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Note

Case report: Mass mortality of olive flounder (*Paralichthys olivaceus*) caused by acute gas bubble disease

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This is the first report describing acute mass mortality occurred in juvenile olive flounder (*Paralichthys olivaceus*) caused by gas bubble disease (GBD). A total of 610 fish (average weight = 35 g), which were more than half of the fish acclimated at 17°C in an aquarium, were killed within two days of acclimation. The dead and moribund fish showed excessively opened opercula and mouths, and occasionally, severe exophthalmia. Through microscopic observation, numerous gas emboli were found in the gills of the dead and live fish, while the fish were not infected with any microbial pathogens. The dissolved oxygen (DO) saturation level of the rearing water and seawater nearby the facility reached 145% and 286%, respectively, whereas other water quality parameters (such as salinity, pH, and chemical oxygen demand) were normal. The extreme saturation rate of seawater in the shore nearby seemed to be due to an enormous algal bloom that occurred there. Through molecular identification based on 18S rDNA sequences, the most dominant algal species was most closely related to *Ulva californica* (99.87% sequence identity) followed by *U. prolifera*, *U. linza,* and *U. curvata* (99.81%). Therefore, it can be concluded that supersaturated seawater due to mass algal bloom caused gas bubble disease in the olive flounder, leading to mass mortality. After technical adjustment, such as increased aeration, lowered water circulation rate, and inlet water filtration using micro-pore carbon filters, the DO level became normal, no further mortality occurred and the status of the fish was stabilized.

Key words: Gas bubble disease, Hyperoxia, Olive flounder, Acute mortality, Algal bloom, *Ulva* sp.

Introduction

Dissolved oxygen (DO) is one of the most essential factors for the survival of aquatic organisms. Both hypoxia and hyperoxia could have severe impacts on physiology, growth, and the survival of fish (Mallya, 2007; Wang *et al*., 2018 and Roh *et al*., 2020). The supersaturation of water can be induced through excessive water pressure (e.g., hydroelectric plants) and photosynthesis in algae (Coffin *et al*., 2018 and Pleizier *et al*., 2020). When fish are exposed to water with supersaturated gas, air emboli can be formed in the skin, eyes, and gills, causing gas bubble disease (GBD) (Grahn *et al*., 2007). Previously, cases of acute mass mortality occurred in wild as well as various

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farmed fish species (e.g., Grahn *et al*., 2007; Machova *et al*., 2017 and Midilli *et al*., 2019). In general, over 110% gas saturation is known to be capable of causing GBD (Xue *et al*., 2019 and Pleizier *et al*., 2020). However, those studies showed that the severity of the disease could vary depending upon fish species, sizes, life stages, and environmental conditions. In this study, we report on GBD that occurred naturally in olive flounder (*Paralichthys olivaceus*) acclimated in an aquarium.

A total of approximately 1,200 healthy olive flounder (average weight = 35 g) were acclimated in six 1,500 L-sized cylindrical flow-through tanks in the aquarium of the Fisheries Science Institute, Pukyong National University (Busan, Korea). Inlet seawater was pumped inshore to the facility from 250 m at a depth of 10 m (Fig. 1) and, after sand filtration treatment, the water was introduced into the tank at a flow rate of ~1,000 L $hour^{-1}$ (circulation rate of 25 cycles per a day). More than 600 fish were dead within two days after they were housed in the facility (water temperature, 17°C). Ten dead fish and five moribund fish were sampled for microscopic observation and the presence of infection. The water quality in the rearing water in the tank and inlet seawater was measured including temperature, salinity, pH, DO, nitrite, ammonia, chemical oxygen demand), and phosphate according to the Water Pollution Standard method (KME, 2011). Additionally, the seawater near the facility was sampled to measure DO levels. Algal samples taken from seawater near the facility were moved to the laboratory and used for species identification based on 18S rDNA gene sequencing. Briefly, the genomic DNA of the algae was extracted using a DNeasy PowerSoil kit (Qiagen, Germany), and 18S rDNA was amplified using universal primer sets (SS5, GGT GAT CCT GCC AGT AGT CAT ATG CTT G; SS3, GAT CCT TCC GCA GGT TCA CCT ACG GAA ACC) according to a previously described method (Khaw *et al*., 2020). The PCR amplicon was purified using an AccuPrep PCR/Gel Purification Kit (Bioneer, Korea) and sequenced using a 3730XL DNA Analyzer (Thermo Fisher-Applied Biosciences, USA) by Bionics Co. (Korea).

