

Studies on the behavioural ecology and physiology of a hypogean loach, *Nemacheilus evezardi*, from the Kotumsar Cave, India

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The Kotumsar Cave, one of the largest known caves of India, has been successfully colonized by a species of loach, *Nemacheilus evezardi*. This species is found in both hypogean and epigean form. This paper summarizes the current knowledge on the behavioural ecology and physiology of the hypogean form as a result of 15 years of field and laboratory studies. Most studies have concentrated on metabolism, biological rhythms, general behaviour, ecophysiology and the biochemical composition of its metabolically important tissues. Yet, little is known on the evolutionary history of this species.

HYPOGEAN fishes that have yet to show complete troglomorphy are becoming the focus of studies aimed to understand the evolution of cave organisms. One of these species is the hill-stream loach, *Nemacheilus evezardi*. Studies with special reference to this species were carried out only 27 years after its discovery¹. Since then, our group at the Pt. Ravishankar Shukla University, Raipur has been actively engaged in studying various aspects of the ecology of the cave, as well as the behavioural ecology and physiology of the hypogean loach. This paper provides an updated account of these studies. The results obtained were compared with those obtained on its epigean counterpart, whenever it was possible.

Kotumsar Cave

The Kotumsar Cave is located along the bank of the River Kanger, flowing in the Kanger Valley National Park (KVNP) (18°52'09"N; 81°56'05"E), at an altitude of 560 m, in Jagdalpur, Bastar, India (Figure 1). It is one of the largest caves in India, and one among several explored and unexplored natural limestone caves of KVNP that is open to tourists. The cave was discovered in 1958.

The entrance to the cave is a vertical fissure in the wall of a hill. It is a narrow but twisted opening measuring about 15 m in length. The cave in general contains irregular chambers that make it appear honeycombed in

structure (Figure 2). The main tunnel of the cave extends for nearly half a kilometre and there are many sideward and downward passages that have been partially explored. The roofs and walls of the different chambers are lined with colourful dripstone formations as result of precipitation of calcite-dissolved carbonate of lime. The chambers of this cave are floored with either rocks or pebbles of varying dimensions or surface-derived soil/clay deposits.

The air temperature and several other abiotic parameters of Kotumsar Cave were monitored during six different months between May 1987 and March 1988. The air and water temperatures of the cave remain relatively stable at an annual average of $28.25 \pm 1.23^\circ\text{C}$ and $26.33 \pm 0.96^\circ\text{C}$ respectively (range = $25.0\text{--}32.7^\circ\text{C}$ for air; $22.9\text{--}29.3^\circ\text{C}$ for water). The water pools are distinctly alkaline. The annual average of pH values is 8.04 ± 0.36 . Further, a statistically significant annual variation in the conduc-

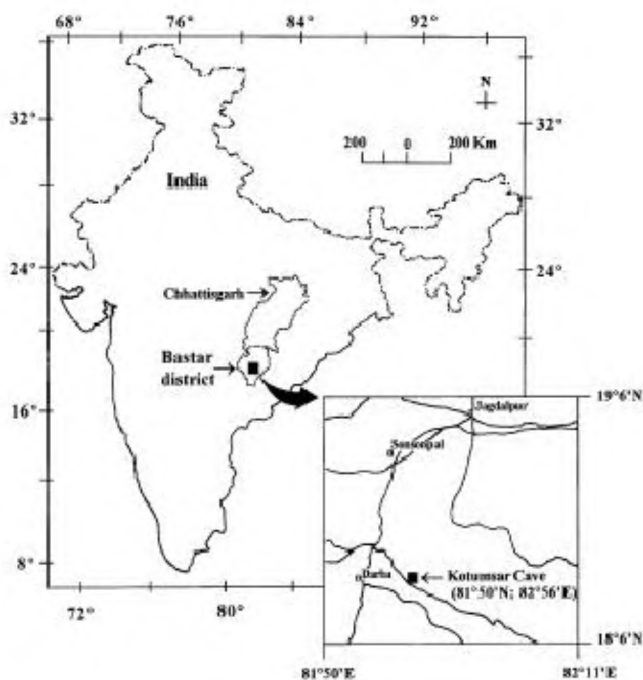


Figure 1. Location map of the Kotumsar Cave located in the Kanger Valley National Park, Jagdalpur, Bastar, India.

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tivity of water was found, with a peak during December and an annual average of 0.27 ± 0.03 m Mhos (ref. 2). The annual mean for dissolved oxygen and percentage saturation for oxygen in the cave water are 0.42 ± 0.52 ppm and $74.83 \pm 5.91\%$ respectively. This cave is subjected to frequent flooding when monsoon begins in the middle of June.

