Features of the exogenic development of *Passalurus ambiguus* (Nematoda, Oxyuroidea) at different temperature regimes

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**Abstract**

Passalurus is a prevalent disease among helminthiasis of domestic rabbits. This invasion is caused by the nematode *Passalurus ambiguus* (Nematoda, Oxyuroidea), which is cosmopolitan and localized in the cecum and colon of rabbits. Passalurus is highly contagious and capable of unlimited spread, due to the biological characteristics of its pathogen, such as the conditions that ensure maximum preservation of parasites at exogenous stages of their development. Experimental research in the laboratory established the timing of development of *P. ambiguus* eggs isolated from the gonads of female helminths, depending on temperature regimes and features of their growth and development. According to the morphological features of *Passalurus* eggs, four stages were distinguished in their exogenous development: zygote, cleavage and formation of blastomeres, formation of larva and motile larva. Depending on the cultivation temperature, the duration of embryogenesis ranged from 4 to 9 days, and egg viability ranged from 59.3% to 72.7%. The most favourable temperature regime for the development of *P. ambiguus* eggs was the temperature of 35 °C, at which 72.7% of eggs with motile larva were formed within 4 days. At this temperature, the zygote stage lasted for 1 day of cultivation, the stage of cleaving and formation of blastomeres occurred on days 1–2, the stage of larval formation on days 1–3, and the stage of formation of motile larva on days 3–4. At lower temperatures, the term of development of *Passalurus* eggs increased, and the number of viable eggs decreased. At temperatures of 30 °C and 25 °C, the development of *Passalurus* eggs took place during 5 and 7 days, respectively, and the viability was 66.7% and 62.7%, respectively. At these temperatures, the zygote stage lasted 1–2 days, and 1–3 days, the stage of cleaving and formation of blastomeres occurred on days 1–3 and 1–4, the stage of larval formation lasted from days 2–4 and 2–6, and the stage of formation of motile larva took place on days 3–5 and 4–7, respectively. The least favourable temperature for the development of *P. ambiguus* eggs was the temperature of 20 °C, at which the formation of motile larva occurred in 9 days, and their viability was only 59.3%. At this temperature, the zygote stage lasted 1–4 days, the stage of cleaving and blastomere formation occurred on days 2–6, and the larval formation stage on days 3–8, and the motile larval stage happened on days 5–9. The growth and development of *P. ambiguus* eggs was accompanied by significant changes in morphometric parameters, such as the increase in egg width and thinning of egg shell at the egg shell plug. The obtained data will allow preventive measures to be effectively implemented on rabbit farms that are susceptible to pinworms, taking into account the terms of exogenous development of pathogens in different seasons.

**Keywords**: passalurosis; rabbits; helminth eggs; abiotic factor; biological features; metric parameters.

**Introduction**

Passalurus is a widespread pinworm infestation of rabbits in many countries. It is recorded in Ukraine, the Azores, Belarua, Great Britain, Bulgaria, the Canary Islands, European Turkey, France, Hungary, Ireland, the Italian mainland, Madeira Islands, Poland, the Portuguese mainland, the Spanish mainland (De Jong et al., 2014; Myklutiutenko et al., 2019; Khorolskyi et al., 2021). This spread of the infection is due to the peculiarities of the development cycle of parasites. Their transition to a parasitic lifestyle was accompanied by the emergence of a number of biological adaptations that facilitate the helminths’ existence, development and reproduction in various habitats. In particular, such adaptations include high reproductive activity, as well as the ability of helminth eggs to withstand adverse environmental conditions (McSorley, 2003; Blaxter, 2003; Blaxter & Koutsovoulos, 2015).

The formation and functioning of parasitic systems are in the process of constant transformation. At the same time, in natural and anthropogenically modified ecosystems, the patterns of processes in the “parasite–host” system differ significantly. In local, historically formed biogeocenoses, parasites are associated with their hosts by evolutionarily established parasite–host relationships, where the parasite and the host act as elements of a stable and stable structure. Parasites, as well as free-living organisms, are integral components of any ecosystem, any biosystem. The consequences of interference of powerful anthropogenic factors in the functioning of parasitic systems are the activation of local foci of natural invasions, increasing pathogenicity of parasites and activations of their adaptive properties to environmental conditions at all stages of development. This determines the adaptation of the life cycle of parasites to new, changed living conditions (Lafferty, 1997; Perry, 2011; Benesh et al., 2021).

