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

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The European Starling (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 cause damage in dairy farms and feedlots by consuming and contaminating food and spreading diseases. Damage can be partly mitigated by the use of scare devices, which can disperse flocks until they become habituated. Large-scale trapping and euthanizing before starlings move to fields and farms could be a 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-Similkameen region, we used 21 trace elements in bone tissue to discriminate the spatial distribution of juvenile starlings and to reveal the geographic origin of the problem birds in fall. Stepwise discriminant analysis of trace elements classified juveniles to their natal origin (minimum discrimination distance of 12km) with 79% accuracy. In vineyards and orchards, the majority (55%) of problem birds derive from northern portions of the valley; and the remaining 45% of problem birds were a mixture of local and immigrant/unassigned birds. In contrast, problem birds in dairy farms and feedlots were largely immigrants/unassigned (89%) and 11% were local from northern region of the valley. Moreover, elemental signatures can separate starling populations in the Valley yielding a promising tool for identifying the geographic origin of these migratory birds.

ORIGIN IDENTIFICATION OF MIGRATORY PESTS (EUROPEAN STARLING)

USING GEOCHEMICAL FINGERPRINTING

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1 **Abstract**

2   The European Starling (Sturnidae: *Sturnus vulgaris* L.) is an invasive bird in North

3 America where it is an agricultural pest. In British Columbia (Canada), the starling population

4 increases in orchards and vineyards in autumn, where they consume and damage ripening fruits.

5 Starlings also cause damage in dairy farms and feedlots by consuming and contaminating food

6 and spreading diseases. Damage can be partly mitigated by the use of scare devices, which can

7 disperse flocks until they become habituated. Large-scale trapping and euthanizing before

8 starlings move to fields and farms could be a practical means of preventing damage, but requires

9 knowledge of natal origin.

10   Within a small (20,831 km^2), agriculturally significant portion of south-central British

11 Columbia, the Okanagan-Similkameen region, we used 21 trace elements in bone tissue to

12 discriminate the spatial distribution of juvenile starlings and to reveal the geographic origin of

13 the problem birds in fall. Stepwise discriminant analysis of trace elements classified juveniles to

14 their natal origin (minimum discrimination distance of 12km) with 79% accuracy. In vineyards

15 and orchards, the majority (55%) of problem birds derive from northern portions of the valley;

16 and the remaining 45% of problem birds were a mixture of local and immigrant/unassigned

17 birds. In contrast, problem birds in dairy farms and feedlots were largely immigrants/unassigned

18 (89%) and 11% were local from northern region of the valley. Moreover, elemental signatures

19 can separate starling populations in the Valley yielding a promising tool for identifying the

20 geographic origin of these migratory birds.

Introduction

22   European starling (*Sturnus vulgaris* L.) is one of the most successful non-native species

23 in North America (Vuilleumier 2009). A few hundred birds were introduced in New York City in

24 1890 (David 2010; Kerpez T. A. 1990; Linz et al. 2007). Surviving descendant birds spread

25 rapidly throughout North America in the following years (Cabe 1993; Linz et al. 2007) and have

26 now grown to a population of many millions. The perception of starlings changed along with

27 population size from a beautiful, robin size 'Chunky' songbird into an aggressive and costly pest.

28   Starling is listed as one of the three worst invasive birds in the World Conservation

29 Union List of '100 of the world's worst invasive alien species' (Lowe S., Browne M., Boudjelas

30 S. 2004). According to the International Union for Conservation of Nature, its conservation

31 status is considered to be of least concern on the basis of its probability of extinction. It is

32 considered as an agricultural pest, especially in fruit crops such as cherries, berries, and grapes

33 (Pimentel et al. 2005, BCGA 2013; Conover et al. 2007; Virgo 1971).

34   The migration pattern of European starlings varies regionally and individually. In some

35 areas, most of the breeding individuals are sedentary (Kessel 1953; Suthers 1978). Some

36 individuals migrate in some years and not in others. Some juveniles migrate, but even their nest

37 mates (siblings) might not (Kessel 1953). Generally, spring migration occurs between mid-

38 February to end of March and fall migration occurs from September to December (Dolbeer 1982;

39 Kessel 1953). Starlings can cover up to 1,000-1,500 km if needed in search of food especially

40 during winter (Linz et al. 2007).

41   The Okanagan-Similkameen region is a nationally significant agricultural region in

42 Canada due to climatic and geological suitability. The valley is ideal for several tree fruits such

43 as apples, peaches, and other soft fruits, like cherries, berries, and grapes, and for livestock

44   operations including both dairy farms and beef cattle feedlots. Around 97.5 % of British

45   Columbia's vineyard and 98% of the province's apples and grapes are grown in the south-central

46   Okanagan-Similkameen region region (Neuhauser 2013; BC Growers 2015). In the northern

47   Okanagana region, livestock operations, such as dairy farms and feedlots, grains and forage

48   crops predominate (BC Growers 2015; BCGA 2013). The cold short winters, warm summers,

49   rural/urban interface, and availability of year-round food sources in the Okanagan-Similkameen

50   region provide excellent starling habitat.

51   Large flocks of up to 1000s starlings are known to destroy fruits in the vineyards,

52   orchards and depredate cattle feed at dairy farms (Glahn 1981). Annually around $800 million

53   USD worth of agricultural crops were destroyed by starlings in the United States, based on crop

54   losses of $5/ha (Pimentel et al. 2000). In livestock facilities, starlings consume and contaminate

55   around 15-20 tons of cattle feed per day (Linz et al. 2007). Annually, around $3 million USD per

56   year is lost in vineyards and tree fruit farms in the Okanagan-Similkameen region alone (BCGA

57   2012).

