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# Comparative performances of non-visual food search in the hypogean cyprinid *Phreatichthys andruzzii* and in the epigean relative *Barbus filamentosus* *

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## SUMMARY

The anophthalmic hypogean cyprinid *Phreatichthys andruzzii* Vinciguerra shows a more efficient food searching behaviour than blinded specimens of the epigean species *Barbus filamentosus* Cuvier and Valenciennes. This difference seems to be related to a tendency to swim near the bottom of the aquarium and the behavioural stereotypes in food localization and intake. Both in *P. andruzzii* and in the blinded *B. filamentosus* food search is guided by chemical stimulation. Other data indicate that mechanical stimulation could play an important role in the feeding behaviour of the hypogean form. The behavioural analogies in food localization between *P. andruzzii* and the cave forms of the characid *Astyanax fasciatus* Cuvier are briefly discussed.

## INTRODUCTION

Fish adaptation to a subterranean life is characterized by a clear evolutionary convergence in regressive features such as eye and optic lobe regression and pigment reduction. Besides these regressive traits, which are common to almost all the subterranean fish (Thinès, 1969), a few forms also show some constructive traits. These appear as a result of natural selection based on the scarce supply of trophic resources, which generally characterizes the subterranean waters (Poulson, 1963; Barr, 1968; Culver, 1982; Wilkens, 1988). An example is represented by the characid *Astyanax fasciatus* Cuvier, where constructive features shown by the cave dwelling

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populations involve an improved efficiency in feeding behaviour, in resisting starvation and in energy economy (Wilkens, 1988). Moreover the constructive traits show some variability in intensity according to the degree of cave adaptation shown by each population. Similar relationships have been pointed out in amblyopsid fish such as *Chologaster agassizi* Putnam, *Typhlichthys subterraneus* Girard, *Amblyopsis spelaea* Dekay and *A. rosae* Eigenmann which are differently adapted to cave life (Poulson, 1963).

The cyprinid *Phreatichthys andruzzii* Vinciguerra, a phreatic species from Somalia closely related to the genus *Barbus*, is thought to represent (Vandel, 1965) one of the forms of most ancient adaptation to the subterranean habitat as shown by the importance of both regressive and constructive features. Scales and pigmentation are fully absent (Vinciguerra, 1924), and the anophthalmia, accompanied by the loss of the optic nerves, is complete (Ercolini and Berti, 1975). A strong reduction of the entire encephalon, and in particular of the optic lobes, is also present (Ercolini and Berti, 1975). The high degree of adaptation to a subterranean life is also confirmed by the presence of an extremely reduced metabolic rate, as seen by comparing closely related epigean and hypogean cyprinids (Ercolini et al., 1987).

In such a well adapted hypogean form, the existence of other evolutionary constructive traits appeared very probable. The aim of the present study was to verify if adaptation to hypogean life has been accompanied, in *P. andruzzii*, by an increase in the efficiency of food searching behaviour and intake of necessity based on and coordinated by non-visual sensory stimulation.

## MATERIALS AND METHODS

For the present study a group of *Phreatichthys andruzzii* Vinciguerra (Pisces Cyprinidae), collected in December 1982 in a well at Gheriale (4°07'N - 46°29'15''E; Central Somalia), and a group of *Barbus filamentosus* Cuvier and Valenciennes, bought in November 1987 from a dealer, were used. The latter species belongs to the epigean genus considered the closest to *Phreatichthys* (Vinciguerra, 1924).

In order to test the basic hypothesis, the efficiency of the non-visual feeding activity of both species has been compared. In the epigean fish, any optic-motor coordination was excluded not only during the tests but also for a long period before the experiments. Such a pre-experimental treatment appeared essential to permit the fish to be accustomed to finding food without the aid of sight and to allow any possible sensory-motor coordination, based on non-visual stimulation, to be enhanced.