The life cycles of parasites differ from those of free-living organisms. Complication or simplification of the development cycle is also a form of parasite adaptation to habitat to sustain the parasite as a species. Thus, the life cycle of a species consists of the changing life forms that perform three main functions: reproduction, settlement, and self-preservation. Also, many known cycles of parasite development can transform depending on living conditions and the manner of adaptation. Special and very diverse ecological and physiological adaptations, often amounting to significant changes in life cycles, occur in parasites that have mastered agrocenoses with domestic and synanthropic animals (Germill et al., 1999; Auld & Tinsley, 2015; Benesh et al., 2017; Blascoe-Costa & Poulin, 2017).

The development of many parasites, including helminths, characteristically occurs in stages. During life, helminths go through a number of
successive stages: egg, larva, adult. The helminth enters the body of the host at one stage of development, and leaves at another. The ultimate goal of any living being is to preserve itself as a member of a species. The viability of parasites at the exogenous stages of their development also determines their ability to survive as species (Danheim & Ackert, 1929; Anderson, 2008; Gibson et al., 2014). Therefore, despite helminths belonging to the same genus or family sharing a certain genetic relationship, their developmental cycles, especially exogenous stages in the environment differ significantly. This determines their prevalence (Beveridge et al., 1989; Yevstafieva et al., 2016, 2019).

The nematode *P. ambiguus* Rudolphi, 1819 has direct development typical of oxyurids. In particular, mature females under the action of peristalsis of the rabbit intestine migrate into the host’s rectum and, independently or passively with fecal masses, out of the anus. In the external environment, the muscles of the pinworm’s uterus contract in the area around the rabbit’s anus. The uterus turns out, and the eggs are released on the skin of the host. The female sticks these eggs to the hair and skin of the host. After the eggs hatch, the female’s body shrinks, and the parasite soon dies (Taffs, 1976; Manning et al., 1994; Fayek et al., 1995). As of now, the scientific data on the peculiarities of the exogenous development of *Passalurus* parasites of rabbits are extremely scarce. It is reported that under favourable conditions (temperature, humidity and free access of oxygen), invasive larvae develop in the eggs of *P. ambiguus* in 7–8 days. Moreover, some authors note that the optimal temperature for the development of invasive larvae in *P. ambiguus* eggs is 35–36 °C. In the temperature range of 25–40 °C, the development of *Passalurus* eggs can also occur, but at different times. The temperatures up to 15–17 °C are critical temperatures at which the eggs die (Skriabin et al., 1976). Other researchers point out that the temperature range of 35–38 °C is the most optimal for the development of *P. ambiguus* eggs, and the critical temperatures are more than 40 °C and less than 20 °C (Grozdev et al., 1970).

Therefore, an important condition for effective prevention of passalurosis in rabbit farms is the knowledge of the specifics of exogenous development of *Passalurus* eggs, especially given the climate change and ongoing anthropogenic impact on biocenoses.

The aim of this work was to establish the features of exogenous development of eggs of *P. ambiguus* nematodes that parasitize domestic rabbits, depending on the temperature regime.

**Materials and methods**

Experimental studies were conducted in the Laboratory of the Department of Parasitology and Veterinary Medicine of Poltava State Agrarian University (Ukraine) in 2021. The pinworms were collected by complete helminthological dissection of the large intestine of slaughtered rabbits (Skriabin, 1928). The species of *Passalurus* was determined according to identification keys (Skriabin et al., 1967; Grozdev et al., 1970). In laboratory conditions, the eggs from *P. ambiguus* females were washed in Petri dishes (at least 50 specimens) with Thioglycollate medium and cultured according to the method (Yevstafieva et al., 2021).

Each individual egg culture was divided in four subcultures that were cultured at 20, 25, 30 and 35 °C until a motile larva appeared in the egg. Every 24 hours, the degree of development, morphological changes and structure of eggs were determined in experimental egg cultures, and the presence of eggs that stopped developing was noted. The number of eggs at each stage of development and egg mortality were counted. Experimental cultivation of *Passalurus* eggs was performed in triplicate.

