

has also been reported in the brain and optic ganglia of *Caridina fossarum*.

Periodic acid-Schiff (PAS) positive neuro-secretory material has been reported by many workers^{1,3,6,7,11,12}. A, B and C type neurosecretory cells of the optic lobe of *P. magnum magnum* show weak to moderate positivity to PAS; type D cells show significant positivity. Similar results have been reported in *C. fossarum*¹³.

Different results have been obtained for lipid components of neurosecretory material in Arthropoda. Earlier workers have found little or no lipid in the neurosecretory cells of different arthropods. However, a little to moderate amounts of lipids are detected in the neurosecretory cells of the eyestalk of *P. magnum magnum*.

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LACTATE DEHYDROGENASE IN THE GRASS CARP *CTENOPHARYNGODON IDELLA* (PISCES)

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CTENOPHARYNGODON IDELLA, commonly known as grass carp, an exotic fish introduced into Indian

aquaculture in 1959 to control submerged vegetation in freshwater bodies, has since become an important constituent in polyculture practices¹.

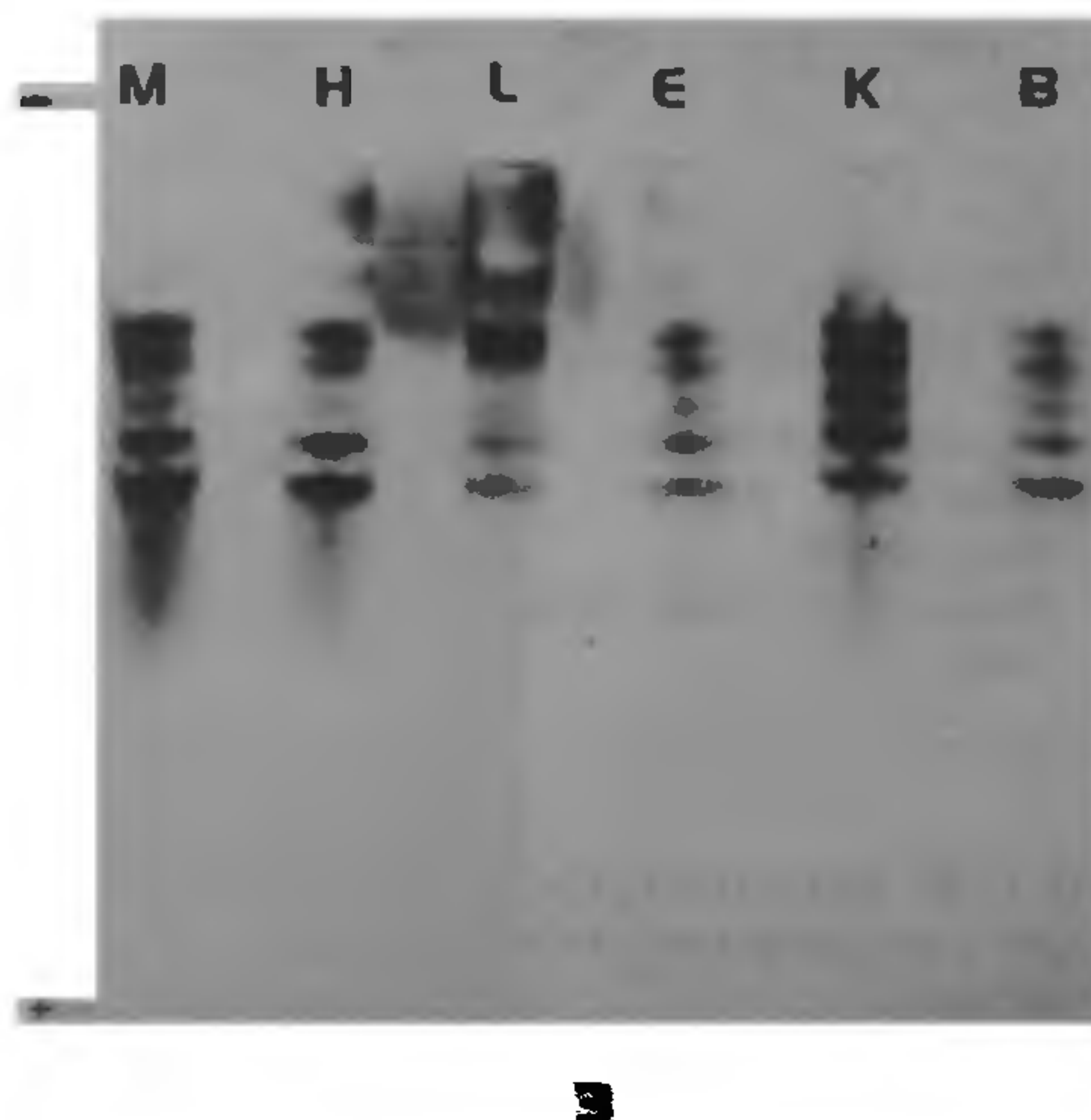
Lactate dehydrogenase (LDH, L-lactate: NAD oxidoreductase, E.C. 1.1.1.27), a polymorphic, multi-locus enzyme, is a useful system in the understanding of differential gene expression in developmental stages and in different tissues of adults^{2,3}. In most vertebrates, LDH has a tetrameric structure, and usually two different codominant loci have been identified which code for two subunits, A and B. These two subunits randomly associate with each other to form five tetrameric isozymes, viz. A₄, A₃B₁, A₂B₂, A₁B₃ and B₄. However, in many teleosts, there occurs a third locus, C, which may be expressed as a highly anodal band in eye and brain (eye-specific)⁴⁻⁶ or as a slightly anodal (cathodal) band in liver (liver-specific)⁶⁻⁸. So far as the authors are aware, the expression of LDH isozymes in *C. idella* has not been studied earlier, either in developing stages or in adult tissues.

