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Freshwater larval fish from Lake Trichonis (Greece)

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The larvae of 15 freshwater fish species from Lake Trichonis (western Greece) are described from field samples and laboratory-raised fish. Larval morphologies are compared to identify distinguishing characters. The potential utility of these data sets for assessing phylogenetic relationships is discussed.

Key words: freshwater fishes; reproduction; eggs; larvae; taxonomy; Greece.

I. INTRODUCTION

There are 105 fish species, possibly 110, and very many subspecies in the fresh waters of Greece (Economidis, 1991). Seventy-eight are native, and found exclusively in fresh water, 47·4% of these are endemic to Greece or adjacent countries. This high degree of endemism is attributable to geographic isolation, complicated geological history and climate. Most endemic species have a restricted distribution, some taxa are now extinct, and more than one-third are classified as endangered, vulnerable, potentially threatened or indeterminate (Economidis, 1991).

Unfortunately, fish conservation features little in the management of the limited and exploited water resources of Greece. The projected diversion of the Acheloos River will alter dramatically four natural lakes, four artificial lakes, numerous pools, marshes, springs, etc., endangering the entire system, rich in endemic aquatic species. Conservation actions often fail if not based on sound biology, so in 1988 we started to study the biology of the fishes of Lake Trichonis, the largest lake of the Acheloos basin. Data have been published on embryonic and larval development of four of these species (Economou *et al.*, 1991; Daoulas *et al.*, 1993). This paper provides information on diagnostic characters of 11 more, on larval ecology and behaviour in the field and in aquaria, and on adult biology and ecology.

THE STUDY AREA

Lake Trichonis (Fig. 1) [the largest (97 km^2) and deepest (maximum 58 m, average 30·5 m) natural lake in Greece], with Lakes Lyssimachia, Amvrakia and Ozeros, are the remnants of the ancestral Lake Aetoloakarnania which covered the Acheloos basin before the end of the Pliocene (Koussouris, unpublished). Lake Trichonis was originally oligotrophic, but through human perturbations is now mesotrophic (Overbeck *et al.*, 1982). A narrow channel allowed outflow from surface effluents to the adjacent smaller and more

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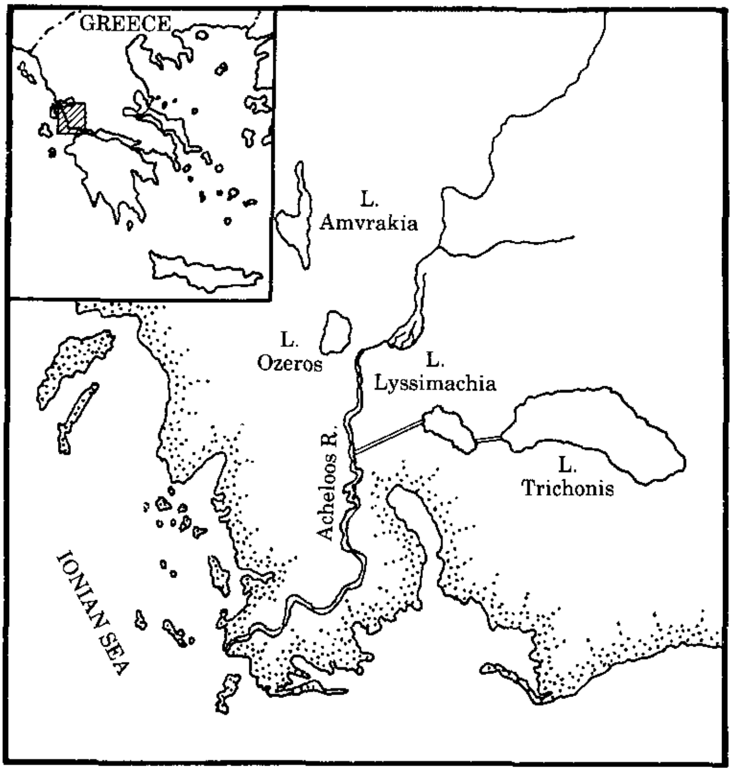

FIG. 1. Trichonis Lake and the Acheloos basin system.

shallow Lake Lyssimachia which in turn maintains an open connection to the sea through the Acheloos River. Since 1961, a dam has prevented the movement of fish and other organisms from Lake Lyssimachia to Lake Trichonis.

Lake Trichonis was characterized by 'Project Aqua' (Luther & Rzoska, 1971) as an area of high potential research value, because of its endemic diatoms, Chrysophyceae, Cyanophyceae, molluscs and fishes, some of which now face extinction.

THE FISH FAUNA

The 20 exclusively freshwater species of Lake Trichonis are listed below (taxonomy follows Economidis, 1991):

CYPRINIDAE

Leuciscus cephalus (Linnaeus, 1758)*
Rutilus ylikiensis Stephanidis, 1939*†
Scardinius acarnanicus Stephanidis, 1939*†
Barbus albanicus Steindachner, 1870*†
Barbus peloponnesius peloponnesius Valenciennes, 1842*†
Pseudophoxinus stymphalicus (Valenciennes, 1844)*†
Tropidophoxinellus hellenicus (Stephanidis, 1939)*†
Phoxinellus pleurobipunctatus (Stephanidis, 1939)†
Tinca tinca (Linnaeus, 1758)

Cyprinus carpio Linnaeus, 1758
Carassius auratus gibelio (Bloch, 1783)
ANGUILLIDAE
Anguilla anguilla (Linnaeus, 1758)
GOBIIDAE
Economidichthys pygmaeus (Holly, 1929)*†
Economidichthys trichonis Economidis & Miller, 1990*†
Knipowitschia caucasica (Kawrajsky, 1899)*
ATHERINIDAE
Atherina boyeri Risso, 1810*
SILURIDAE
Silurus aristotelis (Agassiz, 1856)*†
POECILIIDAE
Gambusia affinis Baird & Girard, 1858*
BLENNIIDAE
Salaria fluviatilis Asso, 1801*
COBITIDAE
Cobitis trichonica Stephanidis, 1974*†

*Larval description is provided in this paper.
†Greek endemic species.

II. MATERIAL AND METHODS

Trichonis Lake larval fishes were sampled by plankton nets, bottom-towing instruments, beakers, scoop nets, fry nets, seining and traps. Larvae of fluvial species were sampled in the small tributary stream Myrtia with scoop nets, fry nets and electrofishing. Samples were taken at intervals of 5 to 30 days from June 1988 to June 1991. The material was preserved in 4% formalin in water, neutralized with sodium phosphate.

For laboratory rearing, we utilized embryos and larvae from artificial fertilizations, nests, naturally fertilized eggs attached to weeds or solid substrata, and wild larvae collected from the field. The larvae were reared in 10–80-l aerated glass aquaria and fed on wild plankton (when available), nauplii of *Artemia salina* (L.), dried *Daphnia* and commercial foodstuffs. Undeveloped eggs, dead larvae, unused food and debris were pipetted-off, and part of the water was replaced daily. Whenever fungal infections appeared, the cultures were treated with malachite green. Samples of developing embryos and larvae were preserved at regular intervals in 4% formalin in water.