Hypogean loach, *Nemacheilus evezardi*

N. evezardi is a bottom-dweller and has successfully colonized subterranean streams and water pools of limestone caves along the Kanger River flowing inside the KVN. Its epigeal populations inhabit rapidly flowing water in hill streams³.

Systematic position

The nomenclature of the fish inhabiting Kotumsar Cave, herein named *N. evezardi* Day, has been a source of confusion. Kottelat⁴ put it under the generic name *Indoreonectes* in place of *Nemacheilus*, without specifying any reasons. However, Proudlove⁵ still continues to describe it as *Indoreonectes evezardi*. It was also called

Oreonectes evezardi for some time^{6,7}. In some of our earlier publications we described *Nemacheilus* as *Oreonectes*^{3,8}. There were also disagreements regarding its systematic position. For example, Day⁹ included it within the family Cyprinidae, Biswas¹⁰ included it within the family Cobitidae, and Eschmeyer and Bailey¹¹ placed it with the family Balitoridae. Prior to this, it was within the family Homalopteridae (Singh, pers. commun.). We believe it should be kept within the family Balitoridae.

Distinctive morphological features

N. evezardi has a cylindrical body with dorsally located eyes, small in size. It has eight barbels; one pair is nasal, two rostral and one maxillary. The lateral line is indistinct. The hypogean form is short (3–4 cm) in size compared with the epigeal form (5–7 cm). There is also a remarkable difference in the pigment distribution pattern. The cave form is totally or partially depigmented. Thus, it lacks the longitudinal dark/pale strips of the epigeal form. The hypogean form has reduced eyes¹⁰. The spines are absent on the head in both the forms. It has yet to be ascertained whether or not the hypogean and epigeal forms are currently reproductively isolated.

Distribution

N. evezardi has an ample distribution in the Godavari and Kolab watersheds, of which the Kanger River is a part. It has been reported from Pune, India⁹ and may inhabit rivers in the the Deccan Plateau.

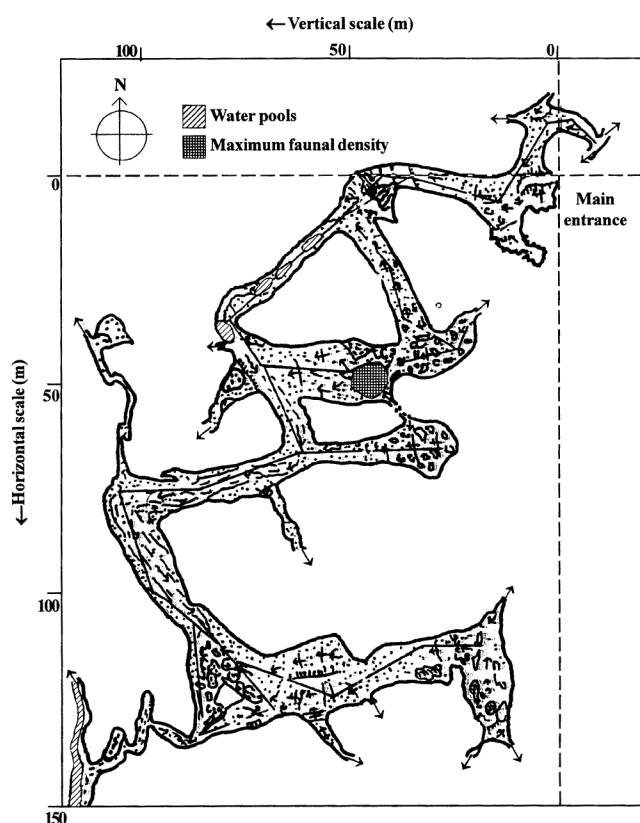


Figure 2. Plan of Kotumsar Cave indicating location of perennial water pools and area of maximum faunal density. Arrows indicate position of blowholes.