Healthy female and male brood fishes of *C. idella* were hormonally induced to release gametes in a specially designed 'breeding pool' in the Ganga Matsya Utpadan Kendra, Rathtala, West Bengal. The fertilized eggs were immediately collected and removed to a 'glass-jar hatchery' or to a 'Chinese hatchery' for smooth progress of development. The developing embryos were collected at suitable times and examined under the microscope to ascertain the developmental stage by identifying characteristic features (designated by serial numbers in table 1). The embryos were thoroughly washed in double-distilled water and then homogenized. The homogenates were centrifuged at 11,000 g for 30 min at 4°C. A known amount of the supernatant was used for electrophoresis. Polyacrylamide slab gel was prepared by following the methodology of Ornstein⁹ and Davis¹⁰. Electrophoresis was carried out at 200 V and 3 mA/slot and the gel was then stained for LDH bands following the procedure of Nakano and Whiteley¹¹. The relative mobility values of the bands were then calculated.

A single LDH band (figure 1) was observed in nine pre-hatching stages. But just before hatching, 5 electrophoretic bands appeared (figure 2) and persisted for 72 h after hatching. In the adult fish also, these five LDH bands were observed in muscle, heart, liver, kidney, eye and brain (figure 3). In addition, there was a lowly anodal band in the liver of adult. The staining properties and other details of the bands are listed in table 2.

Table 1 Arbitrary designation of different developmental stages of *Ctenopharyngodon idella*

Arbitrary stage	Hours (h) after fertilization (f)/hatching (H)	Morphology
G-1	$\frac{1}{2}$ h f	Non-adhesive and non-floating zygote, cleavage started
G-2	$\frac{3}{4}$ h f	Cleavage continued
G-3	1 h f	Mass of cell over the yolk
G-4	2 h f	Blastodisc appeared
G-5	3 h f	Embryo enlarged in size and covered two thirds of the yolk surface
G-6	5 h f	— do —
G-7	7 h f	Eye-bud initiation
G-8	9 h f	The embryo with yolk-sac bean-shaped, head and tail distinguishable
G-9	11 h f	Twisting movement of the embryo inside the egg case
G-10	13 h f	Hatching
G-11	3 h H	Vitelline circulation started
G-12	6 h H	— do —
G-13	12 h H	No pigmentation over eyes
G-14	18 h H	Pigmentation over eyes
G-15	24 h H	Pigmentation over the eyes dense
G-16	36 h H	Dorsal fin developed
G-17	48 h H	— do —
G-18	60 h H	Yolk-sac reduced, gills appeared
G-19	72 h H	Complete dissolution of yolk-sac, free swimming fries



Figures 1 and 2. 1, LDH zymograms of pre-hatching and 2, post-hatching stages showing 1 and 5 bands respectively.

Figure 3. LDH zymograms of different tissues in adult *C. idella*. (M, muscle; H, heart; L, liver; E, eye; K, kidney; B, brain.)

Table 2 Lactate dehydrogenase isozyme patterns in developmental stages and in different tissues of *Ctenopharyngodon idella*

Stage no./tissue	No. of bands	Rd values	Staining intensity
In developing embryo			
G-1 through G-10 (pre-hatching)	1	0.261 ±	2+
G-11 through G-19 (post-hatching)	5	0.131 ± 0.156 ± 0.187 ± 0.212 ± 0.250 ±	2+ 2+ 2+ 2+ 3+
In adult			
Muscle	5	0.141 0.173 0.206 0.233 0.282	3+ 2+ 2+ 3+ 3+
Heart	5	0.141 0.173 0.206 0.233 0.282	3+ 3+ 2+ 3+ 3+
Liver	6	0.092 0.141 0.173 0.206 0.233 0.282	3+ 3+ 1+ 2+ 2+ 2+
Eye	5	0.141 0.173 0.206 0.233 0.282	2+ 1+ 1+ 2+ 2+
Kidney	5	0.141 0.173 0.206 0.233 0.282	3+ 3+ 3+ 3+ 3+
Brain	5	0.141 0.173 0.206 0.233 0.282	2+ 2+ 2+ 2+ 2+

1+, faint staining (marginal presence); 2+, moderate staining (intermediate abundance); 3+, intense staining (most abundant).

