Significant and emerging parasitic diseases of finfish

KAZUO OGAWA

Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan

ABSTRACT

Fish parasites constitute widely diversified groups of unicellular and multicellular organisms. Recently, less known parasite groups have emerged as serious pathogens. Firstly, infection with dinoflagellates of the genus *Ichthyodinium* has been found during seed production of leopard coral grouper, *Plectoropomus leopardus*, in Japan and yellowfin tuna, *Thunnus albacares*, in Indonesia. Schizonts multiply in host ova, leading to burst of yolk sacs of embryos or hatched larvae. The complete life cycle is unknown, but there is evidence that the infective stage was present in the rearing water. Secondly, heavy infections of endoparasitic turbellarians have been noticed in hatchery-reared stonefish, *Inimicus japonicus*, fry and cage-cultured sea perch, *Lateolabrax* sp. and greater amberjack, *Seriola dumerili*. Furthermore, wild Japanese sea perch, *Lateolabrax japonicus*, and cultured red sea bream, *Pagrus major*, were concurrently infected. It is not clear whether these turbellarians, probably belonging to the genus *Paravortex*, comprise a single species. Hitherto unknown parasitic diseases among wild fish should also be noted: the monogenean, *Neoheterobothrium hirame*, has been a serious threat to wild olive flounder, *Paralichthys olivaceus*, since its sudden appearance in the early 1990s. Circumstantial evidence and molecular data strongly suggest its natural host is southern flounder, *Paralichthys lethostigma*, in North America, and that infected fish was introduced into Japanese waters. Among freshwater fishes, metacercariae of the bucephalid trematodes, *Parabucephalopsis parasiluri* and *Bucephalopsis ozakii*, and the leech, *Limnotrachelobdella sinensis*, have been found in wild cyprinid fishes of Japan. It is suspected that these parasites were introduced through importation of live shellfish and fish, respectively. These cases in the wild are more serious than those occurring in aquaculture, as it is unrealistic to take control measures for the containment of diseases of wild fish.

Key words: emerging disease, parasite, *Ichthyodinium*, *Paravortex*, *Neoheterobothrium hirame*, *Parabucephalopsis parasiluri*, *Limnotrachelobdella sinensis*

Ogawa, K. 2011. Significant and emerging parasitic diseases of finfish, pp. 03-12. *In* Bondad-Reantaso, M.G., Jones, J.B., Corsin, F. and Aoki, T. (eds.). Diseases in Asian Aquaculture VII. Fish Health Section, Asian Fisheries Society, Selangor, Malaysia. 385 pp.

Corresponding author: Kazuo Ogawa, aogawak@mail.ecc.u-tokyo.ac.jp

INTRODUCTION

With increasing demands for specific pathogen-free seeds in aquaculture, more and more species of culture seeds have recently been produced in an ever larger scale than before. This practice is accompanied by frequent outbreaks of infectious diseases in hatcheries. While most diseases are caused by viral agents, protozoan and metazoan infections do occur in hatcheries, but have not yet been sufficiently documented.

In contrast to aquaculture, diseases of wild fish and shellfish have been recorded much less frequently. This is mainly because diseased animals are easy prey to predators before we notice their presence. Diseases caused by parasites are no exceptions, and only a few cases have so far been documented in wild populations (Jones, 2005). Although much attention is focused on the economic loss due to parasitic diseases in aquaculture, impacts of the parasitic infections on wild populations must not be neglected.

