

1 **An evaluation of the role of “biological evidence” in zoo and aquarium enrichment practices**

2 **Running title:** *Evidence use in zoo enrichment*

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4 **Abstract**

5 Evidence-based approaches are key to advancing all areas of zoo and aquarium practice. Output from
6 empirical study must be disseminated to those within the industry so that results can support changes
7 to husbandry and management for individual species. Information on enrichment techniques is
8 published in a range of sources, including papers in the peer-reviewed and ‘grey literature’ (i.e.
9 professional but non-reviewed publications). To investigate how evidence is implemented into
10 enrichment practices, we sampled all enrichment studies identified in one online repository of peer-
11 reviewed papers and two grey literature publications across an 11-year period. We recorded whether
12 the enrichment was supported with biological evidence (whether it was developed using published
13 enrichment-focussed research for that species and/or with the species’ ecology and behaviour
14 information) alongside analysis of the type of enrichment used and the chosen study species.
15 Enrichment articles were more likely to be supported by biological evidence in peer-reviewed than
16 grey literature. Taxonomic differences in the use of evidence were noted; for example, enrichment
17 provided to carnivores and parrots was more likely to be supported with biological evidence
18 compared to that used for penguins. Of the five enrichment types, nutritional enrichment was most
19 often based on biological evidence. Multi-category and physical enrichment types were more common
20 across all literature sources whereas social enrichment was less common, suggesting barriers to
21 implementation of all enrichment types in zoological facilities. Our research suggests that zoo and
22 aquarium professionals are considering species-specific welfare needs by ensuring that enrichment
23 protocols are supported by biological evidence. However, opportunities to diversify the enrichment
24 types being offered and species being researched are identified.

25 **Keywords:** Animal welfare; Environmental enrichment; Evidence-based approaches; Zoo animal
26 management; Zoo research

27 **1. Introduction**

28 The concept of evidence-based captive animal management has been gathering momentum in recent
29 years (Melfi 2009; Kaufmann et al 2019; Rose et al 2019a) and zoological facilities are increasingly
30 using empirical study to inform their husbandry practices. One of the underpinning roles of modern
31 animal collections is to uphold practices that promote animal welfare. Evidence from zoo literature
32 can inform professionals about successful or relevant management techniques, which can provide
33 support for the development of more advanced, species-specific husbandry approaches (Shyne 2006).
34 A key area of captive animal husbandry where application of evidence is integral to improving
35 welfare (and ameliorating poor welfare) states is the use of species-appropriate environmental
36 enrichment (EE).

37 EE is described as the provision of novel stimuli into an animal's environment (Swaigood &
38 Shepherdson 2005) and its use has been identified as an important component of good husbandry for
39 many captive-housed species (Fernandez et al 2019). While animal enclosures may sometimes be
40 sufficiently stimulating that EE is not necessary, EE can provide numerous benefits for animals
41 including cognitive challenge (Meehan & Mench 2007; Hopper et al 2016), opportunities to express
42 natural behaviour, reduction of abnormal repetitive behaviour (Mason et al 2007), improvements to
43 physical and psychological health and enhanced behavioural flexibility (Swaigood & Shepherdson
44 2005). Since its development as a husbandry practice during the 20th century, the body of literature on
45 EE has grown such that researchers can initiate structured analysis of EE topics to further refine its
46 application to the zoo and aquarium (Riley & Rose 2020; Shyne 2006; Swaigood & Shepherdson
47 2005).

48 All taxa may benefit from an enriched captive environment that allows performance of behavioural
49 diversity and that promotes positive affective states (Rose et al 2017a, 2017b, 2019a). As some
50 taxonomic groups are particularly susceptible to the development of abnormal repetitive behaviour
51 when housed in captivity, EE has often been implemented as a preventative or treatment strategy
52 (Shyne 2006). Focus on the necessity of EE for big cats (Felidae), bears (Ursidae) and primates
53 (Regaiolli et al 2019; Fernandez & Timberlake 2019) shows the importance of empirical study of EE

54 techniques to improve and enhance animal welfare at the species and individual level. Taxa such as
55 primates and Carnivora have received more research attention in response to an increased likelihood
56 of performing abnormal behaviour in the zoo (Clubb & Mason 2003; Mason et al 2007). Behavioural
57 indicators of good welfare may be easier for welfare researchers to interpret for such taxa, as a bias
58 towards the study of mammals in pure and applied behavioural science suggests a greater familiarity
59 with their ecology (Melfi 2009; Mather 2019; Rose et al 2019a), therefore increasing the research
60 attention they receive.

61 Inference of welfare state in reptiles, amphibians, fish or invertebrates appears more challenging than
62 for endotherms. This challenge may be because outward visible signs of welfare state are often subtle
63 and environmental variables (such as temperature) strongly influence activity levels and behaviour
64 (Burghardt 2013; Bashaw et al 2016; Rose et al 2017, 2017b). If key welfare indicators are unknown
65 or hard to identify, the difficulties for researchers in their attempts to identify effective EE strategies
66 are increased (Greenway et al 2016). An animal's personality may also influence the way in which it
67 interacts with EE (Pich et al 2019). Personality dimensions are not well studied in many taxa (Pich et
68 al 2019) so the requirements of some taxonomic groups for EE complexity may be underestimated
69 (Riley & Rose 2020).

70 *1.1. Increasing the impact of EE research and applying evidence more widely.*

71 A key aim of published EE literature is to inform practitioners of its effectiveness in enhancing animal
72 welfare (Rose et al 2019a). Reports on effective and ineffective EE strategies have merit as they both
73 inform practitioners on the suitability of current EE techniques (Claxton 2011). Any improvement and
74 diversification of EE can be measured in the scientific literature; for example, Lutz and Novak (2005)
75 proposed the use of several forms of EE for primates, including touch-screen technology. At the time
76 of publication, touch-screen technology was relatively novel and its use for non-human primates had
77 rarely been proposed. At the time of writing, Lutz and Novak's 2005 paper has received 159 citations,
78 several of which have applied touch-screen computers to non-human primates (e.g. Ritvo & Allison
79 2017; Wooddell et al 2019; Huskisson et al 2020). This shows how EE concepts, once communicated
80 within the scientific literature, may be developed, modified and shared globally with practitioners.

81 Evidence for EE practices that enhance animal welfare can be obtained from the literature and
82 implemented into animal husbandry techniques. The sharing of EE research can allow practitioners to
83 adapt existing EE techniques to novel subjects (Rose et al 2016). For example, auditory EE, which
84 had originally been trialled for use in kennel-housed domestic dogs (*Canis lupus familiaris*) and
85 primates can be successfully repurposed to use for parrots and great apes (Ritvo & Macdonald 2016;
86 Williams et al 2017). Such work provides a foundation for further EE research to fully realise the
87 welfare benefits of evidence-based EE.