The morphometric parameters of the eggs of *P. ambiguus* (length, width, thickness of egg shell in different areas) were studied in the process of their growth and development using Image for Windows® software (version 2.00) in interactive mode with ×10, ×40 eyepiece and ×10 photo eyepiece. Microphotography was performed using digital camera to the microscope Sigeta M3CMOS 14000 14.0 MP (China).

Statistical processing of the experimental results was carried out using Statistica 10 (StatSoft Inc., USA) software. Standard deviation (SD) and average values (x) were calculated. Significance of difference between average values in the studied cultures of *Passalurus* eggs was established using one-way analysis of variance and F-test for 95% confidence level.

**Results**

Experimental studies showed that *P. ambiguus* eggs obtained from the gonads of female helminths can be divided by their morphological features into four stages of the exogenous development: zygote, cleavage and formation of blastomeres, formation of larvae, and formation of motile larva. In particular, all eggs of *Passalurus* obtained from the gonads of female nematodes were at the stage of the zygote, which was morphologically characterized by the presence of an embryo in the form of an amorphous mass that filled almost the entire egg (Fig. 1a). Eggs at the next stage were characterized by gradual cleavage of the embryo into blastomeres. First, two large blastomeres, unequal in size, were formed, then their number increased and their sizes decreased (Fig. 1b). The stage of larval formation in the egg was morphologically characterized by the presence of a cylindrical immobile larva, located along the egg, but not reaching the poles of the egg. The posterior end of the larva was slightly narrowed. The internal structure of the larva was not visible (Fig. 1c).

In contrast, eggs at the stage of motile larva were morphologically characterized by the fact that the actively moving larva with a visible intestine reached the edges of the egg and was more formed (Fig. 1d).

With increasing temperature from 20 to 35 °C, the time of exogenous development gradually reduced, and the number of viable eggs with motile larvae gradually increased. In particular, 59.3% of viable eggs at the stage of motile larva formed at a temperature of 20 °C for 9 days (Table 1).

The zygote stage lasted for days 1–4 of cultivation, the number of eggs at this stage gradually decreased from 100.0% to 13.3% and no eggs were detected at this stage of development at the fifth day. The stage of cleavage and formation of blastomeres lasted from days 2–6 of cultivation, the maximum proportion of such eggs (39.3–36.0%) was detected on the third and fourth days, and the minimum on the sixth day (6.0%). The larval stage lasted from day 3 to day 8. Moreover, the maximum share of eggs at this stage was observed on the fifth day (36.7%), after which it decreased to the eighth day (from 33.3% to 10.0%) and on the ninth day such eggs were no longer detected. The motile larva was found in eggs during day 5 to day 9 of cultivation, and their share gradually increased from 8.0% to 59.3%. Egg death was recorded from day 1 (22.0%), the proportion of such eggs gradually increased to day 6 (40.7%) and remained at this level until the end of cultivation.

At a temperature of 25 °C, the development of *Passalurus* eggs occurred in 7 days, and 62.7% of viable eggs were formed at the stage of motile larva (Table 2). Thus, the zygote stage lasted for days 1–3 of cultivation, the number of eggs gradually decreased from 100.0% to 5.3%, no eggs were found at this stage of development at the fourth day. The stage of cleavage and formation of blastomeres lasted for days 1–4 of cultivation. The maximum number of eggs at this stage of development (42.0–45.3%) was found on days 2 and 3, and the minimum on day 4 of cultivation (15.3%). The stage of larval formation occurred on days 2–6. The maximum number of eggs at this stage was noted on day 4 (44.0%), after which their number decreased to day 6 (from 24.7% to 8.7%) and no such eggs were detected on day 7. Motile larvae were found in eggs during days 4–7 of cultivation. The number of such eggs gradually increased from 10.0% to 62.7%. Egg death was observed from day 1 (18.7%), the number of such eggs gradually increased to day 5 of cultivation (37.3%) and remained at this level until the end of cultivation.

At 30 °C, *P. ambiguus* eggs developed within 5 days, and the percentage of viable eggs formed during cultivation was 66.7% (Table 3).