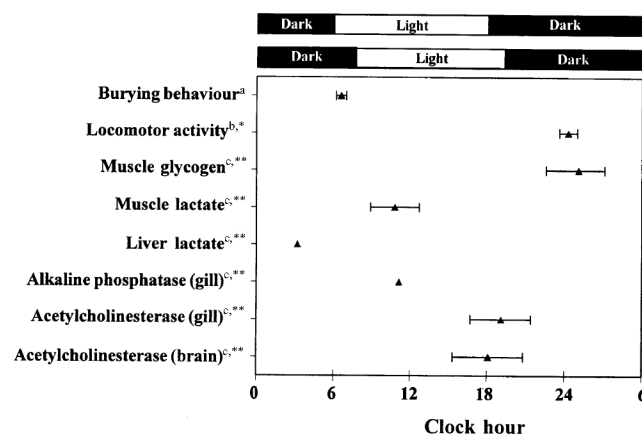


Figure 3. Each point represents the estimated maximum of each variable studied over a 24 h time-scale with midnight as phase reference. Horizontal line defines the 95% confidence limits of the peak timing. Absence of horizontal line indicates non-detection of statistically significant circadian rhythm. Burying behaviour has been studied under constant darkness (DD), while other variables have been studied under LD 12 : 12 photoperiod. *LD 12 : 12 (light on 6:00 h); **LD 12 : 12 (light on 8:00 h): Based on Pradhan¹, Biswas *et al.*³, Pati³².

Population studies

Full population studies of the epigeal *N. evezardi* have yet to be performed. However, two surveys one year apart were carried out to ascertain seasonal frequency of adults (pigmented or depigmented) and juveniles (pigmented or depigmented) in the Kotumsar Cave. They showed that the population of hypogean adults and juveniles increases every year soon after monsoon, in the middle of June¹⁰. Our personal observations based on several visits to the cave during different seasons of the year suggest that at any given time of the year, the stock of depigmented fish does not exceed 10% of the entire hypogean stock. The hypogean juveniles are observed only soon after the monsoon, i.e. from the middle of June onwards, suggesting that for the hypogean form flooding of the cave acts as a temporal cue for the onset of breeding.

Biochemical studies

Pradhan¹ evaluated a number of biochemical variables of protein metabolism (amino acids, proteins and transaminases activities); carbohydrate metabolism (glycogen, lactate, pyruvate, and lactate and succinic dehydrogenases activities); activities of nonspecific phosphomonoesterases and acetylcholinesterase; and the tissue levels of certain inorganic cations, such as Na^+ , K^+ , Mg^{++} , and Ca^{++} . Most of the biochemical variables, excepting the enzymes, were determined in the muscle tissue of the hypogean *N. evezardi*. Unfortunately, Pradhan did not study the same parameters for the epigeal form, but compared his results with those of the surface *Lepidocephalichthys guntea*, a species sympatric with the epigeal *N. evezardi*.

Energy and metabolism

During a starvation period of 29 days, both hypogean and epigeal fish have shown a body mass loss. The loss was estimated to be only 9.5% in the former compared to 16.5% in the latter. When food was supplied at the rate of 1 mg g^{-1} body weight, the hypogean *N. evezardi* lost less weight compared to its epigeal counterpart. Biswas¹² concluded that in a food-limited environment, the hypogean *N. evezardi* has developed some mechanism to reduce energy expenditure.

The rate of oxygen consumption by muscle tissue of the epigeal fish is remarkably higher (about 3-fold) than the hypogean *N. evezardi*^{8,12}. These results are similar to others that document lower metabolic rate in fish and non-fish hypogean species compared with their epigeal relatives¹³⁻²⁰.

Biswas and Pati⁸ compared the *in vivo* and *in vitro* responses of muscle tissue (over 70% of total body mass)

to calorogenic hormones, viz. thyroxine (T_4) and 3,5,3'-triiodothyronine (T_3) of both hypogean and epigeal individuals of *N. evezardi*. In hypogean fish, T_4 or T_3 *in vivo* did not stimulate the rate of oxygen consumption by muscle tissue. In contrast, in epigeal fish, both T_4 and T_3 significantly increased the rate of oxygen consumption by the muscle tissue. One explanation is that the muscle tissue of the hypogean fish lost its sensitivity to exogenous thyroid hormones during adaptation to the subterranean environment. The alternative hypothesis that there may be some other hormone(s) and/or substance(s) buffering the action of thyroid hormones, could not be rejected based on the fact that both T_4 and T_3 *in vitro*, irrespective of the dose level employed, increase the rate of oxygen consumption by the muscle tissue of both hypogean and epigeal fish. These findings coupled with those from starvation and oxygen consumption studies, strongly suggest that hypogean fish may have developed an endocrine mechanism that helps them exercise energy economy, even if the level of thyroid hormones abruptly shoots up in the circulation.