58   A Starling Control Program (SCP) was initiated in the Okanagan-Similkameen region of

59   British Columbia in 2003 (BCGA 2010) to control the starling population and the damage they

60   cause. Agricultural industries, environmental agencies, and regional districts supported the

61   program (BCGA 2010). The SCP traps birds in various locations in the Okanagan-Similkameen

62   region throughout the year. From 2003 to 2013 around 544,000 birds were trapped by the control

63   program (OSSCP 2014). Although the control program has been trying to reduce the starling

64   population by aggressively trapping all year round, numbers appear to be increasing from 996 to

65   23592 from 1974 to 2013 (Christmas Bird Count, Birds Canada), especially in the fall season

66   due to migration and natal dispersal. Starling numbers increase in vineyards and orchards in fall

67 (BCGA 2013), but dispersion and movement patterns of starlings has not been well understood

68 in the Okanagan-Similkameen region. It is necessary to find the origin of these migrant birds to

69 enhance program effectiveness. Thus, understanding the natal origin and movement pattern of

70 migratory pests, like starlings, is important for developing successful management (Cabe 1993;

71 Neuhauser 2013). The main objective of this research is to identify the origin of the migratory

72 starlings using a geochemical (trace element) fingerprinting approach in the Okanagan-

73 Similkameen region so that starling might be managed at sources.

74 Biogeochemical markers such as stable isotopes (Hobson 1999, 2005a, 2005b; Szép et al.

75 2009) and trace elements analyses (Szép et al. 2003; Szép et al. 2009) are potential methods to

76 trace the origin and migration of such birds because the markers are indicators of the

77 environment of origin. Starlings can disperse long distances and retain stable isotopes and trace

78 element signatures in tissues reflecting natal food (Hobson 1999), location, and habitat (Szép et

79 al. 2009). These chemical signatures can be applied as tools to reconstruct animal movement

80 pathways (Ethier et al. 2013).

81 We chose trace elements over light isotope ratios for this study for two reasons. First,

82 isotope ratios in birds can be homogeneous for thousands of kilometres (Inger and Bearhop

83 2008), an order of magnitude larger than the Okanagan-Similkimeen region. In contrast, the

84 province of British Columbia and Okanagan-Similkameen region are geochemically diverse

85 across spatial scales of tens of kilometres or less (Okulitch 2013), making trace elements

86 potentially much more sensitive for tracking regional starling movements (Neuhauser 2013).

87 Second, trace elements can provide a higher specificity than isotopes because of the larger

88 number of potential variables (Szép et al. 2009). We hypothesized that the elemental

89 composition of starling bones depends on geographic region. Because birds acquire trace

90   element signatures in their tissues from their diet, and these signatures reflect the chemical

91   composition of the area in which the tissue was generated, or keep the record of past feeding

92   history as recorded in long turnover tissues (Hobson 1999; Szép et al. 2009).

93   We used samples of starling leg bone tissue (tarsometatarsus) commonly known as tarsus

94   or metatarsus to fingerprint natal origins of individual starlings. Feather tissue is more

95   convenient to use but we chose bone because feathers moult seasonally shedding the evidence of

96   natal origin, whereas the turnover rate of bone tissue is as much as 30 years (Tieszen et al. 1983,

97   Lanocha and Kalisinska 2012). Bone tissues are very useful for origin analysis as the mineral

98   phase in the bone matrix grows rapidly when an individual is a juvenile and then turns over very

99   slowly (Neuhauser, 2013). Most bone mass consists of type I collagen and apatite (a mineral

100   composed mainly of calcium and phosphate) (Boonen et al. 2010). The bone mineralization is

101   analogous to the geological mineral (Boonen et al. 2010) and is known as hydroxyapatite, Ca_5

102   $(PO_4)_3OH$ (Boskey 2007; Pasteris, Wopenka et al. 2008). Other cations and anions easily

103   substitute into the mineral matrix of apatite. Thus, other elements, derived from the diet can be

104   incorporated into bone to create a fingerprint, representing the food an individual has ingested

105   (Grynpas et al. 1993). The decision of using bone is also supported by an earlier study of six

106   European starling tissues (bone, liver, heart, muscle, brain, and feather) that identified bone as

107   the tissue for statistically separating populations of starlings across much of its range in British

108   Columbia (Neuhauser 2013).

109   In this study, we evaluated the geochemical fingerprint of juvenile starlings throughout

110   the Okanagan-Similkameen region, and compared these signatures to that of problem birds

111   caught in the fall trapped in fruit crops in and dairy farms. By fingerprinting starling natal origin,

112   appropriate management to reduce starling populations could be implemented in focused areas to

113   reduce the damage created by starlings.

114   **Materials and Methods**

115   **Study Area**

116   British Columbia (BC) is the western-most province of Canada and has high geological

117   diversity in rock types (Canada 2012) (Fig. 1). The Okanagan-Similkameen region in south-

118   central British Columbia is about 200 km long and 20 km wide (Fig. 1). It lies in between the

119   Columbia and Cascade Mountain ranges in the southern interior of BC. The valley bedrock is

120   comprised of volcanic, sedimentary, metamorphic and intrusive rocks (Okulitch 2013). Southern

121   Okanagan-Similkameen region is heterogeneous, made up of volcanic, metamorphic, plutonic,

122   and sedimentary rocks, and overlain by glacial sediments (Okulitch 2013). Central Okanagan is

123   made up lava, andesite with quartz-filled amygdales. The northern Okanagan is made up of

124   Paleozoic phyllite, argillite, schist, amphibolitic, quartzite, carbonaceous, micaceous chlorite and

125   other minor constituents (Table S1) (Okulitch 2013).

126   Starling habitat is generally the lower elevation portion of a long valley, approximately

127   between 300 and 700 m elevation. Above 700 m, the valley rises quickly to a plateau of >1200

128   m (Fig. S1), greatly constraining distribution and likely movement and migration to a relatively

129   small area (Fig. S1).

130   **Sampling**

131   Sampling was performed in summer (May-July) and in fall/winter of 2015. In summer,

132   only juveniles, having little prior chance to disperse beyond their natal habitat, were collected to

133   establish fingerprints of source populations. Thus, their bone tissue will reflect the elemental

134   fingerprint of the sites where they were collected. To make our trace element fingerprint library

135   more representative, juveniles were collected from almost all the Okanagan-Similkameen

136   cities/towns, and lands used for different agricultural activities, such as vineyards, orchards,

137   dairy farms, feedlots, grasslands, and landfill sites.

138   In fall, problem birds (both adult and juveniles (<1year) were collected in

139   vineyard/orchards and dairy farms/feedlots. The age of the birds was determined from their

140   plumage (Szép et al. 2003; Szép et al. 2009). Additionally, aging by plumage was validated by

141   the chronology of skull development (skull ossification - skulling) in juveniles (McKinney,

142   2004). Five randomly selected samples, which were considered as juvenile through plumage,

143   were dissected to observe evaluate developmental stage by skulling (Mueller and Weise 1996).

