RESEARCH ARTICLE

Wildlife mortality	on an Ecuadorian	coastal highway

3	Catherine L. Woodward ^{1*} , Luis M. Fernández, and Joe E. Meisel
4	Ceiba Foundation for Tropical Conservation, 301 S. Bedford St, Suite 7A, Madison, WI
5	53703, United States; Phone: +1 608 230-5550. Juan Ramírez N36-14 y Germán Alemán,
6	170504, Quito, Ecuador; Phone: +593 2603-5904
7	29 September 2016

Transportation networks have direct and indirect impacts on the ecosystems they traverse. Roads have become an omnipresent element that modify and fragment ecosystems and divide animal populations. The most direct effect of roads is the death of the animals that try to cross them as they move between habitat patches. We studied wildlife mortality along a 63-km stretch of coastal highway in Manabí, Ecuador. We conducted 12 surveys from April 2014 to July 2015 recording every animal carcass visible within the roadway and its GPS coordinates. We found a total of 916 wild animal carcasses, comprised of 501 amphibians (54.7%), 225 birds (24.6%), 103 reptiles (11.2%), and 77 mammals (8.4%). Ten animals (1.1%) could not be identified. Assuming these surveys are representative of mortality rates throughout the year, we estimated that the total number of animals killed on this stretch of road is between 1,860 (assuming a 15-day carcass turnover rate (CTR)) and 9,300 (assuming a 3-day CTR). Our results indicate a wide variety of taxa affected, including several threatened

species. We identified road mortality "hotspots" in the region, and recommend

¹*Corresponding author. Email: cwoodward@ceiba.org

mitigation strategies such as traffics signs and wildlife crossing structures to reduce wildlife mortality in these locations.

Keywords: roadkill, road ecology, fauna, Ecuador.

Las redes de transporte tienen impactos directos e indirectos sobre los ecosistemas que atraviesan. Las carreteras se han convertido en elementos omnipresentes que modifican y fragmentan los ecosistemas y dividen las poblaciones de animales. El efecto más directo de las carreteras es la muerte de los animales que tratan de cruzarlas para moverse entre fragmentos de hábitat. Estudiamos la mortalidad de fauna silvestre a lo largo de 63 km de la autopista costera en Manabí, Ecuador. Realizamos 12 muestreos entre abril de 2014 y julio de 2015 registrando cada cadáver animal encontrado en la vía y sus coordinadas GPS. Encontramos un total de 916 animales silvestres atropellados, conformados por 501 anfibios (54,7%), 225 aves (24,6%), 103 reptiles (11.2%) y 77 mamíferos (8.4%). Diez animales (1,1%) no pudieron ser identificados. Asumiendo que estos muestreos son representativos de las tasas de mortalidad a lo largo del año, estimamos que el total de animales muertos por atropellamientos en este segmento de vía está entre 1860 (asumiendo una tasa de renovación del cadáver (CTR) de 15 días) y 9300 (asumiendo una CTR de 3 días). Nuestros resultados indican que una gran variedad de taxones están afectados, incluyendo varias especies amenazadas. Identificamos "hotspots" de mortalidad en la región y recomendamos varias estrategias de mitigación, tales como señales de tráfico y estructuras de paso de fauna para reducir la mortalidad de la fauna silvestre en estas localizaciones.

Palabras clave: mortalidad en la vía, ecología de carreteras, fauna, Ecuador.

Introduction

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

- 45 Transportation networks are usually planned based upon the potential economic and social
- development of the places they connect, and environmental impacts are rarely taken into

account unless they cross through natural or protected areas. These networks however, and roads in particular, result in major modifications of the ecosystems they pass through, with animal-vehicle collisions killing millions of animals globally each year [1-3].

Impacts of roads on abiotic ecosystem components include altered hydrology and sedimentation, increased noise level, and pollution [3–5]. Roads also have direct and indirect effects on the biotic components of ecosystems [6], including spread of invasive plants [3], and behavioral changes in animals [7], but animal mortality due to vehicular collisions is the main effect of roads on vertebrates [2,3], leading to the reduction of population sizes in amphibians [8], reptiles [9], birds [10], and mammals [11]. This direct impact is especially important in megadiverse countries like Ecuador due to the higher number of affected species and large numbers of rare endemics [5,12].