88 It is likely that EE is implemented for a wider array of species than appear in peer-reviewed literature
89 (Rose & Riley 2020). There may be barriers that prevent practitioners from publishing their work with
90 EE in peer-reviewed journals, for example time to write up outside of their working day. Other
91 platforms may be more commonly utilised for sharing EE concepts and their effectiveness. Such
92 platforms document case studies on specific populations or individual animals and provide valuable
93 assessment of EE efficacy (Hoy et al 2010) because they showcase the strategies most commonly
94 used by animal keepers to implement and assess EE, and they provide information on welfare
95 assessment strategies across taxa. To further bridge the gap between EE theory and EE practice, first
96 identified by papers such as Shyne (2006), investigation of what the gap currently is would be
97 beneficial.

98 Papers published in the grey literature (e.g. non-peer-reviewed articles written by professionals or
99 subject specialists for publications such as the *Shape of Enrichment*
100 <https://theshapeofenrichmentinc.wildapricot.org/> or *Wild Welfare* <https://wildwelfare.org/>) may
101 provide an overview of the current state of practice for EE use in zoos and aquaria, as such platforms
102 may be more accessible to animal keepers to both submit their work and to gain ideas and knowledge.
103 Other forms of non-peer reviewed literature available to zookeepers include zoo association
104 magazines and the proceedings from relevant symposia and workshops. All of these sources are noted
105 as containing information on EE usage across zoo-housed taxa.

106 In order to determine how relevant EE is for improving husbandry standards and welfare states in zoo
107 and aquarium housed species, this research evaluates the extent that EE is informed by evidence from

108 available literature. We summarised the current trends in published research regarding types of EE
 109 investigated and the taxonomic groups most commonly investigated/provided with EE. We also
 110 investigated the use of such published studies by other researchers, evaluating the impact (based on
 111 citations of a specific piece of work) of peer-reviewed EE-focussed science. We analysed the
 112 prevalence of EE studies in (zoo-specific) “grey literature” to determine whether EE practices are
 113 being informed by existing research output.

114 **2. Materials and methods**

115 EE papers from 1 January 2008 to 31st December 2019 were Sourced from three repositories: the
 116 database Web of Science©, *Ratel* (the journal produced by the Association of British and Irish Wild
 117 Animal Keepers, ABWAK 2020), and *Animal Keepers’ Forum* (the journal of the American
 118 Association of Zoo Keepers, AAZK 2020). Web of Science represents peer-reviewed literature,
 119 whereas *Ratel* and the *Animal Keeper’s Forum* represent more practical information available to
 120 keepers and aquarists, which is referred to throughout the course of the paper as grey literature.

121 Results were first categorised by the animal Class studied in each paper (mammal, bird, reptile,
 122 amphibian, fish and invertebrate). A final category, “multi”, consisted of papers with a focus on more
 123 than one taxonomic group. Next, the number of papers using each type of EE as per Bloomsmith et
 124 al’s (1991) five categories, which are nutritional, occupational, physical, sensory and social (Table 1).
 125 Two further categories, training and multi, were identified. Debate amongst authors (Melfi 2013;
 126 Westlund 2014) stipulates that operant conditioning and positive reinforcement training in the zoo can
 127 be EE but should be provided alongside of conventional EE techniques for the specific species.

128 *Table 1. Enrichment categories and examples.*

Enrichment category	Example	Reference
Nutritional	A novel food source or different feed presentation type, requiring the animal to spend more time processing its meal.	Wooddell et al 2019

Occupational	A puzzle or task provided to an animal that requires it to solve a problem.	Field and Thomas 2000
Physical	Enclosure features (fixed or temporary) that require the animal to exert effort, such as climbing ropes.	Bloomsmith et al 1991
Sensory	A scent trail, using herbs or spices to guide animal around exhibit.	Resende et al 2011
Social	Visual and auditory contact with conspecifics that the animal is not normally exposed to.	Reinhardt et al 2013
Training	Use of a stick (target) to guide an animal around its exhibit, using food rewards for positive reinforcement.	Melfi 2013
Multi	Use of multiple forms of enrichment, such as when comparing the use of a puzzle feeder and a scent trail.	Swaigood and Shepherdson 2005

129

130 For all suitable articles, irrespective of their Source, the species, Order, Family, Aim and Outcome
131 were recorded. Consideration was also given as to whether EE had been developed in light of the
132 species' natural behaviour and biology. Biological evidence (BE) was defined as the use of natural
133 history and behavioural ecology evidence when developing EE. Use of BE was determined when the
134 EE described in a paper was based on behavioural ecology and/or on previous empirical study of EE
135 usage for that species. An example would be EE that enabled naturalistic foraging behaviours for
136 captive parrots being based on key natural history information (Field & Thomas 2000). Papers that
137 described EE that did not clearly explain natural history or encourage natural behaviours (e.g. use of
138 submerged disco balls for penguins with no link to the encouragement of natural behaviour) were
139 classed as non-BE.

140 The Aims (i.e. the point of doing the EE research) and Outcomes of the article were identified, as
141 specified by each paper's authors. These Aims and Outcomes were then categorised as per Rose et al
142 (2019a). The Aim categories used for this study were Behaviour, Cognition, Husbandry & Training,

143 Nutrition, Veterinary Medicine, or Methods (how to collect data in a specified situation). Where a
144 study had multiple Aims, the primary aim (as stated by the paper’s author) was used.

145 Outcomes were described as suggested consequences of the paper. The categories for Outcomes were
146 Animal and Ecosystem Health, Husbandry and Welfare, Pure Biology and Scientific Validity (Rose et
147 al 2019a). The Outcome gains were categorised as advancing knowledge or practical application,
148 either at a specific (single population or species) or general level.

149 *2.1. Web of Science®*

150 Raw data were used retrospectively from a previous study published in Palgrave Communications in
151 2019 (Rose et al 2019b) where the dataset for this paper is publicly available
152 (<https://doi.org/10.24378/exe.1903>). To collect these data, Web of Science was searched from 2008 to
153 2019 using the key words of either “zoo” or “aquarium”, followed by the term “enrichment”.
154 Additionally, the terms “mammal OR bird OR reptile OR amphibian OR fish OR invertebrate” were
155 added to “zoo enrichment” or “aquarium enrichment”, to ensure that all relevant papers were
156 identified. Papers were included in the dataset if their focus was on some form of EE in animals held
157 in zoos, aquariums or wildlife parks. Purely theoretical papers and reviews were also included within
158 the dataset. From each relevant paper, the species or taxa being covered was included, in addition to
159 the year, journal, journal’s impact factor, the number of citations that the article has received, and the
160 number of times other authors used the article to develop further EE studies.

