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Seasonal Metabolic Strain: Tracking Glycogen Disruption in Cercariae-Infected Bellamya Bengalensis

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Abstract:
This study examines the relationship between cercarial infection and the seasonal change in the freshwater snail *Bellamya bengalensis's* glycogen level. During a 12-month period spanning four seasons, snails were gathered, and environmental factors including dissolved oxygen and water temperature were noted. The shedding method was used to evaluate cercarial infections, while the Anthrone method was used to test glycogen levels. The glycogen content of infected snails was shown to have significantly decreased, especially during the monsoon season when infection incidence was maximum. The study emphasizes how environmental stress and parasite burden combine to affect *B. bengalensis's* metabolic health.

Keywords: *Bellamya bengalensis*, cercarial infection, glycogen content, seasonal variation, Anthrone method, parasitic stress

1. Introduction:

In aquatic environments, freshwater snails are essential because they are both grazers and prey, and they act as intermediate hosts for many parasitic trematodes. On the Indian subcontinent, *Bellamya bengalensis* (Lamarck, 1822) is a common prosobranch snail that is found in both lotic and lentic freshwater bodies. The snail's function in disease transmission and ecosystem nutrient cycling is making its ecological and physiological responses to parasite infections—particularly by larval trematodes like cercariae—more significant (Subba Rao, 1989; Agarwal & Singh, 2001).

Mollusks with parasitic infections frequently have serious physiological abnormalities, especially in the area of energy metabolism. Trematode cercariae use the resources of their host to grow and reproduce during their intramolluscan development. The host's energy storage systems are interfered with by this exploitation, particularly the metabolism of glycogen, an essential energy store for invertebrates (Cheng, 1986; Das & Ray, 2008). A common biochemical indicator for evaluating metabolic stress and energy imbalance under parasite load is glycogen depletion (Kumar et al., 2012).

Another significant element influencing host metabolism and parasite life cycle is seasonality. *B. bengalensis's* physiological condition is controlled by temperature, photoperiod, and food availability,

which affects its immunological responses, growth, and reproduction (Ghosh & Chandra, 2006). In addition, cercarial emergence and infection rates are very seasonal, typically reaching their peak during warmer months when parasite development and snail activity are at their best (Khokhar & Sharma, 1990; Prasad et al., 2011). Therefore, to properly understand the host's metabolic dynamics, one must grasp how seasonal fluctuation and parasitic infection interact.

Mollusks' primary store polysaccharide, glycogen, acts as an instantaneous energy source needed for immunological responses, osmoregulation, motility, and reproduction (Livingstone et al., 1981; Bayne et al., 2001). The host's and the parasite's growing energy needs both rise dramatically during parasitic infections. In order to meet their own metabolic needs, trematodes frequently use the host's glycogen stores, which causes infected snails' glycogen reserves to be significantly depleted (Das & Ray, 2008; Upadhyay & Singh, 2014). In addition to weakening the host, this metabolic drain jeopardizes its ecological competitiveness, reproductive fitness, and survival (Minchella & LoVerde, 1981).

The biochemical effects of trematode infection in freshwater snails, including decreased protein, lipid, and glycogen levels, have been reported in a number of studies (Mukherjee et al., 2010; Upadhyay & Singh, 2014). Integrative research on the effects of seasonal cercarial infection patterns on *B. bengalensis* energy storage, specifically in relation to glycogen metabolism, is still lacking, nevertheless.

Seasonal fluctuations additionally influence this dynamic interplay. Environmental factors that have a substantial impact on parasite life cycle and host physiology include temperature, photoperiod, and nutrient availability (Ghosh & Chandra, 2006; Khokhar & Sharma, 1990). For instance, warmer months tend to see more cercarial output from infected snails because of ideal temperature and photoperiod circumstances that promote parasite establishment and development (Pelecanda & Fried, 1997; Prasad et al., 2011). The snail's feeding habits, metabolic status, and glycogen accumulation or use are also impacted by seasonal variations, particularly when the snail is under stress from an infection (Singh & Agarwal, 2012).

By evaluating *Bellamya bengalensis's* seasonal variations in glycogen content in connection to cercarial infection, the current study seeks to close this information gap. This study sheds light on the degree of metabolic stress caused by parasitism as well as the host's physiological adaptations to changing environmental circumstances. Understanding host-parasite interactions and how they affect disease ecology in freshwater environments depends on this kind of research.

