Habitat, distribution, ecology and behavior of cave balitorids from Thailand
(Teleostei: Cypriniformes)

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Data on the ecology and behavior of balitorid cavefishes from Thailand are presented. Schistura oedipus and Cryptotora thamicola, both troglobites, coexist in Mae Lan cave (Mae Hong Son), where they have apparently diverged ecologically, specializing for slow and fast waters, respectively. Total populations are estimated to be on the order of $10^8-10^9$ vs. $10^7-10^8$. These are among the highest and lowest population sizes for cavefishes estimated. Schistura oedipus retains some melanin pigmentation and has behavioral responses to light, including avoidance and perhaps a residual diurnal rhythmicity. We show that two separate populations differ in degrees of eye regression and residual pigmentation. Cryptotora thamicola lacks melanin, is anophthalmic, and appears indifferent to light stimulus. The troglobitic species Nemacheilus troglotaractus (from Kanchanaburi) has a low population density, yet individuals exhibit agonistic behavior upon encounter. Individuals of other species with apparently "normal epigean" morphology occur in all of the caves visited. Their role in the cavefish ecology awaits clarification.

Introduction

Thailand is remarkable for its magnificent karst areas harboring a particularly rich troglobitic cavefish fauna (DS, unpub.). Troglobites are species restricted to the subterranean habitat, usually showing specializations such as reduction of eyes and pigmentation, called troglomorphisms. Five cypriniform species reported as troglobites have been described from Thailand since 1988: the cyprinid Poropuntius speleops (Roberts, 1991), and the balitorids Cryptotora thamicola (Kottelat, 1988) (Fig. 1), Nemacheilus troglotaractus Kottelat & Géry, 1989 (Fig. 2), Schistura oedipus (Kotte-

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Fig. 1. Cryptotora thamicola, approx. 35 mm SL; Thailand: Susa cave. Photograph by D. Belliveau.

Fig. 2. Nemacheilus trogloctaraactus, approx. 50 mm SL; Thailand: Wang Badan cave.

lat, 1988) (Figs. 3-4), and S. jarutanitie Kottelat, 1990a. In addition, a depigmented population of Pterocryptis huceata (Siluriformes: Siluridae) was found in Kanchanaburi, Western Thailand (Ng & Kottelat, 1998). Other trogloomorphic undescribed species have been reported, including Schistura sp. (Balitoridae) and Neolissoschilus sp. (Cyprinidae) from Phitsanulok province in Central Thailand (RB, unpub.; CV, unpub.; Smart, 1998). For a revision of general cave fauna from Thailand, see Deharveng & Bedos (2001).

Knowledge of this important fish fauna is scant. Published accounts are limited to descriptions of species, with some comments on relationships, distribution and a little data on habitat (Kottelat, 1988, 1990a; Kottelat & Géry, 1989). Brief references to trogloomorphic (blind and/or depigmented) fish seen in several caves may also be found in the general speleological literature on Thailand (Dunkley, 1995). Thus, much basic information about the biology, ecology and behavior of troglobitic fishes from Thailand is yet to be learned, in contrast to some other cavefishes that have been intensively investigated (cf.,

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Wilkens, 1988; Parzefall, 1993; Trajano, 1997a). Published data on the ecology and behavior of epigean (surface) balitorids from southeast Asia is also scarce, and consists primarily of notes on biology and habitat embedded in species descriptions (e.g., Alfred, 1969; Kottelat, 1990b). In this paper, we try to provide some of this basic information, distilled from a series of field trips conducted from 1996 through 1999.

Consistent qualities of the subterranean habitat are permanent darkness, lack of photoperiodicity, and tendencies towards climatic stability and food scarcity. Ecological and behavioral studies focusing on hypogean fishes from the Americas and Africa reveal several convergences in behavior and life history traits apparently related to these characteristics. For example, in comparison to their epigean relatives, many troglobitic fishes tend to present K-selected life history traits probably related to energy economy, such as larger and fewer eggs, and increased life span. Also, there are reductions in photophobia (especially in catfishes), circadian rhythms, cryptobiotic habits and agonistic behavior, and a more efficient feeding behavior (e.g., Parzefall, 1993; Weber et al., 1998; Trajano & Bockmann, 1999). Cavefishes from Thailand, only distantly related to the previously studied species, offer good opportunities to enlarge our knowledge of evolutionary convergence in subterranean fishes.

Specific goals of the 1999 field work were to get data on population sizes and densities of cave balitorids and to perform behavioral observations both in the cave habitat and in aquaria. Caves from two karst areas in western Thailand were visited and populations of three troglobitic balitorids were investigated (C. thamico, S. oedipus, N. trogladactatus). In addition, we recorded the presence in caves of fishes with epigean morphology (normal eyes and pigmentation). Herein, we present data on the distribution, ecology, behavior and variation of troglomorphic traits in three species of Thai balitorid cavefishes.
Fig. 5. Cave locations in the Mae Hong Son region of north-western Thailand. Huet, Tham Huet; MLN, Tham Mae Lana; BLKL, Tham Ban Louk Kow Larm; NPC, Tham Ban Nong Pha Cham; NL, Tham Nam Lang. Known cave passages shown diagrammatically in fine line, hypothesized connections in dotted line. Cave collection sites are marked with dots. Approximate elevations are given in meters above sea level.

Study sites

Our activities were concentrated in two karst areas: Mae Hong Son province in the northwest where we visited caves in the Salween river drainage that have populations of *S. oedipus* and *C. thamicola*. We also worked in Kanchanaburi province, where we visited two caves of the Lam Khlong Ngua system (Mae Khlong basin), from which a troglobitic population of *S. jarutani* has been described. Further south in Kanchanaburi we visited Tham Wang Badan, from which *N. troglotaractus* has been described. In addition to the above, many other river and stream caves in Mae Hong Son, Kanchanaburi and other parts of Thailand were visited over a three year course of study. These caves are identified, where relevant.

