한국어병학회지 제 18 권 제 2 호 (2005)
J. Fish Pathol., 18(2) : 125 ~ 131 (2005)

Neoparamoeba sp. Infection on Gills of Olive Flounder, *Paralichthys olivaceus* in Korea

Hyoung Jun Kim, Jae Bum Cho, Mu Kun Lee, Min Do Huh and Ki Hong Kim†

Department of Aquatic Life Medicine, Pukyong National University,
Pusan 608-737, Korea

Amoebic gill disease of flounder, *Paralichthys olivaceus* was diagnosed at commerical culture facility in South Korea. The amoeba was identified as a species of the genus *Neoparamoeba* based on the morpholgical characteristics of trophozoites. Transmisson electron microscopy revealed the presence of a symbiotic organism, parasome in the cytoplasm and dense glycocalyx on the surface of the trophozoites. They lacked the boat-shaped microscales on the surface and contained numerous vacuoles and channels, mitochondria in the cytoplasm. Colonization of amoebae on gill tissue elicited extensive fusion and hyperplasia of gill lamella.

Key words : *Neoparamoeba*, Amoebic gill disease, Olive flounder

Introduction

The majority of amoebae infecting marine fish are opportunistic pathogens that, under certain conditions such as immunodepression and suboptimal environmental conditions, can become parasites and cause the disease outbreaks (Nash *et al*., 1988; Noble *et al*., 1997). Kent *et al*. (1988) and Dykova *et al*. (1995) have suggested that amoebae could play a primary role in the development of gill disease in cultured fish. Amoebic gill disease (AGD) has been reported for a number of cultured fish species including Atlantic salmon, *Salmo salar* (Roubal *et al*., 1989; Adams and Nowak, 2001), rainbow trout, *Oncorhynchus mykiss* (Munday *et al*., 1990), coho salmon, *Oncorhynchus kisutch* (Kent *et al*., 1988), European seabass, *Dicentrarchus labrax*, sharpsnout seabream, *Diplodus puntazzo* (Dykova *et al*., 2000; Dykova and Novoa, 2001) and turbot, *Scophthalmus maximus* (Dykova *et al*., 1995, 1998). AGD has been a major problem in Tasmania once salmon production bacame intensive and more full strength salinity rearing sites came into use (Munday *et al*., 1990), and mortalities appeared to be related to elevated water temperature and salinity (Munday *et al*., 1993). Dykova *et al*. (1995) published a histopathological study of an amoebic infection that caused severe gill tissue damage in turbot *Scophthalmus maximus*. Such AGD of turbot associated with mortalities also reported from culture facilities in NW Spain (Dykova *et al*., 1998).

In Korea, although AGD has been noticed in olive flounder, *Paralichthys olivaceus*, farms, little information is available on the amoeba species and histopathological effects on olive flounder. In the present study, we investigated the morphological characteristics of the amoeba using light- and elec-

†Corresponding Author : Ki Hong Kim, Tel : 051-620-6145,
Fax : 051-628-7430, E-mail : khkim@pknu.ac.kr

tron-microscopies, and the histopathology of infected gills of olive flounder.

Materials and Methods

Juvenile cultured olive flounder *Paralichthys olivaceus* (average body weight 8.2 g) were collected from a culture facility located in southern coastal area of Korea. The gills of moribund fishes were excised to examine the presence of amoeba and other parasites. The gill tissue was smeared on a slide, air-dried and stained with Diff-Quik (International Reagents Co., Japan). Isolated amoebae were examined by light microscopy and transmission electron microscopy.

For the transmission electron microscopical observation, isolated amoebae were washed with PBS (pH 7.0) 3 times by centrifugation, and were fixed in 2% v/v glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) at 4℃ overnight, postfixed in 1% w/v cacodylic OsO_4 for 2 h. The specimens were dehydrated, embedded in epoxy resin (Spurr) and ultrathin-sectioned, stained with uranyl acetate and lead citrate, and examined by a JEOL JEM1200 transmission electron microscope (JEOL LTD., Japan).

For histological study, the gill tissues were fixed in Bouin's solution and embedded in paraplast. Sections 4 ㎛ thick were stained with hematoxylin and eosin.