161 *2.2. Ratel and Animal Keeper’s Forum*

162 Archived copies of ABWAK’s magazine *Ratel*, and the AAZK’s *Animal Keeper’s Forum* were
163 manually searched for articles investigating the use of EE. All issues were searched from January
164 2008, to December 2018, resulting in 10 years of data for each Source. Articles were included if the
165 title or methods included consideration of the use of EE.

166 *2.3. Data Analysis*

167 Data were analysed in Minitab v.19 (www.minitab.com/en-us/products/minitab/). A total of 295 data
168 entries were generated from the three different Sources of EE literature: Web of Science (167,
169 56.61%), *Ratel* (45, 15.25%) and *Animal Keeper's Forum* (83, 28.13%).

170 To analyse the number of papers that used BE for the EE documented, a binary logistic regression
171 was run that compared BE in the peer-reviewed and grey literature across time. Age of the paper
172 (years), taxonomic Class, type of EE described and Source (i.e. peer-reviewed or grey literature) were
173 predictors of use of BE. The consideration (1) or lack (0) of BE in each type of publication (peer-
174 reviewed or grey literature) was the Outcome variable.

175 To investigate the predictors of 'Aim category', a nominal logistic regression was run. The Aim
176 categories of Behaviour, Husbandry & Training and Welfare were used in the model and any
177 remaining categories were excluded as they were not found in both literature types. The Outcome
178 variable was the 'Aim category' of each paper, and the predictors were the age of paper (years), paper
179 Source (peer-reviewed or grey literature), BE, taxonomic Class, and type of EE described.

180 To analyse the predictors of a paper's total number of citations (i.e. the number of times a paper was
181 cited in other peer-reviewed Sources), a Poisson regression was run. The outcome variable was the
182 paper's total number of citations, and the predictors included were age (years), taxonomic Class, type
183 of EE and the journal's Impact Factor. The interaction between the age of a paper and its impact
184 factor was also included as a predictor as impact factor changes over time. This analysis was run only
185 on the peer-reviewed literature, as citation numbers were not available for grey literature.

186 For models where multiple P values were compared, a corrected level of significance was calculated
187 using the Bejamini-Hochburg method to test for any false discovery of significance (Benjamini &
188 Hochberg 1995). Based on this corrected significance level, new probability values were calculated (Q
189 values) and presented alongside of the original P values. Significant Q values in all cases are
190 highlighted with the asterisk symbol. In each case, model fit was determined using generated r^2
191 values.

192

193 **3. Results**

194 3.1. *Biological evidence*

195 The output of the binary logistic regression revealed that a paper’s Source (i.e. the repository where
 196 the paper was found) and the type of EE were significant predictors of whether or not a paper used
 197 BE enrichment, whereas paper age and taxonomic Class were not ($\chi^2(286) = 271.15, r^2 = 13.31\%, P =$
 198 0.004). P values for each predictor were compared to a corrected alpha level of 0.03 and significant Q
 199 values are marked with a * (Table 2). Papers published in Web of Science were more likely to contain
 200 EE based on evidence compared to those published in AKF.

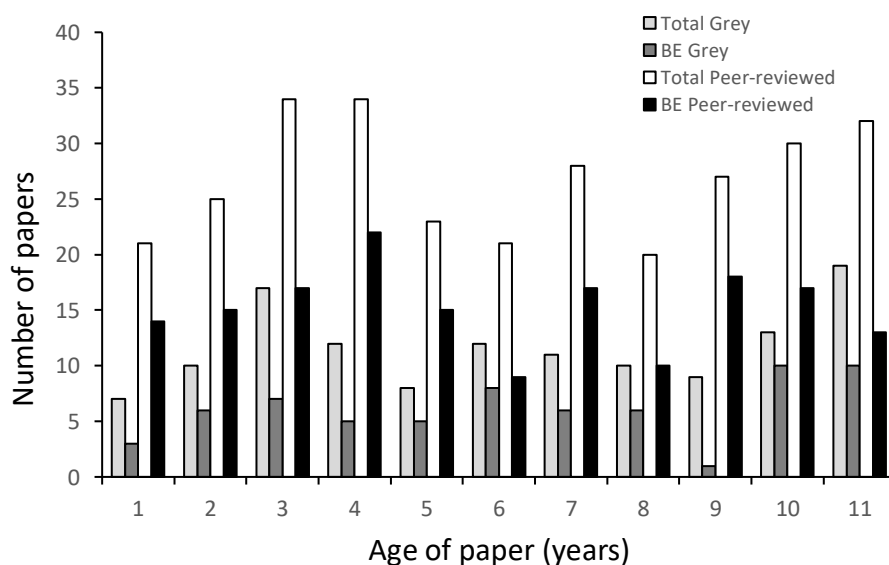
201 *Table 2. Output of binary logistic regression on use of BE for documented EE practices.*

Predictor	Variable	Estimate	Standard error	DF	P value	Q value
Age (years)	1	0.480	0.769	10	0.131	0.0375
	2	0.391	0.675			
	3	-0.235	0.664			
	4	-0.176	0.704			
	5	0.972	0.771			
	6	0.011	0.698			
	7	1.209	0.821			
	8	0.621	0.733			
	9	1.387	0.743			
	10	-0.532	0.680			
Class	Amphibian	19	0.197	7	0.506	0.05
	Bird	10	0.197			
	Fish	8	0.197			
	Invertebrate	21	0.241			
	Mammal	9	0.197			

	Reptile	11	0.279			
Source	Web of Science	1.357	0.324	2	<0.001	0.0125*
	Ratel	0.773	0.455			
	Animal	-0.578	0.346			
	Keeper's forum					
Type of enrichment	Nutritional	0.626	0.507	7	0.003	0.025*
	Occupational	-1.146	0.731			
	Physical	-0.050	0.418			
	Sensory	-0.554	0.452			
	Social	-0.350	0.708			
	Training	-2.221	0.581			