Thai caves often have several names. To clarify matters somewhat, we list the principal caves discussed herein, along with brief synonyms and cave ID numbers from their most recent cataloguing (Dunkley, 1995). We also give the years of our visits, the known species of troglobitic fishes they contain, and the sites within the cave where they are known to occur. All information presented herein was obtained during the 1999 visit, unless stated otherwise.

**Mae Hong Son caves.** Cave locations and hypothesized underground passages from this region are shown in Figure 5. Tham Huet (= Tham Hud, Tham Phah, Blind Fish Cave; MH33 in Dunkley, 1995; visited 1996, 1997) is a seasonal stream sink cave which gives access to a small perennial stream about 400 m in from the entrance. It contains a population of *S. oedipus*. The stream is accessible for some 900 m. A large spring located on the north bank of the Nam Lang valley, 5 km down river from Soppong village and 8 km from the end of the cave is believed to be its resurgence. This has never been directly confirmed.

Tham Ban Louk Kow Larm (= Tham Ham, Tham Ban Pak Kut, Porch Pit; MH16; 1997) is a seasonal stream sink cave descending via five vertical drops to 113 m depth. At the bottom, pools fed by rainy season floods and perennial drips contain *S. oedipus*. Most likely, this cave drains to Tham Nam Lang.

Tham Nong Pha Cham (MH91; 1997, 1999) presents a small perennial stream accessed via a 40 m deep doline some 200 m down valley from the impenetrable sink. A population of *S. oedipus* lives in the short section of observable stream. Its resurgence is unknown.

Tham Nam Lang (MH57; 1996, 1997) is a large cave carrying the Nam Lang river underground for more than 8 km. Access is via the resurgence in the Nam Khong valley. Populations of *S. oedipus* have been found, so far, at four sites within the cave, all in small, perennial tributary streams. The internal tributaries cannot be followed very far.

Tham Mae Lana (MH42; 1996, 1997, 1999) contains *S. oedipus* and *C. thamicola* at several sites. It is a river cave accessible from sink to resurgence, a subterranean distance of over 8 km. Both species are found in the two main internal tributaries. The “northern” tributary is 1600 m long and joins the main stream in the middle of the cave while the “southern” tributary is 2400 m long and 2 km from the sink end of the cave. *Cryptota thamicola* has also been observed at a rapids locality in the main cave stream. We focused our ecological and behavioral observations on the southern tributary populations.

Tham Susa (MH59; 1996) is a large cave carrying a sizeable, perennial stream. Access is via the resurgence in the Nam Khong valley. A pop-
ulation of *C. thamicaola* is found at the only rapids and waterfalls in the cave, about 740 m upstream of the entrance. Slow moving, deep water with a muddy bottom characterises the rest of the cave. The source of the water is unknown, but is thought to be mostly percolation from the extensive karst plateau above.

**Kanchanaburi caves.** Tham Nam (DS, unpub. data) is part of the Tham Nok Nang En system (Tham Nam Khlong Ngu 2-6, Swallow Cave; KA172-177). It is the lowest resurgence for that system, located 2 km southeast of the 6th karst window. Apart from two gravel shallows near the entrance, the cave contains slowly moving water, 13 m deep for the cave’s entire length (357 m). From Tham Nam, the river flows down a deep gorge for 7 km before sinking again before its final resurgence from Tham Sao Hin. Here we sought *S. jaratunani*.

Tham Sao Hin (= Tham Lot, Column Cave; KA178; 1996, 1998, 1999). Downstream from Tham Nam, the final resurgence of the Khlong Ngu river flows from large river cave with fast moving water. Tham Sao Hin is the type locality of the troglobitic *S. jaratunani*.

Tham Wang Badan (= Tham Vang Ba Dahl, Tham Sai Yok Noi, Tham Nam Tok; KA84; 1996, 1999) contains the only known population of *N. troglobataractus* in its small perennial stream. The cave is a popular attraction for tourists, most of whom are deterred from its full exploration by a climb down a small hole and the high carbon dioxide levels found at stream level. The resurgence is some 2 km down valley and the water source is unknown.

**Material and methods**

All caves were visited once or twice (Tham Mae Lana) towards the end of the dry season in March 1999. Tham Nong Pha Cham was visited in January by an advance party, and some specimens of *S. oedipus* were measured, marked and released.

Specimens were located by visual inspection of the habitat, and hand-netted after observation and counting. Collection of *S. oedipus* in the southern tributary of Tham Mae Lana was hindered by the presence of a silty substratum, making the water turbid as the collectors walked, but in most situations waters within the caves were highly transparent. Several specimens of *S. oedipus* captured during the first visit to Tham Mae Lana (March 18) were noted, measured, marked and released. Among the cavefishes collected on other occasions, some were kept alive for behavioral observations in an aquarium and others fixed in formalin (for morphology) or in ethanol (for DNA studies).

The standard lengths (SL) of all specimens collected were recorded to 0.1 mm; those kept alive for marking or behavioral observations were also weighed (W) with a dynamometer (Pesola) to the nearest 0.1 g. Data were tested for goodness of fit to the normal distribution by the Anderson-Darling test (Stevens, 1974). Allometric condition factors (*K* = *W* · *SL*^−1*; LeCren, 1951) were calculated for *S. oedipus* from Mae Lana, Nong Pha Cham (March sample) and Nong Pha Cham (January sample) caves. For these calculations, units of mm and g were used, and all values of *K* were scaled by 10^5 for presentation. We estimated the power coefficient, *b*, for the growth equation from the slope of the regression of log weight on log standard length.

