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Sarconesiopsis magellanica (Diptera: Calliphoridae) life-cycle, reproductive and population parameters using different diets under laboratory conditions

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ABSTRACT

Sarconesiopsis magellanica is a forensically relevant necrophagous blowfly that can aid in determining the post-mortem interval (PMI) as it is the first to colonise decomposing corpses. The blowfly has been reported in several South-American countries including Colombia, in high-altitude regions ranging from 1200 to 3100 m above sea level. The present study reports this blowfly's life cycle and an analysis of its reproductive and population parameters under laboratory conditions for the first time. Six successive generations of flies were produced with an average of 65.38% adults emerging with respect to the total number of puparia. The shortest life cycle from egg to adult emergence was found in individuals fed on a lyophilised liver (LL) diet, while the longest one was found in individuals fed with an egg-powdered milk (E-PM) diet; intermediate values were found when the pig liver (PL) diet was tested. The greatest adult longevity was achieved when the PL diet was used, the LL diet giving the shortest. The population parameters based on the horizontal life table were: net reproductive rate (R_o) = 447.752 ± 9.9, mean generational time (T_c) = 18.18 ± 0.38, natural population increase rate (r_m) = 0.145 and finite population increase rate (λ) = 1.398. This blowfly colony represents a valuable asset for both basic and applied studies. Members of the *S. magellanica* colony so established were used for analysing the life-cycle, reproductive and population parameters, and further medical and forensic application studies are currently underway.

Sarconesiopsis magellanica (Le Guillou 1842) (Diptera: Calliphoridae) is a necrophagous and hemisynanthropic blowfly [1,2]. These flies are important in forensic research because they can aid in determining the post-mortem interval (PMI), being one of the first insects to colonise a decomposing corpse [3]. Furthermore, this species is important in medicine as it is a potential mechanical vector for pathogens, such as virus, bacteria, fungi, protozoa and helminths [4,5].

The fly has been reported in several South-American countries such as Argentina [1], Bolivia, Chile, Ecuador and Peru [6,7]. It has been found in the Antioquia, Boyacá, Cundinamarca and Norte de Santander departments in Colombia, particularly in sites located from 1200 to 3550 m above sea level [7,8].

Analysing such organisms' life-cycle stages and their colonisation under laboratory conditions might provide access to valuable biological material for undertaking basic and applied research in medicine and forensic sciences. Additional studies of colonised insects have been related to systematics, bionomy, genetics, insecticide susceptibility, vector competence, vector capacity, vaccine development and the establishment of cell cultures [9,10].

Life tables have been used to describe the development, survival and fecundity for a cohort of individuals and to provide basic information about a particular population's growth [11,12]. A vertical life table is used for determining survival and mortality whilst a horizontal life table is useful for estimating species' reproductive and population parameters such as net reproductive rate, natural population increase rate, mean generational time and finite population increase rate [13].

Insect feeding is one of the key factors in mass fly breeding under laboratory conditions. Two of the most important diet quality indicators are biomass accumulation and fecundity [14]; organic tissues are amongst the optimal diets for necrophagous insects as they are able to satisfy both requirements. However, artificial diets have also been used (though infrequently) and, when evaluated, have proved optimal for supplying nutrients for flies throughout their life cycle [15]. The first such diets had the disadvantage of producing offensive odour and contamination [16]. It has also been shown that the presence of toxins in

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decomposing tissues from natural diets can alter the development rate and lead to errors in estimating the PMI when necrophagous insects are used for forensic study under laboratory conditions [17]. Artificial diets can be a reliable alternative for breeding and maintaining strains, that is, continuous generations of an insect strain [17–19].

Even though work on the life-cycle, colonisation, reproductive and population parameters of some species from the Calliphoridae family has been carried out in different parts of the world [11,20,21], just one report describing the *S. magellanica* life cycle in samples collected from Perú has been published to date [7]. The present work was thus aimed at determining the biological cycle of *S. magellanica*, as well as analysing the blowfly's reproductive and population parameters for the first time in specimens raised on both a natural and two artificial diets under laboratory conditions. Life tables from this species were also constructed.

