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Oxygen consumption of *Astyanax fasciatus* (Characidae, Pisces): a comparison of epigean and hypogean populations

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Synopsis

The standard and routine oxygen consumptions of *Astyanax fasciatus* from one surface population (Rio Teapao) and three cave populations (Chica, Micos and Pachon caves: '*Anoptichthys jordani*', the 'Micos-fish' and '*Anoptichthys antrobius*') were determined individually over 24 hours by the use of a flow-through respirometer and polarographic oxygen electrodes. The phylogenetically oldest Pachon fish had a significantly lower standard metabolic rate (0.230 ± 0.036 mg $O_2 g^{-1} h^{-1}$) than the epigean Teapao fish, the hybrid Chica fish and the phylogenetically younger Micos fish (0.314 ± 0.081 mg $O_2 g^{-1} h^{-1}$, 0.284 ± 0.048 mg $O_2 g^{-1} h^{-1}$, 0.277 ± 0.063 mg $O_2 g^{-1} h^{-1}$). No significant differences could be determined among the latter three populations. A significant difference in routine metabolic rate existed only between the Pachon fish (0.309 ± 0.056 mg $O_2 g^{-1} h^{-1}$) and the Teapao fish (0.415 ± 0.071 mg $O_2 g^{-1} h^{-1}$). The Chica fish (0.356 ± 0.084 mg $O_2 g^{-1} h^{-1}$) and the Micos fish (0.355 ± 0.080 mg $O_2 g^{-1} h^{-1}$) could not be separated from either the Teapao or the Pachon fish, but a decreasing trend from the surface population through the Chica and the Micos to the Pachon population was obvious. During a starvation period of 29 days the metabolic rate of epigean Teapao and hypogean Pachon fish decreased significantly by 32.5% and 34.8% (standard oxygen consumption rate) and 27.5% and 28.2% (routine oxygen consumption rate), respectively. Body mass loss during the starvation period was 16.3% for the Teapao fish and 9.5% for the Pachon fish.

Introduction

Cave environments, besides having relatively stable physical, chemical and biological conditions, are often characterized by food scarcity due to the lack of primary producers (Poulson 1964, Barr 1968, Culver 1982). This requires several adaptations of animals living in caves, especially with regard to energy demand, usually expressed by metabolic rate. The first examinations of the metabolic rates of troglobites were made with amphipods (Gal 1903). Many other authors showed that food restricted hypogean species of several systematic groups have lower metabolic rates than their epigean relatives (Eigenmann 1909, Burbanck et al. 1948, Dérouet 1949, 1952, 1953a, 1953b, Heuts 1951, Troiani 1954, Dresco-Dérouet 1959, 1960, 1967, Eberly 1960, Wautier & Troiani 1960, Poulson 1963, Jegla 1964, H.J. Koch [unpublished data, cited in Poulson 1964], Weingartner 1977, Caine 1978, Dickson & Franz 1980, Franz 1978, Hadley et al. 1981). A summary on energy economy in cave animals was given by Hüppop (1986). Poulson (1963) showed that amblyopsid cave fish in particular have a lower metabolic rate resulting from low food supply. However, all previous comparisons were made among different species or even different genera.

Astyanax fasciatus (Cuvier 1819), common in Mexican surface waters, colonized some of the limestone caves in Tamaulipas and San Luis Potosi provinces, Mexico, not earlier than 2 million years ago (Kosswig 1960, 1967, Myers 1966, Wilkens 1971, 1977, Mitchell et al. 1977). Colonization continues and there exist cave populations of different phylogenetic ages and differing stages of morphological reduction, especially pigmentation and eye size as a result of the lack of light. *A. fasciatus* has given rise to many investigations of evolutionary processes, particularly as a cave animal and especially because of the interfertility of its populations (Sadoglu 1956).

The object of the present investigation was to supplement these studies and to test the supposition that the cave populations of *A. fasciatus* like other cave animals, have their metabolic rate adapted to the cave environment. There has been one investigation of the oxygen consumption of *A. fasciatus* by Schlagel & Breder (1947), who found an oxygen consumption rate higher in cave fish than in surface fish. However, their results are not representative since they investigated only Chica fish. They are hybrids between epigean and hypogean fish (Breder 1942, 1943, Sadoglu 1956, Wilkens 1972b, Peters & Peters 1973, Mitchell et al. 1977, Romero 1983) and therefore represent a very atypical occurrence of the cave characins.