In many cases, importation of foreign fish and shellfish has been deeply involved in the emergence of new diseases among indigenous species. In Japan, legislation was introduced in 1996 to prevent foreign fish and shellfish pathogens from entering into Japanese waters. Under the law for conservation of aquatic resources, we request the exporting countries to issue specific pathogen-free certificates for selected diseases of selected aquatic animals. At present, they are Spring Viremia of Carp (SVC) and Koi herpesvirus (KHV) disease for carp *Cyprinus carpio* (common carp and koi carp), SVC for some cyprinid fish including goldfish *Carassius auratus*, Viral Hemorrhagic Septicemia, Epizootic Hematopoietic Necrosis, Piscirickettsiosis and Enteric Redmouth Disease for salmonid eyed-eggs and juveniles, Nuclear Polyhedrosis Baculoviroses, Yellow Head Disease, Infectious Hypodermal and Hematopoietic Necrosis and Taura Syndrome for penaeid shrimp juveniles. Further, once introduced, we try to contain these specified diseases under the law for sustainable aquaculture production. For KHV, since the introduction of the virus in 2003, efforts have been directed to the prevention of the virus from spreading to still uncontaminated areas in Japan. In other words, we do not have any legislation to stop importation of foreign aquatic animals harbouring other pathogens than the listed ones above, as long as they are apparently healthy upon importation.

In this short review, I would like to present examples of lesser known parasitic diseases in the wild and in hatcheries, which I recently encountered. It is almost certain that newly emerging diseases in wild fish described here are all caused by pathogens introduced from abroad. Diseases in hatcheries occur in close association with those in the wild and possibly also in growout facilities. Thus, for the establishment of effective control measures in hatcheries, it will be important to demonstrate the life cycle and to investigate the entire infection cycle among hatcheries, growout facilities and wild environments.

PARASITIC DISEASES IN SEED PRODUCTION FACILITIES

Infection of fertilized eggs by the dinoflagellate, *Ichthyodinium chaberardi*, has been known to occur among marine fish including Atlantic sardine, *Sardina pilchardus*, and similar infections have been described in the eggs of Atlantic cod, *Gadus morhua*, and turbot, *Scophthalmus maximus*, in hatcheries (Noga and Levy, 2006). These occurrences have so far been limited in Europe, but, since 2000, very similar infections have been occurring in the seed production of leopard coral grouper, *Plectropomus leopardus*, on Ishigaki Island, southwestern Japan (Mori *et al*., 2007) and of yellowfin tuna, *Thunnus albacares*, in Indonesia (Yuasa *et al*., 2007). There were considerable fluctuations in the annual occurrence, but once it occurred, high mortality was induced. Schizonts of the parasite multiplied in the yolk sac of fertilized eggs (Fig. 1) or hatched larvae (Fig. 2) and parasite-filled yolk sac eventually burst. The parasite released into the water transformed into the dinoflagellate stage, the fate of which is unknown. Although the infective stage has not been specified, there is evidence that their life cycle is established among local wild fish populations, and fertilized eggs of the grouper in hatcheries got infected through contaminated water from outside. This infection was effectively controlled by treating the rearing water with ozone at 0.5 mg/l for more than one minute (Table 1) (Mori *et al*., 2007).

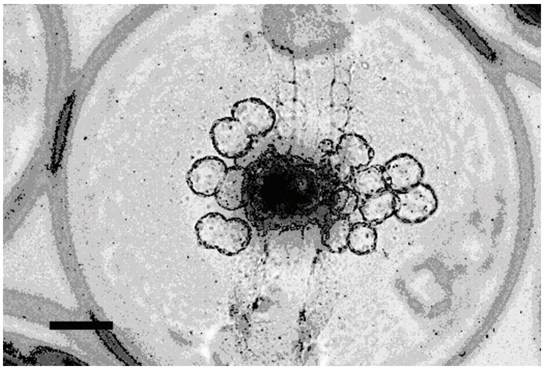


Figure 1. Dinoflagellate, *Ichthyodinium* sp. in the egg of coral trout, *Plectropomus leoparadus* (15 h post-fertilization). Scale: 0.1 mm. Reproduced from Mori *et al.* (2007).

Some groups of turbellarians are fish parasites, while the majority are free-living or commensal with other animals. Among them, *Paravortex* sp. (possibly *Paravortex* spp.) infects many wild fish species (Cannon and Lester, 1988), and, in captivity, caused mortality among aquarium fish (Kent and Olson, 1986). This parasite is viviparous (Fig. 3), and, when mature, leaves host to give birth to its offspring in the water.