In the clinical observations, most carcasses showed excessively opened opercula and mouths (Fig. 2A), and occasionally, severe exophthalmia (Fig. 2B). Although there were no pathogens isolated from the fish samples, numerous gas emboli were found in the lamellar arterioles of all sampled moribund and dead fish (Fig. 2C and D), which are common clinical traits of GBD in fish (Grahn *et al*., 2007). The concentration of DO in the rearing water, inlet water, and seawater near the facility was 11.4, 11.5, and 22.5

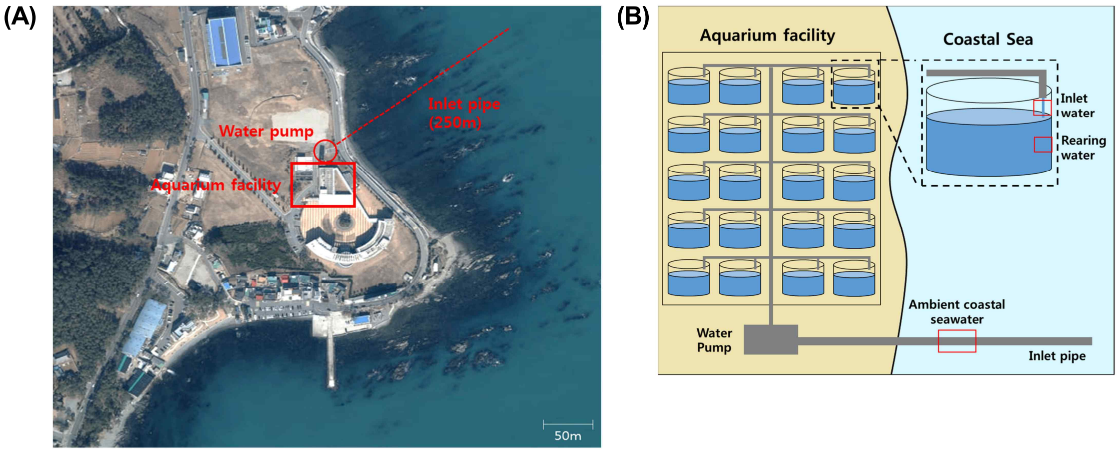

Fig. 1. Satellite image (A) and schematic diagram of the aquarium in the Institute of Fisheries Sciences (B).

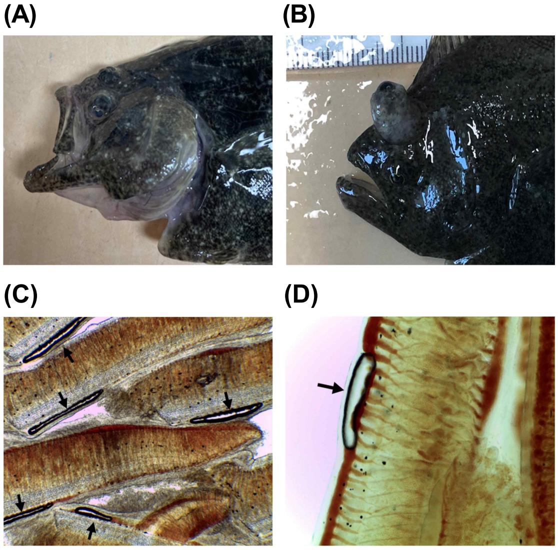

Fig. 2. Clinical signs of gas bubble disease in olive flounder. Almost all dead fish showed excessively opened opercula and mouths (A) and unilateral exophthalmos (B). Gas emboli (black arrows) in the lamellar arterioles were observed in the wet mount preparations under a light microscope (C, x100 magnification and D, x200 magnification).

mg L^{-1}, respectively, which corresponded to 145, 146, and 286% saturation at 17°C and 34 PSU (Table 1). Except for the DO level, the other water quality parameters were normal (Table 1). These results clearly indicated that the excessively supersaturated DO levels in the inlet water caused GBD in fish.

In an effort to identify the causative agent of the supersaturated seawater near the facility, we found an enormous algal bloom along the coast near the facility (Fig. 3). As a result, 1,588 base pairs of 18S rDNA sequence were retrieved (Genbank accession: OL 333871), which were most closely related to *Ulva californica* (99.87% sequence identity), followed by *U. prolifera*, *U. linza,* and *U. curvata* (99.81%). In recent years, massive blooms of green algae have been increasing in frequency worldwide due to coastal eutrophication and global warming (Smetacek and Zingone, 2013 and Kang and Chung, 2017). *Ulva* species are the typical macroalgae species responsible for green tides, causing tremendous ecological and economical losses worldwide (Ye *et al*., 2011). A previous study (Coffin *et al*., 2018) showed that the DO levels in estuaries were highly likely to be supersaturated (up to 25 mg L^{-1}) due to the presence of *Ulva* species. Similarly, a heavy algal bloom composed principally of *Chlamydomonas* induced extremely high supersaturation (32 mg L^{-1}), which even-

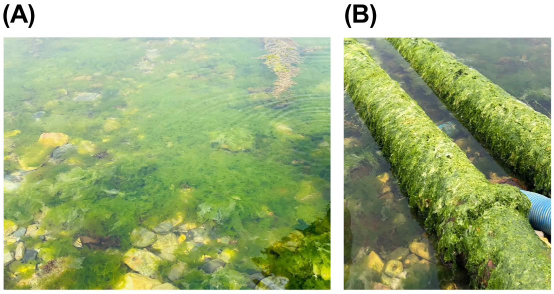

Fig 3. Algal bloom observed along the coastline (A) and the nearby inlet pipeline (B).