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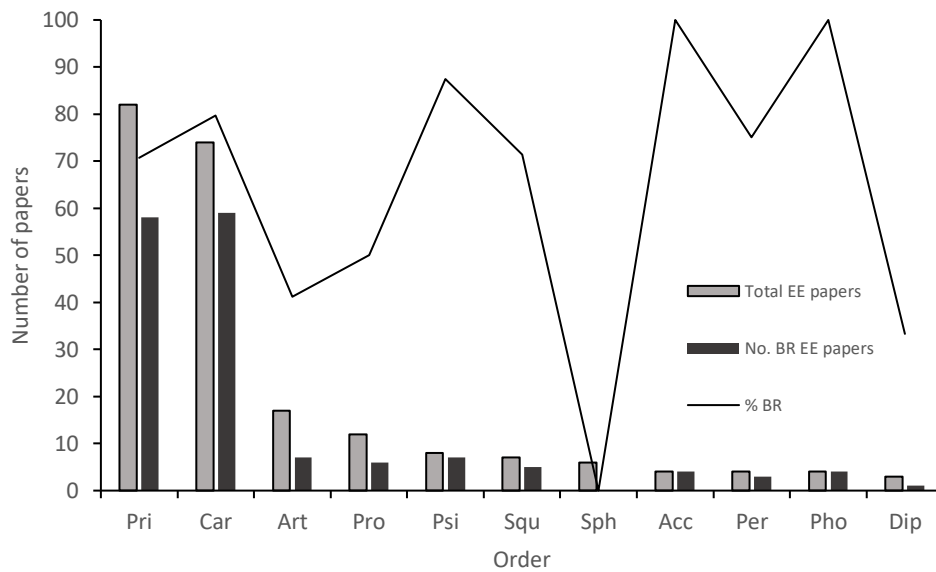
203 The number of papers with BE enrichment was tracked across time for both peer-reviewed and grey
 204 literature (Figure 1). EE papers in peer-reviewed literature were more likely to have a biological basis.
 205 Whilst taxonomic Class was not a significant predictor of BE use, there were differences apparent in
 206 the publication trend for different taxonomic Orders (Figure 2) with some species (e.g.
 207 Sphenisciformes, penguins) showing no use of BE and others (e.g. Phoenicopteriformes, flamingos,
 208 and Accipitriformes, birds-of-prey) showing that all described EE in a paper was based on BE.



209

210 *Figure 1. Total number of papers per year for grey and peer reviewed literature, and the number of*
 211 *papers that use BE EE. The ‘total grey’ and ‘total peer-reviewed’ boxes show the total number of*
 212 *papers published per year on enrichment for these sources. The ‘BE grey’ and ‘BE peer-reviewed’*
 213 *boxes show the number of papers that used BE enrichment in that year.*

214



215

216 *Figure 2. Total number of papers for the most frequently investigated taxonomic orders (as defined*
 217 *by any order that has been the focus of three or more papers), and the number of papers which*
 218 *included BE enrichment. Key: Pri = Primates, Car = Carnivora, Art = Artiodactyla, Pro =*
 219 *Proboscidea, Psi = Psittaciformes, Squ = Squamata, Sph = Sphenisciformes, Acc = Accipitriformes,*
 220 *Per = Perissodactyla, Pho = Phoenicopteriformes, Dip = Diprotodontia.*

221

222 3.2. Characteristics of peer-reviewed and “grey” literature

223 The “husbandry and training” category was the most common aim for EE papers, irrespective as to
 224 whether they were from peer reviewed or grey literature sources (Table 3). Similarly, the “husbandry
 225 and welfare” category was the most common output for EE studies regardless of their source.

226 *Table 3. Number of papers with each Aim and Outcome category for all sources of information.*

	Category	Animal Keeper's Forum number of papers (%)	Ratel number of papers (%)	Web of Science number of papers (%)	Total number of papers (%)
Aim	Behaviour	4 (1.36)	2 (0.68)	10 (3.39)	16 (5.42)
	Cognition	0	0	2 (0.68)	2 (0.68)
	Husbandry & training	63 (21.36)	34 (11.53)	139 (47.12)	236 (80.0)
	Methods	0 (0)	0 (0)	9 (3.05)	9 (3.05)
	Physiology	0 (0)	0 (0)	3 (1.02)	3 (1.02)
	Visitor studies	1 (0.34)	0 (0)	2 (0.68)	3 (1.02)
	Welfare	15 (5.08)	9 (3.05)	2 (0.68)	26 (8.81)
Outcome	Behaviour change (Human)	0 (0)	0 (0)	1 (0.34)	1 (0.34)
	Conservation & sustainability	0 (0)	0 (0)	5 (1.69)	5 (1.69)
	Husbandry & welfare	81 (27.46)	45 (15.25)	151 (51.19)	277 (93.90)
	Pure biology	0 (0)	0 (0)	6 (2.03)	6 (2.03)
	Scientific validity	2 (0.68)	0 (0)	4 (1.36)	6 (2.03)

227 The results of the nominal logistic regression investigating the predictors of Aim category indicated
228 that only the source, Web of Science, was significant with all other predictors non-significant ($\chi^2(243)$
229 = 327.192, P = 0.007) (Table 4). However, once multiple P values were inputted into a Benjamini-
230 Hochburg correction factor to check for false discovery at a new significance level of 0.019, this
231 significant value for Web of Science now only approaches significance.

232 *Table 4. Output of nominal logistic regression on the Outcome of Aim categories of Behaviour,*
233 *Husbandry & Training, and Welfare.*

Predictor	Variable	Estimate	Standard error	DF	P value	Q value
Age (years)	1	-1.307	1.466	10	0.373	>0.05
	2	-1.773	1.313		0.177	>0.05
	3	19.117	0.676		0.998	>0.05
	4	-1.994	1.418		0.160	>0.05
	5	-2.177	1.386		0.116	>0.05
	6	18.515	0.783		0.998	>0.05
	7	-1.309	1.530		0.393	>0.05
	8	-2.303	1.453		0.113	>0.05
	9	-0.176	1.668		0.916	>0.05
	10	-0.480	1.441		0.760	>0.05
Class	Amphibian	-24.166	65760.7	7	1.000	>0.05
	Bird	21.626	0.461		1.000	>0.05
	Fish	-21.273	0.461		1.000	>0.05
	Invertebrate	-1.023	0.065		1.000	>0.05
	Mammal	21.225	0.461		1.000	>0.05
	Reptile	22.571	0.461		1.000	>0.05
Source	Web of Science	2.514	0.837	2	0.003*	0.002
	Ratel	-0.480	0.648		0.526	>0.05
	Animal	-0.492	0.624		0.459	>0.05
	Keeper's forum					
Type of enrichment	Nutritional	-17.498	0.109	7	0.761	>0.05
	Occupational	34.930	0.055		0.999	>0.05
	Physical	0.191	1.899		0.106	>0.05
	Sensory	17.945	0.611		0.509	>0.05
	Social	4.101	1.693		0.110	>0.05

	Training	-16.5543	0.756		0.841	>0.05
Biological basis	Yes/No	0.831	0.576	1	0.149	>0.05

234

235 *3.3. Predictors of citations in peer-reviewed literature*

236 Results of the Poisson regression ($\chi^2(155) = 629.58, r^2 = 63.32 P < 0.001$) indicated that taxonomic
 237 Class, impact factor, type of EE and the interaction between impact factor and age of paper were
 238 significant predictors of total citation number (Table 5). For each predictor, multiple P values were
 239 compared to a corrected alpha level of 0.04 and significant Q values highlighted. Whilst not a
 240 significant factor under the corrected alpha level for this model, the model estimates for age show an
 241 increase in the number of times a paper is cited over time. Species representation is similar across the
 242 study period with the exception of Psittaciformes (parrots), which only appeared in more recent (<6-
 243 year-old) papers.

