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Research article

Colonization pattern and thermal needs of immature phases of *Sarcophaga argyrostoma* (Diptera: Sarcophagidae): Significance for estimating postmortem interval

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ABSTRACT

Members of Sarcophagidae are necrophagous and are commonly found on decaying carcasses; and their developmental forms are important indicators for the approximation of lowest post-mortem interval (PMImin). This work describes the biological characteristics of *Sarcophaga argyrostoma* from Tehran, Iran. Various temperature regimes were applied to estimate the thermal summation constant (k) and thermal requirements for development of *S. argyrostoma.* Five growth proceedings, containing 1st ecdysis, 2nd ecdysis, wandering, pupariation and eclosion, were investigated under eight fixed temperature regimes (6–30 °C). The effects of fly age, freshness, and availability of oviposition substrate on oviparity and viviparity was studied. At 6 °C, no development occurs, and at 8 °C only the first ecdysis occurs. At temperatures between 10 and 30 °C, all immature stages proceeded to the adult stage, and thus immature development was analyzed at these six remaining temperatures. The development phases needed minimum (Dz ± SE) 5.4(0.4), 8.5(0.26), 5.3(0.44), 3.8(0.1), and 6.6 (0.6)°C to attain one of the succeeding developmental occasion, correspondingly. The approximated K for those five occasions were 15.04 ± 1.12, 12.62 ± 0.65, 140.36 ± 4.35, 14.59 ± 0.6, and 222.8 ± 4.18°-day (DD) accordingly. When the food substrate is available and fresh, the female prefers to lay eggs, no matter how old she is. However, the chance of ovoviviparity increased when no oviposition substrate was available. The truth that *S. argyrostoma* able to either larviposit or to lay eggs encompasses serious consequences for the precise estimation of the PMImin, as the existence of larvae resulting from eggs laid on the carcass could add hours (based on ambient temperature) to the PMImin.

1. Introduction

Sarcophagidae flies, also known as flesh flies, are found worldwide and include more than 100 genera and 3100 species [1–3]. Flesh flies are grouped into 3 subfamilies: Sarcophaginae, Paramacronychiinae, and Miltogramminae, each of which shows diverse breeding habits and strategies [3]. They are usually considered to be sarcosaprophagous scavengers, however, in addition to saprophagy, their

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biological habits and breeding strategies also include necrophagy, and the parasitizing and predation of insects [3–5]. In addition, flesh flies implement ecological services such as pollination and carrion decomposition and play a role as possible biological control agents [6,7]. The fertility strategy of these flies is ovoviviparity; often, instead of laying eggs, they deposit 10–40 maggots directly on corpses or in superficial wounds on the body [8–10], which can result in myiasis. Much interest has been rewarded to Sarcophagidae flies due to their myiasis potential and their pathogen-carrying abilities [11–13].

Sarcophagid flies are typical early colonizers at various stages of carrion decomposition [1,8,12–17]. The synanthropic behavior of flesh flies, includes visiting and breeding carrions, let them to colonize human cadavers, that emphasizes their significant for forensic disciplines, and facilitates their being efficiently analyzed for minimum post-mortem interval (PMImin) [3,17–19]. In criminal entomology, *Sarcophaga (Liopygia) argyrostoma* (Robineau-Desvoidy, 1830) has great significance, and it is also one of the most prevalent Sarcophagid species in Iran [20–22]. Recent studies have identified it as a cause of skin (wound) and genital (vaginal) myiasis [27–29]. *Sarcophaga argyrostoma* is distributed worldwide including USA, Canada, Europe, Asia, and the Middle East. Its larvae consuming human corpses equally in indoor and outdoor sites and from those at different points of decomposition [18,23–29]. The maggots similarly feed on the dead larvae of other Dipterans, in addition to spoiled organic matter, while adults feed on both spoilage and flowers [26]. Adults of this species could sense fresh meat hidden at 20 cm in relaxed soil and deposit larvae on the earth surface. The maggots creeped via the soil to contact the meat and finalized their growth beneath ground. Baits or meat via gauze bandages can be infested. They can invade baits or meat via gauze bandages but not sore bandages [26].

PMImin commonly is calculated based on the association between ambient temperature and insect growth [30–32]. A knowledge of heat constraints, in particular the lower development threshold (LDT) temperature, the ambient temperature below which the growth of a cold-blooded organism ceases, is of required value to determine Accumulated Degree Days (ADD). Except at the two extremes, close to the minimal and higher temperature limits, the association between growth and temperature is nearby straight. Estimation of growth time of a species can be provided by a fitting linear regression model. Thus, using such a model, the LDT and the thermal summation constant (k) can be provided for each species individually [30]. Because each growth stage needs a specific accumulated degree day (ADD) or accumulated degree hour (ADH) the data are stage-specific [31]. Immature stages growth of *L. argyrostoma* was investigated at 30, 25, and 20 °C in Cairo, Egypt [33]; whereas Grassberger and Reiter (2002) investigated the entire growing period of the species reared at 8, 15, 20, 25, 30, and 35 °C, but they also determined a general minimum development threshold (LDT) for total immature development stages [31]. Later, Sert et al. (2020 and 2021) examined pupal development at fluctuating and fixed temperatures of 30, 25, and 20 °C in Turkey [34,35]. Nonetheless, no comprehensive developmental records are existing for the various immature stages of *L. argyrostoma*.