One year after that the *B. filamentosus* were purchased, twenty of them, with an equal number of *P. andruzzii*, were anaesthetized with Ms 222 and subjected to surgery. The *B. filamentosus* were blinded by removing the eyeballs, whereas the *P. andruzzii* were lanced on both sides of the head in the areas corresponding *Barbus* eyes, so as to cause trauma as similar as possible to that experienced by *B. filamentosus*[1]. Both the trauma of the operation and the effects of the anaesthesia were quickly overcome. Regular feeding was observed four days after the operation, when food was given for the first time. For the three following months nourishment consisted of dried food commonly used for tropical ornamental fish and thawed chironomid larvae. Later only the larvae were supplied. Food was supplied daily, in a single dose. After a few hours any remaining food was removed. The aquaria were kept in a room weakly illuminated by orange lights so as to enable spot checks and routine maintenance. Light was kept continuous and constant.

Approximately six months after surgery, 16 fish were randomly chosen from each of the two groups. The 32 specimens were distributed in four aquaria so as to make four experimental groups: a monospecific group of 8 *P. andruzzii*, a monospecific group of 8 *B. filamentosus* and two mixed groups, each of 4 *P. andruzzii* and 4 *B. filamentosus*. The test aquaria (100 × 40 × 50 cm) were subdivided in two sections. A carbon filter and a heating system were installed in the smaller section (20 × 40 × 50 cm) whereas the fish were placed in the larger compartment (80 × 40 × 50 cm). Water depth was 40 cm, water temperature 27 °C. Four horizontal layers 10 cm thick were indicated by thin strips of coloured tape applied to the acquaria walls.

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[1] Alternative procedures that excluded surgical injuries were considered. The only one wich appeared able to meet the basic requirement of the experimental design (i.e. the exclusion of any optic-motor coordination in the epigean fish during both the tests and the pre-experimental training) was to keep and test fish in complete darkness, using an infra-red night-sight device for both tests and maintenance operations of the aquaria. The procedure was unworkable: the infra-red apparatus, perfectly fit to carry out the experiments, was not suitable for the routine maintenance operations.

The observations started about three months from the formation of the four groups, nine months after surgery. These were conducted using a method similar to the one already adopted in a study of the food finding ability of the hypogean and epigean forms of the characid *Astyanax fasciatus* Cuvier (Hüppop, 1987).

Each group of fish was starved for a 24 hr period, and then single chironomid larvae were successively offered. A time interval of 10 minutes was strictly kept between an eaten larva and the next one supplied. Each larva was offered in such a way as to ensure that it would sink to the bottom of the aquarium and not float on the water surface. The latency, from the moment the larva was introduced in the aquarium to the moment it was eaten, was recorded. If the larva was eaten before hitting the bottom of the tank, the layer in which it had been taken was registered. Obviously in the mixed fish groups the species that ate the prey also was recorded. At the end of each experimental session all fish were fed *ad libitum*.

From September to October 1989, during a four week period, 24 experimental sessions were carried out with each group. During each experimental session a total of 16 larvae were successively supplied, giving a total of 384 food tests performed with each group.

## RESULTS

Observations conducted on both the monospecific and mixed groups revealed a greater efficiency in food localization in the hypogean form.

Quantitative results of the tests carried out on the monospecific groups are shown in Table 1. In 384 food tests, food was eaten before hitting the bottom 23 times by *Phreatichthys andruzzii* and only twice by *Barbus filamentosus*. The mean latency of the fish feeding on the sunken larvae was of 33.6 sec for the former group and of 76.5 sec for the latter, a 1 : 2 ratio. The one-tailed Kolmogorov-Smirnov test for large samples (Siegel, 1956) was used for the statistical analysis of the latency distributions. The test shows a highly significant difference ($D = .604$; $p < .001$) between the two species.

The data relative to the test conducted on the mixed groups are shown in Table 2, and substantially confirm the results obtained

Table 1 — Comparative food finding performance in *Phreatichthys andruzzii* and blinded *Barbus filamentosus* (monospecific groups). Number of larvae presented to each group: 384.

