Golondrinas Handbook Supplement

The fieldwork is replicated at each Golondrinas study site to yield a robust set of information for testing hypotheses regarding geographic variation in clutch size. We have grouped these hypotheses into FOUR primary categories, as illustrated in Figure 1. To test many of the hypotheses requires the field technicians to collect more data than in the standard Golondrinas Handbook. This Supplement describes the additional field

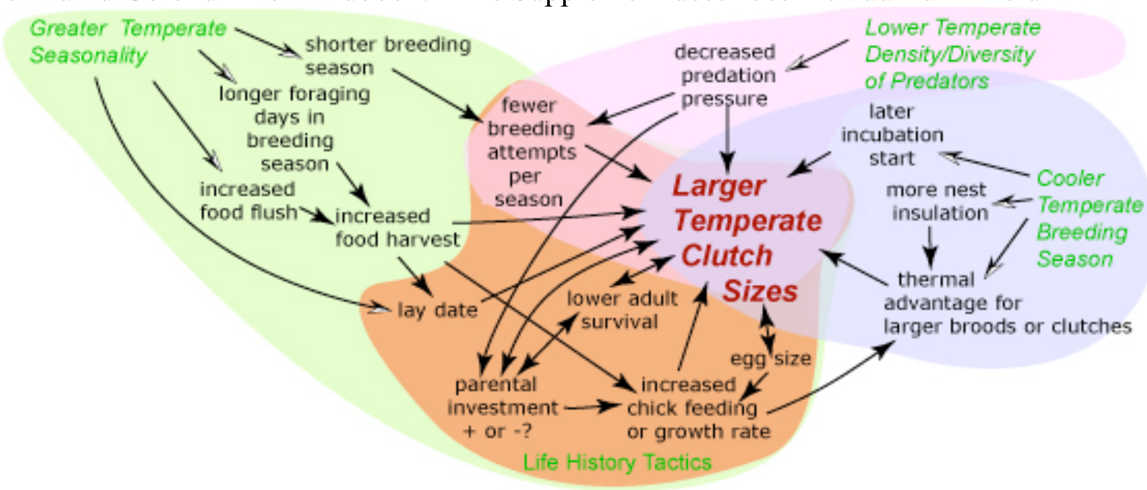

Figure 1. Sketch of the relationships among the various potential causes of latitudinal variation in clutch size. Arrows indicate hypothesized direction of causation among factors when these are clear. All arrows on this figure will be tested with data collected in this project. The causative factors in green italics are those directly affected by latitude per se.

protocols, in relation to the hypotheses, to assist field technicians in carrying out data collection effectively.

Focal Nests

The basic Golondrinas protocols are carried out on all nests. Supplemental data for sections on **Greater Temperate Seasonality** and **Cooler Temperate Breeding Season**, as described below, should be gathered from a set of **20 focal nests**. It is important that the nests selected to be part of the focal 20 have laying dates that span the breeding season. Given the expected number of nests at a site (e.g., 60), field technicians should include nests at the appropriate interval (e.g., every 3rd nest as it is initiated) to yield 20 temporally spaced focal nests. If in doubt, err on the side of more nests than 20, in case the expected 60 nests aren't all successful.

Data Management

All data collected must be clearly identified by the unique combination of site identity (study site name), year, nest box identity, and attempt number <site_id.year.nest_box_id.attempt>.

Greater Temperate Seasonality

Background: The seasonality hypothesis predicts higher food availability in the north leading to larger clutches. Increased food availability for nestlings could occur because of greater food resources or because of increased time during the day in order to forage or both. Central to this idea is that greater food resources in northern latitudes allow birds to lay larger clutches and to raise them successfully. In other words, southern species and populations are limited in the resources available to them and thus lay and produce smaller clutches. In addition to the role of resources, it may be that the longer potential breeding season in the low latitudes allows for more breeding attempts or the greater ability to relay if a breeding attempt fails. We test these hypotheses by measuring food supply, food deliveries and parental feeding effort.

The key prediction that will allow us to differentiate between the role of food supply and foraging day is whether total hourly feeding rates (i.e. mean feeding rates per hour) vary across latitudes. If food supply increases with increasing latitude, then total feeding visits/unit time should increase as well. However, if food supply is constant, then longer days should allow for increased time spent foraging but no difference in effort/hour.

High latitudes have a shorter breeding season, thus there are no opportunities to breed multiple times in a single breeding season and fewer opportunities to renest if a breeding attempt fails. Thus the life history value of a single breeding attempt is higher leading to a greater investment (i.e. more eggs) per breeding attempt.

