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GARRAFÃO PROJECT: ORIGIN, HISTORY AND MAIN ASPECTS OF THE DEVELOPMENT OF THE LONGEST LONG-TERM STUDY OF ECOLOGY OF SMALL MAMMALS IN BRAZIL

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Abstract: This article introduces the special volume dedicated to one of the longest wildlife monitoring programs ever carried out in Brazil: the long-term study on small mammals developed by the Laboratório de Vertebrados at UFRJ from 1996 to 2019 in Garrafão, municipality of Guapimirim, Rio de Janeiro. The guiding ideas of this study emerged from a previous project developed at the Restinga de Barra de Maricá, RJ, in the 1980s on the ecology of small mammal populations and communities. The main research objectives were related to community structure, population growth and population dynamics, aiming to understand the ecological processes and the mechanisms explaining the fluctuation of population size in several species over time. Initially, models of genetic population structure and population dynamics were tested. Further developments in the project allowed other aspects to be studied, such as diet and nutritional requirements of the animals, population genetics, use of space, habitat, biogeography, taxonomy, and systematics. These approaches, based on relevant ecological questions, have provided advances in knowledge in several areas of biology, especially in population ecology. The study resulted in dozens of articles, book chapters, dissertations and theses and was interrupted by conjuncture factors and abrupt changes in the funding policy for the development of scientific research in Brazil.

Keywords: habitat selection; litterfall production; marsupials, population biology; rodents.

INTRODUCTION

This special volume of Oecologia Australis is dedicated to the Garrafão Project, one of the longest and uninterrupted monitoring studies of small mammals ever carried out in Brazil, developed by the Laboratório de Vertebrados (LabVert) at the Universidade Federal do Rio de Janeiro (UFRJ), coordinated by Professor Rui Cerqueira. Considering long-term studies of the ecology of small mammals with durations of more than five years in Brazil, we can cite Cerqueira *et al.* (1993), Gentile *et al.* (2000), Marinho (2003), Castro & Fernandez (2004) and Vieira *et al.* (2009) in the Atlantic Forest; Alho *et al.* (1986) in the Cerrado; Cáceres *et al.* (2011) in the Pantanal; and Emmons (1984), Malcolm (1997) and Rosa *et al.* 2021 in the Amazon. However, some of them were not uninterrupted (Cerqueira *et al.* 1993, Cáceres *et al.* 2011, Emmons 1984, Malcolm 1997 and Rosa *et al.* 2021). Thus, the Garrafão Project can be considered a landmark or reference for long-term ecological studies, given its uniqueness and complexity. The entire context and production related to the Garrafão Project can be used as an incentive and guide for other long-term studies with small mammals (and other taxa) in Brazil.

The project was carried out between 1996 and 2019 in the locality of Garrafão, municipality of Guapimirim, state of Rio de Janeiro, in southeastern Brazil (Figure 1). In this article, we report the development of the scientific ideas that triggered this project and its origin, the state of the art at that initial moment, the global context, the main results and subsequent development, the main scientific knowledge acquired, as well as the technical and scientific restraints of this project. The project involved several aspects of the ecology and genetics of small mammal populations, such as demography, community structure, habitat selection, litterfall production and its relationship with population dynamics, and use of space, among other topics.

Since its inception, LabVert has always had a thematic proposal related to population biology studies. This theme originated from the development of ecological theory around the idea that evolution is an integral part of the phenomena related to ecological studies (Begon *et al.* 1996). Since the 1930s, evolutionary theories, in turn, have been developed in terms of interactions of organisms from a hereditary basis and the environment. However, it was not until the 1980s when ecology finally began to be incorporated into this synthesis (Shorrocks 1984). Such a synthesis must incorporate

