Dietary studies in birds: testing a non-invasive method using digital photography in seabirds

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Summary

1. Dietary studies give vital insights into foraging behaviour, with implications for understanding changing environmental conditions and the anthropogenic impacts on natural resources. Traditional diet sampling methods may be invasive or subject to biases, so developing non-invasive and unbiased methods applicable to a diversity of species is essential.

2. We used digital photography to investigate the diet fed to chicks of a prey-carrying seabird, and compared our approach (photo-sampling) to a traditional method (regurgitations) for the greater crested tern *Thalasseus bergii*.

3. Over three breeding seasons, we identified >24,000 prey items of at least 47 different species, more than doubling the known diversity of prey taken by this population of terns. We present a method to estimate the length of the main prey species (anchovy *Engraulis encrasicolus*) from photographs, with an accuracy < 1 mm and precision ~0.5 mm. Compared to regurgitations at two colonies, photo-sampling produced similar estimates of prey composition and size, at a faster species accumulation rate. The prey compositions collected by two researchers photo-sampling concurrently were also similar.

4. Photo-sampling offers a non-invasive tool to accurately and efficiently investigate the diet composition and prey size of prey-carrying birds. It reduces biases associated with observer-based studies and is simple to use. This methodology provides a novel tool to aid conservation and management decision-making in light of the growing need to assess environmental and anthropogenic change in natural ecosystems.

Key-words: diet, digital photography, non-invasive monitoring, prey-carrying birds, rarefaction curves, *Thalasseus bergii,* regurgitation

Introduction

Dietary studies are essential to understand animal ecology, temporal changes in the environment, and to establish sustainable management strategies for natural resources (Jordan 2005). In complex natural systems, top-predators can act as indicators of environmental conditions, and their diet, in particular, can provide important information on prey species abundance, occurrence and size, which may reflect processes over short time-frames (e.g. Suryan *et al.* 2002; Parsons *et al.* 2008). As such, outcomes from diet studies are important tools for monitoring changes in demographic parameters or behaviour, themselves a product of changing diet (Sherley *et al.* 2013). Moreover, dietary studies can provide powerful indicators of anthropogenic impacts and environmental change on food-webs (e.g. Piatt *et al.* 2007; Green *et al.* 2015), facilitating conservation biology and ecosystem-based management (Grémillet *et al.* 2008; Sherley *et al.* 2013). The importance of monitoring diet thus demands the development of simple, efficient, non-invasive methods applicable to a diversity of species.

Numerous techniques exist to investigate bird diets (Jordan 2005; Inger & Bearhop 2008; Karnovsky, Hobson & Iverson 2012). Invasive techniques include induced regurgitations (Diamond 1984), stomach flushing of live birds (Wilson 1984), application of neck-collars on chicks (Moreby & Stoate 2000) and the dissection of birds collected specifically for this purpose (Doucette, Wissel & Somers 2011). These methods describe short-term diet composition accurately (González-Solís *et al.* 1997), despite some errors introduced by differential prey regurgitation or digestion (e.g. Jackson & Ryan 1986). More recent biochemical methods involving isotopic, lipid and DNA analyses provide complementary approaches, but generally cannot be used alone due to their coarse taxonomic resolution (Karnovsky, Hobson & Iverson 2012). Moreover, these approaches typically require disturbance or capture of birds, which can impact their physiology and behaviour (e.g. Ellenberg *et al.* 2006; Carey 2009).

Accurate, non-invasive diet sampling is therefore required to give fine-scale indicators of prey availability or prey selection. One of the least non-invasive methods is to observe birds carrying visible prey with binoculars or video recording systems, from a safe distance. This typically involves birds feeding offspring or incubating partners (e.g. Safina *et al.* 1990; Redpath *et al.* 2001; Tornberg & Reif 2007). Such studies are generally limited to assessing chick diet, but have the potential to reveal changes in prey communities (Anderson *et al.* 2014). However, observer-based diet studies are subject to several

67 methodological limitations (Cezilly & Wallace 1988; González-Solís *et al.* 1997; Lee & Hockey 2001)

68 calling for further development of this approach.

