Origin identification of migratory pests (European Starling) using geochemical fingerprinting (#41793)

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Origin identification of migratory pests (European Starling) using geochemical fingerprinting

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The European Starling (EUS(Sturnidae: *Sturnus vulgaris* L.) is an invasive bird in North America where it is an agricultural pest. In British Columbia (Canada), the starling population increases in orchards and vineyards in autumn, where they consume and damage ripening fruits. Starlings also create damage in dairyfarms and feedlots by consuming and contaminating food, and spreading diseases. Damage can be partly mitigated by the use of scare deterrents, which only can divert flocks until they become acclimated. Large-scale trapping and euthanizing before they move to fields and farms is the most practical means of preventing damage, but requires knowledge of natal origin.

Within a small (20,831 km^2), agriculturally significant portion of south-central British Columbia, the Okanagan Valley, we used 21 trace elements in bone tissue to discriminate the spatial distribution of juvenile starling and to reveal the geographic origin of the problem birds. Stepwise discriminant analysis of trace elements classified juveniles 79 % accurately to their natal origin, with minimum discrimination distance of 12 km. The majority of problem birds (55%) caught in vineyards and orchards, mainly in the southern portion of the valley were derived from the northern portion of the valley. In contrast, only 11% of problem birds caught at dairy farms and feedlots were local and most (89%) were likely from outside of the region. Moreover, elemental signatures can separate starling populations with a high degree of spatial accuracy, yielding a promising tool for identifying the geographic origin of migratory birds even over small geographic scales.

ORIGIN IDENTIFICATION OF MIGRATORY PESTS (EUROPEAN STARLING) USING GEOCHEMICAL FINGERPRINTING

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Abstract

The European Starling (EUST) (Sturnidae: *Sturnus vulgaris* L.) is an invasive bird in North America where it is an agricultural pest. In British Columbia (Canada), the starling population increases in orchards and vineyards in autumn, where they consume and damage ripening fruits. Starlings also create damage in dairy farms and feedlots by consuming and contaminating food, and spreading diseases. Damage can be partly mitigated by the use of scare deterrents, which only can divert flocks until they become acclimated. Large-scale trapping and euthanizing before they move to fields and farms is the most practical means of preventing damage, but requires knowledge of natal origin.

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Introduction

European starling (EUST, *Sturnus vulgaris* L.) is one of the most successful non-native species in North America (Vuilleumier 2009). According to the International Union for Conservation of Nature, its conservation status is considered to be of least concern. It is considered as an agricultural pest (BCGA 2012), especially in fruit crops such as cherries, berries, and grapes (Pimentel et al. 2005, BCGA 2013). Starlings are a highly successful pest species for five main reasons. First, they are very adaptive in nature and omnivorous in feeding habit (dietary generalist) (Cabe 1993) which makes them resilient and adaptive to extreme and diverse habitat conditions. Second, they brood several times in a year (Dunnet 1955; Tinbergen 1981) with a 3-6 egg clutch size with 48-79% success (Linz et al. 2007). Third, they lay eggs in other birds nests and intraspecific brood parasitism is common in starlings, which increases the success rate and also the number of chicks that fledge (Kennedy and Harry 1999). Fourth, natural predators, parasites and diseases are not sufficiently inhibitory to control the population. Finally, they migrate individually and in huge flocks for food and survival. All these factors together make starlings very resilient, adaptive generalist birds. They maintain a population size of over 200 million in North America.

Starling cause significant financial losses for fruit growers and dairy farmers in the Okanagan Valley. The Okanagan Valley is a nationally significant agricultural region due to climatic and geological suitability. The valley is ideal for several tree fruits such as apples, peaches, and other soft fruits, like cherries, berries, and grapes, and for livestock operations including both dairy farms and beef cattle feedlots. For example, around 97.5 % of BC's vineyard and 98% of the province's apples are grown in the Okanagan-Similkameen region (Neuhauser 2013;BC Growers 2015). The orchards and vineyards are predominantly in the

44 southern portion of the valley, and dairy farm and feedlots in the northern portion of the valley.
45 The cold short winters, warm summers, rural/urban interface, and availability of year-round food
46 sources in the Okanagan Valley provide excellent starling habitat. Large flocks destroy fruits in
47 the vineyards, orchards and dairy farms in the Okanagan Valley. Annually, over $4 million
48 worth of crops are damaged in the Okanagan region alone and $800 million worth of agricultural
49 crops are damaged in North America (BCGA 2013).
50 Various types of eradication techniques such as falconry, noise deterrents (propane
51 cannons), electronic distress calls, bird netting, and visual repellents have been applied but none
52 of these techniques has proven effective (BCGA 2008). In 2003 a Starling Control Program
53 (SCP) was initiated in the Okanagan region of British Columbia, Canada to control its population
54 and subsequent damage. Agricultural industries, environmental agencies, and local governments
55 supported the program. The SCP traps birds in various locations in the Okanagan Valley
56 throughout the year. From 2003 to 2013 around 544,000 birds were trapped by the control
57 program (OSSCP 2014). Although the control program has been trying to reduce the starling
58 population by aggressively trapping all year round, numbers appear to be increasing, especially
59 in the fall season due to migration and natal dispersal. This is possibly because the origin of these
60 pest birds was unknown and none of the applied techniques addressed the problem at the source.
61 It is necessary to find the origin of these migrant birds to enhance program effectiveness. Thus,
62 understanding the natal origin and movement pattern of migratory pests, like starlings, is
63 important to develop successful management (Cabe 1993; Neuhauser 2013). The main objective
64 of this research is to identify the origin of the migratory starlings using a geochemical (trace
65 element) fingerprinting approach in the Okanagan-Similkameen region so that starling might be
66 managed at sources.

Biogeochemical markers such as stable isotopes (Hobson 1999, 2005a, 2005b; Szép et al. 2009) and trace elements analyses (Szep et al. 2003; Szép et al. 2009) are the potential methods to trace the origin and migration of such birds because the markers are indicators of the environment of origin

Starlings can disperse long distances and retain stable isotopes and trace element signatures in tissues reflecting natal food (Hobson 1999), location, and habitat (Szép et al. 2009). These chemical signatures can be applied as tools to reconstruct animal movement pathways (Ethier et al. 2013). We chose trace elements over light isotope ratios for this study for two reasons. First, isotope ratios in birds can be homogeneous for thousands of kilometres (Inger and Bearhop 2008). Whereas, the province of British Columbia (BC) is geochemically diverse across spatial scales of tens of kilometres or less (Okulitch 2013), making trace elements potentially much more sensitive for tracking regional starling movements (Neuhauser 2013). Second, trace elements can provide a higher specificity than isotopes because of the larger number of potential variables (Szép et al. 2009).