Prediction 1: Average daily food availability measured at automated insect samplers operating at the sites will increase with latitude
Prediction 2: Estimated feeding effort (mean visits/hour) increases with latitude.
Prediction 3: Total time spent active increases with increasing latitude. (difference between photoperiod and active day increases with latitude – i.e., southern birds face day length constraint)

<u>Methods</u>
We will test the seasonality hypothesis using four main methods:

1. Insect sampling
2. Feeding observations
3. Diet samples
4. Determining active day

Insect sampling: At each site will have at least one automated insect sampler. These samplers suck a column of air above a circulating fan blade. Any insect that flies above the column is drawn into a bottle of alcohol (percentages of mixture?) and captured.

Clearly label each bottle with the Site ID and complete Date (month, day, year) and keep a log of each sample name in your field notebook.

By changing the sampling bottle daily, we are able to determine daily fluctuations in insect activity and availability. This dataset can reveal differences in food availability among sites, as well as providing insight into insect thermal tolerances, differences in prey size among sites, and the role of climate change on insect populations. The daily routine of changing the insect sampler is building a unique and powerful dataset.

Feeding observations: Field technicians will measure feeding activity of our birds on standardized intervals all across the western Hemisphere. Each of the 20 focal nests will be observed at least twice (at nestling age 7 and 10) for a minimum of 60 minutes in the morning. During the observations, field technicians must note the time of each visit and the sex of the parent. The day prior to feeding observations are days when the parents should be trapped, in accordance with the Standard Golondrinas Handbook. Technicians should use that opportunity to mark (place white-out on the upper back of) the first adult captured at each nest. It is critical to clearly note exactly which sex was marked for each nest. During each feeding observation, it is also important to record the weather (temperature, wind speed, cloud cover, precipitation) so as to correct for environmental effects. Use accompanying Feeding Observation Data Sheet. Assign boxes to observation time slots prior to going in the field (don't decide to watch a box because you see lots of activity).

Before starting a feeding observation, settle into a location at least 100m from the box in a location that does not interfere with other nests. Wait no less than 10 minutes after placing yourself in location to begin the observation. The most efficient manner of recording your data while keeping focused on the nestbox is to circle notation on the data sheet and attach a timing device to your clipboard. This will minimize the time with your eyes away from observing.

Diet samples: When adult swallows are captured during the nestling phase, they are often holding a bolus of food in their mouth. It is critical that we remove these diet samples whenever possible. Each field technician should carry forceps and a few vials filled with alcohol, so that when a diet sample presents itself, we can remove and store it. Data samples should be labeled with date, site identity (study site name), year, nest box identity, and attempt number <site_id.year.nest_box_id.attempt> in pencil on a piece of paper or notecard in the vial. In addition, each sample should be recorded on the data sample log.

Active day: We will extract time of activity from the iButton dataloggers placed in each nest (as part of the investigations into Cooler Temperate Breeding Season (see below). The temperature traces contained within these data files indicate when females shift their behavior from day-time to night-time incubation revealing how closely activity patterns match daylight hours.

Cooler Temperate Breeding Season

Incubation Start

Background: The egg-viability hypothesis suggests that the reduction in egg-viability of unattended eggs during the laying period selects for smaller clutches in warmer environments. Birds lay at most one egg per day, and passerine embryos can remain viable for extended periods before development is triggered. High (above 26°C) ambient temperatures raise the risk of unsynchronized tissue growth, abnormal development and embryo mortality before the start of incubation and thus could select for relatively ***earlier incubation start*** at lower latitudes leading to smaller clutches. Comparisons of Tree Swallows breeding in Tennessee and Alaska revealed that females were more likely to start incubation early when temperatures exceeded 26°C, irrespective of location and lay date. These results from North American sites are especially interesting since the hypothesized mechanism (loss of embryo viability) is expected to be of increasing importance at tropical latitudes as daily temperatures exceed 26°C for much of the day.

Prediction 1: Within and between sites, warm temperatures lead to incubation beginning earlier in the laying sequence.
Prediction 2: Under warm temperatures, early-laid eggs will be less likely to hatch if females don't begin incubation earlier in laying sequence.

Methods

For each of the 20 focal nests, visit every day during laying, marking each egg as it appears. Using a pencil, held tangential (not point-on) to the shell, eggs should be numbered as they appear. The pencil markings permit technicians to record hatching order (if possible) and to identify the laying order of any unhatched eggs.