| | | *Phreatichthys andruzzii* | | *Barbus filamentosus* | |
|---|---|---|---|---|---|
| Water layer | Height above bottom (cm) | No. of eaten food items | Mean time of intake (sec) | No. of eaten food items | Mean time of intake (sec) |
| IV | 30-40 | 0 | | 0 | |
| III | 20-30 | 0 | | 1 | 10.8 |
| II | 10-20 | 5 | 10.9 | 1 | 10.2 |
| I | 0-10 | 18 | 17.3 | 0 | |
| bottom | —— | 361 | 33.6 | 382 | 76.5 |

with the monospecific groups. Once again, better performances were revealed by the hypogean fishes as compared to the blinded epigean ones. In the 768 food tests, conducted on the two mixed groups, sinking food was eaten 39 times by *P. andruzzii*, 3 times by *B. filamentosus*. Of the 726 sunken larvae, 714 were eaten by the former species and only 12 by the latter. While the mean latency of feeding on the bottom recorded for *P. andruzzii* is substantially similar to the one registered during the tests conducted on the monospecific group, the mean latency for *B. filamentosus* was greatly reduced and appears equal to that recorded for the hypogean species. An hypothetical improvement in the food finding

Table 2 — Comparative food finding performance in *Phreatichthys andruzzii* and blinded *Barbus filamentosus* (mixed groups). Total number of larvae presented to the two groups: 768.

| | | *Phreatichthys andruzzii* | | *Barbus filamentosus* | |
|---|---|---|---|---|---|
| Water layer | Height above bottom (cm) | No. of eaten food items | Mean time of intake (sec) | No. of eaten food items | Mean time of intake (sec) |
| IV | 30-40 | 0 | | 2 | 2.7 |
| III | 20-30 | 6 | 5.8 | 1 | 6.6 |
| II | 10-20 | 10 | 11.0 | 0 | |
| I | 0-10 | 23 | 18.4 | 0 | |
| bottom | —— | 714 | 35.4 | 12 | 33.8 |

ability of *B. filamentosus* due to the interspecific competition can be excluded as an artefact of the testing procedure. In fact, in consequence of the greater capacity in food finding shown by the hypogean species, almost all the food items were consumed by the *P. andruzzii* specimens and the epigean fishes could eat only when they exceptionally localized the prey during the earlier phases of the test. Since the probability that a similar event could happen is evidently very low, both the remarkable decrease of the mean latency and the very small amount of food items eaten appear fully explained. Given the great differences in performance registered between the two species, a statistical analysis of the data appeared to be superfluous.

No particular tendency to occupy the lower part of the aquarium was shown by the blinded *B. filamentosus*. Generally the fish swam in each layer with the same frequency and only in some cases a single specimen would swim near the bottom of the tank, skimming it ventrally. Food presence was detected at mid-water by crossing the odorous trace left by the sinking larva and a sudden increase in swimming movements was generally registered. The whole group was then seized with a frantic locomotory activity, characterized by horizontal and non-orientated movement patterns and by sudden short vertical excursions. Food search would start when one individual, later followed by other specimens, swam towards the bottom. At this point the fish would start to swim frequently touching the ground with the tip of their snouts and interrupting their locomotory activities with quick snappings, in the course of which their body axis was considerably inclined to the bottom. As the search proceeded the initially rare snappings would become more frequent and would be characterized by larger angles of inclination. Near the food a long series of snappings would be performed by the fish, while maintaining a steep angle of about 90° with the bottom rotating around their body axis. Food intake seemed to depend on a largely random contact between the lips and the larva. Sensorimotor coordinations did not seem very effective and sometimes food was hit or moved by the fish's snout without being eaten.

The tendency of *P. andruzzii* to swim near the bottom of the aquarium and to skim it ventrally was greater than in *B. filamentosus*. Consequently food presence was traced not only at mid-water by crossing the odorous trace left by the sinking larva but also by passing near the point where the larva had hit the bottom surface.

In this second case, once the fish had realized the presence of food, it would suddenly slow down its swimming movements. Then the fish would proceed slowly skimming the ground with the ventral parts of the head forming a 20° angle and frequently it would change direction. The search for food lasted a few seconds and seemed to pinpoint the prey's location given that ingestion did not necessarily involve prior contact with the larva itself. When, instead, food presence was detected at mid-water by crossing the scented track left by the sinking larva the fish would change direction abruptly and spiral almost vertically down around the scent, often capturing the larva before it reached the bottom. A great sensory capacity and sensori-motor coordination was demostrated by the fact that the fish, swimming along the bottom of the aquarium at a certain distance from a food item placed laterally, would suddenly stop and rapidly turn towards the food without missing the mark. If the fish causally encountered the spot where the food was present, it could be ingested even before it was contacted. The food intake by a fish was usually followed by a sudden increase in the locomotory activity of its tank mates which converged on the spot and started an intense food search.