This study defines, first-ever, the biological characteristics of *S. argyrostoma* from the city of Tehran, Iran. The species is highly abundant worldwide including Iran, and since it is associated with human cadavers, detailed developmental data can be used to reckon PMImin. This study will strengthen the utilization of *S. argyrostoma* in forensic studies and permit a further correct prediction of the PMImin to be prepared when it is retrieved from the murder scene. Here, different temperature regimes (6–30 °C), were used to estimate the thermal summation constant (k) and thermal requirements (LDT or Dz), using linear models for each development event of *S. argyrostoma.* Also, the sex ratio, the reproductive rate, and the influences of egg-laying source readiness, age, and meat freshness on ovoviviparity and viviparity were studied.

2. Materials and methods

Specimens of *S. argyrostoma* (n = 33), were collected in Tehran city by means of a chicken liver as lure and recognized using morphological characters including the male genitalia [3]. The classification was approved using the COI and COII molecular markers [36] resulting from a primary specimen (GenBank ID: MG913301). The average temperature and relative humidity on collecting days were 33 °C and 13%, respectively, with an average wind speed of 15 km/h from the south/southwest and 26% cloud cover.

2.1. Mass rearing of flies

Sarcophaga argyrostoma specimens were reared in a fly insectary in the Department of Vector Biology and Control of Diseases, School of Public Health (SPH), Tehran University of Medical Sciences with 10:14 h dark: light photoperiod, 27 ± 1 °C of temperature, and 50 ± 10% of relative humidity (RH). Automated photoperiodic controller, humidifier and a heating blower was used for control of photoperiod, humidity, and temperature. In a screen cage (36 by 36 by 36 cm), 300 adult flies were kept and nourished a mix of ground milk and dry granulated sugar. Water was supplied by cotton wicks inside the bottle. Chicken liver was used to rear the larvae. When the larvae reached the post feeding stage, wood shaving substrate for pupariation and intra-puparial periods was provided [37], and they were monitored until they were fully mature. During this study we have maintained a colony of *S. argyrostoma* under laboratory conditions for 12 generations.

2.2. Thermal requirements of immature stages

To afford coordinated larvae batches, the fresh liver was put in the raising cage for about 5 min and females were permitted to larviposit. The existence of maggots was checked and confirmed by stereomicroscope (Nikon SMZ-745, Japan) observation. In the case of observing eggs, only the fresh livers with larvae batches were used for experiments. Then, for each experiment, 600 synchronized larvae gathered over fewer than 30 min were allotted in 40 (15 per cup) Styrofoam® cups (200 ml) with a soaked cotton pad and 15 g of new chicken liver. The upper inner wall of the cups was coated with white petroleum jelly (Shimi Taghtiran Co., Tehran, Iran) to

avoid larval escape a few hours before larvae reach to post feeding stage. The cups of larvae were placed in an incubator (Lab-Line Instrument Inc., Tripunithura, India) already arranged at one of the eight fixed temperatures to be attested (6, 8, 10, 15, 20, 25, 28, and 30 °C) at 50 ± 10% RH and under a 10:14 h dark: light photoperiod. The incubators were provided with cooling and heating systems with accurateness of ±1 °C. The cups were set on two rock shelves interior the incubator. To shrink the influence on insect development of the cup position interior the incubator, the cups were casually reorganized daily. Almost 1 g food per larva was supplied from the commencing of the second day of the trial forward. Based on the developing phase and trial temperature, specimens were collected every 4, 6, 8, 12, or 24 h. At each sampling period, as many as eight immature specimens were collected from an accidently picked cup. The cup with left maggots or pupae was return into the incubator instantly after sampling. As the trial progressed, those previously tested cups were accidently selected for inspection and a second random sample of the left specimens for the purpose of experimental endurance. Sampled maggots were placed in 90 °C water to prevent larval curving after killing them, prior to leaving them in 70% ethanol. The larvae that had departed from the chicken liver and were nearby on the cup's side were deemed as wandering stage (post-feeding maggots). Larval stages were classified using a stereomicroscope. As explained above, the post feeding larvae were added to wood shaving substrate into 100 × 100 mm plastic containers. Pupa was indicated when it reached a completely rigid state with no elasticity. Plastic containers of post feeding larvae and pupae were incubated, and monitored every 4, 6, 8, 12, or 24 h, based on the trial temperature, to record the epoch of pupariation and intra-puparial periods and adult emergence. The sampling procedure lasted till the entire post-feeding maggots changed to pupae and afterward pupae changed to adult.

2.3. Impact of food resource availability on ovoviviparity and oviparity

For this experiment, cages containing 10 pairs each of one day old adult female and male flies were chosen and split into three clusters. Adult flies were offered a mix of ground milk and dry granulated sugar, and fresh water. Each group (three replicates) was deprived of chicken liver for one, two, or three days, respectively; after which, a cup containing an average of 15 g of chicken liver was placed in each cage. Then, the number of larvae or eggs were examined daily until the first batch of eggs or larvae appeared.