In Japan, *Paravortex* infection is a serious threat in the seed production of a stonefish, *Inimicus japonicus* (Family Synanceiidae). The parasite is endoparasitic, invading the gills, skin and fins of fry. The source of infection is thought to be spawner stonefish, which

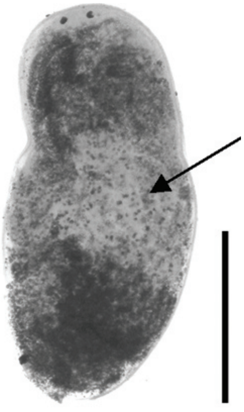
Table 1
Experimental control of Ichthyodinium infection of fertilised eggs of Plectropomus leopardus by sterilisation of seawater used to rear broodstock (data based on Mori *et al.* (2007).

Experimental group	**Occurrence (%) (Infected egg batches/total egg batches)**	
	2003	**2004**
Spawning in disinfected seawater (Treated with oxidant)	0% (0/82)	0% (0/136)
Spawning in untreated seawater	7.30% (6/82)	10.30% (7/68)

got infected in the wild. This may have been the result of fry being reared in sea water contaminated with young parasites released from adult parasites. This turbellarian was also found in wild rockfish, *Sebastiscus marmoratus* (Ogawa, unpublished data) in 1980s and wild Japanese sea perch, *Lateolabrax japonicus*, in 2006 (N. Akao, pers. comm.). *Paravortex* infection has also recently been noticed among cage-cultured fish in 2006: amberjack, *Seriola dumerli*, imported from China, Chinese sea perch, *Lateolabrax* sp., red sea bream, *Pagrus major*, and the stonefish, *I. japonicus*. Fecundity of the parasite is still largely

Figure 2. Coral trout, *Plectropomus leoparadus* immediately after hatch with full of schizont stage dinoflagellates. Scale: 0.5 mm. Reproduced from Mori *et al.* (2007).

Figure 3. *Paraveotex* sp. infecting stonefish, *Inimicus japonicus*. Arrow indicates larvae in the uterus. Heidenhain's iron hematoxylin stain. Scale: 0.5 mm.



unknown except that the number of offspring an adult produced each time ranged from 62 to 248 (mean: 133) (Fukuda, Y., Miyoshi, Y. and Ogawa, K. 2006). It caused mortality among *I. japonicus* fry reared in hatcheries and in culture tanks. Chemotherapy seems ineffective because of its endoparasitism, and prevention of infection by using parasite-free water will be a realistic control method in the hatchery.

PARASITIC DISEASES OF WILD FISHES

A new diclidophorid monogenean, *Neoheterobothrium hirame*, was found on the gills and buccal cavity wall of wild olive flounder, *Paralichthys olivaceus*, collected from the Sea of Japan (Ogawa, 1999) (Fig. 4). Infected fish sometimes show severe anaemia caused by the blood feeding of the parasite (Yoshinaga *et al*., 2000). Infection was first noticed among wild olive flounder in 1995, but subsequent examination of flounder specimens collected in 1989-1999 and preserved in formalin (n=841; 0- and 1- year old) in Niigata Prefecture on the Sea of Japan side confirmed the infection of fish collected in 1993 and later, but not from fish in 1989-1992 (Anshary *et al*., 2001).

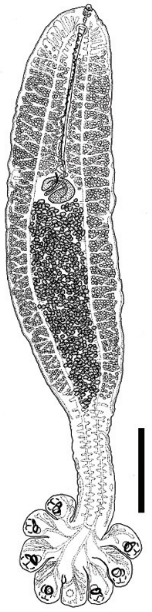


Figure 4. Line drawing of *Neoheterobothrium hirame* infecting wild olive flounder. Scale: 3 mm. Reproduced from Ogawa (1999).

