Universidade de Évora - Escola de Ciências e Tecnologia

Mestrado em Modelação Estatística e Análise de Dados

Dissertação

Demographic behavior of social insect populations: the specific case of Formicidae

Laura Magnani Machado

Orientador(es) | Filipe José Ribeiro
Dulce Maria de Oliveira Gomes

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A dissertação foi objeto de apreciação e discussão pública pelo seguinte júri nomeado pelo Diretor da Escola de Ciências e Tecnologia:

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Dedico esta dissertação aos meus pais, Márcia e Machado, por me apoiarem nos meus sonhos (mesmo os mais loucos, como esse de fazer estatística de formigas)

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Por último, agradeço aos meus pais, a quem dedico esta dissertação, por todo o apoio, não só nos últimos anos, mas sempre. Obrigada por estarem ao meu lado em cada etapa, por apoiarem meus projetos e por cultivarem em mim um certo amor pela matemática e estatística. Vocês são para mim um exemplo de pessoas trabalhadoras e justas, mas que, acima de tudo, buscam ser felizes no que fazem e ver o lado bom da vida, mesmo nos momentos mais difíceis.

Abbreviations and acronyms

GEE: *Generalized Estimating Equations* (Equações de Estimação Generalizadas)

LAR: *Lifetable aging rate* (Velocidade de envelhecimento da população)

PDF: *Probability Density Function* (Função Densidade de Probabilidade)

GLM: *Generalized Linear Model* (Modelo Linear Generalizado)

GLMM: *Generalized Linear Mixed Model* (Modelo Linear Generalizado Misto)

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Resumo

Monomorium pharaonis é uma das muitas espécies invasoras de formigas que podem estar associadas à bactéria endossimbionte *Wolbachia*. Essa associação ainda está a ser estudada, mas sabe-se que confere uma vantagem reprodutiva à colónia. O presente trabalho teve como objetivo utilizar a biodemografia para avaliar o efeito da *Wolbachia* nas colónias, no seu potencial reprodutivo e, se possível, na sua taxa de envelhecimento. Os resultados obtidos mostraram que a bactéria aumenta as taxas reprodutivas das colónias. Também verificamos que a utilização de abordagens interdisciplinares contribui significativamente para a obtenção de resultados mais precisos e quantificáveis. Essas abordagens metodológicas podem ser novas ferramentas extensíveis a outras colónias e espécies de formigas. Mais estudos são necessários para compreender completamente a relação simbiótica, mas este pode ser um primeiro passo para a utilização de metodologias diferenciadas que, no futuro, possibilitem o desenvolvimento, quer de melhores métodos de controlo para *M. pharaonis*, quer de inovação na área da biodemografia, utilizando uma abordagem com grande foco estatístico.

Palavras-chave: taxa intrínseca de crescimento; biodemografia; formigas *M. pharaonis*; modelação estatística; GEEs.

Demographic behavior of social insect populations: the specific case of Formicidae

Abstract

Monomorium pharaonis is one of the many invasive ant species which can be associated with the endosymbiont bacteria *Wolbachia*. This association is still being studied but is known for giving the colony a reproductive advantage. The present work aimed to use biodemography analysis to check the effect of *Wolbachia* in the ant colonies, regarding its reproductive potential and, if possible, rate of aging. The results obtained in the present work showed that the bacteria increase the reproductive rates of the colonies. We also found that the employment of interdisciplinary approaches highly contributes to obtaining more accurate and quantifiable results. These methodological approaches could be new tools extendible to other ants' colonies or even other species. More studies are needed to fully understand symbiont relations, but this could be a first step in using a different methodology to, in the future, develop better control methods for *M. pharaonic*, and contribute with innovative approaches for biodemographic studies, with a strong statistical basis.

Keywords: intrinsic growth rate; biodemography; *M. pharaonis* ants; statistical modelling; GEEs.

Introduction

The ants *Monomorium pharaonis*, known as "Pharaoh's ants," are cosmopolitan, inhabiting buildings in temperate regions of the globe, and are characterized as a highly polygynous[1] species that reproduces by budding (Passera, 1994, apud. Buczkowski & Bennett, 2009). Their reproduction is rapid, producing new broods at intervals of a few months through mating within the colony, and their queens become reproductively active quickly (Schmidt et al., 2011). This species is known for producing foraging trails that persist for up to 48 hours and can be altered according to the nutritional demands of the brood (Jackson et al., 2006).

Regarding the reproductive cycle of this species, as in other ant species, the fertile females of the colony, which are the queens, lay eggs throughout their lives after mating with a male. The larval stage lasts from 7 to 18 days, followed by the prepupal and pupal periods, which range from 9 to 19 days. The pupae then differentiate into fertile adults (queens or males) or sterile adults, known as colony workers (Alvares, Bueno & Fowler, 1993).

The Pharaoh's ant has become an invasive species with massive pest infestations inside homes, being dependent on human activity for its survival (Buczkowski & Bennett, 2009). For this reason, many studies on the mechanisms of colony growth have been conducted in recent years. Previous studies have shown that pharaoh ants adjust resource allocation according to their reproductive capacity and the colony's workforce, altering the number of queens depending on the number of eggs present in the colony (Warner, Lipponen & Linksvayer, 2018). In some cases, workers may collect more proteins to prioritize colony growth, rather than individual survival, when the colony is divided into smaller parts (Krabbe et al., 2019).

In addition to resource allocation, ants use other reproductive mechanisms linked to their evolution and natural history, such as associations with endosymbiotic[2] bacteria (Singh & Linksvayer, 2020). *Wolbachia* is a genus of bacteria that, among many others, infects

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[1] Polygynous ant colonies are characteristic of species that have more than one queen, alive and reproducing, within a single colony. In the case of *M. pharaonis*, the ideal minimum colony size consists of an average of 30 queens (Buczkowski and Bennett, 2009).

[2] The concept of endosymbiosis was developed by the researcher Lynn Margulis, in 1938, regarding the establishment of a symbiotic relationship in which one organism is engulfed by another, meaning a relationship that benefits all parties involved, in the evolution of cells such as those of animals. This type of mutually beneficial relationship between bacteria and certain insects, for example, is called endosymbiosis because of the effect that this obligatory association has on the biological, nutritional, and survival functions of the insects that partner with the bacteria, known as endosymbionts (Eleftherianos et al. 2013).

about 34% of the known ant species worldwide and is associated with reproductive strategies (Russell et al., 2009). The association between *M. pharaonis* ants and *Wolbachia* is still being studied, but it is already known to confer a reproductive advantage to the colony, depending on the life stage of the queens and workers, as colonies produce reproductive individuals earlier when infected with the endosymbiont (Singh & Linksvayer, 2020).

The study of population growth and reproductive dynamics of insects can benefit from the use of classical tools adapted to demography. Biodemography is a subdiscipline of classical demography that emerged in the early twentieth century to better understand the reproductive mechanisms of animal populations, going beyond the creation of specific tools for biological problems (Carey, 2001). Social insects are ideal for evolutionary studies because queens and workers have different lifespans (Kramer et al., 2016). Birch (1948) developed the concept of an Intrinsic Rate of Natural Increase (*r*) for insect populations, which is more appropriate for these studies compared with analyses of "biotic potential" and other classical growth rates. This rate defines the intrinsic capacity of a population to grow, given its particular regimes of fecundity and mortality. Besides that, this concept is not widely used in biological studies, nor in research on the association between *M. pharaonis* and *Wolbachia*, making it a significant asset in terms of scientific innovation.

The present study aimed to use a statistical–biodemographical analytical framework to examine the effect of *Wolbachia* on *M. pharaonis* ant colonies, evaluating their reproductive potential through the application of biodemographic concepts and rates, as well as the use of methodologies that are novel in biology, such as modeling through the application of Generalized Estimation Equations (GEE) models, and classical demographic concepts to analyze the data and results obtained. This dissertation is structured like a scientific article, which is preceded by this general overview and a detailed methodology, and followed by final considerations.

The dataset used in the present work was published by Singh along with other authors in 2023, titled "*Wolbachia*-infected pharaoh ant colonies have higher egg production, metabolic rate, and worker survival" (Singh et al., 2023). The data used came from an experiment conducted in 2019 that compared the fertility rates of queens in colonies infected and uninfected with *Wolbachia*. Thirty-one *M. pharaonis* ant colonies were observed, and population data and the number of eggs produced were collected every three days, covering a total of 46 days of observation. Twenty colonies were infected with *Wolbachia*, while eleven composed the uninfected group.

Methodology

Age-specific survival (l_x) and fertility (m_x) rates were calculated. Considering the reproductive and colony life type of the ants (Hymenoptera: Formicidae), the “females” were considered to be only the queens, since although workers are also female individuals, they lack reproductive capacity. Therefore, x was considered as the age of the queen in days. Before calculating *r*, two important comparison parameters were obtained: the net reproductive rate (R_0), which is the ratio between the total number of female births in two successive generations, and the mean generation time (*T*). R_0 can be calculated using the following formula:

$$R_0 = \int_0^{\infty} l_x m_x \, dx \tag{1}$$

A value for each queen age was obtained for each variable through a simple average of the values from the 31 different colonies observed. R_0 was then calculated, along with an estimate of the mean generation time (T), obtained using the following formula:

$$T = \frac{\sum x \, l_x m_x}{\sum l_x m_x} \tag{2}$$

The calculation of *r* was made for infected and uninfected colonies, using a procedure described in the article for the exponential function (e).

$$\sum e^{-rx} \, l_x m_x = 1 \tag{3}$$

To detect the deceleration of fertility and possible plateaus at older ages, the first derivative, on a logarithmic scale, of the force of mortality (here treated as the “force of fertility”) is calculated. This demographic measure is known as the population aging rate (LAR – Lifetable Aging Rate), defined as: $LAR(x) = \frac{dl_n \mu_x}{d_x}$, and in this work, interpreted as the fertility velocity of a colony/queen.

Empirical LAR values were calculated by age-specific differentiation of log-transformed hazard rates, while model-based LARs were derived analytically from fitted hazard functions. Following biodemographic approaches (Horiuchi & Wilmoth, 1998; Vaupel et al., 1979), we defined the onset of fertility deceleration as the first age at which the LAR reached non-positive values for consecutive sustained ages.

Bootstrap resampling was employed to assess the uncertainty in estimates of the deceleration age. This work was performed using R version 4.2.2, with a script based on the ‘Tidyverse’ library. The dataset consisted of the life tables of the colonies. *r* values were calculated for subsequent comparison with Birch's methodology described in Section 2.2. The life table aging rate (LAR) was obtained based on formula (4) from Horiuchi and Coale (1990), where $\overline{b}$(x) represents the LAR and M(x) is the central mortality rate at age x (Ribeiro & Missov, 2016).

$$\bar{b}^{*}(x) = ln\,(M(x)) - ln\,(M(x-1)) \tag{4}$$

To avoid large fluctuations caused by variations in mortality rates at older ages, Horiuchi and Coale proposed the empirical formula (5), which applies a nine-year moving average to the results of formula (4), after applying a five-year moving average to $\mu\,(x)$.

$$\bar{b}_{emp}(x) = \sum_{n=-4}^{4} \frac{(5-|n|)}{25} * \bar{b}^{*}(x+n) \tag{5}$$

However, due to the short number of observation days, this two-step smoothing procedure could not be applied.

To quantify fertility patterns and detect fertility deceleration at older ages in ants, we combined the empirical construction of life tables with parametric mortality models. Our analysis was based on classical biodemographic approaches (Vaupel et al., 1998; Carey, 2001; Mueller & Rose, 1996), adapted for colony-level insect data.

In this dissertation, we fitted four parametric mean functions m(x) representing fertility per queen at age x. These models provide alternative characterizations of age-specific reproductive schedules, and their parameters are estimated via maximum likelihood under a Poisson framework.

