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Production of *Benedenia*-resistant Yellowtail (*Seriola quinqueradiata*) Families –A Preliminary Approach to the Broodstock Candidates–

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Abstract: The skin fluke *Benedenia seriolae* is a parasite specific to *Seriola* species. It feeds on the epidermal tissues of yellowtail (*Seriola quinqueradiata*) and causes external injuries that render the fish susceptible to bacterial and viral infection. Infection with this parasite is a serious problem for yellowtail aquaculture because it also leads to a reduction in fish growth. A previous study has reported the existence of an inherited *Benedenia* disease-resistance factor in yellowtail. Any yellowtail families resistant to *Benedenia* disease have not yet been produced, although the production of such families for aquaculture would potentially help to reduce not only infectious diseases but also the labor costs to eradicate *Benedenia*. In this study, we investigated time series changes of the number of *Benedenia* parasites on each host yellowtail for the purpose of selecting *Benedenia* resistant broodstock candidates.

In September 2014, the number of individual parasites on 100 fish that were selected randomly from 10,000 wild-caught 0-age yellowtail at an aquaculture farm was investigated and it ranged from 1 to 48 individuals / fish. Thus we selected 961 fish that has the lowest 10% parasite susceptibility (3 or fewer *Benedenia*). The selected fish were then cultured in sea net cages and the number of parasites on each was counted at five times between November 2014 and July 2015. The average number of parasites per fish had a wide range from 0.2 to 39.4 individuals / fish over this period, and the overall mean was 8.9 individuals / fish. One hundred sixty fish with lower than 3 parasites were selected as broodstock candidates.

We are now using these broodstock candidates as parents to produce F1 yellowtail families for *Benedenia*-resistant analysis using DNA marker-assisted-selection breeding methods.

Key words: Yellowtail (*Seriola quinqueradiata*), Skin fluke (*Benedenia seriolae*), Breeding, *Benedenia*-resistant family.

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Introduction

Many fish species are farmed in Japan, and yellowtail is one of the most important species for aquaculture production (Fig. 1). In yellowtail aquaculture, wild- caught juveniles are farmed to marketable size (Nakada 2008). However, fishing season, abundance and size of wild juveniles are unstable. Additionally, fishing pressure on wild juveniles for aquaculture could impact the natural resources of yellowtail. For these reasons, using artificial seed is anticipated to enable a more stable and sustainable aquaculture production. Furthermore, farmers require seedlings with additional value such as disease resistance and rapid growth during culture (Yoshida *et al.* 2012).

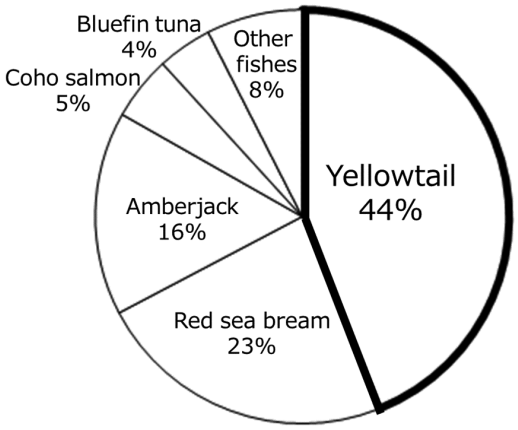

Fig. 1. Species composition of marine finfish aquaculture production in Japan 2013. Total production is approximately 240,000 tons (adapted from the 2013 census of fisheries by Fisheries Agency of Japan).

In yellowtail aquaculture, one of the serious problems is the parasitic disease caused by the skin fluke, *Benedenia seriolae*. It parasitizes the fish body surface and feeds on the epidermal tissues. The parasitization causes external injuries and increases the risk of viral or bacterial infections, as well as growth reduction of the host fish. At aquaculture farms, the most common method to eradicate the parasite is the freshwater bathing treatment. However, new parasites resurface within a few weeks after the treatment. Thus, the farmers have to treat the cultured fish many times during the aquaculture period and the treatment requires a great deal of effort, as well as causing stress to the fish via both handling and the freshwater treatment.

A previous study of genetic analysis reported a heritable *Benedenia* resistance factor in yellowtail (Ozaki *et al.* 2013). However, *Benedenia*-resistant yellowtail families have not yet been produced. The production of such families would help reduce both risks of infectious disease and efforts to eradicate the parasite in aquaculture facilities. In the present study, broodstock candidates with a low parasite count were selected for breeding value-added "*Benedenia*-resistant" yellowtail families.

