of intra-cellular phenylalanine under this condition cannot make the synergistic inhibition of DAHP synthase operative as tyrosine and tryptophan level in the medium for these double auxotrophs are maintained at a level far below the required level.

1 Gibson, F. and Pittard, J., *J. Bacteriol. Rev.*, 1968, **32**, 465–492.
2. Jensen, R. A., Nasser, D S. and Nester, E. W., *J. Bacteriol.*, 1967, 94, 1582–1593.
3. Jensen, R. A. and Nassar, D. S., *J. Bacteriol.*, 1968, **95**, 188–198.
4. Maiti, T. K. and Chatterjee, S P, *Hind. Ant. Bull.*, 1990, **32**, 3–27.
5. Brown, K. D and Doy, C. H, *Biochim. Biophys. Acta*, 1965, **104**, 377–389
6 Smith, L. C., Ravel, J M, Lax, S. R. and Shieve, W., *J. Biol. Chem*, 1962, **237**, 3566–3570.
7. Wallance, B. J. and Pittard, J, *J. Bacteriol*, 1967, **93**, 237–244.
8 Halsal, D. M and Doy, C. H, *Biochim Biophys. Acta*, 1969, **185**, 432–440
9. Gollub, E., Zalkin, H. and Sprinson, D. B., *J Biol Chem.*, 1967, **242**, 5323–5328
10. Lingens, F., Goebel, W. and Uesseler, H, *Eur. J. Biochem*, 1967, **2**, 442–447
11. Lingens, F, Goebel, W. and Uesseler, H, *Biochem Z.*, 1966, **346**, 357–367.
12. Doy, C. H, *Biochim. Biophys. Acta.*, 1968, **151**, 293–299
13 Jensen, R. A. and Nester, E. W., *J. Mol. Biol.*, 1965, **12**, 468–481.
14. Jensen, R. A. and Trentini, W. C., *J. Biol. Chem.*, 1970, **245**, 2018–2022.
15. Gorisch, H. and Lingens, F., *Biochim Biophys Acta.*, 1971, **242**, 617–629.
16. Sugimoto, S., Miyajima, R. and Shiio, I, *Agril. Biol Chem*, 1973, **37**, 2327–2336.
17. Hagino, H and Nakayama, K., *Agril Biol. Chem*, 1974, **38**, 2125–2134
18. Maiti, T. K. and Chatterjee, S. P., *Acta Biotechnol*, 1991, **11**, 249–254.
19. Maiu, T. K. and Chatterjee, S P., *Folia Microbiol.*, 1991, **36**, 234–239.
20. Klausner, A., *Bio/Technology*, 1985, **3**, 301–307.
21. Roy, D. K. and Chatterjee, S. P, *Acta Microbiol. Polon.*, 1982, **3**, 117–122.
22. Alfoldi, L., *Ann. Inst. Pasteur*, 1958, **94**, 474–484.
23. Lowry, O. H, Rosenbrough, N. J, Farr, A L. and Randall, R J, *J Biol. Chem*, 1951, **193**, 265–275.
24. Srinivasan, P. R. and Sprinson, D. B., *J Biol Chem.*, 1959, **234**, 716–722.
25. Shetty, K., Crawford, D. L. and Pometto III, A. L., *Appl. Environ Microbiol.*, 1986, **52**, 637–643.
26 Kurahashi, O, Noda–Watanabe, M, Sato, K., Morinaga, Y. and Enei, H, *Agril. Biol. Chem.*, 1987, **51**, 1785–1792.
27 Huang, L, Monotoya, A. L, Nester, E. W., *J. Biol. Chem*, 1975, **250**, 7675–7686.
28 Sugimoto, S and Shiio, I., *Agril. Biol Chem.*, 1982, **46**, 2711–2720
29 De Boer, L., Vrijbloed, J W., Grobben, G. and Dijkhuizen, L, *Arch Microbiol.*, 1989, **151**, 319–325
30 Adelberg, E. A., *J. Bacteriol*, 1958, **76**, 326,
31. Ezekiell, D. H, *Biochim Biophys Acta.*, 1965, **95**, 54–62.
32. Coats, J H and Nester, E. W., *J. Biol Chem*, 1967, **242**, 4948–4955.