Using iThermo running for a Palm Pilot, technicians should program pairs of iButton data loggers (DLs) Mission to record every 2 minutes. The memory of DLs recording every 2 minutes will reach capacity in 3 days. Technicians should download data from DLs and reprogram them every 3rd day throughout laying. Information on how to program and use the Palm software and the iButtons is included in a separate guide. It is critical to save files so that the name includes the Site Identity, Year, Nest Box Identity, and Attempt Number. This information will enable the DL data to be matched up with the monitoring data such as clutch size, first egg date, etc.

Clutch cooling rates

Background: The thermal dynamics of incubation and brooding are dependent on the number of eggs and chicks, respectively. The clutch-cooling hypothesis suggests that for intermittent incubators (like swallows), a ***thermal advantage for larger clutches*** arises from the greater thermal inertia of

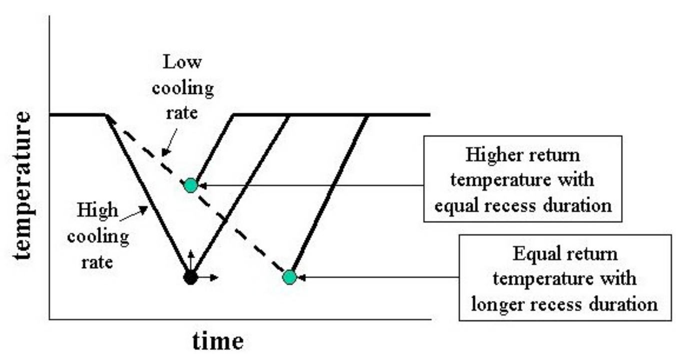

Figure 2. Clutch temperature vs . incubation recesses and the relative cooling rates of small (solid line/black dot) and large (dotted line/green dots) clutch sizes.

larger clutches in cooler weather. Incubation rhythms represent the optimal balance females create between meeting their own energy demands while maintaining the thermal requirements of the embryos. This hypothesis predicts that reduced cooling rates could affect female decisions regarding the balance of parent-embryo demands in several ways. The hypothesis is complicated by the likelihood that reduced cooling rates may result predominantly from warmer ambient temperatures, and secondarily from larger clutches and greater nest insulation. Reduced cooling rates could benefit females by permitting longer foraging bouts without any costs to embryos (no decrease in average egg temperature), or could benefit the embryos (increase average egg temperature) without any costs to the female (no change foraging bout duration) (see Figure 2). Reductions in cooling rates that stem from larger clutches and nest insulation might be advantageous in higher latitudes and in cooler environments, while reductions in cooling rates from higher ambient temperatures in the tropics may swamp out any effects of clutch size or nest insulation on cooling rates.

Overall cooling rates are determined by the interaction of three factors (clutch size, ambient temperatures, and nest insulation) that may vary latitudinally, sometimes in opposite directions. We will gather the first systematic data set on all three of these factors, together with the key behavioral component, incubation rhythm, to understand how latitude affects incubation and selection on clutch sizes.

Prediction 1: when environmental conditions are cooler, females have short recesses, with shortest recesses among females with smaller clutches, or
Prediction 2: when environmental conditions are cooler, females of all clutch sizes have equally short recesses, and so females with larger clutches have higher return temperatures

Methods

As during the laying phase, technicians should place iButtons in nests recording at 2 min intervals. The memory of DLs recording every 2 minutes will reach capacity in 3 days. Technicians should download data from DLs and reprogram them every 3rd day until all eggs have hatched. It is critical to save files so that the name includes the Site Identity, Year, Nest Box Identity, and Attempt Number. This information will enable the DL data to be matched up with the monitoring data such as clutch size, first egg date, etc.

Geographic variation in nest insulation

Refer to the Golondrinas Handbook, which outlines methods for measuring nest material.

Lower Temperate Density/Diversity of Predators

Alexander Skutch proposed one of the most influential hypotheses for clutch size reduction in the tropics by connecting the frequency of ***parental feeding visits*** to the ***predation risk*** of the brood, later demonstrated by Martin and colleagues. Skutch claimed that the number and diversity of nest-predators was much higher in the tropics, and reasoned that parents visiting the nest served as a location-cue for would-be predators. Thus, parents outside the tropics, encountering ***lower densities or diversities of predators*** could afford to risk rearing the chicks from ***larger temperate clutches.*** There is a clear need to monitor nest-predation intensity with latitude, and we will do so in natural nests in this project. There are also very few direct comparisons of the patterns of prey delivery to the offspring across latitudes. Orians found that females visited the nest less often and fed lower amounts of food per hour in Costa Rican blackbirds than in the same species in Washington, yet Martin et al. found that mid-latitude birds in the southern hemisphere, with smaller clutches, actually delivered more food to their broods, more frequently, than do birds at higher latitudes in the north. Clearly, further systematic data collection in a single clade of birds is warranted over a larger geographic scope to determine whether feeding rates are higher in temperate areas and whether the strength of the link between feeding rates and predation risk varies geographically.