Table 1. Water quality measured in rearing water, inlet water, and coastal water

Parameters	Rearing water	Inlet water	Coastal water
Dissolved oxygen (mg L^{-1}) / Saturation rate (%)	11.4 / 145	11.5 / 146	22.5 / 286
Temperature (°C)	17	17	17
Salinity (psu)	34	34	NT*
pH	7.94	7.93	NT
Chemical oxygen demand (mg L^{-1})	0.8	0.6	NT
Ammonia (mg L^{-1})	0.025	0.019	NT
Nitrate (mg L^{-1})	0.019	0.019	NT
Phosphate (mg L^{-1})	0.010	0.007	NT

* NT, Not tested.

tually resulted in the occurrence of GBD and massive mortality in various wild fish (Woodbary, 1942). Consistent with those findings, the DO level of the inlet water in this study was 22.5 mg L^{-1}, indicating supersaturation, causing GBD and acute mass mortality.

To reduce DO concentrations in the seawater used, we temporarily minimized the introduction of inlet water from 1,000 to 100 L $hour^{-1}$ and maximized the aeration rate inside the tank, resulting in decreased DO levels from 11.4 to 8.8 mg L^{-1} (112% saturation). Similarly, Machova *et al*. (2017) showed that removing the cover of the holding tank and increasing the aeration level could successfully lower the saturation level from 136% to 110%. Additionally, the inlet water was filtered using triple carbon filters with 50, 10, and 1 um pore sizes. As the inlet water passed through the micro-pores, the supersaturated gas was de-aerated, which decreased the amount of dissolved gas in the water to under 100% and completely ceased the mortality.

This case report described acute mass mortality in olive flounder due to GBD caused by the supply of supersaturated seawater into an aquarium. The culprit in the gas supersaturation of seawater was a mass bloom of photosynthetic organisms (*Ulva* species in this case report).

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References

Coffin, M. R., Courtenay, S. C., Pater, C. C., &van den Heuvel, M. R. (2018). An empirical model using dissolved oxygen as an indicator for eutrophication at a regional scale. *Marine pollution bulletin*, 133, 261-270.

Grahn, B. H., Sangster, C., Breaux, C., Stephen, C., &Sandmeyer, L. (2007). Case report: clinical and pathologic manifestations of gas bubble disease in captive fish. *Journal of Exotic Pet Medicine*, 2(16), 104-112.

Kang, J. W., &Chung, I. K. (2017). The effects of eutrophication and acidification on the ecophysiology of Ulva pertusa Kjellman. *Journal of Applied Phycology*, 29(5), 2675-2683.

Khaw, Y. S., Khong, N. M. H., Shaharuddin, N. A., & Yusoff, F. M. (2020). A simple 18S rDNA approach for the identification of cultured eukaryotic microalgae with an emphasis on primers. *Journal of microbiological methods*, 172, 105890.

Korean Ministry of environment (KME). The Water Pollution Standard Method (2011)

Machova, J., Faina, R., Randak, T., Valentova, O., Steinbach, C., Kroupova, H. K., &Svobodova, Z. (2017). Fish death caused by gas bubble disease: a case report. *Veterinární medicína*, 62(4), 231-237.

Mallya, Y. J. (2007). The effects of dissolved oxygen on fish growth in aquaculture. *The United Nations University Fisheries Training Programme, Final Project*.

Midilli, S., Çoban, D., Güler, M., &Küçük, S. (2019). Gas bubble disease in Nile tilapia and hybrid red tilapia (Cichlidae, Oreochromis spp.) under culture conditions. *Journal of Fisheries and Aquatic Sciences (Su Ürünleri Dergisi)*, 36(3), 285-291.

Pleizier, N. K., Algera, D., Cooke, S. J., &Brauner, C. J. (2020). A meta-analysis of gas bubble trauma in fish. *Fish and Fisheries*, 21(6), 1175-1194.

Roh, H., Kim, B. S., Kim, A., Kim, N., Lee, M. K., Park, C. I., &Kim, D. H. (2020). Changes in hematoserological profiles and leukocyte redistribution in rainbow trout (Oncorhynchus mykiss) under progressive hypoxia. *Journal of fish pathol*ogy, 33(1), 23-34.

Smetacek, V., &Zingone, A. (2013). Green and golden seaweed tides on the rise. *Nature*, 504 (7478), 84-88.

Ye, N. H., Zhang, X. W., Mao, Y. Z., Liang, C. W., Xu, D., Zou, J., ... &Wang, Q. Y. (2011). 'Green tides' are overwhelming the coastline of our blue planet: taking the world's largest example. *Ecological Research*, 26(3), 477-485.

Wang, Y., Li, Y., An, R., &Li, K. (2018). Effects of total dissolved gas supersaturation on the

swimming performance of two endemic fish species in the upper Yangtze River. *Scientific reports*, 8(1), 1-9.
Woodbury, L. A. (1942). A sudden mortality of fishes accompanying a supersaturation of oxygen in Lake Waubesa, Wisconsin. *Transactions of the American Fisheries Society*, 71(1), 112-117.
Xue, S., Wang, Y., Liang, R., Li, K., &Li, R. (2019). Effects of total dissolved gas supersaturation in fish of different sizes and species. *International journal of environmental research and public health*, 16(13), 2444.

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