We estimated minimum population size of *S. oedipus* in Tham Mae Lana after visual censuses in which all fishes seen in the southern tributary were counted at different occasions. In March 1999 we also recorded the number of *S. oedipus* seen at Tham Nong Pha Cham, *C. thamicola* at the southern tributary in Tham Mae Lana, and *N. troglobataractus* at Tham Wang Badan. In an attempt to estimate population sizes using mark-recapture methods, specimens of *S. oedipus* were marked in Nong Pha Cham (11 fish, January 1999) and Mae Lana (17 fish, March 1999) caves by subcaneous injection of pigmented latex microspheres using a Photonic Marking device (New West Technology). One marked individual was kept alive during several days in an aquarium to check the effects of marking.

Field observations focused on the distribution of fishes in the habitat and their preferences for microhabitats (e.g., substratum or water flow). We also recorded interactions between individuals, their positions in the water column, spontaneous activity, and reaction to stimuli such as light from lamps and mechanical disturbance of the water.

Small groups (maximum ten individuals) of *S. oedipus* from Mae Lana and Nong Pha Cham caves were also observed in a 5-l aquarium (30 × 15 × 15 cm) with hiding places provided by small rocks. They were checked several times.
during the day-time and during the first hours of the night for activity, position (hidden or not, close to the bottom or at midwater), reaction to stimuli, and interactions between individuals. These specimens were kept under natural temperature cycles and photoperiods, i.e., light/dark cycles of approximately 12:12 hs, with intermittent aeration.

Reaction to light in S. oedipus was tested during the daytime using the choice-chamber methodology (Romero, 1985; Trajano & Gerhard, 1997). For tests, the aquarium was divided along its long axis with one half covered by opaque black sheeting. The number of fish in the lighted compartment was counted every 5 min during a test period of either one or two hours. Before each test, the number of fishes present in the aquarium half that would stay illuminated was counted as control for preferences unrelated to illumination conditions. The statistical significance of the differences in distribution of fishes between the sides in the test and control series was determined by $\chi^2$ (a=0.05). Fishes were tested under light intensities of 200-250 lux (natural light in side rooms) and 500-700 lux (light from hand lights positioned over the aquarium). Water temperature was periodically measured and stayed within the air temperature range, not exceeding 24 °C during the tests.

**Results**

**Fish distribution and habitat preferences: ‘epigean taxa’**. In all caves visited, we observed fishes with epigean morphology swimming in isolation or in small groups, up to ten individuals. These ‘epigean’ fishes are found all the way through most caves, often many kilometers from the nearest entrance in the typical aphytic subterranean habitat, and are taxonomically diverse. In the caves of the Lam Khlong Ngue system, for example, we observed representatives of several cyprinid subfamilies, including Danio sp., Tor sp. and Poropuntius sp., and balitorids including Schistura sp., Hemaloptera sp., and Balitora sp. In

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other caves we also saw the cyprinid, *Garra* sp., the sisorid *Glaptolithorax* sp., and an unidentified silurid. Identifications were usually made by sight. The cyprinids were usually seen in slow-moving water of variable depths. Both adults and juveniles were recorded. Netted specimens seemed to be well fed, in good condition, and healthy. The eyed balitorids found inside caves were in habitats typical for these fishes—swimming over or maintaining position on rocks in fast-flowing, turbulent water. One siluriform catfish, probably *Pterocryptis*, was observed at Tham Khao Roi Sak–KA299. We did not observe co-occurrence of ‘epigean’ and troglobitic fishes.

During our dry season visits these runoffs were rapidly flowing rivulets, no deeper than several centimeters (Fig. 7). In the northern tributary the second waterfall is over a basalt intrusion, about 1.5 km from the tributary’s downstream end. In the mainstream, *C. thamicola* has been observed on limestone bedrock in a fast flowing section above small waterfalls created by basalt or andesite intrusions. In 1997 a representative of the epigean fast-water species *Balitora burmanica* was collected at the same spot. These statements incorporate not only our own observations but also the accumulated experience of local cavers, most notably John Spies, who discovered the Tham Mae Lana population and has made scores of trips through this cave and others in the area and has actively sought the fish.

Thus, the habitat of *C. thamicola* is typical of a balitorine’s preference for fast moving waters. Perhaps because such areas are relatively uncommon in the accessible portions of its home caves, the species appears extremely limited in its distribution.

In contrast, the second troglobitic fish in Tham Mae Lana, *Schistura oedipus*, tends to occur in
slow moving water. It is found in the same two internal tributaries as *C. thamicola*, but usually in the main stream of the tributaries or in gours (rimstone dammed basins atop flowstone). These have still or slowly moving water over silty substratum, at least during the dry season (Fig. 8). It is noteworthy that *S. oedipus* has never been observed in the main cave stream, in spite of the fact that local cavers have looked for them there intensively.

Thus, although syntopic in Tham Mae Lana, *S. oedipus* and *C. thamicola* present an obvious habitat segregation: the latter seems to be more adapted to fast-flowing water on rocky substrate, whereas the former seems to prefer water with lentic characteristics. Of course, our observations were made during the dry season, and conditions of flow must be very different during the rainy season and might afford less opportunity for this separation. Nevertheless, the ecological separation of the two species during the dry season is clear-cut.

In other caves also, *S. oedipus* tends to be found in still waters. In Tham Nam Lang, this species is found in at least four distinct locations, two of which we visited. Both are in slow moving waters of internal tributaries. We found none in the faster moving waters of the main stream. In Huet, Ban Louk Kow Larm and Nong Pha Cham caves the populations are found in the largest streams in the accessible areas, but these streams are significantly smaller than those in Nam Lang and Mae Lana caves. During the dry season the waters in Tham Huet move more slowly than in the larger caves, and in the others there is little or no flow. In Tham Ban Louk Kow Larm the fish survive in shallow pools held back by gours and in Tham Nong Pha Cham they crowd together in shallow muddy pools. This latter population seems to be particularly resistant to overcrowding conditions, surviving transport and maintenance in captivity well.