69   Digital photography represents an excellent alternative tool to study the diet fed to chicks of prey-

70 carrying birds, because 1) there is virtually no limit to the number of pictures that can be taken, 2)

71 species identification is possible in most cases, 3) prey can potentially be measured accurately and

72 precisely, 4) images can be re-analysed without loss of data quality, i.e. samples do not deteriorate over

73 time and 5) storage is simple. Over the last decade, the use of digital photography for dietary studies has

74 included the use of camera-traps to investigate the diet of nesting raptors (García-Salgado *et al.* 2015;

75 Robinson *et al.* 2015), and the combined use of digital compact cameras with spotting scopes

76 (digiscoping) to assist prey identification (made primarily by observations) for Caspian terns

77 (*Hydroprogne caspia*) and common murres (*Uria aalge*) (Larson & Craig 2006, Gladics *et al.* 2015).

78 However, both techniques have limitations including poor image quality and difficultly in capturing

79 images of birds carrying prey in flight or during fast delivery to chicks (see Larson & Craig 2006,

80 García-Salgado *et al.* 2015).

81   Recent advances in performance and price reductions of digital single lens reflex (DSLR) cameras

82 combined with autofocus telephoto lenses makes digital photography an affordable option for prey

83 identification, even for birds in flight. In the last few years, DSLRs have been used opportunistically to

84 identify items carried by a variety of birds (e.g. Woehler *et al.* 2013; Gaglio, Sherley & Cook 2015, Tella

85 *et al.* 2015) but a systematic approach and an accurate method to estimate prey dimensions are lacking.

86 We developed a standardised application of digital photography using DSLR cameras and telephoto

87 lenses to investigate chick diet composition and prey size in prey-carrying birds. We tested the method

88 on the colonial breeding greater crested tern *Thalasseus bergii* in South Africa. We compared the

89 efficacy of photo-sampling to the more traditional used regurgitations (Walter *et al.* 1987) using prey

90 identified to species level collected from chicks, and assessed the accuracy and precision of length

91 measurements of the main prey made from photographs. We also evaluated the potential for observer

92 bias in this system. Finally, we discuss the validity of applying our non-invasive approach to any prey-

93 carrying bird and the potential to develop a simple and effective tool-box to accurately identify and

94 estimate the size of any carried item.

Methods

STUDY SPECIES AND SITES

The greater crested tern (hereafter 'tern') is distributed from the Namibian coast eastwards to the central Pacific. It feeds mostly at sea by dipping onto the surface or plunge diving up to ca 1 m (Crawford, Hockey & Tree 2005). During breeding, adults usually return from foraging with a single prey item, which is either offered to the partner during courtship or delivered to the offspring (Crawford, Hockey & Tree 2005). In South Africa, the sub-species *Thalasseus bergii bergii* breeds mostly on islands in the Western Cape (Crawford 2003). Since 2008, Robben Island (33°48'S, 18°22'E), Table Bay, has hosted the largest southern African colony, reaching ~13,000 breeding pairs in 2010 (Makhado *et al.* 2013). A few hundred pairs breed in the Eastern Cape, mostly on Seal Island (33°50'S, 26°17'E), Algoa Bay (Makhado *et al.* 2013). We studied their diet at both Robben and Seal Islands.

PHOTO-SAMPLING

We investigated the diet of breeding terns at Robben Island during 2013 (February–June), 2014 (January–June) and 2015 (February–June) and at Seal Island during June 2015. Adult terns returning with prey were photographed from a vantage point of 50–80 m from the edge of their colony (Fig. 1a). At Seal Island (~300 pairs) we were able to photograph all adults returning to the colony during our photo-sampling sessions. At Robben Island, colonies were much larger (> 6,000 pairs) so we could not photograph all individuals. However, every attempt was made to not bias selection to individuals carrying particularly conspicuous prey items. The distance to the flying birds ranged between 6.5 and 25 m. Total sampling effort represented ~ 50 h of photography per year. For each individual, we typically took a sequence of 3 photos (a "photo set") for identification and prey measurements (Fig. 1b). We found by trial and error that 3 images provided the best trade-off to balance processing time with obtaining at least one sharp image. To avoid biasing the results and maintain independence among photo sets, ad-hoc image analysis was performed for each sampling session to discard repeated photo sets of the same adults carrying the same prey item. Recurrent birds were identified using distinguishable feather patterns,

122   presence of colour or metal rings, type and position of prey in the bill while flying, and distinctive

123   markings on the prey.

