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Evidence for seasonal changes in population structure of Reed Buntings (*Emberiza schoeniclus*) in the Lake Neusiedl Region: a stable-isotope approach

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Barbara Kofler, BSc

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TABLE OF CONTENT

Evidence for seasonal changes in population structure of Reed Buntings (*Emberiza schoeniclus*) in the Lake Neusiedl Region: a stable-isotope approach

1) ABSTRACT

Migrating species and populations are affected by conditions existing across large geographical scales. The degree to which populations mix at stop-over sites and wintering grounds is variable and can involve important fitness consequences. Thus, the link between breeding and wintering grounds and seasonal shifts in population structure are important to understand population demographics, local adaption and diversification patterns. This work aims to infer migration patterns and population connectivity in the Reed Bunting (*Emberiza schoeniclus*) community of a Ramsar-designated wetland, Lake Neusiedl – Seewinkel. Stable isotope markers (hydrogen, δ^2H, carbon, δ^{13}C) in feathers were analysed to assign individuals that inhabit the area during migration and winter to a specific breeding or natal area. Furthermore, on the basis of the geographic assignment, birds were compared with respect to their ecomorphological traits and their habitat selection during winter in order to assess potential ecological differences and possibly associated morphological divergence. Stable isotope data revealed that the local Reed Bunting community undergoes seasonal changes in population structure. More precisely, three different populations that vary in their δ^2H signature and to some extent in morphological characteristics as well as in δ^{13}C values, were identified. Moreover, differential habitat selection among co-occurring populations was found during winter. The results give evidence that different populations mix during migration and during winter at Lake Neusiedl and the extent to which they are specialized to a specific habitat varied. This finding could be linked to their morphological attributes, where bill size and shape apparently plays a decisive role. Indications for differential migration according to age and/or sex classes were additionally found.

Keywords: Reed Bunting, *Emberiza schoeniclus*, stable isotope analysis, hydrogen isotopes, carbon isotopes, geometric morphometrics, ecomorphology, Lake Neusiedl, habitat selection, migratory connectivity, differential migration

2) ZUSAMMENFASSUNG

Wandernde Arten oder (Teil-) Populationen, unterliegen aufgrund ihrer teilweise sehr großen Aktionsradien den Umweltbedingungen, welche sie in einem weiträumigen geografischen Gebiet vorfinden. Dieses umfasst für ziehende Vogelarten nicht nur ihr Brutgebiet, sondern auch Rast- und Überwinterungsgebiete. Zudem kann die Art und Weise sowie das Ausmaß in dem sich Populationen und Teilpopulationen einzelner Arten in diesen Gebieten mischen weitreichende Konsequenzen für ihr Überleben und ihre Fitness haben. Um spezifische Anpassungsmuster und demografische Aspekte besser zu verstehen ist es deshalb essentiell Zugbewegungen nachzuvollziehen und saisonale Veränderungen in der Zusammensetzung der Vogelgemeinschaften eines Gebiets zu ergründen. In diesem Zusammenhang soll die vorliegende Studie weiterführende Erkenntnisse zum Zugverhalten und der Konnektivität von Rohrammer (*Emberiza schoeniclus*) Populationen im Ramsar-Schutzgebiet Neusiedler See – Seewinkel liefern. Durch die Analyse von stabilen Isotopen (Wasserstoff, δ^2H, Kohlenstoff, δ^{13}C) in Federn wurde die geografische Herkunft von rastenden und überwinternden Rohrammern ermittelt. Individuen welche unterschiedlichen Brutgebieten zugeordnet werden konnten, wurden anschließend hinsichtlich ihrer ökomorphologischen Eigenschaften sowie ihrer Habitatwahl während des Winters verglichen um damit zusammenhängende phänotypische und ökologische Unterschiede zu ermitteln. Die Analyse der stabilen Isotope zeigte, dass die am Neusiedler See vorkommenden Rohrammern saisonalen Veränderungen in ihrer Populationszusammensetzung unterliegen. Genauer gesagt wurde festgestellt, dass sich während der Zugzeit sowie im Winter drei Populationen verschiedener Herkunft im Gebiet mischen, welche sich zu gewissem Grade sowohl in ihren Isotopenwerten als auch in ihrer Morphologie unterscheiden. Die im Gebiet überwinternden Rohrammern waren zudem in ihrer Habitatwahl verschieden. Dies deutet auf einen unterschiedlichen Spezialisierungsgrad der Populationen für gewisse Ressourcen hin, was in Zusammenhang mit ihren morphologischen Eigenschaften, v.a. mit der Schnabelform, stehen könnte. Des weisen die Ergebnisse dieser Studie auf mögliche geschlechts- und/oder altersspezifische Unterschiede im Wanderverhalten der Populationen hin.

3) INTRODUCTION

In contrast to animals that spend their entire lives within a local home range, migrant species are affected by jointly more variable conditions existing across larger geographical areas (Arizaga et al. 2015). It was found that migratory species and populations differ in the degree to which individuals from distinct breeding localities mix on different stop-over sites and wintering grounds (Chabot et al. 2012; Marra et al. 1998), and the link between breeding, stop-over and wintering sites can have important fitness consequences for migrating individuals. These consequences are many-fold and include the interaction of direction, distance and duration of migration, the use of stop-over sites and wintering grounds that may vary in quality and the physiological state (Veen et al. 2014). Patterns of connectivity between breeding and wintering grounds of migrating species are thus highly significant in terms of their evolution, ecology and conservation (Arizaga et al. 2015; Webster and Marra 2005; Woodworth et al. 2016). Hence, to understand population demographics, local adaptation and causes of diversification, the spatial characteristics (i.e. which populations mix on breeding and on wintering grounds) and the strength of migratory connectivity must be quantified (Chabot et al. 2012; Webster et al. 2002).

Connectivity is conceptually of particular interest in specialist species. Habitat specialists are species, which occupy narrow ecological niches and therefore, they usually utilize similar habitat types throughout the entire annual cycle. As these species have strict habitat requirements, they are potentially vulnerable to changes in environmental conditions and land use, especially in small, local populations (Briedis et al. 2016).

Although, globally over 85% of wetlands have already been lost, they are still being drained and have yet come under considerable pressure (IPBES 2019). Population trends of reed-dwelling bird species are closely tied to the condition and the state of maintenance of those habitats. As habitat fragmentation continues, the conservation of remaining wetlands has become crucial, especially to a number of highly sensitive or specialized species, whose occurrences have already been reduced to small insular and isolated populations. This also applies to the Reed Bunting (*Emberiza schoeniclus*) (Pasinelli and Schiegg 2012), which is primarily associated with wetlands (Glutz von Blotzheim and Bauer 1997).

The Reed Bunting is a small Palearctic short- to medium-distance migratory songbird, whose distribution is wide-ranging and covers most of Europe and large parts of temperate and northern

Asia (Cramp and Perrins 1994; Glutz von Blotzheim and Bauer 1997). The Reed Bunting is one of the most variable species of the large bunting family, the Emberizidae (Neto et al. 2013). Historically over 30 subspecies have been described on the basis of bill size, body size and plumage colour (Glutz von Blotzheim and Bauer 1997; Vaurie 1956; Vaurie 1958). As reported by Neto et al. (2013) the variation in phenotype is complex and to a large extent clinal. Specifically, there is a latitudinal gradient in beak thickness (and body size) increasing towards the south; additionally, in the southern part of the distribution range there is a longitudinal gradient of beak thickness, increasing towards the east. An additional longitudinal gradient exists with respect to plumage, which is darkest in the west and becomes increasingly light towards the east (Neto et al. 2013). While southern populations are resident, those further north (partially) migrate towards various directions over short- and medium distances; consequently, during winter thin-billed individuals often co-occur with thick-billed birds (Prŷs-Jones 1984; Neto et al. 2013).

The main objective of this study was to assess the provenance of wintering and migratory Reed Buntings as well as the phenological patterns of the Reed Bunting population in a Ramsar-designated wetland, Lake Neusiedl – Seewinkel, and furthermore, to appraise the role of the region as stop-over site and non-breeding residency area in terms of migratory connectivity for Reed Bunting populations.

In Austria the Reed Bunting is a common breeder, especially in the Lake Neusiedl region (Dvorak et al. 1993), which harbours the second largest reed stand (*Phragmites australis*) in Europe. According to biometric measurements the breeding population of the Lake Neusiedl region was designated to the subspecies (1) *E. s. stresemanni* (Bauer et al. 1955; Glutz von Blotzheim and Bauer 1997), whereas Dornberger (1979) assigned the local breeders to the subspecies (2) *E. s. intermedia*, which in contrast, has been referred to as a wintering subspecies by other authors (Bauer et al. 1955; Glutz von Blotzheim and Bauer 1997). In addition, northern migrants of the nominate form, (3) *E. s. schoeniclus*, and (4) *E. s. ukrainae* may complement local residents during the non-breeding season (Bauer et al. 1955; Dornberger 1979).

(1) The subspecies' *E. s. stresemanni* (Steinbacher 1930) breeding range reaches from eastern Austria southwards, over south-east Czech Republic and southern parts of Slovakia through Hungary to western Romania. Bill depth in this subspecies varies between 5.7-6.4 mm (Cramp and Perrins 1994).

(2) *E. s. intermedia* (Degland 1849) belongs to the so-called thick-billed Reed Bunting subpopulations, showing heavy beaks and an intensely curved culmen. Bill depth is ranging between 12.0-14.5 mm. This subspecies mainly breeds in Italy and along the Dalmatian coast (Cramp and Perrins 1994).

(3) The breeding range of the nominate form, *E. s. schoeniclus* (Linnaeus 1758), stretches over northern and western Europe, Britain, Fennoscandia, Russia and the Ural Mountains, south to northern Iberia and southern France, including the Alps and Italian foothills, the Carpathians and Belarus. The bill of *E. s. schoeniclus* is comparably small with bill depths between 4.3-5.8 mm. The culmen of the bill is straight or only very slightly convex (Glutz von Blotzheim and Bauer 1997; Shirihai and Svensson 2018).

(4) *E. s. ukrainae* (Zarudny 1917) inhabits regions in northern Ukraine and south-central European Russia. The culmen of this subspecies is clearly curved, but still fine and pointed compared to the thick-billed subspecies (Glutz von Blotzheim and Bauer 1997; Shirihai and Svensson 2018). Bill depth ranges between 5.2 and 6.5 mm (Cramp and Perrins 1994).

Despite the well-studied phenotypical variation among Reed Bunting subpopulations, the study of subspecies is complicated because of individual variation and the existence of intermediate populations (Neto et al. 2013; Vaurie 1956).

Ring recoveries indicate main migration periods in adjacent Hungary from mid-September to mid-November and from mid-February to April (Csörgő et al. 2009). A similar migration phenology is assumed for the Lake Neusiedl region. When Reed Buntings depart from their breeding grounds at Lake Neusiedl in late August, the number of records decreases steadily until the beginning of September. At that time, a wave of northern migrants passes through the region, dropping again to very low numbers until mid-November. Subsequently, Reed Bunting flocks are again encountered regularly (Glutz von Blotzheim and Bauer 1997) (with individuals of the subspecies *E. s. intermedia* among them) (Bauer et al. 1955). The local breeding population is partly sedentary, and partly migrates to winter grounds in coastal regions along the northern Adria in Italy and Croatia (Glutz von Blotzheim and Bauer 1997) and possibly in France (Csörgő et al. 2009). Song period of the Reed Bunting at Lake Neusiedl lasts from mid-February/ mid-March till the end of July (Glutz von Blotzheim and Bauer 1997).

In this study intrinsic stable isotope markers (hydrogen, δ^2H, carbon, $\delta^{13}C$) were used to assign Reed Bunting individuals to groups with different geographic provenance in order to unravel movement patterns of Reed Buntings that occur at Lake Neusiedl in the course of one year. Potential geographic variation in morphology (e.g. bill shape and bill size, wing shape) between these groups were assessed using geometric morphometrics and linear measurements of morphological traits.

3.1) Stable isotope analysis

Assessing connectivity and establishing specific migration patterns requires individuals of populations to be tracked as they move between breeding and wintering grounds (Clegg et al. 2003). Tracking migratory terrestrial animals has involved numerous techniques over the years (Hobson et al. 2019). The traditional method of bird banding has been informative for some avian groups, but in many passerines the recovery rate is below 1% (Berthold 1993). Similarly, radio and satellite tracking can only be used in large bodied migrants, which are capable of carrying heavy transmitters, but cannot be applied to small song birds (Clegg et al. 2003). The difficulties associated with the use of these techniques has led to the use of intrinsic markers to track populations. As pointed out by Hobson et al. (2019), the primary advantage of intrinsic markers is that initial marking of individuals is not necessary and that every capture provides information on origin. Isotope tracking of migratory terrestrial animals relies on the assimilation and incorporation of intrinsic stable isotopic markers (stable isotopes of C, H, N, O, and S) from the environment into animal body tissues. The basic mechanism that makes stable isotopes so useful for animal migration studies relates to the extent and pattern of spatial isotope ratio variation in the environmental substrates from which they are assimilated (Bowen and West 2019). Mass-dependent isotopic fractionation occurs whenever a chemical, biological or physical process drives a change in the isotope ratios due to slight chemical differences (Wassenaar 2019). After growth, bird feathers are metabolically inert. Therefore, they retain the isotopic signature of their place of growth. Consequently, feathers sampled during one period of the annual cycle provide a rough estimate of the location where the individual stayed in the period when the feather was grown (Hobson et al. 2004; Hobson and Wassenaar 1997).

In the present study stable hydrogen and stable carbon isotope values from Reed Bunting feathers, collected at Lake Neusiedl, are used to infer the approximate geographic origin of sampled individuals.

For animal migration studies, stable hydrogen isotopes are of paramount interest (Wassenaar 2019). Stable hydrogen isotope values in precipitation (δ^2H_P) vary predictably according to large-scale variation in temperature, rainfall and evaporation. Spatial patterns in precipitation isotope ratios are associated with isotope ratios found at the base of all terrestrial food webs via the linkage of water and primary production (Bowen and West 2019). In addition, stable carbon and stable nitrogen isotope assays, which are indicating types of habitat (diet and trophic relationship), can be used in order to improve the differentiation and spatial resolution of migratory assignments. The strong correspondence between carbon isotope ratios in primary production and photosynthetic pathway (C3, C4, CAM) provides strong linkages to climate in terrestrial systems (Hobson 2019).

3.2) Geometric morphometrics

For birds, there is a large body of literature on geographic variation in plumage and morphological characters (Hobson et al. 2019), which can be used to assess directly migratory connectivity whenever phenotypes are geographically segregated within either their breeding or non-breeding range (Webster et al. 2002). Morphological diversity in adaptive radiations have long been quantified utilizing univariate measurements of key traits (Foster et al. 2008). Traditional methods usually employ the analysis of linear distance measurements; the first combined axis (PC1) is related to overall size, the others are related to basically something that is 'not size' (Foster et al. 2008; Klingenberg 1996). However, as these axes do not correspond to any particular interpretable 'shape', the actual form cannot be visualized. Such limitations involved with traditional univariate measurements can be circumvented through the application of geometric morphometrics (Foster et al. 2008; Zelditch et al. 2004), which is the statistical analysis of shapes based on Cartesian landmark coordinates instead of measurements (Mitteroecker and Gunz 2009). The geometric morphometric approach was used – in addition to linear bill measurements (bill depth and bill length) – to determine possible beak shape variation among Reed Buntings of different geographic provenance.