Few rules are without exceptions, however, and some individuals of *S. oedipus* have been observed climbing rapids in Tham Nam Tok (MH48, part of the Tham Mae Lana system), probably moving between pools (J. Spies, pers. comm.). Also, in Tham Mae Lana, *C. thamicola* has been observed rarely in slower moving waters in both tributaries (J. Spies, pers. comm.).

The third troglobite, *N. troglobataractus*, inhabits a typically headwater stream in Tham Wang Badan, with a relatively low volume of water flowing over rocks, alternating with small pools and gravel-bottomed shallow reaches. The few individuals observed were either adhering to rocks in fast-flowing, well-aerated water, or swimming in the pools. As in Tham Susa, air in this cave has high levels of carbon dioxide (Dunkley, 1995), and this caused noticeable respiratory discomfort during both of our visits.

Three visits were made to the Lam Khlong Ngu system, in particular to Tham Sao Hin, the type locality for the fourth troglobite, *S. juratanti* (1996, 1998, 1999). On none of these occasions were we able to find specimens of blind and/or depigmented fish, although we did net fully pigmented, eyed members of this species. Our efforts were diligent, but we may not have been able to locate the correct habitat within the cave system.

The only aquatic invertebrate species we observed in the caves were shrimp (*Macrobrachium* sp.), found at Tham Mae Lana and Tham Wang Badan. The larger individuals may be significant predatory threats to *S. oedipus* and *N. troglobataractus*, respectively. In Tham Mae Lana we recorded the following animals: three species of cockroaches, including an unidentified epigean species, a very common red-yellow troglobite with short wings, and a troglobite (*Speleoblatus* sp.). Raphidophorid crickets were common for the first hundred meters near the entrance, but rare deeper in the cave. There were at least four species of surface-dwelling spiders, one troglobiont nestcid spider (*Nesticella* sp.), one troglobitic opilionid, and a troglobitic uropygid. Two species of oniscoid isopods were common, one was white and blind, the other was eyed and pigmented. Finally, we saw two species of diplododa, one of which was troglomorphic. These species were not distributed evenly between the main stream passage of the cave and the side tributaries that contained the cave fishes. The main stream has organic matter derived from the river, and its abundant invertebrate fauna is typical of epigean litter dwellers (uropygi, winged cockroach, spiders and millipedes). The southern tributary was oligotrophic, with much less exogenous organic matter, and its sparser invertebrate fauna consisted mainly of troglomorphic populations (including the small pink cockroaches, white millipedes (*Diplododa*), white oniscoidea, the *Nesticella* sp., and the troglomorphic harvestmen. No observations were made of the invertebrates of the northern tributary.
Estimates of population sizes. A visual census in March 1999 of *S. oedipus* in the southern tributary of Tham Mae Lana resulted in 134 fishes counted along 150 m of stream at its downstream end. The full length of the tributary is 2400 m and its width in the studied section averages about 2 m. Thus, minimum population density in the studied section is around 0.45 fish·m⁻². A concurrent visual census of *S. oedipus* in the northern tributary was 241 individuals over 900 meters at its downstream end (width varying from 0.2 to 1 m), corresponding to a minimum density of 0.3 fish·m⁻². The full length of the tributary is 1600 m. In contrast, a visual census made in the early 1990s by J. Spies and T. Roberts counted about the same number of individuals along the full length of the southern tributary (“in the low 200’s”, J. Spies, pers. comm.) as we did in its lowest 150 m. *Schistura oedipus* is more common at the downstream ends of the two tributaries, but there may also be temporal fluctuations in population size.

Thus, we counted a total of 375 individuals in 1999. Because the habitat of this species in Tham Mae Lana is probably not restricted to the cave’s accessible passage, and because visual censuses can never detect all individuals in the passages walked, the cave’s population must number in the thousands, or more.

This figure is consistent with our mark-recapture data. None of the 17 fish marked in Tham Mae Lana or the 11 fish marked in Tham Nong Pha Cham were among the 32 and 47 individuals captured in March 1999 in these two caves, respectively. A heavy mortality caused by marking is unlikely, because tattooing has been successfully used in fish for decades, and the Photonic marking system has been tested in several species, both in laboratory (including a *S. oedipus* specimen) and in the field, without any significant harmful effect over the fish populations (Tranito, pers. obs.). Low recapture rates are more probably related to a high dilution factor from large population sizes and/or high rates of fish movements.

Only a small part of the habitat of *S. oedipus* is accessible in Tham Nong Pha Cham. The stream was interrupted in March 1999 due to the drought, and the fishes were observed in great numbers in a few discontinuous pools, presumably concentrated by stream contraction. In one of those pools, 1.5 long by 1.0 wide, about 100 individuals were counted and 47 were netted; dozens of individuals were seen in two other pools. On previous visits (1997, January 1999) the stream was freely flowing and fewer (tens or scores) were seen. No precise figure is possible for the number of individuals of this species in Tham Nong Pha Cham, but here too it is clear that it must be in the thousands, or more.

The five caves in which we studied *S. oedipus* outline an area of over 100 km² (Fig. 5). Mitochondrial DNA sequence data (Control Region and 16S rRNA) show that the populations of Mae Lana, Ban Louk Kow Larm and Nam Lang caves are very similar to one another and differentiated from the populations of Huet and Nong Pha Cham caves (Borowsky & Mertz, 2001). This suggests recent gene flow among the three western caves over a distance of about 10 km. With opportunity for a wide distribution in the subterranean habitat, the aggregate population size of this species is probably large, likely on the order of 10⁴ to 10⁵.

