systat Software, Richmond, Calif.).

Previous studies have shown that food resources are limited for the eagles in Lapland and, probably as a result, nestlings tend to be thinner during the investigated nestling period (Helander 1983). To avoid potential confounding effects of the different habitat characteristics, data analysis was performed treating the two populations separately: methods for nestling sex identification were developed based on the population in southern and central Sweden. In a second step, these methods were evaluated for use in the Lapland population.

Results

Sex identification in southern and central Sweden

Morphometric measurements were obtained from 185 nestlings from southern and central Sweden and from 26 nestlings from Swedish Lapland. Molecular sex identification revealed that there were 84 males and 98 females among the samples from southern and central Sweden, and 13 males and 13 females among the nestlings from Lapland. For two individuals, the PCR remained unsuccessful despite three amplification attempts, probably due to low-quality DNA. For another individual, the banding pattern remained inconclusive after repeated amplification attempts, precluding its sex identification (contrary to results from other individuals, the W band in this individual was present, but weaker than the band from the longer Z fragment). These three individuals, all from southern and central Sweden, were discarded from further analyses.

We used the samples from southern and central Sweden to investigate methods to identify the nestling's sex, and we then evaluated the performance of these methods on individuals from the population in Lapland. Among the samples from southern and central Sweden, female nestlings as a group were significantly larger than males for all measurements and indices ($P < 0.001$ in all cases) except for wing length (wing, $P = 0.759$; Table 1). Single measurements – tars1, tars2, and weight – allowed the correct sex identification (larger values than a certain cut-off point corresponding to females, and smaller values corresponding to males) of a very large proportion of the individuals, ranging from 76% for weight and 96% for tars1. The best sex discriminator for both sexes was tars1, which allowed the correct determination of 95% of the females and 98% of the males based on a cut-off point of 13.8 mm (see Table 1, Fig. 1); given the measurement accuracy, this implies that individuals with tars1 smaller than or equal to 13.5 mm are classified as males, whereas a measure of 14 mm or larger classifies an individual as female. The product tars1 × tars2 has similar discriminatory powers as

Table 1 Morphometric characteristics of white-tailed eagle nestlings from southern and central Sweden

Measure	Females (n = 98)		Males (n = 84)		Sexual dimorphism[a]	% Females/males correctly classified[b]
	Average	SD	Average	SD		
1. tars1 (mm)	14.8	0.6	12.8	0.5	$P < 0.001$	95/98
2. tars2 (mm)	16.9	0.8	15.6	0.7	$P < 0.001$	81/92
3. Weight (kg)	4.48	0.66	3.76	0.50	$P < 0.001$	77/74
4. Wing (mm)	326.6	72.1	323.5	66.8	$P = 0.759$	48/49
5. tars1 × tars2	249.86	20.31	196.78	15.12	$P < 0.001$	91/96
6. tars1/tars2	0.87	0.03	0.84	0.03	$P < 0.001$	68/65
7. All (1–6)	–	–	–	–	–	95/98
8. tars1 × tars2/wing	0.80	0.17	0.63	0.13	$P < 0.001$	63/80
9. Weight/wing	0.014	0.002	0.012	0.001	$P < 0.001$	71/82
10. tars1 × weight/wing	0.207	0.026	0.152	0.016	$P < 0.001$	82/92

n denotes sample size

[a] As assessed by two-tailed Student t-tests

[b] Based on discriminant function analysis

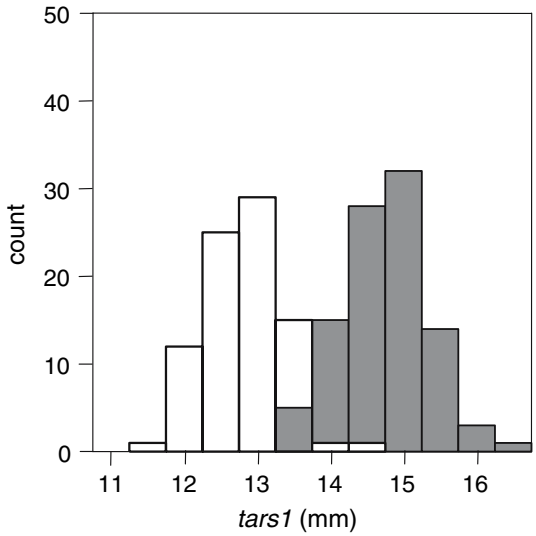

Fig. 1 Distribution of measurements of tars1 for 84 male and 98 female white-tailed eagles from southern and central Sweden. *White* and *grey bars* correspond to males and females, respectively

