## Study on Noto Deep Ocean Water with Fish Stress Reduction Effect

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## Summary

Deep ocean water (DOW) is found 200 m below the surface of Earth's oceans. DOW has a very uniform low temperature  $(0-3^{\circ}C)$ , a salinity of about 3.5%, rich nutrients, and is clean. These characteristics may be useful for aquaculture. In fact, it has been reported that the growth of seaweeds and shrimp was promoted by breeding in DOW. Also, fish culture has been attempted using a closed recirculating aquaculture system with DOW. However, until now, there has been little scientific evidence regarding the mechanism of effectiveness for aquaculture. In the present study, therefore, we noticed the stress response of fish and measured plasma cortisol levels in fish bred in DOW.

Nibbler fish (*Girella punctata*) were caught by fishing in Tsukumo Bay of the Noto Peninsula (Ishikawa Prefecture). In addition, flounder (*Paralichthys olivaceus*) were purchased from a commercial source (Marinetech Co. Ltd., Aichi, Japan). These fish were used in the present experiments after acclimation for approximately two weeks. Nibbler fish and flounders were anesthetized with a 0.04% 2-phenoxyethanol (Wako Co. Ltd., Osaka, Japan) solution. To determine initial cortisol levels, blood sampling was performed. A heparinized syringe was used to collect blood samples from the caudal vessels of individual, anesthetized nibbler fish and flounders. The collected blood was put into 1.5 ml tubes. The tubes were then centrifuged at 15,000 rpm for 3 min. The separated plasma was immediately frozen and kept at -80°C until use. Every 5 and 10 days after both fish were bred in surface seawater and DOW, blood samples were taken again, and the plasma was separated by centrifugation as described above. Thereafter, the plasma cortisol level was determined using an ELISA kit (Cosmo Bio Co. Ltd., Tokyo, Japan).

Five days after breeding nibbler fish, the plasma cortisol levels of nibbler fish kept in the surface seawater were higher than those of nibbler fish kept in DOW, although there were no significant differences between plasma cortisol levels of fish kept in surface seawater and those kept in DOW. At 10 days, the plasma cortisol concentrations had increased remarkably in nibbler fish kept in surface seawater, although the plasma cortisol level in nibbler fish kept in DOW did not change from the initial level. A significant difference between the plasma cortisol levels of nibbler fish kept in DOW was obtained 10 days after breeding. In the case of the flounder, similar results were obtained. At 10 days after breeding, the plasma cortisol levels of flounders kept in surface seawater were significantly higher than those kept in DOW, just as with the nibbler fish. Experience has caused us to believe that long-term breeding without stressing fish is possible when we breed fish in DOW. We are the first to demonstrate that DOW influences stress responses in marine teleosts and may contribute to aquaculture.