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Morphology and reproduction of the cavefish *Trichomycterus chaberti* and the related epigean *Trichomycterus cf. barbouri*

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Hypogean and epigean populations of *Trichomycterus* catfishes inhabit streams from different environments (cave, headwater, canyon and valley) in the Torotoro National Park in the Andes, Bolivia. A significant reduction in the diameter of the eyes and in the surface area of the mesencephalon was observed in subterranean populations, along with an increase in the surface area of the telencephalon. Contrary to expectations, the barbel did not appear to be longer in hypogean populations. The observed pattern of modification of the other variables (pigmentation, eye asymmetry, surface area of the cerebellum and rhombencephalon, fecundity and egg diameter) corresponded to a gradient of values from valley to canyon, headwater and subterranean populations. This result argues not for a simple distinction between epigean and hypogean populations but for an adaptation to an environmental gradient of constraints in which caves correspond to an extreme situation.

Key words: brain; cave life; environmental gradient; morphology; reproduction; *Trichomycterus.*

INTRODUCTION

Trichomycterus is a catfish genus (Trichomycteridae) including *c.* 100–120 species commonly found in neotropical headwater streams (Eigenmann, 1918). This genus presents a high colonization potential in constraining environments such as high altitude streams (>4000 m, M. Pouilly pers. obs.), warm thermal water (>35° C, G. Miranda pers. obs.) and subterranean streams. Romero & Paulson (2001) reported on three troglobitic (cave-restricted) species: *Trichomycterus chaberti*, Durand in Bolivia, *T. conradi* (Eigenmann) in Venezuela and *T. itacarambiensis* Trajano & de Pinna in Brazil (region of Minas Gerais). Hypogean populations (not necessarily cave-restricted) have been reported in the Colombian Andes (Sket, 1988) and in the Brazilian region of Goiás (Trajano & Souza, 1994), and in other neotropical regions (Trajano, 1997).

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Permanent darkness (Langecker, 2000) and food scarcity (Hüppop, 2000) are the main limiting factors in caves, and troglobitic species have had to be pre-adapted or to profoundly change their physiology, biology and ecology to adapt to these extreme conditions. Some features, such as eyes and pigmentation, lose their function because of darkness and consequently regress (Culver & Wilkens, 2000). To compensate for the loss of vision, troglobitic organisms may develop non-optic sensory structures and appendages (organs that are receptive to chemical and mechanical stimuli). The sensorial information is gathered in the brain and the size of the different brain lobes provides an indirect indication of the level of activity of each type of sensorial reception (Kotrschal *et al.*, 1998). The relative size of the different brain lobes may differ between epigean and troglobitic fishes (Poulson & White, 1969). These adaptations are essential to improve food-finding capacity and reproductive contact in the dark. In addition, the limited food supply in the cave environment may lead to a reduction in the metabolic rate and to a modification in both the life cycle and the reproductive strategy to increase resistance to starvation (Culver, 1982; Hüppop, 2000; Trajano, 2001). Most catfishes show behavioural (nocturnal activity and cryptobiotic habitat) and morphological characters (presence of barbels, small eyes and chemo-sensory orientation) that could be interpreted as pre-adaptation to hypogean life (Bertin, 1958; Romero & Paulson, 2001) and that facilitate colonization of caves.

Trichomycterus chaberti is endemic to the Torotoro National Park, Bolivia, and has only been observed in the Umajalanta Cave. Populations of epigean *Trichomycterus cf. barbouri* (Eigenmann) are present in the same area. They are geographically isolated from each other by natural waterfalls in the streams that flow in different canyons and valleys. The isolation of the streams makes it possible to conduct a comparative study of phylogenetically related populations that live independently in different environmental conditions (cave *v.* superficial streams, canyon and headwater rocky streams *v.* valley gravel-bed streams).

The aim of the study was to compare morphological (eye, barbel, pigmentation and brain characteristics) and reproductive (fecundity and egg diameter) features that are known to be affected by hypogean life, in eight populations of *Trichomycterus* from valley, canyon, headwater and subterranean streams in the Torotoro National Park. This comparison revealed what kinds of specialization the population of *T. chaberti* underwent after colonization of the cave environment, and whether these specializations are specifically linked with a cave environment or rather with a gradient of changes between the different environments found in the different types of streams.

MATERIALS AND METHODS

STUDY AREA

Torotoro National Park is located in the Bolivian Andes (18°15′ S; 65°45′ W) at an altitude of between 1950 and 3850 m. The area corresponds to a small massif (15 by 3 km) of Cretaceous limestone characterized by karstic phenomena (caves and canyons). The local hydrographic network shows four principal rocky streams located in canyons parallel to each other and perpendicular to the valley of the Caine Stream into which they drain (Fig. 1). They have a high slope (*c.* 10%) with a steep profile, and are