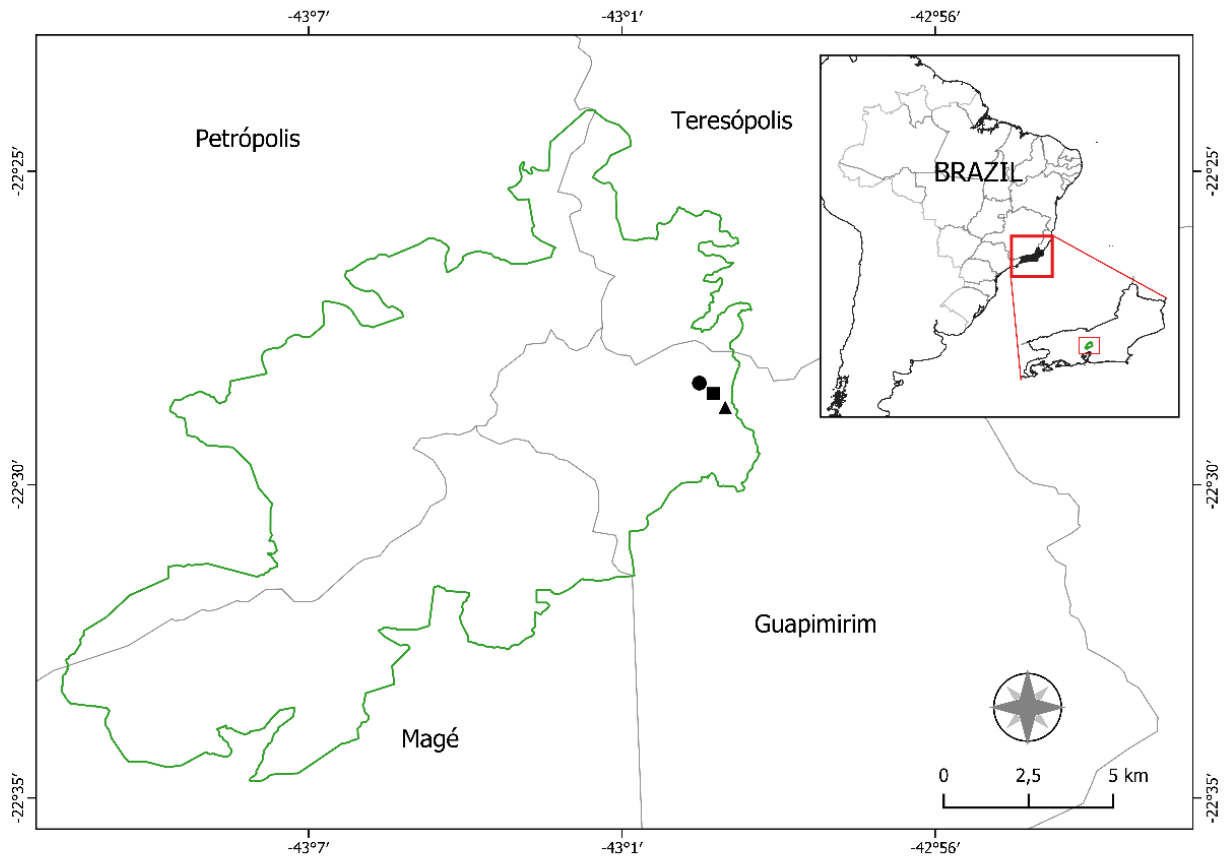

Figure 1. Map of the study area indicating the three grids of small mammal captures of the Garrafão Project inside the Parque Nacional da Serra dos Órgãos (PARNASO; green line) and the municipalities. On the right side is the location of PARNASO in the state of Rio de Janeiro and the location of the state in Brazil. Grid A: circle; Grid B: square; Grid C: triangle.

the evolutionary concepts of classical population genetics (Roughgarden 1995) and their approach in ecology (Roff 1992). Heritability, a necessary condition for evolution, despite still being poorly investigated, gains importance with an agreement, therefore, between ecological factors and life history strategies (Lemos *et al.* 2001). These approaches were the basis of the Garrafão project, explicitly integrating genetic, ecological and demographic methods, aiming for a comprehensive and unifying Population Biology study.

Much of the ideas of the project arose from the knowledge about evolutionary and community ecology published in previous decades (McArthur 1972, Cody & Diamond 1975), in addition to aspects related to the former views of Clements (1916) and Gleason (1917) about how communities can be structured. Identifying the processes governing spatial and temporal variation in biological populations and the structuring of communities were the main aims in population and community ecology studies worldwide in the 1980s and 1990s. Thus, the questions related to these governing processes could be understood based on local and regional approaches.