Materials and methods

Preliminary selection

The preliminary process focused on selecting yellowtail with the lowest 10% parasite count among 10,000 wild-caught 0-age fish at Shimaura Aquafarm Branch, Maruha Nichiro Corporation, Miyazaki, Japan. Because there was no data available on the parasite intensity for the selection, the initial step of our study was to investigate the infection levels of the yellowtail.

In September 2014, the frequency distribution of the parasite number for 100 randomly selected fish was examined (the procedure of the parasite counts is described later). The parasite count per fish ranged from 1 to 48 individuals / fish, and the lowest 10% of them ranged from 1 to 3 individuals / fish (Fig. 2). From these data, we decided to select fish with 3

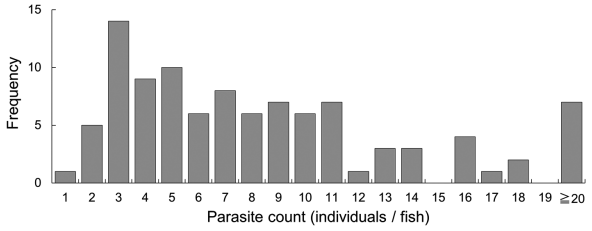

Fig. 2. Frequency distribution of the parasite count among the 100 wild-caught 0-age yellowtail. The frequencies from 20 to 48 (the highest value) individuals / fish range from 0 to 2.

or fewer parasites per fish, and 961 fish were preliminary selected from the sample of 10,000 yellowtail at the aquaculture farm. The mean of fork length and body weight of these fish were approximately 30.0 cm and 370 g, respectively.

Selection for broodstock candidates

In November 2014, these preliminary selected fish were transferred from the aquaculture farm to floating sea net cages at Goto Laboratory, Seikai National Fisheries Research Institute, Nagasaki, Japan. The mean (± SD) of fork length and body weight of these fish were 36.5 ± 1.5 cm and 828 ± 109 g, respectively. Of all the 961 fish, 800 fish that had no obvious physical damage were selected and passive integrated transponder (PIT) tags (Biomark Inc.) were inserted for individual identification. The fish were reared in 4 groups of 200 individuals in 4 net cages (A–D).

The examinations of parasite count on the experimental fish (detailed in Yoshida *et al*. 2012) were performed at 5 times during November 2014 and July 2015. Because the number of *Benedenia* parasites tends to decrease during winter, the counting of them was not conducted between December and March. At each counting, the experimental fish were individually captured in a mesh bag and then the bags were placed in a freshwater tank for 4 minutes (all *Benedenia* died within 4 minutes). After that, the fish was identified from the PIT tag, and the total number of parasites was obtained by summing the remaining dead parasites on the fish body surface and the removing ones in the bag.

Results and Discussion

Fig. 3 shows the changes in parasite count for 40 yellowtail with the lowest parasite count and 10 individuals with the highest parasite count in each net cage. Among 5 examinations, the average number of parasites per fish had a wide range from 0.2 to 39.4 individuals / fish over this period, and the overall mean parasite count was 8.9 individuals / fish.

Based on the results, a total of 160 fish (40 fish with the lowest parasite count were selected from each cage) were selected for broodstock candidates with low parasitic susceptibility. The mean (range) of parasite count on these candidates was 3.3 (0.2–7.0) individuals / fish. Additionally, the average parasite count for 9 fish was lower than 1.0 individual / fish during the experimental period, and these fish showed remarkably less-susceptibility.

The purpose of further study is to establish *Benedenia*-resistant yellowtail families. In 2017, F1 yellowtail hatchery juvenile will be produced from these selected broodstock candidates. Moreover, we are developing DNA markers for marker-assisted selection breeding methods. In the F1 generation, we will carry out both trait evaluation and also marker-assisted selection by genetic analyses. The goal of this study is fixation of the trait in the F2 generation and to produce *Benedenia*-resistant yellowtail families for development of sustainable yellowtail aquaculture