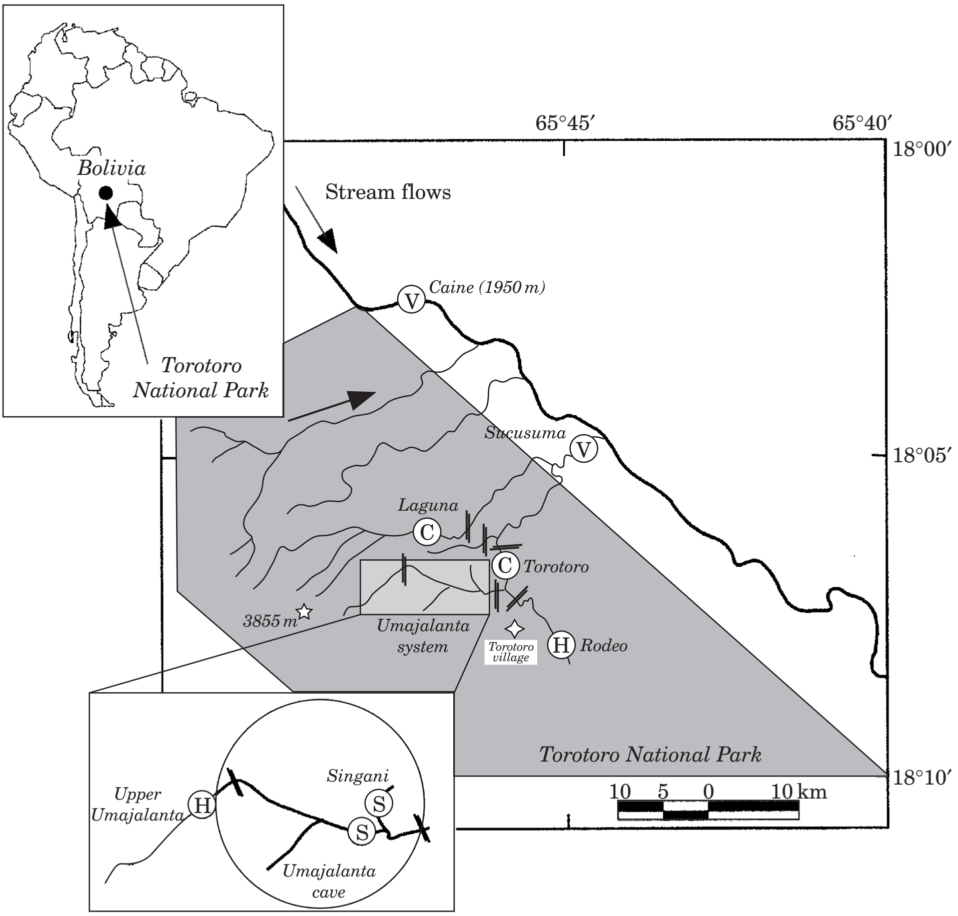

FIG. 1. Torotoro National Park. Schematic map showing the location of the eight sampled streams. V, valley stream; C, canyon stream; H, headwater stream; S, subterranean stream; ═ waterfall.

characterized by a series of waterfalls (>5 m high) that biologically isolate stream segments by preventing the migration of fishes upstream. The Caine is an unstable gravel-bed stream connected to the Amazonian hydrographic network (Mamoré-Madeira watershed).

Local climatic conditions are semiarid with *c.* 950 mm of annual precipitation, a mean annual temperature of 18° C, and are characterized by the alternation of a warm wet season (October–March) and a cold dry season (April–September).

SAMPLING LOCATION AND TECHNIQUE

Six superficial streams were sampled in three different kinds of environments (Fig. 1). The Caine and Sucusuma streams are located in the valley, at an altitude of 1980 and 2020 m, respectively. The Laguna and Torotoro streams are located in canyons, at an altitude of 2200 and 2300 m, respectively. The Rodeo and Umajalanta streams, both tributaries of the Torotoro canyon, are located in small headwater valleys, at an altitude of 2740 and 2820 m, respectively. The Umajalanta stream has a subterranean segment of *c.* 3 km and flows into the Torotoro canyon by means of a 20 m waterfall. The Upper Umajalanta is located in the superficial upper reaches of the Umajalanta stream before the stream is lost in the Umajalanta cave. Two streams, the Umajalanta and Singani, were sampled in the subterranean environment of the Umajalanta cave. These are two distinct subterranean streams that converge in the cave (altitude *c.* 2700 m).

Populations were sampled on two dates: November 1998 (early wet season) and July 1999 (dry season). Due to concern for conservation, and not having any previous information, a maximum of 30 individuals per population on each sampling occasion was captured, or even fewer if the density was low. Fishes were collected with a backpack electrofishing gear delivering a current of between 300 and 600 V (Smith-Root Inc., http://www.smith-root.com). Electrofishing is a satisfactory method for this kind of habitat made up of rocky streams as well as for studies of fragile or small populations. Individuals were preserved in buffered formaldehyde (4%) before transport to the laboratory.

MORPHOLOGICAL MEASUREMENTS

Four attributes were selected among those most commonly used in studies of fish adaptation to cave life (Ginet & Decou, 1977; Culver, 1982; Weber, 2000). They were measured in seven to 30 individuals from each population. The diameter of the eyes was measured horizontally and expressed as a ratio between the average of the diameter of the right and left eye and the standard length (L_S) of the individual. The asymmetry of eyes was estimated using the same measurements and corresponded to the ratio of the diameter of the right and left eye. The average length of the two maxillary barbels was measured from insert to tip and expressed as a ratio with L_S of the individual. The pigmentation was estimated by counting under a microscope the number of chromatophores on 1 mm^2 of three body parts: the right post-cephalic region, and the region of the lateral line at the tip of the pectoral and pelvic fins.