124   Photos were taken using Canon 7D and 7D Mark II cameras, fitted with Canon EF 100–400 mm

125   f/4.5-5.6L IS USM zoom lenses. We set the cameras to (i) shutter speed priority (1/2500 s); (ii)

126   automatic ISO (or aperture priority mode that provided shutter speeds of at least 1/2500 s); (iii) high-

127   Speed Continuous Shooting; (iv) Autofocus on AI Servo (for moving subjects) using the AF point

128   expansion; and (v) large Jpeg file format for high-speed recording. We set the telephoto lens to

129   autofocus, the image stabilizer to on and the closest focal point to 6.5 m to increase autofocus speed.

130

131   IDENTIFICATION OF PREY SPECIES

132   All blurred or otherwise non-identifiable images (due to e.g. distance, an unfavourable position of prey in

133   the bill or lighting) were discarded. From the remaining photographs (e.g. Fig. 1), we determined the

134   numerical abundance (Duffy & Jackson 1986) of prey (usually at species level) using fish guides (Smith

135   & Heemstra 2003; Branch *et al.* 2010) and assistance from experienced observers (see

136   Acknowledgements). In some instances, good quality photographs contained prey that could not be

137   identified (< 0.01% of total prey items). For example, some adults returned with pieces of fish flesh,

138   possibly originating from kleptoparasitism disputes or scavenging. These images were excluded from our

139   analyses. Approximately 45% of photo sets were suitable for prey identification; there was no evidence

140   of bias towards particular prey types among discarded images.

141

142   ESTIMATION OF PREY STANDARD LENGTH

143   Dietary studies of piscivorous birds commonly measure the standard length (SL) of the fish (length from

144   the tip of the snout to the posterior edge of the hypural plate) to compare prey size (Barret 2002, Smith &

145   Heemstra 2003). We estimated SL from photographs for anchovy *Engraulis encrasicolus*, the most

146   common species in the tern's diet. As prey tended to flex to differing degrees in the adults' bills, direct

147   SL measurement from the image underestimates fish length. Thus, we estimated SL from measurements

148   of individual body parts (eye diameter, operculum width and head width, all measured dorsoventrally),

149 which were less distorted in the image and generally in a plane parallel to the bird's bill and the camera

150 (Figs 1b and 2).

151   To do this we first assessed the accuracy of predicted SLs based on these morphological

152 measurements using cross-validation by fitting log-linear allometric regressions to a training dataset (n =

153 50) and comparing model predictions to a test dataset (n = 20) of anchovies measured by hand (see

154 Appendix S1). Next, we measured 37 additional anchovies with Vernier callipers (to the nearest 0.1 mm)

155 and then photographed them held in the bill of a dead tern, for which the culmen length was known (Fig.

156 2 in Appendix S1). For each image, we used the 'line selection tool' in ImageJ (Schneider *et al*. 2012) to

157 estimate eye diameter ($\hat{E}$), operculum width ($\hat{O}$) and head width ($\hat{H}$) for each fish by scaling the pixel

158 length in the image to (1) the length of the dead tern's culmen (62.1 mm; measured with Vernier

159 callipers), (2) the mean culmen length for this species (61.2 mm, n = 128; Crawford, Hockey & Tree

160 2005) and (3) the minimum and maximum recorded culmen lengths (range: 54.5–67.6 mm, Crawford,

161 Hockey & Tree 2005). We used the estimates of $\hat{E}$, $\hat{O}$ and $\hat{H}$ to obtain three estimates of SL ($\widehat{SL}$) using

162 the log-linear allometric regressions (see also Appendix S1), and calculated their arithmetic mean

163 (combined $\widehat{SL}$) and used this value in further analyses (since it was generally most accurate; Appendix

164 S1).

165   To determine the accuracy (γ) of the combined $\widehat{SL}$ estimates from the images, we compared them to

166 the known SL of each fish. We defined the mean percentage accuracy ($\bar{\gamma}$) of the combined $\widehat{SL}$ estimates

167 as:

$$\bar{\gamma} = \frac{100}{n} \sum_{i=1}^{n} \left(1 - \frac{|SL_i - combined\ \widehat{SL}_i|}{SL_i} \right)$$

168   (eqn 1)

169 where *i* indexes each of the *n* = 37 fish. As the absolute difference was computed, both overestimates and

170 underestimates of e.g. 2% would yield γ = 98%. In addition, we assessed the mean difference between

171 the known SLs and the combined $\widehat{SL}$ estimates using permutation tests with 10,000 Monte Carlo

172 iterations (*perm* library v. 1.0-0.0 for R).