In this context, the first study of population and community ecology carried out by LabVert was the Maricá Project (1986 to 1990). A review of some aspects of the population structure of small mammals in Maricá can be accessed in Cerqueira (2000a), as well as the biogeographical setting (Cerqueira 2000b). Among the results, we highlight that some species, such as the marsupials *Didelphis aurita* and *Metachirus myosuros* (formerly *M. nudicaudatus*), showed high mobility, low aggregation coefficients, and absence of permanent population centres, in addition to irregular patterns of temporal dynamics in the study area (Cerqueira *et al.* 1993, Gentile & Cerqueira 1995). In contrast, species such as the marsupial *P. quica* (formerly *P. opossum*) and the rodent *A. cursor* showed higher levels of aggregation and local constancy in the study area. Therefore, the research group hypothesised that some species could be structured into metapopulation systems, given that animal movements are species-specific and that local populations are undergoing processes of extinction and recolonisation over time. Thus, the possibility of testing these hypotheses on larger spatial and temporal scales began to be discussed at LabVert.

The Maricá study came to an end due to political changes in the Brazilian science financing policies. When the conditions changed, an opportunity for a study in the Serra dos Órgãos arose, mainly because there were lodging facilities. The Maricá Project was originally designed as a long-term project; thus, the same ideas that based the Maricá Project, also based the Garrafão Project. As long as there was funding available, the project grew, incorporating an increasing number of objectives throughout its existence. The main initial objective was to elaborate and evaluate population models to integrate population ecology, demography and population genetics knowledge. The studies of habitat selection, litterfall production and use of space merged with the posted hypotheses and took place in parallel throughout the population study.

POPULATION BIOLOGY: ECOLOGY AND GENETICS

Traditional approaches in population dynamic studies focused on estimating population sizes and the factors affecting their fluctuation (Cole 1954). In turn, population genetics began as an experimental and theoretical discipline in the first half of the 20th century (Provine 1971). Field studies commenced in the United States and Europe, and the analysis of the genetic structure of populations soon became an important tool to help understand the patterns of genetic population structure associated with population ecology studies. Considering that the degree of connectivity among local populations can be measured with population genetic methods - using the gene frequencies of a given molecular marker - the idea of the Garrafão project regarding Population Biology was to test ecological hypotheses and models using molecular markers as tools, along with traditional methods of population ecology studies.

We conducted two species surveys in Garrafão, one in 1992 and one in 1996, to identify the species present and deposit voucher specimens in the mammal collection of Museu Nacional do Rio de Janeiro before the beginning of the long-term study. The ecological approach was carried out using the capture-mark-recapture method in three fixed trapping sites (Grid A: 22°28’15”S 42°59’52”W; Grid B: 22°28’26”S 42°59’37.4”W; Grid C: 22°28’40”S 42°59’25.2”W) established since April 1997 (Gentile *et al.* 2004; Figure 2). The sampling campaigns were carried out bimonthly for five consecutive days until February 2019, following a standardised methodology at all sites and over time. Each grid had 25 capture points (5 x 5), spaced at 20 m. Initially, traps were placed only on the ground, using a Tomahawk® (40.64 cm x 12.70 cm x 12.70 cm) and a Sherman® (7.62 cm x 9.53 cm x 30.48 cm) live-trap at each point. In addition, five large Tomahawk® live-traps (50.80 cm x 17.78 cm x 17.78 cm) were placed on each grid, arranged in cross. From April 2000, platforms were installed in the canopy, in 13 points of each grid, in an alternating way. Each platform had a Tomahawk® and a Sherman® trap (Figure 3). From February 2009, Sherman® traps were settled in the understory at every point. This design remained until the end of the study. Population studies included the data from all traps.

Initially, the target was to analyse the population dynamics and the basic demographic aspects of the *D. aurita* population, the most abundant species in the study area, among sites and over time, as well as to quantify its genetic variability at each site and among them. Genetic samples of the animals were collected over time and preserved in LabVert. The genetic approach was carried out using microsatellite molecular markers to determine the degree of genetic differentiation in *D. aurita* population within and between sites.