2.4. Impact of meat freshness on ovoviviparity and oviparity

Chicken liver at three different decaying stages: fresh, semi-rotten (kept out of the refrigerator for one day), and rotten/semi-dried (kept outside the refrigerator for two days) in laboratory condition with 10/14 h dark: light photoperiod, 27 ± 1 °C of temperature, and 50 ± 10% of RH). The semi-rotten or rotten liver were put in a plastic container, kept hydrated, and then were used to induce the flesh flies to give birth. The livers were offered in separate cups simultaneously in a cage (three replicates) containing 10 pairs of the *S. argyrostoma* flies. The rotten liver was used to induce the flesh flies to give birth. The cups contained an average of 15 g of each type of chicken liver. The cups were checked daily for the existence of eggs/larvae. The experiments were carried out for 6 consecutive days and every two days the cups were replaced with new cups containing new fresh or rotten liver. The number of eggs or larvae per batch were counted every day.

2.5. Impact of population size on sex ratio and reproductive rate of S. argyrostoma

In this experiment, to understand the impact of population size on the reproduction rate and sex ratio of *S. argyrostoma*, we provided three replicate groups of adult flies, in cages containing 1, 5, 10, and 15 pairs of flies of same ages in individual cages. Cups containing approximately 15 g of fresh chicken liver were placed inside each cage until the first batch of eggs or larvae appeared. Then, the cups including eggs/larvae were replaced with new ones (with new fresh meat). The cups with offspring were transferred to new cages and kept in an incubator under optimum growth conditions (14/10 h light: dark photoperiod, 27 ± 1 °C, and 50 ± 10% RH). The offspring larvae grew until adult emergence, and then the total number of the offspring and their sex ratio were determined for each group. The experiment was continued until the death of the last parent fly in each group.

2.6. Data analysis

The LDT (Dz) and thermal summation constant (k) of each growth occasion were assessed using the linear model advised by Ikemoto and Takai (2000) [30]. The thermal requirements (Dz and k) were valued from the linear regression obtained from the points on the plot of the mean time to reach onset of a developmental stage at given temperature (D) on the X-axis, and D multiplied by T (mean time of development multiplied by rearing temperature (°C) of development) on the Y-axis. From Ikemoto and Takai we know that DT = k + DzD; so, y = DT and X = D. So, the thermal summation constant (k) on the plot is intercept and Tmin (or Dz) is slope. The k (Mean ± SE) was determined for temperatures 10, 15, 20, 25, 28, and 30 °C for each maggot, or pupal stage and the entire growth stages. Association between temperature points (six nominated temperatures) and growing ratio has been produced by Reduced Major Axix (RMA) with 95% of confidence level for the five growing occasions of *S. argyrostoma*. Amounts of k correspond to the sum of degree days (DD) above the LDT (Dz) required for maggot and pupal development and entire juvenile progress.

R-Studio software, Excel, and GraphPad Prism were used to prepare graphs. The mean time to accomplish each developing occasion was utilized to assemble these graphs at each of the fixed temperatures examined. The Chi-square analysis was employed to check association between the kind of offspring and time of meat scarcity.

3. Results

All developing phases of *S. argyrostoma* fruitfully developed to the adult phase in six (10, 15, 20, 25, 28, and 30 °C) out of the eight fixed temperatures (Fig. 1, Table 1). The first instar larvae did not grow at 6 °C but could grow and develop only to second instar larvae at 8 °C (Table 1). The development rate of *S. argyrostoma* increased as the temperature improved, as illustrated in Fig. 1 and in Table 1, and the development rate of *S. argyrostoma* was linearly related to temperature between 10 and 30 °C ($R^2 = 0.804–0.998$) (Table 2). At 10 °C, although the immature stages grew, they developed considerably slower (1927 h, equal to more than 80 days) than at the five higher temperatures. The immature stages grew well at 15 °C and higher temperatures and proceeded to the adult stage (Table 1). The longest and shortest mean time ± standard error (SE) for development of first instar larvae were 117 ± 2.1 and 14.3 ± 0.7 h at 8 °C and 30 °C, correspondingly (Table 1). The lengthiest mean time ±SE for entire growth from ovoviviposition to adult emergence was 1927 ± 13.1 h (80.3 ± 0.5 days) at 10 °C, while the quickest time was 401.2 ± 13.3 h (18.6 ± 0.5 days) at 30 °C (Table 1). The wandering and pupal periods constituted 28.9% and 58.4%, respectively, of the overall growth time of the juvenile stages.

The regression line revealed the value of k (degree-day (DD) or degree-hour (DH)) and Dz for different stages of *S. argyrostoma*. The results of these calculations are shown in Table 2. The growth phases needed minimum (Dz (±SE)) 5.4(0.4), 8.5(0.26), 5.3(0.44), 3.8 (0.1), and 6.6 (0.6) °C to attain one of the succeeding developmental occasions, correspondingly (Table 2, Fig. 2). The anticipated thermal summation constant (k) ± SE for each development stage was 15.04 ± 1.12, 12.62 ± 0.65, 140.36 ± 4.35, 14.59 ± 0.6, and 222.8 ± 4.18 DD for the five events, accordingly (Table 2). This value was 404.69 (10.9) DD for total development stages.

