

sufficient reproductive motivation to orient to a chemical stimulus, not itself a food odor, may not have been adequately replaced by the motivation to search for food in 'home' waters.

Finally, comment must be made about the apparently different mechanisms underlying food choice behavior of the hatchlings and chemosensory choice behavior of the juveniles. These results may be due to an underlying biological difference in selecting food versus responding to non-food chemical cues, or to the difference in experimental protocol, i.e., a 'reward' versus a 'non-reward' situation. Because juvenile loggerheads are subject to the same patchy distribution of food in the marine environment as are hatchlings and thus would equally benefit from food choice behavior following the PR model, we speculate that the former is true. This is an easily-testable hypothesis, and is the focus of research planned by the third author.

- 1 This work was supported by the Texas A & M University Sea Grant College Program, grant No. 4A79AA-D-00127. Turtles were collected and maintained under Federal Wildlife Permit PRT 2-3456 and Florida Department of Natural Resources Permit TP-72.
- 2 Grassman, M., and Owens, D., *Anim. Behav.* 35 (1987) 929.
- 3 Grassman, M., and Owens, D., *Copeia* 1982 (1982) 965.
- 4 Owens, D. W., Grassman, M. A., and Hendrickson, J. R., *Herpetologia* 38 (1982) 124.
- 5 Carr, A. F., *So Excellente a Fische*. Natural History Press, New York 1967.
- 6 Cooper, J. E., Scholz, A. T., Horrall, R. M., Hasler, A. D., and Madison, D. M., *J. Fish. Res. Bd. Can.* 33 (1976) 307.
- 7 Dawkins, R., *Anim. Behav.* 17 (1969) 120.
- 8 Guthrie, D. M., *Neuroethology*, p. 34. John Wiley, New York-Toronto 1980.

0014-4754/89/020202-04\$1.50 + 0.20/0

© Birkhäuser Verlag Basel, 1989

Locomotory response of *Phreatichthys andruzzii* Vinciguerra (Pisces, Cyprinidae) to chemical signals of conspecifics and of closely related species

R. Berti, R. Vezzosi and A. Ercolini

Dipartimento di Biologia Animale e Genetica dell'Università, Via Romana 17, I-50125 Firenze (Italy)

Received 27 July 1988; accepted 7 November 1988

Summary. The phreatic cyprinid *Phreatichthys andruzzii* Vinciguerra, tested singly in a choice-apparatus, is strongly attracted to the chemical signals of unfamiliar conspecifics, while it is completely indifferent to the chemical cues of *Barbus semifasciatus* var. *schuberti*, a species that is close systematically. The stimulus and response are evidently species-specific.

Key words. Cave fish; chemoreception; locomotory response.

The attraction of isolated specimens to conspecific odor has long been known in several species of epigean fish¹⁻⁴. Recent experiments verified the existence of an analogous phenomenon in the blind cave forms of *Astyanax mexicanus* Filippi^{5,6} and in the hypogean species *Caecobarbus geertsi* Boulenger⁷ and *Phreatichthys andruzzii* Vinciguerra⁸. Specimens of the cave forms of *A. mexicanus* can discriminate not only between the odor of conspecific groups of different size (4, 8, 16, 32 individuals) tested in pairs, systematically preferring the larger ones^{9,10}, but also between familiar and unfamiliar groups of equal size, where they show a preference for the familiar group⁶. Similarly, in *P. andruzzii* the attraction to chemical information of conspecifics is more immediate and intense when they are familiar to the test fish⁸. In the light of these findings we decided to investigate whether the reaction of this species – which shows very marked morphological regression and functional adaptations related to the subterranean habitat^{11,12} – has remained species-specific or has become merely a response to generic fish-odor. The loss of the species-specificity of the chemical stimulus and/or of the response to this, brought about in the process of their adaptation to a subterranean habitat, where there are no known sympatric species, would not have compromised either the biological function of the stimulus or the significance of the biological response. The locomotory response of *P. andruzzii* to the chemical traces of unfamiliar conspecifics and of *Barbus schuberti*, the golden variety^{13,14} of *B. semifasciatus* Günther, which is an epigean species close to it systematically, was tested using the method employed in earlier experiments^{7,8}.