We used samples of starling leg bone tissue, tarsometatarsus, commonly known as tarsus or metatarsus to fingerprint natal origins of individual starlings. Feather tissue is more convenient to use but we chose bone because feathers moult seasonally shedding the evidence of natal origin, whereas the turnover rate of bone tissue is as much as 30 years (Tieszen et al. 1983, Lanocha and Kalisinska 2012). Most bone mass consists of type I collagen and apatite (a mineral composed mainly of calcium and phosphate) (Boonen et al. 2010). Other cations and anions easily substitute into the mineral matrix of apatite. Thus, other elements, derived from the diet can be incorporated into bone to create a fingerprint, representing the food an individual has ingested (Grynpas et al. 1993). The bone mineralization is analogous to the geological mineral

(Boonen et al. 2010) and is known as hydroxyapatite, $Ca_5(PO_4)_3OH$ (Boskey 2007; Pasteris, Wopenka et al. 2008). Finally, an earlier study of six European starling tissues (bone, liver, heart, muscle, brain, and feather) in BC identified bone as the best tissue for tracing bird origins (Neuhauser 2013). Since BC and the Okanagan Valley are geologically heterogeneous, potential starling source populations were identified from different areas in the valley, to compare with problem birds caught in the fall in fruit crops and dairy farms. By fingerprinting starling natal origin, appropriate management to reduce starling populations could be implemented in focused areas to reduce the damage created by the starling.

Materials and Methods

Study Area

British Columbia (BC) is the western-most province of Canada and has high geological diversity in rock types (Canada 2012) (Fig. 1). The Okanagan Valley in south-central British Columbia is about 200 km long and 20 km wide (Fig. 1). It lies in between the Columbia and Cascade Mountain ranges in the southern interior of BC. The valley bedrock is comprised of volcanic, sedimentary, metamorphic and intrusive rocks (Okulitch 2013). The valley is characterized by hot summers and mild winters with an average daily maximum temperature of 27.2 ˚C in July and a minimum average daily temperature of -5.7 ˚C in December and January (Canada 2013). Around 2000 hours of sunlight per year and 250-400 mm of precipitation are received in most of the valley (Marsh 2015). There are several large to small lakes in the valley, which helps to moderate extreme temperatures.

Due to climatic and geological suitability, the Okanagan Valley is an agricultural hub of the Province. Around 96% of BC's soft fruits, apples, and grapes are grown in the south-central

Okanagan, whereas livestock operations such as dairy farms and feedlots, grains and forage crops predominate in the north Okanagan (BC Growers 2015; BCGA 2013). Thus, vineyards and orchards are dominantly located in the southern region; and dairy farms and feedlots are dominantly located in the northern region of the valley.

Sampling

Sampling was performed in collaboration with the Starling Control Program (SCP), British Columbia Grapesgrowers' Association (BCGA). Since 2003 a team of professional trappers contracted by the BCGA, has been trapping and euthanizing starlings following the guidelines of the Canadian Council on Animal Care. We obtained fresh and frozen sample and each bird represents a single sample. Juvenile starlings (n=105) were collected in early summer (May-July) from different locations situated at distances from 12 to 190 km apart throughout the Okanagan Valley in 2015. Age of the birds was determined from their plumage (Szep et al. 2003; Szép et al. 2009). Additionally, aging by plumage was validated by a chronology of skull development (skull ossification) in juveniles. Five randomly selected samples which were considered as juvenile through plumage, were dissected in skull to observe the skulling (Mueller and Weise 1996). Juveniles collected shortly after fledging were considered as source populations because they were collected with little prior chance to disperse beyond their natal habitat. Thus, their bone tissue will reflect the elemental fingerprint of the sites where they were collected. Additionally, adult problem birds (n=118) were collected in the Valley during fall to winter (August-December) 2015.

Sample preparation

Samples were prepared by methods described by Norris et al. (2007) with some modification. Briefly, the bone was cleaned by removing outer skin and marrow. Bones were

washed repeatedly with ultra-pure water (18 MΩ cm^{-1}) (Szep et al. 2003; Norris et al. 2007; Szép et al. 2009), dried at room temperature (20 ˚ C) (Szep et al. 2003; Szép et al. 2009) and weighed (Chamberlain et al. 1997; Norris et al. 2007). The clean fragments of the bone sample, weighing between 30 mg to 80 mg each, were put separately into Teflon tubes (Chamberlain et al. 1997; Norris et al. 2007). Trace element grade concentrated nitric acid (2ml) was added to each tube and placed in a heating block 75˚ C (±5˚ C) (Norris et al. 2007) until the bone was completely dissolved. Samples were then cooled to room temperature. Afterward, one milliliter of trace metal grade hydrogen peroxide was added to further digest the dissolved organic compounds (Norris et al. 2007), after which samples were evaporated on a hot plate at 85˚C (±5˚ C) (Szep et al. 2003; Neuhauser 2013). After cooling, 2 ml of 1 % HNO_3 with 1 ppb indium internal standard was added to each vial to dissolve the residue over a period of 2 hours at room temperature. Samples were transferred quantitatively with three washes of the HNO_3 indium solution into acid-washed high-density polyethylene tubes to a final volume of 10 mL (Norris et al. 2007;Neuhauser 2013). Sample blanks were prepared in the same way for every set of digestions to monitor contamination (Donovan et al. 2006; Norris et al. 2007; Szép et al. 2009). Sub-samples were further diluted 100 fold with 1 ppb indium-1% HNO_3 for ICP-MS analysis and 5 and 10 fold with 1ppm yttrium-1% HNO_3 for ICP-OES analysis. Samples were diluted to fall within the detection limits of the instruments.