173   To determine the precision (or repeatability) of the method, we repeated the measurement process in

174 ImageJ to obtain six $\hat{E}$, $\hat{O}$ and $\hat{H}$ values and the corresponding combined $\widehat{SL}$ values for 17 of the 37 fish

175   (using a known length on the ruler in each photograph). We calculated the combined $\widehat{SL}$ as above and

176   used this to assess precision. Precision (τ) was defined as:

$$\tau_{f,j} = \left| \left(\frac{1}{n} \sum_{j=1}^{n} combined\ \widehat{SL}_{f,j} \right) - combined\ \widehat{SL}_{f,j} \right|$$

177   (eqn 2)

178   where j indexes each of the $n = 6$ combined $\widehat{SL}$ values for the $f = 17$ fish. We report mean precision (in

179   mm) of all (6 x 17 = 102) values of $\tau_{f,j}$.

180   In addition, we examined whether either precision or accuracy were influenced by the SL of a fish.

181   For accuracy, we used a linear model of the form:

182    $$logit(\gamma_i) = \alpha + \beta \times SL_i + \varepsilon_i$$

183   (eqn 3)

184   where α and β are estimated from the data, γ_i are the accuracy estimates (as proportions), SL_i the known

185   standard length for fish i and $\varepsilon_i \sim N(0,\sigma)$ the residual error, with σ estimated from the data. For precision

186   we used a linear-mixed model (LMM: *lme4* library for R) of the form:

187    $$\tau_{fj} = \beta \times SL_{fj} + \delta_{fj} \times \eta_j + \varepsilon_{fj}$$

188   (eqn 4)

189   where β is the fixed effect parameter, $\eta_j \sim N(0, \varsigma)$ the random effect parameter, $\varepsilon_{fj} \sim N(0, \sigma)$ the residual

190   error, δ_{fj} the vector of fish IDs, τ_{fj} the vector of precision values and SL_f the vector of known standard

191   lengths for each measurement j of fish f, with β, σ and ς estimated from the data.

192   Finally, we used the above approach to estimate SL of the prey in a subset of the digital images

193   collected in the field where the bird's bill and the head of the prey were clearly visible and approximately

194   parallel to the camera (Fig. 1b). For each image, we used combined $\widehat{SL}$ and assumed the length of the

195   bird's culmen to be 61.2 mm (see above).

196

197   COMPARISON BETWEEN PHOTO-SAMPLING AND REGURGITATION-SAMPLING

198   To compare photo-sampling and regurgitation-sampling, we collected images of adults carrying prey and

199   regurgitations from chicks concurrently on 18 and 19 April 2015 at Robben Island (photo-sampling

effort: 600 min) and on 9 June 2015 at Seal Island (photo-sampling effort: 132 min). Regurgitates were collected from the ground, while chicks were inside a pen during ringing operations (chicks often regurgitate when disturbed). Prey were later identified from whole-prey or diagnostic prey remains resistant to digestion such as otoliths and squid beaks using Clarke (1986), Smith & Heemstra (2003), Smale, Watson & Hecht (1995), Branch *et al.* (2010) and the Port Elizabeth Museum's reference collection. Prey items that were not identified mainly consisted of fish flesh and were excluded from our analysis. The SL of whole anchovies collected from regurgitations was measured using a ruler.

We compared the number of prey items from different taxa between methods using χ^2 tests and assessed differences in the estimated anchovy SLs using permutation tests (10,000 iterations) for each island separately as the SL variance between islands was heterogeneous (Levene's test: $W_{(1,164)} = 5.8$, p = 0.017).

We examined prey diversity using sample-based rarefaction curves as these allow for standardized comparison across collections that differ in sample size (Gotelli & Colwell 2001). Using 1,000 random permutations of both the photo-samples and regurgitations from 18 and 19 April 2015, we produced curves of the mean (± asymptotic 95% confidence intervals, CI) species accumulation rate (species identified per sample made). We then compared this rate at samples sizes of n = 190. In addition, by fitting a Generalised Additive Model (GAM) to the photo-sample means and by assuming equal accumulation rates for extrapolation, we also compared the predicted species accumulation rate for regurgitations to the mean rate for photo-sampling at n = 1500. The chosen sample sizes approximate those obtained in the field.