In contrast, *C. thamicoela* seems to present very low population sizes, probably related to its extremely specialized and restricted habitat. We counted four individuals on the flowstone at the southern tributary and seven at the northern tributary. On another occasion, three were counted at the rapids locality in the mainstream. All three locations in Tham Mae Lana are exposed to easy visual inspection. We observed only five individuals in the rapids below the waterfall in Tham Susa in 1996, although here detection is far more difficult because the water is deep and moves swiftly (Fig. 6). On other occasions, up to 20 individuals were counted in these rapids, where this species attains the highest density recorded (J. Spies, pers. comm.). Because the habitat available for these specialized fishes has scattered occurrence in these caves, we conclude that the total number of *C. thamicoela* specimens is low, on the order of 10² individuals, possibly reaching a few thousands.

*Nemacheilus troglodotaractus* is known only from Tham Wang Badan. A total of 621 m of accessible stream passage has been surveyed in the cave (Roche, 1992) but its full size is unknown. In March 1999, only three specimens were observed by four observers walking along 250 m of stream passage (average width 1.5 m) during two hours. In March of 1996, we observed eight individuals during a more limited walk along the stream bed. Twelve specimens were collected during the French Thai-Maros expedition in June
1986 (Kottelat & Géry, 1989). If we take 12 as the maximum observable in the 621 meters, this corresponds to an approximate population density of 0.01 ind·m⁻². Population size and density in the known habitat of the species is very low, perhaps artificially so because the fish has been collected commercially on occasion for the aquarium trade.

**Size, condition factor and feeding in Schistura oedipus.** For the March 1999 period, fish from Tham Mae Lana were larger than from Tham Nong Pha Cham (range 20.7–74.0 mm SL, mean = 45.6, n = 49 versus range = 21.3–57.4 mm SL, mean = 40.4, n = 36), but not significantly so (Kruskal-Wallis H = 3.17, P > 0.05). The size ranges are within that recorded in the species description (Kottelat, 1990b, max. 74.3 mm SL). Size distributions from both collections departed from normal (Anderson-Darling statistics of 0.82 and 0.83 respectively, P = 0.05), and were distinctly bimodal (Fig. 9).

The studied populations are characterized by a nearly isometric relationship between weight and length, with the calculated power coefficient of the growth equation, b = 2.96. Using this coefficient, we calculated the allometric condition factor, K, for all specimens of *S. oedipus* measured and weighed alive. Mean values of $K \pm S.E.$ are as follows: Mae Lana (March) = 14.78 ± 0.36; Nong Pha Cham (March) = 10.43 ± 0.27 (N = 29); Nong Pha Cham (January) = 14.46 ± 1.00. The Nong Pha Cham March sample differs significantly from both the Nong Pha Cham January and the Mae Lana samples ($t_{55} = 14.78, P < 0.05$ and $t_{55} = 5.54, P < 0.05$), whereas the latter two do not differ from one another ($t_{55} = 0.37$). Condition of fishes from Tham Nong Pha Cham in March was significantly lower than in the other collections (Fig. 10).

The proportion of fishes that appeared on external examination to have stomach contents and their apparent fullness (both used as indices of food availability in the cave habitat) varied between the studied populations: seven out of 17 specimens captured in Mae Lana had full to half full stomachs, and several others had some stomach contents, whereas only 11 of the 30 specimens examined on March in Tham Nong Pha Cham had any stomach contents, none had full stomachs, and the others seemed to have empty digestive tracts. Digestion seems to be very slow: digestive contents were visible in Mae Lana fishes kept alive more than 48 h after capture.
Out of seven specimens that were dissected, five had some material in their stomachs. This consisted of highly fragmented remnants of arthropods, basically insects. Every examined stomach contained fragments of small adult lepidopterans (scales, fragmented wings), in addition to non-identifiable insects. One stomach contained a cf. Psocoptera, and in another we found sediment (sand fragments); a roundworm, probably an endoparasite, was found in a third stomach. Minimum number of prey individuals per stomach varied from one to three. No stomach was completely full.

Tineid lepidopterans are abundant in many caves from Thailand (Deharveng & Bedos, 2001). Moths accidentally falling on the water surface (as other terrestrial invertebrates like pscopterans) would be taken by the fish, and could constitute an important part of their diet. On the other hand, small-sized aquatic fauna seems to be rather poor in most Thai caves (apparently the case with the presently studied ones). Macrobrachium shrimps, the commonest aquatic macroarthropods in Thai caves (Deharveng & Bedos, 2001), which we observed in Tham Mae Lana and Tham Wang Badan among others, may be eaten by fish as young individuals, but are more probably fish predators as adults.

Pigmentation and eyes. In S. oedipus, coloration varied from light gray, due to melanic pigmentation, to pink-yellowish, in individuals nearly devoid of melanin in the skin. Two distinct pigmentation polymorphisms were exhibited by the Tham Nong Pha Cham population. Of individuals examined in observation dishes, some presented a distinct yellow color as opposed to the more normal light pink of troglobitic fishes with reduced melanin. Of 25 fish scored for pigmentation, seven were yellow, 18 were pink. In addition, some individuals visibly fluoresced under 340 nm UV light: two over the whole body, four in patches along the flanks and caudal peduncle, and 19 not at all. Additional observations made in the cave revealed a continuous range of population variation in the extent of the fluorescence. Fewer than 10% were fluorescent all over the body, slightly more with partial fluorescence, and the majority with none.

In contrast, the Tham Mae Lana population seems not to be polymorphic for coloration. We checked for but saw no evidence of UV fluorescence in the Tham Mae Lana fish, although we examined far more of these (30 in hand and 110 in the stream) than from the Tham Nong Pha Cham population. All 30 of the Mae Lana specimens examined in hand were yellow in coloration. The yellow pigment may be diet dependent, because some specimens lost the color when retained in the laboratory (compare Figs. 3 and 4).