Trace element analysis

We measured 21 elements: aluminium (Al), silver (Ag), barium (Ba), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), molybdenum (Mo), manganese (Mn), lead (Pb), sulphur (S), scandium (Sc), selenium (Se), tin (Sn), strontium (Sr), vanadium (V), zinc (Zn), magnesium (Mg), sodium (Na), and potassium (K) in bone tissue. The elements were

158 selected on the basis that they are present at levels high enough for reliable quantification and
159 have been used in fingerprinting various species of birds to identify origin and migratory
160 movements (Szép et al. 2009). Seventeen of the 21 elements were analysed by Thermo-Fisher
161 Element XR sector field Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) (Poesel et al.
162 2008; Neuhauser 2013). A subset of four highly-abundant elements, Ca, Mn, Na, and K, were
163 analysed via a Thermo-Electron Corporation, iCAP 6000 Series XR Inductively Coupled
164 Plasma-Optical Emission Spectrometer (ICP-OES) (Szep et al. 2003; Szép et al. 2009;
165 Neuhauser 2013). For ICP-MS, elements were analysed in either low resolution or medium
166 resolution mode to resolve polyatomic interferences. Both instruments were calibrated by
167 external multi-element standards. Four different concentrations of multi-element standards were
168 run to produce calibration curves (Poesel et al. 2008). Indium (Norris et al. 2007) and yttrium
169 (Neuhauser 2013) were used as an internal standard for ICP-MS and ICP-OES respectively to
170 correct for instrument sensitivity drift, sample density and to improve accuracy and repeatability.
171 The blank and reference solutions were run at every 20-sample interval throughout the analysis.
172 Three replicate analyses were done for each sample. Relative Standard Deviation (RSD) of the
173 analysis was between 1-2%.

174 **Statistical Analysis of Data**

175 The elemental concentration was normalized to the concentration of calcium rather than
176 mass to minimize variability in the ratio of mineral:collagen content. The collagen structure of
177 bone bonds relatively poorly with metals, whereas the mineral apatite substitutes cations and
178 anions relatively easily into its structure. As birds develop, the relative content of apatite
179 increases (Rogers and Zioupos 1999; Currey 2004; Currey et al. 2004; Pasteris et al. 2008).
180 Normalizing with calcium, therefore, removes this variability due to collagen content. After

calcium normalization, the data were then standardized via z-scoring (Equation 2) to prevent individual elements from having a disproportionate influence on the grouping (Fowler et al.1998; Norusis 2016).

Trace Element Multivariate Analysis

A multivariate analysis of variance (MANOVA) was used to analyse trace element profiles of juvenile bone tissue collected in different locations (Norusis 2016). A multiple discriminant analysis (Hair et al. 2010) of juvenile samples was performed to discard elements (variables), which were little related to group distinction, and also to develop the predictive model of group membership based on trace elements (chemical profile of bone). A discriminant function (equation) was developed based on the linear combination of the predictor variables (trace elements) that provides the best discrimination (correctly separating individual birds) among the predetermined groups (Lachenbruch and Goldstein 1979; Greenough et al. 1997; Szép et al. 2009). The predetermined groups were the sites where the birds were caught. The stepwise method and Mahalanobis distance (Hair et al. 2010) were used for the analysis because of the large number of variables (20 different element concentrations).

The stepwise method looks at each element (variable), one at a time, and determines which element is the best predictor of group membership, and ultimately generates the best set of variables to predict group membership. Cross-validation assessed the success of the proper bird groupings via the discriminant function and rules. In order to classify objectively the problem birds with respect to origin, the value obtained from each function for each problem bird was normalized (ratio of sample/ratio of centroid) by the centroid value of each site. Thus, if an individual problem bird were to lie at the centroid for a particular site, its value with respect to

that site would be one. Each bird's probable origin was identified by how closely that bird's normalized value approximated the centroid values of each site. For some of the local problem birds, the discriminant function value was too high to assign a specific juvenile source population. For such birds, we have categorized them as non-specific Okanagan problem birds.

Cluster analysis (Hartigan 1975) was performed to evaluate the spatial separation of the elemental fingerprint of starlings and the identification of likely immigrants. In this study, the ward.D method (Ward 1963; Murtagh and Legendre 2014) with Euclidean distance measures was used for analysis, which provided better separation of individual birds with similar signatures and geographic location. Additionally, an average method with correlation distance was used to compute a test of significance of the clusters (by calculating the p-value of the cluster). The R package "*pvclust*" (Suzuki and Shimodaira 2014) was used; it uses bootstrap resampling techniques to compute the p-value for each cluster. This method generates thousands of bootstrap replications by randomly sampling elements of the data. For each of the clusters, Approximately Unbiased (AU) and Bootstrap Probability (BP) values were calculated. The AU probability values (p-values) are computed by multiscale bootstrap resampling where AU $\geq$ 95 % are considered to be strongly supported by the data, while BP corresponds to the frequency that the cluster is identified in bootstrap copies (Suzuki and Shimodaira 2014).

Statistical analysis was conducted in SPSS ver. 24.0 and R statistical software version 3.3.1 (R Development Core Team n.d.) with the *cluster* (Maechler et al. 2017) *and pvclust* (Suzuki and Shimodaira 2014) packages. Map projections of data were done in ArcGIS 10.4.

Results

Spatial separation of source population of starlings in the Okanagan Valley

The source population trace element fingerprint library was developed from samples from 105 juvenile starling collected from 10 different locations. The number of juvenile samples varied depending on availability at each location, Kelowna (n=20), Hullcar (n=10), Salmon Arm (n=10), Armstrong (n=9), Mara (n=9), Vernon (n=18), Oliver (n=10), Osoyoos (n=8), Penticton (n=6) and Keremeos (n=5) (Table 1).

Multivariate comparison of 20 elements in the juvenile population using MANOVA shows there is a significant difference in the trace elemental composition of juvenile bone among locations (Wilks' λ <0.0001, F=6.655, df= 180, 645.230, power=1, p<0.0001).

A stepwise discriminant analysis of 20 trace elements based on the juveniles (n=105) collected at 10 different locations, provided four canonical discriminant functions with significantly high eigenvalues (>1), and canonical correlations (rc> 0.70), that separated juvenile samples from respective sites (Wilks' λ = 0.001, 0.11, 0.73, χ^2 = 633, 428, 247, p<0.0001) (Fig. 2). Out of 20 elements, 10 elements (K, Mg, Na, Ag, Cd, Ba, Sc, Cr, Cu, and Se) were used as the best predictor variables in discriminating group membership. The probabilities of correctly classifying juveniles to their respective locations using stepwise discriminant analysis are shown in Table 1. The overall probability of correctly classifying juveniles was 79% (P<0.0001, Press's Q; Table 1). The juveniles were grouped 60%-100% correctly, with Osoyoos as an exception. Only 25% of the Osoyoos juveniles were correctly grouped by location. Mostly the misclassification pattern that occurred was among nearby locations. For example, a high rate of misclassification, 30% and 37% (Table 1), occurred between Oliver and Osoyoos (~17 km apart), 33% between Penticton and Keremeos (~45 km apart), and 33% between Mara and

Armstrong (Table 1). Hence, almost all the misclassification occurs between sites that are very close to each other, with one exception, between Osoyoos and Vernon in 2015 (37%, ~165 km apart, Table 1).