244 *Table 5. Model output to identify significant predictors of the number of citations per peer reviewed*
 245 *paper.*

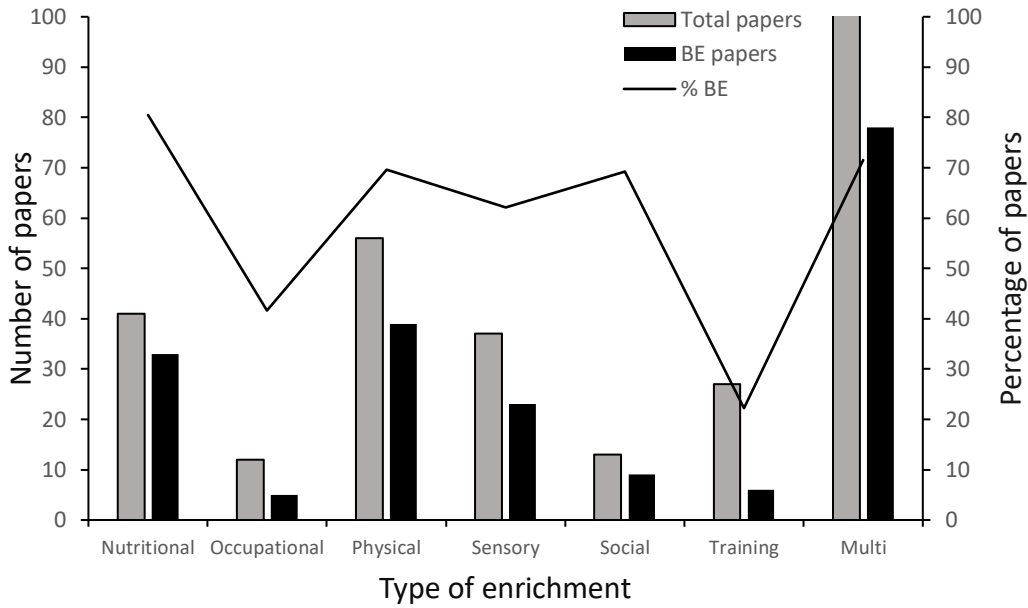
Predictor	Variable	Estimate	Standard error	DF	P value	Q value
Age (years)	1	2.161	0.739	10	0.192	0.05
	2	2.761	0.726			
	3	2.709	0.728			
	4	2.589	0.735			
	5	3.469	0.733			
	6	3.864	0.725			
	7	3.726	0.729			
	8	3.546	0.725			
	9	3.866	0.725			
	10	4.237	0.726			

Class	Amphibian	0.046	0.165	5	<0.001	0.01*
	Bird	-1.910	0.215			
	Fish	0.455	0.169			
	Invertebrate	-2.14	1.02			
	Mammal	-1.111	0.0883			
	Reptile	-0.988	0.275			
Impact factor	Impact factor	1.43	0.496	17	<0.001	0.02*
Type of enrichment	Nutritional	-0.341	0.095	6	<0.001	0.03*
	Occupational	0.053	0.118			
	Physical	-0.453	0.088			
	Sensory	0.306	0.083			
	Social	0.436	0.093			
	Training	0.180	0.094			
Impact factor *Age	Interaction between predictors	0.1532	0.0172	10	<0.001	0.04*

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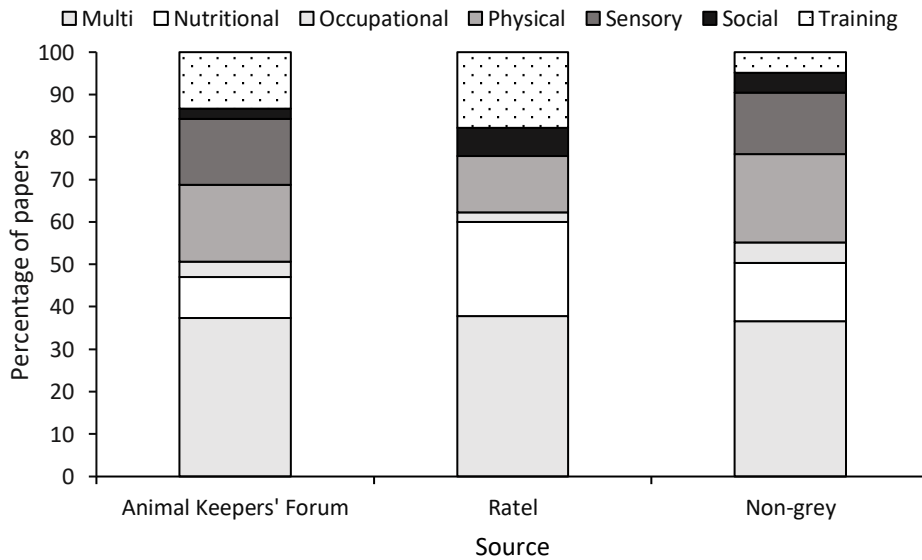
247 *3.4. Type of enrichment*

248 Of the EE types, multi-category (109, 36.94%) appeared most frequently in this sample of the
249 literature (109, 36.94%), followed by physical EE (56, 18.98%) nutritional (41, 13.89%), sensory (37,
250 12.54%), training (27, 9.15%), social (13, 4.4%) and occupational (12, 4.06%). The representation of
251 each EE category also differed between the types of literature (Figure 3), with training studies
252 appearing more frequently in grey literature.