ACKNOWLEDGEMENT. The first author thanks CSIR, New Delhi for a research fellowship.

Received 31 December 1992, revised accepted 20 November 1993

Observations on fungal infection of *Chela laubuca* Ham. with special reference to deep mycoses

G. C. Srivastava, S. K. Sinha* and S. K. Prabhuji**

Department of Botany, St. Andrew's College, Gorakhpur 273 001, India
*U.P State Fisheries, Gorakhpur 273 015, India
**Department of Botany, U.N Postgraduate College, Padrauna 274 304, India

Some specimens of *Chela laubuca* Ham., bearing fungal infection on the body surface and eye, were collected from Domingarh Pond and Garden Water Tank of the Office of N.E. Railway, Gorakhpur, UP, India. The causal watermoulds have been identified as *Achlya orion* Coker & Couch, *Saprolegnia diclina* Humphrey, *S. ferax* (Gruith.) Thuret and *Pythium aphanidermatum* (Ed.) Fitz. Histopathological studies of infected skin have shown a varying degree of destruction of epidermis, hypodermis and the underlying musculature whereas infected eye has shown profuse hyphal growth, inflammation of cornea, disintegrated iris and deshaped retina. The pathogenic nature of the fungal isolates has also been proved under laboratory conditions.

THE pioneering work in the field of fish-mycopathology in India is that of Gopalakrishnan[1] who described fish mycoses caused by *Saprolegnia parasitica* in Indian waters. Subsequently, many other workers reported numerous watermould species parasitizing different species of fish and their eggs[2–11]. However, a perusal of the literature indicates very few reports on the deep mycoses in fish, in India[9,11].

During the course of investigations on fungi associated with fish diseases some specimens of *Chela laubuca* Ham., showing hyphal tufts protruding out through eyes and body surface, were collected during November 1982 to February 1983 and October 1990 to January 1991 from Domingarh Pond and Garden water tank of the Office of N.E. Railway, Gorakhpur, U.P. The infected living and dead specimens of fish were collected using hand-nets and brought to the laboratory in large-sized polythene bags, half-filled with fresh water. The infected fish, when placed in clean water, showed white cottony patches with hyphal tufts on body surface and eyes (Figure 1). Small bits of mycelium were taken out from white cottony patches and rinsed thoroughly in distilled water and were then placed in petri-dishes containing 10 ml of sterile distilled water on boiled hemp-seed cotyledons. Unifungal, bacteria-free cultures of the fungi were raised on the lines described earlier[12–14]. The fungi were identified as *Achlya orion* Coker & Couch (November 1982, October 1990; from body surface), *Saprolegnia diclina* Humphrey (December 1982, December 1990; from eye and body surface), *Saprolegnia ferax* (Gruith.)

Thurct (February 1983, January 1991; from eye) and *Pythium aphanidermatum* (Ed.) Fitz. (January 1983; from body surface) using earlier published monographs[14–17]. The identification of the fish specimen was confirmed as *Chela laubuca* Ham. with the key provided by Jhingran[18].

To ascertain the parasitic ability of the fungi isolated, controlled reinfection experiments were conducted using the healthy individuals of *Chela laubuca* on the lines of Scott and O'Warren[19] at room temperature ranging between 20 and 25°C. Covered Pyrex baking dishes (8 cm × 30 cm × 20 cm size) were wrapped in aluminium foil and sterilized in a hot-air oven at 200°C for 2 h. Filtered, sterile (bacteria-free) distilled water (1,500 ml per dish) was added aseptically to two dishes per experiment. Experiments were performed in duplicate for each fungus. A vibrator pump was used for aeration of the water throughout each experiment. Four fungus inoculated blocks and four uninoculated blocks of SPS-medium (1 sq. cm each) were placed at opposite ends of each infection dish. After two days oven-dried fish food was added and experimental fish (three in number) were put in the dishes. Three days prior to exposure to the fungal inoculum, test fish were dipped in 0.5 ppm solution of malachite green for 2 h and placed in trough containing sterile distilled water to eliminate any possible fungal contamination. After segregation (three days) test fish were inflicted injury in the following three steps:

(i) the fish was anesthetized in a solution of tricaine-methano-sulphonate (8 ml of 0.5% solution in 500 ml of sterile distilled water) for 1–2 minutes.

