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# Effect of temperature on gametocyst development and dehiscence of *Stylocephalus hoffmannseggi* devdhar and amoji 1977 (Apicomplexa: Stylocephalidae), parasitizing *Gonocephalum hoffmannseggi*

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## Abstract
Temperature is a pivotal environmental factor influencing the physiology and behaviour of organisms, including parasites. The life cycle of many parasitic species involves susceptible external stages subject to environmental conditions. In the case of septate gregarines, which live in the digestive tracts of arthropods, the gametocyst, which is responsible for parasite transmission, is part of the external stage. Mature gametocysts release infective oocysts, surviving in the environment until ingestion by a suitable host.
This study examines the impact of temperature on gametocyst development and dehiscence in *Stylocephalus hoffmannseggi* Devdhar and Amoji 1977 [4]. Gametocysts were collected from beetle faeces, subjected to temperatures between 10°C and 55°C, and monitored for development and dehiscence. Results show an optimal range of 20°C to 40°C for gametocyst dehiscence. Extremely low (≤15°C) and high (≥40°C) temperatures inhibit gametocyst development and dehiscence, suggesting a critical threshold. Infections in *S. hoffmannseggi* exhibit seasonal variations, with increased prevalence during the winter likely linked to temperature fluctuations.
The intricate relationship between temperature, gametocyst development, and infection dynamics highlights the interplay of environmental factors in host-parasite interactions. This research advances our understanding of parasitic ecology and sheds light on the delicate balance required for successful parasite maturation and transmission.

**Keywords:** Apicomplexa, septate gregarines, *Stylocephalus hoffmannseggi,* gametocysts

## Introduction
Temperature is an important environmental component that has a significant impact on both the physiology and behaviour of organisms, including parasites. In the life cycle of many parasitic species, there are frequently external stages that are susceptible to the conditions of their surrounding environment (Anderson and May, 1979) [1]. This stage of the parasite's life cycle normally takes place outside of the host organism, which enables the parasite to disseminate and come into contact with new prospective hosts.
Septate gregarines are parasitic protozoans that belong to the phylum Apicomplexa, generally inhabiting the digestive tracts of their invertebrate hosts (Bhoopathy, 1996) [2]. In the case of septate gregarines, the external stage often involves the formation of structures known as gametocysts. These gametocysts, which are produced within the digestive tract of the host and are eventually expelled into the external environment through the host's faeces, are responsible for the transmission of the parasite.
As the gametocysts mature, they eventually undergo dehiscence to release infective oocysts. Oocysts are able to survive in the environment, where they could be subjected to a variety of environmental factors, such as temperature and humidity levels, as well as the availability of hosts that are ideal for them to infect (Harry, 1971) [5]. When ingested by a suitable host oocyst undergoes excystation within the intestinal tract and releases eight sporozoites, the infective stage specialized for invading intestinal epithelium where they undergo development.
Infection rates in septate gregarines were shown to fluctuate considerably throughout the year. In the case of *Stylocephalus hoffmannseggi* Devdhar and Amoji 1977 [4], during the winter season, there was a significant increase in the number of infections. Seasonal shifts in the intensity of gregarine infections may be attributable, in part, to the wide range of atmospheric temperatures. According to the literature, not much has been done to advance this particular area. The purpose of this study is to determine the effect of temperature on gametocyst development and dehiscence of *S. hoffmannseggi* Devdhar and Amoji 1977 [4].

## Materials and Methods
### Collection of host insect
Adult *G. hoffmannseggi* were collected from the grass fields of Purulia district where the beetles are infected only by S. *hoffmannseggi and Unilobus* sp. from September 2022 to March 2023. Individuals were collected by handpicking method in the first three hours following sunrise. Adult beetles were brought into the laboratory for the collection of gametocysts, and the next morning they were released in their habitat.

**Collection of gametocysts**

The gametocysts of the gregarine were collected from the beetle faeces by simply leaving the beetles out on a petridish overnight and their faecal matter in the next morning were analysed. Faeces were submerged in a saline solution to soften the adhering faeces, and then gametocysts were removed. To assure surface sterility, gametocysts were submerged in 70 PPM methyl paraben (p-hydroxybenzoic acid, methyl ester: distilled water) for 5 minutes (Clopton et al., 1992) [3].

**Development and dehiscence of gametocyst**

In septate gregarines, once the gametocyst development is complete, the gametocyst ruptures, leading to the dehiscence of the cyst and the release of mature spores ready to be dispersed and initiate infection in a new host. The dehiscence of the gametocyst and the release of the mature spores of a septate gregarine are indications that the development of the gametocyst is now complete.