Material and methods

In total, 54 individuals belonging to five groups of healthy adult epigean and hypogean *A. fasciatus* were used for the experiments, including some fish originally caught in the Mexican caves (Table 1). One epigean population from the Rio Teapao was compared to four hypogean forms, two of which used to be classified as independent species of a different genus. The four hypogean forms are (a) the hybrid Chica fish (*'Anoptichthys jordani'* Hubbs & Innes 1936), living in the Chica cave where food is abundant, provided from bat guano (Breder 1942, Mitchell et al. 1977), (b) the phylogenetically younger (Wilkens & Burns 1972, Peters et al. 1975, Wilkens 1976, Mitchell et al. 1977) Micos fish (*'Micos-fish'* Wilkens & Burns 1972) from the Micos cave, and (c) two groups of the phylogenetically older (Wilkens 1976, 1980) Pachon fish (*'Anoptichthys antrobius'* Alvarez 1946) from the Pachon cave. The Micos and the

Table 1. Oxygen consumption rates of seven groups of epigean and hypogean *Astyanax fasciatus* (0 = caught wild, 1 = first generation from O, F = fed, S = starved).

	Teapao-1F	Teapao-1S	Chica-0	Micos-0	Pachon-0	Pachon-1F	Pachon-1S
Mean standard ox. cons.	0.314	0.212	0.284	0.277	0.215	0.243	0.150
(mg $O_2 g^{-1} h^{-1}$)	±0.081	±0.079	±0.048	±0.063	±0.035	±0.033	±0.025
Range	0.230	0.150	0.231	0.200	0.152	0.202	0.118
(mg $O_2 g^{-1} h^{-1}$)	to 0.481	to 0.361	to 0.349	to 0.444	to 0.260	to 0.284	to 0.185
Mean routine ox. cons.	0.415	0.301	0.356	0.355	0.296	0.319	0.222
(mg $O_2 g^{-1} h^{-1}$)	±0.071	±0.084	±0.084	±0.080	±0.073	±0.038	±0.076
Range	0.328	0.221	0.244	0.251	0.163	0.245	0.150
(mg $O_2 g^{-1} h^{-1}$)	to 0.544	to 0.435	to 0.498	to 0.513	to 0.405	to 0.383	to 0.323
Range of wet body mass	4.29	3.22	3.35	3.03	3.17	4.03	3.89
(g)	to 9.86	to 6.99	to 6.82	to 11.33	to 9.20	to 6.66	to 8.11
n, sex	2♀, 5♂	3♀, 3♂	5♀, 1♂	6♀, 6♂	5♀, 3♂	4♀, 5♂	3♀, 3♂
Born/caught	1978	1978	1982	1982	1982	1975	1975

