

## Studies on Ichthyophonus Disease of Ayu

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Ichthyophonus disease broke out among cultured ayu *Plecoglossus altivelis*, in the summer through spring of 1979. Diseased fish displayed pale body coloration, small open ulcers on the body surface, swollen abdomen due to accumulation of ascitic fluid and the production of small nodular lesions in visceral organs. Histopathological aspects were dissemination of multinucleate spherical bodies of Ichthyophonus and reactions against the spherical bodies by macrophages, multinucleate giant cells and granulomas in the affected visceral organs of diseased fish.

### Introduction

In recent years, *Ichthyophonus hoferi* infection (ichthyophonus disease) has been found among cultured rainbow trout, *Salmo gairdneri*, (ONO *et al.*, 1966; MIYAZAKI *et al.*, 1977a, b, c), yellowtail, *Seriola quinqueradiata*, (CHIEN *et al.*, 1979a), black sea bream, *Acanthopogrus schlegelli* (CHIEN *et al.*, 1979a) and ayu, *Plecoglossus altivelis* in Japan. This disease has been also found among cultured Japanese eel, *Anguilla japonica* (CHIEN *et al.*, 1979a) in Taiwan. Histopathological aspects were revealed in infected rainbow trout, yellowtail, black sea bream and Japanese eel by above research reports. But no histopathological study has been made on ayu with ichthyophonus disease.

This paper describes the histopathological aspects of ayu with ichthyophonus disease.

### Materials and Methods

Ten diseased ayu (10 g, average body weight) were collected from commercial fish culturing ponds in Tokushima Prefecture in the summer of 1979. After observation of clinical signs of diseased fish, affected visceral organs and lateral musculature were fixed with Bouin's fluid and sectioned with routine histological procedures. Then tissue sections were stained with Mayer's hematoxylin and eosin stain, Azan stain, PAS reaction and silver impregnation.