Cluster analysis of juveniles shows broadly similar results. The clusters (Ward. D method) grouped juveniles from different sites into different clusters, even separating individuals derived from locations ≤12 km (Fig. 3) apart. Since there is no particular rule for choosing the height in the Ward.D method dendrogram, the rule of thumb height of 10 was applied. Between 33 – 100% of juveniles from the same locations were grouped together at the height of 10 (Fig. 3). Approximately unbiased (AU) values obtained via the average method were used to test the significance of the clusters. Ten major significant clusters with red boxes (AU p-value >0.95) separated juveniles from different geographic locations (EMS. 1). Thus, juvenile grouping with respect to geographic location where they were collected in cluster analysis supports the predicted membership results derived from stepwise discriminant analysis. Thus, our results are robust to different multivariate statistical methods.

Identification of the source population of problem birds in the Okanagan Valley

To distinguish between immigrant and local problems birds in the Valley, cluster analysis was performed. Cluster analysis of all birds including both juvenile (n=105), and problem birds (adults, n=118) depicts two distinct clusters, separating most problem birds into one cluster and another cluster comprising a mix of the remaining problem birds and juvenile birds. Around half of the problem birds (n=52, 44%) clustered separately, indicating chemical composition distinct from the Okanagan Valley birds (Fig. 4). These problem birds were considered as likely immigrants to the valley.

To identify the origin of the remaining (n= 66, 56%) local problem birds in the Okanagan Valley, the same four canonical discriminant functions were used. The first function explained 44.2 % of the total variance among locations. The second function explained 33.0%, and the third and fourth explained 11.7 % and 6.1 %, respectively of the total variance among locations. To make the identification easier from a management perspective, the problem birds collected in fall were divided into two groups. First, problem birds collected in vineyards and orchards and second, those collected at dairy farms and feed lots.

The majority (55%) of problem birds caught in vineyards and orchards were consistent with trace element signatures from the North Okanagan. Specifically, the highest contributing site was Vernon (41%) (Fig. 5). The remaining 45% of problem birds were a mixture of local and immigrant birds (29% southern local, 2% central local). Out of which 6% of problem birds were immigrants and the origin of 8% of problem birds could not be more closely defined (Fig. 5).

In contrast to vineyards and orchards, the problem birds caught on dairy farms and feedlots were largely immigrants (89%) and only 11% were local (Fig. 6). Like in vineyard/orchards, trace element signatures were consistent with a single northern site (Vernon).

284 **Discussion**

285 **Spatial separation of starlings**

286 The spatial separation of starling populations in this study via bone tissue trace elemental
287 fingerprinting is comparable to feather tissue results obtained on other avian species. Trace
288 element fingerprinting of feather tissues has been used to separate avian populations on local to
289 regional scales. For example, white-crowned sparrow (*Zonotrichia leucophrys* F.) samples
290 collected from four dialect populations were distinguished within 400km (Poesel et al. 2008).
291 Similarly, sand martin (*Riparia riparia* L.) and barn swallow (*Hirundo rustica* L.) populations
292 were separated within and between continents (i.e., across Europe and between European and
293 African moulting sites) (Szep et al. 2003; Szép et al. 2009). In our study, the shortest distance
294 separating sites with distinct chemical signatures was 12 km (between Vernon and Armstrong).
295 This spatial resolution is similar to that reported for western sandpiper (*Calidris mauri* Cabanis)
296 using trace element composition of feathers from two sites less than 3 km apart (Norris et al.
297 2007) in the USA.

298 Site-specificity of the elemental compositions of bones from juvenile starling is similar
299 to analyses of natal origin from nestlings and juvenile sand martins (*Riparia riparia* L.) (Szep et
300 al. 2003) and for western sandpiper (*Calidris mauri* Cabanis; Norris et al. 2007) from feathers.
301 This suggests, for both feathers and bone, the elemental composition depends on colony micro-
302 geographic position. Thus, the elemental composition of juveniles might represent the site where
303 the nestlings' food was collected (Szep et al. 2003) and/or where the juveniles fed while growing
304 bone and feather.

305 The similarity in the elemental composition of juveniles collected within sites could be
306 due to the similarity in the phenotypic quality of adults, such as breeding at the same site, and

use of communal or cooperative breeding (feeding by multiple adults) (Pinxten, Eens, and Verheyen 1994; Szép et al. 2009). This type of adult behaviour could yield similar nestling chemical profiles during bone formation. Moreover, a high degree of similarity in feeding and foraging habitat of juveniles in the colony could be factors maintaining the similarity of juvenile bone trace element composition in a colony. Since most juveniles collected within sites were chemically similar, it appears that these juveniles had not dispersed. Thus, the juvenile fingerprint library adequately represents the fingerprints of source populations at particular sites in the valley.

However, the chemical signature of a few juvenile birds caught within a site suggest short range dispersion because their fingerprint matched with fingerprints from other sites according to the predicted group membership derived from discriminant analysis. But then again, the misclassification of juveniles among the sites that are geographically proximate such as Oliver and Osoyoos may also have arisen due to geological similarity, soil types, very similar agricultural practices and food choice. Additionally, misclassification noted in a few birds between the sites that are 100s km geographically apart might be due to differences in food choice and early short distance dispersal or geochemical similarity between remote sites. Starlings are generalist birds with extremely diverse diets. Their diet varies geographically, seasonally, and with age (Tinbergen 1981; Cabe 1993).

The overall degree of spatial separation in juvenile fingerprints was 79% among sites. This is similar to the degree of differentiation reported for shearwaters (*Calonectris* Cory & Oustalet) 75-89.9% (Gómez-Díaz and González-Solís 2007) and lower than that in white-crowned sparrows 100% at 400 km stretch (Poesel et al. 2008). The dissimilarity of chemical composition between locations within the Okanagan Valley may be due to either geologic

(Ethier et al. 2013), or irrigational (Poesel et al. 2008) factors, or both. The underlying geology of the Okanagan watershed is very diverse within small spatial scales (Okulitch 2013). For instance, bedrock underlying the southern Okanagan is heterogeneous, made up of volcanic, metamorphic, plutonic, and sedimentary rocks, and overlain by glacial sediments (Okulitch 2013).