Larvae were measured by means of a calibrated eyepiece to the nearest 0·1 mm. Before the completion of notochord flexion, body length was measured from the snout to the tip of the notochord (NL, notochord length). In post-flexion specimens, body length was measured to the posterior edge of the hypural plate (S.L., standard length). Illustrations were drawn using a binocular microscope fitted with a camera lucida. Line drawings, morphological descriptions and morphometric data were based on preserved specimens. Terminology of the morphological characters and the pigmentation patterns follows Russell (1976). Information on the geographical distribution of species is from Economidis (1991). Information on spawning seasons and the characteristics of reproduction is from our unpublished data on the gonadal maturation cycle of the Trichonis Lake species.

III. RESULTS

CYPRINIDAE

Most of the 11 cyprinid species are endemic to Greece. Their larvae are similar morphologically, distinguished as a family by a bilobed yolk sac, two-chambered swimbladder and the anus always behind the midpoint of the body.

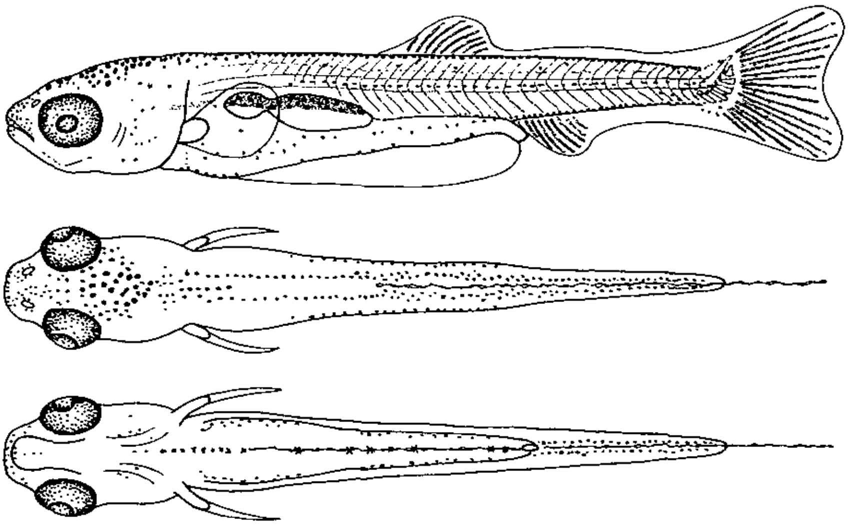

FIG. 2. *Leuciscus cephalus* laboratory reared. Larva 9·8 mm, day 15 (lateral, dorsal and ventral view).

Leuciscus cephalus *(Fig. 2)*

Chub, moderately abundant in Lake Trichonis and abundant in its tributaries, is a fractional spawner, matures at 2 or 3 years, and lays demersal, adhesive, yellow-green eggs of around 2-mm diameter, after water hardening and fertilization, on gravel substratum in the tributaries between mid-April and mid-June.

The unpigmented larvae hatch at about 6·3 mm NL. A characteristic cyprinid pigmentation pattern soon appears: internal melanophores in the dorsal area of the peritoneum, an array of head melanophores, a mediolateral stripe running along the body, and dorsal and ventral double contour rows of melanophores. In addition, there is an abdominal ventral single row of melanophores which is interrupted by a gap, and two abdominal lateral rows. These two characters separate the larvae of *L. cephalus* from those of other cyprinids. (For a more detailed description, see Economou *et al.*, 1991.)

Scardinius acarnanicus *(Fig. 3)*

The lacustrine, chiefly phytophagous *S. acarnanicus* is endemic in the lakes of the Acheloos basin, matures at 14–18 cm (2 to 3 years), and grows to at least 30 cm over 7 years (unpublished data; Iliadou & Ondrias, 1980). It is a fractional spawner, reproducing between late March and early July, and depositing adhesive, spherical, yellowish eggs, around 1·4 mm after water hardening and fertilization, on vegetation.

Eggs and sperm stripped from wild spawners developed from fertilization to hatching in 5 days at 23·5° C in the laboratory. The larvae hatched at about 5·1 mm NL and the mouth and anus were closed. Yolk was fully absorbed by day 3, at about 6 mm NL. Flexion occurred on day 15 at 7·5 mm. Larvae between 8 and 8·6 mm formed dorsal and anal fin rays. The budding of pelvic fins started at 10·5 mm. The larvae metamorphosed between 13 and 15 mm at age 36–82 days, suggesting that the size is less variable than the age of metamorphosis (Barbieri-Tseliki, unpublished).

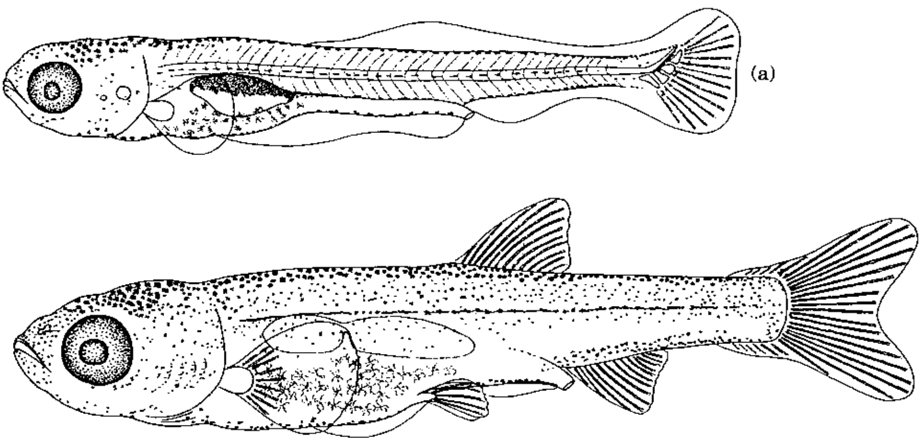

FIG. 3. *Scardinius acarnanicus* from the field. (a) Larva 7·9 mm; (b) larva 12·0 mm.

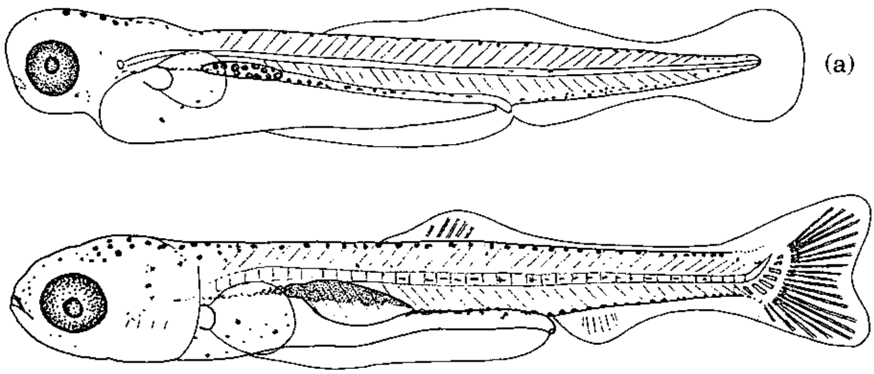

FIG. 4. *Rutilus ylikiensis* laboratory reared. (a) Embryo 7·0 mm NL, day 1; (b) larva 10·9 mm, day 16.