Materials and methods. Three groups of native *P. andruzzii* and one group of *B. schuberti* were used. Two of the three groups of *P. andruzzii* (A1: 32 specimens; A2: 33 specimens) came from wells in the locality of Bud-Bud (Central Soma-

lia) and one (B: 31 specimens) from a well in Gheriale, 7 km south of Bud-Bud. The group of *B. schuberti* (C: 35 specimens) was purchased from a pet store. Both the groups of *P. andruzzii* and *B. schuberti* were kept in similar glass aquariums (100 × 40 × 50 cm, water height 40 cm) with an adsorbing charcoal filter and aerator, taking all the necessary precautions so that there was no passage of chemical information between the various aquariums either during normal maintenance or testing. Sixty days before testing, the aquariums were emptied and washed, and both the filter material and water renewed. Six days prior to testing the operation was repeated without renewing the water.

The test aquarium was a narrow glass corridor (105 × 8 × 20 cm, water height 8 cm) divided by removable partitions into three compartments 35-cm long. The test fish was selected from either group A1 or A2 and placed in the central section of the test aquarium where it was allowed to acclimatize for 9 to 13 h, considered sufficient time for the animal to overcome the stress of transfer. Then two 300 cc samples of water – one odorless and one taken from either the home aquarium of group B (A × B tests) or group C (A × C tests) – were simultaneously poured into the two end compartments. For each experiment, the introduction of the 'odorous' sample in the right or left end was determined using a table of random numbers. For both types of tests (A × B; A × C) the odorous sample was poured the same number of times in either end. After 5 min, sufficient for the two samples to spread in the end compartments, the partitions were removed and the fish was then free to swim the length of the test aquarium. At this point the presence of the fish in each of the three compartments, whose borders were indicated by the position of the partition slots, was recorded every 30 s for 30 min for a total of 60 successive registrations. During each experimental session an equal number of both types of tests

($A \times B$; $A \times C$) were performed contemporaneously. At the end of the test, the test aquariums were hand-scrubbed and then rinsed with a high pressure (70 atm) spray. The water used to fill the test aquariums and for the odorless samples was obtained from a tank with an adsorbing charcoal filter like the ones in the home aquariums. The tank, test and home aquariums were in the same room, constantly illuminated by filament lamps which emitted a weak red light corresponding to the wavelengths to which *P. andruzzii* is least photophobic¹⁵. The lamps and test aquariums were arranged so that there were no differences in luminosity at the ends of each aquarium. The temperature of the water was maintained at 27°C.

The fish were fed thawed chironomid larvae in quantities that were consumed in a few minutes. The usual frequency of feeding in the keeping conditions was twice weekly for all three *P. andruzzii* groups, and daily for *B. schuberti*. Six months before testing, the *P. andruzzii* of groups A1 and A2 were fed twice a week, while the *P. andruzzii* of group B were fed once a day as were the *B. schuberti* of group C. The usual low frequency of feeding of *P. andruzzii* was maintained for the test fish (groups A1 and A2) in order to enhance any possible component of their oriented response dependent on food odors eventually present in the home aquarium water of the two (B and C) stimuli-supplying groups. No food was given during the 18 h preceding testing.

A total of 76 tests – 38 of type $A \times B$ and 38 of type $A \times C$ – were performed in 16 consecutive days between February and March 1988. As the total number of specimens in groups A1 and A2 was 65, some specimens were tested again after an interval of at least six days.

Results and discussion. The overall results are shown in terms of absolute frequency of presence of the test fish in each one of the three sections of the experimental aquarium. A preference for the compartment containing the chemical traces is clearly evident when these were supplied by the conspecific

group (fig. 1). Even though the test fish generally did not remain in the section with the conspecific chemical cues throughout the test but often swam back and forth along the entire corridor, it spent more time in the compartment with chemical cues than in the other two. Their oriented response in this experimental condition seems to be comparable to an orthokinetic reaction. The situation differs when the chemical signals were supplied by the group of *B. schuberti*, where no preference was shown for either end section (fig. 2). In both cases only the external compartments can be considered as equivalent and thus comparable, the central section being not only familiar to the test fish at the beginning of the test but also not defined by any terminal walls. Furthermore, it was the obligatory path between the two ends of the corridor.