Groove slides holding gametocysts were placed in the moist chamber (Sprague, 1941) [8] constructed using the following method: The inside of the petridishes were wiped down with two pieces of blotting paper that had been dampened with water. To prevent the petridishes from drying out, white gelly wax was used as a sealant (Patil et al., 1983)

In order to study the effect of temperature on gametocyst development and dehiscence of *S. hoffmannseggi* Devdhar and Amoji 1977 [4], fifty gametocysts, five for each set temperature, were subjected to development at temperatures ranging from 10 to 55°C. The B.O.D. incubator is set to maintain 10, 15, 20, 25, 30, 35, 40, 45, 50, and 55°C temperatures for development. Gametocysts were incubated for ten days and observed every 4 hours to assess and record the gametocyst development and dehiscence. The time it took for the dehiscence of the gametocyst was recorded.

**Results and Discussion**

Table 1 shows how temperature affects gametocyst development and dehiscence in *S. hoffmannseggi* Devdhar and Amoji 1977 [4]. No gametocyst dehiscence was seen at 10°C. This indicates that the process of gametocyst release may not be optimal at 10°C. The time needed for gametocyst dehiscence was reduced at higher temperatures. A trend formed between 15 and 35 degrees Celsius. Within this range, the release time of gametocysts reduced as the temperature climbed. This suggests that increased temperatures within this range may have a beneficial correlation with hastening the gametocyst release process. Even though the incubation time at 40°C was constant at 84 hours, 4 gametocysts out of five actually dehisced. This may suggest that gametocyst formation is affected by high temperatures, temperature of 40°C may interfere with the maturation or discharge of gametocysts. As with the 10°C condition, neither 45°C nor 50°C caused gametocyst dehiscence. This suggests that extremely high temperatures, like extremely low ones, can prevent the release of gametocysts. The table as a whole show that there is a correlation between heat and the shedding of gametocysts. Temperatures between 15 and 35 degrees Celsius appear to be ideal for gametocyst dehiscence. Temperatures above 40 degrees Celsius and below 10 degrees Celsius appear to impede this process.

**Table 1:** Effect of temperature on gametocyst development and dehiscence in *S. hoffmannseggi* Devdhar and Amoji 1977 [4]

| Temperature | Duration (hours) | No of gametocyst dehiscence out of 5 |
|---|---|---|
| 10°C | 0 | 0 |
| 15°C | 112 | 3 |
| 20°C | 96 | 5 |
| 25°C | 92 | 5 |
| 30°C | 84 | 5 |
| 35°C | 84 | 5 |
| 40°C | 84 | 4 |
| 45°C | 0 | 0 |
| 50°C | 0 | 0 |

According to current observations, it has been noted that the optimal development and dehiscence (release) of gametocysts in *S. hoffmannseggi* Devdhar and Amoji 1977 [4] tend to transpire within a specific temperature range spanning from 20°C to 40°C. However, the scenario shifts at low temperatures (below 15°C) and exceedingly high temperatures (above 40°C), where the process of dehiscence does not take place.

This observation implies the existence of an ideal temperature range crucial for the progression of gametocyst development and subsequent dehiscence in S. hoffmannseggi Devdhar and Amoji 1977 [4]. It becomes apparent that the conditions become less favourable as temperatures plummet below the lower limit or rise above the upper limit of this optimal range, ultimately hindering the process.

In their separate investigations involving various species of septate gregarines, Kundu et al. (1980) [6] and Patil et al. (1983) [7] documented similar findings. In their respective studies, the influence of varying temperatures on gametocyst development was explored. Interestingly, the results converged: when the temperature was elevated

to 45°C and 50°C, a conspicuous absence of gametocyst development was observed. However, an intriguing discrepancy arose between the two research teams. While Kundu et al. (1980) [6] noted instances of gametocyst development even at 45°C, Patil et al. (1983) [7] proposed an alternative explanation. Patil et al. speculated that the apparent development at 45°C, as reported by Kundu et al., might have been a consequence of examining the gametocysts during advanced stages of development. Collectively, the data suggests a critical threshold. Temperatures exceeding 45°C appear to exert a detrimental impact on the developmental processes of gametocysts. This insight sheds light on the susceptibility of these organisms to temperature extremes, thereby underscoring the delicate balance required for their successful maturation.

The infection rates in septate gregarines exhibit noticeable variations over the course of a year. Specifically, when observing the behaviour of *S. hoffmannseggi* Devdhar and Amoji 1977 [4], it becomes evident that the occurrence of infections undergoes distinct changes during different seasons. Notably, during the winter season, there is a marked upsurge in the prevalence of infections.

This intriguing phenomenon could potentially be linked to the wide spectrum of atmospheric temperatures that characterise various seasons. The wide range of temperatures experienced throughout the year might have an impact on infection intensity fluctuations, at least in part. In the end, the changes in infection rates among septate gregarines, like those seen in *S. hoffmannseggi* Devdhar and Amoji 1977 [4], give us important information about how environmental factors and parasitic dynamics work together. The heightened infections during the winter season underscore the potential impact of colder temperatures on the prevalence and propagation of these gregarine parasites. This scenario highlights the complex nature of host-parasite interactions and how external environmental conditions can influence the dynamics of infectious diseases in natural ecosystems. Further research and investigation into these seasonal shifts could yield a deeper understanding of the ecological and physiological factors that govern the patterns of infection among septate gregarines.

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