Behaviour

In both hypogean and epigeal *N. evezardi*, burying behaviour has been investigated as a function of time of the day, type of substratum, water current and body size³. Studies revealed that: (1) the frequency of burying is less in the hypogean compared to its epigeal counterpart; (2) although both hypogean and epigeal populations prefer to bury themselves under the stones, the hypogean form may also bury themselves in the mud; (3) the burying behaviour in the hypogean fish is the same regardless of the water flow speed, while the epigeal fish unbury themselves immediately after water flow ceases; (4) the frequency of burying is directly proportional to body size in the epigeal *N. evezardi*.

Burying behaviour in loaches helps them to avoid strong light, extreme temperature variations and predators²¹. These factors are irrelevant for the hypogean *N. evezardi*. Burying frequency in hypogean *N. evezardi* is considerably lower when compared with its epigeal counterpart. The fact that the hypogean form still exhibits burying behaviour may be explained as part of their resting behaviour. The burying in mud for the hypogean form could be explained as part of their feeding behaviour, because they prey predominantly on soil nematodes. An alternative explanation is that this behaviour is a vestigial trait.

The hypogean fish *N. evezardi* has been reported to exhibit surfacing behaviour²². The term 'surfacing behaviour' is synonymous with the term 'air-breathing behaviour' or 'air-gulping behaviour' or 'surfacing activity' or 'air-breathing activity'²³. The habit of air gulping is characterized sequentially by a fast upward movement, a

quick air gulping at the water–air interface, and an equally swift descent. The rate of air-breathing behaviour is usually considered as one of the most important parameters in respiratory physiology of those fishes that habitually breathe atmospheric air. Unlike its hypogean counterpart, the epigeal *N. evezardi* never exhibits air-gulping behaviour either in its natural habitat or under laboratory conditions¹⁰. This behavioural novelty found also among other fish species inhabiting low-oxygen waters, can be explained for the hypogean loach by the fact that it inhabits water pools that are hypoxic for long periods of time.

Responses to light in the hypogean *N. evezardi* have been studied^{24–26}. Biswas²⁷ demonstrated that the depigmented *N. evezardi* is more photophobic (scotophilic) compared to the sparsely pigmented hypogean fish. He argued that the preference to stay away from light in the depigmented form could be due to the disappearance of the protective layers of pigments from its integument. A marked scotophilia in the blind phreatic fish *Phreatichthys andruzzii* has been described to possess adaptive values. Probably this helps the species to stay within the borders of the subterranean niche^{28,29}. Romero³⁰ has suggested that the response to light among troglomorphic fish may only represent a behavioural relict from the ancestral surface stock, rather than a behavioural adaptation to the cave environment. We further suggest that probably depigmented (regardless the level) individuals of the hypogean *N. evezardi* possess extraocular photoreceptors. However, studies are yet to be performed to confirm this hypothesis.

Many of the physiological and behavioural functions of fishes are rhythmic in nature³¹. In both epigeal and hypogean stocks of *N. evezardi*, the peak burying time takes place at dawn (Figure 3). This was true even for hypogean individuals kept and observed under complete darkness^{3,10}. A multifrequency rhythm in air-gulping behaviour has been documented in the hypogean *N. evezardi*²². Circadian rhythm has been observed for air-gulping activity during nine out of 14 months of observation under constant darkness (DD). Circannual rhythm in air gulping has also been reported for the hypogean form. Although air-gulping rhythms were studied under carefully simulated cave conditions in the laboratory in relation to darkness, temperature and humidity, the method employed to record air-gulping activity was subjective and the duration of the study was short. Therefore, the conclusion drawn in one of our earlier papers²², that the air-gulping rhythm in the hypogean *N. evezardi* is endogenous, is questionable.

We have reported a high amplitude circadian phototactic rhythm in the hypogean fish, *N. evezardi*²⁴. This rhythmic behaviour may be considered as truly phototactic as the peak does not coincide with the peak in 24 h air-gulping rhythm reported by Biswas¹⁰. The group of fishes being fed between 7:00 and 11:00 h exhibited a

peak in phototactic rhythm at 17:00 h, whereas the peak was noticed at 2:42 h in groups of fishes receiving food between 19:00 and 23:00 h. This strongly suggests that the phase of this rhythm is modulated by the imposition of a periodic yet restricted feeding regimen²⁴.