144   These starlings are considered pests and hereafter referred to as "problem birds".

145   Sampling was performed in collaboration with the Starling Control Program (SCP),

146   British Columbia Grapesgrowers' Association (BCGA). A team of professional trappers

147   contracted by the BCGA, has been trapping and euthanizing starlings following the guidelines of

148   the Canadian Council on Animal Care every year. We obtained frozen sample from SCP trappers

149   and each individual bird is considered as a single sample. Altogether, 105 juveniles were

150   collected from ten different locations situated at distances from 12 to 190 km apart throughout

151   the Okanagan-Similkameen region in 2015. The number of juvenile samples collected in summer

152   varied depending on availability at each location, 20 from Kelowna, 10 from Hullcar, 10 from

153   Salmon Arm, 9 from Armstrong, 9 from Mara, 18 from Vernon, 10 from Oliver, 8 from

154   Osoyoos, 6 from Penticton and 5 from Keremeos. In fall, 118 problem birds (including young

155   adults <1 year, n=20; and adults: 1+ years, n=98) were collected from vineyards/orchards and

156   dairy farms and feedlots having major starling problem. Some of the problem bird sampling

157   locations are the same where juveniles were collected like Vernon (n=4), Penticton (n=27),

158 Keremeos (n=5), Kelowna (n=14), and Oliver (n=15); however, Okanagan falls (n=4),

159 Summerland (n=30), Coldstream (n=10), and Lumby (n=9) were added in fall. These locations

160 spread across Okanagan-Similkameen region from south to north (Fig. 1).

161 **Sample preparation**

162   Samples were prepared by methods described by Norris et al. (2007) with some

163 modification. Briefly, the bone was cleaned by removing outer skin and marrow. Bones were

164 washed repeatedly with ultra-pure water (18 MΩ cm^{-1}), dried at room temperature (20 ˚C) (Szép

165 et al. 200) and weighed. The clean fragments of the bone sample, weighing between 30 mg to 80

166 mg each, were put separately into Teflon tubes. Trace element grade concentrated nitric acid

167 (2ml) was added to each tube and placed in a heating block 75˚C (±5˚C) until the bone was

168 completely dissolved. Samples were then cooled to room temperature, and then one milliliter of

169 trace metal grade hydrogen peroxide was added to further digest the dissolved organic

170 compounds (Norris et al. 2007). Samples were then evaporated on a hot plate at 85˚C (±5˚C)

171 (Szép et al. 2003; Neuhauser 2013). After cooling, 2 ml of 1 % HNO_3 with 1 ppb indium internal

172 standard was added to each vial to dissolve the residue over a period of 2 hours at room

173 temperature.

174   Samples were transferred quantitatively with three washes of the HNO_3 indium solution

175 into acid-washed high-density polyethylene tubes to a final volume of 10 mL (Norris et al.

176 2007;Neuhauser 2013). Sample blanks were prepared in the same way for every set of digestions

177 to monitor contamination (Donovan et al. 2006; Norris et al. 2007; Szép et al. 2009). Sub-

178 samples were further diluted 100-fold with 1 ppb indium-1% HNO_3 for ICP-MS analysis and 5

179 and 10 fold with 1ppm yttrium-1% HNO_3 for ICP-OES analysis. Samples were diluted to fall

180 within the detection limits of the instruments.

181   **Trace element analysis**

182   We measured 21 elements: aluminium (Al), silver (Ag), barium (Ba), calcium (Ca),

183   cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), molybdenum (Mo), manganese (Mn),

184   lead (Pb), sulphur (S), scandium (Sc), selenium (Se), tin (Sn), strontium (Sr), vanadium (V), zinc

185   (Zn), magnesium (Mg), sodium (Na), and potassium (K) in bone tissue. The elements were

186   selected on the basis that they are present at levels high enough for reliable quantification, and

187   could also vary in space.

188   Seventeen of the 21 elements were analysed by Thermo-Fisher Element XR sector field

189   Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) (Poesel et al. 2008; Neuhauser 2013).

190   A subset of four highly-abundant elements, Ca, Mn, Na, and K, were analysed via a Thermo-

191   Electron Corporation, iCAP 6000 Series XR Inductively Coupled Plasma-Optical Emission

192   Spectrometer (ICP-OES) (Szép et al. 2003; Szép et al. 2009; Neuhauser 2013). For ICP-MS,

193   elements were analysed in either low resolution or medium resolution mode to resolve

194   polyatomic interferences. Both instruments were calibrated by external multi-element standards.

195   Four different concentrations of multi-element standards were run to produce calibration curves

196   (Poesel et al. 2008). Indium (Norris et al. 2007) and yttrium (Neuhauser 2013) were used as an

197   internal standard for ICP-MS and ICP-OES respectively to correct for instrument drift, sample

198   density and to improve accuracy and repeatability. The blank and reference solutions were run at

199   20-sample intervals throughout the analysis. Three replicate analyses were done for each sample.

200   Relative Standard Deviation (RSD) of the analysis was between 1-2%.

201   **Statistical Analysis of Data**

202   The elemental concentration was normalized to the concentration of calcium rather than

203   mass to minimize variability in the ratio of mineral:collagen content. The collagen structure of

204   bone bonds relatively poorly with metals, whereas the mineral apatite substitutes cations and

205   anions relatively easily into its structure. As birds develop, the relative content of apatite

206   increases (Rogers and Zioupos 1999; Currey 2004; Currey et al. 2004; Pasteris et al. 2008).

207   Normalizing with calcium, therefore, removes this variability due to collagen content. After

208   calcium normalization, the data were then standardized via z-scoring to prevent individual

209   elements from having a disproportionate influence on the groupings derived from multivariate

210   analyses (Fowler et al.1998; Norusis 2016).

211   A multivariate analysis of variance (MANOVA) was used to analyse trace element

212   profiles of juvenile bone tissue collected in different locations (Norusis 2016). A multiple

213   discriminant analysis (Hair et al. 2010) of juvenile samples was performed to discard elements

214   (variables), which were little related to group distinction, and also to develop the predictive

215   model of group membership based on trace elements (chemical profile of bone). A discriminant

216   function (equation) was developed based on the linear combination of the predictor variables

217   (trace elements) that provides the best discrimination (correctly separating individual birds)

218   among the predetermined groups (Lachenbruch and Goldstein 1979; Greenough et al. 1997; Szép

219   et al. 2009). The predetermined groups were the sites where the juvenile starlings were caught.

220   The stepwise method and Mahalanobis distance (Hair et al. 2010) were used for the analysis

221   because of the large number of variables (20 different element concentrations).

