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Correspondence

Mark-resight estimates confirm a critically small population size in threatened marine iguanas (*Amblyrhynchus cristatus*) on San Cristóbal Island, Galápagos

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Amblyrhynchus cristatus, the world's only extant marine lizard, occurs on all 13 major islands of the Galápagos Archipelago. Relatively little is known with regard to the current census size of populations, with the most recently published estimates being acknowledged as "very rough" by the authors of the study (Wikelski & Nelson 2004). Despite its wide range within the archipelago, *Amblyrhynchus cristatus* is an endemic species that has been listed as 'vulnerable' to extinction by the International Union for Conservation of Nature and Natural Resources (IUCN) since 1996. Although the total population of marine iguanas may be as high as several hundred thousand individuals (Rassmann 1996a, Wikelski & Nelson 2004), some local populations are of conservation concern (Wikelski & Nelson 2004, MacLeod & Steinfartz 2016). In recognition of the strong between-island population structure, and the mismatch between genetic clusters and the current taxonomy of this species, genetically based management units (MUs) have recently been delineated (MacLeod & Steinfartz 2016). On most islands, there is a single MU per island, but one island – San Cristóbal – has two.

San Cristóbal, the most eastern island of the archipelago, is home to two well-studied populations of marine iguanas: the southwestern population found at La Loberia, and the northeastern population around Punta Pitt. Analyses of microsatellite loci have revealed that these populations represent two reproductively isolated and highly divergent genetic clusters (Steinfartz et al. 2009), which have consequently been classified as separate MUs (MacLeod & Steinfartz 2016). Individuals of the Punta Pitt MU are the most genetically distinct marine iguanas presently known (Rassmann 1996b, Steinfartz et al. 2009), and the population appears to be undergoing a speciation process (MacLeod et al. 2015). Future work to revise the subspecies-level taxonomy within this system could well recognise them as a distinct taxon. *Amblyrhynchus* population-size estimates for this island vary widely, with 50–400 estimated by Wikelski & Nelson (2004), and 5,032 by Snell & Marquez (2002). However, low densities at Punta Pitt have been regularly reported for more than three decades (Laurie 1983, Rassmann 1996a, Wikelski & Nelson 2004); this is reflected in the heterozygosity values for this population, which are the lowest across the archipelago (Rassmann et al. 1997, Steinfartz et al. 2009). San Cristóbal is also considered to be the most heavily human-modified island of the archipelago (Watson et al. 2010), and anthropogenic threats include marine pollution, urban development, and predation by invasive species (Wikelski & Nelson 2004). Taken together, these data suggest that this population should not only be a high priority for research, but also for conservation management.

The genetic effective sizes (N_e) of the San Cristóbal populations appear to be alarmingly low, with both being estimated at around or below 100 (MacLeod & Steinfartz 2016), which is just one tenth of the minimum size thought necessary to maintain evolutionary potential (Frankham et al. 2014). The N_e of a population reflects the rate at which genetic diversity is lost through genetic drift, and is usually a much smaller number than the actual (census) size in a non-idealized population. While N_e is a useful parameter for conservation issues, the ratio between N_e and census (N_c) is unknown for most species, and thus N_c cannot be reliably predicted from N_e. An accurate estimate of the actual population size is essential for conservation purposes, particularly for monitoring the effectiveness of management practices, assessing population trends, or measuring the influence of anthropogenic threats. Given the high conservation priority and lack of current census data, reassessing population sizes on San Cristóbal Island is a matter of urgency. Typically, simple counts are undertaken by the management authority of the Galápagos National Park for monitoring and assessment purposes. However, marine iguana movements during the day (Buttemer & Dawson 1993) mean that it can be difficult to see all iguanas in a colony at any point of time; for this reason, simple counts may miss large numbers of individuals. In order to obtain better estimates of the population size, we employed a mark-release-resighting (MRR) method. Choosing resighting, rather than recapture, allowed us to gain a large sample size during the second sampling stint, as it would increase the number of marked resighted animals, and thus improve the reliability of the population estimate. We chose a basic two-stint sampling method and employed Chapman's bias-reduced modification of the Lincoln-Petersen index for calculating population size. The aims of the study were to obtain population size estimates for one colony from each San Cristóbal MU using a MRR technique, and use this information as a basis for future monitoring and conservation practices.