The Mae Lana and Nong Pha Cham cave populations also differ in their reduction of eyes and melanic pigmentation. All specimens from Tham Nong Pha Cham had vestigial eyes, visible externally as small black dots of variable size (smaller diameter about ½ the larger one), and scattered melanophores, at least on the dorsal region of head (especially on the brain region) and body. In contrast, specimens of Mae Lana had only traces, if any, of melanin, and the proportion of individuals with visible eyes seems to be distinctly lower - for instance, only 11 among 17 fish captured for marking had eyes visible as black dots.

The few specimens of C. thamicola observed and collected were totally depigmented and anophthalmic. Three specimens (25-33 mm SL), two from Tham Mae Lana and one from Tham Susa, were subsequently examined by thin section and found to lack even rudimentary eyes (RB, unpub.).

The measurements for the three specimens of N. troglotataractus collected in Tham Wang Badan are as follows: 58.0 mm SL, 2.3 g; 53.0 mm SL, 2.0 g; and 25.0 mm SL, 0.2 g. The skins of these individuals appeared totally depigmented, and showed a pink-yellowish colour. The 53.0 mm long fish had a single vestigial eye (the right one) externally visible as a melanic dot; the other ones did not have visible eyes.

Behavior. Schistura oedipus: Basic aspects of this species' behavior were determined in Mae Lana cave, although close observation for long periods was made difficult due to the habitat characteristics (relatively deep water with soft bottom). Most S. oedipus specimens observed were stationary on the muddy bottom, at depths from 20 cm to up to 1.0 m. In the southern tributary, a few undisturbed individuals were seen calmly swimming in the midwater and near the surface. When disturbed, stationary fishes swam unhurriedly away to rest somewhere else, or occasionally made incursions into the midwater. Small groups of individuals (most commonly three or four, sometimes up to eight fish), showing similar or differ-
ent sizes, were usually seen in the pools; isolated individuals were more common in rapids.

While the fishes seemed indifferent to moderate or indirect lighting from headlamps, and even slightly photopositive to instantaneous moderate illumination, they were distinctly reactive and photophobic to direct illumination by more powerful hand held diving lights. In this brighter light, they exhibited a shimmery behavior and/or swam away.

Observed in aquariums, the fishes generally remained near the bottom of the aquarium, also alone or in small groups of two or three individuals close to each other, sometimes in body contact. Differences in activity apparently related to the phases of light/dark cycles were observed. During the day-time and under moderate light, these fishes were intermittently active. Among groups with seven to ten individuals, at least one fish moved every 1-2 min. During focal-animal observations, most individuals moved at least once every 5-10 min. When inactive, individuals remained stationary on the bottom, over or under the rocks provided as hiding places. Small movements of a few cm were seen, along with more active swimming, usually near the aquarium bottom and walls, occasionally crossing the water column (especially the larger individuals). During the night, the fishes were mostly inactive. In all observation occasions, the individuals were stationary on the bottom, generally under or next to and in contact with the rocks.

The results of tests of light reaction in *S. eotipus* are in Table 1. The studied fishes were indifferent to slightly negative to moderate light, corroborating the observations in the cave habitat, but displayed highly photophobic behavior under brighter light.

*Schistura eotipus* is weakly or not at all cryptobiotic. With a few exceptions, the observed fishes did not remain under rocks for more than 5 min during the day-time. Besides, the large numbers of fishes seen in the cave habitat staying freely on the bottom or swimming indicate that cryptobiotic habits, if present, are not accentuated in the species.

No agonistic interactions between *S. eotipus* were observed, except for some lateral shoves when two individuals met inside dens. When two moving individuals met by chance, they simply avoided each other, changing direction of swimming. No clear size-related dominance was observed on these occasions. Stationary individuals were isolated or in groups of two or three fishes, frequently in body contact.

*Nemacheilus trosococarticus*: Components of agonistic behavior were distinguished for the three *N. trosococarticus* specimens kept alive and observed over a period of two days. The larger individual (58.0 mm SL) was clearly dominant, patrolling all the available space, whereas the second larger fish (53.0 mm SL) remained most of time stationary at a corner of the observation dish. When the two fishes met, usually due to the swimming activity of the larger one, this individual exhibited zig-zag swimming, lateral and ventral shoving (as if to push the other fish off from the bottom), and biting. These are agonistic behaviors typically seen in the epigean balitorid *N. seloteoricus* (Wickler, 1959) and both epigean and cave catfishes (Todd, 1971; Berti et al., 1983; Trajano, 1991a). The smaller fish (25.0 mm SL) was active most of the time, avoiding the larger ones when they met by chance; it also displayed some tail-beating.

*Cryptotora thamnica*: Observation of the behavior of this fish in situ is hampered by the reflections of light in the running water. In March 1999, the few individuals located on the flowstone at the northern tributary of Tham Mae Lana in the shallow water lamina were stationary and facing upstream; Fig. 7). We observed frequent spontaneous movements. When touched, the fish would make a rapid movement that appeared to be a flip from the substrate and would be carried by the flow to a new position several tens

Table 1. Results of tests on light reaction in *Schistura eotipus*, expressed as counts of the number of fishes in the illuminated and dark sides of the chamber during the tests and in the controls. Lux, approximate light intensity; ε, Yates’ corrected Chi-Square value with df = 1.