1. Materials and methods

1.1. Obtaining and breeding S. magellanica

Adult *S. magellanica* were captured in Bogotá, Colombia, specifically in the upper part of the city's Parque Nacional (4°37′8.90″ N, 74°3′27.73″ W, 2800 m above sea level, 14 °C). About 1.5 kg of decomposing pig's liver was used as bait for attracting the specimens, which were then trapped by using entomological nets. The blowflies were transported (in plastic jars covered with a veil) to the Universidad del Rosario's Medical and Forensic Entomology laboratory, where they were identified taxonomically using previously described keys [8]. Adult forms were kept in 45 cm × 45 cm × 45 cm Gerberg cages under controlled environmental conditions in the laboratory at 24 °C with 70% relative humidity and a12-h photoperiod (light/darkness). The blowflies were initially fed on pig's liver (PL) and a carbohydrate source, sugar solution (30% sucrose), supplemented with vitamin B12 [22].

1.2. Colonisation

Eggs of *S. magellanica* were collected from parental females' first oviposition (two masses each containing around 100 eggs) deposited on the food substrate to start the colonisation. The eggs were then placed on Petri dishes with fresh food substrate (decomposing pig's liver). The larvae which emerged were used for the colony's continuity. The blowflies were placed in Gerberg cages on reaching the adult phase where the biological cycle continued. Six continuous insect generations were analysed. The feeding, physical and environmental conditions necessary for guaranteeing biological cycle continuity throughout different generations were verified. Pertinent safety measures were taken to avoid one strain contaminating another within the insectariums, using veils to cover the Gerberg cages and respectively labelling each cage and plastic jar.

1.3. Life cycle of S. magellanica

The *S. magellanica* life cycle was evaluated using a natural diet (PL) and two artificial diets: lyophilised liver (LL) and egg-powdered milk (E-PM) (Table 1) [21,23]. The LL diet has previously been described [21] as a source having a high protein percentage to provide better adaptation and development of the insects; it also contains phosphate that promotes larval growth; the E-PM diet, despite containing a lower protein proportion when compared to LL, is rich in casein, lactoalbumin and cholesterol that are also essential for developing and maintaining the insect's life cycle [23]. This biological analysis was begun from three ovipositions (each one of around 150 eggs) which were obtained from the previously established colony (i.e., this was the fourth generation) and each oviposition was given its respective diet. Larvae that hatched from the eggs and passed from one instar to another were counted and maintained under the same environmental conditions as the adult organisms. The following aspects were taken into account when analysing each diet's efficacy: developmental stage duration (in days; the I, II and III larval instars, puparia and adult), sex ratio and adult longevity. The size of immature and adult stages was also determined using a stereomicroscope (Nikon, Tokyo, Japan; SMZ1500) linked to a high-resolution digital camera (DS-Ri1-U2) and NIS-Elements software. Immature stages were measured from front to back using a stereoscope (Leica; Solms, Germany) with specialised software (Application Suite) LAS EZ version 2.0.0. The lengths of random samples taken from 15 specimens belonging to each stage and from each pot were averaged. Adult size was inferred by measuring wing length, from the basicosta to the mid-costal vein [24]. A total of 15 adults were randomly taken for each plot.

1.4. Vertical life table and estimating mortality

The vertical life table was constructed from a cohort of 100 virgin female adults which emerged the same day. Following a settling period, the parental females suitable for oviposition were grouped according to each selected diet and placed in separate cages. The eggs oviposited daily on the substrate were collected and counted, as were the different stages of the development cycle up to the adults' deaths for evaluating the effect of the diets. The vertical life table was constructed, its parameters were established and survival curves were drawn following the procedure proposed by Rabinovich [13].

Table 1
The composition of two artificial diets used to rear *Sarconesiopsis magellanica* (Diptera: Calliphoridae) under laboratory conditions.

Ingredients	Lyophilised liver (Rueda et al., 2010) [21]	Egg-powdered milk (modified from Álvarez et al., 2005)
$Na_2HPO_4{\cdot}12H_2O$	0.012 g	–
KH_2PO_4	0.012 g	–
NaCl	1.2 g[a]	–
Powdered liver	6 g	–
Glucose	1.2 g	–
Nutritive agar	4.6 g[a]	2.5 g
Brain heart infusion (BHI)	4.2 g	9 g[a]
Blood	25 mL	–
Whole powdered milk	–	6 g
Powdered egg	–	6 g
Distilled water	200 mL	100 mL

[a] Ingredient modified regarding the amount of grams.