The zygote stage lasted for days 1–2 of cultivation, the number of eggs at this stage gradually decreased from 100.0% to 16.7% and no eggs were found at this stage of development on day 3. The stage of cleavage and formation of blastomeres lasted for days 1–3 of cultivation, the maximum number of such eggs (45.3–40.0%) was detected on days 1 and 2, and the minimum on day 3 (13.3%). The stage of larval formation occurred from days 2 to 4. The maximum number of eggs at this stage was recorded on day 3 (46.7%), after which their number decreased to day 4 (11.3%) and by day 5 such eggs were no longer found. Motile larvae were found in eggs during days 3–5 of cultivation. The number of such eggs gradually increased from 12.7% to 66.7%. Egg death was recorded from day 1 (16.0%), and the number of such eggs gradually increased to day 4 (33.3%) and remained at this level until the end of cultivation.

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Fig. 1. Morphological characteristics of the development of *Passalurus ambiguus* eggs, obtained from the gonads of female helminths and maintained in experimental culture: *а* – zygote; *b* – cleavage and formation of blastomeres; *с* – formation of immotile larva; *d* – formation of motile larva

Table 1
Parameters of the growth and development of eggs of *Passalurus ambiguus* in laboratory culture at the temperature of 20 °C (n = 3, x ± SD)

| Day of cultivation | zygote  | cleavage and formation of blastomeres | formation of larva | formation of motile larva | Egg death, number of eggs |
|-------------------|---------|--------------------------------------|-------------------|--------------------------|--------------------------|
| before culture    | 50.0    | –                                    | –                 | –                        | 11.0 ± 1.0               |
| 1                 | 34.0 ± 1.7 | 5.0 ± 1.0                        | –                 | –                        | 12.7 ± 2.1               |
| 2                 | 23.0 ± 1.0 | 14.3 ± 2.5                       | –                 | –                        | 14.3 ± 2.5               |
| 3                 | 12.3 ± 2.5 | 19.7 ± 3.2                       | 3.7 ± 1.5         | –                        | 15.0 ± 3.0               |
| 4                 | 6.7 ± 1.5  | 18.0 ± 2.7                       | 10.3 ± 0.6        | –                        | 17.3 ± 2.5               |
| 5                 | –        | 10.3 ± 2.5                       | 18.3 ± 0.6        | 4.0 ± 2.0                | 20.3 ± 1.5               |
| 6                 | –        | 3.0 ± 1.0                        | 16.7 ± 3.1        | 10.0 ± 2.0               | 20.3 ± 1.5               |
| 7                 | –        | –                                 | 12.7 ± 5.1        | 17.0 ± 3.6               | 20.3 ± 1.5               |
| 8                 | –        | –                                 | 5.0 ± 1.0         | 24.7 ± 2.1               | 20.3 ± 1.5               |
| 9                 | –        | –                                 | –                 | 29.7 ± 1.5               | 20.3 ± 1.5               |

Note: “–” – no eggs at the stage of development.

Table 2
Parameters of the growth and development of eggs of *Passalurus ambiguus* in laboratory culture at the temperature of 25 °C (n = 3, x ± SD)

| Day of cultivation | zygote  | cleavage and formation of blastomeres | formation of larva | formation of motile larva | Egg death, number of eggs |
|-------------------|---------|--------------------------------------|-------------------|--------------------------|--------------------------|
| before culture    | 50.0    | –                                    | –                 | –                        | 9.3 ± 0.6                |
| 1                 | 22.0 ± 2.0 | 18.7 ± 1.5                       | –                 | –                        | 13.0 ± 1.0               |
| 2                 | 10.7 ± 3.5 | 21.0 ± 1.7                       | 5.3 ± 1.5         | –                        | 14.7 ± 2.1               |
| 3                 | 2.7 ± 1.2  | 22.7 ± 1.5                       | 10.0 ± 1.7        | –                        | 15.3 ± 1.5               |
| 4                 | –        | 7.7 ± 1.5                        | 22.0 ± 1.0        | 5.0 ± 4.0                | 18.7 ± 2.1               |
| 5                 | –        | –                                 | 12.3 ± 1.5        | 19.0 ± 2.7               | 18.7 ± 2.1               |
| 6                 | –        | –                                 | 4.3 ± 1.5         | 27.0 ± 18.7              | 18.7 ± 2.1               |
| 7                 | –        | –                                 | –                 | 31.3 ± 2.1               | 18.7 ± 2.1               |

Note: see Table 1.

The temperature of 35 °C turned out to be optimal for the development of *P. ambiguus* eggs. At this temperature, the formation of motile larvae in the egg occurred in 4 days and the percentage of viable eggs was 72.7% (Table 4).