3.3) Wing shape

Ecomorphological studies frequently tried to relate wing morphology, species' flight aerodynamics and the associated energetic costs to the evolution of migratory behaviour and to find out how related species utilize different ecological niches and avoid predatory pressure (Nowakowski and Chruściel 2008). On the one hand, migratory birds should minimise the costs of migration, which can be achieved by long and pointed wings because these reduce energy expenditure and increase endurance during long distance flights. On the other hand, outside the migratory period, when short flights with frequent take offs prevail, a short and rounded wing reduces both energy expenditure and predation risk (Pérez-Tris and Tellería 2001). Hence, in view of potential differences in migration distance and habitat use between Reed Bunting populations that occur in the region of Lake Neusiedl during migration and winter, wing pointedness and wing convexity were calculated as additional morphological measures.

The present study is a case study that examined the local Reed Bunting community at Lake Neusiedl over one year. Of main interest to this research was the species' community composition during migration times and during winter. Stable isotope markers were used to roughly assign individuals to a specific breeding or natal area. Furthermore, on the basis of the geographic group assignment, birds were compared with respect to their ecomorphological traits and their habitat selection during winter.

The project was funded by the German Ornithologists' Society (DO-G).

4) MATERIALS & METHODS

Ethics statement

All federal permits for data collection and the use of feather material were issued by the Austrian Federal Ministry of Education, Science and Research and the Department for Nature Conservation of the provincial government of Burgenland. The capture and ringing of birds followed standard protocols and birds were released unharmed on site.

4.1) Fieldwork

In the course of one year, from February 2017 till February 2018, Reed Buntings (n=372) were caught at the eastern shore of Lake Neusiedl in Illmitz (mean annual precipitation = approximately 600 mm, mean annual temperature = 10.1 °C, elevation= 117 m a.s.l.). The main field site (*reed*) was located along a dam (west-east orientation) that leads through an approximately 1 km wide reed bed at the eastern shore of the lake (47.769375 N, 16.756356 E). A second site was also located east of the lake but in a more open habitat at the shore of an alkaline lake (*salt pan*), with less extensive reed stands, abundant halophytic Chenopodiaceae species and early successional vegetation with oleaster and other shrubs, close to some wine yards, where wintering Reed

Buntings are regularly observed (47.769375 N, 16.756356 E). This field site was additionally studied during winter in order to assess whether different populations or morphotypes that potentially mix in their wintering area are locally segregated between microhabitats.

Birds were captured with mist-nets and during winter they were additionally attracted using a playback mix of song records of all relevant subspecies. Each bird was marked with a metal ring, and sex and age were determined using published criteria (Jenni and Winkler 1994). Wing length (maximum chord) and the length of P8 were measured with a metal ruler to the nearest 0.5 mm. Bill depth (at nostrils), bill length (to skull) and tarsus were measured with plastic callipers to the nearest 0.1 mm. The birds were weighted with a digital balance to the nearest 0.1 g. The development of subcutaneous fat deposits was assessed with a nine-class scale following Kaiser (1993). Moreover, the development of the flight muscle was assigned to one of four classes (Oschadleus 2012).

4.2) Stable isotope analysis

For stable isotope analysis a feather sample of each bird was obtained. With regard to the species-specific moulting patterns the 5th secondary was determined to reflect the stable isotope signature of the local food web of the breeding or natal area of the birds. According to Jenni and Winkler (1994), in 0% of 1st-year individuals this feather is moulted during their post-juvenile moult and adult Reed Buntings are known to undergo a complete post-nuptial moult at their breeding site directly after breeding.

Collected feathers were stored separately in clean paper envelopes for later isotope analysis. The samples were then randomly subsampled in order to maintain enough data when subdividing the dataset into sex, age and seasonal classes. Stable isotope analyses were performed at the stable isotope laboratory of the Leibniz Institute for Zoo and Wildlife Research (IZW, Berlin, Germany). Prior to isotope-ratio mass spectrometry, the samples were cleaned for 24 hours in a 2:1 chloroform:methanol solution on an RS-LS 5 shaking platform (Phoenix Instrument, Garbsen, Germany), to remove surface contaminants and oils. Then the samples were dried in a drying oven over 24 hours at 50 °C.

For stable carbon isotope analysis, 0.3-0.4 mg of cleaned feather vanes were placed into 4 mm x 6 mm tin capsules (OEA Laboratories, Callington, Cornwall, UK), which were burnt in an elemental analyser (Flash EA 1112 Series, Thermo Fisher Scientific, Bremen, Germany). Carbon stable isotopes were measured with a Delta V-Advantage stable isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen), connected to the analyser in continuous mode. The stable isotope ratios were calculated as per mil (‰) deviations from the ratios of the international standard, Vienna Pee Dee Belemnite (V-PDB) and expressed in the delta (δ) notation. The laboratory standard of known $^{13}C/^{12}C$ ratio tyrosine was used in conjunction with other international standards (NBS 19, NBS 22, USGS 24 and L-SVEC).

For stable hydrogen isotope analysis, 0.27 ± 0.01 mg of cleaned feather vanes were placed into 3.5 mm × 5 mm silver capsules (IVA Analysetechnik e.K. Meerbusch, Germany). The capsules were folded and sorted into 96 port microtiter trays and stored for at least 24 hours at 50°C in a drying oven. The comparative equilibration method described by Wassenaar and Hobson (2003), in which samples were analysed together with previously calibrated keratin hydrogen isotope reference materials, was used for stable hydrogen isotope analysis. This method overcomes the problem of uncontrolled hydrogen isotopic exchange between keratin and isotopically variable ambient moisture in the laboratory. On each microtiter tray a sample sequence consisting of spaced repetitions of four keratin standards, interspersed with 66 feather samples was analysed. Hair standards (sheep, goat, human) with the following δ^2H-values were used to determine non-exchangeable hydrogen isotope ratios: -167.9 ± 1‰ for SWE-SHE (powdered wool from Swedish sheep), -108.3 ± 1‰ for ESP-SHE (Spanish sheep), -66.3 ± 0.9‰ for AFR-GOAT (African goat, Tanzania) and -74.2 ± 0.5‰ for USGS42 (Tibetan hair). More details of the preparation of these in-house keratin standards are provided by Popa-Lisseanu et al. (2012) and Voigt and Lehnert (2019). The next steps follow Kravchenko et al. (2019). The samples and standards were loaded into a Zero Blank autosampler (Costech Analytical Technologies Inc., Cernusco sul Naviglio, Italy) and flushed with helium for 1 h. As carrier gas chemically pure helium with a flow rate of 100 mL/min was used. Next, samples and standards were pyrolysed in a high-temperature elemental analyser (HEKAtech GmbH Analysentechnik, Wegberg, Germany), operating at 1350 °C. A gas chromatograph (HTO Element Analyzer, HEKAtech GmbH Analysentechnik) separated H_2, N_2 and CO at 80°C and the resolved H_2 sample pulse was introduced into the stable isotope ratio mass spectrometer (Delta V Advantage, Thermo Fisher Scientific). The stable hydrogen isotope ratios were expressed in the delta notation as per mil (‰) deviations from the international standard V-SMOW.

4.3) Geometric morphometrics

For geometric morphometric analysis a photograph of the bill in profile (n=109) was captured on a millimetre grid using a Nikon D7000 ® camera (Nikon, Melville, New York, USA). Prior to analysis the photographs were edited in Adobe Photoshop CS2. A tps file was built from each image using *tpsUtil* version 1.74 (Rohlf 2016a). *TpsDig2* version 2.30 (Rohlf 2016b) was then used to place seven landmarks (discrete homologous points), and eight semi-landmarks (points on a curve, determined by extrinsic criteria) (Zelditch et al. 2004) on each beak image (Fig. 1). The semi-landmarks were placed by reference to a standardized grid superimposed onto each image, as referred in Foster et al. (2008).

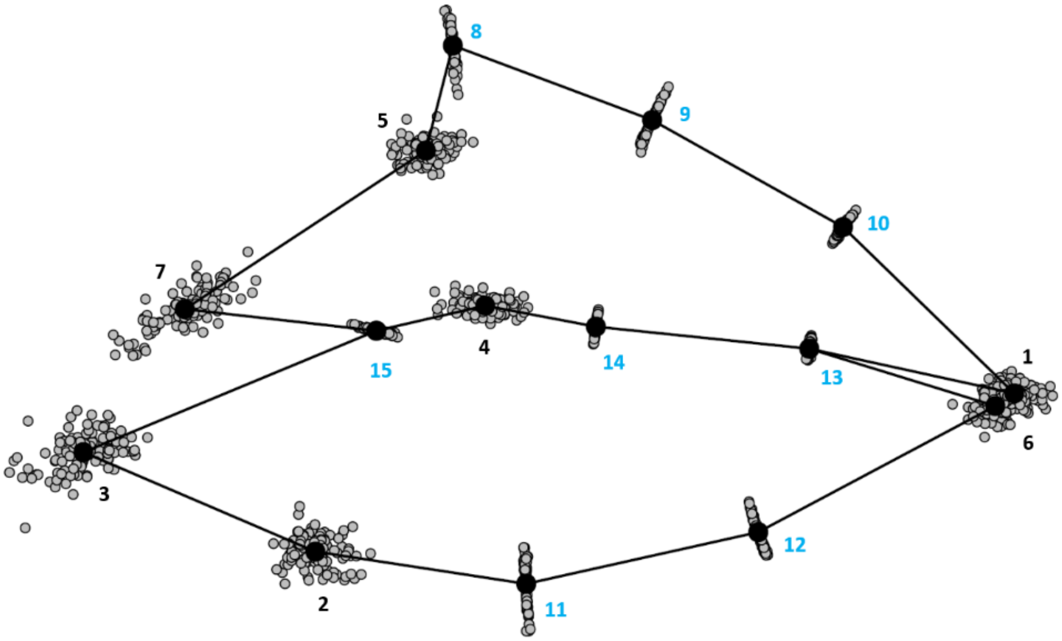


Fig. 1: Position of placed landmarks (black number) and semi-landmarks (blue numbers) on each beak image.

Extracted landmark data contain a lot of variation in position, orientation and scale between specimens. These non-shape variation has to be removed before further analysis (Brookstein et al. 1985; Brookstein 1997). Therefore, the landmark coordinates were rotated, translated and scaled through Generalized orthogonal least-squares Procrustes Analysis (GPA) as a 'standardization' step implemented in the the R package *geomorph* (Adams et al. 2018). The grand mean was calculated (i.e. the consensus of all specimens) and shape variables were then generated (Brookstein 1997). The centroid size (Csize) was computed as the square root of the sum of the squares of the distances from all landmarks to their centroid (Bookstein 1991).

4.4) Wing shape

For each bird, a digital picture (Nikon D7000 camera®, Nikon, Melville, New York, USA) was taken of its fully extended right wing. For each individual, all measurements were performed on the same picture. A millimetre grid was placed in the background to provide a scale. The length of primary 1-9 (most distal primary was ignored) was digitally measured, as described in (Vanhooydonck et al. 2009), using the image processing program ImageJ (Schneider et al. 2012). Wing length was measured as the distance between the shoulder joint and the tip of the outstretched wing. Wing area was quantified by digitizing the outer edge of the wing and the length of each primary was measured as distance between the shoulder joint and the tip of the respective primary (Vanhooydonck et al. 2009). The measured length of the primary feathers 1-8 was used to calculate wing pointedness (C2-index) and convexity (C3-index) according to Lockwood et al. (1998). The C2 Lockwood index does not consider the total wing length and is independent from body size. It calculates wing pointedness according to the location of the longest primary. The concept of convexity is described as a measure of the decrease in the acuteness of the handwing, brought about by a more rapid lengthening of primaries close to the wingtip compared to the most proximal primaries (Lockwood et al. 1998). Higher values of C2 relate to a relatively more rounded wing, whilst higher values of C3 correspond to a more convex wing (Arizaga et al. 2006).

4.5) Statistical analyses

Statistical analyses were conducted in R 3.5.2 (R Core Team 2018). Unless specified otherwise in the methods, required packages for this analysis included: *car* (Fox and Weisberg 2011), *ggpubr* (Kassambara 2019), *geomorph* (Adams et al. 2018), *stats* (R core Team 2018), *IsoriX* (Courtiol et al. 2019) and *densityClust* (Pedersen et al. 2017). Statistical significance was accepted at $p < 0.05$.

First, the relationship between δ^2H and δ^{13}C values of feathers was evaluated with Spearman´s rank correlation. Feather δ^2H and δ^{13}C values were then used to perform cluster analysis provided by the R-package *densityClust* (Pedersen et al. 2017). This function takes a distance matrix and calculates the values necessary for clustering based on the algorithm proposed by Rodrigues and Laio (2014). *densityClust* provides robust tools to generate the initial ρ and δ values for each observation as well as to use these thresholds to assign observations to clusters. The cluster centres are recognized as local density maxima that are far away from any points of higher density (Pedersen et al. 2017). The algorithm depends only on the relative densities rather than their absolute values (Pedersen et al. 2017). The assigned cluster membership of all observations was extracted and used as grouping factor in univariate and multivariate analysis.

To estimate the geographic provenance of the sampled feathers (moulting locality/natal origin), the R package *IsoriX* (Courtiol et al. 2019) was used. A spatial mixed model was calculated by predicting a δ^2H isoscape based on rainfall δ^2H_p values (corrected for altitudinal changes) from the Global Network of Isotopes in Precipitation (GNIP). All available precipitation data from June till October, when Reed Buntings undergo their complete or post-juvenile moult (Jenni and Winkler 2007), were used and the feather δ^2H_f values were converted to rainfall isotope $\delta 2H_p$ values using the transfer equation ($\delta^2H_f = 1.28, \delta^2H_{ISOSCAPE} - 10.29$) of Eurasian Reed Warblers (*Acrocephalus scirpaceus*) from Procházka et al. (2013). Based on this calibration model the geographic assignment of feathers of unknown origin (δ^2H_f) was performed and a probability map of geographic origin was created.

Statistical analysis and visualization of beak shape variation was performed using the R package *geomorph* (Adams et al. 2018). This data was then used in multivariate analysis to assess shape variation. A covariance matrix from GPA-aligned Procrustes coordinates was used to perform a number of statistical tests to appraise beak-shape differences in predefined geographical groups (cluster 1-3). However, GPA does not take account of the relationship between size and shape (i.e. allometry). Therefore, allometric effects were assessed by reference to centroid size (Bookstein, 1996) to check for potential size related effects on shape variation. An allometric regression was performed using Procrustes ANOVA with permutation procedure (function *procD.allometry* of the R-package *geomorph*) in order to assess the association between log centroid size and shape. Furthermore, Procrustes ANOVAs were calculated to assess the relative amount of shape and size variation attributable to a categorical predictor (cluster), with age and sex as covariates. In these analyses, significance was evaluated with a residual randomization permutation procedure of 1000 iterations. A principal component analysis (PCA) of shape variation was performed to visualize morphological differences among groups in a two dimensional plot of tangent space for a set of Procrustes shape variables (PC1 and PC2). Deformation grids were additionally computed to display the shape of specimens at the end of the range of PC1 and PC2.

Linear measurements of beak shape were reduced to one variable which represents an overall bill shape index (bill shape_{IND}) by dividing bill depth by bill length. Overall body size, estimated as the first principal component of an analysis including the variables tarsus and wing length ($PC1_{BODY\ SIZE}$ 71.3% explained variance), was included as covariate in linear regression models to test the relationship of body size and linear bill measurements. An additional grouping variable (season) was defined in relation to the date of capture. In the dataset seasons were specified according to the Reed Buntings' phenology at these latitudes, as referred in Csörgő et al. (2009) and Glutz von Blotzheim and Bauer (1997) (spring 16.2.-30.4., summer 1.5.-11.9., autumn 12.9.-18.11., winter 19.11.-16.2.). Further, age classes were reduced to two groups (1st-year, adults). Undetermined individuals were not considered in the analysis.