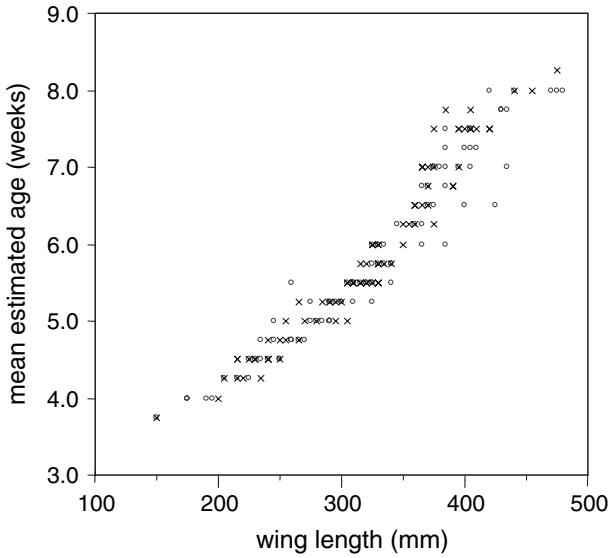

Fig. 2 Nestling age and wing length in white-tailed eagle from southern and central Sweden. *Circles* Females, *crosses* males

tars1 alone (see Table 1), allowing the correct identification of the sex for 93% of the samples. We also evaluated the possibility that shape differences in the tarsus may correspond to sexes by using the ratio tars1/tars2. However, the discriminatory power of this ratio was clearly lower and only allowed the correct identification of the sex in 67% of the cases. Combining all measurements and these two indices in a stepwise discriminant analysis did not improve the proportion of correct sex identifications compared to tars1 alone and allowed us to assign the correct sex to 95% of the females and 98% of the males (Table 1).

The estimated age of nestlings was highly correlated with wing ($r = 0.95$ for females, $r = 0.96$ for males; Fig. 2). Thus, wing length could be used as a proxy for age. In both males and females, all individual measurements were significantly ($P < 0.05$) correlated with wing. This correlation was weakest for tars1 ($r = 0.38$ and 0.44 for females and males, respectively), indicating that this measure was least affected by growth during the post-hatching period studied. On the other hand, the correlation between wing and weight reached values of $r = 0.84$ and 0.88 for females and males, respectively (Fig. 3). Since both tars1 and tars2 slightly increase with age, we also evaluated the power of tars1 × tars2/wing in an attempt to correct for differences associated with growth, but the performance to determine sex was clearly worse (Table 1). We also examined the usefulness of weight/wing and tars1 × weight/wing, but neither proved to be reliable measures by which to discriminate between the sexes.

To specifically account for the fact that all of the morphological characters we evaluated for sex identification were correlated with wing length – i.e. were growing during nestling development – we calculated a linear

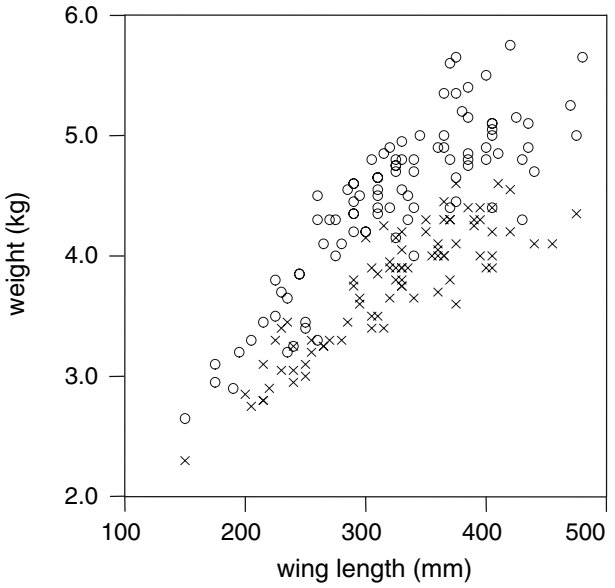

Fig. 3 Relationship between weight and wing length in white-tailed eagle nestlings from southern and central Sweden. *Circles* females, *crosses* males

regression for each one measure of tars1, tars2, weight, tars1 × tars2 and tars1/tars2 in females using wing as the independent variable. The resulting expressions were used on males and females, and the residuals were calculated (difference between the observed value and the expected value – from the regression – for a female with that wing length). These residuals were used in a stepwise discriminant analysis to separate the sexes. The discriminant function allowed the correct identification of sex for 95% of the females and 98% of the males, the same percentage that had been obtained for tars1 alone or in combination with other measures (Table 1).