We therefore fitted a Gaussian approach (bell-shaped) to the queen's fertility:

$$\mu(x) = A * \exp\left(-\frac{(x-\mu)^2}{2\sigma^2}\right), \tag{6}$$

With the parameters: amplitude A ≥ 0, center μ, and spread σ > 0. Next, we used the Probability Density Function (PDF) of the gamma distribution, i.e., a random variable that follows a gamma distribution with parameters k and γ where$\big(X \sim \Gamma(k,\gamma)\big)$:

$$\mu(x) = \frac{\lambda^{k} x^{k-1} e^{-\lambda x}}{\Gamma(k)}\ . \tag{7}$$

where, in case $k = 1$, the *gamma* distribution becomes exponential.

Third, we fitted a Weibull PDF, which contains two positive parameters $Weibull\ (\lambda, \gamma)$:

$$\mu(x) = \lambda\gamma x^{\gamma-1}e^{-\lambda x^{\gamma}} \tag{8}$$

If $\lambda = 1$, an exponential distribution is also obtained; however, for $\lambda < 1$, a monotonic decline is observed, whereas for $\lambda > 1$, the opposite occurs.

Finally, we also employed a Gompertz–Makeham structure:

$$\mu(x) = ae^{bx} + c \quad , \tag{9}$$

The parameters are also positive, where a ≥ 0, c ≥ 0. Here, the Gompertz–Makeham function, typically used for mortality, is repurposed to describe fertility patterns, combining an exponential trend with a baseline level.

All models were fitted using a Maximum Likelihood Estimation approach, where the counts per age class y_i are modeled according to a Poisson model with mean $\lambda_i = E_i\, m(x_i)$, where E_i is the exposure and $m(x_i)$ is the fertility (m_x) rate predicted by the model (log-likelihood):

$$\ell(\theta) = \sum_i [y_i \log\big(\lambda_i(\theta)\big) - \lambda_i(\theta) - log(y_i!) \tag{10}$$

Additionally, the model-based LAR was calculated using:

$$ln(M(x)) - ln(M(x - 1)) \tag{11}$$

The figures include empirical LAR, smoothed trends (LOESS, GAM spline, cubic polynomial), and model-predicted LAR. Comparisons were made between colonies to test the robustness of the observed patterns, while the residuals between empirical mortality rates and model predictions were calculated to assess model fit. Error distributions were analyzed to explore potential systematic deviations. This combined framework integrates classical demographic models with empirical risk estimates, allowing for rigorous testing of whether insect populations exhibit fertility plateaus analogous to those observed in human and other animal demography.

Another methodology used in this study for data analysis was model building. Model building is a tool frequently used in various fields to study relationships between a dependent variable and one or more independent variables. In many studies, regression models of the GLM type (acronym for Generalized Linear Models) are constructed, introduced by John Nelder and Robert Wedderburn in 1972. These models assume that the response variable belongs to a broad family of probability distributions (e.g., Poisson, Binomial, Gamma, Normal) and that observations are independent. In other words, the response variable is said to follow a distribution in the exponential family of distributions. GLMs represent a broad class of models that generalize multiple linear regression models, offering great flexibility because, unlike the latter, the response variable does not need to be continuous or normally distributed.

That is, due to the relationship between the response variable Y and its assumptions in multiple linear regression models with p independent variables $X_1, X_2, \dots, X_p$ and the observations,

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \cdots + \beta_p X_{pi} + \epsilon_i \, , i = 1,2, \dots, n \quad , \tag{12}$$

the expected value of Y, conditional on the independent variables, results in the following linear relationship

$$E\left[Y_i \middle| X_1 = x_{1i}, X_2 = x_{2i}, \dots X_p = x_{pi}\right] = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \cdots + \beta_p X_{pi} \; . \tag{13}$$

If we define $\mu_i = E[Y_i \,|\boldsymbol{X}]$ for the previous conditional value, it is common to simply use the notation

$$\mu_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \cdots + \beta_p X_{pi} \, , i = 1,2, \dots, n \tag{14}$$

In GLMs, there is a link function, denoted by g, which connects the mean of the response variable Y to the independent (or explanatory) variables, such that

$$g(\mu_i) = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \cdots + \beta_p X_{pi} \, , i = 1,2, \dots, n \tag{15}$$

and this function is differentiable and strictly monotonic (and therefore invertible) (Agresti, 2007). When $g(x) = x$, the link function is the identity function, which is equivalent to stating that the multiple linear regression model is a special case of the GLM. In other words, GLMs model the expected value of the response variable through the equation:

$$\mu_i = g^{-1} \left(\beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \cdots + \beta_p X_{pi}\right) \tag{16}$$

These models have three fundamental components:

- Random component: the response variable, which can be continuous, discrete, or a factor, from which n independent observations are collected, and whose distribution belongs to the exponential family of distributions, not necessarily normal.

- Systematic component: p covariables, which can be numeric and/or factors, from which n independent observations are collected for each, and which are expressed through a linear relationship.
- Link function: an invertible function that relates the expected value μ_i (of the response variable) to the explanatory variables through the relationship given by $g(\mu_i)$ (12).

As we have seen, GLMs are much more flexible models than multiple linear regression models, being particularly suitable for count data (Agresti, 2007).

When the response variable is not independent, a requirement in standard GLMs, one can instead use GLMMs (Generalized Linear Mixed Models). These models include both fixed effects and random effects, the latter allowing the incorporation of non-independence in the data, such as repeated measures (longitudinal data).

Another option, when the response variable is not independent but longitudinal (i.e., when repeated observations are made over time for the same individual or study unit), was proposed by Liang & Zeger (Agresti, 2007). These authors introduced the GEE method (Generalized Estimating Equations), which extends the GLM methodology to the context of longitudinal data.

The GEE methodology models the mean response as a function of the set of explanatory variables, taking into account the correlation (or dependence) between observations. Additionally, results from a GEE model allow us to evaluate the relationship between the response variable and the explanatory variables at the population level, rather than at the individual level as in mixed-effects models.

Correlation between data can occur in different types of studies, for instance, when observed individuals belong to the same family, live in the same neighborhood, are patients in the same hospital, or when data consist of multiple observations of the same individual over time. In such cases, it is necessary to account for the dependence of observations, highlighting the importance of using GEEs.

As mentioned, GEEs are based on GLMs, so they also assume that the dependent variable belongs to the exponential family, but they include a correlation structure in the estimation of the regression parameters $\boldsymbol{\beta} = [\beta_0, \beta_1, \beta_2, \dots, \beta_p]$.

The proposal by Liang & Zeger (1986) consisted of solving the following equation to obtain the parameter estimates $\boldsymbol{\beta}$

$$S_{\boldsymbol{\beta}}(\boldsymbol{\alpha},\boldsymbol{\beta}) = \sum_{i=1}^{n}(\frac{\partial \mu_i}{\partial \boldsymbol{\beta}})^T \, V_i^{-1}(y_i - \mu_i) = 0 \, , \tag{17}$$

where V_i is a specific matrix that incorporates a given correlation structure, and $\boldsymbol{\alpha}$ represents the parameters of that correlation structure,

$$V_i = \sigma \, A_i{}^{1/2} \, R(\alpha) \, A_i{}^{1/2} \tag{18}$$

where A_i is a diagonal matrix of the outcomes' variance, $R(\alpha)$ is the correlation matrix specified and σ is a dispersion parameter.

Liang & Zeger (1986) demonstrate that if the structure for the mean is correctly specified, then, regardless of the chosen V matrix, the estimator $\widehat{\boldsymbol{\beta}}$ is unbiased and consistent. The distribution approaches to a multivariate normal distribution, that is

$$\sqrt{n}\,(\widehat{\boldsymbol{\beta}} - \boldsymbol{\beta_0}) \sim^{a} N\,(0, \Sigma) \tag{19}$$

where Σ is the empirical estimation of variance. This property allows us to calculate p-values and confidence intervals using the Wald test.

The equations used in the correlation matrix are extensions of those used in the quasi-likelihood method. These likelihood methods are not Maximum Likelihood, since the distribution of Y is not specified, and therefore there is no likelihood function; the alternative computational method for clustered data is a generalization of quasi-likelihood (Agresti, 2007).

The GEE method uses the quasi-likelihood methodology to create equations that link marginal means to linear predictors in order to provide a variance–covariance matrix (Agresti, 2007). Therefore, GEEs do not require an assumed distribution for the response variable, provide more robust inference results, and do not specify any likelihood (Hubbard et al., 2010).

In the present study, the observations made colony by colony form a dependency structure because, although the colonies are independent, each one, considered an individual, was observed over time. Therefore, despite being widely used in biological and ecological studies, the use of GLMs is not suitable here, as they assume independence among observations and would not provide robust results appropriate to the *M. pharaonis* population and its relationship with infection by the endosymbiont *Wolbachia*.

Hence, GEE models were applied, considering a Poisson distribution, a logarithmic link function, and a first-order autoregressive correlation structure (AR(1)). A significance level of 5% was considered for the models. All analyses were performed in R (version 4.2.2) using the 'geepack', 'dplyr', and 'ggplot2' packages.

A better understanding of the true effect of *Wolbachia* on longevity, and especially on the colony's ability to grow within a single generation, can help develop more effective control methods. Given that knowledge about the effect of this bacterium on ants is still very limited (Ramalho et al., 2021), finding new analytical methodologies that can provide more robust and novel results, through models not widely used and demographic methodological approaches, is essential.

Scientific article

Demographic behavior of social insect populations: the specific case of Formicidae

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Abstract

Monomorium pharaonis is one of the many invasive ant species which can be found associated with the endosymbiont bacteria of the genus *Wolbachia*. The association of *M. pharaonis* ants with *Wolbachia* is still being studied but is already known for giving the colony a reproductive advantage. The present work aimed to use biodemography analysis to check the effect of *Wolbachia* in the colonies of *M. pharaonis* ants, regarding its reproductive potential and rate of fertility (fertility pace). We took advantage of Birch (1948) methods to assess the effect of the bacteria in the whole colony, using data of the Dryad project. We evaluated the mean length of the generation, its capacity to multiply and the intrinsic rate of increase (*r*) and if the presence of the bacteria favors the longevity of the colony. The results obtained in the present work confirmed our initial hypothesis that the presence of the endosymbiont bacteria *Wolbachia* increased the reproductive rates of *M. pharaonis* colonies. We also found that the employment of interdisciplinary approaches highly contributes to obtaining more accurate and quantifiable results. The application of this methodological approach, highly contributed to obtain more accurate and directly driven results. For example, colonies infected with *Wolbachia* showed higher intrinsic growth rate (*r*) and thus enlightening with a new methodological approach results already presented in previous research. This "new" methodological approach revealed itself as a new tool extendible to other ants' colonies or even other species. The use of statistical and biodemographic formulas and the adaptation of classical demography concepts for the study of the growth and reproduction of ant colonies revealed to be very useful.

1. Introduction

Monomorium pharaonis (Hymenoptera: Formicidae) is an ant species known as

“Pharaoh's ants”, are cosmopolitan, inhabiting buildings in the temperate region of the globe and are characterized by being a highly polygynous species that multiplies by budding (Passera, 1994, apud. Buczkowski & Bennett, 2009). Its reproduction is rapid, producing new offspring every few months through crossings within the colony, in addition to its queens becoming reproductive quickly (Schmidt et al., 2011). Ants are eusocial organisms which live in colonies, organized in the reproductive castes, such as the queens and males, and the sterile castes, such as the workers and, in some cases, soldiers. Allocation to different castes is regulated by the ratio between the number of adult workers and the number of eggs in the colony, and not by the number of queens (Warner, Lipponen & Linksvayer, 2018).