Results

Heavily infected fish with amoebae secreted excess mucus on the gills (Fig. 1A). The gill filaments had irregular outlines and abnormal greyish coloration. The trophozoites of amoebae were observed on the secondary lamella of gills by light microscopy (Fig. 1B, C). A nucleus and a parasome were observed clearly by differential microscopy (Fig. 1D, F). Body shape of trophozoites was spherical with smooth outline (Fig. 1E, F, H) or short blunted projections (lobose pseudopod) from hyaloplasmic zone (Fig. 1C, D, G). Trophozoite diameter was averaged 38.29 ± 5.03 ㎛. A nucleus (lighter) and a parasome (dark) were also distinguished clearly by Diff-Quik stain (Fig. 1I). Cytoplasm of trophozoites had numerous vacuoles in the fresh and methylene blue stained amoebae (Fig. 1F, G, H, J).

In transmission electron microscopy, a distinct parasome was presented in the cytoplasm of amoebae (Fig. 2A, B, C). Channels and vacuoles presented in the center of cytoplasm (Fig. 2D). Also, several mitochondria were found in the cytoplasm of amoebae (Fig. 2E). Surface of amoebae was surrounded by glycocalyx (Fig. 2F). Scales or microscales were not present on the cell surface.

In histopathological observation, a number of amoebae were found in the gill, which showed a variety of pathological changes. The amoebae were irregular in shapes and had highly-vacuolated cytoplasm giving them foamy appearance, and a nucleus with a large, distinct nucleolus (Fig. 3A). Main lesion was extensively fused secondary lamella with hyperplasia of the lamella epithelium. The hyperplastic changes were especially marked over fused tips of secondary lamella where a number of amoebae existed. In heavily infected cases, primary filaments were also fused (Fig. 3B). Fusion of lamellar tips resulted in the formation of a number of discrete interlamellar cavities (Fig. 3A, B). Desquamated epithelial cells, edematous albuminous fluid, or various developing stages of amoeba were found in the interlamellar cavities. Inflammation with the appearance of a few numbers of necrotic cells was occasionally noted in secondary lamella around the parasites (Fig. 3A).