The reliability of MRR results depend upon non-violation of the inherent assumptions. The assumptions of the method we employed include the following: (1) the population must be effectively closed; (2) marked individuals are able to mingle freely and are not affected by the mark; (3) the mark is not lost or overlooked; and (4) individual detection probability is the same on both occasions. Since the sampling took place in an area selected as to restrict the daily home range of individuals within the specific colonies, and resampling occurred only three days after the initial marking session, we can assume that the population is effectively closed (i.e., with a negligible probability of births, deaths, or migration between sessions). We know from previous field seasons that the marks used will remain clearly visible for several weeks and do not appear to alter the behaviour of their bearers in any way. Experience has taught us that marine iguanas are not easily perturbed by handling and we have seen no evidence of capture-shy behaviour developing in colonies consistently sampled over successive years. Furthermore, the fact that animals were only resighted (not recaptured) meant that iguanas could be sampled from a distance, thus reducing the probability of 'shy' animals escaping detection by hiding or fleeing, and therefore reducing the probability of introducing bias through an uneven individual detection probability. Both MRR stints took place during the mid-morning, when the iguanas have emerged from their overnight shelters but are still fairly sedentary, and when large males have not yet reached the thermal optimum at which they leave the intertidal area to forage in the sub-tidal zone (Buttemer & Dawson 1993). During sampling, which began at low tide and ended at mid-tide, we moved through the colony in a systematic manner, covering all areas between the rocks and bushes utilised overnight and the waterline. This ensured that we sampled both intertidal and sub-tidal foragers, and covered all zones utilised by iguanas during the morning (Buttemer & Dawson 1993). In this manner, we maximised our ability to sample animals randomly, and ensured that sampling was without replacement (i.e., we did not sample the same animal twice during a single sampling session).

The fieldwork took place in May/June of 2013, in two separate colonies on San Cristóbal Island. For the Loberia MU, we sampled the La Loberia colony (0°55'19.80" S, 89°37'15.04" W; SRL in Fig. 1) close to the town of Puerto Baquerizo Moreno. For the Punta Pitt MU, we sampled at Playa Blanca (0°41'42.04" S, 89°15'27.08" W; SRPB in Fig. 1). These colonies were chosen because they represented the largest of each population, and are characterised by distinct and well-defined geographic limits. The work was undertaken by observers who had already spent more than two months working intensely at these localities, and team composition was identical during all sampling occasions. For both colonies, the two sampling stints took place during the same time of day (approximately 08:00–12:00 h), and each session was spaced by three days. During the first stint, iguanas were captured using a specially modified lasso loop or by hand, marked with white paint on the lower flank (Fig. 2), and measured before release. Age and gender were recorded in three categories: adult male, adult female, and juvenile. We sexed only adult iguanas, using external features such as enlarged dorsal crests, comparatively larger body size, and well-developed femoral pores to indicate males, with those adults lacking such features being identified as females. Animals below the size of adult females (total length < 70cm) were classed as juveniles. On the resighting round (session two), we used the same route as during session one. We scanned the entire area visually, taking care to walk within 5 m of all possible basking and hiding places. During resighting, we only closely approached and handled animals if the body area used for marking was not otherwise visible, but this was not usually necessary.