The reproductive cycle of the Pharaoh’s ants, such as other ant species, is summarized in Figure 1. The fertile females of the colony, which are the queens, lay eggs throughout their life, after mating with a male. The larvae phase lasts from 7 to 18 days, followed by the pre-pupae and pupae period, that vary from 9 to 19 days. The pupae then differentiate into the fertile adults (queens or male individuals) or sterile adults, known as the workers of the colony (Alvares, Bueno & Fowler, 1993).

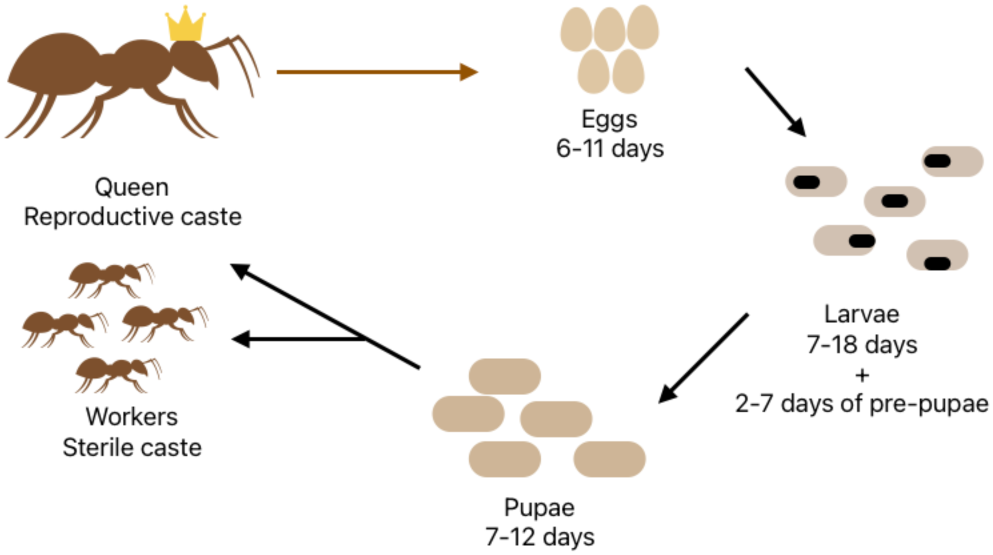


Figure 1: Reproductive cycle of *M. pharaonis*, with the mean time of each stage after the laying of the eggs by the queens. (Own elaboration, Alvares, Bueno & Fowler 1993).

The pharaoh’s ant has become an invasive species with massive pest infestations inside houses, being dependent on human activities for its survival (Buczkowski & Bennett, 2009) (Figure 2). Because of that, many studies regarding its colonies’ growth mechanisms have been done on the past years. Previous studies showed that pharaoh's ants alter the allocation of resources according to its reproductive capacity and workforce of the colonies, altering the number of queens depending on the number of the eggs present at the colony (Warner, Lipponen & Linksvayer, 2018). In some cases, workers can forage for more proteins to prioritize the growth of the colony, instead the survival in an individual level, when the colony is divided in smaller propagules (Krabbe et al., 2019).

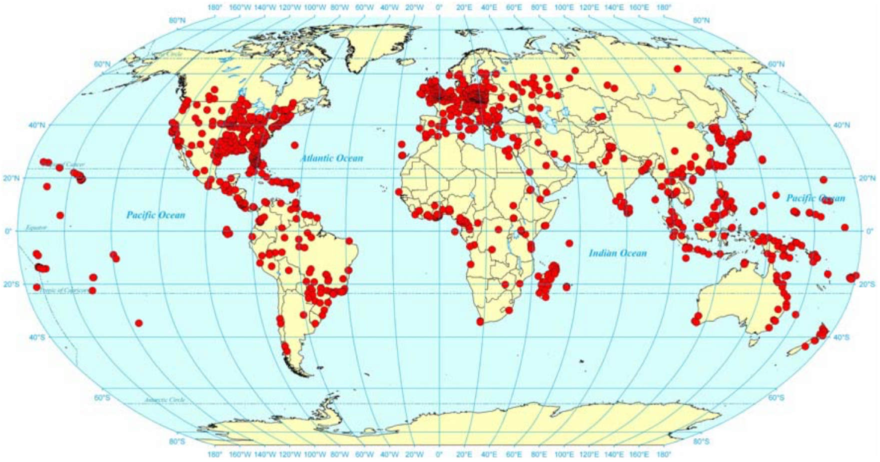


Figure 2: Distribution of *M. pharaonis* worldwide, according to Wetterer (2010).

Besides resource allocation, ants use other reproductive mechanisms related to their evolution and natural history, such as the association with endosymbiont bacteria (Ramalho et al., 2021; Singh & Linksvayer, 2020; Singh et al., 2023). *Wolbachia* is a bacteria genus which, among many others, infect around 34% of the ant species known in the world and is associated with reproductive strategies (Russell et al., 2009). The association of *M. pharaonis* ants with *Wolbachia* is still being studied but is already known for giving the colony a reproductive advantage, depending on the life stage of the queen and workers, as the colony produces more reproductive individuals earlier when infected with the endosymbiont (Singh & Linksvayer, 2020).

The study of insects' populations' growth and reproductive dynamics can benefit from the use of classic demography adapted tools. Biodemography is a subdiscipline of the classic demography, which emerged in the beginning of the century to a better understanding of the reproductive mechanisms of animal populations, going beyond the creation of specific tool for biology problems (Carey, 2001). Social insects are ideal for evolution studies as queens and workers have different lifespans (Kramer et al., 2016). Birch (1948) has developed the concept of an Intrinsic Rate of Natural Increase (*r*) for insect populations, which should be more appropriate for these studies in comparison to ‘biotic-potential’ analysis and other classical growth rates. This rate defines the intrinsic capacity of a certain population to increase, with its particulars regimes of fecundity and mortality. Besides that, this concept is not widely used in the biology studies, nor in the pharaoh’s ants-*Wolbachia* association studies.

The present work aimed to use biodemography analysis to check the effect of *Wolbachia* in the colonies of *M. pharaonis* ants, regarding its reproductive potential, with the use of biodemography concepts and rates, as well as the application of bold methodologies, such as GEE modelling and classical demography concepts to analyze the data. The database used in this work had previous studies published which stated that *Wolbachia* increased the life days of the queens and workers, as the egg laying rates (Singh et al., 2023). Having a better knowledge of the true effect *Wolbachia* has on the life span and, mainly, on the capacity it gives the colony to grow in only one generation, may help to develop more effective control methods.

2. “Bug’s life”, deceleration and Plateaus in Insect colonies

Following the introductory section, this study about fecundity dynamics in insect colonies provides a unique opportunity to explore fundamental questions about fecundity, reproduction rate (pace) and survival. The key concept of this biodemographic procedure is to modulate fecundity deceleration observed at older ages by employing almost exclusively human mortality models such as the Gompertz model (Gompertz, 1825). Nevertheless, mortality deceleration observed at advanced ages, where the exponential increase in mortality predicted by models such as the Gompertz law no longer holds (Vaupel et al., 1998; Horiuchi & Wilmoth, 1998) in human populations, but could be useful for innovative approaches.

In humans, the force of mortality tends to a plateau, and that indicates that the rate of aging slows down or stabilizes among survivors (Kannisto, 1994). This mortality behavior is widely documented in humans and other species, but evidence also suggests that it can be also validated for social insects as ants. In this peace of research, we follow Birch’s (1948) work as a first step to explore this connection.

From a theoretical perspective, Birch (1948) developed a pioneering approach to study intrinsic rates increase in insect populations providing the foundation for understanding how fecundity patterns shape insect population growth and reproductive strategies. It is known that, in colonies, where the division of labor and social organization influences individuals' survivorship, mortality plateaus may reflect both biological heterogeneity and colony-level buffering mechanisms (Carey, 2001; Hughes & Reynolds, 2005), but it will be also possible to be observed in ant fecundity patterns?

Heterogeneity models, such as frailty distributed based ones, explain mortality deceleration because of differential survival of more robust individuals within a cohort (Vaupel et al., 1979). In insect colonies, more precisely for ants, this may translate into survival advantages for certain worker subgroups or castes that disproportionately contribute to late-life survival and reproductive success.

Despite that empirical analysis of insect mortality and reproduction are considered very important and informative, within this piece of research, we believe that the application of advanced demographic methods will major benefit the outcome. For example, the usually denominated hazard function estimates (fecundity rates' hazard, in our approach) will be calculated, as well as fecundity deceleration and model comparison between Gaussian, Gamma,, Weibull and Gompertz-Makeham distributions. This follows findings that hazard function estimation, survival analysis, and model comparisons between Gompertz, Gompertz–Makeham, and Weibull distributions enable precise identification of the age at which deceleration occurs (Promislow et al., 1996; Baudisch, 2011).

We strongly believe thus that these approaches, when combined with life-history data from controlled colony experiments (infected vs. uninfected colonies of ants), make it possible to assess whether insect populations display longevity plateaus like those described in human demography. Understanding the onset and magnitude of fecundity deceleration in ants is not merely of theoretical interest but has practical implications for evolutionary biology and research in a broader way. Linking empirical findings to Birch's (1948) classical framework and modern biodemographic insights, we intend to contribute to broader debates on the universality of aging laws and the existence of biological limits to longevity (Mueller et al., 2011; Jones et al., 2014) and fecundity in insect colonies.

3. Materials and Methods

The present study investigates fecundity dynamics and late-life fecundity deceleration in ant colonies using an integrative biodemographic framework. Data was collected from controlled laboratory experiments in which queen ants were isolated and observed over their lifespans under standardized conditions. Daily records of survival and reproduction (egg counts) were maintained for each colony, allowing the construction of detailed age-specific mortality and fertility schedules.

3.1. Database collection and description

The data was collected from the Dryad database, an open data publishing platform, (https://datadryad.org) in December of 2023. The dataset used in the present work was published by Sachin and collaborators in 2023 (Sachin et al., 2023), entitled "*Wolbachia*-infected pharaoh ant colonies have higher egg production, metabolic rate, and worker survival". From the dataset, the data used was from an experiment made in 2019 which compared egg-laying rates of queens in *Wolbachia*-infected and -uninfected colonies. Thirty-one colonies of *M. pharaonis* ants were observed and population data and egg laying rates were collected each three days, consisting of 46 days of experiment. Twenty colonies were infected with *Wolbachia*, and eleven composed the uninfected group. The experiment started when the queens were four days old.

The data downloaded from the Dryad was converted to an Excel document, having the following variables collected on the experiment:

- **date:** Date at which the data was collected
- **wolbachia:** Refers to *wolbachia* infection status.
- **day:** Number of days since the start of experimental protocol
- **colonyID:** Unique identifier for the colonies
- **q.age.days:** Age of queen(s) in days
- **males:** Number of males in the colony
- **workers:** Number of workers in the colony
- **queens:** Number of queens in the colony
- **eggs:** Number of eggs laid by the queens
- **yl:** Number of young larvae
- **ol:** Number of old larvae
- **worker.pp:** Number of workers pre-pupae
- **worker.pup:** Number of workers pupae

3.2. Biodemography analysis

To verify the effect of the *Wolbachia* in the colonies using the formulas proposed by Birch, the variables ‘wolbachia’, ‘q.age.day’, ‘colonyID’, ‘queens’ and ‘eggs’ were selected for the analysis. For the calculation of the Intrinsic Rate of Increase (*r*), two rates were previously calculated, all with the use of the Excel software:

1. The age-specific survival rates (l_x): in the case of this study, as all the colonies started with 20 queens, the l_x was equal to the ‘nqueens’ divided by 20.
2. The age-specific fecundity rate (m_x): equal to 0.5 multiplied by the ‘neggs’ and divided by the number of queens at the age x.