Eye size and pigmentation are known to present regression in cave species. An increase in eye asymmetry is also a characteristic of some cave species (Wilkens, 1988). An increase in barbel length is considered to be an adaptation to obtain more chemical and mechanical information (Weber, 2000; Wilkens, 2001).

The surface area of the four major distinctive parts of the encephalon was estimated for five individuals from each population (except from the Upper Umajalanta): telencephalon, mesencephalon, cerebellum and rhombencephalon (Fig. 2; Nieuwenhuys, 1982; Nalbant & Linares, 1987; Trajano, 1994). The surface area was estimated using the NIH Image free software (http://rsb.info.nih.gov/nih-image/) on a numerical photograph of the dorsal view of the dissected encephalon. Compared to epigean fishes, the main modifications that can be observed in the brain of troglobitic fishes are a reduction of the optic lobes (mesencephalon), and an enlargement of the telencephalon and the cerebellum (Poulson, 1963; Trajano, 1994).

REPRODUCTION

The trend towards more K-selected features of life history (or equilibrium strategy; Winemiller, 1989) is one of the main adaptations reported for cave species (Culver, 1982; Weber, 2000). Fecundity and egg diameter are often modified and cave fish species tend to have fewer but larger eggs (Poulson, 1963; Hüppop & Wilkens, 1991). Fecundity and egg diameter were determined using mature females (stage V; Bagenal, 1978). After the extraction of the intact gonads, all the eggs were counted to estimate fecundity. Finally, the diameter of 30 randomly selected eggs was measured with a micrometric scale under a microscope.

DATA ANALYSIS

Values of morphological and reproductive attributes were compared between the eight sampled populations using ANOVA. Only a small number of individuals was used to measure the encephalon and the reproductive attributes (Appendix), and this could bias ANOVA results. In these cases, the ANOVA tests were confirmed by a non-parametric

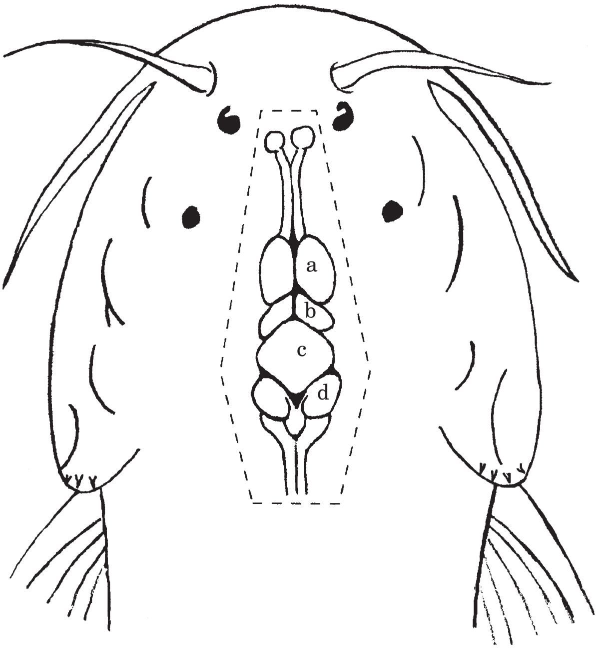

FIG. 2. Dorsal view of the dissected encephalon of a *Trichomycterus chaberti* from Umajalanta cave (120 mm L_S). Measured brain parts are indicated: a, telencephalon, b, mesencephalon, c, cerebellum and d, rhombencephalon.

Kruskal–Wallis test. When populations were significantly different, a Tukey pair-wise test was performed to see which populations differed from one another. All the tests were performed with SYSTAT software (Wilkinson *et al.*, 1992). A critical level of 5% ($P < 0{\cdot}05$) was used in determining the significance of the tests.

RESULTS

MORPHOLOGY

Eyes

Significant differences existed between eye diameters in the eight populations [ANOVA, d.f. = 7 and 169, $P < 0{\cdot}001$, Fig. 3(a)]. Pair-wise Tukey tests allowed three groups of populations to be distinguished: the populations living in the Umajalanta system (Umajalanta cave, Singani cave and Upper Umajalanta) had a smaller eye diameter; fishes belonging to the Laguna population had the largest eye diameter and the other populations represented intermediate values.

Reduction in eye diameter was asymmetric within populations and, depending on the population, affected one eye or the other [Fig. 3(b)]. Fishes from the Singani showed a reduction in the diameter of the right eye, whereas fishes from the Umajalanta showed a reduction in the size of the left eye. In canyon and headwater environments, fishes from the Upper Umajalanta, Laguna and Torotoro showed a reduction in the size of the left eye while fishes from the Rodeo showed a small but constant reduction in the size of the right eye.