222   The stepwise method looks at each element (variable), one at a time, and determines

223   which element is the best predictor of group membership, and ultimately generates the best set of

224   variables to predict group membership. Cross-validation assessed the success of the proper bird

225   groupings via the discriminant function and rules. In order to classify objectively the problem

226   birds with respect to origin, the value obtained from each function for each problem bird was

227 normalized (ratio of sample/ratio of centroid) by the centroid value of each site. Thus, if an

228 individual problem bird were to lie at the centroid for a particular site, its value with respect to

229 that site would be one. Each bird's probable origin was identified by how closely that bird's

230 normalized value approximated the centroid values of each site. For some of the problem birds,

231 the discriminant function value was too high to assign a specific juvenile source population. For

232 such birds, we have categorized them as immigrant/unassigned birds.

233   Cluster analysis (Hartigan 1975) was performed to evaluate the spatial separation of the

234 elemental fingerprint of starlings and the identification of likely immigrants. In this study, the

235 ward.D method (Ward 1963; Murtagh and Legendre 2014) with Euclidean distance measures

236 was used for analysis, which provided better separation of individual birds with similar

237 signatures and geographic location. Additionally, an average method with correlation distance

238 was used to compute a test of significance of the clusters (by calculating the p-value of the

239 cluster). The R package "*pvclust*" (Suzuki and Shimodaira 2014) was used; it uses bootstrap

240 resampling techniques to compute the p-value for each cluster. This method generates thousands

241 of bootstrap replications by randomly sampling elements of the data. For each of the clusters,

242 Approximately Unbiased (AU) and Bootstrap Probability (BP) values were calculated. The AU

243 probability values (p-values) are computed by multiscale bootstrap resampling where AU $\geq$ 95 %

244 are considered to be strongly supported by the data, while BP corresponds to the frequency that

245 the cluster is identified in bootstrap copies (Suzuki and Shimodaira 2014).

246   Statistical analysis was conducted in SPSS ver. 24.0 and R statistical software version

247 3.3.1 (R Development Core Team n.d.) with the *cluster* (Maechler et al. 2017) *and pvclust*

248 (Suzuki and Shimodaira 2014) packages. Map projections of data were done in ArcGIS 10.4.

249 **Results**

250 **Spatial separation of source population of starlings in the Okanagan-Similkameen region**

251   The source population trace element fingerprint library for the Okanagan-Similkameen

252 region was developed from juvenile starlings collected from 10 different locations. Multivariate

253 comparison of 20 elements in the juvenile (n=105) population using MANOVA shows there is a

254 significant difference in the trace elemental composition of juvenile bone among locations

255 (Wilks' λ <0.0001, F=6.655, df= 180, 645.230, power=1, p<0.0001).

256   A stepwise discriminant analysis of 20 trace elements based on the juveniles (n=105)

257 collected at 10 different locations, provided four canonical discriminant functions with

258 significantly high eigenvalues (>1), and canonical correlations (rc> 0.70), that separated juvenile

259 samples from respective sites (Wilks' λ = 0.001, 0.11, 0.73, χ^2 = 633, 428, 247, p<0.0001) (Fig.

260 2). Ten elements (K, Mg, Na, Ag, Cd, Ba, Sc, Cr, Cu, and Se) out of 20, were used as the best

261 predictor variables in discriminating group membership. The probabilities of correctly

262 classifying juveniles to their respective locations using stepwise discriminant analysis are shown

263 in Table 1. The table shows the predicted classification (group membership) using cross-

264 validation from different locations compared to the apparent or actual observation. The overall

265 probability of correctly classifying juveniles was 79% (P<0.0001, Press's Q; Table 1). The

266 juveniles were grouped 67%-100% correctly, with Osoyoos as an exception. Only 25% of the

267 Osoyoos juveniles were correctly grouped by location. Mostly the misclassification pattern that

268 occurred was among nearby locations. For example, a high rate of misclassification, 30% and

269 37% (Table 1), occurred between Oliver and Osoyoos (~17 km apart), 33% between Penticton

270 and Keremeos (~45 km apart), and 22% and 33% between Mara and Armstrong (Table 1).

271 Hence, almost all the misclassification occurs between sites that are very close to each other,

272 with one exception, between Osoyoos and Vernon in 2015 (37%, ~165 km apart, Table 1).

273   Cluster analysis of juveniles shows broadly similar results. The clusters (Ward. D

274   method) grouped juveniles from different sites into different clusters, even separating individuals

275   derived from locations ≤12 km (Fig. 3) apart. Since there is no particular rule for choosing the

276   height in the Ward.D method dendrogram, the rule of thumb height of 10 was applied. Between

277   33 – 100% of juveniles from the same locations were grouped together at the height of 10 (Fig.

278   3). Approximately unbiased (AU) values obtained via the average method were used to test the

279   significance of the clusters. Ten major significant clusters with red boxes (AU p-value >0.95)

280   separated juveniles from different geographic locations (Fig. S2). Thus, juvenile grouping with

281   respect to geographic location in cluster analysis supports the predicted membership results

282   derived from stepwise discriminant analysis. Thus, our results are robust to different multivariate

283   statistical methods.

284   **Identification of the source population of problem birds in the Okanagan-Similkameen**

285   **region**

286   The source population of problem birds was identified by comparing their trace element

287   fingerprint with the juvenile fingerprint library. Cluster analysis of all birds including both

288   juvenile (n=105), and problem birds (< 1 year adults (n=20), and 1 + year adults (n=98)) depicts

289   two distinct clusters, separating most problem birds into one cluster and another cluster

290   comprising a mix of the remaining problem birds and juvenile birds (Fig.4). Around half of the

291   problem birds (n=52, 44%) clustered separately, indicating chemical composition distinct from

292   the juvenile fingerprint library (Fig. 4). Problem birds having trace element fingerprints distinct

293   from the Okanagan-Similkameen populations (doesn't match our library) were considered as

294   immigrant/unassigned birds, likely originating from unsampled sites within the Valley or from

295   outside of the Valley.

296   To identify the origin of the remaining (n= 66, 56%) local problem birds in the

297   Okanagan-Similkameen region, the same four canonical discriminant functions were used. The

298   first function explained 44.2 % of the total variance among locations. The second function

299   explained 33.0%, and the third and fourth explained 11.7 % and 6.1 %, respectively of the total

300   variance among locations. The problem birds collected in fall were divided into two groups.