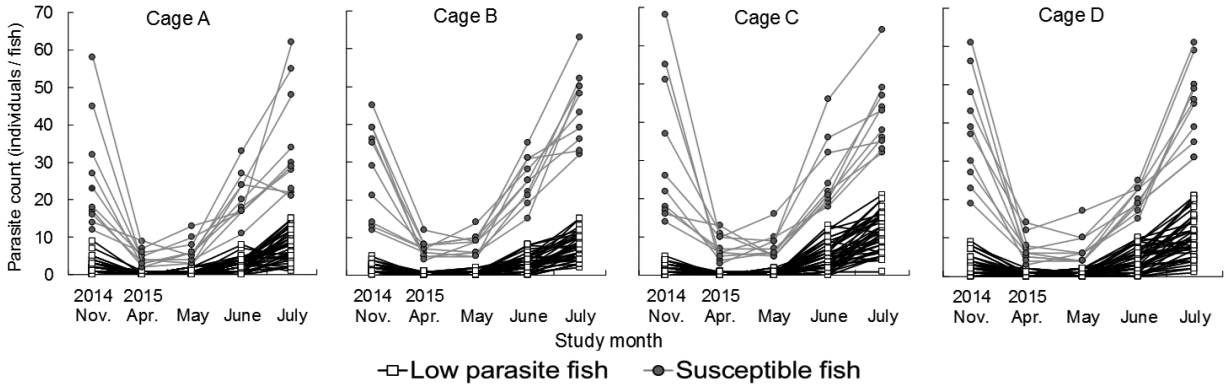

Fig. 3. Time series changes of parasite count per yellowtail by the fish cage. The lowest 40 and the highest 10 fish of parasite susceptibility were drawn. Because the number of *Benedenia* parasites tends to decrease during winter, counting of them was nod conducted between December and March.

production.

Acknowledgment

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Mushiake K., Yamazaki H., and Fujimoto H., 2007: Current Situation of Technical Developments in Seed Production of Yellowtail (*Seriola quinqueradiata*) in Japan. Proceedings of the thirty-fourth U.S.-Japan aquaculture panel symposium (ed. by Stickney R., Iwamoto R., and Rust M.), NOAA Technical Memorandum NMFS-F/SPO-85, Seattle, pp. 1-4.

Nakada M., 2008: Capture-based aquaculture of yellowtail. Capture-based aquaculture. Global overview (ed. By Lovatelli A. and Holthus P. F.), FAO Fisheries Technical Paper 508, FAO, Rome, pp. 199–215.

Ozaki A., Yoshida K., Fuji K., Kubota S., Kai W., Aoki J., Kawabata Y., Suzuki J., Akita K., Koyama T., Nakagawa M., Hotta T., Tsuzaki T., Okamoto N., Araki K., and Sakamoto T., 2013: Quantitative Trait Loci (QTL) Associated with Resistance to a Monogenean Parasite (*Benedenia seriolae*) in Yellowtail (*Seriola quinqueradiata*) through Genome Wide Analysis. *PLoS ONE* **8(6)**: e64987.

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Annotated Bibliography

(1) Ozaki A., Yoshida K., Fuji K., Kubota S., Kai W., Aoki J., Kawabata Y., Suzuki J., Akita K., Koyama T., Nakagawa M., Hotta T., Tsuzaki T., Okamoto N., Araki K., and Sakamoto T., 2013: Quantitative Trait Loci (QTL) Associated with Resistance to a Monogenean Parasite (*Benedenia seriolae*) in Yellowtail (*Seriola quinqueradiata*) through Genome Wide Analysis. *PLoS ONE* **8(6)**: e64987.

Benedenia infections caused by the monogenean fluke ectoparasite *Benedenia seriolae* seriously impact marine finfish aquaculture. Genetic variation in host has been inferred to play a significant role in determining the susceptibility to this parasitic disease. To evaluate the genetic basis of Benedenia disease resistance in yellowtail (*Seriola quinqueradiata*), a genome-wide and chromosome-wide linkage analyses were initiated using F1 yellowtail families (n = 90 per family) based on a high density linkage map with 860 microsatellite and 142 single nucleotide polymorphism (SNP) markers. Two major quantitative trait loci (QTL) regions on linkage groups Squ2 (BDR-1) and Squ20 (BDR-2) were identified. These QTL regions explained 32.9–35.5% of the phenotypic variance. On the other hand, the relationship between QTL for susceptibility to B. seriolae and QTL for fish body size were investigated. The QTL related to growth was found on another linkage group (Squ7). As a result, the authors present first genetic evidence that contributes to detailing phenotypic resistance to Benedenia disease, and the results will help resolve the mechanism of resistance to this important parasitic infection of yellowtail.