Finally, to evaluate any possible observer effect on photo-sampling, two different researchers (observer-A and observer-B) simultaneously collected photographs at Robben Island on 18 and 19 April 2015. The two observers used the same equipment (Canon 7D Mark II camera, Canon 100–400 mm lens) and had similar experience in wildlife photography. All other procedures were the same as described above. We compared the samples from the two observers using χ^2 tests. Unless otherwise stated, all means are presented ± 1 SD and all statistics were performed using R v.3.2.1.

Results

PHOTO-SAMPLING *VS*. REGURGITATION-SAMPLING

In total ~160,000 photos were taken during the three breeding seasons on Robben Island, yielding images of 24,211 prey items identifiable to species (96%, 48 species) or family (98%, 49 families) level (total of 51 prey taxa; Table 1). During the regurgitation comparison trial at Robben Island, we identified 27 species from 1,510 photo-samples compared to 11 species from 198 regurgitated prey items. At Seal Island, we identified 11 species from 157 photo-samples and 6 species from 103 regurgitated prey items (Appendix S2). The mean species accumulation rate at 190 samples was 0.075 (95% CI: 0.058–0.089) for photo-sampling and 0.057 (95% CI: 0.053–0.058) for regurgitations; however, at this sample size, the 95% CIs overlapped (Fig. 3). The number of species predicted from 1,500 regurgitations was 23.4 (based on the GAM extrapolation) versus 27.0 for photo-sampling (Fig. 3). The diet composition of main prey did not differ significantly between the two methods for Robben Island ($\chi^2 = 47$, d.f. = 42, p = 0.26) or Seal Island ($\chi^2 = 18$, d.f. = 15, p = 0.26; Table S3 in Appendix S2).

ACCURACY AND PRECISION IN ESTIMATING ANCHOVY STANDARD LENGTH

Mean SL of the 50 anchovy used to calculate the allometric regressions between the morphometric measurements (training set) was 109.6 ± 13.5 mm (range = 83.3–130.5 mm), similar to the 20 anchovy in the test set (SL 112.8 ± 3.0 mm; range = 107.6–116.8 mm). The predicted $\widehat{SL}$s of the test set predominantly fell within the 95% prediction intervals for all three specific body part models (Fig. S1, Appendix S1). The mean accuracy ($\bar{\gamma}$) for the combined $\widehat{SL}$ was 97.9 ± 1.7% (range 93.0–99.9%) for the training set and 97.3 ± 1.8% (range 92.5–100%) for the test set. Accuracy was not affected by SL in either case (linear models: p > 0.05, see Appendix S1).

The mean SL of the 37 photographed anchovy was 113.4 ± 6.7 mm. With the culmen length of the dead tern (62.1 mm) as the reference, mean accuracy ($\bar{\gamma}$) for the combined $\widehat{SL}$ was 98.3 ± 1.5% (range 93.8–100%), yielding a mean combined $\widehat{SL}$ of 114.0 ± 7.1 mm (Table S2 in Appendix S1). With the species' mean culmen length (61.2 mm) as the reference, the mean combined $\widehat{SL}$ = 112.7 ± 7.0 mm ($\bar{\gamma}$ = 98.1 ± 1.5%, range 92.2–99.9%; Fig. 4, Table S2). The length of a fish (actual SL) did not influence the accuracy in either case (linear models: p > 0.05, Fig. 4) and neither of the combined $\widehat{SL}$s differed

255 significantly from the actual SL (permutations tests: p > 0.05). The mean accuracy ($\bar{\gamma}$) reduced to 88.9 (±

256 3.3)% and 91.3 (± 3.2)% for the minimum (54.5 mm) and maximum (67.6 mm) recorded culmen lengths

257 respectively (Table S2) and these combined $\widehat{SL}$ series did differ significantly from the actual SLs

258 (permutations tests: p < 0.001; see Appendix S1).

259   The mean precision of the combined $\widehat{SL}$ estimates was 0.52 (± 0.38) mm or 99.6 (± 0.3)%, with an

260 absolute range of 0.02–1.58 mm or 98.6–99.99%. Precision was not related to the actual SL of the fish

261 being measured (LMM: χ^2 = 0.02, p = 0.89).