This strongly suggests that the monogenean appeared suddenly in Japanese waters in early 1990s. Since the first confirmation of infection in 1993, *N. hirame* was found in wild flounder from Hokkaido to Kyushu to cover the whole area of the host distribution in six years (Fig. 5). This rapid expansion of the parasite's geographical distribution was probably due not only to host migration, but also to human activities by transporting flounder between the Sea of Japan side and the Pacific side. Flounder of the year are quite susceptible to infection, and the catch has drastically declined especially in western Japan (Anshary

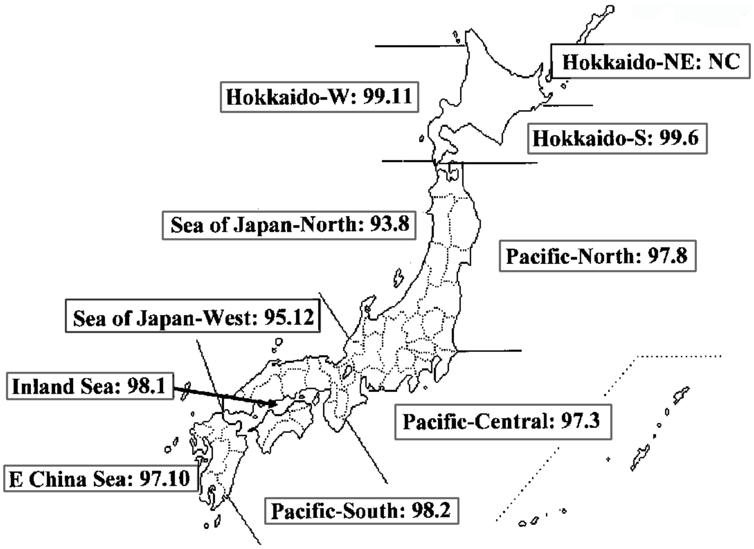


Figure 5. Record of the first confirmation of *Neoheterobothrium infection* of wild flounder from 10 coastal areas of Japan. Infection was first confirmed in Northern Sea of Japan in August 1993 and last confirmed in Western Hokkaido in November 1999.

et al., 2002). Infection experiments suggest that *N. hirame* induces behavioural changes in infected flounder, making them vulnerable to predation by larger flounder (Shirakashi *et al*., 2008). This may be responsible for the recent decline in the flounder populations of Japan.

The Yodo River system originates in Lake Biwa, passing through two dams to lead into the Uji River and then, the Yodo River, finally flowing into Osaka Bay. Since 2000, hitherto unknown parasites have appeared almost coincidentally: metacercariae of bucephalid trematodes, *Parabucephalopsis parasiluri* and *Bucephalopsis ozakii*, were found encysted in many cyprinid fish, most notably in *Zacco platypus* and *Squalidus chankaensis* in the Uji River and Yodo River (Ogawa, K., Nakatsugawa, T. and Yasuzaki, M. 2004; Urabe *et al*., 2007) (Fig. 6), and a leech, *Limnotrachelobdella sinensis*, infecting crucian carps, *Carassius cuvieri* and *Carassius langsdorfii*, in the Yodo River (Ogawa, K., Rusinek, O. and Tanaka, M. 2007) (Fig. 7). Heavy metacercarial infection in the eye and fins caused hemorrhages. These trematodes involve a freshwater bivalve, *Limnoperna fortunei*, as the first intermediate host with fish, mostly cyprinids, as the second intermediate hosts and Lake Biwa catfish, *Silurus biwaensis*, and Amur catfish, *Silurus asotus*, as the final hosts (Ogawa, K., Nakatsugawa, T. and Yasuzaki, M. 2004; Urabe *et al*., 2007). It is probable that the trematode was introduced from eastern Asia to Japan through infected bivalve, *L. fortunei*. With the presence of potential second and final hosts in this river system, the life cycle of these bucephalid trematodes started to be completed there. Since the first confirmation, the distribution of the parasite has been restricted to the Uji River-Yodo River area, but very recently in 2007, infected bivalves were found in the upstream of the two dams between