The main conclusions of this first stage of the Garrafão project carried out during the first three years were as follows: 1) genetic and geographic distances were correlated, indicating that dispersal was an important factor in the degree of genetic variation; 2) ecological or geographic isolation might have caused population differences; and 3) *D. aurita* showed a population structure model characterised by a continuous landscape formed by patches of heterogeneous environment, with the absence of permanent population centres, persisting regionally (Gentile 2000, Gentile *et al.* 2004, Gentile & Kajin 2015). Thus, the set of subpopulations formed a metapopulation, corroborating the hypothesis raised in the Maricá Project. However, the regional structure of the *D. aurita* population followed the model of spatially extended populations (Freckleton & Watkinson 2002).

After seven years of study, we also observed that the density and biomass dynamics of *D. aurita* indicated a tighter regulating process acting on female biomass, which was less variable among years compared to their density (Mendel *et al.* 2008). However, further studies with fifteen years of data showed that the pattern is rather complex, different from the initially observed pattern (Sá 2013).

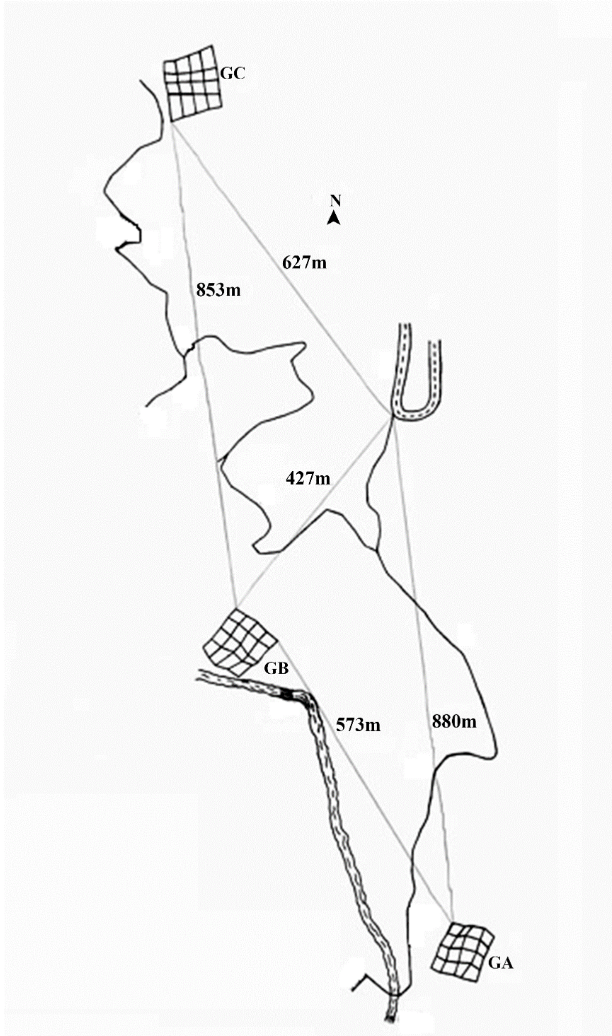

Figure 2. Schemes of the grids of trapping points of small mammals of the Garrafão Project with distances between them. The solid black line indicates the dirt road that gives access to the areas. The dotted line indicates the Rio-Teresópolis highway. The dashed line between GB and GC indicates a stream. GA: Grid A; GB: Grid B; GC: Grid C. Adapted from Freitas (1998).

From then on, the project had its objectives, as well as the applied approaches, methodologies and investigated species expanded, giving rise to the project entitled “Biology of Mammal Populations with Emphasis on the Atlantic Forest of Rio de Janeiro” (Biologia de Populações de Mamíferos com

Figure 3. Sherman and Tomahawk live traps used in the capture-mark-recapture study of small mammals of the Garrafão Project. Author: Rosana Gentile.

Ênfase na Mata Atlântica do Rio de Janeiro - 2006-2008). The continuation of the Garrafão project increased the time series length of population sizes, and consequently, species demography could be analysed using more complex methods of mathematical modelling.