In the overall dataset, the variables $\delta^{13}C$, wing length and body size were not normally distributed. Thus, non-parametric tests were applied to assess trait and stable isotope differences among clusters, as well as between age and sex classes (Wilcoxon-Mann-Whitney test, Kruskal-Wallis test). Bill depth, after normalization through log-transformation, and all other variables were approximately normally distributed and there were no obvious deviations from variance homogeneity. Therefore, these variables were subjected to one-way ANOVA and Student's *t*-test in order to evaluate the effects of age, sex and cluster on each of the morphological traits and feather stable isotope values. Whenever there were significant differences between the two sex and age classes, tests were performed for males and females or for adults and 1st-year individuals separately. As post-hoc analysis Tukey´s tests or Dunn´s tests with Benjamini-Hochberg adjustment (1995) were applied to determine which groups differed from others.

A data-subset containing all observations from winter ($\text{data}_{\text{WINTER}}$) was used to test for differences of morphological traits as well as feather stable isotope ratios of wintering Reed Buntings that were caught at different sampling locations (*reed*, *salt pan*). In the $\text{data}_{\text{WINTER}}$ dataset (n=66) all variables (except for $\delta^{13}C$) were normally distributed, thus Students *t*-tests were conducted to reveal trait differences among sampling sites. In the case of $\delta^{13}C$ a Wilcoxon-Mann-Whitney test was applied.

5) RESULTS

5.1) Cluster analysis

A dataset containing the relevant variables δ^2H, $\delta^{13}C$, age, sex, season, wing length, bill depth, Lockwood C2 and Lockwood C3 remained with a sampling size of 110 complete cases. This data frame was subjected to cluster analysis. As preliminary analysis, Spearman´s rank correlation revealed a strong positive linear relationship between feather δ^2H and $\delta^{13}C$ signatures (1st-year R=0.73, p<0.001; adult R=0.52, p<0.001). Thus, not only δ^2H but also $\delta^{13}C$ can be considered to contain information on the origin of the captured birds. Subsequently, on the basis of stable hydrogen and carbon isotope values, a distance matrix was calculated and three clusters could be defined (distance cut-off: 0.169, threshold for local density when detecting cluster peaks ρ=3.5, threshold for minimum distance to higher density when detecting cluster peaks δ=0.3). The distance matrix was used to perform multidimensional scaling and a MDS scatterplot of cluster assignments was generated (Fig. 2, App. 1). 50 Individuals were assigned to the cores of the clusters (*core)* and 60 individuals were assigned as *halo*, i.e. points with low-density.

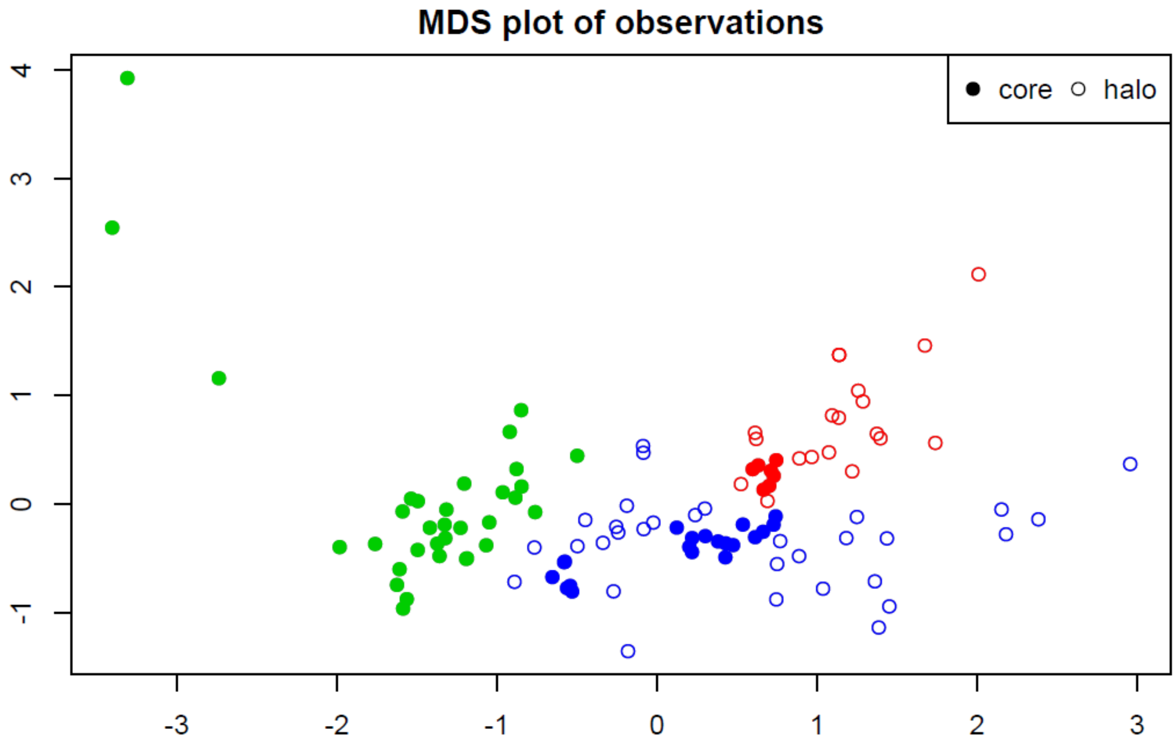


Fig. 2: MDS ordination of the distance matrix based on δ^2H and δ^{13}C values. 110 observations were assigned to one of 3 clusters; green=cluster1, blue=cluster2, red=cluster3. Observations belonging to a cluster core are plotted with filled circles and observations assigned as halo are plotted with hollow circles.

The *halo*-observations were kept in the dataset for further analysis. Only for the visualization of relative frequencies of clusters per season (Fig. 3) the dataset was filtered for core assignments alone. In spring the first two clusters were present at Lake Neusiedl. All core assignments from summer are belonging to cluster 1, thus cluster 1 is assumed to represent the local breeding population. During autumn, again individuals of cluster 2 were found. Further, the local Reed Bunting assembly was extended by cluster 3. As well as in autumn, the assembly during winter was composed of individuals from all three clusters, with cluster 3 having a higher relative frequency than in autumn (Fig. 3).

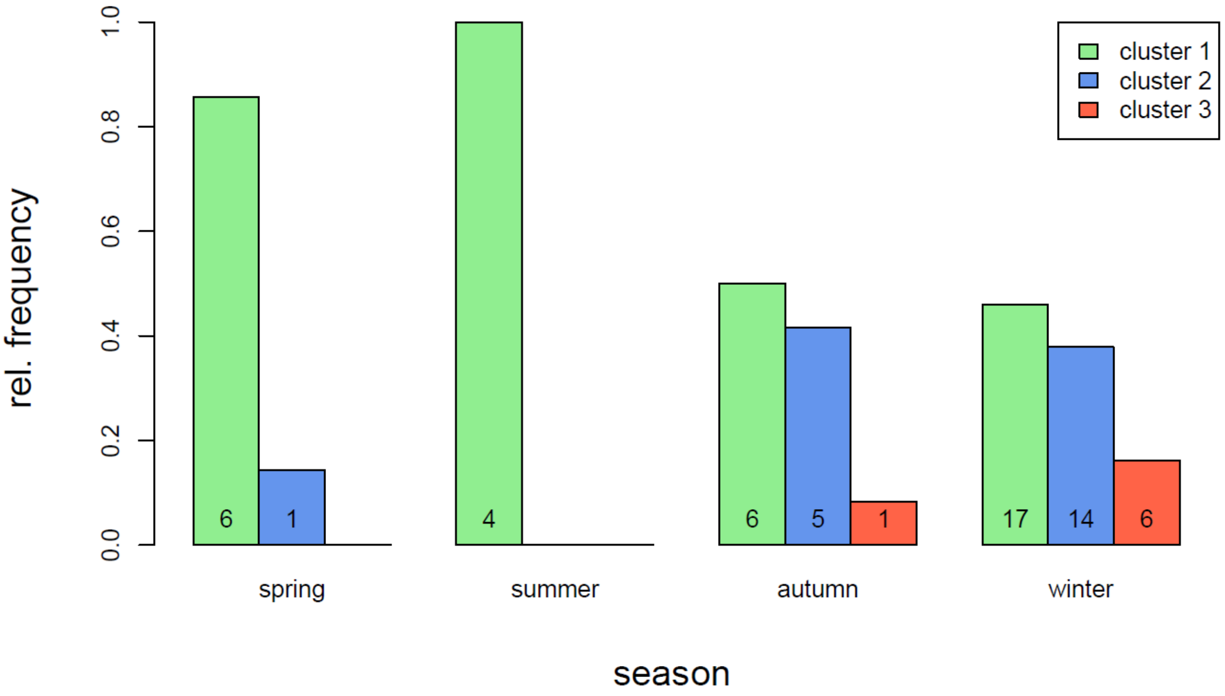


Fig. 3: Relative frequencies of Reed Bunting clusters per season (numbers refer to individuals belonging to the cluster cores).

5.2) Stable isotopes and morphological traits

The results of univariate analysis are summarized in Table 2. Since the clustering was based on the two stable isotope variables δ^2H and δ13C, first the divergence of the clusters in their stable isotope values was assessed to control clustering results. δ^2H values differed between age and sex classes and one-way ANOVAs and following post-hoc tests revealed highly significant differences between all three clusters in both sex- and age classes (Fig. 4). Feathers from birds of cluster 1 had the highest δ^2H values and feathers from cluster 3 were the most depleted in $\delta\,^2$H.

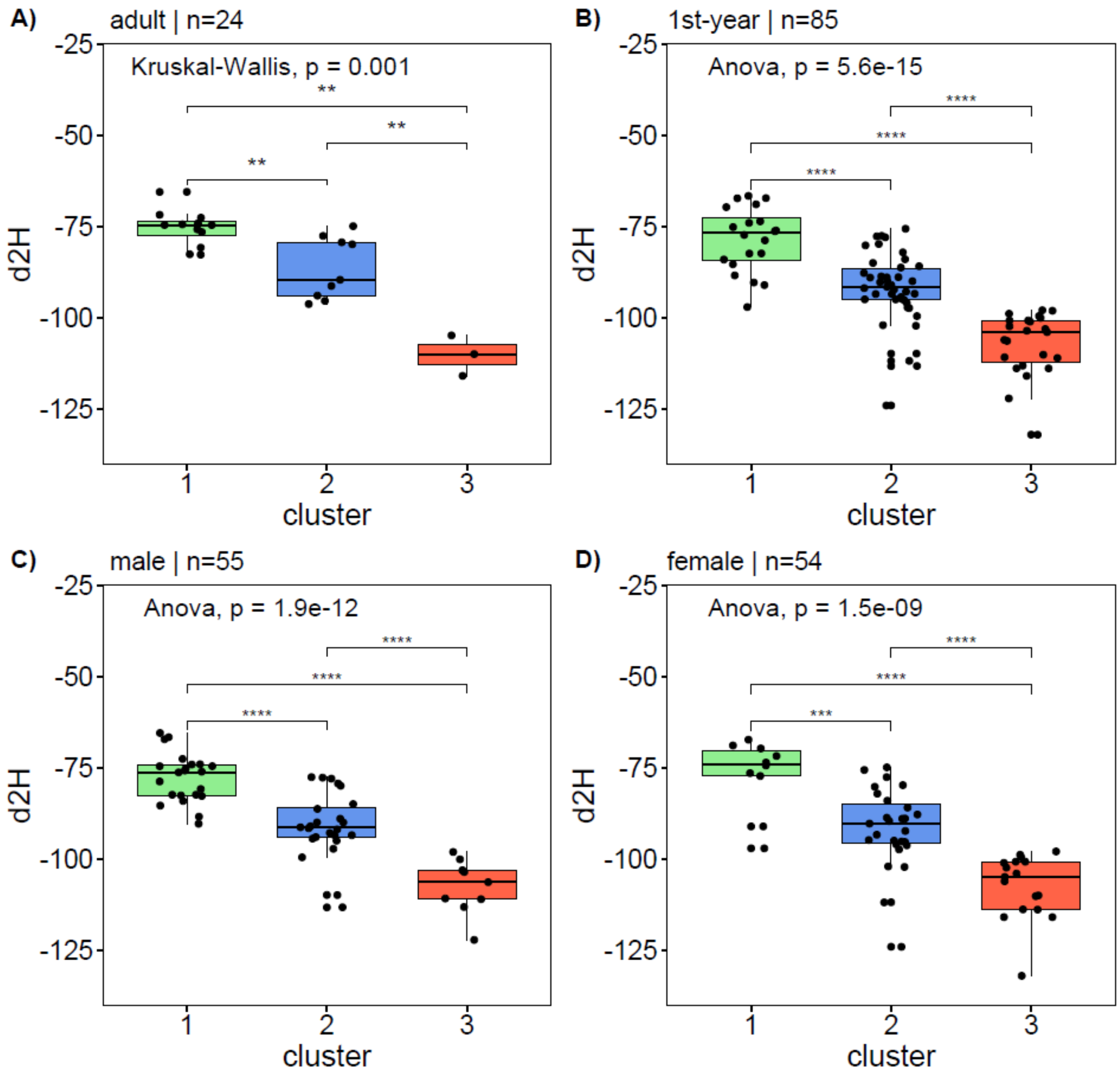


Fig. 4: Boxplots of stable hydrogen isotope values by cluster, age class (A = adult, B = 1^{st}-year) and sex class (C = male, D = female); statistical significance levels from pairwise comparisons (post-hoc tests) are displayed above brackets (p > 0.05 (ns), p < 0.05 (*), p < 0.01 (**), p < 0.001 (***), p < 0.0001 (****)).

Regarding feather $\delta^{13}C$ values, cluster 1 differed significantly from cluster 2 and cluster 3, having the highest $\delta^{13}C$ values in both, 1^{st}-year birds and adults (Fig. 5). Moreover, $\delta^{13}C$ showed a positive linear relationship with $PC1_{BODY\ SIZE}$ (R=0.24, p=0.012). Therefore, a linear model with $PC1_{BODY\ SIZE}$ as covariate was calculated to quantify the variance in $\delta^{13}C$ explained by clustering and body size. The interaction term was also taken into account. The model outcome (Tab. 1) showed significant differences in feather $\delta^{13}C$ between clusters. In contrast, this was not the case for $PC1_{BODY\ SIZE}$, nor for the interaction of cluster and body size.

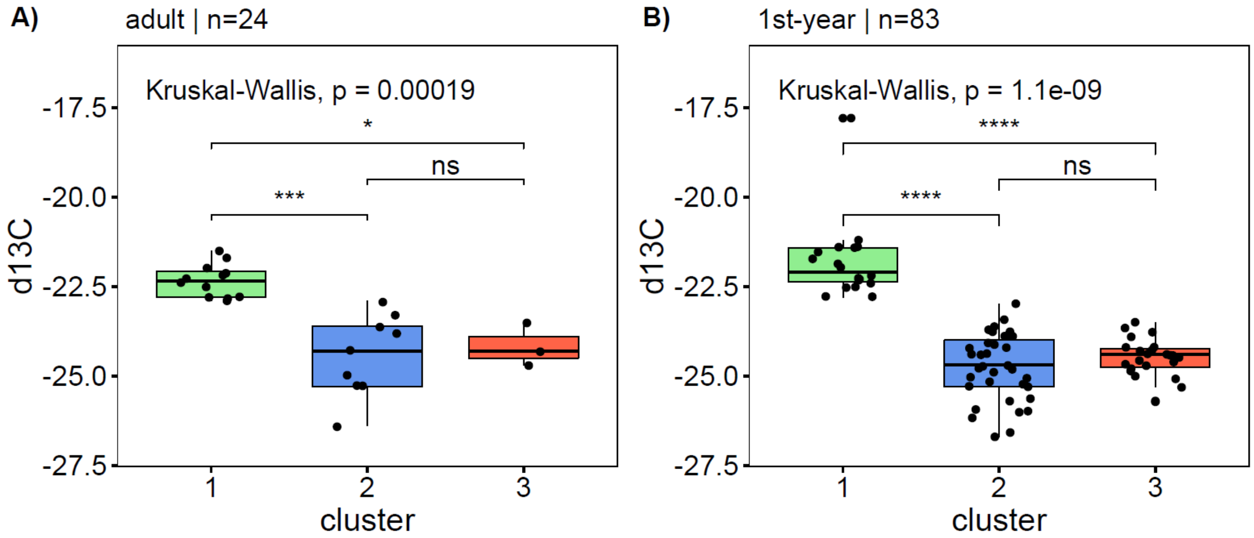


Fig. 5: Boxplots of stable carbon isotope ratios of different clusters and age classes (A = adult, B = 1st-year); statistical significance levels from pairwise comparisons (post-hoc tests) are displayed above brackets as described in caption of Fig. 4.