The statistical analysis of the data was conducted classifying each test as positive, neutral or negative according to whether, out of the total of 60, the number of registrations occurring in the end sector into which the sample of water containing chemical traces of group B or C had been poured were greater than, equal to, or less than those registered in the opposite end sector into which odorless water had been poured. For each type of test the significance of the difference between the number of positive and negative tests was tested with the chi-square one-sample test¹⁶. The evolution of the response in time was analyzed by subdividing each 30-min observation into 6 subperiods of 5 min and applying an analogous procedure to each of these. The results of the statistical analysis (table), show the response of *P. andruzzii* in the two test situations to be very different. When tested with chemical cues of unfamiliar conspecifics, specimens of *P. andruzzii* appear significantly attracted to these. Their reaction is immediate, reaching a maximum intensity in the second and third 5-min periods and then gradually tapering off. On the contrary they appear to be completely indifferent to the chemical signals of *B. schuberti*.

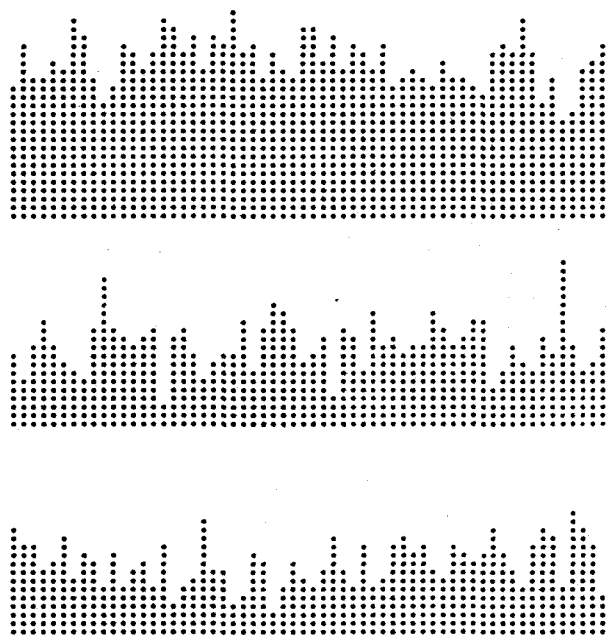


Figure 1. Absolute frequencies of presence of the test fish in the three compartments of the experimental aquarium in the $A \times B$ tests (chemical traces of unfamiliar conspecifics). Top row: end section containing chemical information; middle row: central section; bottom row: end section containing odorless water. Abscissa: observation time (30 min; 60 counts). Each point on the ordinate corresponds to a single count (total per column: 38 points).

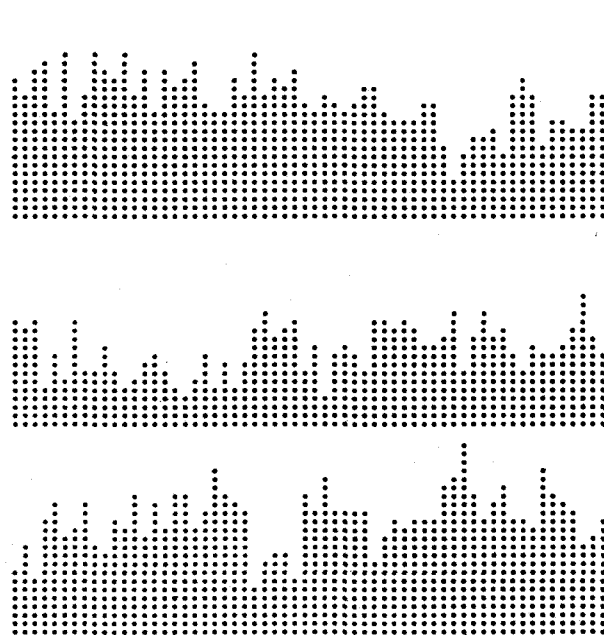


Figure 2. Absolute frequencies of presence of the test fish in the three compartments of the experimental aquarium in the $A \times C$ tests (chemical traces of *B. schuberti*). For further explanations, see fig. 1.

Locomotor response of *Phreatichthys andruzzii* to chemical traces of unfamiliar conspecifics (A×B tests) and of *Barbus schuberti* (A×C tests): statistical analysis of results.