Fragmentation of habitat by roads increases edge effects, reducing the effective habitat area especially for edge-sensitive species [13]. Roads act as a barrier for the movement of wildlife [3, and references therein] thereby reducing genetic exchange and increasing the probability of local extinctions [14,15]. Although road ecology has developed into a discipline in its own right, data from South America are scarce [5]. Existing studies indicate that road mortality is a global threat to wildlife [5,16,17].

The main goal of this study is to determine the direct impact of an Ecuadorian coastal highway on terrestrial vertebrate fauna, and provide a baseline for monitoring future wildlife mortality as traffic increases to this developing tourism area. Specifically, we sought to identify the most affected taxa and the most critical wildlife crossing points along the highway in order to make recommendations for mitigating faunal mortality.

Material and methods

We conducted this study along the Pacific coastal highway in Ecuador (E-15) between April 2014 and July 2015. This 741 km long highway crosses three out of the five coastal provinces of the country (Esmeraldas, Manabí, and Santa Elena), from the Chocó region in the north to the Tumbes region in the south. We surveyed a 63-km stretch of highway between the towns of Pedernales and Rambuche, in northern Manabí province (Figure 1), that traverses threatened seasonally deciduous tropical forests and transitional ecosystems bridging these two ecoregions. The survey area lies within the Tumbes-Chocó-Magdalena biodiversity hotspot, and two Important Bird Areas (EC010 Hacienda Camarones and EC011 Tito Santos [18]). Although this road has been in place for several years it was paved and widened in 2013 [19]. In the area surveyed, the road is two lanes, with an average width of 9 m and an unenforced speed limit of 80 km/h. New housing and tourism developments in the region suggest that traffic will increase along this road in the future.

We carried out 12 surveys in a car moving at a constant speed of 30 km/h, with two observers checking both sides of the road, from curb to curb. For each carcass found, we classified it at least to Class whenever possible (e.g., amphibian, reptile, bird, or mammal) and recorded its coordinates (Garmin® GPSMap 78). We assumed that all animals found dead in the road were killed by collisions with vehicles. All domestic animals identified, mostly dogs, were not included in the analyses.

To estimate the annual roadkill rate (individuals killed/km/year) we used the following formula: (number of carcasses/length of road in km) * (365 days/number of days monitored). A prior ad hoc study in the region determined that carcasses decompose or are removed or consumed by scavengers in fewer than 15 days (J. Meisel, unpubl. data), and published studies have utilized a range of carcass turnover rates (CTR) [e.g., 2,16]. We therefore provide three estimates of annual roadkill reflecting high (CTR = 3 days), medium

(CTR = 7 days), and low (CTR = 15 days) carcass turnover rates, that are multiplied by the number of surveys to obtain "number of days monitored" in our formula.

To identify mortality hotspots, we overlaid each road kill point on a georeferenced map of the road system using QGIS (v 2.14) and ArcMap (v 10.3). Using a spatial join, we obtained the number of kills in each 0.5 km segment of road (n=126) for all kills combined as well as for each Class separately, and applied a square-root transformation to the count data to achieve a normal distribution. We defined hotspots as 0.5 km segments where number of kills exceeded two standard deviations (S.D.) above the mean (\overline{X}) of the transformed data. To create a map showing mortality hotspots, we assigned a color ramp using five bins separated by values equal to \overline{X} + 2 S.D., \overline{X} + 1 S.D., \overline{X} , and 0 (zero roadkills).

Results

In 12 surveys of this 63 km roadway we observed a total of 916 wild animal carcasses. The most affected taxa were amphibians (501 individuals; 54.7%), followed by birds (225 individuals; 24.6%), reptiles (103 individuals; 11.2%), and mammals (77 individuals; 8.4%). There were 10 individuals (1.1%) that were too decomposed or fragmented to be classified (Figure 2). We also encountered 23 domestic animals (21 dogs, one horse, and one chicken) that were excluded from the data analysis.

Assuming constant kill rates over the year, the lowest estimate of the annual roadkill rate (CTR = 15 days) is 29.5 roadkills/km, the medium estimate (CTR = 7 days) is 63.2 roadkills/km, and the highest estimate (CTR = 3 days) is 147.4 roadkill/km on this stretch of the road per year. These rates translate, respectively, to annual totals of 1857, 3980, and 9287 animals killed just on the 63 km length of the road we sampled. If roadkill rates are similar along its entire 741 km length, the number of wild animals killed each year on the Pacific

coastal highway of Ecuador could be as high as 109,235. Roadkill rates and annual mortality estimates by taxonomic groups can be found in Table 1.