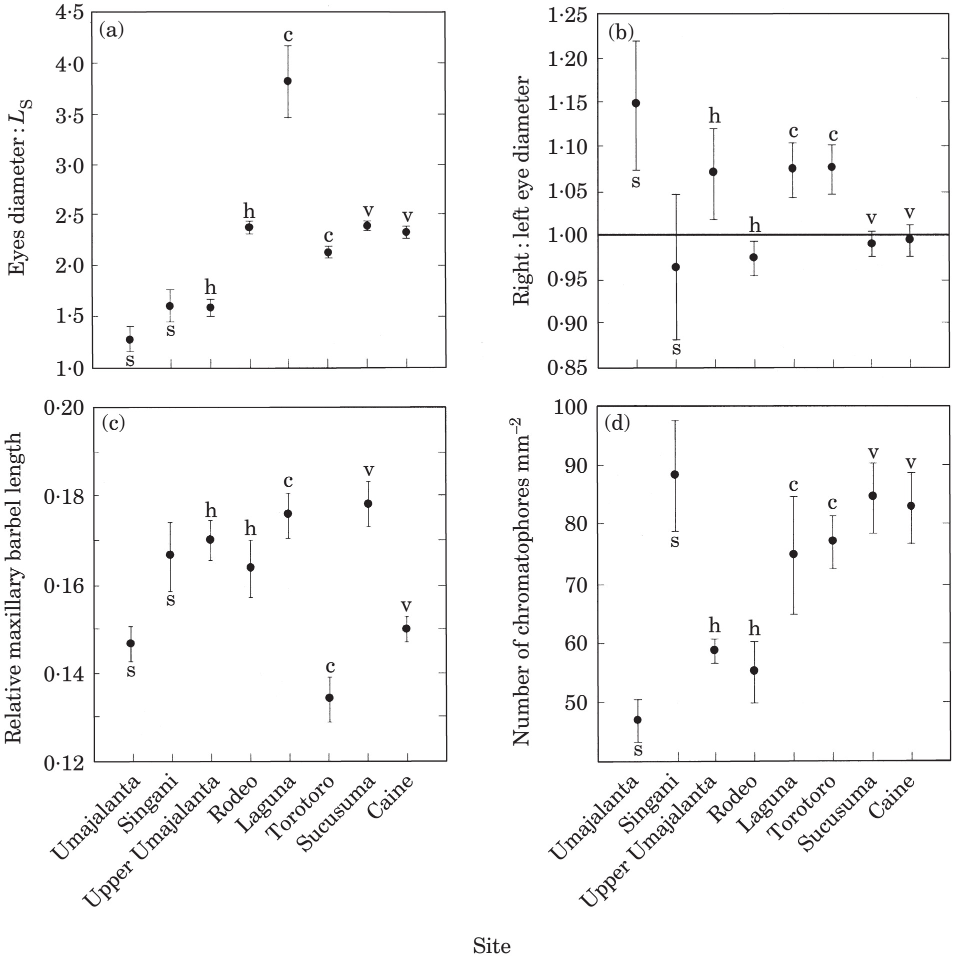

FIG. 3. Mean ± S.D. of (a) eye diameter : standard length ratio, (b) eye diameter asymmetry (horizontal line represents symmetry), (c) relative maxillary barbel length and (d) pigmentation for eight *Trichomycterus* spp. populations from Torotoro National Park. s, subterranean; h, headwater; c, canyon; v, valley populations. See Appendix for the number of individuals measured.

Populations living in valley streams displayed symmetric eyes. The level of variation appeared to be higher in cave populations than in epigean populations (S.D. of 0·32 for the Singani cave and 0·28 for the Umajalanta cave) and lower in valley populations (S.D. of 0·08 for Sucusuma and 0·09 for Caine). Canyon and headwater populations showed intermediate values (ranging from 0·11 to 0·15).

Barbels

There was a significant difference in the length of maxillary barbels in the eight populations [ANOVA, d.f. = 7 and 169, $P < 0{\cdot}001$). These differences, however, did not correspond to an environmental or spatial pattern. Pair-wise Tukey tests allowed three groups of populations to be distinguished [Fig. 3(c)]. The Laguna and Sucusuma populations had the longest barbels and these were significantly different (Tukey test probabilities $P < 0{\cdot}05$) from the Torotoro, Umajalanta cave and Caine populations. Populations from the Rodeo, the Singani cave and the Upper Umajalanta formed an intermediate group, which only showed a significant difference from Torotoro populations.

Pigmentation

All eight populations displayed significant differences in the density of chromatophores [ANOVA, d.f. = 7 and 169, $P < 0{\cdot}001$). Excepting the Singani cave population, the values of pigmentation followed a gradient from cave to headwater, canyon and valley populations [Fig. 3(d)]. A pair-wise Tukey test allowed three groups of streams to be distinguished. The Umajalanta cave and Rodeo populations showed significantly lower densities of chromatophores than the three populations from the Caine, Sucusuma and the Singani cave. The other populations showed intermediate and highly variable densities of chromatophores. The highest density of chromatophores was observed in the Singani cave population. In addition, uncoloured individuals were observed during sampling, as mentioned in the original description of the *T. chaberti* (Durand, 1968), but this was reversible if the individuals were exposed to sunlight for a few minutes. In this case, lightening seems to be produced by the concentration of melanin in the central part of chromatic cells.