Test type	Intervals of analysis (min)	Experiments Positive	Neutral	Negative	Chi-square values	Significance
A×B	0–30	32	1	5	19.703	p < 0.001
	0–5	25	3	10	6.429	p < 0.02
	5–10	28	4	6	14.235	p < 0.001
	10–15	28	4	6	14.235	p < 0.001
	15–20	22	6	10	4.50	p < 0.05
	20–25	25	1	12	4.568	p < 0.05
	25–30	21	4	13	1.882	n.s.
A×C	0–30	20	0	18	0.105	n.s.
	0–5	23	1	14	2.189	n.s.
	5–10	18	4	16	0.118	n.s.
	10–15	19	5	14	0.758	n.s.
	15–20	17	4	17	0.00	n.s.
	20–25	12	5	21	2.455	n.s.
	25–30	17	4	17	0.00	n.s.

At present no hypothesis can be formulated about the nature of the attractive substance, except that this does not seem to consist of catabolism products presumably similar in both species. Moreover, the reaction does not appear to be dependent on food odor possibly present in the water of the home aquariums of the B and C groups. Furthermore, no patterns of food search were observed during the tests. Therefore the reaction appears to be dependent on some specific chemical signal. The biological significance of the presence of a species-specific response in a species which in the course of its evolution has undergone marked morphological regression and functional adaptation, and inhabits an environment free of any known sympatric species where presumably no selective pressure exists for its retention, has yet to be established. Nonetheless, it is further proof of both the complexity of the mechanisms regulating the oriented locomotory response in hypogean forms and the importance of chemical communication in their biology.

- 3 Hemmings, C. C., J. exp. Biol. 45 (1966) 449.
- 4 Höglund, L. B., and Åstrand, M., Rep. Inst. Freshwat. Res. Drottningholm 53 (1973) 21.
- 5 Quinn, T. P., Behav. neur. Biol. 29 (1980) 123.
- 6 de Fraipont, M., and Thinès, G., Experientia 42 (1986) 1053.
- 7 Berti, R., and Thinès, G., Experientia 36 (1980) 1384.
- 8 Berti, R., Thinès, G., and Lefèvre, B., Int. J. Speleol. 12 (1982) 103.
- 9 de Fraipont, M., Annls Soc. r. zool. Belg. 117 (1987) 63.
- 10 de Fraipont, M., Stygologia 2 (1986) 276.
- 11 Ercolini, A., Berti, R., Chelazzi, L., and Messana, G., Monitore zool. ital. (N.S.) Suppl. 17 (1982) 219.
- 12 Ercolini, A., Berti, R., Chelazzi, L., and Messana, G., Monitore zool. ital. (N.S.), Suppl. 22 (1987) 23.
- 13 Innes, W. T., Innes Publ. Co., Philadelphia 1951.
- 14 Hervey, G. F., and Hems, J., Batchworth Press, London 1952.
- 15 Ercolini, A., and Berti, R., Monitore zool. ital. (N.S.) Suppl. 6 (1975) 29.
- 16 Siegel, S., Kogakusha Co. Ltd., Tokyo 1956.

- 1 Wrede, W. L., Z. vergl. Physiol. 17 (1932) 510.
- 2 Keenleyside, M. H. A., Behaviour 8 (1955) 183.

0014-4754/89/020205-03\$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1989

Experimental evidence for endogenously programmed differential migration in the blackcap (*Sylvia atricapilla*)

S. B. Terrill and P. Berthold¹

Max-Planck-Institut für Verhaltensphysiologie, Vogelwarte Radolfzell, D-7760 Schloss Moeggigen (Federal Republic of Germany)

Received 9 September 1988; accepted 31 October 1988

Summary. Female hand-raised blackcaps (*Sylvia atricapilla*) held in constant conditions (except for simulated seasonal changes in photoperiod) showed significantly more, and significantly longer, autumnal migratory activity as well as significantly later initiation of spring migratory activity than males from the same population (including siblings) held under identical conditions.

Key words. Migration; differential migration; endogenous program; blackcap; *Sylvia atricapilla*; warbler.

Migration is a relatively widespread attribute among both vertebrates and invertebrates that enables organisms to escape detrimental ecological conditions in one region while exploiting more benign conditions in another^{2,3}. Migration, however, is a highly diverse behavioral phenomenon^{4,5} and it has been well established that migratory behavior varies from highly facultative responses to immediate environmen-

tal change² to endogenously controlled behavior with high heritability values⁶. Of considerable interest from an evolutionary, ecological, and physiological standpoint are the relative roles of exogenous and endogenous factors in the regulation of migratory behavior^{7,8}. Currently, the most persuasive experimental evidence for a high degree of endogenous regulation derives from an extensive, long-term investi-