Given the reproductive and colony life type of ants (Hymenoptera: Formicidae), the ‘females’ were considered the queens only, besides the fact that ant workers are also female individuals, as the only reproductive ants are the queens.

Before the calculation of *r*, two important parameters of comparison were obtained, the Net reproduction rate (R_0), which is the ratio of total female births in two successive generations, and the Mean length of a generation (T). The R_0 can be calculated with the use of the following formula:

$$R_0 = \int_0^\infty l_x m_x \, dx \tag{1}$$

The $l_x m_x$ factor was calculated before a simplification of the dataset. One value for each queen age was obtained for each variable by a simple mean of the values of the 31 different observed colonies. The R_0 was then calculated, along with the approximation of the Mean length of a generation (T), obtained with the following formula:

$$T = \frac{\sum x \, l_x m_x}{\sum l_x m_x} \tag{2}$$

The calculation of the *r* was made with formula 3, for infected and uninfected colonies, using a procedure described in the article for the power of e. ‘x’ here was used as the queen age in days.

$$\sum e^{-rx} \, l_x m_x = 1 \tag{3}$$

All the formulas were used for infected and uninfected colonies, for posterior comparison and analysis. Summing up, the analytical framework follows the life table approach pioneered by Lotka (1907) and Birch (1948), adapted to insect populations. Age-specific egg (“birth”) counts were transformed into fecundity rates (m_x) and “survival” probabilities (l_x), from which force of fecundity (μ_x) was derived. Empirical fecundity trajectories were then compared with classical parametric models explained in the following subsection.

2.3. Classical demography

To detect fecundity deceleration and potential plateaus at later times, we estimated the logarithmic derivative of the force of mortality, the demographic measure known as the log-aging rate (LAR), defined as: $LAR(x) = \frac{dl_n\mu_x}{d_x}$.

Empirical LAR values were computed by age-specific differencing of log-transformed hazards, whereas model-based LARs were derived analytically from fitted hazard functions. Following approaches in biodemography (Horiuchi & Wilmoth, 1998; Vaupel et al., 1979), we defined the onset of fecundity deceleration as the first age at which LAR reached non-positive values for sustained consecutive ages. Bootstrap resampling was employed to assess uncertainty in estimates of the deceleration age. This work was done in R version 4.2.2 with a ‘Tidyverse’ library-based script. The dataset consisted of the life tables of both colonies (Tables 2 and 3). The values of *r* were calculated for posterior comparison with the Birch methodology described in 2.2. The life-table aging rate (LAR) was obtained based on Horiuchi and Coale’s (1990) formula (4), where $\bar{b}(x)$ represents the LAR and $M(x)$ is the central death rate at age x (Ribeiro and Missov, 2016).

$$\bar{b}^{*}(x) = ln\,(M(x)) - ln\,(M(x - 1)) \tag{4}$$

To avoid high fluctuations caused by variations in the death rates of older ages, Horiuchi and Coale proposed the empirical formula (5), which takes nine-year moving averages on the results of (4), after applying a five-year moving average to $\mu(x)$.

$$\bar{b}_{emp}(x) = \sum_{n=-4}^{4} \frac{(5-|n|)}{25} * \bar{b}^{*}(x+n) \tag{5}$$

Nevertheless, due to the low number of observation days, this second-step smoothing procedure was unable to be employed.

To quantify fecundity patterns and detect late-life fecundity deceleration in ants, we combined empirical life table construction with parametric mortality models. Our analysis was grounded in classical biodemographic approaches (Vaupel et al., 1998;

Carey, 2001; Mueller & Rose, 1996), while adapting them to colony-level insect data.

In the present work, we fit four parametric mean functions $\mu(x)$ representing per-queen fecundity at age x. These models provide alternative characterizations of age-specific reproductive schedules, and their parameters are estimated via maximum likelihood under a Poisson framework.

We thus fit a Gaussian (bell-shaped) approach to the queen's fertility:

$$\mu(x) = A * \exp\left(-\frac{(x-\mu)^2}{2\sigma^2}\right) \tag{6}$$

with parameters' amplitude A ≥ 0, center μ, and spread σ > 0.

Next, a gamma Probability Density Function (PDF) was employed, with parameters $k\ e\ \gamma$ where $\left(X \sim \Gamma(k,\gamma)\right)$:

$$\mu(x) = \frac{\lambda^k x^{k-1} e^{-\lambda x}}{\Gamma(k)} \tag{7}$$

where, if $k = 1$, the gamma distribution is reduced to the exponential distribution.

Third, we fit a Weibull PDF, with two positive parameters $Weibull\ (\lambda, \gamma)$:

$$\mu(x) = \lambda\gamma x^{\gamma-1} e^{-\lambda x^{\gamma}} \tag{8}$$

if $\lambda = 1$, we end up again with an exponential distribution, but with $\lambda < 1$the function increases monotonously, and if $\lambda > 1$, the opposite.

Lastly, we also employ a Gompertz–Makeham framework:

$$\mu(x) = ae^{bx} + c \tag{9}$$

Parameters are also positive, where a ≥ 0, c ≥ 0. Here, the Gompertz–Makeham function – typically used for mortality – is repurposed to describe fecundity shapes, combining an exponential trend with a baseline floor.

All models were fitted following a Maximum Likelihood Estimation methodological approach where counts per age class yi are modeled as Poisson with mean λi = Ei $\mu(xi)$, where Ei is exposure and $\mu(xi)$ is the model-predicted fecundity rate (log-likelihood):

$$\ell(\theta) = \textstyle\sum_i [y_i \log\left(\lambda_i(\theta)\right) - \lambda_i(\theta) - log(y_i!) \tag{10}$$

Additionally, model-based LAR was calculating using:

$$ln(M(x)) - ln(M(x - 1) \tag{11}$$

Evaluating plots include empirical LAR, smoothed trends (LOESS, GAM spline, cubic polynomial), and model-predicted LAR.

Comparisons were conducted across colonies to test for robustness of the observed patterns, while Residuals between empirical mortality rates and model predictions were computed to assess goodness-of-fit, and error distributions were analyzed to explore potential systematic deviations. This combined framework integrates classical demographic models with empirical hazard estimation, making it possible to rigorously test whether insect populations display fecundity plateaus analogous to those found in human and other animal demography.

2.4. GEE modelling

To understand the influence of the presence of the bacteria and the time on the number of queens, eggs and workers, we used Generalized Estimating Equations (GEE), typical of marginal models (or population average models). This kind of model consider the non-independence of the observations, while not requiring assumptions about the distribution. While the linear models assume no correlation between the observations, the population average model provide a more robust inference and it does not specify any likelihood, being suitable for analyzing dependent observations, throughout time, between different treatments.

Although it is a useful analytical tool for evaluating relationships between different variables, Generalized Linear Models (GLM) can only be used when there is no correlation among the data. The GEE method uses quasi-likelihood methodology to create equations that link marginal means to linear predictors to provide a variance-covariance matrix (Agresti, 2007).

As mentioned, GEEs are based on GLMs, so they also assume that the dependent variable belongs to the exponential family, but they include a correlation structure in the estimation of the regression parameters $\boldsymbol{\beta} = [\beta_0, \beta_1, \beta_2, \dots, \beta_p]$.

The proposal by Liang & Zeger (1986) consisted of solving the following equation to obtain the parameter estimates $\boldsymbol{\beta}$

$$S_{\boldsymbol{\beta}}(\boldsymbol{\alpha}, \boldsymbol{\beta}) = \sum_{i=1}^{n} (\frac{\partial \mu_i}{\partial \boldsymbol{\beta}})^T \, V_i^{-1}(y_i - \mu_i) = 0 \ , \tag{12}$$

where V_i is a specific matrix that incorporates a given correlation structure, and $\boldsymbol{\alpha}$ represents the parameters of that correlation structure,

$$V_i = \sigma A_i^{1/2} R(\alpha) A_i^{1/2} \qquad (13)$$

where A_i is a diagonal matrix of the outcomes' variance, $R(\alpha)$ is the correlation matrix specified and σ is a dispersion parameter.

Liang & Zeger (1986) demonstrate that if the structure for the mean is correctly specified, then, regardless of the chosen V matrix, the estimator $\widehat{\boldsymbol{\beta}}$ is unbiased and consistent. The distribution approaches to a multivariate normal distribution, that is

$$\sqrt{n}\,(\widehat{\boldsymbol{\beta}} - \boldsymbol{\beta}_{\mathbf{0}}) \sim^{a} N\,(0, \Sigma) \qquad (14)$$

where Σ is the empirical estimation of variance. This property allows us to calculate p-values and confidence intervals using the Wald test.

The equations used in the correlation matrix are extensions of those used in the quasi-likelihood method. These likelihood methods are not Maximum Likelihood, since the distribution of Y is not specified, and therefore there is no likelihood function; the alternative computational method for clustered data is a generalization of quasi-likelihood (Agresti, 2007).

The GEE method uses the quasi-likelihood methodology to create equations that link marginal means to linear predictors in order to provide a variance–covariance matrix (Agresti, 2007). Therefore, GEEs do not require an assumed distribution for the response variable, provide more robust inference results, and do not specify any likelihood (Hubbard et al., 2010).

In the present study, the observations made colony by colony form a dependent structure, since although the colonies themselves are independent, each one—considered an individual—was observed over time. Therefore, despite being widely used in biological and ecological studies, the use of GLM is not appropriate here, as they assume independence between observations and would not yield robust or reliable results for the *M. pharaonis* population and its relationship with infection by the endosymbiont *Wolbachia*. As the dependent variables analyzed consisted of counts, the distribution used was 'Poisson', which link function is the logarithmic, and the correlation structure considered was autoregressive of the first order (AR (1)). The analysis was done in the R software (version 4.2.2) with the packages 'geepack', 'dplyr' and 'ggplot2'.

4. Results and discussion

4.1. Descriptives of the data

The variables ‘wolbachia’, ‘day’, ‘colonyID’, ‘queens’, ‘eggs’ and ‘workers’ were selected for the analysis done in this work, as they are the variables related to the biodemography formulas used and describe the reproduction rates of the ant colonies, as well as the infection status by the *Wolbachia* bacteria. Since the data is not normally distributed and the variances between the infected and not infected group are not equal, the non-parametrical test of Mann-Whitney was made for each caste of the colony.

The mean number of queens infected with *Wolbachia* was 19.04, and the uninfected was 17.69 (Fig.3). The Mann-Whitney test showed that we can reject the null hypothesis that these means are equal ($p<0.00$).

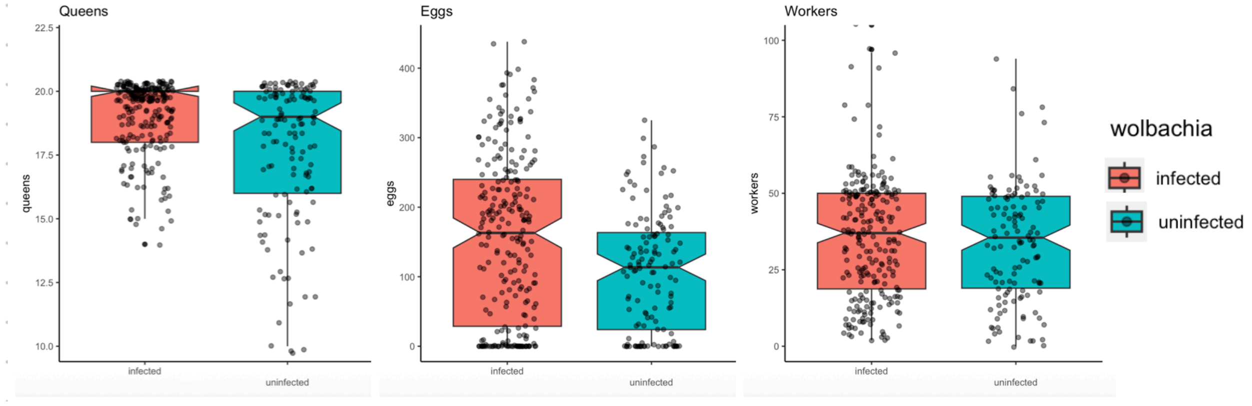


Figure 3: Boxplot of the number of queens, eggs and workers, respectively, in the infected (orange, left) and uninfected (green, right) colonies. Queens and eggs showed significant differences between treatments, while worker numbers showed no difference between treatments.