Already at hatching, conspicuous double rows of melanophores run along the dorsal and ventral sides of the body. Stellate melanophores occur on the head region, a mediolateral stripe extends up to the tail and branched melanophores are scattered over the yolk sac. Larvae over 10·5 mm develop side melanophores that progressively cover the whole dorsolateral region and form small aggregations in the ventrolateral region.

Early larvae of *S. acarnanicus* frequently form dense aggregations in quiet parts of the lake, especially during storms. Schools of late larvae often travel along the shoreline. While retaining the same melanophore pattern, field-collected larvae appeared conspicuously more heavily pigmented than the laboratory reared ones, reflecting differences in the ambient light intensity in the field and the aquarium (Kendall *et al.*, 1985).

Rutilus ylikiensis *(Fig. 4)*

The endemic *R. ylikiensis* is closely related to the Mediterranean complex of *Rutilus* species (Rincon & Lobon-Cervia, 1989; Economidis, 1991). In Lake Trichonis, *R. ylikiensis* feeds on both animal and plant food (Daoulas & Economidis, 1984) and spawns once a year from age 1 (*c.* 90 mm F.L.) to a maximum age 10 (max. 258 mm F.L.) in females and 7 (max. 202 mm F.L.) in males (Daoulas & Kattoulas, 1985). The eggs are deposited in March on plants or on gravel. No eggs were obtained from Lake Trichonis, but fertilized eggs (*c.* 2·05 mm) firmly adhered to the stones of a coastal spring of Lake Amvrakia were collected on 19 March 1991 and incubated in the laboratory at 22°C. The larvae hatched at 6·5 mm NL with no functional mouth and anus. The swimbladder filled on the second day. Flexion occurred on day 9 at 9·5 mm. The anal and dorsal fin rays appeared on days 15–18 at *c.* 10·5–11 mm, and the pelvic fins budded at 12·5 mm, along with the development of pectoral fin rays. The larvae metamorphosed at *c.* 15 mm. Rearing through the juvenile stage permitted positive identification as *R. ylikiensis*, based on adult characters.

Their melanophore patterns are similar to most other cyprinid larvae. At hatching, only the eyes and an area above the yolk-sac are pigmented. From day 2 a double row of melanophores develops along the dorsal side of the body, a double ventral row from the anus backwards to the tail, a weak mediolateral stripe and large head melanophores. The gut is distinctly pigmented and large melanophores cover the swimbladder. With development, pigment increases on the head, snout, otocyst and in the preanal region, new melanophores emphasize the mediolateral line, and progressively more and larger scattered melanophores appear in the dorsolateral area.

In the light pigmented *R. ylikiensis*, the melanophores are smaller and more widely spaced than in other cyprinid larvae. Larvae and juveniles were rather infrequent near the shore, but more frequent than other cyprinid larvae in plankton hauls or bottom-tows away from the shore.

Tropidophoxinellus hellenicus *(Fig. 5)*

T. hellenicus is endemic to the Acheloos basin and to one river of the western Peloponnese. Its phylogenetic relationships are still a question of investigation (Stephanidis, 1974). It grows to 11 cm F.L. over 4 years, shoals semipelagically, and feeds mainly on zooplankton, but seasonally on benthos (Daoulas, 1985, 1986). It is a fractional spawner maturing at age 2, and lays adhesive, yellowish eggs (1·0–1·6 mm after spawning but before fertilization) on aquatic vegetation, from April to July (Daoulas, 1984).

Larvae below 5·5 mm NL have similar body morphologies and pigment characteristics to those of *S. acarnanicus*. Eggs deposited on aquatic vegetation on known spawning grounds of *T. hellenicus* were incubated in the laboratory, but these also contained eggs of *S. acarnanicus*, causing confusion about the identity of the subsequent larvae. For larvae above 5·5 mm NL, safe diagnostic characters were established from wild larvae. In particular, flexion and fin ray development in *T. hellenicus* occurs at a smaller size, the body is more slender, and the anus, dorsal, anal and pelvic fins are more anteriorly situated than in *S. acarnanicus*. Pigment variation starts to develop at 6 mm, with *T. hellenicus* becoming progressively more heavily pigmented dorsally and ventrally than *S. acarnanicus*. Furthermore, the dorsal melanophores in *T. hellenicus* appear to extend slightly laterally, whereas in *S. acarnanicus* they do not. Also, *T. hellenicus* has melanophores on the base of the pectoral fins, and *S. acarnanicus* has fewer melanophores between the end of the double ventral row and the tail. The pigmentation patterns diverge more prominently after 7·5 mm,

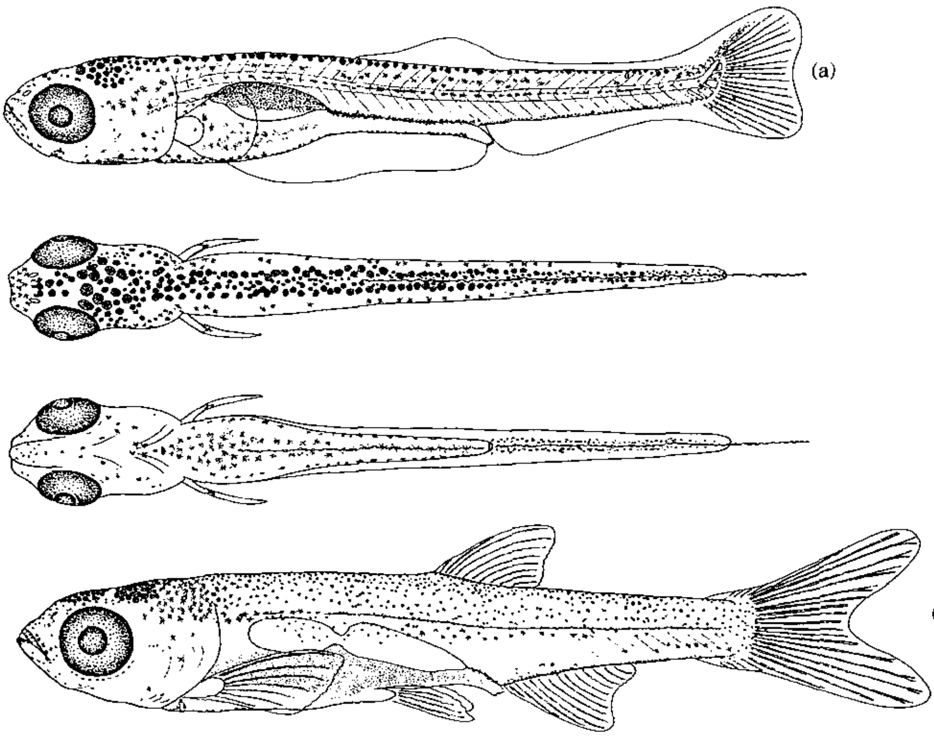

FIG. 5. *Tropidophoxinellus hellenicus* from the field. (a) Larva 7·6 mm (lateral, dorsal and ventral view); (b) larva 9·6 mm.

when *T. hellenicus* develops side melanophores, whereas in *S. acarnanicus* such melanophores develop after 10·5 mm. Further, the dorsolateral melanophores in *T. hellenicus* are uniform, while those of *S. acarnanicus* are larger stellate with smaller ones among them.