2. Materials And Method:

2.1 Study Site Selection and Environmental Conditions:

In order to capture seasonal fluctuations, sampling was done throughout a 12-month period (January 2023 to December 2023) that included pre-monsoon (March–May), monsoon (June–August), post-monsoon (September–November), and winter (December–February). Three permanent freshwater ponds in the Amravati region were used to collect freshwater snails (Bellamya bengalensis). These ponds had varying degrees of aquatic vegetation and human activity, but all shared comparable biological characteristics.

- Portable probes were used to measure water quality parameters on-site at each sampling location:
- A digital thermometer is used to measure the water's temperature.
- The Winkler titration method is used to quantify dissolved oxygen (DO) (APHA, 2012).
- A digital multiparameter meter (Model: Hanna HI 98194) is used to measure conductivity and pH.

These metrics were utilized to correlate metabolic responses and infection dynamics, and they were monitored on a monthly basis.

2.2 Collection and Acclimatization of Snails: Using a hand-held scoop net or by hand-picking in the early morning hours when snails are most active, a total of 100–120 adult snails (average shell length: 3.5–5.0 cm) were randomly collected from each site once a month (Ghosh & Chandra, 2006; Subba Rao, 1989).

Upon collection:

- Snails were rinsed in pond water and transported in aerated containers to prevent hypoxia.
- In the laboratory, snails were **acclimatized for 48 hours** in large glass aquaria (20–25 L capacity) with **aerated, dechlorinated tap water** at room temperature (26 ± 2°C).
- Aquaria were maintained under a **12:12 h light:dark photoperiod**.
- Snails were fed **with spinach leaves and soft algae** during acclimatization.
- Dead or inactive snails were discarded.

2.3 Screening for Cercarial Infection:

To detect cercarial infection, the **individual shedding method** was used (Kendall & McCullough, 1951; Frandsen & Christensen, 1984):

- Each snail was placed in a 100 mL beaker with **30 mL of filtered dechlorinated water**.
- The beakers were exposed to **natural sunlight for 2–3 hours between 9:00 a.m. and 12:00 p.m.**, a time known to promote cercarial emergence.
- After exposure, water from each beaker was examined under a **stereomicroscope** (Magnus MSZ-Bi) for **emergent cercariae**.
- Cercariae were identified morphologically using standard keys (Frandsen & Christensen, 1984, Yamaguti, 1958; Schell, 1970).
- Snails were classified into two groups:
  - **Infected group** (visible cercarial emergence)
  - **Uninfected control group** (no cercariae detected after 2 screening sessions over 48 hours).

2.4 Estimation of Glycogen Content:

Glycogen levels in snail tissues were estimated using the **anthrone reagent method** described by Carroll et al. (1956), with modifications for molluscan tissue as per Singh & Agarwal (2012).

Procedure:

- 10–15 infected and 10–15 uninfected snails were randomly selected each month.
- After shell removal, soft tissues were blotted, weighed, and homogenized in 80% ethanol.
- The homogenate was centrifuged at 5,000 rpm for 15 minutes, and the supernatant was discarded.
- The pellet was hydrolyzed with 30% KOH in a boiling water bath for 20 minutes.
- After cooling, glycogen was precipitated with 95% ethanol, centrifuged again, and the residue dissolved in distilled water.
- Anthrone reagent was added, and the mixture was heated in a boiling water bath for 10 minutes.
- Absorbance was read at 620 nm using a spectrophotometer (UV-Vis).
  Glycogen concentration was calculated using a standard glucose calibration curve and expressed in **mg/g wet tissue**.

2.5 Environmental Parameters:

Monthly data on **water temperature, pH, dissolved oxygen (DO), and conductivity** were recorded using portable field meters. This helped correlate snail metabolic changes with seasonal environmental conditions (Boyd, 1981).

2.6 Statistical Analysis:

Data analysis was performed descriptively and compared with previous studies

3. Result And Discussion:

Table 1. Seasonal Variation in Environmental Parameters, Cercarial Infection, and Glycogen Content in *Bellamya bengalensis.*

Season	Water Temp.(^{0}C)	DO (mg/L)	Total snails Examined (n)	No. Of Infected Snails (n)	Infection Prevalence (%)	Mean wet weight of snail (g)	Glycogen in Uninfected Snails (mg/g ± SD)	Glycogen in Infected Snails (mg/g ± SD)
Pre-Monsoon	30.2 ± 1.1	5.4 ± 0.3	**60**	18	30.0%	2.4 ± 0.2	7.85 ± 0.45	5.32 ± 0.39
Monsoon	28.7 ± 0.8	5.8 ± 0.4	**60**	24	40.0%	2.6 ± 0.3	8.12 ± 0.51	4.98 ± 0.41
Post-Monsoon	26.4 ± 1.0	6.1 ± 0.5	**60**	15	25.0%	2.3 ± 0.2	7.68 ± 0.47	5.62 ± 0.36
Winter	19.6 ± 0.7	6.8 ± 0.4	**60**	9	15.0%	2.1 ± 0.2	6.93 ± 0.42	5.91 ± 0.40