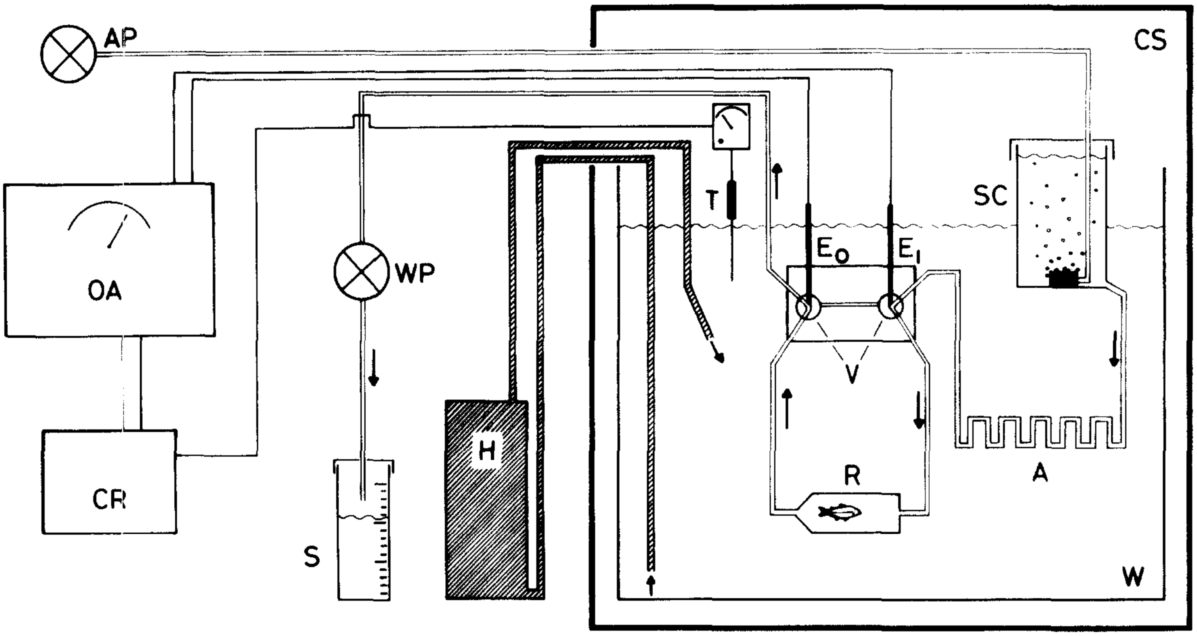

Fig. 1. Flowing-water respirometer arrangement (A = acclimation tube, AP = air-pump, CR = linear chart recorder, CS = camera silens, E_i = inflow electrode, E_o = outflow electrode, H = heater element, OA = oxygen analyzer, R = respiration chamber, S = sample water, SC = supply container, T = temperature measuring instrument, V = valves, W = water bath, WP = water pump).

Pachon caves are relatively poor in food, so that inwashed epigean *A. fasciatus* cannot survive in competition with their hypogean relatives as they can in the Chica cave (Wilkens & Burns 1972, Peters et al. 1975, Mitchell et al. 1977, Parzefall 1983, Wilkens & Hüppop 1986). Two groups of Pachon fish were tested to determine whether a difference exists in oxygen consumption between fish originally caught in the Mexican cave (Pachon-0) and fish born and reared in the laboratory (Pachon-1) (Table 1).

All fish were held for at least one and a half years in the Zoological Institute, Hamburg, in tap water at 25° C and were fed daily. Except for the Micos fish, which were held in total darkness, all fish were exposed to a constant photo period of 12:12 hours maintained throughout the year. Usually the test fish were caught randomly out of the group, but obviously gravid females had to be excluded from the experiment. The oxygen consumption was measured continuously (Fig. 1) as the difference in oxygen content of the water flowing through a respirometer with two polarographic oxygen electrodes, one in front of the respiration chamber and one behind it. During experiments the water flowed through the respiration chamber along the long path. To calibrate the electrodes, the two valves were turned so that the water took the shortest path between the two electrodes (Fig. 1). Zero calibration was made with a 3% solution of Na_2SO_3, second-point calibration with aerated tap water whose oxygen content was determined with the unmodified Winkler method. The advantages of the open-circuit method to the closed-bottle method were described in Kamler (1969).

All experiments took place in the total darkness of the *camera silens* (CS in Fig. 1), a sound-proofed, cave-imitating room. During operations in darkness an infra-red viewing apparatus was used. Oxygen consumption and water temperature (25 ± 0.1° C) were recorded simultaneously on a two channel recorder. Every fish was acclimatized to darkness and silence in the *camera silens* for about two days without being fed. The volume of the respiration chamber was about 420 ml, fitted to the size of the animals so that they could turn around but not move extensively. The experiment started at least 48 h after the last feeding and continued about 24 h following Erckens (1981b) who found that even hypogean *A. fasciatus* have a circadian rhythm (see also Fry 1957). After every experiment the fishes were weighed in several layers of cellulose and the weight of the wet cellulose subtracted.