The larvae school along the shoreline together with individuals of *S. acarnanicus*. During storms, dense aggregations of young larvae of the two species occurred in shallow protected pools formed by accumulated rotten vegetation and broken reeds.

Barbus albanicus, Barbus peloponnesius peloponnesius *(Fig. 6)*

B. albanicus is fluvio-lacustrine, endemic in Lake Trichonis and its tributaries. *B. p. peloponnesius* is an entirely rheophilic endemic barbel of western Greece and Peloponnese with related subspecies found in northern Greece, Albania and SW Yugoslavia. It is found commonly in streams discharging into Lake Trichonis.

B. albanicus matures at age 3–4, reaching a maximum of 29 cm F.L. at maximum age of 14 (Daoulas & Economidis, 1989). *B. p. peloponnesius* matures at age 2–3 years and does not exceed 16 cm. Both species are fractional spawners breeding between May and July. *B. albanicus* spawns partly in the tributaries and partly in the lake. Individual females may spawn with three to seven males in sandy beaches of the lake, especially near stream estuaries. The eggs of both species are spherical, yellowish and non-adhesive. Ripe eggs of *B. albanicus* and *B. p. peloponnesius* in the gonad measured *c.* 2·5 and 2·1 mm respectively.

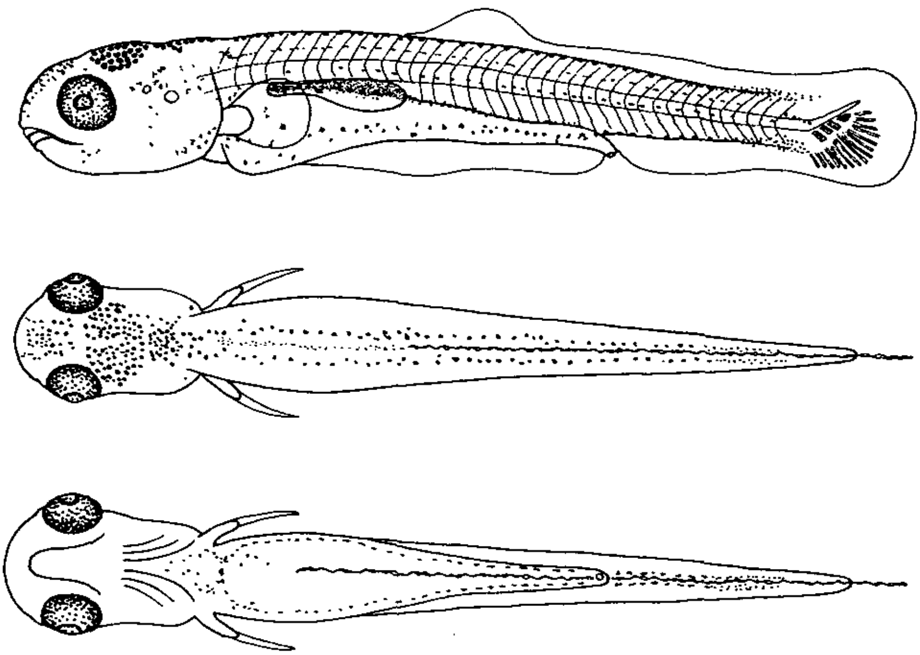

FIG. 6. *Barbus* sp. from the field. Larva 10·6 mm (lateral, dorsal and ventral view).

The larvae of the two species are indistinguishable in the field and have not been reared yet. The mouth migrates ventrally early, and the primordial fin in the tail region is almost rectangular. The pigmentation pattern consists of double dorsal and ventral post-anal rows of melanophores, a mediolateral stripe which persists during development despite the addition of numerous lateral melanophores, and the presence of a characteristic preanal parabolic row of abdominal melanophores. The barbels appear between 16 and 17 mm and scalation develops at 18 mm. The two species become distinct at 15 mm, when *B. albanicus* larvae develop serration on the last single ray of the dorsal fin. [The larvae of *B. barbus* (L.) develop serration on the same ray at about the same size (see Kryzhanovsky, 1949; Koblitskaya, 1981)].

In streams, the sympatric *Barbus* and *L. cephalus* larvae were distinguished by the absence in *Barbus* of the pre-anal single row and the presence of a parabolic row in the abdominal region. Late *Barbus* larvae differ from *L. cephalus* larvae in that their body melanophores become arranged in bars, the mouth is more ventral and the anus and unpaired fins are more anteriorly situated. In the lake, larvae and juveniles of *B. albanicus* occurred predominantly in small single species shoals in bays and fishing harbours.

Pseudophoxinus stymphalicus *(Fig. 7)*

P. stymphalicus is endemic to Greece and Lake Ohrid (Yugoslavia). In Lake Trichonis it aggregates in littoral pools and marshes, enclosed bays, protected harbours and the estuaries of tributaries. It is small (up to 65 mm F.L.), feeds on diatoms, algae, cladocerans, copepods, amphipods, insects and larvae of *Dreissena polymorpha* (Pallas), and spawns adhesive spherical eggs (1·3 ± 0·05 mm)

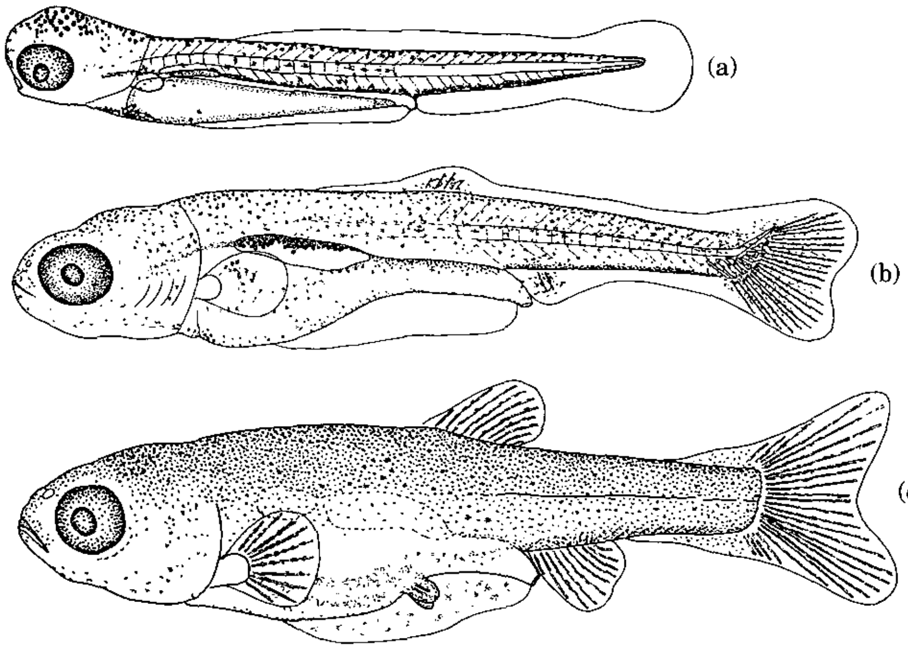

FIG. 7. *Pseudophoxinus stymphalicus* laboratory reared. (a) Embryo 4·8 mm NL, day 1; (b) larva 8·0 mm, day 15; (c) late larva 10·6 mm, day 22.

repeatedly on vegetation from December to early April, peaking in February and March.