Assigning problem starlings to natal populations

Problem birds were assigned to potential natal populations through discriminant analyses. The results can be compared to those of other studies, for example, in the marine environment, for the geographic assignment of shearwaters (*Calonectris*) to breeding colonies (Gómez-Díaz and González-Solís 2007); and in the terrestrial environment, to assigning non-local-dialect white-crowned sparrows (*Zonotrichia*) to populations that matched their song dialect (natal populations) (Poesel et al. 2008) using trace elemental analysis of feather and discriminant functions.

The problem bird contribution to vineyards and orchards in the southern part of the Okanagan Valley was mostly from the northern end of the valley. It is likely that the birds reared in the northern part of the valley move south as fruit ripens in the fall. The remaining problem birds in vineyards and orchards are a mixture of local (southern) and immigrant birds. This implies that the population increase in the south (vineyards/orchards) in the fall is likely due to migration especially from the north. This is consistent with documented migration patterns in starlings. Generally, fall migration occurs from September to December (Kessel 1953; Dolbeer 1982).

The problem birds on dairy farms and feedlots were a mixture of immigrants (from outside of the valley) and local birds. The majority of the problem birds in the northern region

353 were immigrants, indicating that birds from other (Northern BC) regions likely enter the northern
354 end of the valley. During winter, dairy farms and feedlots provide, adequate shelter and warmth
355 and a diverse supply of food and water (Palmer 1976). The high immigrant population in the
356 north Okanagan is likely attributable to the higher number of dairy farms and feedlots, and
357 spatial proximity to unsampled populations. Several past studies have noted that the flocks of
358 starlings increase in number and concentrate on dairy farms and feedlots in winter (Palmer 1976;
359 Glahn 1981).

360 Problem birds having trace element fingerprints distinct from the Okanagan populations
361 were considered immigrant birds. These immigrant birds were detected through cluster analysis.
362 Immigrant bird fingerprints display a chemical composition distinct from Okanagan Valley
363 juveniles. It is unlikely that starlings from outside the region were misidentified as Okanagan
364 Valley starlings because the geochemical fingerprints of starling populations sampled hundreds
365 of kilometers outside of the valley are very distinct (Neuhauser 2013).

366 Some problem birds are likely from the Okanagan valley but could not be assigned to
367 sites of natal origin because the discriminant function values for these birds were too different
368 from the centroid values for natal locations. It is likely that they are from areas between our
369 sampling locations because trace element signatures varied over small spatial scales and we only
370 had 10 reference distributed through a valley of more than 100km in length.

371 **Conclusion**

372 The results provide evidence that bone tissue elemental fingerprints of migratory pest
373 starling can be used to identify the natal origin of starling with a high spatial resolution (10s of
374 km). The technique can be very effective in an area like the Okanagan Valley with highly diverse
375 geology. The selected trace elements may provide the best marker for tracing the origins of

starlings and similar birds. This study also demonstrates the robustness of using trace elements in the bone tissue to track small-scale movements with a high degree of spatial precision

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Figure 1

Map of the study area (Okanagan-Similkameen region) with sampling sites in British Columbia (BC), Canada.

Filled black circles represent sampling sites for juveniles, whereas, unfilled black circles represent sampling sites for adults. Black circles with black dots represent the sampling sites where both adults and juveniles were collected.

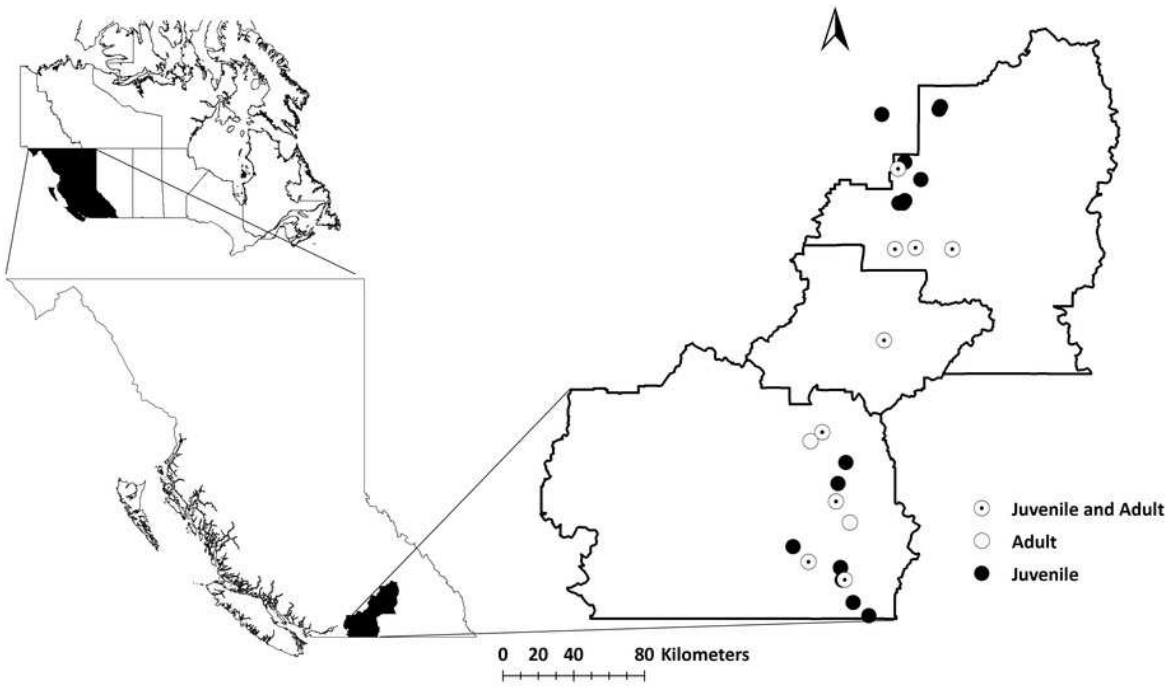

Figure 2

Distribution of juvenile birds caught in different locations.

Stepwise discriminant analysis of juvenile birds (n=105) from 10 different locations based on first two canonical discriminant functions (rc=0.07, P<0.0001) calculated from 20 trace elements. Different color represents different sampling area, and the black square represents the centroid of each sampling area.