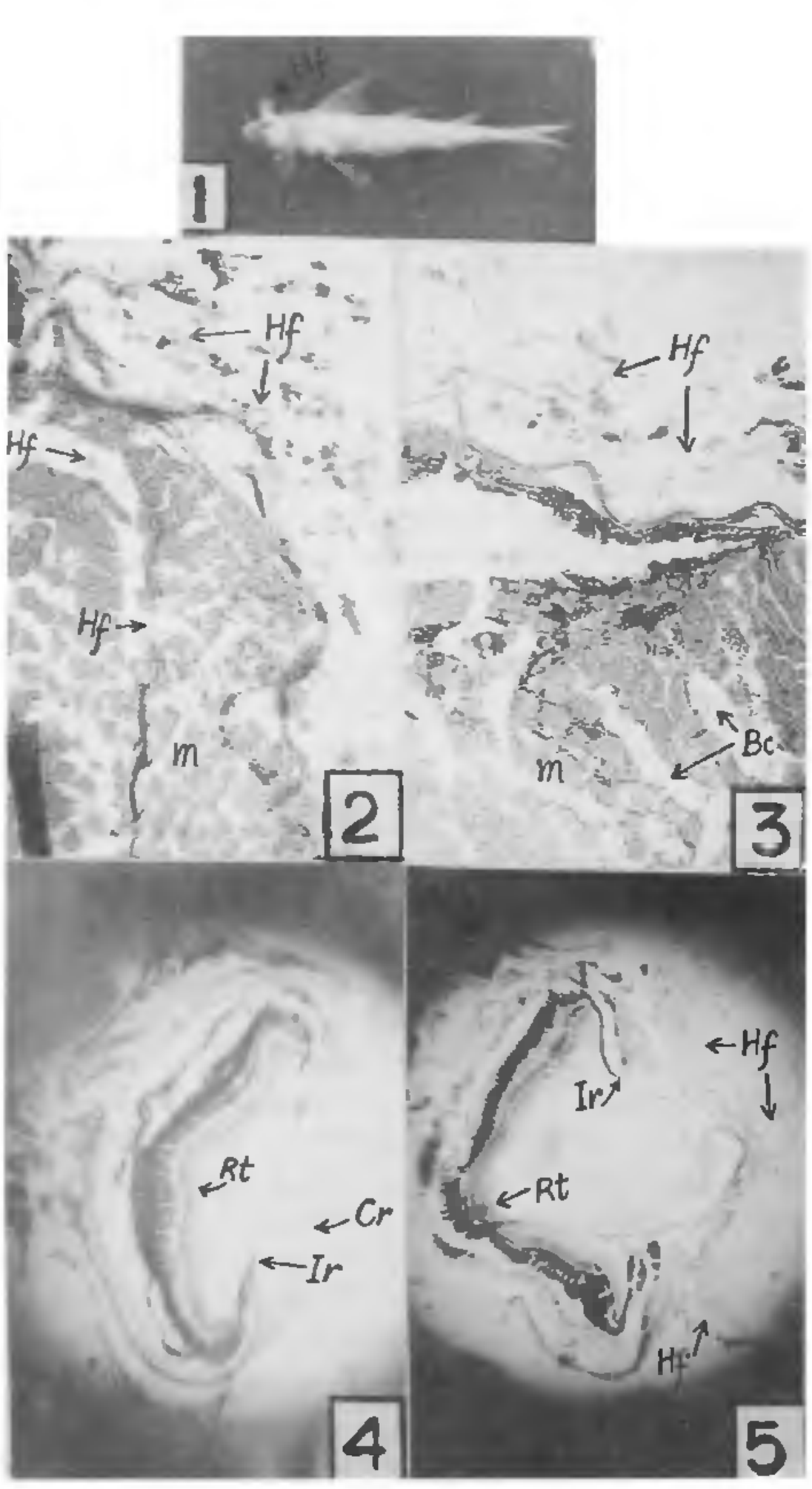
(ii) the unconscious specimen was placed on a paraffin block under a dissecting microscope and injury was inflicted by scrapping scales from the left side of the front area of the caudal peduncle and by clipping the caudal fin.

(iii) the fish was then revived by placing it in sterile, fresh aerated water and put in the infection dish.