On 15 February 1990, unidentified eggs at an early stage of development attached to weeds were collected and incubated in an aquarium at 19° C. The larvae hatched after 5–7 days at 4·7 mm NL. Yolk was absorbed by 5·8 mm NL. Notochord flexion occurred between 6·9 and 7·4 mm. Anal and dorsal fin development started at 7·7–8 mm. The pelvic fins budded at 10–10·5 mm. Transformation to the juvenile began around day 35 at 10·8 mm and was completed at 12 mm.

The larvae are immediately distinguishable from other cyprinids by their more robust body and darker appearance. At hatching they are heavily pigmented and are characterized by dorsal and ventral double rows of numerous close-spaced melanophores, scattered lateral melanophores and sometimes a very weakly developed, almost inconspicuous, mediolateral stripe. At 10 mm, the body is laterally more compressed than in other cyprinids. By day 90 the juveniles reached 20–34 mm and were then identified as *P. stymphalicus*.

GOBIIDAE

Three freshwater gobies inhabit Lake Trichonis, *Economidichthys trichonis*, *E. pygmaeus* and the provisionally ascribed *Knipowitschia caucasica* (Economidis & Miller, 1990). The two *Economidichthys* species possess a perianal dermal organ, unique among teleosts, the function and phylogenetic significance of which have yet to be clarified (Bianco *et al.*, 1987; Daoulas *et al.*, 1993). All three species mature during their first year, display sexual dimorphism, spawn successive batches of eggs during only one breeding season, exhibit parental care and die

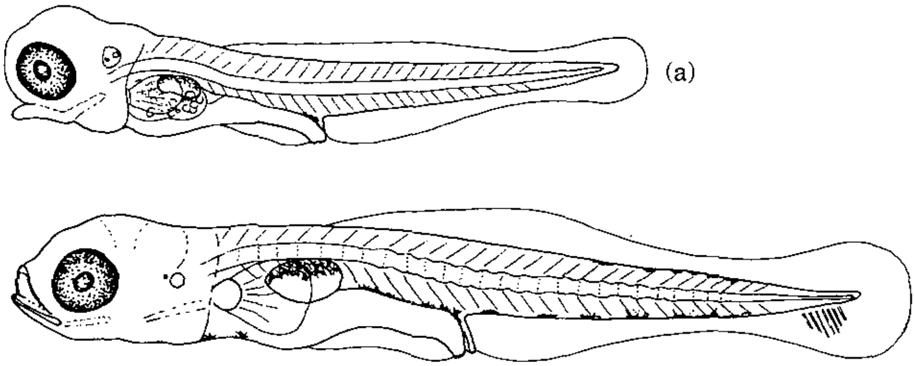

FIG. 8. *Economidichthys trichonis.* (a) Embryo laboratory reared 2·2 mm NL, day 1; (b) larva from the field 6·4 mm.

soon after spawning. The eggs are elliptic with characteristic structure and appearance and are laid in a single layer in nests guarded by the male. The larvae are distinguishable by body shape, fin formulae (separate dorsal fins, pelvic fins united, rounded caudal fin), prominance of the swimbladder and position of the anus.

Economidichthys trichonis *(Fig. 8)*

This smallest freshwater European teleost is known only from Lake Trichonis (Economidis & Miller, 1990). It spawns ovoid eggs (0·65 × 0·6) from late February–mid-May on the inner surface of broken reeds, previously cleaned by males. Planktonic larvae hatch at 2·1–2·5 mm NL, almost completely unpigmented.

Recently constructed male-guarded nests were brought to the laboratory where larvae hatched. The yolk sac was absorbed within 1 day and the larvae died within 4 days having reached 2·6 mm, and exhibited increased pigmentation. Wild specimens up to 7 mm had a single row of ventral post-anal melanophores, a single row of dorsal melanophores, pre-anal pigment cells and melanophores above the gut and on the swimbladder. Later, new melanophores are added on the head, the lower jaw and on the sides of the body, finally taking the form of transverse bars (Daoulas *et al.*, 1993).

The early larvae of *E. trichonis* are smaller than any other species in the lake. Larvae above 4 mm resemble those of *K. caucasica*, and separation is not always possible from badly preserved material (see below).

Economidichthys pygmaeus *(Fig. 9)*

E. pygmaeus is endemic to western Greece. It breeds in Lake Trichonis in March and April. The spherical ripe egg becomes oblong (2·4 × 0·9 mm) after fertilization. The species displays the same hole-nesting behaviour and parental care activities as *E. trichonis*. Numerous larvae were collected in tows on or just above the bottom, but few with plankton nets, suggesting that they are mainly demersal. Larvae were also reared from nests. They hatch at *c.* 4·3 mm NL, at a more advanced stage than larvae of *E. trichonis*, and their pigmentation pattern, already apparent during the embryonic stage, is fully developed. At all stages, the embryos and larvae have double dorsal and ventral rows of

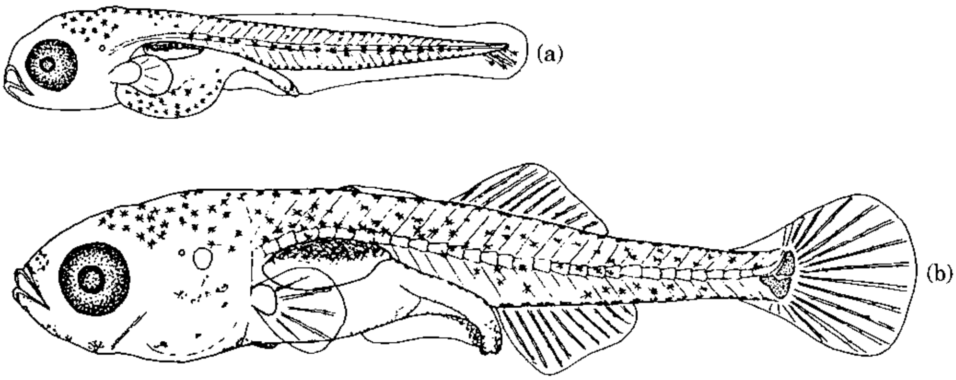

FIG. 9. *Economidichthys pygmaeus* laboratory reared. (a) Embryo 4·1 mm, day 1; (b) larva 6·6 mm, day 13.

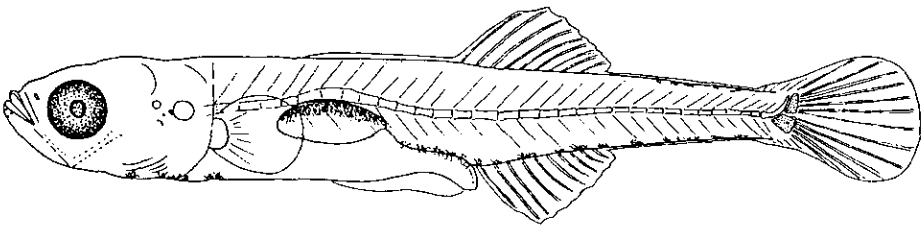

FIG. 10. *Knipowitschia caucasica* from the field. Larva 6·9 mm.

melanophores and aggregation of pigment cells on the head, the throat and the tail. By metamorphosis at 8–8·5 mm, lateral aggregations of melanophores had appeared, finally becoming short transverse bars (Daoulas *et al.*, 1993).