4. Effects of meat deprivation and freshness on ovoviviparity and oviparity

Our results show that the female flies preferentially initiate oviparity (3–4 egg batches, 8–10 eggs per batch) in the presence of meat. However, a lack of oviposition substrate (i.e., fresh meat) induces ovoviviparity gradually: after one day of meat deprivation, the females laid eggs (1–3 batches, 15–35 eggs per batch) as well as depositing larvae (15–45) and, although they laid less eggs, the differences between ovoviviparity and oviparity were not significant. After two days' meat deprivation, the female flies showed completely ovoviviparous behavior, depositing only larvae (35–55) on the third day (Fig. 3). The ANOVA analysis showed that there is a meaningful relationship ($P = 0.0001$) concerning the kind of progeny and period of meat deprivation.

The female flies did not lay eggs nor deposit larvae on semi-rotten or rotten chicken livers at all, during the six consecutive days of experiment. They always exhibited a preference for fresh meats on which to lay eggs or deposit larvae. Increasing age of the female insects did not affect their oviparity tendency, and if the meat was available and fresh, the females preferentially laid eggs.

5. Impact of population size on reproduction rate

In this experiment we used cages containing either 1, 5, 10, or 15 pairs of flies (males = females), with three replicates. Our results show that, in cages containing just one single pair, the flies did not lay eggs or deposit larvae, and after 18–20 days, the flies died without progeny.

In cages with five or more pairs, egg production started between 3 and 6 days after eclosion, however, as the population of females increase, the rate of population reproduction decreases. When the female population was 5, 10 and 15, the mean number of progeny (based on the number of third instar larvae), was 77, 43 and 44 larvae per female respectively (Fig. 4). The post hoc Tukey and ANOVA test displayed a statistically substantial alteration ($P < 0.0001$) between all the groups except for the comparison between groups containing 10 and 15 females ($p = 0.6786$).

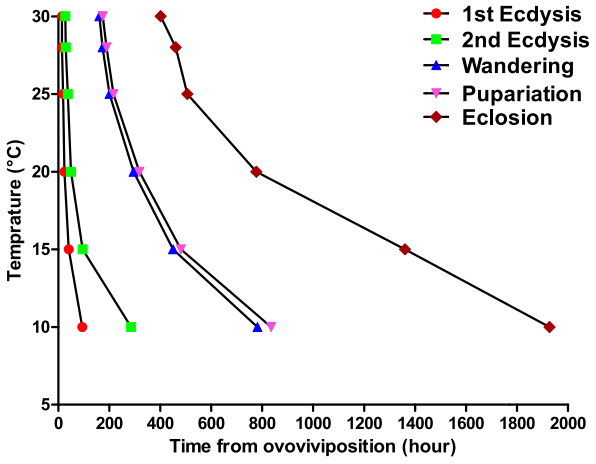

Fig. 1. Isomorphen graph for *S. argyrostoma*. Records spots display the mean time to start appearance of each of five growing occasions (1st ecdysis; 2nd ecdysis; wandering; pupariation; and eclosion) which have been shown by different symbols.

Table 1
Mean ± SE (hour) period between two consecutive development stage and total time (hour: H or day: D) to reach adult stage for *S. argyrostoma* at eight constant temperatures. SE: standard errors.

Temperature (°C)	1st instar (SE)	2nd instar (SE)	3rd instar (SE)	Wandering (SE)	Intrapuparial stage (SE)	Total (H) (SE)	Total (D) (SE)
6	a	a	a	a	a	a	a
8	117 (2.1)	a	a	a	a	a	a
10	94 (1.0)	192 (1.5)	495 (3.6)	54 (1.2)	1092 (5.8)	1927 (13.1)	80.3 (0.5)
15	41 (3.5)	54.3 (1.8)	355 (2.6)	30.7 (0.7)	879 (6.9)	1360 (15.5)	56.7 (0.6)
20	24 (1.0)	26 (1.0)	245 (0.6)	23 (1.2)	459 (5.2)	777 (9.0)	32.4 (0.4)
25	19 (1.2)	18 (0.6)	164 (2.1)	15.7 (0.9)	289.7 (5.4)	506.4 (10.2)	21.1 (0.4)
28	14.7 (0.3)	14.3 (0.3)	145 (2.9)	15 (1.2)	271.7 (8.7)	460.7 (13.4)	19.2 (0.6)
30	14.3 (0.7)	13.6 (0.3)	133.3 (3.3)	13 (0.6)	227 (8.4)	401.2 (13.3)	18.6 (0.5)

[a] Immature phases did not end progress at the determined temperature.

Table 2
Thermal needs for five developing stages for *S. argyrostoma* computed from regression line model [30,31].