Hyphae of the fungi were observed protruding out from the experimentally injured areas of all the test fish within 9–20 h of placing the fish in the infection dish. These individuals died of infection resulting in dermal ulceration within 18–34 h of exposure to the fungal inoculum (Table 1). The fungi, growing on the artificially infected fish, were isolated and compared with the cultures of the original inocula which was found identical to the original isolates. To maintain a control for the experiment, three test fish of *Chela laubuca* were kept under identical conditions, but were not exposed to the fungal inocula.

To study the development and extent of damage caused by *Achlya orion* and *Saprolegnia diclina* to the host, the small pieces of infected skin together with underlying muscles and eyes with their healthy counterparts, were fixed in aqueous Bouin's fluid[20]. After decalcification, the samples were dehydrated, embedded in paraffin and sectioned at 8–10 µm The sections were stained with Ehrlich's acid haematoxylin and eosine-Y and permanent slides of serial sections were prepared.

Histopathological studies on *Chela laubuca* have shown a varying degree of destruction of epidermis, hypodermis and the underlying musculature:



Figures 1–5. *Chela laubuca* Ham. **1,** White cottony patches on the body surface and hyphal tuft protruding out of an eye (× ½), **2, 3,** Cross sections showing complete disintegration of epidermis and dermis with invasion of blood cells (erythrocytes) between muscle fibre bundles. (× 1200), **4,**Cross sections of eye: Uninfected eye (× 600); Infected eye showing distinct hyphae over cornea, disintegrated iris and deshaped retina (times~600). [m = muscle fibre bundles, Hf = hyphae; Bc = blood cells (erythrocytes); Cr = cornea; Ir = iris; and Rt = retina].

Table 1. Controlled reinfection studies on *Chela laubuca* Ham. demonstrating the parasitic ability of different fungal isolates

Name of the pathogen	Mycosis evident within hour	Death occurred within hour
Achlya orion Coker & Couch	9–10	18–21
Saprolegnia diclina Humphrey	9–11	18–21
Saprolegnia ferax (Gruith) Thuret	18–20	30–34
Pythium aphanidermatum (Ed) Fitz.	10–14	23–26

Number of test fish studied = 3; Mycosis evident and number of fish dead = 3; Number of fish used as control = 3.

Achlya orion: The lesions were completely dominated by the profuse growth of fungal mycelia. Epidermis and dermis were completely missing and the hypodermal layer also showed disintegration exposing the underlying musculature. Some of the hyphal fragments penetrating into the muscle tissues were also observed (Figure 2). In addition, there was a massive invasion of blood cells (erythrocytes) into the superficial muscle layers (Figure 3).

Saprolegnia diclina: This fungus attacked the ocular region making it milky white and completely opaque. Histopathological study of the infected eye in comparison to the healthy one (Figure 4) showed profuse hyphal growth and inflammation of the cornea. The iris was completely disintegrated and the concavity of the retina increased making it deshaped (Figure 5).

During the present investigations all the four fungal pathogens, viz. *Achlya orion*, *Saprolegnia diclina*, *S. ferax* and *Pythium aphanidermatum*, have been reported for the first time as parasites (naturally occurring) of *Chela laubuca* Ham. Since long, watermoulds are known to attack body surface of the fish and progressively grow deeper in the tissues[21–23]. The present histopathological studies indicate, in general, the growth of mycelia over epidermis, destruction of scales and gradually passing through the dermis causing necrosis of muscle layers. These observations suggest that the pathogenic watermoulds are not tissue-specific and are capable of attacking virtually any tissue. This conclusion agrees with the observations of other workers[24–29]. During the present studies invasion of blood cells (erythrocytes) was observed in the muscle fibres which indicates the penetration of fungal mycelia in the blood vessels causing subsequent haemorrhage. This undesirable presence of fungal fragment in blood vessels may hinder the free passage of blood. This conclusion is in agreement with the observations of Papatheodorou[29].

Achlya klebsiana Pieters has been reported to cause eye infection in *Poecilia reticulata*[30]. Corresponding with the observations of Dukes[27], *Saprolegnia diclina* has been found to disorganize the ocular tissues of *Chela laubuca* during the present investigations. The eye infection, as it appears, is a localized infestation of certain watermoulds.