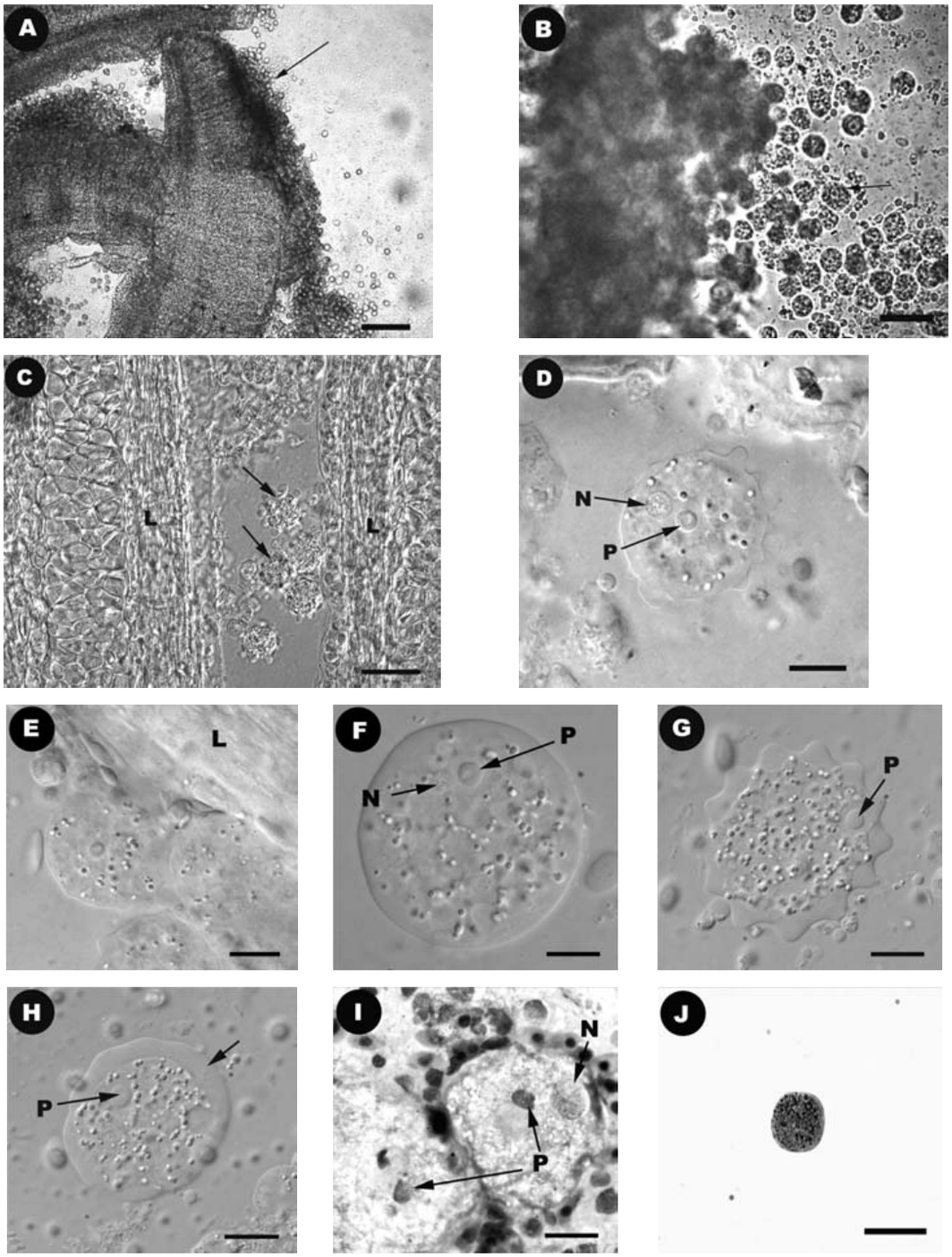

Fig. 1. Light microscopic observations of *Neoparamoeba* sp. infecting gills of olive flounder *Paralichthys olivaceus*. (A) Gill lamellae heavily infected with *Neoparamoeba* sp., Bar=1 mm. (B) Magnification of arrowed region of figure A, Bar=100 ㎛. (C) *Neoparamoeba* sp. between gill lamellae (L), Bar=45 ㎛. (D) *Neoparamoeba* sp. showing nucleus (N) and parasome (P), Bar=9 ㎛. (E) *Neoparamoeba* sp. attached to the gill lamellae, Bar=9 ㎛. (F, G, H) High magnification of *Neoparamoeba* sp., Bar= 9 ㎛. An arow in figure H indicate hyalinic ectoplasm. (I) *Neoparamoeba* sp. stained with Diff-Quik. N, Nucleus; P, Parasome, Bar= 9 ㎛. (J) *Neoparamoeba* sp. stained with methylene blue, Bar= 40 ㎛.