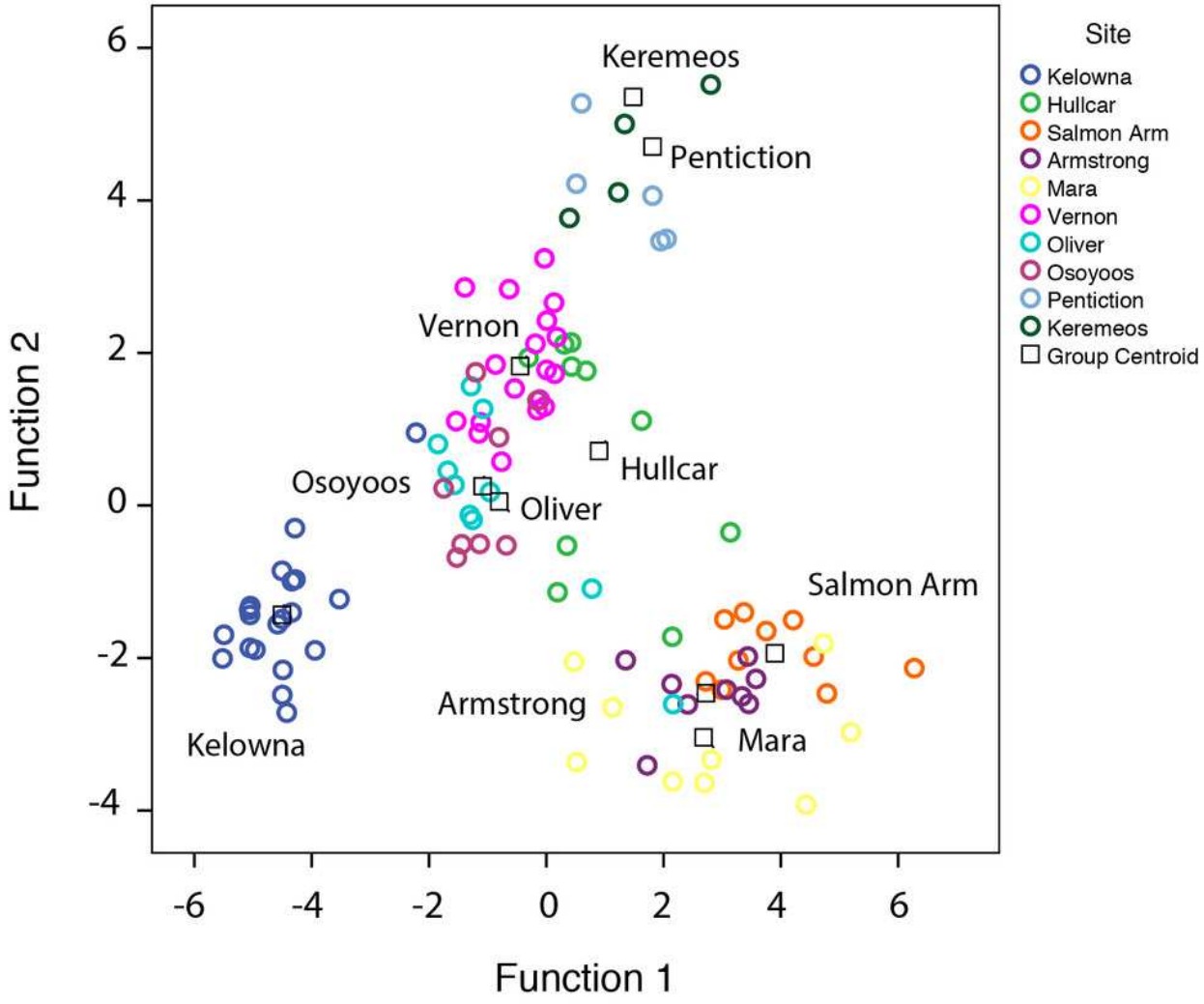

Figure 3

Cluster analysis of juvenile birds.

Cluster analysis of juvenile birds (n=105) sampled in the Okanagan Valley based on Ward.D method derived from the Euclidean distance using 20 elements. The distance provides a relative measure of how different the clusters are. Dark blue colour represents the samples from the northernmost area, and the colour fades as the location moves towards the south.

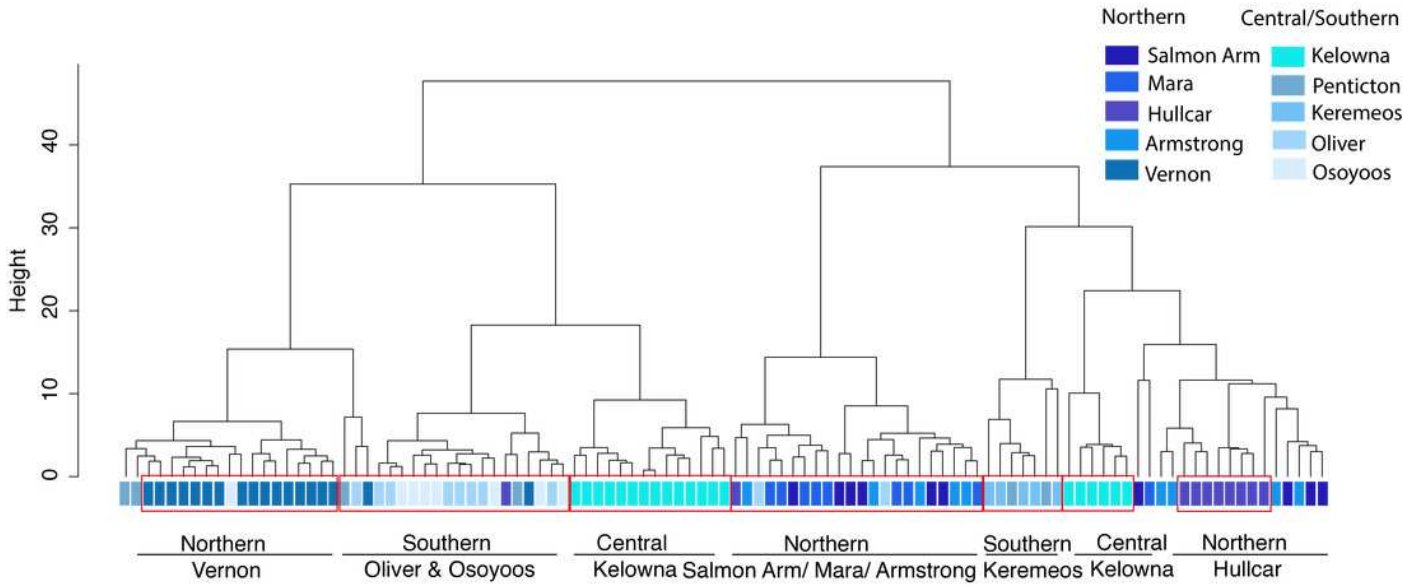

Figure 4

Cluster analysis of juvenile and adult birds.