Both the standard and routine oxygen consumptions were calculated by using the formula:

$$VO_2 = \frac{PO_2 \cdot V}{G},$$

where VO_2 = standard or routine oxygen consumption (mg $O_2 g^{-1}h^{-1}$), PO_2 = mean or smallest oxygen consumption (mg $O_2 l^{-1}$), V = water flow rate (lh^{-1}), G = wet mass of the test animal (g). Usually routine oxygen consumption is defined as the mean oxygen consumption over a 24 h-period (Fry 1957). For standard oxygen consumption the smallest oxygen consumption which could be measured during the test was taken (Poulson 1963, Kausch 1968). Accompanying observations with the infra-red viewing apparatus confirmed that at these times activity was nearly zero. The oxygen consumption rates of six Pachon fish and six Teapao fish, three males and three females in each case, which had been starved in darkness for 29 days, were measured in the same manner. The fish were starved in four groups of three beginning at one week intervals since measurement of one fish took about two days. Each trio was weighed together every fourth day in a calibrated water-filled flask to avoid injuries due to handling the animals frequently with cellulose. Duncan's multiple range test (Duncan 1955), extended by Kramer (1956) to group means with unequal numbers of replications, was used to compare the mean metabolic rates of all groups simultaneously. For the comparison of two groups only the Mann-Whitney U-test was chosen. If not stated otherwise, a statistically significant difference always means $p<0.05$.

Results

No statistically significant difference between the Pachon-0 fish, originally caught in the Mexican cave, and the Pachon-1 fish, the laboratory progeny, could be determined (Table 1). From this it was concluded that the level of the metabolic rate is genetically fixed and not merely a response to immediate conditions. Thus, the Pachon-0 fish and the Pachon-1 fish were combined as 'Pachon' with standard metabolic rate = 0.230 ± 0.036 mg $O_2 g^{-1}h^{-1}$ and routine metabolic rate = 0.309 ± 0.056 mg $O_2 g^{-1}h^{-1}$ ($p>0.2$) (Fig. 2). Furthermore there was no difference between male and female oxygen consumption rates in all investigated groups.

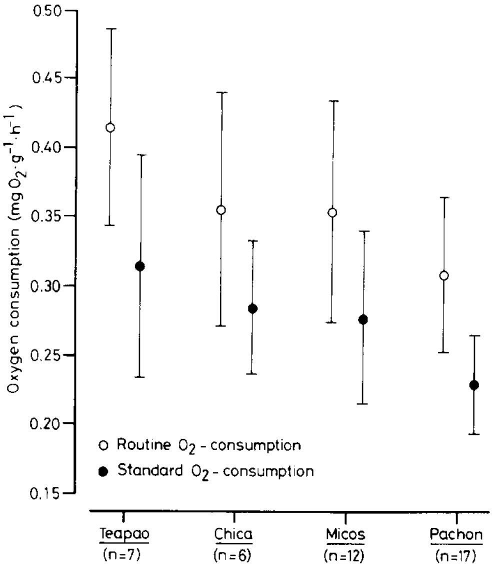

Fig. 2. Mean standard oxygen consumption rates (closed circles) and mean routine oxygen consumption rates (open circles) (± 1 s.d.) of one epigean population (Teapao) and three cave populations (Chica, Micos, Pachon) of *Astyanax fasciatus*.

The mean routine metabolic rate of the epigean *A. fasciatus* (Table 1) accords well with that of *A. eigenmanniorum* (Cope 1894), a close relative (average routine oxygen consumption = 0.44 mg $O_2 g^{-1}h^{-1}$, Freyre et al. 1982) and other epigean fish species at comparable weights and temperatures. In comparison to the cave populations, the mean routine oxygen consumption rate of the epigean Teapao fish could be distinguished statistically only from that of the Pachon fish in being significantly higher. The Chica fish as well as the Micos fish did not show any statistically significant difference either from the Teapao fish or from the Pachon fish. However, Figure 2 shows the decreasing tendency in metabolic rate from the epigean fish to the

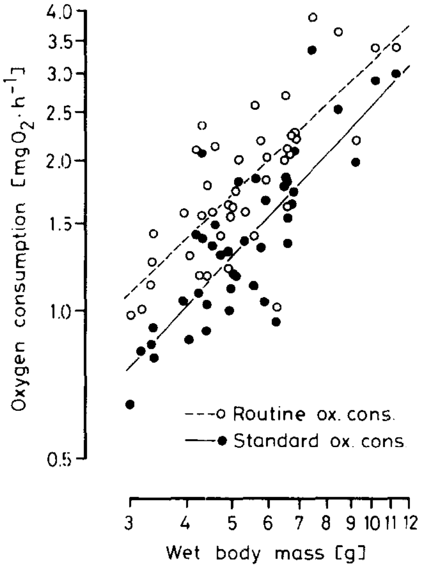

Fig. 3. Relationship of body mass to oxygen consumption in *Astyanax fasciatus* (all data of all groups together). Standard ox. cons.: $y = 0.247 \cdot x^{1.031}$, $r = 0.827$. Routine ox. cons.: $y = 0.411 \cdot x^{0.884}$, $r = 0.767$.