Reed Buntings showed significant body size variation among sex classes (p<0.001). Males of cluster 1 differed in body size from males belonging to cluster 2. In females a different pattern occurred, with females from cluster 3 differing from the other two clusters (Fig. 6). Prior to dividing the data between males and females, comparisons showed significant differences between cluster 2 and cluster 1 (p<0.001) and between cluster 2 and cluster 3 (p=0.026) (see Tab. 2).

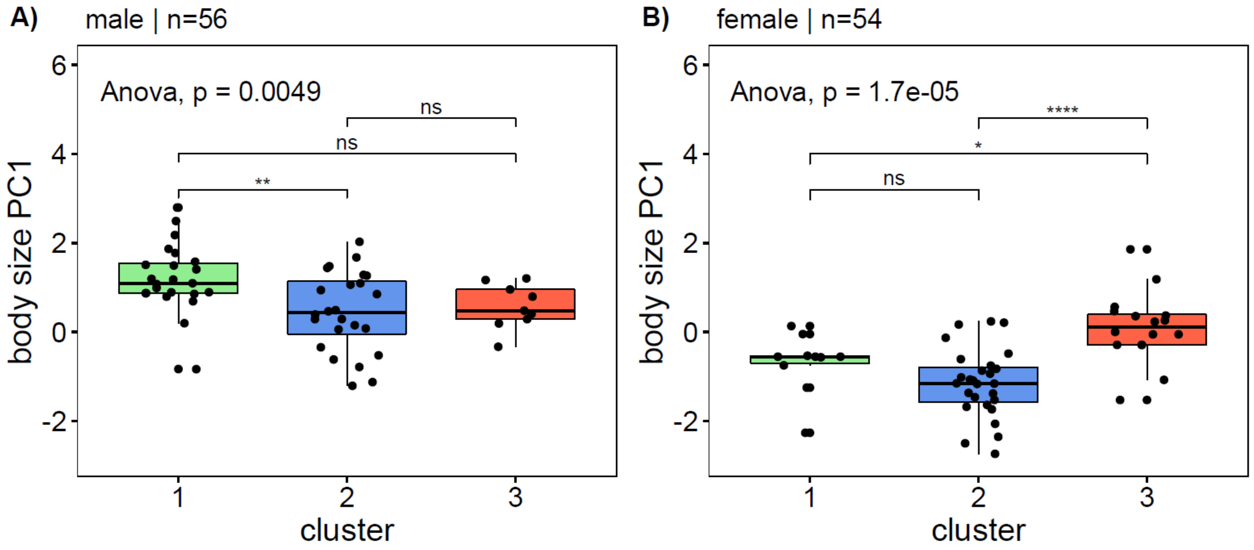


Fig. 6: Boxplots of the overall body size, estimated as the first principal component of an analysis including the parameters tarsus and wing length ($PC1_{BODY\ SIZE}$ 71.3% explained variance) by cluster and sex class (A = male, B = female); statistical significance levels from pairwise comparisons (post-hoc tests) are displayed above brackets as described in caption of Fig. 4.

$PC1_{BODY\ SIZE}$ was not only related to $\delta^{13}C$, but also correlated with bill depth ($R^2=0.46$, $p<0.001$). Bill shape$_{IND}$, however, showed no significant relationship with $PC1_{BODY\ SIZE}$ ($R^2=-0.18$, $p=0.069$). As in $\delta^{13}C$, a linear model including $PC1_{BODY\ SIZE}$ as covariate only showed significant effects of cluster on the response variables bill depth and bill shape$_{IND}$ (Tab. 1).

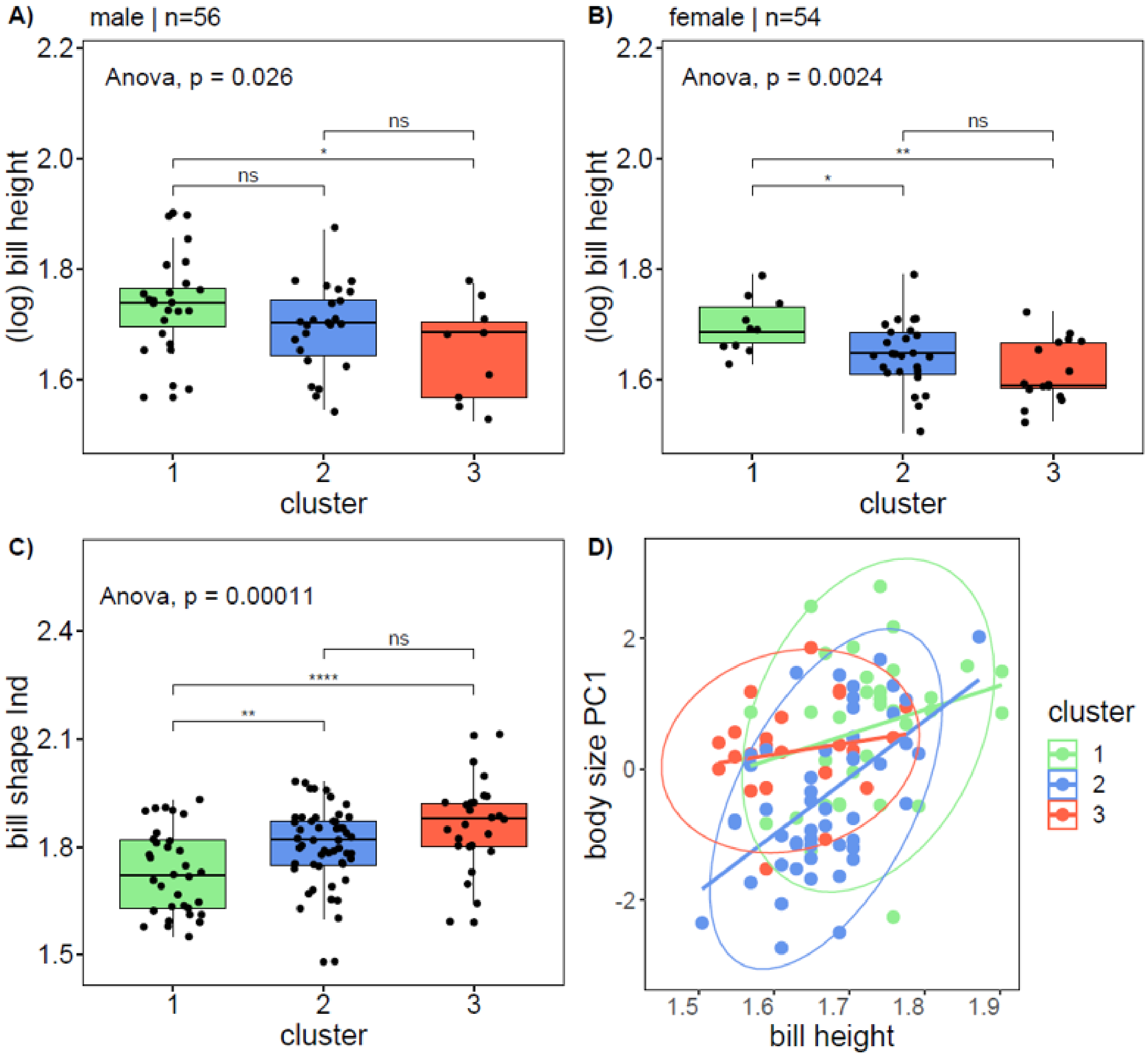


Fig. 7: Boxplots of the measured bill height (i.e. bill depth) by cluster and sex class (A = male, B = female); and boxplot of bill shape$_{IND}$ (bill depth/bill length) of different clusters (C); scatterplot of bill height and $PC1_{BODY\ SIZE}$ of different clusters, with a fitted linear regression line and an ellipse drawn at 95% confidence level; statistical significance levels from pairwise comparisons (post-hoc tests) are displayed above brackets as described in caption of Fig. 4.

In both sex classes Reed Buntings that were assigned to cluster 1 had the thickest bills and the lowest bill shape$_{IND}$, i.e. more massive bills in general. Cluster 2 and 3 did not diverge significantly from one another but individuals from cluster 3 showed a tendency to have the smallest beaks (Tab.

2, Fig. 7). The effect of body size as covariate turned out to be insignificant when analysing bill depth variation (Tab. 1).

Linear regression (LM) PC1$_{BODYSIZE}$ as covariate			
variable	**Estimate±SE**	**t**	**p**
d13C Residual standard error: 1.11 on 60 DF; Multiple R²: 0.6486; Adj. R²: 0.619; F-statistic: 22.15 on 5 an 60 DF, p-value:<0.001***			
(Intercept)	-22.023±0.308	-71.315	<0.001***
cluster2	-3.028±0.376	-8.711	<0.001***
cluster3	-2.442±0.434	-5.624	<0.001***
body.size.PC1	0.209±0.243	0.861	0.393
cluster2:body.size.PC1	-0.091±0.3055	0.301	0.765
cluster3:body.size.PC1	-0.367±0.501	-0.734	0.466
bill height Residual standard error: 0.3006 on 60 DF; Multiple R²: 0.3697 Adj. R²: 0.3172 F-statistic: 7.04 on 5 an 60 DF, p-value:<0.001***			
(Intercept)	0.5496±0.083	65.73	<0.001***
cluster2	-0.022±0.101	-2.204	0.031*
cluster3	-0.5131±0.118	-4.362	<0.001***
body.size.PC1	0.098±0.066	1.48	0.144
cluster2:body.size.PC1	0.016±0.083	0.19	0.576
cluster3:body.size.PC1	-0.002±0.136	0.013	0.703
bill shape$_{IND}$ Residual standard error: 0.09012 on 60 DF; Multiple R²: 0.2445 Adj. R²: 0.1815 F-statistic: 3.883 on 5 an 60 DF, p-value: 0.004**			
(Intercept)	1.759±0.025	70.19	<0.001***
cluster2	0.076±0.030	2.513	0.015*
cluster3	0.141±0.035	4.004	<0.001
body.size.PC1	0.027±0.019	1.379	0.173156
cluster2:body.size.PC1	-0.047±0.025	-1.896	0.062729
cluster3:body.size.PC1	0.041±0.041	-1.011	0.316057

Tab. 1: Results of linear models with δ^{13}C, bill depth and bill shape$_{IND}$ as response variables, cluster assignment as predictor and PC1$_{BODY SIZE}$ as covariate (the interaction term was also taken into account).

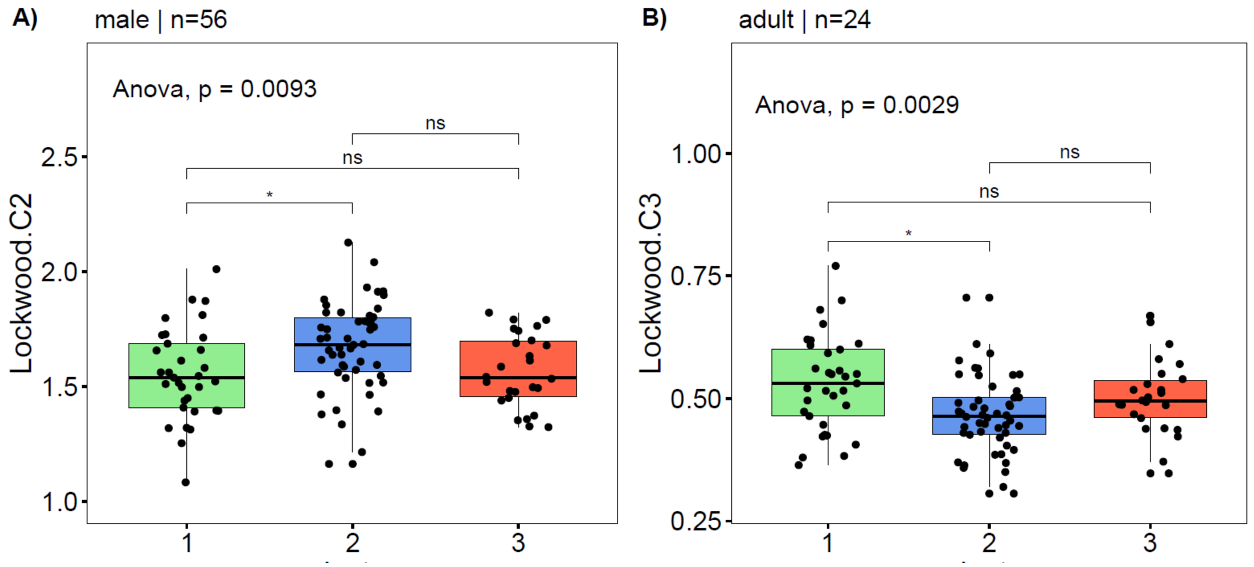
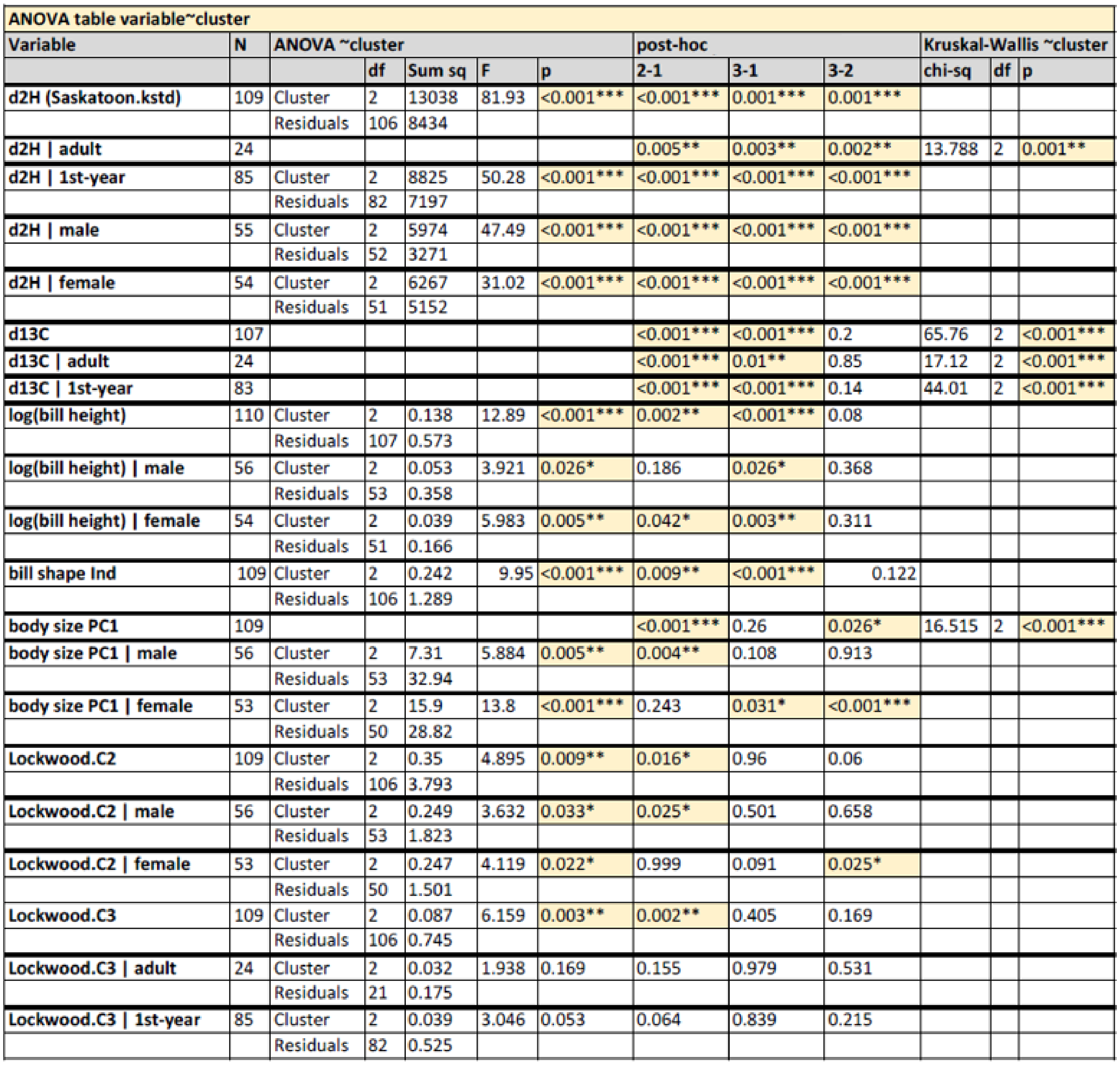


Fig. 8: Boxplots of Lockwood indices (C2, C3) of different clusters; statistical significance levels from pairwise comparisons (post-hoc tests) are displayed above brackets as described in the caption of Fig. 4.