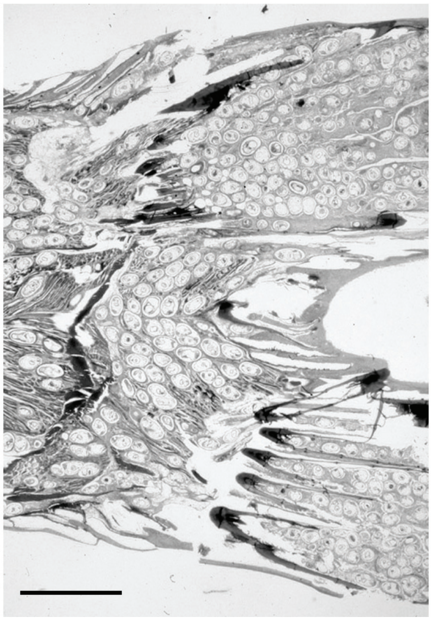


Figure 6. Histological section of the caudal part of *Squalidus chankaensis* with numerous metacercariae collected in Uji River. Azan stain. Scale bar: 1 mm. Reproduced from Ogawa *et al*. (2004)

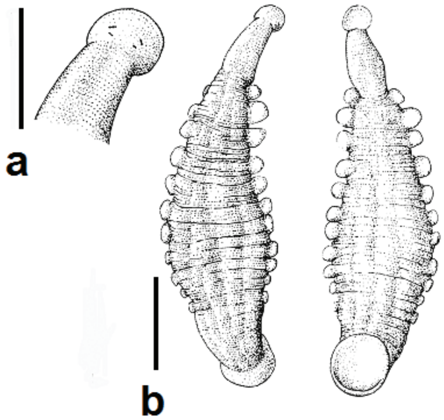


Figure 7. Line drawing of *Limnotrachelobdella sinensis* infecting crucian carps. Scale a: 0.5 cm; Scale b: 1 cm. Reproduced from Ogawa *et al*. (2007).

Lake Biwa and the Uji River (M. Urabe, personal communication) (Fig. 8). The parasite has expanded its distribution upstream beyond the Uji River possibly by accidental introduction of infected cyprinids or catfish. Now that the parasite is present just downstream of the Lake Biwa, where many unique indigenous cyprinid fish are inhabited, it will be a great risk to such fish, which may be as susceptible as the cyprinid fish in the Uji River.

The origin of the leech, *L. sinensis*, found in the Yodo River, is not clear since it suddenly appeared in 2000 (Ogawa, K., Rusinek, O. and Tanaka, M. 2007). It is quite unlikely that the parasite, up to 5 cm in body length, has long been overlooked, assuming that it *is* an indigenous species to Japan. It is rather natural to consider that the leech was introduced

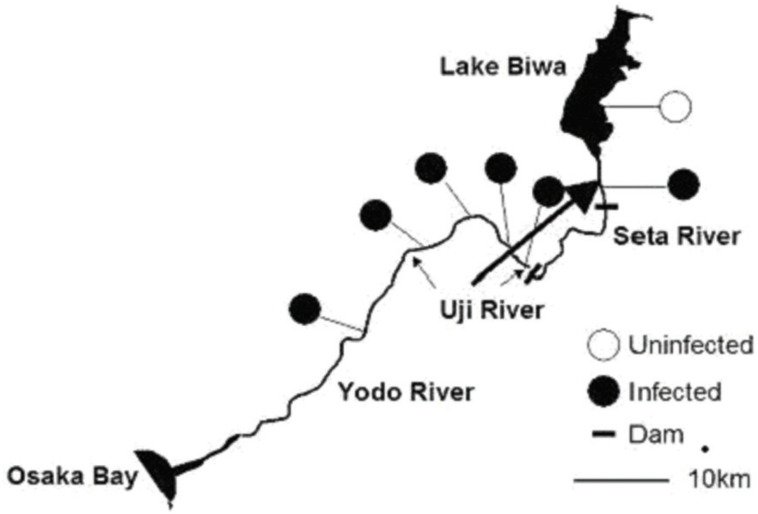


Figure 8. Infection of the first intermediate host with bucephalid trematodes in the Lake Biwa water system. Arrow indicates possible upstream transportation of infection, which was confirmed in 2007.