We estimated population size according to Chapman's modified Lincoln-Petersen index:

$$N = \left[\frac{(M+1)(C+1)}{(R+1)}\right] - 1 \qquad (1)$$

where 'N' is the estimate of abundance, 'M' is the number marked in the first sampling session, 'C' is the total number captured in the second session, and 'R' is the number of marked animals captured (here: sighted) in the second session (recaptured/resighted).

$$\mathrm{Var}(N) = \frac{(M+1)(C+1)(M-R)(C-R)}{(R+1)^2\,(R+2)} \qquad (2)$$

Following Seber (1970), standard errors (SE) and 95% confidence intervals (CI) were estimated as:

$$SE = \sqrt{Var(N)} \quad (3)$$

$$CI = \pm 1.965 \sqrt{Var(N)} \quad (4)$$

A total of 114 animals were marked at SRL and 75 at SRPB (Table 1). The proportion of marked iguanas that were resighted was high, with an overall total of 0.47 at SRL and 0.73 at SRPB. The estimated population sizes were 400 (CI: 350–449) and 183 (155–210) at SRL and SRPB, respectively (Table 1). Excluding juveniles yielded estimates of 300 (265–335) at SRL and 147 (127–168) at SRPB. Confidence intervals were narrow, giving limits of within ± 12% of the estimated abundance of adults at SRL and 15% at SRPB.

If we use the most recently published estimates for population size (up to 400 animals; Wikelski & Nelson 2004), then the number of marked and resighted animals compared to population size was sufficient to provide the ± 10% accuracy recommended for research purposes and well above the ± 25% level of accuracy suggested for management purposes (Krebs 2014). Although estimates calculated by the local authorities are not routinely published, those from 2002 happen to be available (Snell & Marquez 2002). This report estimates the total *Amblyrhynchus* population on San Cristóbal at just over 5,000 animals. The reasons for such a large discrepancy between the estimates in 2002 and 2004 are unclear, and details on methodology used in both reports are sparse. Our experience suggests that the actual number is somewhere between these extremes. Typically, simple counts are utilised for estimates by wildlife managers, but these may underestimate the population size. The numbers of animals recorded in session two of our study included all *Amblyrhynchus* visible at the colonies, and they are therefore likely to be similar to the numbers obtained through simple counts. However, the number of animals counted at Loberia and Punta Pitt was only 60% and 56% of the estimated total size, respectively. This indicates that simple counts significantly underestimate the number of marine iguanas in a colony.

When comparing the population-size estimates provided by this study to estimates available elsewhere it is important to note that the numbers given here concern only one colony of a population, whereas other studies give estimates for a whole population (e.g., MacLeod & Steinfartz 2016) or an entire island (Snell & Marquez 2002, Wikelski & Nelson 2004). However, observations made through several years of fieldwork suggest that the two colonies estimated here are the largest of each population, and although the total population estimate will be higher, it is very unlikely to be more than two or three times larger. Similar studies are now needed in order to provide estimates of abundance at other major colonies. Our finding that SRL (of the Loberia MU) has more animals than SRPB