Knipowitschia caucasica *(Fig. 10)*

Daoulas *et al.* (1993) described wild larvae of *K. caucasica* measuring 5 mm and larger, and remarked that at *c.* 5 mm they resemble *T. trichonis* in shape and melanophore distribution. In well preserved material, distinction is possible on account of slight differences in robustness, intensity of pigmentation, placement of individual melanophores at comparable sizes and size of the pelvic disc. Also, caudal flexion had occurred in *K. caucasica* by the size of 5 mm, while in *E. trichonis* it occurs at 6·5–7 mm. Larvae definitely identified as *K. caucasica* occurred mainly in the bottom tows during April and May. Descriptions of the spawning behaviour under aquarium conditions and development of early larvae of *K. caucasica* are in preparation.

SILURIDAE

Silurus aristotelis *(Fig. 11)*

S. aristotelis is endemic to the Acheloos lakes but has been introduced to Janina Lake (Epirus) and Volvi Lake (Macedonia). It looks like a dwarf *S. glanis* L. but is distinguished by two pairs of barbels instead of three. Little is known of its biology. It feeds at night predominantly on fish, and secondarily on crustaceans, gastropods, insects, frogs and aquatic snakes (Iliadou & Ondrias,

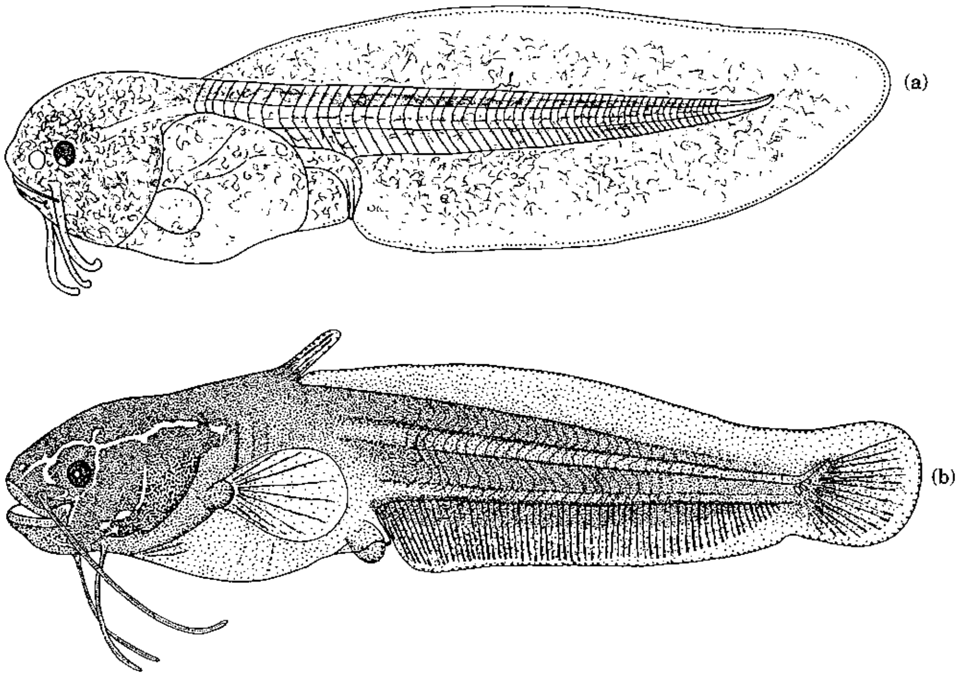

FIG. 11. *Silurus aristotelis* from laboratory rearing. (a) Embryo 7·2 mm NL, day 1; (b) larva 15·2 mm, day 10.

1986). The females mature at 2–3, spawn repeatedly from April to July, and attain maxima of 10 years and 40 cm (Iliadou & Ondrias, 1986). They excavate nests or attach eggs to tree roots (Breder & Rosen, 1966). Fishermen in Lake Trichonis have reported nest-building with pieces of aquatic plants at depths of *c*. 2–3 m.

We have failed to find nests or larvae in the field. A mature female was stripped on 18 May 1990 and the eggs were artificially fertilized. They measured around 2·7 mm, and 13 embryos hatched after 5 days at 23° C. The newly hatched larvae were *c*. 7 mm NL, and were toad-like, with small eyes. The greenish yolk-sac was ovoid, the mouth and anus were open, and three pairs of barbels were present, one on the maxilla and two on the mandibula, in contrast to two pairs possessed by the adults. The body was covered by numerous branched melanophores and surrounded by a large pigmented primordial fin. Growth was exceptionally fast and differentiation rapid. On day 2 the yolk of an 8·5 mm NL larva was almost absorbed, the maxillary barbel was relatively larger than the mandibular barbels, and notochord flexion was under way. On day 5 the larvae had reached 11·5–12·5 mm, the hypurals were well-developed, and rays were differentiated in the anal, pectoral and dorsal fins. On day 8, the last survivor reached 15·5 mm, its primordial fin was restricted to its dorsal surface, the dorsal, anal and pectoral fin rays were further developed, and the pelvic fins had budded. From juveniles caught with scoop nets and bottom tows in thick vegetation, it was documented that one mandibular pair of barbels was lost at *c*. 95 mm.

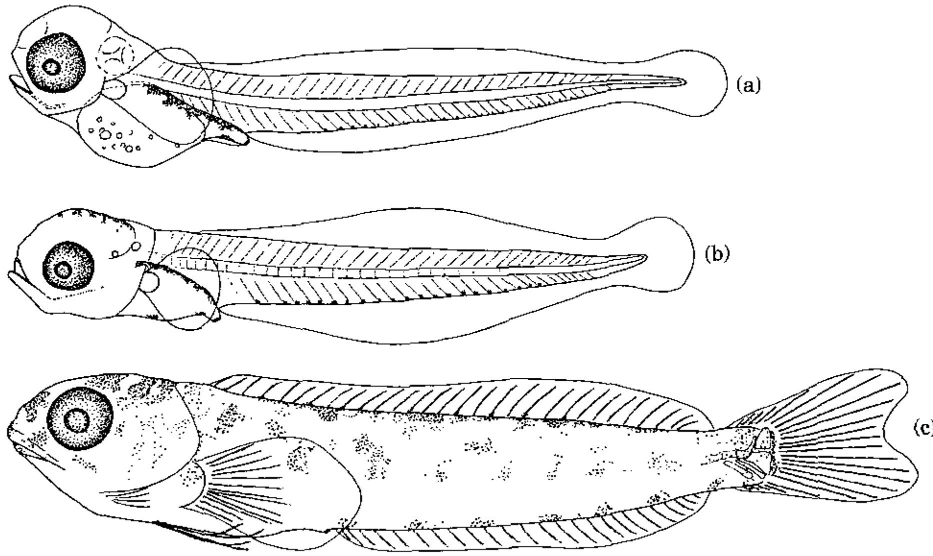

FIG. 12. *Salaria fluviatilis*. (a) Embryo laboratory reared 3·7 mm NL, day 1; (b) larva from the field, 6·4 mm NL; (c) larva from the field, 15 mm.