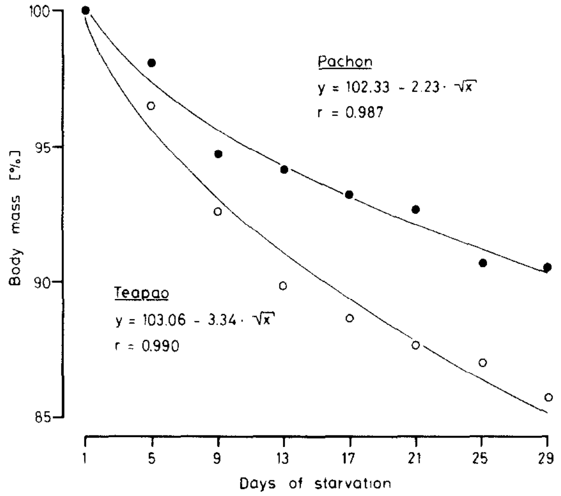

Fig. 4. Body mass loss of epigean (Teapao) and hypogean (Pachon) *Astyanax fasciatus* during 29 days of starvation. One point = six fish weighed together.

hybrids and the phylogenetically younger to the phylogenetically older cave fish. According to standard metabolic rate the Pachon group differed significantly from the Teapao and the Micos groups but not from the Chica.

The relationship of body mass to oxygen consumption was tested in regression analyses with $y = a \cdot x^b$ [y = oxygen consumption (mg $O_2 h^{-1}$) and x = wet body mass (g)]. Since there were no statistically significant differences between the regression coefficients of epigean and hypogean fish separately, all data were combined (Fig. 3). The regression coefficients for standard oxygen consumption as well as for routine oxygen consumption were relatively high. However, according to Heusner (1984) values of b different from the theoretical value of 2/3 can be interpreted as changes in body composition, form, function etc. with changes in size. Only if the metabolic change is due to a change in mass alone, will b be 2/3 (Heusner 1984).

The comparison of the oxygen consumption rates of the Pachon and Teapao fish, before and after the starvation period, showed a statistically significant decrease of standard and routine metabolic rates in both groups. The Pachon fish reduced their standard metabolic rate by 34.8% and their routine metabolic rate by 28.2% on average. The starved Teapao fish showed a reduction by 32.5% and 27.5%, respectively (Table 1). The body mass loss during the starvation period represents a preliminary result only (Fig. 4). Since no sexual differences within one population could be determined, the body masses of all six fish of each population were summarized and the percent decrease of body mass calculated. The starting body mass for each group of six fish was nearly the same at 41.56 g (Teapao) and 39.27 g (Pachon). From this the epigean Teapao fish lost 16.3% during the 29 days of starvation while the hypogean Pachon fish lost only 9.5% (Fig. 4). Body mass loss in both groups followed best the square root regression ($y = a + b\sqrt{x}$). According to the comparison of regression coefficients of Sachs (1984) the two curves differed significantly ($P < 0.001$).

Discussion

The high variability in oxygen consumption rates does not allow many interpretations of the metabolic rates established here, since the sample size was relatively small. The fish showed very different individual reactions to the conditions of the experiment, especially to captivity in the small respiration chamber. Therefore some values for the routine oxygen consumption as well as for the standard one may be unusually high due to increased activity, but on the whole at least a trend is obvious. Of all the four investigated groups the phylogenetically older and most food restricted Pachon fish are best adapted to the cave environment. The metabolic rate of the Micos fish corroborates their phylogenetically younger cave character as genetically established by Peters et al. (1975) and Wilkens (1976). The reduction in the metabolic rate in the Pachon and Micos fish seems to be an indicator of the phylogenetic age of cave populations, in accordance with the conclusions of Poulson (1963, 1964) Peters & Peters (1966), and Wilkens (1972b, 1973), based on morphological reductions of eye size and pigmentation, and the trend in a great number of morphological, ethological and physiological alterations (see summaries in Schemmel 1980, Parzefall 1983, 1984a, 1984b, Wilkens 1984).

