

Review of

“Power analyses to inform clutch sampling design to determine the breeding sex ratio in populations with multiple paternity”

submitted to PeerJ

The authors in this paper employ simulations to quantify the effect of genetic sampling effort (within clutch and within season) on the estimated number of fathers in oviparous species that exhibit polygamy, e.g. sea turtles. The paper is well-written, straightforward and easily understandable. The results are not surprising though, in fact the problem can be essentially posed as an exercise combinatorial probability. Nevertheless, the simulations are clear, and they provide useful sampling guidelines. Furthermore, the fact that there is an associated publicly available code (which I did not check in detail) where one can adapt several parameters makes the paper valuable to the community. Thus I support its publication. However, the paper contains some elements that they should be corrected/discussed in more detail.

Major remarks:

- Please define clearly the terms *Breeding Sex Ratio* (BSR) and *Operational Sex Ratio* (OSR) as well as situations where they can be different. A few times these terms are interchanged in the text. Aligned with the authors, at the timescale of a breeding season like here, these are defined as:

$$\text{OSR} = \frac{\text{Number of males available to breed}}{\text{Number of females available to breed} + \text{Number of males available to breed}}$$

$$\text{BSR} = \frac{\text{Number of males managed to breed}}{\text{Number of females managed to breed} + \text{Number of males managed to breed}}$$

Under these definitions OSR cannot be tracked by genetic sampling or beach surveys. The authors argue that in species which exhibit polygamy OSR could be very different than BSR. However, I postulate that this might not be the case at least for sea turtles: If many available females do not manage to breed that would mean a lot of unfertilised clutches which is not the case in most sea turtle populations (unless the authors can argue that these females will not even try to lay clutches). For males, it is less clear if there are many available individuals that do not manage to breed. In their simulations, because the authors assume no polygyny and much less males than females, they adopt an exhaustive approach where all males manage to breed and most of the females do not. This is not so realistic. For instance in Figure 1: “*Females 7 through 90 did not mate in this scenario as there were no males left in the male breeding pool vector after female 6 mated with males 8, 9, and 10*”. I found this scenario not realistic given the mating behaviour of male sea turtles. In general, I find the figure not representative for a sea turtle population (i.e. 84% of the females not mating).

That brings me to the other main remark which is if absence of polygyny makes sense. The authors rightfully claim that even though there is a general expectation that polygyny is prevalent, a few genetic studies have shown it. However this remains paradoxical due to my argument above. I suggest at least not having the polygyny example only in the supplementary but having it in the main text instead and not give special focus in the case where there is no polygyny.

- *This is especially important given that green turtles are listed as globally endangered with a declining population trend (IUCN, 2024):* This is incorrect. The authors actually cite a paper that indicates the opposite (Chaloupka et al. 2008). Furthermore, in this paper <https://www.nature.com/articles/s44358-024-00011-y> it is mentioned: “*Owing to these positive nesting trends in many areas, the global population of green turtles is expected to be downlisted*”

in 2025 to Least Concern from their prior IUCN listing as Endangered (IUCN Marine Turtle Specialist Group)”.

- Discussing potential preferences of males to larger females is worthwhile, please see Zbinden et al. 2007 Figure 2. That could indicate that it is preferable to sample nests of larger females and thus (since there is a correlation) clutches of larger size.
- I do not understand why the analysis should be constrained to a particular green sea turtle population. It looks like the only reason is to have some fixed means and variances for number of nests per season and eggs per clutch. But the authors can make these free parameters (plus play with varying polyandry and polygyny rates) and thus handle essentially all sea turtle species and populations. There is nothing in the methodology which constrains it to a particular species/population.
- Line 108: I understand why in simulations is it important to state that you assume that sampling did not cause adult mortality (so they can “lay” all the nests they are “supposed to”). But why is it relevant to state that for hatchlings as well?
- While the use of normal distributions is probably ok for the number of eggs per clutch, I am a little bit worried about doing the same for the number of clutches per female. Can you show that the empirical distribution that results from that assumption + rounding to integer aligns well with what is observed at the field? Furthermore, it well-known that not all clutches of a given female are detected and thus not all of them can be potentially sampled. I would also discuss this as a limitation.

Some more minor remarks:

- (1) Line 52: Reference Botsford et al. 2019, is missing from the list of references at the end.
- (2) Try to reduce the sentence length in lines 59-66.
- (3) Lines 67-68: Please add the review <https://doi.org/10.1016/bs.amb.2017.09.004> to the list of references. Also, are the Reina et al. 2005, Thornhill and Alcock 2013 really relevant here? I did not read them but from the titles they seem not.
- (4) Is there any reason why you fixed 100 individuals in the population? Do the graphs change when you consider other numbers of higher orders of magnitude?
- (5) It is assumed that the paternal contributions to clutches of a given female will be similar in all the nests she lays during the season. Is this realistic?
- (6) Line 172: Referencing Figure 1 seems not so suitable since you are talking about the sufficiency of 10,000 simulations.
- (7) Line 182: State the Bayes theorem in that context. This sentence should be made more precise as probabilities should be stated with respect to events not numbers.
- (8) Make it clear upfront whether you “sample” eggs or hatchlings. In that context discuss whether multiple paternity affects hatching success or not (see Zbinden et al. 2007).
- (9) Lines 280-281: It seems that there is no difference to what was simulated before as in the main simulations also females were equally likely to mate with any number of males between one and five.
- (10) Lines 375: The paper <https://doi.org/10.1111/1365-2435.12930> should be also cited.
- (11) Line 395: What do you mean “not detected in many more populations”. Maybe rephrase?
- (12) Table 1: Instead of “Probabilities that a mother/father mates with 1-5 fathers/mothers” is better to state it in terms of fertilising eggs, since mating might not result in fertilised eggs which is what is used here.