Encephalon

There was no difference between the total surface area of the encephalon in the populations (ANOVA, d.f. = 6 and 28, $P = 0{\cdot}397$; Kruskal–Wallis test, KW, d.f. = 6, $P = 0{\cdot}511$). The telencephalon (ANOVA, d.f. = 6 and 28, $P < 0{\cdot}001$; KW, d.f. = 6, $P = 0{\cdot}001$), mesencephalon (ANOVA, d.f. = 6 and 28, $P < 0{\cdot}001$; KW, d.f. = 6, $P = 0{\cdot}004$) and cerebellum (ANOVA, d.f. = 6 and 28, $P = 0{\cdot}005$; KW, d.f. = 6, $P = 0{\cdot}006$) presented different relative surface areas between populations. Tukey tests showed that there was a significant difference in the telencephalon and mesencephalon between the cave populations (Umajalanta and Singani) and the epigean populations [Fig. 4(a), (b)]. The Tukey test performed on the cerebellum did not reveal significant differences between the populations, but a trend of declining values was observed from hypogean to headwater, canyon and valley populations [Fig. 4(c)]. There was no significant difference in the surface area of the rhombencephalon between the populations (ANOVA, d.f. = 6 and 28, $P = 0{\cdot}063$; KW, d.f. = 6, $P = 0{\cdot}065$), but, as in the case of the cerebellum, a trend of increasing values was observed toward the epigean populations from the valley [Fig. 4(d)].

REPRODUCTION

Due to the small number of individuals captured and the limited number of sampling occasions, the study failed to provide valid values for reproduction variables such as seasonality, sex ratio and size at the onset of maturity. The minimum size recorded for a female that had started a maturity cycle (stage II, Bagenal, 1978), ranged from 33 to 47 mm L_S in both epigean and hypogean populations. Except for the Singani and Sucusuma populations, the percentage of mature females appeared higher during the dry season (from 30 to 100% in

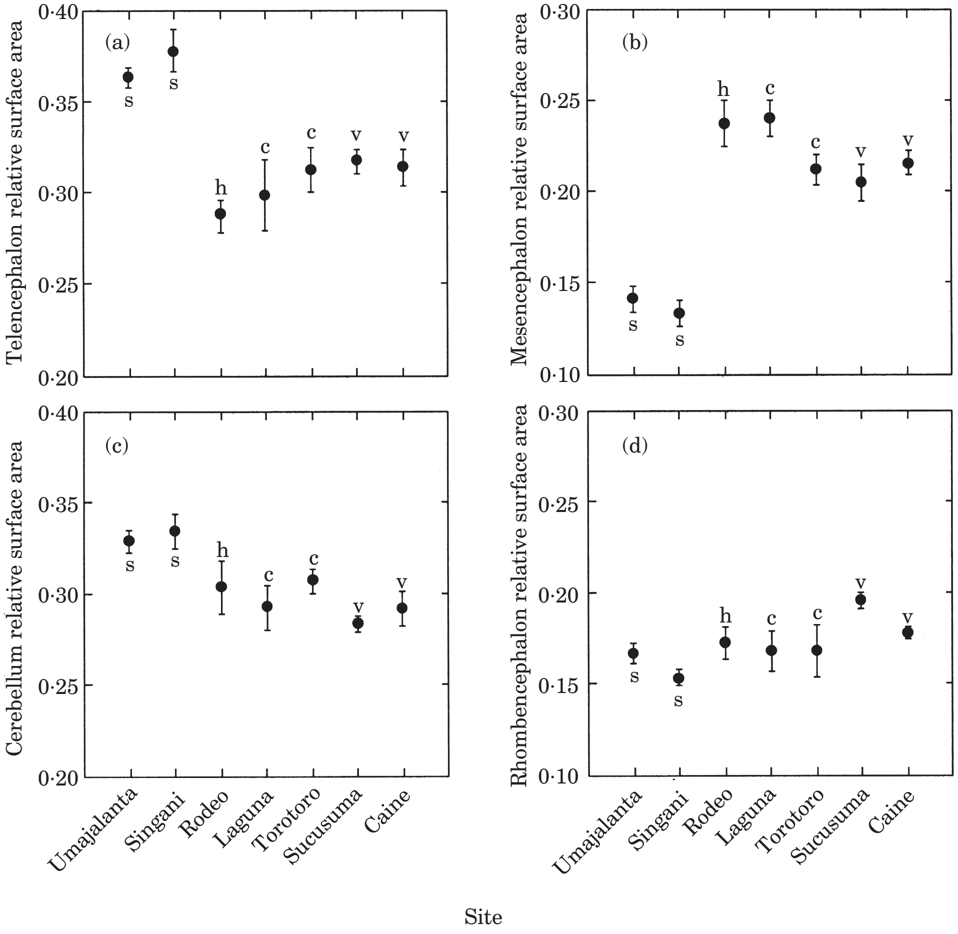

FIG. 4. Mean ± S.D. relative surface area of (a) telencephalon, (b) mesencephalon, (c) cerebellum and (d) rhombencephalon for seven *Trichomycterus* spp. populations from Torotoro National Park. s, subterranean; h, headwater; c, canyon; v, valley populations. See Appendix for the number of individuals measured.