The chromatophore-dependent colour changes have shown that when the hypogean *N. evezardi* is kept under LD 12 : 12 photoperiod, the number of different types of chromatophores, such as punctate (stage-I), punctostellate (stage-II), stellate (stage-III), reticulostellate (stage-IV) and reticulate (stage-V) appeared to vary as a function of time (Figure 4). All types of chromatophore, except stellate, exhibited a 24 h rhythm. A maximum dispersion of pigments was noticed in the latter half of the light phase, and the maximum aggregation was observed in the dark phase of the LD cycle^{1,26}. However, in contrast, a significant rhythm only in the punctate-type chromatophore was observed when the hypogean *N. evezardi* was kept under DD. This suggests that the hypogean fish still retains the ability to respond to light in its chromatophore system. However, the presence of a rhythm at the basal level (punctate-type) under DD is difficult to explain.

Other studies have revealed the presence of a 24 h rhythm in the contents of muscle glycogen, muscle lactate and in the acetylcholinesterase enzyme activities of gill and brain tissues of hypogean form under LD 12 : 12 photoperiod¹ (Figure 3). This indicates that the variation in the above-mentioned biochemical variables could be entrained to an LD 12 : 12 photoperiod. However, variations in these parameters have not been studied in the hypogean fish, either under DD or in its natural habitat.

Studies conducted earlier on biological rhythms in the hypogean loach *N. evezardi* do not clearly establish if any of these rhythms are endogenous. Further, most of the rhythms studied in the hypogean form under LD 12 : 12 photoperiod have not been compared with those

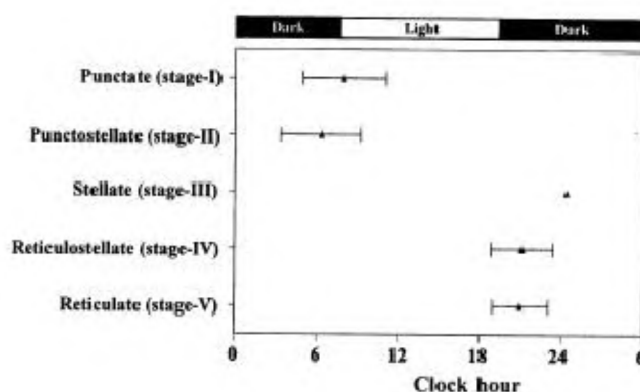


Figure 4. Each point represents the estimated maximum of each variable studied over a 24 h time-scale with local midnight as phase reference. Horizontal line defines the 95% confidence limits of the peak timing. Absence of horizontal line indicates non-detection of statistically significant circadian rhythm. Based on Pradhan¹.

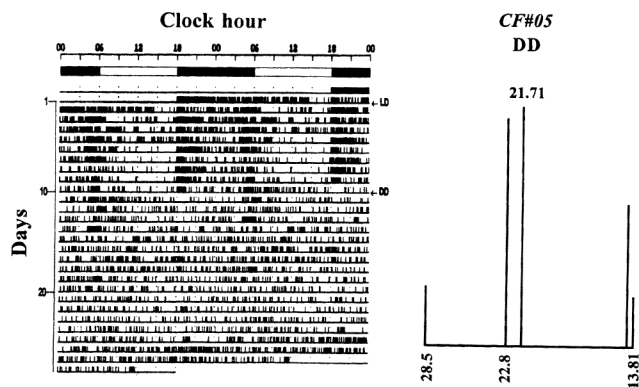


Figure 5. Activity records in double plot showing locomotor activity rhythm and power spectra of hypogean fish *Nemacheilus evezardi*. The fish were maintained first under LD cycles and then DD. The hypogean fish exhibits bimodality under LD and a weak circadian component ($\tau = 21.71$ h) under DD. From Pati³².

of the epigeal form. Recent studies suggest that the hypogean form still retains functional circadian oscillators³² (Figure 5). Similar conclusions have also been drawn for cave-dwelling catfish from Brazil^{33,34}. In contrast, loss of circadian activity has been reported in *Poecilia mexicana* (*sphenops*)³⁵.

Conclusions

Hypogean individuals of *N. evezardi* display convergent features shared by many other hypogean fish, such as reduction of eye size and pigmentation, lower levels of metabolism, and reduction in the frequency of the burying behaviour, while retaining a number of behavioural features from their epigeal ancestors, namely circadian rhythms and the ability to change colour. Among the new features found in the hypogean form is the acquisition of air-gulping behaviour. Seasonal flooding in the cave influences breeding. Yet, a number of questions remain unanswered. For example, to what extent is preadaptation in the epigeal form important to understand the evolution of the hypogean form? When did the hypogean form start to evolve? Are the hypogean and epigeal forms currently reproductively isolated? These and many other questions are yet to be answered.

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