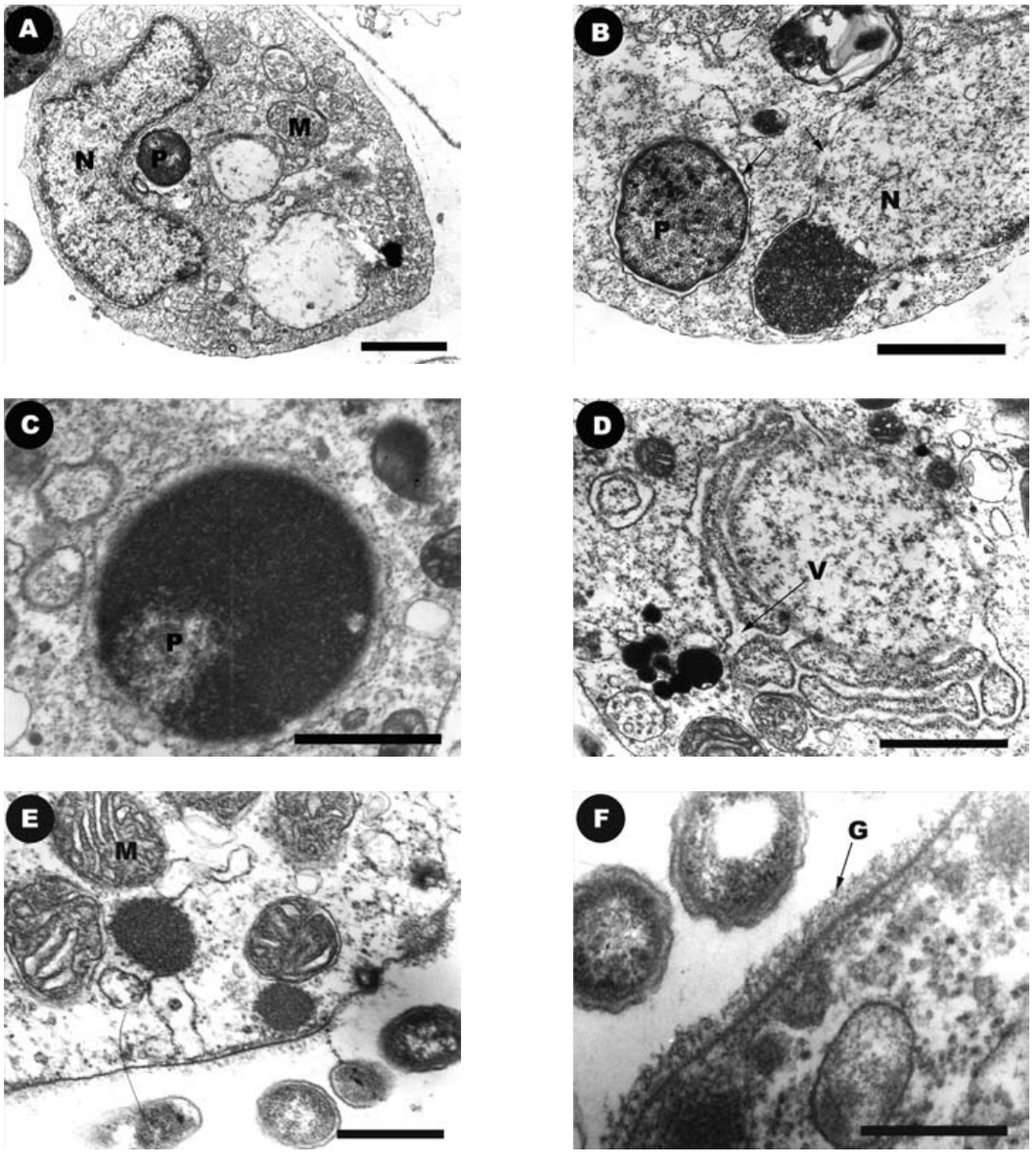

Fig. 2. Electron micrographs of *Neoparamoeba* sp. (A) Cytoplasm containing a large nucleus (N), parasome (P) and small mitochondria (M). (B) Nucleus and parasome with 2 membranes, Bar=1 ㎛. (C) Morphology of parasome, Bar=1 ㎛. (D) Channels and vacuoles (V) in the center of cytoplasm, Bar=1 ㎛. (E) Mitochondria in the cytoplasm, Bar=400 nm. (F) Glycocalyx (G) of cell surface, Bar=334 ㎛.

Discussion

The parasitic agent of amoebic gill disease (AGD) of olive flounder in the present study was identified as a species of the genus *Neoparamoeba* Page, 1987 based on the morphological and ultrastructural characteristics of trophozoites. The presence of a parasome in the trophozoites of the present study was visualized by the fresh preparations as well as by the ultrstructural sections. The para-