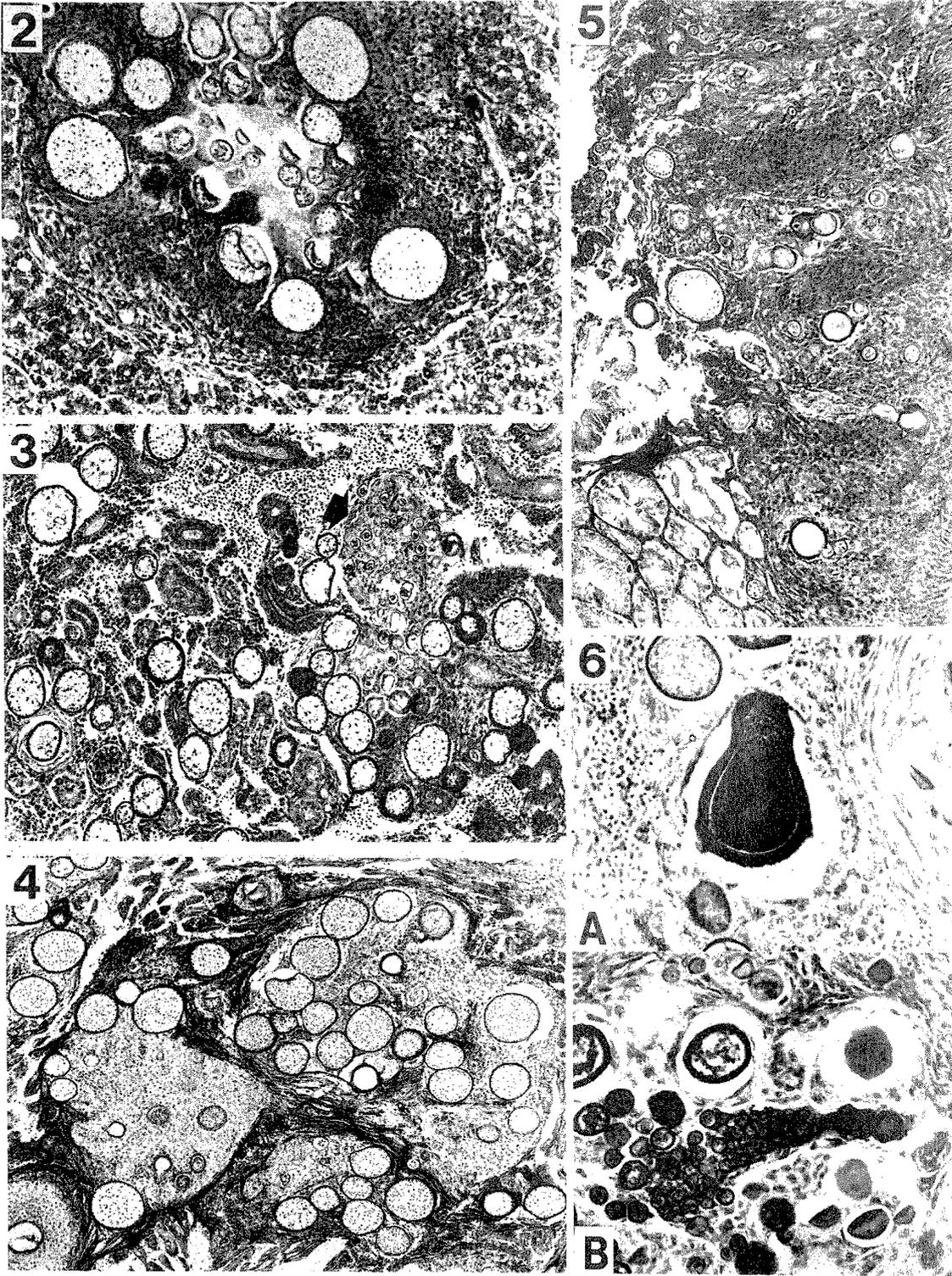
### Results

#### 1. Occurrence of ichthyophonus disease and clinical signs of diseased fish

Ichthyophonus disease broke out among young ayu (10 g, average body weight) cultured in freshwater ponds in the spring of 1979. The fish groups were caught off the sea shore and transported to the land to be reared in freshwater ponds. Mortalities had lasted to summer since spring and resulted in 10% of a total loss. Diseased fish displayed slow swimming under the water surface, pale body coloration, small open ulcers on the body surface and the swollen abdomen. Internally, accumulation of ascitic fluid and the production of small nodular lesions in the



Fig. 1. A dissected view of an infected ayu shows nodular lesions in the heart and liver.



heart, liver, spleen, kidney, stomach, intestine and peritoneum were commonly observed (Fig. 1).

## 2. Histopathological aspects

Ichthyophonus was extensively disseminated into the liver (Fig. 2), spleen, kidney (Fig. 3), heart (Fig. 4), gill, brain, intestine, pyloric caecum, pancreas, peritoneum and lateral musculature. Ichthyophonus mainly shaped multinucleate spherical bodies and moreover hyphal bodies producing spores. In the affected tissue, multinucleate spherical bodies were commonly disseminated in capillaries and sinusoids, causing circulatory disturbances. With growth of spherical bodies, they pressed the surrounding tissue and they were individually or massively walled off by macrophages, fibrocytes and multinucleate giant cells. Massive accumulation of these inflammatory cells around spherical bodies developed to granulomas. Numbers of spherical bodies had extended hyphal bodies and produced spores to be released at the tops of hyphal bodies, all of which were enclosed by granulomas.

On the other hand, the affected stomach displayed granulomas with Ichthyophonus, involving the gland layer, submucosa and muscle layers (Fig. 5). Such granulomatous lesions destroyed the overlying mucous layer and gland layer, resulted in ulcerative lesions. These granulomas of the stomach contained frequently empty spherical bodies with hyphal bodies of Ichthyophonus indicating the post sporogony. Spherical bodies extending hyphal bodies were also in the same granulomas. The size of spherical bodies extending hyphal bodies were smaller in lesions of the stomach than that in other affected organs.

The life cycle of Ichthyophonus was traced as follows: spores with one to several nuclei, multinucleate spherical body, spherical bodies of pregermination, extension of hyphal bodies and

production of spores in hyphal bodies (Fig. 6).