<table>
<thead>
<tr>
<th></th>
<th>Lux lighted darkened</th>
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<th>χ²</th>
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<tr>
<td><strong>Tham Mae Lana</strong></td>
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<tr>
<td>200</td>
<td>57 39</td>
<td>55 41</td>
<td>0.02</td>
<td>ns</td>
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<td>500</td>
<td>57 39</td>
<td>33 95</td>
<td>24.4</td>
<td>&lt;0.05</td>
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<tr>
<td><strong>Tham Nong Pha Cham – no hiding places</strong></td>
<td></td>
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<tr>
<td>500</td>
<td>48 36</td>
<td>14 77</td>
<td>31.5</td>
<td>&lt;0.05</td>
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<tr>
<td><strong>Nong Pha Cham cave – with hiding places</strong></td>
<td></td>
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<tr>
<td>200</td>
<td>53 43</td>
<td>77 115</td>
<td>5.3</td>
<td>&lt;0.05</td>
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<tr>
<td>500</td>
<td>53 43</td>
<td>74 70</td>
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of centimeters downstream. We interpret this as a stereotyped escape behavior. It is difficult to judge the extent to which the rapid lateral motion was due to exertion by the fish or the movement of the water. John Spies (pers. comm.) has observed quick spontaneous sideways movements of the fish in which they quickly (“instantly”) move from one spot to another 10-20 cm away, as well as the “gecko style” upstream walking, from which the name “waterfall climbing fish” is derived.

We have no observations of interactions among individuals and left all specimens we observed in the cave. In one location, three individuals were sited within 10 cm of each other, but may have been so close because the area was a preferred spot. They exhibited no movement while under observation for about ten minutes.

Discussion

Distribution and habitat. The poor condition of *S. oedipus* collected in Tham Nong Pha Cham in March 1999, compared to that of fish collected there in January 1999, or in March 1999 in Tham Mae Lana, reflects the crowding caused by low water. Due to the pronounced drought that disrupted the stream flowing in Tham Nong Pha Cham after January, this population was subject to severe food shortage, as indicated by the low frequency of specimens with digestive contents. On our visit to the same locality in March of 1996, the stream was freely flowing. Periodic drought and seasonal variation in food availability may be factors of importance in the biology of this species. Water levels in the tributaries of Mae Lana were noticeably lower in 1999 than in previous years (March 1996, March 1997). According to local information, such particularly dry years are not uncommon and are becoming more frequent as progressive clearing of Thai forests allows faster, more complete run off. This could pose a threat to these populations.

*Nemacheilus thamicola* appears to be restricted to two caves: Tham Mae Lana and the type-locality, Tham Susa (Kottelat, 1988). Waters from the Mae Lana resurgence flow above ground for 30 km before merging with those from Tham Susa and it is unlikely that the two caves have a current underground connection. Inside the caves, *C. thamicola* presents a highly disjunct distribution, associated only with waterfalls and rapids. Nevertheless, there must be movements between favored sites, possibly during floods, when the waterfalls are submerged and strong currents could carry fishes to other sites.

Although competition among similar species may be more intense in the subterranean environment than on the surface because of a limited trophic base, several examples of cavefish species coexisting in a system are known. Proudlove (1997) listed 72 species of cavefishes, and noted instances of syntopy. Updating his compilation with the information on *S. oedipus* and *C. thamicola*, of the 72 species, 44 appear to be the sole troglobitic fish occupants of their cave systems, while eleven pairs and two triplets of species cohabit their cave systems. The ways in which sympatric cavefishes subdivide niche space deserves more attention, but in the case of *S. oedipus* and *C. thamicola*, habitat separation on the basis of flow rate and/or slope is likely an important factor in limiting competition.

*Nemacheilus troglobotractus* is known from only Tham Wang Badan, the full extent of which remains unknown. Thus, while only a few individuals have ever been observed, not enough information exists to reliably estimate its population size.

Apart from troglobites, organisms found in the subterranean habitat may be troglophilic (organisms able to complete their life cycle either in the hypogean or in the epigean habitat), troglo xenes (organisms regularly found in the hypogean habitat, but which must return periodically to the surface to complete their life cycle), or accidentals (organisms that generally exist in caves only temporarily) (Holsinger & Culver, 1988). Such organisms show an "epigean morphology," including normally developed eyes and pigmentation, and are frequently difficult to categorize.

To distinguish between troglophilic and trogloxenes reliably requires knowledge of their life cycles, although distribution inside caves is helpful information, because trogloxenes generally are not found too far from cave entrances. Nevertheless, the status of the cypriniforms with epigean morphology found in caves of Thailand is difficult to ascertain. Their frequency, including the occurrence of juveniles, and good physical condition observed deep inside caves indicate that some species could form troglophilic populations. On the other hand, these relatively large, mobile organisms could move periodically to the epigean habitat. The possibility of fish
being washed into the caves during floods and their survival for variable periods also cannot be ruled out. Ecological studies focusing on distribution and life cycles of cave cypriniforms in Thailand are needed to elucidate this point.

Population sizes. It is well known that visual censuses produce underestimates of the real population sizes. As a rough guide to the potential magnitude of this factor, the proportion between the numbers of fishes observed or captured and of those estimated in the few studies on cavefish population ecology using mark-recapture techniques was calculated (Trajano, 2001). On average, about three individuals were estimated from mark-recapture techniques for each one actually observed. Applying that correction factor of 3:1 to the number of fish counted at the downstream end of southern tributary in Tham Mae Lana (134 individuals along 150 m) produces an estimated population of 400 individuals, which would be consistent with the lack of recaptures due to the low number of fish marked in this section. This illustrates the importance of preliminary studies in order to determine minimum numbers of marked individuals necessary in mark-recapture programs, which depends on the total population sizes.