through infected crucian carp from eastern Asia, where it is known to infect common carp and crucian carps. The parasite appears to be pathogenic, since infected fish showed anemia. For the moment, there is no evidence that the leech has expanded its distribution beyond the Yodo River, but the host fish, *C. cuvieri* and *C. langsdorfi*, are widely distributed in Japanese waters, and once introduced, there is a risk that the parasite life cycle becomes established in a new locality.

Since control of infections of wild fish is not possible, care should be taken not to transport such potential hosts from these contaminated areas to naïve areas.

CONCLUDING REMARKS

It is almost certain that the causative parasites of the emerging diseases among wild fish populations described here were of foreign origin. In the case of *Neoheterobothrium hirame* infection of olive flounder, it is strongly suggested that the monogenean is originally the parasite of southern flounder, *Paralichthys lethostigma*. This means that the parasite has switched its host from an American flounder to the native Japanese flounder. Such host switch would never have happened without the human activity to introduce the foreign flounder from North America to the Far East, which was totally an unexpected event. It was also unexpected that the bivalve, *L. fortunei*, probably attaching to freshwater bivalves imported for food consumption (Urabe *et al*., 2001), was infected with trematodes new to Japan, which have established their life cycles in Japanese waters.

It should be noted that *N. hirame* has spread over the whole area where olive flounder is naturally distributed, in just six years. Similarly in the Yodo River system, the bucephalid trematodes (or at least one of the two species) have expanded their distribution upstream, posing a great threat to wild fish populations in the Lake Biwa, while the geographical distribution of the leech, *L. sinensis*, is still limited. It seems unlikely that olive flounder dispersed from the Sea of Japan side to the Pacific side in such a short period, and that cyprinids swam upstream from the Uji River across the two dams. It is more likely that human activities may have been involved in the expansion of these parasites' distribution.

Since control measures against diseases in wild fish populations are almost impossible and since we cannot anticipate the consequence of any host switch as in the case of *N. hirame* infection of olive flounder, it is clear that introduction of live foreign aquatic animals should be done responsibly and cautiously. Domestic production of culture seeds may prevent further introduction of foreign pathogens.

Knowledge about outbreaks of parasitic diseases in hatcheries and in the wild has been very limited, compared with those occurring in growout facilities. Two new parasitic diseases in hatcheries were described here, one by the dinoflagellate, *Ichthyodinium* sp., and the other by the turbellarian, *Paravortex* sp. (or *Paravortex* spp.). In both infections, the causative agents transmit horizontally, i.e. the source of infection was thought to be contaminated water containing the infectious agent in the *Ichthyodinium* infection and newly born offspring from matured turbellarians which had infected spawners in the *Paravortex* sp./spp. infection.

It is very important to understand the infection cycle of these parasites in the hatcheries. In the case of *Ichthyodinium* infection, there may be a persistent infection among wild fish, and rearing water pumped into the facilities is contaminated with the infectious agent. Thus, transmission can be prevented by disinfecting the rearing water. In the case of *Paravortex* infection, it is likely that the spawner had already been infected with the turbellarian and the infection cycle started to be completed within the culture facility involving not only the spawner but also its progeny. Since eradication of the parasite from spawners is very difficult, prevention measures should be taken by rearing fry using a different water source from that of the spawner.

REFERENCES

Anshary, H., Ogawa, K., Higuchi, M. and Fujii, T. 2001. A study of long-term change in summer infection levels of Japanese flounder *Paralichthys olivaceus* with the monogenean *Neoheterobothrium hirame* in the central Sea of Japan, with an application of a new technique for collecting small parasites from the gill filaments. *Fish Pathology* 36:27-32.