Nevertheless, this must be considered with caution. First, the reduction of the oxygen consumption rate in the Micos fish was not statistically significant enough to allow hard and fast interpretations. Secondly, a comparison of the ages of cave fish populations through metabolic rates is only permissible for caves where food scarcity is the main restricting factor and selection pressure on energy economy is equal. Even here it can be a complementary point of view and used for relative statements only. The decreasing tendency of the metabolic rate from the epigean Teapao fish through the phylogenetically younger Micos fish to the phylogenetically older Pachon ones is supplemented by the hybrid Chica fish. In the Chica cave the abundant food supply reduces the selective advantages of the cave forms to a point where the surface forms can survive sufficiently long to interbreed with the cave forms (Mitchell et al. 1977). The intermediate results of the Chica fish, between those of pure epigean and pure hypogean fish, also reflect their hybrid nature.

This appears to be in contrast to Schlagel & Breder (1947) who compared the activity of Chica and epigean fish, in light and darkness, using oxygen consumption measurements. Under both conditions, they found a higher oxygen consumption in the hybrid cave fish than in epigean ones. However, the different aims of Schlagel & Breder (1947) and the present study, and the nonhomogenous experimental conditions due to varying group size in Schlagel & Breder (1947), make a comparison difficult. Furthermore, in both investigations the differences in oxygen consumption between the surface fish and the hybrids were not statistically significant, due to high individual variability, so that all interpretations should be viewed with caution. Recently is has been shown that hypogean *A. fasciatus* actually are less active than their epigean relatives (Erckens 1981a). Thines et al. (1965) supported Schlagel & Breder (1947), but they included an unusually high activity value in their calculations which, according to the test on outliers of Sachs (1984) should be eliminated. Without this value there is no difference at all in activity between the cave fish and the surface fish.

As the mean metabolic rate of the surface form was not lower than that of other surface fish species (see results), *A. fasciatus* appears not to be preadapted to cave life in metabolic rate as it is in the form of its highly developed and dense system of neuromasts, which can to a large degree take over the orienting function of the eyes (Schemmel 1967, 1974), and the chemical orientation of spawning behavior (Wilkens 1972a). However, it should be emphasized that the oxygen consumption of some individuals was within the range of the Pachon fish, the cave population with the most reduced energy demand (Table 1). Wilkens & Hüppop (1986) hypothesize that, at the beginning of cave colonization, these individuals might have been and still are able to survive and reproduce when washed into a cave, and could be considered potential cave pioneers. Presently, survival seems to be possible only in the Chica cave, where food is abundant, and in otherwise uninhabited caves. Neither in the Micos cave nor in the Pachon cave, where food inputs are low, can inwashed epigean fish survive in competition with the cave fish already present (Mitchell et al. 1977), although some individuals with low energy demand could be expected to be among the inwashed. In the beginning of cave colonization there would have been no need to compete with better adapted inhabitants and these first colonizing fish could have survived.

However, a lower metabolic rate is not the only, or even the dominant adaptation to cave life. Other cave features develop relatively quickly at the same time, since adaptation to a short food supply has a high selective value. Such adaptive features are the enlarged and predominantly ventrally spread gustatory areas in the skin covering the head (Schemmel 1967, 1974), the reduction of headstanding when searching for food (Schemmel 1980), and, as a consequence, the improved food finding ability in darkness (Hüppop, unpublished).

Poulson (1963) found a direct relationship between the rate of body mass loss of amblyopsid fish during starvation and standard metabolic rate, and also an increase in survival time with increasing adaptation to cave life. Culver (1982) made a lowered standard metabolic rate responsible for a greater resistance to starvation, while a lowered routine metabolic rate would make more energy available for reproduction. In this investigation, the statistically significantly lower body mass loss observed for the Pachon fish during starvation in comparison to that of the epigean fish might be related to their lower energy demand as expressed by their lower oxygen consumption rate even during starvation.