The mean number of eggs in the infected colonies with *Wolbachia* was 156, and in the uninfected was 107 (Fig.3). The Mann-Whitney test showed that we can reject the null hypothesis that these means are equal ($p<0.00$). The mean number of workers in the infected colonies with *Wolbachia* was 39.1, and in the uninfected was 39.7 (Figure 3). The Mann-Whitney test showed that we do not reject the null hypothesis that these means are equal ($p=0.536$).

4.2. Biodemographic analysis

The mean number of queens and eggs per day of observation and the age-specific survival (l_x) and fecundity (m_x) rates were summarized in Table 1. The t test made with the number of queens from infected and uninfected colonies showed that the number of queens is significantly different between these two groups (p-value = 0.04629), but not the number of eggs laid by the queens (p-value = 0.2003).

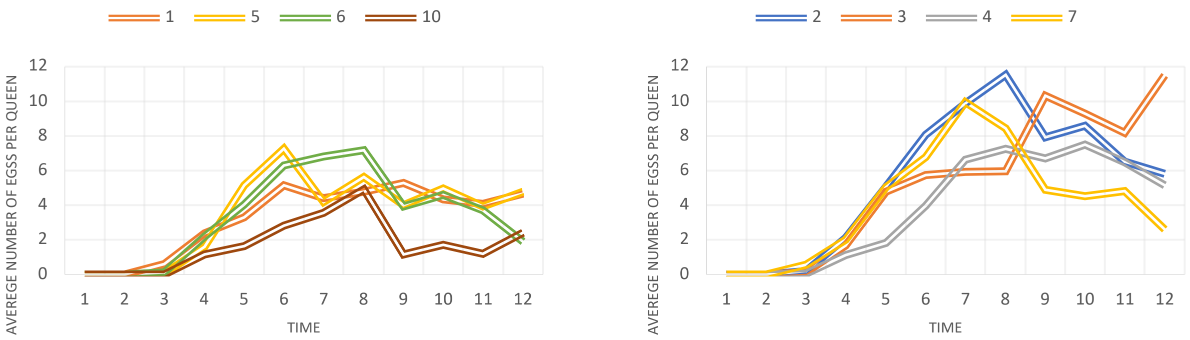
Table 1: Simplified life table with the mean of each observation and the age-specific survival and fecundity rates for the infected ad uninfected colonies. ‘Wolbachia’ indicates the state of infection of the colonies, the ‘Number of queens’ represents the mean observed queens in the colonies at the age x and the ‘Number of eggs’ represents the mean number of eggs laid by the queens at the age x (Own elaboration, data from Singh et al., 2023)

Wolbachia	*Queen age in days (x)*	l_x	m_x	$l_x m_x$	*Number of queens*	*Number of eggs*
infected	4	1	0	0	20	0
	5	1	0	0	20	0
	8	0,9900	0,3484	0,3463	19,8000	13,8500
	11	0,9825	1,8847	1,8550	19,6500	74,2000
	14	0,9750	3,9605	3,8900	19,5000	155,6000
	17	0,9725	5,7588	5,6213	19,4500	224,8500
	20	0,9625	6,6190	6,4025	19,2500	256,1000
	23	0,9425	7,5671	7,1663	18,8500	286,6500
	35	0,9225	6,2659	5,7938	18,4500	231,7500
	39	0,9100	5,9102	5,3763	18,2000	215,0500
	43	0,8975	5,9074	5,3213	17,9500	212,8500
	50	0,8725	5,7165	5,0150	17,4500	200,6000
uninfected	4	1	0	0	20	0
	5	0,9909	0,0000	0,0000	19,7273	0,0000
	8	0,9816	0,1822	0,1799	19,5455	7,1818
	11	0,9679	1,5958	1,5437	19,2727	61,5455
	14	0,9452	3,3345	3,1218	18,8182	124,3636
	17	0,9361	4,6915	4,3437	18,6364	173,0909
	20	0,9089	5,2934	4,8438	18,0909	192,8182
	23	0,8952	5,4073	4,8712	17,8182	194,1818
	35	0,7993	4,4626	3,5286	15,9091	140,5455
	39	0,7672	4,6698	3,6202	15,2727	144,2727
	43	0,7581	4,5810	3,5348	15,0909	140,6364
	50	0,7170	3,7412	2,7053	14,2727	107,7273

The calculation of the R_0, by the sum of the values of $l_x m_x$, showed different values for the two colony types. While the infected ones had R_0 = 47, the uninfected ones had a R_0 = 32,29. This means that the presence of the *Wolbachia* bacteria makes the capacity the *M. pharaonis* colonies must grow in each generation go from 32,29 times to 47 times its original size. The values of T also favor the presence of *Wolbachia*, increasing the mean length of a generation by 1,31 days (from 27,71 to 29,02 days). The infinitesimal rate of increase, *r*, of the infected colonies (*r*= 0,18) was also higher when compared to the uninfected ones (*r*=0,16).

4.3. Classical demography insights

Following the methodology specified in sections 3.2 and 3.3, it was possible to calculate diverse parameters trying to explain the evolution on the average number of eggs produced by the queen each day, i.e., queens' fecundity. This analysis is possible to differentiate by infected and non-infected colonies. The next figures present an example of 8 random ant colonies: 4 infected and 4 uninfected.



a) Uninfected random sample colonies

b) Infected random sample colonies

Source: Own calculation

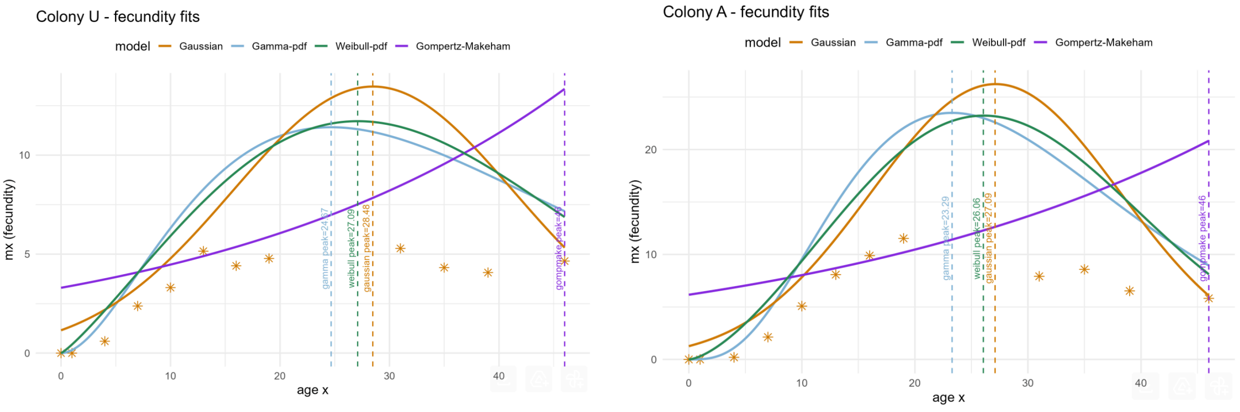
Figure 4: Average fecundity of queens over time.

From figure 4, on overall it is possible to identify a peak in the average number of eggs by queen in the middle of the observed time. Nevertheless, the fecundity of infected colonies is much higher than for uninfected.

Combined with the approaches exposed in equations (6) to (9), we can also try to find a proximity between laws of human demographic behavior and insect reproduction, in this case: ant colonies. Figure 5, presents, as example, empirical LAR and model fitting for an infected (labeled as colony "A") colony a an uninfected (labeled as colony "U") one.

a) Uninfected random sample colonies

b) Infected random sample colonies

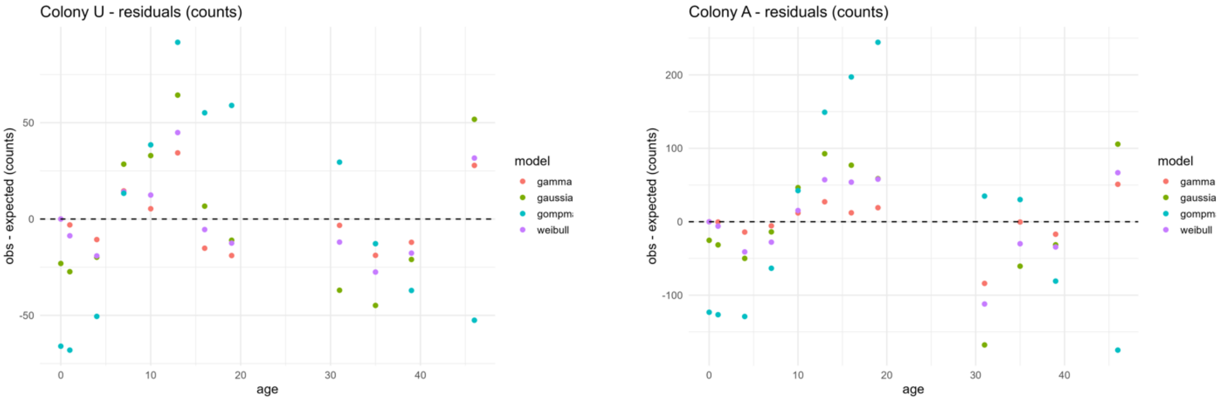


Source: Own calculation

Figure 5: Life Table Aging Rate (empirical and theoretical)

The presented results show that there is some difficulty to follow closely the empirical LAR (dots) but the two main models that visually present better results are the Weibull and the Gaussian. The left axis still shows the empirical differences in fecundity, with infected colonies presenting higher values, but the age at which fecundity starts to decelerate is later for uninfected ones.

In Figure 6, we can now confirm which of the models produces more accurate results. As we expected from Figure 5 observation, it is possible to conclude, for the presented examples, the gamma model is the one with lower residuals, i.e., the differences calculated between observed and estimated LAR values ($r_i = y - \hat{y}$).


a) Uninfected random sample colonies

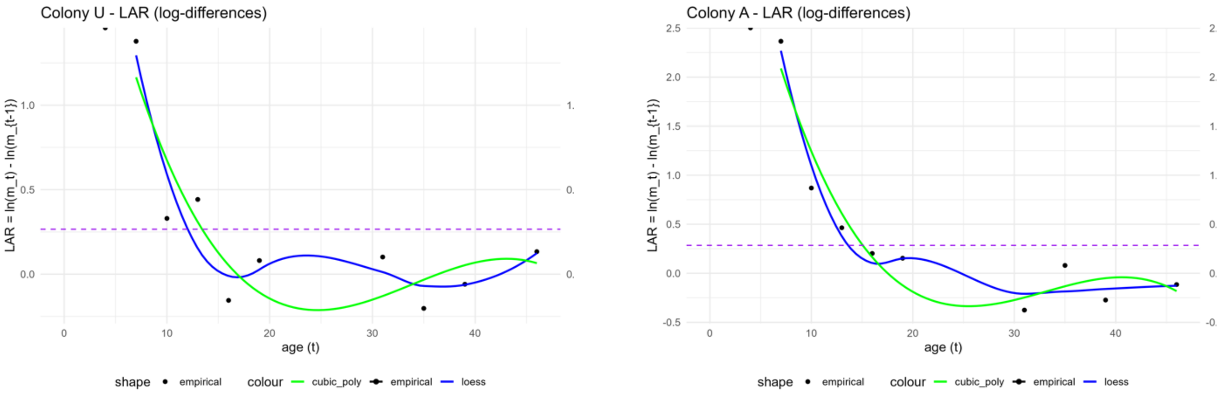
b) Infected random sample colonies

Source: Own calculation

Figure 6: Fecundity Residuals (empirical - theoretical)

The gamma model estimates the age of fecundity deceleration for both colonies (infected and uninfected) a little lower than the Weibull and Gaussian Models. Nevertheless, our sensibility shows that for colony A (Figure 5 – infected) fecundity keeps increasing until age/day 20, and in colony U (uninfected) starts at day 13. So, besides if the gamma model estimates better the fecundity behavior, the Weibull and Gaussian models estimate more accurate the age at which deceleration starts.