Although most carcasses could be identified to broader taxonomic group (e.g., opossums), we were able to identify only 90 individuals definitively to genus or species, comprising 30 different taxa. Of them, the most commonly found in each Class were the cane toad (*Rhinella marinus*), the boa constrictor (*Boa constrictor*), anis (*Crotophaga* spp.), and the common opossum (*Didelphis marsupialis*). There were several examples of vulnerable species that were identified among the kills, including the Pacific royal flycatcher (*Onychorhynchus occidentalis*), the rufous-headed chachalaca (*Ortalis erythroptera*), and the Northern tamandua (*Tamandua mexicana*) [20, 21].

Hotspot mapping identified five 0.5-km segments with combined mortality across all taxa exceeding two standard deviations above the mean of the transformed data (global $\bar{X} = 2.36$, S.D. = 1.30, n = 126); some segments were contiguous, however, resulting in a total of two distinct hotspots (Figure 3A). When amphibians were excluded, the largest of the two hotspots disappeared, but new hotspots were revealed, reflecting road segments with high mortality of other taxa (Figure 3B). Hotspots separated by Class are shown in Figures 3C-F.

Discussion

Our study confirms that roads have a direct harmful effect on wildlife populations across many taxa. Concordant with other studies [22-24], amphibians were the hardest hit, representing over half of all roadkills. Because CTR may vary widely based on the abundance of scavengers, climate and weather conditions, removal by people, and inherent characteristics of the animal bodies, as well as the strongly aggregated pattern of mortality, amphibian roadkill may be most prone to underestimation. Amphibian carcasses are small in size and more likely to be missed by observers, to be removed in one piece by scavengers, or

to rapidly decompose [24-26]. In our study, however, we observed toad carcasses (*Rhinella marina*) to be extremely persistent, remaining on the road as desiccated crusts for several weeks, particularly in the dry season. Better CTR data are needed on a variety of taxa to improve the reliability of roadkill estimates. Still, we believe the CTR values we used offers a realistic range (21,847 to 109,235) for the number of wild animals killed each year along Ecuador's E-15 coastal highway.

The 23 domestic animal carcasses encountered represent just 2.4% of the total roadkills observed, but 25.9% of the identified mammals (22 out of 85). Our total roadkill estimates for wild animals would be slightly overestimated if a similar percentage of the 14 mammals in our sample that we could not identify plus the ten carcasses that could not be identified to any taxonomic level were in fact domesticated animals (ca. 6 animals). While it is obviously important to obtain accurate species identifications whenever possible and exclude domestic roadkills to obtain precise estimates, we found the vast majority of animal collisions with cars to involved wild animals.

Mapping the distribution of kills through hotspot analysis allowed us to identify critical crossing points where risk of mortality is high. Roadkills occurred throughout the entire length of road surveyed (only four 0.5-km segments had zero mortalities), but because amphibians comprised 54% of the data, they masked mortality hotspots of other taxa. Analysis of hotspots by Class revealed that mammal and reptile mortality were more evenly distributed, while amphibian and bird mortality were more strongly aggregated. There was no overlap between hotspots for amphibians and hotspots for mammals, suggesting that these groups utilize different parts of the landscape.

Analysis of the hotspot maps allow us to identify factors that may be predictive of high wildlife mortality. For example, the two hotspots for amphibian mortality are located in low-lying areas, where amphibians may congregate to breed. Hotspots for mammals seem to coincide with areas where forest is close to the road, although further analysis is needed to evaluate the relative contributions of proximity to forest versus other factors on roadkill rates. Mortality rates tended to be higher on straight stretches, perhaps because cars are travelling faster.

Identification of factors that affect mortality rates of specific taxonomic groups from hotspot maps can lead to efficient implementation of targeted mitigation strategies. Integrated approaches, such as speed control and signage may help reduce wildlife road mortality [27,28], although the success of road signs by themselves is limited [29,30]. Wildlife crossing structures, such as culverts, underpasses, or overpasses may be particularly beneficial to wildlife on roads through protected areas, where the higher diversity and abundance of animals, including threatened and/or endemic species, may translate to elevated impacts on biodiversity [31,32]. In a landscape already fragmented, like on the Ecuadorian coast, reducing barriers to the movement of wildlife becomes an urgent necessity to prevent decline and local extinction of animal populations [13,33,34].