DEMOGRAPHIC MODELLING IN POPULATION ECOLOGY

Mathematical models are fundamental in ecological studies, as they help to describe ecological concepts and mechanisms, representing components and functions of biological systems. However, the use of mathematical models in population dynamics requires long time-series data, which was only possible a few years after the beginning of the Garrafão Project. In addition, marking pouch young marsupials in their first life stage (Loretto *et al.* 2013) allowed a more detailed demographic analysis of this taxon, including the understanding of development stages, which are easily recorded in the field, with great consistency among species and genera (Macedo *et al.* 2006).

Previous empirical studies of LabVert regarding reproduction showed markedly different patterns for rodents (Cerqueira & Lara 1991) and marsupials (Cerqueira & Bergallo 1993), suggesting that reproduction, as well as population ecology, could be addressed with mathematical modelling. Therefore, the demographic approach involved the construction of life tables, analysis of population projection matrices and population dynamics modelling (Kajin 2008). The long time series data allowed the construction of one of the first life tables of a Brazilian mammal, the opossum *D. aurita* (Kajin *et al.* 2010a, 2010b). This method requires demographic data from different cohorts of individuals, followed from birth to their disappearance, and enables the estimation of several population parameters, including the population growth rate. The opossum population was also analysed using population projection matrices (Kajin *et al.* 2008), and sensitivity and elasticity analyses, which allowed the quantification of the importance of each vital rate (such as age-specific survival and fecundity) for the population growth rate (Kajin 2008). The survival rate of pouch young individuals indicated weaning as the most crucial moment in the life cycle of *D. aurita* (Kajin *et al.* 2008), corroborating the results of traditional population analyses for marsupials.

Intrinsic and extrinsic population dynamics mechanisms were also investigated to understand the interplay of density-dependent and density-independent processes as factors governing population regulation. After 13 years, the main results showed an outstanding importance of intrinsic mechanisms related to first-order feedback for *D. aurita*, indicating that density-dependent factors were more important than density-independent factors for the opossum population dynamics (Brigatti *et al.* 2016). Thus, a delayed density-dependence model was created for this species, which was partially explained by the opossum's mode of reproduction (Brigatti *et al.* 2016). After detecting the principal temporal structures of population dynamics, an autoregressive model, which included the main dynamic drivers, was elaborated and revealed the crucial importance of the time of attaining sexual maturity for the dynamics of the opossum's population (Brigatti *et al.* 2016).

The influence of local climatic factors, such as rainfall, compared to regional factors, such as the Southern Oscillation Index (El Niño), was also investigated. The latter was an important factor for the population growth rate and survival of the opossum (Kajin 2008). After 16 years, we concluded that *D. aurita* had a population dynamic characterised by density-dependent feedback on population growth rates with negative effects of minimum and maximum temperatures and rainfall in the dry season (Ferreira *et al.* 2016b).

Subsequently, mathematical models were applied in the demographic analysis of other marsupial species occurring in the study area, such as *Marmosops incanus*, *P. quica*, *Marmosa paraguayana* (formerly *Micoureus paraguayanus*) and *M. myosuros* (Ferreira *et al.* 2016a,b). Predominant density-dependent factors were also observed in all these species. The influence of the El Niño phenomenon was observed for *P. quica*, while local climatic factors seemed to have greater importance for *M. incanus* and *M. myosuros* (Ferreira *et al.* 2016a,b).

These studies were pioneers using such demographic analysis for Brazilian marsupials, showing the importance of endogenous and exogenous factors in population dynamics, as well as local and regional factors. These analyses would not have been possible without the existence of uninterrupted long-term data, one of the main characteristics of the Garrafão Project.