Anshary, H., Yamamoto, E., Miyanaga, T. and Ogawa, K. 2002. Infection dynamics of the monogenean *Neoheterobothrium hirame* infecting Japanese flounder in the western Sea of Japan. *Fish Pathology* 37: 131-140.

Cannon, L.R.G. and Lester, R.J.G. 1988. Two turbellarians parasitic in fish. *Diseases of Aquatic Organisms* 5:15-22.

Fukuda, Y., Miyoshi, Y. and Ogawa, K. 2006. A species of turbellarian infecting cultured stonefish, *Inimicus japonicus*, with mortality. Abstract of oral presentation at autumn meeting of Japanese Society for Fish Pathology, Nagasaki, Japan.

Jones, B. 2005. Mass mortalities in the oceans. pp. 371-374. *In* Rohde K. (ed.). Marine Parasitology. CSIRO Publishing, Collingwood.

Kent, M.L. and Olson, Jr. A.C. 1986. Interrelationships between a parasitic turbellarian (*Paravortex* sp.) (Graffillidae: Rhabdocoela) and its marine host fishes. *Fish Pathology* 21:65-72.

Mori, K., Yamamoto, K., Teruya, K., Shiozawa, S., Yoseda, K., Sugaya, T., Shirakashi, S., Itoh, N. and Ogawa, K. 2007. Endoparasitic dinoflagellate of the genus *Ichthyodinium* infecting fertilized eggs and hatched larvae observed in the seed production of the coral trout *Plectropomus leoparadus*. *Fish Pathology* 42:49-57.

Noga, E.J. and Levy, M.G. 2006. Phylum Dinoflagellata. pp. 16-45. *In* Woo, P.T.K. (ed.). Fish diseases and disorders, Vol. 1. Protozoan and metazoan infections. Second ed. CAB Internat., Wallingford.

Ogawa, K. 1999. *Neoheterobothrium hirame* sp. nov. (Monogenea: Diclidophoridae) from the buccal cavity wall of Japanese flounder *Paralichthys olivaceus*. *Fish Pathology* 34:195-201.

Ogawa, K., Nakatsugawa, T. and Yasuzaki, M. 2004. Heavy metacercarial infection of cyprinid fishes in Uji River. *Fisheries Science* 70:132-140.

Ogawa, K., Rusinek, O. and Tanaka, M. 2007. New record of the leech *Limnotrachelobdella sinensis* infecting freshwater fish from Japanese waters. *Fish Pathology* 42:85-89.

Shirakashi, S., Teruya, K. and Ogawa, K. 2008. Altered behaviour and reduced survival of juvenile olive flounder infected by an invasive monogenean parasite *Neoheterobothrium hirame*: a possible cause for depletion of flounder population in Japan. *International Journal for Parasitology* 38:1513-1522.

Urabe, M., Ogawa, K., Nakatsugawa, T., Imanishi, Y., Kondo, T., Okunishi, T., Kaji, Y., Tanaka, H. 2001. Newly recorded gasterostome trematode (Digenea: Bucephalidae) in the Uji River: the life history, distribution and damage to fishes. *Bulletin Kansai Organisation for Nature Conservation* 23:13-21.

Urabe, M., Ogawa, K., Nakatsugawa, T., Nakai, K., Tanaka, M. and Wang, G. 2007. Morphology of two bucephalid trematodes collected from freshwater fishes in the Uji River, Kyoto, Japan. *Parasitology International* 56:269-272.

Yoshinaga, T., Kamaishi, T., Segawa, I., Kumagai, A., Nakayasu, C., Yamano, K., Takeuchi, T. and Sorimachi, M. 2000. Hematology, histopathology and the monogenean *Neoheterobothrium hirame* infection in anemic flounder. *Fish Pathology* 35:131-136.

Yuasa, K., Kamaishi, T., Mori, K., Hutapea, J. H., Permana, G.. N. and Nakazawa, A. 2007. Infection by a protozoan endoparasite in embryos and yolk-sac larvae of yellowfin tuna *Thunnus albacares*. *Fish Pathology* 42:59-66.