Methods

Exploring the role of predators requires estimates of parental feeding rates (estimated for 20 focal nests as described under section on Greater Temperate Seasonality) and estimates of natural predation rates. For this objective, field technicians should assess the likelihood of natural nests occurring near the study site. If natural nests are likely, technicians should search for natural nests. If any natural nests are found, they should be monitored in a manner analogous to nest boxes, but with inferring phases based on parental observations. If nest entrances can be reached, technicians should use telescoping mechanics mirrors to view inside cavities. If ladders are used, technicians MUST wear helmets.

Life History Tactics

The central set of interactions among effects in Fig. 1 involves the intriguing set of tactical trade-offs among different forms and amounts of reproductive effort, many of which are remote from environmental influences. It is extremely likely that tactical trade-offs vary with latitude, and elucidating these patterns to test the following hypotheses will likely provide many of the missing pieces to the clutch size puzzle.

*Adult Survival-Reproduction Trade-off (across years).*Latitudinal differences in ***adult survival*** could constrain or mediate latitudinal variation in clutch size. Trade-offs between parental survival and fecundity are a basic building block of life history theory. The traditional generalization is that survival rates are higher in the tropics, yet there is considerable debate about the evidence because many studies mix the return rates of different segments of the populations (e.g. floaters vs. territorial breeders vs. dispersing young). The proposed study is focusing on the survival and ecology of the breeding segment of swallow populations throughout the Americas. The project will produce the first comparative mark-recapture dataset on a single genus of birds across the entire range of possible latitudes, making it possible to test for a latitudinal gradient in breeding adult survival and its correlation with clutch size.

An association of ***larger clutches*** with ***lower adult survival*** is often presumed to exist for latitudinal gradients and has been demonstrated for contrasts between northern and southern hemisphere passerines from mid-latitudes. After we explore the generality of this association, the next step will be to determine whether such an association results from selection for reduced clutch sizes in response to higher adult survival, or higher adult survival in response to reduced fecundity, a fundamental question in life history theory.

Breeding attempts per season (trade-off within year). If there are ***fewer temperate breeding attempts per season***, this raises the possibility that ***larger temperate clutch sizes*** could arise from a within-season reallocation of effort. In *Tachycineta*, breeding seasons are shorter in the temperate, both within and across species, but it is not clear whether this is due to infrequent multiple attempts by individuals or greater synchrony of laying dates at higher latitudes. Separating multiple brooding from highly de-synchronized breeding is critical to accurately measuring latitudinal gradients in fecundity (for *Adult Survival-Reproduction Trade-off*; see above) and to weighing accurately the ultimate and proximate causes of regional variation in parental tactics and their effects on clutch size variation. Few studies have examined systematic latitudinal variation in multiple-broodedness within a single bird species, but comparative studies suggest that the number of broods, rather than clutch size, is the primary influence on annual fecundity.

Egg Size-Clutch Size allocations (trade-off within breeding attempt). One of the most straightforward correlates of reduced clutch size may be a parental repackaging of gametic effort into fewer but larger eggs. The meager data available on egg sizes of *Tachycineta* species suggests that birds in lower latitudes may actually be laying larger eggs for their body size (Winkler et al. unpubl. data) without changing relative egg length. Increases in ***egg size*** between species are correlated with increases in incubation and nestling periods, and they thus may be associated with the intriguing negative relationship between clutch size and these other reproductive traits.

Methods

These hypotheses will be addressed using data collected from all nests as described in the standard Golondrinas Handbook. It is very important that technicians trap and band all adults and offspring. It is equally important that technicians re-capture all adults banded in previous years in order to make a documented re-sighting (i.e., record band numbers). The Golondrinas Handbook contains the protocols for measuring egg size.

Feeding Observation Data Sheet

Site:		**Box:**		**Date:**		**Observer Name:**	

Start time:		Temperature	
End time:		Wind	
Confidence in determining (M vs F)		Cloud	
		Precipitation	

Time Entering Box:	Sex	Time Entering Box:	Sex
	F M U		F M U
	F M U		F M U
	F M U		F M U
	F M U		F M U
	F M U		F M U
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