HABITAT

Studies of habitat preference by small mammals began in the Maricá Project, similar to population dynamics studies, aiming to understand which habitat characteristics determine or intensively influence species occurrence and investigate its possible relationship with population sizes (Rosenzweig 1981, August 1983, Morrison *et al.* 1992). In the Maricá project, the restinga vegetation was mostly shrubby with short trees; thus, we developed a method adjusted for such type of habitat (Fernandez *et al.* 1997, Freitas *et al.* 1997, Cerqueira & Freitas 1999). For the Garrafão Project, we developed a simple and fast method that could be used in denser and steeper topography. It consisted of measuring the habitat structure using a square wooden frame device (0.25 m^2; 0.50 x 0.50 m) divided into 100 open squares made of wire mesh (Freitas 1998, Freitas *et al.* 2002) (Figure 4). Seven variables were measured: vegetation cover, litter cover and rock cover on the ground, canopy cover, and vertical leaf obstruction at 0-0.5 m, 0.5-1.0 m and 1.0-1.5 m heights. Measurements were taken at each trapping point in all campaigns.

There was a concern about making the method replicable; that is, different people using the method should obtain the same measurements (Freitas *et al.* 2002). This allowed the habitat study to be carried out from 1997 to 2009. Scale-dependent habitat preferences were evaluated for three small mammal species: *D. aurita, P. quica*, and *M. myosuros* (Moura *et al.* 2005). All species were more selective at the largest spatial scale, the meso-habitat (compared to areas of 188 m^2): *D. aurita* and *P. quica* were associated with rock cover, whereas *M. myosuros* was associated with open canopy cover (Moura *et al.* 2005). Later, Costa (2014) modelled the small mammal presence as a function of microhabitat preference using occupancy and detectability analyses.

TEMPORAL VARIATION IN LITTER PRODUCTION

Litterfall studies in Garrafão were planned to represent a measure of resource and habitat

Figure 4. Habitat measurement during the study of small mammals of the Garrafão Project. Author: LabVert collection – Paula Aprigliano.

availability for small mammals. Many previous studies indicated that invertebrates found in litter are important food items for small mammals (Santori *et al.* 2012) and are associated with their habitat characteristics (Freitas *et al.* 1997). Positive correlations were found between litter accumulation and arthropod density (mites, collembolans, spiders and others) in many studies carried out in different ecosystems, such as forests, tree commercial plantations (Pellens & Garay 1999), grasslands (Knapp & Seastedt 1986) and horticultural sites (Riechert & Bishop 1990). These studies led to the hypothesis that variation in litterfall over time could influence the population dynamics of small mammals.

Litterfall measurements are a good proxy for the production of leaves, twigs, seeds, and fruits over a given period (Kouri & Hokkanen 1992, Huebschmann *et al.* 1999, Liu *et al.* 2003, Rowland *et al.* 2018). These measures can also indicate temporal variation in resource production and habitat availability for a given species (Malhi *et al.* 2011, Wu *et al.* 2016). Long-term litterfall studies are scarce and were mainly conducted in temperate regions (Kouki & Hokkanen 1992 - 24 years, Huebschmann *et al.* 1999 - seven years, Liu *et al.* 2003 - nine years, Rowland *et al.* 2018 - 15 years).

The study of litterfall in Garrafão started with a sampling effort of five collectors (Figure 5) arranged diagonally in each sampling site of small mammals due to logistical limitations. An experiment was carried out at one of these sites to assess the suitability of the sampling method. Litter collectors were placed at each of the 25 sampling points at that site, where litter was collected during three months (August, September and October 2000). These data were compared with different numbers of collectors using bootstrap methods to calculate means and coefficients of variation. The results indicated that five litter traps were sufficient to estimate the litterfall variation over time, showing no increment either in accuracy or in precision with more litter collectors (Finotti *et al.* 2003), so that the originally proposed sample design was maintained.

First, we investigated the relationship between the variation in population sizes of *D. aurita, P. quica, M. incanus, M. paraguayana,* and *M. myosuros* and

Figure 5. Litterfall collector used in the study of small mammals of the Garrafão Project. Author: LabVert collection – Ramon Campos.