When looking at the two Lockwood indices C2 (wing pointedness) and C3 (wing convexity), cluster 1 differed from cluster 2 (Tab. 3, Fig. 8). This indicates that Reed Buntings from cluster 2 are having more rounded and less convex wings than individuals belonging to cluster 1. When analysing Lockwood C2 for sex classes separately, contrasting result were found. Females from cluster 3 were different from cluster 2 and males that belonged to cluster 1 differed from males which were assigned to the second cluster. Cluster 1 and 2, instead, did not differ significantly (Tab. 3). The difference in wing convexity (Lockwood C3) was not consistent when analysing sex categories independently (Tab. 3).



ANOVA table variable~cluster												
Variable	N	ANOVA ~cluster					post-hoc			Kruskal-Wallis ~cluster		
			df	Sum sq	F	p	2-1	3-1	3-2	chi-sq	df	p
d2H (Saskatoon.kstd)	109	Cluster	2	13038	81.93	<0.001***	<0.001***	0.001***	0.001***			
		Residuals	106	8434								
d2H | adult	24						0.005**	0.003**	0.002**	13.788	2	0.001**
d2H | 1st-year	85	Cluster	2	8825	50.28	<0.001***	<0.001***	<0.001***	<0.001***			
		Residuals	82	7197								
d2H | male	55	Cluster	2	5974	47.49	<0.001***	<0.001***	<0.001***	<0.001***			
		Residuals	52	3271								
d2H | female	54	Cluster	2	6267	31.02	<0.001***	<0.001***	<0.001***	<0.001***			
		Residuals	51	5152								
d13C	107						<0.001***	<0.001***	0.2	65.76	2	<0.001***
d13C | adult	24						<0.001***	0.01**	0.85	17.12	2	<0.001***
d13C | 1st-year	83						<0.001***	<0.001***	0.14	44.01	2	<0.001***
log(bill height)	110	Cluster	2	0.138	12.89	<0.001***	0.002**	<0.001***	0.08			
		Residuals	107	0.573								
log(bill height) | male	56	Cluster	2	0.053	3.921	0.026*	0.186	0.026*	0.368			
		Residuals	53	0.358								
log(bill height) | female	54	Cluster	2	0.039	5.983	0.005**	0.042*	0.003**	0.311			
		Residuals	51	0.166								
bill shape Ind	109	Cluster	2	0.242	9.95	<0.001***	0.009**	<0.001***	0.122			
		Residuals	106	1.289								
body size PC1	109						<0.001***	0.26	0.026*	16.515	2	<0.001***
body size PC1 | male	56	Cluster	2	7.31	5.884	0.005**	0.004**	0.108	0.913			
		Residuals	53	32.94								
body size PC1 | female	53	Cluster	2	15.9	13.8	<0.001***	0.243	0.031*	<0.001***			
		Residuals	50	28.82								
Lockwood.C2	109	Cluster	2	0.35	4.895	0.009**	0.016*	0.96	0.06			
		Residuals	106	3.793								
Lockwood.C2 | male	56	Cluster	2	0.249	3.632	0.033*	0.025*	0.501	0.658			
		Residuals	53	1.823								
Lockwood.C2 | female	53	Cluster	2	0.247	4.119	0.022*	0.999	0.091	0.025*			
		Residuals	50	1.501								
Lockwood.C3	109	Cluster	2	0.087	6.159	0.003**	0.002**	0.405	0.169			
		Residuals	106	0.745								
Lockwood.C3 | adult	24	Cluster	2	0.032	1.938	0.169	0.155	0.979	0.531			
		Residuals	21	0.175								
Lockwood.C3 | 1st-year	85	Cluster	2	0.039	3.046	0.053	0.064	0.839	0.215			
		Residuals	82	0.525								

Tab. 2: Results of one-way ANOVAs and Kruskal-Wallis tests comparing stable isotope ratios and morphological variables among clusters; and results from post-hoc tests (Tukey test, Dunn´s test with Benjamini-Hochberg adjustment).

To evaluate whether Reed Buntings that were caught at two different sampling sites (*reed*, *salt pan*) during winter differ in their biometric measurements and stable isotope ratios, the data subset data$_{\text{WINTER}}$ was used. The birds caught in the reed stand of Lake Neusiedl differed significantly from those captured near the salt pan regarding their bill shape$_{\text{IND}}$ ($p=0.03$) and bill depth ($p=0.009$). When analysing bill depth for the two sex classes separately, only in males evidence for that difference was found ($p=0.008$). Moreover, birds form the two habitats differed in their feather δ^{2}H ($p=0.01$) and δ^{13}C values ($p<0.001$). Birds that were caught at the alkaline lake had thinner bills and were more depleted in ^{2}H and ^{13}C (Tab. 3, Fig. 9).

***t*-test results (variable~habitat) data$_{\text{WINTER}}$**					
variable	**t.test ~habitat**			**Man-Whitney U test ~habitat**	
	t	**df**	**p**	**W**	**p**
d2H (Saskatoon.kstd)	2.65	57.51	0.01*		
d13C				743	<0.001***
bill height	2.756	38.642	0.009**		
bill height | male	2.953	18.87	0.008**		
bill height | female	1.437	14.952	0.171		
bill shape Ind	-2.253	38.406	0.03*		
Lockwood.C2	1.484	53.081	0.144		
Lockwood.C3	-0.928	55.809	0.357		
wing	-0.917	57.284	0.3626		
body size PC1	0.385	44.938	0.702		

Tab. 3: Results from Students t-tests and Man-Whitney U-test comparing stable isotope ratios and morphological variables among the two different habitat types (reed and salt pan)

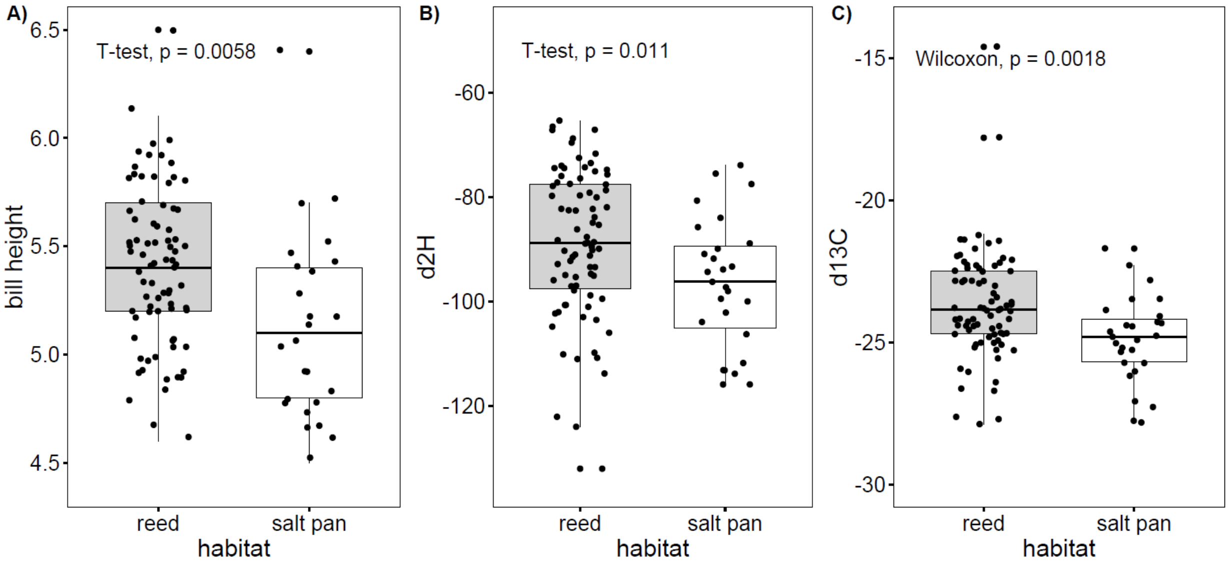


Fig. 9: Boxplots of bill height and stable hydrogen and carbon isotopes at different habitat types (reed, salt pan).

5.3) Geometric morphometrics

There were no differences in (log) centroid size among clusters and age and sex classes in the overall dataset (Tab. 4 top panel). Allometric regressions revealed a small size related effect in shape variation among clusters and cluster with age as covariate, which explained only 5% (cluster) and 3% (cluster:age) of the total variation (Tab. 4 bottom panel). Beak shapes differed significantly between clusters (p=0.002) and the interaction between cluster and age had also a significant effect on beak shape variation (p=0.034) (Tab. 4 middle panel). Procrustes ANOVA on shape with cluster as predictor and age as covariate was highly significant (F= 2.177, p=0.001) and post-hoc analysis (function *advanced.procD.lm*) indicated shape differences, mainly in beak curvature, between cluster 1 and the other two clusters. Among cluster 2 and cluster 3 no significant beak shape divergence was found. The visualization of shape differentiation in the principal component plot was grouped by cluster and age classes. The first principal component of shape variation (PC1) explained 27.5% of the total variation, while PC2 explained 24.2% (Fig. 10).

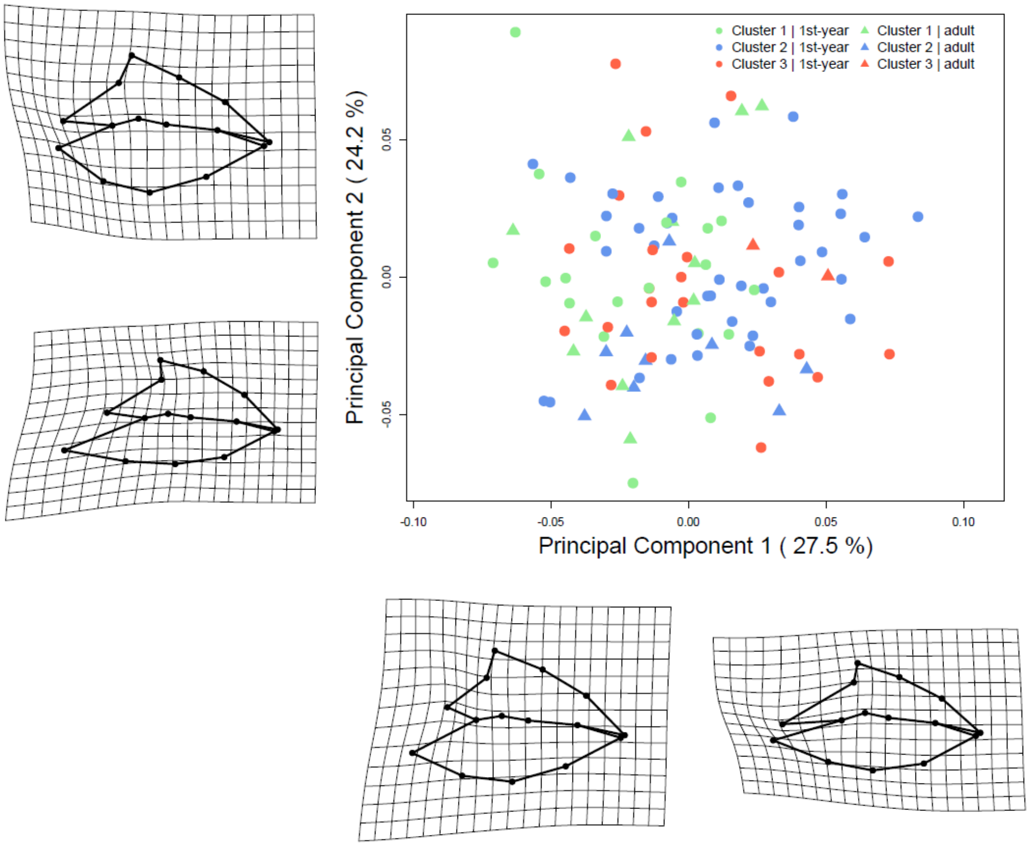


Fig. 10: Morphological difference among groups (cluster and age class) plotted in a two-dimensional plot of tangent space of Procrustes shape variables (PC1 and PC2). Deformation grids display the shape of specimens at the end of the range of the principal component axes.

Comparisons of the data$_{WINTER}$ beak (log) centroid size indicated no difference in size among the predictor variable habitat with age and sex as covariates (Tab. 5 top panel). Allometric regression revealed a small size related effect on shape variation associated with habitat (p=0.001) as well as with (log) centroid size and sex (p=0.023) (Tab. 5 bottom panel). Approximately 10% of the beak shape variation of wintering Reed Buntings could be explained by the different habitats they were captured at (p=0.001) (Tab. 5 middle panel). Procrustes ANOVA on shape variance returned a significant effect (F=3.133, p=0.001) associated with habitat as predictor and age as covariate. This shows a clear divergence in beak shape among Reed Buntings caught at different habitats during winter. The first principal component of shape variation (PC1) explained 31.4% of the total variation, while PC2 explained 21.4% (Fig. 11). Birds from cluster 1 were only caught in the reed bed of the Lake.