301   First, problem birds collected in vineyards and orchards and second, those collected at dairy

302   farms and feed lots. The majority (55%) of problem birds caught in vineyards and orchards were

303   consistent with trace element signatures of juvenile birds from the North Okanagan. Specifically,

304   the highest contributing site was Vernon (41%) (Fig. 5). The remaining 45% of problem birds

305   were a mixture of local and immigrant/unassigned birds (29% southern local, 2% central local),

306   14% of problem birds in vineyards and orchards were immigrants/unassigned (Fig. 5). In

307   contrast to vineyards and orchards, the problem birds caught on dairy farms and feedlots were

308   largely immigrants/unassigned (89%) and only 11% were local from northern site (Vernon) (Fig.

309   5).

310   **Discussion**

311   **Spatial separation of starlings**

312   Resolving populations in space revealed distinct trace elemental signatures within the

313   200 km length of the Okanagan-Similkameen region. The spatial separation of starling in this

314   study using bone tissue trace elemental fingerprinting is comparable to spatial separation of other

315   birds at scales ranging between kilometers to continental distances using feather tissue trace

316   elemental fingerprinting (Poesel et al. 2008; Szép et al. 2009). For example, white-crowned

317   sparrow (*Zonotrichia leucophrys* F.) samples collected from four dialect populations were

318   distinguished within 400km (Poesel et al. 2008). Similarly, sand martin (*Riparia riparia* L.) and

319   barn swallow (*Hirundo rustica* L.) populations were separated within and between continents

320   (i.e., across Europe and between European and African moulting sites) (Szép et al. 2003; Szép et

321   al. 2009). In our study, the shortest distance separating sites with distinct chemical signatures

322   was 12 km (between Vernon and Armstrong).

323   Site-specificity of the elemental compositions of bones from juvenile starling is similar to

324   analyses of natal origin from nestlings and juvenile sand martins (*Riparia riparia* L.) (Szép et al.

325   2003) and for western sandpiper using feather tissue (*Calidris mauri* Cabanis; Norris et al. 2007).

326   Moreover, a high degree of similarity in feeding and foraging habitat of juveniles could be

327   factors maintaining the similarity of juvenile bone trace element composition in local

328   populations. Since most juveniles collected within sites were chemically similar, it appears that

329   these juveniles had not dispersed. Thus, the juvenile fingerprint library adequately represents the

330   fingerprints of source populations at particular sites in the valley.

331   The chemical signature of a few juvenile birds caught within a site matched with

332   fingerprints from other sites according to the predicted group membership derived from

333   discriminant analysis. Such misclassification of juveniles among nearby sites such as Oliver and

334   Osoyoos may have arisen due to overlapping foraging ranges of the juveniles or adults feeding

335   chicks, geological similarity between nearby sites, similar agricultural practices and food choice.

336   These mechanisms are not mutually exclusive and all may have been operating. Finally,

337   misclassification noted in a few birds between the sites that are 100s of kilometers apart

338   geographically might be due to differences in food choice and early short distance dispersal or

339   geochemical similarity between remote sites.

340   The overall degree of classification of juvenile fingerprints was 79% among sites. This is

341   similar to the degree of differentiation reported for shearwaters (*Calonectris* Cory & Oustalet)

342   75-89.9% (Gómez-Díaz and González-Solís 2007) and lower than that in white-crowned

343   sparrows 100% across 400 km (Poesel et al. 2008). The dissimilarity of chemical composition

344   among sites within the Okanagan-Similkameen region may be due to geologic factors (Ethier et

345   al. 2013), and/or hydrologic factors including irrigation (Poesel et al. 2008). The underlying

346   geology of the Okanagan-Similkameen watershed is very diverse within small spatial scales

347   (Okulitch 2013).

348   Similarly, soil chemistry is non-uniform over the landscape (Goitom Asfaha et al., 2011).

349   Water, mineralogy and soil microbiology affect soil chemistry. Solutes present in soil water can

350   be derived from weathering of rocks and minerals locally or could have come from water

351   (precipitation and irrigation). The trace elements move from minerals to soil water (Galgano,

352   Favati, Caruso, Scarpa, & Palma, 2008) where they are taken up by plants. In turn, plants form

353   the base of the food web, eventually accumulating in animal tissues, forming a unique marker.

354   The trace element availability in soil can be further modified by factors like soil pH, water

355   content and porosity (Kelly, Heaton, & Hoogewerff, 2005; Kim & Thornton, 1993).

356   Trace element composition in bone tissues can yield a very specific biogeochemical

357   marker. Out of 21 elements analysed, ten elements (K, Mg, Na, Ag, Cd, Ba, Sc, Cr, Cu, and Se)

358   were used in a discriminant analysis that classified juveniles source populations. All are cations

359   (Se is also an oxyanion) and could potentially substitute into apatite. For example, Pb substitutes

360   for Ca in hydroxyapatite in bone with a turnover rate ranging from 1-8% per year depending on

361   the type of bone tissue (Martin, Naftel, Nelson, & Sapp, 2007). The shortlisted elements from

362   the discriminant analysis are therefore a mixture of those elements, which could easily be present

363   in bone, and could also vary in space.

364   **Assigning problem starlings to natal populations**

365   Problem birds were assigned to potential natal populations through discriminant analyses.

366   However, some of the problem birds could not be assigned to its source population and were

367   designated as immigrant/unassigned problem birds. Failure to assign origins for a portion of the

368   problem birds could be caused by continued addition of elements to bone over large unknown

369   space over time. Second, adult starlings >1 year old, natal fingerprints may differ inter annually,

370   especially if foraging by such a generalist might change for different climatic years. Third,

371   problem birds could come from outside the region.

372   The problem bird contribution to vineyards and orchards in the southern part of the

373   Okanagan-Similkameen region was mostly from the northern end of the valley. It is likely that

374   the birds reared in the northern part of the valley move south as fruit ripens in the fall. The

375   remaining problem birds in vineyards and orchards are a mixture of local (southern) and

376   immigrant/unassigned birds. This is consistent with documented migration patterns in starling.

377   Generally, fall migration occurs from September to December (Kessel 1953; Dolbeer 1982).

378   The problem birds on dairy farms and feedlots were a mixture of immigrants/unassigned

379   and local birds. The high immigrant/unassigned population in the north Okanagan is likely

380   because these sites are close to unsampled source populations further to the north. During winter,

381   dairy farms and feedlots provide, adequate shelter and warmth and a diverse supply of food and

382   water (Palmer 1976). Several past studies have noted that the flocks of starlings increase in

383   number and concentrate on dairy farms and feedlots in winter (Palmer 1976; Glahn 1981).