Cluster analysis of juvenile and adult (n=223) birds collected in different locations in the Okanagan Valley based on Ward.D method derived from the Euclidean distance using 20 elements. Orange color represents immigrant adult adults, dark blue colours represent samples from the northernmost area, and the colour fades as the location moves towards the south.

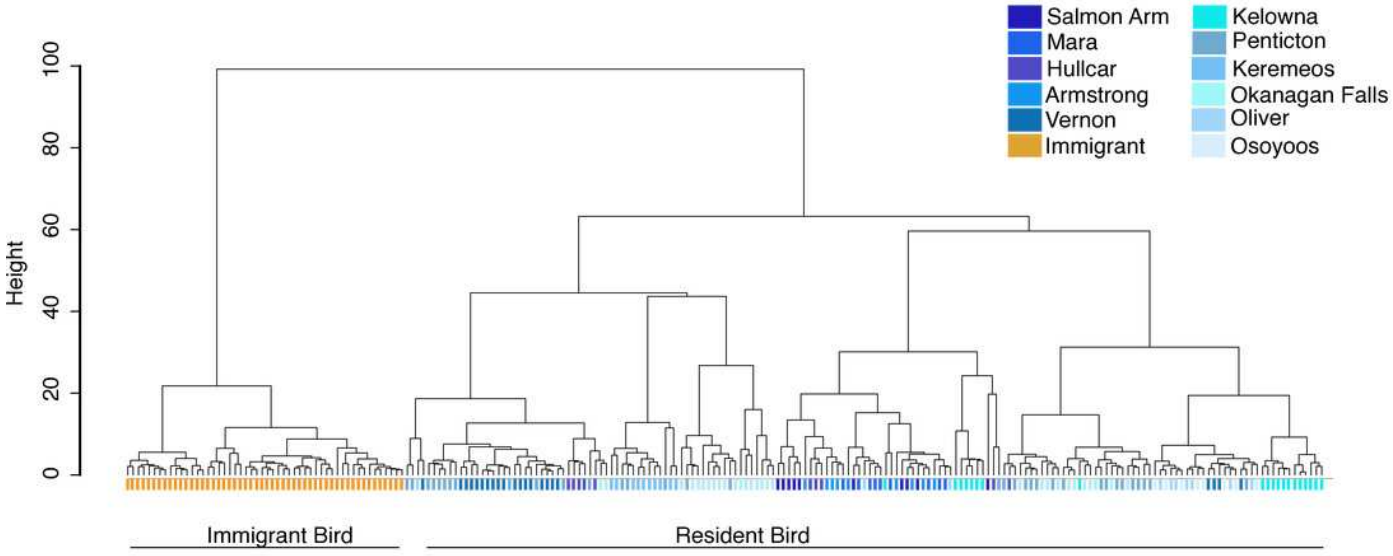

Figure 5

Heat map of the origin of the source population of problem birds caught in vineyards and orchards in the Okanagan Valley.

The radius of the circle depends on the percentage contributed from particular sites.

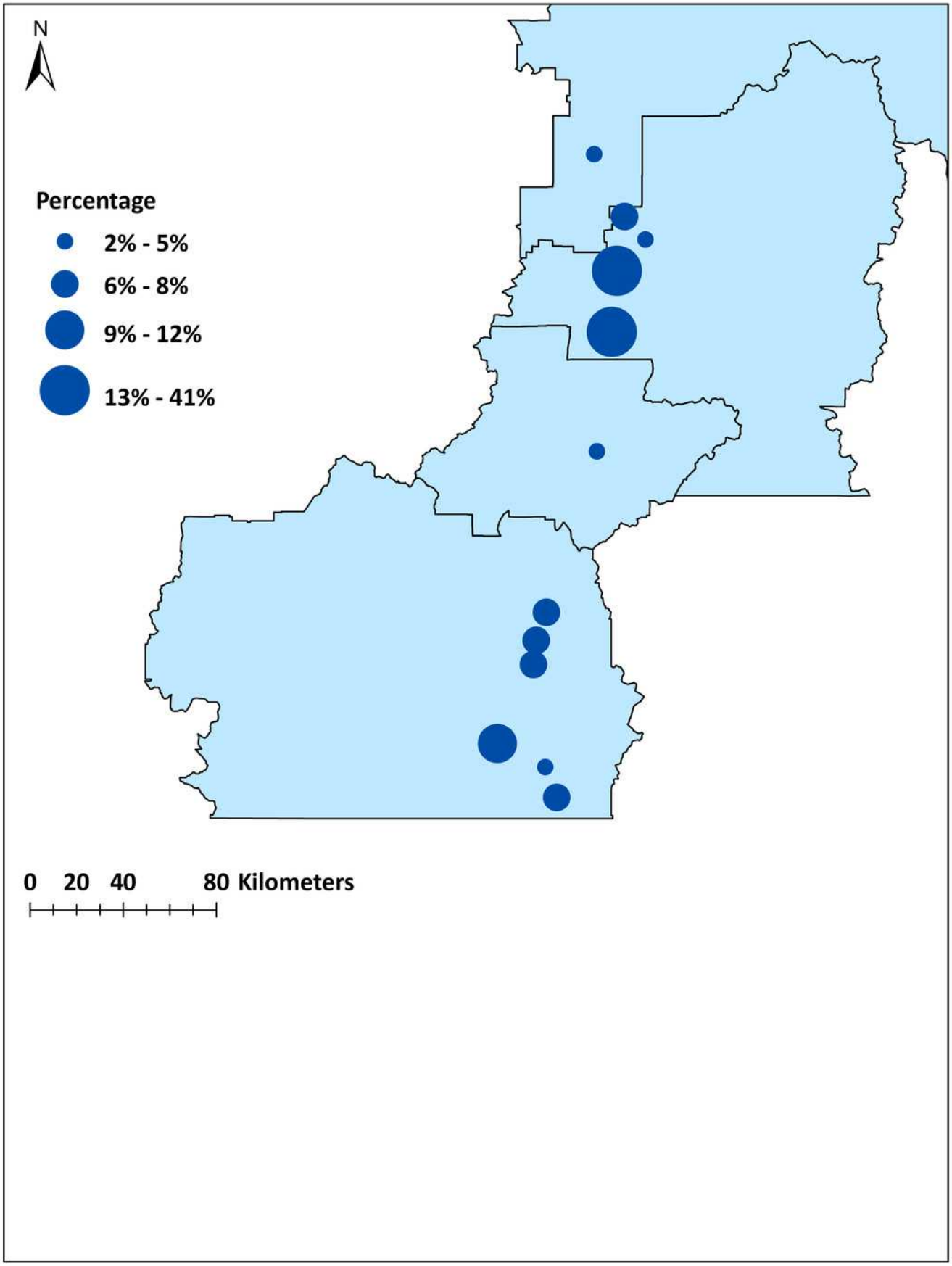
N
Percentage
2% - 5%
6% - 8%
9% - 12%
13% - 41%
0 20 40 80 Kilometers