Developmental events	LDT or Dz °C (SE)	K, degree °C hour or ADH (SE)	K, Degree °C Day or ADD (SE)	R2
1st instar	5.4 (0.4)	360.96 (26.9)	15.04 (1.12)	0.967
2nd instar	8.5 (0.26)	302.9 (15.6)	12.62 (0.65)	0.998
3rd instar	5.3 (0.44)	3368.64 (104.4)	140.36 (4.35)	0.988
Wandering	3.8 (0.1)	350.16 (14.4)	14.59 (0.6)	0.937
Intra-puparial	6.6 (0.6)	5329.92 (100.392)	222.08 (4.183)	0.804
Overall		9712.58 (261.69)	404.69 (10.9)	

LDT or Dz: the lower development threshold (LDT) or development zero (Dz) temperature, at which the development of a species ceases.

As the density of females increases, it seems that breeding takes place in turn and females that have not had a chance to oviposit or to deposit will breeds in the following days. In this case, at high female density, breeding was observed every day and it was not clear how many times the individual females oviposited. However, when the female population was small ($n = 5$) and there was little or no competition for spawning, it seems that the females oviposited easily every 3–5 days. It seems most likely that each fly spawns 2 to 3 times during her life (19–22 days) in the laboratory under cage conditions (Fig. 5). The average (±SE) reproductive rate (growth) of flies was 0.66 ± 0.43, 0.71 ± 0.29, and 0.56 ± 0.34, respectively, for groups of 5, 10, and 15 females, giving a mean rate of 0.643 ± 0.076. In general, it was observed that almost half of spawning or larvae laying (a mean of 46.9%) occurred in the first week and gradually this rate decreases with increasing life-span. The mean number of eggs or larvae laid by a female *S. argyrostoma* fly during her lifetime was 24.65.

6. Male: female ratio and its association with population size

The findings of this experiment showed that the optimal male: female ratio of the progeny varies, depending on parent population size. Totally the sex ratio was in favor of males under laboratory conditions (Chi-Squire test, $p < 0.001$). Generally, this ratio was greater than 1 at lower population sizes (5 or 10 females in cage), whereas the ratio was in favor of females for the cages with 15 females. When there were 10 females in cage, the number of males was almost twice that of females. This rate (Mean ± SE) was 1.4 ± 0.25, 1.92 ± 0.39, and 0.94 ± 0.16 for cages with 5, 10, and 15 females respectively (Fig. 6). Chi-Square analysis revealed that significant ($P < 0.001$) bias toward male progeny in the cages with 5 or 10 females but in the cages with 15 females ($P = 0.2$). When male to female ratios was compared between the groups, the ANOVA and post hoc Tukey test revealed that these variations were significant only between the cages with 10 and 15 females ($P = 0.0356$).

7. Discussion and conclusion

This study has revealed fundamental biological data on *S. argyrostoma*, an ovoviviparous flesh fly of considerable forensic importance globally. We report the different development periods under various temperature regimes, and the lower developmental thresholds (LDT/Dz) for different immature stages of *S. argyrostoma*. To our understanding, only three reports have previously tried to verify the development periods and or general LDT for *S. argyrostoma* [31,33,34].

We found that the mean lifespan of a generation, from first instar larvae to adult emergence, was 21.1 and 18.6 days at 25 and 30 °C, consistent with Grassberger and Reiter (2002) who calculated it to be 22 and 16 days at 25 and 30 °C, respectively [31]. In our current study, the LDT values we have determined are consistent with those reported by Grassberger and Reiter [31]. Although first instar larvae could survive at 8 °C, larval progress was not finalized at 8 °C. Sert (2021) investigated the effect of changing (mean 25.2 °C) and constant (25 °C) temperatures on the timing of *S. argyrostoma* development [35], and reported a development period requirement for adult emergence of 289.5 h (±1.52) at a constant 25 °C, and 319 h (±1.41) at fluctuating temperatures. Previously, Sert et al. (2020) had shown the development times for adult emergence to be 459 h (±1.41), 289.5 h (±1.52), and 227 h (±1.89) at 20, 25, and 30 °C, respectively [34]. Here, we show that *S. argyrostoma* can fulfill its lifespan at fixed temperatures between 10 °C and