These results imply that in caves poor in food, inhabited by both hypogean and inwashed epigean *A. fasciatus*, competition might occur as already suggested by Mitchell et al. (1977) and Wilkens & Burns (1972). Recent investigations (Wilkens & Hüppop 1986) showed that competition in such a case, for example in the Micos and Pachon caves, really does occur and that the epigean fish are at a disadvantage in comparison to the hypogean ones. Food finding ability in darkness was much better in the Pachon cave fish than in the epigean Teapao ones for food lying on the bottom as is typical in caves (Hüppop, unpublished). Epigean fish washed into the Micos cave, which is poor in food, looked undernourished and had lower condition factors than the hypogean Micos fish, and where also poorer than fish from other caves or than epigean fish not washed into the Micos cave (Wilkens & Hüppop 1986). The reduction of the metabolic rate even in the phylogenetically older cave population of Pachon, although statistically significant, was not very great compared to amblyopsid fish (Poulson 1963, for example). However, since temperate caves, where the amblyopsids live, have a lower total energy input than the tropical ones where the hypogean *A. fasciatus* live (Mitchell 1969), stronger selection pressure upon energy economy will be found in temperate caves and will lead to highly adapted species faster than in tropical caves. Furthermore, all previous investigations concerning metabolism in food restricted cave animals were made at least between different species, but mainly between different genera or families. Therefore it was most interesting to find any difference at all in oxygen consumption within the same species, although the separation of the populations was rather recent as has been shown biochemically by Avise & Selander (1972). This unique example of recently separated populations presents an opportunity to investigate the genetics of the metabolic rate in crosses between epigean and hypogean fish as has been done for the reduction of eye size, pigmentation and aggressive behaviour and improved gustatory apparatus (Wilkens 1970a, 1970b, 1971, 1972b, Schemmel 1974, 1980, Burchards et al. 1985).

Culver & Poulson (1971) described a 'life history hypothesis' which assumes that caloric output per lifetime is constant so that 'fast living' results in early death and 'slow living' confers increased longevity. The advantage of a decreased metabolic rate in food restricted cave animals could be an increased life expectancy and therefore a prolonged time for reproduction, thus compensating for the rarity of the reproductive event due to a lack of food by a longer life (Culver & Poulson 1971). This hypothesis was supported by Dickson & Franz (1980), who found that the ATP-turnover rates were significantly lower in cave-crayfish gills than in epigean crayfish (see also, Craig 1985). This strategy has not yet been observed in *A. fasciatus* but it could be expected. At least a general reduction of activity, typical of cave animals (e.g. Verrier 1929, Burbanck et al. 1948, Dresco-Dérouet 1959, Eberly 1960, Ginet 1960, Poulson 1963, Thines

1969, Thines & Piquemal 1978) and the strategy with the earliest success according to energy economy, was also found in the Pachon form of *A. fasciatus* (Erckens 1981a). This contrasts with some previous investigations (Schlagel & Breder 1947, Thines et al. 1965 and Brust personal communication), but the method and the analyses of Erckens (1981a) seem to be the most accurate. The reduced routine metabolic rate can be explained by reduced spontaneous activity, and the reduced standard metabolic rate attributed, at least partly, to reduced aggressive behavior (Burchards et al. 1985) in *A. fasciatus* living in darkness. The reduction of the aggressive behavior in some, but not all cave animals, cannot be interpreted as a phenomenon only of regressive evolution, but must also be seen as an advantage in the adaptation to food scarcity, i.e. as a phenomenon of constructive evolution. Aggressive behavior still exists in several cave-dwelling animals, but is based on other than optical cues. Either the absence of stabilizing selection or energy economy might be responsible for a change in aggressive behavior (see also Parzefall 1982). Reduced aggressive behavior might reflect a lower endogenous spontaneity, meaning lower energy consumption resulting from lower adrenalin release. It might be more economic for hypogean *A. fasciatus*, living in food poor caves, to reduce their aggressive behavior also, in particular since it no longer has a function in darkness (Burchards et al. 1985). Evidence that increased oxygen consumption based on 'emotional activity' is possible without any increase in physical activity, was given by the investigations of metabolism and activity of the hypogean amphipod *Niphargus rhenorhodanensis* (Mathieu 1980) and *Carassius auratus* (Smit 1965), respectively.

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