384   Moreover, one of the challenges to this type of studies is that the trace element profiling is more

385   difficult for wide-ranging, continuously distributed species as the number of potential source

386   areas is vast. If the organism migrates long distances, then trace elements alone might not be

387   sufficient to track their origin. Also trace elements alone would not be sufficient for tracking the

388   origin of migratory organisms that undertake large-scale movements and forage in diverse

389   geochemical environments. However, the combined use of trace elements and isotopes could

390   help track continental-scale movement patterns. Besides identifying bird origins, the trace

391   elemental signature could be used in several other ecological studies of birds, including matching

392   breeding populations with overwintering populations, tracking fine-scale dispersal (Poesel et al.

393   2008), identifying migratory connectivity (Szép et al. 2009) and identifying moulting areas (Szép

394   et al. 2003).

395

396   **Conclusion**

397   The results provide evidence that bone tissue elemental fingerprints of migratory pest

398   starlings can be used to identify the natal origin of starlings with a high spatial resolution (10s of

399   km). The technique can be very effective in an area like the Okanagan-Similkameen region with

400   highly diverse geology. Most of the problem birds in vineyards and orchards of the south were

401   identified as local birds; very few were immigrants/unassigned. In contrast, in dairy farms and

402   feedlots in the north, most of the problem birds were identified as immigrants/unassigned to the

403   valley, probably because migration in fall/winter is generally southerly and the populations to the

404   north were not sampled. This study reveals that trace element fingerprints, a natural

405   environmental tracer, can track the origin of mobile organisms like starlings. Knowing the

406   origins of problem birds enables managers to make more informed decisions about how and

407   where to concentrate control efforts.

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

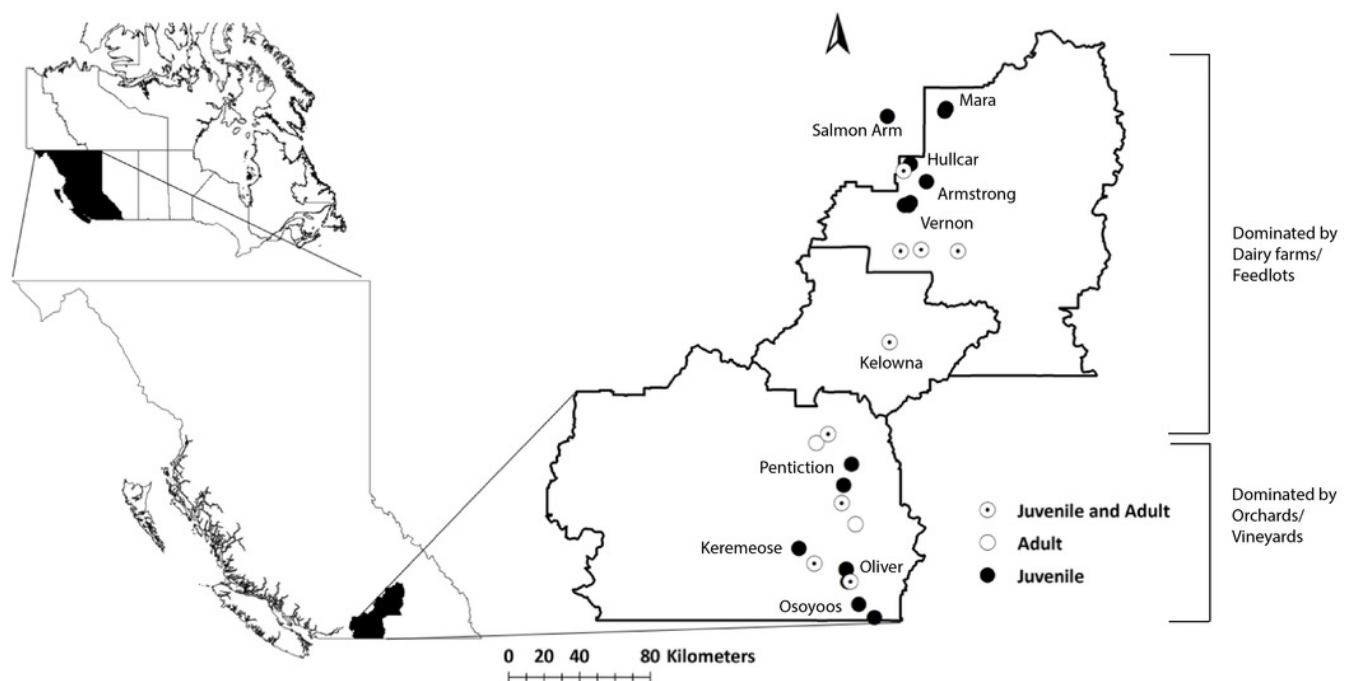



Figure 2

Distribution of juvenile starlings caught in different locations in the Okanagan-Similkameen region, British Columbia.

Stepwise discriminant analysis of juvenile starlings (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.