We recommend culverts and underpasses be installed in hotspots of amphibian and mammal mortality. These are particularly effective when combined with fences along the road to guide the animals toward them [35]. Camera trapping has shown such structures are used by wildlife, and are an effective means of reducing animal-vehicle collisions and connecting wildlife habitat [36], although few "before" and "after" studies exist to allow direct evaluation of impact [37]. We recommend the installation of signage and velocity reduction devices (e.g., speed bumps) in straightaways with mortality hotspots. We hope that our study will provide the needed baseline for monitoring if our recommendations for mitigating wildlife mortality in coastal Ecuador are implemented.

189	Geolocation information
190	The northern end of the study area is the city of Pedernales (UTM 10007232 N, 0605361 E;
191	WGS84 zone 17S) and the southern end of the survey is the community of Rambuche
192	(9966571 S, 0574460 E). The approximate center is the Lalo Loor Dry Forest Reserve
193	(9991478 S, 0594199 E).
194	Acknowledgements
195	We are grateful to field assistants who assisted with data collection, including Stephanie
196	Bianco, Ryan Grist, Clea Harrelson, Michael Li, Marin Oschmann, Tomas Setubal, Kayla
197	Theis, Grace White, and Kylie Vanchena. We also thank the donors to the Ceiba Foundation
198	for Tropical Conservation who made this study possible, and the ESRI Nonprofit
199	Organization Program for reduced-cost software license.
200	Author contribution
201	Catherine L. Woodward co-designed the study, obtained funding, supervised field assistants,
202	conducted part of the surveys, and co-wrote the paper.
203	
204	Luis M. Fernández performed data preparation, carried out GIS and statistical analyses, and co-wrote
205	the paper.
206	
207	Joe E. Meisel co-designed the study, obtained funding, supervised field assistants, conducted some of
208	the surveys, carried out GIS analysis, and provided revisions on the paper.
209	References
210	[1] Lalo J. The problem of roadkill. Am For. 1987;50:50–52

[2] Forman RT, Sperling D, Bissonette JA, Clevenger AP, Cutshall CD, Dale VH, Fahrig L, 211 France R, Goldman CR, Heanue K, Jones JA, Swanson FJ, Turrentine T, Winter TC. 212 Road Ecology: Science and Solutions. Washington DC (CO): Island Press; 2003. 213 [3] Coffin AW. From roadkill to road ecology: A review of the ecological effects of roads. J 214 of Transp Geogr. 2007;15:396-406. 215 [4] Riley SJ. Effect of clearing and roading operations on the permeability of forest soils, 216 Karuah catchment, New South Wales, Australia. For Ecol and Manag. 1984;9(4):238– 217 218 293. 219 [5] Bager A, da Silva P, Bourscheit A, Kuczach A, Maia B. Os caminhos da conservação da biodiversidade brasileira frente aos impactos da infraestructura viária. Biodiversidade 220 221 Brasileira. 2016;6(1):75-86. [6] Bennett AF. Roads, roadsides and wildlife conservation: a review. In: Saunders DA, 222 223 Hobbs RJ, editors. Nature Conservation 2: The Role of Corridors. Chipping Norton: Surrey Beatty; 1991. p. 99–117. 224 [7] Jaeger JA, Bowman J, Brennan J, Fahrig L, Bert D, Bouchard J. Predicting when animal 225 populations are at risk from roads: an interactive model of road avoidance behavior. 226 Ecol Model. 2005;185(2-4):329-348. 227 228 [8] Fahrig L, Pedlar JH, Pope SE, Taylor PD, Wegner JF. Effect of road traffic on amphibian density. Biol Conserv. 1995;73:177-182. 229 230 [9] Boarman WI, Sazaki M. 1996. Highway moratality in desert tortoises and small vertebrates: success of barrier fences and culverts. In: Evink G, Zeigler D, Garrett P, 231 Berry J, editors. Transportation and wildlife: reducing wildlife mortality and 232