262

263 COMPARISONS OF PREY SIZE BETWEEN PHOTO-SAMPLING AND REGURGITATIONS

264 At Robben Island, 116 anchovy from photo-samples (10% of anchovy photographed) and 20 from

265 regurgitates (12%) could be measured, while at Seal Island, the corresponding values were 21 (18%) and

266 nine (9%) respectively. Overall, the anchovy were longer at Seal Island (mean = 120.3 ± 8.2 mm, n = 30)

267 than at Robben Island (91.2 ± 13.2 mm, n = 136; p < 0.001; Fig. 5). For Robben Island, the mean

268 combined $\widehat{SL}$ of anchovy in the photo-samples was 91.3 ± 13.6 mm compared to 90.8 ± 11.1 mm for

269 regurgitates (Fig. 5). At Seal Island, they were 121.6 ± 9.3 mm and 117.4 ± 3.6 respectively. The SL

270 estimates from the two methods did not differ statistically for either Robben Island (p = 0.85) or Seal

271 Island (p = 0.21).

272

273 COMPARISON BETWEEN OBSERVERS

274 We identified 1,510 prey items of 22 species from the photographs taken by observer-A and 1,625 of 21

275 species from observer-B. Prey composition did not differ significantly between the two (χ^2 = 72, d.f. =

276 64, p = 0.23). However, three species were not recorded in common; observer-A photographed one

277 horsefish Congiopodidae sp. and one eel Ophichthidae sp., while observer-B recorded three individuals

278 of Cape hake *Merluccius capensis*.

279

280 **Discussion**

281 Photo-sampling offers an effective, low-impact alternative to traditional diet studies for birds that carry

282 prey items in their bill, with accurate prey identification and size estimates possible. Samples can be

283 acquired quickly and equivalent diet compositions obtained with relatively low effort (Fig. 3). In three

284 breeding seasons, we sampled 24,211 prey items and identified 51 prey taxa (Table 1) with this

285 approach; the most comprehensive diet analysis for terns in southern Africa prior to our study identified

286 25 species from 1,311 regurgitated prey items in 10 breeding seasons (1977–1986; Walter *et al*. 1987).

287 Despite ~55% of photos being discarded, our approach yielded an order of magnitude more samples and

288 identified twice as many species, with minimal disturbance to breeding birds.

289   The photo-sampling approach has several other advantages over traditional diet sampling. First, terns

290 often regurgitate only the posterior body and caudal fin of a fish, making identification of similar species

291 difficult (McLeay *et al*. 2009). Photo-sampling records the entire prey, and if there is doubt as to the

292 identification, images can be shared easily with global experts or on specialized websites (e.g. I-spot).

293 Second, photo-sampling can be used in a range of situations (e.g. on land or from a boat), by one

294 individual (collection of regurgitations often involves many people), with minimal training in

295 photography (cameras can be pre-set). Third, the photographic equipment is relatively affordable and

296 once purchased can be used for several years, at multiple colonies and for several species. Also, although

297 processing the photographs can be time-consuming, taking about 30 min for an average of 100 prey

298 identified, the images can be stored and analysed multiple times if needed, without the loss of data

299 quality or metadata (e.g. date and location).

300   Possible drawbacks associated with photo-sampling include the repeated photography of prey items,

301 especially those with long handling times, leading the frequency of these items being over-estimated.

302 This is predominately a problem in larger colonies, where it is difficult to follow the fate of individual

303 prey items, and one that could be countered using delays (e.g. 5 mins) between photosets. When only a

304 subset of prey is sampled, large or conspicuous prey items may induce an observer bias if they are easier

305 to photograph, more readily identified to species level or more interesting to the photographer. Training

306 photographers to randomise the photo-sampling as much as possible should help reduce this potential

307 bias. Differences in photographic experience between different observers could create a potential bias

308 and should be examined in future studies. Photo-sampling is difficult in bad weather (strong wind, rain or

309 mist) and this may also introduce bias in some situations. Finally, one constraint of our study is that

310 photo-sampling was applied to study chick diet. Although this can provide important insights into

311 changes in prey communities (Anderson *et al.* 2014), it may not always represent adult diet, or diet

312 outside the breeding season (McLeay *et al.* 2009). We thus suggest implementing indirect methods such

313 as measuring stable isotope ratios in e.g. blood and feathers of adults (Inger & Bearhop 2008)

314 concurrently with photo-sampling. Moreover, applying both methods concurrently on marked individuals

315 would allow the development of trophic discrimination factors in wild animals (Newsome *et al.* 2010).