253

254 *Figure 3. Total number of papers using each enrichment type for both grey literature and peer*
 255 *reviewed literature combined. The number of papers which contain BE enrichment are shown in*
 256 *black, and the percentage of papers that are biologically informed is shown as a line.*



257

258 *Figure 4. Percentage of papers covering each singular type of enrichment and multi (for papers that*
 259 *discussed several EE types).*

260

261 *3.5. Taxonomic representation*

262 Mammals (227, 76.95%), birds (34, 11.52%) and reptiles (12, 4.07%) were the most frequently
263 documented taxonomic Classes in this sampled literature. Amphibians (2, 0.68%), fish (1, 0.34%) and
264 invertebrates (1, 0.34%) were the subject of fewer studies and 18 studies (6.10%) had a multi-taxa
265 focus. Supplementary information on taxonomic representation is available in the appendix
266 (Appendix 1, 2).

267

268 **4. Discussion**

269 This study has identified that biological evidence was significantly more likely to be found in peer-
270 reviewed papers than in grey literature articles. Differences were noted in how BE was used for
271 different categories of EE, with papers focussing on nutritional and multiple forms of EE more likely
272 to be supported with BE. Some taxonomic groups, such as carnivores, primates, ungulates and
273 elephants, were more often represented in this sample of EE literature compared to other taxa.

274 Husbandry and Training was the most common aim for papers, irrespective of their Source (Tables 3
275 and 4), which reflects the growing research output from zoological collections (Loh et al 2018) that is
276 aimed at improving animal care standards (Barber 2009), which in turn advances animal welfare. A
277 paper's Source (peer-reviewed or grey literature) was a predictor of aim category, with peer-reviewed
278 literature showing a wider range of possible Aim categories (e.g. cognition, methods and physiology)
279 which were not identified in grey literature. This difference in Aim categories is likely a reflection of
280 the target audience, as grey literature sources targeted zoo professionals whereas peer-reviewed
281 journal articles tended to target a more academic audience. Reviews of multiple EE strategies, and
282 application of novel methods were more likely to appear in peer-reviewed sources, suggesting that
283 larger meta-analyses or gap analyses, as well as papers documenting new ways of measuring or
284 evaluating EE have a more theoretical rather than practical audience.

285 *4.1. Biological evidence*

286 Our research demonstrates that many published EE strategies are biologically relevant to the taxa they
287 are used on, but there is scope for more widespread BE use in EE development. Nutritional EE was

288 significantly more likely to be biologically informed (Figure 4), which likely reflects the push towards
289 evidence-based zoo nutrition and a reduction in unhealthy or inappropriate dietary alternatives in zoo
290 foods (Less et al 2014; Britt et al 2015). Likewise if nutritional EE aims to increase foraging or food
291 handling and processing time, then knowledge of a species' behavioural and evolutionary ecology
292 regarding feeding and foraging strategies will be needed for such EE to work successfully (Stoinski et
293 a. 2000; Altman et al 2005).

294 Difference in BE usage across papers and EE categories may be a direct result of how EE is utilised
295 for and the ease of providing EE to a given species. For example, it is more challenging to incorporate
296 natural history information into training regimes or occupational EE. However, training, particularly
297 when it is used to encourage species-specific behaviours, can be biologically informed. Training-
298 based EE appeared frequently in the grey literature (Figure 1) and this may be a cause of a lower use
299 of BE in these papers. Training studies have considerable value for sharing knowledge on good
300 practice, animal management, and potentially may improve human-animal interactions (Melfi 2013;
301 Ward & Melfi 2013). The greater occurrence of training research in the grey literature suggests that
302 authors are targeting a specific audience (i.e. other zookeepers) to share important information on
303 species-specific training techniques. Whilst the grey literature was less likely to contain articles that
304 featured BE, it probably better reflected the actual use of EE within zoos and aquariums (i.e. written
305 by zookeepers for zookeepers).

306 The grey literature is more difficult to systematically search for specific-subject content compared to
307 peer-reviewed articles. Consequently, areas of EE practice that appear less frequently (and therefore
308 might receive less attention) may have appeared in the scientific literature but indexing and
309 cataloguing issues could limit their overall readership. A study of zoo and aquarium professionals
310 noted that conference delegates considered EE to be essential for non-parrot birds, fish and
311 invertebrates (Rose & Riley 2020) but our study revealed limited numbers of papers on these taxa.
312 The positive attitude of zoo professionals to widespread use of EE, as stated by Rose & Riley (2020),
313 suggests that EE may be commonplace throughout zoological collections but is not always being
314 shared in or read from the literature.

315 There may be occurrences of when an EE provided is not based on BE but still provides positive
316 welfare outcomes, for example the use of touch-screen computers for zoo-housed primates (Kim-
317 McCormack et al 2016; Schmitt 2018) or the use of music as sensory stimulation (Ritvo &
318 MacDonald 2016). Measurement of any positive behavioural indicators (for example play, exploration
319 or enhanced positive social interaction) in conjunction with the use of the EE could be analysed and
320 published to then provide situational or individual evidence for why this type of “non-BE” enrichment
321 is actually relevant, beneficial for the welfare of zoo-housed species, and effective at providing an
322 output for the performance of welfare positive behaviours. Clearly, the need for individual interaction
323 with such forms of non-BE enrichment is crucial; research on mouse lemurs (*Microcebus murinus*)
324 showed that as animals age, their interaction with touchscreen technology declined in comparison to
325 younger animals (Joly et al 2014). This finding may be relevant to various BE enrichment methods
326 too, and provides clear reasons for evaluation of EE (in any of its forms) so that it continues to be
327 relevant to the animals it is provided to for all of their life stages.

328 *4.2. Predictors of citations in peer-reviewed literature*

329 Our research revealed that a paper’s age, the animal’s taxonomic Class, the interaction between age
330 and journal Impact Factor, and type of EE were all significant predictors of total paper citation
331 number. The significant interaction between the age of a paper and its impact factor in our study
332 supports the idea that the longevity of an article enhances its value to others, particularly as journal
333 impact grows over time as they are available for longer. The growth in open access scientific
334 publishing is a helpful development that should bring high impact academic research to more zoo and
335 aquarium researchers who may have struggled to reach such publications in the past. A positive
336 correlation was noted between a paper’s age and increasing citation rate in other disciplines
337 (Tahamtan et al 2016), so it may take time for the findings from a paper to be digested and then
338 applied to industry by others working on the topic of said paper.

339 Work on amphibians and fish were positive predictors of total citation number, whereas other
340 taxonomic Classes were negative predictors. There may be a restricted pool of EE evidence for fish
341 and amphibians, researchers are actively using the available content to write high-impact papers that

342 are useful to those working with such species. The limited research evidence for some amphibians and
343 fish might result in the papers that are available being cited extensively, particularly as practitioners
344 and scientists work more closely on defining and examining welfare states and quality of life
345 measurement for such species (Michaels et al 2014; Graham et al 2018).