Thus, the zygote stage lasted for 1 day of cultivation, the number of eggs at this stage changed from 100.0% to 23.3%. At day 2, eggs at the zygote stage were not found. The stage of cleaving and formation of blastomeres lasted for days 1–2 of cultivation. The maximum number of eggs
at this stage of development (59.3%) was also found on day 1 of cultivation, and the minimum on day 2 (12.0%). The stage of larval formation occurred in 1–3 days. The maximum number of eggs with immotile larva was seen on day 2 (65.3%), after which it decreased to day 3 (16.0%) and no such eggs were detected on day 4. The motile larvae were found in eggs during days 3–4 of cultivation, and their number gradually increased from 56.7% to 72.7%. Egg death was noted from day 1 (9.3%), and the number of such eggs gradually increased to days 3–4 of cultivation (to 27.3%).

The metric parameters of *P. ambiguus* eggs significantly changed during embryogenesis. There was an increase by 3.8% (Fig. 2a) in the width of eggs with motile larvae (45.42 ± 1.06 μm, *P* < 0.01), and the egg shell thinned by 19.5% (Fig. 2b) in the area of the plug (2.15 ± 0.15 μm, *P* < 0.001) compared with similar parameters of eggs at the zygote stage (43.70 ± 1.68 and 2.67 ± 0.33 μm, respectively). Other indicators did not change significantly. In particular, during cultivation from the zygote stage to the motile larva, the egg length ranged from 107.46 ± 4.23 to 106.72 ± 3.88 μm (Fig. 2c) The length of the egg plug ranged from 8.51 ± 0.78 to 8.31 ± 0.76 μm (Fig. 2d), the egg plug width changed from 3.04 ± 0.28 to 2.84 ± 0.48 μm (Fig. 2e), the thickness of the shell in its middle part ranged from 1.06 ± 0.17 to 1.01 ± 0.17 μm (Fig. 2f), the thickness of the shell in the region of the extended end ranged from 2.52 ± 0.25 to 2.39 ± 0.22 μm (Fig. 2g).

**Table 3**

Parameters of the growth and development of eggs of *Passalurus ambiguus* in laboratory culture at the temperature of 30 °C (*n* = 3, *x ± SD)

| Day of cultivation | Stage of development, number of eggs | Egg death, number of eggs |
|-------------------|-------------------------------------|--------------------------|
|                   | zygote                              | cleavage and formation of blastomeres | formation of larva | formation of motile larva | |
| before culture    | 50.0                                | –                        | –                | –                       | |
| 1                 | 17.0 ± 1.0                          | 22.7 ± 1.2              | –                | –                       | 8.0 ± 1.0 |
| 2                 | 8.3 ± 3.1                           | 20.0 ± 2.0              | 11.3 ± 1.5       | –                       | 10.3 ± 1.5 |
| 3                 | –                                   | 6.7 ± 3.1               | 23.3 ± 3.1       | 6.3 ± 1.5               | 13.7 ± 1.5 |
| 4                 | –                                   | –                       | 5.7 ± 2.5        | 27.7 ± 3.8              | 16.7 ± 2.1 |
| 5                 | –                                   | –                       | –                | 33.3 ± 2.1              | 16.7 ± 2.1 |

Note: see Table 1.

**Table 4**

Parameters of the growth and development of eggs of *Passalurus ambiguus* in laboratory culture at the temperature of 35 °C (*n* = 3, *x ± SD)

| Day of cultivation | Stage of development, number of eggs | Egg death, number of eggs |
|-------------------|-------------------------------------|--------------------------|
|                   | zygote                              | cleavage and formation of blastomeres | formation of larva | formation of motile larva | |
| before culture    | 50.0                                | –                        | –                | –                       | |
| 1                 | 11.6 ± 2.5                          | 29.7 ± 1.2              | 40.0 ± 2.0       | –                       | 4.7 ± 1.5 |
| 2                 | –                                   | 6.0 ± 2.0               | 32.7 ± 2.1       | –                       | 11.3 ± 0.3 |
| 3                 | –                                   | –                       | 8.0 ± 1.0        | 28.3 ± 3.2              | 13.7 ± 2.5 |
| 4                 | –                                   | –                       | –                | 36.3 ± 2.5              | 13.7 ± 2.5 |

Note: see Table 1.