improving wildlife passageways across transportation corridors. Washington DC

(CO): U.S. Department of Transportation, Federal Highway Administration; 1996. 234 p.169–173. 235 236 [10] Ramsden DJ. 2003. Barn Owls and Major Roads: results and recommendations from a 15-year research project. Ashburton (UK): The Barn Owl Trust; 2003. 237 [11] Ferreras P, Aldama JJ, Beltrán JF, Delibes M. Rates and causes of mortality in a 238 fragmented population of Iberian Lynx Felis pardina Temminck, 1824. Biol Conserv. 239 1992;61(3):197–202. 240 241 [12] Mittermeier RA, Robles P, Mittermeier CG. Megadiversity. México City: CEMEX; 1997. 242 [13] Fahrig L. 2003. Effects of habitat fragmentation on biodiversity. Annu Rev of Ecol, 243 Evol, and Syst. 2003;34:487-515. 244 [14] Johnson WC, Collinge SK. Landscape effects on black-tailed prairie dog colonies. Biol 245 Conserv. 2004;115:487-497. 246 247 [15] Simmons JM, Sunnucks P, Taylor AC, van der Ree R. 2010. Beyond roadkill, 248 radiotracking, recapture and F_{ST} – A review of some genetic methods to improve understanding of the influence of roads on wildlife. Ecol and Soc. 2010;15(1):9. 249 [16] Quintero-Ángel A, Osorio-Domínguez D, Vargas-Salinas F, Saavedra-Rodríguez CA. 250 2012. Roadkill rate of snakes in a disturbed landscape of Central Andes of Colombia. 251 Herpetol notes. 2012;5:99-105. 252 253 [17] Seijas AE, Araujo-Quintero A, Velásquez N. Mortalidad de vertebrados en la carretera Guanare-Guanarito, estado Portuguesa, Venezuela. [Vertebrate mortality in the 254 Guanare-Guanarito road, Portuguesa State, Venezuela]. Rev de Biol Trop. 255 256 2013;61(4):1619–1636.

257	[18] Santander T, Freile JF, Loor-Vela S. Ecuador. In: Devenish C, Díaz-Fernández DF, Clay			
258	RP, Davidson I, Yépez-Zabala, I, editors. Important Bird Areas Americas – Priority			
259	sites for biodiversity conservation. Quito: Birdlife International; 2009. p. 187–196.			
260	[19] Secretaría Nacional de Planificación y Desarrollo. Proyectos emblemáticos en Manabí.			
261	[Emblematic projects in Manabí]. Montecristi: Secretaría de Planificación y			
262	Desarrollo. Gobierno del Ecuador; 2003;p.88.			
263	[20] Tirira DG. Libro rojo de los mamíferos del Ecuador. [Red book of Ecuadorian			
264	mammals]. 2nd ed. Quito: Fundación Mamíferos y Conservación, Pontificia			
265	Universidad Católica del Ecuador y Ministerio del Ambiente. Publicación especial			
266	sobre los mamíferos del Ecuador 8; 2011.			
267	[21] Birdlife International. The IUCN Red List of Threatened Species 2012:			
268	e.T22678318A37858144. http://dx.doi.org/10.2305/IUCN.UK.2012-			
269	<u>1.RLTS.T22678318A37858144.en</u> . Downloaded on 12 September 2016.			
270	[22] Puky M. Amphibian road kills: a global perspective. In: Irwin CL, Garrett P, McDermott			
271	KP, editors. Proceedings of the 2005 International Conference on Ecology and			
272	Transportation. Raleigh: Center from Transportation and the Environment, North			
273	Carolina State University; 2006. p. 325–338.			
274	[23] Glista DJ, DeVault TL, DeWoody JA. 2008. Vertebrate road mortality predominantly			
275	impacts amphibians. Herp Cons and Biol 2008;3(1):77-87.			
276	[24] D'Anunciação PE, Lucas PS, Silva VX, Bager A. Road ecology and Neotropical			
277	amphibians: contributions for future studies. Acta Herp 2013;8(2):129-140.			
278	[25] Antworth RL, Pike DA, Stevens EE. Hit and run: effects of scavenging on estimates of			
279	roadkilled vertebrates. Southeast Nat 2005;4(4):647-656.			