346 *4.3. Type of enrichment*

347 Multi-category EE studies, in which several different EE types are featured, appeared most frequently
348 in all forms of publication (Figure 4). Review papers that summarise and compare EE across
349 situations and taxonomic groups (Swaisgood & Shepherdson 2005) may be especially useful for zoo
350 professionals, enabling comparison of the effectiveness of EE types before a choice is made to apply
351 EE to a specific species in the zoo or aquarium. Developing such review papers for understudied taxa
352 (e.g. fish or invertebrates) may increase engagement with EE for such species as well as overcome
353 some of the challenges with how it is used in this species that could be a barrier to EE being provided.
354 Of single category EE papers, the most commonly documented in all literature sources were physical,
355 nutritional, sensory and training. Social and occupational EE were rarely included in EE articles. EE
356 types are not mutually exclusive, and as such, social and occupational forms of EE may be covered in
357 other ways. Introduction of new animals into an exhibit might be investigated under the topics of
358 animal compatibility, breeding or social network analysis, and may not be labelled as EE. Similarly,
359 group housing for social animal species may be enriching, yet this is often considered part of routine
360 husbandry and is therefore not always covered as EE research (Rose et al 2016). Occupational EE,
361 similarly, may either be provided as part of normal husbandry routines as well as by enclosure
362 fixtures, fittings, planting and substrates. Consequently, the role of the enclosure itself as a form of
363 occupational EE, with the associated welfare benefits that this brings, may be forgotten as it is not
364 “given or provided” specifically to the animal as a form of EE, and may be constantly available to the
365 animal.

366 Both occupational and social EE can be provided without nutritional rewards, allowing keepers to
367 move away from food as EE in situations where dietary provision needs to be carefully monitored.

368 Social EE could be provided as opportunities to interact with individuals from another social group in
369 a safe, controlled manner using appropriate barriers where necessary (Lutz & Novak 2005). Many
370 sociable species are highly motivated to seek out social interaction (Hopper et al 2016) and therefore
371 the natural behaviour of the animal could be manipulated and managed in conjunction with enclosure
372 change or modification to enhance the enriching nature of the social environment provided (Rose et al
373 2016).

374 *4.4. Taxonomic representation*

375 Over 75% of papers focused on mammals (with primates and carnivores being especially popular
376 research subjects); amphibians, fish and invertebrates were the focus of less than 1% of studies
377 respectively. This taxonomic bias is represented in research fields such as general zoo science (Melfi
378 2009; Rose et al 2019a), animal behaviour (Rosenthal et al 2017), conservation (Bautista and Pantoja
379 2005; dos Santos et al 2020), and is also reflected in how popular such taxa are with the general
380 public (Courchamp et al 2018).

381 Taxonomic Classes less common in this sample of the EE literature are well-represented in zoos and
382 aquariums globally (Brereton & Brereton 2020) and therefore diversification of research output as
383 well as the replication of EE experiments across institutions could be possible (Rose et al 2019a).
384 Some of these taxa are featured in the literature but in other topics, such as conservation or breeding
385 (Rose et al 2019a). Many Orders, such as the amphibians Caudata and Gymnophiona, and almost all
386 fish Orders, were not represented in our dataset. Basic behavioural ecology knowledge of these
387 Orders may be lacking, inhibiting the development of new research initiatives. There is evidence to
388 suggest that some of the EE strategies are not yet appearing in the published literature, and therefore it
389 is possible that EE for these taxa is more widespread than our literature search would suggest.

390 *4.5. Extending this research question*

391 Previous papers (Melfi 2009) and more recent journal special editions that have focussed on the use of
392 evidence in the zoo (e.g. *Animals*, MDPI 2020) have illustrated gaps in husbandry knowledge for
393 many taxa in the zoo and consequently still call for zoo animal management to be evidence based at

394 the species-specific level. A requirement for using evidence to underpin practice is further emphasised
395 by zoo and conservation organisations themselves as they implement changes to existing species
396 management protocols. For example, the European Association of Zoos and Aquaria, EAZA (2020),
397 Best Practice Guidelines and the IUCN’s Conservation Planning Specialist Groups, CPSG (2020),
398 One Plan Approaches can integrate management of species between the wild and zoo environments
399 (Traylor-Holzer et al 2019). For such evidence to be available, it is important for zoos and academic
400 institutions continue to work together to identify what research questions need to be posed, and where,
401 to ensure that evidence gathered is credible and relevant to the practical application it links to. Our
402 research on BE for EE provides examples of how evidence has been gathered on EE for particular
403 species, as well as where more information is needed for specific taxonomic groups and EE styles. To
404 further enhance evidence gathering on EE use (e.g. species relevance and application) surveys,
405 workshops and training could be used to garner information on: how EE is decided upon, how it can
406 be adapted for the promotion of key behavioural and ecological needs within a species, and how it can
407 be reassessed and re-evaluated as a cyclic process of action research (Kirkey 2005) to audit its
408 continued efficacy over time (Therrien et al 2007; Woods et al 2020). Increasing the understanding of
409 what EE is and the capacity for its use by animal care staff can be successfully undertaken via the use
410 of species-specific workshops, with learning objectives centred on linking species ecology with EE
411 needs in the zoo (Melfi & Hosey 2011; Rose et al 2016). Research that investigates the best
412 methodologies for systematic scoring and categorisation of “how much evidence was used to develop
413 the EE” should be developed, trialled and analysed. Such scoring methods could rate the final EE
414 output against a scale of evidence usage.

415 Such descriptors on a scale of this kind could be linked to a numeric score (e.g. a score of 0 for no BE
416 at all; a score of 1 for no BE put the potential for its inclusion) to enable a full evaluation of all stages
417 of EE design and application to a specific species within a specific animal collection. This approach
418 would help with welfare auditing, provide useful information on the “normal characteristics” of the
419 individual animals to support health and wellbeing records kept by vets or curatorial staff, and be
420 useful for animal welfare policies and zoo licencing documentation.

421 Further extension of this research to develop and test a BE scoring system to assess the species-
422 specific relevance of different EE types could also reduce the effect of any confounds to this research,
423 notably the need for reference material and citations to be used within the peer-reviewed literature as
424 a requirement of publishing in this medium. It may well be that EE devices documented in the grey
425 literature were originally developed using information on species' ecology and behaviour but the lack
426 of requirement for referencing means this information is lost from any final publication. Scoring the
427 BE from each paper, in a Likert scale (where 1 and 5 equate to 'no consideration of BE' and 'full
428 consideration of BE throughout development of EE' respectively) would provide greater depth on the
429 integration of BE in enrichment provision.