On other hand, applying equation (4) it is possible to identify properly the time at which fecundity starts to decelerate. Thus, Figure 7 presents the Horiuchi Life Table Aging rate, but very conditioned once that the second step smoothing procedure from the original model is not possible to implement. In this case, it is only possible to make a very general conclusion: fecundity deceleration starts much earlier than expected by fitting the models to fecundity curves (Figure 5). Lastly, but still very interesting and important conclusion, is that the Birch intrinsic growth rate is higher for infected colonies when compared with uninfected.



a) Uninfected random sample colonies

b) Infected random sample colonies

Source: Own calculation

Figure 7: Empirical Life Table Aging Rate (observed and smoothed) and Birch intrinsic growth rate.

4.4. GEE model analysis

One marginal model for each life stage used for the biodemography analysis was constructed, showing different results regarding the influence of the *Wolbachia* for each caste of the ants' colony. The GEE models were constructed considering the family Poisson, with the logarithmic link function. The constraints of "independence" and "ar1" were tested and, since the coefficients were equal, we concluded that the correlation between the responses within the subjects is practically inexistent.

In order to build more realists models, that take into consideration the effect of time in the *Wolbachia* infection in the colonies, a term of interaction between time and the

Wolbachia infection was included in the models. The coefficients and p values for the queens model are in Table 2.

Table 2: Summary of the GEE model for the queen number in infected and uninfected colonies.

Coefficients	**Estimate**	**p-value**
(Intercept)	2,99	< 2e-16
day	-0,02	< 2e-16
Day*wolbachiauninfected	-0,004	5.7e-07

This was the only model that showed a significant interaction between time and the bacteria infection, meaning that, in uninfected colonies, the decrease in queen number is more pronounced than in the infected colonies, indicating that, for the queens, the state of infection has an influence in the queen decrease throughout time.

Considering the coefficients of Table 2 and the state of infection by *Wolbachia* as 0= ‘Infected’, and 1= ‘Uninfected’, this model indicates that the exponential expected number of queens in the infected colonies on day 0 is exp (2,99) =19,89, and the exponential expected number of queens decreases in a rate of exp (-0,02), or 2%, each day in infected colonies, and exp (-0,024), or 2,3%, in uninfected colonies.

For the eggs, a life stage related to the reproductive potential of the colony, the coefficients and p values is in Table 3.

Table 3: Summary of the GEE model for the eggs number in infected and uninfected colonies.

Coefficients	**Estimate**	**p-value**
(Intercept)	4,48	< 2e-16
wolbachiauninfected	-0,37	1.4e-06
day	-0,02	< 2e-16

Considering the coefficients of Table 3 and the state of infection by *Wolbachia* as 0= ‘Infected’, and 1= ‘Uninfected’, this model indicates that the exponential expected number of eggs decreases in a rate of exp (-0,02), or 2%, each day in infected colonies, and exp (-0,39), or 32,29%, in uninfected colonies.

The coefficients were significant at a 5% level and show a positive influence of the presence of the bacteria on the number of eggs, but the infected and uninfected colonies have the same effect throughout time. The assumptions of the model residuals were evaluated.

For the workers, a caste not directly related to the colony's reproduction, the following results were obtained (Table 4):

Table 4: Summary of the GEE model for the worker number in infected and uninfected colonies.

Coefficients	**Estimate**	**p-value**
(Intercept)	4,15	< 2e-16
day	-0,03	< 2e-16

The coefficient for the *Wolbachia* presence on the colony did not show a statistical significance for the worker numbers for this ant population, which indicates that the state of infection of the colony does not have an influence in the worker numbers, which decrease naturally through time. This model indicates that the exponential expected number of workers in the infected colonies on day 0 is exp $(4{,}15)$ =63,43, and the exponential expected number of workers decreases 2,3% each day.

5. Discussion

It is already known that *Wolbachia* infections in *M. pharaonis* colonies have an effect in some reproductive dynamics (Singh & Linksvayer, 2020). Besides that, the data used in the present work was analyzed in the original study (Singh et al., 2023) only in the scope of the number of queens and eggs laid and the expected age of the queens. The effect of this bacteria in ants is not widely known, and Singh and collaborators' (2020, 2023) work was one of the first experiments with the Pharaoh's ants and the *Wolbachia* effect in the colony's life cycle and growth (Ramalho et al., 2021).

Here, formulas based on demography studies of Birch (1948) were used to check, within the scope of the biodemography, the effect of *Wolbachia* in the length of the generations, the capacity the colony has to grow in each generation and, mostly, if it had an effect on the infinitesimal rate of increase. The effect of *Wolbachia* in each caste throughout the days was also studied with the GEE modelling, a novel methodology in biology and ecology studies, as well as the application of classical demography concepts for this analysis.

The use of biodemography formulas, especially the Intrinsic Rate of Increase (*r*), is not frequent in ant colonies growth' studies, so far. Until 2009, the knowledge on the *M.*

pharaonis demography was not existent, although it is essential for the study of the population dynamics of this invasive species (Buczkowski & Bennett, 2009). The results obtained from the original study of the data, as the ones obtained in the present work, favor the presence of the bacteria for the longevity of the colony (Singh et al. 2023). However, it shows that the survival probability is similar in some stages of the colony life and that there is a similar proportion of alive queens over time.

The use of the rates calculated based on the work of Birch (1948) presented results related to the reproductive capacity each colony type has, and, because of that, the use of biodemography formulas could be a way of having more specific analysis of the effect on the capacity the colony has as a whole, and not only the queens, the workers and the eggs alone. When thinking about control methods for ants, the calculation of the intrinsic rate is an interesting approach, as it takes in consideration the number of reproductive individuals and its individual and group capacity of producing eggs in the conditions the colony is in. This is a valuable tool on quantifying different variables on the colonies' growth and on comparisons between different groups/treatments, in the scope of thinking more environmentally friendly control methods.

The results obtained in the present work show that the presence of *Wolbachia* increase the mean length of the generation, as well as the capacity it has to grow in each generation. With that, it becomes clearer that, along with the higher intrinsic rate of increase, the bacteria may give a reproductive acceleration to the Pharaoh's ants colonies, giving it a capacity to grow more in a shorter period of time. As previous studies have shown that the size of the colony may influence the queen-worker ratio (Schmidt et al. 2011), an important next step could be studying the relation between colony size and the presence of *Wolbachia*, calculating the ratios used in the present work.

An important approach that should be considered is the use of biodemography concepts for workers analysis. As Kramer and collaborators (2016) have shown, the lifespan of the colony influences the individual lifespan of workers and its sizes in *Lasius niger* - another ant species- colonies. Workers are responsible for the colony dispersion and a key factor on the invasion capacity of *M. pharaonis*. Adding the workers factor on the biodemography analysis can bring a new light on the understanding of *Wolbachia* and its use as a control mechanism for the Pharaoh's ants.

The demography analysis of the LAR for the ant colonies indicates that the *Wolbachia* infection, besides giving the colonies a later peak on the eggs-per-queen numbers, a higher intrinsic rate of increase, generation length and the capacity the colony has to grow in each generation, the queen's fecundity starts to decrease in earlier ages. This may indicate that the bacterial infection can act as a reproductive 'boost', leading to a rapid increase in the colony's reproductive capacity, followed by a decline in fecundity at a younger age compared to the absence of *Wolbachia*. This knowledge may aid future studies on the *Wolbachia*–*Monomorium pharaonis* mutualism, as well as on control methods for invasive ants that maintain relationships with these endosymbiotic bacteria, by supporting the development of strategies targeting the most appropriate life stages of the colonies.

Still within the scope of demographic analyses, the fitting of queen number over experimental time points (t) and their ages in days (x) using different models, i.e., Weibull, Gamma, and Gompertz, represents an uncommon practice in studies on ant colony growth and reproduction. To date, the application of such models in myrmecological research has been limited to studies on ant foraging activity (Yamanaka et al., 2019). Based on the results obtained in the present study, this methodology is believed to offer substantial added value to research on colony growth in different ant species, particularly by enabling more accurate estimates of the ages at which queen fertility begins to decline, an important factor in studies aiming to develop control methods for invasive ants. It is worth emphasizing that data collection must be continuous and conducted at regular, closely spaced intervals in order to ensure more reliable model estimates.

The original study of the data, as well as other reproduction and growth studies, was analyzed with Generalized Linear Mixed Models (GLMM) for analyzing the differences in egg laying through time. Here, using GEE models, accurate and robust results were obtained regarding the influence of *Wolbachia* in the numbers of queens, workers and eggs during the days of the experiment. The presence of *Wolbachia* influenced the numbers of eggs and queens, corroborating the findings of previous studies (Singh et al. 2023; Singh & Linksvayer 2020), but not the worker numbers, which could indicate another evidence that *Wolbachia* is related to the reproductive capacity of the ants' colonies. The GEE model for the queens also showed a significant interaction between the bacteria infection and the days of the experiment. This type of interaction, as other aspects of the *Wolbachia* infection in ants, has not been well studied, especially in a separated way for each caste and, given the simplicity of the GEE model construction and analysis, this could be a way to start analyzing this interaction and increase the knowledge on this symbiosis.

The understanding of *Wolbachia* interactions still need to increase (Ramalho et al., 2021). As *M. pharaonis* is an invasive species, many studies focus on control strategies and an understanding of the resource allocation (Schmidt et al. 2011), giving studies that have the objective of understanding the relations between the colonies and the bacteria's presence an importance for the biological control of the ant species. The suppression of *Wolbachia* in *M. pharaonis* invaded places as a control method should decrease the reproduction capacity of the colonies and, by that, diminish the use of pesticides and the environmental impact.

6. Conclusions

The results obtained in the present work confirmed our initial hypothesis that the presence of the endosymbiont bacteria *Wolbachia* increased the reproductive rates of *M. pharaonis* colonies. Besides that, it is also important to highlight the importance of using rates and concepts that go beyond the classical ecology and biology indexes and formulas. The use of classical demography adapted concepts showed a more quantifiable and integrated analysis of the reproductive differences between the colony types and, by that, a clearer vision of the effects *Wolbachia* has on *M. pharaonis*. Besides that, more studies are needed to fully comprehend this symbiotic relation and more interdisciplinary works need to be done on this and other biology themes.

The infection of the bacteria had a different influence in each caste. For the queens, the infected colonies showed to have a smaller decrease in their number throughout time, and the age in which their fecundity starts to decrease is earlier when compared to uninfected colonies. For the eggs, the peak if the number of eggs per queen happens in older ages in infected colonies, which also have more eggs when compared to uninfected colonies. For the workers, the infection state did not show a significant influence on their number. Considering the colony as a whole, the infection of *Wolbachia* increased the capacity the colony has to grow in each generation, its length and the colonies' intrinsic rate of increase.