280	[26] González-Gallina A, Benítez-Badillo G, Rojas-Soto OR, Hidalgo-Mihart MG. The
281	small, the forgotten and the dead: highway impact on vertebrates and its implication
282	on mitigation strategies. Biodivers Cons 2013;22:325–342.
283	[27] Forman RT, Alexander LE. Roads and their major ecological effects. Annu Rev of Eco
284	and Syst 1998;29:207–231.
285	[28] Colino-Rabanal VJ, Lizana M, Peris SJ. 2011. Factor influencing wolf Canis lupus
286	roadkills in Northwest Spain. Eur J of Wildl Res 2011;57:399-409.
287	[29] Sullivan TL, Messmer TA. Perceptions of deer-vehicle collision management by state
288	wildlife agency and department of transportation administrators. Wildl Soc Bull
289	2003;31:163–173.
290	[30] Grilo C, Bissonette JA, Cramer PC. Mitigation measures to reduce impacts on
291	biodiversity. In: Jones SR. Highways: Construction, Management, and Maintenance.
292	Nova Science Publishers, Inc.; 2010. p. 73-114.
293	[31] Bager A. Repensando as medidas mitigadoras impostas aos empreendimentos viários
294	associados às unidades de conservação. [Rethinking the mitigation measures imposed
295	on road projects associated with the conservation units] In: Bager A., editor. Áreas
296	Protegidas. Conservação no âmbito do Cone Sul. [Protected Areas. Conservation in
297	the Southern Cone]. Pelotas: Editor's edition; 2003. p. 159–172.
298	[32] Bager A, Fontoura V. Evaluation of the effectiveness of a wildlife roadkill mitigation
299	system in wetland habitat. Ecol. Eng. 2013:53:31-38.
300	[33] Yanes M, Velasco J, Suarez F. Permeability of roads and railways to vertebrates: the
301	importance of culverts. Biol Cons. 1995;71(3):217–222.

302	[34] De Meester L, Declerck S, Stoks R, Louette G, Van de Meutter F, Bie TD, Michels E,
303	Brendonck L. Ponds and pools as model systems in conservation biology, ecology and
304	evolutionary biology. Aquat Cons: Mar and Freshw00 Ecosyst. 2005;15:715-725.
305	[35] Dodd CK, Barichivich WJ, Smith LL. 2004. Effectiveness of a barrier wall and culverts
306	in reducing wildlife mortality on a heavily traveled highway in Florida. Biol Cons.
307	2004;118:619-631.
308	[36] Donaldson BM. Use of highway underpasses by large mammals and other wildlife in
309	Virginia and factors influencing their effectiveness. In: Proceedings of the
310	International Conference of Ecology and Transportation. San Diego (CA); 2005. p.
311	433–441.
312	[37] Glista DJ, DeVault TL, DeWoody JA. 2009. A review of mitigation measures for
313	reducing wildlife mortality on roadways. Landsc and Urban Plan. 2009; 91:1-7.

Table 1. Total number of carcasses found, roadkill rates (individuals/kilometer/year), and annual estimates of kills per year (individuals) for each taxonomic group based on low (3 days), medium (7 days), and high (15 days) carcass turnover rates along the 63-km stretch of road sampled.

Taxa	Carcasses		Roadkill rate (ind/km/year)	Annual estimate (individuals)
	501	Low	16.1	1015.9
Amphibians		Medium	34.6	2177.0
		High	80.6	5079.6
		Low	3.3	208.9
Reptiles	103	Medium	7.1	447.6
		High	16.6	1044.3
	225	Low	7.2	456.3
Birds		Medium	15.5	977.7
		High	36.2	2281.3
	77	Low	2.5	156.1
Mammals		Medium	5.3	334.6
		High	12.4	780.7
	916	Low	29.5	1857.4
Total		Medium	63.2	3980.2
		High	147.4	9287.2

319 **Figures**

318

321

314

315

316

- Figure 1. Study area. This satellite image shows the sampled 63 km length of the Ecuadorian 320 coastal highway (E-15, highlighted in yellow) in the province of Manabí.
- Figure 2. Percentage of carcasses (out of 916 total) found during roadkill surveys, by Class. 322
- Figure 3. Hotspot maps of wildlife mortality for: A) All taxa, B) All minus amphibians, C) 323
- Amphibians, D) Mammals, E) Reptiles, and F) Birds. 324