Like the adults, the larvae were photophobic and hid under stones in the aquarium. They were extremely voracious and fed by scooping the bottom and the sides of the tank.

BLENNIIDAE

Salaria fluviatilis *(Fig. 12)*

S. fluviatilis is a blenny common in fresh waters around the Mediterranean and Black Seas (Moosleitner, 1988; Papakonstantinou, unpublished). It is cryptobenthic, feeds on aquatic insects, exhibits breeding colouration, reproduces in spring and summer, the female deposits small eggs underneath rocks and the male guards the eggs (Freeman *et al.*, 1990; Tortonese, 1975).

In Lake Trichonis, spherical eggs which compress to $0{\cdot}68 \pm 0{\cdot}04 \times 0{\cdot}94 \pm 0{\cdot}04$ mm when attached, are laid from May–July. They contain numerous yellowish oil droplets and vary from white to bright violet, depending on the stage of development. The newly hatched larvae (*c.* 3·5 mm NL) are transparent, narrow-elongated, and lack a functional mouth. They are very noticeable from their large, black, reflective eyes, and have a spherical yolk sac, the anus positioned anteriorly, a large primordial fin, large stellate melanophores on the dorsal side of the digestive system, and a single row of ventral punctuate melanophores from the sixth post-anal myomere to near the tail, each melanophore corresponding to one myomere. This differs from Cipria's (1936) description of *Blennius inaequalis* C. & V. (=*S. fluviatilis*, [Moosleitner (1988); Tortonese (1975)]) in having no large melanophore midway between the end of the ventral row of melanophores and the end of the notochord.

Early larvae were attracted at night by artificial light. They remained planktonic throughout the larval stage, until settlement at 14–15 mm. Newly settled individuals exhibited increased juvenile pigmentation.

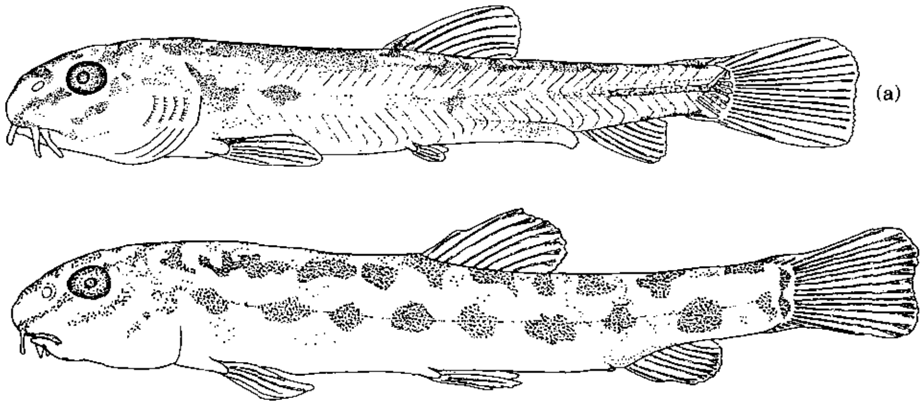

FIG. 13. *Cobitis trichonica* from the field. (a) Late larva 16·5 mm; (b) post-metamorphosed juvenile 25·2 mm.

COBITIDAE

Cobitis trichonica *(Fig. 13)*

C. trichonica is endemic to the Acheloos basin. Adults occur in shallow water in muddy areas or among aquatic vegetation, ranging from 45–85 mm (90 mm T.L. maximum recorded). It is inferred from gonadosomatic index variation and oocyte size distribution that the eggs are released in batches from April–June. Ripe eggs in the gonads are yellowish, spherical, and measure between 1·1 and 1·3 mm.

One late larva and one recently metamorphosed juvenile were found among submerged vegetation in the shallow delta of a slow-flowing stream with a muddy bottom. These had characteristic cobitid body shape, position of anus, presence of upper jaw barbels and pigmentation patterns (Koblitskaya, 1981; Saitoh, 1990). At 16·5 mm long, fin rays were complete, lateral aggregations of melanophores were under way, but no lower jaw dermal appendages had yet appeared. In the 25·2 mm juvenile, scale development had initiated and all characters were differentiated. It is inferred from Koblitskaya's (1981) description of *C. taenia* L. larvae that the morphological characters of *C. trichonis* larvae correspond to those of smaller *C. taenia*.

ATHERINIDAE

Atherina boyeri *(Fig. 14)*

The schooling, planktophagous, euryhaline sand smelt, *A. boyeri* has invaded Lake Trichonis, presumably via the Acheloos River. This landlocked population is now extremely abundant and supports an important commercial purse-seine fishery.

The fish live up to 3 years (exceptionally 4) reaching 135 mm T.L. They breed from March to October with peak spawning in April. Peak larval abundance occurred in April–May and in September. The eggs, deposited in depths from 2–6 m, are spherical, *c.* 1·45 mm, containing several oil globules, and have long hairy appendages attaching them to filamentous aquatic plants. The larvae hatch at *c.* 5·8 mm NL and the yolk is completely absorbed at 6·3 mm NL. From

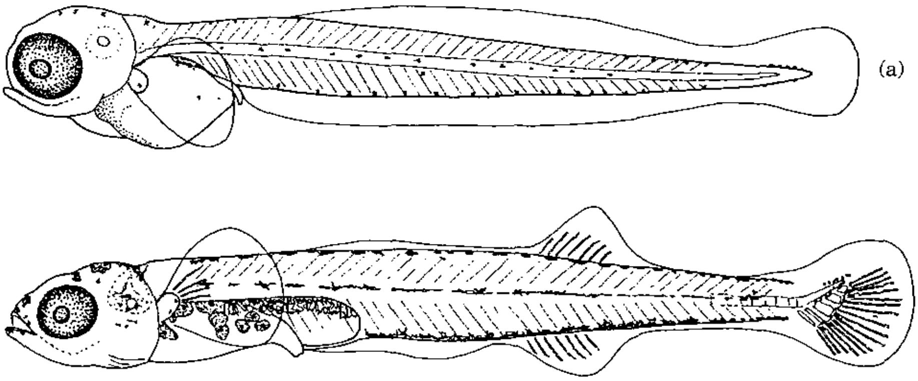

FIG. 14. *Atherina boyeri* from the field. (a) Embryo 5·8 mm NL; (b) larva 11·2 mm.