July 1999) than at the beginning of the wet season (from 0 to 66% in November 1998). This may correspond to an increase in reproductive activity during the dry season (July).

It was impossible to undertake analysis of mature females in the Torotoro and Umajalanta cave populations, because of the low number of mature individuals (two and one, respectively). Fecundity (ANOVA, d.f. = 5 and 32, $P = 0{\cdot}027$; KW, d.f. = 5, $P = 0{\cdot}011$) and egg diameter (ANOVA, d.f. = 5 and 32, $P < 0{\cdot}001$; KW, d.f. = 5, $P < 0{\cdot}001$) differed significantly in the six populations. Fecundity values showed a trend to an increase in the number of eggs from subterranean (Singani) to headwater (Rodeo, Upper Umajalanta), to canyon (Laguna) and to valley populations (Caine, Sucusuma) [Fig. 5(a)]. Conversely, a reduction in egg size was observed from Singani and Upper Umajalanta populations to Laguna population, to Rodeo and to valley populations (Caine,

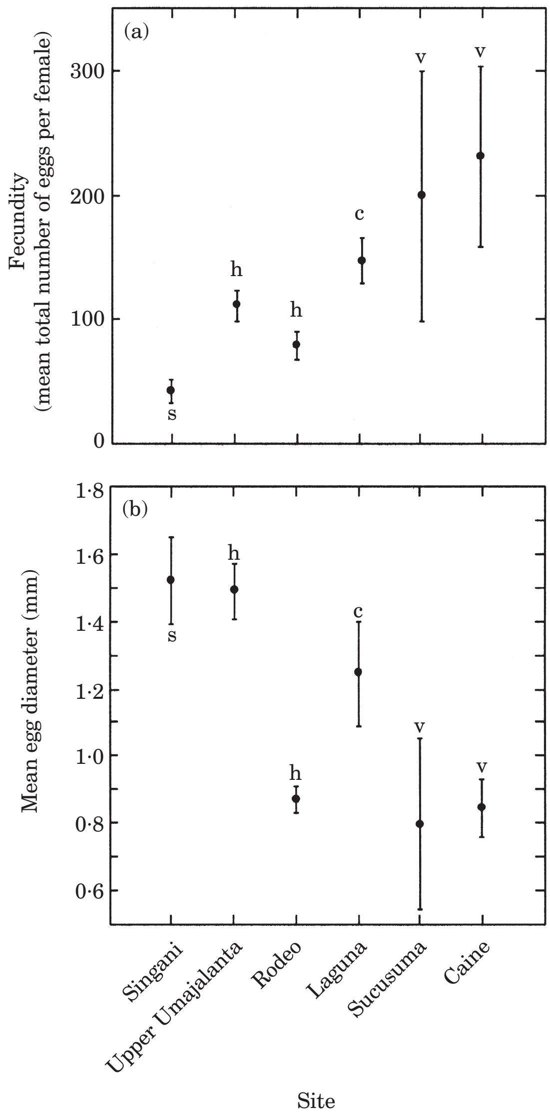

FIG. 5. Mean ± S.D. (a) fecundity and (b) egg diameter of 38 mature females from six *Trichomycterus* spp. populations from Torotoro National Park. s, subterranean; h, headwater; c, canyon; v, valley populations. See Appendix for the number of individuals measured.

Sucusuma). The Tukey test showed a significant difference between two extreme groups: the Singani and Upper Umajalanta populations had the largest eggs while Rodeo, Sucusuma and Caine had the smallest [Fig. 5(b)].

DISCUSSION

A reduction in eye diameter is the most obvious morphological feature of cave-restricted animals (Eigenmann, 1909; Culver, 1982; Weber, 2000). The eye regression process begins by a reduction in the size of the optic apparatus (eyes and optic lobes in the brain) that results in partial or total blindness. Depending on the length of isolation of the population, the regression process can be seen to evolve to total loss of the optic apparatus (anophthalmy) and, in the case of fishes, to profound modifications in the structure of the skull (Breder, 1944; Wilkens *et al.*, 1989). Eyes of hypogean *Trichomycterus* from Umajalanta and Singani streams were still present but showed a significant reduction in diameter in comparison to epigean populations. Moreover, this reduction in eye diameter was associated with a reduction in the surface area of the mesencephalon, clearly indicating the first step in a regression process as a result of the decrease in optical activity. In addition, right : left asymmetry was higher and more variable in hypogean than in epigean *Trichomycterus*. Wilkens (2001) observed the same pattern for the blind Pimelodidae, *Rhamdia reddelli* Miller, and noted that this phenomenon is correlated with the process of ontogenetic regression. Symmetry is likely to be an important feature for the efficient functioning of a paired organ, thus asymmetry may be considered as an indication of the beginning of a regression process due to loss of function. In another way, the fluctuating asymmetry hypothesis (FA) assumes that the differences in the expression of a character between the right and left side of a symmetric organism provide a measure of developmental instability (Palmer & Strobeck, 1986; Parsons, 1992). This measure could be indicative of a stress that can result from internal genetic or external environmental factors. In the present study, the observed patterns of eye diameter showed increased asymmetry from valley populations to canyon and headwater populations and finally to hypogean populations. According to the FA, it can be assumed that the asymmetry of the eyes is not only due to the contrast between epigean and hypogean environments but that it is also the consequence of a gradient of environmental constraints.