In *Catla catla*, *Cirrhina mrigala* and *Labeo rohita*, 3 LDH bands were expressed in both pre- and post-hatching stages while 5 bands were observed throughout the developmental stages of *Labeo bata*¹². However, during the initial stages of development, some species have been reported to

express only one LDH band depicting B₄ isozyme (e.g. in trout)¹³ while in some others, only A₄ is expressed (e.g. in *Micropterus salmonoides salmonoides*)¹⁴. In others, both LDH A and B sub-units may be expressed equally¹⁵. It seems that the single LDH band appearing during the pre-hatching stages represented B₄ isozyme as indicated by the relative anodal mobility. The five bands expressed in both post-hatching stages and in adult tissues presumably represent five tetrameric associations, viz. A₄, A₃B₁, A₂B₂, A₁B₃ and B₄.

The additional lowly anodal band in the liver has presumably liver-specific C-gene expression. Incidentally, the appearance of LDH C₄ has been reported to coincide with either retinal development^{16,17} in some species, where eye-specific C-gene expression occurs, or with liver embryogenesis in some others, where liver-specific expression of the gene occurs¹⁸. In the present study, the LDH C gene expression is lacking in embryos even up to 72 h after hatching, presumably because functional activity of the liver did not start, though it had been formed. Further immunochemical studies may be helpful in confirming the genetic involvement speculated through the expression of the LDH bands.

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AEROMONAS HYDROPHILA SEPTICAEMIA OF INDIAN MAJOR CARPS IN SOME COMMERCIAL FISH FARMS OF WEST GODAVARI DISTRICT, ANDHRA PRADESH

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AEROMONAS HYDROPHILA is an important pathogen of warm water fishes¹. Gopalakrishnan² reported many instances of entire populations of Indian major carps being wiped out by epidemics of *A. hydrophila* infection in stocking tanks in West Bengal, India. Snieszko and Axelrod³ classified disease symptoms caused by *A. hydrophila* under four categories, viz. acute, rapidly fatal septicaemia, with a few gross symptoms; an acute form with dropsy, blisters, abscesses and scale protrusion; chronic ulcerous form with furuncles and abscesses; and latent form with no symptoms. An ulcerative form of *A. hydrophila* infection in *Catla catla*⁴ has

been reported earlier. An acute septicaemia due to *A. hydrophila* in some commercial fish farms of West Godavari District of Andhra Pradesh is discussed here.

Following complaints from some fish farmers in Eluru, West Godavari District, Andhra Pradesh, of mortality of fish in their farms during April 1988, infected fish were collected.

Farm A: Water spread area of 7 acres, stocking three Indian major carps, Catla, Rohu and Mrigal, in a ratio of 1:2:0.8. At the time of sampling, average weights were Catla 0.8 kg, Rohu 1 kg, Mrigal 0.5 kg. Mortality was noticed in Catla and Rohu at the rate of 6–7/day and 1–2/day respectively.

Farm B: Water spread area of 20 acres, stocking Catla, Rohu, Mrigal and grass carp in the ratio 1:3:0.5:0.125. Average weights of fish at the time of sampling were Catla 0.75 kg, Rohu 1.25 kg, Mrigal 1 kg and grass carp 2.0 kg. Mortality was noticed only in Rohu at the rate of 10–15/day.

Fish from both farms showed dark patches on the body. Live fish were transported to the laboratory for collection from surface lesions for culturing. Fish were anaesthetized by keeping cotton dipped in 70% alcohol under the operculum. The fish was then cut open using sterile instruments. Blood was drawn from the heart, and pieces of liver, kidney and spleen were removed, taking care to avoid contamination from the alimentary canal. All samples were plated on trypticase soy agar and incubated at ambient temperature. Isolates were purified and initial identification of the isolates was made using the diagnostic scheme suggested by Plumb and Bowser⁵. Identification of *A. hydrophila* was by a series of biochemical tests described earlier⁴.

Surface swabs from infected fish yielded predominantly *A. hydrophila*. This organism was isolated in pure culture from blood, liver and kidneys of infected fish. This indicated that there was acute septicaemia due to *A. hydrophila*. The organism's presence in blood, liver and kidney is a clear indication of its causative role. In both farms, the species that was in larger number was affected.

Factors contributing to virulence of *A. hydrophila* have been investigated earlier⁶. Allan and Stevenson⁷ demonstrated that crude extracellular preparations of *A. hydrophila* containing haemolytic and proteolytic activities could produce pathological symptoms in trout. Thune *et al.*⁸ also demonstrated that crude extracellular preparations from *A. hydrophila* containing haemolysin and heat-stable

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