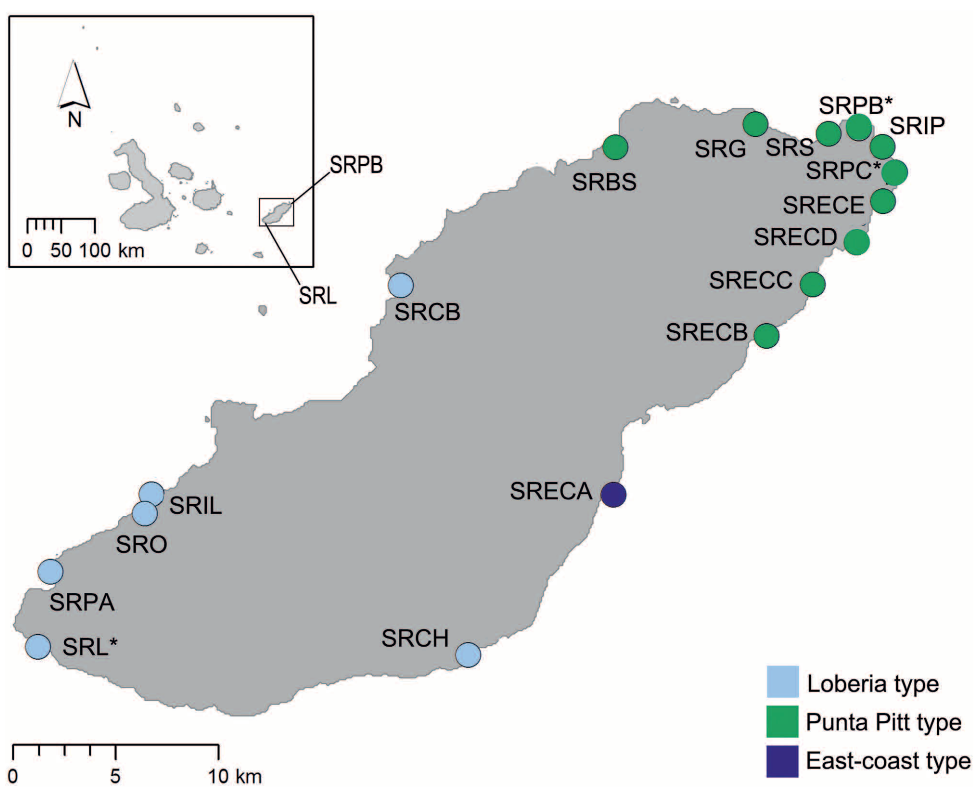

Figure 1. Schematic map of San Cristóbal Island, Galápagos, showing the results of a genetic structure analysis in *Amblyrhynchus cristatus* adopted from MacLeod et al. (2015). The three genetic types are indicated by differently coloured dots and in general have well-separated ranges on the island. Investigated sites studied for the data presented herein are marked with an asterisk and additionally indicated on the inset map.

Table 1. Results of the mark-resighting study on marine iguanas at two colonies on San Cristóbal island in June of 2013, showing the numbers of animals marked in the primary session (M), numbers of marked and unmarked animals (C), and numbers of marked animals only (R) detected in the secondary session, population size estimates (N) according to a Chapman's modified Lincoln-Petersen index with standard errors (SE) and 95% confidence intervals (CI)

	Loberia (SRL)						Playa Blanca (SRPB)					
	Raw data			Estimates			Raw data			Estimates		
	M	C	R	N	SE	CI	M	C	R	N	SE	CI
Adults	103	190	65	300	18	265–335	65	91	40	147	10	127–168
Males	98	160	62	252	15	223–281	58	80	34	136	11	114–157
Females	5	30	3	46	11	23–68	7	11	6	13	1	11–15
Juveniles	11	53	4	129	38	53–204	10	12	2	47	18	12–82
Total	114	243	69	400	25	350–449	75	103	42	183	14	155–210

(of the Punta Pitt MU) is consistent with previous studies, with Loberia demonstrating a larger N_e than Punta Pitt (MacLeod & Steinfartz 2016), along with higher values across a number of measurements of genetic richness (Rassmann 1996a, Steinfartz et al. 2009). More generally, the estimates obtained herein confirm that the N_e estimates for this island, although alarmingly small, are nonetheless reasonable, and that both these estimation methods can provide important information for managers.

Since San Cristóbal harbours two separate MUs, treating marine iguanas on this island as a single population is no longer appropriate for conservation purposes, and management should take into account the range and size of each population. Genetic analysis has revealed that each distinct colony on San Cristóbal can be categorised as belonging to one of three populations: Punta Pitt, Loberia, or a third 'East-coast' type that was probably established by migrants from Española Island (MacLeod et al. 2015).

Figure 2. Individuals of *Amblyrhynchus cristatus* on San Cristóbal Island, Galápagos, showing the temporary marks used during the mark-resighting study.

These populations exist in well-defined and genetically separate colonies (Fig. 1). Whilst both native San Cristóbal populations are small and vulnerable to anthropogenic threats (Wikelski & Nelson 2004), Punta Pitt consistently ranks as a higher priority for conservation management than Loberia (Rassmann 1996a, Steinfartz et al. 2009, MacLeod & Steinfartz 2016). Considering their genetic distinctiveness and small population size, highlighted now by three independent studies (Wikelski & Nelson 2004, MacLeod & Steinfartz 2016), the iguanas on San Cristóbal should henceforth be regarded as the highest priority for *Amblyrhynchus* conservation in the Galapagos.

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