As compensation for the loss of vision, cave animals may enhance their chemical and mechanic sensory perception. *Trichomycterus chaberti* did not show any evidence of increased barbel length as an adaptation to cave life, as has been observed in two species of blind Pimelodidae, *Rhamdia reddelli* Miller and the blind form of *Rhamdia laticauda* (Kner) (Wilkens, 2001). Trichomycteridae, like the majority of siluriform fishes, possess well developed barbels, which is considered to be a pre-adaptation to cave life (Bertin, 1958; Weber, 2000). One reason for the lack of pattern in barbel length could be that in the case of the *Trichomycterus*, these organs are already sufficiently long to sustain an increase in activity.

The relative size of a peripheral organ is positively correlated with the size of the related brain centre, and can give an indication of the relative importance of a particular sensory capacity (Kotrschal *et al.*, 1998). Bolivian *Trichomycterus* showed similar brain structure to *Trichomycterus guianensis* (Eigenmann) from Venezuela (Nalbant & Linares, 1987) except for the mesencephalon which appeared to be reduced in the present observations. Besides the reduction in the size of the optic lobes (mesencephalon), *T. chaberti* showed a marked enlargement of the telecenphalon in comparison to the epigean *T. cf. barbouri*. Similar results have been observed for several troglobitic fishes and interpreted as an improvement in olfaction, topographical memory and social behaviours

(Trajano, 1994). In the present results, there was a marked difference in the mesencephalon and the telencephalon between epigean and hypogean populations, although there was only a gradual modification in the rhombencephalon and cerebellum along the environmental gradient. Thus a direct sensory compensation for loss of vision by the senses associated with the telencephalon (mainly olfaction and topographic memory) may be hypothesized in the case of *T. chaberti*, completed by a gradual enlargement of the cerebellum (complex integrative centre for equilibrium and mechano-sensitivity; Poulson, 1963). Although not significant, the decreasing size of the rhombencephalon toward the hypogean populations, associated with the observed stability in barbel length may indicate that taste (one of the senses associated with this organ; Nieuwenhuys, 1982) was not improved in the case of *T. chaberti*.

The second most obvious morphological feature associated with cave animals is the reduction in melanic pigmentation (Ginet & Decou, 1977; Culver, 1982; Weber, 2000). In *Trichomycterus* populations from Torotoro, values for the density of chromatophores follow a gradient of decreasing values in fishes from valley streams to canyon, headwater and subterranean streams. Because of this gradient, and because of the high level of pigmentation of the hypogean population of *T. chaberti* inhabiting the Singani stream [Fig. 3(d)], the reduction in pigmentation cannot only be explained by the contrast between hypogean and superficial environments. Cryptic colouration in fishes is interpreted as a protection against visually orientated predation (Endler, 1980). *Trichomycterus* populations of valley streams are part of a multispecific assemblage, and could thus be subject to predation by aquatic, aerial and terrestrial predators. *Trichomycterus* populations in mountain streams (canyon and headwater) are in a monospecific situation and are *a priori* only subject to predation by aerial and terrestrial predators. *Trichomycterus chaberti* populations are in a monospecific situation with no visual predators. Thus, a loss of pigmentation associated with a decrease in predation may be hypothesized at the same time as a consequence of the absence of light.

For logistic reasons, the study of reproduction could not be conducted through a complete seasonal cycle, limiting the interpretation of temporal variation in reproductive activity. In contrast with observations for the cave species *T. itacarambiensis*, whose reproductive activity occurs during the rainy season (Trajano, 1997), in Torotoro populations, more mature females were observed during the dry season.

Changes in the values of fecundity and egg size resulted in a change in the reproductive strategy of the populations, which could be linked to the environmental conditions of the streams. The population of *T. chaberti* in the Umajalanta cave laid larger but fewer eggs than epigean *T. cf. barbouri* populations. A similar difference in egg size was found between epigean and hypogean populations of amblyopsid fishes (Poulson, 1963). This trend characterises a K-strategy in the r-K continuum. A similar trend toward K-selected features has been described for the troglobitic Amblyopsidae (Poulson, 1963), *Pimelodella kronei* (Miranda-Ribeiro), (Trajano, 1991) and *T. itacarambiensis* from Brazil (Trajano, 1997) based on growth, longevity and frequency of reproduction. In their analysis of 216 North American fish species, Winemiller & Rose (1992) considered that the cave fishes *Amblyopsis rosae* (Eigenmann) and *Amplyopsis* *spelaea* DeKay constitute the extreme forms of the equilibrium strategy, equivalent to the K-strategy (Winemiller, 1989). The trend towards K-strategy features of reproduction in the troglobitic species could be interpreted as an adaptation to food scarcity in a hypogean environment (Culver, 1982; Hüppop, 2000). The increase in egg size could provide better autonomy at the beginning of the larval stage and could subsequently be favourable for the development of the individual in streams with a low food supply by providing higher resistance to starvation and higher mobility for food foraging (Poulson & White, 1969).