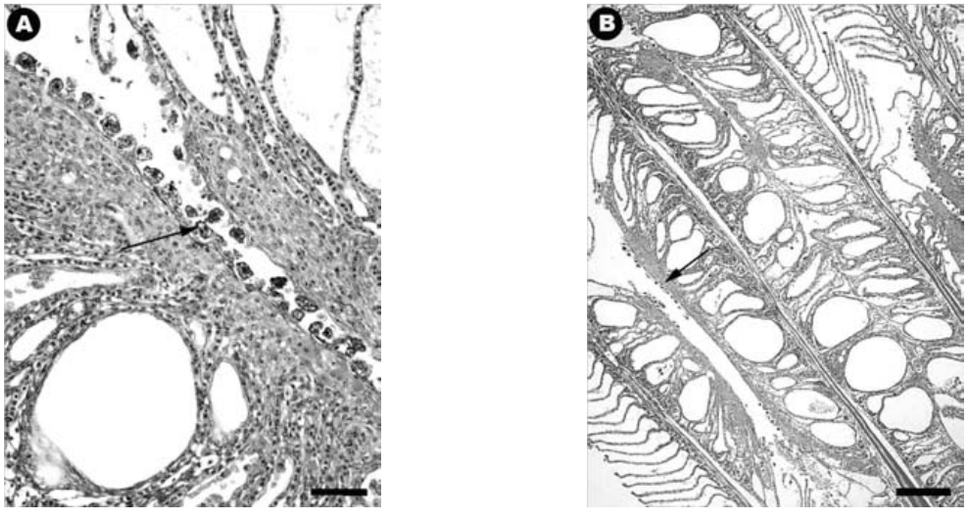

Fig. 3. Hyperplastic and edematous gill tissue associated with *Neoparamoeba* sp. (A) *Neoparamoeba* (arrowed) attached to the hyperplastic region of the gill epithelium, Bar=160 ㎛. (B) Extensive gill hyperplasia and lamellar fusion of gill filament with *Neoparamoeba* trophozoites (arrowed), Bar=1 mm.

somes in amoebae have been described as endosymbionts of the family Paramoebidae (Dykova *et al*., 2000, 2003, 2005). The presence of parasome separates species of *Paramoeba*, *Neoparamoeba* and *Janickina* from all other amoebae except *P. eilhardi* which sometimes lacked parasomes (Dykova *et al*., 2000), and has been taken as the pivotal diagnostic feature of the genus Paramoeba. Among parasome containing amoebae, *Janickina* lacked finger-like lobose pseudopodia (dactylopodia) and showed monopodial morphology under light microscope (Hollande, 1980). Amoeba trophozoites in the present study had several dactylopodia, similar to the species of the genera *Paramoeba* and *Neoparamoeba*.

Cell surface structure of amoebae was extremely imporant for the taxonomy of genus *Paramoeba* (Page, 1983), because it includes species with surface microscale or with surface glycocalyx. Page (1987) transferred *P. pemaquidensis* and *P. aestuarina* to the newly established genus *Neoparamoeba* Page, 1987, based on their lacking of microscales on the surface. In the ultra-thin sections, the surface of *Neoparamoeba* species is covered with dense glycocalyx consisting of tightly packed tubular element without microscale.

Based on the presence of parasome, dactylopodia, and surface glycocalyx structure, we assigned the present AGD agent of olive flounder to a species of the genus *Neoparamoeba*.

The detailed description for the lesions of amoebic gill disease (AGD) were previously made by Dykova *et al*. (1995) and Leiro *et al*. (1998) in turbot, Zilberg and Munday (2000) and Adams *et al*. (2001, 2003) in salmonids. AGD is generally characterized by the extensive fusion and hyperplasia of primary and secondary lamella. In the present study, the histopathological findings of AGD in farmed flounder were quite similar to those reported previously from other species. Extensive fusion and hyperplasia of gill lamella will lead to increase respiratory distance, which could be contributable to respiratory distress, especially under unfavorable environments. However, failure of artificial infec-

tion to olive flounder with the isolated amoebae and lack of data on the epizootiological characteristics of the amoeba make it ambiguous to determine whether the amoeba can act as a primary pathogen of olive flounder. Therefore, further studies are needed to elucidate the pathogenic potential of *Neoparamoeba* sp. in olive flounder.

Acknowledgements

This work was supported by the Brain Korea 21 Project in 2005, Republic of Korea.

References

Adams, M. and Nowak, B. F.: Distribution and structure of lesions in the gills of Atlantic salmon (*Salmo salar* L.) affected with amoebic gill disease. J. Fish Dis., 24: 535-542, 2001.

Adams, M. and Nowak, B. F.: Amoebic gill disease (AGD): sequential pathology in cultured Atlantic salmon, *Salmo salar* L. J. Fish Dis., 26: 601-614, 2003.

Dykova, I. and Novoa, B.: Comments on diagnosis of amoebic gill disease(AGD) in turbot, *Scophthalmus maximus*. Bull. Eur. Assoc. Fish Pathol., 21: 40-44, 2001.