**Fig. 2.** Morphometric parameters of *Passalurus ambiguus* eggs in the process of exogenous development at a temperature of 35 °C: a – egg width, b – thickness of the shell in the egg plug area, c – egg length, d – length of egg plug, e – width of egg plug, f – thickness of egg shell in its middle part, g – thickness of egg shell in the area of the extended end (μm); A – zygote stage, B – stage of motile larva; ** – *P* < 0.01, *** – *P* < 0.001 – relative to the eggs in the zygote stage; the small square in the centre corresponds to the median, the lower and upper borders of the large rectangular correspond to the first and the third quartiles, respectively, vertical line segments, directed up and down from the rectangular, correspond to minimum and maximum values (n = 10).
Thus, the growth and development of *P. ambiguus* eggs were characterized by the dependence of their maturation and viability on the abiotic factor such as temperature of the external environment, and were accompanied by morphological and metric changes.

**Discussion**

Passalurosis of rabbits is highly prevalent in most countries of the world. The prevalence rates can reach more than 50% (Boog & Iason, 1986; Frank et al., 2013; Hajipour & Zavarshani, 2020). The authors note that this prevalence is due to many factors, such as insanitary conditions for keeping rabbits on constant bedding or dense floors, coprophagy in hosts, and the direct development of the parasite (Fayek et al., 1995; Fondona et al., 2003; Rinaldi et al., 2007; Komasa et al., 2015). Therefore, it is important to study the features of exogenous development of the pathogen of rabbits, *Passalurus ambiguus* Rudolphi, 1819, the effect of temperature on the timing and viability of its eggs due to lack of information on this issue.

We identified four stages of exogenous development of *Passalurus* eggs, which are characterized by morphological differences: zygote, cleavage and formation of blastomeres, formation of larvae and formation of motile larvae. At the same time, there are scientific reports which also distinguish four stages of *Passalurus* development, namely: gastrula, cylindrical embryo formation (stage 1 larva), spindle-shaped embryo formation (stage 2 larva) and invasive stage formation (stage 3 larva). These larvae also differ in morphological structure, especially in the structure of larval stages in formation of intestines, bulbus, pharynx, anus, bend of the tail end and molting (Skjabin et al., 1967). This difference in stages can be explained by the fact that the authors studied the development of *Passalurus* eggs obtained from feces or animal skin. Such eggs are already at the gastrula stage, and the zygote stage and its cleavage had already happened. Also, it is very difficult to determine the stage of development of eggs without the experimental destruction of the egg shell and the study of the structure of the larvae. Therefore, we identified the motile and immobile larvae that correspond to non-invasive (stages 1 and 2) and invasive (stage 3) stages of parasite development.

We found that with increasing temperature from 20 to 35 °C, the time of exogenous development is gradually reduced, and the number of viable eggs with motile larva gradually increases. In particular, 59.3% of viable eggs at the stage of motile larva were formed at a temperature of 20 °C in 9 days; the development occurred in 7 days at a temperature of 25 °C and 62.7% of viable eggs were formed; the development happened in 5 days and 66.7% of viable eggs formed at a temperature of 30 °C. Lastly, the development occurred in 4 days and 72.7% of viable eggs formed at a temperature of 35 °C. Thus, according to research, the optimal temperature of 35 °C is optimal for exogenous development of *Passalurus*: the embryogenesis with the formation of 72.7% of viable eggs with motile larva occurs in 4 days in laboratory conditions. With decreasing temperatures to 30, 25 and 20 °C, the exogenous development of *P. ambiguus* is extended to 5, 7 and 9 days, respectively, and the percentage of viable egg formation decreases to 66.7%, 62.7% and 59.3%. Four stages of development of *Passalurus*, namely the zygote, the cleavage and formation of blastomeres, and the formation of larvae and motile larvae are distinguished by significant morphological differences. The duration of each stage depends on the temperature at which *P. ambiguus* eggs are cultured: the stage of zygote occurs from 1 to 4 days, the cleavage and formation of blastomeres happens from 1 to 6 days, the larval formation from 1 to 8 days, and the formation of motile larva from 3 to 9 days. The growth and development of *Passalurus* eggs from the zygote stage to the stage of mobile larva formation in the egg is accompanied by metric changes, characterized by an increase in egg width by 3.8% and thinning of the shell of egg plug by 19.9%.

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