The results of the three areas of this study (biology, demography and statistics) proved to be complementary in providing a more holistic understanding of the effects of the endosymbiotic bacteria on both the distinct castes and the colony as an integrated unit. The methodologies applied in this work should be replicated to other ant species also mutualist to *Wolbachia* and to other experimental settings (as the comparison between food treatments), to study invasive species control methods, or even develop conservation actions and understand mutualistic relations between *Wolbachia* and the Formicidae.

References

Agresti, Alan. 2007. *An Introduction to Categorical Data Analysis Second Edition*. Second. John Wiley & Sons, Inc., Hoboken, New Jersey.

Alvares, Lucia E., O. C. Bueno, and H. G. Fowler. 1993. "Larval Instars and Immature Development of a Brazilian Population of Pharaoh's Ant, *Monomorium Pharaonis* (L.) (Hym., Formicidae)." *Journal of Applied Entomology* 116(1–5): 90–93. doi:10.1111/j.1439-0418.1993.tb01171.x.

Baudisch, A. (2011). The pace and shape of ageing. Methods in Ecology and Evolution, 2(4), 375-382.

Birch, L C. 1948. 17 Source: Journal of Animal Ecology *The Intrinsic Rate of Natural Increase of an Insect Population*.

Buczkowski, Grzegorz, and Gary Bennett. 2009. "Colony Budding and Its Effects on Food Allocation in the Highly Polygynous Ant, Monomorium Pharaonis." *Ethology* 115(11): 1091–99. doi:10.1111/j.1439-0310.2009.01698.x.

Carey, J. R. 2001. "Insect Biodemography." *Annual Review of Entomology* 46: 79–110. doi:10.1146/annurev.ento.46.1.79.

Horiuchi, S., & Wilmoth, J. R. (1998). Deceleration in the age pattern of mortality at older ages. Demography, 35(4), 391–412.

Hubbard, Alan E., Jennifer Ahern, Nancy L. Fleischer, Mark Van Der Laan, Sheri A. Lippman, Nicholas Jewell, Tim Bruckner, and William A. Satariano. 2010. "To GEE or Not to GEE: Comparing Population Average and Mixed Models for Estimating the Associations between Neighborhood Risk Factors and Health." *Epidemiology* 21(4): 467–74. doi:10.1097/EDE.0b013e3181caeb90.

Hughes, W. O., & Reynolds, S. E. (2005). Evolutionary instability of insect societies. Proceedings of the Royal Society B: Biological Sciences, 272(1561), 1817–1822.

Jones, O. R., Scheuerlein, A., Salguero-Gómez, R., et al. (2014). Diversity of ageing across the tree of life. Nature, 505(7482), 169–173.

Kannisto, V. (1994). Development of the oldest-old mortality, 1950–1990: Evidence from 28 developed countries. Monographs on Population Aging, 1. Odense University Press.

Krabbe, Birla A., Xavier Arnan, Pol Lannes, Christoffer Echtvad Bergstedt, Rasmus Stenbak Larsen, Jes Søe Pedersen, and Jonathan Z. Shik. 2019. “Using Nutritional Geometry to Define the Fundamental Macronutrient Niche of the Widespread Invasive Ant *Monomorium Pharaonis*.” *PLoS ONE* 14(6): 1–17. doi:10.1371/journal.pone.0218764.

Kramer, Boris H., Ralf Schaible, and Alexander Scheuerlein. 2016. “Worker Lifespan Is an Adaptive Trait during Colony Establishment in the Long-Lived Ant Lasius Niger.” *Experimental Gerontology* 85: 18–23. doi:10.1016/j.exger.2016.09.008.

Liang, K., Zeger, S. Longitudinal data analysis using generalized linear models, *Biometrika*, Volume 73, Issue 1, April 1986, Pages 13–22, https://doi.org/10.1093/biomet/73.1.13

Lotka, A. J. (1907). Relation between birth rates and death rates. Science, 26(653), 21–22.

Mueller, L. D., & Rose, M. R. (1996). Evolutionary theory predicts late-life mortality plateaus. Proceedings of the National Academy of Sciences, 93(26), 15249–15253. https://doi.org/10.1073/pnas.93.26.15249

Mueller, L. D., Rauser, C. L., & Rose, M. R. (2011). Does aging stop? Oxford University Press.

Promislow, D. E. L., Tatar, M., Khazaeli, A. A., & Curtsinger, J. W. (1996). Age-specific patterns of genetic variance in Drosophila melanogaster. I. Mortality. Genetics, 143(2), 839–848.

Ramalho, Manuela De O., Zoe Kim, Steven Wang, and Corrie S. Moreau. 2021. “Wolbachia across Social Insects: Patterns and Implications.” *Annals of the Entomological Society of America* 114(2): 206–18. doi:10.1093/aesa/saaa053.

Ribeiro, F. e Missov, T.I. 2016. Revisiting mortality deceleration patterns under a gamma-Gompertz-Makeham framework. In Robert Schoen (Ed.) Dynamic Demographic Analysis, Springer, ISBN 978-3-319-26601-5.

Russell, Jacob A., Benjamin Goldman-Huertas, Corrie S. Moreau, Laura Baldo, Julie K. Stahlhut, John H. Werren, and Naomi E. Pierce. 2009. “Specialization and Geographic Isolation among Wolbachia Symbionts from Ants and Lycaenid Butterflies.” *Evolution* 63(3): 624–40. doi:10.1111/j.1558-5646.2008.00579.x.

Schmidt, A. M., T. A. Linksvayer, J. J. Boomsma, and J. S. Pedersen. 2011a. “Queen-Worker Caste Ratio Depends on Colony Size in the Pharaoh Ant (Monomorium Pharaonis).” *Insectes Sociaux* 58(2): 139–44. doi:10.1007/s00040-010-0126-x.

Schmidt, A. M., T. A. Linksvayer, J. J. Boomsma, and J. S. Pedersen. 2011b. “Queen-Worker Caste Ratio Depends on Colony Size in the Pharaoh Ant (Monomorium Pharaonis).” *Insectes Sociaux* 58(2): 139–44. doi:10.1007/s00040-010-0126-x.

Singh, Rohini, and Timothy A. Linksvayer. 2020. “Wolbachia-Infected Ant Colonies Have Increased Reproductive Investment and an Accelerated Life Cycle.” *Journal of Experimental Biology* 223(9). doi:10.1242/jeb.220079.

Singh, Rohini, Sachin Suresh, Jennifer H Fewell, Jon F Harrison, and Timothy A Linksvayer. 2023. “Wolbachia-Infected Pharaoh Ant Colonies Have Higher Egg Production, Metabolic Rate, and Worker Survival”. doi:10.1101/2023.01.31.526493.

Vaupel, J. W., Manton, K. G., & Stallard, E. (1979). The impact of heterogeneity in individual frailty on the dynamics of mortality. Demography, 16(3), 439–454.

Vaupel, J. W., Carey, J. R., & Christensen, K. (1998). Biodemographic trajectories of longevity. Science, 280(5365), 855–860.

Warner, Michael R., Jessica Lipponen, and Timothy A. Linksvayer. 2018. “Pharaoh Ant Colonies Dynamically Regulate Reproductive Allocation Based on Colony Demography.” *Behavioral Ecology and Sociobiology* 72(3): 1–13. doi:10.1007/s00265-017-2430-1.

Wetterer, James K. 2010. “Worldwide Spread of the Pharaoh Ant, Monomorium Pharaonis (Hymenoptera: Formicidae)”. *Myrmecol. News* 13: 115-129.

Yamanaka O., Shiraishi M., Awazu A., Nishimori H. 2019. “Verification of mathematical models of response threshold through statistical characterisation of the foraging activity in ant societies”. Scientific reports, 9:8845. doi: 10.1038/s41598-019-45367-w

Final considerations

This dissertation allowed for a deeper understanding of the application of classical statistical and demographic methodologies in the study of ant colony growth, presenting a novel methodological approach for studies on invasive ant species control, nature conservation, and other areas of biology and ecology.

It is already known that *Wolbachia* infections in *M. pharaonis* colonies affect certain reproductive dynamics (Singh & Linksvayer, 2020). Moreover, the data used in the present study were analyzed in the original study (Singh et al., 2023) considering only the number of queens, eggs laid, and the expected age of queens. The effect of this bacterium on ants is still not widely understood, and the work by Singh and collaborators (2020, 2023) is among the pioneering studies conducting experiments with pharaoh ants to investigate the effects of *Wolbachia* on the life cycle and colony growth (Ramalho et al., 2021).

In this study, formulas based on Birch's demographic studies (1948) were used to evaluate, within a biodemographic framework, the effect of *Wolbachia* on generation duration, colony growth capacity per generation, and, most importantly, whether there is an effect on the infinitesimal population growth rate. The effect of the bacteria on each caste over time was also analyzed using GEE models (Generalized Estimating Equations), a novel methodology in biological and ecological studies, alongside the application of classical demographic concepts for this analysis.

The use of biodemographic formulas, especially the Intrinsic Rate of Increase (r), is still uncommon in studies of ant colony growth. Until 2009, there were no data on the demography of *M. pharaonis*, despite its essential role in studying the population dynamics of this invasive species (Buczkowski & Bennett, 2009). The results obtained in the original study of the data, as well as those from the present work, support the presence of the bacteria for colony longevity (Singh et al., 2023). However, they also show that survival probability is similar at certain stages of the colony life cycle, as is the proportion of living queens over time.

The use of rates calculated based on Birch's work (1948) provided results related to the reproductive capacity of each colony type. Therefore, the use of these biodemographic formulas can be a way to obtain more specific analysis regarding the effect on the reproductive capacity of the colony as a whole, and not just on queens, workers, or eggs individually. When considering ant control methods, calculating the intrinsic rate of increase proves to be an important approach, as it accounts for the number of reproductive individuals and their individual and collective ability to produce eggs under the conditions in which the colony exists. This represents a valuable tool for quantifying different variables in colony growth, within the context of developing more environmentally sustainable control methods.

The results obtained in the present study show that the presence of *Wolbachia* increases the mean generation duration, as well as the colony's growth capacity per generation. This makes it clearer that, together with a higher intrinsic growth rate, the bacteria can act as a reproductive accelerator in Pharaoh's ant colonies, providing greater growth potential in a shorter time. Since previous studies have shown that colony size can influence the ratio of queens to workers (Schmidt et al., 2011), an important next step could be to study the relationship between colony size and the presence of *Wolbachia*, using the indices calculated in the present work.

A relevant approach to consider is the application of biodemographic concepts to the analysis of workers. As demonstrated by Kramer and collaborators (2016), colony longevity influences the individual longevity of workers and their body size in *Lasius niger* colonies (another ant species). Workers are responsible for colony dispersal; therefore, including workers in the biodemographic analysis could provide a new perspective on understanding *Wolbachia* and its potential use as a control mechanism for *M. pharaonis*.

The demographic analysis of the LAR for ant colonies indicates that *Wolbachia* infection results in a later peak in the number of eggs per queen, a higher intrinsic growth rate, longer generation duration, and greater growth capacity per generation, but causes queen fertility to begin declining at earlier ages. This may suggest that the infection acts as a reproductive "boost," leading to a rapid increase in the colony's reproductive capacity, followed by a decline in fertility at younger ages compared to uninfected colonies. This knowledge can contribute to future studies on the *Wolbachia-M. pharaonis* mutualism, as well as to invasive ant control methods that involve these endosymbiotic bacteria, by supporting the development of strategies targeting the most appropriate life stages of colonies.