Comparison of populations

We assessed whether the morphology-based sex identification method devised, based on samples from southern and central Sweden, also yields correct results in nestlings from another geographic area, Swedish Lapland. To do this, we applied the discriminant functions obtained from nestlings from the southern and central Swedish population on nestling measurements from Lapland. The resulting sex classification was then compared with the correct sex as revealed by the molecular analyses.

None of the measurements or indices derived from the southern population provided a reliable way to identify the sex of the nestlings from Lapland (Table 2). This could be due to the fact that nestlings from Lapland were often leaner than those in southern and central Sweden, and the differences were significant for almost all individual measurements for both males and females as well as for the product tars1 × tars2 (Table 2). Many female nestlings from the northern population were so thin that they were mistakenly identified as males based on the criteria derived from the southern population. For example, using the cut-off point for tars1, the measurement that best discriminated between males and females in central and southern Sweden, led to the erroneous identification of sex for 85% of the females from Lapland.

In a further attempt to take into account differences in size between nestlings from the two populations during the studied period of growth, we applied the discriminant functions built upon the residuals calculated from the

Table 2 Morphometric characteristics of white-tailed eagle nestlings from Lapland and their differentiation from nestlings in southern and central Sweden (Table 1)

Measure	Females (n = 13)		Males (n = 13)		% Females/males correctly classified[a]
	Average	SD	Average	SD	
1. tars1 (mm)	13.2***	0.8	12.3*	0.7	15/92
2. tars2 (mm)	15.3***	1.0	14.4*	1.3	23/92
3. Weight (kg)	3.42***	0.80	3.10*	0.83	23/85
4. Wing (mm)	285.7 NS	77.3	269.6*	79.3	31/62
5. tars1 × tars2	203.48***	23.00	178.02*	24.05	15/92
6. tars1/tars2	0.86 NS	0.05	0.86 NS	0.06	46/54
7. All (1–6)	–	–	–	–	23/85
8. tars1 × tars2/wing	0.77 NS	0.24	0.71 NS	0.18	38/62

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significantly different from nestlings in southern and central Sweden (as assessed by Student's t-tests)

The proportion of Lapland nestlings whose sex was correctly identified was estimated using the classification functions developed for southern and central Sweden (Table 1). n denotes sample size

[a] Based on discriminant function analysis

regression for females in the central and southern population. This discriminant function improved the correct identification of females, since 92% were correctly sexed. However, the percentage for males only reached 46%. In conclusion, none of the criteria for sex identification usable in central and southern Sweden yielded reliable results when directly applied on nestlings from Swedish Lapland.

Discussion

The PCR-based method of Fridolfsson and Ellegren (1999) was suitable for sex discrimination of white-tailed eagles, yielding unambiguous banding patterns in 208 out of 211 tests (98.6%). The Z chromosome fragment is amplified in both males and females, thus serving as an internal control to verify PCR amplification success. Absence of the W fragment, but presence of the Z fragment allows an individual to be classified as a male. The usefulness of this internal control is augmented by the fact that the W band is shorter than the Z band: shorter fragments amplify more easily than longer ones (Wandeler et al. 2003). Therefore, if the Z copy has amplified, then the W fragment should also have amplified – if that individual is a female. The uneven sex ratio obtained among the nestlings from southern and central Sweden could be a sampling effect (see Methods).

When only the samples from central and southern Sweden are considered, most single measurements, the product tars1 × tars2 as well as a combination of measurements in a discriminant analysis provided good separation between males and females. Among the measurements studied, tars1 proved to be the most efficient in separating sexes. It is also the measurement that is least prone to increase with body growth after the nestlings reach 4 weeks of age (see Bortolotti 1984c for the closely related bald eagle *Haliaeetus leucocephalus*), which illustrates the usefulness of characters or indices that level off early during nestling growth. We tried to obtain such indices using the ratios tars1/tars2 and tars1 × tars2/wing, but neither proved more helpful in discriminating between the sexes. The use of weight, a potentially useful discriminator in sexually dimorphic species, did not improve the performance to discriminate between the sexes, most likely due to variation among nestlings of similar age within each sex (Fig. 3). A more complex approach using the residuals of regressions versus wing length and a discriminant analysis provided better power to separate sexes, but it was still not better than tars1 alone. Among the ten variables measured in bald eagle nestlings, tarsus width also showed the highest sexual dimorphism index (Bortolotti 1984b).