## DISCUSSION

The greater efficiency in food search shown by *Phreatichthys andruzzii* as compared to the blinded *Barbus filamentosus* indicates that adaptation to the hypogean habitat was accompanied, in this species also, by the acquisition of behavioural constructive traits.

The better performances of the hypogean form do not seem to be due to a precocious discovery of the food presence, which took about the same time in both species. The higher ability shown by *P. andruzzii* to find food seems to be mainly related to its tendency to occupy the lower levels of the tank and to swim along the bottom surface, as well as to its greater efficiency in locating and grabbing the prey. In the blinded *B. filamentosus* the polarisation of the locomotory activity on the bottom of the aquarium, registered in blinded *B. conchonius* (Heuts and Thinès, 1971; Thinès and Capon, 1975), did not appear. *B. filamentosus* rarely swims along the bottom surface and in this way differs greatly from *P. andruzzii*. Consequently, while in *P. andruzzii* food search on the bottom starts immediately after the discovery of its presence, in *B. filamentosus* a certain amount of time elapses between the two events.

In the blinded *B. filamentosus* specimens an important role for food localization seems to be played by the direct contact of the fish's snout with the ground surface. This is achieved by a strong inclination of the fish's body axis with the bottom. The headstanding position, a feeding behavioural pattern previously described for other epigean species experimentally deprived of visual coordination (Schemmel, 1967; Glaser, 1968; Heuts and Thinès, 1971; Thinès and Capon, 1975; Hüppop, 1987), probably facilitates the perception of chemical stimulation through taste (Schemmel, 1980).

In *P. andruzzii* food localization does not seem to depend on the contact of the fish's mouth with the bottom surface, which is merely skimmed even when the fish's body axis tends to lean, and appears to be characterized by a small inclination of the body axis with the bottom. The searching behaviour is substantially similar to the one shown by the cave dwelling populations of the characid *Astyanax fasciatus* (Schemmel, 1980; Hüppop, 1987). Also the latter forms show, as compared to the relative epigean populations, a clear reduction of the subtended angle, which seems to be related to the great improvement of their taste apparatus (Schemmel, 1980). As in *A. fasciatus* (Schemmel, 1967), the adaptation of *P. andruzzii* to cave life is not associated with an improvement of the olfactory organ (Delfino et al., 1981). No data regarding the development of the taste apparatus of *P. andruzzii* are at present available, but the fact that both hypogean forms have a much more efficient food finding behaviour than the relative epigean forms and that an extraordinary similarity between their food localization behavioural patterns is shown, suggests that in *P. andruzzii* also the gustatory equipment is particularly developed.

The similar behavioural stereotypes that characterize food search in *P. andruzzii* and in the cave populations of *A. fasciatus*, two species rather far apart from a systematical point of view, represent a clear example of evolutionary convergence. The fact that the angles subtended by the fish's body axis with the bottom surface are much smaller in *P. andruzzii* than in the hypogean populations of *A. fasciatus* suggests that the behavioural divergence from the corresponding epigean form appears to be larger in the former than in the latter. This suggests that adaptation to subterranean life is characterized, from a behavioural point of view, by some variability related to the phylogenetical age of cave colonization.

Both in the blinded *B. filamentosus* and in the anophthalmic *P. andruzzii* food search seems to be released and guided only by chemical stimulation. But other considerations make us hypothesize that mechanical stimulation may play an important role in the feeding behaviour *sensu lato* of the hypogean form. In fact, both the immediacy and the simultaneity with which the group converges on the spot where the food item was eaten on the ground indicates that the response is presumably guided by mechanical stimulations released by the fish when ingesting the prey. The ingestion of a prey clearly signals the presence of a potential food source. The acquisition of a behaviour that facilitates its immediate exploitation has a highly adaptive significance in a habitat, so poor in food as the subterranean one. Moreover, the accuracy shown in converging on the spot seems to indicate a highly efficient mechanoreceptor apparatus. Only future observations with live prey will be able to clarify the role played by mechanical stimulation in food localization.

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