Figure 6

Heat map of the origin of the source populations of problem birds caught in dairy farms and feedlots in the Okanagan Valley.

The radius of the circle depends on the percentage contributed from particular sites.

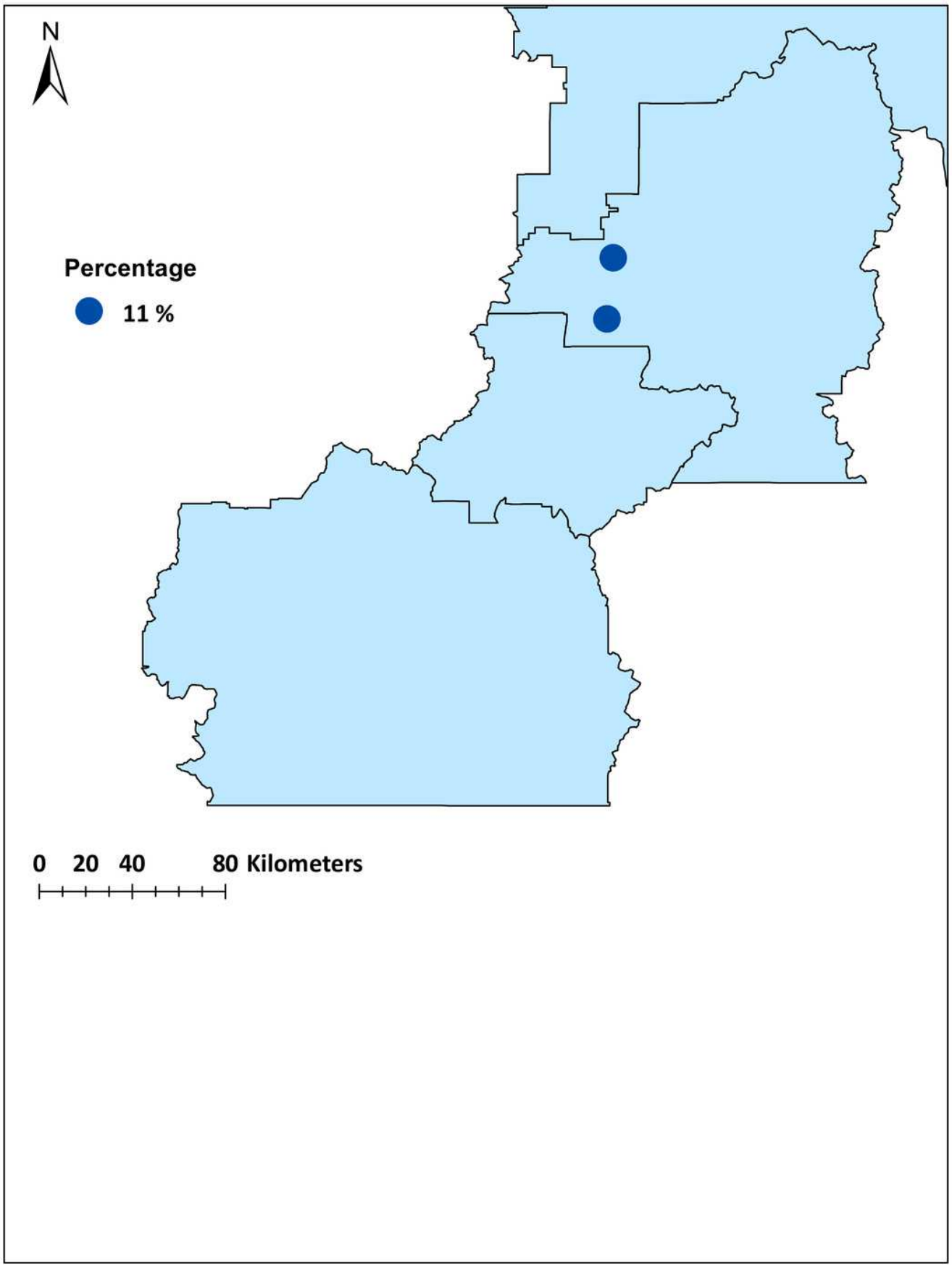
N
Percentage
11 %
0 20 40 80 Kilometers

Table 1(on next page)

Classification results for juveniles to its predicted locations

The predicted group membership shows the percentage of correctly classified samples, based on the cross-validation function in the SPSS software package where 79.0% of original grouped cases correctly classified.

1
2

Year	Site	Predicted Group Membership (%)										
		Kelowna	Hullcar	Salmon Arm	Armstrong	Mara	Vernon	Oliver	Osoyoos	Penticton/ Summerland	Keremeos	No of Sample
20	Kelowna	**95**	0	0	0	0	5	0	0	0	0	20
	Hullcar	0	**90**	0	0	0	0	0	10	0	0	10
	Salmon Arm	0	0	**70**	20	10	0	0	0	0	0	10
	Armstrong	0	0	0	**78**	22	0	0	0	0	0	9
	Mara	0	0	0	33	**67**	0	0	0	0	0	9
	Vernon	0	0	0	0	0	**100**	0	0	0	0	18
	Oliver	0	0	0	0	10	0	**60**	30	0	0	10
	Osoyoos	0	0	0	0	0	37	37	**25**	0	0	8
	Penticton	0	0	0	0	0	0	0	0	**67**	33	6
	Keremeos	0	0	0	0	0	0	0	0	0	**100**	5

3