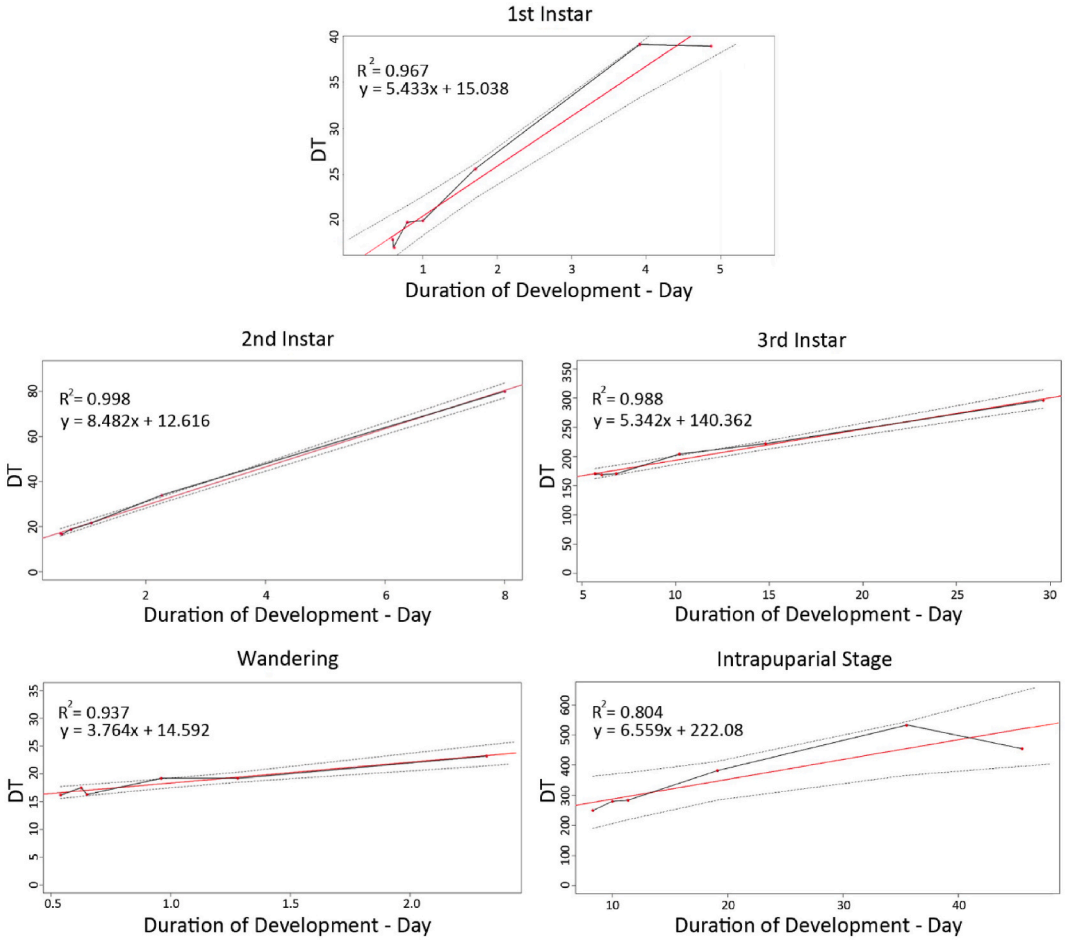

Fig. 2. Regression lines with 95% confidence intervals (spotted lines) produced by reduced major axis (RMA) which was applied to estimate the minimum development threshold (Dz) and k values for the five biological events of *S. argyrostoma* at six/seven constant temperatures.

30 °C, with a complete growth period of 80.3 days at 10 °C, 21.1 days at 25 °C, and 18.6 days at 30 °C. These findings are similar to those reported by Grassberger and Reiter (2002) [31], but lengthier than those found by Sert et al. [34,35], who conveyed 12.05 and 13.3 days at fixed (25 °C) and fluctuating temperatures (mean 25.2 °C), respectively. These differences may be owing to the distinct food intake, genetic backgrounds, and procedures employed, or the environmental circumstances in which these trials were supervised.

Grassberger and Reiter (2002) also determined that the LDT for entire juvenile growth was 7.4 °C, and the complete thermal constant (K) for *S. argyrostoma* was 396.4 ± 19.18 (Mean ± SE) day degrees (DD) above the LDT [31]. Here, for the first time, we have determined the LDT for all immature developmental stages separately: apart from the second ecdysis stage, the LDT values we determined for the immature stages were lower than the previously estimated value [31]. However, the thermal constant (K) for *S. argyrostoma* reported here was very close to those reported by other researchers [18,31].

Knowledge of the parameters influencing the oviposition or ovoviviposition behavior of *S. argyrostoma* is important for the utilizing of this fly in forensic studies. In this study we observed the females prefer to oviposit (lay eggs) if a source of food is available but will change to ovoviviposition after one to two days' food deprivation. Thus, reproduction is possible, even when, under otherwise optimal environmental conditions, there is no cadaver or oviposition substrate available. However, many researchers have suggested that *S. argyrostoma* females prefer ovoviviposition [18,38–40]. This could relate to egg-shell structure: when the egg-shell is delicate, reflecting low food availability, and not suitable for embryo protection, oviposition would be ineffective; whereas ovoviviposition, in which the embryos grow to the 1st instar larva inside the female's reproductive region, and hatch just prior being placed or nearly instantly later would be an additional effective reproductive strategy.

The results of this study are important for approximating the PMImin in forensic cases, indicating that forensic entomologists should consider the time required for *S. argyrostoma* hatching, if the species lays eggs on the cadaver. It is generally believed that *S. argyrostoma* directly deposits live larvae on corpses; consequently, the time for egg hatching has not been considered in calculations of PMImin. In this study, we found a few hours' lapse between egg laying and egg hatching, thus the omission of the egg hatching period in legal cases could result in noticeably wrong estimates (from a few hours to more than a day, depending on the ambient