- Water Temperature & DO: Seasonal averages of in situ measurements of water temperature and dissolved oxygen.
- Total Snails & Infected Snails: The quantity of snails gathered and tested, using the shedding method, for cercarial infection.
- Infection Prevalence (%): The percentage of infected people that shows the severity of seasonal transmission.
- Mean Wet Weight: The average weight of tissue per snail, which is used to normalize glycogen.
- Glycogen Content: Determined using the Anthrone technique, indicating the effect of cercarial infection on seasonal metabolism.

3.1 Seasonal Cercarial Infection Prevalence:

There was a noticeable seasonal change in the incidence of cercarial infection in *Bellamya bengalensis.* The monsoon season had the highest infection rate (40%) followed by the pre-monsoon (30%), post-monsoon (25%), and winter (15%) (Table 1).

This seasonal pattern is in line with previous research (Ghosh & Chandra, 2006; Prasad et al., 2011), which found that warmer and wetter times were associated with higher rates of snail infection and cercarial emergence. During the monsoon, higher water temperatures and humidity probably encourage the growth of parasites in the intermediate host, which increases cercarial production (Frandsen & Christensen, 1984).

3.2 Glycogen Content in Uninfected vs. Infected Snails:

In every season, the amount of glycogen in uninfected snails was consistently higher than that of infected

snails. Infected snails had significantly lower glycogen levels, ranging from 4.98 ± 0.41 mg/g (monsoon) to 5.91 ± 0.40 mg/g (winter), whereas uninfected snails had levels ranging from 6.93 ± 0.42 mg/g (winter) to 8.12 ± 0.51 mg/g (monsoon).

These results support earlier studies (Das & Ray, 2008; Upadhyay & Singh, 2014) and demonstrate that the host incurs metabolic costs as a result of cercarial infection. The growing larval trematodes divert or deplete glycogen, which is the main glucose resource in molluscs (Minchella & LoVerde, 1981).

The infection-related decline in glycogen levels may also be attributed to:

- Increased energy demand for immune defense,
- Tissue damage from larval migration,
- Host energy redirection to support parasite metabolism.

The monsoon season, when illness prevalence and ambient temperatures were high, showed the most noticeable depletion. This implies that parasitic load and environmental stress have a combined effect on the depletion of energy reserves (Singh & Agarwal, 2012).

3.3 Environmental Influence on Metabolic Trends:

Seasonal fluctuation was also observed in environmental parameters as dissolved oxygen (DO) and water temperature:

- Pre-monsoon had the highest temperature (30.2°C), while winter had the lowest (19.6°C).
- DO was highest during the winter (6.8 mg/L) and lowest during the pre-monsoon (5.4 mg/L).

The physiological burden on infected snails may be exacerbated by higher temperatures and lower DO during the pre-monsoon and monsoon seasons, which may increase metabolic activity and oxygen demand. Particularly in hosts that are already weakened by parasites, this stress makes glycogen consumption and depletion even worse (Mukherjee et al., 2010; Livingstone, 1981).

3.4 Statistical Significance and Trends:

- Within each season, there were notable variations in the glycogen content between infected and uninfected snails.
- There is a positive relationship between infection prevalence and ambient temperature.
- A negative relationship exists between the amount of glycogen and the severity of the infection. These trends imply that *Bellamya bengalensis's* energy metabolism is severely hampered by cercarial infection, which is made worse by seasonal environmental stressors.

3.5 Ecological and Epidemiological Implications:

The results suggest that:

- The host-parasite relationship is seasonal, with the monsoon causing the highest metabolic strain.
- The infected snails' ability to reproduce, grow, and survive may be impacted by the depletion of their energy stores.
- Predicting the dynamics of trematode transmission, particularly in disease-prone freshwater settings, requires an understanding of these patterns.
- Seasonal monitoring of glycogen content can function as a bioindicator of parasite stress and ecosystem health.

4. Conclusion:

This study demonstrates that *Bellamya bengalensis's* energy metabolism is considerably changed by cercarial infection, with seasonal environmental factors having a substantial impact on the extent of

glycogen disruption. Due to a combination of high infection intensity and environmental stress, the monsoon season became a crucial time for the host snail's metabolic fragility. Our knowledge of host-parasite ecology is improved by these discoveries, which could also influence future approaches to ecological risk assessment and freshwater disease management.

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