316 More broadly, ecologists now use digital photography to study animals across a wide range of taxa

317 (e.g. Morrison *et al.* 2011; Marshall & Pierce 2012; Gregory *et al.* 2014). Opportunistic observations

318 have documented novel behaviours and trophic interactions (e.g. Gaglio, Sherley & Cook 2015; Tella *et*

319 *al.* 2015), suggesting that standardised approaches to study species bringing items to a known location

320 have great potential for ecological monitoring. This approach could also be applied to a diversity of taxa

321 in addition to birds that carry prey (e.g. carnivores bringing prey to their offspring, or ants and termites

322 carrying items to their nests). In any of these applications photo-sampling could provide high quality

323 photographic data to complement the now extensive use of camera-traps.

324 The ecological information provided by prey size is almost as important as prey species, giving

325 information on the targeted prey cohort and the predator's energetics. We demonstrated that prey size

326 (anchovy SL) can be estimated accurately (~98%) and precisely (~99%) from images. The approach

327 could be used with a wide variety of predators and prey species to eliminate biases associated with *in situ*

328 visual observation (Lee & Hockey 2001). Even if photo-sampling is unlikely to obtain measurements as

329 accurately or precisely as regurgitated/dropped prey, the sample size from photo-sampling is always

330 likely to be greater than the number of prey found undigested. A crucial step to estimate absolute prey

331 size is identifying a reference object (e.g. culmen, eye diameter) of known size, to provide a scale for

332 prey measurements. These reference objects should be chosen carefully and the degree to which the

333 selected trait varies within the population assessed to constrain and minimise errors where possible (see

334 Results). Additional studies could photograph birds of known bill length, age and sex (e.g. colour banded

335 individuals) with prey held with different angles to the body and compare larger numbers of observers

336 photo-sampling concurrently to further quantify the errors associated with prey measurements. For prey

337 species that are not distorted in images (e.g. some insects do not bend over a bird's bill), size can be

338 estimated directly and even when absolute estimates are not possible, the method still can be used to

339 assess changes in relative prey size, allowing for spatial and temporal comparisons.

340   Crucially, the photo-sampling method caused little if any disturbance to the nesting birds. Distances

341 from animals can be selected to balance each species' sensitivity against image quality. The opportunity

342 to record the number and size of prey brought to offspring remotely and in real time without influencing

343 behaviour, allows for accurate monitoring of temporal variability. For threatened or declining species

344 (e.g. many seabirds; Croxall *et al.* 2012), such non-invasive methods can help elucidate functional links

345 between population dynamics, environmental variability and anthropogenic pressures (Saraux *et al.*

346 2011). Incorporating these observations into detailed information on species composition and energy

347 content for energetic models offers great potential for indicators of long-term and large-scale ecosystem

348 change (Furness & Cooper 1982). Furthermore, with standardized protocols, digital images can be shared

349 easily using digital platforms (e.g. I-spot, Google Images) to facilitate global collaborations (e.g.

350 González-Solís *et al.* 2011; Lynch *et al.* 2015), encourage community involvement in citizen science

351 projects (e.g. Newman *et al.* 2012), and develop data archives to answer as yet unforeseen questions.

352 Given the growing need to assess environmental changes and human impacts on natural ecosystems

353 (Hobday *et al.* 2015), our methodology offers a novel tool for collaborative efforts in conservation.

354

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368

369 ## Data accessibility

370 All data used in this article are available in the Supplementary materials or Dryad Digital Repository

371 (Gaglio *et al*. 2016) http://dx.doi.org/10.5061/dryad.j647p.

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373 ## References

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503

Supporting Information

505   Additional Supporting Information may be found in the online version of this article.

506   **Appendix S1. Additional methods and results for estimation of prey standard length**.

507   **Appendix S2. Results of the comparison between photo-sampling and regurgitation.**

508

509 **Table 1.** Prey families in the greater crested tern diet identified by photo-sampling on Robben Island during the

510 2013, 2014 and 2015 breeding seasons. N = number of prey items identified.