## Discussion

Histopathological aspects of ichthyophonus disease have been revealed in infected herring, *Clupea herengus*, (SINDERMANN *et al.*, 1954), rainbow trout, (AMLACHER, 1965; MIYAZAKI *et al.*, 1977a, b; CHUN *et al.*, 1981), yellowtail flounder, *Limanda ferruginea*, (RUGGIERI *et al.*, 1970), yellowtail (CHIEN *et al.*, 1979a), Japanese eel (CHIEN *et al.*, 1979a). The histopathological aspects of ayu infected with *Ichthyophonus hoferi* were common to those of above fishes with ichthyophonus disease. Inflammation with macrophages, multinucleate giant cells and granulomas is a common reaction against *I. hoferi*. The life cycle of *I. hoferi* infected ayu was same as that traced in infected rainbow trout (MIYAZAKI *et al.*, 1977c), yellowtail and Japanese eel (CHIEN *et al.*, 1979b). The origin of *I. hoferi* infecting ayu was not traced in this investigation. But ichthyophonus disease has not been found among ayu population coming from Lake Biwa. In Tokushima Prefecture, the fish culturing ponds which ichthyophonus disease occurred in, reared larval ayu caught off the sea shore. There was no pond rearing other species of freshwater fishes suffering from ichthyophonus disease around the ponds which attracted the present investigation. These facts suggest that *I. hoferi* in ayu is the marine origin.

## References

- AMLACHER, E. (1965): Pathologische und histochemische Befunde bei Ichthyosporidienbefall der Regenbogenforelle (*Salmo gairdneri*) und am Aquarienfisch Ichthyophonus. *Zeit. Fisch.*, **13**, 85–112.
- CHIEN C., T. MIYAZAKI and S. S. KUBOTA (1979a): Studies on ichthyophonus disease—IV. Comparative study on naturally infected fishes. *Bull. Fac.*

- Fig. 2. Affected liver shows granulomas enclosing spherical bodies of *I. hoferi*. Azan,  $\times 100$ .
- Fig. 3. Affected kidney shows extensive dissemination of spherical bodies of *I. hoferi* into sinusoids of the hematopoietic tissue and formation of granulomas (arrow). Azan,  $\times 80$ .
- Fig. 4. Affected heart shows formation of granulomas enclosing spherical bodies and necrosis of cardiac muscle around the granulomas. Azan,  $\times 50$ .
- Fig. 5. Affected stomach shows ulcerative and granulomatous lesions enclosing empty spherical bodies and hyphal bodies from mucous membrane through muscle layers. Azan,  $\times 160$ .
- Fig. 6A. *I. hoferi* showing germination. PAS,  $\times 200$ .
- Fig. 6B. *I. hoferi* showing extension of a hyphal body and release of many spores. PAS,  $\times 200$ .

- Fish., Mie Univ.*, 6, 129-146.
- CHIEN, C., T. MIYAZAKI and S. S. KUBOTA (1979b): ———VII. Morphology and life cycle. *Ibid.*, 6, 161-172.
- CHUN, S. and Y. KIM (1981): Infection by an *Ichthyophonus* sp. fungus in rainbow trout. *Bull. Korean Fish., Soc.*, 14(1): 37-42.
- MIYAZAKI, T. and S. S. KUBOTA (1977a): Studies on ichthyophonus disease I. Rainbow trout fry. *Bull. Fac. Fish., Mie Univ.*, 4, 45-56.
- MIYAZAKI, T. and S. S. KUBOTA (1977b): ———II. Yearling rainbow trout-Chronic infection. *Ibid.*, 4, 57-65.
- MIYAZAKI, T. and S. S. KUBOTA (1977c): ———III. Life cycle of *Ichthyophonus* affected rainbow trout. *Ibid.*, 4, 67-80.
- ONO, T., M. KANEKO, T. AWAKURA and A. AOMI (1966): Pathological studies on ichthyosporidium disease in rainbow trout (*Salmo gairdneri*). *Sci. Rep. Hokkaido Fish Hatch.*, 21, 43-53.
- RUGGIERI, G. D., S. G. ROSS, F. NIGRELLI, P. M. POWLES and D. G. GARNETT (1970): Epizootics in yellowtail flounder, *Limanda ferruginea* Storer, in the Western North Atlantic caused by *Ichthyophonus*, an ubiquitous parasitic fungus. *Zool.*, 55(3), 57-72.
- SINDERMANN, C. J. and L. W. SCATTERGOOD (1954): Diseases of fishes of the Western North Atlantic—II. Ichthyosporidium disease of the sea herring (*Clupea herengus*). *Bull. Dep. Sea Shore Fish.*, 19, 3-40.