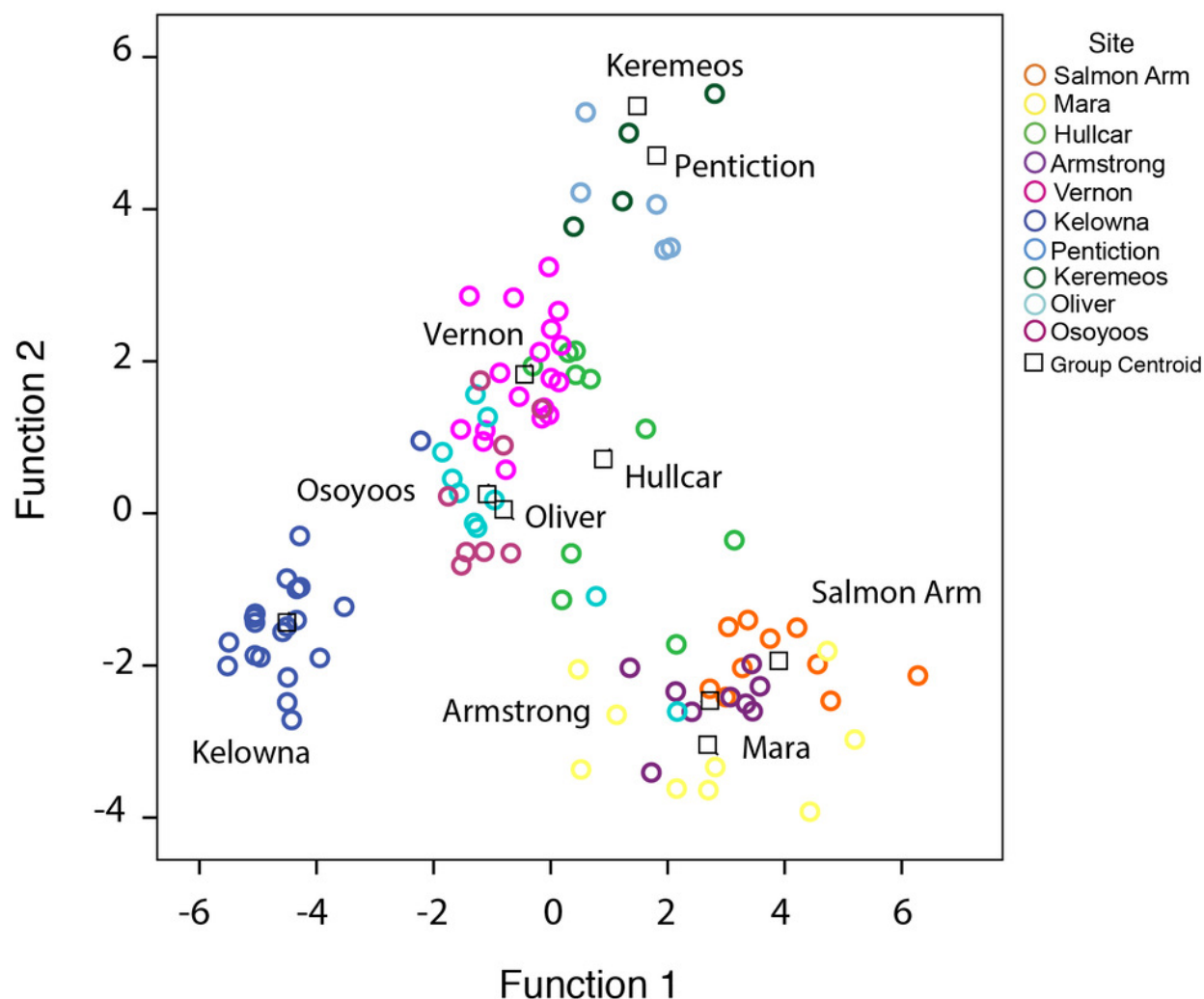



Figure 3

Cluster analysis of juvenile starlings.

Cluster analysis of juvenile starlings (n=105) sampled in the Okanagan-Similkameen region 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 in relation to each other. Different colour represents different locations in the Okanagan-Similkameen region, British Columbia.

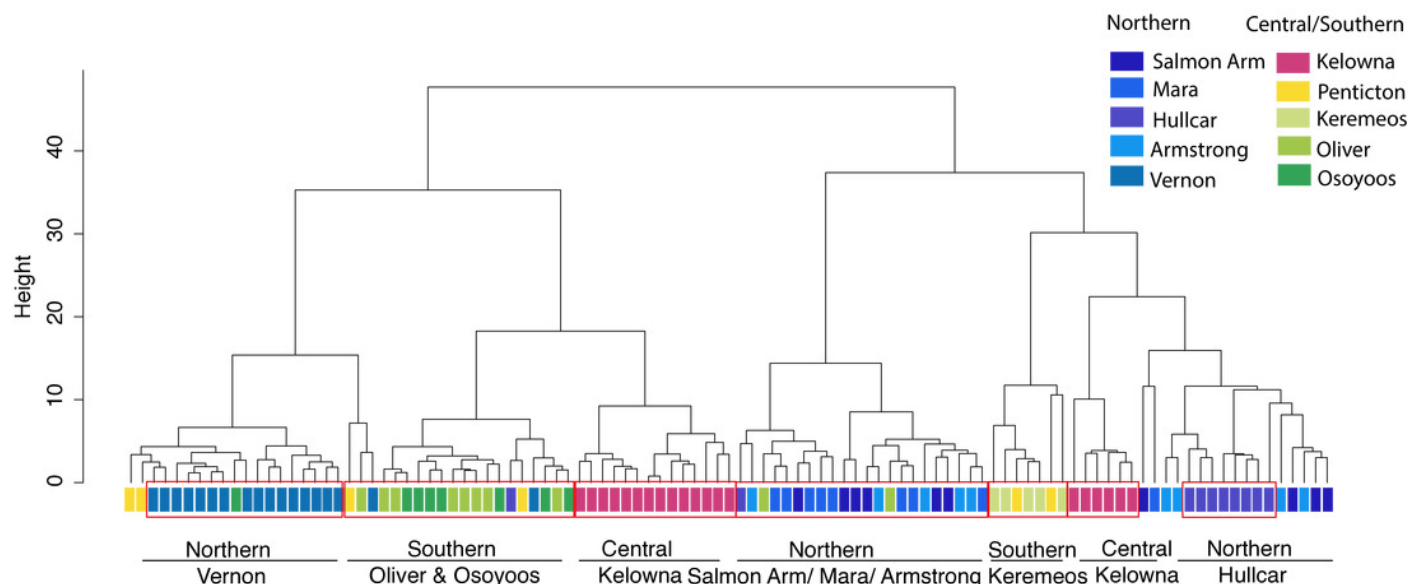



Figure 4

Cluster analysis of juvenile and adult starlings.

Cluster analysis of juvenile and adult (n=223) birds collected in different locations in the Okanagan-Similkameen region, British Columbia 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.