Comparing values from the visual censuses, the population density of S. oedipus in Mae Lana cave (0.5 ind. m\(^{-2}\)) is much lower than those recorded by Parzefall (1993) for Mexican poeciliids, Poecilia mexicana (100-200 ind. m\(^{-2}\)) and the cave populations of Astyanax mexicanus (max. dens. 5-15 ind. m\(^{-2}\)) but comparable to that recorded for the North American ambylopsid, Amblyopsis rosea (0.15 ind. m\(^{-2}\); Parzefall, 1993) and the Brazilian armoured catfish, Ancistrus cryptophthalmus (0.2 ind. m\(^{-2}\)), and higher than in the Brazilian catfishes, T. itacarambiensis (about 0.05 ind. m\(^{-2}\)), a new heptapterine genus from northeastern Brazil (0.03 ind. m\(^{-2}\)) and Tautuapia sp. (0.01 ind. m\(^{-2}\)) (Trajano, 2001), the ambylopsids A. spelaea (0.05 ind. m\(^{-2}\)) and Typhlichthys subterraneus (0.03 ind. m\(^{-2}\)) (Parzefall, 1993), and also N. troglodactaractus (less than 0.01 ind. m\(^{-2}\)).

The high population densities of P. mexicana and A. mexicanus appear exceptional among troglobitic fishes and may be related to high food availability represented, respectively, by sulfur bacteria and bat guano and non-benthic distribution. Therefore, except for those cases of populations living under conditions of exceptionally high food availability or concentrated in pools due to periodic droughts (as in Tham Nong Pha Cham during March 1999), density of S. oedipus in Tham Mae Lana is among the highest recorded for troglobitic fishes.

The total population size of S. oedipus also appears to be high compared to other studied cavefishes. It is possibly comparable to those of the ambylopsids T. subterraneus and A. rosea, and the ictalurids Trogoeglantis pattersoni and Satan eurystomus, which occur in large karst areas in the United States, and also to A. cryptophthalmus and P. mexicana, but certainly larger than the populations of T. itacarambiensis, P. kornei, Taumaria sp., A. rosea, Speoplatyrhinus poulsoni and others (Trajano, 2001). In contrast, the total population sizes estimated for C. thamicola, from hundreds to a few thousand, are among the lowest observed for cavefishes. N. troglodactaractus appears to have a low population size, but undiscovered habitat may harbor considerable reserves of this species.

The smallest individuals collected in both populations of S. oedipus sampled were just over 20 mm SL and size distributions in both were bimodal, with the lower modes in the range of 25-35 mm SL (Fig. 9). This suggests active reproduction and recruitment in both populations. In the absence of data on growth rates in S. oedipus, we cannot determine whether recruitment is annual or less frequent.

Ecology and behavior. Many behavioral specializations have been described for troglobitic fishes (e.g., Parzefall, 1993), based on comparison with their closest epigean relatives. It is generally accepted that a phylogenetic scheme is needed for a full understanding of the evolution of behavioral patterns (Pinna, 1997). Phylogenetic hypotheses are not yet available for the species groups comprising the presently studied cavefishes, limiting the evolutionary interpretation of the observed traits.

Data on the ecology and behavior of balitorids in general are scanty and discordant in some aspects. Our observations, made in the course of extensive collecting of surface species for other studies, are that Thai epigean balitorids are generally diurnal, not cryptobiotic nor territorial. During the night time, these fishes stay motionless on the bottom, usually under rocks. On the other hand, the Indian balitorid, Indoreovetes everardi, is described as a bottom dweller mainly active at dusk that hides under stones during

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most of the day time (Biswas, 1991). *Nemacheilus selangoricus* has been reported to live in holes dug under stones (Wickler, 1959). The European *Barbatula barbatula* is described as a strictly nocturnal, non-visual predator by several authors (e.g., Neveu, 1981), but with a peak of activity at dusk and declining during the dark period (Wetton et al., 1983).

Analysis of stomach contents of *S. oedipus* collected from Tham Mae Lana indicate some feeding stress and dependence on alloglottic prey items, at least during the season of collection. This is consistent with observations on other cave species that show feeding stress and dependence on terrestrial food items during the dry season (Trajano, 1997b). The diets of epigean balitorids from SE Asia are generally reported as consisting mainly of benthic insects. Nemacheilines feed mostly on insect larvae, worms and some algae (Wetton et al. 1983; Kottelat, 1990b). Carnivory is considered a preadaptation to subterranean life.

A general pattern for this family seems to be a benthonic habit, with periodic bouts of midwater activity. In aquariums, *I. evezardi* specimens maintain stationary positions, although these may be changed frequently, and they occasionally undertake vertical swimming bouts (Biswas, 1991; Pradhan et al., 1989). Our observations are that *S. oedipus* exhibits the same general pattern, perhaps with a greater midwater activity. Increased midwater activity of troglobitic fishes has been noted previously for Brazilian blind catfishes (e.g., Trajano, 1997b). This hypothesis needs testing through quantitative comparative study. Likewise, feeding tactics herein suggested for *S. oedipus* (chemically oriented predation of bottom animals, grubbing and surface picking) were also recorded for other troglobitic fish, such as the catfishes *Trichomycterus labirintus* (Trajano, 1997b) and *Pimelodella tronca* (Trajano, 1989). Surface picking is considered an autapomorphy of these troglobitic species, related to the increased midwater activity, as an adaptation to enhance chances of finding food in a food-poor environment, in the absence of interspecific competition at midwater and under low predation rates (Trajano & Bockmann, 1999).

Observations on activity levels of *S. oedipus* under different light intensities indicate that this activity is passively induced by moderate, but not by strong light, or that these fishes originated from ancestors which were active at dusk. These alternatives can only be decided when this species is put in phylogenetic context. Many cavefishes have epigean relatives that are active at dusk and/or night, showing photonegative responses, such as Neotropical ophiidiids (Farzeff, 1986), heptapterine catfishes (Trajano & Gerhard, 1997), and North American amblyopsids (Langecker, 1992).

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