(2) Yoshida K., Ozaki A., Nakagawa M., Hotta T., Aoki J., Koyama T., Araki K., Okamoto N., Sakamoto T., and Tsuzaki T., 2013: Characteristic Evaluation Method Relating to Benedenia Disease of Yellowtail (*Seriola quinqueradiata*). Proceedings of the forty U.S.-Japan aquaculture panel symposium (ed. by Rust M., Olin,P. Bagwill A., and Fujitani M.), NOAA Technical Memorandum NMFS-F/SPO-136, Seattle, pp. 96-99.

Benedenia is a parasitic disease caused in Seriola species by *Benedenia seriolae*. This parasite can cause growth reduction and external injuries in yellowtail, increasing the risk of secondary viral or bacterial infection. The main method of parasite removal is to soak the fish in a freshwater bath. However, this method requires a great deal of time,

cost, and effort. We have been studying DNA Marker-Assisted Selection (MAS) breeding, to select for resistance to Benedenia disease. Three components ("Reproduction technology", "Character evaluation", and "DNA analysis") are critically important to promote MAS breeding success. We focus on one of the key components, "Characteristic evaluation method" relating to Benedenia disease in yellowtail.

(3) Mushiake K., Yamazaki H., and Fujimoto H., 2007: Current Situation of Technical Developments in Seed Production of Yellowtail (*Seriola quinqueradiata*) in Japan. Proceedings of the thirty-fourth U.S.-Japan aquaculture panel symposium (ed. by Stickney R., Iwamoto R., and Rust M.), NOAA Technical Memorandum NMFS-F/SPO-85, Seattle, pp. 1-4.

The National Center for Stock Enhancement (NCSE, formerly Japan sea-farming Association), of the Fisheries Research Agency, introduced the stock enhancement program for yellowtail (*Seriola quinqueradiata* and *Seriola lalandi*) in 1977. Technical developments in induced spawning as well as larval and juvenile rearing techniques have increased the population of this species to 1 million juveniles per year at NCSE. This project faced three major drawbacks: high mortality of larvae, cannibalism, and the smaller size of released juveniles in comparison with their wild counterparts. The high mortality of larvae was overcome by utilizing strong aeration during the early larval stage, while cannibalism was controlled by grading juveniles by size selection. The two-month delay in the spawning season of reared broodstock (the usual spawning season is late April to early May), which caused the smaller size of released juveniles, was solved by developments in advanced spawning techniques. Photoperiod and water temperature manipulations were used to produce eggs in February, thus producing yellowtail juveniles that can be released into the wild at a size similar to that of the wild stock.

(4) Nakada M., 2008: Capture-based aquaculture of yellowtail. Capture-based aquaculture. Global overview (ed. By Lovatelli A. and Holthus P. F.), FAO Fisheries Technical Paper 508, FAO, Rome, pp. 199–215.

The 2004 production of cultured yellowtail (*Seriola* spp.) in Japan from 1,288 enterprises was 150,028 tonnes valued at ¥111.2 billion (US$1.334 billion). Yellowtail mariculture has developed remarkably due to the abundant supply and low price of wild-caught juveniles (Mojako) and sardines used as the main fish feed of fishmeal component. Hatchery produced yellowtail seed are far more expensive. Other critical elements that supported the growth of yellowtail farming include the existence of abundant suitable culture sites along the Japanese coast and innovative technical developments.

The history of yellowtail culture in Japan began over 70 years ago. Before that, fishers cultured undersized fish in ponds and sold them when they reached marketable size. This utilization of bycatch (undersized fish) was accepted by the public, particularly as unmarketable fish were often used as fertilizer or livestock feed. Currently aquaculture production for many species exceeds that landed from capture fisheries.

Some commercial culture trials on amberjack have been undertaken in Taiwan, Province of China, Mexico and Vietnam, but no successes have been achieved with raising yellowtail. The main constraints include diseases and low production costs in tropical areas. In contrast, the culture of *Seriola* spp. is promising due to their strong vitality and rapid growth, and may well expand at the global level through hatchery-produced juveniles.