the variation in total litterfall and its components (leaves, twigs and reproductive structures) at different time lags (Gentile *et al.* 2004). The results showed a delayed response in small mammal abundance to increased litterfall production, highlighting the importance of using time lags to understand temporal variation in population sizes. Significant correlations were found for all the species with enough data for the analysis. Some small mammal populations were correlated with total litterfall and others with the production of litter components, such as leaves, twigs and reproductive structures. Positive correlations with leaf and twig fall were observed in *D. aurita, M. myosuros* and *P. quica*, and with reproductive structures in *M. incanus*, with different time lags. These results are in accordance with the diet of these small mammals and the differences in their diet composition (Ceotto *et al.* 2009, Santori *et al.* 2015), as well as their use of forest strata (Delciellos & Vieira 2006). There is an evolutionary association between increasing frugivory and increasing use of arboreal strata among didelphid marsupials (Finotti *et al.* 2018). Thus, the relationship between *M. incanus* population sizes (a more arboreal marsupial than the other three species) and plant reproductive structures, and the relationships between the other three species analysed (which are more terrestrial than *M. incanus*) with leaf and twig fall can be a consequence of this association (Vieira & Camargo 2012). However, local associations between population densities and litterfall are also influenced by density-dependent processes and may differ between sexes. Relationships between population density and litterfall and rainfall were found only for females, which showed lower levels of vagility, whereas variation in male population density appeared to be random (Mendel *et al.* 2008).

The study of litterfall variation in Garrafão provided important findings to explain the fluctuation in small mammal population sizes. Long-term studies of litterfall are required because population fluctuations can be affected by seasonal and long-term climatic variations. In addition, short-term responses to climatic anomalies were also observed (Finotti *et al.* 2020). These relationships might bring important outcomes for animal populations, especially under the effects of global climatic changes.

LOCOMOTION, SPATIAL DYNAMICS, AND INNOVATIVE DETECTION METHODS (SPOOL-AND-LINE, PLATFORMS AND USE OF ARTIFICIAL NESTS)

Traditional population dynamics focus on temporal variation in population parameters, such as genetic structure and habitat selection, presented previously. Those population parameters have space as an essential component, depending on estimates of individual movements and their dispersal, which are the distances the animals move to settle in new areas. Studies of movement distances and home range areas were developed in Garrafão to gain a deeper understanding of the spatial dynamics of these populations. In addition, the alternative methods of artificial nests (Figure 6) and spool-and-line tracking (Figure 7) provided insights and information that were not possible to obtain with live-traps. These methods included nest site selection (Cobra *et al.* 2023), sampling by trapping grids (Mendel & Vieira 2003), scale-dependent habitat selection (Moura *et al.* 2005), and more accurate estimates of density and other demographic parameters (Mendel *et al.* 2008). These studies also quantified potential bias in studies based on live-traps and baits (Loretto & Vieira 2011, Loretto & Vieira 2023).

From 1998 to 2006, we tracked 333 individuals of four species in 405 tracks using spool-and-line devices (Cunha & Vieira 2002, 2005, unpublished data). This technique allowed us to determine the use of the forest by small mammals in unprecedented detail. Some scansorial species, such as *P. quica*, actually left the forest ground only occasionally, despite having the potential ability to do so (Delciellos & Vieira 2006, 2007, 2009), while others used predominantly the understorey but never the canopy, such as *M. incanus* (Cunha & Vieira 2002, 2005). In addition to the use of vertical strata, the diameters and incline of branches and supports used to climb were also measured and compared with their climbing and arboreal walking performances in controlled conditions (Delciellos & Vieira 2006, 2009).

The movement areas of males and females were clearly distinct for most species, with males moving longer distances and using larger areas than females (Loretto & Vieira 2005, 2008). This is a common pattern for mammals, especially in species with sexual dimorphism in body size, with males being larger than females (*e.g.*, Russel 1984, Moraes-Júnior & Chiarello 2005). For *D. aurita* and *M. incanus*, movements of females were determined by resource availability, whereas movements of males were determined by reproductive season (Loretto & Vieira 2005, Vieira *et al.* 2019), but for *M. nudicaudatus*, only climatic seasons seemed to affect movements (Ferreira *et al.* 2017). Despite the relatively small variation in body size within a species, the effects of body size on movements could still be detected after accounting for other effects, such as reproductive and climatic seasons (Vieira *et al.* 2019).