1 Gopalakrishnan, V., *Proc. Zool. Soc., Calcutta*, 1964, **17**, 95–100
2. Bhargava, K. S., Swarup, K and Singh, C. S., *Indian Biol*, 1971, **3**, 65–69.
3. Gopalakrishnan, V and Jhingran, V. G., *Proc. Symp. Sci. & India's Food Problem*, New Delhi, 1972.
4. Srivastava, G. C. and Srivastava, R. C., *Geobios*, 1976, **3**, 139–140.
5. Srivastava, G. C. and Srivastava, R. C., *Curr. Sci.*, 1976, **45**, 874.
6. Srivastava, G. C. and Srivastava, R. C., *Curr. Sci.*, 1977, **46**, 87.
7. Khulbe, R. D. and Sati, S. C., *Geobios*, 1979, **6**, 178.
8. Prabhuji, S. K. and Srivastava, G. C., *J. Indian. Bot. Soc.*, 1981, **60**, (suppl), 9
9 Sinha, S. K., Ph.D. thesis, Gorakhpur University, 1985, pp. 136.
10 Srivastava, G. C., Sinha, S. K. and Prabhuji, S. K., *Curr. Sci.*, 1986, **55**, 256–258.
11. Prabhuji, S. K., Srivastava, G. C. and Sinha, S. K., *Proc. Nat. Sem. Persp. Aquatic Biol.*, 1988, 361–368.
12. Raper, J. R., *Science*, 1937, **85**, 342.
13. Tiffney, W. N., *J. Elisha Mitchell Sci. Soc.*, 1939, **55**, 134–151.
14. Johnson, T. W. Jr., *The Genus Achlya. Morphology and Taxonomy*, Univ. Michigan Press, Ann Arbor, 1956, pp. 180.
15. Middleton, J. T., *Mem. Torrey Bot. Club*, 1943, **20**, 1–171.
16 Johnson, T. W. Jr., *J. Elisha Mitchell Sci. Soc.*, 1968,, 84, 179–183.
17. Seymour, R. L, *The genus Saprolegnia, Nova Hedwigia*, 1970, **19**, 1–124.
18. Jhingran, V. G., *Fish and Fisheries of India*, Hindustan Publ. Corp., India, 1983, pp. 666.
19. Scott, W. W and O'Warren, C., *Tech. Bull. Virg. Polytech. Inst.*, 1964, **171**, pp. 24
20 Roberts, R. J., in *Fish Pathology* (ed. Tindall, B. Londres), 1978, pp. 203–215.
21 Huxley, T. H., *Nature*, 1882, **25**, 437–440
22 Vishniac, H. S and Nigrelli, R. F., *Zoologica*, 1957, **42**, 131–134.
23 Egusa, S., *Bull. Jap. Soc. Sci. Fish.*, 1963, **29**, 27–36.
24. Bootsma, R., *Aquaculture*, 1973, **2**, 385–394
25 Nolard-Tintigner, N., *Bull. Acad. Belg. Cl. Sci.*, 1971, **57**, 185–191
26. Wolke, R. E., in *Pathology of Fishes* (eds. Ribelin, W. E. and Migaki G.), Univ. Wisconsin Press, Madison, 1975, pp. 33–116
27. Dukes, T. W., in *Pathology of Fishes* (eds. Ribelin, W. E and Migaki, G.), Univ. Wisconsin Press, Madison, 1975, pp 383–398.
28 Neish, G. A., *J. Fish Biology*, 1977, **11**, 513–522
29. Papatheodorou, V., Les mycoses chez les poissons· etiologie-Pathogenie-Traitment, These le Grade de Docteur de., L'Institut Nat. Polytech. de Toulouse, 1980, pp. 188.
30 Nolard-Tintigner, N., in *Disease of Fishes Book. 6 Fungal Diseases of Fishes* (eds. Snieszko, S. F. and Axelrod, H. R.), T.F.H. Publ., Neptune, N.J., 1980, pp. 159.

ACKNOWLEDEGMENTS We thank the UGC, New Delhi, for financial assistance and to the Principal, St. Andrew's College, Gorakhpur, for facilities to work and encouragement

Received 25 February 1993, revised accepted 22 October 1993