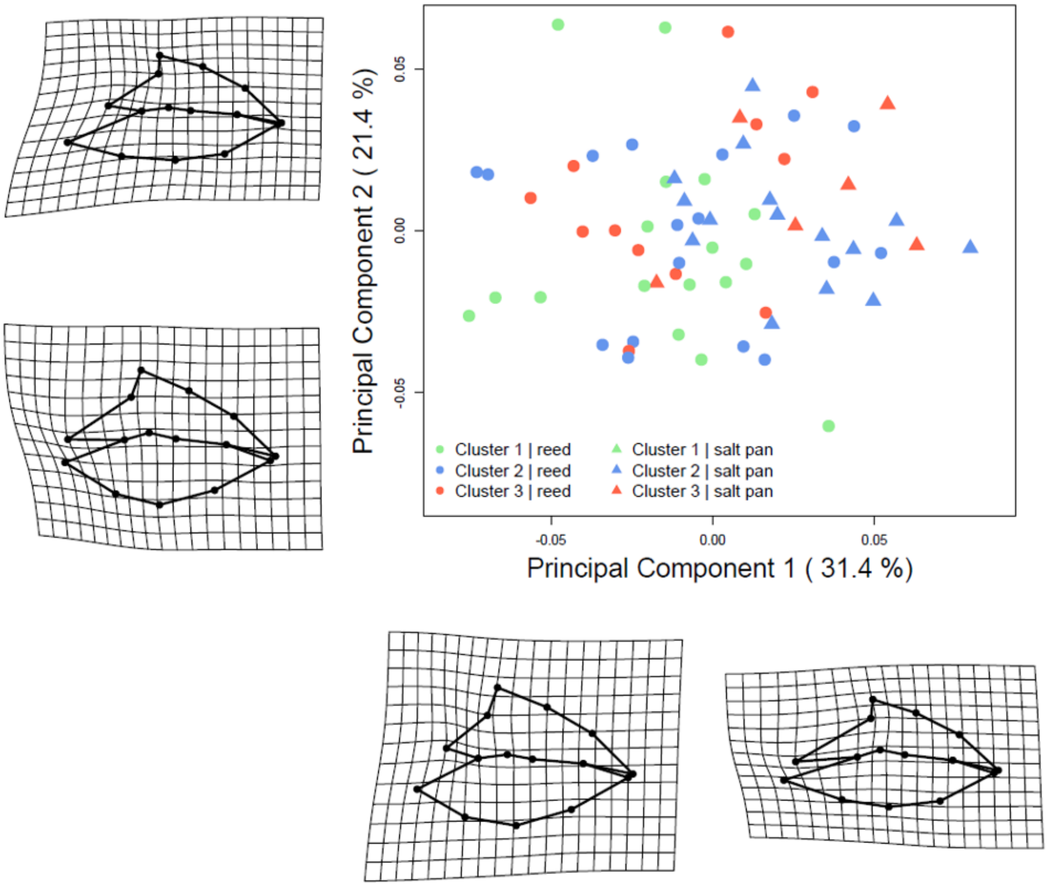
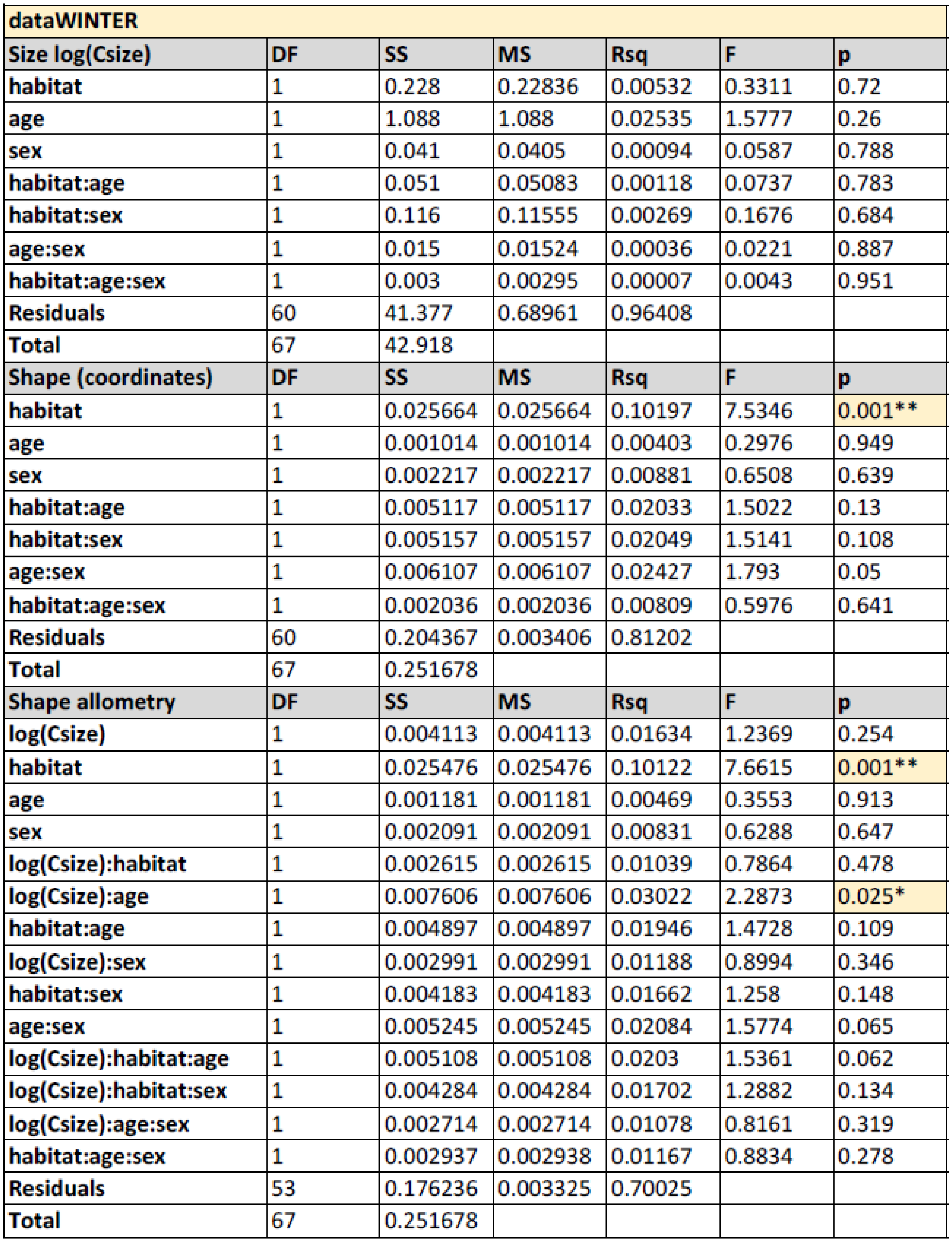


Fig. 11: Morphological difference among groups (cluster and habitat type) plotted in a two-dimensional plot of tangent space of Procrustes shape variables (PC1 and PC2). Deformation grids display the shape of specimens at the end of the range of the principal component axes.

Size log(Csize)	**DF**	**SS**	**MS**	**Rsq**	**F**	**p**
cluster	2	1.533	0.76655	0.026	1.3604	0.297
age	1	1.513	1.51347	0.02567	2.6859	0.091
sex	1	0.04	0.04046	0.00069	0.0718	0.77
cluster:age	2	0.074	0.03684	0.00125	0.0654	0.921
cluster:sex	2	1.61	0.80495	0.0273	1.4285	0.241
age:sex	1	0.013	0.01327	0.00022	0.0235	0.878
cluster:age:sex	1	0.081	0.08144	0.00138	0.1445	0.711
Residuals	96	54.095	0.56349	0.91748		
Total	106	58.96				
Shape (coordinates)	**DF**	**SS**	**MS**	**Rsq**	**F**	**p**
cluster	2	0.024	0.121	0.056	2.879	0.002**
age	1	0.004	0.004	0.009	0.349	0.376
sex	1	0.005	0.005	0.013	0.979	0.157
cluster:age	2	0.014	0.007	0.032	1.843	0.034*
cluster:sex	2	0.006	0.003	0.012	-0.358	0.653
age:sex	1	0.004	0.004	0.009	0.503	0.326
cluster:age:sex	1	0.001	0.001	0.003	-1.565	0.939
Residuals	96	0.376	0.004	0.864		
Total	106	0.435				
Shape allometry	**DF**	**SS**	**MS**	**Rsq**	**F**	**p**
log(Csize)	1	0.00497	0.004967	0.01142	1.2694	0.247
cluster	2	0.02425	0.012124	0.05578	3.0988	0.001**
age	1	0.00338	0.003376	0.00777	0.8628	0.466
sex	1	0.00562	0.005624	0.01294	1.4374	0.148
log(Csize):cluster	2	0.00761	0.003805	0.0175	0.9725	0.358
log(Csize):age	1	0.00141	0.001412	0.00325	0.3609	0.911
cluster:age	2	0.01341	0.006704	0.03084	1.7135	0.029*
log(Csize):sex	1	0.00699	0.006993	0.01609	1.7872	0.051
cluster:sex	2	0.00689	0.003447	0.01586	0.881	0.42
age:sex	1	0.00378	0.003776	0.00869	0.965	0.308
log(Csize):cluster:age	2	0.00675	0.003375	0.01552	0.8625	0.36
log(Csize):cluster:sex	2	0.00923	0.004614	0.02123	1.1793	0.132
log(Csize):age:sex	1	0.0034	0.003395	0.00781	0.8678	0.315
cluster:age:sex	1	0.00101	0.001014	0.00233	0.2592	0.939
log(Csize):cluster:age:sex	1	0.00347	0.003467	0.00797	0.886	0.336
Residuals	85	0.33257	0.003913	0.765		
Total	106	0.43474				

Tab. 4: Results of Procrustes ANOVAs, considering the effects of cluster, age and sex on centroid size (top), shape (middle) and shape, while accounting with variation in centroid size (bottom).



dataWINTER						
Size log(Csize)	**DF**	**SS**	**MS**	**Rsq**	**F**	**p**
habitat	1	0.228	0.22836	0.00532	0.3311	0.72
age	1	1.088	1.088	0.02535	1.5777	0.26
sex	1	0.041	0.0405	0.00094	0.0587	0.788
habitat:age	1	0.051	0.05083	0.00118	0.0737	0.783
habitat:sex	1	0.116	0.11555	0.00269	0.1676	0.684
age:sex	1	0.015	0.01524	0.00036	0.0221	0.887
habitat:age:sex	1	0.003	0.00295	0.00007	0.0043	0.951
Residuals	60	41.377	0.68961	0.96408		
Total	67	42.918				
Shape (coordinates)	**DF**	**SS**	**MS**	**Rsq**	**F**	**p**
habitat	1	0.025664	0.025664	0.10197	7.5346	0.001**
age	1	0.001014	0.001014	0.00403	0.2976	0.949
sex	1	0.002217	0.002217	0.00881	0.6508	0.639
habitat:age	1	0.005117	0.005117	0.02033	1.5022	0.13
habitat:sex	1	0.005157	0.005157	0.02049	1.5141	0.108
age:sex	1	0.006107	0.006107	0.02427	1.793	0.05
habitat:age:sex	1	0.002036	0.002036	0.00809	0.5976	0.641
Residuals	60	0.204367	0.003406	0.81202		
Total	67	0.251678				
Shape allometry	**DF**	**SS**	**MS**	**Rsq**	**F**	**p**
log(Csize)	1	0.004113	0.004113	0.01634	1.2369	0.254
habitat	1	0.025476	0.025476	0.10122	7.6615	0.001**
age	1	0.001181	0.001181	0.00469	0.3553	0.913
sex	1	0.002091	0.002091	0.00831	0.6288	0.647
log(Csize):habitat	1	0.002615	0.002615	0.01039	0.7864	0.478
log(Csize):age	1	0.007606	0.007606	0.03022	2.2873	0.025*
habitat:age	1	0.004897	0.004897	0.01946	1.4728	0.109
log(Csize):sex	1	0.002991	0.002991	0.01188	0.8994	0.346
habitat:sex	1	0.004183	0.004183	0.01662	1.258	0.148
age:sex	1	0.005245	0.005245	0.02084	1.5774	0.065
log(Csize):habitat:age	1	0.005108	0.005108	0.0203	1.5361	0.062
log(Csize):habitat:sex	1	0.004284	0.004284	0.01702	1.2882	0.134
log(Csize):age:sex	1	0.002714	0.002714	0.01078	0.8161	0.319
habitat:age:sex	1	0.002937	0.002938	0.01167	0.8834	0.278
Residuals	53	0.176236	0.003325	0.70025		
Total	67	0.251678				

Tab. 5: Results of Procrustes ANOVAs performed on the data_{WINTER} *dataset, considering the effects of habitat, age and sex on centroid size (top), shape (middle) and shape while accounting with variation in centroid size (bottom).*

6) DISCUSSION

Although clustering of the target variables feather δ^{2}H and δ^{13}C gave only few core assignments for the clusters 2 and 3 (Fig. 2, App. 1), group comparisons of hydrogen and carbon stable isotope ratios showed that the method is nonetheless powerful to separate groups that differ in their isotopic signature. Especially in the case of δ^{2}H the three defined clusters diverged significantly in both age and sex classes. Therefore, it can be considered that the specific clusters indicate groups of different geographic provenance. Cluster 1 was least depleted in deuterium and represents the local birds. When exclusively looking at core assignments (Fig. 3), cluster 1 was the only group present during the breeding season (summer). Moreover, individuals assigned to cluster 1 were encountered throughout of the whole year, indicating that at least part of the local breeding population stays resident year-round at the breeding site. A first autumn migration wave additionally brought individuals from cluster 2 and cluster 3, while cluster 2 in comparison to cluster 3 reached a higher relative abundance within the autumn Reed Bunting assemblage. These findings indicate that first migrants in autumn arrive from comparatively short distances, which corresponds to specifications on the Hungarian Reed Bunting population provided by Csörgő et al. (2009). The authors further noted that at this time, exchange can also be observed between neighbouring populations. Later, during winter, observations from cluster 3, which comprises individuals with lowest feather δ^{2}H ratios (furthest north), became relatively more abundant. Feather δ^{2}H values were most useful for the differentiation of Reed Bunting clusters.

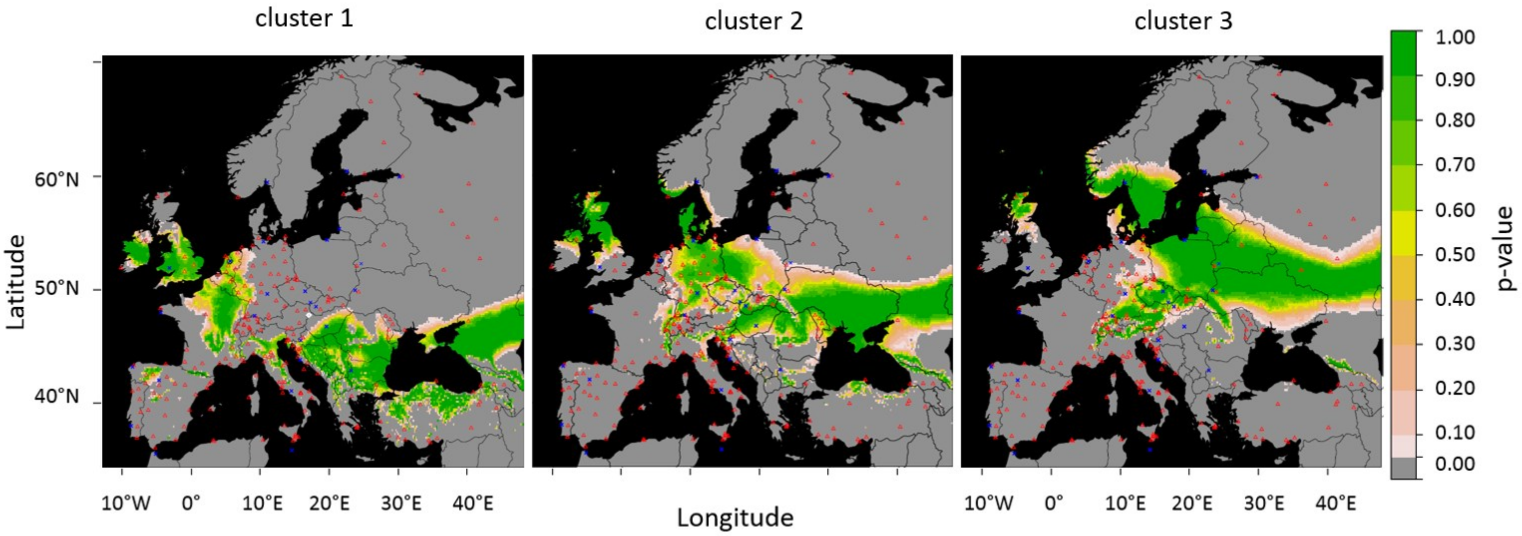


Fig. 12: Geographic assignment of core individuals of each cluster (red triangles indicate sampling locations of the sources; blue crosses indicate the sampling locations of the calibration samples; white diamond indicates the sample location of the assignment samples), significant p-values are strong evidence that the sample does NOT come from the candidate location.