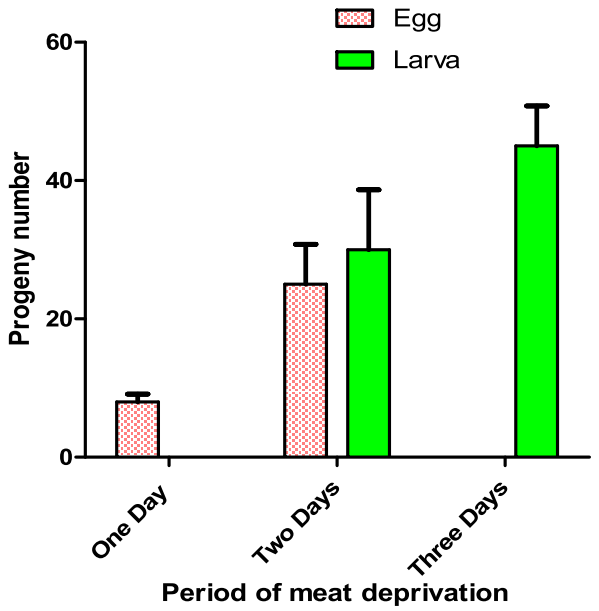

Fig. 3. Effect of oviposition source availability on ovoviviparity and oviparity of *S. argyrostoma*. There is a significant relationship between the type of progeny and the period of meat deprivation (ANOVA test, P = 0.0001). Bars show Mean ± SE.

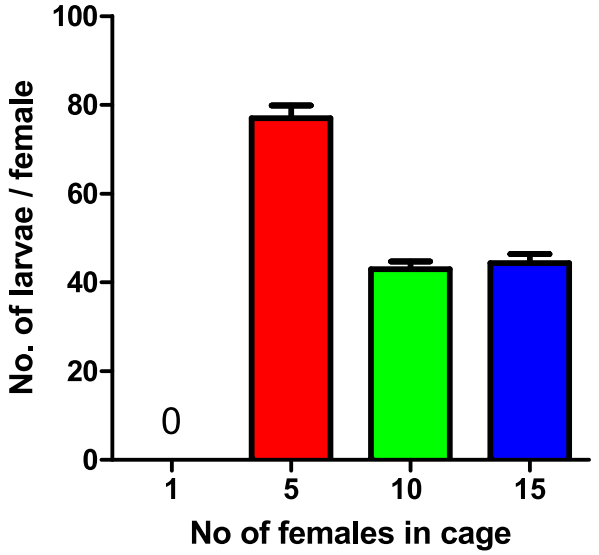

Fig. 4. Mean ± SE progeny number of *S. argyrostoma* flies at four population sizes in cage conditions tested by the ANOVA and post hoc Tukey test which exhibited a statistically substantial variation (P < 0.0001) between all the sets apart from the groups containing 10 and 15 females (p = 0.6786).

temperature) of the PMImin in case of using *S. argyrostoma*. Similarly, it has been suggested that the species should be identified, and the viability of eggs found on a carrion should be confirmed, as some ovoviviparous fly species lay faint and bare eggs before regular larviposition happens [41,42]. Shifting between oviparity and ovoviviparity has been observed in *Calliphora* spp [41,43]. For example, the ovoviviparous blowfly of *Calliphora dubia* Macquart (Diptera: Calliphoridae), sometimes lays eggs [42]: 70% *C. dubia* females exclusively laid live larvae, but 30% laid at least some eggs, although none were viable. Here, we have observed that *S. argyrostoma* eggs are viable. However, most authors have recorded *S. argyrostoma* as being solely ovoviviparous [44]. We suggest that *S. argyrostoma*'s ovoviviparous status in other parts of the world should be more thoroughly inspected.

In this study, females laid a mean of 25 larvae over their life-span in laboratory cage conditions, which closely accords with the lower range of other ovoviviparous blowflies such as *C. dubia* [42], *C. varifrons* and *C. maritima* [45], which have been stated to lay 22–83, 23–80, and 33–78 live maggots, correspondingly. We also found that the *S. argyrostoma* often larviposited in 2–3 discrete bouts

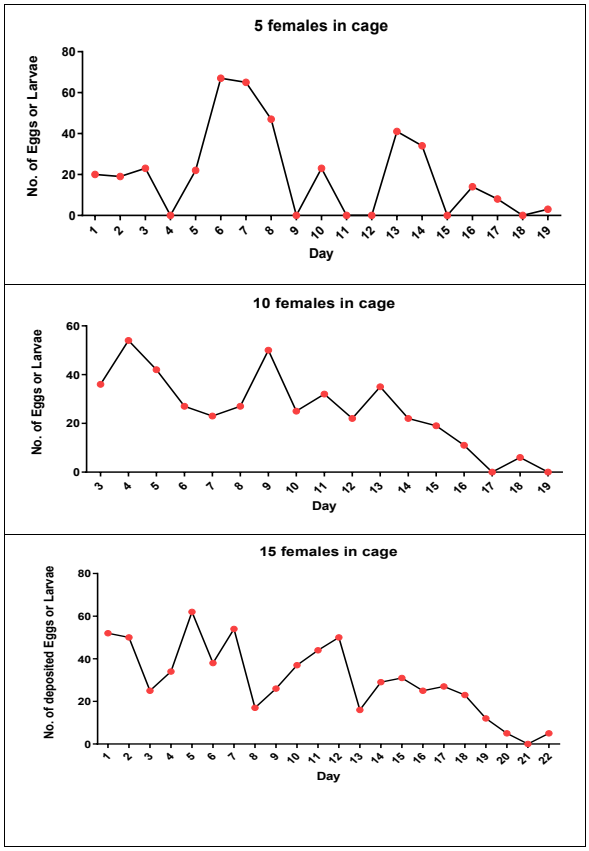

Fig. 5. Trends of progeny production when different numbers of female *S. argyrostoma* flies were present in the cage.