Dykova, I., Figueras, A. and Novoa, B.: Amoebic gill infection of turbot, *Scophthalmus maximus*. Folia Parasitol., 42: 91-96, 1995.

Dykova, I., Figueras, A., Novoa, B. and Fernandez-Casal, J.: *Paramoeba* sp., an agent of amoebic gill disease of turbot *Scophthalmus maximus*. Dis. Aquat. Org., 33: 137-141, 1998.

Dykova, I., Figueras, A. and Peric, Z.: *Neoparamoeba* Page, 1987: light and electron microscopic observations on six strains of different origin. Dis. Aquat. Org., 43: 217-223, 2000.

Dykova, I., Fiala, I., Lom, J. and Lukes, J.: *Perkinsiella amoebae*-like endosymbionts of *Neoparamoeba* spp., relatives of the kinetoplastid *Ichthyobodo*. Europ. J. Protistol., 39: 37-52, 2003.

Dykova, I. Nowak, B. F., Crosbie, P. B. B., Fiala, I., Peckova, H., Adams, M. B., Machackova, B. and Dvorakova, H.: *Neoparamoeba branchiphila* n. sp., and related species of the genus *Neoparamoeba* Page, 1987: morphological and molecular characterization of selected strains. J. Fish Dis., 28: 49-64, 2005.

Hollande, A.: Identification du parasome (nebenkern) de *Janickina pigmentifera* a un symbionte (*Perkinsella amoebae* nov. gen. - nov. sp.) apparente aux flagelles kinetoplastidies. Protistologica, 16: 613-625, 1980.

Kent, M. L., Sawyer, T. K. and Hedrick, R. P.: *Paramoeba pemaquidensis* (Sarcomastigophora: Paramoebidae) infestation of the gills of coho salmon *Oncorhynchus kisutch* reared in seawater. Dis. Aquat. Org., 5: 163-169, 1988.

Leiro, J., Paniaqua, E., Ortega, M., Parama, A., Fernandez, J. and Sanmartin, M. L.: An amoeba associated with gill disease in turbot, *Scophthalmus maximus* (L.). J. Fish Dis., 21: 281-288, 1998.

Munday, B. L., Foster, C. K., Roubal, F. R. and Lester, R. J. G.: Paramoebic gill infection and associated pathology of Atlantic salmon, *Salmo salar*, and rainbow trout, *Salmo gairdneri* in Tasmania. (ed. by Perkins F. O. and Cheng T. C.), *Pathology in Marine Science*. Academic Press, Lodon, UK, pp. 215-222, 1990.

Munday, B. L., Lange, K., Foster, C., Lester, R. T.

G. and Handlinger, J.: Amoebic gill disease of sea-caged salmonids in Tasmania waters. Tasmanian Fish Res., 28: 14-19, 1993.

Nash, G., Nash, M. and Schilotfeld, H. J.: Systemic amoebiasis in cultured European catfish, *Silurus glanis* L. J. Fish Dis., 11: 57-71, 1988.

Noble, A. C., Herman, R. L., Noga, E. J. and Bullock, G. L.: Recurrent amoebic gill infestation in rainbow trout cultured in a semi-closed water recirculation system. J. Aquat. Anim. Health, 9: 64-69, 1997.

Page, F. C.: Marine Gymnamoebae. Institute of Terrestrial Ecology, Culture Centre of Algae and Protozoa Cambridge, England, 54 pp, 1983.

Page, F. C.: The classification of "naked" amoebae of phylum Rhizopoda. Arch. Protistenkd., 133: 199-217, 1987.

Roubal, F. R., Lester, R. J. G. and Foster, C. K.: Studies on cultured and gill-attached *Paramoeba* sp. (Gymnamoebae: Paramoebidae) and the cytopathology of paramoebic gill disease in Atlantic salmon, *Salmo salar* L., from Tasmania. J. Fish Dis., 12: 481-493, 1989.

Zilberg, D. and Munday, B. L.: Pathology of experimental gill disease in Atlantic salmon, *Salmo salar* L., and the effect of pre-maintenance of fish in sea water on the infection. J. Fish Dis., 23: 401-407, 2000.

Manuscript Received : June 08, 2005
Revision Accepted : August 02, 2005
Responsible Editorial Member : Myung-Joo Oh (Yosu Univ.)