**B.11. Animal Procedures:**

See B.10. Experimental Procedures.

**Justification for numbers:**

I will use generalized linear mixed models to compare diet as determined by the two techniques. A mixed model is necessary to account for pseudoreplication. Although I will likely pool fecal sacs from siblings to reduce the cost of prey DNA sequencing, I would still need to account for temporal pseudoreplication if the same chicks are sampled at different ages. However, I am not aware of a power analysis to determine sample size for such mixed model. A new R package (SIMR) has been developed to calculate the power of such analysis, but this can only be done with the collected data. Therefore, I determined my sample sizes based on other nestling prey DNA barcoding studies: Trevelline et al. (2018a,b) used between 78 and 137 nestlings from 10 and 43 nests, respectively, whereas Jedlicka et al. (2016) used 169 nestlings. For bluebirds, I will sample 20 nests, which hold 4 nestlings on average, totaling 80 nestlings. Other species are not as common as bluebirds in nest boxes, so I will have to limit my sampling to 5 nests, with usually more nestlings per nests (5-7), totaling 25 nestlings per species. If the sample size is too small for these other species to obtain a power of 0.80 or more, I will only test for differences between bluebirds and non-bluebirds as a whole.

All individuals fall in category C because there is no pain and only temporary stress involved. No euthanasia is needed for this study; zero bird will be euthanized at the end of this study.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| GROUP | TREATMENT | NUMBER OF ANIMALS | PAIN CATEGORY | EUTHANIZED AT END OF EXPERIMENT |
| Bluebird | N/A | 80 | C | 0 |
| Chickadee | N/A | 25 | C | 0 |
| Titmouse | N/A | 25 | C | 0 |
| Wren | N/A | 25 | C | 0 |

To avoid bird-bird and human-bird contamination, we will make sure that thorough cleaning of the hands will be done using wet wipes with alcohol or hand sanitizer after processing each bird.

**Permits**:

Federal Bird Banding Permit: 23811

State Scientific Collection Permit: 121820174