In the present study, eye diameter and brain structure presented values that opposed epigean and hypogean populations. Eye regression clearly appears as a distinctive character of troglobitic organisms that may involve compensation by other sensorial systems (chemical and mechanical). The observed pattern of modification of the other variables (pigmentation, eye asymmetry, fecundity and egg diameter), however, corresponded to a gradient of values from valley to canyon, headwater and subterranean streams. Specialization of *T. chaberti* to cave life appears to be the result of two kinds of environmental constraints, a distinct one between epigean and hypogean habitats (light and darkness) and a gradual one that may result from the combination of multiple factors (*e.g.* predation and food supply), in which caves correspond to an extreme situation.

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APPENDIX. Summary of data of number of individuals, standard length range and mean ± S.D. of morphological, brain and reproductive attributes for eight populations of *Trichomycterus* spp. from Torotoro National Park, Bolivia (see Fig. 1).

Morphology

Environment	Stream	n	L_S range (mm)	Eye diameter : L_S	Eye asymmetry[1]	Barbel length : L_S	Pigmentation[2]
Valley	Caine	30	33·9–68·2	2·34 ± 0·32	0·99 ± 0·10	0·15 ± 0·02	82·81 ± 32·48
Valley	Sucusuma	30	34·3–65·9	2·40 ± 0·30	0·99 ± 0·08	0·18 ± 0·03	84·62 ± 32·76
Canyon	Laguna	20	30·2–81·6	3·82 ± 1·62	1·07 ± 0·14	0·18 ± 0·02	74·80 ± 44·50
Canyon	Torotoro	30	31·1–81·6	2·14 ± 0·32	1·08 ± 0·15	0·13 ± 0·03	76·98 ± 25·08
Headwater	Rodeo	30	32·6–75·4	2·39 ± 0·35	0·97 ± 0·11	0·16 ± 0·04	54·84 ± 29·10
Headwater	Upper Umajalanta	7	68·0–87·5	1·59 ± 0·22	1·07 ± 0·14	0·17 ± 0·01	58·53 ± 5·12
Cave	Umajalanta Cave	15	41·5–103·7	1·27 ± 0·50	1·15 ± 0·28	0·15 ± 0·02	46·55 ± 13·96
Cave	Singani	15	41·7–82·8	1·61 ± 0·66	0·96 ± 0·32	0·17 ± 0·03	88·23 ± 36·71

[1] Right eye diameter : left eye diameter.
[2] Number of chromatophores mm^{-2}.

Brain				Relative surface (%)			
Environment	Stream	n	L_S range (mm)	Telencephalon	Mesencephalon	Cerebellum	Hombencephalon
Valley	Caine	5	33·9–68·2	0·31 ± 0·02	0·22 ± 0·02	0·29 ± 0·02	0·18 ± 0·01
Valey	Sucusuma	5	41·4–65·9	0·32 ± 0·02	0·20 ± 0·02	0·28 ± 0·01	0·20 ± 0·01
Canyon	Laguna	5	45·3–66·0	0·30 ± 0·04	0·24 ± 0·02	0·29 ± 0·03	0·17 ± 0·02
Canyon	Torotoro	5	43·7–81·6	0·31 ± 0·03	0·21 ± 0·02	0·31 ± 0·02	0·17 ± 0·03
Headwater	Rodeo	5	32·6–60·0	0·29 ± 0·02	0·24 ± 0·03	0·30 ± 0·03	0·17 ± 0·02
Cave	Umajalanta Cave	5	41·5–93·9	0·36 ± 0·01	0·14 ± 0·02	0·33 ± 0·01	0·17 ± 0·01
Cave	Singani	5	41·7–82·8	0·38 ± 0·03	0·13 ± 0·02	0·33 ± 0·02	0·15 ± 0·01

Reproduction					
Environment	Stream	*n*[1]	L_S range (mm)	Fecundity[2]	Egg diameter[3]
Valley	Caine	7	61·0–70·9	231·29 ± 192·76	0·84 ± 0·23
Valley	Sucusuma	3	44·7–61·7	199·33 ± 174·73	0·80 ± 0·44
Canyon	Laguna	4	58·7–77·8	147·50 ± 36·79	1·25 ± 0·31
Headwater	Rodeo	9	37·3–47·1	79·00 ± 35·24	0·87 ± 0·12
Headwater	Upper Umajalanta	11	53·9–97·6	111·64 ± 41·69	1·49 ± 0·27
Cave	Umajalanta Cave	4	44·0–68·8	42·25 ± 18·37	1·52 ± 0·26

[1] Mature female of stage V (Bagenal, 1978).

[2] Total number of eggs per female.

[3] Estimation from 30 eggs per female.