Among the misclassified nestlings based on a cut-off point for tars1 of 13.8 mm were those with a wing measure of 150, 195, 235, 260 and 440 mm (females) and 305 and 330 mm (males), corresponding to ages ranging from approximately 3 weeks up to 8 weeks. The misclassified female with wing = 150 mm was the single youngest nestling in the sample. Allowing for the possibility that young age contributed to the misclassification in this case, we suggest that tars1 should be used for the sexing of white-tailed eagle nestlings from an age of 4 weeks and older.

When these approaches based on the discriminant functions derived from the southern population were applied to the Lapland population, they proved to be of very limited value as a tool for sex identification. This northern population is characterized by lower productivity and thinner nestlings during the studied growth period (Table 2), probably a result of limited food resources and starvation (Helander 1983, 2003a). Therefore, the methods devised on the southern and central Swedish samples misclassified a substantial proportion of Lapland nestlings of the larger sex– females – as males (Table 2). Moreover, none of the indices intended to take nestling growth into account significantly improved sex discrimination. Only the discriminant analysis on the residuals of the regressions against wing length provided reasonable power to separate males and females, but the method is unpractical to implement, and the total proportion of correct assignments was still below 80%. These results reflect the difficulty in designing simple robust methods for sex identification that could be applicable to populations living under different ecological conditions. Given our results from southern and central Sweden (Table 1) and Lapland (Table 2) and those of Bortolotti (1984b), tars1 (i.e. tarsus width) can also be expected to be useful in the Lapland white-tailed eagles. However, a larger sample size from Lapland would be necessary to identify an appropriate cut-off point for tars1 to separate males and females in this population.

Conclusion

Working in the top of a tree and with a bird species that is sensitive to disturbance imposes limitations to the number of measurements that can be recorded from nestlings. Although we only investigated a rather limited number of morphological variables, we observed that a simple method to sex white-tailed eagle nestlings allowed the correct classification of 96% of the individuals. However, this high percentage is dependent on high consistency while taking the measurements (in our case, they were always taken by the same person, BH). Other traits that have not been measured in this study could yield even more accurate sex

determination, perhaps also useful across populations. Further exploration of the best discriminatory measurements may provide better resolution in the cases where nestlings can be handled for longer, and other measurements can be taken (Shephard et al. 2004). In field conditions where this is not possible, the present study shows that even a simple measurement like tarsus thickness, easily taken when ringing, can provide efficient sex identification of white-tailed eagle nestlings following local calibration in the study population.

Zusammenfassung

Morphologische und genetische
Geschlechtsbestimmung von Nestlingen des Seeadlers
Haliaeetus albicilla

Kenntnis über das Geschlechtsverhältnis von Vogelnestlingen liefert wichtige Grundlagen für ethologische und andere ökologische Untersuchungen und ist ein zentraler Aspekt beim Schutz und Management von bedrohten Populationen. Der Seeadler *Haliaeetus albicilla* war bis kürzlich ein in weiten Teilen Europas bedrohter Greifvogel, der in vielen Ländern Teil breit angelegter Monitoringprogramme ist. Ein Grund hierfür ist, daß sich der Seeadler gut als Indikatorart eignet, um die Anreicherung von Schadstoffen in limnischen und marinen Ökosystemen zu überwachen. In der vorliegenden Arbeit wurden verschiedene morphometrische Maße auf ihre Eignung zur Geschlechtsbestimmung von Nestlingen hin untersucht. Diese Maße können von einer einzelnen Person beim Beringen, sowie direkt am Nest (häufig in der Baumkrone) erhoben werden. Darüber hinaus wurde zur Kontrolle das Geschlecht jedes Adlernestlings mit molekulargenetischen (DNS-basierten) Methoden bestimmt. Eine einzelne Meßvariable, Tarsusbreite *(tars 1)*, gewährleistetete die korrekte Geschlechtsbestimmung von über 96% der Nestlinge in Süd- und Mittelschweden. Diese Kriterien ließen sich jedoch nicht direkt auf Nestlinge in Nordschweden (Lappland) anwenden. Nestlinge in dieser Region sind in der Regel dünner, vermutlich aufgrund von Nahrungsknappheit während der Brutzeit. Unsere Ergebnisse zeigen, dass Geschlechtsbestimmung von Seeadlernestlingen mit hoher Genauigkeit möglich ist, auch wenn nur wenige morphometrische Merkmale aufgenommen werden. Jedoch scheinen die Bestimmungskriterien lokal an die untersuchte Population angepaßt werden zu müssen.