The core assigned individuals of each cluster were subjected to geographic assignment and the resulting probability maps were considered to reflect the clusters' geographic origin (Fig. 12).

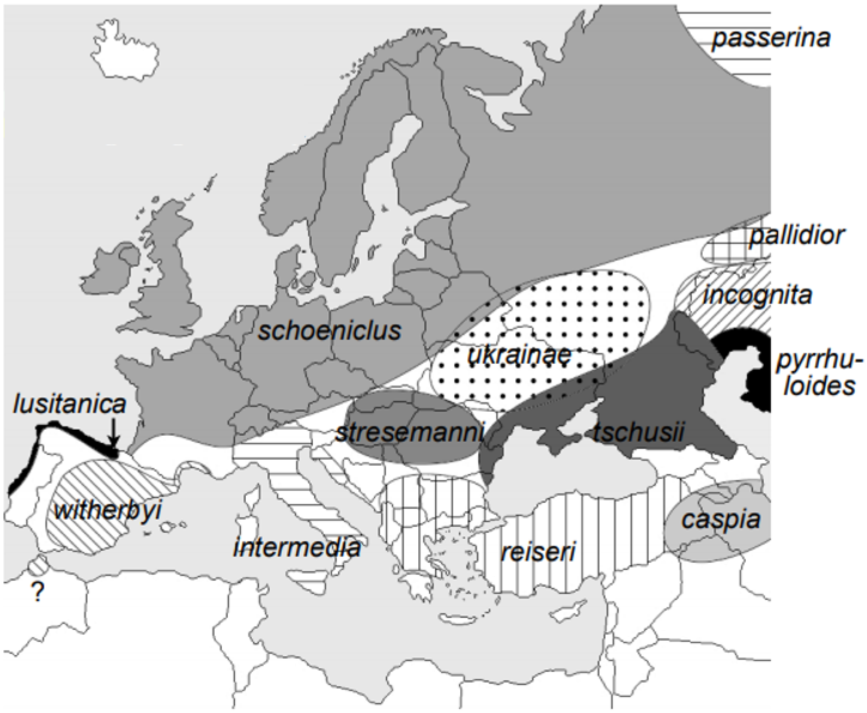


Fig. 13: Approximate breeding range of *Emberiza schoeniclus* subspecies (Demongin 2016).

Considering published information on the approximate geographic breeding range of *Emberiza schoeniclus* subspecies (Cramp and Perrins 1994; Glutz von Blotzheim and Bauer 1997, Demongin 2016) and variation in bill depth of core assigned observations of the different clusters (cluster 1: 4.8-6.7, cluster 2: 4.7-5.9, cluster 3: 4.6-5.6) indicates that cluster 1 represents individuals of the subspecies *E. s stresemanni*, cluster 2 the subspecies *E. s. ukrainae*, and cluster 3 could be assigned to *E. s. schoeniclus*. However, the methodological approach of this study is not suitable for unambiguously identifying subspecies and thus clusters cannot be accurately assigned to subspecies.

Interestingly, adult males that overwintered at Lake Neusiedl were only assigned to cluster 1 and 2. No adult males that were caught during that time of the year were assigned to cluster 3. In contrast, adult females which spent their winter in the study area were only assigned to cluster 2 and 3 and no adult female of the local breeding population was observed within the local Reed Bunting assemblage during winter. This pattern could simply be an artefact of overall low numbers of adults that were caught during winter and assigned to the cluster cores. Another possible explanation could be differential age and sex specific wintering distributions among individuals of the species. In birds, spatial segregation during the non-breeding period is a common phenomenon, in which distances travelled differ between portions of a population (Arizaga et al. 2015; Cristol et al. 1999). By spending the non-breeding season in separate areas, different members of a population may face varying factors that potentially affect survivorship (e.g. interspecific competition, predation, harsh weather conditions, and food availability) and/ or sexual selection (e.g. benefits of earlier arrival at breeding areas, mate choice at non-breeding areas) (Cristol et al. 1999). For Reed Buntings that spend the winter on the Iberian Peninsula, an increase in the proportion of males close to obligate migratory pathways was found (Arizaga et al. 2015). This pattern is proposed to be associated with the advantage to males that overwinter as close as possible to breeding quarters (Arizaga et al. 2015). However, untangling the hypotheses for age or sex-ratio bias during the non-breeding season in songbirds is difficult as predictions tend to overlap (Macdonald et al. 2015). Larger birds are considered to tolerate cold weather conditions better (body size hypothesis) (Ketterson and Nolan Jr 1976), while also being socially dominant (social dominance hypothesis) (Gauthreaux Jr 1978; Macdonald et al. 2015). The coldest wintering sites are also usually closer to the breeding grounds of northern migrants, which makes it difficult to determine the relevant factors for differential wintering distribution of passerines (arrival time hypothesis, body size hypothesis) (Ketterson and Nolan Jr 1976; Cristol et al. 1999; Macdonald et al. 2015). Irrespective of possible reasons for sex or age dependent intraspecific migration patterns, differential migration can have considerable implications for the conservation of migratory birds (Cristol et al. 1999).

While in Europe precipitation δ^2H showed clear gradients with increasingly negative values towards the northeast (Bowen et al. 2005; Brattström et al. 2008), European precipitation isoscapes generally appear to be more complex compared to North America. Moreover, isotope studies on migrating animals originating from Europe still remain few (Brattström et al. 2008). Stable carbon isotope ratios in animal tissues closely reflect those of their diet (Körner 1991; Hobson 1999; Rubenstein and Hobson 2004; Brattström 2008) and undergo fairly limited changes (generally increase by 0-1‰) between trophic levels or from diet to consumers (Inger and Bearhop 2008). More importantly, δ^{13}C values of primary producers tend to respond to a number of environmental variables and photosynthetic processes (Inger and Bearhop 2008). Among terrestrial plants, C3, C4 and CAM plants show divergent δ^{13}C ratios because of their different primary carboxylases (Lajtha and Marshall 1994; Inger and Bearhop 2008, Hobson 2019). Moreover, several studies found less negative δ^{13}C values of C3 plants with increasing latitude and altitude (e.g. Hobson and Wassenaar 1999; Staddon 2004, Brattström 2008). Less discrimination against ^{13}C was observed in plant populations adapted to drier and warmer habitats (Lajtha and Marshall 1994). Cluster 1 differed in their feather $\delta^{13}C$ significantly from the other two clusters. This variation in $\delta^{13}C$ could be linked to the different geographic origin, to differential habitat use and/or an altered preference of food sources among clusters during feather growth. Although a positive relationship between $\delta^{2}H$ and $\delta^{13}C$ signatures of Reed Bunting feathers was found and cluster 1, which showed the southernmost breeding distribution, was least depleted in ^{13}C, further investigations that include measurements on potential food sources and foraging ecology during feather growth are clearly required to shed more light onto this pattern.

Reed Buntings that belonged to different clusters did not only show differences in their stable isotope ratios but also in their morphological traits, such as in bill depth, bill shape and wing shape. The local breeding population differed in bill depth and bill shape from northern migrants and wing shape variation was most remarkable between cluster 2 and the other two clusters.

It is well-known that morphological traits such as bill, wing and tarsus length are shaped by the behaviour and the ecological niche of a species, allowing to make inferences about these niches (Dawideit et al. 2009). High polymorphism in beak size is rather unusual in passerine birds but exceptions do exist, e.g. in Darwin's finches from the Galapagos archipelago (Grant 1986; Grapputo et al. 1998). Bill shape variation is expected to be subject to strong natural selection for feeding (e.g. Grant 1985, Dawideit et al. 2009) and can further be linked to climatic conditions, to sexual selection or to biogeographic patterns (Neto et al. 2016). While Reed Buntings are mainly insectivorous during spring and summer, during winter thin-billed birds feed almost exclusively on small seeds, whereas thick-billed subspecies seem to feed on dormant insects located inside the reed stems (Neto et al. 2016). For instance, in the western Palearctic, the southern, resident subspecies *E. s. intermedia* breaks reed stems and extracts insect larvae (e.g. Stegmann (1948) cited by (Prŷs-Jones 1984), (Matessi et al. 2002; Neto et al. 2013; Orlowski et al. 2013). This behaviour has also been documented in *E. s. pyrrhuloides*, in the west-Mediterranean subspecies *E. s. witherby* and possibly in *E. s. stresemanni* at Lake Neusiedl (Glutz von Blotzheim and Bauer 1997). Neto et al. (2016) proposed that differences in bill size and shape between northern migratory and southern resident subspecies in Spain are a consequence of natural selection through competition during the winter. They suggested for the *Emberiza schoeniclus* subspecies to be in an early stage of speciation. Similarly, the Reed Bunting community at Lake Neusiedl, where birds of different geographic origin mix, could circumvent intraspecific competition through the use of different food sources and habitats, which is probably the main driver for beak shape divergence among clusters.

This is substantiated by the fact that birds belonging to the local breeding population, during winter, were exclusively encountered in the reed bed of Lake Neusiedl, whereas Reed Buntings that were assigned to cluster 2 and 3 used both habitats, the large reed stand as well as more open areas (Fig. 11). Also, birds at the salt pan had significantly thinner beaks, and lower δ^2H and δ^{13}C values than those caught in the reed (Fig. 9, Tab. 4). These results show that there is considerable ecological differentiation between the clusters, and indicate higher specialization and local adaption in the local breeding population compared to migrants coming from breeding areas further north. Additionally, a sexual dimorphism in bill depth was found, which could not be explained by body size variation, while age had an effect on beak shape. It is possible that bill size as well as bill shape differences between sex and age categories are a consequence of different food preferences driven by competition (Radford and du Plessis 2003; Neto et al. 2016).

Adaptations to ecological contexts have also been noted for bird wings, whose morphology and function may be shaped in part by natural or sexual selection (Vanhooydonck et al. 2009). In this study individuals of cluster 2 had the most rounded wings and again sexual dimorphism was found. The development of short and rounded wings is reported to enhance manoeuvrability (Kaboli et al. 2007) and manoeuvrability and wing loading could correspond with foraging behaviour, microhabitat use and sexual display (Vanhooydonck et al. 2009). Also, because escape speed and angle is associated with low wing loadings, less pointed wings could be an adaption to high ground predation rates (Burns and Ydenberg 2002; Swaddle and Lockwood 2003, Vanhooydonck et al. 2009). However, in view of sexual differences, the pattern found appears to be more complicated. Related ecomorphological associations, which could involve habitat use or sexual selection (Swaddle and Lockwood 2003), cannot be further discussed at this point, because of lack of detailed information on effects of environmental and ecological conditions on behavioural properties of northern clusters (2 and 3) at their home range. This also applies to the variation found in body size. Reed Buntings in cluster 2 were the smallest, but again not only sex classes differed in body size, they also showed differences when clusters were compared in males and females separately. In this context, apparently not enough detail is provided by a single-site study like this and the present data therefore do not enable conclusive interpretation of the mentioned patterns.

7) CONCLUSION

In conclusion, the present findings provide valuable insights into the phenology, population structure and ecology of the local Reed Bunting community at Lake Neusiedl, giving evidence that the region is not important to the local breeding population alone. A mixture of European populations can be encountered during migration and during winter. This emphasises the importance of the area on a much bigger geographic scale. Moreover, a variety of habitats is used by northern migrants spending their winter in the region, whereas the local breeding population is more specialized and adapted to the reed bed at the lake. This implies that conservation measures must protect and restore landscape diversity to provide sufficient resources that allow co-existence of populations that mix during the non-breeding season.

cluster	sex	age	season	core	halo	all		1st-year	adult	
1	1	1st-year	spring	4	0	4	43	male	male	13
2	1	1st-year	spring	0	1	1	43	female	female	11
3	1	1st-year	spring	0	1	1	6	6		
1	1	adult	spring	1	0	1				
2	1	adult	spring	0	0	0				
3	1	adult	spring	0	0	0	1		1	
1	2	1st-year	spring	1	0	1				
2	2	1st-year	spring	0	2	2				
3	2	1st-year	spring	0	1	1	4	4		
1	2	adult	spring	0	0	0				
2	2	adult	spring	1	0	1				
3	2	adult	spring	0	1	1	2		2	
1	1	1st-year	summer	0	0	0				
2	1	1st-year	summer	0	0	0				
3	1	1st-year	summer	0	1	1	1	1		
1	1	adult	summer	2	0	2				
2	1	adult	summer	0	0	0				
3	1	adult	summer	0	0	0	2		2	
1	2	1st-year	summer	0	0	0				
2	2	1st-year	summer	0	2	2				
3	2	1st-year	summer	0	0	0	2	2		
1	2	adult	summer	2	0	2				
2	2	adult	summer	0	1	1				
3	2	adult	summer	0	0	0	3		3	
1	1	1st-year	autumn	3	0	3				
2	1	1st-year	autumn	2	4	6				
3	1	1st-year	autumn	0	1	1	10	10		
1	1	adult	autumn	3	0	3				
2	1	adult	autumn	1	1	2				
3	1	adult	autumn	0	0	0	5		5	
1	2	1st-year	autumn	0	0	0				
2	2	1st-year	autumn	2	2	4				
3	2	1st-year	autumn	1	1	2	6	6		
1	2	adult	autumn	0	0	0				
2	2	adult	autumn	0	1	1				
3	2	adult	autumn	0	0	0	1		1	
1	1	1st-year	winter	7	0	7				
2	1	1st-year	winter	8	5	13				
3	1	1st-year	winter	1	5	6	26	26		
1	1	adult	winter	3	0	3				
2	1	adult	winter	2	0	2				
3	1	adult	winter	0	0	0	5		5	
1	2	1st-year	winter	6	0	6				
2	2	1st-year	winter	3	11	14				
3	2	1st-year	winter	5	6	11	31	31		
1	2	adult	winter	1	0	1				
2	2	adult	winter	1	1	2				
3	2	adult	winter	0	2	2	5		5	
				60	**50**	**110**	**110**	**86**	**24**	

App. 1: Table of cluster assignments of Reed Bunting individuals.