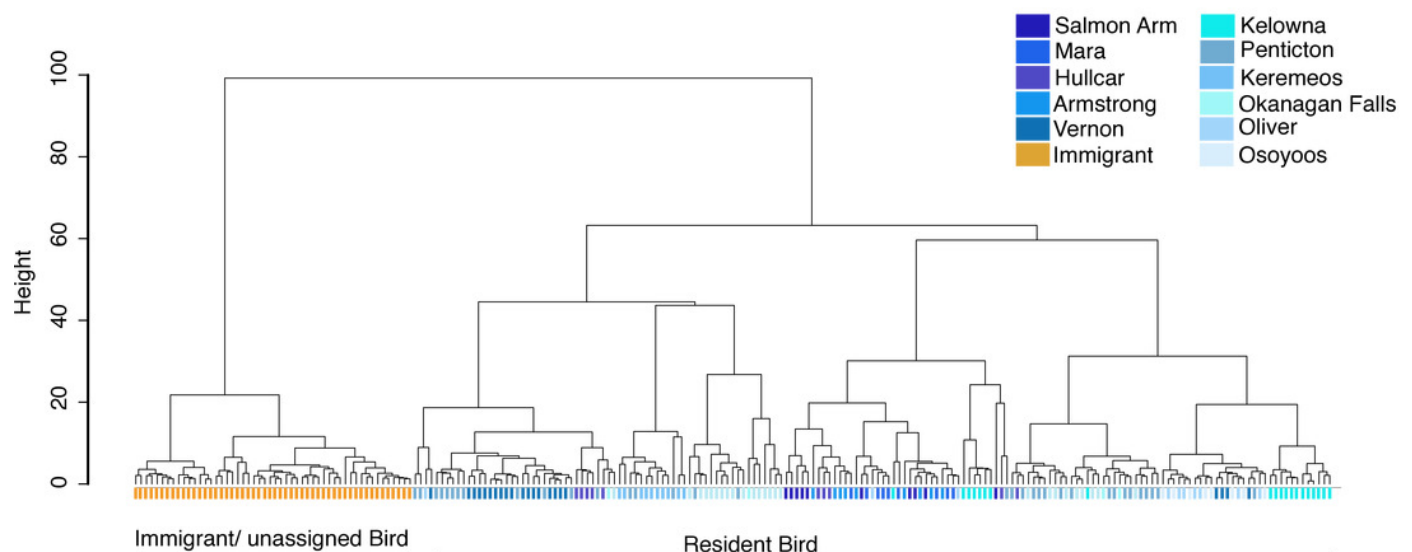



Figure 5

Map of the origin of the source population of problem birds caught in vineyards /orchards and dairy farms/feedlots in the Okanagan-Similkameen region, British Columbia.

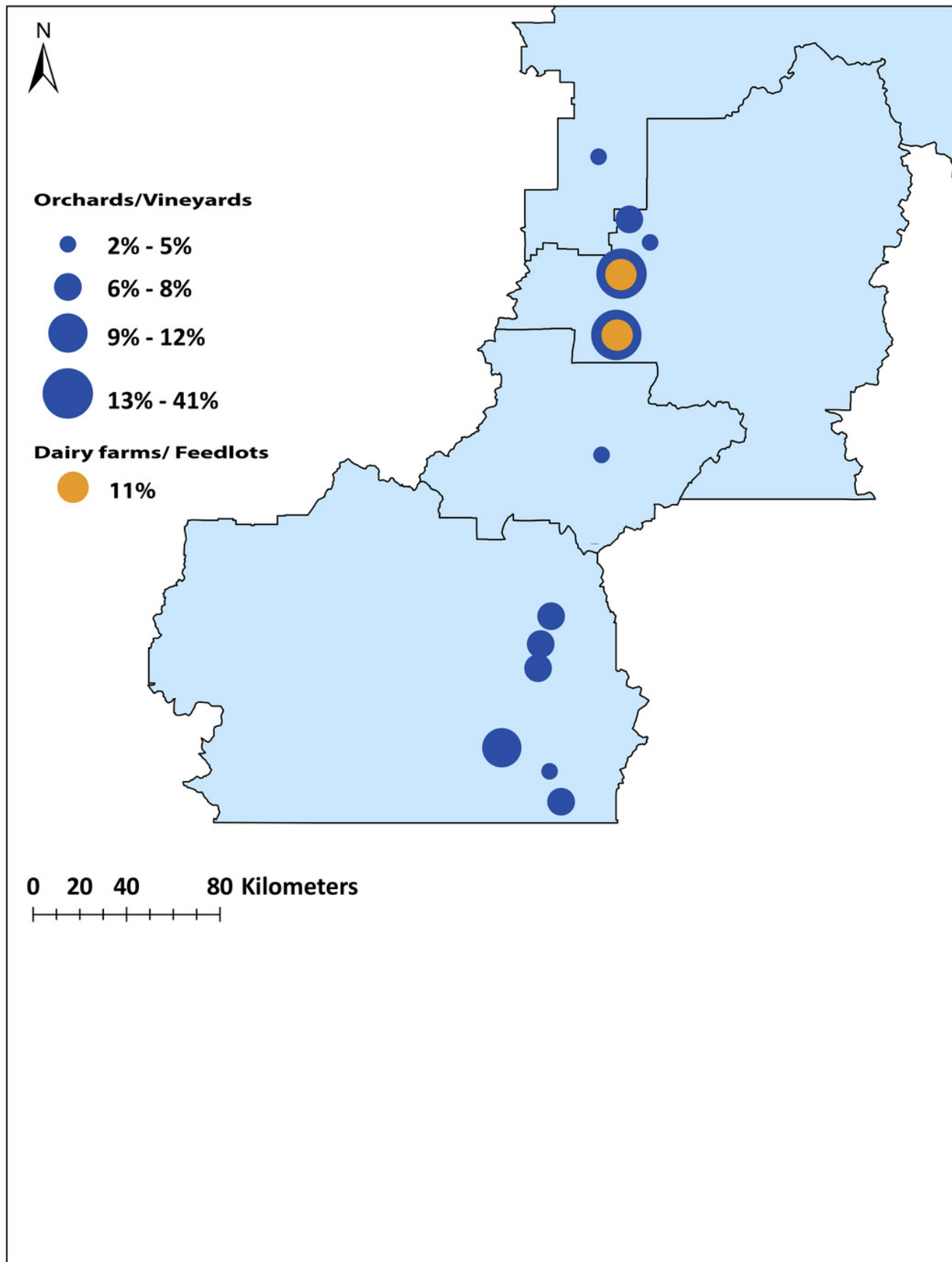
The radius of the circle depends on the percentage contributed from particular sites.

Orchards/Vineyards

- 2% - 5%
- 6% - 8%
- 9% - 12%
- 13% - 41%

Dairy farms/ Feedlots

- 11%

0   20   40   80 Kilometers

Classification results for juvenile starlings to its predicted locations in the Okanagan-Similkameen region, British Columbia.

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.

Year	Site	Predicted Group Membership (%)										No of Sample
		Kelowna	Hullcar	Salmon Arm	Armstrong	Mara	Vernon	Oliver	Osoyoos	Penticton/ Summerland	Keremeos	
2015	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