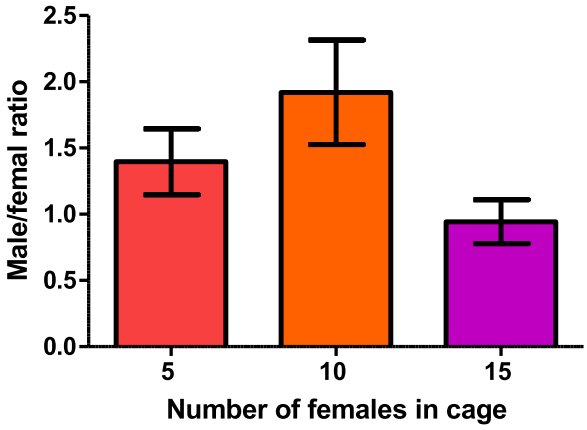

Fig. 6. Progeny sex ratio (males to females) of *S. argyrostoma* in the cages with different parent population sizes. Bars show Mean ± SE. Chi-Square analysis revealed that significant ($P < 0.001$) bias toward male progeny in the cages with 5 or 10 females but in the cages with 15 females ($P = 0.2$). Comparison between the groups revealed significant variation only between the cages with 10 and 15 females (ANOVA and post hoc Tukey test, $P = 0.0356$).

and laid an average of 46.9% live larvae in the first bout; it is probable that larviposition over two to three different times, and hence at 2–3 locations, would enhance their likelihood of strength and/or decrease possible predation [42].

Parameters affecting the egg-laying behavior of necrophagous flies include abiotic and biotic factors (RH, temperature, season, or precipitation); physical barriers; the habitat (indoor or outdoor, the type of oviposition substrate, the preceding attendance of prey and predator maggots on the carcass, the existence of pheromones, and inter- and intra-specific competition): all may influence the ovipositing activity of *S. argyrostoma* flies [46–54].

It has been suggested that rotting phase and earlier larval colonization are influencing species colonization models on cadavers, and the incidence of flesh fly oviposition [55]. Our results show that fresh substrate is colonized extensively by *S. argyrostoma*, whereas no colonization occurs on the decayed substrate, which agrees with previously reported results for forensically important flies [50]. We also cannot rule out the fact that the water loss, in combination of rotten substrate, may contribute to the fly's failure to give birth. Moreover, Wessels et al. (2011) found that the availability of protein-rich food sources had no effect on reproductive allocation (number of eggs or egg growth rate) in the *S. crassipalpis* flesh fly [56].

In this study we observed that, as the density of females raises, the sum of offspring reduces. It seems that, at high female density, some females have no chance to oviposit or deposit reducing the mean number of offspring per female. However, when the female density is low (n = 5), there is little or no competition for spawning. This phenomenon was reported in *Bactrocera tau*, with the survival ratios at high densities being substantially lower than that at low densities [57]. The availability of fresh medium also appears to affect the chronological production and fundamental number of offspring in *Drosophila melanogaster* [58]. The authors suggested that reduction in offspring was linked to both female adjustment of gamete release and to declined larval persistence under crowded settings. Further studies should examine larval survival rate in uncrowded and crowded conditions, as well as the reproductive conflicts between females and offspring, as factors that may influence the number of offspring in *S. argyrostoma*.

In our study the sex ratio seemed to be in favor of males under laboratory conditions, with the male to female ratio being affected by population size: groups of 20 and 30 flies having the highest and lowest male: female ratios. It is noteworthy this rate might be quite different in natural populations attracted to carrion or baits.

8. Conclusion

The data obtained in this research concerning the thermal requirements of *S. argyrostoma,* and other biological characteristics, are valuable for forensic investigators to estimate PMImin. The information that *S. argyrostoma* can lay either live larvae or eggs could also have important implications for the exact determination of the PMImin, as the existence of maggots resulting from oviposition on the carcass could increase significant time to the PMImin.

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Declarations

This study followed the guidelines of the institutional ethical committee (Tehran University of Medical Sciences, TUMS). The protocols were approved by TUMS ethical committee under registry IR. TUMS.SPH.REC.1399.256.

Data availability

The sequence data obtained in this study are deposited in GenBank database under accession number MG913301. All other data of this study are included in the article.

CRediT authorship contribution statement

Saba Hediyeloo: Writing – original draft, Visualization, Methodology, Investigation. **Kamran Akbarzadeh:** Supervision, Resources, Formal analysis, Conceptualization. **Majid Rezaei:** Software, Methodology, Formal analysis, Data curation. **Mohammad Ali Oshaghi:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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