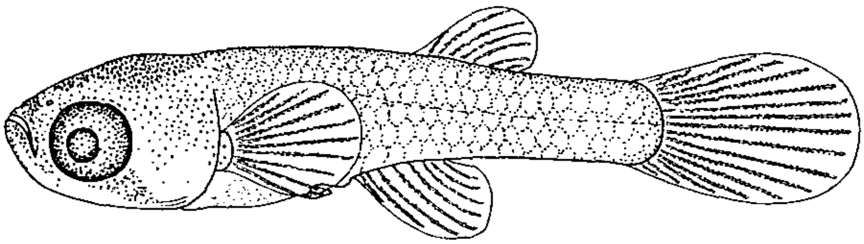

FIG. 15. Recently extruded juvenile of *Gambusia affinis*, 7·8 mm.

field material, flexion occurs between 10 and 12 mm, the second dorsal and the anal fins develop at *c.* 10–11 mm, the pelvic fins bud at *c.* 11 mm (and are complete at 12–12·5 mm), the first dorsal fin rays appear at 14–15 mm and scalation begins at 19–20 mm.

The larvae are identified by the narrow-elongated body, the anterior position of the anus, the characteristic pattern of head melanophores, the long mediolateral stripe with many elongated melanophores and the ventral single row of post-anal melanophores (divided into two by the marginal fin). These descriptions conform with those of Boscolo (1970). *A. boyeri* larvae and post-metamorphosed fish schooled close to the coasts, but were also plentiful in ichthyoplankton samples, suggesting a long stay in the pelagic habitat. They were positively phototactic throughout larval development.

POECILIIDAE

Gambusia affinis *(Fig. 15)*

The introduced American viviparous mosquitofish, *G. affinis*, is widely distributed in marshes, small harbours and generally stagnant waters of Lake Trichonis. It feeds on insects and small crustaceans, reaches 45 mm T.L., males being smaller than females, and is notably sexually dimorphic. *G. affinis* does not have a larval stage. Its development is in accordance with earlier descriptions (e.g. Flegler-Balon, 1989).

OTHER SPECIES

The larvae of four other cyprinids are likely to occur in Lake Trichonis, because adults have been recorded.

Tinca tinca

Tench is native to northern Greece, but a rare introduction in Lake Trichonis. Its larvae have only an intense ventral row of melanophores and lack a dorsal row and a mediolateral stripe (Koblitskaya, 1981).

Cyprinus carpio

Carp is native to northern Greece, but a rare introduction in Lake Trichonis. Carp larvae display only a dorsal and ventral row of melanophores and lack a mediolateral stripe (Jones *et al.*, 1978; Koblitskaya, 1981).

Carassius auratus gibelio

This European subspecies of *C. auratus* (L.) is native to north-eastern Greece and a relatively abundant introduction in Lake Trichonis. Like the larvae of carp, the larvae of *C. a. gibelio* display only a dorsal and a ventral row of melanophores (Koblitskaya, 1981). A small number of such larvae were found in Lake Trichonis but have not been identified with certainty.

Phoxinellus pleurobipunctatus

This is endemic to rivers and streams of western Greece and Peloponnese, but is also likely to occur in south Albania. Larvae provisionally ascribed to *P. pleurobipunctatus* are almost identical to those of *L. cephalus*, and differ only in having one melanophore on the pectoral fin and usually a continuous rather than an interrupted row of preanal abdominal melanophores. However, larvae must be reared to confirm these findings.

IV. DISCUSSION

There is generally little information on the taxonomy and early life-histories of European freshwater fishes, especially those of southern Europe. Before the present surveys, specific identification of fish larvae from Lake Trichonis was impossible, except for *Gambusia affinis*, *Atherina boyeri* and a few cyprinids.

Moser & Ahlstrom (1974) have stressed that because fish embryos and larvae live in entirely different habitats than the adults, a functional independence of larval and adult characters is expected, which may provide independent assessments of phylogeny. Since it is often difficult to evaluate character states for primitiveness or degree of derivation in adults, it has repeatedly been suggested that larval characters may sometimes have a stronger bearing to phylogeny than adult characters (Ahlstrom & Moser, 1976; Cohen, 1985; Fuiman, 1985).

For example, the presence of three barbels in larval *S. aristotelis* suggests a common ancestry with *S. glanis*. However, morphologically and developmentally, *S. aristotelis* larvae resemble *Parasilurus* larvae more closely than other *Silurus* larvae. Kryzhanovsky (1949) stated that the eggs of *P. azotus* had a longer incubation period than those of *S. glanis* (5 days *vs* 3, at *c.* 24° C), and that the larvae of *P. azotus* hatch with three pairs of barbels, subsequently losing one, while *S. glanis* retains three pairs of barbels throughout life. Also, *S. glanis* larvae have only rudimentary barbels at hatching. Our data suggest that the ontogenetic development of *S. aristotelis* resembles that of *P. azotus* more than of *S. glanis*. Karyologically, *S. aristotelis* also resembles the *Parasilurus* group of species (Iliadou & Rackham, 1990). Based on such evidence, we suggest that the taxonomic status of *S. aristotelis* at the generic level should be re-examined.

Our data on the modes of reproduction and the embryonic and larval morphologies of the fishes of Lake Trichonis, coupled with relevant information taken from the literature (e.g. Kryzhanovsky, 1948; Balon, 1975, 1981; Koblitskaya, 1981; Flegler-Balon, 1989), indicate that the breeding habits and an array of embryonic and larval characters, for example the body form, the position of the anus, the fin structure and the shape of the swimbladder, have a potential systematic value at a high (e.g. family) taxonomic level. The underlying assumption is that such characters respond little to local environmental pressures, and are likely to be present in a group of taxa because of common ancestry rather than as a result of convergent evolution through natural selection acting independently on these taxa.

At a low taxonomic (e.g. generic or specific) level, the pigmentation pattern offers the most promising array of systematic characters. Nevertheless, the larval pigmentation patterns are highly adaptive and deviate quite rapidly within a group of taxa of common ancestry. However, it is this adaptiveness which may potentially reveal genealogical lineages within a high taxonomic group. According to this view, pigment divergence from a basic pattern reflects the most recent derivations from a common ancestor, while there has been insufficient evolutionary time since derivation for a sufficient divergence in more conservative characters to accumulate.

Do classifications of larval fishes based on the pigmentation pattern approximate phylogenetic relationships? Koblitskaya (1981) classified cyprinid genera into three groups according to the presence of one, two or three rows of melanophores along the larval body. The first group includes larvae with a dorsal, a ventral and a mediolateral row, and is represented in Lake Trichonis by larvae of the genera *Rutilus*, *Leuciscus*, *Barbus*, *Tropidophoxinellus*, *Scardinius* and *Phoxinellus*. The second group, with a dorsal and a ventral row, is represented by the genera *Cyprinus* and *Carassius*. The mediolateral row in *P. stymphalicus* larvae is extremely weak and hardly noticeable, and perhaps the genus *Pseudophoxinus* should also be placed in this group. Larvae of the third group, with only a ventral row, is represented by tench only.

It is not certain whether such a classification reflects phylogenetic relationships or the results of convergent evolution. At present, it is not possible to assess the relative contribution of the phylogenetic effects and the environment in the formulation of pigmentation patterns of the Trichonis Lake species. The utility of the larval pigment patterns in taxonomy is also limited by the changing melanophore pattern with the growth of larvae, the variable state of melanophore contraction due to the effects of lighting, and the extent of individual variability in melanophore distribution within species. To arrive at classifications with a potential systematic value, comparative data on embryonic and larval ecology and morphological development are needed, especially at the population level, where most evolutionary change is expected to occur.

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