8) REFERENCES

Adams DC, Collyer ML, Kaliontzopoulou A (2018) Geomorph: Software for geometric morphometric analyses. R package version 3.0.6. https://cran.r-project.org/package=geomorph

Arizaga J, Campos F, Alonso D (2006) Variations in wing morphology among subspecies might reflect different migration distances in Bluethroat. Ornis Fennica 83:162-169

Arizaga J, Alonso D, Cortés JA, Eggenhuizen T, Foucher J, Franz D, García J, Koning F, Leconte M, Rguibi H, Valkenburg T, Vera P, Hobson KA. (2015) Migratory Connectivity in European Bird Populations: Feather Stable Isotope Values Correlate with Biometrics of Breeding and Wintering Bluethroats *Luscinia svecica*. Ardeola 62:255-267 doi:10.13157/arla.62.2.2015.255

Bauer K, Freundl H, Lugitsch R (1955) Weitere Beiträge zur Kenntnis der Vogelwelt des Neusiedlersee Gebietes. Wiss Arb Bgld 7:1-123

Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society. Series B (Methodological). 57:289–300

Berthold P (1993) Bird Migration. A General Survey. Oxford University Press, New York

Bookstein FL; Chernoff B, Elder R, Humphries J, Smith G, Strauss R (1985) Morphometrics in Evolutionary Biology. Academy of Natural Sciences, Philadelphia, PA

Bookstein FL (1991) Morphometric tools for landmark data; geometry and biology (Cambridge, UK: Cambridge University Press, /New York/Port Chester/Melbourne/Sydney)

Bookstein FL (1997) Morphometric Tools for Landmark Data: Geometry and Biology. Cambridge University Press, New York

Bookstein FL (1996) Combining the tools of geometric morphometrics. In: Advances in Morphometrics (eds. Marcus LF, Corti M, Loy A, Naylor G, Slice DE), pp. 131–151. Plenum Press, New York

Bowen GJ, Wassenaar LI, Hobson KA (2005) Global applications of stable hydrogen and oxygen isotopes to wildlife forensics. Oecologia 143:337-348 doi 10.1007/s00442-004-1813-y

Bowen GJ, West JB (2019) Isoscapes for Terrestrial Migration Research. In: Tracking animal migration with stable isotopes - Second Edition (eds. Hobson KA, Wassenaar LI) London: Academic Press

Brattström O, Wassenaar LI, Hobsin KA, Åkesson S (2008) Placing butterflies on the map - testing regional geographic resolution of three stable isotopes in Sweden using the monophagus peacock *Onachis io*. Ecography 31:490-498. doi: 10.1111/j.2008.0906-7590.05267.x

Briedis M, Beran V, Hahn S, Adamík P (2016) Annual cycle and migration strategies of a habitat specialist, the Tawny Pipit *Anthus campestris*, revealed by geolocators. J Ornithol 157:619-626 doi:10.1007/s10336-015-1313-3

Burns JG, Ydenberg RC (2002) The effects of wing loading and gender on the escape flights of least sandpipers (*Calidris minutilla*) and western sandpipers (*Calidris mauri*). Behavioral Ecology and Sociobiology 52: 128–136

Chabot AA, Hobson KA, Van Wilgenburg SL, McQuat GJ, Lougheed SC (2012) Advances in linking wintering migrant birds to their breeding-ground origins using combined analyses of genetic and stable isotope markers. PLoS ONE 7:e43627 doi:10.1371/journal.pone.0043627

Clegg SM, Kelly JF, Kimura M, Smith TB (2003) Combining genetic markers and stable isotopes to reveal population connectivity and migration patterns in a Neotropical migrant, Wilson's warbler (*Wilsonia pusilla*). Mol Ecol 12:819–830

Courtiol A, Rousset F, Rohwäder M, Soto DX, Lehnert L, Voigt CC, Hobson KA, Wassenaar LI, Kramer-Schadt S (2019) Isoscape computation and inference of spatial origins with mixed models using the R package IsoriX. In: Tracking animal migration with stable isotopes - Second Edition (eds. Hobson KA, Wassenaar LI) London: Academic Press

Cramp S, Perrins CM (1994) Handbook of the birds of Europe, the Middle East and North Africa. Oxford University Press, Oxford 9

Cristol DA, Baker MB, Carbone C (1999) Differential Migration Revisited: Latitudinal Segregation by Age and Sex Class. In: Current ornithology (eds. Nolan VJ, Ketterson ED, Thompson CF) Vol 15 Academic, New York 15:33-88

Csörgő T et al. (2009) Magyar madárvonulási atlasz. Kossuth kiadó, Budapest: 635-637

Dawideit BA, Phillimore AB, Laube I, Leisler B, Böhning-Gaese K (2009) Ecomorphological predictores of natal disperal distances in birds. J. Animal Ecol. 78:388-395 doi: 10.1111/j.1365-2656.2008.01504.x

Demongin L (2016) Identification Guide to Birds in the Hand. Beauregard-Vernon, p. 266

Dornberger W (1979) Zur Biometrie der Rohrammer (*Emberiza schoenidus*) am Neusiedler See und Koronia See. Vogelwarte 30:28-32

Dvorak M, Ranner A, Berg H-M (1993) Atlas der Brutvögel Österreichs. Ergebnisse der Brutvogelkartierung 1981 - 1985 der Österreichischen Gesellschaft für Vogelkunde. Umweltbundesamt, Wien

Foster DJ, Podos J, Hendry AP (2008) A geometric morphometric appraisal of beak shape in Darwin's finches. J. Evol. Biol. 21:263-275

Fox J, Weisberg S (2011) An {R} Companion to Applied Regression, Second Edition. Thousand Oaks CA: Sage. URL: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Gauthreaux Jr SA (1978) The ecological significance of behavioural dominance. – In: Perspectives in ethology (eds. Bateson PPG, Kopfer PH). Plenum Press, pp. 17 – 54

Glutz von Blotzheim U, Bauer KM (1997) Handbuch der Vögel Mitteleuropas. Aula, Wiesbaden

Grant PR (1985) Selection on bill characters in a population of Darwin's finches: *Geospiza conirostris* on Isla Genovesa, Galapagos. Evolution, 39, 523–532

Grant PR (1986) The ecology, evolution and behaviour of Darwin's Finches. Princeton University Press

Grapputo A, Pilastro A, Marin G (1998) Genetic variation and bill size dimorphism in a passerine bird, the reed bunting *Emberiza schoeniclus*. Mol Ecol 7:1173-1182

Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120:314-326. doi:10.1007/s004420050865

Hobson KA (2005) Using stable isotopes to trace long-distance dispersal in birds and other taxa. Divers Distrib 11:157-164. doi:10.1111/j.1366-9516.2005.00149.x

Hobson KA (2019) Application of Isotopic Methods to Tracking Animal Movements. In: Tracking animal migration with stable isotopes - Second Edition (eds. Hobson KA, Wassenaar LI). London: Academic Press

Hobson KA, Bowen GJ, Wassenaar LI, Ferrand Y, Lormee H (2004) Using stable hydrogen and oxygen isotope measurements of feathers to infer geographical origins of migrating European birds. Oecologia 141:477-488. doi:10.1007/s00442-004-1671-7

Hobson KA, Norris DR, Kardynall KJ, Yohannes E (2019) Animal Migration: A Context for Using New Techniques and Approaches. In: Tracking animal migration with stable isotopes - Second Edition (eds. Hobson KA, Wassenaar LI) London: Academic Press

Hobson KA, Wassenaar LI (1997) Linking Breeding and Wintering Grounds of Neotropical Migrant Songbirds Using Stable Hydrogen Isotopic Analysis of Feathers. Oecologia 109:142-148

Hobson KA, Wassenaar LI (1999) Stable isotope (δD and δ ^{13}C) are geographic indicators of natal origins of monarch butterflies in eastern North America. Oecologia 120:398-404

Inger R, Bearhop S (2008) Applications of stable isotope analysis to avial ecology. Ibis 150:447-461

IPBES (2019): Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. IPBES Secretariat, Bonn.

Jenni LW, Winkler R (1994) Moult and ageing of European Passerines. Academic Press, London

Jenni LW, Winkler R (2007) Alters- und Geschlechtsbestimmung europäischer Singvögel. Schweizerische Vogelwarte Sempbach

Kaiser A (1993) A new multi-category classification of subcutaneous fat depositis of songbirds. J. Field Ornithol. 64:246-255

Kassambara A (2019) ggpubr: 'ggplot2' Based Publication Ready Plots. R package version 0.2.3. https://CRAN.R-project.org/package=ggpubr

Ketterson ED, Nolan Jr V (1976) Geographic variation and its climate correlates in the sex ratio of eastern-wintering darkeyed juncos (*Junco hyemalis hyemali*s). Ecology 57: 679 – 693

Klingenberg CP (1996) Multivariate allometry. In: Advances in Morphometrics (eds. Marcus lF, Corti M, Loy A, Naylor G, Slice DE), pp. 23–49. Plenum Press, New York

Körner C, Farquhar GD, Wong SC (1991) Carbon isotope discrimination by plants follows latitudinal and altitudinal trends. Oecologia 88:3

Kravchenko KA, Lehnert LS, Vlaschenko AS, Voigt CC (2019) Multiple isotope tracers in fur keratin discriminate between mothers and offspring. Rapid communications in Mass Spectrometry 33:907-913.

Lajtha K, Marshall JD (1994) Sources of variation in the stable isotopic composition of plants. In: Stable isotopes in ecology and environmental science (eds. Lajtha K, Michener RH). Blackwell, pp. 1-21

Lockwood R, Swaddle JP, Rayner JMV (1998) Avian wingtip shape reconsidered: wing tip shape indices and morphological adaptions to migration. J Avian Biol 29:273-292

Macdonald CA, McKinnon EA, Gilchrist HG, Love OP (2015) Cold tolerance, and not earlier arrival on breeding grounds, explains why males winter further north in an Arctic-breeding songbird. J Avian Biol 46:001-009

Marra PP, Hobson KA, Holmes RT (1998) Linking winter and summer events in a migratory bird by using stable-carbon isotopes. Science 282:1884-1886. DOI 10.1126/science.282.5395.1884

Matessi G, Griggio M, Pilastro A (2002) The geographical distribution of populations of the large-billed subspecies of reed bunting matches that of its main winter food. Biol J Linn Soc 75:21-26. DOI 10.1046/j.1095-8312.2002.00003.x

Mitteroecker P, Gunz P (2009) Advances in Geometric Morphometrics. Evol Biol 36:235. https://doi.org/10.1007/s11692-009-9055-x

Neto JM, Gordinho L, Belda EJ, Marin M, Monros JS, Fearon P, Crates R (2013) Phenotypic divergence among west European populations of Reed Bunting *Emberiza schoeniclus*: the effects of migratory and foraging behaviours. PLoS ONE 8:e63248. doi:10.1371/journal.pone.0063248

Neto JM, Gordinho LD, Vollot B, Marin M, Monros JS, Newton J (2016) Stable isotopes reveal differences in diet among reed bunting subspecies that vary in bill size. J Avian Biol 48:284-294. doi:10.1111/jav.01069

Nowakowski JN, Chruściel J (2008) An index to estimate the wing area in small Passerine, using the Blue tit (Cyanistes caeruleus) as a case study. The Ring 30 1/2: 19-30

Orlowski G, Karg J, Czarnecka J (2013) Substantial contribution of invertebrates to the diet of a winter seed-eater, the reed bunting *Emberiza schoeniclus*, wintering in a sewage farm in south-western Poland. Biol J Linn Soc 108:429-433. doi:10.1111/j.1095-8312.2012.02026.x

Oschadleus D (2012) Measuring Birds – Vögel vermessen. By Siegfried Eck, Jürgen Fiebig, Wolfgang Fiedler, Iris Heynen, Bernd Nicolai, Till Töpfer, Renate van den Elzen, Raffael Winkler and Friederike Woog, Ostrich 83(2):117. DOI:10.2989/00306525.2012.703802

Pasinelli G, Schiegg K (2012) Die Bedeutung kleiner Feuchtgebiete für den Artenschutz: Synthese einer Populationsstudie an der Rohrammer *Emberiza schoeniclus*. Der Ornithologische Beobachter 109:201-220

Pedersen TL, Hughes S, Qiu X (2017) densityClust: Clustering by Fast Search and Find of Density Peaks. R package version 0.3. https://CRAN.R-project.org/package=densityClust

Pérez-Tris J, Telleria JL (2001) Age-related variation in wing shape of migratory and sedentary Blackcaps *Sylvia atricapilla*. Journal of Avian Biology 32:207-213

Popa-Lisseanu AG, Sörgel K, Luckner A, et al. (2012) A triple-isotope approach to predict the breeding origins of European bats. PLoS ONE 7(1): e30388. https://doi-org.uaccess.univie.ac.at/10.1371/journal.pone.0030388

Prŷs-Jones RP (1984) Migration patterns of the reed bunting, *Emberiza schoeniclus schoeniclus*, and the dependence of wintering distribution on environmental conditions. Gerfaut 74:15–37

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Radford AN, du Plessis MA (2003) Bill dimorphism and foraging niche partitioning in the green woodhoopoe. J. Anim. Ecol. 72: 258 – 269

Rodriguez A, Laio A (2014) Clustering by fast search and find of density peaks. Science, 344:1492-1496. doi:10.1126/science.1242072

Rohlf FJ (1993) Relative warp analysis and an example of its application to mosquitos. Contributions to morphometrics 8, 131.

Rohlf FJ (2016a) TpsUtil, Version 1.74. StonyBrook: Department of Ecology and Evolution, State University of New York. http://life.bio.sunysb.edu/morph/index.html

Rohlf FJ (2016b) TpsDig2, Version 2.3. StonyBrook: Department of Ecology and Evolution, State University of New York. http://life.bio.sunysb.edu/morph/index.html

Rubenstein DR, Hobson KA (2004) From birds to butterfies: animal movement patterns and stable isotopes. Trends Ecol. Evol. 19:256-263

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671-675

Shirihai H, Svensson L (2018) Handbook of Western Palearctic Birds, Volume 2: Passerines: Flycatchers to Buntings. Bloomsbury Academic

Staddon PL (2004) Carbon isotopes in functional soil ecology. Trends Ecol. Evol. 19: 148-154

Stegmann B (1948) Functional significance of subspecific characters among Reed Buntings

(*Emberiza schoeniclus* L.). Zoologische Zhurnal 27:241–244

Swaddle JP, Lockwood R (2003) Wingtip shape and flight performance in the European starling *Sturnus vulgaris*. Ibis 145: 457–464

Vanhooydonck B, Herrel A, Gabela A, Podos J (2009) Wing shape variation inthe medium ground finch (*Geospiza fortis*): an ecomorphological approach. Biol J Linn Soc 98:129-138

Vaurie C (1956) Systematic notes on Palearctic birds. N. 22 Fringilidae: *Emberiza schoeniclus*. American Museum Novitates 1795:1–13

Vaurie C (1958) Systematic notes on Palearctic birds. N. 33. A second review of *Carpodacus puniceus*, with a study of its plumages, and a supplementary note on *Emberiza schoeniclus*. Am Mus Novit 1898:1-10

Veen T, Hjernquist MB, Van Wilgenburg SL, Hobson KA, Folmer E, Font L, Klaassen M (2014) Identifying the African Wintering Grounds of Hybrid Flycatchers using a Multi-Isotope (δ^{2}H, δ^{13}C, δ^{15}N) Assignment Approach. PLoS ONE 9:e98075 doi:10.1371/journal.pone.0098075

Voigt CC, Lehnert LS (2019) Tracking of movements of terrestrial mammals using stable isotopes. In: Tracking animal migration with stable isotopes - Second Edition (eds. Hobson KA, Wassenaar LI) London: Academic Press

Wassenaar LI (2019) Introduction to Conducting Stable Isotope Measurements for Animal Migration Studies. In: Tracking animal migration with stable isotopes - Second Edition (eds. Hobson KA, Wassenaar LI) London: Academic Press

Wassenaar LI, Hobson KA (2003) Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. Isot Environ Health Stud 39: 211–217

Webster MS, Marra PP (2005) The importance of understanding migratory connectivity and seasonal interactions. In: Birds of two worlds (Greenberg R, Marra PP). John Hopkins University Press, Baltimore, Maryland,USA.

Webster MS, Marra PP, Haig SM, Bensch S, Holmes RT (2002) Links between worlds: unraveling migratory connectivity. Trends Ecol Evol 17:76-83

Woodworth BK et al. (2016) Differential migration and the link between winter latitude, timing of migration, and breeding in a songbird. Oecologia 181:413-422. doi:10.1007/s00442-015-3527-8

Zelditch ML, Swiderski DL,Sheets HD, Fink WL (2004). Geometric Morphometrics for Biologists: A Primer. Elsevier Academic Press, London.