Prey type	Family	Species	N
Fish	Engraulidae	1	16206
	Dussumieriidae	1	2557
	Scomberesocidae	1	1658
	Syngnathidae	2	866
	Clupeidae	1	545
	Carangidae	2	409
	Gonorynchidae	1	351
	Atherinidae	1	198
	Mugilidae	1	117
	Merlucciidae	1	76
	Pomatomidae	1	67
	Soleidae	Unid.	63
	Champsodontidae	1	58
	Clinidae	Unid.	63
	Clinidae	5	25
	Holocentridae	1	47
	Nomeidae	2	46
	Triglidae	Unid.	43
	Blenniidae	Unid.	38
	Myctophidae	1	23
	Gobiidae	Unid.	9
	Gobiidae	1	23
	Scombridae	1	22
	Scyliorhinidae	Unid.	16
	Macrouridae	Unid.	12
	Congridae	1	12
	Coryphaenidae	1	10
	Sebastidae	Unid.	6
	Gobiesocidae	1	6
	Trichiuridae	2	9
	Tetraodontidae	Unid.	5
	Cheilodactylidae	1	4
	Ophichthidae	Unid.	5
	Bregmacerotidae	Unid.	4
	Ophidiidae	1	3
	Ophidiidae	Unid.	1
	Sparidae	2	3
	Congiopodidae	Unid.	2
	Berycidae	1	2
	Centriscidae	1	1
	Chlorophthalmidae	1	1
	Batrachoididae	1	1
	Aulostomidae	Unid.	1
Cephalopods	Loliginidae	2	54
	Sepiidae	1	85
	Octopodidae	1	11
Crustaceans	Squillidae	1	244
	Brachyura*	Unid.	2
	Portunidae	1	1
	Palinuridae	1	3
Insects	Gryllidae	1	191
	Gryllotalpidae	1	2
	Sphingidae	1	2
	Sphingidae	Unid.	1
	Coleoptera **	Unid.	1

511 *Infraorder, **Order

512 **Figure Legeneds**

513 **Fig. 1 a)** Examples of capturing a photo-sample of an adult greater crested terns carrying prey to the colony without

514 causing disturbance to nesting birds and **(b)** the resulting close-up image of the prey used for identification

515 (anchovy) and standard length measurements. **From c to n:** Examples of tern prey items: **c)** sardine *Sardinops*

516 *sagax*; **d)** Atlantic saury *Scomberesox saurus;* **e)** multi-prey load (3 anchovy and 1 sardine); **f)** dolphinfish

517 *Coryphaena hippurus;* **g)** snake eel *Ophichthidae* sp.; **h)** sole *Austroglossus* sp.; **i)** longsnout pipe fish *Syngnathus*

518 *temminckii;* **l)** shyshark *Haploblepharus* sp**.**; **m)** cuttlefish *Sepia vermiculata;* **n)** common squid *Loligo vulgaris;* **o)**

519 rock lobster *Jasus lalandii*; **p)** two-spotted cricket *Gryllus bimaculatus*.

520

521 **Fig. 2** Example of the application (in ImageJ) of the 'line selection tool' to measure the linear distances for the

522 three morphometric parameters: (1) eye diameter (E); (2) head width (H) and (3) operculum width (O).

523

524 **Fig. 3** Sample-based rarefaction and species accumulation curves for greater crested tern diet at Robben Island.

525 Accumulation curves show the observed species accumulation from 1510 photo-samples (orange points) and 198

526 regurgitations (blue points) collected on 18 and 19 April 2015. Rarefaction curves (solid lines) and 95% asymptotic

527 confidence intervals (shaded areas) are based on 1,000 random permutations (shown as light grey points) of the

528 observed data. The rarefaction curve for regurgitations is extrapolated (blue dashed line) based on a GAM fit to the

529 photo-sampling, assuming an equal species accumulation rate beyond the range of the observed data. Vertical

530 dotted lines show sample sizes of 190 and 1500 used to compare the methods.

531

532 **Fig. 4** Accuracy of estimated standard length ($\widehat{SL}$) (y-axis) compared with actual SL values (x-axis) of anchovy

533 from photographs in ImageJ using allometric regressions based on estimates of eye diameter ($\hat{E}$, open orange

534 circles), operculum width ($\hat{O}$, open blue circles), head diameter ($\hat{H}$, purple open circles) and the mean of all three

535 (mean $\widehat{SL}$, black closed circles). The mean culmen length of greater crested terns (61.2 mm) was used as the

536 reference length to scale the pixel-based length estimates in ImageJ. The grey dashed line represents 100%

537 accuracy.

538

539 **Fig. 5** Frequency distribution of anchovy standard length from photo-samples and regurgitations (A = Robben

540 Island; B = Seal Island).