430 **5. Animal welfare implications and conclusions**

431 EE is considered integral to the improved welfare states experienced by many zoo-housed animals
432 (Swaisgood & Shepherdson 2005). EE presented in both Source types showed the use of BE that
433 demonstrates the value and relevance of the EE to the species receiving it. It appears that, as a general
434 observation, zoos and aquariums are using an evidence-based approach to develop EE strategies for
435 the animals in their care. Some EE types were referred to less frequently in the literature (e.g. social,
436 occupational), and this could be an opportunity for zoos to diversify their EE strategies as newer BE
437 becomes available to those guiding EE programmes, specifically for species whose welfare may be
438 improved by use of social or occupational enrichment.

439 Not all EE types necessarily need to be supported by BE, and in such cases, attention should be paid
440 to the intended welfare outcome for the animals involved. For example, training programmes are not
441 always based on BE, yet they can provide measurable welfare benefits for animals involved by
442 enabling coping mechanisms, reducing stress during husbandry and management or by enhancing the
443 animal's feelings of autonomy over its current situation (Laule et al 2003; Westlund 2014). However,
444 where possible an animal's natural history and behaviour should be taken into account to ensure that
445 EE is biologically appropriate for that species; an especially important consideration for animals in
446 conservation programmes where the promotion of adaptive traits, essential for survival of future
447 generations in the wild, is a key requirement (Greggor et al 2018). Enhancing animal welfare outputs

448 for individuals within conservation programmes is possible with the correct use of relevant EE
449 (enabling “opportunities to thrive”) and therefore the welfare relevance of our review, and our call to
450 encourage more evidence gathering on how EE is developed, is applicable to the many roles of the
451 zoo or aquarium’s animal collection (Greggor et al 2018).

452 With some taxonomic groups appearing more frequently in the literature, there are opportunities for
453 practitioners to diversify and adapt their EE strategies to new subjects. It is likely that a wide range of
454 EE types are already being used for a much more diverse variety of taxa than is actually being
455 published. As the actual scope of EE being practiced by the zoo and aquarium community is clearly
456 challenging to measure, we encourage those practitioners already using novel EE strategies or who are
457 conducting EE on under-represented taxa to consider sharing their findings. Increased dissemination
458 of studies by animal care staff would provide more evidence for future work that aims to fill current
459 gaps in knowledge relating to EE.

460 Active online social media groups, widely accessible to animal care staff, may allow new EE ideas to
461 be shared more rapidly than via traditional media. There are benefits to the rapid sharing of
462 information online but some sources may lack repeatability. Unlike information presented in the “grey
463 literature” whose articles often require some evaluation of the suitability of EE (ABWAK 2020), the
464 instantaneous communication within social media can reduce the changes of such important reflection
465 and review of suggested practice. However, these online forums could provide greater insight into the
466 EE strategies commonly used by animal care staff, particularly if they document EE targeted for
467 welfare improvements in “poorly researched” taxa. Future research should include an assessment of
468 the types of EE and species advertised in these media, with a comparison against what is being
469 published in grey and peer-reviewed sources.

470 Given the scope for developing EE at the species-specific level, to enhance welfare using BE within
471 the EE protocol, alongside the likelihood that relevant and useful EE approaches exist in the grey
472 literature publications of many zoo organisations, we suggest that such professional zoo organisations
473 consider how they share and archive the articles from their newsletters, magazines or journals. An
474 enhanced, and searchable, online repository of past articles would increase the readership of

475 information that has been submitted to the publication and, from a research standpoint, enable more
476 vigorous assessment of the content and application of the results contained within these articles.

477 Overall, we have shown that key aspects of a zoo or aquarium animal welfare programme, notably the
478 use of EE as a means of enhancing the lives of the animals at the institution, is more often than not,
479 based on facets of ecological or biological evidence that relate to the species being enriched or the
480 design or type of EE protocols being used to enhance welfare through behavioural means (i.e. the
481 promotion of specific activities or behavioural events). Zoos need to consider increasing their research
482 outputs to show the use of evidence for a wider range of species, and they should continue to re-
483 evaluate current EE practices to ensure that they remain relevant to the animal's behaviour patterns
484 and attainment of positive welfare states.

485

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680

681 **Appendix:**

682 *Appendix 1. Table containing number (and proportion) of papers per taxonomic group from each*
 683 *literature Source*

	Animal Keeper's Forum	Ratel	Web of Science	Total
Amphibian	0 (0)	0 (0)	2 (0.68)	2 (0.68)
Bird	8 (2.71)	13 (4.41)	13 (4.41)	34 (11.53)
Fish	1 (0.34)	0 (0)	0 (0)	1 (0.34)
Invertebrate	1 (0.34)	0 (0)	0 (0)	1 (0.34)
Mammal	64 (21.69)	25 (8.47)	138 (46.78)	227 (76.95)
Reptile	6 (2.03)	3 (1.02)	3 (1.02)	12 (4.07)

Multi	0 (0)	4 (1.36)	11 (4.75)	18 (6.10)
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685 *Appendix 2. Table of most represented taxonomic Orders in this enrichment literature*

Class	Order	Number of papers (proportion)
Amphibians	Anura	2 (0.68)
Birds	Psittaciformes	8 (2.71)
	Sphenisciformes	6 (2.03)
	Accipitriformes	4 (1.36)
	Phoenicopteriformes	4 (1.36)
	Pelecaniformes	2 (0.68)
	Cathartiformes	1 (0.34)
	Galliformes	1 (0.34)
	Gruiformes	1 (0.34)
	Passeriformes	1 (0.34)
	Strigiformes	1 (0.34)
Fish	Perciformes	1 (0.34)
Invertebrates	Octopoda	1 (0.34)
Mammals	Primates	82 (27.80)
	Carnivora	74 (25.08)
	Artiodactyla	17 (5.76)
	Proboscidaea	12 (4.07)
	Perissodactyla	4 (1.36)
	Diprotodontia	3 (1.02)
	Chiroptera	2 (0.68)
	Pilosa	2 (0.68)
	Rodentia	2 (0.68)

	Cetartiodactyla	1 (0.34)
	Lagomorpha	1 (0.34)
	Macrosceliformes	1 (0.34)
Reptiles	Squamata	6 (2.03)
	Crocodylia	2 (0.68)
	Testudines	2 (0.68)
Multi-taxa		55 18.64)

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