Still within the scope of demographic analyses, the adjustment of the number of queens over the experimental time (t) and their ages in days (x), using different models, Using models such as Weibull, Gamma, and Gompertz represents an uncommon practice in studies on ant colony growth and reproduction. To date, the application of these models in myrmecological research has been limited to studies on ant foraging activity (Yamanaka et al., 2019). Based on the results obtained in the present study, this methodology is believed to represent a significant advancement in research on colony growth in different ant species, especially as it allows for more precise estimates of the ages at which queen fertility begins to decline, an important factor for the development of invasive ant control methods. It is emphasized that data collection should be continuous and at regular, closely spaced intervals between observation days (unlike what was done ion the experiment) to ensure better model estimates and more accurate and scientifically relevant conclusions.

The original study of these data, as well as other analyses of reproduction and growth, used Generalized Linear Mixed Models (GLMMs) to investigate differences in oviposition over time. In the present work, using GEE models, more precise and robust results were obtained regarding the influence of *Wolbachia* on the numbers of queens, workers, and eggs over the days of the experiment. The presence of the bacteria significantly influenced the numbers of eggs and queens, corroborating the results of previous studies (Singh et al., 2023; Singh & Linksvayer, 2020), but it did not significantly affect the number of workers, which may be further evidence that *Wolbachia* is related to the reproductive capacity of the colonies. The GEE model for queens also showed a significant interaction between bacterial infection and the experimental days. This type of interaction, as well as other aspects of *Wolbachia* infection in ants, is still little studied, especially separately for each caste. Given the simplicity of constructing and analyzing GEE models, this approach may be a pathway for a deeper analysis of this interaction and for advancing knowledge of this symbiosis. Singh and collaborators (2023) also found that colonies infected with older queens had higher metabolic rates compared to uninfected colonies.

Further studies are still needed to better understand the interactions between *Wolbachia* and ants (Ramalho et al., 2021). Since *M. pharaonis* is an invasive species, many studies focus on control strategies and understanding resource allocation (Schmidt et al., 2011), highlighting the importance of research aimed at understanding the relationships between colonies and the presence of the bacterium for the biological control of this species. Suppressing *Wolbachia* in areas invaded by *M. pharaonis* as a control method could reduce the reproductive capacity of colonies, thereby decreasing pesticide use and environmental impact.

The results obtained in the present study confirmed our initial hypothesis that the presence of the endosymbiotic bacteria *Wolbachia* increases the reproductive rates of *Monomorium pharaonis* colonies. Furthermore, it is important to highlight the relevance of using rates and concepts that go beyond classical indices and formulas in ecology and biology. The application of concepts adapted from classical demography allowed for a more quantifiable and integrated analysis of reproductive differences among colony types, providing a clearer view of the effects that *Wolbachia* have on *M. pharaonis*. Nevertheless, further studies are needed to fully understand this symbiotic relationship, as well as more interdisciplinary work in this and other areas of biology.

The bacterial infection had distinct effects on each caste. For queens, infected colonies showed a smaller reduction in their numbers over time, and the age at which their fertility begins to decline occurs earlier compared to uninfected colonies. Regarding eggs, the peak number of eggs per queen occurs at older ages in infected colonies, which also present a higher number of eggs compared to uninfected colonies. In the case of workers, infection status did not show a significant influence on their numbers. Considering the colony as a whole, *Wolbachia* infection increased the colony's growth capacity per generation, the duration of generations, and the intrinsic growth rate of colonies.

The results obtained across the three areas of this study (biology, demography, and statistics) were not only complementary in building a more realistic understanding of the effects of the endosymbiotic bacteria, but also provided relevant information about the different castes, offering a more comprehensive analysis of the colony itself as an integrated unit. The methodologies applied in this work, in addition to being interdisciplinary and innovative, can and should be replicated in other ant species that also maintain mutualistic relationships with *Wolbachia*, as well as in other experimental contexts (such as comparisons between different dietary treatments), as a way to investigate invasive species control methods, develop conservation actions, and deepen the understanding of mutualistic relationships between *Wolbachia* and Formicidae. Additionally, the advancement of biodemographic studies with a strong statistical foundation also allows for obtaining cross-cutting insights into both animal and non-animal populations, including, as discussed here, human populations.

References

Agresti, Alan. 2007. *An Introduction to Categorical Data Analysis Second Edition*. Second. John Wiley & Sons, Inc., Hoboken, New Jersey.

Alvares, Lucia E., O. C. Bueno, and H. G. Fowler. 1993. "Larval Instars and Immature Development of a Brazilian Population of Pharaoh's Ant, *Monomorium Pharaonis* (L.) (Hym., Formicidae)." *Journal of Applied Entomology* 116(1–5): 90–93. doi:10.1111/j.1439-0418.1993.tb01171.x.

Baudisch, A. (2011). The pace and shape of ageing. Methods in Ecology and Evolution, 2(4), 375-382.

Birch, L C. 1948. 17 Source: Journal of Animal Ecology *The Intrinsic Rate of Natural Increase of an Insect Population*.

Buczkowski, Grzegorz, and Gary Bennett. 2009. “Colony Budding and Its Effects on Food Allocation in the Highly Polygynous Ant, Monomorium Pharaonis.” *Ethology* 115(11): 1091–99. doi:10.1111/j.1439-0310.2009.01698.x.

Carey, J. R. 2001. “Insect Biodemography.” *Annual Review of Entomology* 46: 79–110. doi:10.1146/annurev.ento.46.1.79.

Eleftherianos, Loannis, Jaishri Atri, Julia Accetta, and Julio C. Castillo. 2013. “Endosymbiotic Bacteria in Insects: Guardians of the Immune System?” *Frontiers in Physiology* 4 MAR. doi:10.3389/fphys.2013.00046.

Horiuchi, S., & Wilmoth, J. R. (1998). Deceleration in the age pattern of mortality at older ages. Demography, 35(4), 391–412.

Hubbard, Alan E., Jennifer Ahern, Nancy L. Fleischer, Mark Van Der Laan, Sheri A. Lippman, Nicholas Jewell, Tim Bruckner, and William A. Satariano. 2010. “To GEE or Not to GEE: Comparing Population Average and Mixed Models for Estimating the Associations between Neighborhood Risk Factors and Health.” *Epidemiology* 21(4): 467–74. doi:10.1097/EDE.0b013e3181caeb90.

Hughes, W. O., & Reynolds, S. E. (2005). Evolutionary instability of insect societies. Proceedings of the Royal Society B: Biological Sciences, 272(1561), 1817–1822.

Jackson, Duncan E., Stephen J. Martin, Mike Holcombe, and Francis L.W. Ratnieks. 2006. “Longevity and Detection of Persistent Foraging Trails in Pharaoh’s Ants, *Monomorium Pharaonis* (L.).” *Animal Behaviour* 71(2): 351–59. doi:10.1016/j.anbehav.2005.04.018.

Jones, O. R., Scheuerlein, A., Salguero-Gómez, R., et al. (2014). Diversity of ageing across the tree of life. Nature, 505(7482), 169–173.

Kannisto, V. (1994). Development of the oldest-old mortality, 1950–1990: Evidence from 28 developed countries. Monographs on Population Aging, 1. Odense University Press.

Krabbe, Birla A., Xavier Arnan, Pol Lannes, Christoffer Echtvad Bergstedt, Rasmus Stenbak Larsen, Jes Søe Pedersen, and Jonathan Z. Shik. 2019. “Using Nutritional Geometry to Define the Fundamental Macronutrient Niche of the Widespread Invasive Ant *Monomorium Pharaonis*.” *PLoS ONE* 14(6): 1–17. doi:10.1371/journal.pone.0218764.

Kramer, Boris H., Ralf Schaible, and Alexander Scheuerlein. 2016. “Worker Lifespan Is an Adaptive Trait during Colony Establishment in the Long-Lived Ant Lasius Niger.” *Experimental Gerontology* 85: 18–23. doi:10.1016/j.exger.2016.09.008.

Liang, K., Zeger, S. Longitudinal data analysis using generalized linear models, *Biometrika*, Volume 73, Issue 1, April 1986, Pages 13–22, https://doi.org/10.1093/biomet/73.1.13

Lotka, A. J. (1907). Relation between birth rates and death rates. Science, 26(653), 21–22.

Mueller, L. D., & Rose, M. R. (1996). Evolutionary theory predicts late-life mortality plateaus. Proceedings of the National Academy of Sciences, 93(26), 15249–15253. https://doi.org/10.1073/pnas.93.26.15249

Mueller, L. D., Rauser, C. L., & Rose, M. R. (2011). Does aging stop? Oxford University Press.

Promislow, D. E. L., Tatar, M., Khazaeli, A. A., & Curtsinger, J. W. (1996). Age-specific patterns of genetic variance in Drosophila melanogaster. I. Mortality. Genetics, 143(2), 839–848.

Ramalho, Manuela De O., Zoe Kim, Steven Wang, and Corrie S. Moreau. 2021. “Wolbachia across Social Insects: Patterns and Implications.” *Annals of the Entomological Society of America* 114(2): 206–18. doi:10.1093/aesa/saaa053.

Ribeiro, F. e Missov, T.I. 2016. Revisiting mortality deceleration patterns under a gamma-Gompertz-Makeham framework. In Robert Schoen (Ed.) Dynamic Demographic Analysis, Springer, ISBN 978-3-319-26601-5.

Russell, Jacob A., Benjamin Goldman-Huertas, Corrie S. Moreau, Laura Baldo, Julie K. Stahlhut, John H. Werren, and Naomi E. Pierce. 2009. “Specialization and Geographic Isolation among Wolbachia Symbionts from Ants and Lycaenid Butterflies.” *Evolution* 63(3): 624–40. doi:10.1111/j.1558-5646.2008.00579.x.

Schmidt, A. M., T. A. Linksvayer, J. J. Boomsma, and J. S. Pedersen. 2011a. “Queen-Worker Caste Ratio Depends on Colony Size in the Pharaoh Ant (Monomorium Pharaonis).” *Insectes Sociaux* 58(2): 139–44. doi:10.1007/s00040-010-0126-x.

Schmidt, A. M., T. A. Linksvayer, J. J. Boomsma, and J. S. Pedersen. 2011b. “Queen-Worker Caste Ratio Depends on Colony Size in the Pharaoh Ant (Monomorium Pharaonis).” *Insectes Sociaux* 58(2): 139–44. doi:10.1007/s00040-010-0126-x.

Singh, Rohini, and Timothy A. Linksvayer. 2020. “Wolbachia-Infected Ant Colonies Have Increased Reproductive Investment and an Accelerated Life Cycle.” *Journal of Experimental Biology* 223(9). doi:10.1242/jeb.220079.

Singh, Rohini, Sachin Suresh, Jennifer H Fewell, Jon F Harrison, and Timothy A Linksvayer. 2023. “Wolbachia-Infected Pharaoh Ant Colonies Have Higher Egg Production, Metabolic Rate, and Worker Survival”. doi:10.1101/2023.01.31.526493.

Vaupel, J. W., Manton, K. G., & Stallard, E. (1979). The impact of heterogeneity in individual frailty on the dynamics of mortality. Demography, 16(3), 439–454.

Vaupel, J. W., Carey, J. R., & Christensen, K. (1998). Biodemographic trajectories of longevity. Science, 280(5365), 855–860.

Warner, Michael R., Jessica Lipponen, and Timothy A. Linksvayer. 2018. “Pharaoh Ant Colonies Dynamically Regulate Reproductive Allocation Based on Colony Demography.” *Behavioral Ecology and Sociobiology* 72(3): 1–13. doi:10.1007/s00265-017-2430-1.

Wetterer, James K. 2010. “Worldwide Spread of the Pharaoh Ant, Monomorium Pharaonis (Hymenoptera: Formicidae)”. *Myrmecol. News* 13: 115-129.

Yamanaka O., Shiraishi M., Awazu A., Nishimori H. 2019. “Verification of mathematical models of response threshold through statistical characterisation of the foraging activity in ant societies”. Scientific reports, 9:8845. doi: 10.1038/s41598-019-45367-w