The study of movements in the field led to unprecedented records on the shelter sites of the studied species, in addition to the behavioural biology linked to shelters (*e.g.*, *M. myosuros*; Loretto *et al.* 2005). This led us to seek a direct understanding of the reproductive biology of the species while leaving aside the traps that captured them during the foraging moment. From 2003 to 2010, we developed a new line of research to directly assess not only the use of space but also the reproductive behaviour of the species by using artificial nests on the same grids as in the seminal study (Loretto 2006). During this approach, we found that the forest strata used for nesting and shelter were the same as those described in the study of movements, showing that both foraging and reproductive aspects followed the same niche partition. Thus, it became evident that the studied species also built nests in a species-specific way in terms of shape and size, corresponding to the size of each respective species (Loretto 2005, 2006). We successfully studied in detail the reproduction of *Caluromys philander* (Loretto & Vieira 2011), which was abundant and almost exclusively recorded in the nests. For this species, we could understand unique reproductive characteristics, such as a single litter per reproductive season, few offspring by brood and long-term parental care. We still recorded a longevity of more than five years in two individuals (Loretto 2012).

Figure 6. Artificial nest used in the study of small mammals of the Garrafão Project. Author: LabVert collection – Diogo Loretto.

CONCLUSION

The standardization of the sampling methods involving the trapping effort and the collection of age-specific data over time with two months regular intervals allowed the analysis of several consecutive generations of the studied species. In the case of marsupials, marking the animals inside the maternal pouch was also one of the

Figure 7. Spool and line tracking technique used in the study of small mammals of the Garrafão Project. Author: LabVert collection – Maíra Moura.

determining factors for the demographic analyses (Loretto *et al.* 2013). This sampling design allowed inferences about population regulation processes and demographic patterns in small mammals (Gentile & Kajin 2015). Population genetics studies have proven to be of great importance for understanding ecological processes on a broader scale. In addition, gaining knowledge of the biology of the studied species was essential because it allowed estimating the animals' age in the field, for example, making demographic studies possible. In this way, the Garrafão Project may be considered a model for future studies of the population biology of small mammals.

Despite the enormous contribution of the project in terms of information and novel results, answering several relevant questions in ecology, the project, like any other, presented limitations and some problems, some of which led to its end. The main limitation was the high financial cost for its long-term maintenance, respecting a standardized methodology. This type of study requires a large team of people in the field, hosting logistics, a vehicle for fieldwork, inputs for collecting data and samples, material for genetic analysis, among other expenses. The quality of the data collection and samples is also a factor that involves some complexity. There is a need for methodological rigor and constant teaching of the new students, with transmitted knowledge among the various successive generations of students and post-docs who worked in the laboratory. In addition, the management of the collected database also requires specialized people directly involved in it.

The long-term scale of the Garrafão project was challenging because grants had to be obtained constantly from different sources. Several new lines of research were created, incorporating them into broader projects, which increased its original objectives. Therefore, all the LabVert students were influenced by this project at some moment. The project resulted in many products. A perfunctory list includes articles (76), annals of congresses (8), book chapters (7), master's dissertations (26) and doctoral theses (6). Approximately 90 students worked directly on the project, which was part of their academic training.

Long-term studies are necessary. The results summarised here showed that increasing the time series of collected data leads to the acquisition of new knowledge. LabVert tried to carry out a long-term ecological study of small mammals in Maricá in the 1980s, but the unclear science policies in Brazil at that time made it impossible to continue that study. In the 1990s, the laboratory already had a high academic status, and we were confident that it was worth the risk of trying again a long-term project. We succeeded for twenty-two years, but we did not expect governments whose policy guidelines for science and technology would lead to irreversible damage to Brazilian science (Barbosa *et al.* 2021, Grelle *et al.* 2022). Although there are some scientific research grants directly aimed at long-term studies in Brazil, such as the CNPq's Long Term Ecological Research Program, which exists since 1999, this type of funding is still incipient in Brazil. The maintenance of this type of project requires a certain political stability and maintenance of regularity in the financial programs offered.

One of the main learnings of this project was the need to create an effective integrated network of cooperation among several laboratories addressing the issues in a multidisciplinary way, with publications in collaboration, use of common equipment, and funding continuously obtained by groups of researchers from different institutions. We believe that this is an effective strategy to address the limitations of a long-term study and to add value to the quality of scientific research.

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