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References

Bent AC (1961) Life histories of North American birds of prey. General Publishing Company/Constable and Company, Toronto/London, pp 316–334

Bortolotti GR (1984a) Sexual size dimorphism and age-related size variation in Bald Eagles. J Wildl Manage 48:72–81

Bortolotti GR (1984b) Criteria for determining age and sex of nestling bald eagles. J Field Ornithol 55:467–481

Bortolotti GR (1984c) Physical development of nestling bald eagles with emphasis on the timing of growth events. Wilson Bull 96:524–542

Cederberg H, Sandström C, Rannug U, Helander B (2003) Genetic variation based on DNA fingerprinting in two white-tailed sea eagle populations in Sweden. In: Helander B, Marquiss M, Bowerman W (eds) Sea eagle 2000. Swedish Society for Nature Conservation/SNF & Åtta.45 Tryckeri AB, Stockholm, pp 219–221

Ellegren H, Sheldon BC (1997) New tools for sex identification and the study of sex allocation in birds. Trends Ecol Evol 12:255–259

Fridolfsson A-K, Ellegren H (1999) A simple and universal method for molecular sexing of non-ratite birds. J Avian Biol 30:116–121

Gilpin ME, Soulé ME (1986) Minimum viable populations: processes of species extinction. In: Soule ME (ed) Conservation biology: the science of scarcity and diversity. Sinauer Assoc, Sunderland, pp 19–34

Griffiths R, Double MC, Orr K, Dawson RJG (1998) A DNA test to sex most birds. Mol Ecol 7:1071–1075

Hailer F, Helander B, Folkestad AO, Ganusevich SA, Garstad S, Hauff P, Koren C, Nygård T, Volke V, Vilà C, Ellegren H (2006) Bottlenecked but long-lived: high genetic diversity retained in white-tailed eagles upon recovery from population decline. Biol Lett 2:316–319

Heinroth O, Heinroth M (1967) Die Vögel Mitteleuropas. II. Band. Verlag Harri Deutsch, Frankfurt/Main & Zürich, pp 72–77

Helander B (1981) Nestling measurements and weights from two White-tailed Eagle populations in Sweden. Bird Study 28:235–241

Helander B (1982) Sea eagle experimental studies. National Swedish Environment Protection Board SNV PM 1386, Stockholm

Helander B (1983) Reproduction of the White-tailed Sea Eagle *Haliaeetus albicilla* in Sweden, in relation to food and residue levels of organochlorine and mercury compounds in the eggs. PhD thesis, GOTAB, Stockholm

Helander B (2003a) The White-tailed Sea Eagle in Sweden – reproduction, numbers and trends. In: Helander B, Marquiss M, Bowerman W (eds) Sea eagle 2000. Swedish Society for Nature Conservation/SNF & Åtta.45 Tryckeri AB, Stockholm, pp 57–66

Helander B (2003b) The international colour-ringing programme – adult survival, homing, and the expansion of the white-tailed sea eagle in Sweden. In: Helander B, Marquiss M, Bowerman W (eds) Sea eagle 2000. Swedish Society for Nature Conservation/SNF & Åtta.45 Tryckeri AB, Stockholm, pp 145–154

Masterov VB (2000) Postnatal development of Steller's sea eagles in East Asia. In: Ueta M, McGrady MJ (eds) First symposium on Steller's and white-tailed sea eagles in East Asia. Wild Bird Society of Japan, Tokyo, pp 17–28

Merz JE, Merz WR (2004) Morphological features used to identify Chinook Salmon sex during fish passage. Southwest Nat 49: 197–202

Morris WF, Doak DF (2002) Quantitative conservation biology: theory and practice of population viability analysis. Sinauer Assoc, Sunderland

Nebel S (2006) Latitudinal clines in sex ratio, bill, and wing length in Least Sandpipers. J Field Ornithol 77:39–45

Salomonsen F (1979) Ornithological and ecological studies in SW Greenland. Meddelelser om Groenland, 204(6). Nyt Nordisk Forlag Arnold Busck, Copenhagen, pp 66–78

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor

Setiawan AN, Darby JT, Lambert DM (2004) The use of morphometric measurements to sex Yellow-eyes Penguins. Waterbirds 27:96–101

Shephard JM, Catterall CP, Hughes JM (2004) Discrimination of sex in the white-bellied sea-eagle, *Haliaeetus leucogaster*, using genetic and morphometric techniques. Emu 104:83–87

Sutherland WJ (2002) Conservation biology: Science, sex and the kakapo. Nature 419:265–266

Wandeler P, Smith S, Morin PA, Pettifor RA, Funk SM (2003) Patterns of nuclear DNA degeneration over time—a case study in historic teeth samples. Mol Ecol 12:1087–1093